Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978

Editors: George Losos and Amy Chouinard
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Contents

Participants .......................................................... 5

Foreword B.L. Nestel .................................................. 11

Introduction
Welcoming address W. Masiga ..................................... 13
Opening address J. Muliro .......................................... 14
Vote of thanks B.L. Nestel ......................................... 15
Theme and objectives of the conference L. Goodwin ....... 16

The Organism
The metabolism of African trypanosomes in relation to pathogenic mechanisms B.A. Newton ............................. 17
Biology and ultrastructure of trypanosomes in relation to pathogenesis K. Vickerman and L. Tetley 23
Biochemistry of variant antigens G.A.M. Cross .................. 32
Cross-reacting determinants in trypanosome surface antigens A.F. Barbet, T.C. McGuire, A.J. Musoke, and H. Hirumi 38
Mechanisms of antigenic variation in salivarian trypanosomes J.J. Doyle, H. Hirumi, and A.L.W. de Gee 44
Genetic basis of antigenic variation R.O. Williams .......... 46
Cyclical transmission and antigenic variation L. Jenni ........ 49
Antigenic heterogeneity of bloodstream and metacyclic forms of T. brucei J.D. Barry and S.L. Hajduk ......... 51
Discussion summary B.A. Newton and K. Vickerman ........ 57

Infections
Infections caused by pathogenic African trypanosomes G.J. Losos .... 59
Rodent trypanosomiases P. A. D’Alesandro .................... 63
Parasitemia and host susceptibility to African trypanosomiases M. Murray and W.I. Morrison 71
Immunity in the bovine to T. congolense induced by self-cure or chemotherapy B.T. Welde, W.T. Hockmeyer, R.M. Kovatch, and M.S. Bhogal 82
Trypanosomiases of game animals R. Olubayo .................. 87
Discussion summary F.E.G. Cox and G.A.M. Cross .......... 89

Mechanisms of Cellular Injury: Blood and Circulatory System
Is the anemia in bovine trypanosomiases caused by immunologic mechanisms? H. Tabel, F.R. Rurangirwa, and G.J. Losos 91
Complement in experimental trypanosomiases K.H. Nielsen, I.R. Tizard, and J. Sheppard 94
Biologically active lipids generated by autolysis of *T. con golense*

Pharmacologically active substances in *T. vivax* infections
D. Zwart and G.H. Veenendaal 111

Pharmacologically active substances in *T. brucei* infections
P.F.L. Boreham 114

Discussion summary P.F.L. Boreham and F.E.G. Cox 120

**Blood and Hematopoietic Tissue Responses**

Anemia of bovine African trypanosomiasis: an overview M. Murray 121
Erythropoietic response in bovine trypanosomiasis J.D. Dargie 128

Pancytopenia in bovine trypanosomiasis
M.G. Maxie and V.E.O. Valli 135

Effect of bovine trypanosomiasis on hematopoiesis
G.P. Kaaya, G.J. Losos, M.G. Maxie, and V.E.O. Valli 137

Effects of *T. con golense* and *T. brucei* on the circulatory volumes of cattle J.D. Dargie 140

Hemodilution in bovine trypanosomiasis
M.G. Maxie and V.E.O. Valli 145

Discussion summary J.D. Dargie and P.A. D’Alesandro 149

**Lymphoid Tissue Responses**

Serum protein changes in bovine trypanosomiasis: a review H. Tabel 151

Lymphoid changes in African trypanosomiasis
W.I. Morrison and M. Murray 154

Changes in the immune system during experimental African trypanosomiasis T.W. Pearson, G. Roelants, and W.I. Morrison 161

Immunosuppression of humoral immune response in bovine trypanosomiasis F.R. Rurangirwa, H. Tabel, and G.J. Losos 165

Discussion summary L. Karstad and V.E.O. Valli 169

**Tissue Lesions**

Pathogenesis of tissue lesions in *T. brucei* infections
W.I. Morrison, M. Murray, and P.D. Sayer 171

Organ and tissue weights in diseases caused by *T. vivax* and *T. con golense* G.J. Losos and P.M. Mwambu 178

Pathology of *T. con golense* in calves V.E.O. Valli, C.M. Forsberg, and J.N. Mills 179

Ultrastructural changes in blood vessels of tissues of cattle experimentally infected with *Trypanosoma con golense* and *T. vivax*: a preliminary report P.M. Mwambu and G.J. Losos 184

Discussion summary V. Houba and G.J. Losos 186

**Conclusions**

The trypanosome revisited: a summary of the conference L. Goodwin 187

**References** 189
Holstein calves injected with the TREU 112 strain of *Trypanosoma congolense* developed a chronic debilitating disease characterized by anemia, pyrexia, intermittent diarrhea, and poor weight gain. Pancytopenia persisted throughout the period of observation and fibrinogen levels were reduced and turnover increased. Pathologically there was thymic atrophy with enlargement of the heart, lungs, liver, spleen, kidneys, and lymph nodes. The contents of the small intestines were increased in weight and there was red marrow in the midfemoral shaft. Histologically there was widespread microvascular dilatation with decreased cellularity in all lymphoid tissues and thymic-dependent atrophy. Chronic mononuclear inflammatory foci were present in the heart, liver, and kidneys, and renal glomeruli were diffusely enlarged and hypercellular. Hemosiderin deposition was prominent in the lungs, liver, spleen, renal epithelium, and bone marrow.

The principal lesion caused by *T. congolense* infection in cattle has been described as dilatation of capillaries in all organs of the body (Losos et al. 1973; Fiennes 1950). The strictly intravascular habitat of *T. congolense* has been described by Fiennes (1946), although most pathologic descriptions appear to include changes due to mixed infections and due to autolysis.

Anemia has been recognized as an important symptom of *T. congolense* infection and has been quantified by modern methods (Maxie, Losos, and Tabel 1976). It appears to be hemolytic and complicated by terminal hypoproliferation and vascular dilatation and likely sludging (Valli, Forsberg, and McSherry 1978). The mechanism of this dilatation has not been identified, although it may be similar to the kallikrein activation in bovine babesiosis (Wright 1973) and mediated by material released by intravascular trypanosomes (Tizard and Holmes 1977).

### Materials and Methods

Twenty-four castrated Holstein calves were used in our experiment. They were 3–5 months of age and weighed 80–100 kg at the beginning of the experiment. Thirteen calves were given $1 \times 10^6$ *T. congolense* TREU 112 intravenously and were observed for the next 6 months. Hematologic studies were carried out as well as a variety of coagulation tests, and the animals were weighed weekly. Necropsy procedures consisted of inducing recumbency by intravenously injecting chloral hydrate and killing by electrocution. Necropsy was done immediately.

### Results

Animals infected with *T. congolense* had persistent, moderate pyrexia and gained weight more slowly than did control animals. Following infection, they experienced a drop in hemoglobin value (initial mean, 11 mg/dl; mean at 16 days post infection, 7.8 mg/dl), and the red cell count continued to decrease to a low of $5.2 \times 10^6/\mu l$. On the 23rd day of infection, it was stabilized by a macrocytic response that was normocytic and almost fully saturated. During the next 6 months, the infected calves remained significantly but not critically anemic. The red cell life span decreased to about half that of the control animals, and the marrow myeloid-to-erythroid ratio changed from a normal of 1:2 to less than 1:5 in the infected calves (Valli, Forsberg, and McSherry 1978). There was a mild reduction in red cell mass and by indirect measurement an apparent increase in plasma volume in the infected calves.

Platelet levels in infected calves were about half those of the control animals but seldom dropped below $10^3/\mu l$ and were never sufficiently
Pathological Changes

Infected calves had pendulous abdomens because of a greater quantity of fluid in the small intestine. They had much less body fat and body flesh and a greater proportion of viscera to carcass weight. Significant changes in the thoracic viscera indicated a marked atrophy of the thymus gland of infected calves that weighed 0.21% of body weight as compared to 0.35% for control calves (Valli, Forsberg, and Robinson 1978). The hearts from the infected calves were more globose and tended to have less fat around coronary vessels; they weighed 0.67% of body weight, significantly higher than the 0.53% for control calves. Lungs from infected calves did not collapse completely, had a rubbery texture, and were tan; they weighed 1.5% of body weight compared to 1.2% of body weight for control animals.

The liver was grossly enlarged in infected calves and weighed 2.69% of body weight; the mean for the control calves was 1.67%. The livers were dark red and had a faint lobular pattern on cut surface and fine white striations extending out from the larger vessels into the lobular parenchyma. Livers of infected calves had a greatly increased resistance to digital pressure, and when placed on a necropsy table with the diaphragmatic surface down, the edges remained elevated from the surface. The spleens were large and significantly heavier in infected calves (mean of 0.47% of body weight). Due to the induction of anesthesia in calves before electrocution, the spleens of control animals were congested and oozed blood from the cut surface after removal. In contrast, the parenchyma of the spleens from infected calves were dry, and the organs were turgid, maintaining their shape when placed on the table. The cut surface of the spleen had a dark sinus area and prominent white lymphoid follicles.

The kidneys of all the calves were normal in outline but heavier in infected calves with a mean of 0.53% body weight, compared to 0.39% of body weight for control calves. The external capsule and cut surface of the infected animals' kidneys had the same tan discoloration as the lung. All body nodes were enlarged, although they were not weighed. The medullae were discoloured with reddish-brown striations extending deep into the subcapsular areas. Hemal lymph nodes became prominent in the flank areas after several weeks of infection and were especially prominent in the abdominal cavity, often forming a continuous chain from the renal node back to the iliac nodes at the bifurcation of the aorta. These structures were spherical, from 0.5 to 1 cm in diameter, and dark red on cut surface. The most prominent change in the hematopoietic system was marked conversion of fat to hematopoiesis in femoral marrow with almost all but a small central area of medullary fat converted to hematopoiesis in most animals (Valli, Forsberg, and Robinson 1978).

Histological Changes

The changes in the hematopoietic organs of infected calves constitute a remarkable spectrum of stimulation and depletion. Thymic lobules were smaller than those in control animals, and there was a prominent reduction in cortical width. Interlobular septa were infiltrated with fat and frequently eosinophils, and there was a noticeable reduction in density of lymphocytes in both the cortex and medullary areas. Furthermore, lymphocytes that were present in the cortex changed in mean size from small (with a nuclear diameter slightly larger than that of a red cell) to medium (with a nuclear diameter close to two red cells) (Valli and Forsberg in press).

The changes in lymph nodes were similar in all body sites and consisted of follicular hyperplasia with the presence of follicles in the medullary areas and a consistent and marked atrophy of thymic-dependent areas, which in some cases extended through to the capsule and left the follicles without a normal cuff of small lymphocytes. The follicles...
of infected calves were generally larger than those in control animals, though the cell density within the germinal centres themselves was lower, and in many cases there was follicular hyalinosis and surprisingly fewer mitoses in infected animals than in controls. The areas normally occupied by thymic-dependent small lymphocytes had a marked increase in stromal collagen and a dense collection of large macrophages, often with prominent hemosiderin accumulations. Focal plasmacytosis occurred around small vessels in the medullae, and the postcapillary venules were prominent with high vesicular endothelium. The follicular changes were similar in the spleen with relative lymphocytic depletion in both follicular and thymic-dependent areas, but there was a marked increase in fixed cells in the sinus areas, many of which were hemosiderin-bearing macrophages.

The liver changes consisted of hepatocellular atrophy with sinusoidal dilation and endothelial and Kupffer-cell proliferation. Periportal lymphoid accumulations occurred but were not prominent, and no areas of necrosis were present.

Renal changes consisted of lymphoid aggregates occasionally including germinal centres around arcuate arterioles and occasionally in juxta glomerular locations. There was a moderate overall atrophy of tubular epithelium without areas of necrosis and a generalized increase in glomerular size and mesangial density. Hemosiderin stainable with Prussian blue was present in basilar areas of the glomeruli. There was a diffuse and global increase in glomerular cellularity, and luminal area was decreased occasionally with hemosiderin-bearing macrophages within capillaries. Synechiae frequently occurred between the visceral and parietal layers of Bowman’s capsule; otherwise the parietal capsules were of normal thickness.

Consistent mononuclear infiltrates occurred within the myocardium, and there was an overall increase in numbers of myocardial nuclei per unit area. Nuclei were larger and more vesicular than in control animals.

Pulmonary changes consisted of a marked and consistent increase in width of alveolar walls in all areas of the lung. There were increased numbers of inflammatory cells within the alveolar walls and stainable iron was present throughout the alveolar septa in all infected calves. There were more inflammatory cells in the alveoli of infected calves but peribronchiolar lymphoid cuffs were not prominent. Trypanosomes could occasionally be seen within the pulmonary capillaries.

The adrenal gland had an overall increase in cortical width, which was largely due to an increase in width of the zona fasciculata. Nuclear density was greater in the zona fasciculata indicating that the cells within, although more numerous, were smaller than those of control animals.

Changes in the brains of infected animals were not marked and consisted of a mild dilatation of the perivascular spaces and increased numbers of lymphocytes in these areas. The most marked change was a uniform dilation of capillary lumina, many of which contained hemosiderin-bearing macrophages apparently fixed in situ. Trypanosomes were frequently seen in these areas and were most numerous in the vessels of the corpora quadrigemina.

Pituitary glands contained increased numbers of sinusoidal inflammatory cells and occasional actual germinal centres. Nuclear density was greater in the infected animals, indicating some reduction in the size of the pituitary cells.

Changes in the enteric tract consisted of a thickening of the squamous epithelium of ruminal villi in both the germinal and keratinized layers. There was mild reduction in thickness of glandular mucosa in the abomasum of infected calves and a shortening of villi in the small intestine. A greater area of lamina propria in the intestinal villi of infected calves was occupied by eosinophils and plasma cells. In addition there was a mild and consistent dilation of the central lymphatics of the intestinal villi. There was a mild reduction in pancreatic acinar cell size and in cellular area occupied by zymogen granules.

The bone marrow of infected calves was much more cellular than that of normal animals, generally 70–95% cellular as against 10–20% in control animals, with the remaining area being occupied by lipocytes. The fat cells in infected calf marrows were only half the size of those in control animals. Roughly 5 times as many megakaryocytes were found per unit area of marrow in infected calves as were found in controls, and these cells were roughly equal in diameter, although the nuclei of the infected calves were larger. Lymphoid nodules (and occasionally germinal centres) were frequently present in the marrow of infected calves and were never seen in the marrow of control animals. Hemosiderin was increased in marrow of infected calves and tended to be larger and more coarse than in controls. Plasma cells were much more numerous in marrow of infected calves as were vascular endothelial cells — a finding that indicates a mild degree of myelofibrosis. There were approximately three times as many myeloid cells per unit area in infected calves and about 15 times as many erythroid cells. Red blood cells were 6 times as numerous per unit area in the marrows of infected calves, indicating capillary dilation in this tissue.
The omentum of infected calves had red discoloured areas that did not blanch on digital pressure. Histologically these foci were due to capillary and venular dilatations with a marked increase in stromal pericytes and small lymphocytes.

**Discussion**

Changes in the tissues of calves with trypanosomiasis are in many ways like those of horses with equine infectious anaemia where Jubb (1970) noted that in individual organs the lesions themselves were not specific but, taken collectively, produced a spectrum of change not seen in any other disease. *T. congolense* in calves caused anaemia and neutropenia followed by a degree of recovery, the sequence resulting in generalized reticuloendothelial hyperplasia. This change is remarkable in that it results in a gross increase in volume of tissue in node and spleen and marrow at the expense of a reduction in cell density. The atrophy begins in the thymus and moves concurrently to the thymic-dependent areas of lymphoid tissues throughout the body. Although germinal centres are larger in infected calves, their cellular density is lower, and, most significantly, the number of mitoses per unit area in these areas is lower than in control calves, indicating incipient lymphoid depletion.

The outcome of *T. congolense* infection in calves appears to reflect the rapidity with which marrow hyperplasia occurs. In fulminating diseases, the calf responds poorly when marked increase in demand is made on both myeloid and erythroid marrow production (Valli, Forsberg, and Lumsden in press). The major differences between the results from Guelph, Canada, and those from Muguga, Kenya, are that the infected calves at Guelph developed marked marrow hypertrophy with extension of red marrow throughout most of the medullary cavity, whereas those in Kenya did not. Neutropenia was more severe in the African animals, and death often resulted from secondary bacterial infection. These differences may be due to age: the animals in Guelph were younger and may have been able to reactivate their neonatal hematopoietic sites with greater rapidity than were the more mature animals.

Vascular dilation is a consistent and remarkable finding in *T. congolense* infection and involves virtually all organs. The parasites themselves are only found with any regularity in the brain and skeletal muscle and occasionally in lung and myocardium if the parasitemia is high. The intravascular parasites undoubtedly interfere with circulation and are responsible for the rapid onset of depression and incoordination in animals dying of infection. The reduction in level of plasma fibrinogen with shortened life span and the increased uptake of isotope indicate a consumptive error associated in fibrinogen kinetics. Surprisingly, platelets are not consumed at a greater rate, but their reduced numbers in the peripheral blood with normal life spans are associated with an increased marrow megakaryocytic mass with increased nuclear size and reduced cytoplasmic volume, indicating ineffective thrombopoiesis. It is likely that these changes are accelerated when infection is acute rather than chronic, prompting a full-blown consumption coagulopathy. Whether infection due to *T. congolense* will be peracute or chronic is related to each animal's resistance, its nutritional plane, its freedom from other diseases, and the age at which it is infected (Forsberg et al. in press).

The two most productive areas for future research appear to be hemodynamics and the mechanisms by which the organisms produce microvascular dilation. In addition to the increase in size of hearts in infected animals, there is an increase in width of the media of the muscular arterioles of the lung and of the kidney and increased prominence of the juxta glomerular cells of the renal glomeruli, all suggestive of both pulmonary and systemic hypertension. Anemia, which is hemolytic and initially responsive, is seldom severe enough to be life threatening but appears to be complicated by vascular dilation, which is likely an adaptive mechanism to increase flow through areas obstructed by intraluminal parasites and margined red cells.

The homing of the trypanosomes to brain and skeletal muscle is remarkable and likely related to endothelial changes in these organs. There are regional differences in the mitochondria of endothelial cells throughout the body, and this may interact with the energy system of the parasite such that it finds some areas more conducive to growth than others. $^{35}$Cr-labeled trypanosomes given to normal calves and calves that had been infected and were sterilized with Berenil caused anaphylactic reactions in the previously infected calves and the sludging of parasites in the lungs of all animals. These changes are likely hemodynamic and related to jugular vein injection and not an indication of the parasite-homing process. The mechanism by which the parasites adhere to the endothelium is unclear and deserving of further study. Macromolecular compounds such as dextran, which will sweep sequestered red cells back into axial flow, have no effect on parasitemia due to *T. congolense*. Thus the reaction does not appear to be due to electrical
charge on the parasites. Similarly heparin, which
has an antisludging effect on red cells has no effect
on the attachment of parasites to vascular
endothelium. Berenil rapidly releases the
organisms into axial flow where they are still motile
in wet mounts. The parasitemia is transient, and
apparently the free organisms are rapidly removed
by the reticuloendothelial system. In contrast,
reticuloendothelial blockade with corticosteroids
causes a pronounced and continued increase in
parasitemia likely due to a reduction in phagocytic
removal. The effects of the organism on the host
cannot be explained simply as a toxic phenomenon,
because young calves infected with *T. con golense*
occuriously develop very high parasitemias of
10 000–80 000/μl of blood during the first 10
days of infection and are completely asymptomatic.
They may very suddenly become depressed and
recumbent associated with a reduction in
parasitemia. It is tempting to speculate that this
reduction in parasitemia is coincident with the
appearance of antibody that causes the parasite to
home in on muscle and brain and, thus, to produce
cerebrovascular blockage and functional depres-
sion.

In summary, it seems that the study of the kinin
system in calves infected with *T. con golense* should
be undertaken along with an intensive,
hemodynamic workup on the animal dying of the
uncomplicated disease.