STUDIES OF POLYMORPHONUCLEAR LEUCOCYTE FUNCTION AND OF GROSS AND MICROSCOPIC LESIONS IN SHEEP EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI* AND *T. CONGOLENSE*

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

Masters of Science

in the Department of Pathology and Microbiology

Faculty of Veterinary Medicine

University of Prince Edward Island

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Charlottetown, P.E.I.

April, 1994

ISBN 0-315-88448-7

Canada

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ABSTRACT

Trypanosomiasis in animals is associated with immunosuppression that manifests clinically as increased susceptibility to opportunistic infections. Although immunosuppression has been shown to result from depression of antibody production and suppression of cell-mediated immunity, in some cases there is no obvious impairment of these functions. For effective immune response, all of the three effector systems; the mononuclear phagocytic system, the polymorphonuclear phagocytic system, and the complement cascade, must be intact. Polymorphonuclear leucocyte (PMN) function was assessed in trypanosome-infected sheep to establish its possible contribution to lowering of body defence. *Trypanosoma congolense* (Broden, 1904) and *T. brucei* (Plimmer and Bradford, 1899) were obtained from American Type Culture Collection, Maryland USA., and amplified in rats. Fourteen castrated, eight-month old, male sheep were purchased locally. Initially, four Suffolk x Finnish sheep were inoculated with *T. congolense* while two acted as controls. Later, six North Country Cheviot sheep were inoculated with *T. brucei* while two acted as controls. Each infected sheep received eight million trypomastigotes intravenously. The sheep were monitored daily for clinical disease and tests of PMN function were assessed over two months. PMNs were isolated from ethylenediamine tetra-acetic acid anti-coagulated blood obtained from each sheep and superoxide anion production assessed by cytochrome C reduction, every second week in the first experiment and weekly in the second experiment. In addition, heparinized blood was obtained weekly and used to perform nylon wool adherence and phagocytosis of opsonized zymosan in the second experiment.

Although sheep infected with *T. congolense* developed parasitaemia, there were no clinical signs of trypanosomiasis, no gross or microscopic lesions and results of cytochrome C assay were not significantly different between infected and control sheep. Sheep infected with *T. brucei* developed parasitaemia, clinical signs of trypanosomiasis and alteration in PMN function. PMNs obtained from infected sheep had significant decrease in superoxide anion production ($p < 0.01$) and nylon wool adherence ($p < 0.01$), but enhanced phagocytosis of opsonized zymosan ($p < 0.01$).

Clinical disease of *T. brucei* infection in sheep was characterized by pyrexia, anorexia, weight loss, anaemia, and inflammatory edema of skin of the head, neck, brisket, distal limbs, and tail. At the end of the trial, the sheep were euthanised and examined for lesions. There was edema of the skin and enlargement and edema of superficial lymph nodes. Microscopic lesions were observed in the skin, superficial lymph nodes, spleen and kidneys. Skin lesions consisted

of severe edema accompanied by diffuse perivascular and periadnexal dermatitis, perineuritis, lymphangitis, distension of lymphatic vessels with fluid, and lymphatic thrombosis in the dermis and subcutis. Inflammatory reaction extended deep into subcutis leading to severe panniculitis and myositis. Inflammatory cells were mainly macrophages, plasma cells, and lymphocytes although polymorphonuclear leucocytes were also present. Numerous trypanosomes were seen in skin lesions. Superficial lymph nodes had severe capsular lymphadenitis and lymphoid hyperplasia. The spleen had lymphoid hyperplasia. Lesions in the kidney consisted of mild to moderate glomerulonephritis and a few tubular casts.

In summary, sheep infected with *T. congolense* for two months were not clinically sick and had no alteration in PMN function. Sheep infected with *T. brucei* for two months were clinically sick and had significant alteration in PMN function. Such alteration may be responsible for the increased occurrence of opportunistic infections in trypanosomiasis. Microscopic appearance of skin lesions described in *T. brucei*-infected sheep confirms that the inflammatory reaction is similar to that previously described in other organs.

DEDICATION

To members of my family who had to accept my absence from home as a way of life and especially to my mother who can not share this life with us.

ACKNOWLEDGMENTS

I would like to thank my supervisors; Dr. Barbara Horney and Dr. Basil Ikede, for their immeasurable help and advice and especially Dr. Ikede for facilitating my admission into this university and department. I also thank other members of my supervisory committee, Dr. R. Cawthorn, Dr. R. Long, and Dr. J. Pringle, for their immense interest in my work, and especially Dr. Pringle for providing veterinary advice and care to the experimental sheep.

My research work would not have been done without the competent help and support of Mr. Allan MacKenzie, who showed me many new laboratory techniques and went out of his way to assist me with the sampling of the experimental animals and specimen preparation in the laboratory. I would also like to acknowledge the technical help provided by Linda Ruschkowski-Kerr and Dennis Olexson from Diagnostic services, Dr. Alfonso Lopez on taking photomicrographs and Dr. R. Bildfell on interpretation of histology sections. I thank Dr. Frederick Kibenge for allowing me to store trypanosomes in the liquid nitrogen tank in the virology laboratory and Judy Sheppard for allowing me to use equipment in the immunology laboratory. I would like to thank the staff of the research animal facility, post mortem, histology section, audio visual services, and diagnostic services for their support and understanding.

I would like to acknowledge the financial assistance provided by the KENYA-CANADA GENERAL TRAINING FUND III PROGRAM for providing my stipend and part of my research funds. Finally I would like to thank the Department of Pathology and Microbiology for the support given to me in my program.

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ABBREVIATIONS

Abbreviation	Term
ATCC	American Type Culture Collection
BLAD	Bovine leucocyte adherence deficiency
CFT	Complement Fixation Test
CBC	Complete blood count
cc	Cubic centimetres
CNS	Central nervous system
CSF	Cerebro-spinal fluid
DMSO	Dimethylsulfoxide
DPI	Days post infection
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FMD	Foot-and-Mouth disease
g	Centrifugal force
HBSS	Hanks' balanced salt solution
H&E	Haematoxylin and Eosin
HPETE	Hydroperoxyeicosatetraenoic acid

Abbreviations continued...

ICAM	Intercellular adhesion molecule
IFAT	Indirect fluorescent antibody test
IgG	Immunoglobulin G
IL-1	Interleukin 1
ILRAD	International Laboratory for Research on Animal Diseases
LAD	Canine leucocyte adherence deficiency
LTB4	Leukotriene B4
mg	milligrams
ml	millilitre
NADPH	Nicotinamide adenine dinucleotide phosphate
PAF	Platelet activating factor
PBS	Phosphate buffered saline
PCV	Packed cell volume
PGA	Prostaglandin A
PGE	Prostaglandin E
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PMA	Phorbol 12-myristate 13-acetate
PMN	Polymorphonuclear leucocyte
RBC	Red blood cells

Abbreviations continued...

RES	Reticuloendothelial system
SE	Standard error of the mean
SOD	Superoxide dismutase
TNF	Tumour Necrosis Factor
TxA_2	Thromboxane A_2
VATs	Variable antigenic types
VSG	Variant surface glycoprotein
WBC	White blood cells
μL	Microlitres

CHAPTER 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

Trypanosomiases are a group of serious and often fatal parasitic diseases that occur in large areas of Africa, Latin America, the Middle East, and Asia. These diseases affect most species of domestic livestock, many wild animals, and human beings. In Africa the most important trypanosomes, both in human beings and livestock, are transmitted cyclically by tsetse flies (*Glossina* spp). The occurrence of African trypanosomiasis is closely related to the distribution of tsetse flies which are found between latitudes 14°N and 29°S (Brown et al, 1990). In total, 40 African countries are infested with tsetse flies, and the International Laboratory for Research on Animal Diseases (ILRAD) estimates that this is an area covering approximately 10 million square kilometres of land (ILRAD, 1993).

Trypanosomiasis manifests in various ways in animals, but is generally characterized by poor growth, weight loss, low milk yield, reduced capacity to work, infertility, abortion, and if left untreated, high mortality. Sick animals have intermittent pyrexia, relapsing parasitaemia, swollen lymph nodes, anaemia and wasting (Brown et al, 1990). Although similarities exist in clinical and

pathological features of diseases caused by different trypanosomes, the underlying host-parasite interactions are significantly different (Brown et al, 1990).

Trypanosomiasis has particular economic importance in Africa because of the detrimental effect it has on livestock production. Programs for control of tsetse flies and trypanosomiasis are very expensive. Recently, ILRAD estimated total current annual costs of the disease, including both production losses and control costs, to be more than US$ 500 million (ILRAD, 1993). This is more than 10% of the total value of the livestock industry in tropical Africa, estimated at 5 billion dollars (Jahnke, 1982).

Transmission of trypanosomes involves tsetse flies ingesting parasites along with blood from an infected animal. Ingested trypanosomes undergo cyclical development for 8-35 days (Brown et al, 1990). The blood stream forms (trypomastigotes), undergo several developmental stages referred to as amastigotes, promastigotes, epimastigotes and metacyclics. These stages can be differentiated based on appearance and position of flagellum, kinetoplast and nucleus (Stephen, 1986). Development occurs in the midgut and proboscis of the tsetse fly, and involves multiplication and differentiation of trypanosomes to the metacyclic stage, which is infectious to mammalian hosts. Once infected, a fly can transmit trypanosomes for the rest of its life. When an infected fly feeds, metacyclic trypanosomes are injected into the skin of the host with tsetse saliva. In mammals, parasites develop to trypomastigotes and multiply in blood vessels

and other organs causing a variety of disease syndromes. Although tsetse flies are the most important transmitters of trypanosomiasis in Africa, other biting insects can mechanically transmit the disease (Stephen, 1986).

1.2 Etiology

Trypanosomiasis is caused by protozoal parasites of the genus *Trypanosoma*. The genus is divided into several subgenera, the most important being *Schizotrypanum, Duttonella, Nannomonas, Trypanozoon*, and *Pycnomonas*. Classification of the genus *Trypanosoma* was established by Hoare, when he recognised that the mammalian species of trypanosomes can be divided into two sections, Stercoraria and Salivaria, depending on their development in the vector and host (Hoare, 1964). Parasites in the section Stercoraria complete their vector developmental cycle in the "posterior station" of the vector thus, the infective stage for mammals appears in the faeces of the vector, and transmission is by the contaminative route. Parasites of the section Salivaria complete their developmental stages in the "anterior station", i.e. in the salivary medium, and transmission from vector to host occurs by inoculation of the metacyclic stage in saliva (Stephen, 1986).

According to Stephen's (1986) classification, the Stercoraria section contains three subgenera, the most important being the *Schizotrypanum*. This subgenus contains the species *T.* (*S.*) *cruzi* that causes Chagas disease in human

beings and occasionally in dogs. The section Salivaria contains four subgenera, *Duttonella*, *Nannomonas*, *Trypanozoon*, and *Pycnomonas*, all causing disease in domestic animals; only *Trypanozoon* spp affects human beings. The subgenus *Duttonella* contains two species, *T.* (*D.*) *vivax* and *T.* (*D.*) *uniforme* that cause disease in domestic animals. The subgenus *Nannomonas* contains two species important in domestic animals, *T.* (*N.*) *congolense*, and *T.* (*N.*) *simiae*. The subgenus *Trypanozoon* encompasses trypanosomes affecting a wide range of mammals, including human beings. These include *T.* (*T.*) *rhodesiense* and *T.* (*T.*) *gambiense* that cause sleeping sickness in human beings. and *T.* (*T.*) *brucei*, *T.* (*T.*) *equiperdum* and *T.* (*T.*) *evansi* that affect domestic animals. The subgenus *Pycnomonas* has only one species, *T.* (*P.*) *suis* that affects both wild and domestic pigs.

1.3 Pathogenesis and Pathology

Pathogenesis and pathology of tsetse transmitted trypanosomiasis has been extensively studied and yet it is not fully understood. It depends on the species causing the infection and the species of host affected (Losos and Ikede, 1972). Following inoculation and multiplication of trypanosome metacyclics in the skin, the animal reacts with inflammation at the point of inoculation. This characteristic, raised, cutaneous swelling called "chancre" may persist for several days (Willett and Gordon, 1957; Roberts et al, 1969). Trypanosomes subsequently

spread via draining lymph nodes (Luckins and Gray, 1979) and lymph vessels (Emery et al, 1980) to the blood stream and later to other organs of the body. After the local inflammatory skin reaction, there is local lymph node enlargement (Emery and Moloo, 1981), followed by generalized lymph node enlargement and splenomegaly (Losos and Ikede, 1972). This is due to marked lymphoid proliferation (Murray, 1974; Masake, 1980).

Invasion of the blood with actively dividing trypanosomes is accompanied by an increase in body temperature for several days before a trypanolytic crisis occurs, when parasites become scanty in the blood and the temperature returns to normal. This first trypanolytic crisis is followed by further intermittent periods of parasitaemia, with associated febrile episodes, and remissions of infection. The subsequent course and outcome of the disease varies considerably as it is influenced by several factors, including species and breed of the host, nature and severity of trypanosome challenge, pathogenicity of infecting trypanosomes, and duration of infection (Brown et al, 1990; Ikede, 1994).

1.3.1 *Trypanosoma congolense*

Clinical signs of *T. congolense* are variable and depend on the strain of trypanosome and species of the affected host. The prepatent period averages 15-17 days (Stephen, 1986). After trypanosomes appear in the blood, there is an increase in body temperature to about 39.5-40°C. Temperature fluctuates along

with parasitaemia as the disease progresses, and with time anaemia sets in. Although pathognomonic signs are not seen, the haircoat is usually lustreless and ruffled, and there is gradual but severe loss of body condition (Stephen, 1986). Superficial lymph nodes may be enlarged, and death occurs in weeks or several months to a year.

Haematological examination of trypanosome-infected animals demonstrates anaemia and variable white blood cell kinetics (Anosa, 1988). Usually, there is leucopenia associated with neutropenia, eosinopenia, lymphopenia and monocytosis (Naylor, 1971; Anosa, 1988). Anaemia is due to haemolysis and erythrophagocytosis by mononuclear phagocytic system in spleen, bone marrow, haemolymph nodes, lung, kidney and liver (Mammo and Holmes, 1975; Ikede et al, 1977; Anosa, 1988). Increased erythrophagocytosis is associated with trypanosomal antigens on the surface of erythrocytes (Kobayashi et al, 1976; Mackenzie et al, 1978). Intravascular haemolysis is due to a lytic factor produced by trypanosomes (Wellde et al, 1974; Valli and Forsberg, 1979).

Neutropenia and eosinopenia result from bone marrow granulocyte hypoplasia as shown by reduced myeloid:erythrocyte ratio (Valli et al, 1979; Anosa, 1988). Another factor contributing to neutropenia and possibly eosinopenia is splenic sequestration, especially in animals with splenomegaly. Splenomegaly is accompanied by hypersplenism which usually results in neutropenia (Anosa, 1988). Lymphopenia can be caused by lymphocyte redistribution external to the

vascular system. Despite intense antigenic stimulation and accompanying lymphoid hyperplasia in the early phases of infection, lymphopenia still occurs presumably because lymphocytes are immobilized in lymphoid organs and in organs with inflammatory response (Anosa, 1988). In chronic cases, lymphopenia results from severe lymphoid hypoplasia characterized by depopulation of nodules and disappearance of germinal centres.

Trypanosome-infected animals have monocytosis accompanied by proliferation of macrophages in several tissues (Ikede and Losos, 1972b; Murray et al, 1974b; Morrison et al, 1982; Anosa, 1983; Anosa and Kaneko, 1984b). The presence of monocytosis indicates that depression of the myeloid colony formation (Kaaya et al, 1979) occurs after developmental divergence of granulocyte and monocyte cell lines. Increase in macrophage numbers is accompanied by their activation (Murray et al, 1974b; Fierer and Askonas, 1982). Increased monocyte and macrophage proliferation and activation result from increased demands to remove particulate matter, including trypanosomes, RBCs, WBCs, and dead tissue (Anosa, 1988). This is because the cells of the reticulo-endothelial system (RES) proliferate when the work load increases (Jandl et al, 1965).

At necropsy, the carcass from *T. congolense*-infected animal is emaciated and body fat stores are depleted or gelatinous. In acute cases the lymph nodes may be swollen and haemorrhagic, but in chronic cases, they may be smaller and

fibrotic (Stephen, 1986). Changes in the liver and heart are inconsistent. When present, these two organs are slightly enlarged and pale. The spleen is usually enlarged, with blunt edges, its cut surface is dry, and the follicles prominent with enlarged germinal centres (Losos and Ikede, 1972). Changes in the bone marrow are variable; in acute cases, there may be extension of the marrow into medullary cavity of long bones while in chronic cases, there is little evidence of haemopoiesis (Losos and Ikede, 1972; Stephen, 1986). There are no pathognomonic gross lesions in *T. congolense* infections.

Histopathological findings in *T. congolense* infection in ruminants are variable and unremarkable (Fiennes, 1970; Losos and Ikede, 1972). There may be lesions in bone marrow, heart, liver, kidneys, testes and blood vessels. In acute cases the bone marrow may show erythroid hyperplasia and haemosiderosis (Naylor, 1971; Losos et al, 1973). Naylor (1971) reported slight focal hyaline degeneration in myocardium while Kaliner (1974) also reported slight interstitial infiltration with macrophages and lymphocytes in the heart. The liver may show haemosiderosis, erythrophagocytosis, foci of haemopoiesis, hepatocellular atrophy and congestion of sinusoids and central veins (Naylor, 1971; Kaliner, 1974; Valli and Forsberg, 1979). Focal infiltration with either macrophages or lymphocytes can occur in renal cortex, and glomeruli may be hypertrophic and hypercellular (Losos et al, 1973; Kaliner, 1974; Valli and Forsberg, 1979). Kaaya and Oduor-Okello (1980) found that seminiferous tubules in the testicles

were markedly shrunken and devoid of spermatozoa. Also, the spermatogenic cells had degenerated, epididymal lumina were empty, and there was increased connective tissue around epididymal tubules.

Changes in the circulatory system involved widespread occlusion of venules, generalized capillary dilation and occlusion of the lumina due to swelling of endothelial cells (Fiennes, 1950; Losos et al, 1973). The tunica elastica of small arteries was swollen and lysed, whereas the tunica media was necrotic and swollen causing occlusion of the lumen (Kaliner, 1974). Recently, Abebe et al (1993) demonstrated the presence of numerous trypanosomes in the microvasculature of the pituitary gland in *T. congolense*-infected cattle. This was accompanied by localized degenerative changes in the secretory cells of the adenohypophysis.

In summary, *T. congolense* is primarily intravascular, usually eliciting little specific tissue or organ damage. The main pathological findings are anaemia and emaciation.

1.3.2 *Trypanosoma brucei*

The prepatent period of *T. brucei* in natural infections in ruminants is variable, but may be as short as 3-4 days. Dwinger (1985) found a prepatent period of 4 days in goats when infected by *Glossina morsitans centralis*. Natural infections in cattle result in parasitaemia and mild pyrexia, but clinical

signs are seldom seen (Stephen, 1986). Clinically, natural *T. brucei* infections in sheep and goats are chronic with low or medium parasitaemia, and loss of body condition. The chief signs are progressive anaemia, moderate pyrexia, loss of body condition, edema of subcutaneous tissues, and ocular opacity. Although naturally infected ruminants do not develop severe lesions, experimental infection with *T. brucei* causes more severe pathological changes than with *T. congolense* infection (Losos and Ikede, 1972; Stephen, 1986).

Ikede and Losos (1975a) described the main clinical signs in sheep infected experimentally with *T. brucei* as fever, loss of weight, dullness, weakness, enlarged superficial lymph nodes, and edema of the head, scrotum, limbs, tail, ventral wall of thorax and abdomen. Edema of the head involved the lips, bridge of the nose, eyelids and ears. This facial edema was so severe that it interfered with prehension and breathing. The sheep developed progressive anorexia and moderate pallor of the mucous membranes. Some sheep showed neurological signs characterized by paresis, tremors and shaking of the head and neck, twitching of eyelids and ears, and generalized shivering. Terminally, neurological signs became severe, involving chewing movements, foaming from the mouth, grinding of teeth, walking backwards, staggering, somnolence, and incoordination.

Haematological changes found in ruminants infected with *T. brucei* are very mild and have received little attention (Ikede and Losos, 1972a; Stephen,

1986). Anaemia may be mild or absent and WBC kinetics are frequently ignored. Within tissues, there is a greater inflammatory cell infiltration compared to *T. congolense* infection, and more frequent demonstration of trypanosomes. Ikede and Losos (1972b) described the basic reaction in affected tissues and organs as an interstitial and perivascular mononuclear cell infiltration associated with extravascular localization of trypanosomes. Inflammatory cells were medium-sized to large lymphocytes, macrophages and plasma cells. In some cases, there were also polymorphonuclear cells and fibrinous exudate. There was generalized lymphoid hyperplasia in spleen and lymph nodes with prominent germinal centres, numerous macrophages, and reticuloendothelial hyperplasia. Lesions observed in the liver and kidneys were similar although relatively more severe than those seen in *T. congolense*. Severe interstitial myocarditis, extensive cellulitis in the dermis and subcutaneous tissues, widespread necrotizing vasculitis, lymphatic thrombosis and fibrinous exudation have also been observed (Stephen, 1986). Ikede (1979) described severe orchitis and testicular degeneration that resulted in complete aspermatogenesis in experimentally infected rams. Similar lesions developed in *T. brucei* infections in male rabbits (Ikede and Akpavie, 1982), in mice (Anosa and Kaneko, 1984a), and in monkeys (Peruzzi, 1928). Overall the lesions observed in the male reproductive organs cause infertility (Ikede et al, 1988).

Neurological signs in *T. brucei*-infected animals are accompanied by central nervous system (CNS) lesions (Ikede and Losos, 1972a,b; Losos and Ikede, 1972; Ikede et al, 1977; Morrison et al, 1981; Onah and Uzoukwu, 1991), which include meningoencephalomyelitis in the brain stem, hypothalamus and thalamus. There was perivascular mononuclear cell infiltration in the meninges and in Virchow-Robin spaces of the brain and spinal cord. Trypanosomes were occasionally found in meninges and in perivascular spaces of the brain. Changes observed in cerebrospinal fluid (CSF) included infiltration with trypanosomes, increase in total cell counts and elevated total protein concentration (Ikede and Losos, 1975b). The predominant cells in CSF were lymphocytes, macrophages, occasional plasma cells, and morular cells of Mott.

Ikede and Losos (1975c) observed endocrine lesions consisting of edema, mononuclear cell infiltration, necrosis and fibrosis of the adenohypophysis in sheep experimentally infected with *T. brucei*. There was inflammation of the neurohypophysis and surrounding meninges, enlargement of the adrenal cortex, and atrophy of the thyroid gland. These lesions were accompanied by massive localization of trypanosomes in the interstitial tissue of the adeno- and neuro-hypophyses. Trypanosomes were not detected in other endocrine glands.

Infiltration and multiplication of trypanosomes in the eye caused severe ocular lesions in experimentally infected sheep (Ikede, 1974). There was out-pouring of fibrinous, non-purulent exudate into the anterior chamber of the eye.

Other components of the exudate included mononuclear cells and trypanosomes, and resulted in the corneal opacity.

Although *T. brucei* infection in ruminants produces mild anaemia, extravascular localization of trypanosomes in the heart, brain, testes, skin and eye is accompanied by interstitial and perivascular mononuclear cell infiltration. Similar lesions occur in other hosts, including dogs (Morrison et al, 1981), donkeys (Ikede et al, 1977), and pigs (Onah and Uzoukwu, 1991). In these species, moderate to severe anaemia was observed (Stephen, 1986). The inflammation, edema, and necrosis that occurred in the above organs resulted in the clinical signs and lesions.

1.4 Diagnosis

Diagnosis of trypanosomiasis in the absence of laboratory facilities is difficult because clinical signs are not pathognomonic. The most frequently used method is parasite detection by examination of blood films. With low parasitaemia, parasites can be concentrated by centrifugation (Paris et al, 1982; Murray et al, 1983). Serological tests have been developed but have not attained widespread use because they detect antibodies and not trypanosome antigens. Serum antibodies are not detectable until 14 days post infection and remain detectable for long periods after treatment and recovery. Therefore, these tests do not detect early infections and a positive antibody test result does not always

indicate patent infection. The three serological tests most frequently used are Indirect Immunofluorescent Antibody Test (IFAT) (Wilson, 1969), Complement Fixation Test (CFT) (Perie et al, 1975), and the Enzyme-Linked Immunosorbent Assay (ELISA) (Luckins, 1977).

Rae and Luckins (1984) developed an ELISA technique that detects circulating trypanosomal antigens. This was improved by use of monoclonal antibodies that distinguish the three main groups of African trypanosomes, i.e, the vivax, congolense and brucei (Nantulya et al, 1987). This new antigen-ELISA technique is simpler than the other serological tests, is positive soon after infection, and becomes negative within 2-3 weeks of cure (ILRAD, 1988).

1.5 Disease Resistance

One of the unique features of trypanosomes is their ability to undergo antigenic variation (Borst et al, 1983). The antigenic determinants of trypanosomes are located on the surface coat, which is composed of a single glycoprotein, the Variant Surface Glycoprotein or VSG (Cross, 1975; Borst and Cross, 1982). The highly immunogenic VSG molecule changes during antigenic variation (Vickerman, 1978) resulting in numerous variable antigenic types (VATs). As the host mounts antibody response to the first VSG, some parasites will have changed their VSG, thus initiating another wave of parasitaemia (Barry

and Emery, 1984). Therefore, antigenic variation keeps trypanosomes always ahead of the host's immune response (Gray, 1967).

Another important aspect of trypanosomiasis is lowered resistance of the host to other diseases (Urquhart, 1980). This immunosuppression has been well demonstrated in laboratory animals (Goodwin et al, 1972; Murray et al, 1974a), although mechanisms involved are not well documented. In trypanosome-infected ruminants, derangement of the immune system is less marked than in small laboratory animals (Dwinger, 1985). Hypergammaglobulinemia occurs consistently in cattle (Kobayashi and Tizard, 1976) with a dramatic increase in immunoglobulin catabolism (Nielsen et al, 1978). Mwangi et al (1990a) found that antibody production to anthrax vaccine was severely depressed in goats infected with *T. congolense*. When these goats received trypanocidal therapy 14 days post infection, they developed antibody levels similar to uninfected controls. Although significant suppression of antibody responses to *Brucella* spp vaccine was found in *T. congolense*-infected cattle (Rurangirwa et al, 1983) and in goats challenged with anthrax vaccine (Mwangi et al, 1990a), in some cases the impairment does not compromise the immune response to all infectious agents. The lowered antibody titres were still able to confer sufficient protection with Foot-and-Mouth disease (FMD) vaccine against FMD live virus challenge (Sharpe et al, 1982), while it resulted in impairment of protective immunity after Mycoplasma vaccination (Ilemobade et al, 1982). Interestingly, antibody

response to the variant-specific antigen of trypanosomes is sufficient to clear parasitaemia repeatedly during chronic infection (Diggs, 1982).

Mechanisms of immunosuppression in experimental trypanosomiasis in mice, rats, and cattle have been reviewed (Murray, 1975; Mansfield, 1978; Rurangirwa et al, 1979; Diggs, 1982). Briefly, immunosuppression was attributed to three mechanisms, namely loss or suppression of helper T-lymphocyte function, non-specific polyclonal activation of B-lymphocytes, and stimulation of non-specific suppressor T-cells. Immunosuppression in ruminant trypanosomiasis is reversible after live trypanosomes or their antigens have been eliminated (Nantulya, 1985; Mwangi et al, 1990a). Clinically, immuno-suppression in trypanosome-infected animals manifests as reduced body defence leading to increased secondary bacterial and other opportunistic infections.

For an effective immune response, the effector systems must be intact. The three effector systems that participate in immune response are the mononuclear-phagocytic system, the complement cascade, and the polymorpho-nuclear phagocytic system (Tizard, 1992a). In addition to phagocytosis, mononuclear cells also process antigens and present them to T-lymphocytes and B-lymphocytes. In trypanosomiasis, the mononuclear phagocytic system is stimulated and highly expanded (ILRAD, 1990). In depth studies have not been done on the complement cascade and the polymorphonuclear phagocytic systems in trypanosome-infected animals. Polymorphonuclear leucocytes (PMNs) are

important in host defence against invading microorganisms (Quie and Cates, 1977) and decreased neutrophil function often leads to bacterial infections (Melech and Gallin, '987).

The polymorphonuclear phagocytic system in trypanosomiasis may have various abnormalities that could contribute to the decreased body defence. Valli and Forsberg (1979) suggested that the susceptibility of calves with *T. congolense* infection to other diseases, especially bacterial, was caused by the neutropenia rather than immunosuppression. There were lymphoid follicles and germinal centres in bone marrow indicating no obvious B-cell depression. They concluded that death was probably from super infection caused by the neutropenia. PMNs are primarily involved in destruction of bacteria and bacterial proteins. Their destruction, impaired production or function would play a large role in the effectiveness of the immunological response of the host to bacteria. Naylor (1971) noted reduction of eosinophil precursors and eosinophils, vacuolation of cytoplasm and nucleus of granulocytic cells and disintegration of mature granulocytes in the bone marrow of cattle experimentally infected with *T. congolense*. Whether PMNs remain functionally normal during trypanosome infection is unknown. This thesis reports studies of quantitative and some functional aspects of polymorphonuclear leucocytes in *T. congolense*- and *T. brucei*-infected sheep.

1.6 Research Objectives

Trypanosomiasis is associated with lowered body defence characterized by increased incidence of opportunistic infections. Although T-lymphocyte and B-lymphocyte derangement contribute to reduced body defence, in some cases they have no obvious alteration. Murray (1975) noted that immunosuppression in animals chronically infected with trypanosomes developed because such animals often have morphologically depleted lymphoid systems. In the acute stage, the existence of immunosuppressed state with massive plasma cell hyperplasia is difficult to explain. The main objective of this study was to initiate and follow the course of clinical trypanosomiasis in sheep and examine the functional status of one effector system, the polymorphonuclear phagocytic system. Results of the study would determine the possible role of the polymorphonuclear phagocytic system in the weakening of body defence.

Local and widespread skin lesions often occur in *T. brucei*-infected animals and may be the most debilitating clinical sign in experimentally infected sheep (Ikede and Losos, 1975a). A second objective of this study was to describe such skin lesions, and to characterise their histologic appearance.

CHAPTER 2. POLYMORPHONUCLEAR LEUCOCYTE FUNCTION IN TRYPANOSOME-INFECTED SHEEP

2.1 Introduction

2.1.1 Immunosuppression in Trypanosomiasis

Animals infected with trypanosomes often have secondary bacterial and other parasitic infections. These animals have reduced ability to mount detectable immune responses to certain antigens (Murray, 1975). This has been demonstrated in rabbits (Goodwin et al, 1972) and in laboratory rodents (Goodwin et al, 1972; Murray et al, 1974a,b) infected with *T. brucei*, and in cattle infected with *T. congolense* (Holmes et al, 1974). Breakdown of body defence mechanisms results from immunosuppression during these infections (Murray, 1975; Rurangirwa et al, 1978; Mansfield, 1978). The role of *T. congolense* in causing suppression of immune response to viral and bacterial vaccines in ruminants is inconclusive. There was minimal evidence of immunosuppression in response to viral antigens (Scott et al, 1977; Rurangirwa et al, 1980). In contrast, with bacteria or bacterial antigens, there was strong evidence of immunosuppression (Holmes et al, 1974; Scott et al, 1977; Whitelaw et al, 1979; Mwangi et al, 1990a).

Several hypotheses explaining immunosuppression in trypanosomiasis have been put forward. One hypothesis proposes that the B cell system of the host with trypanosomiasis undergoes generalized activation and thus proceeds to a stage of differentiation limiting additional responses to antigenic stimulation (Clayton et al, 1979; Diggs, 1982). Mansfield (1978) suggested that the major defect is in the T-lymphocyte subpopulation but others (Askonas et al, 1979) stressed generalized dysfunction of lymphoid tissue including T and B memory cells and T cells responsible for mixed lymphocyte reactivity. Another report implicated macrophage dysfunction to be important in the development of immunosuppression (Grosskinsky et al, 1981). We assessed the role of one previously overlooked effector system of the immune response, i.e., the polymorphonuclear phagocyte function during trypanosome infection.

2.1.2 Polymorphonuclear Leucocyte Function

PMNs are produced in bone marrow and move into peripheral blood circulation where one half are circulating and the other half are adherent to endothelial surfaces of capillaries (Wade and Mandell, 1983). A major function of PMNs is phagocytosis and destruction of invading microorganisms, thereby protecting the host from microbial infection. To function effectively, PMNs must complete the following processes: chemotaxis, adhesion, migration, adherence, ingestion, and destruction of ingested substances (Van der Valk and Herman,

1987). Congenital or acquired defects in any of the processes will result in increased susceptibility to infection with bacterial or fungal pathogens (Baehner, 1972).

During infection, certain molecules which exhibit chemotactic activity for neutrophils and mononuclear phagocytes are produced. These include bacterial products (e.g formylpeptides), C5a, platelet activating factor (PAF), leukotriene B4 (LTB4), and interleukin-8 (Snyderman and Uhing, 1992). In response to these chemoattractants, PMNs first adhere to the surface of endothelial cells before migrating into tissues towards the site of infection. Once PMNs reach the source of chemotactic factors, contact with the offending agent initiates adherence and phagocytosis. Adherence and phagocytosis are facilitated by prior opsonization (Wade and Mandell, 1983). Opsonization is achieved when the invading substance is coated with opsonins, e.g., antibody (IgG), C3 and C5, tuftsin, and properdin (Najjar, 1972; Stossel et al, 1973).

Migration of PMNs is facilitated by formation of pseudopodia. When pseudopodia contact an opsonized particle, they flow over and around the particle and binding occurs between ligands (opsonins) on the particle and receptors on PMNs (Tizard, 1992a). This process ensures that PMNs' surface is bound firmly to the particle, which is drawn into the cell. The particle finally becomes enclosed in a phagosome. After the particle is ingested, two processes are initiated by PMNs to neutralise or kill the organism. First, there is release of

lytic enzymes (lysozyme, myeloperoxidase, hydrolases) from specific and azurophil granules into the phagocytic vacuole (Laszlo and Rundles, 1977). The second process involves generation of highly reactive oxygen radicals during the respiratory burst (Van der Valk and Herman, 1987). The combination of myeloperoxidase and oxygen radicals ensures effective killing of microorganisms (Clark and Klebanoff, 1979).

Although no studies applying PMN function tests methods have been done in trypanosome-infected animals, the use of these assays in other diseases have shown that impairment in PMN function leads to increased susceptibility to microbial infections. Significant impairment of neutrophil function during the postpartum period can occur in cows with retained fetal membranes, increasing their susceptibility to various infections (Gilbert et al, 1993).

2.1.3 Polymorphonuclear Leucocyte Function Testing

The activities of PMNs in controlling microbial infection are complex. To adequately evaluate PMN function, various *in vitro* tests are utilized concurrently (Roth and Kaeberle, 1981a). There are two standard tests commonly used to assess the chemotactic, migrational, and/or adherence properties of PMNs: migration under agarose (Nelson et al, 1975) and nylon wool adherence (MacGregor et al, 1974). Ingestion and phagocytosis are assessed by two tests: ingestion of *Staphylococcus aureus* (Roth and Kaeberle, 1981a), and phagocytosis of opsoni-

zed zymosan (Buchta, 1990). To assess digestion/killing properties, tests measuring ability of PMNs to generate reactive oxygen products are used. They include nitroblue tetrazolium reduction (Baehner and Nathan, 1968), chemiluminescence (Allen et al, 1972), iodination (Klebanoff and Clark, 1977), and cytochrome C reduction (Bellavite et al, 1983).

In this study we used three PMN function assays covering three critical aspects of PMN function, i.e., adherence, phagocytosis, and generation of reactive oxygen products. The assays were cytochrome C reduction, nylon wool adherence and phagocytosis of opsonized zymosan.

2.1.4 Research Objectives

Alteration of immunoregulation in trypanosomiasis involves multiple components of the immune system, although no unifying hypothesis is available to explain all of them. The objective of this study was to determine if experimental infection with *T. congolense* and *T. brucei* cause dysfunction of PMNs in sheep. Results might explain some aspects of immunosuppression in trypanosome-infected animals.

2.2 Material and Methods

2.2.1 Trypanosomes

Trypanosoma brucei (Plimmer and Bradford, 1899) strain ATCC 30118 and *T. congolense* (Broden, 1904) strain ATCC 30018 were acquired from American Type Culture Collection Maryland, USA. Each strain was injected intraperitoneally into male CD Sprague Dawley rats weighing 250 grams. After four days when peak parasitaemia was observed, the rats were anaesthetized by intraperitoneal injection of barbiturate and bled by cardiac puncture using a syringe containing heparin. The blood was diluted 1:4 with phosphate buffered saline (PBS) and trypanosomes counted in a haemocytometer before injection into each sheep.

2.2.2 Sheep

Fourteen 7-8 months old castrated male sheep, were locally purchased. The sheep were injected intramuscularly with Ivermectin (Ivomec®, Merck Agvet, Canada), vitamin E/selenium (Dystosel®, Rogar/STB Inc., Canada) and clostridial vaccine (Tasvax-8®, Coopers Agrochem Canada). Animals were acclimatised for at least one month to the housing conditions at the Atlantic Veterinary College.

In the first experiment, four Suffolk x Finnish sheep were injected with *T. congolense*-infected rat blood while two acted as controls. In the second experiment, six sheep of the North Country Cheviot breed were injected with *T. brucei*-infected rat blood while two sheep acted as controls. Each infected sheep received 8 million trypomastigotes suspended in one ml of PBS and rat blood intravenously. The control sheep were given one ml PBS. Body weight, parasitaemia, and packed cell volume were determined as detailed in chapter three section 3.2.2.1, 3.2.2.2 and 3.2.2.3. Sheep were monitored daily for clinical signs and were also under constant veterinary care to prevent unnecessary suffering. The Canadian Council on Animal Care (CCAC) guidelines were followed in the handling of the experimental animals (CCAC, 1993).

In *T. congolense*-infected sheep, in addition to cytochrome C reduction assay, bone marrow biopsies were obtained every two weeks and examined by light microscopy. In *T. brucei*-infected sheep, in addition to cytochrome C reduction assay, nylon wool adherence and phagocytosis of opsonized zymosan were also performed. At the end of the trials, all the sheep were euthanised by barbiturate overdose and post mortem examination done.

2.2.3 Total and Differential White Blood Cell Counts

Blood was collected from the jugular vein of each sheep into a 2 ml EDTA vacutainer tube (Beckton Dickinson, USA). Total White blood cell

(WBC) counts were determined three times a week by an electronic particle counter (model T 660, Coulter Electronics Inc., USA), which had been calibrated for sheep and goat blood cells. Smears were made from EDTA anti-coagulated blood and stained with Wright's stain in an automatic stainer (model Hematek 1000, Miles Scientific Lab. Inc., USA). Differential cell counts were determined from at least 200 white blood cells examined with 100X objective.

2.2.4 Polymorphonuclear Leucocyte Function Tests

2.2.4.1 Cytochrome C Reduction Test

Polymorphonuclear leucocytes were isolated from sheep blood as described by Carlson and Kaneko (1973) and modifications of Buchta (1990). Briefly, 30-40 ml of blood were collected from each sheep by jugular venipuncture into 3-4 ml of 1% EDTA and 0.9% NaCl (pH 7.2). The blood was centrifuged at 1000 *g* for 15 min and the plasma and buffy coat discarded. The remaining packed cells were lysed at 4°C with two volumes of buffered 0.2% NaCl for 45 seconds. Isotonicity was restored with addition of two volumes of buffered 1.6% NaCl solution. To recover PMNs the tube was centrifuged at 300 *g* for 10 min. The supernatant was discarded, the cell pellet re-suspended in 10 ml of PBS and the lysis process repeated. The cell pellet was washed once in 35 ml of PBS (pH 7.2). The pellet was suspended in 10 ml of PBS and viability, purity and cell

counts determined. Viability was assessed by Trypan blue exclusion test (Metcalf et al, 1986). Finally, the cells were centrifuged and suspended in sterile PBS containing 1mM Ca^{2+}, 1mM Mg^{2+} and 5mM glucose at a concentration of 2 X 10^7 cells/ml. In all the isolations viability and purity averaged 89% and 87%, respectively.

Cytochrome C from horse heart (Sigma Chemical Co., St. Louis, MO), was freshly prepared each day of test in two forms as described by Bellavite et al (1983). Solution one (S1) contained 3.75 mg of cytochrome C/ml of PBS containing 1mM Ca^{2+} + 1mM Mg^{2+} + 5mM glucose. Solution two (S2) was made up of 3.75 mg cytochrome C and 0.125 mg of Superoxide Dismutase (SOD) (Sigma Chemical Co., St. Louis, MO) in one ml of PBS containing 1mM Ca^{2+} + 1mM Mg^{2+} + 5mM glucose. Phorbol 12-myristate 13-acetate preparation (PMA) (Sigma Chemical Co., St. Louis, MO) was used as a stimulant of superoxide anion production and was prepared as described by Nagahata et al (1991). It was dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Co., St. Louis, MO) at a concentration of 0.2 mg/ml. This was divided into 50 µL aliquots and stored at -20 °C until needed.

The procedure for cytochrome C reduction assay was developed based on the method described by Bellavite et al (1983). In *T. congolense*-infected sheep PMN function was assessed every second week while in *T. brucei*-infected sheep

this was done weekly. For each sheep the reagents were prepared in labelled glass tubes as follows:

tube A: cells + PBS + Cytochrome C

B: cells + PBS + Cytochrome C + SOD

E: cells + PBS + Cytochrome C + PMA

F: cells + PBS + Cytochrome C + PMA + SOD

For each experiment the following cell free controls were also prepared:

tube a: PBS + Cytochrome C

b: PBS + Cytochrome C + SOD

e: PBS + Cytochrome C + PMA

f: PBS + Cytochrome C + PMA + SOD

The reagents were added to the tubes in the following sequence:

0.4 ml of S1 was added to tubes a, e, A, E

0.4 ml of S2 was added to tubes b, f, B, F

2.5 µl (0.5 µg) of PMA to tubes e, f, E, F

The tubes were pre-incubated for 5 min at 37°C. The isolated granulocytes in solution (2 X 10^7 cells/ml) were pre-incubated for 5 min before 0.1 ml was added to tubes (A to F) for each sheep. Control tubes (a-f) received 0.1 ml of PBS. The tubes were capped and incubated (while being agitated) for 15 min. Subsequently, 2 ml of cold (4°C) PBS was added to stop the reaction and the

tubes centrifuged at 1500 *g* for 10 min at 4°C. The absorbance of the supernatant was measured at 550 nm and then at 468 nm.

Superoxide anion produced was calculated following the method of Bellavite et al (1983). The absorbance values of the supernatants at 550 nm and 468 nm in tubes with SOD were subtracted from the absorbance values of the respective tubes without SOD. This was done to correct for the superoxide anion-independent reduction of cytochrome C. The resulting absorbance value was multiplied by 2.5 (dilution factor) and divided by 0.0245 (the extinction coefficient μmoles/litre of cytochrome C at 550-468 nm). The result was expressed as nanomoles of superoxide anion produced by 2 X 10^6 cells.

2.2.4.2 Nylon Wool Adherence Assay

Six ml of peripheral blood was collected from the jugular vein into a 10 ml syringe containing heparin (100 units/ml of blood). The blood was utilized within three hours for nylon wool adherence assay and phagocytosis of zymosan assay. Nylon wool adherence of PMNs in whole blood was examined weekly by the method of Nagahata et al (1991) and Metcalf et al (1986) with modifications. Each adherence column was constructed from a disposable tuberculin syringe, a 20 gauge disposable needle and 40 mg of nylon wool (3 denier type 200, Polysciences Inc., USA). The nylon fibres were packed to a volume of 0.4 cc with the syringe plunger. Heparinized blood and the columns were pre-

warmed at 37°C for 15 min before 1 ml of blood was added to each column. Duplicate columns were set up for each sheep.

Total WBC counts were performed by an electronic particle counter (T 660, Coulter Electronics Inc., USA). Differential leucocyte counts on Wright stained blood smears were performed before and after the blood was allowed to percolate through the nylon fibre columns by gravity in a 37°C humidified chamber. Granulocyte adherence was calculated by the formula:

$$\% \text{ PMN adherence} = 100 - \left[\frac{\text{PMNs/ml effluent sample}}{\text{PMNs/ml initial sample}} \text{ X } 100\right]$$

2.2.4.3 Phagocytosis of Zymosan Assay

Opsonised zymosan was prepared as described by Buchta (1990). Zymosan (Sigma Chemical Co., St. Louis, MO) was hydrated by boiling for 30 min in 0.9% NaCl. Blood was coagulated by warming for one hour at 37°C, followed by centrifugation at 1000 *g* for 15 min. Serum was decanted and added to zymosan at 5 mg of zymosan/ml of serum. This mixture was incubated at 37°C for 20 min, and then centrifuged at 500 *g* for 10 min at 4°C. The zymosan was washed twice with PBS, aliquoted and stored at -20°C. Prior to use, the zymosan was re-suspended in Hank's balanced salt solution (HBSS) with Ca^{2+}, Mg^{2+} and glucose.

Phagocytosis assay was done weekly and was based on the methods of Buchta (1990) and Nagahata et al (1991). Briefly, 0.2 ml of heparinized whole blood was placed in a small test tube. To this was added 0.1 ml of opsonized zymosan suspended in HBSS with Ca^{2+} and Mg^{2+} at a concentration of 5 mg of opsonized zymosan/ml of blood. The reagents were mixed and incubated at 37°C for 15 min. The contents were mixed and blood smears prepared. The smears were stained with Wright's stain and examined with a light microscope.

In total, 200 granulocytes were examined under oil immersion (100X objective) and classified as either (A) neutrophils and eosinophils that had phagocytozed zymosan, or (B) neutrophils and eosinophils that had not phagocytozed zymosan. The percentage of granulocytes [A/(A+B)] that had phagocytozed zymosan was calculated.

2.2.5 Statistical Analysis

To test whether there was significant difference in weight gain, white blood cell counts, cytochrome C reduction, nylon wool adherence and phagocytosis of opsonized zymosan, between infected and control sheep, a two tailed t-test was applied (Ryan et al, 1985). A value of $p \leq 0.05$ was considered a significant difference.

2.3 Results

2.3.1 Clinical Signs and Pathological Changes

Sheep infected with *T. congolense* became parasitaemic between 4 and 11 days post inoculation with an average of 7 days. Parasitaemia was scanty and in most cases was detectable only on buffy coat. Parasites were detected in peripheral blood of infected sheep until termination of the experiment 60 days post infection (dpi). Infected sheep did not develop clinical signs of trypanosomiasis and there were no significant changes in rectal temperature, packed cell volume and weight gain between experimental and control sheep. Infected sheep had a higher average weight gain of 255 grams/day compared to 214 grams/day for the controls; this difference was not statistically significant. There were no gross and histological lesions in both infected or control sheep. Bone marrow biopsies obtained from both infected and uninfected sheep showed no microscopic changes.

Sheep infected with *T. brucei* became parasitaemic between 2 and 16 dpi with an average of 8 days. Parasitaemia was scanty and in most cases was detectable only on buffy coat examination early in the experiment. Infected sheep developed pyrexia, weight loss, reduced packed cell volume, edema and inflammation of the skin. The first edematous swellings appeared on the neck at the site of trypanosome inoculation and throughout the course of infection, the

swellings regressed and recurred repeatedly at this site. Infected sheep developed inflammatory edema of the head 35 dpi. This edema was prominent on the jaws (both lower and upper), above the nostrils, on the ears, and the eyelids. Later the edema involved distal parts of the limbs in all infected sheep, the scrotal area in one, and the tail in two others. The inflammatory edema was so severe in one sheep that it interfered with proper prehension of food. This necessitated euthanising the most severely affected animal 48 dpi and others subsequently. Gross and histological changes are described in chapter three sections 3.3.4 and 3.3.6.

2.3.2 White Blood Cell Counts

In sheep infected with *T. congolense*, there was no significant difference in total and differential white blood cell counts as compared to control group (Figs. 1, 2, and 3). Sheep infected with *T. brucei* had a mild leucocytosis characterized by a mild lymphocytosis in the early part of the infection and a mild neutrophilia in the terminal part of the experiment (Figs. 4, 5, and 6). The neutrophilia was not accompanied by appearance of immature neutrophils in peripheral circulation.

2.3.3 Cytochrome C Reduction Assay

There was no significant difference between *T. congolense*-infected and control sheep in the production of superoxide anion (Fig. 7). In *T. brucei*-infected sheep there was significant decrease ($p<0.01$) in the ability of PMNs from infected sheep to reduce inhibitable cytochrome C compared to controls (Fig. 8). This decrease was noticeable by 21 dpi, became more pronounced as the disease progressed and correlated well with the severity of the disease. Four severely affected sheep had a greater decrease compared to two mildly affected sheep. During the study, the number of PMNs in infected sheep increased from the fourth week of infection; however the number of PMNs recovered during isolation procedures from the blood decreased.

2.3.4 Nylon Wool Adherence

In *T. brucei*-infected sheep, nylon wool adherence by PMNs was not significantly different between controls and infected sheep until the third week of infection (Fig. 9). From the fourth week onwards the ability of PMNs to adhere to nylon wool in the infected sheep was significantly lower ($p<0.01$) than in the controls.

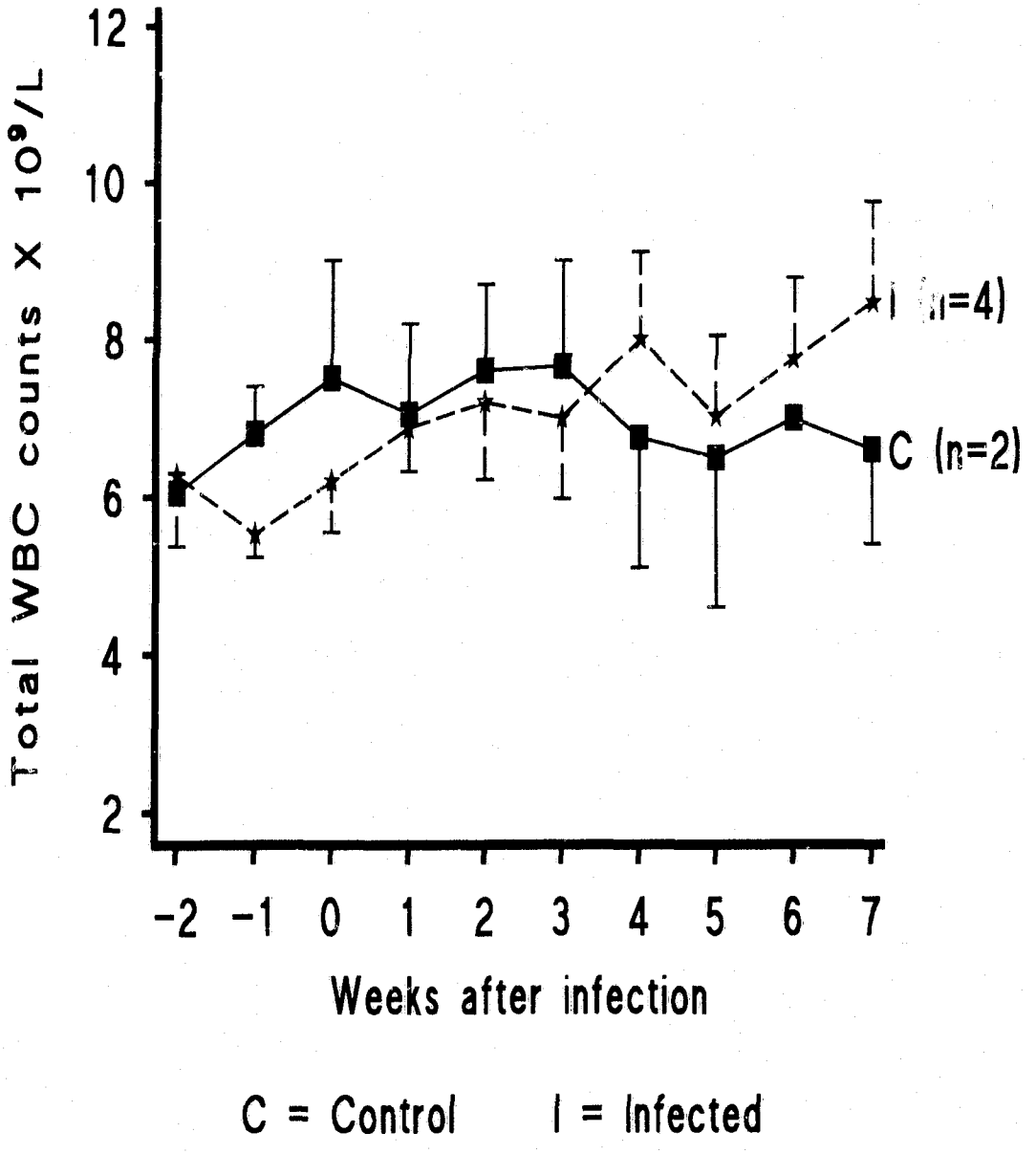

Fig. 1 Total white blood cell counts ($\bar{x}$ ± standard error of the mean (SE)) in *Trypanosoma congolense*-infected and uninfected sheep.

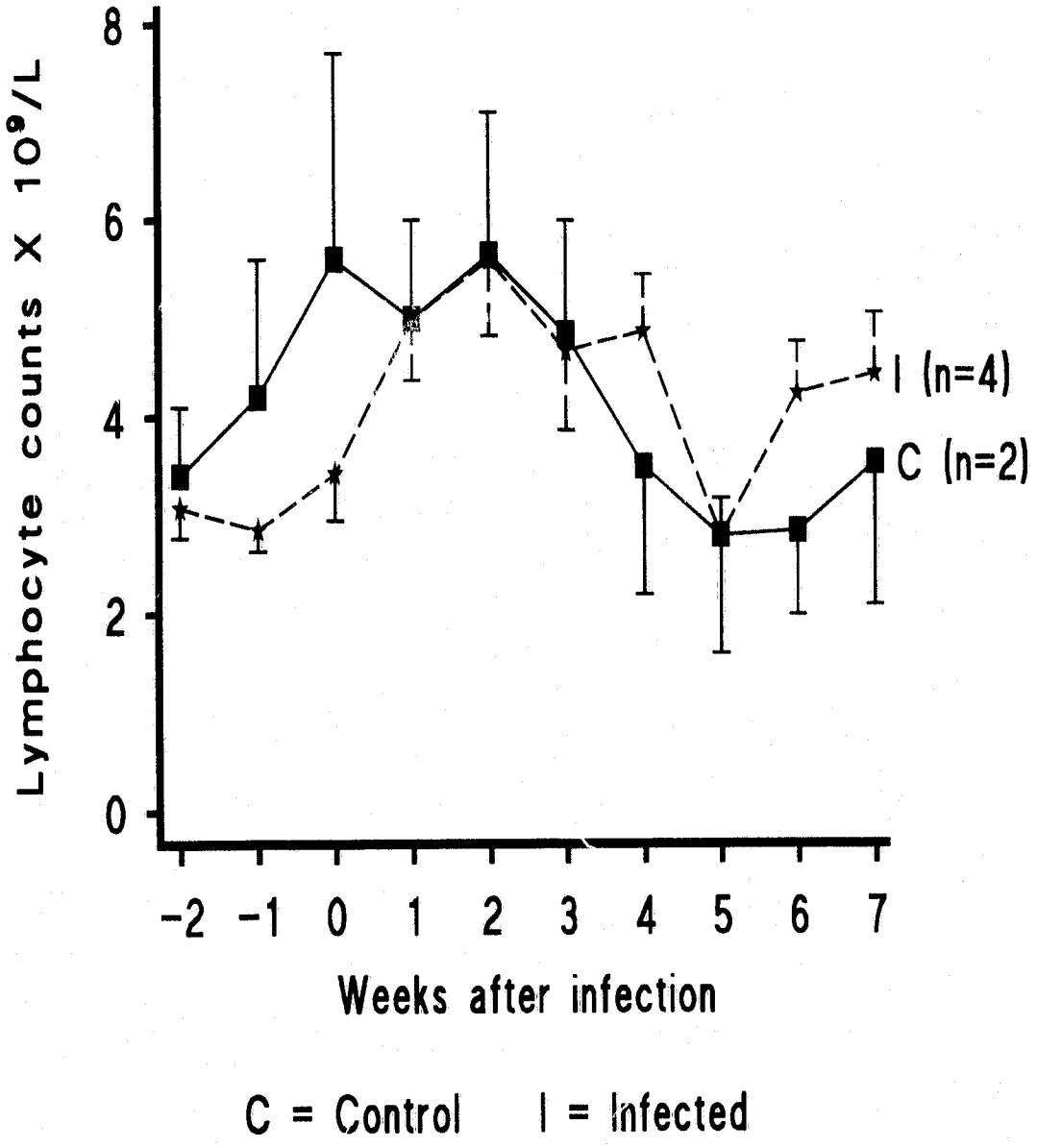

Fig. 2 Lymphocyte counts ($\bar{x}$ ± SE) in *Trypanosoma congolense*-infected and uninfected sheep.

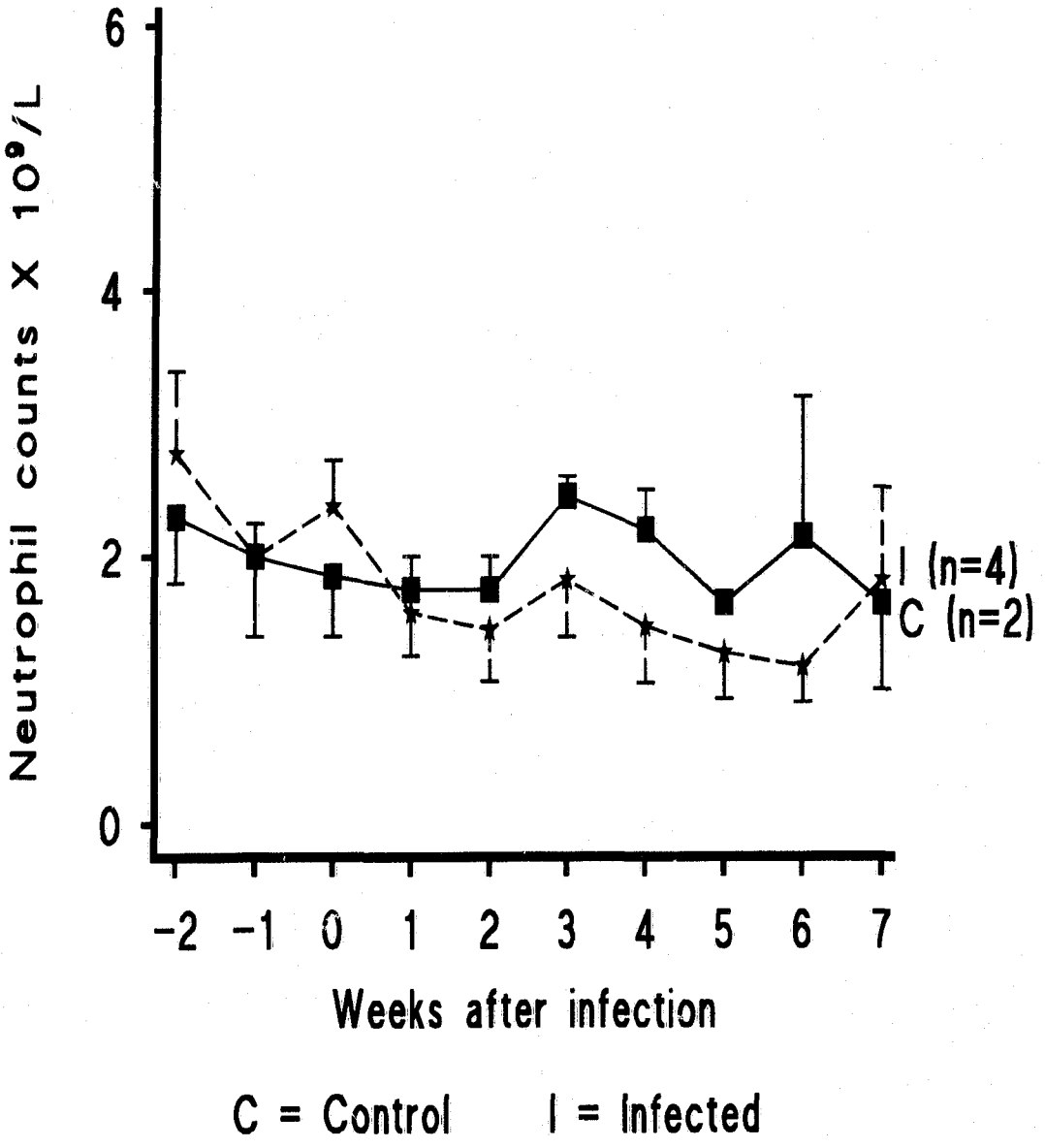

Fig. 3 Neutrophil counts ($\bar{x}$ ± SE) in *Trypanosoma congolense*-infected and uninfected sheep.

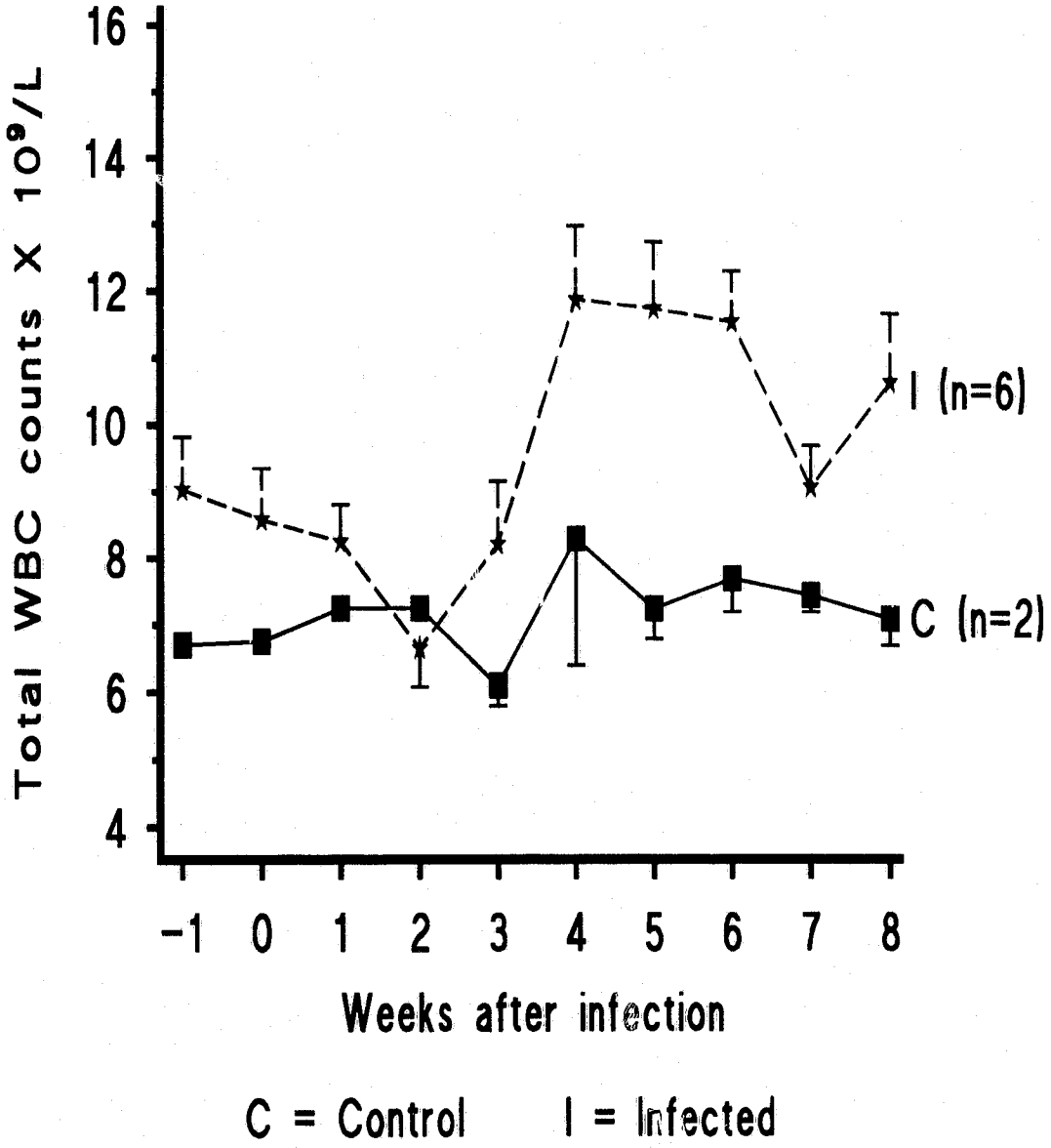

Fig. 4 Total white blood cell counts ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep.

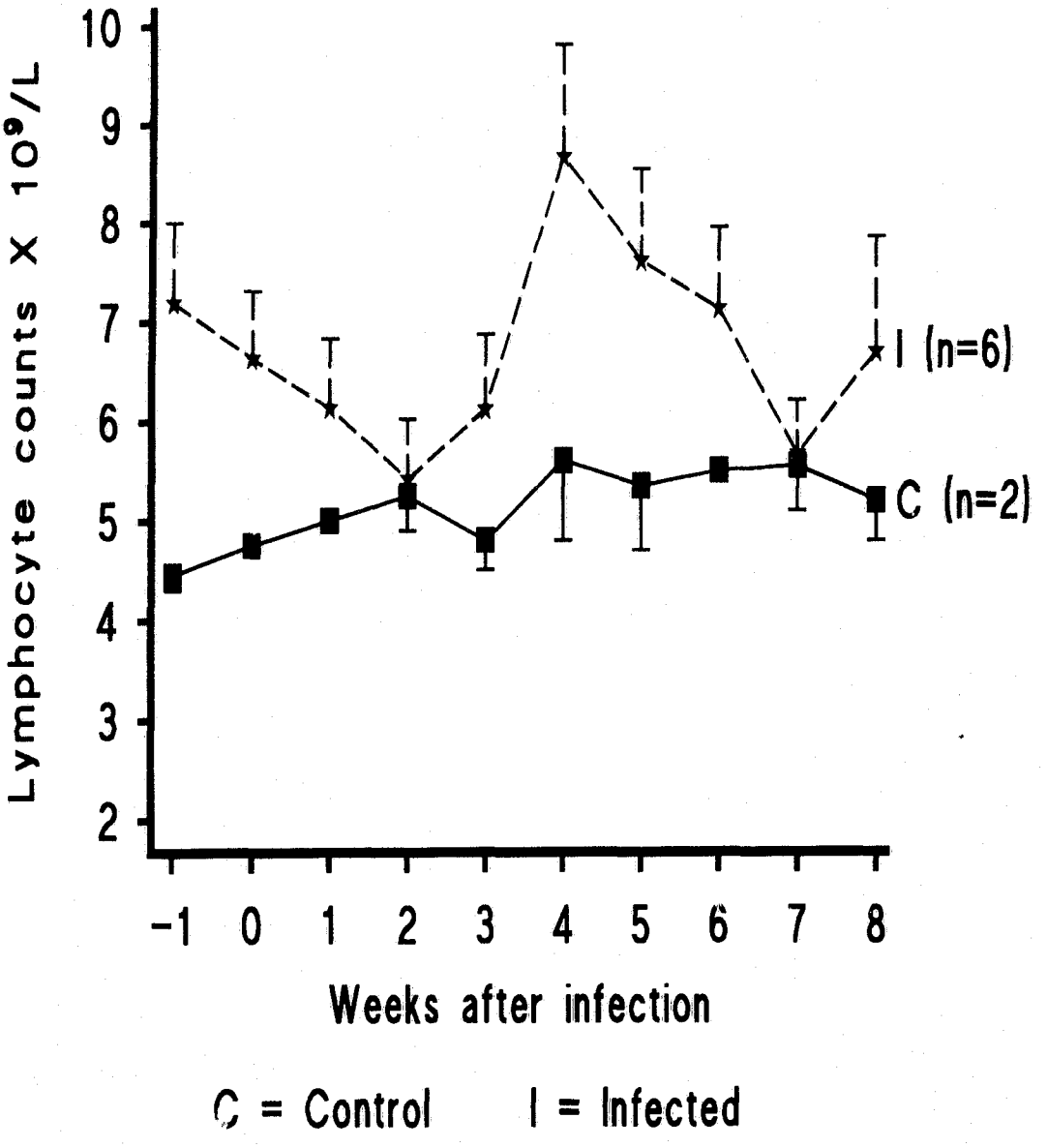

Fig. 5 Lymphocyte counts ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep.

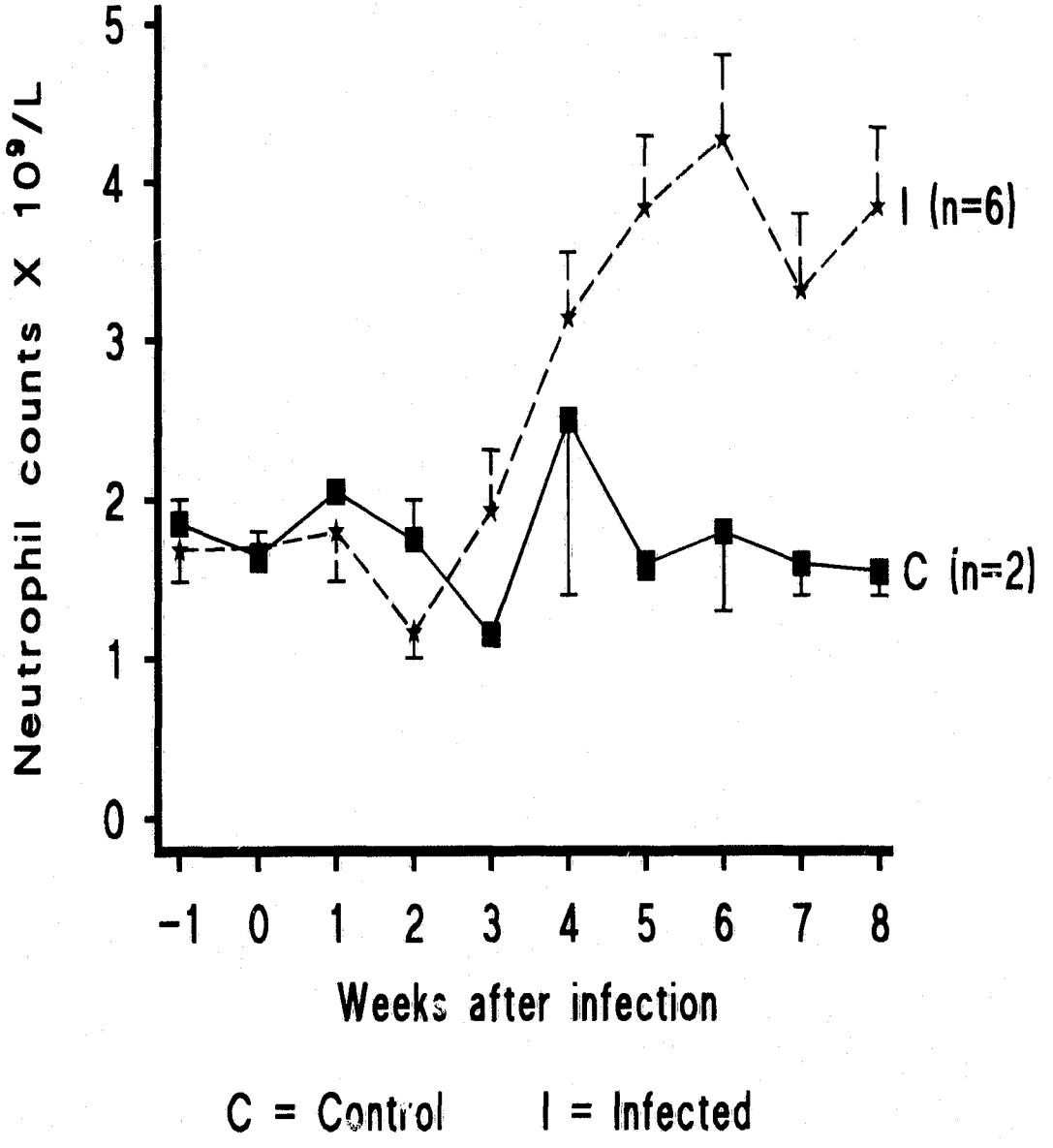

Fig. 6 Neutrophil counts ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep.

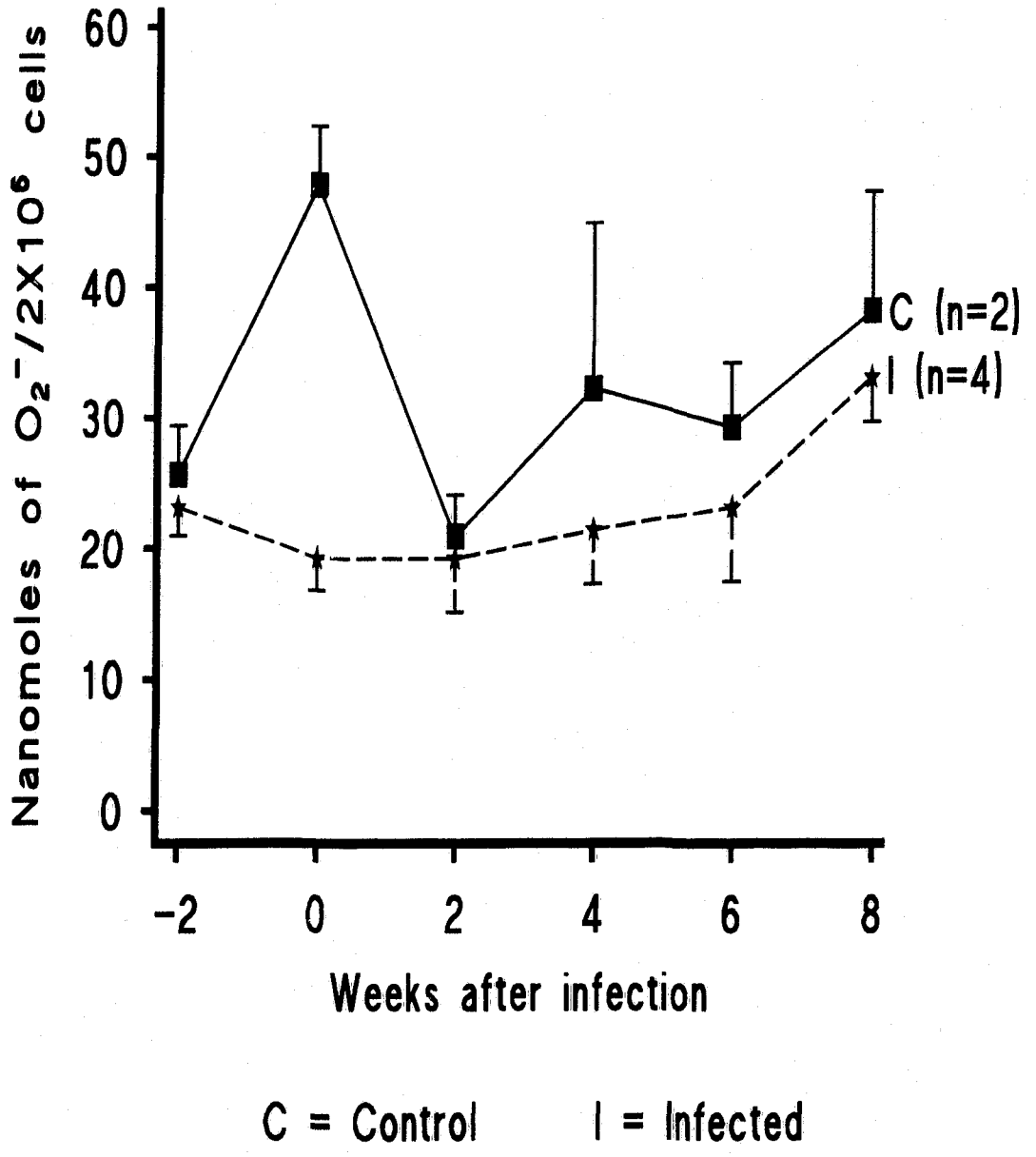

Fig. 7 Cytochrome C reduction ($\bar{x}$ ± SE) in *Trypanosoma congolense*-infected and uninfected sheep.

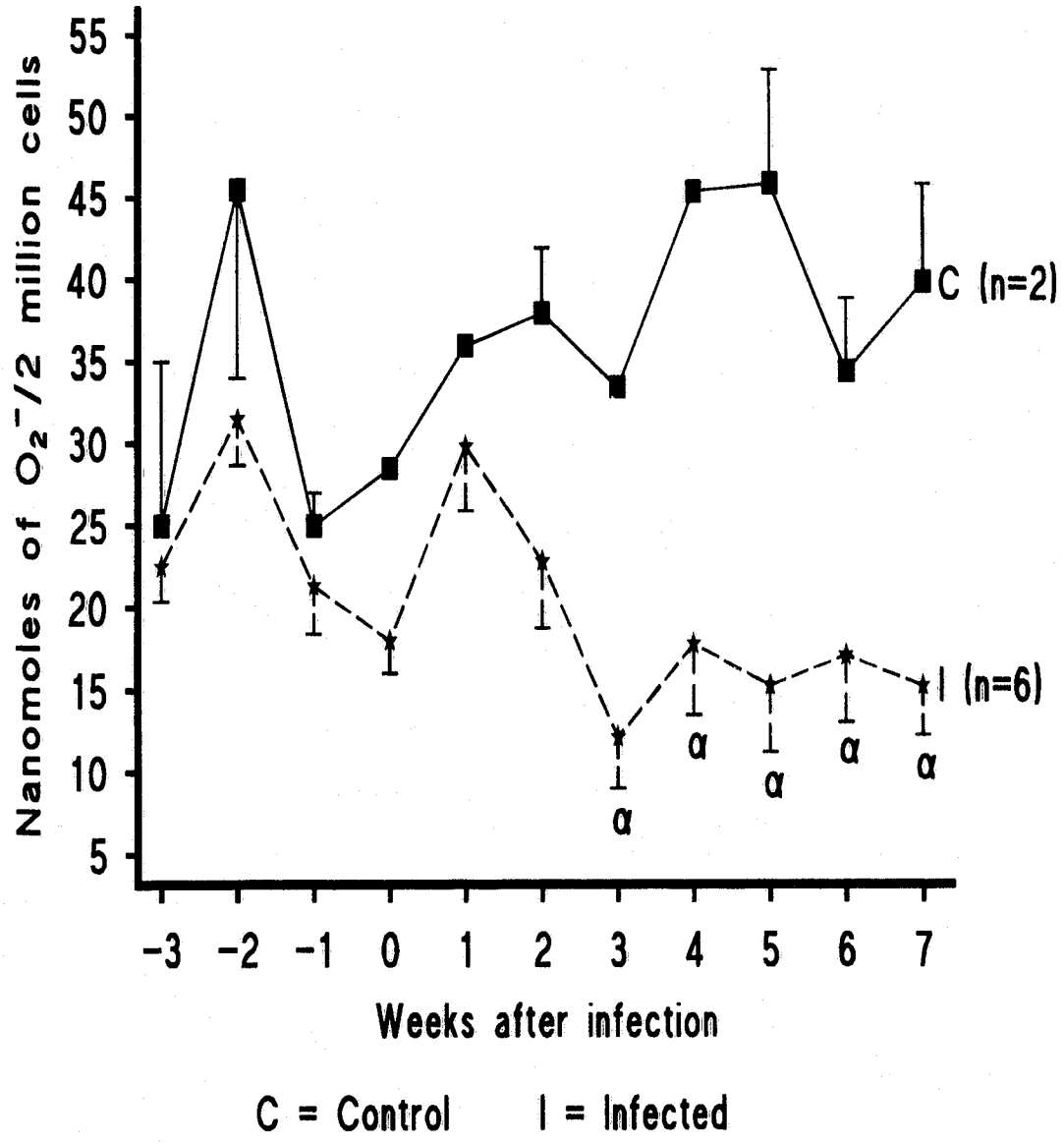

Fig. 8 Cytochrome C reduction ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.01$).

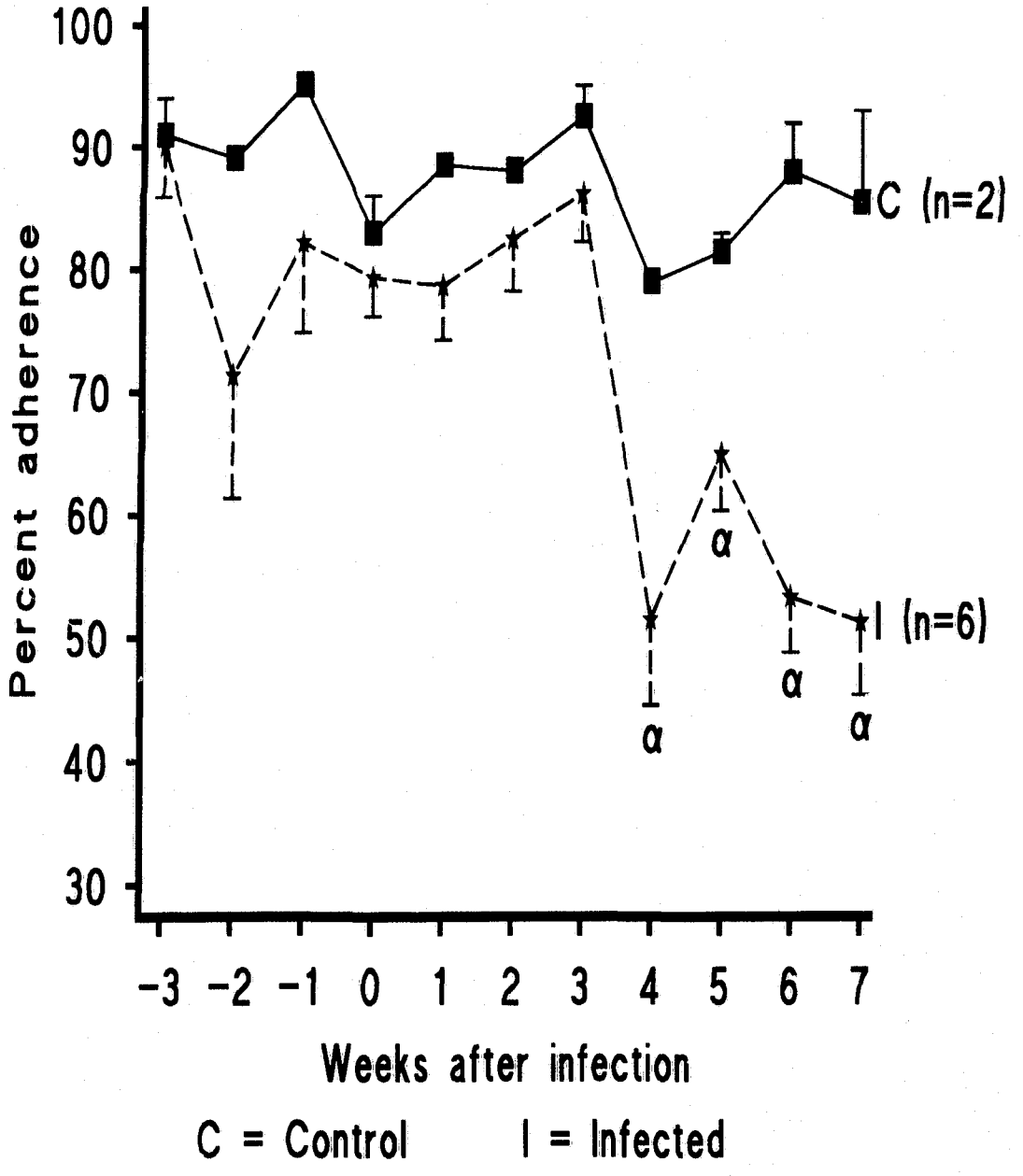

Fig. 9 Percentage adherence of PMNs to nylon wool ($\bar{x} \pm$ SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.01$).

2.3.5 Phagocytosis of Zymosan Assay

The ability of PMNs to phagocytoze opsonized zymosan was not different between infected and control sheep during the first three weeks of infection (Fig. 10). Previous to this there had been progressive decrease in phagocytosis of opsonized zymosan in both the control and infected sheep. In the fourth week, a fresh batch of opsonized zymosan was prepared and the phagocytic index of both control and infected sheep rebounded to pre-infection levels. Subsequently, there was a significant difference ($p<0.01$) in the percent phagocytosis between infected and uninfected sheep; the infected animals had a higher phagocytic index (Fig. 10). The difference between moderately affected and severely affected sheep was not significant. Platelet clumping in infected sheep increased compared to control sheep and examination of smears showed that platelet clumps were in close association with neutrophils. Phagocytosis due to eosinophils was negligible in both control and infected sheep.

2.4 Discussion

Sheep infected with *T. congolense* did not develop clinical trypanosomiasis. This was reflected in results of cytochrome C reduction assay, which were not significantly different between the control and the infected groups. In contrast, sheep infected with *T. brucei* developed clinical trypanosomiasis and had significant changes in PMN function as the disease progressed. The decrease

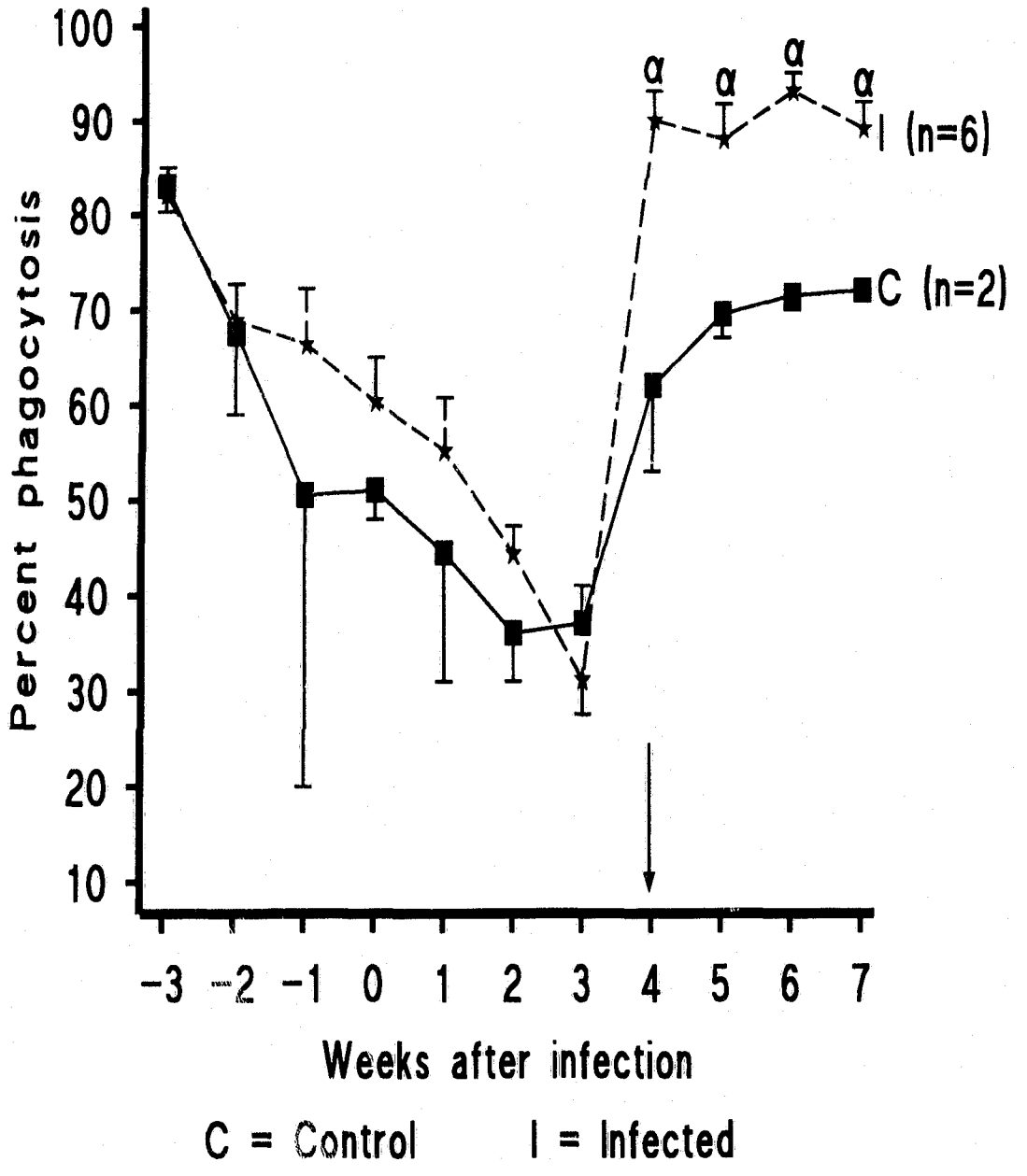

Fig. 10 Phagocytosis of opsonized zymosan ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.01$). Fresh batch of opsonized zymosan was prepared 4 weeks (arrow) post infection.

in cytochrome C reduction was proportional to the severity of disease (data not shown). The number of PMNs recovered during isolation procedures from infected sheep decreased suggesting that the ability of the cells to remain intact progressively decreased, causing increased destruction during isolation. Naylor (1971) observed increased disintegration of mature granulocytes in *T. congolense* infection in cattle, although the functional status of the granulocytes was not determined. The observation in this study of decreased recovery of granulocytes during the isolation process seems to support Naylor's observation.

There was a significant decrease in the ability of PMNs from infected sheep to adhere to nylon wool, however, their PMNs had increased phagocytosis of opsonised zymosan. Despite increased phagocytosis, if other functional aspects of PMNs, e.g., killing ability and adherence are compromised, the net effect would be reduced capacity of these cells to mount effective body defence. The reduction in body defence may result in increased susceptibility to bacterial and fungal infections, a common feature of trypanosomiasis. Increased phagocytic capacity accompanied by impairment of other neutrophil functions has been reported by Hoedemaker et al (1992) during the last month of pregnancy in cows. They found that although ingestion capacity of bovine neutrophils was increased at parturition, other neutrophil function assays, especially bactericidal activity and chemotaxis, were significantly impaired. The net effect rendered the cows more susceptible to bacterial infections, such as mastitis and metritis.

Cytochrome C reduction assay measures the amount of superoxide anion produced. Superoxide anion is one of the highly reactive oxygen products formed during respiratory burst and is essential for killing of microorganisms (Babior, 1978). This process is triggered by the attachment of a foreign particle to an Fc receptor (CD32). This activates nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and electrons are transferred from glucose to oxygen via cytochrome *b*-245 (also referred to as cytochrome *b*-558) (Van der Valk and Herman, 1987). At the same time, the hexose monophosphate shunt is activated to supply NADPH (Babior, 1978; Segal, 1985). When oxygen accepts two donated electrons, superoxide anion is formed (Tizard, 1992a).

For a decrease of superoxide anion production to occur, the processes leading to its generation must be compromised. It is possible that trypanosomes interfere with the above metabolic process by producing substances or inducing the host to produce substances that interfere with this pathway. This may result in decreased production of superoxide anion. Alteration of neutrophil oxidative metabolism is a common pathogenic mechanism among many infectious agents. Depression of oxidative burst has been demonstrated in bovine neutrophils after infection with bovine viral diarrhoea virus (Roth et al, 1986), parainfluenza virus (Briggs et al, 1988), and *Brucella abortus* (Canning et al, 1988). A change in neutrophil function at parturition in cows has been associated with increased concentration of blood plasma cortisol (Guidry et al, 1976; Peter and Bosu, 1987)

and the synthetic glucocorticoid, dexamethasone, impairs the oxidative metabolism of bovine neutrophils *in vivo* (Roth and Kaeberle, 1981). Significantly higher cortisol concentrations were detected in cows destined to retain fetal membranes compared to controls, these changes started six days prepartum and peaked three days before parturition (Peter and Bosu, 1987). Defective leucocyte function in periparturient cows, and in particular cows with retained fetal membranes, may be mediated by elevated cortisol levels (Gilbert et al, 1993). The effect of trypanosome infection on corticosteroid production has not been well studied, but Mutayoba et al (1989) found high cortisol levels in *T. congolense*-infected goats. If cortisol levels are raised in trypanosome-infected animals, this may contribute to the reduction in superoxide anion production.

This study showed that PMNs from *T. brucei*-infected sheep have reduced ability to adhere to nylon wool. Adherence is important for PMN function because before PMNs attack invading pathogens, they migrate from blood circulation to sites of infection in tissues. The first step in this process is adherence of PMNs to capillary endothelium. A congenital abnormality in this process in dogs and cattle results in canine leucocyte adherence deficiency (LAD) and bovine leucocyte adherence deficiency (BLAD) (Tizard, 1992b) respectively. The animals have severe recurrent bacterial infections that usually prove to be fatal. Assessment of leucocyte function in dogs with LAD revealed profound abnormalities in adherence-dependent activities including impaired granulocyte

adhesion to glass, plastic surfaces or to nylon wool fibres (Tizard, 1992b). There was diminished response to chemotactic stimuli although PMN oxidative activity was normal.

The ability of PMNs to adhere to surfaces is mediated by heterodimeric adherence glycoproteins CD11/CD18 (integrins) (Tizard, 1992a). The integrins regulate granulocyte adherence and locomotion *in vitro*, diapedesis and migration into inflammatory sites *in vivo* (Wallis et al, 1986; Harlan et al, 1987; Arnaout, 1990). In human patients with a history of recurrent infections and impaired neutrophil function, this molecule is lacking (Ricevuti et al, 1993). Interference in granulocyte function, especially inhibition of chemotaxis and adherence, has been observed in other diseases in human beings such as Hodgkin's disease, rheumatoid arthritis, leprosy, cirrhosis and infections with the dental organism *Capnocytophaga* (Ward and Berenberg, 1974; Shurin et al, 1979; Wade and mandell, 1983). If the adherence function is compromised as in trypanosome-infected animals, then the defect most likely involves the CD11b/CD18 molecule.

Results of phagocytosis of opsonized zymosan showed that PMNs from *T. brucei*-infected sheep had a higher phagocytic index at the end of the trial compared to the control sheep. Phagocytosis is important because it initiates the killing process of the ingested material. Impairment of phagocytosis leads to reduced ability of PMNs to clear bacteria. The reason for the increased phagocytic index in the infected sheep while the other PMN function assays were

decreased is not clear. The observed increase in platelet clumping may have influenced this assay. Activation of platelets during clumping is accompanied by release of 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and thromboxane A_2 (TxA_2) that may enhance PMN activity (Goetzl et al, 1977; Spagnuolo et al, 1980; Mehta et al, 1988). This is an area that needs further research.

Sheep infected with *T. brucei* had mild leucocytosis characterized by a mild lymphocytosis and mild neutrophilia. Lymphocytosis was probably caused by the proliferative response of lymphoid organs to excessive trypanosome-antigen stimulation. The severe inflammation and edema observed in the skin may have resulted in increased neutrophil demands to combat bacterial invasion in damaged skin. This may have resulted in the mild neutrophilia.

In conclusion, this study indicates that there is interference in PMN function that may contribute to increased susceptibility to bacterial infection. To confirm this, PMN function should be studied in several trypanosome-infected hosts, and the period of monitoring be extended to the terminal stage of the disease when immunosuppression is clinically evident. This study supports the view that administration of antibiotics concurrently with trypanocides to debilitated animals suffering from trypanosomiasis is beneficial. Such animals may have reduced PMN function and are likely to suffer from opportunistic bacterial infections.

CHAPTER 3. PATHOLOGICAL CHANGES IN SHEEP EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA BRUCEI*

3.1 Introduction

Animals infected with *T. brucei* show clinical signs which correspond with the extent of trypanosome localization in the tissues. During feeding, infected tsetse flies deposit metacyclic trypanosomes in the skin. Multiplication and differentiation of metacyclics in the skin results in formation of a chancre (Roberts et al, 1969; Emery and Moloo, 1980; Barry and Emery, 1984). The development of the chancre occurs within a few days after fly bites, reaches its peak during the second week and regresses by the third week (Morrison et al, 1985). At its peak, the chancre shows all the features of inflammation characterized by swelling, redness, heat and pain, and a twofold increase in skin thickness (Dwinger, 1985). Histologically, the reaction is characterized by infiltration of neutrophils and lymphocytes in the papillary dermis and around vascular trunks in dermis and hypodermis. At the peak of the reaction, the majority of the cells are lymphocytes, lymphoblasts, and monocytes, although neutrophils can still be encountered (Dwinger, 1985; Dwinger et al, 1987).

Before animals can mount significant immunological responses, trypanosomes leave the chancre and invade lymph vessels (Emery et al, 1980), drain to the local lymph nodes (Luckins and Gray, 1979), and eventually enter the blood

stream. The appearance and multiplication of trypanosomes in the blood stream is accompanied by a rise in body temperature and a generalized lymph node enlargement (Ikede and Losos, 1972a,b; Emery and Moloo, 1981). The initial parasitaemia is followed by a trypanolytic crisis in which trypanosomes are rapidly cleared from the blood and the temperature returns to normal. Later, parasitaemia starts to fluctuate, pyrexia becomes intermittent and anaemia develops. As disease progresses, parasites localize in various tissues and organs where they cause a predominantly inflammatory reaction and clinical signs corresponding to organ involvement.

Clinical signs associated with *T. brucei* infection include intermittent fever, pale mucous membranes, enlargement of superficial lymph nodes, weakness, wasting, dullness, abortion and edema of head, scrotum, limbs, tail, ventral wall of thorax and abdomen (Losos, 1986; Brown et al, 1990; Ikede, 1994). In the late stages, trypanosomes may invade the brain and spinal cord and cause neurological signs characterized by paresis, twitching of the eyelids, chewing movements, grinding of teeth, staggering and incoordination (Ikede and Losos, 1972b). Parasites may invade the eye and cause intermittent corneal opacity, lacrimation, photophobia and sometimes total blindness (Losos and Ikede, 1972).

Animals that die from the disease show emaciation and subcutaneous edema. Emaciation is accompanied by serous atrophy of fat, especially around the heart, perirenal region, superficial lymph nodes, and in bone marrow.

Subcutaneous edema is most prominent around the lips, face, ears, limbs, scrotum and tail (Ikede and Losos, 1972a,b). There is serous effusion into body cavities and enlargement and edema of lymph nodes. The spleen is enlarged and firm; on cut surface, the white pulp is prominent. Gross lesions in other organs are associated with localization of trypanosomes in these organs or development of intercurrent infections (Ikede and Losos, 1972b).

Microscopically, there is extensive mononuclear infiltration in the skin and subcutaneous tissue, skeletal muscles, heart, brain, epididymis and testis, liver, pituitary gland, eye, and kidneys (Ikede and Losos, 1972a,b; Losos and Ikede, 1972; Ikede, 1974; Ikede and Losos, 1975b; Moulton and Sollod, 1976; Ikede, 1979). Usually these inflammatory reactions are accompanied by severe edema and there may be numerous trypanosomes in the interstitial tissues. The basic reaction is an interstitial and perivascular mononuclear cell infiltration. Inflammatory cells are mainly medium-sized and large lymphocytes, macrophages, and plasma cells as well as polymorphonuclear cells (Losos and Ikede, 1972).

In the heart, there is severe interstitial carditis involving the atrium, ventricular walls, endocardium, epicardium and the coronary fat (Ikede and Losos, 1972b; Moulton and Sollod, 1976; Morrison et al, 1981). Inflammatory cells are mainly mononuclear. Trypanosomes are usually found in the interstitial connective tissue of the heart as well as in the epicardial fat. The reaction in the

skeletal muscles is similar to that in the myocardium. There is extensive cellulitis in the dermis and subcutis of the head, sternum, ventral abdomen and limbs. This cellulitis is accompanied by acute necrotizing vasculitis, lymphangitis, lymphatic thrombosis and edema. The skin lesions are associated with large numbers of trypanosomes in the connective tissue (Ikede and Losos, 1972b). Lymphoid follicles in the lymph nodes are markedly hyperplastic, with prominent germinal centres. There is lymphoid and reticuloendothelial cell hyperplasia in the red and white pulp of the spleen. Lesions in the kidney and liver are similar to those found in the heart and lymph nodes.

Localization of trypanosomes in the central nervous system results in meningoencephalomyelitis of the brain stem, hypothalamus, and thalamus (Ikede and Losos, 1972a,b; Onah and Uzoukwu, 1991). These lesions are accompanied by extensive mononuclear cuffs and focal gliosis. Trypanosomes may localize in the pituitary gland leading to focal coagulative necrosis and mononuclear inflammatory cell accumulation in the interstitial connective tissue (Losos and Ikede, 1970; Ikede and Losos, 1975b). Other endocrine glands such as adrenals, thyroid, parathyroid and pancreas are less affected and trypanosomes are not usually found in these glands. Ocular lesions are characterized by fibrinous and mononuclear exudate into the aqueous and vitreous humour, iridocyclitis, choroiditis, retinitis, optic neuritis and ocular myositis (Ikede, 1974). Trypanosomes are readily seen in the above mentioned regions of the eye. Genital lesions

associated with *T. brucei* infection include scrotal edema and granulomatous periorchitis accompanied by extravascular localization of trypanosomes (Ikede, 1979).

Overall, *T. brucei* readily invades tissues resulting in extensive tissue damage. The extent and severity of the lesions are variable, depending on the strain of *T. brucei*, the species or breed of animal affected, and their individual susceptibility. While the chancre has been well described (Roberts et al, 1969; Emery and Moloo, 1980; Barry and Emery, 1984, Dwinger et al, 1987; Mwangi et al, 1990b; Luckins et al, 1991), there are only limited descriptions of the pathology of the generalized skin lesion in ruminants (Ikede and Losos, 1972b). This chapter reports clinical and histological findings in experimental *T. brucei* infection in sheep in which the significant lesion was severe dermal and subcutaneous edema accompanied by focal to diffuse perivascular dermatitis. Numerous trypanosomes were demonstrated in the edema fluid and in the interstitial tissue.

3.2 Material and Methods

3.2.1 Trypanosomes and Experimental Animals

The acquisition of trypanosomes, the experimental sheep, and the procedure used to infect the sheep, have been described in chapter two, sections 2.2.1

and 2.2.2 of this thesis. Briefly, *T. brucei* (Plimmer and Bradford, 1899) strain ATCC 30118 was obtained from American Type Culture Collection, Maryland, USA. Trypanosomes were amplified in CD Sprague Dawley rats before inoculation into six male castrated sheep, each receiving 8 million trypomastigotes intravenously. Two sheep were injected with phosphate buffered saline (PBS) as controls. Canadian Council on Animal Care guidelines were followed in handling of the experimental animals (CCAC,1993).

3.2.2 Temperature and Clinical Observations

Rectal temperature was monitored daily before 9.00 a.m. Other clinical features of the disease including appetite and alertness were also observed daily while weight measurements were done weekly. Health of the sheep was closely monitored and the experiment was to be terminated at 60 days p.i. or when the sheep would show signs of discomfort or inability to eat, which ever came first.

The presence of trypanosomes in blood was monitored daily for the first 3 weeks after infection or until patent parasitaemia was established. Thereafter, it was monitored twice a week. Parasitaemia was checked on both wet preparation and the buffy coat technique (Murray et al, 1977).

3.2.3 Packed Cell Volume and Red Blood Cell Counts

Packed blood cell volume (PCV) and red blood cell (RBC) counts were determined at least twice a week from jugular venous blood collected into 2 ml vacutainer EDTA bottles. A capillary tube was filled with blood, sealed and centrifuged at 12,000 rpm for 5 min before the PCV was read. To determine RBC counts an electronic particle counter (Model T660, Coulter Electronics Inc., USA) was used.

3.2.4 Post Mortem Examination

At the end of the trial, the sheep were euthanised by barbiturate overdose and examined for gross lesions. Samples were taken from several organs including the brain, heart, liver, spleen, lymph nodes, kidney, adrenals, lungs, thyroid, eye, pituitary gland and skin. Skin tissues were taken from the ear, face, lips, submandibular area, neck, brisket, tail and distal parts of limbs for histology. The samples were fixed in 10% neutral buffered formalin, routinely embedded and sectioned at 6 μm thickness. Sections were stained with Haematoxylin & Eosin and examined with light microscopy. Gross and microscopic skin lesions were subjectively graded as severe, moderate, mild or absent. Selected skin sections were cut at 3 μm thickness and stained with Giemsa for trypanosomes.

3.2.5 Statistical Analysis

Temperature, weight changes, PCV, and RBC counts were compared between infected and uninfected sheep by a two tailed t-test in a computer statistical program, Minitab version 9.1, Minitab Inc., (Ryan et al, 1985). A value of $p \leq 0.05$ was considered significant.

3.3 Results

3.3.1 Temperature

Infected sheep developed pyrexia by 2-3 days post infection (dpi). The temperature continued to rise and peaked at 40.5 °C by the fourth week post infection (Fig. 11). This high temperature was maintained with minor fluctuations throughout the trial. The temperature difference between experimental and control sheep was statistically significant ($p<0.01$).

3.3.2 Parasitaemia

Trypanosomes were detectable in the blood of infected sheep between 2 and 16 dpi with an average of 8 days. Parasitaemia was scanty and detectable intermittently only on buffy coat (Table I). There was no relationship between frequency of parasite detection and severity of the disease. Edema fluid from

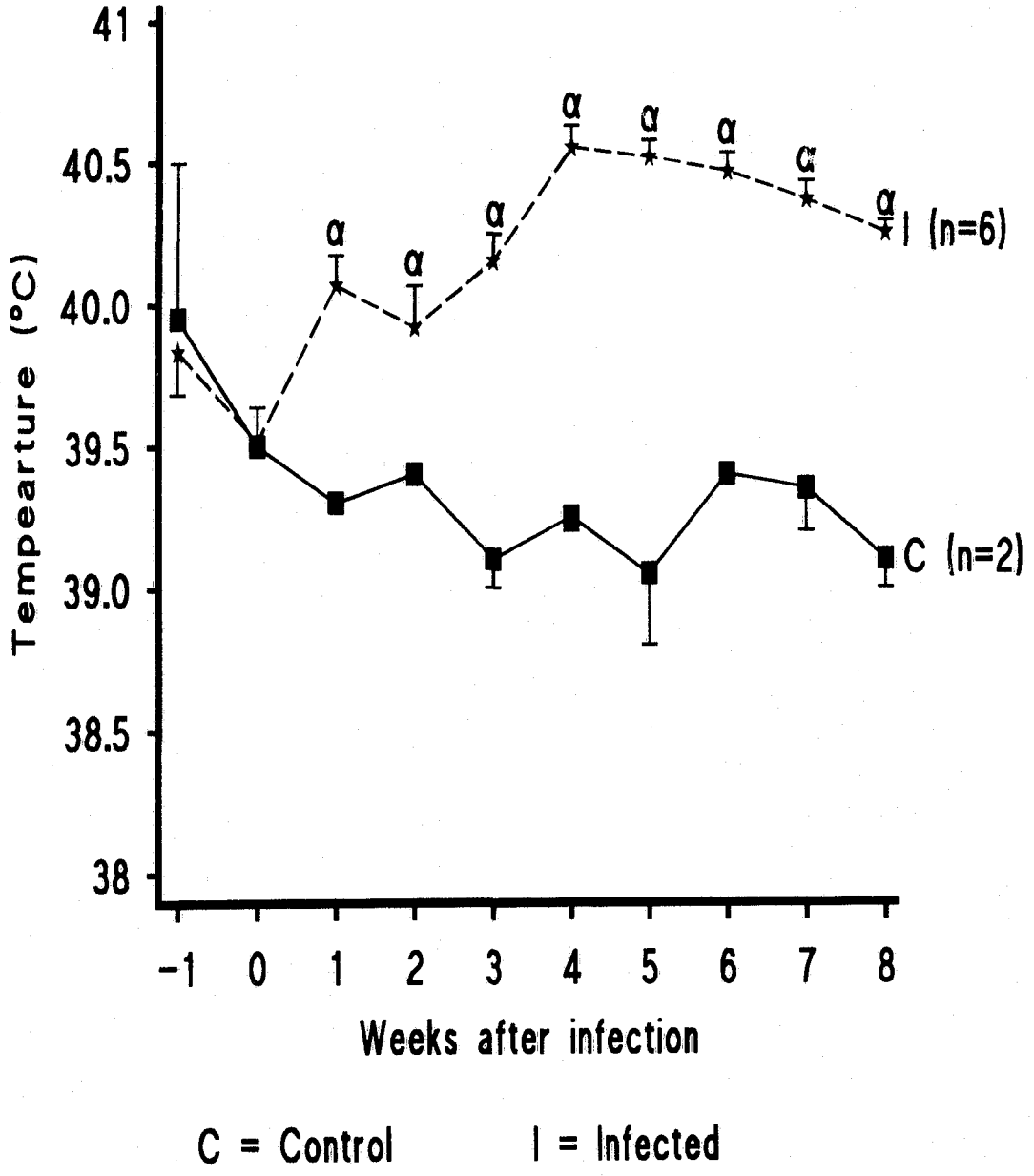

Fig. 11 Temperature changes ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.01$).

Table I. Number of days per week trypanosomes were detected in peripheral blood of *Trypanosoma brucei*-infected sheep using the buffy coat method

	Weeks								
	1	2	3	4	5	6	7	8	
Sheep No.									Total No. of days
2	2/6	2/7	4/5	0/2	0/2	0/2	0/2	0/2	8
3	2/6	2/7	4/5	1/2	0/2	1/2	1/2	0/2	11
4	1/6	2/7	4/5	0/2	0/2	0/2	1/2	0/2	8
5	0/6	4/7	5/5	0/2	1/2	0/2	1/2	0/2	11
6	0/6	0/7	3/5	0/2	1/2	0/2	0/2	D	4
7	0/6	0/7	3/5	0/2	1/2	0/2	0/2	0/2	4

D - sheep had been euthanised.

subcutaneous swellings usually contained trypanosomes even when none could be detected from jugular blood.

3.3.3 Body Weight Changes

By 21 dpi, infected sheep started to gain less weight than controls (Fig. 12). Weight changes were significantly different ($p<0.03$) between infected and control sheep. Weekly weight changes for each sheep are shown in Table II. On the basis of weight change, infected sheep could be classified into those that lost weight (sheep #2, #6 and #7) and those that gained less (sheep #3, #4 and #5) (Table II). The severity of the disease was highly reflected in the weight changes.

3.3.4 Clinical Observations

Four dpi, all infected sheep had developed swellings on the neck. The swellings first appeared on the side of the neck used for trypanosome inoculation and later spread to the other side of the neck. These swellings regressed and recurred in all infected sheep. Sometimes multiple swellings were observed on the neck. Each swelling increased in size gradually, reaching a peak at about 3-5 days and subsiding to unnoticeable levels by the seventh day of infection. At the peak, swellings measured approximately 3-4 cm in diameter and 1.5-2.5 cm in

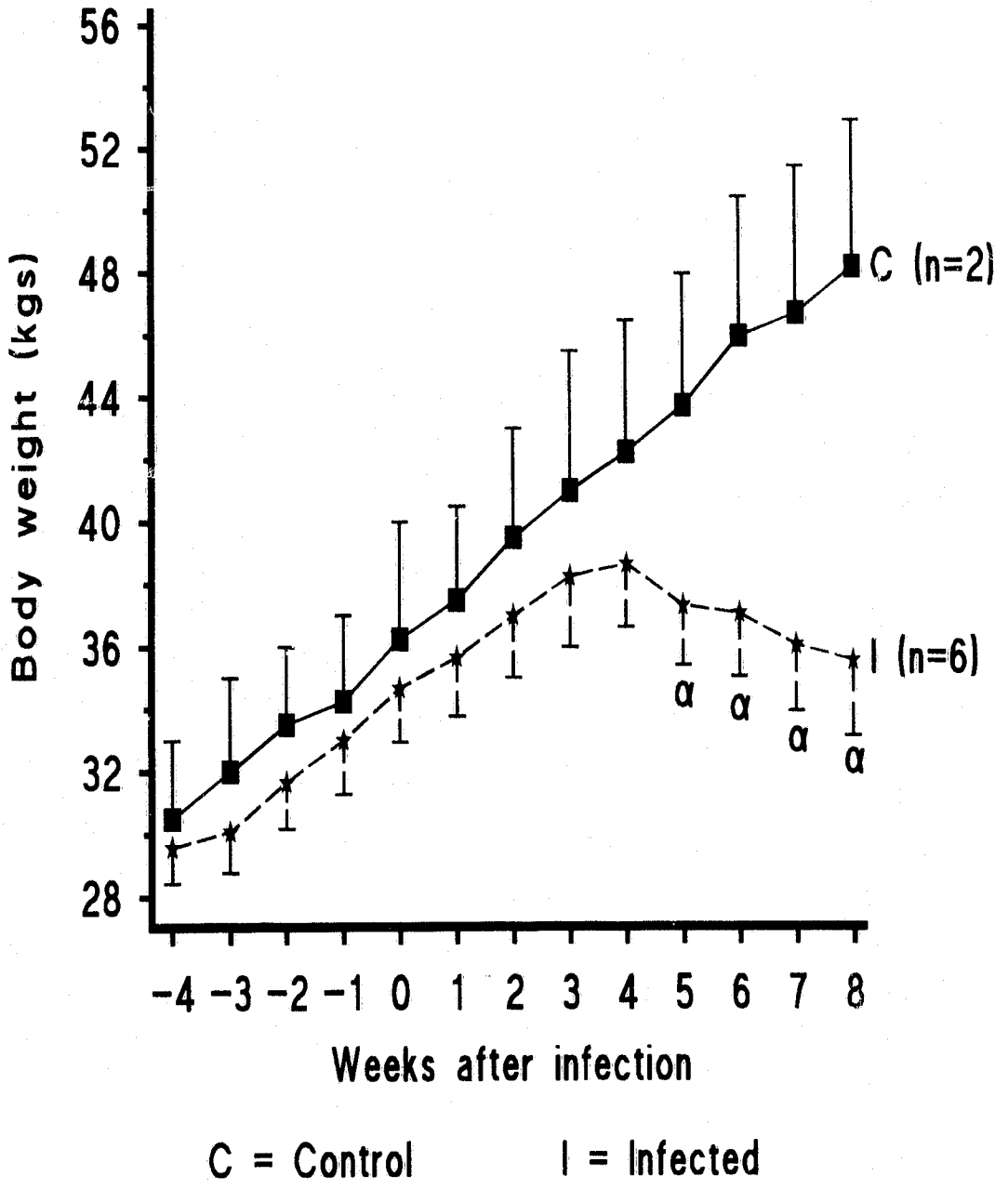

Fig. 12 Body weight changes ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.03$).

Table II. Individual weekly weight changes (kgs) in *Trypanosoma brucei*-infected and uninfected sheep

	Weeks								
	1	2	3	4	5	6	7	8	
Sheep No.									ADG
2	+0.5	+2	0	-0.5	-1	-1	0	-2.5	-42
3	+1.5	-1	+2	+1	+0.5	+0.5	+1	+1	+105
4	+0.5	+2	0	+3.5	-1.5	-1	-0.5	-1	+32
5	0	+3	+1.5	0	0	0	0	0	+78
6	+2	+1.5	+2.5	-2.5	-1.5	-2.5	-4	-1.5	-113
7	+0.5	+0.5	+1.5	+1	-0.5	-1.5	-2.5	-0.5	-25
1	+2	+1.5	+1.5	+1.5	+1.5	+2	+0.5	+1.5	+188
8	+0.5	+2.5	+2.5	+1	+1.5	+2.5	+2	+1.5	+230

Sheep 2-7 - Infected, 1 and 8 - Controls. ADG - Average daily gain in grams

thickness (Fig. 13). Swellings were firm and warm on palpation. Numerous trypanosomes were observed in fluid obtained from these swellings.

Three weeks after infection, infected sheep developed a rough hair coat which could only be noticed on the head as the rest of the body was covered by wool. Skin around the head became hyperaemic and this was followed by swelling of the eyelids and congestion of the conjunctiva (Fig. 14). One sheep (# 6) had lacrimation. By 30 dpi, all the infected sheep developed facial swelling which was prominent on the jaws (both lower and upper), above the nostrils, the ears and the eyelids. Concomitant with the facial swelling, parotid and superficial cervical lymph nodes became markedly enlarged. These changes were not observed in the control sheep (Fig. 15). Infected sheep had diminished appetite and appeared depressed.

Thirty nine days post infection, two sheep (#6 and #7) exhibited lameness of the front legs, without visible leg swelling or inflammation. Three days later lameness of the right leg was observed in another sheep (# 4) and edema of the distal parts of the limbs was observed in all infected sheep. One sheep (#5) developed swelling of the scrotal area while sheep #3 and #5 had edema of the tail. These swellings only appeared on areas of the skin that were not covered by wool i.e head, ears, neck (shaved area only) brisket, distal limbs and tail. Swellings became so severe that the infected sheep could not keep their ears

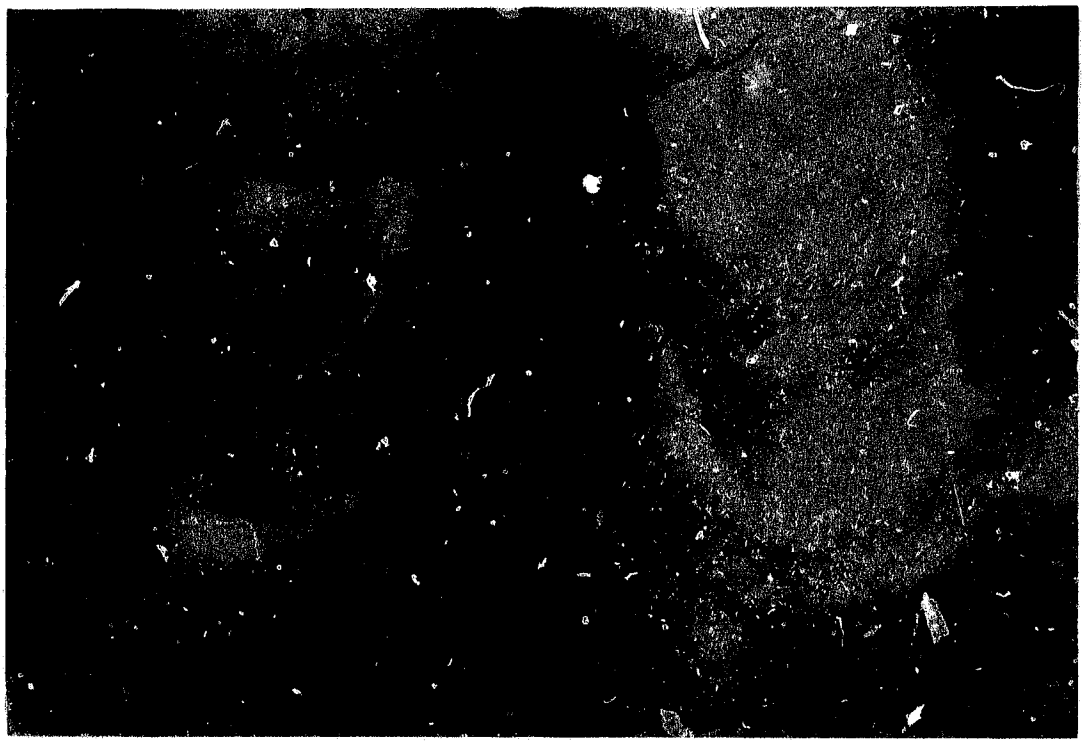

Fig. 13 Sheep #6 and #1. Initial swellings (S) at inoculation site on the neck of *Trypanosoma brucei*-infected sheep four days post infection. C = Control sheep. Bar = 7 cm.

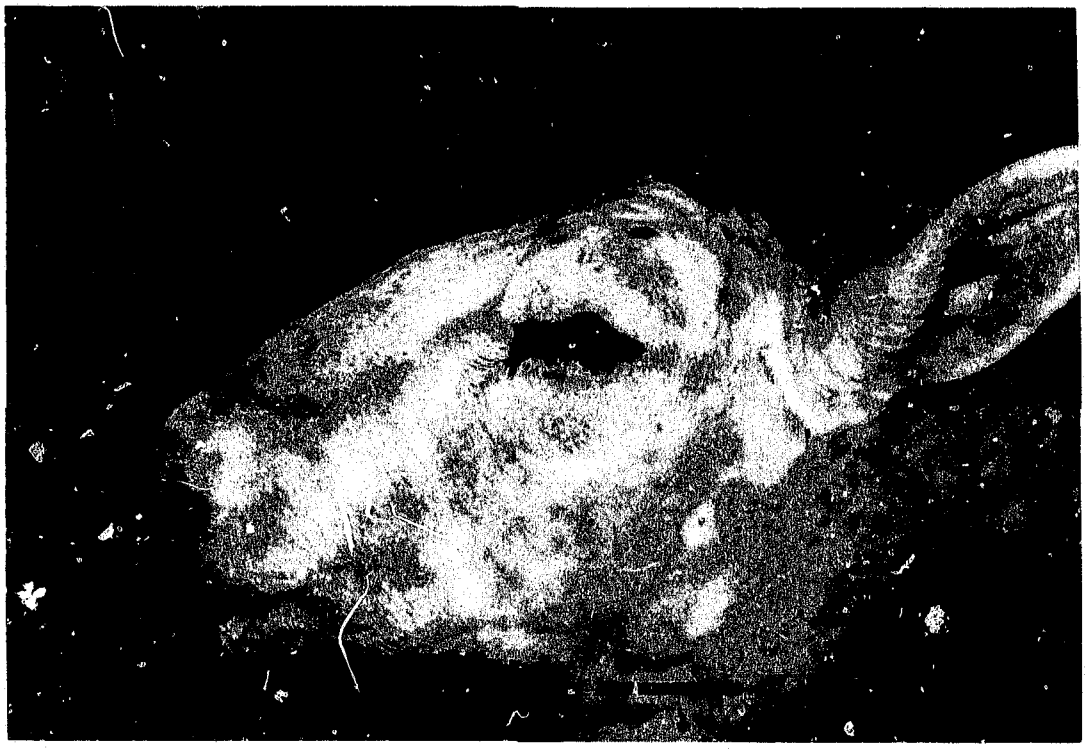

Fig. 14 Sheep #5. Trypanosome-infected sheep showing rough haircoat, swollen eyelids, and hyperaemia of the face and external ear three weeks post infection. Bar = 5 cm.

Fig. 15 Sheep #8. Control sheep showing alert eyes and raised ears. Bar = 5 cm.

upright (Fig. 16). Forty five days post infection, the facial swelling had markedly increased and spread to involve both lower and upper lips (Fig. 16 and 17). This was so severe in one sheep (#6) that it interfered with proper prehension of food. This sheep became very depressed and was euthanised 48 dpi. The condition of the other sheep (#2, #3, #4, #5 and #7) also deteriorated; they were euthanised between 55 - 59 dpi.

3.3.5 Packed Cell Volume and Red Blood Cell Counts

Packed cell volume was not significantly different between experimental and control sheep until 3 weeks post infection. Thereafter, infected sheep had significant drop in PCV values compared to controls ($p<0.03$) (Fig. 18). Although control sheep had a mild reduction in RBC counts between week zero and week three post infection, the counts had returned to pre-infection levels by week five post infection (Fig. 19). In contrast, infected sheep had a steady reduction in RBC counts and the values were significantly different ($p<0.01$) from those of the control sheep.

3.3.6 Post Mortem Findings

The most consistent finding was severe edema of the face, lips, ears, submandibular area, distal parts of the limbs, tail and brisket skin (Table III).

Fig. 16 Sheep #7. Severe inflammatory edema of the ear, face, nose, and upper lip in *Trypanosoma brucei*-infected sheep 45 days post infection. The ears are heavy and drooping due to severe edema. Bar = 2 cm.

Fig. 17 Sheep #5. Severe inflammation and swelling of the face, upper lip and the eyelids in *Trypanosoma brucei*-infected sheep 45 days post infection. Notice the loss of hair and encrustation of the facial skin. The sheep was unable to open the eyes fully due to the swelling of the eyelids. Bar = 5 cm.

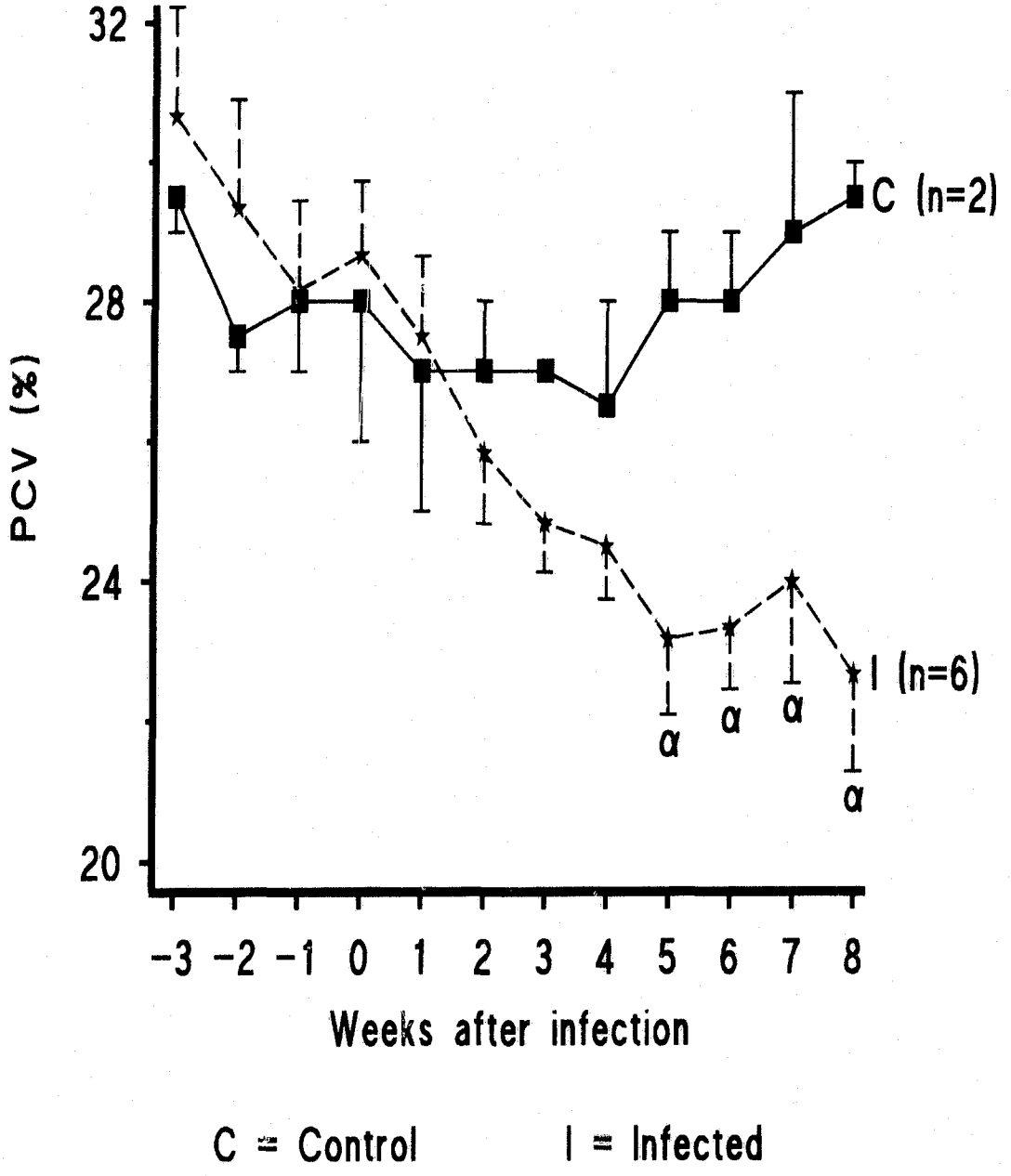

Fig. 18 Packed red blood cell volume ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.03$).

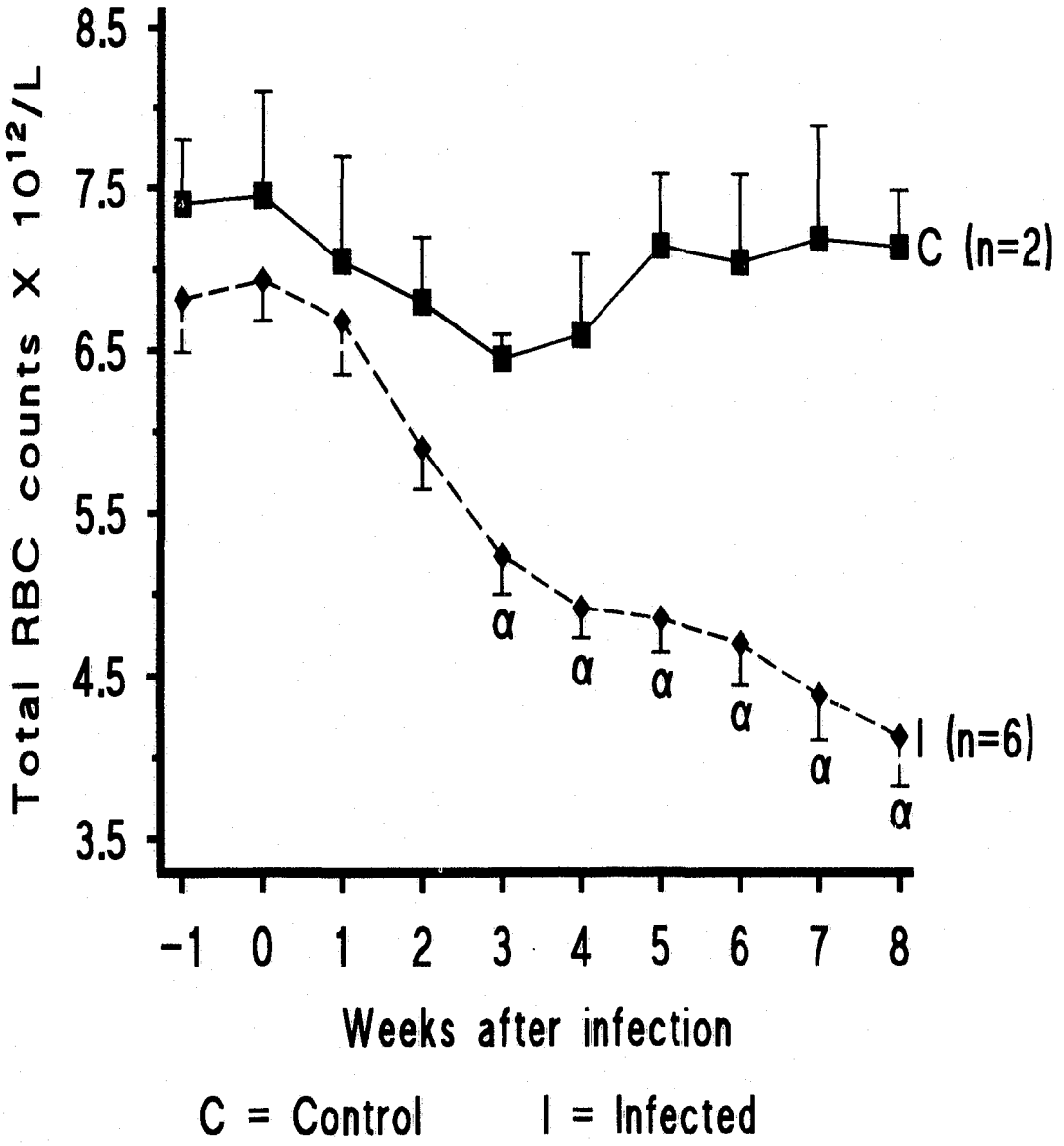

Fig. 19 Total red blood cell counts ($\bar{x}$ ± SE) in *Trypanosoma brucei*-infected and uninfected sheep. α - represents values significantly different from those of the controls ($p<0.01$).

Table III. Extent of edema and inflammation of the skin from various sites in *Trypanosoma brucei*-infected sheep based on gross and microscopic lesions

	Source of Skin Sample							
	Lips	Face	Ear	Submandibular	Neck	Limbs	Brisket	Tail
Sheep No.								
2	2+	2+	2+	ND	2+	1+	ND	ND
3	2+	2+	1+	ND	-	1+	ND	3+
4	2+	2+	1+	3+	2+	1+	ND	-
5	2+	2+	2+	3+	1+	1+	ND	3+
6	3+	3+	2+	3+	2+	2+	2+	2+
7	2+	2+	1+	ND	2+	1+	ND	ND

3+ = severe edema and inflammation, 2+ = moderate, 1+ = Mild, - = none
ND = not done. There was no edema and inflammation in the skin of the control sheep.

Skin in these areas was 2-3 cm thick compared to 0.5 cm in the control sheep. The superficial cervical and parotid lymph nodes were enlarged 2-3 times while internal lymph nodes were only slightly enlarged. All lymph nodes were edematous. Cerebro-spinal and pericardial fluid contained no trypanosomes in direct wet preparation and in smears of centrifuged deposit.

3.3.7 Histological Findings

3.3.7.1 Changes in the Skin

The external ears (pinna) of all infected sheep except sheep #4 and control sheep (Fig. 20), had severe edema and inflammation of the superficial and deep dermis and subcutis (Fig. 21). There was multifocal to diffuse perivascular (Fig. 22) and periadnexal (Fig. 23) accumulation of mainly lymphocytes, plasma cells and macrophages (Fig. 24) in the dermis and subcutis, although occasional neutrophils and eosinophils were present. Both the outer and the inner skin of the pinna was affected except sheep #6, in which only the outer side was affected. There was marked acanthosis of the epidermis and in sheep #6 there was evidence of degeneration of the epidermis and crust formation (Fig. 25). There was accumulation of inflammatory cells in lymphatics and distension of lymph vessels and veins with fluid (Fig. 26). There was severe lymphangitis

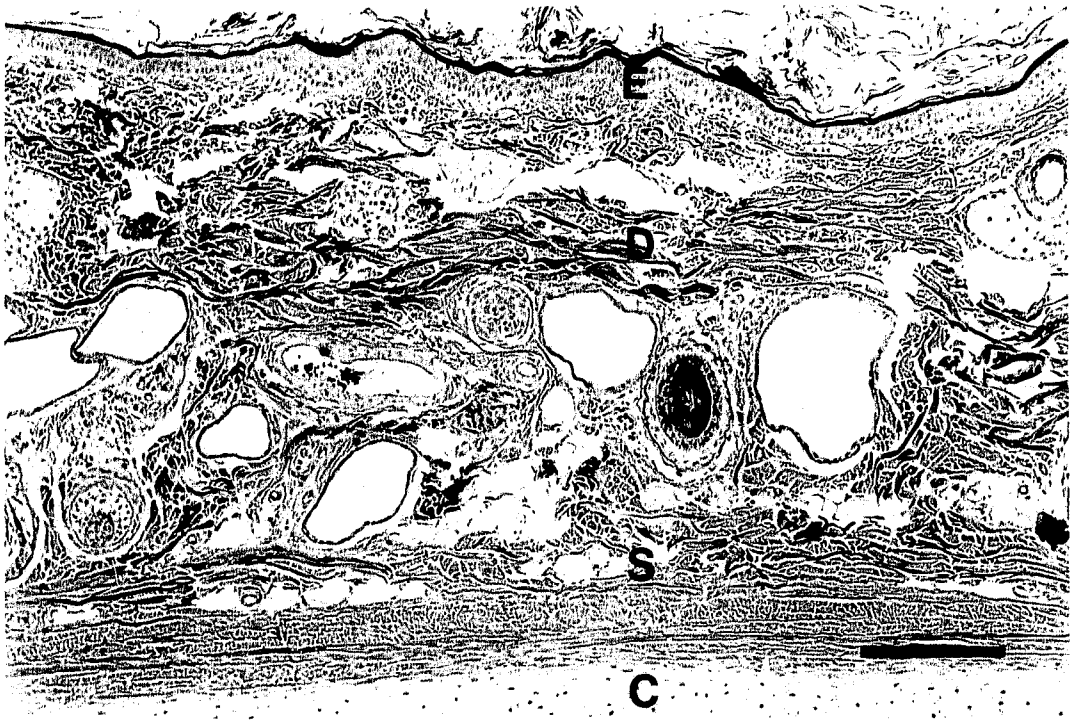

Fig. 20 Sheep #8, Control. Section from ear showing normal epidermis (E), dermis (D), subcutis (S), and ear cartilage (C). H&E. Bar = 300 μm.

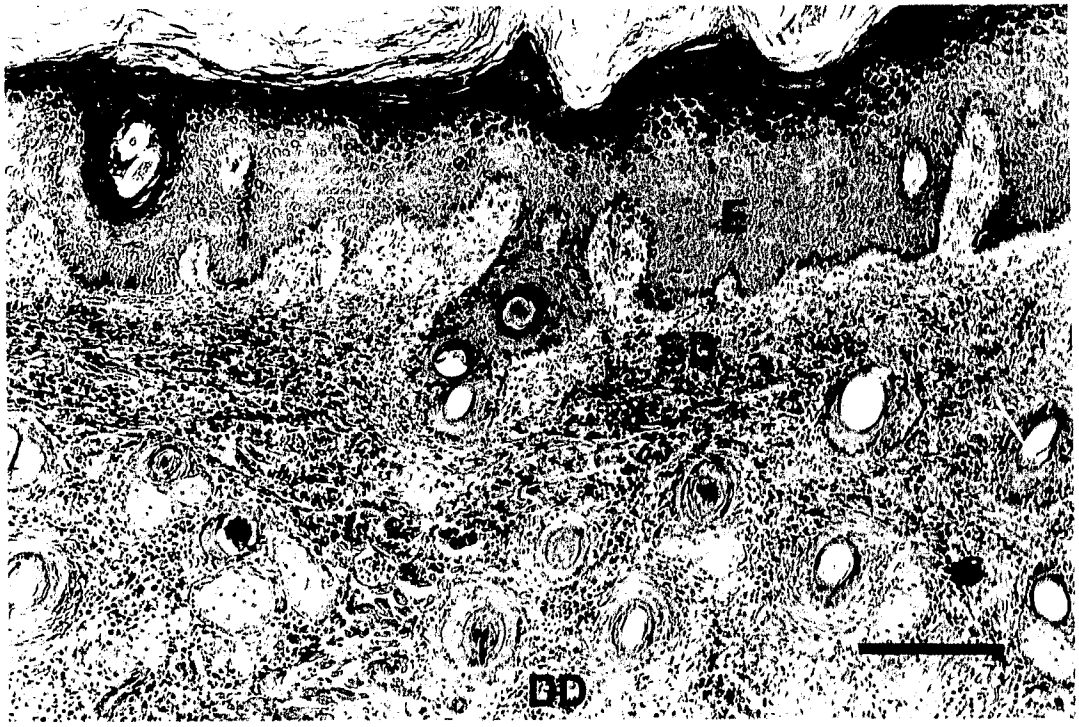

Fig. 21 Sheep #5, Ear. Severe infiltration with inflammatory cells in the skin involving superficial (SD) and deep dermis (DD) in *Trypanosoma brucei*-infected sheep. The epidermis (E) is markedly hyperplastic. H&E. Bar = 300 μm.

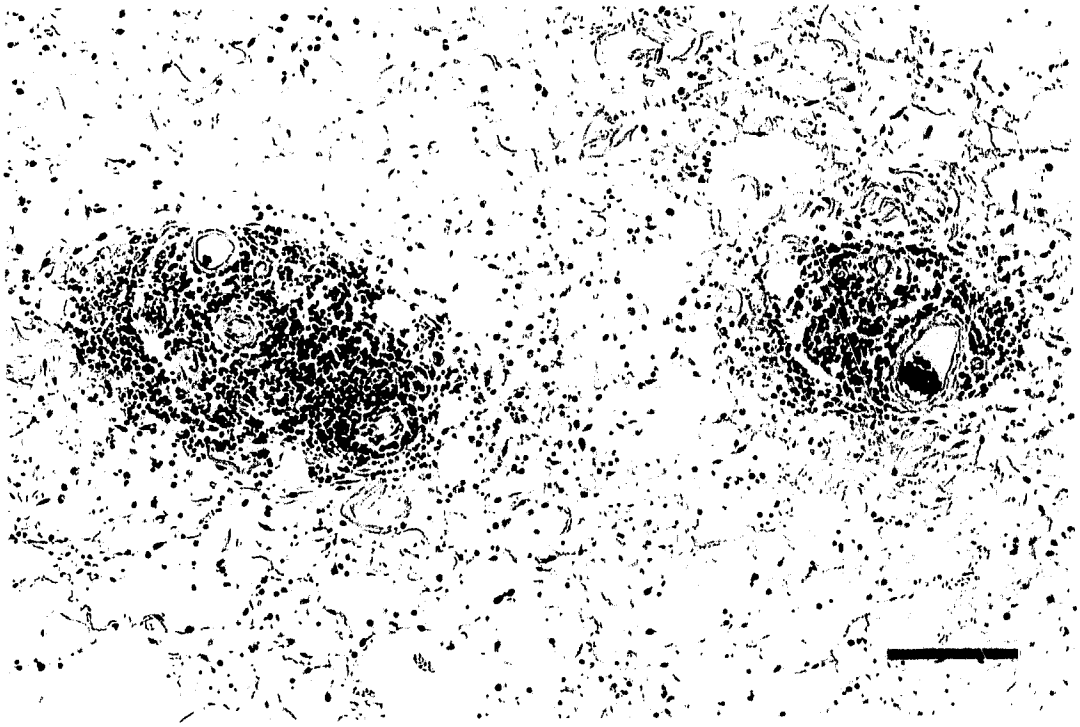

Fig. 22 Sheep #5, Ear. Focal perivascular infiltration with mononuclear inflammatory cells in the subcutis in *Trypanosoma brucei*-infected sheep. H&E. Bar = 100 μm.

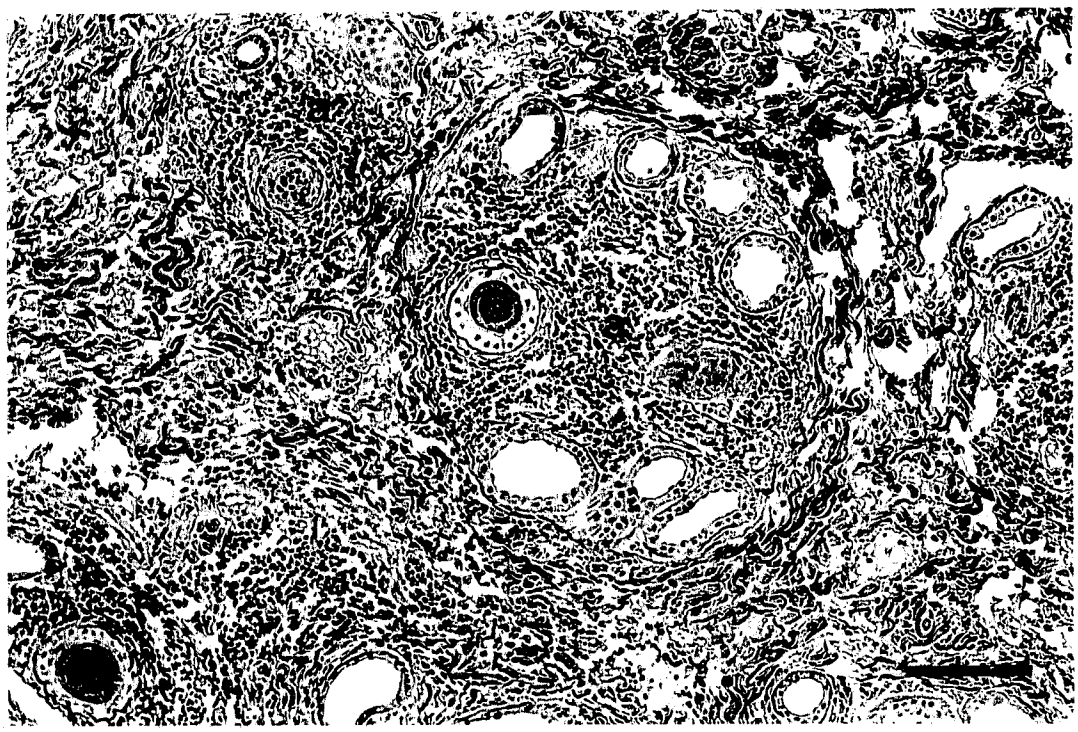

Fig. 23 Sheep #3, Ear. Severe periadnexal accumulation (a) of mononuclear inflammatory cells in the dermis in *Trypanosoma brucei*-infected sheep. H&E. Bar = 100 μm.

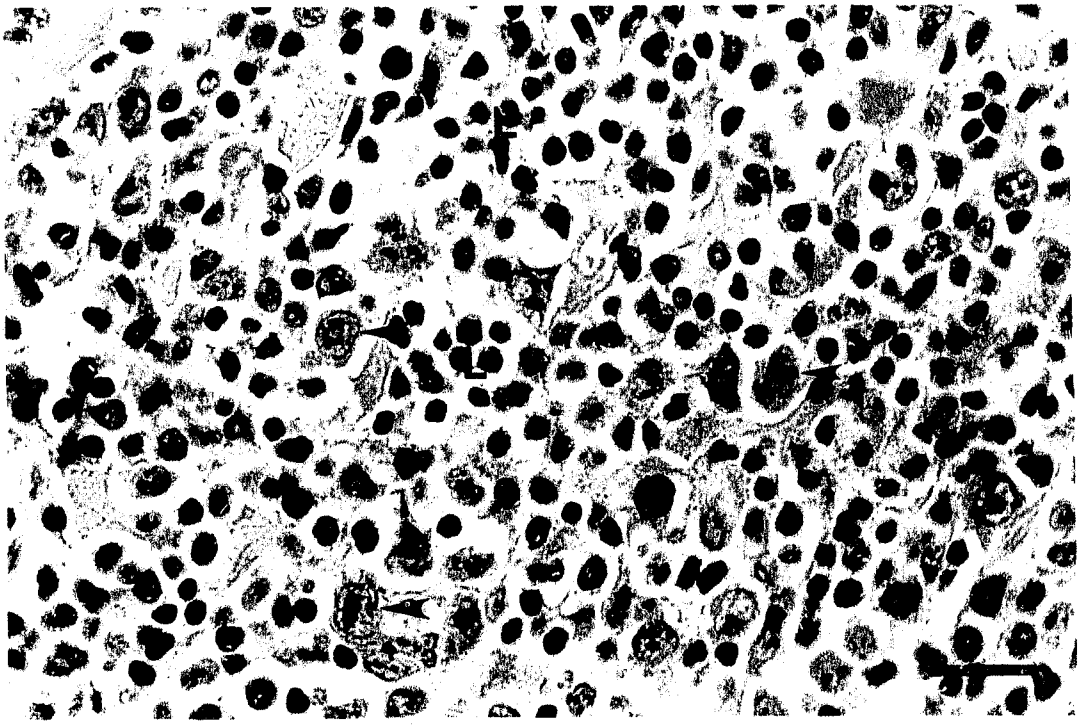

Fig. 24 Sheep #6, Ear. Diffuse infiltration with inflammatory cells composed mainly of lymphocytes (L), and macrophages (arrowheads) in subcutis, *Trypanosoma brucei*-infected sheep. H&E. Bar = 15 μm.

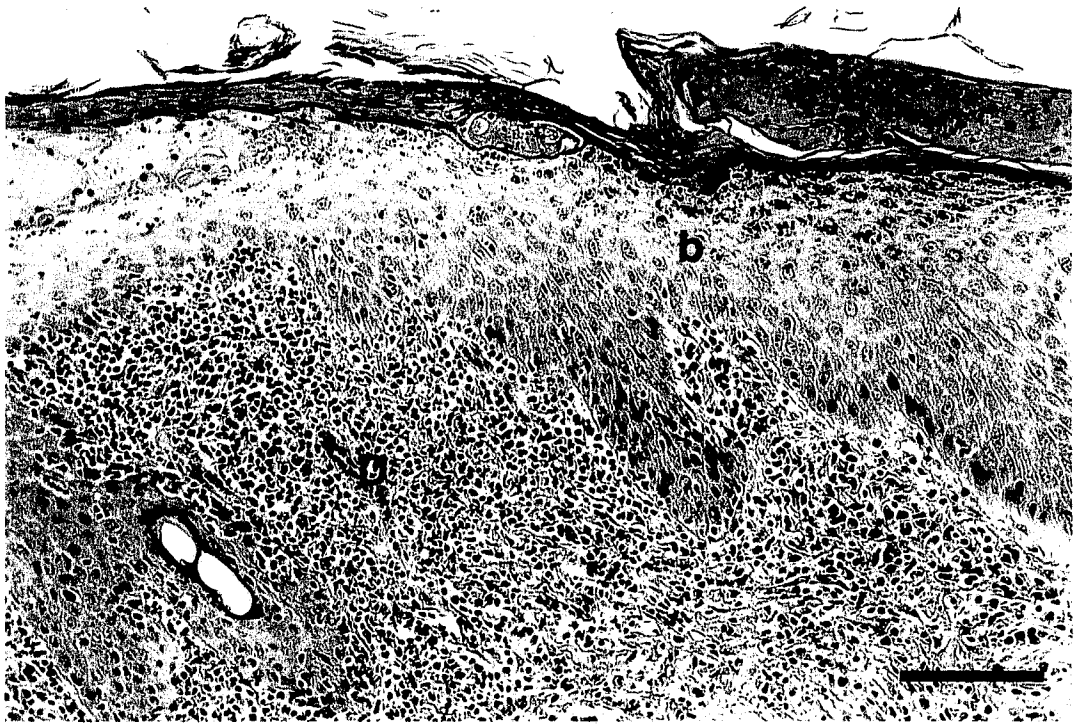

Fig. 25 Sheep #6, Ear. Epidermis showing slight acanthosis (b) and encrustation (c) in *Trypanosoma brucei*-infected sheep. Inflammation is restricted to the superficial dermis (D). H&E. Bar = 75 μm.

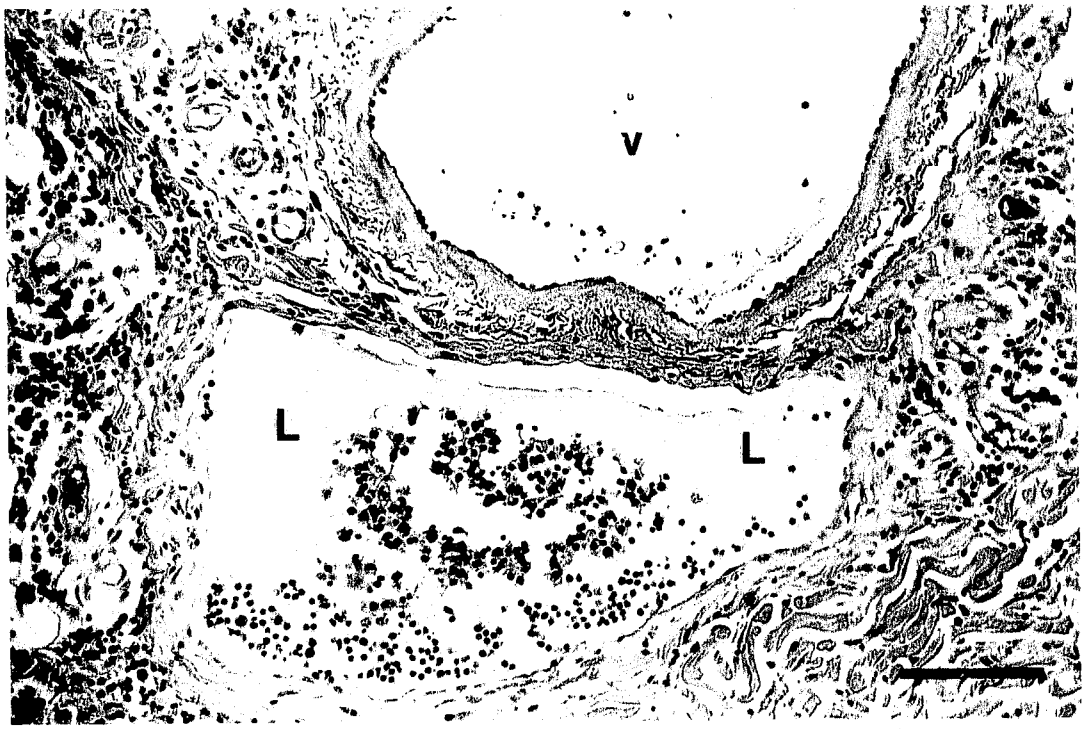

Fig. 26 Sheep #6, Ear. Distension and accumulation of inflammatory cells and fluid in and around lymph vessel (L) and vein (v) in the skin of *Trypanosoma brucei*-infected sheep. H&E. Bar = 75 μm.

accompanied by lymphatic thrombosis (Fig. 27). Walls of some arteries in sheep #3 and #6 were thickened and there was fibrinoid degeneration in the tunica media (Fig. 28). In all infected sheep, there was severe perineuritis in both the superficial and deep dermis. In sheep #2 and #7 neutrophils and eosinophils were prominent in the dermis of the ear. Numerous trypanosomes were seen in the edematous areas of the ear in 5 cases especially where infiltrating cells were scanty (Fig. 29), and occasionally in distended lymphatics. They were absent in controls and sheep #4 which had no ear lesions.

The skin on the bridge of the nose and lips had similar changes to those of the ear except that edema and cellular infiltration was more severe, extending deep into facial and lip muscles (Fig. 30). The epidermis of submandibular skin in one sheep (#4) had severe degeneration in stratum spinosum and stratum granulosum (Fig. 31). This resulted in the formation of vesicles and pustules. Vesiculation and pustule formation in the epidermis was accompanied by infiltration with neutrophils, macrophages and eosinophils. Trypanosomes were not seen in epidermal edema but were abundant in the dermis, subcutis and intermuscular tissue. Deeper areas of the submandibular skin had similar changes to those seen in the ear.

Changes in other areas of the skin including the face, brisket, distal limbs, and tail in all infected sheep were essentially similar to those seen in the ear.

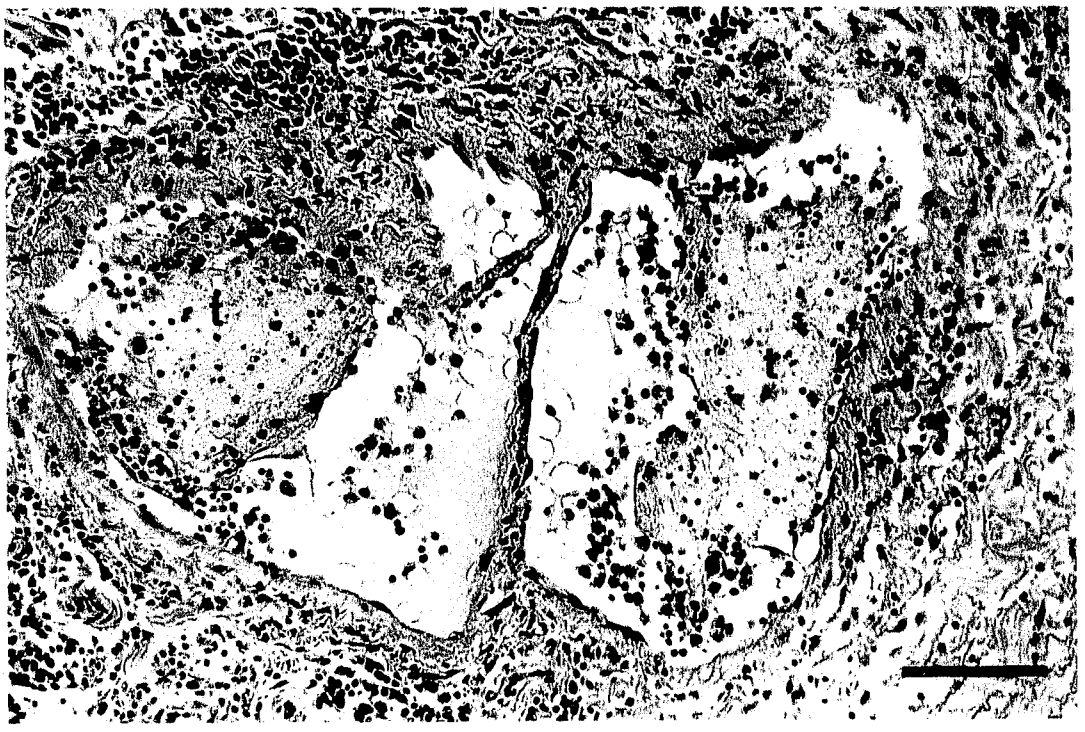

Fig. 27 Sheep #6, Ear. Two lymphatic vessels showing severe distension with fluid and lymphangitis accompanied by fibrinous thrombosis (t) in dermis of *Trypanosoma brucei*-infected sheep. H&E. Bar = 65 μm.

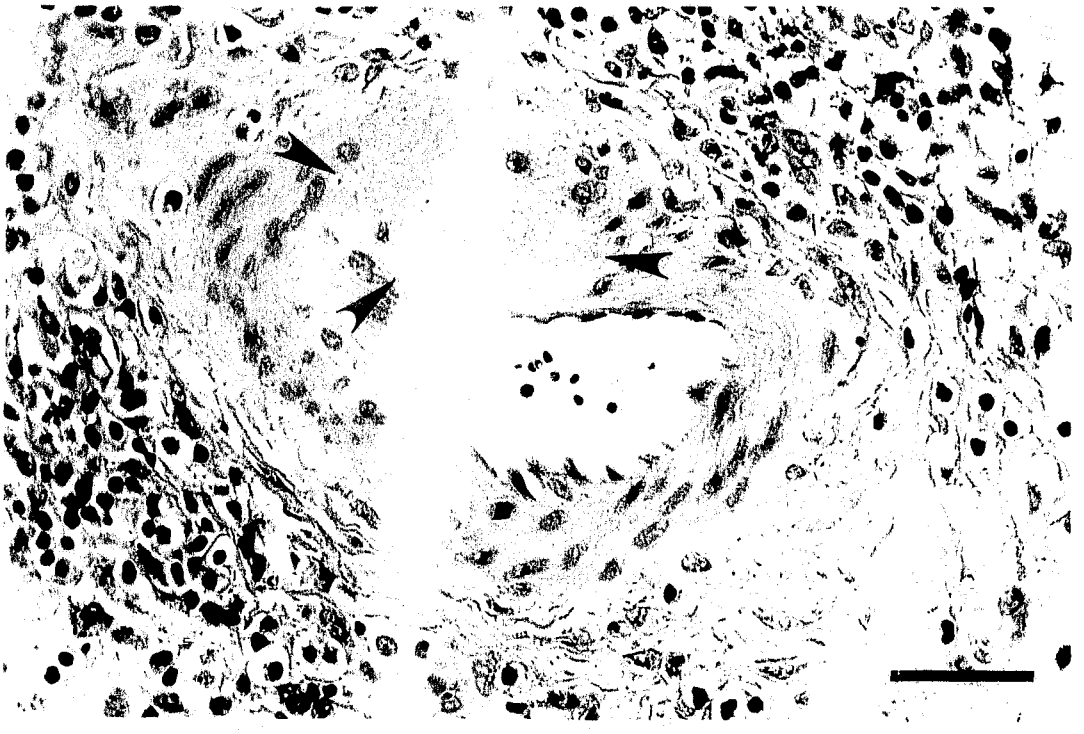

Fig. 28 Sheep #6, Ear. Thickening and degeneration (arrowheads) of tunica media in arterial wall and perivascular mononuclear infiltration in *Trypanosoma brucei*-infected sheep. H&E. Bar = 30 μm.

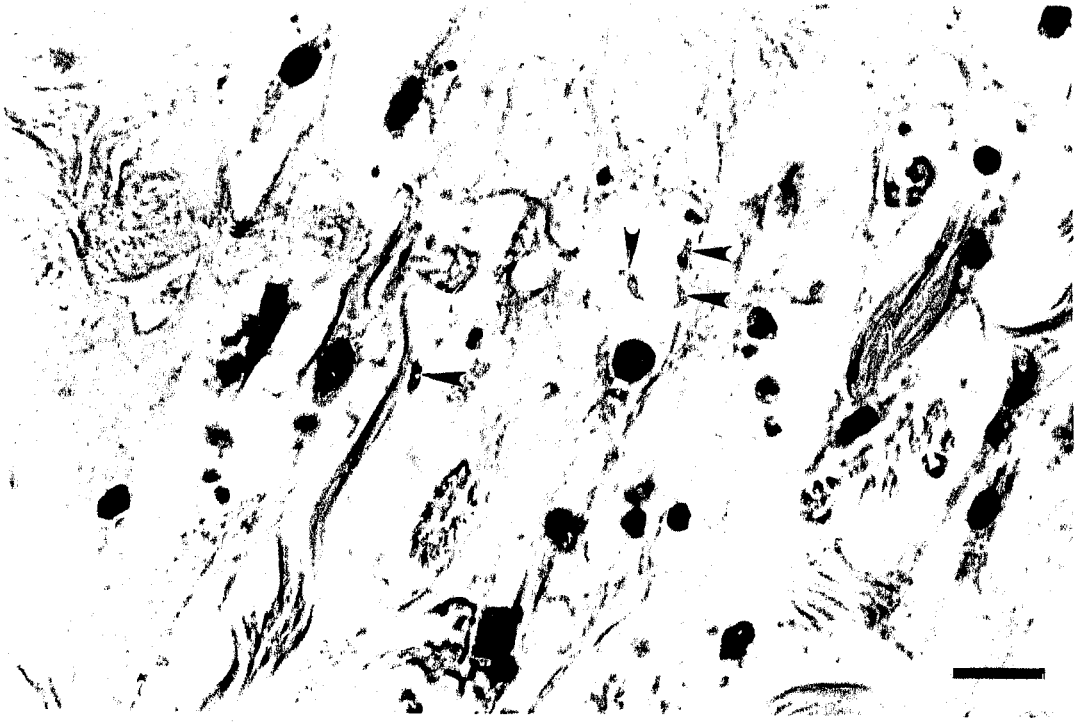

Fig. 29 Sheep #6, Ear. Trypanosomes (arrowheads) with a few mononuclear cells in a markedly edematous area of the ear in *Trypanosoma brucei*-infected sheep. H&E. Bar = 10 μm.

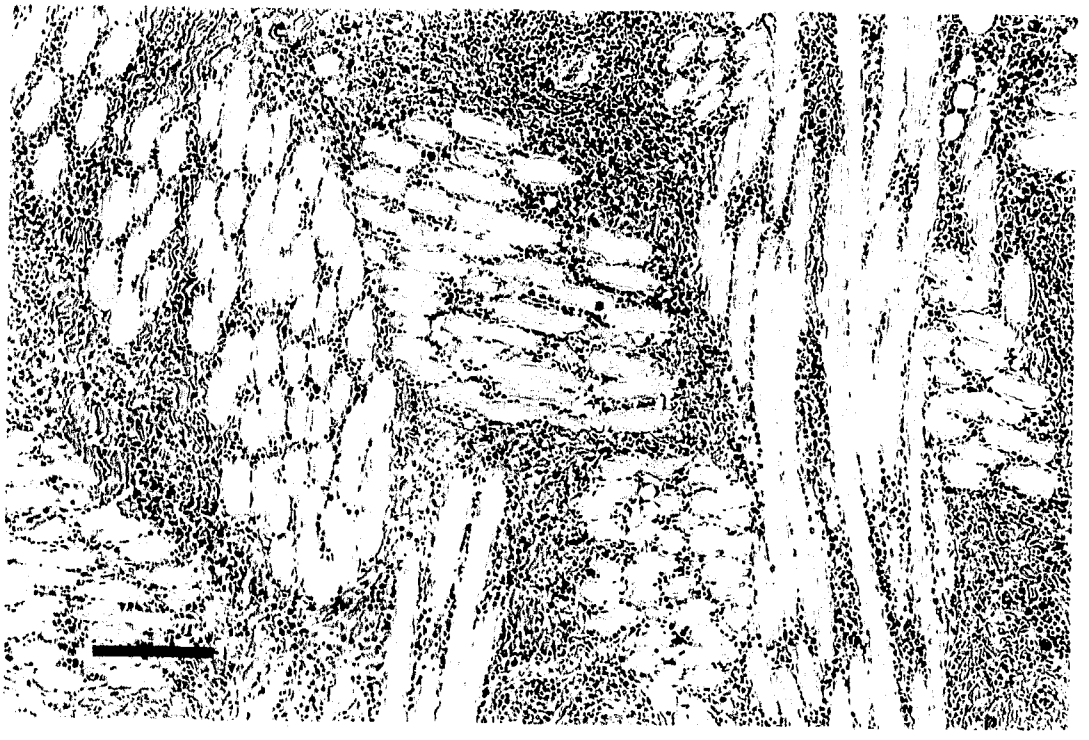

Fig. 30 Sheep #6, Lips. Severe mononuclear cell infiltration in the striated muscles of the lip in *Trypanosoma brucei*-infected sheep. The reaction extended from the superficial dermis through the subcutis (not shown). H&E. Bar = 120 μm.

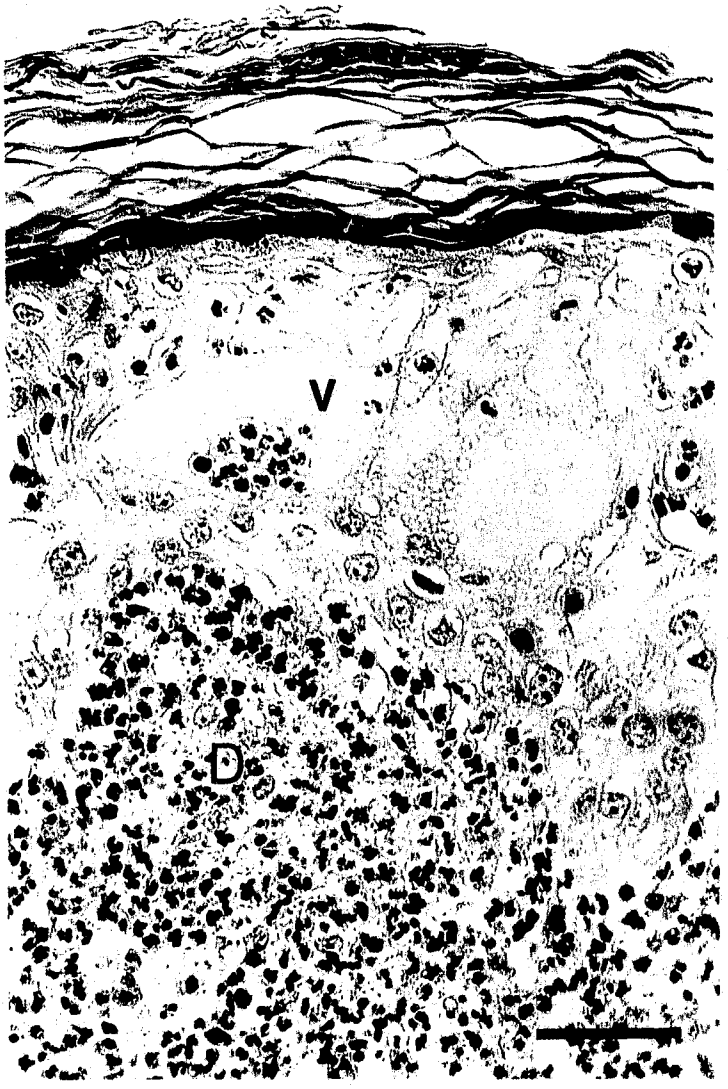

Fig. 31 Sheep #4, Submandibular Skin. Ballooning degeneration, vesiculation and pustule formation (V) in epidermis, *Trypanosoma brucei*-infected sheep. Inflammatory cells in superficial dermis (D) are mostly neutrophils. H&E. Bar = 30 μm.

In tail skin, the epidermis was not affected but the dermal and subcutaneous lesions extended deep into the thick adipose tissue causing severe panniculitis (Fig. 32). In all infected sheep, there was severe perineuritis in both superficial and deep dermis of the leg skin (Fig. 33). Trypanosomes were not readily observed in inflamed areas of adipose tissue, but numerous trypanosomes were seen in edematous areas of skin of infected sheep, especially where infiltrating cells were scanty, and in distended lymphatics (Fig. 34).

Changes in skin from the neck, especially around the inoculation site were slightly different from other skin lesions. In sheep #2, #3, #5, #6 and #7, there was marked acanthosis and necrosis of the epidermis accompanied by crust formation. Severe inflammatory reaction in the epidermis was characterized by accumulation of mainly neutrophils, macrophages and eosinophils. However, dermal and subcutaneous tissue reactions were similar to those in the ear. Although trypanosomes were not observed in the inflamed areas of the epidermis, they were numerous in the dermis and subcutaneous areas especially where edema was severe.

3.3.7.2 Changes in Other Organs

Severe lesions other than those of the skin were confined to superficial lymph nodes and spleens of infected sheep. There was marked mononuclear cell

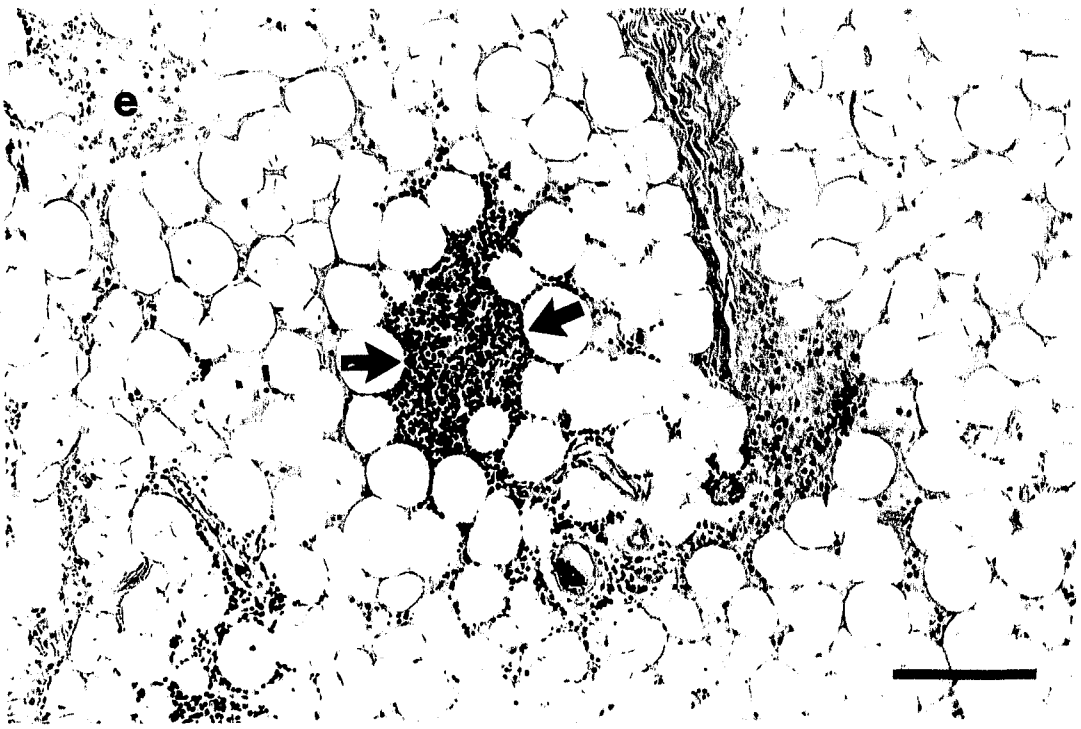

Fig. 32 Sheep #3, Tail. Extension of inflammation (arrows) and edema (e) into the adipose tissue of tail in *Trypanosoma brucei*-infected sheep. H&E. Bar = 75 μm.

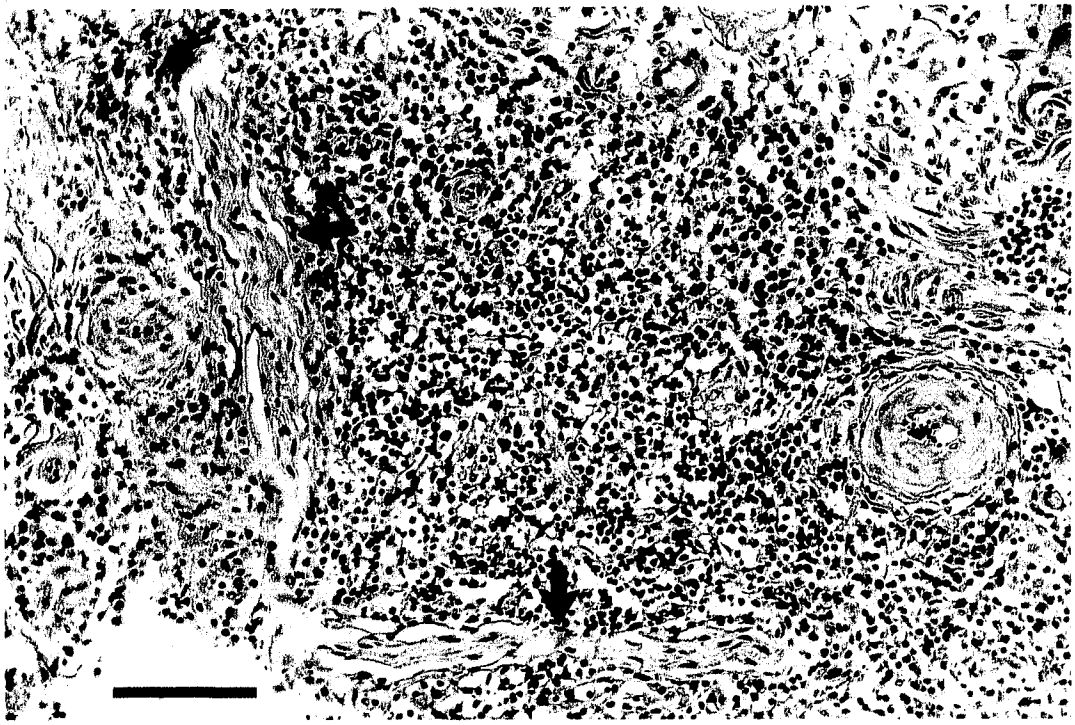

Fig. 33 Sheep #5, Tail. Severe perineuritis (arrows) in the dermis of tail in *Trypanosoma brucei*-infected sheep. H&E. Bar = 25 μm.

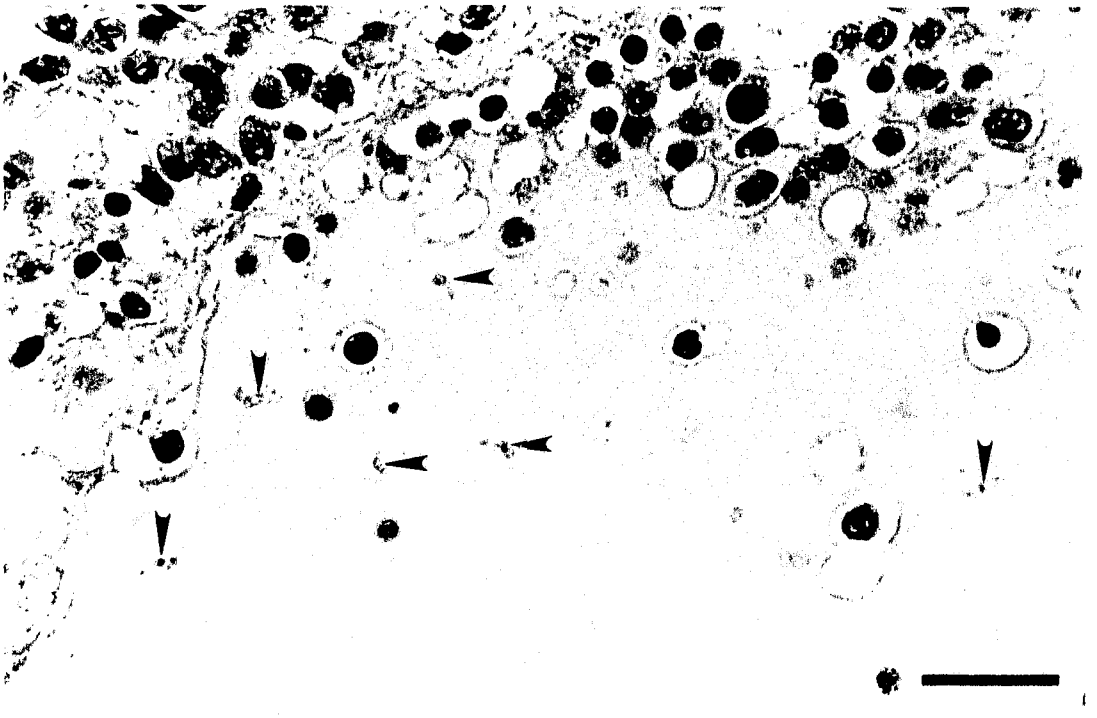

Fig. 34 Sheep #3, Skin, Distal Limbs. Trypanosomes (arrowheads) in distended lymphatic in *Trypanosoma brucei*-infected sheep. H&E. Bar = 25 μm.

infiltration and edema around the capsule of the peripheral lymph nodes. This was accompanied by severe distension of lymphatics with fluid containing macrophages, plasma cells and lymphocytes (Fig. 35). Some lymphatics contained fibrin thrombi and had severe lymphangitis. Subcapsular sinuses were distended with fluid mixed with macrophages and plasma cells. Lymphoid follicles in the cortex of lymph nodes were markedly expanded with prominent germinal centres primarily composed of proliferating lymphoblasts, lymphocytes and macrophages. Numerous mitotic figures were observed in germinal centres. The increase in the B-lymphocyte area was accompanied by an apparent reduction in the T-lymphocyte dependent area, which was invaded by proliferating B-lymphoblasts (Fig. 36). Medullary cords were markedly thickened due to increased numbers of plasma cells. Medullary sinuses were distended with fluid and numerous macrophages. Control sheep did not show the lesions observed in the lymph nodes of infected animals.

Changes in the spleen were less marked than those in the lymph nodes. In all infected sheep, there was enlargement and proliferation of lymphoid follicles. Enlargement of the germinal centres was similar to that in lymph nodes described above. The red pulp had diffusely increased cellularity due to proliferation of macrophages and plasma cells. Many of these macrophages had hemosiderin granules in their cytoplasm, although increased erythrophagocytosis

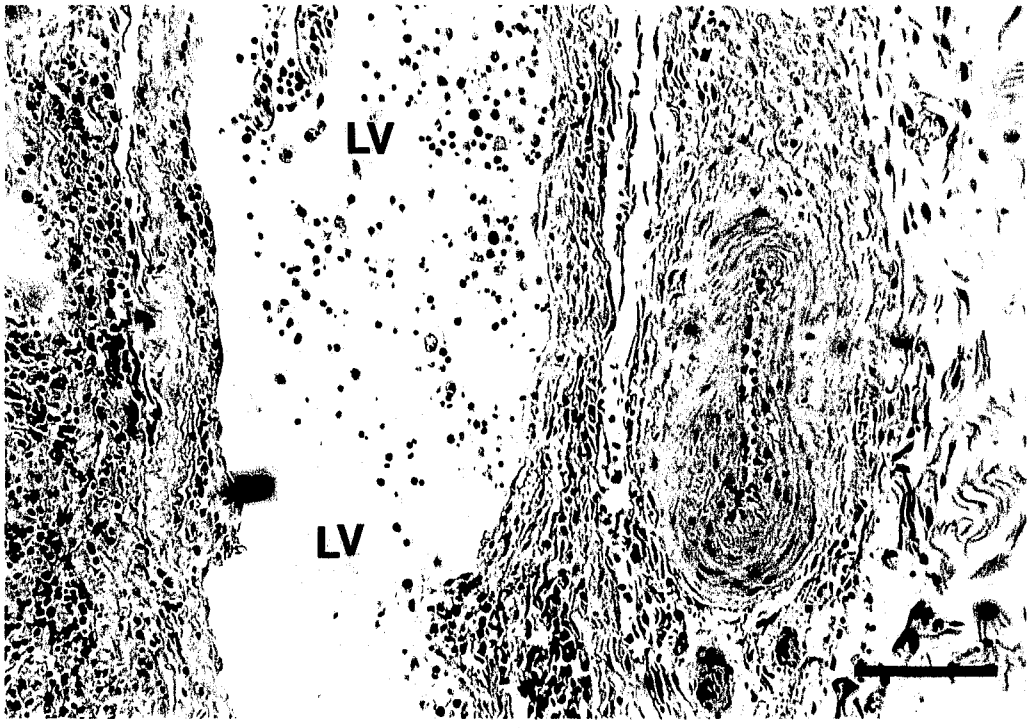

Fig. 35 Sheep #2, Lymph node. Marked distension of afferent lymphatic (LV), with fluid containing mixed leucocytes in capsule, in *Trypanosoma brucei*-infected sheep. H&E. Bar = 65 μm.

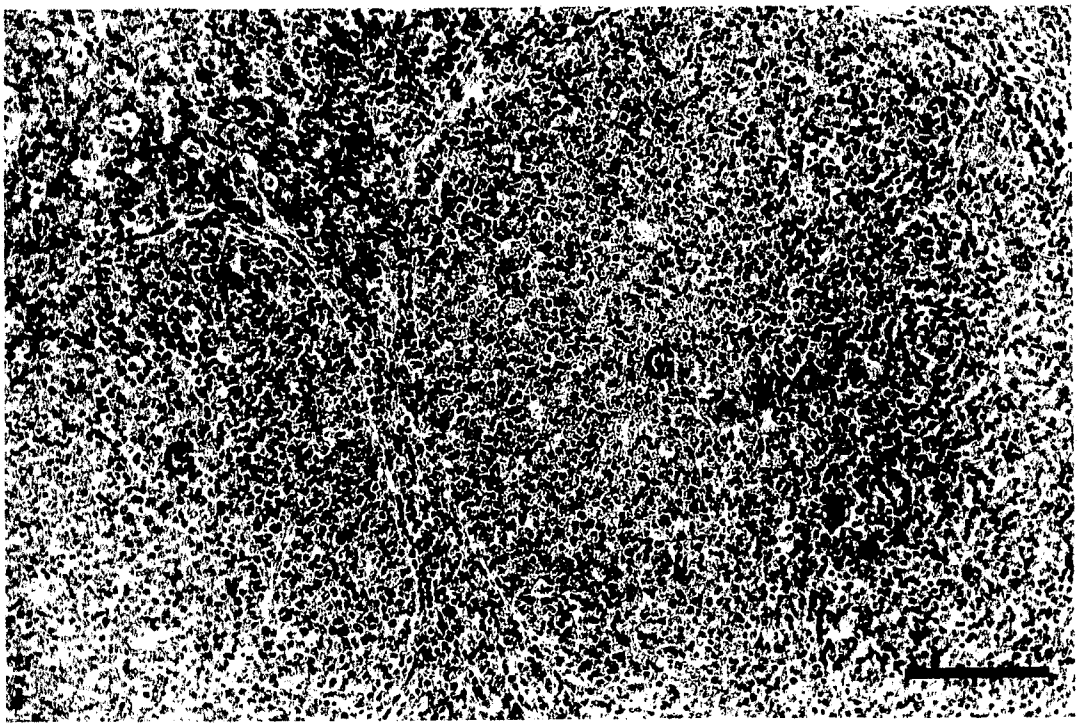

Fig. 36 Sheep #2, Lymph node. The cortex showing lymphoid follicle hyperplasia accompanied by enlarged germinal centres (G) and decreased T-dependent areas (p) in *Trypanosoma brucei*-infected sheep. H&E. Bar = 75 μm.

was not observed. No lesions were observed in the spleen of control sheep. The liver and kidneys of infected sheep had mild to moderate changes. The liver had mild periportal mononuclear infiltration. The kidneys had mild to moderate glomerulonephritis accompanied by occasional tubular casts in the renal medulla. There were no gross or microscopic lesions in the heart, brain, thyroid gland, kidneys, pituitary gland, adrenal gland, and lungs of infected sheep.

3.4 Discussion

Sheep infected with *T. brucei* developed clinical trypanosomiasis characterized by pyrexia, scanty parasitaemia, reduction in PCV values and RBC counts, and loss of weight/reduced weight gain. Weight loss correlated well with the severity of clinical observations in that sheep (#6) with the greatest weight loss had the most severe clinical picture. The most notable change was progressive development of subcutaneous edema of the neck, ears, face, lips, submandibular space, brisket, distal extremities of the limbs and tail. This edema was associated with edematous enlargement of superficial lymph nodes. Microscopically, there was severe edema and mononuclear dermatitis in the skin of the affected regions. There was diffuse perivascular and periadnexal accumulation of lymphocytes, plasma cells, macrophages and occasionally polymorphonuclear cells. Superficial lymph nodes had marked lymphoid proliferation in the germinal centres and severe inflammation of the lymph node capsule. The spleen

had marked proliferation of the lymphoid follicles accompanied by increased cellularity of the red pulp.

Although subcutaneous edema had been noted in sheep infected with *T. brucei* (Ikede and Losos, 1972b), detailed histopathology had not been previously described. Results of this study showed that localization and multiplication of trypanosomes in the skin resulted in severe dermal and subcutaneous edema, accompanied by extensive perivascular to diffuse dermatitis. Inflammatory cells were mainly mononuclear although polymorphonuclear cells were present. Although trypanosomes only localised in the skin, the infection none the less resulted in severe clinical disease.

Trypanosoma brucei has long been shown to localise in extravascular sites more readily than either *T. congolense* or *T. vivax*, both in ruminants and laboratory rodents (Ikede and Losos, 1970; Goodwin, 1970; Ikede and Losos, 1972b). In previous studies, trypanosomes were found to invade and localize in many tissues and organs, including the eye (Ikede, 1974), the testes (Ikede, 1979), brain and spinal cord (Ikede and Losos, 1975a; Ikede and Losos, 1975b), heart and connective tissue, resulting in severe inflammation and directly contributing to the clinical signs. In this study, the severe edema in the skin and subcutaneous tissue occurred in the absence of cardiac lesions and appeared to result from the presence of trypanosomes in these areas. Previously, Losos and Ikede (1972) had postulated among other things, that the edema of the skin and subcutaneous

tissues could have resulted from heart failure caused by the extensive myocarditis. The absence of cardiac pathology in the infected sheep of this study suggests that mechanisms other than heart failure are responsible for the skin lesions observed. The lameness observed in the infected sheep was probably due to severe edema and inflammation in the dermis and subcutis accompanied by perineuritis.

At the time of euthanasia, the initial chancre reaction at the site of inoculation in the neck was not markedly different from the edema in the other areas of the skin. The only exception was necrosis and encrustation of the epidermis. This resulted in the composition of inflammatory cells becoming predominantly granulocytic. Frequent piercing of the skin when obtaining blood may have resulted in bacterial infection leading to necrosis and crust formation. Previous studies have shown that injection of blood stream trypanosomes intradermally resulted in swellings which were indistinguishable from the chancre seen in natural infections (Manson-Bahr and Charters, 1963).

Although the inflammatory response in the skin was similar to that previously described in other organs (Ikede and Losos,1972a,b; Ikede, 1974; Ikede, 1979), the events leading to these lesions have not been clearly established. Severe edema could be caused by release of vasoactive substances from either the trypanosomes or the inflammatory cells (Tizard and Holmes, 1976; Tizard et al, 1978). Activation of macrophages and interaction of antibodies and

antigens can activate complement with subsequent release of C5a and histamine that increase vascular permeability. Infiltration of mononuclear cells in the skin of *T brucei*-infected animals was probably a cell-mediated immunological reaction of the delayed hypersensitivity type (Goodwin and Hook, 1968; Ikede and Losos, 1972b). However, phenotypic typing and immunohistochemical analysis with monoclonal antibodies has not been done. T-lymphocyte phenotyping has been done in *T. congolense*-infected sheep for the chancre reaction (Mwangi et al, 1990b), for the lymph nodes draining the local skin reaction (Mwangi et al, 1991), and in lymph nodes in *T. congolense*-infected N'Dama and Boran cattle (ILRAD, 1992). The type of lymphocyte phenotype generated determines the response of the animal to trypanosome infection, and determines trypanotolerance in N'Dama cattle (ILRAD, 1992). Similar studies should be done on the chancre in *T. brucei* infection and also on subcutaneous lesions observed in *T. brucei*-infected animals. This strain of *T. brucei* multiplied and produced edema in areas without wool, i.e., head, ears, legs, tail and neck (shaved). There were no gross lesions in wooled parts of the skin. We postulated that the higher temperature in internal organs and other body skin (covered with wool) prevented localization and multiplication of trypanosomes in these areas. This phenomenon requires further investigation.

Weight loss and reduced appetite could be due to reduced feed intake as a result of fever and inflamed lips. They can also result from dysfunction of the

endocrine system. Abebe and Eley (1992) found a significant reduction in total thyroxine hormone (T_4) during acute stage of *T. congolense* infection in cattle and Mutayoba et al (1988) found reduced thyroxine levels in *T. congolense*-infected goats. They suggested that this was due to a dysfunction of the hypothalamic-pituitary-thyroid axis. Plasma concentration of thyroid hormones correlate positively with energy intake (Blum et al, 1980; Dauncey et al, 1983). The fall in T_4 levels in trypanosome-infected animals could have an adverse effect on feed intake and efficiency of feed conversion. In this study there was no pathological change in the thyroid gland, pituitary gland or hypothalamus. Perhaps trypanosomes release biologically active substances such as phospho-lipase A (Tizard et al, 1978), proteases and peptidases (Knowles et al, 1989), that act on the hypothalamus-pituitary-thyroid axis without morphological change to these organs. A similar effect can be achieved via hyper-activation of the immune system, especially macrophages, to produce tumour necrosis factor (TNF) which is known to inhibit the secretion of pituitary hormones (Walton et al, 1989; Pang et al, 1989). TNF has been implicated in causing impairment of hypothalamic-pituitary-thyroid function in rats (Pang et al, 1989) that can lead to cachexia. Interference with the hypothalamic-pituitary-thyroid axis has been demonstrated in humans affected by African trypanosomiasis (Boersma et al 1989; Reincke et al 1993) no similar studies have been done in ruminants. Dysfunction in the hypothalamus-pituitary-thyroid axis can also result from direct

structural changes in any of these glands. *Trypanosoma brucei* can localize in the pituitary gland leading to focal coagulative degeneration (Ikede and Losos, 1975b). Recently, Abebe et al (1993) noted that *T. congolense* localized in the microvasculature of the pituitary gland of experimentally infected Boran cattle. This localization was associated with focal degenerative changes in the pituitary gland which they concluded could lead to its dysfunction. Although we found no lesions in the thyroid or pituitary glands, the anorexia and weight loss could be due in part to interference with the hypothalamic-pituitary-thyroid axis function. The relationship between trypanosomiasis and the hypothalamic-pituitary-thyroid axis function should be investigated, and the factors and mechanisms involved identified.

In this study, sheep infected with *T. brucei* showed intermittent parasitaemia, pyrexia and moderate anaemia. Intermittent parasitaemia, pyrexia and severe anaemia are common in African trypanosomiasis and their pathogenesis is fairly well understood. Intermittent/fluctuating parasitaemia is due to competition between the circulating trypanosomes and the animals' immune response.

Actively multiplying trypanosomes produce pyrogens or induce host cells to produce endogenous pyrogens that act on the hypothalamic temperature regulatory centre (Davis and MacIntyre, 1992). Prostaglandins (e.g PGE, $PGF_{2\alpha}$ and PGA) act as endogenous and exogenous pyrogens (Milton and Wendlandt,

1970; Dinarello and Wolff, 1978) and their action is mediated by IL-1 (Lachman, 1983; Slauson and Cooper, 1990). Increase in body temperature during infection may be one way animals alter their internal environment so that it becomes unfavourable for pathogen multiplication (Slauson and Cooper, 1990). During trypanosome infections, phagocytes, especially monocytes and macrophages, come into contact with trypanosome antigens. Interaction of the phagocytic cells with parasite antigen induces pyrogen reduction (Stephen, 1986). In addition, there are excess trypanosomal antigens which cause production of large quantities of antibodies. The resultant formation of antigen-antibody immune complexes is a potent stimulus for pyrogen release from phagocytic cells (Stephen, 1986).

In this study there was moderate anaemia. Although erythrophagocytosis in the reticuloendothelial system was not pronounced, the presence of numerous macrophages filled with hemosiderin in the spleen indicated that this anaemia was due to extravascular haemolysis.

In conclusion, *T. brucei*-infected sheep had severe inflammation and edema of the skin and subcutaneous tissues. The major difference observed in this study was that this reaction was not accompanied by inflammation and localization of trypanosomes in internal organs, especially the heart and brain. The pathogenesis of edema of the skin and subcutaneous tissues in this study was not cardiogenic.

CHAPTER 4. GENERAL DISCUSSION AND CONCLUSIONS

4.1 Review of results

4.1.1 *Trypanosoma congolense*

Although sheep infected with *T. congolense* developed parasitaemia seven days post infection, they did not develop clinical trypanosomiasis within 60 days. All parameters monitored including PCV, temperature, weight changes, total and differential blood cell counts were not significantly different from the control sheep. Gross and microscopic examination of tissues showed no lesions. Results of cytochrome C reduction assay were not significantly different between infected and control sheep.

4.1.2 *Trypanosoma brucei*

Sheep infected with *T. brucei* developed clinical trypanosomiasis characterized by fever, weight loss, moderate anaemia, mild leucocytosis, and inflammatory edema of the skin of the head, neck, brisket, distal limbs and tail within two months. Microscopically, the skin reaction was characterized by perivascular, periadnexal and diffuse inflammation involving both the dermis and subcutis. Inflammatory cells were mainly lymphocytes, plasma cells and macrophages with fewer polymorphonuclear leucocytes. Dermatitis was

associated with severe edema, lymphangitis, distension of the lymphatic vessels with proteinaceous fluid, lymphatic thrombosis as well as perineuritis. Numerous trypanosomes were seen in the skin reactions, especially in areas where edema was severe. Superficial lymph nodes were enlarged and edematous; microscopically, there was severe capsular lymphadenitis characterized by lymphangitis, distension of lymphatics with fluid and inflammatory cells, and diffuse infiltration with mononuclear cells in the capsule. The lymph nodes had severe lymphoid hyperplasia characterized by proliferation of lymphocytes in germinal centres and of plasma cells and macrophages in the sinuses. The spleen had hyperplastic reaction similar to that seen in peripheral lymph nodes. The hyperplastic reaction of the lymphoid elements in the spleen resulted in a reduction of the red pulp.

Assessment of PMN function showed that PMNs from sheep infected with *T. brucei* had significant decrease in the ability to reduce inhibitable cytochrome C ($p < 0.01$). In addition, as the disease progressed, PMNs became more fragile, and more were lost during isolation. Nylon wool adherence assay showed that PMNs from infected sheep had significant reduction ($p < 0.01$) in their ability to adhere to nylon wool compared to control sheep. In contrast to results of cytochrome C reduction and nylon wool adherence, phagocytosis of opsonized zymosan was significantly enhanced ($p < 0.01$) in trypanosome-infected sheep.

4.2 General Discussion

4.2.1 *Trypanosoma congolense* Infection

The observation that *T. congolense* did not elicit disease in the breed of sheep used in this experiment despite the establishment of parasitaemia was unusual. The same strain of trypanosomes was very pathogenic to rats used to amplify parasites before inoculation into the sheep (unreported observation). It was speculated that either this particular strain of *T. congolense* had lost its virulence for sheep, or the type of sheep used had natural resistance to this strain. The observation that cytochrome C reduction by PMNs from these sheep was not significantly different from the controls was not surprising, as the infected sheep did not develop clinical trypanosomiasis. The absence of clinical disease was confirmed on microscopic examination of tissues from organs of infected sheep. As a result of this, it was unlikely there would be any demonstrable alteration in PMN function.

4.2.2 *Trypanosoma brucei* Infection

The primary objective of the thesis was to experimentally produce clinical trypanosomiasis in sheep and assess quantitative and some qualitative aspects of PMNs isolated from these sheep. This objective was satisfactorily achieved in *T. brucei*-infected sheep. The clinical disease produced in *T. brucei*-infected

sheep was similar to that previously reported by other investigators in sheep and other hosts (Ikede and Losos, 1972a,b; Moulton and Sollod 1976; Morrison et al, 1981). The only difference observed between our findings and the earlier reports was that in previous reports, the development of subcutaneous inflammatory edema was usually accompanied by similar and severe reactions in other vital internal organs such as the heart, liver, kidney and brain. Apart from the reaction described in the superficial lymph nodes and spleen, there were no pathological changes in other internal organs. The significance of this finding is not apparent, although skin lesions were only observed in areas of the skin that did not have wool. Based on these two findings; namely the absence of localization of trypanosomes in internal organs, and the appearance of skin lesions only in areas without wool, we postulated that this strain of trypanosome probably requires cooler temperatures to multiply. This observation requires further investigation. The basic skin reaction was similar to that reported in other organs (Ikede and Losos, 1972b; Losos and Ikede, 1972, Moulton and Sollod, 1976).

We were able to isolate PMNs from trypanosome-infected sheep and assess their function. All the three tests showed that there was significant alteration in PMN function. Two tests, cytochrome C reduction assay and nylon wool adherence showed a reduction in function, while one test, phagocytosis of opsonized zymosan, was enhanced during the infection. The reason for enhanced phagocytosis was not clear, though we observed that platelets from infected

sheep had increased tendency to clump. Platelet clumping may have resulted in activation of complement and generation of substances e.g C5a, histamine, that have chemotactic and stimulatory effect on PMNs. Although the phagocytic aspect of PMN function was enhanced, the fact that the cells may not migrate effectively to sites of infection as indicated by reduced nylon wool adherence, and may not effectively kill ingested pathogens due to reduced superoxide anion production, means that the overall result is likely to be reduced ability to protect the host against invading pathogens. To our knowledge no studies had been done to evaluate PMN function in trypanosome infection and their possible contribution to the lowering of body defence in trypanosomiasis. Immunosuppression in trypanosomiasis has been extensively studied, and although much knowledge has been acquired, it is not yet possible to formulate a unified hypothesis to explain all the aspects of trypanosome immunosuppression (ILRAD, 1990). This study further supports that immunosuppression in trypanosomiasis is complex and involves functional derangement of polymorphonuclear phagocytic system in addition to the other established modes of immunosuppression.

4.3 Conclusions

Sheep infected with *T. brucei* have significant alteration in PMN function. This alteration can lead to reduced ability of polymorphonuclear phagocytes to protect the host against invading pathogens. The interference in PMN function

may contribute to the increased occurrence of opportunistic infections in trypanosomiasis. The results substantiated prior observations that adminstration of antibiotics concurrently with trypanocides to animals debilitated by trypanosomiasis is beneficial. Development and microscopic appearance of skin lesions in *T. brucei*-infected sheep confirmed that the basic reaction was similar to that previously described in other organs. In the absence of lesions in the heart, the skin lesions appeared to result from localization and multiplication of trypanosomes at the site.

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