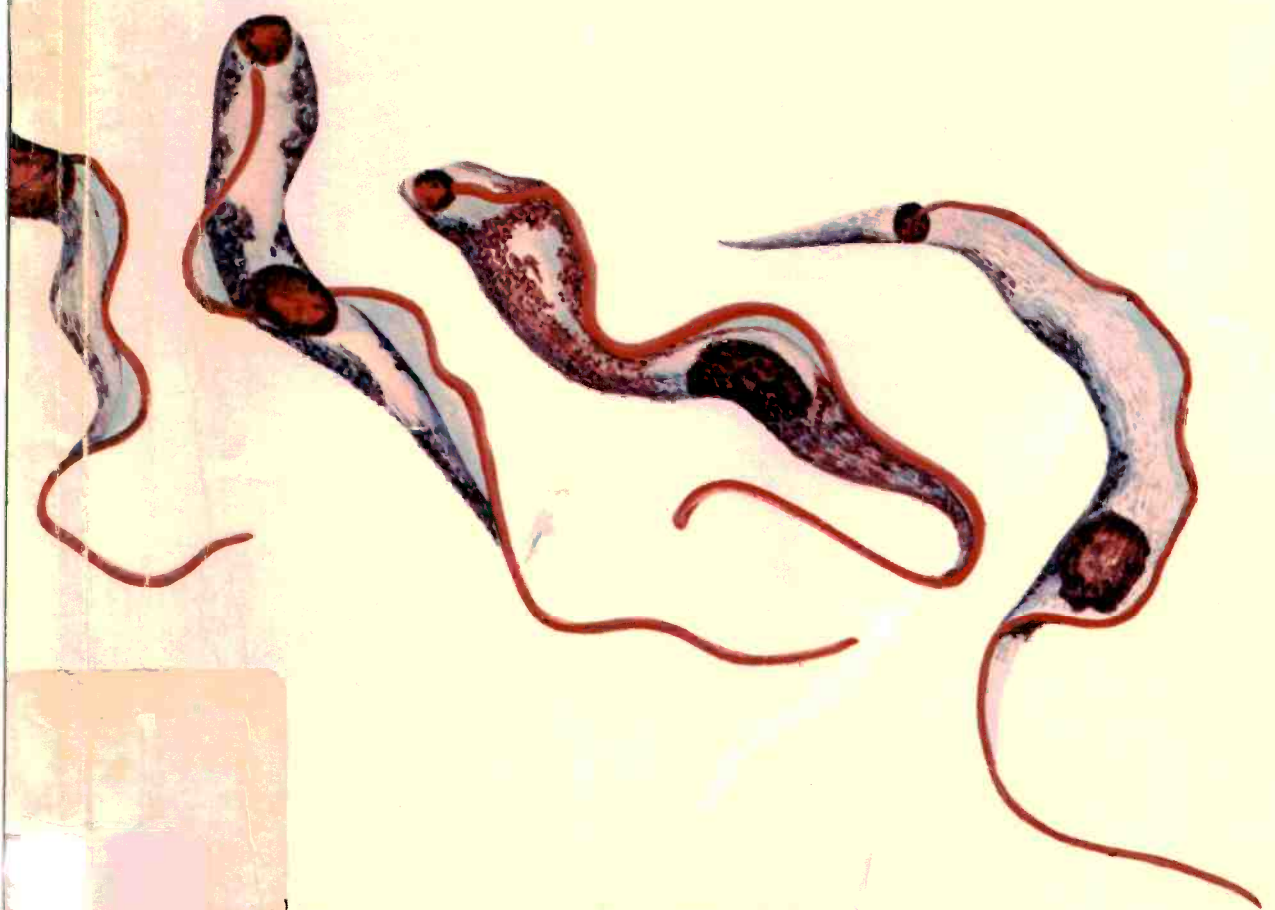
IDRC-132e

Pathogenicity of Trypanosomes

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



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Editors: **George Losos[1] and Amy Chouinard[2]**

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Contents

Pathogenesis of tissue lesions in *T. brucei* infections

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Abstract. *T. brucei* is capable of producing tissue damage in a large number of mammalian hosts including the bovine. However, in our experience, the dog is particularly severely affected. In the dog, *T. brucei* causes an acute disease syndrome characterized by high levels of parasitemia and invasion of a wide range of tissues by the trypanosomes. This is associated with marked cellular infiltration and tissue cell degeneration and death. The heart, choroid plexus, and eyes are consistently and severely affected, and a striking finding in some animals is a necrotizing vasculitis affecting the coronary vessels. The mechanisms involved in tissue injury are open to speculation but possibly include immunologic mechanisms, biologically active factors produced by the trypanosomes, physical swelling and disruption of tissue architecture, and increased vascular permeability. Associated with tissue lesions, dramatic lymph node changes occur: initially these are proliferative but later lymphoid depletion occurs.

Organisms of the *T. brucei* subgroup live both extravascularly and intravascularly in their mammalian hosts. In extravascular sites they cause cellular infiltration and, often, tissue cell degeneration and death. The degree to which they invade extravascular sites varies considerably from one host species to another as does the extent of tissue damage. The number of organisms present in the tissues does not always correlate with the number in the blood. In our experience, working with several laboratory and domestic animal species, the most severe tissue lesions occur in dogs infected with *T. brucei* (Morrison et al. in preparation). The purpose of this paper is to describe the main features of tissue lesions found in *T. brucei* infected animals, with particular reference to the dog, and to discuss the possible mechanisms involved in the tissue injury.

Dogs Infected with *T. brucei*

Using two different isolates of *T. brucei*, we have experimentally produced an acute disease in dogs invariably leading to death 3–4 weeks after infection. Both the clinical and pathological findings closely parallel those found in natural cases of the disease. Infected dogs show high levels of parasitemia, and during the 2nd and 3rd weeks of infection there is invasion of a wide range of tissues by large numbers of organisms. In animals examined post mortem during the 3rd and 4th weeks of infection, trypanosomes can readily be found in fluid taken from the peritoneal, thoracic, and pericardial cavities; synovial fluid; cerebrospinal fluid; aqueous humour; and subcutaneous edema fluid. Large numbers of trypanosomes can be seen histologically in many tissues. However, despite the widespread distribution of the organisms, there appears to be some predilection for certain tissues: we have most consistently found severe lesions in the heart, central nervous system, eyes, skin and subcutis, skeletal muscle, kidney, nasal mucosa, testicles, and pituitary gland. Some of the tissues, in particular the heart, central nervous system, skeletal muscle, and pituitary gland are also severely damaged in other species infected with *T. brucei* (Ikede and Losos 1972, 1975; Murray 1974; Murray, P.K. et al. 1974; Poltera, Owor, and Cox 1977).

In the dog, the heart, central nervous system, and eyes are always severely affected. There is a diffuse myocarditis (Fig. 1), which in most terminal cases is judged to be the cause of death. In the central nervous system, the choroid plexus is particularly involved, although lesions are also

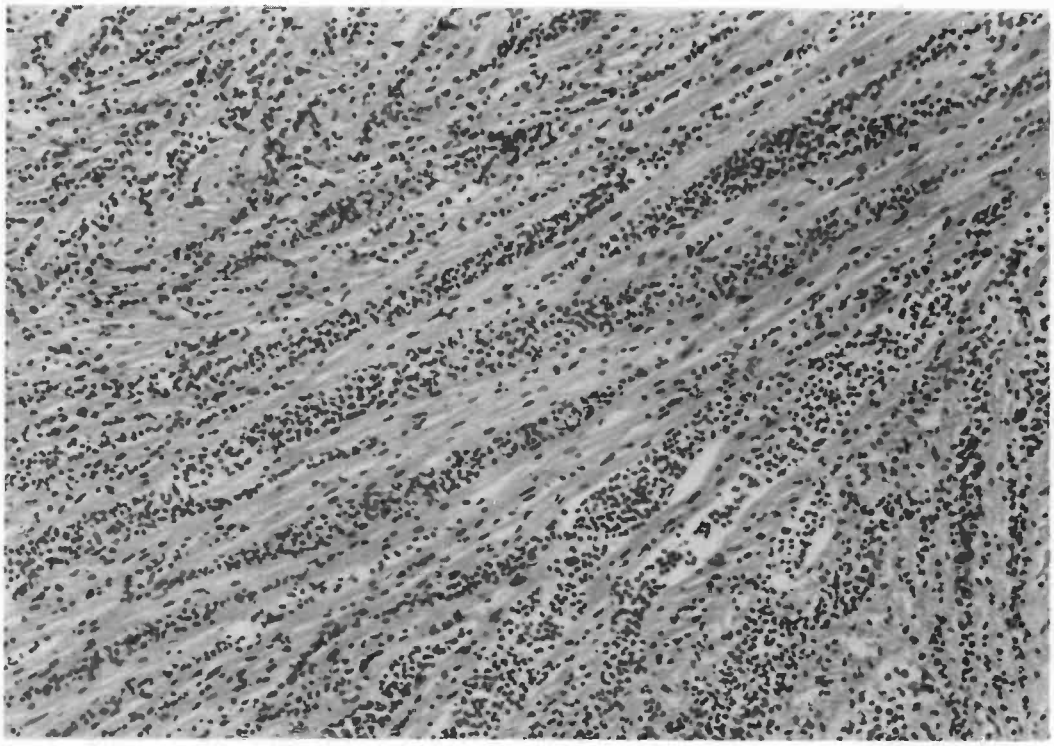

Fig. 1. *Ventricular myocardium of dog examined 23 days after inoculation with* T. brucei. *Marked cellular infiltration can be seen throughout the myocardium resulting in separation of the muscle fibres.*

found elsewhere, mainly in the meninges. In the eyes, there is a dramatic anterior uveitis with exudation of cells and fibrin and sometimes hemorrhage into the anterior chamber.

The tissue lesions vary in severity with the numbers of trypanosomes present but, in general, are similar in different tissues. Initially, the cellular infiltrate is composed predominantly of lymphocytes and plasma cells with occasional macrophages. As extravascular trypanosomes become more numerous, large numbers of macrophages and polymorphonuclear leukocytes are found (Fig. 2). In different sites within the same tissue, either cell type may predominate. In the heart, lesions are initially found beneath the epicardium and endocardium and are more severe in the atria than in the ventricles; in more advanced cases the entire myocardium is involved (Fig. 1); there is marked distortion and degeneration of myocardial fibres; and in some sites frank necrosis is apparent. The lymphatics in the epicardium are distended and contain numerous macrophages and polymorphonuclear leukocytes and sometimes lymphocytes and trypanosomes. On occasion, the lymphatics are completely occluded by thrombi that contain fibrin as well as many of the above cell types. An additional feature in the heart is a severe necrotizing vasculitis affecting both arteries and veins in the epicardium (Fig. 3). Affected vessels often exhibit necrosis of the entire vessel wall, which contains large numbers of polymorphonuclear leukocytes and numerous trypanosomes.

Mechanisms of Tissue Injury

Although much has been written on the lesions that develop in trypanosome-infected animals, there have been few attempts to investigate the mechanisms involved in tissue injury.

Physical damage: In infected dogs, the degree of interstitial edema and cellular infiltration observed in many tissues must affect the normal physiological functioning of the tissues. For example, swelling of the choroid plexus causes the vessels in the plexus to separate from the ependymal lining and must affect its normal specialized secretory function. Similarly, in the eye, severe lesions in the choroid, iris, and ciliary body will affect the fluid balance within the eyeball, and indeed some animals suffer from a transient glaucoma. The

anemia, although moderate, is likely to lead to tissue anoxia and contribute to the cellular degeneration. However, these physical effects hardly account for all of the degenerative changes in the tissues.

Increased vascular permeability: As evidence of increased vascular permeability in trypanosome-infected animals, the blood vessel walls are often swollen, with expanded perivascular spaces. This may be, at least partially, related to increased levels of kinins (Boreham 1968a, 1970; Goodwin 1970) possibly brought about by the interaction of antigen–antibody complexes and Hageman factor (Goodwin 1970). In *T. brucei* infection, the local inflammatory response initiated by extravascular trypanosomes is also likely to be an important mechanism, not only in increasing vascular permeability but also in allowing extravasation of leukocytes. Although a localized increase in vascular permeability is a normal physiological response to an invading organism, it may impair normal functional activity if it is severe and prolonged. Its effect could be particularly relevant to highly specialized vascular areas such as the choroid plexus and the uveal tract of the eye. Also, interstitial edema in the heart may interfere with the conducting system by separating Purkinje's fibres from the myocardial fibres.

Direct toxic damage: Until recently, the hypothesis that trypanosome "toxins" are involved in the pathogenesis of trypanosomiasis has been out of favour, especially as early reports were difficult to confirm. However, there are now several studies that describe the existence of biologically active substances produced by either dead (dying) or living trypanosomes. A hemolytic factor, a heat-stable protein with a molecular weight of 10 000 daltons, has been shown to be produced by *T. brucei* (Huan et al. 1975) as well as by *T. congolense, T. vivax, T. gambiense,* and *T. rhodesiense* (Murray, Huan, Lambert et al. in press). In addition, permeability and inflammatory factors have been demonstrated in association with *T. gambiense* (Seed 1969) and *T. congolense* (Tizard and Ringleberg 1973; Tizard and Holmes 1977), the inflammatory factor being a polypeptide with molecular weight 1500 daltons. Musoke and Barbet (1977) have shown that purified variable antigen of *T. brucei* is capable of activating complement and inducing increased vascular permeability, and Nielsen and Sheppard (1977) have shown that factors extracted from *T. congolense* also activate complement. Davis et al. (1974) demonstrated a heat-labile, non complement-dependent, platelet aggregating factor produced by *T. rhodesiense*, and Ackerman and Seed (1976) found that tryptophol, a substance known to be

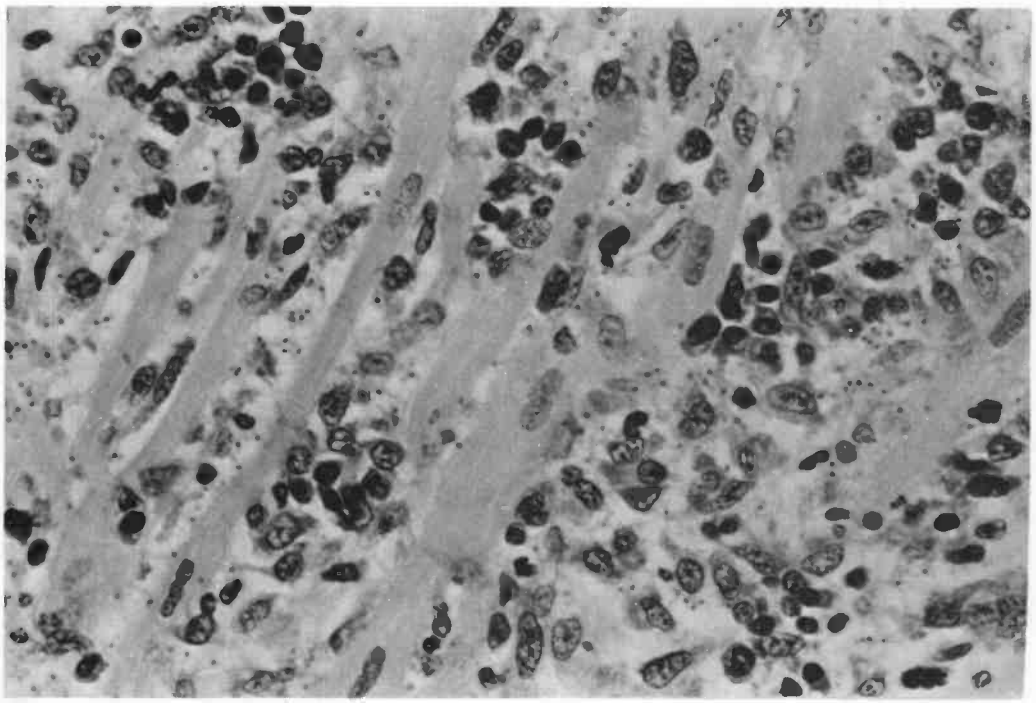

Fig. 2. *Myocardium of dog examined 23 days after inoculation with* T. brucei. *Large numbers of trypanosome nuclei, lymphocytes, plasma cells, and macrophages can be seen between the myocardial fibres.*

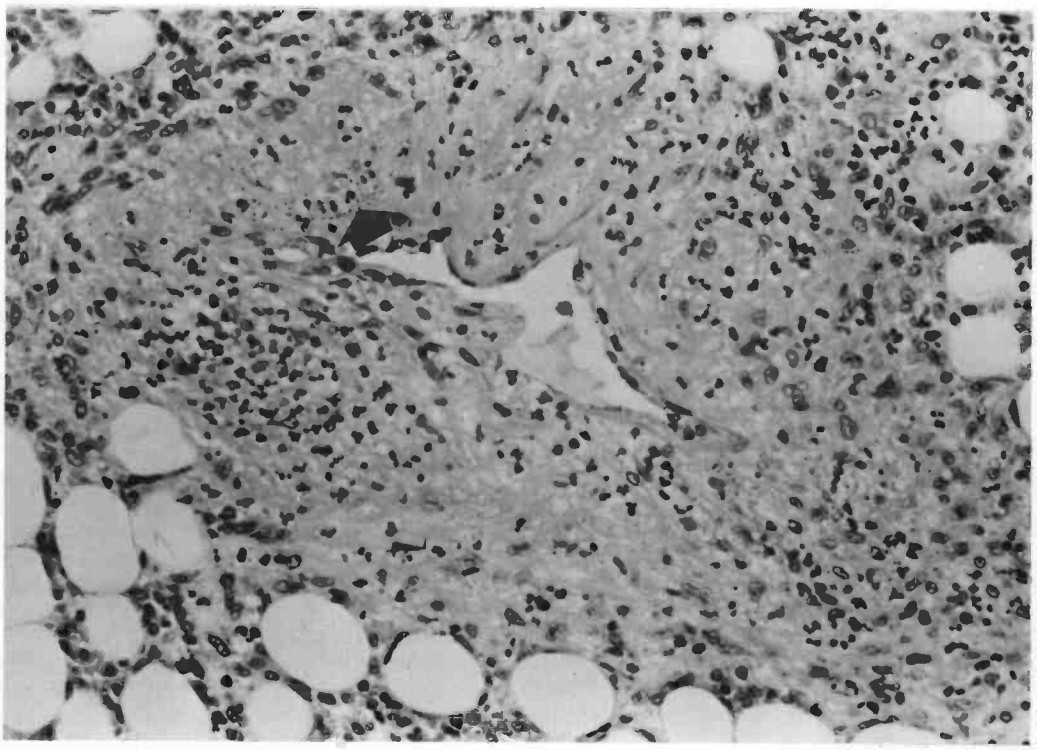

Fig. 3. *Artery in epicardium of dog examined 24 days after inoculation with* T. brucei. *There is diffuse necrosis of the arterial wall, which contains large numbers of polymorphonuclear leukocytes. Foci of trypanosome nuclei can also be seen (arrow).*

synthesized by *T. gambiense*, induced immunosuppression in laboratory rodents. There is now preliminary evidence that *T. brucei, T. congolense,* and *T. rhodesiense* or their extracts (Esuruoso 1976; Mansfield, Craig, and Stelzer 1976; Campbell and Phillips 1976) act as polyclonal B-lymphocyte mitogens. In a series of papers, Tizard and his colleagues (Tizard and Holmes 1976; Tizard et al. 1978) demonstrated that autolyzing *T. congolense* generate phospholipase A activity and free fatty acids that have hemolytic as well as cytotoxic activity and have been incriminated in the induction of immunosuppression (Assoku, Tizard, and Nielsen 1977). Thus, trypanosomes per se generate factors that may contribute to some of the major lesions known to be involved in the disease process of African trypanosomiasis, including anemia, increased permeability of the peripheral vasculature, complement reactivity, immunosuppression, polyclonal mitogenicity (reviewed by Murray 1974), and thrombocytopenia (Davis et al. 1974), although in many instances their activity in vivo remains to be determined. At present, there is little evidence that trypanosome toxins are involved in the development of tissue lesions. Studies in neonatal mice or sublethally irradiated mice infected with *T. brucei* have demonstrated large numbers of organisms in the tissues but scanty cellular infiltration and virtually no tissue cell degeneration or death (Galvao-Castro, Hochmann, and Lambert 1978). Although these findings are open to interpretation, they suggest that live trypanosomes by themselves are not responsible for severe tissue injury. However, it is possible that when many organisms die in the tissues they release substances capable of initiating tissue damage.

Immunologic mechanisms: The presence of a foreign antigen or organism extravascularly in the tissues initiates a localized inflammatory response that allows serum components and cells to enter the tissues; antigens or antigen-bearing cells leave the tissue by afferent lymphatics and initiate an immunologic response in the drainage lymph node. The resultant antibody or antigen-primed cells may then recirculate from the lymph stream and enter the site of foreign antigen from the bloodstream. In this way, nonspecific inflammatory reactions and specific immune responses complement each other in combatting foreign antigenic challenge. The cells present in the tissues at a particular time

represent a net balance between those entering from the circulation and those leaving in the afferent lymphatics. The balance is dependent on vascular permeability, on stimuli for cells to enter the tissues, and on stimuli for retention of the extravasated cells within the tissues. The type of antigen, the quantity of antigen, and the quality of the host's immune response influence the entire process. In most infectious diseases there is a distinct lack of information on these aspects of tissue reactions in relation to pathogenesis of disease. This may reflect the practical difficulties in carrying out the in vivo studies.

Although in most instances the host's response eliminates a foreign antigen, it is confounded in trypanosome infections by antigenic variation. The immune reaction, which at the outset is beneficial to the host, may actually have adverse effects when it is sustained and intensified.

Support for this possibility comes from recent work carried out in mice infected by *T. brucei* (Galvao-Castro, Hochmann, and Lambert 1978). *T. brucei* produces relatively severe myocardial and skeletal muscle lesions in mice, associated with extravascular trypanosomes and a cellular infiltrate composed of lymphocytes, plasma cells, macrophages, and a few polymorphonuclear leukocytes (Murray 1974; Galvao-Castro, Hochmann, and Lambert 1978). Galvao-Castro, Hochmann, and Lambert (1978) demonstrated granular deposits of immunoglobulin and trypanosome antigen between the muscle fibres in sections of heart and skeletal muscle examined by immunofluorescence. They also found that acid eluates of the tissues contained high levels of antitrypanosome antibodies. In addition, immunologically incompetent mice, including neonates, sublethally irradiated mice, and athymic nude mice, showed lower levels of Ig deposits in the tissues and much less severe tissue lesions despite the presence of large numbers of trypanosomes in the tissues. Furthermore, following the transfer of normal syngeneic spleen cells or an Ig-negative fraction of spleen cells to nude mice, the tissue lesions found, following *T. brucei* infection, were similar to those in intact infected mice. Athymic nude mice that received antitrypanosome antibody 5–7 days after infection showed only slightly more severe lesions than the infected nude controls.

Several mechanisms may be involved: antigen–antibody reactions in the circulation, antigen–antibody reactions within the tissues, cell-mediated reactions, and autoimmune reactions. It is well established that immune complexes are present within the circulation of animals suffering from trypanosomiasis (Galvao-Castro, Hochmann, and Lambert 1978) and that in some instances immune complex deposition occurs in the renal glomeruli (Nagle et al. 1974; Lambert and Houba 1974; Murray 1974; Murray, Lambert, and Morrison 1975). However, such deposits are often detected only after several weeks of infection. In the dogs we examined, only scanty deposits of immunoglobulin were found in the kidneys in a few of the terminal cases. Thus, preformed immune complexes deposited from the circulation are probably not a major mechanism of tissue injury. Much more likely is the local interaction of antibody with trypanosomes or trypanosome antigens within the tissues. This is supported by the observation of Galvao-Castro and colleagues that the severity of the lesions was related quantitatively to the amounts of Ig deposits found in the tissues. The antibody may enter the tissues from the circulation or may be produced locally by plasma cells in the cell infiltrates. Further evidence for local immunologic reactivity was the necrotizing vasculitis that we found in dogs infected with *T. brucei*; this was characteristic of an Arthus reaction. Because large numbers of trypanosomes were found within the vessel walls, it is likely that the vasculitis developed as a result of antibody from the circulation interacting with trypanosome antigen in the vessel walls. We want to emphasize that only in the dog have we seen a true necrotizing lesion, although we have observed a range of vascular degenerative changes in other animals infected with *T. brucei*.

Galvao-Castro and his colleagues' finding that Ig-negative spleen cells increased the severity of lesions in nude mice may have been due to enhanced lymphocyte cooperation that caused greater production of antibody and, hence, larger deposits of immunoglobulin in the tissues. Whether or not conventional cell-mediated reactions are important in the pathogenesis of the tissue lesions remains to be determined. However, a delayed hypersensitivity-type reaction to trypanosome antigen has been demonstrated in rabbits infected with *T. brucei* (Tizard and Soltys 1971).

Various autoantibodies have been detected in the sera of trypanosome-infected rabbits (Mansfield and Kreier 1972; Mackenzie and Boreham 1974c), monkeys and humans (Houba and Allison 1966; Houba, Brown, and Allison 1969; Lindsley, Kysela, and Steinberg 1974), and it has been suggested that they are involved in the development of the tissue lesions. However, the role played by autoantibodies awaits evaluation; it is not known whether they are involved in initiating the tissue lesions or whether they are by-products of tissue antigen being released at sites of injury or, alternatively, are produced during polyclonal B cell activation. In studies of *T. brucei*-infected mice, autoantibodies

to heart and skeletal muscle were not detected (Galvao-Castro, Hochmann, and Lambert 1978). Thus, autoimmune reactions, if they are involved at all, may only be operative in long-standing infections when there are severe changes in the lymphoid system.

In dogs infected with *T. brucei*, large numbers of macrophages and polymorphonuclear leukocytes are found in sites of tissue injury. Both cell types are capable of phagocytosing antigen–antibody complexes. Indeed, complement fixation by immune complexes is one of the mechanisms by which polymorphonuclear leukocytes may be attracted into sites of inflammation, the best example of this being the Arthus reaction. Furthermore, during phagocytosis of immune complexes by polymorphonuclear leukocytes, there is leakage of enzymes from their granules into the surroundings (Henson 1971; Weissmann et al. 1971). If sufficient enzymes are released, either during phagocytosis or from dying polymorphonuclear leukocytes, they can be cytolytic to tissue cells.

It has been shown recently that trypanosomes or their variant surface antigen are capable of activating complement by the conventional pathway, in the absence of antibody (Musoke and Barbet 1977; Nielsen and Sheppard 1977). This mechanism may contribute to the hypocomplementemia observed in infected animals (reviewed by Assoku, Tizard, and Nielsen 1977), although its relative importance compared to fixation of complement by immune complexes remains to be determined. Whatever causes the decrease in available complement, the effect is likely to favour the trypanosome by lessening the efficiency of antibody-complement-mediated lysis of trypanosomes and possibly by reducing the ability of macrophages to phagocytose by means of the C3 receptor.

Influence of Tissue Lesions on the Lymph Nodes

In addition to causing tissue injury, the presence of *T. brucei* organisms within the tissues has profound effects on the drainage lymph nodes. The cellular changes in lymph nodes are greatly influenced by the amount and type of antigenic challenge presented to the nodes in the afferent lymph. In dogs infected with *T. brucei*, trypanosomes can be seen in the lymph node sinuses from about 2

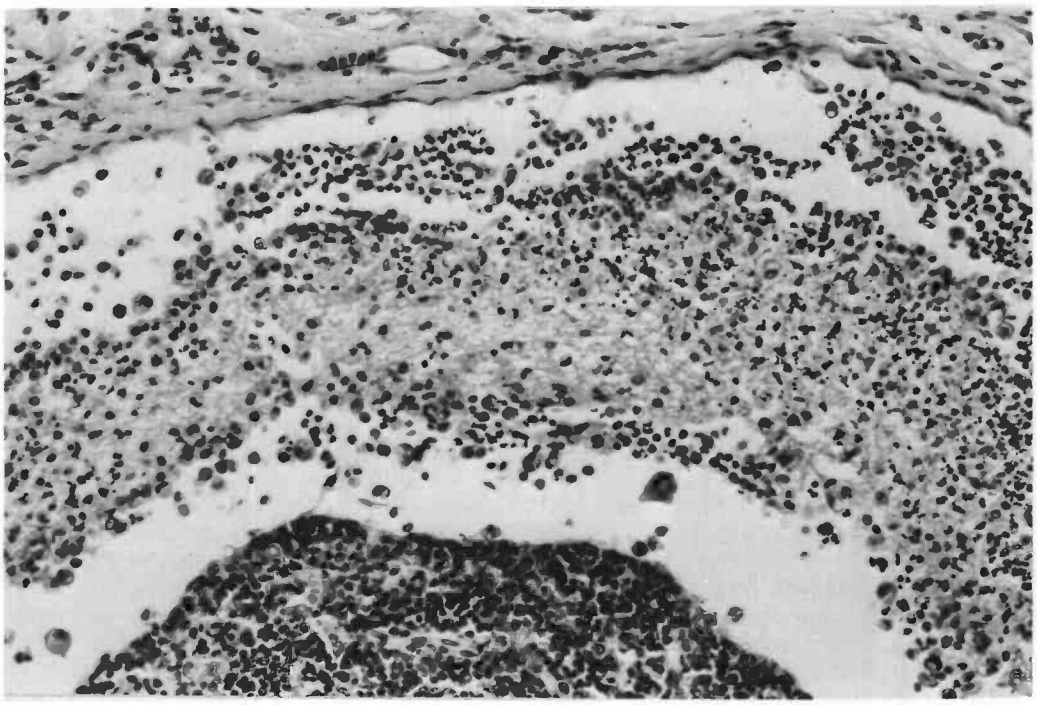

Fig. 4. *Lymph node of dog examined 24 days after inoculation with* T. brucei. *There is distension of the subcapsular sinus, which contains numerous macrophages, polymorphonuclear leukocytes, and cell debris within a network of fibrin. There is no longer a distinguishable fixed network of phagocytic cells in the sinus.*

weeks post infection. At 2 weeks, the sinuses also contain numerous lymphoid cells, many of them lymphoblasts, indicating active traffic of lymphocytes through the tissues. There is also widespread proliferation in the lymph node cortices, and large numbers of lymphoblasts and plasma cells are present in the medullary cords. From about 3 weeks post infection, the picture in the lymph nodes is much different. The cortices are much narrower and show less active proliferation; the number of lymphoid cells in the sinuses is much reduced; and the sinuses are distended and contain large numbers of macrophages and polymorphonuclear leukocytes. As in peripheral lymphatics, a mixture of these two cell types is found in the subcapsular sinuses; fibrinous thrombi similar to those observed in the lymphatics are also present (Fig. 4). By contrast, the medullary sinuses contain mainly macrophages, which show abundant eosinophilic vacuolated cytoplasm and sometimes contain granular phagocytosed material. A striking feature of the sinuses is the disruption of the normal fixed network of phagocytic cells; the majority of the macrophages present in the sinuses are rounded and show no attachment to the sinus lining.

Thus, in terminal cases of *T. brucei* infection in the dog, there appears to be a depletion of the lymph node cortices, a reduction in traffic of lymphoid cells entering in afferent lymph from the tissues, an accumulation of macrophages in the sinuses, and disruption of the normal sinus phagocytic network. These changes undoubtedly arise partly from the severe tissue lesions and the resultant drainage of trypanosomes, cells, and large quantities of antigen via afferent lymphatics into the lymph nodes. Ultimately, because of the severe alterations in the lymph nodes, they may no longer be able to respond adequately to antigenic challenge; this may be particularly important with regard to the appearance of new variable-antigen-type trypanosomes within the tissues.

Tissue Lesions in Trypanosome-Infected Cattle

In cattle infected with *T. brucei*, the heart is one of the most consistently affected organs. The lesions are similar to, but less severe than, those found in the dog and must be considered as functionally significant. In cattle, *T. congolense* and *T. vivax* also produce myocardial damage which is fairly distinctive for the organism involved. With *T. congolense*, perivascular and interstitial edema usually predominates and is accompanied by a scanty infiltrate of small lymphocytes and macrophages. In cattle infected with *T. vivax*, the cellular infiltrate, which is predominated by small lymphocytes, can be intense and, as with *T. brucei*, the organisms may be found in extravascular locations. In the bovine, irrespective of the species of trypanosome, myocardial necrosis is rare, but degenerative changes may be severe and extensive.