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

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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.

La première partie a été publiée dans le numéro 1-1988.

Cette deuxième partie rassemble les données publiées sur la cinétique et les altérations des leucocytes, les conséquences de l'infection sur les mécanismes de la coagulation sanguine (thrombocytopénie, variations des niveaux de fibrinogène et des autres facteurs de la coagulation sanguine), sur ses signes cliniques (temps de saignement et de prothrombine, hémorragie, coagulation intravasculaire), sur les modifications biochimiques du sérum (protéines sériques, acides aminés plasmatiques, haptoglobulines, hormones, glycoprotéines, glucose, lipides, créatinine sérique, kinines, sérotonine et histamine, enzymes sériques, cations et anions du sérum) et sur les altérations du liquide cérébrospinal. Elle se termine par une revue des facteurs qui influent sur la nature et la sévérité des réactions hématologiques et biochimiques chez les hôtes infectés. Dans sa conlusion, l'auteur déduit des données présentées, les caractéristiques communes des trypanosomoses humaines et animales : en premier lieu, il apparaît qu'il existe une relation directe entre le degré de parasitémie et la gravité des altérations hématologiques et biochimiques ; en second lieu, il devient possible de cerner les mécanismes qui permettent aux trypanosomes d'induire ces altérations. Enfin, une troisième caractéristique des trypanosomes est la diversité des facteurs qui concourent à aggraver un symptôme donné (anémie, leucopénie ou thrombocytopénie par exemple). Ces altérations hématologiques et biochimiques induites par les trypanosomes, couplées avec les lésions histopathologiques, sont généralement incompatibles, lorsqu'elles sont sévères, avec la vie de l'hôte. Dans les infections aiguës, la mort est attribuée aux altérations hématologiques et biochimiques. Dans les infections suraiguës, les modifications biochimiques en sont la cause principale, alors que ce sont les lésions organiques et les altérations hématologiques qui entraînent la mort dans les infections chroniques.

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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 trombocytopénie, 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.

LEUCOCYTES

Leucocyte kinetics have received less attention than erythrocyte changes in trypanosomiasis. Leucocytosis has been reported in *T. brucei* infections of highly tolerant deer mice (17) and two chimpanzees with concomitant secondary infections (26), in *T. gambiense* infections of rhesus monkeys (194) and man (59), and in *T. evansi* infections of camels, rats and buffalo calves (109, 219). Leucopenia has, more commonly, been reported in *T. brucei* infection of mice (6), in *T. rhodesiense* infection of chimpanzees (26), in *T. congolense* infection of cattle (119, 122, 144, 145, 163, 209, 212, 222, 224) and in *T. vivax* infections of cattle (67, 118, 144, 215, 221), sheep and goats (12) and mice (104). Leucopenia was associated with periods of high parasitaemia (224). Leucopenia was also reported in a dog infected with an unidentified trypanosome (186). On the other hand no consistent change was observed in monkeys infected with *T. rhodesiense* (182), while normal total leucocyte values were reported in *T. evansi* infections of cattle (219), camels, dogs and rats (161).

Neutrophilia was present in buffalo calves infected with *T. evansi* (191), and was accompanied by left shift in *T. evansi* infections of camels, dogs and rats (161, 219). More commonly, neutropenia has been reported in *T. brucei* infections of mice (111) and monkeys (26), *T. congolense* infection of cattle (122, 131, 144, 163, 209, 212), and in *T. vivax* infections of sheep and cattle (12, 67, 144). Normal neutrophil counts existed in monkeys infected with *T. gambiense* (194) and in *T. brucei* infection of mice in which left shift however occurred (6). Toxic granulation of neutrophils was reported in horses infected with *T. evansi* (98) and was accompanied by cytoplasmic vacuolation in cattle infected with *T. congolense* (209). Bone marrow neutrophil granulocyte reserve was considerably reduced in *T. vivax* infection of sheep (12) and *T. congolense* infection of cattle (209, 210). Phagocytosis of trypanosomes (*T. congolense*) by neutrophils has been reported (122, 224) and has been demonstrated by TEM in mice infected with *T. brucei* (8, 19) and in goats infected with *T. vivax* (22).

Eosinophilia was reported in *T. evansi* infections of camels and buffalo calves (161, 175, 191). Eosinopenia occurred, more consistently, in *T. brucei* infection of mice (6, 17), *T. evansi* infection of buffalo calves (219), *T. congolense* infection of cattle (122, 144, 163) and in *T. vivax* infections of sheep and cattle (12, 67, 144, 167, 221), and it appears to be pathognomonic haematological feature of trypanosomiasis. Basophils are seldom present in the blood of mammals, and are seldom mentioned in haematological studies of trypanosome-infected animals and man. However, a slight basophilia was observed in highly tolerant deer mice infected with *T. brucei* EATRO 110 (17) but was absent in CFLP mice infected with *T. brucei* 667 (6). Basophils were observed in close contact with *T. brucei* in the spleen of deer mice, with degranulation of several basophils in one mouse infected for 7 weeks (Fig. 6). The pathogenetic significance of this event is not immediately obvious. However, it may be at least partly responsible for the elevated histamine levels which was reported in mice infected with *T. brucei* (177).

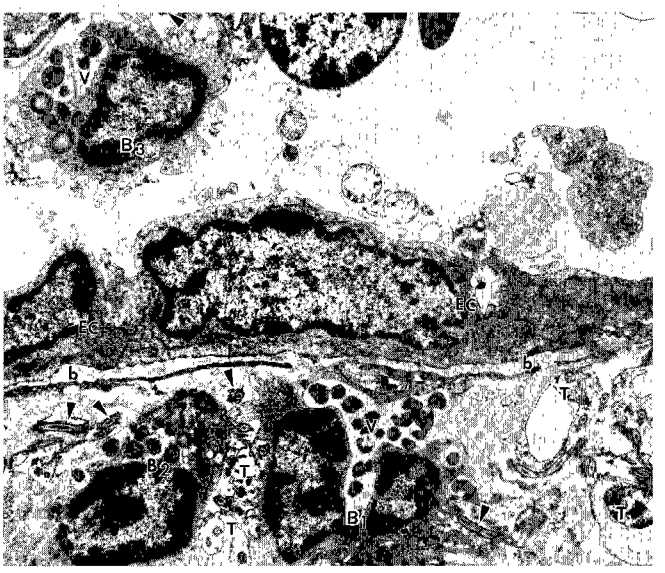

Fig. 6 : TEM of spleen of mouse infected with T. brucei *showing three basophils, two of which (B_1, B_2) lie in the Billroth cord and one (B_3) in the sinusoid, separated by sinus endothelial cells (EC) and basement membrane (b). Several trypanosomes (T) and their flagella (arrows) lie in close contact with the basophils. Two basophils (B_1, B_3) are discharging groups of granules massed in vacuoles (V), while the third basophil (B_2) is discharging two single granules. (x 8,600).*

Lymphocytosis was reported in *T. brucei* infections of tolerant deer mice (17), and rabbits (111), in *T. gambiense* infections of rhesus monkeys (194) and man (151), and in late phases of *T. congolense* infection of calves (209). Lymphopenia occurred, more frequently, in *T. brucei* infection of mice (6), *T. congolense* infection of cattle (131, 144, 163), in *T. vivax* infections of mice (104), sheep (12) and cattle (67), and in *T. evansi* infections of cow calves, buffalo calves, rats, and camels (161, 191). Lymphopenia was also associated with canine trypanosomiasis (186). An increase in numbers of immature and degenerate lyphocytes was reported in cattle infected with *T. congolense* (164).

Monocytosis is a consistent finding in trypanosomiasis and has been reported in *T. brucei* infections of mice and rabbits (6, 17, 112), in *T. vivax* infections of mice (104), sheep and cattle (12, 67) and *T. evansi* infections of camels and rats (161) and buffalo calves (219). No significant changes occurred in monocyte numbers in cattle infected with *T. vivax* and *T. congolense* (144). Erythrophagocytosis by circulating monocytes was reported in one study (224).

These results show that most trypanosome infections are characterized by leucopenia associated with neutropenia, lymphopenia, eosinopenia and monocytosis. Leucopenia becomes obvious as early as 7 days post-infection, and the lowest values are attained between the 7th and 14th days after infection, a period characterized by development of very intense parasitaemia (6, 12, 67, 117, 212, 221). There is a general tendency for the values to improve subsequently and in later phases of infection pre-infection values may be attained. Alleviation of leucopenia preceeded alleviation of anaemia in several studies (6, 12, 131, 221).

The aetiology of these leucocyte aberrations have not been thoroughly investigated, but several deductions can be made. With regards to the neutropenia and eosinopenia, one probable major factor particularly early in infection is marrow granulocyte hypoplasia. Thus total nucleated cell counts of right femurs of *T. brucei* infected mice dropped from 25.15 ± 1.02 ($\times 10^6$) in controls to 8.74 ± 1.30 and 13.10 ± 0.50 on the 7th and 14th days post infection respectively ; when this is coupled with a drop in the M:E ratio, it is obvious that neutrophil and eosinophil precursors are very significantly depressed (3, 6). This depressant action of trypanosome infection is supported by the reports that serum from cattle infected with *T. congolense* or *T. vivax* depressed myeloid colony (CFU-C) formation *in vitro*, the greatest depression occurring at those periods with marked leucopenia and neutropenia in the donor cattle (117). Further studies showed that the inhibitor of leucopoiesis *T. congolense* infection of cattle is probably a protein of molecular weight 100,000 to 200,000 ; however extracts of *T. congolense*, *T. brucei* and *T. theileri* did not depress CFU-C formation indicating that the inhibitor may not be a trypanosome fraction (119). The origin of the inhibitor is not clear but since plasma from infected animals depress colony formation, the inhibitor is found in plasma.

A second mechanism of neutropenia is splenic sequestration, since hypersplenism, which is thought to be associated with the splenomegaly present in trypanosomiasis, usually results in neutropenia (229). The observations that neutrophils increased from 0.4 p. 100 of cells in Billroth cords of the control spleens to 1.7 p. 100 in mice infected with *T. brucei* whose spleens were also enlarged 25.9 fold (20), and that total splenic granulocyte population increased from 3.5×10^6 in control mice to 12.8×10^6, 35.0×10^6, 121.5×10^6 on days 7, 16 and 60 post-infection, respectively, in mice infected with *T. congolense* which also showed a 20-fold increase in spleen size (152) show that marked trapping of neutrophils occurs in the spleen of infected animals. While some of the neutrophils may be present as a result of granulopoiesis, it is noteworthy that most of them were mature, and that neutrophil phagocytosis by macrophages was observed in the spleen and other tissues (19) and was also present in haemolymph nodes of sheep infected with *T. congolense* (134) and of goats infected with *T. vivax* (22). It has been suggested that granulocyte progenitor cells may be coated with trypanosome antigen-antibody as occurred with RBC leading to phagocytosis (117) ; presumably the same mechanism may be responsible for phagocytosis of mature neutrophils. It will be illuminating to study neutrophil kinetics, survival and sites of destruction in trypanosomiasis. Since neutrophils do not constitute any significant proportion of inflammatory cells in tissues and indeed are usually absent in these areas (14, 20, 21, 130), it is obvious that excessive utilization of neutrophils cannot account for neutropenia.

The eosinopenia which occurs frequently may be due to the same mechanisms causing neutropenia, *i.e.* decreased production and splenic sequestration, since the two cell types share a common origin. It was reported that marrow eosinophils were depressed in *T. congolense* infection of cattle (163, 164).

The presence of monocytosis in most infections indicates that the depression of myeloid colony formation reported (118) had no appreciable effects on the monocyte precursors suggesting that the inhibitor acted at a point after the developmental divergence of granulocyte and monocyte cell lines. Monocytosis was matched by a proliferation of macrophages in several tissues in trypanosome-infected animals (8, 13, 14, 19, 20, 21, 96, 152, 153, 158, 159). These macrophages are activated (19, 22, 78, 158) and epithelioid cells (19) and giant cells (148) are also formed. These changes, monocytosis, macrophage proliferation and activation, are stimulated by increa-

sed demands to remove particulate matter including trypanosomes, RBC, leucocytes and dead tissue cells. It has been amply demonstrated that the RES proliferates when its work load increases (110).

The lymphopenia which develops in most infections is presumably due to intense antigenic stimulation which increases the demands for lymphocytes to be transformed into plasma cells and transformed T-lymphocytes in affected tissues, where they usually form the predominant inflammatory cell types, as well as in the spleen and lymph nodes. To meet these demands the lymphoid organs of the spleen and lymph nodes are usually initially hyperplastic with increases in sizes of the lymphoid follicles and germinal centres, appearance of many lymphoblasts and plasma cells ; the lymphoid organs later become hypoplastic or similar to those of controls, in many cases with the loss germinal centres and thinning of the cortex (38, 131, 140, 141, 152, 153). In sheep and goats infected with *T. vivax*, animals that died of acute or subacute infection, which invariably had lymphopenia, usually had marked hypoplasia of lymphoid organs with depopulation of the nodules and disappearance of the germinal centres in the spleen, lymph nodes and haemolymph nodes (14). Despite the lymphoid hyperplasia which develops in the early phase, lymphopenia occurs at this time presumably because the lymphocytes are immobilized in the lymphoid organs as well as in the organs with inflammatory response. The persistence of lymphopenia in the later stages would be due to the hypoplasia of the lymphoid organs. Lymphocyte destruction, which is also a potential cause of lymphopenia appears uncommon although it has been reported in the thymus in one study (38). Phagocytosis of lymphocytes and plasma cells was reported by TEM in mice infected with *T. brucei* (19).

There are fewer reports of leucocytosis, lymphocytosis, neutrophilia and eosinophilia. Lymphocytosis may be associated with inherent enhanced tolerance to trypanosomiasis, as suggested by its development in highly tolerant deer mice which survived infection with *T. brucei* EATRO 110 for as long as 80 days while the same organism kills Swiss Webster mice in 4 or 5 days (17). It may also be associated with the recovery period of infection when parasitaemias have become very mild. Eosinophilia and neutrophilia have been reported in some *T. evansi* infections of buffalo calves, camels, dogs and rats (161, 175, 191) but not in other infections and it is not clear why they developed.

THE BLOOD CLOTTING MECHANISM

Platelets (thrombocytes)

Thrombocytopenia has been reported in several trypanosome infections including *T. rhodesiense* infections of man (29, 181), monkeys (182) and rats (52, 179), in *T. gambiense* infections of man and rats (87), *T. brucei* infections of rats and rabbits (112, 170), *T. vivax* infections of cattle (25, 103, 144, 145, 215, 224) and goats (9, 216) and in *T. congolense* infection of cattle (79, 144, 145, 173, 212, 223). Thrombocytopenia was also present in canine infection with unidentified trypanosome (186). In a recent comprehensive experiment, thrombocytopenia occurred in *T. rhodesiense* infections of rats, cattle, and goats, *T. brucei* infections of rats, cow, goats, water buck and buffalo, *T. gambiense* infection of rats, *T. congolense* infections of rats, cattle, and sheep, and in *T. vivax* infections of rats, cattle and goats, irrespective of whether the animals were infected by syringe intravenous passage or by tsetse challenge, enabling the author to assert that it is a uniform phenomenon in trypanosomiasis (50). Thrombocytopenia usually developed during the first peak of parasitaemia (9, 50, 222, 223), and its development preceeded the onset of anaemia (9, 52, 103, 179, 182, 222). For instance, platelet counts dropped from 1,064,000 ± 106,000 in control rats to 335,000 ± 114,000 at 96 hours after infection with *T. rhodesiense* whereas the haematocrit was the same (41.3 ± 3 p. 100) as the controls (41.0 ± 0.7 p. 100) ; at 120 hours after infection the platelet count dropped further to 18,000 ± 9,000 whereas the haematocrit was just down to 37.0 ± 3.6 p. 100 (52).

An inverse relationship was observed between the level of parasitaemia and the thrombocyte count in several trypanosome infections based on counts made during the first wave of parasitaemia (9, 50, 52, 223). In animals that developed repeated waves of parasitaemia, the thrombocyte counts failed to attain normal values even during the aparasitaemic periods between peaks (9, 50) ; their numbers however later increased (222) and attained low normal levels (215).

Associated with thrombocytopenia, thrombocyte survival decreased in human sleeping sickness caused by *T. rhodesiense* (181) and in cattle infected with *T. congolense* in which the half-life of platelets was 1.3 ± 0.5 days compared to 3.7 ± 0.5 days in control cattle (173). Following treatment with berenil, the mean platelet counts of 7 cows rose above normal in two weeks from their markedly depressed levels and

then subsequently decreased to normal (223). This pattern of response to treatment suggests the existence of marked expansion of thrombopoiesis in the bone marrow, which although surpassed by thrombocytopenic factors during infection, becomes expressed after treatment.

There are several speculations on the aetiology of thrombocytopenia occurring in trypanosomiasis. Three broad mechanisms are possible : decreased production and increased destruction or both. Megakaryocytes have been observed to be abundant (9, 182, 224) and normal (29) in the bone marrow in trypanosome infections. While these observations, and the upsurge of platelet production following berenil treatment (223), suggest normal or even increased thrombopoiesis, one study showed that there was dysthrombopoiesis based on an asynchrony observed between cytoplasmic maturation and megakaryocyte volume (208). These workers concluded in another paper that thrombopoiesis was ineffective based on the co-existence of increased megakaryocyte mass, reduced uptake of S53-methionine into circulating thrombocytes (*i.e.* reduced thrombopoiesis) and a normal platelet life span (79). These conflicting data make a categorical pronouncement on the status of thrombopoiesis difficult, and more studies are required to clear the point. The thickening of surface marginal zones of megakaryocytes of goats (9) may also interfere with platelet release.

Greater attention has been paid to the status of thrombocyte destruction in trypanosomiasis. The close relationship between the presence of trypanosomes in the blood and the degree of thrombocytopenia suggests a causal relationship between the two. Platelet aggregation with resultant thrombocytopenia caused by fresh motile *T. rhodesiense*, dead trypanosomes stored in refrigerator for 1 to 2 weeks, or by supernatant from disrupted trypanosomes has been demonstrated *in vitro*, and this was not blocked by inhibitors of ADP (*), kinins or complement (52). Platelet clumping was also observed in haemocytometer chambers in several trypanosome infections (50) and in *T. congolense* infection of cattle (222). TEM also showed that platelet clumping occurred in blood vessels while platelets showed disorganization of microtubules in *T. vivax* infection of goats (9). However, other workers could not produce platelet aggregation with live *T. gambiense* or its extracts (87). It is not clear whether the success with *T. rhodesiense* and the failure with *T. gambiense* in producing platelet aggregation are due to a basic difference between the two organisms (87). Platelets have been observed to adhere to trypanosomes including *T. equiperdum, T. evansi* and *T. rhodesiense in vitro* (51). It has been suggested that receptor sites on platelets with affinity for trypanosomes may exist and bring about platelet-trypanosome adhesion in the absence of antibody and complement (223). Trypanosome antigen-antibody coating of platelets appears to be another possible mechanism of platelet damage as shown by the ability of *T. vivax* antigen-antibody complexes to induce aggregation and release of radio-labelled serotonin from platelets (193) and by aggregation of platelets coated with trypanosomal antibody in the presence of specific trypanosomes (176). Anti-platelet antibody (IgM, IgG) not related to trypanosomes has been demonstrated in acute *T. vivax* infection of cattle (25, 103), but was not detected in human trypanosomiasis despite the presence of marked thrombocytopenia (181). Some workers have also demonstrated that thrombocytopenia develops rapidly prior to the production of antibody (50, 52), but this observation does not preclude the involvement of antibodies in sustaining the thrombocytopenia in later stages of the infection when antibodies become more abundant. Platelet adhesion and aggregation induces structural changes which may be irreversible in some situations. Thus TEM showed the inducement of degranulation, centralization of granules, presence of micelles and reduction of cytoplasmic density following aggregation (52) as well as loss of granules and disorganization of microtubules (9). Release of radio-labelled serotonin also occurred (193), which is a chemical manifestation of degranulation.

The pathomorphological changes induced by aggregation, coating by immune complexes and adhesion to trypanosomes predispose the platelets to splenic pooling and destruction, which has been demonstrated in human *T. rhodesiense* infection (181), resulting in decreased survival. Platelet phagocytosis has also been demonstrated by TEM in the spleen of mice infected with *T. brucei* (Fig. 7) and in haemolymph

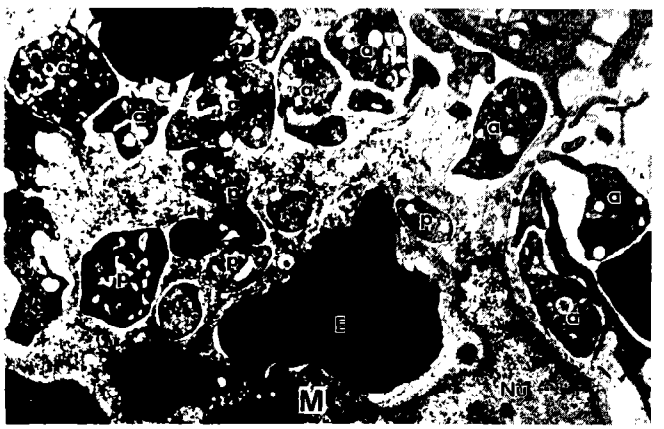

Fig. 7 : TEM of part of splenic macrophage (M) of mouse infected with T. brucei *showing phagocytosis of platelets (p) and one erhytrocyte (E). Nu = nucleus of macrophage. Several other platelets (a) lie outside the macrophage. (x 11,600).*

(*) ADP = adenosine diphosphate.

node of goats infected with *T. vivax* (9). Splenectomy alleviated the thrombocytopenia induced by *T. brucei* in rabbits (111) but not in calves infected with *T. congolense* (173).

Besides platelet damage and phagocytosis, there are two other possible mechanisms of thrombocytopenia. Disseminated intravascular coagulation (DIC) has been reported in several infections such as *T. rhodesiense* infection of man (29, 181), *T. congolense* infection of cattle (79, 223), and *T. vivax* infections of cattle and goats (14, 215, 216), and would contribute to the thrombocytopenia by causing excessive consumption of platelets. Secondly, deposition of platelets on vascular lesions has been described (83) and was suggested as a possible mechanism of thrombocytopenia (52). However, vascular lesions were not reported in *T. rhodesiense* (52) and *T. brucei* infections (20), suggesting that this mechanism may not play any significant role.

The contribution of each of the above mechanisms, and perhaps of others yet unknown, in precipitating thrombocytopenia has not been determined.

Fibrinogen and fibrin/fibrinogen degradation products (FDP)

Elevated fibrinogen levels have been reported in human *T. gambiense* infection (86), in 2 out of 4 persons infected with *T. rhodesiense* (181), in rats with acute *T. rhodesiense* infection (52), during the early phase of acute *T. congolense* infection of cattle (222), and in rabbits infected with *T. brucei* although in this study the values later dropped below normal 5 to 7 weeks post-infection (34, 71). Normal fibrinogen levels were reported in canine trypanosomiasis (186), in *T. rhodesiense* infection of cattle (228), and in *T. congolense* and *T. brucei* infections of cattle (80). Hypofibrinogenaemia occurred in human *T. rhodesiense* infection (29), in *T. brucei* infection of rats (170), and in *T. congolense* infection of cattle (79, 223).

Increased consumption of fibrinogen as judged by decreased half-life of isotope-labelled fibrinogen (79, 181), as well as increased production based on accelerated incorporation of isotope label into fibrinogen (79) have been reported. Increases in the serum concentration of fibrin/fibrinogen degradation products in *T. rhodesiense* infections of man and rats (29, 179, 181), in *T. vivax* infection of cattle (222) and *T. brucei* infection of rabbits (33, 35) have been reported, and further confirm increased consumption of fibrinogen. FDP were also elevated in a cow with acute fatal *T. congolense* infection but not in 3 others with chronic non-fatal infection (215), indicating that the increase in consumption of fibrinogen is proportional to the severity of disease. FDP values were also slightly higher in 18 human patients infected with *T. gambiense* (5.9 ± 4.9 µg/ml) than in non-infected controls (3.7 ± 4.8 µg/ml) (87).

Other blood clotting factors

The concentrations of blood clotting factors VIII, IX, XI doubled while that of factor XII increased six-fold in rabbits infected with *T. brucei* (36). Factor V remained normal in 4 persons infected with *T. rhodesiense* while factor VIII was reduced in one patient, increased in another and normal in two others (181). Factor VIII was also elevated in 4 goats and one horse infected with *T. vivax* or *T. congolense* but was slightly reduced in sheep ; in the same study, factor IX was not altered in sheep and goats (69). Factor V was depressed during acute phase of *T. brucei* infection of rats but not during the chronic phase (170).

Coagulation studies

Bleeding time increased in early acute *T. vivax* infection of cattle while coagulation times were unaltered (224). Prolonged and excessive bleeding has been observed in cut tail tips of rats infected with *T. rhodesiense* but during the last two days (days 8 and 9) of this fatal infection drawn blood clotted despite the presence of adequate anticoagulant (179). Accelerated clotting time of blood taken from sheep infected with *T. vivax* has been reported (14). Prothrombin time (PT) was prolonged in *T. brucei* infection of rats (170), in *T. rhodesiense* infection of a human patient (29) but not in another (181). PT was normal in rats infected with *T. rhodesiense* (179) and in sheep, goats and horses infected with *T. congolense* and *T. brucei* (69). PT was prolonged during the early acute phase of bovine *T. vivax* infection but became normal later when parasitaemia had waned (222). Partial thromboplastin time (PTT) was shortened in human *T. rhodesiense* infection (29) and in sheep, goats and horses infected with *T. brucei* and *T. congolense* (69), but was normal in a human *T. rhodesiense* infection (181). In another study, PTT was prolonged on the 4th and 5th days of infection but decreased terminally on days 8 and 9 in an acute *T. rhodesiense* infection of rats (179). Thus, both hypocoagulability and hypercoagulability states exist in acute trypanosomiasis, the latter often occurring before death.

Clinical disorders of blood coagulation

The aforementioned alterations in platelet numbers and concentrations of coagulation factors indicate that trypanosomiasis produces disorders of the blood clotting mechanism. Clinically, these disorders are manifested as haemorrhages and DIC.

Spontaneous haemorrhages have been reported in several trypanosome infections such as human sleeping sickness caused by *T. rhodesiense* in which bleeding occurred in the skin (29) or in digestive tract in which it was severe (181). They also occurred in *T. vivax* infection of cattle, sheep and goats in different tissues and organs such as the lymph nodes, subcutaneous tissue, peri-renal fat, muscles including the diaphragm, heart, kidneys, tongue, pleura, serosa of intestines, larynx, mucosae of digestive tract, gall bladder and vagina (14, 89, 92, 103, 160, 214, 215, 221, 222). The haemorrhages were associated with acute infection and did not occur in chronic infection. The causes of these haemorrhages include vascular endothelial damage, thrombocytopenia and depletion of some blood clotting factors.

DIC has been reported in several trypanosome infections based on alterations of coagulation factors (fibrinogen, Factors V, IX, X, XII), thrombocytopenia, an increase in FDP, and increased turnover of fibrinogen (14, 29, 79, 181, 215, 216, 223). Further, fibrin thrombi have been encountered in blood vessels of the liver, glomerulus, brain and testes in *T. vivax* infections of mice (104), sheep and goats (14) and cattle (105, 215). The mechanisms by which DIC develops in trypanosomiasis are presently speculative. It is thought to be precipitated by auto-antibodies against fibrinogen (50), procoagulants released by damaged RBC and platelets (29, 181), by antigen-antibody complexes (29, 50, 182), and by vascular injury (29, 181). The major consequences of DIC include fibrin deposition in blood vessels which may lead to vascular occlusion, and haemostatic failure due to the combined effects of excessive consumption with depletion of coagulation factors and platelets, and an interference with the clotting mechanisms by FDP which inhibit platelet function and interfere with fibrin formation and polymerization (229). Vascular occlusion has been reported in *T. vivax* infections, and although infarction is seldom reported, it was present in the testes of infected sheep in which marked coagulative necrosis of testicular tissue occurred (13). The haemorrhages which have been mentioned may at least partly be due to depletion of coagulation factors by DIC.

The usual compensatory mechanisms which accompany DIC include fibrinolysis, repletion of coagulation factors and platelets, and the clearance of FDP, activating/inducing factors and activated coagulation factors by the reticuloendothelial system and liver (229). This review has shown that fibrinolysis with formation of FDP, repletion of coagulation factors, increased production of fibrinogen are operative in various infections. The activated MPS most likely would also be controlling DIC by clearance of the stimuli and activated coagulation factors. Further, since the liver does not develop any severe pathology, its capacity to produce liver-derived coagulation factors is probably high. It has been stated that « the altimate outcome (of DIC) is determined by a dynamic interplay between the various pathologic processes and compensatory mechanisms, *i.e.* fibrin deposition vs fibrinolysis, depletion vs repletion of coagulation factors and platelets, production vs clearance of fibrin, FDP and other products of coagulation » (229). This interplay explains the divergent results and conclusions of many workers on the existence and degree of DIC in trypanosomiasis. Nevertheless it is noteworthy that the manifestations of DIC tended to be associated with acute trypanosome infections, as shown by observations that one cow with acute *T. congolense* infection had DIC while 3 others with chronic infection did not (215), that haemorrhages and/or fibrin thrombi occurred in sheep and goats with acute *T. vivax* infection but not in those with chronic infection (14), and that DIC occurred in some humans but not in others in *T. rhodesiense* infection (181) while it was completely absent in human *T. gambiense* infection (87). *T. rhodesiense* produces a more acute infection in man than *T. gambiense* (60). It would seem, therefore, that where pathologic mechanisms overwhelm the conpensatory, as would occur in acute trypanosomiasis, the signs of DIC are marked. In contrast, in the less severe infections the compensatory mechanisms are sufficient to balance or even outstrip the pathologic so that laboratory tests indicate normal or elevated levels of coagulation factors. This latter alternative corresponds to the chronic or compensated DIC in which « a balance is reached between destruction and production of coagulation factors » (229).

SERUM BIOCHEMICAL CHANGES

Serum proteins

Changes in the serum proteins have received considerable attention. Many investigators have reported normal total protein levels in *T. rhodesiense* infections of monkeys (194) and mice (150), *T. evansi* infections of buffalo calves (190, 218), calves (220) and camels (108), and in *T. congolense* infection of mice (227). Other workers reported elevated total protein in *T. vivax* infections of sheep and goats (11, 44), and in *T. brucei* infection of rabbits (71), while reduced values have been reported in *T. rhodesiense* infection of monkeys (182) and in *T. congolense* infection of cattle (199, 224). Studies with *T. brucei* infection of deer mice showed that infected mice which became anaemic had normal total protein levels while the non-anaemic infected mice, an apparently more resistant

group, had elevated serum protein levels (17), indicating that the severity of disease influences total protein levels and may be responsible for the inconsistency of the above reports. It is further noteworthy that since infected animals usually developed elevated plasma volumes to compensate for decreases in RBC volume, the plasma solute concentration including proteins will be diluted to the extent of such plasma expansion.

Fractionization of plasma proteins showed that albumin values decreased in several infections (17, 44, 71, 99, 108, 182, 190, 192, 194, 218, 220, 224, 227, 231, 233), while total globulin levels were always elevated (17, 71, 99, 190, 194, 218, 227, 232), resulting in a reduced albumin : globulin ratio (99, 190). It is again noteworthy that due to plasma expansion, the actual total albumin and globulin levels will be greater than the apparent values reported. In fact, the decrease in albumin levels may to a large extent be due to this plasma expansion, since the hepatocytes which synthesize albumin do not develop any severe pathology.

Hypergammaglobulinaemia was consistently reported in several infections (11, 17, 44, 56, 108, 150, 182, 192, 220, 231) although in one study low levels were reported early in infection while normal levels occurred later (224). It was also shown that acutely infected muturu cattle with fatal disease developed sub-normal γ-globulin levels while those with chronic non-fatal infection had elevated γ-globulin levels (56). A-globulin levels were either normal (11, 182) or depressed (224). A_1-globulin was elevated in mice infected with *T. brucei* while α_2-globulin levels were normal (17). β-globulin levels were either normal (182) or reduced (11, 44, 224). β_2-globulin levels were normal in *T. brucei* infected deer mice which became anaemic while the non-anaemic infected mice had elevated values ; β_1-globulin levels were unchanged in the two groups (17).

These results show that the most consistent changes in serum protein levels are depression of albumin levels and elevation of globulin levels due to hypergammaglobulinaemia.

Fractionization of the γ-globulins showed that elevation of IgM levels is a very consistent finding in trypanosomiasis, occurring in *T. congolense* infection of mice (153, 227), *T. congolense* and *T. vivax* infections of cattle (124, 132, 141, 200), *T. brucei* infection of mice (2), *T. equiperdum* infection of mice (157), in *T. rhodesiense* infections of mice (55) and monkeys (188), in human sleeping sickness caused by *T. rhodesiense* and *T. gambiense* (40, 41, 126, 143, 149, 181), and in *T. evansi* infection of buffalo calves (192). However, a normal IgM level was recorded in a human *T. rhodesiense* infection (29). A cow with acute *T. congolense* infection showed an initial slight increase in IgM levels but subsequently developed subnormal levels prior to death (132), which is in consonance with the low γ-globulin levels reported in muturu cattle with acute fatal *T. vivax* infection (56).

Serum IgG levels were normal in human sleeping sickness caused by *T. rhodesiense* (29, 181), while elevated values accompanied human *T. gambiense* infection (40, 41, 126) and *T. rhodesiense* infections of monkeys (188) and mice (55). Serum IgG levels were high in one case and within the lower end of normal in another, in human *T. rhodesiense* infection (232). IgG_1 levels were normal in *T. vivax* and *T. congolense* infections of cattle (132, 141, 200) and *T. congolense* infection of mice (227), but subnormal in one *T. vivax* infection (200) and elevated in one study of *T. congolense* infection of cattle (125). IgG_2 levels were normal in *T. vivax* and *T. congolense* infections of cattle (132, 141), but elevated in *T. congolense* infections of mice (153, 227), and cattle (125, 200). Serum IgA was normal in human *T. rhodesiense* infection (29, 181).

In general, therefore, increases in IgM levels are more commonly reported and pronounced than increases in IgG levels. In most instances, except in *T. equiperdum* infection of mice (157), the IgM levels were greater than IgG levels. The increases in these immunoglobulin levels are associated with polyclonal stimulation of B-lymphocytes (139, 226).

The complement C3 was depressed in *T. gambiense* infection of man (87, 126), and *T. vivax* and *T. congolense* infections of cattle (125, 199). The fall in C3 levels in cattle infected with *T. congolense* was associated with the initial parasitaemia, drop in PCV values and increase in complement fixing antibody (125). The ability of mice infected with *T. rhodesiense* to clear IgM-sensitized trypanosomes was not affected by depression of C3 levels by cobra venon factor (55) but a similar study with *T. brucei* demonstrated that mice with depressed C3 levels showed reduced clearance of trypanosomes (146).

Plasma amino acids

Studies with *T. vivax* infections of sheep (106) and cattle (107) and with *T. gambiense* infection of voles (166) demonstrated that trypanosomiasis produces marked alterations in the amino acid (AA) profiles. Thus, *T. gambiense* infection ablated the usual diurnal variation in AA in voles (166), while a general hypoaminoacidaemia occurred (107, 166). The more significant changes in profiles of individual AA included marked decreases in tryptophan (166), and moderate decreases in threonine and tyrosine (106, 107, 166). Decreases in arginine and asparagine also occurred in *T. vivax* infections (106, 107), while serine, valine, isoleucine and leucine decreased in *T. gambiense* infection (166). Alanine values increased (106, 166). The aetiology and implications of these aberrations are not

exactly clear. Some of the changes were attributed to non-utilization by the host of some AA, utilization of others for trypanosome metabolism and secretion of others by trypanosomes (106, 107). The depression of tryptophan and tyrosine was thought to be related to some neuropsychiatric syndromes in trypanosomiasis, while the increase in alanine was attributed to abnormal carbohydrate metabolism (166).

Haptoglobin

Serum haptoglobin decreased in human African trypanosomiasis (40) and completely disappeared in *T. vivax* infection of cattle (68). This was attributed to the possible existence of gradual intravascular haemolysis which depleted haptoglobin (68), a plasma protein with binds free haemoglobulin.

Hormones

Serum levels of follicle stimulating hormone and luteinising hormone were significantly depressed in human *T. gambiense* infection (65). These changes were thought to be related to the marked testicular pathology that has been reported by several workers (13, 21).

Glycoproteins

One study showed that the plasma concentrations of the glycoproteins hexose, hexosamine, sialic acid and seromucoid fraction increased in cattle infected with *T. brucei* and *T. vivax* during the first eight weeks of infection when parasites appeared in the blood, but declined from the tenth week when parasites had become very scanty (136). These increases were thought to result from release of glycoproteins from organs invaded by the organisms. A more recent study demonstrated that erythrocyte surface sialic acid levels decreased in *T. vivax* infection of cattle, coinciding with waves of parasitaemia ; further, the serum sialic acid levels were slightly increased on day 8 of infection coinciding with the decrease in erythrocyte surface sialic acid during the first wave of parasitaemia (66). It was postulated from this study that the reduction in erythrocyte surface sialic acid was caused by its cleavage by trypanosome neuraminidase, and this was assumed to be responsible for destruction of erythrocytes early in infection and for the increase in serum sialic acid levels.

Glucose

Hypoglycaemia occurred in peracute *T. simiae* infection of pigs, and in rodent trypanosomiasis in which the animals succumb to a single wave of massive parasitaemia, such as *T. rhodesiense* infection of mice (150) and *T. congolense* infection of mice (227), in which the hypoglycaemia was maximal terminally. Hypoglycaemia also occurred at periods with high parasitaemia (92) or terminally (224) in cattle with acute *T. congolense* and *T. vivax* infections but did not occur in chronically infected cattle (224), or in chronic *T. rhodesiense* infections of cattle (228), monkeys (182) and man (29). Glucose levels were similarly depressed in the acute phase of *T. evansi* infection of buffalo calves, but fluctuated during the chronic phase (190).

Hypoglycaemia is therefore precipitated by the presence of large numbers of parasites in the circulation presumably because of utilization of the glucose for trypanosome metabolism. It is pertinent that glucose infusion increased the survival time of pigs infected with *T. simiae* from the usual 5 to 12 days to 30 days (92).

Lipids

Total plasma lipids decreased in *T. congolense* infection of sheep (180) and *T. vivax* infection of cattle (215). On the other hand, lipids increased in rabbits infected with *T. gambiense* (57). Plasma cholesterol levels were elevated in *T. gambiense* infection of rabbits (57) and in rats infected with *T. rhodesiense* (217).

Blood urea nitrogen (BUN)

Urea and non-protein nitrogen levels increased in cattle infected with *T. vivax* and/or *T. congolense* (92, 107). High urea values were also reported early in *T. congolense* infection of cattle when parasitaemia was highest while normal values returned in animals that had chronic infection (224). One cow with severe *T. vivax* infection showed high levels of blood urea terminally while 4 others with moderate disease had normal levels (215). Elevated urea levels have also been reported in *T. rhodesiense* infections of monkeys (182) and mice (150). In mice infected with *T. congolense*, blood urea levels rose by 23 p. 100 by the 8th day of infection, but in mice that became moribund between the 8th and 10th days of infection the urea levels had increased by 275.7 p. 100 (227). These results suggest that urea levels are elevated at periods with high parasitaemia and presumably fever which occur in acute infection and sometimes terminally. The causes of elevated BUN levels include kidney disease such as glomerulonephritis, as well as urinary tract obstruction and excessive protein catabolism associated with severe toxic and febrile conditions (127). Fever and glomerulonephritis are common fea-

tures of trypanosomiasis and presumably act together to elevate BUN.

Serum creatinine

Serum creatinine (SC) levels increased in monkeys infected with *T. rhodesiense* particularly in the first week of infection (182), and in *T. congolense* infection of mice (150). SC levels were normal (1.5 mg/dl) in human *T. rhodesiense* infection (29), while normal levels existed in the first 8 weeks of *T. congolense* infection of cattle with slightly subnormal levels occurring between the 11th and 31st weeks of infection (224).

Bromosulphalein (BSP) retention

The only study on this showed that BSP retention rose from 6.3 p. 100 to 15.3 p. 100 in mice infected with acute *T. rhodesiense* for 5 days (150). This suggests liver dysfunction, and it is noteworthy that a small percentage of hepatocytes were degenerate in *T. brucei* infection of mice (20).

Kinins, serotonin and histamine

Bradykinin levels increased in human *T. rhodesiense* infection (32), in *T. brucei* infections of cattle (31) and mice (177), and in *T. vivax* infections of goats (216) and cattle (215). For instance, bradykinin levels rose from 15 ± 6.9 ng in control mice to 84 ± 19.1 ng in mice infected with *T. brucei* (177). The bradykinin peak occurred 2 to 3 days after the peak of parasitaemia (31). Following berenil treatment, the bradykinin levels quickly returned to normal (31, 32). The level of kininogen, the α_2-globulin precursor of bradykinin, was depressed in *T. brucei* infections of mice (177) and cattle (31), and in human *T. rhodesiense* infection (32) ; this change was reversed by berenil treatment in both studies. Kininogen is cleaved to bradykinin by kallikren ; plasma kallikren levels increased while the level of its precursor prekallikren was inversely depressed in rabbits infected with *T. brucei* (31).

Blood serotonin levels decreased during the early acute phase of *T. vivax* infections of goats and cattle, and the decreases were associated with temperature peaks and parasitaemic peaks as well as with thrombocytopenia, fever and platelet aggregation (214, 215, 216). Blood histamine levels increased in mice infected with *T. brucei* (177).

Serum enzymes

Serum glutamic oxaloacetic transaminase (SGOT) activity increased markedly in *T. rhodesiense* infections of mice (150), man (29) and monkeys (182), while slight increases were reported in *T. vivax* infections of cattle and sheep (84, 222) and *T. evansi* infection of buffalo-calves (190). SGOT values decreased during the first 8 weeks of *T. congolense* infection of cattle, remaining stable thereafter except in one animal which developed increasing levels for 3 weeks before death (224). Serum glutamic pyruvate transaminase (SGPT) levels markedly increased in *T. rhodesiense* infections of mice (150) and monkeys (182), rose sharply in *T. vivax* infections of sheep and cattle (84, 196), and increased mildly in *T. evansi* infection of buffalo-calves (190). Serum aspartate transaminase and alanine transaminase markedly increased in *T. congolense* infection of mice (227). Lactic dehydrogenase levels increased markeldy in acute *T. congolense* infection of mice (227) and in *T. rhodesiense* infection of man (29) but decreased in *T. vivax* infection of cattle (222) and was normal in *T. gambiense* infection of rats despite the occurrence of widespread necrotic lesions (57). Tyrosine transaminase (TAT) levels also increased in the serum and liver of voles infected with *T. gambiense*, the serum levels correlating with parasitaemia but poorly with hepatic levels ; the serum TAT was thought to arise from the trypanosomes (198). The causes of the increases in transaminase activities could be specific organ damage and/or release of parasite metabolic products (150, 190, 227). The observation that very marked increases in transaminases occurred in mice infected with *T. rhodesiense* for 5 days (150) or *T. congolense* for 8 days (227), which are both too short for any significant organ damage to occur, suggests that most of the transaminases are derived from the parasites.

Serum cations and anions*

Serum NaCl levels increased in cattle infected with *T. congolense* (77). Hyponatraemia was reported in human *T. rhodesiense* infection (29), and in *T. evansi* infection of camels (175), while blood Na+ levels were normal in acute *T. rhodesiense* infection of mice (150). Serum K+ decreased in *T. evansi* infection of camels (175), was normal in *T. rhodesiense* infection of mice (150) but increased in *T. brucei* and *T. equiperdum* infections of rats (100, 101, 234). The increases in K+ were correlated with decreases in RBC values and were attributed to release of K+ from RBC and damaged tissue coupled with the effects of kidney damage (100). Serum Ca++ levels were normal in acute *T. rhodesiense* infection of mice (150), reduced in *T. evansi* infections of camels (175) and buffalo calves (190), and during relapses in *T. congolense* infection of cattle (77). Phosphate levels decreased in

* Na+ = sodium, K+ = potassium, Ca++ = calcium, Mg++ = magnesium, NaCl = sodium chloride.

Retour au menu

T. evansi infection of camels (175) and *T. congolense* infection of cattle (77) but was normal in *T. rhodesiense* infection of mice (150) and in *T. evansi* infection of buffalo calves (190). Depression of Ca++ and phosphate levels were thought to be due to severe damage to the thyroid gland (77). Serum Mg++ levels decreased during relapses but returned to normal thereafter in cattle infected with *T. congolense* (77). Plasma copper levels fluctuated in cattle infected with *T. vivax* or *T. congolense* but remained within normal limits (184).

CEREBROSPINAL FLUID

The cerebrospinal fluid (CSF) of a patient infected with *T. rhodesiense* had increased pressure (29). The quantity of CSF was thought to be increased in bovine *T. vivax* infection (92). Generally, the colour of CSF was normal except in those samples contaminated with blood during collection (43, 92, 97). Trypanosomes were absent from CSF of sheep and cattle infected with *T. vivax* and *T. congolense* (42, 43) except in one sheep in which blood contamination occurred (43). *T. brucei* was present in the CSF of 18 out of 20 infected sheep although only 9 out of the 18 had the organisms in the blood at the time of collection of CSF (97). *T. brucei* was also present in the CSF of some infected horses (165) but was absent in 3 cattle infected for 9 1/2 months (142). However *T. brucei* appeared at 3, 5 and 5 1/2 months in the CSF of 3 out of 6 cattle simultaneously infected with *T. brucei* and *T. congolense*, while one out of 3 other cattle which received *T. brucei* 1.2 years after infection with *T. congolense* showed *T. congolense* in the CSF (142). *T. rhodesiense* was present in the CSF of all 6 infected persons (54) but was absent in an infected man (29). *T. gambiense* was present in the CSF of 9 out of 18 human patients (85) and in 9 out of another 11 patients (87).

CSF cell counts were normal in some infections (29, 43, 142) but were elevated in others (26, 97, 188). Differential counts showed that small lymphocytes were most numerous, with smaller numbers of macrophages, plasma cells, morula cells of Mott, and neutrophils (87, 96, 188). Protein levels were normal in a case of human *T. rhodesiense* infection (29), in *T. vivax* and *T. congolense* infections of sheep and cattle (43) and in 8 out of 16 sheep infected with *T. brucei* (97). Elevated protein levels occurred in *T. brucei* infection of a chimpanzee (26) and in 8 out of 16 sheep infected with *T. brucei* (97). The albumin and C3 concentrations of CSF were moderately increased in *T. rhodesiense* infection of man (126). IgM levels were elevated in *T. brucei* and *T. rhodesiense* infections of chimpanzee (26), and monkeys (188), in *T. gambiense* infection of man (87), and in human *T. rhodesiense* infection (126, 226). IgG levels were also elevated in these infections (87, 126, 188, 226). IgM and/or IgG anti-trypanosome antibodies were not detected in CSF of cattle infected with *T. brucei* alone but antibodies to *T. brucei* and *T. congolense* were present in CSF of animals infected simultaneously with both organisms (142). Studies with ^{125}I-IgM have demonstrated that the IgM in CSF is locally synthesized and does not originate from the blood (85). The plasma cells and Mott cells in CSF showed immunofluorescence to IgM and less commonly to IgG and seldom to IgA (85). CSF glucose was normal in human *T. rhodesiense* infection (29), while FDP was detected in most patients with *T. gambiense* but not in non-infected persons (87).

These results show that marked alterations of CSF usually occur when the trypanosomes spread to the CSF which occurred fairly commonly in the chronic infections produced by the tissue-invading organisms (*T. brucei, T. gambiense, T. rhodesiense*). The inability of *T. brucei* to appear in the CSF of cattle infected for 9 1/2 months whereas invasion was achieved with simultaneous infections with *T. brucei* and *T. congolense* (142) is noteworthy.

FACTORS AFFECTING HAEMATOLOGICAL AND BIOCHEMICAL REACTIONS

Several factors influence the nature and severity of the haematological and biochemical changes in infected hosts. The strains of a trypanosome species vary in virulence, those with greater virulence inducing more severe haematological and biochemical alterations, as was reported in *T. brucei* infection of rabbits (113, 147) and in mice infected with *T. congolense* (227). Conversely, parasitaemia developed faster, was greater and persisted longer in zebu than in the more tolerant N'Dama cattle exposed to the same field infection ; the anaemia was more severe in the zebu than in the N'Dama (23, 48). Haematological changes in natural outbreaks of haemorrhagic *T. vivax* infection in cattle (160) were similar to those induced by subsequent experimental bovine infections with an isolate from these outbreaks (222), indicating that the mode of transmission did not influence pathogenicity.

The existence of individual variability in susceptibility to a given trypanosome infection produces variations in the severity of haematological and biochemical lesions in groups of animals infected with the same organism as have been reported in *T. vivax* infections of cattle (144, 185, 215, 221, 224), sheep and goats (12), and in *T. congolense* infection of cattle (144). The causes of such individual variability are probably

several, and may include age, nutritional status, individual history of exposure to the trypanosomes, genetic factors such as haemoglobin types, and occurrence of secondary infections. For instance, it was noted that anaemia was less severe in neonatal calves than in 6 month-old calves (212). Cattle with *T. vivax* and concomitant *Anaplasma marginale* and/or *Babesia bigemina* infections developed acute disease with rapid development of anaemia, while other animals in the same study which had only *T. vivax* infection had chronic disease with more gradual decrease in erythrocyte values (183). Two chimpanzees infected with *T. rhodesiense* and *T. brucei*, respectively, which developed secondary bacterial infection had terminal leucocytosis in variance to leucopenia which occurred in two others without secondary infection (26). Contrary to expectations, the superimposition of gastrointestinal nematode infection (*Haemonchus contortus, Oesophagostomum columbianum, Trichostrongylus* sp) did not exacerbate the anaemia caused by *T. vivax* and *T. congolense* infections of sheep and goats (195).

Treatment of infected man and animals with trypanocides generally quickly reversed the pathologic effects of trypanosomiasis, as was observed with anaemia in human *T. rhodesiense* infection (29) and in *T. vivax* infections of horses (197) and goats (171), with thrombocytopenia in human *T. rhodesiense* infection (29) and *T. congolense* infection of cattle (223), and with hypergammaglobulinaemia in *T. rhodesiense* infection of monkeys (231). Elevated IgM levels returned to normal in cattle infected with *T. vivax* and *T. congolense* after treatment (132), while elevated kinin levels also returned to normal following treatment of human *T. rhodesiense* infection (32) and of *T. brucei* infections of cattle and rabbits (31). Red cell and plasma volumes increased after treatment of bovine trypanosomiasis (75). Treatment with nitrofurazone restored elevated plasma levels of SGOT, SGPT and BUN, and BSP retention to normal in mice infected with *T. rhodesiense* (150) while treatment similarly restored elevated SGPT levels to normal in cattle infected with *T. vivax* (84). Although treatment generally leads to rapid recovery, it has been observed in *T. vivax* infection that many goats with severe anaemia die despite the therapeutic elimination of the parasite (7).

CONCLUSION

The data presented here provide considerable insight into the pathogenesis of trypanosomiasis but cannot be assumed to be complete. One particular area of deficiency is the lack of data on the sequential development of haematological and biochemical abnormalities in human infections, a deficiency which will persist for obvious reasons. However, data collected from studies on the pathogenesis of human parasites in non-human primates and rodents will have to be extrapolated in understanding human trypanosomiasis.

Some encompassing features of human and animal trypanosomiasis can be deduced from the data presented above. The foremost of these is the existence of a direct relationship between the degree of parasitaemia associated with an infection and the severity of the haematological and biochemical pertubations that occur during the infection. Thus periods with high parasitaemia, which are usually the early phase (acute crisis) and less commonly terminally, are associated with rapid development of anaemia, leucopenia, thrombocytopenia and such biochemical aberrations as hypoglycaemia, and elevation of BUN. Similarly, the differences in the severity of haematological and biochemical changes associated with various host-parasite combinations depend to a large extent on the level of parasitaemia that develops ; those associated with high parasitaemias also produce marked haematological and biochemical disturbances, and *vice versa*. Such high parasitaemia-severe pathology coexistence bridges host or parasite speciation, being a feature of acute rodent trypanosomiasis caused by *T. brucei, T. congolense, T. rhodesiense* and *T. gambiense*, and acute *T. vivax* and *T. congolense* infections of ruminants. However, some specific pathological phenomena such as thrombocytopenia and hypoglycaemia evolve slightly faster than others such as anaemia and leucopenia. Such parasitaemia-pathology relationships are also manifested as individual variability in studies of intra-host species variation during infection with given parasite strains.

Secondly, various mechanisms by which trypanosomes induce haematological and biochemical aberrations in their hosts become apparent. They may affect individual cells directly or indirectly producing alterations that induce necrosis or more commonly predispose the cells to destruction by phagocytic cells of the MPS ; this is the method of destruction of RBC, WBC and platelets. Trypanosomes may also alter tissues with resultant malfunction ; thus bone marrow precursor cells may be altered with resultant effects on circulating WBC, RBC and platelet counts, while the effects on the lymphoid tissue induces lymphopenia, proliferation of plasma cells and excessive production of immunoglobulins, some of which are auto-antibodies directed against host cells. The alterations induced by trypanosomes in tissues such as the kidneys and liver also affect their level of performance with effects on serum chemistry and haematology. Thus an altered kidney favours the accumulation of toxic metabolites in plasma while hepatic malfunction, which probably does not become serious, affects

detoxification of host and parasite metabolites and the synthesis of some vital metabolites. Trypanosomes also induce alterations due to release of toxic metabolites by living or dead trypanosomes into the plasma. These metabolites such as FFA and haemolysin may be toxic to host cells such as blood cells and their marrow precursors ; they may, like transaminases, simply accumulate in plasma causing elevated plasma levels without inducing any obvious ill effects. Trypanosomes can also induce abnormalities by consumption of materials such as glucose and amino acids for their metabolism, or in tissue lesions which they induce such as the utilization of platelets and blood clotting factors in DIC.

A third general feature of trypanosomiasis is the involvement of several factors in precipitating a given abnormality such as anaemia, leucopenia or thrombocytopenia. However, in these cases where the causation of a lesion is multifactorial, the relative importance of each of the precipitating causes has not been clearly defined but should be. This diversity of pathogenic inputs can be anticipated since the organisms, contrary to most other pathogens causing acute and chronic infections, are not localized but persist in the blood for variable long periods during which they affect several vital organs either directly by tissue invasion and/or indirectly as the products of their metabolism and continuous lysis disseminate. Some of the reactions of the affected tissues summate to induce a particular lesion.

The haematological and biochemical aberrations induced by trypanosomes coupled with the histopathological lesions often become incompatible with life when they are severe, resulting in death of the host. In the acute infections, death could often be attributed to the haematological and biochemical events since severe organ damage may not usually have developed. In the peracute infections, such as in rodent trypanosomiasis in which the animals die within about one week of infection following massive unrelenting parasitaemia, the biochemical changes appear to be the main cause of death since significant haematological changes are usually lacking in such infections. In chronic infection, organ damage and haematological changes become the important causes of death. During the acute or chronic disease, secondary infections may also intervene to terminate an infection.

The possibility of recovery from a trypanosome infection is determined in the main by the severity of the haematological, biochemical and histopathological lesions, the nutritional status of the host, and the presence or absence of secondary infection. When recovery occurs naturally following the subjugation of the parasites by the host's defence mechanisms, the haematological and biochemical parameters return to normal usually more slowly than they developed, and recovery can be prolonged by poor nutrition and secondary infection which are common features in areas with endemic trypanosomiasis. Recovery occurs faster with milder infections that cause less severe pathology. Following successful treatment with trypanocides such as berenil, the reversal of the haematological and biochemical aberrations is very dramatic. The faster recovery following treatment suggests that during natural recovery the parasites may not completely disappear from the blood and tissues but may persist in such small numbers that cannot be easily detected, although complete sterilization is also possible. With treated animals, however, there appears to be level of pathology beyond which death supervenes despite the elimination of the parasite as has been observed in sheep, goats and cattle (7, 135).

Finally, the data reviewed above support the hypothesis on the pathogenesis of trypanosomiasis as most infections can be fitted into the general pattern proposed earlier in this paper (Fig. 1).

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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.

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 bioquímicas 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 bioquimico causados por los tripanosomas provienen de efectos directos o indirectos a través de sus productos de degradación sobre las células del huésped (hematies, leucocitos, plaquetas) y sus tejidos (hígado, riñones, médula osea, órganos linfoideos), de dónde destrucción celular y funcionamiento orgánico defectuoso, sustracción y adición de productos bioquímicos en el huésped ligadas con el metabolismo del parásito. *Palabras claves* : Hombre - Animal - Tripanosomosis - Modificación hematologica - Bioquimia.