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U N I V E R S I T Y O F G L A S G O W.

CLINICAL AND IMMUNOPATHOLOGICAL ASPECTS OF HEART DAMAGE IN DOGS INFECTED WITH *TRYPANOSOMA BRUCEI*.

JOSEPH MATHU NDUNG'U.

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy.

DEPARTMENT OF VETERINARY MEDICINE.

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DEDICATION.

To my family, and all the people at home whose encouragement enabled me to finish this work.

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DECLARATION.

I declare that, apart from the assistance indicated in the acknowledgements, this work was carried out entirely by me after being trained to the necessary level of expertise.

Signed:

Joseph Mathu Ndung'u.

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LIST OF ACRONYMS.

2D - Two dimensional.

ACEI - Angiotensin-converting enzyme inhibitor.

ACTH - Adrenocorticotrophic hormone.

AI - Aortic incompetence.

AII - Angiotensin II.

ALT - Alanine aminotransferase.

ANF - Atrial natriuretic factor.

AP - Alkaline phosphatase.

APP - Acute phase proteins.

AST - Aspartate aminotransferase.

AVN - Atrioventricular node.

AVP - Arginine vasopressin.

BPM - Beats per minute.

BUN - Blood urea nitrogen.

CH - Cholesterol.

CPK - Creatinine phosphokinase.

CPM - Cycles per minute.

CRP - C - reactive protein.

CW - Continuous-wave.

DFMO - DL-α-difluoromethylornithine.

DIC - Disseminated intravascular coagulopathy.

ECG - Electrocardiography.

ELISA - Enzyme linked immunosorbent assay.

EDTA - Ethylenediaminetetraacetic acid.

FFA - Free fatty acids.

FS - Fractional shortening.

HAT - Human African trypanosomiasis.

HbBC - Haemoglobin binding capacity.

Hb - Haemoglobin.

HB - Heart Block.

HDL - High density lipoproteins.

Hp - Haptoglobin.

H&E - Haematoxylin and eosin.

IHB - First degree heart block.

IIHB - Second degree heart block.

IL-2 - Interleukin-2.

LDH - Lactate dehydrogenase.

LDL - Low density lipoproteins.

LPL - Lipoprotein lipase.

LVEDD - Left ventricular end-diastolic diameter.

LVESD - Left ventricular end-systolic diameter.

LVF - Left ventricular function.

MCH - Mean corpuscular haemoglobin.

MCHC - Mean corpuscular haemoglobin concentration.

MCV - Mean corpuscular volume.

MHC - Mean haemoglobin concentration.

MI - Mitral incompetence.

MPS - Mononuclear phagocytic system.

MSB - Martius scarlet blue.

NEFA - Non-esterified fatty acids.

NSAID - Non-steroidal antiinflammatory drug.

PCV - Packed red cell volume.

PRA - Plasma renin activity.

PE - Pericardial effusion.

PW - Pulsed-wave.

RBC - Red blood cells.

ROM - Reactive oxygen metabolites.

SAN - Sinuatrial node.

TEM - Transmission electron microscopy.

TG - Triglycerides.

TGL - Triglyceride lipase.

Th - T-helper cells.

TI - Tricuspid incompetence.

TNF - Tumour necrosis factor.

VLDL - Very low density lipoproteins.

VPB - Ventricular premature beat.

WBC - White blood cells.

SUMMARY.

In an attempt to increase the understanding of the mechanisms of cardiac damage in African trypanosomiasis, a group of beagle dogs were infected with *Trypanosoma brucei*. Cardiac involvement during the course of the disease was monitored using various non-invasive techniques, including auscultation, palpation, echocardiography and electrocardiography (ECG). Parasitological, haematological, endocrinological and biochemical studies were carried out. Two dogs per time point were euthanised at mid-infection on days 10 and 15, and in the terminal stages on days 21, 22 and 26, and tissue samples taken from the heart for histological, histochemical, transmission electron microscopical and immunofluorescence investigations.

The dogs developed an acute disease syndrome, characterised by high parasitaemia, persistent fever, increased plasma levels of acute phase proteins, severe anaemia, lymph node and splenic enlargement, wasting and weight loss. If the dogs were not treated or the experiments terminated by humane euthanasia, death would have invariably occurred during week 4 of infection. Clinical evidence of cardiac damage was demonstrated from the end of week 1 by echocardiography and ECG. The clinical signs included tachycardia, incompetence of all the cardiac valves, and first degree heart block. With progress of the disease, second degree and in some cases complete heart block developed. Towards the end of week 3 and beginning of week 4, cardiac performance deteriorated, as demonstrated by a reduction in left ventricular function, bradycardia,

and accumulation of pericardial effusion.

Histological and ultrastructural studies confirmed a severe pancarditis involving the myocardium, the cardiac valves, and the vasculature. Deposition of fibrinogen and sparse quantities of IgG, IgM and C3 was demonstrated in perivascular and interstitial spaces. A terminal fall in plasma levels of the cardiac hormone atrial natriuretic factor (ANF) occurred, and was associated with decreased numbers, sizes and electron density of the atrial storage granules. The low plasma levels of ANF were accompanied by an inverse increase in plasma renin activity, an indication that the dogs at that time were incapable of controlling blood volume.

From the end of week 2 of infection, a gradually developing hyperlipidaemia and intense deposition of lipids in the myocardium and infiltrating macrophages was demonstrated for the first time. It is possible that cachectin/tumour necrosis factor (TNF) was contributing to the hyperlipidaemic state, following the detection of increased cachectin/TNF activity in monocytes from infected dogs.

The presence of large numbers of trypanosomes, infiltrating cells that consisted mainly of macrophages, neutrophils, plasma cells and a few lymphocytes, in areas of extensive myocardial damage indicated that tissue damage was initiated by excessive immunological responses by the host to this highly antigenic parasite. Tissue damage was probably exacerbated by accumulation of toxic and biologically active substances released from dead

trypanosomes and autolysing inflammatory cells, following obstruction of the lymphatic vessels draining the heart. Anaemia, vascular damage and interstitial oedema reduced tissue perfusion and increased the incidence of myocardial ischaemia. The presence of lipid deposits in ischaemic myocardium indicated that they too could be playing a major role in the pathogenesis of tissue damage. Further, the presence of mesangial deposits of immune complexes in the kidneys confirmed their presence in the circulation, indicating that immune complexes played a role in the pathogenesis of general tissue damage.

Intravenous treatment of terminally ill dogs with the trypanocidal drug suramin at 10 to 20 mg/kg was usually unsuccessful. In most cases, a post-treatment reaction, with increased severity of heart damage, was observed. Nevertheless, the survival period of the dogs was prolonged, precipitating a condition of chronic cardiac failure. The dogs that survived longest were those that were best able to control anaemia after treatment.

When a group of infected dogs were treated with the non-steroidal anti-inflammatory drugs (NSAIDs) cyclosporin A and azathioprine, or a combination of azathioprine and prednisolone, the severity of cardiac damage was reduced, despite intense trypanosome infiltration in the myocardium. These results confirmed the immunological and inflammatory nature of the cardiac disease induced by T.brucei. It would appear that if such drugs were used as an adjunct to trypanocidal drug treatment, the severity of post-treatment myocardial damage, and possibly general tissue damage,

might have been reduced.

The present work has conclusively demonstrated that cardiac damage in canine trypanosomiasis caused by T.brucei is mainly immunologically mediated. The similarity of the disease to human African trypanosomiasis indicated that cardiac damage in humans could be mediated through similar mechanisms. The future of the dog as large monogastric animal model for studying cardiac damage in African trypanosomiasis is promising.

PART I.

INTRODUCTION.

CHAPTER 1.

THE AFRICAN TRYPANOSOMIASES.

1.1. A HISTORICAL PERSPECTIVE.

African trypanosomiasis, referred to as Nagana in animals and sleeping sickness in humans, has now been in existence on the African continent for more than two centuries. The disease is caused by protozoan parasites of the genus Trypanosoma, and transmitted by Glossina (tsetse) flies. Although many of the observations on the effects of African trypanosomiasis in humans and animals were made in the early 1800s (Duggan, 1970), it was not until 1894 when Lt. Col. David Bruce identified trypanosomes in the blood of infected cattle in Zululand and determined them to be the cause of Nagana, eventually tracking them to tsetse as carriers (Kasuke, 1984). Trypanosomes were first discovered in the cerebrospinal fluid of sleeping sickness patients in 1902 by Aldo Castellani, and in the following decade, the trypanosome was incriminated as the cause of the disease in human beings (Kasuke, 1984).

Tsetse-transmitted trypanosomiasis occurs, either at low endemic levels or as localised epidemics in separate foci scattered over the fly belt i.e., between latitudes $15^{o}N$ and $29^{o}S$, an area of Africa 30% larger than continental USA (Murray and Njogu, 1989). The most important tsetse-transmitted trypanosomes and their geographic distribution are shown in Table 1.1. In the case of Trypanosoma vivax, Tabanus species and other biting flies seem to be the primary mechanical vectors outside the tsetse areas, as in Central and South America (Wells et al., 1982).

Animal trypanosomiasis is the main obstacle to a

profitable exploitation of the rich potential that exists for livestock industry in Africa. Currently, the developing countries possess 67%, 52% and 94% of the world's cattle, sheep and goat populations but produce only 21% and 39% of the world's total meat and milk supplies (Murray, 1988). By 1963, it was estimated that the annual loss in meat production due to trypanosomiasis alone was at a value of US$50 billion (WHO/FAO/OIE, 1963); in addition, losses were sustained due to reduction in milk production and unavailability of animals for traction purposes.

With the rapidly growing population in the world, and improved standards of living in Africa, the need for increased animal protein production is growing rapidly. If the problem of trypanosomiasis could be solved, the average carrying capacity of cattle in 18 countries in West and Central Africa, which was 3.4 cows per km^2 in 1974 could theoretically increase to a potential of 20 per km^2, while the small ruminant population would also increase five fold (FAO, 1974).

Non tsetse-transmitted African trypanosomiasis caused by T.evansi is very important among Africa's 12 million camels, 86% of the world's total (Mahmoud and Gray, 1980; Mukasa-Mugerwa, 1981). The nomadic herdsmen of the semi-arid regions of Africa depend on camels for milk and transport.

In addition to the disease in animals, sleeping sickness, caused by T.rhodesiense in East and South Africa and T.gambiense in West and Central Africa, has been a major clinical problem among the human population. In the

period between 1896 to 1906, half a million people died of sleeping sickness in the Congo Basin. On the northern shores of lake Victoria, the disease appeared in Busoga in 1896, and by 1908, the population of this area, originally believed to be 300,000, had been reduced to one third. On the lake islands of Buvuma and Sesse, the population fell from 72,000 to 22,000, and the survivors were evacuated in 1909. In the 1940s, another disastrous epidemic occurred in the Northern shores of lake Victoria, resulting in wholesale evacuation of populations. The disease then spread to Kenya. To the east of the rift valley and the Zambesi Basin, sleeping sickness has been observed only within the last 60 years (Duggan, 1970). Currently, it is estimated that 50 million people in some 200 known foci within the tsetse inhabited areas in Africa are at risk of infection (UNDP/World Bank/WHO, 1989; Kuzoe, 1989), while the number of new cases of sleeping sickness that are recorded every year has increased from 9,000 in 1975 (De Raadt, 1975) to 25,000 (Kuzoe, 1989). Recent reports from Uganda, Sudan and southern Chad indicate that the prevalence of the disease is on the increase, and may be reaching epidemic proportions (Mbulamberi, 1987; UNDP/World Bank/WHO, 1989). For example in 1987, over 5,000 T.rhodesiense-infected patients were reported in a small region of southeastern Uganda, in comparison to only 52 in the same region in 1976 (Mbulamberi, 1989). Indeed, as is the case for many other tropical diseases, these figures are underestimates, as a result of inadequate medical surveillance, diagnostic failures and under-reporting

(Kuzoe, 1989).

Considerable efforts have been made in the last 50 years for the continuous suppression of human sleeping sickness. No vaccine is likely to be available in the foreseeable future, and the risk of epidemics to a large extent dictates the overall control strategy. The main control principles currently being applied include (i) continuous surveillance even when transmission seems under control (ii) systematic case detection and treatment, and where applicable (iii) control of the tsetse fly vector (De Raadt, 1984; UNDP/World Bank/WHO, 1989; Kuzoe, 1989).

In view of the potential danger of sleeping sickness outbreaks as has occurred in Uganda, it is necessary that research on better implementation of current methods of control be intensified.

1.2. THE DISEASE IN DOGS.

1.2.1. THE NATURAL DISEASE.

Naturally occurring tsetse-transmitted canine trypanosomiasis has been known since the turn of the century (Bevan, 1913; Bruce et al., 1913; Duke, 1916), and although its incidence is relatively low in most regions (Losos and Ikede, 1972; Sayer et al., 1979; Omamegbe et al., 1984), in some areas the prevalence of the disease can be very high (Mehlitz, 1985).

Dogs are susceptible to natural infection by various trypanosome species, including T.brucei, T.congolense, T.evansi, T.rhodesiense, T.gambiense (Sayer et al., 1979; Gibson and Wellde, 1985; Mehlitz, 1987) and possibly T.vivax (Ikede and Losos, 1972). Mixed infections involving

two species of trypanosomes have also been reported (Bevan, 1913; Oduye and Dipeolu, 1976; Mehlitz, 1979; Sayer et al., 1979).

In epidemiological surveys, trypanosomes have been demonstrated in the blood of apparently normal dogs (Denecke, 1941; Mwambu, 1979; Gibson and Gashumba, 1983; Mehlitz, 1985), indicating that dogs can act as reservoirs, especially of T.congolense, T.brucei and T.gambiense, and therefore a possible source of infection to man and other domestic animals. This gives the dog an important status in studies on the epidemiology of trypanosomiasis in domestic animals and man.

Natural infection is thought to be either through feeding on freshly killed infected carcasses (Moloo et al., 1973) or through the bites of infected tsetse (Horchner et al., 1985). Although the dog is not a preferred host by tsetse, the palpalis group of tsetse is known to feed on any hosts that enter their environments (Weitz, 1970).

The severity of trypanosomiasis in the dog largely depends on a number of factors, including the species of infecting trypanosome, the age and breed of dog, and possibly previous exposure to the parasite (Morrison et al., 1981a). With regard to the latter, dogs living in endemic regions are known to be more resistant to infection than newly introduced European ones, in which the disease is highly fatal (Mwambu, 1979; Mehlitz, 1985).

The canine disease syndromes caused by trypanosomes usually end fatally. T.brucei and T.rhodesiense cause an acute infection. T.congolense causes either an acute

haemorrhagic syndrome or a chronic progressively anaemic disease (Parkin, 1935; Sayer et al., 1979). On the other hand, T.gambiense has been reported to cause only a mild disease (Bevan, 1913; Mwambu, 1979).

Infections with T.brucei or T.congolense are the most commonly encountered forms of canine trypanosomiasis (Mehlitz, 1979). Dogs infected with T.brucei or T.congolense present signs which are dependent on the stage of the disease. Fever, anaemia, generalised subcutaneous oedema, lymph node enlargement and weight loss are present in most of the cases (Sayer et al., 1979; Omamegbe et al., 1984). A prominent clinical sign is involvement of the eyes. In addition to periorbital oedema, which is part of the generalised oedema, cloudiness, opacity and sometimes rupture of the cornea have been reported in both T.brucei and T.congolense infections (Yorke, 1911; Bevan, 1913; Duke, 1916). This has sometimes led to unilateral or bilateral blindness (Omamegbe et al., 1984).

T.brucei infected dogs that are terminally sick (Omamegbe et al., 1984), and those unsuccessfully treated with diminazene aceturate (Berenil^{R}, Hoechst, Germany) present with signs indicative of central nervous system involvement (Ikede and Losos, 1972a). The signs may include profuse salivation, chapping jaw movements, anorexia, dullness, incoordination, circling, muscular pains and abnormal behavioural patterns such as attempts to bite when approached. The dogs then become comatose and die within the next 24 hours (Ikede and Losos, 1972a; Omamegbe et al., 1984).

In dogs exhibiting the paracute form of T.congolense infection, haemorrhagic gastroenteritis, characterised by stools containing fresh blood, ulcerative and sometimes gangrenous stomatitis and pharyngitis, are often prominent features (Parkin, 1935; Sayer et al., 1979).

1.2.2. EXPERIMENTAL INFECTION.

Most of the information known regarding canine African trypanosomiasis has been derived from experimental infections with either T.brucei (Duke, 1916; Losos and Ikede, 1972; Morrison et al., 1981a,b) or T.congolense (Parkin, 1935; Horchner et al., 1985). Of these, T.brucei infections have been studied most extensively.

The syndrome in dogs due to T.brucei is very similar to the acute form of the disease caused by T.rhodesiense in man (Losos and Ikede, 1972), and because T.brucei is normally not infective to human beings, the dog makes a useful monogastric large animal model for studies on the pathogenesis and chemoprophylaxis of human African trypanosomiasis, while at the same time posing minimal risks of accidental infections to laboratory workers.

Dogs have been infected by intravenous (Losos and Ikede, 1972), subcutaneous (Rodet and Vallet, 1907) and intramuscular (Moloo et al., 1973; Mwambu, 1979) injection with suspensions containing the live parasite, by being fed on carcasses of freshly killed infected animals (Moloo et al., 1973), and by cyclical transmission through infected tsetse flies (Horchner et al., 1985). The prepatent period depends on the infecting dose of parasites and the mode of infection, being shortest when dogs are infected

intramuscularly (Moloo et al., 1973; Mwambu, 1979), and longest when they feed on infected carcasses (Moloo et al., 1973). An increase in the number of infecting parasites results in a shorter prepatent period. Following the appearance of clinical signs, the disease runs a similar course in dogs infected in the various ways, ending fatally after a period of three weeks from the onset of clinical signs.

1.2.2.1. *T.brucei* INFECTION IN DOGS.

Clinical signs.

These resemble those reported in spontaneously infected animals. The initial and prominent sign of the disease is a fever, coinciding with the first parasitaemia peak (Sayer et al., 1979). With progress, fever may occasionally subside, falling to subnormal levels in terminal cases (Sayer et al., 1979; Mwambu, 1979).

In dogs infected by intravenous inoculation, the first wave of parasitaemia occurs between days 5 and 6, reaching a peak on day 9. The number of trypanosomes in the circulation then drops to undetectable levels between days 10 and 12, after which high parasitaemia is re-established, persisting up to termination of the experiment in week 4, either through death of the dog (Mwambu, 1979; Kaggwa et al., 1984) or by humane euthanasia (Morrison et al., 1981a).

One of the hallmarks of canine trypanosomiasis caused by *T.brucei* is a severe, progressive, normocytic and normochromic anaemia that develops beginning the end of week 1. The anaemia is characterised by low packed red cell

volume (PCV), a decrease in total haemoglobin (Hb) and red blood cells (RBC). The mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) do not change appreciably, but a reticulocytosis may be observed at mid-infection (Kaggwa et al., 1984). Pallor of mucous membranes becomes apparent after week 2 of infection, by which time the PCV has dropped by 20 to 30% (Sayer et al., 1979).

The leucocytic responses to infection have been poorly described. Mwambu (1979) observed a general rise in the number of white cells with progress of the disease. Kaggwa et al. (1984), on the other hand, reported a leucocytosis only at mid-infection, and this later tended to subside. The transitory leucocytosis was due an increase in the numbers of circulating neutrophils and was not due to any lymphocytic response.

Signs of cardiac damage begin early in the disease. Initially there is an increase in pulse and heart rate, beginning at the end of week 1, and may go up to 160 beats per minute (BPM). This is followed by changes in pulse regularity and volume. Cardiac arrhythmias, occasional systolic murmurs and variations in the intensity of heart sounds have been reported (Sayer et al., 1979; Morrison et al., 1983).

Following the onset of clinical signs, severe generalised subcutaneous oedema that appears to shift from one region of the body to another is observed (Losos and Ikede, 1972; Sayer et al., 1979; Kaggwa et al., 1984). Most dogs maintain a good appetite (Bevan, 1913; Sayer et al.,

1979). Despite this, all dogs become severely emaciated and lose weight dramatically. In some cases, however, partial or complete anorexia has been reported (Mwambu, 1979).

Ocular changes appear at the end of week 1. Conjunctival oedema, mucopurulent lachrymal discharges, conjunctivitis, photophobia, interstitial keratitis, generalised corneal opacity, total blindness and glaucoma develop with time. Changes inside the eye seem to be localised in the anterior chamber, where hyphema and hypopyon have been observed (Mortelmans and Nectens, 1975; Sayer et al., 1979).

Splenic and lymph node enlargement begins at the end of week 1, is marked in week 2, and may regress slightly towards the terminal stages of the disease. Hepatomegaly may also be observed (Morrison et al., 1981b).

Some dogs show signs of transitory muscle pain, exhibited as lameness on one limb, unwillingness to move, and an inclination to bite when the affected limb is palpated. At the same time, blood-tinged diarrhoea has been observed (Morrison et al., 1981a). Some of the dogs also become dyspnoeic (Rodet and Vallet, 1907; Mwambu, 1979).

Involvement of the central nervous system is clinically apparent in the terminal stages of the disease. Neurological disturbances present as unexplained barking, aggression, ataxia, trembling and convulsions (Morrison et al., 1983). Most of the dogs do not reach this stage and die of heart failure before the end of week 4. The dogs are usually recumbent 1 to 3 days prior to death (Bevan, 1913; Kaggwa et al., 1983).

Little is known about the overall biochemical patterns in dogs infected with T.brucei. In the work reported by Kaggwa et al. (1984), total plasma proteins and albumin decreased without observable changes in total globulins. Interestingly, the plasma levels of the tissue enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were found to increase between days 4 and 12 of infection, but to decrease in the critical stages of the disease (Kaggwa et al., 1984).

In other studies, serum IgM and IgG levels increased in the first three weeks of infection, while IgA declined temporarily (Meirvenne et al., 1972).

Macroscopic pathology.

Studies on the detailed pathology of experimental T.brucei infection in dogs indicate that it resembles that in naturally infected dogs (Sayer et al., 1979; Morrison et al., 1981a,b; Kaggwa et al., 1983). Generally, the disease is associated with severe tissue damage resulting from the invasive nature of the trypanosome. The heart, the brain and the lymphoid organs are the areas most severely affected.

At postmortem examination, the carcass is oedematous, emaciated, and there is loss of muscle mass. Hydrothorax and straw coloured ascitic fluid may be encountered (Morrison et al., 1981a).

The pericardial sac is usually distended with straw coloured fluid containing strands of fibrin. There is oedema and gelatinisation of pericardial fat. The heart is globular, pale and mottled. The mottling is due to the

presence of extensive petechial and ecchymotic haemorrhages in the myocardium of both the ventricles and the atria. The epicardium sometimes has a granular appearance with adhering tags of fibrin (Morrison et al., 1981a).

In the brain, scattered haemorrhages are seen in the meninges (Kaggwa et al., 1983). There is generalised lymph node and splenic enlargement. The liver is enlarged and congested. Oedema and petechial haemorrhages have occasionally been observed in other regions such as the diaphragm and pampiniform plexus of the testes. In the kidneys, generalised oedema, petechial haemorrhages and irregular pale areas may be present in the cortex. Morrison et al. (1981a) found small amounts of dark discoloured blood in the stomach, small intestines and anterior colon of terminal cases but could not locate any discrete bleeding areas.

Microscopic pathology.

Histological examination of tissues from both experimentally and spontaneously T.brucei infected dogs reveals extensive and progressive tissue damage in virtually all organ systems. Of these, the heart is most severely involved. The lesion is a pancarditis that affects the myocardium of all the four heart chambers, the valves and the vasculature (Morrison et al., 1981a; Kaggwa et al., 1983). The atria are affected to a greater extent than the ventricles.

In the myocardium, the extent of tissue damage, trypanosome and cellular infiltration varies with the region and the stage of the infection. Cellular and

trypanosome infiltration is most marked in the subendocardial and subepicardial regions, and in perivascular areas. In the early stages of the disease, infiltration with plasma cells, lymphocytes and a few macrophages is common. Terminally, there is a predominance of foamy macrophages and neutrophils, most often associated with severe necrotising lesions. The distribution of cells varies from area to area and is most marked in the atria.

Terminally, diffuse cardiac damage involving the entire myocardium and the valves, with extensive myocyte degeneration and necrosis, is observed. Focal haemorrhages, oedema and separation of myofibrils is consistently seen.

Involvement of the vascular system presents as swelling of walls of blood vessels and expansion of perivascular spaces. Morrison et al. (1981a) found that necrosis of arterial walls was confined to the heart.

In both the subepicardium and myocardium, lymphatic distention is quite common. The lymphatic vessels are distended with fluid or a mixture of fibrin, trypanosomes and cells, forming plugs. The composition of cells in the lymphatic vessels resembles that in the myocardium.

Immunofluorescence staining of sections of the myocardium demonstrates the presence of trypanosome antigens and immunoglobulins interspersed between muscle fibres and in perivascular areas. The concentration of trypanosome antigen is proportional to IgG and seems to be correlated with the severity of tissue damage in this organ (Kaggwa et al., 1983).

The lesions observed in other organs reflect the

multisystemic nature of the disease, and are most extensive in the kidneys, spleen, lymph nodes, liver, brain, eyes and the testicles (Sayer et al., 1979; Morrison et al., 1981a,b; Kaggwa et al., 1983). Vascular thrombosis has been observed in the spleen, the pampiniform plexus of the testes, the venous plexus of the ovary, and in branches of the renal vein (Morrison et al., 1981a).

Lesions in the brain present as a meningitis with marked infiltration of the leptomeninges with macrophages, neutrophils, plasma cells and a few lymphocytes. Trypanosomes are found in large numbers in the choroid plexus as well as in the meninges. Involvement of the neuropil has not been observed in untreated dogs (Morrison et al., 1981a).

In the kidneys, focal tubular necrosis occurs in the absence of glomerular changes (Kaggwa et al., 1983). Direct fluorescent antibody staining of kidney samples demonstrates the presence of IgG, IgM and trypanosome antigens (Kaggwa et al., 1983). There is marked increase in the number of Kupffer cells in the liver and pronounced venous congestion in terminal stages. In some dogs, foci of fatty degeneration close to the hepatic veins and hepatocyte disorganisation with occasional cell death and mitoses are encountered (Rodet and Vallet, 1907; Morrison et al., 1981a).

Morrison et al. (1981a) found extensive involvement of the gastrointestinal tract, from the stomach to the rectum, in some cases. In such dogs there was marked trypanosome and cellular infiltration involving the submucosa and the

muscular layers, with occasional necrosis of villi.

1.2.2.2. *T.congolense* INFECTION IN DOGS.

Dogs infected with *T.congolense* develop a syndrome more severe than in other animals (Parkin, 1935; Losos and Ikede, 1972; Sayer *et al.*, 1979; Horchner *et al.*, 1985). However, some dogs show a certain degree of tolerance, with only short parasitaemia periods (Horchner *et al.*, 1985). Trypanosomes appear in the blood by day 7 after intravenous inoculation, but unlike in *T.brucei* infections, clinical signs are not seen until days 10 to 12. The dogs develop a fever, splenic and lymph node enlargement (Sayer *et al.*, 1979). Depression sets in rapidly, with inappetence, salivation and mild subcutaneous oedema. Haemorrhagic gastroenteritis, ulcerative and sometimes gangrenous stomatitis and pharyngitis, and ocular lesions are most conspicuous (Parkin, 1935; Sayer *et al.*, 1979). The dogs die within 16 days after infection, with convulsions. In chronic cases lasting up to 6 months, the main signs include emaciation, anaemia and subcutaneous oedema (Losos and Ikede, 1972).

At post mortem, varying degrees of splenomegaly, pulmonary oedema and diffuse haemorrhages in the stomach and intestines have been observed (Sayer *et al.*, 1979). There is a slight excess of fluid in body cavities, and most tissues appear moist on cut section.

Unlike in dogs infected with *T.brucei*, tissue necrosis and cellular infiltration are rarely found at histology. Trypanosomes are only found in blood vessels. In most organs, there is severe oedema particularly around blood

vessels, and mild degenerative changes (Sayer et al., 1979). Gastrointestinal haemorrhage is associated with multiple foci of necrosis on the mucosal surface. In the spleen, focal necrosis has been observed in the periarteriolar lymphoid follicles (Sayer et al., 1979).

1.3. THE DISEASE IN MAN.

1.3.1. CLINICAL FEATURES.

The clinical features of human African trypanosomiasis (HAT), like in dogs, also largely depend on the species of infecting trypanosome. T.rhodesiense causes an acute to subacute illness, with signs of cardiac involvement being most prominent (Manson-Bahr and Charters, 1963; Manuelidis et al., 1965; Francis, 1972; Jones et al., 1975; Harries and Wirima, 1988). The prepatent period before the appearance of clinical signs may be as short as two weeks (Harries and Wirima, 1988) and the duration of the disease before death only 6 weeks (Manuelidis et al., 1965). T.gambiense causes a subacute to chronic infection, and although signs of heart damage are encountered (Bertrand et al., 1971; Adams et al., 1986), the disease is mainly associated with involvement of the central nervous system (Molyneux et al., 1984; Boa et al., 1988).

Following infection from the bite of a tsetse fly, the first sign of disease is the development a chancre at the site of bite. The chancre appears as a small inflamed focus, 5 to 10mm in diameter. It is commonly seen in T.rhodesiense and less so in T.gambiense infections (Molyneux et al., 1984).

In the early stages of the disease, the periodicity of

symptoms is parallel to the presence of trypanosomes in the blood (Onyango et al., 1966), and becomes less with chronicity. Among the initial clinical signs are intermittent fever and tachycardia, progressive headache, malaise, a morbilliform rash, weakness, somnolence (hence the use of the term 'sleeping sickness'), chest pains and body wasting .

T.rhodesiense infection results in generalised oedema with subcutaneous tipping of legs and back. Oedema of the face gives the patient a puffy moonface appearance (Manson-Bahr and Charters, 1963). Cases of severe diarrhoea have also been reported (Basson et al., 1977).

Other clinical signs associated with HAT include incontinence, athralgia and stridor. Signs of endocrine disturbances have also been detected. Pregnant women may have premature births, stillbirths or perinatal deaths. Others become sterile or have arrested menstruation. Men become impotent and may develop gynaecomastis, with a feminine-like distribution of fat (Molyneux et al., 1984; Boersma et al., 1989; Edeghere et al., 1989).

Signs indicative of cardiac damage include intermittent tachycardia (Manuelidis et al., 1965), dyspnoea even at rest, coughing and occasional haemoptysis (Manson-Bahr and Charters, 1963; Koten and De Raadt, 1969; Francis, 1972), marked venous congestion of cervical veins and hepatosplenomegaly (Manson-Bahr and Charters, 1963). Radiography demonstrates cardiomegaly, widening of the superior vena cava and enlargement of hilar vessels. There is hydrothorax and ascites (Manson-Bahr and Charters, 1963;

Francis, 1972; Mbala et al., 1988).

Electrocardiographic (ECG) abnormalities have been demonstrated in patients infected with T.rhodesiense (Manson-Bahr and Charters, 1963; Manuelidis et al., 1965; Jones et al., 1975) and T.gambiense (Bertrand et al., 1971; Francis, 1972; Mbala et al., 1988). The abnormalities described include disorders of ventricular repolarisation, intraventricular conduction defects, heart blocks (HB) and ventricular premature beats (VPB). The presence of pericardial effusion (PE) is demonstrated by decreased voltages of R waves on ECG, and by echocardiography (Mbala et al., 1988). These findings indicate that patients suffering from sleeping sickness develop potentially fatal congestive heart failure. The finding of previous exposure to trypanosomiasis in patients with congestive cardiomyopathy of unknown aetiology have led to the suggestion that it could have been the result of subclinical attacks (Blackett and Ngu, 1976).

Patients develop severe anaemia and lose weight rapidly (Koten and De Raadt, 1969). The anaemia is mostly normocytic and normochromic, sometimes associated with red cell abnormalities, including anisocytosis, polychromacia and hypochromia (Manson-Bahr and Charters, 1963). The anaemia also involves an increase in the erythrocyte sedimentation rate, consistent with coating of RBC with antibodies or immune complexes (Molyneux et al., 1984). Severe hypoproteinaemia and hypoalbuminaemia have also been described (Jenkins and Robertson, 1959; Robertson and Jenkins, 1959; Koten and De Raadt, 1969).

Thrombocytopaenia and decreased serum fibrinogen levels are observed in both T.rhodesiense and T.gambiense disease (Barrett-Connor et al., 1973; Davis et al., 1974; Robins-Browne et al., 1975; Basson et al., 1977; Molyneux et al., 1984). In addition there is increased fibrinogen degradation products in serum and urine, and decreased prothrombin activity in the blood (Basson et al., 1977).

Leucocytes in the blood seem to follow a cyclical pattern, reaching a maximum a few days after peak parasitaemia. The rise is mainly associated with an increase in the number of monocytes (Ross and Thomson, 1911). In other instances, leucocytopaenia with relative lymphocytosis has been observed in T.gambiense patients (Basson et al., 1977).

Among the major features of HAT is an increase in the serum levels of immunoconglutinins (Blackett and Ngu, 1976; Basson et al., 1977; Lambert et al., 1981; Mbala et al., 1988) and immunoglobulins, mainly IgM, and to a lesser extent IgG (Greenwood and Whittle, 1980). This is accompanied a decrease in total haemolytic complement, serum C3 and C4, with indications of high C3 catabolism. Complement-fixing anti-heart antibodies of the IgM and IgG classes have also been demonstrated (Blackett and Ngu, 1976; Mbala et al., 1988), suggesting that the cardiac impairment observed in HAT could be immune mediated.

Trypanosomal infections in man also appear to affect hepatic and renal function to a certain degree. In patients suffering from T.gambiense sleeping sickness, a slight to severe jaudice, bilirubinaemia, increased serum alkaline

phosphatase (AP) and ALT haave been detected (Robertson and Jenkins, 1959; Basson et al., 1977). Clinical features indicating altered renal function include low serum sodium and potassium, low urine volume, high urinary sodium content, mild proteinuria, haematuria and α-ketoaciduria (Basson et al., 1977).

1.3.2. MACROSCOPIC PATHOLOGY.

The major gross changes in HAT caused by T.rhodesiense or T.gambiense are observed in the heart, brain, spleen and liver. Patients dying of acute T.rhodesiense infection portray a picture of congestive heart failure (Hawking and Greenfield, 1941; Manuelidis et al., 1965; Koten and De Raadt, 1969). There is increased fluid in all serous cavities. The heart is soft and flabby. On sectioning, patchy yellowish discoloration of the myocardium may be encountered. Lung congestion and oedema, hepatic and splenic congestion are associated with this form of the disease. A fatty liver has also been reported (Jenkins and Robertson, 1959; Apted, 1970).

In patients dying of T.gambiense infection, the heart may be enlarged, normal or small. In cases with small hearts, there is intense concentric muscular hypertrophy with a reduced ventricular cavity (Bertrand et al., 1971). In addition, the brain appears oedematous.

1.3.3. MICROSCOPIC PATHOLOGY.

Virtually all tissues in the body are found to be involved. In patients dying of acute T.rhodesiense infection, lesions are however found mainly in the heart

(Lavier and Leroux, 1939; Hawking and Greenfield, 1941; Manuelidis et al., 1965; Koten and De Raadt, 1969; Poltera, et al., 1975; Poltera and Cox, 1977). In such patients, the cardiac lesions are similar to those observed in dogs infected with T.brucei, except that in the latter, they tend to be more severe. The lesion is a pancarditis, involving the myocardium, conducting tissue, valves and the vasculature.

The epicardium may be thickened and may show patchy cellular infiltration. This is accompanied by perineuritis and degeneration of parasympathetic ganglion cells.

In the myocardium, myocytes are found at various stages of degeneration. Myocytolysis, contraction band necrosis and granulomata are common findings (Poltera et al., 1976). Sometimes myocytolysis occurs in the absence of inflammatory cells (Poltera and Cox, 1977). Granuloma formation is often found surrounding degenerate and fragmented muscle fibres (Poltera, et al., 1975). In addition, some of the myocytes are hypertrophied. Oedema and deposition of a brown pigment within the myocardium has also been reported (Hawking and Greenfield, 1941).

The endocardium in all four heart chambers may show patchy thickening with or without subendocardial fibrosis. Endocardial microthrombus formation has been observed in the subvalvular, retropapillary and intratrabecular regions (Poltera et al., 1975).

Vascular damage occurs as a periadventitial fibrosis and sometimes congestion, especially of the capillaries. The vessels involved are mainly branches of the coronary

arteries (Lavier and Leroux, 1939; Manuelidis et al., 1965). Vascular damage is accompanied by marked distention of subepicardial and myocardial lymphatic vessels with fluid and cells of a composition similar to that found in the regions which the vessels drain (Hawking and Greenfield, 1941).

Lesions in the heart are associated with marked cellular infiltration that varies from one region to another. Mononuclear cells are predominant, including macrophages, lymphocytes and plasma cells. Occasional morula (Mott) cells are also encountered in some cases. In patients dying of acute T.rhodesiense infection, neutrophils are also found, the number of which may be considerable in some foci (Manuelidis et al., 1965; Koten and De Raadt,1969).

The degree of tissue damage is highest in the subendocardial and subepicardial myocardium, and in perivascular areas where infiltrating cells occur in large numbers. Chronic cellular infiltration also occurs in the subepicardial adipose tissue. In the myocardium itself, focal lipomatosis is observed in areas of chronic cellular infiltration (Poltera and Cox, 1977).

In the conducting system, chronic cellular infiltration, degenerate Purkinje fibres and zones of scarring have been found in the atrioventricular node (AVN), the bundle of His and its branches, raising the question of a possible link between trypanosomiasis and African cardiomyopathies of unknown origin (Amengaud and Diop, 1960).

Cardiac damage is accompanied by a valvulitis, involving all the heart valves. In the valves, cellular infiltration occurs mainly on the flow side (Poltera et al., 1975).

In the brain, meningitis, perivascular mononuclear cellular infiltration, gliosis and focal demyelination are associated with the chronic form of the disease, especially in patients infected with T.gambiense (Haller et al., 1986; Pentreath, 1989). Manuelidis et al. (1965) observed splenic infarcts and glomerular hyalinisation. In the bone marrow, Manson-Bahr and Charters (1963) noted marked normoblastic erythroid hyperplasia with an increase in monocytes and plasma cells. In other organs, focal hepatitis (Francis, 1972), generalised venous congestion and interstitial haemorrhage (Jones et al., 1975) may be encountered. Koten and De Raadt (1969) report two cases in which enteritis was seen.

1.4. TREATMENT.

1.4.1. CANINE TRYPANOSOMIASIS.

The drug most commonly used for treating dogs infected with T.brucei, T.congolense and T.gambiense is diminazene aceturate (Ikede and Losos, 1972a; Okolo, 1986; Kaggwa et al., 1988; Onyeyili and Anika, 1989). Quinapyramine sulphate (antrycide sulphate -ICI) and isometamidium chloride (Samorin^{R} - May and Baker) have also been used where parasites were found to be sensitive (Gitatha and Ogada, 1969; Toure, 1970; Williamson, 1970). More recently, a new drug, DL-α-difluoromethylornithine (DFMO) (Ornidyl^{R} - Merrell Dow pharmaceuticals Inc., Ohio, USA) has been

used in experimental studies on T.brucei-infected dogs with promising results (Onyeyili and Anika, 1989). Occasionally suramin (Moranyl^{R} - Specia) has also been used to treat dogs infected with T.brucei (Sayer, personal communication).

1.4.2. SLEEPING SICKNESS.

Currently, the drugs most extensively used for the treatment of the early stages of HAT when trypanosomes have not penetrated into the central nervous system include suramin for T.rhodesiense, and pentamidine (Lomidine - Specia) for T.gambiense infections. Melarsoprol (MelB, Arsobal - Specia), a highly toxic arsenic compound, is used for treatment of late stage T.rhodesiense and T.gambiense infections when the central nervous system is involved (Freidheim, 1949; Apted, 1970; Adams et al., 1986; Haller et al., 1986; Schechter and Sjoersdsma, 1987; Pepin et al., 1989). DFMO is now established as an effective therapy for T.gambiense sleeping sickness, even with infections that are refractory to MelB (Schechter and Sjoersdsma, 1987; Doua et al., 1987; Kazyumba et al., 1988; UNDP/World Bank/WHO, 1989).

In both dogs and humans, treatment is not always successful, resulting in relapse infections and, sometimes, drug resistance. At the same time, treatment can result in adverse clinical signs, exhibited as either cardiac (Manuelidis et al., 1965; Bertrand et al., 1971; Jones et al., 1975; Junyent et al., 1988; Harries and Wirima, 1988) or neurological (Haller et al., 1986; Arroz, 1987; Doua et al., 1987) abnormalities in 7 to 13% of treated patients,

and deaths in 2 to 6% in all cases treated with various schedules of MelB (Arroz, 1987). Corticosteroids have occasionally been used before, during and after trypanocidal treatment, in an attempt to control the severity of inflammatory reactions (Bertrand et al., 1971; Haller et al., 1986; Harries and Wirima, 1988; Pepin et al., 1989). The beneficial effects of corticosteroid therapy have, however, not been experimentally determined.

1.5. OBJECTIVES.

It appears that cardiac damage and death in African trypanosomiasis is mediated through biologically active substances which are released by the host in immunologically mediated hypersensitivity reactions or generated by dying trypanosomes. The severe pathology induced by trypanosomes appears to be related to their invasive capacity, and to their ability to generate biologically-active mediators (Tizard et al., 1978; Molyneux et al., 1984; Boreham, 1985), or to immunologically mediated reactions stimulated by the persistence of this highly antigenic parasite undergoing antigenic variation (Murray, 1974; Galvao-Castro et al., 1978; Greenwood and Whittle, 1980; Lambert et al., 1981).

It is possible that parasite-derived substances capable of causing cell death are released following drug-related or immunologically induced death of the trypanosomes in the tissues. There appears to be merit in attempting to reduce or prevent hypersensitivity reactions by the strategic use of immunosuppressive agents, including corticosteroids, or blocking inflammatory responses by the use of nonsteroidal

anti-inflammatory drugs (NSAIDs). It is also likely that the basic mechanisms involved in cardiac damage are similar to those responsible for damage to the central nervous system and other tissues and organs; it is possible that any intervention that reduces cardiac damage will have a generalised beneficial effect on the host as a whole.

From the foregoing, it would appear that the generalised disease, and heart damage in particular, in dogs infected with T.brucei closely resembles acute T.rhodesiense infection in man. It is possible that if the disease in dogs is prolonged, for example by subcurative treatment with trypanocidal drugs, the resulting syndrome would be similar to that observed in subacute T.rhodesiense and T.gambiense sleeping sickness.

The objectives of this work were to:

(i) investigate the clinical, physiological, biochemical and immunopathological basis of T.brucei-induced pancarditis in the dog.

(ii) identify parameters that would allow objective assessment of clinical progress and prognosis.

(iii) evaluate the response to trypanocidal drug treatment in the early and late stages of the disease.

(iv) investigate the use of anti-inflammatory drugs and angiotensin-converting enzyme inhibitors (ACEI) in reducing the severity of myocardial damage.

The overall aim of these studies was to improve the clinical management of HAT by:

(i) identification of techniques and assays that would permit the objective evaluation of treatment and subsequent

prognosis.

(ii) reduction of cardiac damage in particular and tissue damage in general by the use of antagonist drugs.

The dog could serve as a good, large monogastric animal model of trypanosomal pancarditis for a number of practical reasons:

(i) Its large size allows for intensive clinical and physiological investigations to be done with ease.

(ii) The dog is easily adapted to a laboratory environment and handling by man.

(iii) As trypanosomiasis does not occur naturally in the U.K., there is no need for intensive quarantine measures before the dog is used for experimental purposes.

(iv) The dog has been used extensively by the pharmaceutical industry for drug screening, with the result that the pharmacokinetics and toxicity of a wide range of anti-inflammatory drugs are well established in this species.

1.6. EXPERIMENTAL DESIGN.

1.6.1. PATHOGENESIS STUDIES.

In order to study, in detail, the pathogenesis of cardiac damage induced in dogs by T.brucei, sequential kill experiments were carried out. A group of clinically normal dogs were intravenously infected with $5x10^3$ T.brucei GVR35/c.1. Before and during the period of infection, clinical, parasitological, biochemical and endocrinological studies were carried out. The number of dogs used varied with the particular study.

Two dogs per time point were euthanised at

mid-infection on days 10 and 15, while another 6 were euthanised on days 21, 22 and 26. After euthanasia, tissue samples were taken from the heart for histopathological, histochemical, immunofluorescence and ultrastructural studies. Tissue samples were taken from all other body systems as well. T.brucei caused tissue damage in virtually all organs, but only those changes directly related to the current work are described. The kidney was of particular importance due to its ability to concentrate immune complexes, and was used as a marker for the presence of circulating immune complexes in infected dogs.

1.6.2. TRYPANOCIDAL TREATMENT.

In an attempt to establish the therapeutic potential of suramin in T.brucei infected dogs, and to induce chronic cardiac damage, 4 dogs were treated with various dosage regimes when terminally ill, and their clinical progress followed up. A dog was euthanised whenever there was no clinical improvement.

1.6.3. IMMUNOSUPPRESSIVE TREATMENT.

A group of 12 dogs were infected with T.brucei. At various stages of the disease, they were treated in pairs with either the steroidal compound prednisolone, the NSAIDs cyclosporin A and azathioprine, or a combination of azathioprine and prednisolone. All the dogs were euthanised on day 15 and tissue samples taken from the heart for histopathological, histochemical, immunofluorescence and TEM studies. Samples were taken from other organs for general histology.

TABLE 1.1.

THE MOST IMPORTANT TSETSE-TRANSMITTED TRYPANOSOMES.

TRYPANOSOME	MAIN HOSTS	MAJOR GEOGRAPHIC DISTRIBUTION
Trypanosoma rhodesiense	Man	Tsetse region of Africa (Kuzoe, 1989)
T.gambiense	Man	Tsetse region of Africa (Kuzoe, 1989)
T.congolense[*]	Cattle, sheep goats, pigs, dogs, camels, horses, most wild animals	Tsetse region of Africa
T.vivax[*]	Cattle, sheep, goats, camels, horses, various wild animals	Africa, Central and South America, West India, Mauritius NB: In non-tsetse areas, transmission is by biting flies (Wells *et al.*, 1982)
T.brucei[*]	All domestic and various wild animals	Tsetse region of Africa
T.simiae[*]	Domestic and wild pigs	Tsetse region of Africa

* - Adapted from: The African Trypanosomiases (1970). H.W. Mullighan (Ed.). Allen and Unwin, London. p751-773.

CHAPTER 2.

GENERAL MATERIALS AND METHODS.

2.1. ANIMALS.

All the dogs used in this work were female beagles, aged 7 months. The dogs were purchased from an approved breeder in groups of 4. This was the largest number on which exhaustive investigations could be carried out at the same time with ease. On arrival, the dogs were weighed and complete clinical examinations, including electrocardiography (ECG) and echocardiography, were performed. Jugular venous blood was collected and haematological, biochemical and endocrinological studies carried out to ensure that the dogs were free of intercurrent infection.

The dogs were dewormed with nitroscanate (Lopatol^{R}, CIBA GEIGY, Switzerland). During the day they were put together in a run and housed in pairs at night. Feeding was done in the afternoons with a standard commercial ration. Water was made available at all times.

2.2. TRYPANOSOMES.

The stabilate of T.brucei used in this work was GVR35/c.1. The stabilate is a derivative of LUMP 1001, isolated from a wildebeest in the Serengeti in 1966 (primary isolate S10) and described by Jennings et al. (1982). The stabilate has been used in the mouse model of T.brucei for screening trypanocidal drugs (Jennings and Gray, 1983). T.brucei GVR35/c.1 was selected from a group of 5 stabilates, including GVR96, GVR23, GVR35 and GVR35/c.2, following pilot studies which showed that it was highly infective in dogs. Before being used for infecting the dogs, the trypanosomes were passaged once in mice. The

dogs were infected by intravenous injection with a 1ml suspension containing approximately 5 x 10^3 trypanosomes.

2.3. GENERAL CLINICAL STUDIES.

The preinfection observation period was two weeks. This allowed the dogs to become accustomed to handling and to establish normal clinical parameters. A detailed clinical examination was done on the dogs every day before and following infection. The appetite was determined from the amount of food consumed after the dogs were fed and the frequency of water intake. The dogs always passed stools within 30 minutes of being released into the run, giving a good opportunity for determination of the amount and character of faeces from each dog. They were weighed at least 3 times every week.

2.4. BIOCHEMICAL STUDIES.

During the course of study, 4ml of jugular venous blood was collected in tubes containing heparin as anticoagulant. From plasma samples, various parameters were assayed as part of routine biochemistry. These included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), blood urea nitrogen (BUN) and creatinine. All the estimations were done using an automated MIRA analyser (Roche). The more specific biochemical techniques have been described in the relevant chapters.

2.5. CARDIOLOGICAL STUDIES.

A detailed examination of the thoracic cavity was done

at regular intervals during the period of study. The methods employed included auscultation, palpation, ECG (described in detail in Chapter 4), and echocardiography.

2.5.1. ECHOCARDIOGRAPHY.

Two-dimensional (2D), M-mode and Doppler echocardiographic studies were carried out on the dogs at regular intervals. A real-time duplex imaging system (Interspec-XL, Pennsylvania), with both pulsed-wave (PW) and continuous-wave (CW) Doppler capabilities was used. All the findings were recorded on VHS video tapes for detailed analysis. The echocardiographic studies were carried out in a dimly-lit room, and to avoid any excitement that could affect the heart rate, the dogs were always handled by people to whom they were accustomed.

2.5.1.1. 2D AND M-MODE ECHOCARDIOGRAPHY.

This was performed using the methods described by Thomas (1984) and O'Grady et al., (1986). The dog was laid in right lateral recumbency on a table in which a hole had been made; the hole coinciding with the lateral thoracic wall (right parasternal) (Fig. 2.1). ECG electrodes were applied on the legs to record the heart rate and rhythm. A 5.0MHz mechanical transducer was passed from under the table and through the hole, to lie on the lateral thoracic wall between the third and fifth intercostal spaces, close to the costochondral junction; an area from which hair had been clipped (Fig. 2.1). Aquasonic gel was applied on the skin to improve contact with the transducer. Assessment of the general appearance and motion of all four heart

chambers, including the cardiac valves, was done from the 2D echocardiograms obtained. The presence or absence of pericardial effusion (PE) was also determined from the 2D and M-mode echocardiograms.

It has previously been demonstrated that linear measurement of cardiac structures in dogs are directly related to body size (Boon et al., 1983; Lombard, 1984; O'Grady et al., 1986). Dogs with a narrow weight margin (9.60 ± 1.67kg: mean ± 1SD) were therefore used for this work. To establish normal values, an M-mode study was carried out on 19 uninfected dogs. Measurements of left ventricular dimensions were obtained at the level of the chordae tendineae, as suggested for adult human echocardiograms (Sahn et al., 1978; Roelandt, 1983) (Fig. 2.2). The dimensions were measured at least from 8 representative cardiac cycles. This was repeated on a second day and the average taken. The left ventricular chamber dimensions at end-diastole (LVEDD) and end-systole (LVESD) were determined as shown on Figure 2.3. The fractional shortening (FS) was calculated using the formula:

$$FS = \frac{LVEDD - LVESD}{LVEDD} \times 100.$$

The mean values obtained are shown in Table 2.1. The FS has previously been used as an index of left ventricular function (LVF) in dogs (Boon et al., 1983; Lombard and Spencer, 1985; Calvert and Brown, 1986; O'Grady et al., 1986) and in humans (Sahn et al., 1978; Roelandt, 1983).

M-mode echocardiograms through the mitral valve leaflets were similar to those obtained in other dogs

(Lombard, 1984; Calvert and Brown, 1986) and in humans (Roelandt, 1983), consisting of an early (D-E), and late (atrial systole) (F-A) filling phases in diastole, as shown in Figure 2.4. Assessment of changes in the E-F slope was used, alongside other parameters, for determination of diastolic function of the left ventricle (Dennis et al., 1978; Roelandt, 1983; Boon et al., 1983; Lombard and Spencer, 1985).

2.5.1.2. DOPPLER ECHOCARDIOGRAPHY.

2.5.1.2.1. The right parasternal window.

To determine the presence or absence of valvular incompetence, the same transducer position as employed for 2D and M-mode studies was used. Investigations were carried out by PW Doppler as described for man by Quinones et al. (1980) and Mark et al. (1986). For the mitral and tricuspid valves, the sample volume was placed on the atrial side of the valve being investigated (Figs. 2.5 and 2.6), and on the left ventricular side of the aortic valve to determine the presence of aortic incompetence (AI). The severity of incompetence was estimated by determining the distance, away from the valve, that spectrals of incompetence could be picked up, and correspondingly graded as either mild, moderate or severe (Adams et al., 1986).

2.5.1.2.2. The subcostal window.

To determine the character and velocity of mitral and aortic blood flow, both PW and CW Doppler techniques were employed. With the dog in a semi-dorsal recumbency position, a 3.5MHz transducer was placed on the left side

of the xiphoid cartilage and directed cranio-medially (Fig. 2.7). A 2D echocardiogram, analogous to the apical 4-chamber view in humans, was obtained (Fig. 2.8). This view was preferred because blood flowing through the aortic and mitral valves would be in line with ultrasound signals emitted by the transducer; hence maximal Doppler shift would occur when the signals are reflected off moving blood particles (Magnin et al., 1981; Lewis et al., 1984).

Mitral blood flow.

Mitral blood flow was investigated by PW Doppler with the sample volume placed approximately 1cm from the valve orifice, inside the left ventricle (Fig. 2.8). This point has previously been demonstrated as optimal for determination of peak mitral blood flow velocity (Delemarre et al., 1988; Fast et al., 1988; Spirito and Maron, 1988). The duplex recording obtained consisted of the early (E) and the late (atrial systole) (A) diastolic filling phases of mitral blood flow (Fig. 2.9). The E and A peaks were measured for at least 8 cardiac cycles, on two days, and the average taken. At the same time, the heart rate was determined. From the results, the A:E ratios were calculated. The values obtained from 16 normal dogs are shown in Table 2.2. A 2.0MHz split-crystal ('stand-alone') transducer was similarly used for CW Doppler of mitral blood flow recordings from the subcostal window (Fig. 2.10). The E, A and A:E values obtained are shown in Table 2.3. In addition, the pressure half-times were determined by both PW and CW Doppler and the values obtained by the two techniques compared (Table 2.4).

The subcostal window, in addition to the parasternal window, was further used to determine the presence or absence of mitral incompetence (MI), by placing the sample volume on the left atrial side of the valve (Figs. 2.11 and 2.12).

Aortic blood flow.

To record aortic blood flow by PW Doppler from the subcostal window, the sample volume was placed in the left ventricular outflow tract, then advanced slowly through the aortic valve into the ascending aorta, to a point at which maximal blood flow velocities were obtained (Figs. 2.13 and 2.14). A similar study by CW Doppler was done with the 2.0MHz transducer. From the subcostal position, aortic blood flows away from the transducer in systole, and hence a negative Doppler shift occurred (Fig. 2.15). The heart rate was recorded along with the blood flow velocity.

The subcostal window was further used to confirm the presence or absence of AI. As for the parasternal window, the sample volume was placed in the left ventricular outflow tract.

The suprasternal window.

Aortic blood flow was further studied from the suprasternal (thoracic inlet) window, as done in humans by Huntsman et al. (1983). The dog was placed in a sitting position and the CW Doppler transducer put on the right side of the manubrium of the sternum, pointing caudally (Fig. 2.16). Since blood flowing in the ascending aorta in systole is towards the transducer, positive Doppler shifts

were recorded (Fig. 2.17). The aortic blood flow velocities obtained from the subcostal and suprasternal windows were compared (Table 2.5).

The thoracic inlet was found to be too narrow for any PW Doppler studies to be performed.

2.5.1.3. PULMONARY BLOOD FLOW.

Blood flow across the pulmonary valve was investigated by PW Doppler from the right parasternal window. A 2D short axis view of the heart was obtained, showing the left ventricular outflow tract, the pulmonary valve, the pulmonary artery and its branches (Fig. 2.18). The sample volume was placed on the arterial side of the valve (Fig. 2.19). The blood flow velocities obtained from a group of 5 dogs are indicated in Table 2.6.

2.5.2. CONCLUSIONS ON ECHOCARDIOGRAPHIC STUDIES.

2.5.2.1. 2D and M-mode studies.

The right parasternal window offered a good 2D view of all the heart chambers and was therefore adopted for the current work. In addition, M-mode echocardiography through the left ventricle and mitral valve was easy to perform, and consistent, repeatable measurements of left ventricular dimensions and FS obtained. The FS was used, in most cases, as an index of LVF.

2.5.2.2. Doppler studies.

Detection of valvular incompetence:

Determination of incompetence of the mitral and aortic valves was to be done by PW Doppler from the parasternal and subcostal windows, while that of the tricuspid and

pulmonary valves would be from the parasternal window only.

Mitral blood flow:

By CW Doppler, the values for peak E velocity (0.755 ± 0.106 m/s) (mean ± 1SD) and peak A velocity (0.531 ± 0.076 m/s) were always higher than the corresponding ones obtained by PW Doppler (E = 0.691 ± 0.075 m/s; A = 0.464 ± 0.064 m/s). Using the two sample T test, this difference was significant at $p< 0.01$ for A but only significant at $p< 0.001$ for E. The A:E ratio obtained by CW Doppler (0.701 ± 0.059) was higher than that obtained by PW Doppler (0.672 ± 0.048). This difference was significant at $p< 0.01$ but not at $p< 0.05$. The pressure half-times obtained by CW Doppler (0.049 ± 0.013 secs) were higher than those obtained by PW Doppler (0.040 ± 0.012 secs) and were significant at $p< 0.01$.

The higher velocities obtained by CW Doppler resulted because CW Doppler records all velocities along the line of investigation, while PW Doppler indicates flow rates only at the sample volume position (Delemarre et al., 1988). PW Doppler is therefore regarded as a more reliable indicator of the velocity of blood flow at the mitral valve orifice (Gardin et al., 1986; Fast et al., 1988). Since the E and the A velocities were affected to the same extent by the method of measurement, the A:E ratios obtained by PW and by CW Doppler were not significantly different. This was similar to observations made in normal humans, in whom the A:E ratio has been used as a reliable indicator of abnormalities in diastolic function (Gardin et al., 1986; Spirito and Maron, 1988; Choong et al., 1988).

The pressure half-times obtained by PW and CW Doppler were not significantly ($p < 0.05$) different . As such, either method could be used reliably to determine the resistance of blood flow across the mitral valve.

Aortic blood flow:

Using the one-way analysis of variance, it was found that, by CW Doppler, peak aortic blood flow velocity obtained from the subcostal position (1.577 ± 0.170 m/s) was not significantly different from that obtained from the suprasternal window (1.564 ± 0.201 m/s). Values obtained by PW Doppler from the subcostal window (1.392 ± 0.129 m/s) were, however, significantly lower. Similar observations have been made for human beings (Teague, 1986). In the present work, all three methods were employed on each dog to assess the character of flow, CW Doppler being used to determine peak aortic blood flow velocity.

Pulmonary blood flow:

From the right parasternal window, it was found difficult to place the transducer and sample volume in a direction approximating that of normal pulmonary blood flow. As a result, the values obtained were only considered as approximate values. Further studies are therefore necessary to determine the optimal transducer position for accurate determination of pulmonary blood flow velocity in dogs.

2.6. POST MORTEM EXAMINATION.

All the studies in this work involved euthanasia of the experimental animals. At the time of euthanasia, the dogs

were sedated by intramuscular injection with 0.5ml etorphine HCl (Immobilon^{R} - CVet. Ltd.). Euthanasia was by intravenous injection with 5ml pentobarbitone sodium (Euthatal^{R} - May and Baker).

The dogs were bled out by severing the axillary artery. The thoracic cavity was opened along the costochondral junction, and after a brief examination, the major thoracic organs were removed intact. The heart was separated from the lungs at the roots of the great vessels and weighed.

Samples for transmission electron microscopy (TEM) were obtained from the walls of both atria and both ventricles (See Ch. 7). After sampling for TEM, a complete post mortem examination was performed. Tissue samples for light microscopy were taken from the walls of both atria, both ventricles, the interventricular septum and the atrioventricular valves (Ch. 8). Representative tissue samples were taken from other body systems for general histology. The tissues were fixed in 10% neutral buffered formalin.

Tissue samples for immunofluorescence were taken from the left ventricular wall, and from the kidneys, and snap frozen at -70^{o}C. From the wall of the left ventricle, a tissue block was taken for lipid histochemistry (See Ch. 6).

TABLE 2.1.

M-MODE VALUES IN NORMAL BEAGLES MEASURED FROM THE RIGHT PARASTERNAL WINDOW.

DOG NUMBER	LVEDD (cm) MEAN SD	LVESD (cm) MEAN SD	FS (%) MEAN SD
1	2.89 ± .730*	1.52 ± .693	47.42 ± 9.79
2	2.86 ± .263	1.53 ± .247	46.85 ± 7.81
3	2.67 ± .381	1.36 ± .292	49.35 ± 7.35
4	2.77 ± .151	1.44 ± .139	48.19 ± 4.37
5	3.20 ± .100	1.90 ± .100	40.63 ± 1.25
6	3.20 ± .346	1.93 ± .208	39.57 ± 1.52
7	3.16 ± .267	1.75 ± .107	44.55 ± 2.45
8	2.72 ± .096	1.67 ± .144	38.94 ± 6.44
9	2.71 ± .196	1.46 ± .256	38.07 ± 5.25
10	3.10 ± .122	1.77 ± .112	46.35 ± 6.02
11	2.38 ± .120	1.34 ± .150	43.58 ± 3.84
12	2.72 ± .158	1.56 ± .192	44.67 ± 4.03
13	2.88 ± .110	1.58 ± .143	43.30 ± 4.72
14	3.00 ± .100	1.92 ± .075	45.90 ± 4.06
15	2.89 ± .030	1.60 ± .060	36.15 ± 2.64
16	2.88 ± .210	1.75 ± .060	44.15 ± 2.40
17	2.88 ± .010	1.85 ± .170	39.10 ± 5.13
18	2.95 ± .061	1.96 ± .117	36.00 ± 5.81
19	2.90 ± .115	1.91 ± .107	35.15 ± 3.68

Group mean: 2.83 ± .293cm 1.59 ± .240cm 44.03 ± 3.57%
Range: 2.38 - 3.20cm 1.34 - 1.96cm 35.15 - 49.35%
Heart rate: 128.61 ± 6.39 beats per minute.

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.
LVEDD - Left ventricular end-diastolic diameter.
LVESD - Left ventricular end-systolic diameter.
FS - Fractional shortening.

TABLE 2.2.

PEAK MITRAL BLOOD FLOW VELOCITY (m/sec) IN NORMAL BEAGLES MEASURED BY PULSED-WAVE DOPPLER ECHOCARDIOGRAPHY.

DOG NUMBER	E PEAK MEAN	E PEAK SD	A PEAK MEAN	A PEAK SD	A:E RATIO MEAN	A:E RATIO SD
1	.762	.047*	.480	.046	.615	.047
2	.764	.042	.508	.084	.666	.097
3	.694	.040	.542	.047	.780	.044
4	.765	.021	.530	.158	.688	.023
5	.534	.015	.318	.022	.594	.044
6	.600	0	.410	.042	.680	.071
7	.768	.074	.538	.055	.700	.050
8	.666	.030	.398	.022	.596	.037
9	.690	.060	.410	.030	.633	.122
10	.650	.040	.460	.040	.712	.028
11	.620	.031	.440	.056	.710	.057
12	.680	.035	.445	.035	.660	.040
13	.710	.050	.490	.010	.680	.030
14	.640	.050	.420	.030	.650	.060
15	.680	.030	.480	.020	.710	.020
16	.830	.040	.550	.040	.670	.040
Group mean:	.691	.075	.464	.064	.670	.040
Range:	.534 - .830		.318 - .550		.594 - .780	

Heart rate: 128.61 ± 6.39 beats per minute.

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.

TABLE 2.3.

PEAK MITRAL BLOOD FLOW VELOCITY (m/sec) IN NORMAL BEAGLES MEASURED BY CONTINUOUS-WAVE DOPPLER ECHOCARDIOGRAPHY.

DOG NUMBER	E PEAK MEAN	E PEAK SD	A PEAK MEAN	A PEAK SD	A:E RATIO MEAN	A:E RATIO SD
1	.820	.020*	.520	.020	.630	.010
2	.665	.060	.580	.069	.820	.035
3	.850	.075	.550	.045	.645	.665
4	1.00	.050	.780	.050	.770	.030
5	.680	.060	.500	.050	.730	.040
6	.700	.030	.520	.040	.740	.050
7	.700	.030	.490	.030	.700	.050
8	.790	.040	.530	.050	.670	.040
9	.910	.020	.550	.030	.600	.030
10	.650	.020	.490	.050	.760	.060
11	.705	.045	.485	.035	.685	.035
12	.695	.040	.465	.030	.660	.035
13	.655	.030	.480	.010	.730	.020
14	.680	.030	.480	.020	.710	.020
15	.830	.040	.550	.040	.670	.040
Group mean:	.755	.106	.531	.076	.701	.059.
Range:	.650 - .910		.465 - .780		.600 - .820.	

Heart rate: 128.61 ± 6.39 beats per minute.

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.

TABLE 2.4.

PRESSURE HALF-TIMES (secs) IN NORMAL BEAGLES MEASURED BY PULSED-WAVE (PW) AND CONTINUOUS-WAVE (CW) DOPPLER ECHOCARDIOGRAPHY.

DOG NUMBER	PW MEAN	PW SD	CW MEAN	CW SD
1	.047	.012*	.050	0
2	.038	.005	.050	.015
3	.044	.012	.070	.010
4	.060	.009	.070	0
5	.058	.013	.040	.010
6	.036	.010	.030	.010
7	.030	0	.040	.010
8	.030	.010	.050	.010
9	.025	.005	.040	.010
10	.030	0	.050	.010
Group mean:	.040	.012	.049	.013.
Range:	.025 -	.058	.030 -	.070.

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.

TABLE 2.5.

PEAK AORTIC BLOOD FLOW VELOCITY (m/sec) IN NORMAL BEAGLES MEASURED BY PULSED-WAVE (PW) AND CONTINUOUS-WAVE (CW) DOPPLER ECHOCARDIOGRAPHY.

DOG NUMBER	PW SUBCOSTAL MEAN SD	CW SUBCOSTAL MEAN SD	CW SUPRASTERNAL MEAN SD
1	1.27 ± .060*	1.42 ± .070	1.83 ± .120
2	1.27 ± .030	1.56 ± .130	1.72 ± .115
3	1.45 ± .045	1.80 ± .060	1.45 ± 0
4	1.65 ± .085	1.77 ± .120	1.58 ± .980
5	1.24 ± .070	1.80 ± .060	1.66 ± .025
6	1.38 ± .040	1.64 ± .040	1.64 ± .030
7	1.60 ± .075	1.60 ± .030	1.65 ± .010
8	1.46 ± .040	1.56 ± .110	1.78 ± .162
9	1.50 ± .057	1.75 ± .040	1.80 ± .100
10	1.38 ± 0	1.69 ± .070	1.43 ± .140
11	1.49 ± .180	1.56 ± .090	1.64 ± .065
12	1.36 ± .070	1.66 ± .080	1.64 ± .100
13	1.34 ± .030	1.61 ± .060	1.48 ± .080
14	1.20 ± .040	1.36 ± .050	1.40 ± .050
15	1.50 ± .100	1.43 ± .080	1.48 ± .030
16	1.28 ± .080	1.39 ± .100	1.41 ± .060
17	1.30 ± .070	1.29 ± .060	1.30 ± .070
18	1.49 ± .060	1.82 ± .010	1.54 ± .100
Group mean:	1.39 ± .129	1.577 ± .170	1.56 ± .201
Range:	1.20 - 1.65	1.29 - 1.82	1.30 - 1.83
Heart rate:	128.61 ± 6.39 beats per minute.		

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.

TABLE 2.6.

PEAK BLOOD FLOW VELOCITY (m/sec) ACROSS THE PULMONARY VALVE IN NORMAL BEAGLES MEASURED BY PULSED-WAVE (PW) DOPPLER ECHOCARDIOGRAPHY.

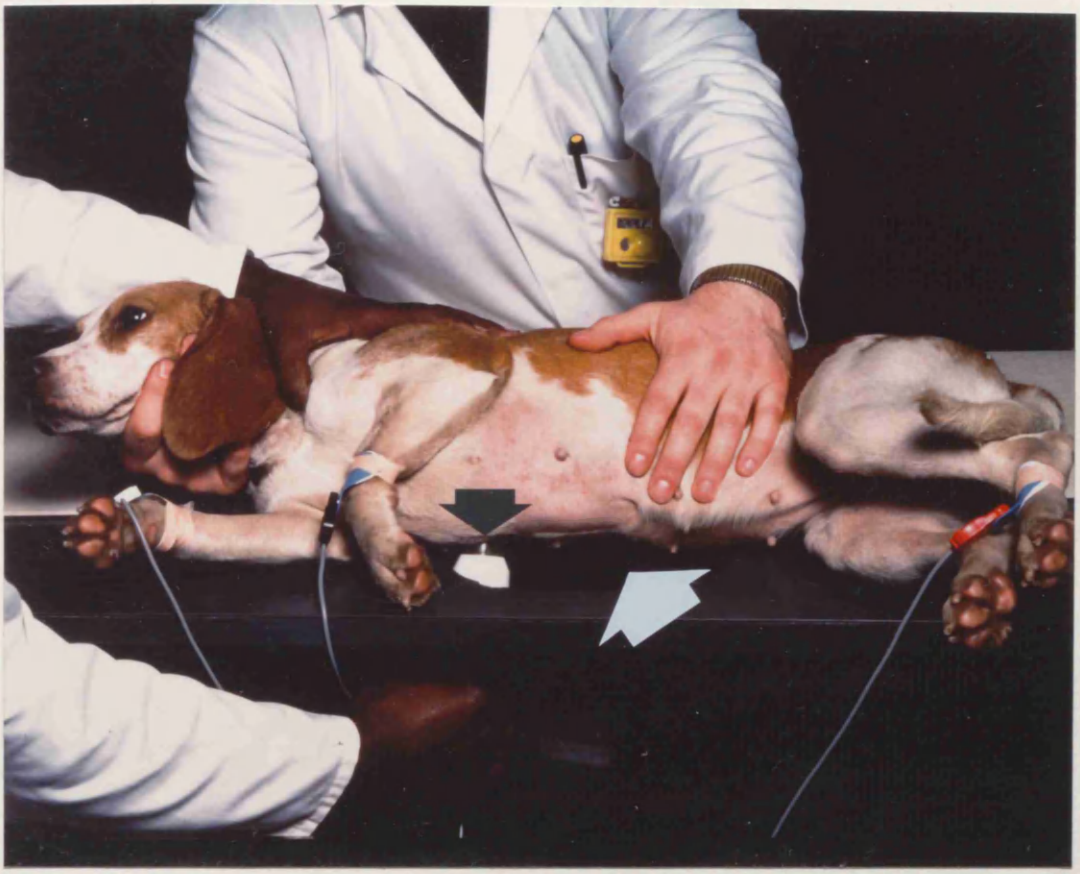
DOG NUMBER	FLOW VELOCITY MEAN SD
1	.920 ± .010*
2	.910 ± .030
3	.840 ± .030
4	.800 ± .100
5	.790 ± .100
Group mean:	.852 ± .061.
Range:	.790 - .920.

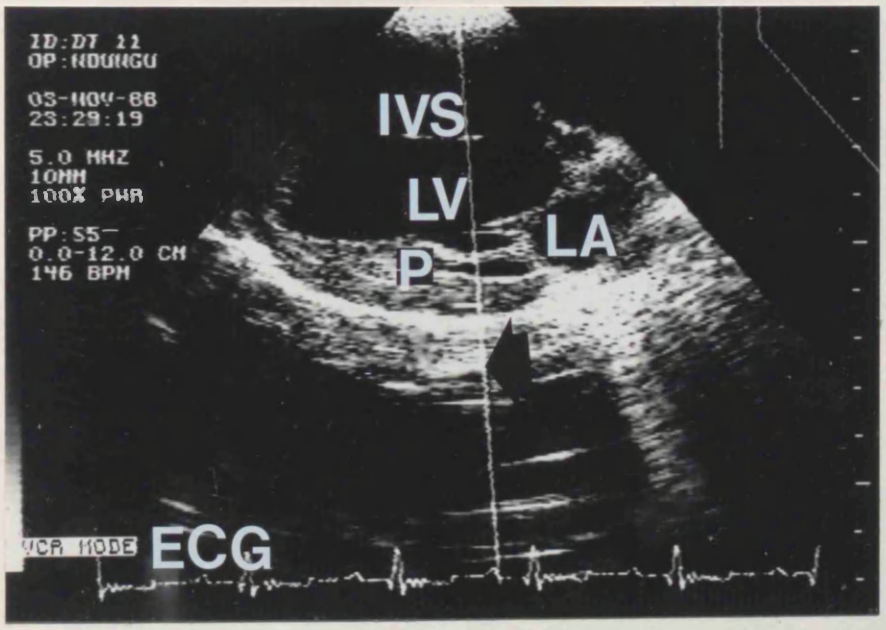
Heart rate: 124 ± 5.66 beats per minute.

* - Values represent the mean ± 1SD of measurements from 8 cardiac cycles on each of 2 days.

Figure 2.1. A dog laid in right lateral recumbency for echocardiographic studies from the right thoracic wall. The transducer (black arrow) is passed through a hole made in the table (white arrow) and comes to lie at the costochondral junction. The hair on the study site has been clipped. Electrodes have been attached to the legs to provide a record of the heart rate and rhythm.

Figure 2.2. A two dimensional long axis view of the heart showing placement of the cursor (arrow) for M-mode echocardiography through the left ventricle. The cursor is between the papillary muscle (P) and the mitral valve.
LV - Left ventricle. LA - Left atrium.
IVS - Interventricular septum

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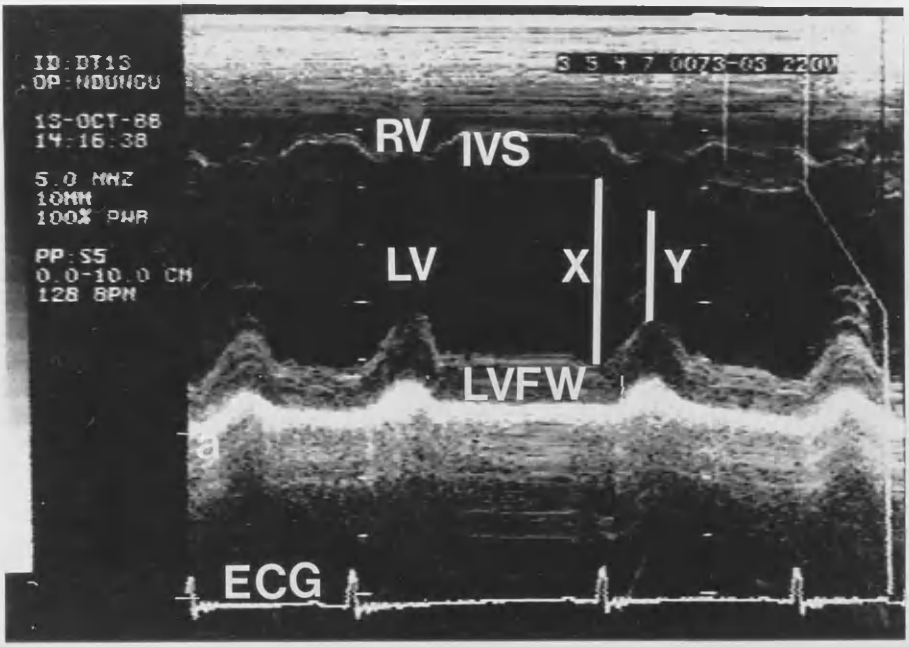
IVS
LV
LA
P
VCR MODE
ECG

Figure 2.3. An M-mode echocardiogram from the right parasternal window taken through the left ventricle, indicating the sites used in measurement of fractional shortening (FS). X - Left ventricular end-diastolic diameter. Y - Left ventricular end-systolic diameter. RV - Right ventricle. IVS - Interventricular septum. LV - Left ventricle. LVFW - Left ventricular free wall. The FS was obtained using the formula.

$$FS = \frac{LVEDD - LVESD}{LVEDD} \times 100.$$

Figure 2.4. An M-mode echocardiogram from the right parasternal window taken through the mitral valve. There is reciprocal motion of the anterior mitral leaflet (AML) and posterior mitral leaflet (PML). The early (D-E) and late (F-A) diastolic filling phases of mitral valve motion are indicated. The E-F slope represents the rate of left ventricular filling. IVS - Interventricular septum. LV - Left ventricle. LVFW - left ventricular free wall.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Y
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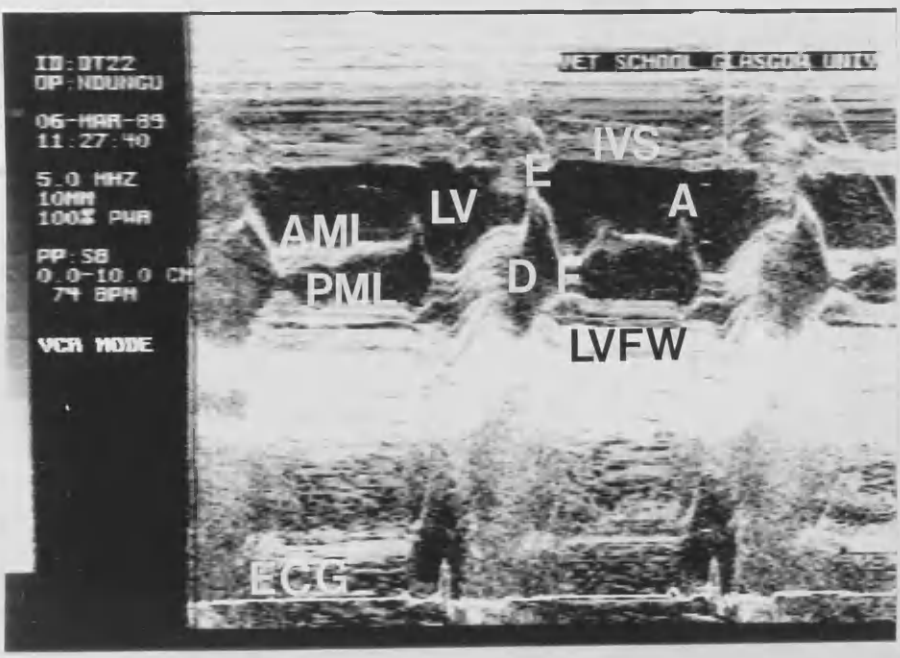
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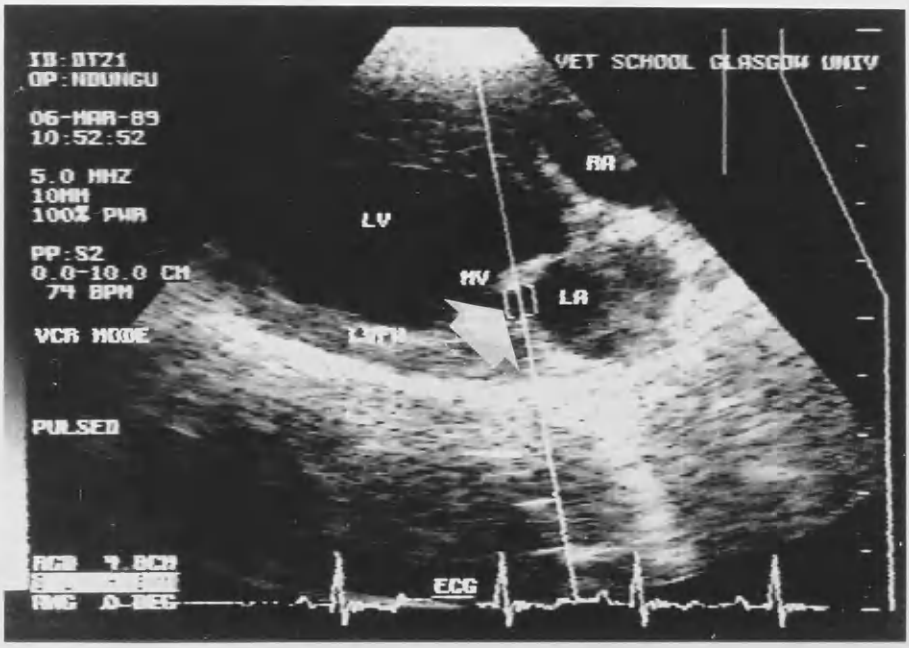
E
LV
A
AML
PML
D
F
LVFW
ECG

Figure 2.5. A two-dimensional right parasternal long-axis view of the heart showing placement of the sample volume (arrow) when investigating the presence of mitral incompetence. The sample volume is placed on the left atrial (LA) side of the mitral valve (MV). LV - Left ventricle. RA - Right atrium. LVFW - Left ventricular free wall.

Figure 2.6. A two dimensional right parasternal long axis view of the heart showing placement of the sample volume (arrow) when investigating the presence of tricuspid incompetence. RV - Right ventricle. RA - Right atrium. LV - Left ventricle.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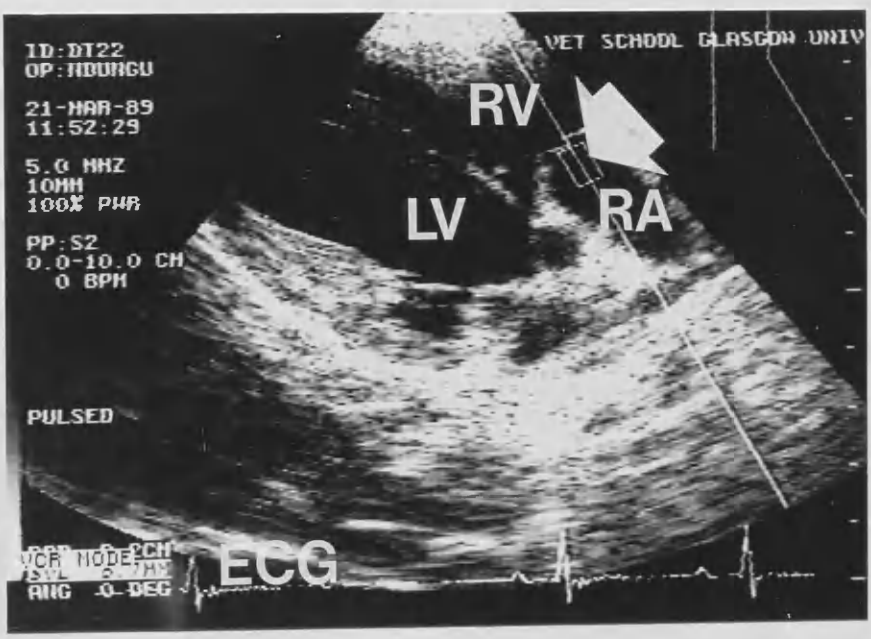
RA
LV
MV
LA
ECG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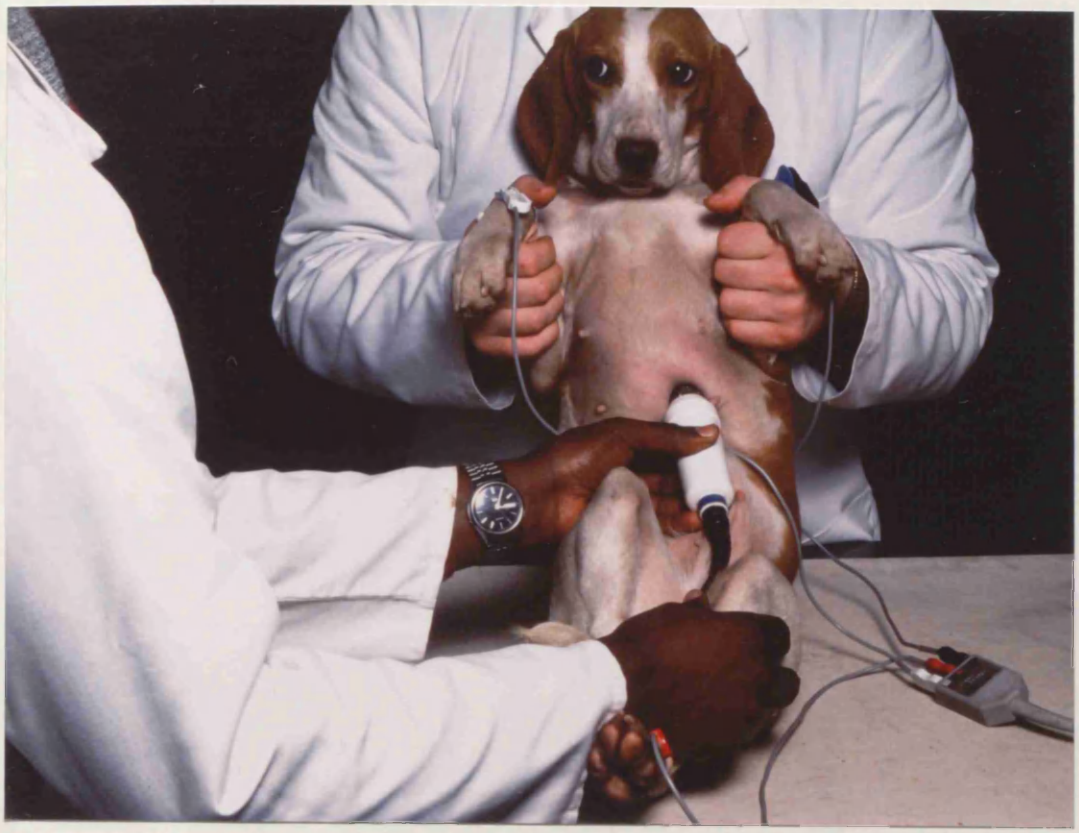
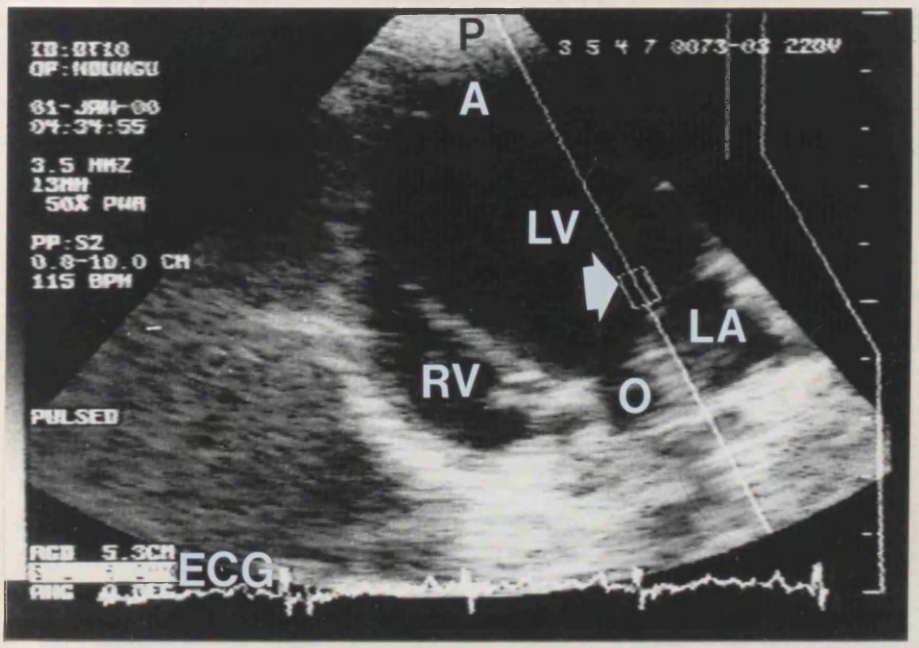
PULSED
VET SCHOOL GLASGOW UNIV
RV
LV
RA
ECG

Figure 2.7. A dog in a semi-dorsal recumbency position and transducer placement for subcostal echocardiography. The transducer is placed on the left side of the xiphoid cartilage and directed cranio-medially.

Figure 2.8. Two-dimensional apical view of the heart of a dog taken from the subcostal window. The apex of the heart (A) is close to the transducer position (P). The sample volume (arrow) is placed on the left ventricular (LV) side of the mitral valve when measuring mitral blood flow by pulsed-wave Doppler. RV - Right ventricle. LA - Left atrium. O - Ascending aorta.

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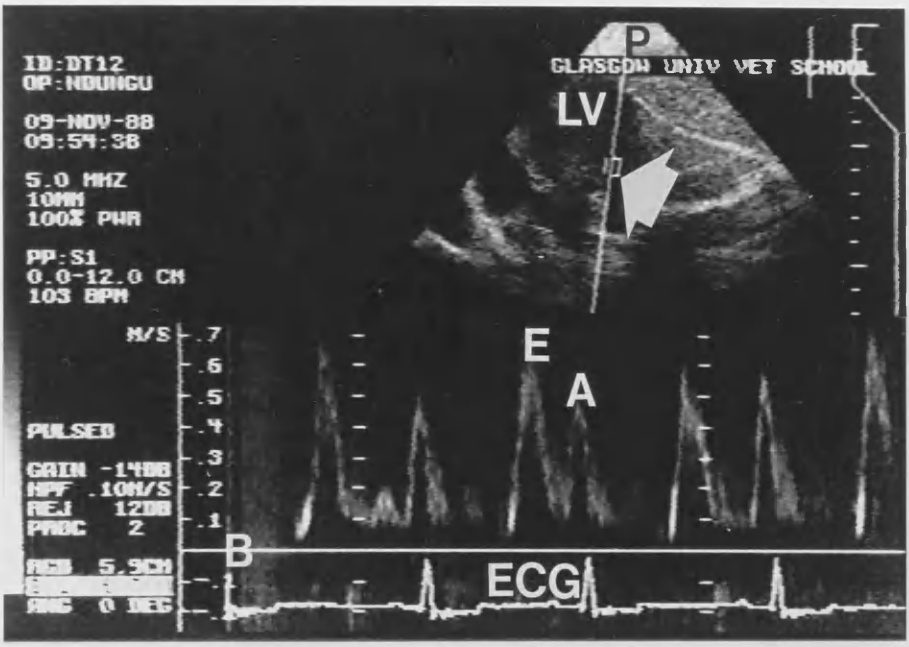
115 BPM
PULSED
RGB 5.3CM
ANG 0.0DEG
P
A
LV
LA
RV
O
ECG

Figure 2.9. Pulsed-wave duplex Doppler recording of normal mitral blood flow from the subcostal window. The sample volume (arrow) is placed on the left ventricular (LV) side of the mitral valve. The early (E) and late (A) phases of mitral blood flow appear as positive Doppler shifts. This is because in diastole, blood flows into the LV and hence towards the transducer position (P). B - Baseline.

Figure 2.10. Continuous-wave Doppler spectral recording of mitral blood flow from the subcostal window. The early (E) and late (A) diastolic phases of mitral blood flow appear as positive Doppler shifts above the baseline (B).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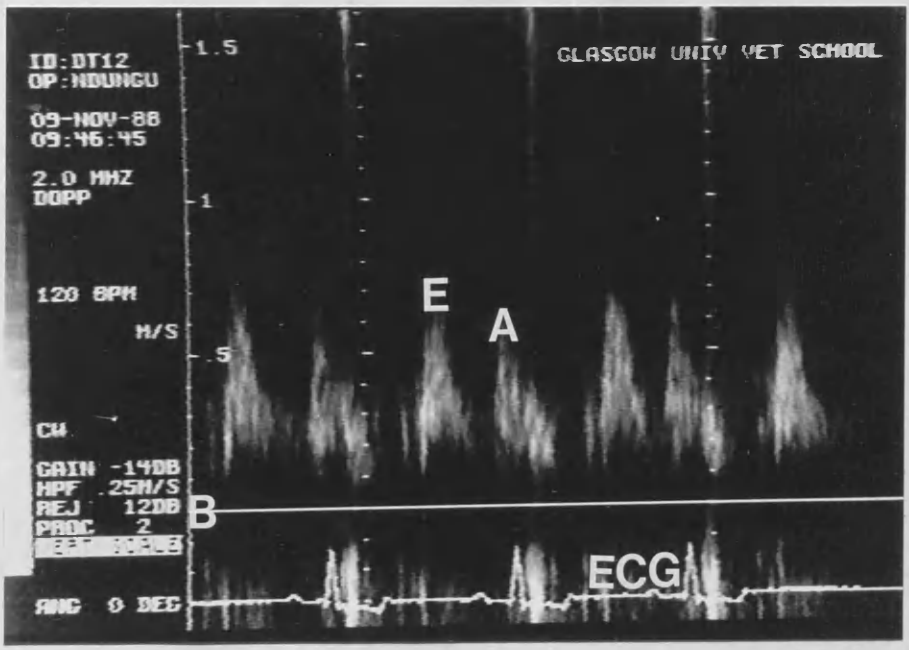
103 BPM
M/S
.7
.6
.5
.4
.3
.2
.1
PULSED
GAIN -14DB
HPF .10M/S
REJ 12DB
PROC 2
ANG 0 DEG
P
GLASGOW UNIV VET SCHOOL
LV
E
A
B
ECG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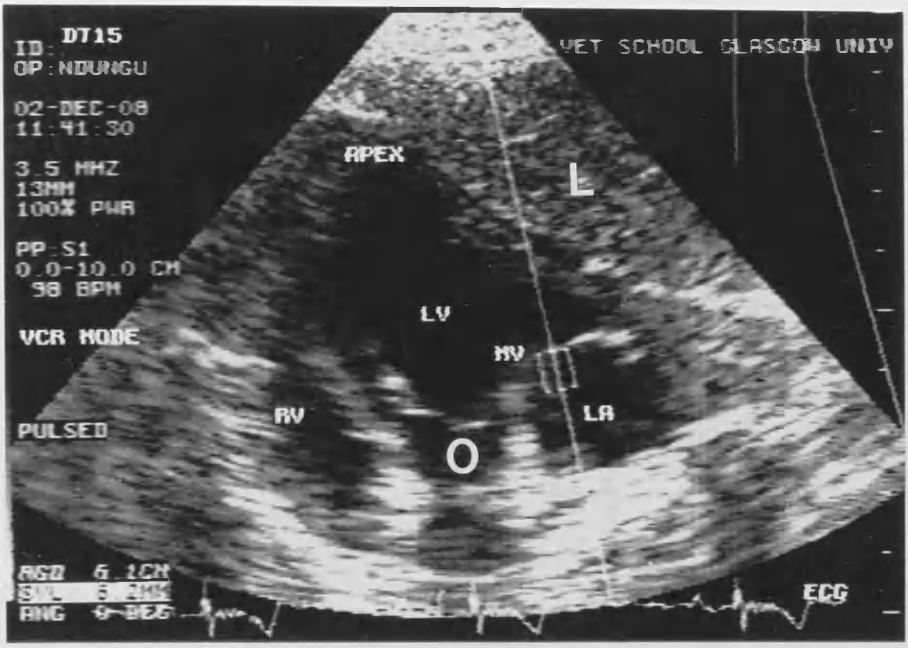
2.0 MHZ
DOPP
120 BPM
M/S
1.5
1
.5
CW
GAIN -14DB
HPF .25M/S
REJ 12DB
PROC 2
ANG 0 DEG
GLASGOW UNIV VET SCHOOL
E
A
B
ECG

Figure 2.11. Investigating the presence of mitral incompetence by pulsed-wave Doppler from the subcostal window. The sample volume (arrow) is placed on the left atrial (LA) side of the mitral valve (MV). RV - Right ventricle. LV - Left ventricle. O - Ascending aorta. L - Liver.

Figure 2.12. Investigating the presence of mitral incompetence by pulsed-wave Doppler from the subcostal window. The E and A peaks of mitral blood flow appear above the baseline (B) in diastole. Any incompetence would be demonstrated below the baseline in systole. Sometimes spectrals representing valve closure appear at the end of diastole (S), which is a normal finding.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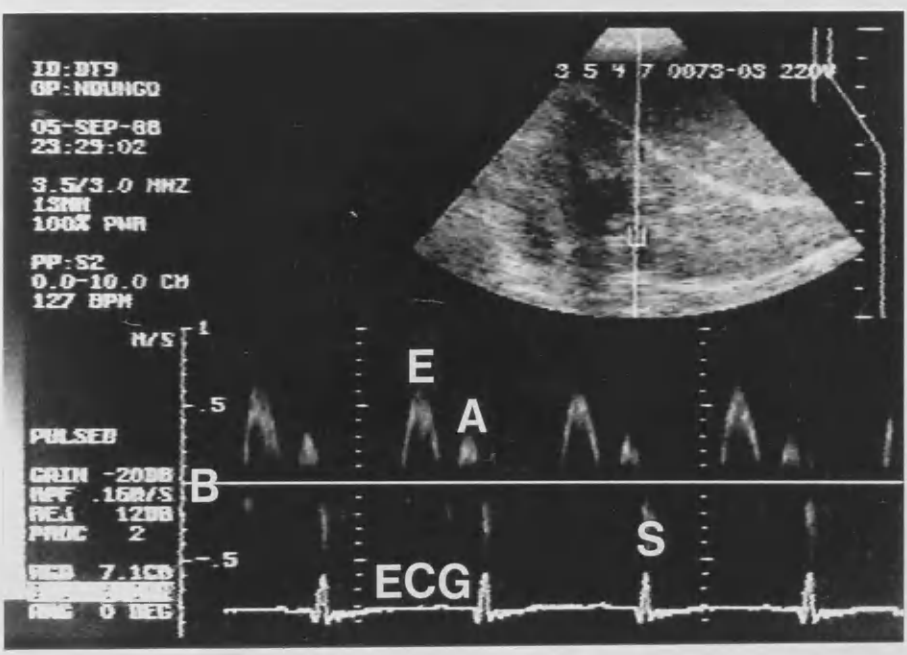
VCR MODE
PULSED
VET SCHOOL GLASGOW UNIV
APEX
L
LV
MV
RV
LA
O
ECG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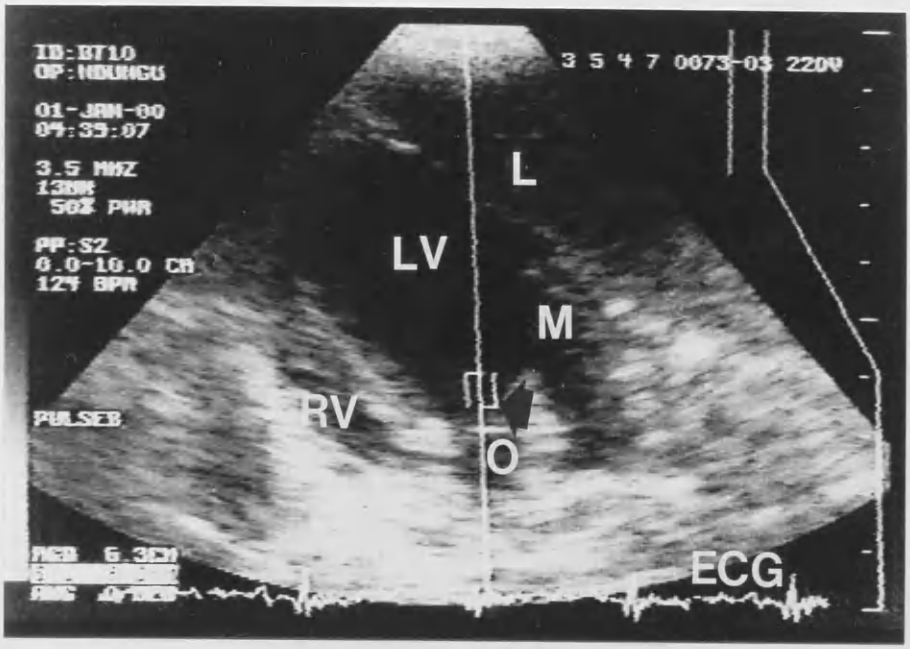
13MM
100% PWR
PP:S2
0.0-10.0 CM
127 BPM
M/S
1
.5
PULSED
E
A
B
S
.5
ECG

Figure 2.13. A two-dimensional apical view of the heart from the subcostal position optimised for measurement of aortic blood flow. The sample volume was placed in the left ventricular outflow tract and advanced into the aorta (O). LV - Left ventricle. L - Liver. RV - Right ventricle. In this frame, the aortic valve is closed (arrow) and the mitral valve is open (M).

Figure 2.14. Pulsed-wave duplex Doppler recording of aortic blood flow from the subcostal window. The sample volume (arrow) was advanced into the ascending aorta (*) to a point from which maximum spectral velocity records were obtained. Aortic blood flow (A) appears as negative velocities since blood flows away from the probe position in systole. The baseline (B) has been moved up to avoid aliasing.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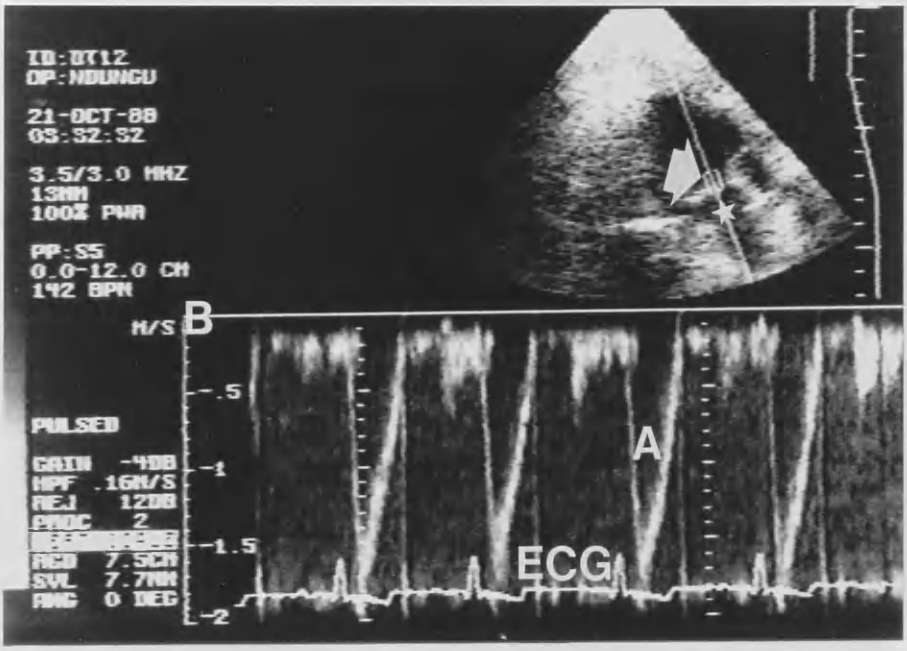
124 BPM
PULSED
L
LV
M
RV
O
ECG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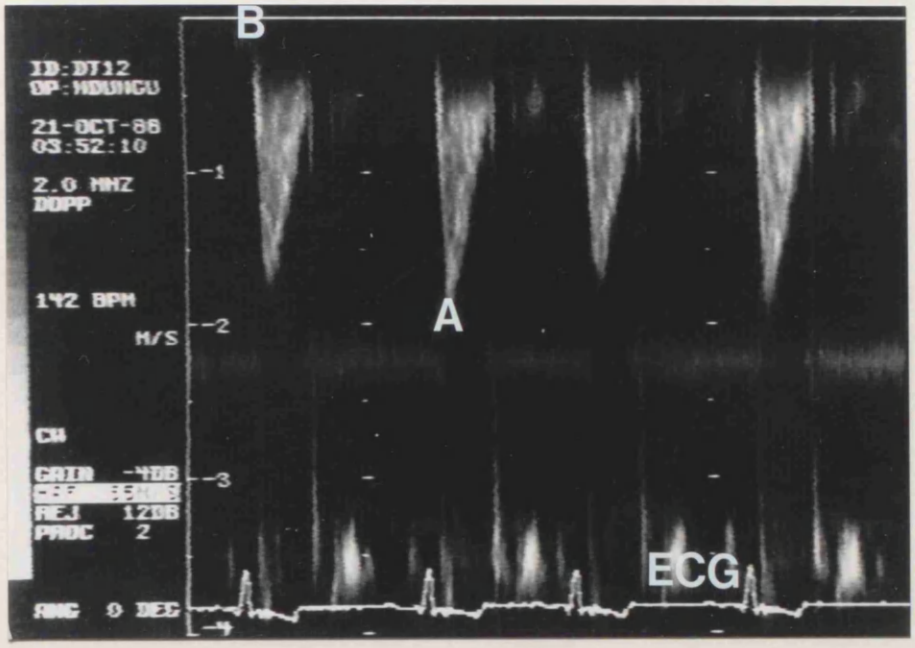
142 BPM
M/S
B
.5
1
1.5
2
PULSED
GAIN -40B
HPF .16M/S
REJ 12DB
PNUC 2
RCD 7.5CM
SVL 7.7MM
ANG 0 DEG
A
ECG

Figure 2.15. Continuous-wave Doppler spectral recording of aortic blood flow (A) from the subcostal window. The Doppler shift is negative because aortic blood is flowing away from the transducer in systole. The baseline (B) has been shifted to the top.

Figure 2.16. Investigating aortic blood flow from the suprasternal window. With the dog in a sitting position, a 2.0 MHz split-crystal ('stand-alone') transducer is placed in the thoracic inlet, on the right side of the manubrium of the sternum, and directed caudally. Electrodes have been attached to the legs to provide a record of the heart rate and rhythm.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M/S
A
CH
GAIN -4DB
REJ 12DB
PROC 2
ECG
ANG 0 DEG

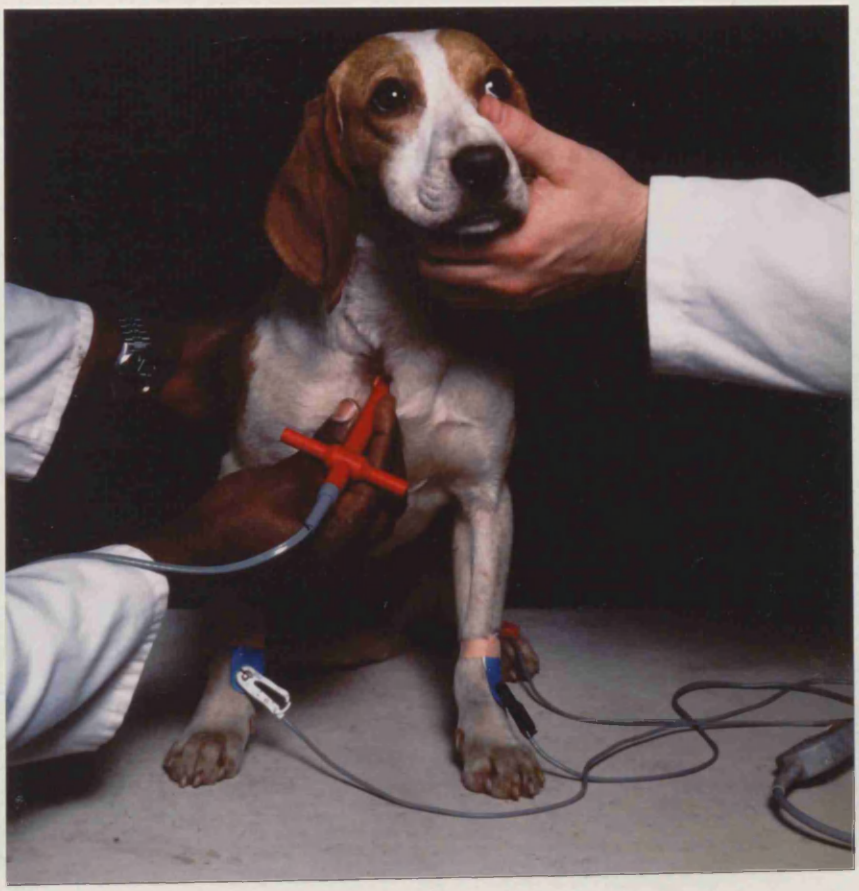
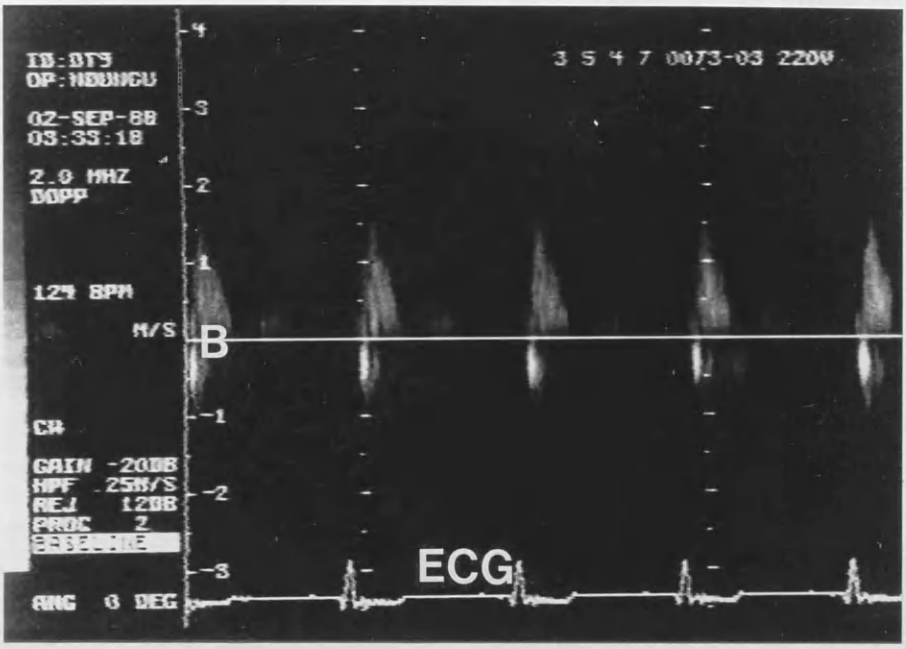


Figure 2.17. Continuous-wave Doppler spectral recording of normal aortic blood flow from the suprasternal window. Positive frequency shifts appear in systole because blood flowing through the ascending aorta is towards the probe position, hence a positive Doppler shift. B - Baseline.

Figure 2.18. A two-dimensional short axis view of the heart base, optimised to show the tricuspid valve (TV), right ventricular outflow tract (RVOT), the pulmonary valve (arrow) and the pulmonary artery (PA). The aorta (AO) is seen in cross-section. rpa - Right pulmonary artery. lpa - Left pulmonary artery.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B
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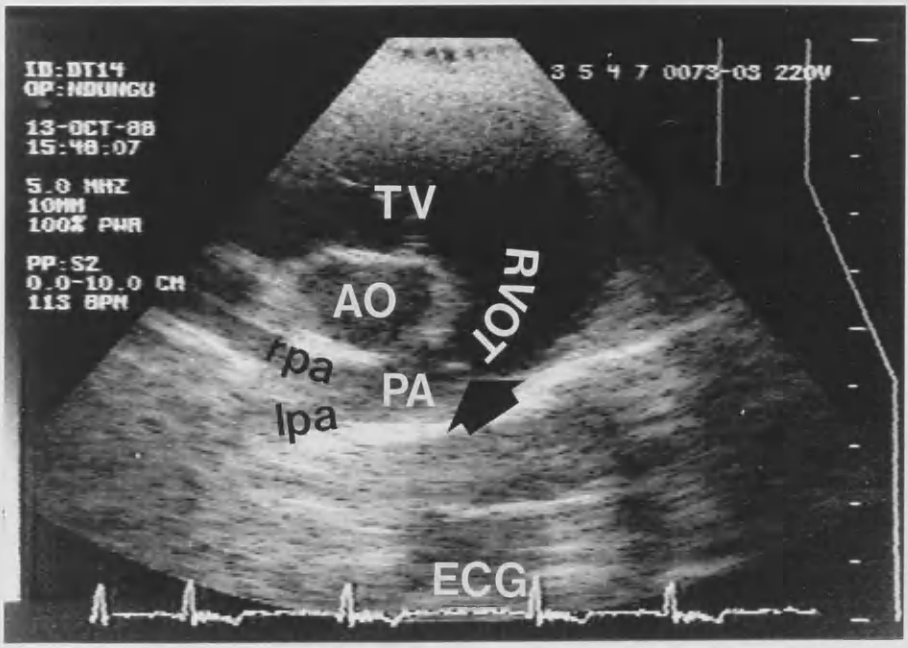
REJ 12DB
PROC 2
ANG 0 DEG
3 5 4 7 0073-03 220V
ECG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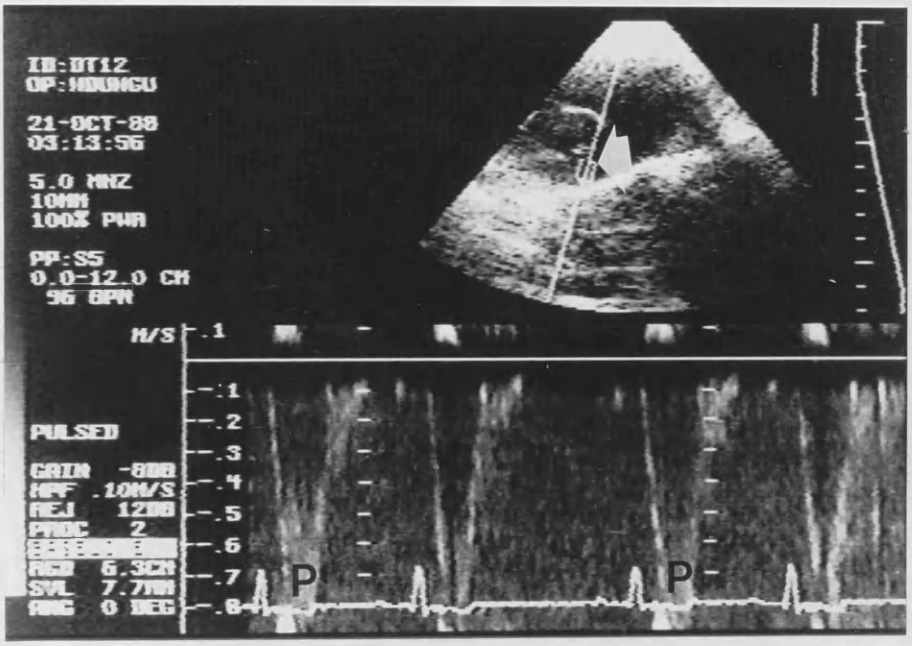
PP:SZ
0.0-10.0 CM
113 BPM
3 5 4 7 0073-03 220V
TV
AO
RVOT
rpa
lpa
PA
ECG

Figure 2.19. Pulsed-wave duplex Doppler recording of pulmonary blood flow (P) from the right parasternal window. The sample volume (arrow) is placed on the arterial side of the pulmonary valve. The direction of sampling is not in line with that of blood flow. The peak velocities obtained are therefore approximate values. The sample volume is moved to the ventricular side of the valve to determine the presence of pulmonary incompetence.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96 BPM
M/S
.1
.2
.3
.4
.5
.6
.7
.8
PULSED
GAIN -8DB
HPF .10M/S
REJ 12DB
PMAC 2
RGD 6.3CM
SVL 7.7MM
ANG 0 DEG
P
P

PART II.

THE PATHOGENESIS OF T.brucei INFECTION IN DOGS.

CHAPTER 3:

CLINICAL, HAEMATOLOGICAL AND PARASITOLOGICAL FEATURES OF *T.brucei* INFECTION IN DOGS.

3.1. INTRODUCTION.

T.brucei infection in dogs results in an acute disease syndrome characterised by fever, persistent parasitaemia, severe anaemia, lymph node and splenic enlargement, cardiac and neurological abnormalities, panophthalmitis, and death in 4 weeks (Losos and Ikede, 1972; Mwambu, 1979; Sayer et al., 1979; Morrison et al., 1981 a,b; Kaggwa et al., 1983; Kaggwa et al., 1984). The severity of the disease in man and animals is affected by various factors, including the virulence of the infecting trypanosome, the breed, age and nutritional status of the host. Among the clinical changes, the severity of anaemia has been recognised as a reliable indicator of the virulence of the infecting trypanosomes and the disease status of the host (Murray and Dexter, 1988).

The purpose of the present part of the study was to establish the clinical, parasitological and haematological changes associated with T.brucei infection in 7-month-old beagle dogs.

3.2. MATERIALS AND METHODS.

3.2.1. CLINICAL STUDIES.

The dogs, their management, and the trypanosome stabilate used in these studies have been described elsewhere (Ch. 2). Briefly, 10 dogs were infected by intravenous inoculation with T.brucei GVR35/c.1. Before and during the course of the disease, daily clinical examination was performed. The dogs were weighed at least twice a week.

Two dogs a time were euthanised at mid-infection on days 10 and 15, and in the terminal stages on days 21, 22 and 26. Tissue samples were taken from the heart for histopathological studies. Four dogs served as uninfected controls and were euthanised after the last pair of infected ones.

3.2.2. HAEMATOLOGICAL AND PARASITOLOGICAL STUDIES.

Two ml samples of jugular venous blood were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. From each sample, a wet film of blood was prepared on a microscope slide and examined for the presence of trypanosomes with a Leitz microscope (eyepiece x10; objective x40). The level of parasitaemia was graded numerically as the $\log_{10}$ of the number of trypanosomes per ml of blood (Herbert and Lumsden, 1976). When no trypanosomes were seen, the blood was centrifuged in a microhaematocrit tube and the dark ground/phase contrast buffy coat technique used (Murray et al., 1977).

From the remaining sample of blood, the total numbers of red blood cells (RBC), white blood cells (WBC) and platelets, total haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined, using a coulter counter (Coulter Electronics, Bedfordshire). The packed red cell volume (PCV) was estimated by the microhaematocrit method. Giemsa stained blood smears were used for differential and reticulocyte counts. For differential counts, a total of 200 WBC were counted on each blood smear.

3.2.3. CACHECTIN/TUMOUR NECROSIS FACTOR ACTIVITY.

Cachectin/tumour necrosis factor (TNF) activity was estimated from plasma using the bioassay method described by Espevik and Nissen-Meyer (1986). In the assay, cachectin/TNF activity is estimated by determining the tumoricidal activity of peripheral blood monocytes after exposure to endotoxin (lipopolysaccharide). Endotoxin at concentrations of 10 ng/ml, 1 ng/ml and 0.1 ng/ml was added to 0.5 ml of fresh blood and incubated for 6 hours. 1 ml saline was added to the 0.5 ml of blood and the diluted plasma fraction stored frozen before the bioassay. The optical density of microtitre wells during the bioassay was determined at 540 nm with a reference wavelength at 690 nm.

3.3. RESULTS.

3.3.1. CLINICAL FINDINGS.

3.3.1.1. GENERAL BODY CONDITION.

Five days after infection the dogs developed signs of disease. These appeared as rapid onset of fever, reaching peak values of up to 40.6^{o}C in some dogs on day 8 of infection (Fig. 3.1). The rise in body temperature corresponded with the demonstration of trypanosomes in the peripheral circulation.

On day 7 of infection, slight subcutaneous oedema of the face and periorbital space was detected. During week 2, subcutaneous oedema increased in severity and became more generalised; the entire head, sternum and the ventral abdomen, lower limbs and the vulva, were involved. In some dogs, oedema appeared to subside in certain regions of the

body, while becoming more severe in others. Limping on one or more limbs was observed when subcutaneous oedema of the legs was most severe, i.e., around day 14 of infection; subsequently, limping disappeared as the disease progressed.

Signs of wasting were obvious by day 12 of infection. At this time the dogs were losing weight rapidly; the drop in weight continued up to the terminal stages of the disease (Fig. 3.2). The dogs became very weak and had a staggering walk. At the time of euthanasia, most of the dogs surviving for more than 23 days were dull and recumbent.

3.3.1.2. PARASITOLOGICAL FINDINGS.

Trypanosomes were detected in the blood 5 to 6 days following intravenous inoculation, corresponding to the onset of fever (Fig. 3.1). The parasitaemia increased rapidly, reaching a peak between days 8 and 9. The highest body temperatures were recorded at this time. There followed a rapid drop in parasitaemia, such that on days 10 and 11, trypanosomes could only be detected on buffy coat smears. Subsequently, high parasitaemia was re-established and persisted up to termination of the study (Fig. 3.1).

3.3.1.3. THE HAEMOPOIETIC SYSTEM.

Red cell parameters.

Changes in red cell parameters appeared around day 6 of infection, at least 24 hours after fever and parasitaemia were detected. Initially, there was a rapid drop in PCV up to day 9, then a gradual and persistent decrease throughout

the rest of the infection period (Fig. 3.3). The number of RBC (Fig. 3.4) and total Hb (Fig. 3.5) followed a similar pattern, decreasing rapidly during week 2, and gradually in weeks 3 and 4.

Transient increases in MCV (Fig. 3.6) and MCH were noted between days 5 and 10, and were associated with the presence of reticulocytes in the blood. Reticulocytes and normoblasts were also observed on day 16 of infection (Fig. 3.6), and persisted up to termination of the study. There were no detectable changes in the MCHC.

Platelet changes.

The changes in platelets before and during the course of the disease are indicated on Figure 3.7. The number of platelets in the blood dropped rapidly beginning from day 5 of infection, coinciding with the onset of fever and the appearance of trypanosomes in the blood. The lowest levels were recorded on day 11, and persisted throughout the rest of the infection period.

Leucocyte changes.

The number of circulating WBC decreased gradually from around day 5 to day 9 of infection (Fig. 3.8). Subsequently, the WBC count remained low, with daily fluctuations, up to termination of the study on day 24. Differential counts showed leucocytopaenia to be the result of reduced numbers of both lymphocytes and neutrophils (Figs. 3.9 and 3.10). While the neutropaenia persisted throughout the infection period, transient increases in lymphocytes towards normal were detected on

days 10 and 17.

The number of monocytes in the circulation varied depending on the stage of infection. Around day 4, an increase in monocytes was noted (Fig. 3.11). The increase appeared to precede the detection of trypanosomes in the blood. Subsequently, the number of monocytes dropped, reaching low levels by day 9, a time when the first peak of parasitaemia was established. Another transient increase in monocytes occurred from day 10 to day 14, returning to low levels thereafter.

From day 18 of infection, band neutrophils and metamyelocytes were occasionally seen. There were no significant changes in the numbers of eosinophils throughout the study period.

Cachectin/TNF activity.

Cachectin/TNF activity was estimated on blood collected from 8 dogs on days 0, 2 and 4 of infection. The results obtained are indicated in Table 3.1. On day 0, response to 0.1 ng/ml endotoxin was 6.250 $\pm$ 4.23 pg/ml (mean $\pm$ 1SD). Increasing the dose of endotoxin to 10 ng/ml resulted in increased cachectin/TNF activity (29.000 $\pm$ 25.5 pg/ml). On days 2 and 4, the activity increased dramatically in response both to 10 ng/ml and 1 ng/ml endotoxin. On day 4, for example, the cachectin/TNF activity in response to 10 ng/ml endotoxin was 103.800 pg/ml (Table 3.1). Response to 0.1 ng/ml endotoxin was less dramatic, increasing to 10.625 $\pm$ 9.09 pg/ml on day 2 and dropping to 9.500 $\pm$ 10.70 pg/ml on day 4.

3.3.1.4. CHANGES IN THE EYES.

Changes in the eyes appeared from day 7 of infection as slight periorbital oedema, accompanied by bilateral serous lachrymal discharge. With progress of the disease, periorbital oedema became more pronounced and the ocular discharge turned mucoid. Marked bilateral conjunctival oedema appeared on days 15 and 16, resulting in prominence of the third eyelid. For most of the dogs, photophobia developed around day 17 of infection. At that time too, irritation of one or both eyes occurred. As a result the dogs kept on pawing and rubbing the affected eyes.

By day 18 of infection the dogs had a staring look and the pupils became markedly dilated. At this time too, the ocular discharge had turned mucopurulent. This was followed one to two days later by unilateral or bilateral corneal opacity.

In week 4 of infection, clouding intensified to involve the entire cornea. In addition, some white flocculent material appeared in the aqueous humour. The material appeared to be suspended in the aqueous without any attachment to the inner walls of the anterior chamber of the eye. These changes were associated with unilateral or bilateral blindness.

3.3.1.5. CHANGES IN THE LYMPHOID ORGANS.

Immediately following the appearance of trypanosomes in the blood on day 6, there was slight enlargement of the retropharyngeal and superficial cervical lymph nodes. In some of the dogs too, the spleen became palpable, although relatively small. Lymph node and splenic sizes increased

rapidly, such that by day 12, the spleen was massive, and all superficial lymph nodes markedly enlarged. On day 16 the spleen was occupying most of the ventral abdomen. During weeks 3 and 4, reduction in the sizes of the spleen and lymph nodes was noted. At termination of the study in week 4, the spleen and lymph nodes were still larger than in control uninfected dogs, but smaller than at day 16 of infection.

3.3.1.6. GASTROINTESTINAL CHANGES.

Around day 11 of infection, the stool in some of the dogs became soft and slightly dark. By day 14, four of the dogs had bloody diarrhoea. In the other dogs the character of the stool varied from soft and mucoid to watery. This situation persisted up to termination of the study in week 4, when the faecal material from most dogs contained either frank or baked blood. At this time too, the dogs had tenesmus.

Throughout the study period, the dogs showed very little change in appetite. In week 4 however, a reduction in the amount of food eaten was observed. The dogs tended to take more water instead, and by day 18 of infection, this was obviously increased.

3.3.1.7. CENTRAL NERVOUS SYSTEM CHANGES.

The central nervous system did not appear to be involved until after day 18 of infection, when the dogs developed a staring look and indifference. Later the dogs became hemiplegic and had a stumbling walk. Some of the dogs showed some aggression when disturbed. This aggression

decreased when the dogs became recumbent.

During the period of the study, the control dogs showed normal clinical, haematological and parasitological parameters similar to the pre-infection ones described here.

3.4. DISCUSSION.

Infection of dogs with T.brucei consistently resulted in an acute disease syndrome, characterised by fever, high persistent parasitaemia, rapid development of anaemia, thrombocytopaenia and leucocytopaenia. The disease was associated with lymph node and splenic enlargement, and latterly, panophthalmitis, wasting and severe weight loss.

A similar course of the disease in dogs infected with T.brucei has been observed in other studies (Sayer et al., 1979; Morrison et al., 1981a,b; Kaggwa et al., 1984). Sleeping sickness in man due to T.rhodesiense takes a longer course, although people are known to have died within 6 weeks of a tsetse bite (Manuelidis et al., 1965).

Anaemia developed rapidly, and appeared to have contributed significantly to the clinical course of the disease. The onset of anaemia, and the rate and extent to which PCV falls, has previously been used as a reliable indicator of the disease status in other animals (Murray and Dexter, 1988). Several factors are known to contribute to the development of anaemia in trypanosomiasis, including increased RBC breakdown, dyshaemopoiesis and haemodilution.

Increased RBC breakdown could occur following haemorrhage or haemolysis. In the present study, however, haemorrhagic gastroenteritis was observed at a stage of the

disease when the anaemia was already well established. At the same time, the degree of gatrointestinal haemorrhage appeared to be too low to account for the severe anaemia. In sequential studies in other dogs, haemorrhagic lesions were only observed in terminally infected dogs (Morrison et al., 1981a). Significant haemorrhages that might affect the PCV only occur in cattle infected with certain strains of T.vivax (Hudson, 1944; Olubayo and Mugera, 1985).

Haemolysis is thought to be the major cause of anaemia in trypanosomiasis. Increased erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) is the main mechanism by which erythrocytes are removed from the blood. This has been demonstrated in studies in dogs (Morrison et al., 1981b), man (Woodruff, 1973), laboratory animals (Jennings et al., 1974; Murray et al., 1974a,b), small ruminants (MacKenzie et al., 1978; Anosa and Kaneko, 1989) and cattle (Murray et al., 1979) infected with various trypanosome species. In mice infected with T.brucei or T.congolense, increased erythrocyte destruction was observed in the spleen from day 3 of patent parasitaemia (Jennings et al., 1974; Ikede et al., 1977). In the current studies, splenomegaly occurred soon after a decrease in PCV was noted. This may have been the result of erythrocyte sequestration and an expanded MPS ingesting RBC.

While intravascular haemolysis is a possible mechanism of erythrocyte destruction, complementary studies in these dogs using haptoglobin as a marker for intravascular haemolysis failed to demonstrate the presence of free Hb in plasma (Ch. 5).

Various mechanisms, occurring alone or in concert, contribute to the increased erythrophagocytosis. Antigen-antibody reactions cause increased trypanolysis, leading to formation of immune complexes (Murray, 1974; Lambert and Houba, 1974) and release of biologically active substances. Immunoglobulins, immune complexes or autoantibodies could attach on erythrocytes, causing their rapid removal from the circulation. In the current studies, the decrease in RBC mass started when parasitaemia was rapidly increasing, indicating that trypanolysis was possibly not taking place at the time. The initial drop in PCV may therefore not have been due to immunological mechanisms.

Living T.congolense and T.vivax are known to cause erythrocyte damage (Banks, 1979; Esievo, 1983). By attaching to the cell surfaces, the erythrocyte membranes could be altered, leading to their recognition by the MPS as foreign, and hence removal from the circulation. T.brucei is, however, not known to attach to erythrocyte membranes. The possibility of causing erythrocyte damage directly is therefore minimal.

Live or dead trypanosomes release biologically active substances which could cause erythrocyte damage and hence phagocytosis by the MPS (Tizard et al., 1978). Among the biologically active substances is a haemolytic factor that has been detected at times of haemolytic crisis in mice infected with T.congolense, T.vivax or T.brucei (Murray, 1979), in rats infected with T.brucei (Murray, et al., 1974), and in cattle infected with T.congolense (Fiennes,

1954) or T.vivax (Murray and Dexter, 1988).

There is evidence that degenerating trypanosomes release lipid soluble substances capable of causing lysis of RBC (Landsteiner and Rubitchek, 1907). Several enzymes capable of causing a haemolytic crisis have also been identified, including proteases, phospholipases and neuraminidases (see review by Mellors, 1985). In the current studies parasitaemia dropped rapidly after the first peak, indicating the presence of a trypanolytic crisis and possible release of large quantities of biologically active substances. It is therefore possible that substances generated from living or dead trypanosomes were a major cause of the drop in RBC mass, at least in the early stages of the disease.

The disease caused by T.brucei in the dogs in the current studies was associated with persistent fever. Studies in man and rabbits have demonstrated that even small alterations in body temperature can have a major effect on erythrocytes, including increased osmotic fragility, increased permeability and decreased plasticity. This could lead to their increased removal from the circulation (Karle, 1974). Increased fragility has been observed in erythrocytes from mice infected with T.brucei or T.congolense (Ikede et al., 1977), and from sheep infected with T.congolense (MacKenzie and Cruickshank, 1973). Increased erythrocyte fragility corresponded to the maximum drop in RBC mass. The high temperatures recorded in the current study may have had an effect on erythrocyte fragility.

Splenomegaly, as was seen in the current study, associated with an expanded MPS, extended travel of RBC through the splenic sinusoids and therefore prolonged contact with activated macrophages, could lead to greater chances of ingestion of normal or defective erythrocytes.

Trypanosomiasis is associated with major changes in body metabolism. Thus, rabbits infected with T.brucei develop a hyperlipidaemia (Rouzer and Cerami, 1980). The hyperlipidaemic state is induced by substances produced by activated macrophages. In another study in these dogs, hyperlipidaemia, increased non-esterified fatty acids (NEFA), and hypoalbuminaemia occurred following infection (Ch. 6). Free fatty acids (FFA) are capable of causing alterations in erythrocyte morphology (Kamada et al., 1987) and could therefore cause their rapid removal by the MPS. It is possible that alterations in lipid metabolism contributed significantly to the anaemia in the current studies.

Dyshaemopoiesis could also contribute to the anaemia in canine trypanosomiasis. In the current study, only a mild reticulocytosis was observed at various stages of the disease. Similar observations have been made in dogs in other studies (Kaggwa et al., 1984). The low reticulocyte numbers, especially in the terminal stages, possibly indicated some degree of bone marrow depression in the face of massive RBC destruction. However, in post mortem studies in other dogs infected with T.brucei (Morrison et al., 1981a) and goats infected with T.vivax (Anosa and Kaneko, 1989), the observation of increased red bone marrow

indicated that erythropoiesis was still taking place. Erythropoietic cells have also been observed in the spleens of dogs euthanised in the terminal stages of a T.brucei infection (Morrison et al., 1981b). The bone marrow response is however too low in comparison with the degree of anaemia (Holmes, 1976; Dargie et al., 1979a), indicating that the bone marrow is either depressed or starved of iron.

The possibility that transportation of iron to the bone marrow for erythropoiesis is blocked by the MPS is indicated by the excessive haemosiderosis observed in the spleens and lymph nodes of cattle infected with T.congolense (Fiennes, 1954), and of mice infected with T.brucei (Jennings et al., 1974). Iron metabolism data have also provided evidence in support of this (Jennings et al., 1974; Dargie et al., 1979a).

Monocytes in the blood of infected dogs were found to be primed for the production of cachectin/TNF. Cachectin/TNF is known to inhibit the release of iron from macrophages (Alvarez-Hernandez et al., 1989). It is likely that, following phagocytosis of erythrocytes by the activated MPS, release of iron by the macrophages was inhibited by cachectin/TNF, making the bone marrow incapable of adequate erythropoietic response. In addition, cachectin/TNF has been shown to cause dyserythropoiesis by its direct effect on the bone marrow (Tracey et al., 1988). It is possible therefore, that cachectin/TNF played a major role in the anaemia observed in these dogs.

Haemodilution has been proposed as one of the causes of

the drop in PCV (Fiennes, 1954; Naylor, 1971; Suliman and Feldman, 1989). Studies using ^{125}I-labeled albumin to measure plasma volume and ^{51}C-labeled erythrocytes to measure total RBC volume have however demonstrated that there is usually no change in total blood volume (Sayer et al., 1979; Dargie et al., 1979a,b; Dargie, 1980).

Thrombocytopaenia developed rapidly and persisted throughout the infection period. This was similar to observations in sleeping sickness patients (Davis et al., 1974; Robins-Browne et al., 1975; Basson et al., 1977), rats and rabbits infected with T.rhodesiense (Davis et al., 1974), goats infected with T.vivax (Anosa and Kaneko, 1989), and in cattle infected with either T.congolense (Wellde et al., 1983) or T.vivax (Olubayo and Mugera, 1985; Assoku and Gardiner, 1989). Thrombocytopaenia persisted for as long as trypanosomes were present in the blood, and the severity was directly related to the height of parasitaemia, similar to the observations made in the current study.

Thrombocytopaenia could have resulted from excessive pooling of platelets in the enlarged spleen, decreased production of platelets by the bone marrow, shortening of platelet lifespan, or disseminated intravascular coagulation (DIC).

Kinetic studies in T.rhodesiense infected human beings have demonstrated increased pooling of platelets in the spleen (Robins-Browne et al., 1975). This, in addition to the expanded MPS, could lead to removal of abnormal platelets by the MPS or removal of normal platelets by an

abnormal MPS, or both. In the present study, thrombocytopaenia preceded splenomegaly, indicating that other mechanisms were involved in the early stages of the disease.

The lifespan of platelets could also be reduced by attachment of antigens, directly or complement mediated, leading to their accelerated removal by the MPS. The recent demonstration of autoantibodies to platelets in plasma from cattle infected with T.vivax (Assoku and Gardiner, 1989) indicated that immunological mechanisms also play a role in the pathogenesis of thrombocytopaenia.

DIC has been implicated as one of the major factors in development of thrombocytopaenia in trypanosomiasis (Davis et al., 1974). Despite the rare occurrence of DIC (Davis, 1982), factors indicating activation of the coagulation system, including a decrease in the plasma fibrinogen concentration, increased fibrinogen degradation products (Robins-Browne et al., 1975; Basson et al., 1977; Molyneux et al., 1984), and changes in the levels of Factors V and VIII of the clotting system have been found in plasma of sleeping sickness patients (Robins-Browne et al., 1975) and in cattle infected with T.congolense (Wellde et al., 1978). In dogs infected with T.brucei, thrombus formation, indicating activation of the clotting mechanism, has been observed in venous plexuses of various organs (Morrison et al., 1981a,b).

Several mechanisms could lead to activation of the coagulation system (Boreham, 1976), including substances from trypanosomes, injury to endothelial cells and tissues

causing release of thromboplastin, erythrocyte or platelet injury with production of phospholipids, or endotoxin.

Some biologically active substances released by dead or dying trypanosomes are capable of causing platelet activation (Tizard, 1978; reviewed by Mellors, 1985). Substances from disrupted trypanosomes, when added *in vitro* to normal rabbit, rat or human blood, have been shown to cause platelet aggregation within 30 minutes (Davis, 1974). The substances are heat-labile and active in the presence of complement inhibitors, suggesting that they could be protein enzymes or toxins. In the present study, the direct relationship between the severity of thrombocytopaenia and the height of parasitaemia suggests a direct effect of trypanosomes or their products on the platelets.

Injury to platelets, unrelated to endothelial damage, could result in intravascular aggregation and disorganisation of the platelet microtubular system, leading to their phagocytosis in the spleen. In this respect, platelet clumping in the blood and sinusoids of haemolymph nodes has been observed in goats (Anosa and Kaneko, 1989) and in cattle (Wellde *et al*., 1983) infected with *T.congolense*.

T.brucei infection in dogs is associated with severe vascular and tissue damage (Morrison *et al*., 1981a; Ch. 8). Such changes might lead to platelet activation and accelerated removal by the MPS.

One of the effects of cachectin/TNF is stimulation of production of platelet activating factor by macrophages, neutrophils and vascular endothelial cells (Camussi *et al*.,

1987; Beutler and Cerami, 1987). Monocytes from the dogs in these studies were shown to be primed to produce cachectin/TNF by day 4 of infection. This coincides with the stage of the disease when thrombocytopaenia begins.

Thrombocytopaenia could also result from decreased platelet production. Nevertheless, experiments in goats infected with T.congolense have demonstrated an increase in the numbers of megakaryocytes in the bone marrow (Anosa and Kaneko, 1989). In other dogs infected with T.brucei, evidence of increased platelet production was also provided by the observation of large numbers of megakaryocytes in the spleen of terminally infected dogs (Morrison et al., 1981b). In dogs infected with T.brucei therefore, it is possible that platelet production took place normally, or even at an increased rate, but could not cope with excessive removal by the expanded MPS.

Leucocytopaenia was one of the major changes observed in the dogs in this study. During week 3, the leucocytopaenia tended to stabilize. Similar observations have been made in other dogs infected with T.brucei (Kaggwa et al., 1984), a leucocytosis only developing at mid-infection and subsiding in later stages. Similarly in rabbits (Nagle et al., 1980) and monkeys (Schmidt and Sayer, 1982) infected with T.rhodesiense, in sleeping sickness patients (Basson et al., 1977), and in cattle infected with either T.congolense or T.vivax (Murray and Dexter, 1988), the acute disease is associated with leucocytopaenia; a leucocytosis only developing in the more chronic cases.

Leucopaenia could result from premature phagocytosis of WBC following their interaction with autoantibodies, preformed antigen-antibody complexes and adsorbed trypanosome antigens (Murray and Dexter, 1988). Premature leucophagocytosis occurs in sheep infected with T.congolense (MacKenzie and Cruickshank, 1973), in goats (Anosa and Kaneko, 1989) and cattle (Logan, 1989) infected with T.vivax, and in dogs infected with T.brucei (Ch. 8). Leucophagocytosis could therefore have contributed to the leucopaenia observed in the current study.

Decreased leucopoiesis in the bone marrow could also lead to leucopaenia. An increase in erythropoiesis at the expense of leucopoiesis has been proposed (Valli and Forsberg, 1979). Indeed, a decrease in the myeloid:erythroid ratio has been observed in T.congolense infected sheep (MacKenzie and Cruickshank, 1973) and cattle infected with T.vivax (Naylor, 1971). In addition, the production of a leucopoietic inhibitor, thought to be either a lymphokine or immunoglobulin, has been observed during the first and second weeks in cattle infected with T.congolense or T.vivax (Kaaya et al., 1979). In the present study, presence of band neutrophils and metamyelocytes in the circulation indicated that the bone marrow was incapable of coping with the leucopaenic state. It is possible that leucocytopaenia was the result of increased removal of WBC from the blood, coupled with decreased bone marrow response.

Changes in the numbers of circulating monocytes, similar to the ones observed in the current study, have

previously been reported in sleeping sickness patients (Ross and Thomson, 1911) and in cattle infected with T.congolense and T.vivax (Moulton and Sollod, 1976; Saror et al., 1981). This might be expected, taking into account the large numbers of monocytes and macrophages present in tissues of infected animals (Morrison et al., 1983). The fact that monocytes primed for the production of cachectin/TNF increase at various stages of the T.brucei infection indicated that they could be playing a central role in the pathogenesis of the disease in these dogs.

In addition to alterations in haemopoietic parameters, dogs infected with T.brucei in the present studies developed marked subcutaneous oedema. Similar observations have been made in other domestic animals infected with trypanosomes (Losos and Ikede, 1972) and human patients infected with T.rhodesiense (Manson-Bahr and Chaters, 1963). Oedema could be caused by increased vascular permeability induced by biologically active substances or following stimulation of the kinin system.

Severe cachexia developed in the dogs, despite maintaining a good appetite. Similar observations have been made in other dogs (Mwambu, 1979; Sayer et al., 1979), cattle (Morrison et al., 1983) and rabbits (Rouzer and Cerami, 1980) infected with T.brucei, and in sleeping sickness patients (Koten and De Raadt, 1969). The cachexia observed in rabbits was associated with production of cachectin/TNF by activated macrophages. Since monocytes in T.brucei infected dogs were also primed for production of cachectin/TNF, this further indicates that cachectin/TNF

could be playing a major role in the pathogenesis of the cachexia observed in the present study.

Infected dogs also developed haemorrhagic gastroenteritis. Similar observations have been made in other dogs infected with T.brucei (Morrison et al., 1981a) and T.congolense (Parkin, 1935). Haemorrhagic gastroenteritis has been reported in sleeping sickness patients (Robins-Browne et al., 1975; Basson et al., 1977), and could therefore be important in the pathogenesis of acute trypanosomiasis in dogs and man.

Marked involvement of the eyes was observed in the present study. This has also been reported in other dogs naturally (Omamegbe et al., 1984) and experimentally (Mortelman and Nectens, 1975; Sayer et al., 1979) infected with T.brucei, cats (Mortelman and Nectens, 1975) and sheep infected with T.brucei (Ikede, 1974), and in horses and goats infected with T.rhodesiense (Yorke, 1910).

Ocular clouding has been associated with leakage of protein into the anterior chamber following increased vascular permeability (Mortelman and Nectens, 1975), blockage of the drainage system of the eyes, and in chronic cases, damage to the optic nerves.

The present study demonstrated that T.brucei causes an acute multisystemic disease in dogs, characterised by major changes in haematological parameters, severe cachexia, lymph node and splenic enlargement, and panophthalmitis. Several mechanisms, some involving the trypanosomes or substances generated by them, and others resulting from the host response to parasitisation, appear to be responsible

for the clinical and haematological observations. While it is impossible to pinpoint the precise mechanisms involved, immunological responses by the dogs, directed to the trypanosomes, would appear to be the most likely basis from which tissue changes begin.

TABLE 3.1.

CACHECTIN/TUMOUR NECROSIS FACTOR ACTIVITY (pg/ml) IN WHOLE BLOOD IN RESPONSE TO ENDOTOXIN DURING THE FIRST FOUR DAYS OF INFECTION WITH T.brucei.

DAY OF INFECTION	DOSE OF ENDOTOXIN		
	10 ng/ml	1 ng/ml	0.1 ng/ml
0	29.000 ± 25.5*	13.875 ± 6.13	6.250 ± 4.23
2	84.000 ± 44.1	27.375 ± 20.3	10.625 ± 9.09
4	103.880 ± 37.1	56.500 ± 20.4	9.500 ± 10.70

* - Values represent the mean ± 1SD of samples from 8 dogs.

Figure 3.1. Mean rectal temperature (●) and parasitaemia (O) in dogs infected with T.brucei. There was rapid rise in body temperature following appearance of trypanosomes in the blood.

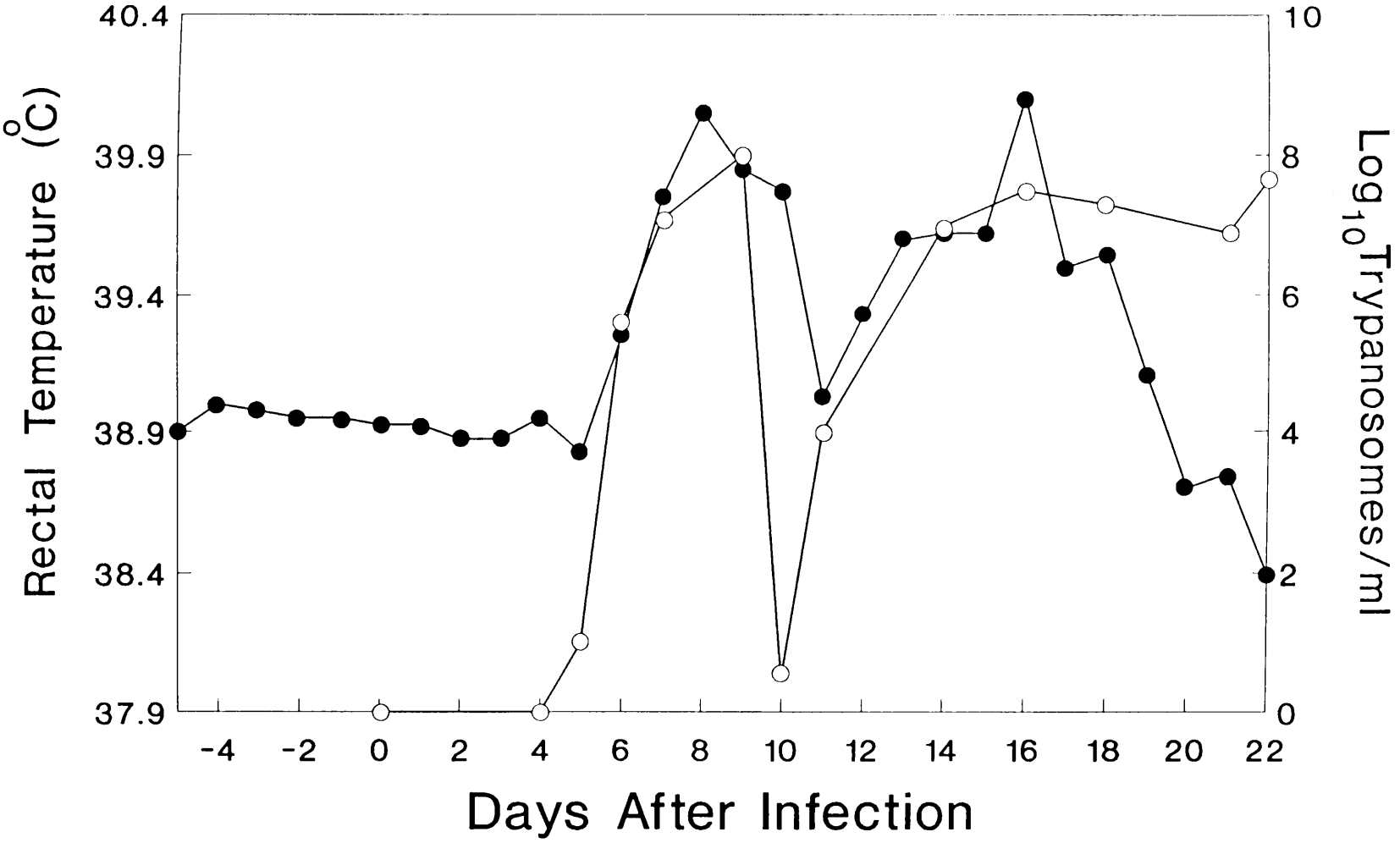

Rectal Temperature (°C)
40.4
39.9
39.4
38.9
38.4
37.9
Log10Trypanosomes/ml
10
8
6
4
2
0
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
Days After Infection

Figure 3.2. Mean body weight changes in dogs infected with T.brucei. There was rapid decrease in body weight after day 4 of infection.

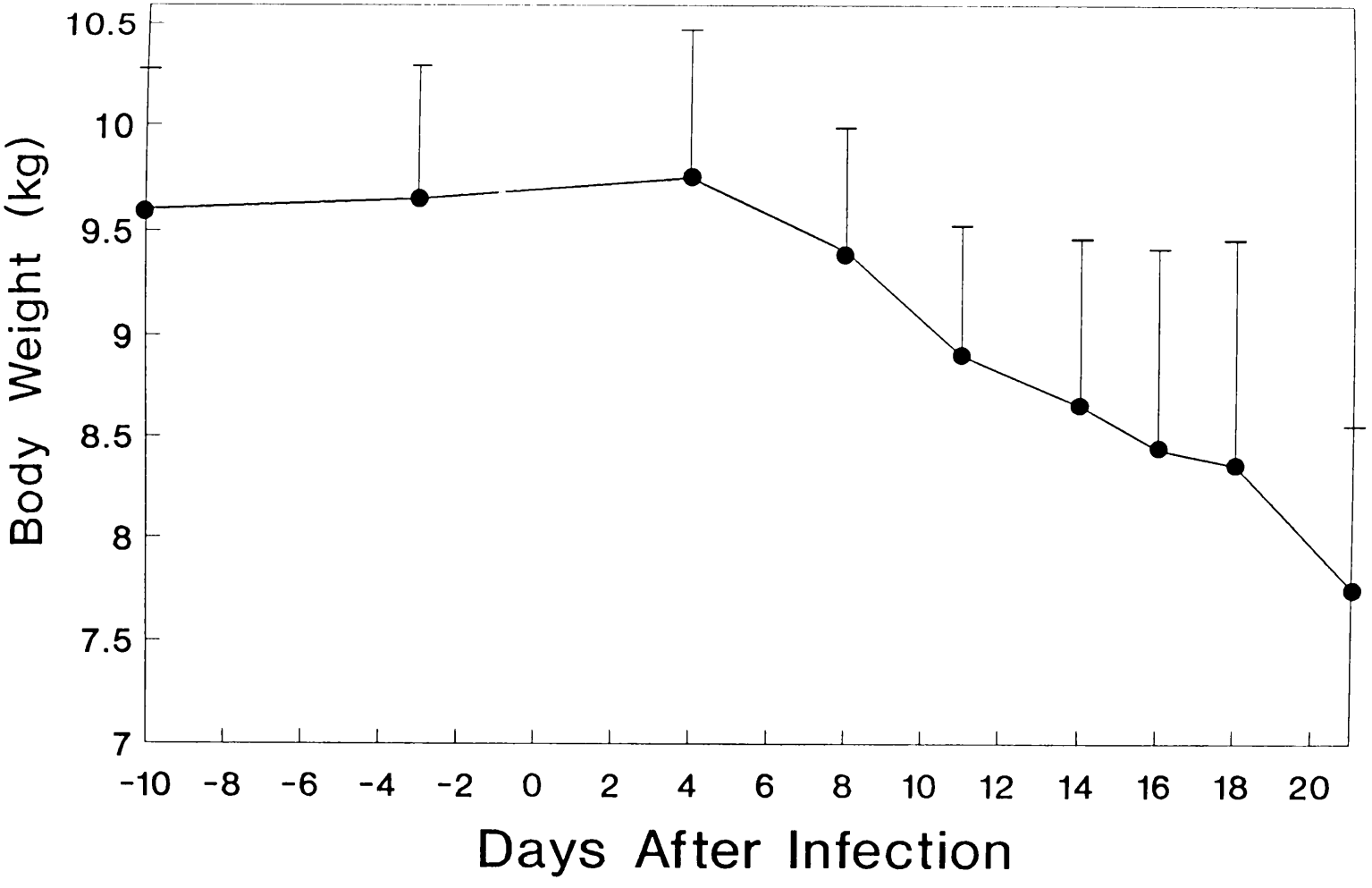

Body Weight (kg)
10.5
10
9.5
9
8.5
8
7.5
7
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Days After Infection

Figure 3.3. Packed red cell volume (PCV) (mean ± 1SD) in dogs infected with T.brucei. The PCV decreased rapidly during week 2 of infection, after which it became more gradual up to termination of the study on day 24.

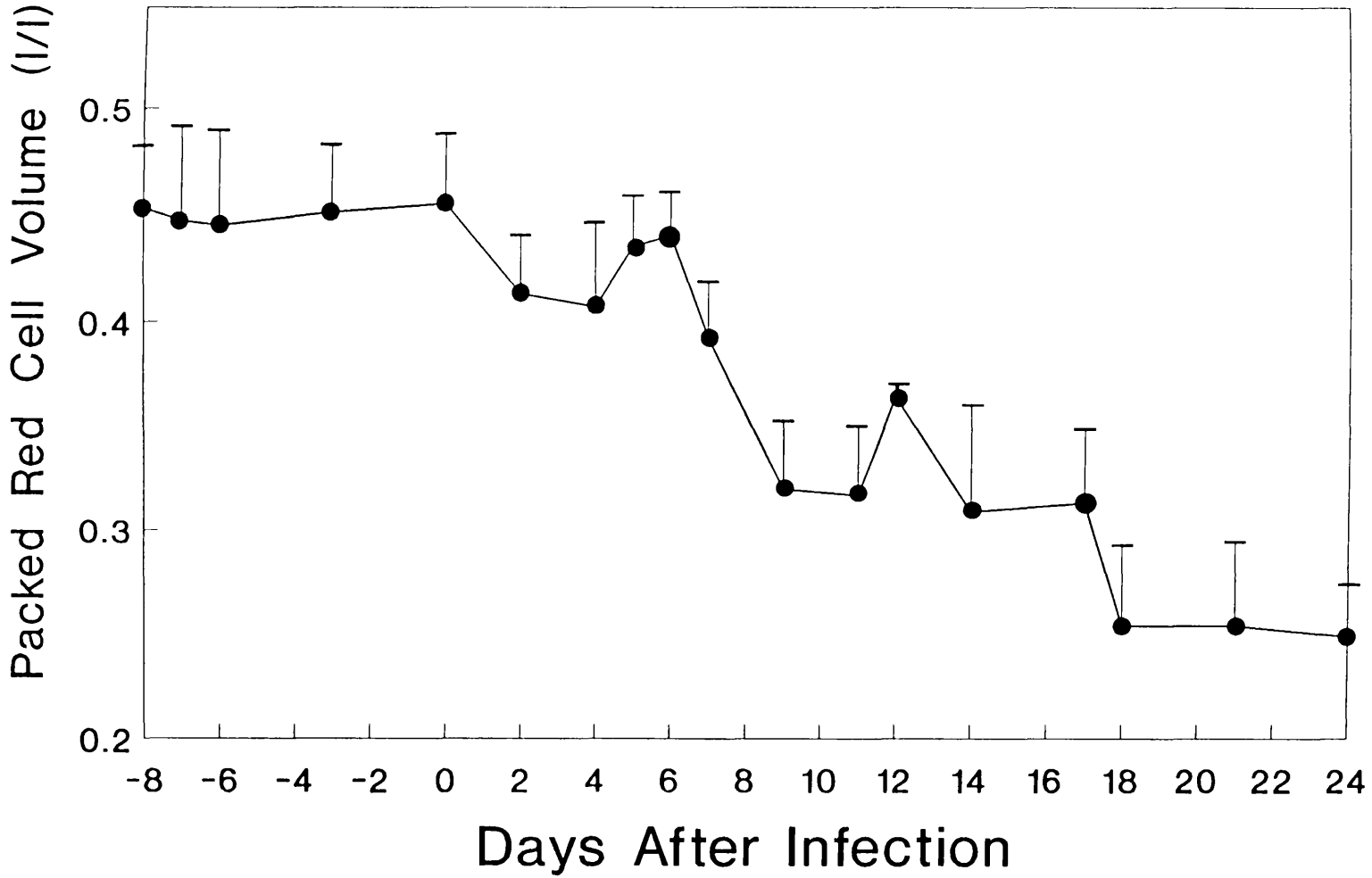

Packed Red Cell Volume (l/l)
0.5
0.4
0.3
0.2
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.4. Number of red blood cells (RBC) (mean ± 1SD) in dogs infected with *T.brucei*.

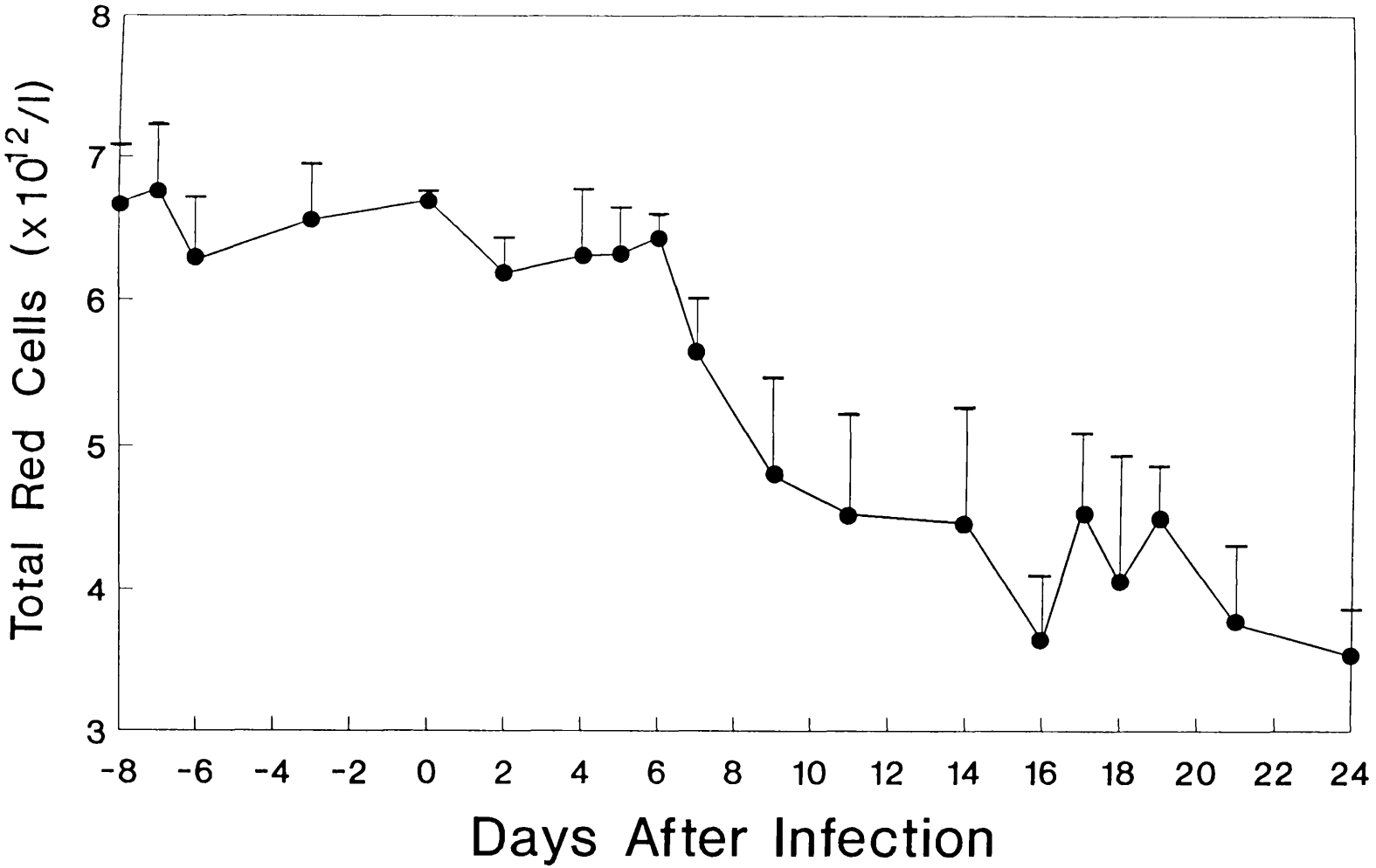

Total Red Cells (x10^12/l)
8
7
6
5
4
3
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.5. Haemoglobin concentration (mean ± 1SD) in dogs infected with T.brucei.

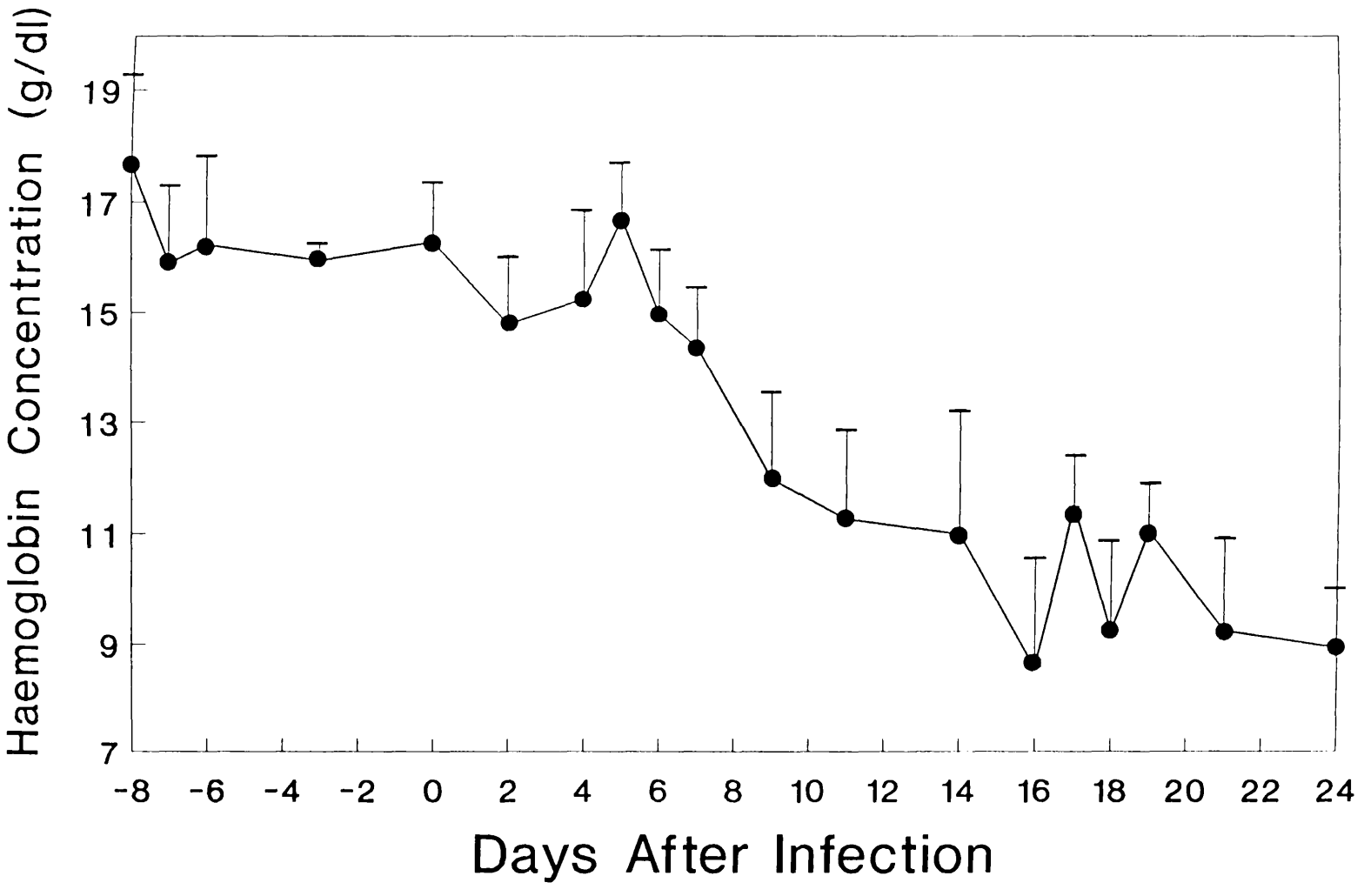

Haemoglobin Concentration (g/dl)
19
17
15
13
11
9
7
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.6. Mean corpuscular volume (MCV) (●) and number of reticulocytes (O) (mean ± 1SD) in dogs infected with T.brucei.

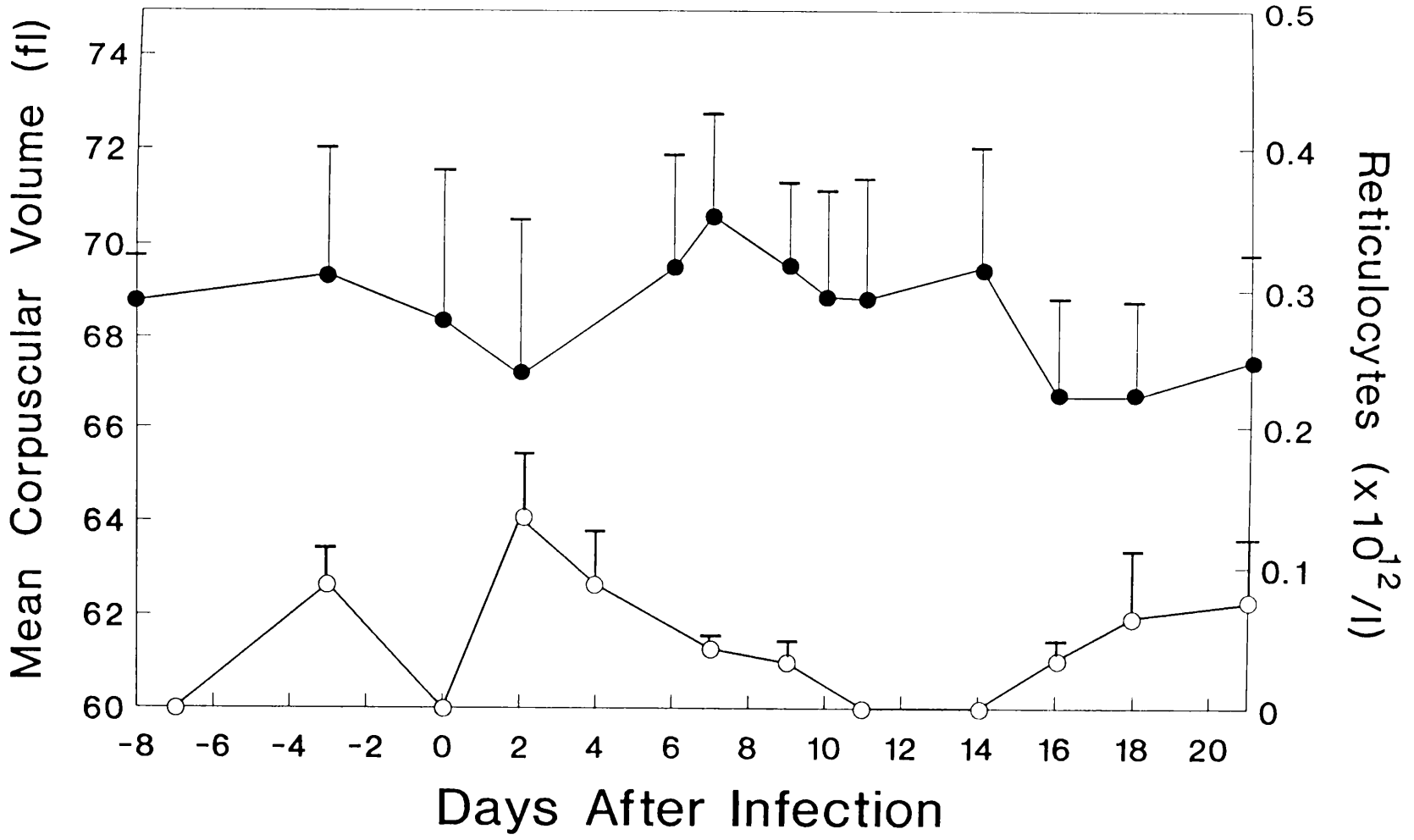

Mean Corpuscular Volume (fl)
74
72
70
68
66
64
62
60
0.5
0.4
0.3
0.2
0.1
0
Reticulocytes (x10^12/l)
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Days After Infection

Figure 3.7. Number of platelets (mean ± 1SD) in the blood of dogs infected with T.brucei. There was rapid decrease in platelets after day 4 of infection. The numbers remained low throughout the rest if the infection period.

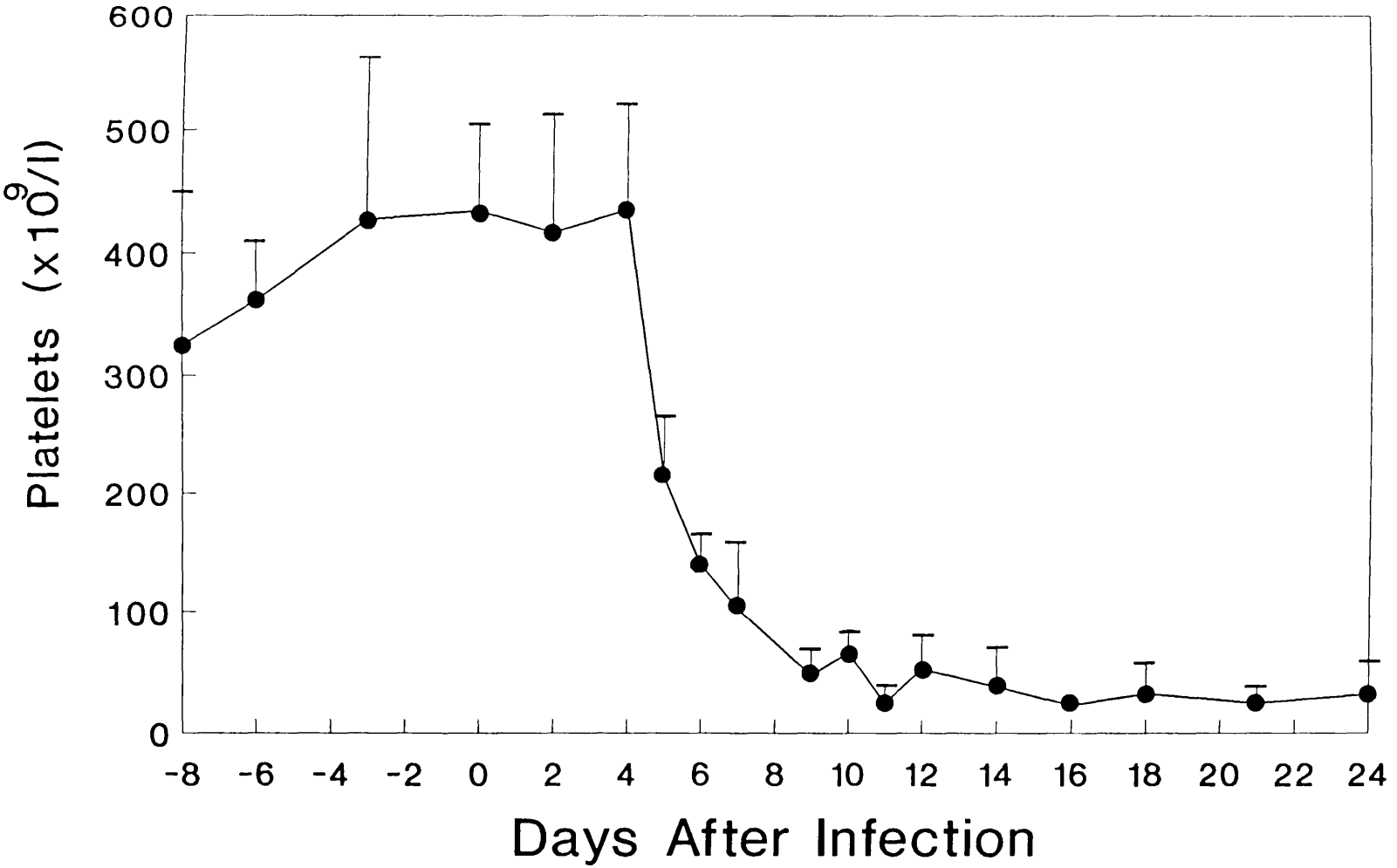

600
500
400
300
200
100
0
Platelets (x10^9/l)
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.8. White blood cells (WBC) (mean ± 1SD) in dogs infected with *T.brucei*. A leucocytopaenia developed after day 4 and persisted throughout the rest of the infection period.

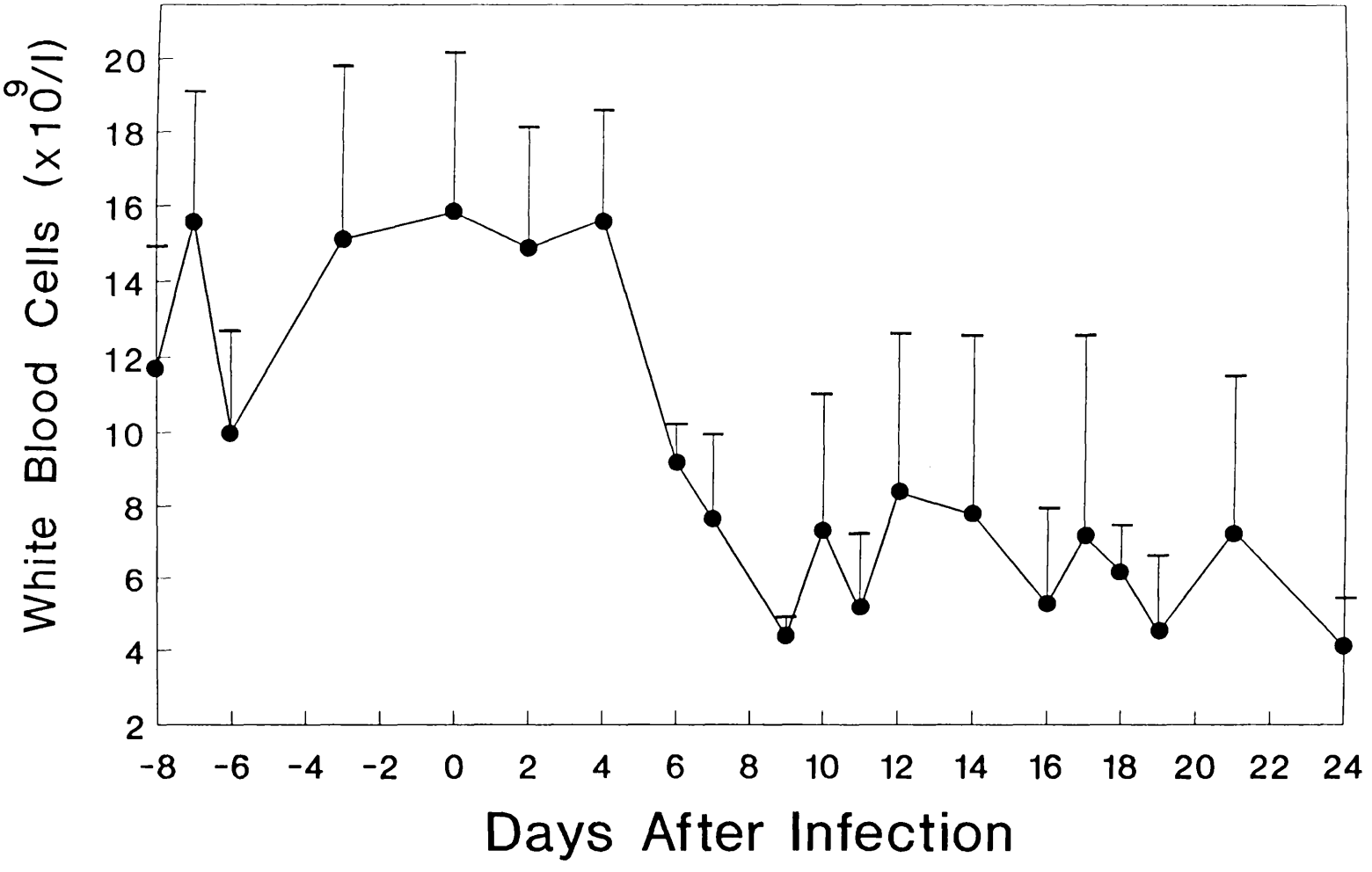

White Blood Cells (x10^9/l)
20
18
16
14
12
10
8
6
4
2
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.9. Number of lymphocytes in the blood of dogs infected with *T.brucei*. Lymphocyte numbers decreased during the course of the disease.

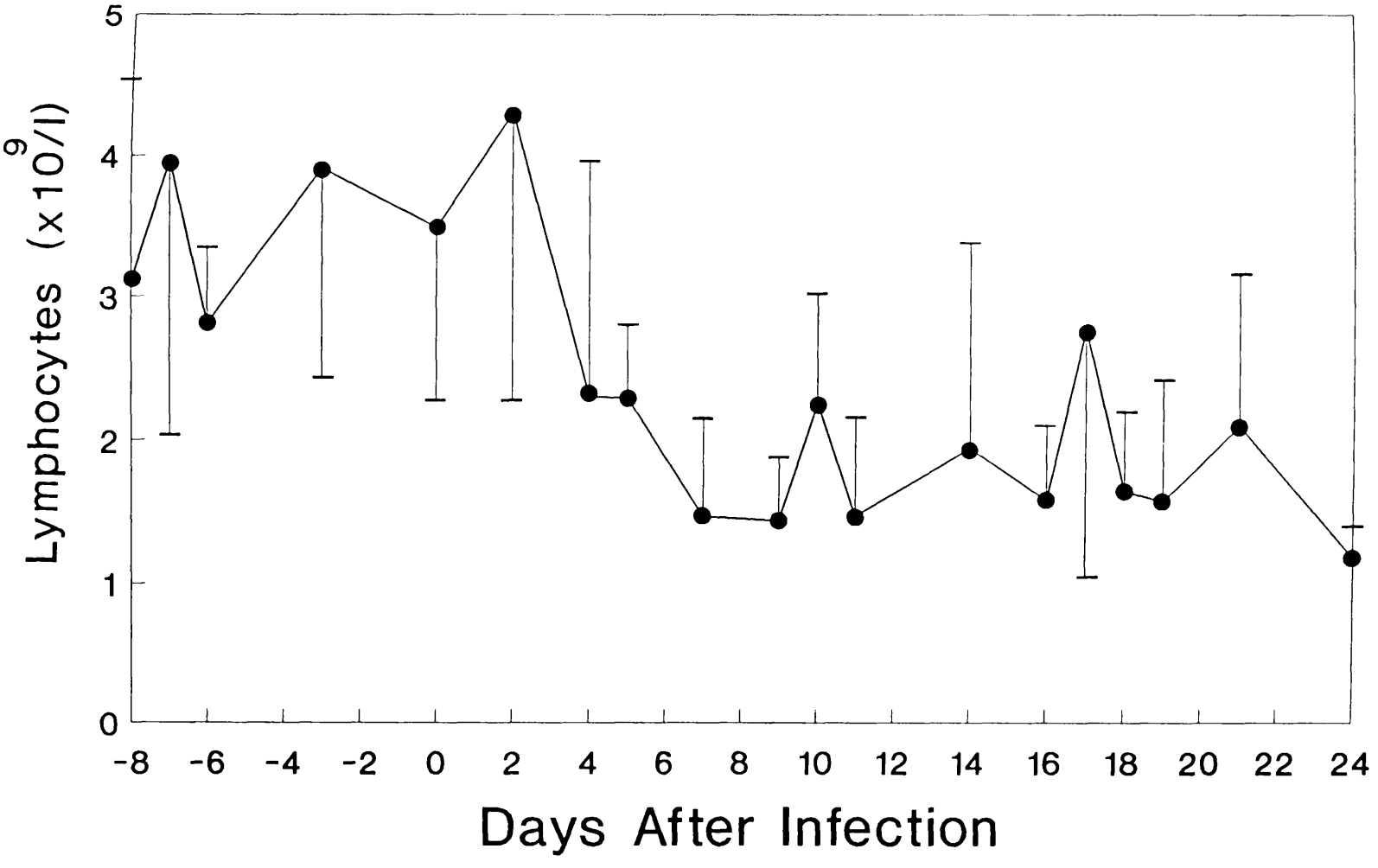

Lymphocytes (x10/l)
9
5
4
3
2
1
0
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.10. Number of neutrophils in the blood of dogs infected with T.brucei. Neutrophil numbers decreased during the course of the disease.

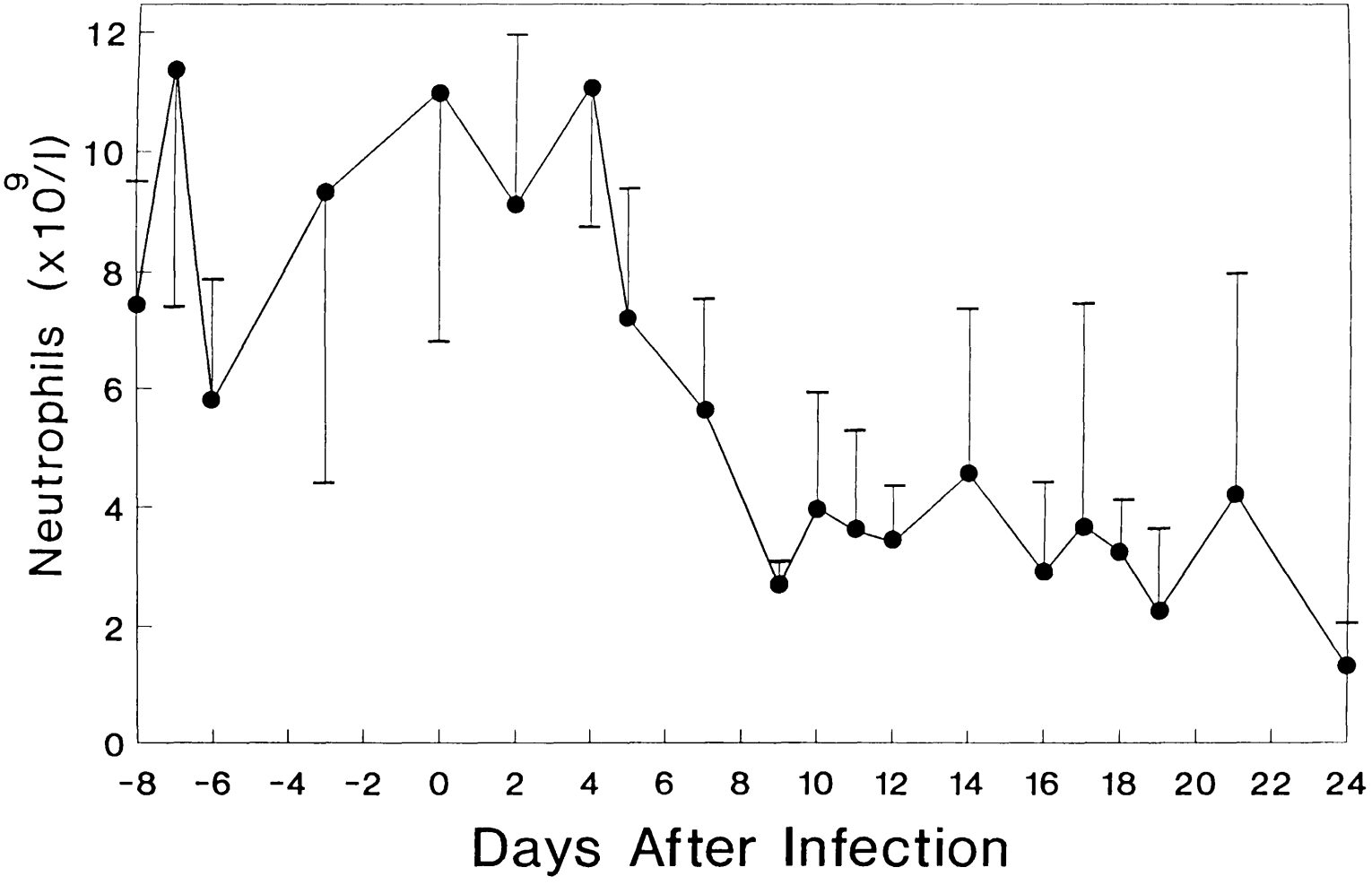
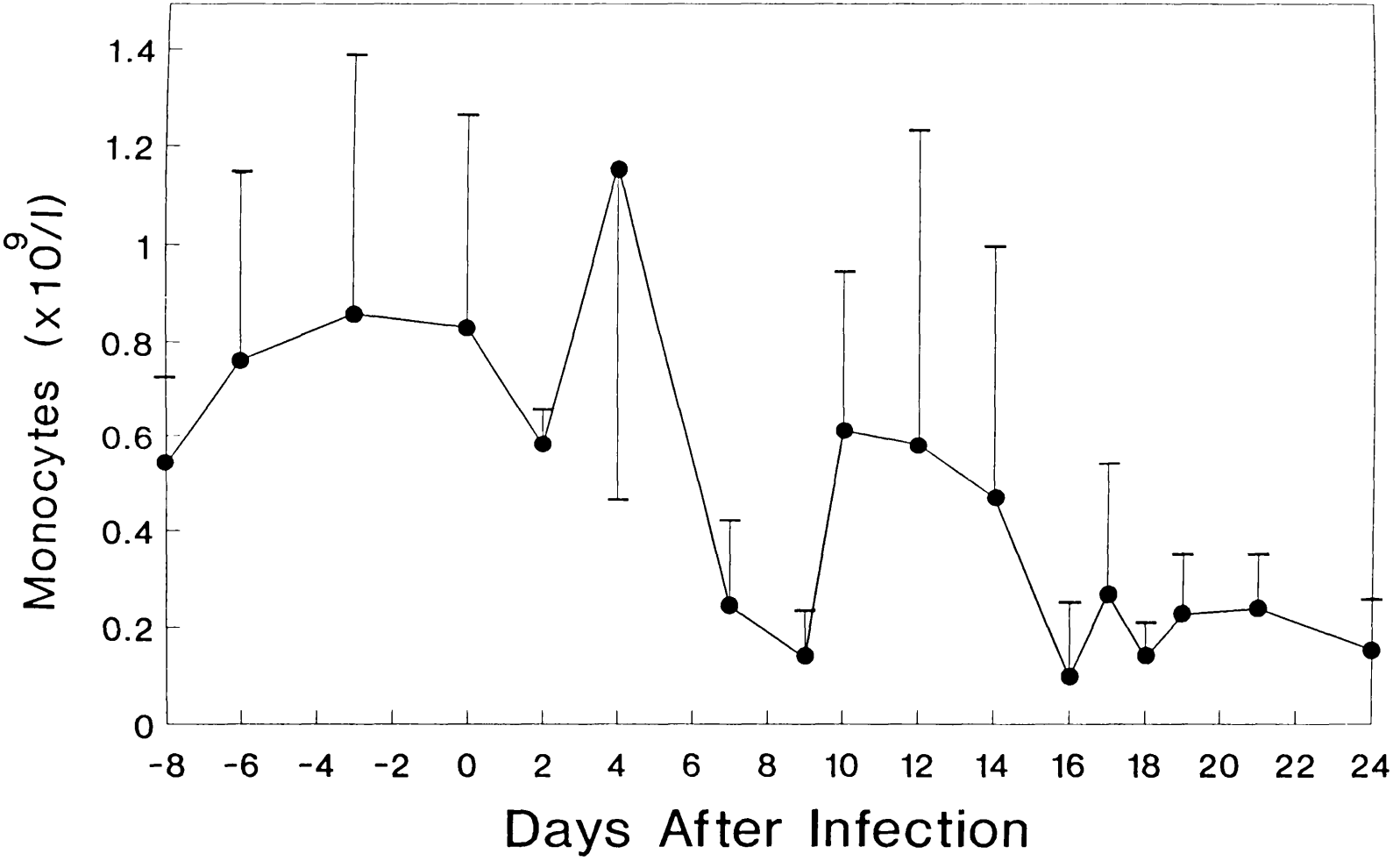

Neutrophils (x10^9/l)
12
10
8
6
4
2
0
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

Figure 3.11. Number of monocytes (mean ± 1SD) in the blood of dogs infected with T.brucei. There was a rapid increase in monocytes on day 4 of infection, followed thereafter by a decrease. Between days 10 and 14, another transient increase in monocyte numbers occurred.


Monocytes (x10^9/l)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Days After Infection

CHAPTER 4.

CLINICAL, ELECTROCARDIOGRAPHIC AND ECHOCARDIOGRAPHIC FEATURES OF CARDIAC DAMAGE IN DOGS INFECTED WITH T.brucei.

4.1. INTRODUCTION.

Infection of dogs with T.brucei results in an acute disease syndrome, affecting most of the body systems (Morrison et al., 1981a,b). During the course of the disease, the dogs develop clinical signs which among others indicate involvement of the cardiovascular system (Sayer et al., 1979). If the dogs are not treated, they invariably die in the fourth week, with pancarditis being a major histopathological finding (Morrison et al., 1981a; Kaggwa et al., 1983).

In veterinary medicine, the clinical signs and pathology of heart damage appear to be similar to some forms of human African trypanosomiasis. Thus, clinical and electrocardiographic (ECG) abnormalities have been demonstrated in human patients infected with T.rhodesiense (Manuelidis et al., 1965; Jones et al., 1975) and T.gambiense (Bertrand et al., 1971; Francis, 1972). In this respect, there have been reports of humans with African trypanosomiasis dying of congestive heart failure (Manson-Bahr and Charters, 1963).

The pathogenesis of cardiac damage in T.brucei infected dogs, and in human African trypanosomiasis, is poorly understood. An understanding of the mechanisms that lead to the trypanosome-induced pancarditis might provide an insight to improved forms of treatment and management of cardiac damage.

In order to identify alterations in cardiac function that accompany the disease at an early stage, and to follow up cardiac response to trypanocidal drug treatment, it is

necessary that improved, non-invasive diagnostic techniques be employed. Until recently, investigations have been limited to auscultation, radiography and ECG. While ECG gives a good indication of the changes in electrical activities in the heart (Tilley, 1985), echocardiography allows for the visual observation of anatomical and functional abnormalities in the heart (Roelandt, 1983; Ch.2).

The purpose of the present section of the work was to determine clinically, the functional and anatomical cardiovascular changes that result from infection of dogs with T.brucei.

4.2. MATERIALS AND METHODS.

4.2.1. ANIMALS.

The dogs, their management, and the stabilate of T.brucei used to infect them have been described before (Ch. 2). Briefly, 10 dogs were infected by intravenous inoculation with approximately 5×10^3 T.brucei GVR35/c.1. Before and during the course of the disease, daily clinical examinations were performed. Two dogs were euthanised on days 10, 15, 21, 22 and 26. A full necropsy was carried out and heart tissue samples were collected for histopathological and ultrastructural investigations (Ch. 8). Four dogs served as uninfected controls and were euthanised after the last pair of infected dogs.

4.2.2. CARDIOVASCULAR STUDIES.

Before and during the course of the disease, a detailed clinical examination of the thoracic cavity was performed

at regular intervals. The methods employed included auscultation and palpation, ECG and echocardiography.

4.2.2.1. Auscultation and palpation:

Auscultation of the thoracic cavity was done daily, with the dog in a standing position. The rate, rhythm and strength of heart beat were determined. The audibility of the heart beat was assessed and any abnormal sounds or murmurs noted. The murmurs were classified according to the method described by Gompf (1985). The intensity of murmurs was graded from 1/6 to 6/6.

Palpation of the thoracic cavity was done to identify the point of maximal intensity of the heart beat, changes in the intensities of heart beat, and the presence or absence of a thrill. In addition, the rate and quality of the pulse was determined by palpation of the femoral artery. The respiratory rate, and character of lung sounds were also evaluated.

4.2.2.2. Electrocardiography:

Electrocardiography was performed at least twice a week with a six-lead electrocardiographic apparatus (Siemens, Germany). The dogs were laid on a non-conducting surface, in right lateral recumbency, with their legs extended perpendicular to the trunk (Ettinger and Suter, 1970). Electrodes to the forelegs were attached on the skin at the region of the olecranon process and those to the hindlegs the stifle joints. Electrode gel was used to improve electrical contact between electrodes and the skin.

Electrocardiograms were recorded at paper speeds of 25

and 50 mm/sec and a voltage of 1mV. Standard bipolar limb-lead II tracings were used for analysis of the electrical activities in the heart (Ettinger and Suter, 1970). The other limb-leads were used for determination of the changes in mean electrical axis of the heart.

4.2.2.3. Echocardiography:

Two-dimensional (2D), M-mode and Doppler echocardiography was performed on the dogs at least twice a week. The equipment and techniques used have been described in Chapter 2. Briefly, the dog was laid in right lateral recumbency on a table. A 5.0 MHz mechanical transducer was placed on the right lateral thoracic wall between the third and fifth intercostal spaces, close to the costochondral junction and 2D and M-mode echocardiograms recorded.

From measurements of left ventricular dimensions, left ventricular function (LVF) was determined by calculation of the fractional shortening (FS). Changes in the anatomy of the heart during the study period were also investigated by 2D and M-mode echocardiography. Involvement of the heart valves was assessed by determination of the presence or absence of incompetence, using pulsed-wave (PW) Doppler (Ch. 2). The characteristics of blood flow through the mitral and aortic valves were studied from the subcostal and suprasternal windows. The results obtained were used to evaluate the changes in cardiac function with progress of the disease.

4.3. RESULTS.

4.3.1. AUSCULTATION.

Clinical signs indicating involvement of the cardiovascular system became apparent 6 days after infection, at which time the dogs developed marked tachycardia. The heart rate increased, to 180 ± 17.2 beats per minute (BPM) (mean ± 1SD) in some dogs, as compared to 131 ± 24.4 BPM in uninfected control dogs. This coincided with the onset of fever and first parasitaemia. The tachycardia was coupled with increased respiratory rate, which at day 6 increased to 45 ± 5 cycles per minute (CPM) as compared to the uninfected control dogs, which had a rate of 32 ± 3 CPM.

On day 10 of infection, in addition to tachycardia, loud heart beats were heard on auscultation of the thoracic cavity. At the same time, sinus arrhythmia was detected in 5 of the dogs. Mild functional systolic murmurs (grade 2/6) were heard in the aortic region (fourth intercostal space on the left side). Three dogs developed a harsh, intermittent choking cough.

From day 12 of infection, systolic murmurs of varying intensity (grades 1/6 and 2/6) were heard in the mitral (fifth intercostal space on the left side) and sometimes the tricuspid (fourth intercostal space on the right side) regions. This indicated the possible presence of mitral incompetence (MI) and tricuspid incompetence (TI). By day 13, the murmurs were more audible, in some dogs the audibility being as high as grade 3/6.

From day 12, the heart beats gradually became

irregular. Six dogs developed marked sinus bradycardia after day 14 of infection (65 ± 10 BPM on day 19), with prolonged periods of asystole, and occasional missed beats were heard from day 13 of infection. At this time, mucous membranes became moderately pale and there was an increase in the capillary refill time to 3 seconds.

By day 16 of infection, the frequency of missed beats had increased, and murmurs of MI and TI were heard in almost all the dogs. In some cases, the periods of asystole had increased to more than 2 seconds. From day 19 of infection, the audibility of the heart sounds on auscultation decreased progressively up to termination of the study on day 26.

By day 18 of infection, 3 dogs developed inspiratory dyspnoea accompanied by a soft, non-productive cough, which later progressed to being productive. The respiratory rate dropped to 15 ± 2 CPM, with marked abdominal breathing. After day 22, both inspiratory and expiratory dyspnoea developed and coughing became more frequent, persisting up to termination of the study on day 26.

4.3.2. ELECTROCARDIOGRAPHIC FINDINGS.

In addition to confirming the changes in heart rate and rhythm heard on auscultation, ECG revealed significant abnormalities in electrical activity in the hearts of dogs infected with T.brucei. There were changes in P-R intervals, QRS complexes, S-T segments and R-R intervals. These changes rarely occurred in one dog at the same time, but were picked up on ECG tracings recorded at different periods. Figure 4.1 shows a normal six-lead ECG tracing

from an uninfected dog.

The first ECG indication of impaired electrical activity in the heart was prolongation of P-R intervals, which in some dogs was seen as early as day 9 of infection. Prolongation of P-R intervals rapidly progressed to first degree heart block (IHB) by day 12. In 3 dogs the IHB persisted throughout the infection period, while the other 6 had developed second degree heart block (IIHB) before day 21 of infection (Fig. 4.2). The IIHB appeared initially as an occasional missed beat, occurring after a period of more than one minute. With progress of the disease, the missed beats were recorded at higher frequencies, sometimes up to 10 missed beats a minute. In one dog there were episodes of complete HB from day 17 of infection (Fig. 4.3).

Prolongation of R-R intervals was noted during week 2 of infection. This appeared earliest in dogs which originally had a normal sinus arrhythmia before being infected. In some dogs, periods of asystole of up to 2.6 seconds were recorded (Fig. 4.4). Prolongation of R-R intervals was recorded in 6 dogs, progressing to episodes of sinus arrest in 2 them.

Towards the end of week 3, the voltage of R waves decreased, gradually at first, and then rapidly in the terminal stages of the disease (Table 4.1). The drop in R wave voltage was directly related to the decrease in intensity of heart sounds noted on auscultation.

Changes in the S-T segment were recorded at the end of week 3 and during week 4 of infection. In week 4

particularly, S-T segment elevation or depression of more than 0.2mV was consistently recorded in most of the dogs (Fig. 4.5). In 2 animals, notching of R waves occurred on leads II, III, aVL and aVF from day 22, becoming more prominent with progress of the disease (Fig. 4.6).

Other ECG abnormalities occasionally and inconsistently recorded included deep Q waves, notched and biphasic P waves.

4.3.3. ECHOCARDIOGRAPHIC FINDINGS.

4.3.3.1. 2D studies:

During the first 3 weeks of infection there were no observable changes in the heart by 2D echocardiography. After day 18, progressive increase in PE occurred. This coincided with the decrease in R wave amplitudes recorded on ECG, and the poor audibility of heart sounds on auscultation. The increase in PE became more conspicuous after day 22 of infection, appearing as an echo-free zone surrounding the heart (Fig. 4.7). In addition, the mitral valve leaflets were thickened from day 15 of infection.

4.3.3.2. M-mode studies:

M-mode investigations revealed changes in mitral valve motion and in LVF. In dogs that did not develop HB or sinus arrest as determined by ECG, normal mitral valve motion on M-mode was maintained up to about day 16 of infection, after which valve motion during periods of early ventricular filling (E peak) became equal to that caused by late ventricular filling (atrial systole) (A peak). The E-F slope was also markedly increased, an indication of poor

diastolic function.

For the dogs that developed sinus arrest, 2D and M-mode echocardiography demonstrated periods of asystole when the mitral valve would remain open for prolonged intervals before finally closing (Fig. 4.8).

From day 10 of infection, an increase in contractility of the heart, indicated by increased FS, was observed on M-mode (Table 4.2). At mid-infection on days 14 and 18, the FS had gone back to normal, but the dogs still had a tachycardia. On day 22, however, a significant reduction in FS occurred, and was associated with the fall in the heart rate (Table 4.2).

4.3.3.3. Doppler studies:

Doppler echocardiography revealed abnormalities in valve function and blood flow dynamics at various stages in the disease process. By PW Doppler, MI and TI were demonstrated in 8 dogs by day 11 of infection (Figs. 4.9, 4.10, 4.11). MI and TI were usually identified by PW Doppler 1 to 2 days before the murmurs of MI, and 3 to 4 days before the murmurs of TI were heard on auscultation. The severity of MI and TI increased with progress of the disease, such that by day 22 of infection, the regurgitant jets were picked up deep in the atria, away from the atrioventricular valves.

Almost coinciding with initial observation of MI and TI, AI was demonstrated by PW Doppler, both from the right parasternal window (Fig. 4.12) and from the subcostal window (Fig. 4.13). As for MI and TI, AI became more intense with increased severity of the disease. From day 14

of infection, pulmonary incompetence (PI) was also detected. The PI jets were more difficult to demonstrate, in comparison with the regurgitant jets of MI, TI and AI.

In dogs that developed IIHB, moderate MI was observed during the periods of prolonged asystole (Fig. 4.14). Similar valvular incompetence occurred in the same dogs on the tricuspid valves but not the pulmonary and aortic valves.

At the same time, PW Doppler demonstrated a gradual increase in the velocity of mitral blood flow during the period of atrial systole (A peak) with increased severity of the disease. This resulted in an increase in the A:E ratio (ratio of atrial systolic blood flow velocity to passive mitral diastolic blood flow velocity), indicating abnormal diastolic function (Table 4.3).

The changes in peak aortic blood flow velocity recorded by CW Doppler during the course of T.brucei infection are shown in Table 4.4. From day 10 of infection, increased aortic blood flow velocity was noted. This coincided with tachycardia and increased LVF observed on M-mode, and development of functional systolic murmurs in the aortic region of the thoracic wall on auscultation.

With progress of the disease, peak aortic blood flow velocity decreased below normal values, and continued to deteriorate up to termination of the study on day 22 of infection (Table 4.4). The decrease in aortic blood flow velocity accompanied the fall in heart rate. In order to minimise stress due to handling, Doppler studies were not performed on the dogs after day 22 of infection.

The control dogs showed normal clinical, ECG and echocardiographic features, similar to the pre-infection ones indicated here.

4.4. DISCUSSION.

The present study demonstrated that T.brucei infection in dogs results in major abnormalities in cardiac function. The abnormalities increased as the disease progressed, and probably contributed significantly to the pathogenesis of the general disease process.

Cardiac damage in dogs infected with T.brucei has previously been clinically identified (Sayer et al., 1979; Morrison et al., 1983). Clinical evidence of cardiac damage, though of a less severe nature than was seen in dogs, has been reported in human patients infected with T.rhodesiense (Manson-Bahr and Charters, 1963; Manuelidis et al., 1965; Ormerod, 1970; Jones et al., 1975; Blackett and Ngu, 1976) and T.gambiense (Bertrand et al., 1971; Francis, 1972; Mbala et al., 1988) and in rats infected with T.brucei (Murray et al., 1974).

Echocardiography and ECG, in addition to confirming observations made on auscultation of the thoracic cavity, revealed functional and anatomical abnormalities in the heart, some of which it would have been impossible to identify using other non-invasive techniques. Although ECG has been widely used as a diagnostic tool in human African trypanosomiasis (Bertrand et al., 1971; Francis, 1972; Bertrand, 1987; Mbala et al., 1988) and in dogs infected with T.brucei (Sayer et al., 1979), echocardiography is a

relatively new technique and only 2D echocardiography has previously been performed in human patients infected with T.gambiense (Mbala et al., 1988).

In the present study, infection of dogs with T.brucei resulted in early tachycardia, soon after the first parasitaemia peak and the development of fever. Similar observations in dogs were made by Sayer et al. (1979). Tachycardia is usually an expected physiological response to pain, emotional stimuli, profound anaemia, or inadequate tissue perfusion caused by hypovolaemia or serious underlying heart disease (Sisson, 1988). Since T.brucei infection in dogs results in massive inflammatory reactions in the heart and other body organs (Morrison et al., 1981a,b; Ch. 8) and fever, this could have caused pain, and hence the tachycardia. In another study in these dogs, the profound anaemia that would cause inadequate tissue perfusion did not occur until after day 8 of infection, at least 2 days after the onset of tachycardia (Ch. 3), indicating that anaemia was probably not the cause of tachycardia in the early stages of the disease.

The tachypnoea observed was probably caused by pulmonary oedema. Tachypnoea has been reported in dogs with mild accumulations of lung fluids (Ware and Bonagura, 1988). In general, pulmonary oedema can be caused by elevated pulmonary capillary hydrostatic pressure secondary to left atrial pressure, excessive pulmonary blood flow secondary to anaemia, severe hypoproteinaemia, or increased capillary permeability (Ware and Bonagura, 1988).

Capillary permeability can increase as a result of a

number of factors such as endotoxic shock, anaphylaxis, immune complex disease, direct damage to lung tissue, or indirect injury mediated by vasoactive components of the clotting cascade (Ware and Bonagura, 1988). T.brucei, due to its ability to invade tissues (Morrison et al., 1983), may have caused direct damage to lung tissue and hence increased capillary permeability. At the same time, immune complexes have been demonstrated in the kidneys of dogs infected with T.brucei (Kaggwa et al., 1983; Ch. 8), while antigen-antibody reactions in T.brucei infected rabbits were shown to cause release of substances that activate the coagulation system (Boreham and Goodwin, 1969). Lysed T.congolense also release vasoactive substances when implanted intraperitoneally in rats (Tizard and Holmes, 1977). In the present study, tachypnoea followed the appearance of parasitaemia, and could therefore have been due to increased capillary permeability resulting from immune reactions, direct damage of lung tissue by the parasites, or mediated by vasoactive components released by parasites.

The loud heart sounds and functional systolic murmurs heard by day 10 of infection were due to increased blood flow velocity through the ascending aorta, as demonstrated by echocardiography. Increased blood flow velocity may be caused by fever, increased contractility of the heart, or anaemia (Gompf, 1988). In dogs infected with T.brucei, increased LVF, fever and severe anaemia were present by day 10 of infection (Ch. 3), and probably accounted for the observed increase in blood flow velocity.

Echocardiography revealed incompetence from all heart valves by day 11 of infection. This was evidence that functional impairment of the heart in dogs infected with T.brucei begins very early in the disease. There is histological evidence of early valve damage in dogs infected with T.brucei, including oedema, connective tissue damage, and cellular and trypanosome infiltration into the valve tissue (Morrison et al., 1981a; Ndung'u et al., 1989; Ch. 8). Similar involvement of all cardiac valves was confirmed histologically in humans infected with T.rhodesiense (Poltera et al., 1976), vervet monkeys infected with T.rhodesiense or T.brucei (Poltera et al., 1985), and in mice infected with T.brucei (Poltera et al., 1980). It is possible that oedema and cellular infiltration in the valves made them poorly flexible, preventing effective closure of valve orifices, and hence incompetence. Valvular oedema and cellular infiltration could also have been the cause of the valve thickening observed by 2D echocardiography.

Abnormalities in generation and conduction of electrical impulses in the hearts of infected dogs were demonstrated by ECG from day 10 of infection. Failure of impulse generation by the sinuatrial node (SAN), eventually leading to sinus arrest, is an indication of possible damage to the nodal tissue, or nodal inhibition by excessive vagal stimulation (Tilley, 1985). In T.brucei pancarditis in dogs, the atria are more severely damaged than the ventricles (Kaggwa et al., 1983; Ch. 8), and since the SAN forms part of the atrial tissue, primary damage to

the node is most likely.

Failure of the SAN to fire electrical impulses on time can cause fainting, especially if a lower pacemaker focus fails to take over the generation of impulses (Edwards, 1987; Miller and Tilley, 1988). In the current study, fainting was not observed, possibly because the episodes of sinus arrest were not prolonged. In another study in these dogs, extreme weakness occurred during week 4 of infection (Ch. 3), possibly because of poor tissue oxygenation resulting from periodic sinus arrest and the severe anaemia present at that time.

Progressive increase in P-R intervals on ECG, eventually leading to complete HB, indicated impaired conduction of impulses through the atrioventricular node (AVN) and the bundle of His. Similar abnormalities in impulse conduction were observed in humans infected with T.rhodesiense (Jones et al., 1975) and T.gambiense (Bertrand et al., 1971; 1987; Mbala et al., 1988).

In dogs, HB can result from bacterial endocarditis, infiltrative or hypertrophic cardiomyopathy, and hyperkalemia, and can cause death (Sisson, 1988). In dogs infected with T.brucei, the marked inflammatory reactions, including cellular infiltration and oedema that result (Morrison et al., 1981a; Ch. 8) could have caused the observed HB. The HB possibly contributed to the features of heart failure observed in terminal stages. Histological evidence of damage to the AVN and the bundle of His was demonstrated in the hearts of humans infected with T.rhodesiense (Poltera et al., 1976) and rats infected with

T.brucei (Poltera et al., 1980).

Echocardiography revealed leakage of blood through the atrioventricular valves in the dogs with IIHB and sinus arrest, due to imperfect closure of the valves during the periods of asystole. Imperfect closure of the mitral valves in dogs with HB, as observed in the present study, has previously been demonstrated by M-mode in other clinical conditions, but no Doppler studies were performed (Moise, 1988). The significance of the leakage is not known. It is possible that leakage of blood into the atria during the period of asystole resulted in decreased ventricular preload, and therefore decreased cardiac output. Considering that the dogs also had MI at this stage of the disease, damming of blood in the left atrium may have resulted in increased left atrial pressure, leading to elevated pulmonary capillary pressure, and hence pulmonary oedema. This possibly contributed to the coughing which occurred after day 18 of infection. Interstitial and alveolar oedema was confirmed histologically in the dogs euthanised from day 21 of infection (Ch. 8).

Rapid accumulation of PE, confirmed by echocardiography, occurred in terminal stages of the disease. This must have been the cause of decreased intensity of heart sounds at the time. Clinical features indicating accumulation of PE have been reported in human patients infected with T.rhodesiense (Manson-Bahr and Charters, 1963; Manuelidis et al., 1965) and T.gambiense (Mbala et al., 1988), and in dogs infected with T.brucei (Morrison et al., 1981a; Sayer et al., 1979; Kaggwa et al.,

1983; Ch. 8), vervet monkeys infected with either T.rhodesiense or T.brucei (Poltera and Sayer, 1983), and in rats infected with T.brucei (Murray et al., 1974).

While accumulation of PE most likely resulted from an inflammatory response to the pericarditis that accompanies the infection, it might also have been exacerbated by impaired drainage of the heart tissue due to obstruction of lymphatic vessels. Obstruction of lymphatic vessels was observed in the hearts of other dogs (Morrison et al., 1981a; Ch. 8) and mice (Poltera et al., 1980) infected with T.brucei, vervet monkeys infected with either T.rhodesiense or T.brucei (Poltera and Sayer, 1983), and human patients infected with T.rhodesiense (Hawking and Greenfield, 1941).

Accumulation of PE could result in cardiac tamponade, characterised by increased intracardiac pressure, progressive limitation of ventricular diastolic filling, and reduced stroke volume. If PE accumulates rapidly, as occurred in the present study, it can cause extremes in pericardial pressure (Reed, 1988). This was possibly one of the causes of decreased LVF detected by echocardiography in the present study.

Infection of dogs for more than 3 weeks resulted in S-T segment changes and notching of R waves on ECG. Similar S-T segment changes were observed in other dogs infected with T.brucei (Sayer et al., 1979), human patients infected with T.rhodesiense (Jones et al., 1975) and T.gambiense (Bertrand et al., 1971; Mbala et al., 1988) and rats infected with T.brucei (Murray et al., 1974). S-T segment elevation occurs in dogs with myocardial infarction,

pericarditis or myocardial hypoxia, while S-T segment depression is seen in cases of myocardial ischaemia, acute myocardial infarction, electrolyte disturbances and trauma to the heart (Miller and Tilley, 1988). Severe pericarditis and myocarditis occur in dogs infected with T.brucei (Morrison et al., 1981a; Ch. 8). The severe anaemia observed in T.brucei infected dogs (Ch. 3) may have led to myocardial hypoxia, and therefore S-T segment changes. The severe inflammatory reactions that accompany infection could also lead to electrolyte disturbances.

Notching of R waves, as observed in the present study, is usually seen on ECGs from dogs with microscopic intramural myocardial infarction (Tilley, 1985). Histological evidence of myocardial infarction was provided in terminally infected dogs (Ch. 8). Infarct-like lesions have also been observed in the hearts of vervet monkeys infected with T.rhodesiense (Poltera and Sayer, 1983).

Features indicating imminent heart failure occurred after day 21 of infection, and were manifest as bradycardia, weak heart beats and femoral pulses, increased capillary refill times, dyspnoea and coughing. The poor LVF and poor diastolic function observed on M-mode, and decreased cardiac output recorded by Doppler echocardiography, further indicated that heart failure may be the possible cause of death in dogs infected with T.brucei. The progressive atrioventricular and aortic incompetence observed might have caused damming back of blood in the heart, precipitating congestive heart failure.

Congestive heart failure has been reported in human

patients infected with T.rhodesiense (Manuelidis et al., 1965; Jones et al., 1975) and T.gambiense (Francis, 1972). At post mortem examination of dogs (Morrison et al., 1981a; Ch. 8) and goats (Whitelaw et al., 1985) infected with T.brucei, the finding of a globular shaped heart, along with venous congestion of the liver, was further evidence of the occurrence of congestive heart failure.

The present study showed that T.brucei infection in dogs results in severe pancarditis involving the myocardium, the conduction system and the valves. Increased cardiac damage resulted in heart failure, and could be the major cause of death. There was marked similarity of cardiac changes in infected dogs to that reported in human African trypanosomiasis and in other animals infected with T.brucei and T.rhodesiense. This indicates that the dog might be a good monogastric animal model for studying the pathogenesis of cardiac damage in human and animal African trypanosomiasis.

TABLE 4.1.

CHANGES IN R WAVE AMPLITUDE (mV) IN DOGS INFECTED WITH *T.brucei*.

DAYS AFTER INFECTION	R-WAVE AMPLITUDE MEAN 1SD
0	2.51 ± 0.501
5	2.37 ± 0.581
10	2.68 ± 0.929
15	2.16 ± 1.150
18	2.13 ± 0.720
21	1.88 ± 0.657
25	1.34 ± 0.365

TABLE 4.2.

CHANGES IN LEFT VENTRICULAR DIMENSIONS IN DOGS INFECTED WITH *T.brucei*, MEASURED BY M-MODE ECHOCARDIOGRAPHY.

DAY OF INFECT	LVEDD (cm) MEAN	SD	LVESD (cm) MEAN	SD	FS (%) MEAN	SD	HR (BPM) MEAN	SD
-2	3.20	±.100*	1.90	±.100	40.63	± 1.25	125	± 1.25
0	3.10	±.122	1.77	±.112	46.35	± 6.02	128	± 12.6
4	3.20	±.346	1.93	±.208	39.57	± 1.52	140	± 18.2
7	3.00	±.100	1.92	±.075	45.90	± 4.06	122.5	± 12.6
10	3.16	±.267	1.52	±.693	51.90	± 4.30	153	± 9.45
14	2.89	±.030	1.60	±.060	36.15	± 2.64	123	± 20.5
18	3.00	±.100	1.97	±.112	34.33	± 3.20	121	± 21.6
22	3.20	±.110	2.20	±.144	31.25	± 2.54	104	± 6.32

LVEDD - Left ventricular end-diastolic diameter.
LVESD - Left ventricular end-systolic diameter.
BPM - Beats per minute.
FS - Fractional shortening.
HR - Heart rate.
* - Measurements for each dog were made on 8 cardiac cycles.

TABLE 4.3.

MITRAL BLOOD FLOW VELOCITY (m/sec) IN DOGS INFECTED WITH *T.brucei*, MEASURED BY PULSED-WAVE DOPPLER ECHOCARDIOGRAPHY.

DAY OF INFECTION	E PEAK MEAN SD	A PEAK MEAN SD	A:E RATIO MEAN SD	HR (BPM) MEAN SD
-2	.690 ±.060*	.410 ±.030	.633 ±.122	125 ± 15.0
0	.620 ±.031	.440 ±.056	.710 ±.122	128 ± 12.6
4	.710 ±.050	.490 ±.010	.680 ±.030	140 ± 18.2
7	.768 ±.074	.538 ±.055	.700 ±.050	122.5 ± 12.6
10	.830 ±.040	.550 ±.040	.670 ±.040	153 ± 9.45
14	.768 ±.074	.625 ±.055	.814 ±.112	123 ± 20.5
18	.688 ±.035	.655 ±.036	.952 ±.056	121 ± 21.6
22	.600 ±.021	.671 ±.045	1.12 ±.013	104 ± 6.32

HR - Heart rate.
BPM - Beats per minute.
* - Measurements for each dog were made on 8 cardiac cycles.

TABLE 4.4.

AORTIC BLOOD FLOW VELOCITY (m/sec) AND THE HEART RATE IN DOGS INFECTED WITH T.brucei, MEASURED BY CONTINUOUS-WAVE DOPPLER ECHOCARDIOGRAPHY.

DAY OF INFECTION	FLOW VELOCITY MEAN SD	HEART RATE (BPM) MEAN SD
-2	1.77 ± .120*	125 ± 15.0
0	1.60 ± .030	128 ± 12.6
4	1.56 ± .110	140 ± 18.2
7	1.56 ± .110	122.5 ± 12.6
10	2.20 ± .115	153 ± 9.45
14	1.94 ± .020	123 ± 20.5
18	1.29 ± .060	121 ± 21.6
22	0.98 ± .178	104 ± 6.32

BPM - Beats per minute.
* - Measurements from each dog were made on 8 cardiac cycles.

Figure 4.1. A normal six lead ECG tracing from an uninfected dog. The PQRST waves of one cardiac cycle are indicated.

Paper speed - 50mm/sec. Voltage - 1mV.

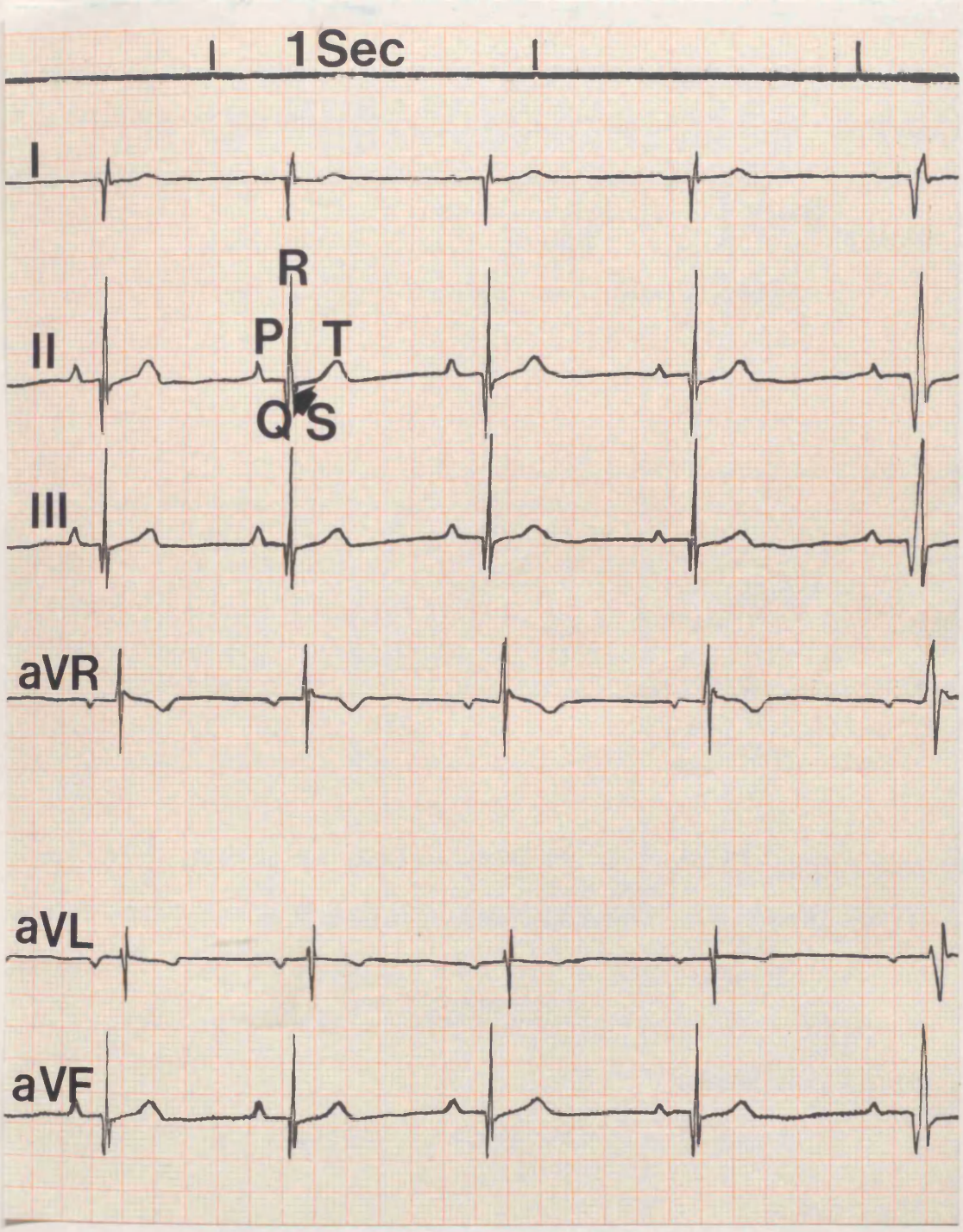
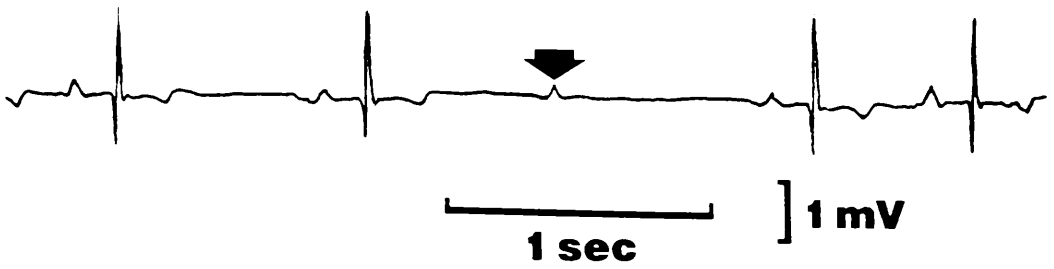

1 Sec
I
II
III
aVR
aVL
aVF
R
P
T
Q
S

Figure 4.2. Limb-lead II ECG tracing of a dog, 16 days after infection, showing second degree heart block (IIHB). One P wave (arrow) is not followed by a QRS complex. The QRS complexes before and after the IIHB are normal.

Paper speed - 50 mm/sec. Voltage - 1 mV.

Figure 4.3. Limb-lead II ECG tracing of a dog, 15 days after infection, showing complete heart block. There are several P waves which are not followed by QRS complexes (arrows). The QRS complexes before and after the heart block are normal.

Paper speed - 50 mm/sec. Voltage - 1 mV.


1 mV
1 sec

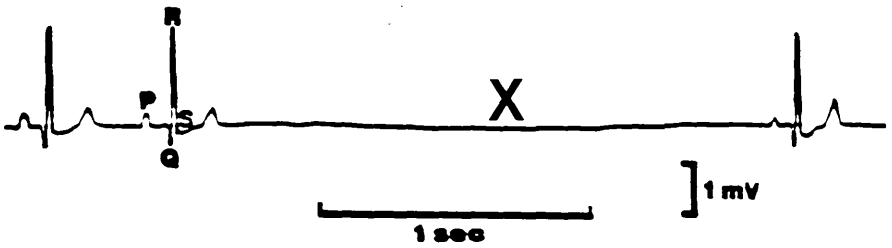

R
P
S
Q
1 mV
1 sec

Figure 4.4. Limb-lead II ECG tracing from a dog infected for 15 days, showing prolongation of the R-R interval. The R-R interval during the period of asystole (X) is more than twice the preceding one.

Paper speed - 50 mm/sec. Voltage - 1 mV.

Figure 4.5. Limb-lead II ECG tracing from a dog infected for 22 days showing S-T segment depression (arrows). The P-R intervals are 0.14 seconds, indicating the presence of first degree heart block. The amplitude of the R waves is 0.4 mV, indicating accumulation of pericardial effusion.

Paper speed - 50mm/sec. Voltage - 1mV.


R
P
S
Q
X
1 mV
1 sec

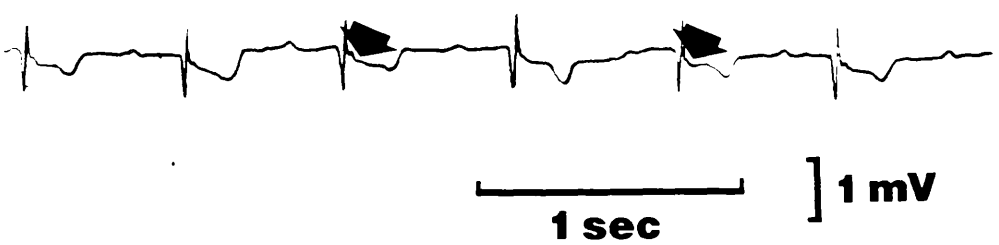

1 mV
1 sec

Figure 4.6. A six-lead ECG tracing from a dog infected with *T.brucei* for 24 days. There is notching of R waves (arrows) on leads II, III, aVL and aVF.

Paper speed - 50mm/sec. Voltage - 1mV.

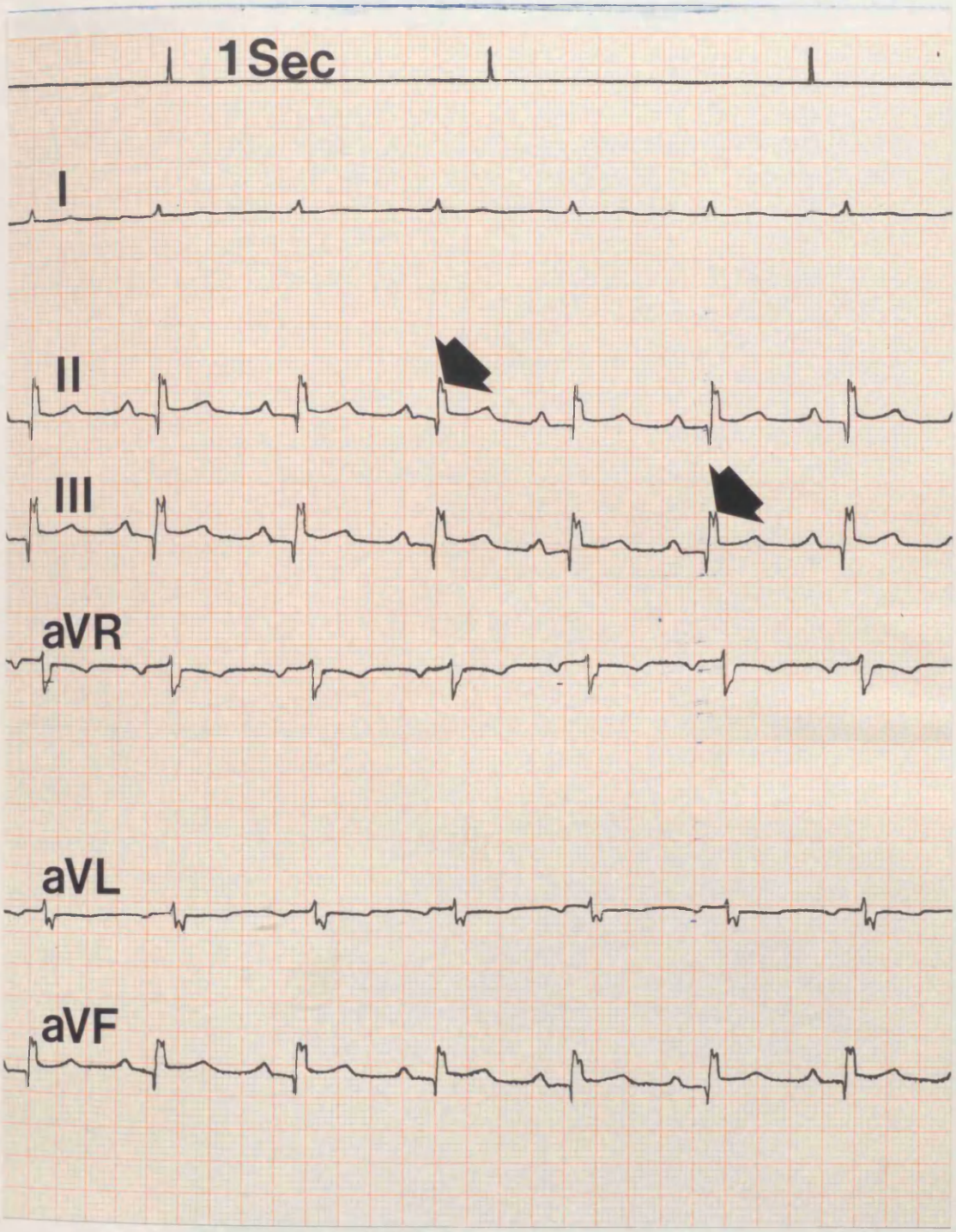
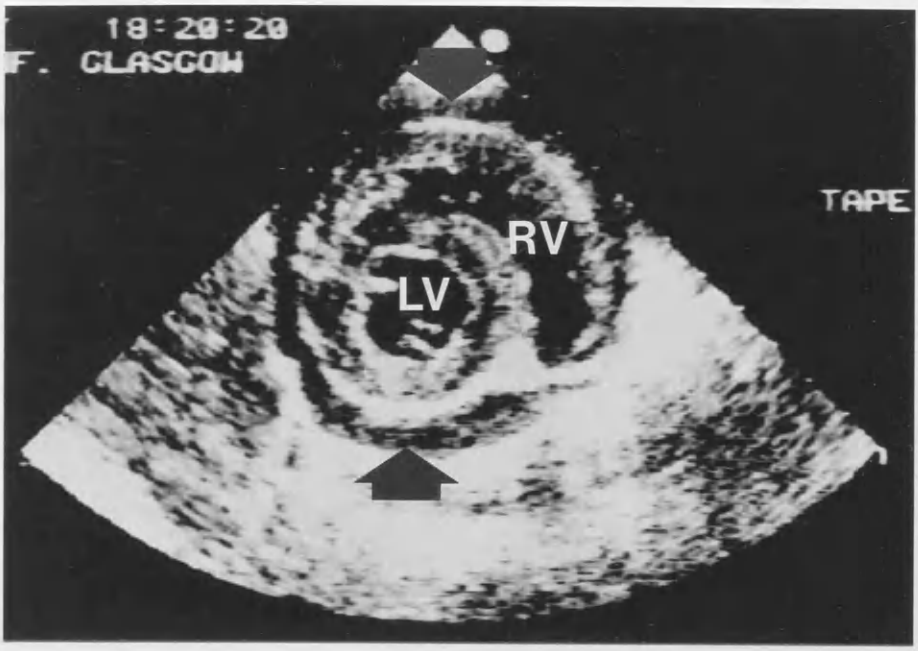
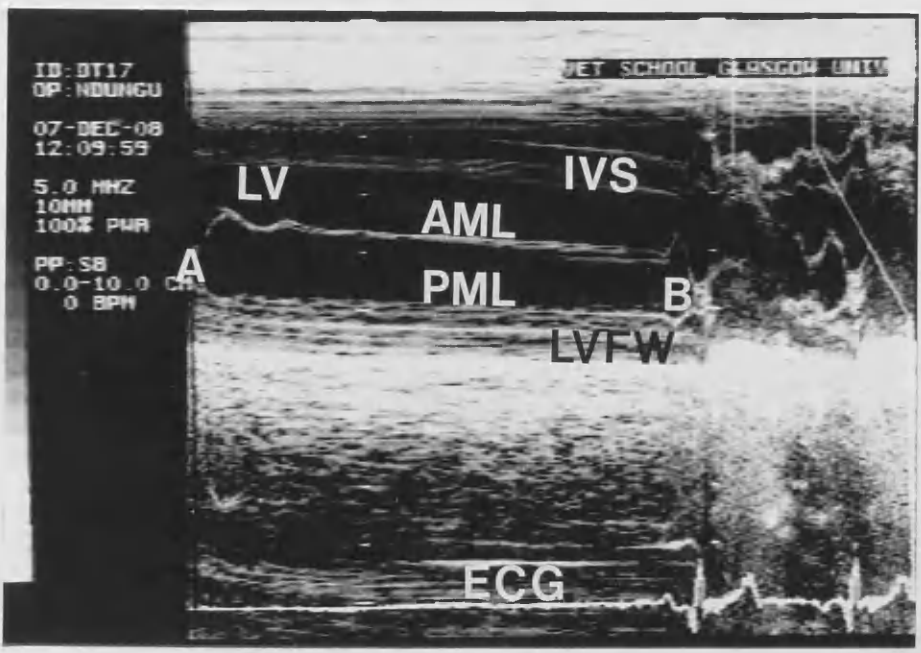

1Sec
I
II
III
aVR
aVL
aVF

Figure 4.7. A 2D parasternal short axis view of the heart of a dog infected with T.brucei for 24 days. Pericardial effusion appears as an echo-free zone surrounding the heart (arrows). LV - Left ventricle. RV - Right ventricle.

Figure 4.8. An M-mode echocardiogram of the heart of a dog infected with T.brucei for 15 days. The dog had sinus arrest. The mitral valve remains open during the period of prolonged asystole (A to B). IVS - Interventricular septum. LV - Left ventricle. AML - Anterior mitral leaflet. PML - Posterior mitral leaflet. LVFW - Left ventricular free wall.


18:20:20
F. GLASGOW
TAPE
RV
LV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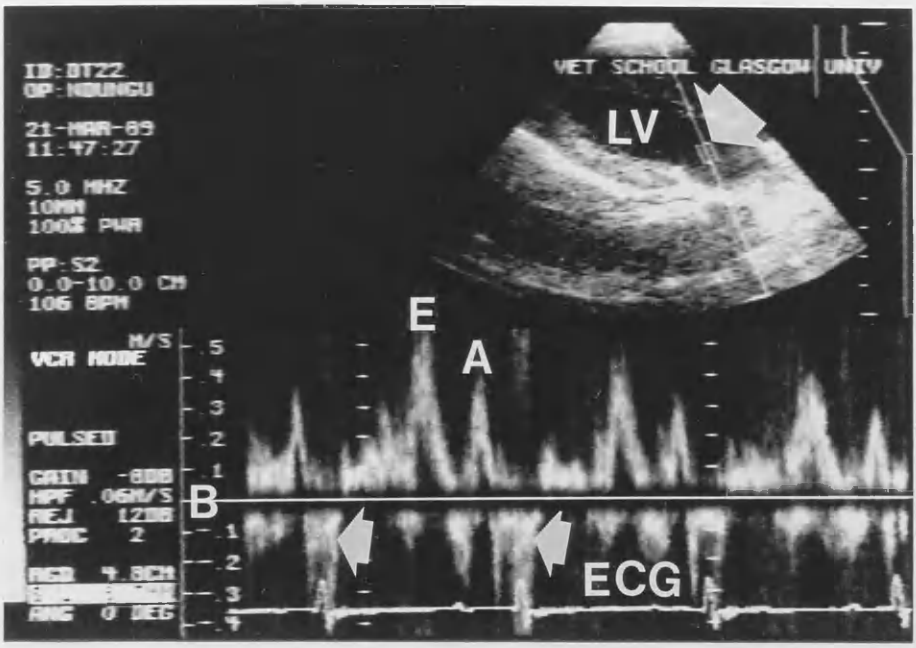
OP:NDUNGU
07-DEC-08
12:09:59
5.0 MHZ
10MM
100% PWR
PP:SB
0.0-10.0 CM
0 BPM
LV
IVS
AML
A
PML
B
LVPW
ECG

Figure 4.9. Mitral incompetence (small arrows) in a dog infected with T.brucei for 12 days, recorded by PW Doppler from the right thoracic wall. The sample volume (large arrow) is placed on the left atrial side of the mitral valve. Spectrals of incompetence are recorded below the baseline (B) in systole. The E and A peaks of normal diastolic flow are above the baseline. LV - Left ventricle.

Figure 4.10. Mitral incompetence (MI) in a dog infected with T.brucei for 14 days, observed from the subcostal window by PW Doppler. The sample volume is on the left atrial side of the mitral valve. Spectrals of MI appear below the baseline (B), in systole, as negative velocities (arrow).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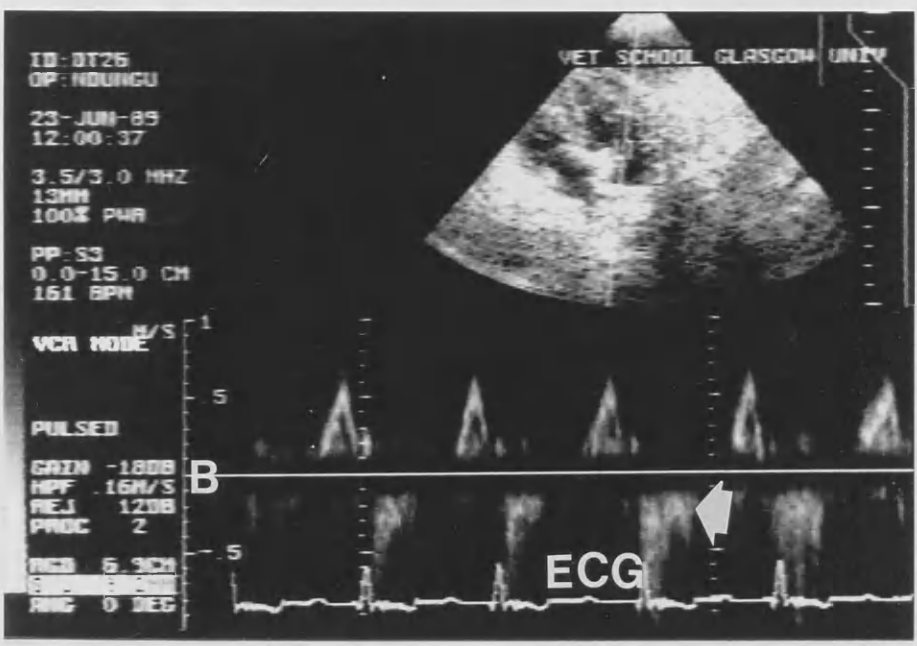
OP:NDUNGU
21-MAR-89
11:47:27
5.0 MHZ
10MM
100% PWR
PP:S2
0.0-10.0 CM
106 BPM
VET SCHOOL GLASGOW UNIV
LV
E
A
B
ECG
M/S
VCR MODE
PULSED
GAIN -8DB
HPF .06M/S
REJ 12DB
PROC 2
ANG 0 DEG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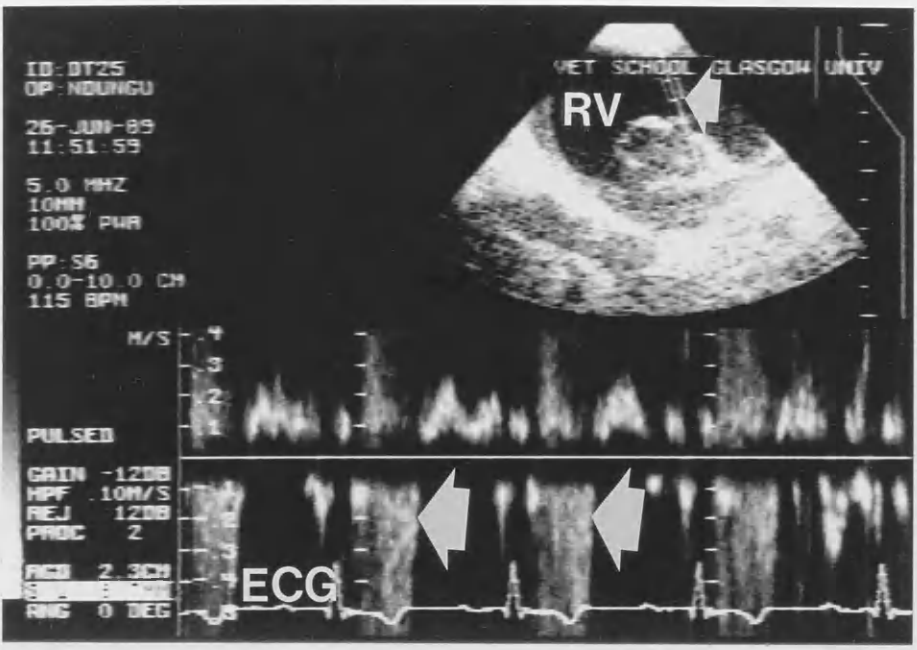
23-JUN-89
12:00:37
3.5/3.0 MHZ
13MM
100% PWR
PP:S3
0.0-15.0 CM
161 BPM
VET SCHOOL GLASGOW UNIV
B
ECG
M/S
VCR MODE
PULSED
GAIN -18DB
HPF .16M/S
REJ 12DB
PROC 2
ANG 0 DEG

Figure 4.11. Tricuspid incompetence in a dog infected with T.brucei for 14 days, demonstrated by PW Doppler from the right thoracic wall. The sample volume (small arrow) is on the right atrial side of the tricuspid valve. The incompetence spectrals (large arrows) are on both sides of the baseline (B) because of aliasing of the high velocity jet. RV - Right ventricle.

Figure 4.12. Aortic incompetence (AI) in a dog infected T.brucei for 10 days, demonstrated from the right side of the thoracic wall by PW Doppler. The sample volume (arrow) is on the left ventricular (LV) side of the aortic valve. X - Spectrals of normal aortic blood flow.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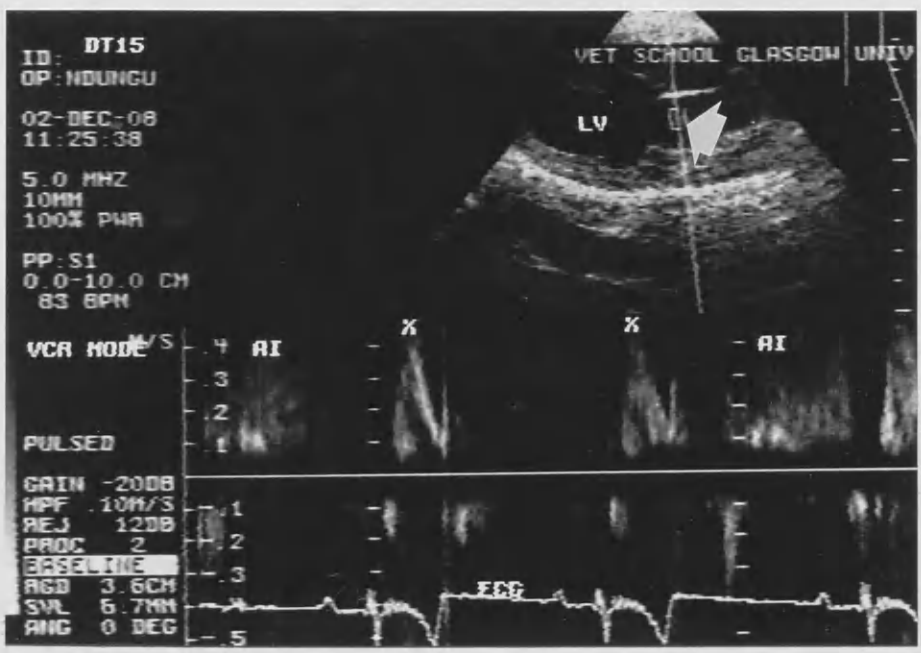
OP:NDUNGU
26-JUN-89
11:51:59
5.0 MHZ
10MM
100% PWR
PP:56
0.0-10.0 CM
115 BPM
VET SCHOOL GLASGOW UNIV
RV
M/S
4
3
2
1
PULSED
GAIN -12DB
HPF .10M/S
REJ 12DB
PROC 2
AGD 2.3CM
ANG 0 DEG
ECG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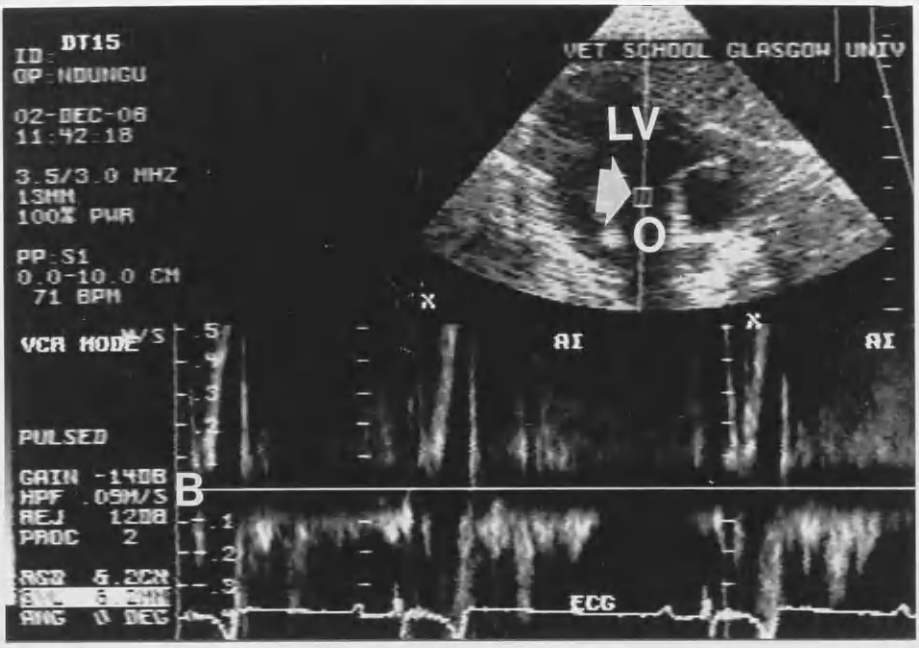
OP:NDUNGU
02-DEC-08
11:25:38
5.0 MHZ
10MM
100% PWR
PP:51
0.0-10.0 CM
83 BPM
VET SCHOOL GLASGOW UNIV
LV
VCR MODE
M/S
4
3
2
1
AI
X
X
AI
PULSED
GAIN -20DB
HPF .10M/S
REJ 12DB
PROC 2
BASELINE
AGD 3.6CM
SVL 6.7MM
ANG 0 DEG
ECG

Figure 4.13. Aortic incompetence (AI) in a dog infected with T.brucei for 10 days, recorded from the subcostal window. The sample volume (arrow) is on the left ventricular (LV) side of the aortic valve. Spectrals of AI are above the baseline (B) as positive velocity recordings because the regurgitant blood flows back into the ventricle in diastole. Spectrals of normal aortic blood flow (X) are on both sides of the baseline due to aliasing. AO - Ascending aorta.

Figure 4.14. Mitral incompetence (MI) in a dog with second degree heart block. The sample volume is in the left atrium. There is no QRS complex after the P wave of the ECG (arrow). The velocity of regurgitant blood is higher than 0.5 m/sec, resulting in aliasing. LV - Left ventricle.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02-DEC-08
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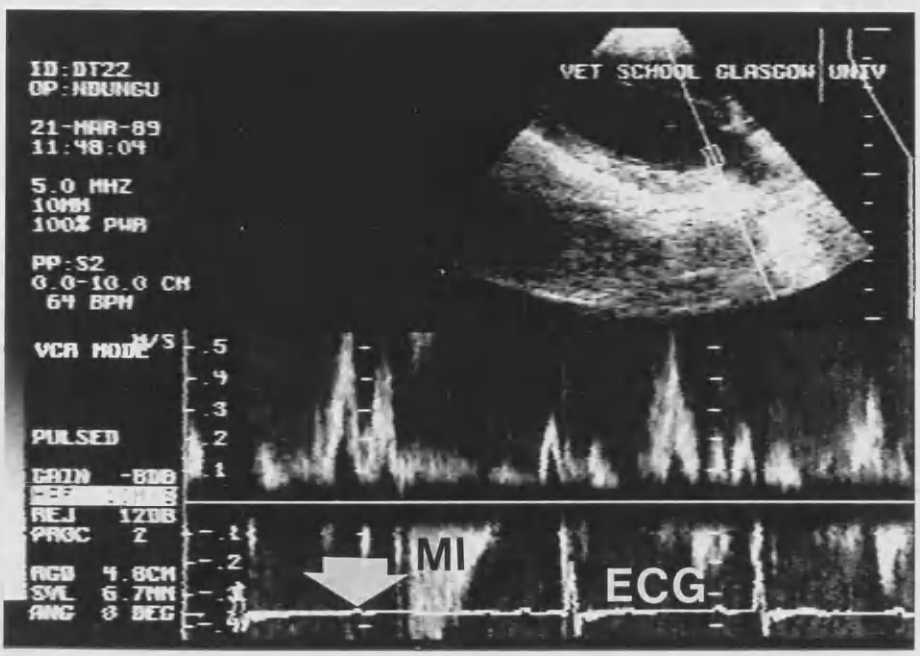
3.5/3.0 MHZ
13MM
100% PWR
PP:S1
0.0-10.0 CM
71 BPM
VET SCHOOL GLASGOW UNIV
LV
VCR MODE M/S
PULSED
GAIN -14DB
HPF .09M/S
REJ 12DB
PROC 2
RGB 6.2CM
SVL 6.2MM
ANG 0 DEG
AI
AI
B
ECG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21-MAR-89
11:48:04
5.0 MHZ
10MM
100% PWR
PP:S2
0.0-10.0 CM
64 BPM
VET SCHOOL GLASGOW UNIV
VCR MODE M/S
PULSED
REJ 12DB
PROC 2
RGB 4.8CM
SVL 6.7MM
ANG 0 DEG
MI
ECG

CHAPTER 5:

ACUTE PHASE PROTEINS IN DOGS INFECTED WITH T.brucei.

5.1. INTRODUCTION.

As previously shown (Ch. 3) dogs infected with T.brucei develop an acute disease syndrome characterised by high persistent parasitaemia, severe anaemia and marked subcutaneous oedema. At the same time, electrocardiographic (ECG) and echocardiographic signs indicating severe cardiac damage were demonstrated (Ch. 4). The disease took a rapid course, and in the terminal stages in week 4, clinical signs of heart failure were found.

In situations that lead to tissue injury, the normal vertebrate homeostatic mechanisms are altered. The characteristic pattern of the alteration is termed the acute phase response (Kuchner and Machiewicz, 1987). Acute phase proteins (APP) are a group of substances secreted by hepatocytes during the acute phase response (Courtoy, et al., 1981; Lamontagne et al., 1984) and are defined as those whose plasma concentration increases by 25% or more following exposure to a stimulus (Kushner and Mackiewicz, 1987).

There are no significant intracellular stores of APP. C-reactive protein (CRP), one of the APP, has a short half life in the circulation (approximately 4 minutes). An increase in plasma concentration during the acute phase response therefore indicates de novo synthesis (Pepys et al., 1985).

Studies on CRP in T.brucei and T.congolense infected rabbits showed major changes during the course of the disease (Thomasson et al., 1973; Cook, 1979). Human patients with sleeping sickness (Basson et al., 1977) and

Plasmodium falciparum malaria (Ree, 1971) also had elevated plasma concentration of CRP.

Haptoglobin (Hp) is another APP secreted by hepatocytes as part of the acute phase response (Kushner and Mackiewicz, 1987; Conner et al., 1988). In the normal vertebrate, Hp exists as a normal plasma protein, but in low concentration. The normal clearance of Hp from the circulation is very slow (half life 3 days). In humans and animals, Hp binds free haemoglobin (Hb) released in plasma following haemolysis of erythrocytes (Esievo et al., 1984). The Hp-Hb complex so formed is rapidly removed from the circulation by the mononuclear phagocytic system (MPS), resulting in a short half life.

The fact that T.brucei infection in the dog resulted in an acute inflammatory syndrome, accompanied by severe anaemia, indicated that measurement of CRP and Hp might be of use in monitoring the course and severity of infection, and the possible mechanisms involved in the development of anaemia.

5.2. MATERIALS AND METHODS.

5.2.1. ANIMALS.

The dogs, their management, and the stabilate of T.brucei used to infect them, have been described (Ch. 2). Briefly, 10 dogs were infected by intravenous inoculation with approximately 5×10^3 T.brucei GVR35/c.1. Before and during the course of the disease, daily clinical examination was performed. Two dogs were euthanised on each of days 10, 15, 21, 22, and 26, and tissue blocks taken

from the heart for histopathological and ultrastructural studies. Four dogs served as uninfected controls and were euthanised after the last pair of infected dogs.

5.2.2. SAMPLE COLLECTION.

At least twice weekly, 6 ml samples of jugular venous blood were collected, 2 ml in EDTA and 4 ml in heparin. From EDTA blood samples, parasitological and haematological studies were carried out as described in Chapter 3.

5.2.3. C-REACTIVE PROTEIN ASSAY.

C-reactive protein was estimated in plasma using an enzyme-linked immunosorbent assay (ELISA), described in detail by Eckersall et al. (1988). Briefly, a conjugate of phosphoryl choline and bovine serum albumin was adsorbed onto microtitre plates. As CRP binds to phosphoryl choline, these plates were used for a specific ELISA for CRP.

The plates were incubated with a diluted sample or standard, followed by rabbit antibody to canine CRP, then donkey antibody to rabbit IgG conjugated to peroxidase, and then tetramethylbenzidine in a pH 5.5 acetate buffer. The absorbance at 450 nm in the microtitre plate wells was read in a Titertek Multiscan plate reader and results calculated using the Immunosoft microcomputer programme (Flow Labolatories, Rickmansworth, Hertfordshire, U.K.).

5.2.4. HAPTOGLOBIN ASSAY.

Haptoglobin was estimated by the method of Makimura and Suzuki (1982), with modifications described by Conner et al. (1988). Tetramethylbenzidine was used in place of the carcinogenic o-dianisidine, requiring a change of the

optimum pH of the reaction to pH 3.8. The blue colour which developed had a maximum absorbance at 370 nm. The results were expressed in Hb binding capacity (HbBC) (mg of Hb bound by Hp in 100 ml plasma).

5.3. RESULTS.

5.3.1. CLINICAL FINDINGS.

The detailed clinical, parasitological and haematological findings in these dogs have been described before (Ch. 3). Trypanosomes appeared in the blood on day 5 of infection and, apart from a transient drop on days 9 to 11, a high parasitaemia was maintained throughout the infection period (Fig. 5.1). Following the appearance of trypanosomes in the blood, the dogs developed clinical signs of disease. This was characterised by sudden rise in body temperature of up to 40.6^{o}C, coinciding with the first wave of parasitaemia, marked subcutaneous oedema, spleen and lymph node enlargement, severe anaemia, wasting and weight loss.

5.3.2. C-REACTIVE PROTEIN.

The changes in plasma concentration of CRP during the course of the disease are shown in Figure 5.1. CRP increased rapidly from day 5 of infection, coinciding with detection of trypanosomes in the blood for the first time. Thereafter, markedly elevated but fluctuating levels of CRP were maintained throughout the infection period. The fluctuations in CRP followed the changes in parasitaemia. In the uninfected control dogs, the concentration of CRP remained low during the period of study.

5.3.3. HAPTOGLOBIN.

On day 4 of infection, a slight decrease in the concentration of Hp occurred (Fig. 5.2). This was subsequently followed by gradual, fluctuating increases in Hp during the next 10 days, after which the concentration increased markedly up to termination of the study on day 22 (Fig. 5.2). The changes in Hp concentration were not related to the numbers of trypanosomes in the circulation or the PCV, and were not as dramatic as those observed for CRP. No significant changes in Hp were observed in the uninfected dogs during the period of study.

5.4. DISCUSSION.

In the present study, infection of dogs with T.brucei resulted in rapid and persistent increase in the concentration of CRP and Hp. The two APP increased at the same time during the infection, with CRP exhibiting the more dramatic response.

A similar increase in the concentration of CRP in plasma was observed in human patients suffering from African trypanosomiasis (Basson et al., 1977), P.falciparum malaria (Ree, 1971) and bacterial infections (Peltola and Jaakkola, 1988), in rabbits infected with T.brucei (Cook, 1979) and T.congolense (Thomasson et al., 1973), in mice infected with Nippostrogylus brasiliensis (Lamontagne et al., 1984), and in rats following turpentine-induced inflammation (Courtoy et al., 1981).

Increased Hp concentration has been noted in rats and

calves following the subcutaneous injection of oil of turpentine (Courtoy et al., 1981; Conner et al., 1988). These increases in APP were associated with increased inflammatory reactions. T.brucei infection in dogs results in severe acute inflammatory reactions and extensive tissue damage (Morrison et al., 1981a; Ch. 8) and could account for the observed increase in CRP and Hp in the current study.

Both CRP and Hp increased simultaneously following the onset of illness. A similar simultaneous increase in several APP has been reported in rats and calves following turpentine-induced inflammation (Courtoy et al., 1981; Conner et al., 1988) and in mice infected with N.brasiliensis (Lamontagne et al., 1984). This was attributed to the nonspecific synthesis of APP by hepatocytes following stimulation. In rabbits, CRP was secreted by progressively increasing numbers of hepatocytes following intramuscular injection of turpentine (Kushner and Feldmann, 1978).

The stimulus for the synthesis of APP by hepatocytes is thought to be by factors belonging to the monokine series, produced by the macrophage-monocyte series after early interaction with parasites or parasite antigens (Lamontagne et al., 1984; Kushner and Mackiewicz, 1987). Such humoral factors are also released following tissue injury and inflammation (Conner et al., 1988). The possibility that humoral factors are the stimulus for APP secretion was supported by observations made by Perlmutter et al. (1986), that recombinant-generated human

cachectin/TNF, in picromolar concentrations, mediated reversible, dose- and time-dependent increases in APP in human hepatoma cell lines. This effect was pre-translational, as was shown by changes in specific mRNA content. Similar increases in mRNAs coding for CRP in hepatocytes occurred in mouse liver following the administration of Escherichia coli lipopolysaccharide (Murakami et al., 1988), one of the most potent stimulus for cachectin/TNF secretion by macrophages (Beutler and Cerami, 1987).

Stimulation of macrophages in bacterial and parasitic infections causes rapid production of cachectin/TNF (Beutler and Cerami, 1987). Since acute trypanosomal infections cause early and marked proliferation of the MPS (Murray et al., 1974; Morrison et al., 1981b), it is possible that cachectin/TNF, secreted by the macrophages, stimulated the early synthesis of both CRP and Hp in dogs infected with T.brucei. Evidence in support of this was provided in a study in dogs infected with T.brucei, in which monocytes were shown to be primed for the production of cachectin/TNF (Ch. 3). Indeed, in other studies, radiolabeled cachectin/TNF was found to be concentrated in the liver after it was intravenously injected (Perlmutter et al., 1986).

In the present study, elevation of CRP occurred at the same time as detection of trypanosomes in the blood and the appearance of clinical signs. Subsequently, the concentration of CRP was directly related to the level of

parasitaemia and severity of the disease. Similar increases in CRP occurred in rabbits infected with T.brucei (Cook, 1979) and T.congolense (Thomasson et al., 1973), and in human patients infected with P.falciparum, the concentration of CRP reached peak values after peak parasitaemia (Ree, 1971).

CRP is not affected by homeostatic control mechanisms that work to maintain a normal value, and therefore the concentration at any one time reflects, closely, the extent of the underlying pathological condition (Pepys et al., 1985), and may have some prognostic value in some circumstances (Kushner and Mackiewicz, 1987). The mechanism and site at which CRP is cleared are not known and there is no particular organ localization.

The overall role of CRP in the inflammatory response is poorly understood. It is thought to participate in modulation of inflammatory and immune responses, as a result of its ability to activate the complement system via the classical pathway by reacting with a number of substrates (Kaplan and Volanakis, 1974), to inhibit platelet aggregation and mediator release (Marder et al., 1977), and to bind to peripheral blood lymphocytes and suppress certain lymphocyte functions. In the presence of pneumococcal C-polysaccharide, CRP exhibits a calcium-dependent binding to T, B and null cell categories which have IgG reactivity (James et al., 1981). CRP also inhibits antigen-induced lymphocyte proliferation and lymphokine production in a dose-dependent manner (Mortensen et al., 1977). Since trypanosomiasis is associated with

severe immunosuppression (Murray et al., 1974) and thrombocytopaenia (Ch. 3), it is possible that CRP contributes to these findings.

At the same time, CRP enhances the tumoricidal activity of murine macrophages and human blood monocytes and macrophages (Barna et al., 1988). The early proliferation of the MPS in trypanosomiasis (Murray et al., 1974; Morrison et al., 1981b) indicates that CRP could be influencing its activation.

Dogs infected with T.brucei also developed marked hyperlipidaemia, characterised by increased concentrations of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) (Ch. 6). In humans, CRP has been shown to exhibit selective, calcium-dependent binding to chylomicrons, VLDL and LDL (Pepys et al., 1985; Hulman, 1988), leading to agglutination of the lipoproteins. It was suggested that if agglutination was severe enough, it could lead to hypoxaemia. The marked increase in lipoproteins and CRP in dogs infected with T.brucei might also result in agglutination of lipoproteins and probably affect tissue perfusion.

The role that CRP plays in direct tissue damage is difficult to determine, since it is not yet clear to what extent it binds to damaged tissue or localises to inflammatory sites, especially in vivo.

In the present study, Hp increased progressively throughout the course of the disease, though less dramatically than CRP. Stimulation of hepatic synthesis of Hp occurs as part of the nonspecific acute phase response

(Courtoy et al., 1981; Conner et al., 1988). Its early removal by the MPS is largely dependent on the formation of complexes with free Hb in the circulation (Esievo, et al., 1984). The removal of bound Hp by the MPS decreases its plasma levels. In calves, for example, Hp falls to undetectable levels in 7 to 10 days following infection with T.vivax, due to intravascular haemolysis (Esievo et al., 1984).

In the absence of free Hb in the circulation, the rate of removal of Hp is very slow. In the present study therefore, the sustained increase in Hp indicated continued secretion by hepatocytes without an accompanying clearance, probably reflecting the absence of free Hb, and indicating that intravascular haemolysis was not contributing to the severe anaemia which occurs in dogs infected with T.brucei.

The present findings, and those of other workers (Thomasson et al., 1973; Pepys et al., 1985; Peltola and Jaakkola, 1988), confirm the value of CRP in determination of progress of acute infections. Due to the short half life, the speed of change, and dynamic range of the concentration of CRP in plasma, its estimation can be employed in following the intensity of active tissue damage during the course of disease, success of therapy, and prediction of re-infections.

Figure 5.1. Plasma concentration of C-reactive protein (CRP) (●) (mean ± 1SD) and the parasitaemia (O) in dogs infected with T.brucei. CRP increased rapidly following the detection of trypanosomes in the blood.

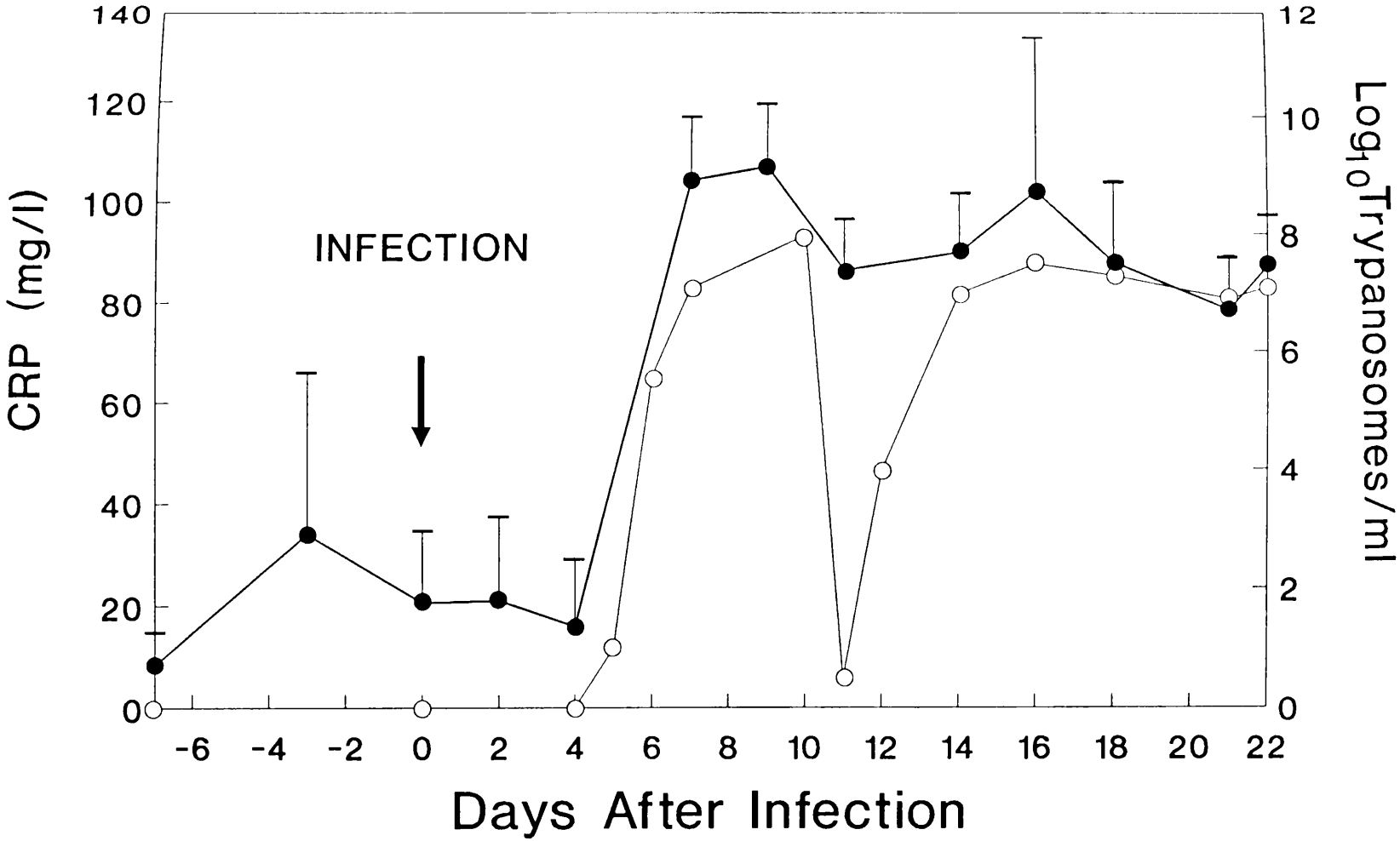
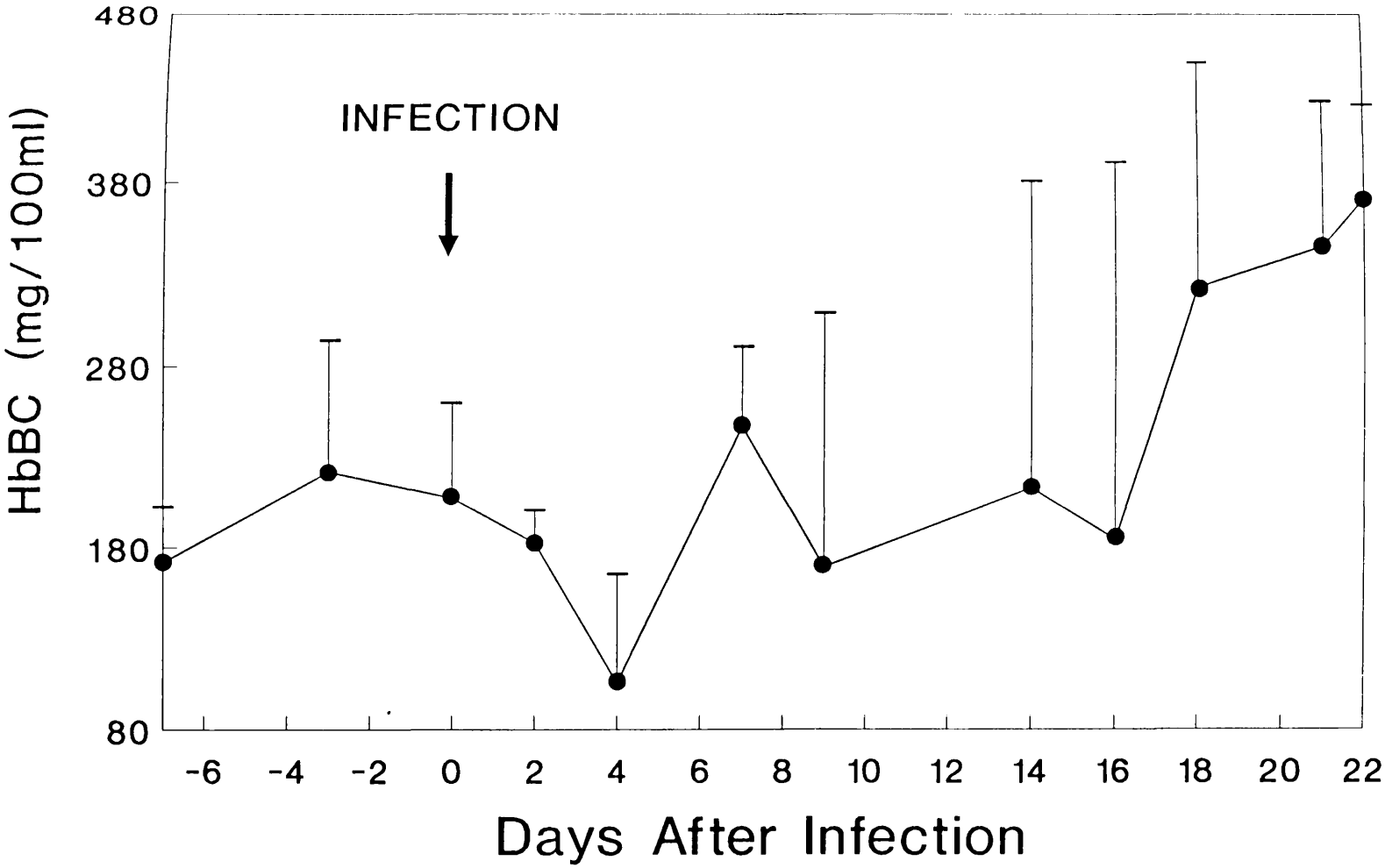

CRP (mg/l)
INFECTION
Log10Trypanosomes/ml
Days After Infection
140
120
100
80
60
40
20
0
12
10
8
6
4
2
0
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22

Figure 5.2. Plasma concentration of haptoglobin in dogs infected with T.brucei (mean ± 1SD). There was gradual increase in Hp after day 4 of infection.


480
380
280
180
80
HbBC (mg/100ml)
INFECTION
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
Days After Infection

CHAPTER 6.

LIPID METABOLISM IN DOGS INFECTED WITH T.brucei.

6.1. INTRODUCTION.

Both animal and human African trypanosomiases are associated with a wide range of blood chemical and tissue changes. Abnormalities in lipid metabolism have been identified in several laboratory and domestic animals infected with various species of trypanosomes. Thus when rabbits were experimentally infected with T.brucei (Guy, 1975; Rouzer and Cerami, 1980) or T.gambiense (Diehl and Risby, 1974), an increase in plasma cholesterol (CH), triglycerides (TG) and total lipids occurred. Increased plasma TG has also been observed in rats infected with T.rhodesiense (Dixon, 1967). At the same time, rabbits (Goodwin and Guy, 1973) and dogs (Kaggwa et al., 1984) infected with T.brucei, and sleeping sickness patients (Jenkins and Robertson, 1959; Ormerod, 1970), become severely hypoalbuminaemic.

Any changes in plasma and tissue lipid content in dogs, and in human beings suffering from trypanosomiasis, have not so far been reported. The purpose of the present section of the work was to investigate the changes in plasma and myocardial lipid content, and total plasma albumin in dogs infected with T.brucei, with a view to evaluating their role in the pathogenesis of cardiac damage.

6.2. MATERIALS AND METHODS.

6.2.1. ANIMALS.

The dogs, their management, and the stabilate of T.brucei used to infect them have been described (Ch. 2).

Briefly, 10 dogs were infected by intravenous inoculation with approximately $5x10^3$ T.brucei GVR35/c.I. At least twice a week before and during the infection, 5ml and 2ml jugular samples of venous blood were collected into separate tubes containing heparin and EDTA, respectively, as anticoagulants. The dogs were always bled before feeding. This ensured that any exogenous lipids derived from the food had been cleared from the circulation. Four uninfected dogs served as controls.

6.2.2. LIPID STUDIES.

6.2.2.1. Triglycerides, cholesterol and non-esterified fatty acids.

The plasma concentrations of TG, CH and non-esterified fatty acids (NEFA) were estimated using a COBAS Mira autoanalyser (Roche). For TG and CH, the absorbance was read at a wavelength of 500nm, and at 550nm for NEFA.

6.2.2.2. Lipoprotein electrophoresis.

An electrophoresis system (Beckman Paragon - Califonia) was used to determine the percentages of lipoproteins present in 5ul of EDTA plasma. Electrophoresis was carried out at 100 volts for 30 minutes. After fixation, the gel was scanned with a densitometer at 600nm and the quantities of lipoproteins present expressed as relative percentages.

6.2.2.3. Tissue processing.

Two dogs were euthanised on days 10, 15, 21, 22 and 26 respectively. The uninfected dogs were euthanised after the last pair of infected ones. Tissue blocks were taken from the atria and the ventricles. Samples for transmission

electron microscopy (TEM) were put into Karnovsky's fixative (Karnovsky, 1965). They were stained with lead citrate and uranyl acetate, and embedded in an Emix resin. Thick sections were cut and stained with toluidine blue for initial viewing under a light microscope, after which ultrathin sections were cut and examined with a JOEL CX100II transmission electron microscope.

Samples for oil red O staining were snap frozen at -70^{o}C. Thin tissue sections were cut with a cryostat and stained for lipids using the method of Casselman (1959).

6.2.3. PLASMA ALBUMIN.

Albumin was estimated using the Bromcresol green method described by Doumas et al. (1971) and the absorbance read at 630nm.

6.3. RESULTS.

6.3.1. CLINICAL FINDINGS.

The detailed clinical and haematological changes in the dogs in this work have been described (Ch. 3). Briefly, the dogs became ill 4 to 5 days after infection, following detection of parasites in the blood. The disease was characterised by rapid onset of fever of up to 40.6^{o}C, and a high persistent parasitaemia (Fig. 6.1), severe anaemia, lymph node and splenic enlargement, severe wasting and weight loss. By day 20 of infection, each of the dogs had lost more than 20% of their original weight.

At the same time changes indicative of cardiac damage were demonstrated by ECG and echocardiography. These included heart blocks (HB), sinus arrest, S-T segment

elevation, atrioventricular and aortic insufficiency, pericardial effusion (PE), and poor left ventricular function (LVF) (Ch. 4).

6.3.2. CHANGES IN PLASMA AND TISSUE LIPIDS.

6.3.2.1. Non-esterified fatty acids, triglycerides and cholesterol.

The changes in the concentration of NEFA before and during the course of the disease are indicated on Figure 6.1. A slight increase in NEFA was noted on day 7, coinciding with the first peak of parasitaemia. The concentration then dropped to below normal by day 14, after which a steady increase followed, and persisted up to termination of the study on day 21.

The concentration of TG in plasma before infection was 0.58 $\pm$ 0.55 mmol/l (mean $\pm$ 1SD) (Fig. 6.2). A drop was noted on day 7, and was subsequently followed by a gradual but fluctuating rise up to termination of the study. Beyond day 14 of infection, plasma TG levels were always higher than normal.

The concentration of CH in uninfected dogs was 3.69 $\pm$ 0.305 mmol/l (Fig. 6.2). An increase in plasma levels of CH was observed soon after the onset of clinical signs on day 5 and persisted up to day 11, followed by a return to normal on day 14 (Fig. 6.2). Afterwards, CH increased gradually up to termination of the study on day 21, corresponding to the changes in TG.

6.3.2.2. Lipoprotein electrophoresis.

Figure 6.3 demonstrates the appearance of a

lipoprotein electrophoretogram of plasma from an uninfected dog. The β peak corresponds to LDL and the α_1 high density lipoproteins (HDL) (DeBowes, 1987). Chylomicrons remain at the origin and VLDL migrate to an α_2 position. In the uninfected dog, HDL represented the major lipoprotein fraction; LDL and VLDL were relatively low and chylomicrons were not demonstrated.

Following infection, changes in relative percentages of the lipoproteins were noted, starting as early as day 9 of infection in some dogs. There was a gradual increase in the relative percentages of VLDL and LDL, and a decrease in HDL (Fig. 6.4).

6.3.2.3. HISTOPATHOLOGICAL FINDINGS.

Histological examination confirmed the presence of a very severe pancarditis, as described in Chapter 8. The lipid content in the myocardium of infected dogs was closely related to the plasma lipoprotein content. On day 10 of infection, oil red O and toluidine blue staining of atrial and ventricular myocardium were both negative for lipid. By TEM, only occasional lipid droplets were present within the myocytes. Similar droplets were seen in the myocardium of control uninfected dogs.

However, in dogs euthanised on day 15, increased myocardial lipid content was demonstrated, and continued to increase with progress of the disease. Oil red O and toluidine blue staining of myocardial tissue from dogs infected for 21 to 26 days was very intense (Figs. 6.5 and 6.6). The lipid appeared both within myocytes and in infiltrating macrophages.

These findings were confirmed by TEM. The lipid droplets were spherical and of variable sizes, scattered throughout the entire sarcoplasm (Figs. 6.7 and 6.8), and in macrophages (Fig. 6.9). Lipid deposition was seen in myocytes that appeared to be relatively normal and others that were undergoing myocytolysis.

6.3.3. ALBUMIN.

The concentration of albumin in the blood before infection was 35.13 ± 2.03 g/dl. Three to 4 days following the appearance of clinical signs, a decrease in albumin concentration was observed. The decrease in albumin was rapid during week 2 of infection, then it became gradual, and persisted up to termination of the study (Fig. 6.10). By day 21 of infection, albumin concentration had gone down to 12.25 ± 1.26 g/dl.

6.4. DISCUSSION.

The present study demonstrated that T.brucei infection in dogs causes marked changes in plasma and myocardial lipid content. The levels of NEFA, TG and CH increased with progress of the disease, and markedly so in the terminal stages. The increase in TG and CH resulted in an increase of the relative percentages of VLDL and LDL. Accompanying these changes was a marked drop in the plasma albumin concentration.

The hyperlipidaemia observed in the present study was similar to that reported in rabbits infected with T.brucei (Guy, 1975; Rouzer and Cerami, 1980) or T.gambiense (Diehl and Risby, 1974), and in rats infected with T.rhodesiense

(Dixon, 1967). In rabbits, the increase in TG and CH was accompanied by high levels of VLDL and LDL (Rouzer and Cerami, 1980), similar to observations in the current study.

Infection of dogs with T.brucei also led to hypoalbuminaemia, as had been reported earlier by Kaggwa et al. (1984). Similar observations have been made in sleeping sickness patients (Jenkins and Robertson, 1959; Ormerod, 1970) and in rabbits infected with T.brucei (Goodwin and Guy, 1973) .

Several factors might have contributed to the hyperlipidaemia and hypoalbuminaemia in dogs infected with T.brucei. Hyperlipidaemia might result from decreased degradation of plasma lipids for energy metabolism or for storage in adipose tissue, increased breakdown of tissue lipids, or increased synthesis of lipoproteins in the liver.

In rabbits infected with T.brucei, marked inhibition of the adipose tissue enzyme lipoprotein lipase (LPL) was demonstrated (Rouzer and Cerami, 1980). LPL is responsible for clearance of lipids from plasma. Later studies showed that lysates from T.brucei, when incubated with peritoneal exudate cells, were capable of inducing release of a mediator which caused up to 39% inhibition of the effect of LPL on pre-adipocyte cells (Hotez et al., 1984). In another section of this work, monocytes from infected dogs were shown to be primed for the production of cachectin/TNF (Ch. 3). Since cachectin/TNF causes marked inhibition of LPL (Sherry and Cerami, 1988), it is possible that its

production in the current study led to decreased lipid clearance from the blood. Production of cachectin/TNF has also been observed in patients with other parasitic diseases (Scuderi et al., 1986).

Losos and Ikede (1972) noted that infection of animals and man with trypanosomes resulted in marked breakdown of body fat. Factors capable of activating the adipose tissue enzyme triglyceride lipase (TGL) could lead to increased breakdown of body fat. Stress of infection leads to production of epinephrine and other catecholamines, which are known to activate TGL (Cheville, 1983). At the same time, cachectin/TNF is capable of stimulating TGL directly (Sherry and Cerami, 1988) and indirectly by its ability to induce increased levels of glucagon, cortisol and catecholamines (Tracey et al., 1987). Infection of dogs with T.brucei results in severe wasting and weight loss (Morrison et al., 1981a; Ch. 3). This could result from the combined effect of cachectin/TNF and stress hormones in activating TGL, leading to increased breakdown of body fat and proteins. The net effect would be an increase in plasma free fatty acids (FFA) and lipids.

Increased lipogenesis was not demonstrated in rabbits infected with T.brucei (Rouzer and Cerami, 1980). Nevertheless, recent in vivo studies have shown that administration of cachectin/TNF to rats leads to increased lipogenesis in the liver, due to the specific stimulation of hepatic LPL (Feingold and Grunfeld, 1987). Cachectin/TNF stimulates hepatic LPL while at the same time inhibiting adipose tissue LPL (Feingold and Grunfeld, 1987). Since

cachectin/TNF can be produced in dogs infected with T.brucei (Ch. 3), it is possible that increased lipogenesis does take place. Thus, it might be that the hyperlipidaemia observed in T.brucei infected dogs was the result of increased lipogenesis, decreased degradation of plasma lipids, and increased mobilization of lipids from the adipose tissue.

The cause of the decrease in albumin is difficult to elucidate. A decrease in plasma albumin could result from decreased synthesis by the liver, increased utilization by the trypanosome as nutrients or by the host for energy metabolism, and excessive loss. Albumin is known to be a negative acute phase protein (Kushner and Mackiewicz, 1987). In acute inflammatory disease, the synthesis of albumin by hepatocytes decreases. Therefore, the acute disease caused by T.brucei in dogs might also cause decreased synthesis of albumin.

Albumin is also required by trypanosomes for optimal survival (Coppens et al., 1987). Since infection of dogs in the present studies resulted in high parasitaemia, increased utilization by parasites might have contributed to the decrease in albumin. At the same time, the possible inability of infected dogs to utilize lipids for energy metabolism due to inhibition of LPL could make them revert to using albumin.

Although it is possible that albumin could also have been lost through the kidneys, previous studies have failed to detect albumin in the urine of infected dogs (Kaggwa et al., 1984). T.brucei infection in dogs leads to

gastroenteritis (Morrison et al., 1981a; Ch. 4). It is possible that loss of albumin could have taken place through the damaged intestinal mucosa.

The changes in plasma and myocardial lipid content demonstrated in the current study are likely to play a major role in the pathogenesis of the disease in dogs. In normal physiological situations, FFA are transported in plasma bound to albumin (Oliver, 1987). In the bound form, FFA are non-toxic. An increase in plasma FFA or a decrease in albumin leads to increased unbound FFA, hence enhancing their cytotoxicity. In the present study, the increase in NEFA was accompanied by severe hypoalbuminaemia. This indicated that the amount of NEFA present in plasma in the unbound form was increased.

Free fatty acids, due to their detergent-like properties, are both haemolytic and cytotoxic (Tizard et al., 1978). On erythrocytes, FFA cause crenation and decreased deformability (Kamada et al., 1987). As a result, blood flow in the microcirculation is impaired. This could lead to pooling of blood, as is seen in the splenic sinusoids of trypanosomiasis infected animals (Murray and Dexter, 1988), and increased removal of erythrocytes by the activated MPS.

Free fatty acids are also capable of causing thrombocytopaenia and thrombosis when administered to experimental animals (Tizard et al., 1978). It is possible that alterations in FFA levels could have contributed to the thrombocytopaenia seen in dogs infected with T.brucei (Ch. 3).

Both saturated and unsaturated fatty acids are immunosuppressive (Mertin and Hughes, 1975; Meade and Mertin, 1976; Mertin, 1976). The immunosuppressive effects are thought to be due to either the direct action of FFA on lymphocyte membranes or by acting as precursors of prostaglandin synthesis. Prostaglandins, by stimulating adenylate cyclase in the lymphocyte membrane, cause an increase in intracellular cyclic adenosine monophosphate (cAMP), which regulates both immediate and delayed hypersensitivity reactions (Mertin and Hughes, 1975; Mertin, 1976). Thus it is possible that the changes in NEFA observed in the current studies might be involved in development of the severe immunosuppression which can occur in T.brucei infected animals (Murray et al., 1974).

Infection of dogs with T.brucei resulted in marked accumulation of lipids in the myocardium and tissue macrophages. Several factors could have contributed to the increase in tissue lipids. Under normal circumstances, there exists an equilibrium between the unbound FFA in plasma, extracellular and intracellular fluid (Kurien et al., 1969). The increase in NEFA and decrease in albumin in the current study might have led to a net flux of fatty acids into the myocardial cells. Here, it is possible that some of the FFA underwent peroxidation for energy metabolism, while the rest was esterified into TG and stored as neutral lipids, thus accounting for the lipid deposition.

At the same time, the rate of FFA oxidation and esterification could be exceeded by the rate of entry into

the myocardium, leading to accumulation of the excess FFA and impaired electrical activities in the heart (Kurien et al., 1969). In the present study, impaired electrical activities in the heart were demonstrated by ECG. It is possible that increased intracellular FFA contributed to the observed ECG changes.

Increased total lipids were associated with increased relative percentages of VLDL and LDL, and decreased HDL. Similarly in rabbits infected with T.brucei, hyperlipidaemia was the result of an absolute increase in VLDL and LDL (Rouzer and Cerami, 1980). An increase in relative percentages in the present study might therefore reflect increased VLDL and LDL, especially as this was accompanied by increased total lipids. Nevertheless, the decrease in relative percentage of HDL cannot be used as an indicator of changes in total HDL. Further studies are therefore necessary to determine the absolute changes.

Trypanosomes require LDL for their survival (Coppens, et al., 1987; Coppens et al., 1988; Black and Vandeweerd, 1989). LDL, by acting as a vehicle for CH transport, favours increased trypanosome survival and multiplication. Evidence in support of this was the persistently high parasitaemia in infected dogs.

In man, hyperlipidaemia and high tissue lipid content is associated with increased incidence of atherosclerosis and myocardial disease (Fredrickson et al., 1972). Atherosclerosis develops when macrophages accumulate modified lipids, become foam cells, and get trapped in the walls of blood vessels (Babiak and Rudel, 1987). In the

present study, lipid-filled macrophages were demonstrated in the heart, where severe myocardial damage occurred (Ch. 8). It is possible that myocardial lipid infiltration in infected dogs exacerbated the degree of damage.

It is also known that VLDL and LDL are highly immunoregulatory, both in vivo (Curtiss et al., 1977) and in vitro (Morse et al., 1977; Chisari, 1977). They inhibit lymphocyte responsiveness to antigenic, mitogenic and allogeneic cell stimulation and transformation. Since trypanosomiasis is associated with marked immunosuppression, the increase in VLDL and LDL might contribute to this effect, at least in animals that develop the hyperlipidaemic state.

The trypanocidal drug suramin, used for the treatment of sleeping sickness patients, being a polyanion, has a high binding capacity for lipoproteins. It is possible that the availability of such a drug to trypanosomes might be affected by the level of lipoproteins in plasma. If abnormalities in lipid metabolism in sleeping sickness patients do occur, this might lead to therapeutic failures. Some evidence for this was obtained when treatment of T.brucei infected dogs with doses of suramin that are normally curative failed to achieve cure (Ch. 9).

The present study demonstrated that T.brucei infection in dogs results in marked abnormalities in lipid metabolism, and hypoalbuminaemia. These abnormalities are likely to be involved in the development of the anaemia, thrombocytopaenia, immunosuppression and tissue damage observed in trypanosomiasis. The hyperlipidaemic state

appears to be related to decreased lipid utilization, increased breakdown of body fats, and lipogenesis in the liver. It is possible that cachectin/TNF, secreted by activated macrophages, played a major role in the initiation of these changes.

Figure 6.1. Plasma concentration of non-esterified fatty acids (NEFA) (●) and parasitaemia (O) in dogs infected with T.brucei. There was a gradual increase in NEFA during week 3 of infection.

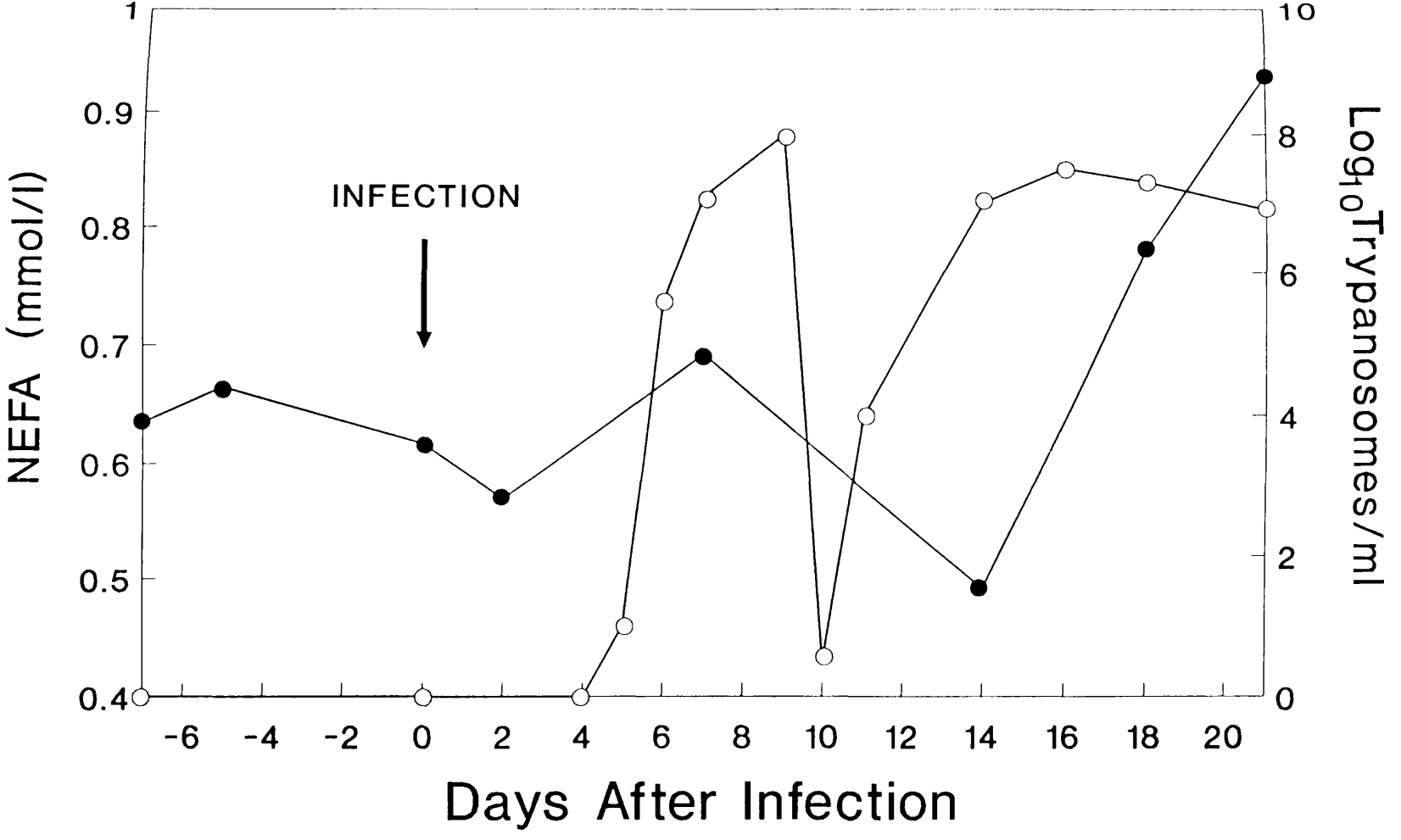

NEFA (mmol/l)
1
0.9
0.8
0.7
0.6
0.5
0.4
INFECTION
Log10Trypanosomes/ml
10
8
6
4
2
0
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Days After Infection

Figure 6.2. Total plasma cholesterol (●) and triglycerides (○) in dogs infected with T.brucei. After day 14 of infection, there was a fluctuating increase in both lipid components.

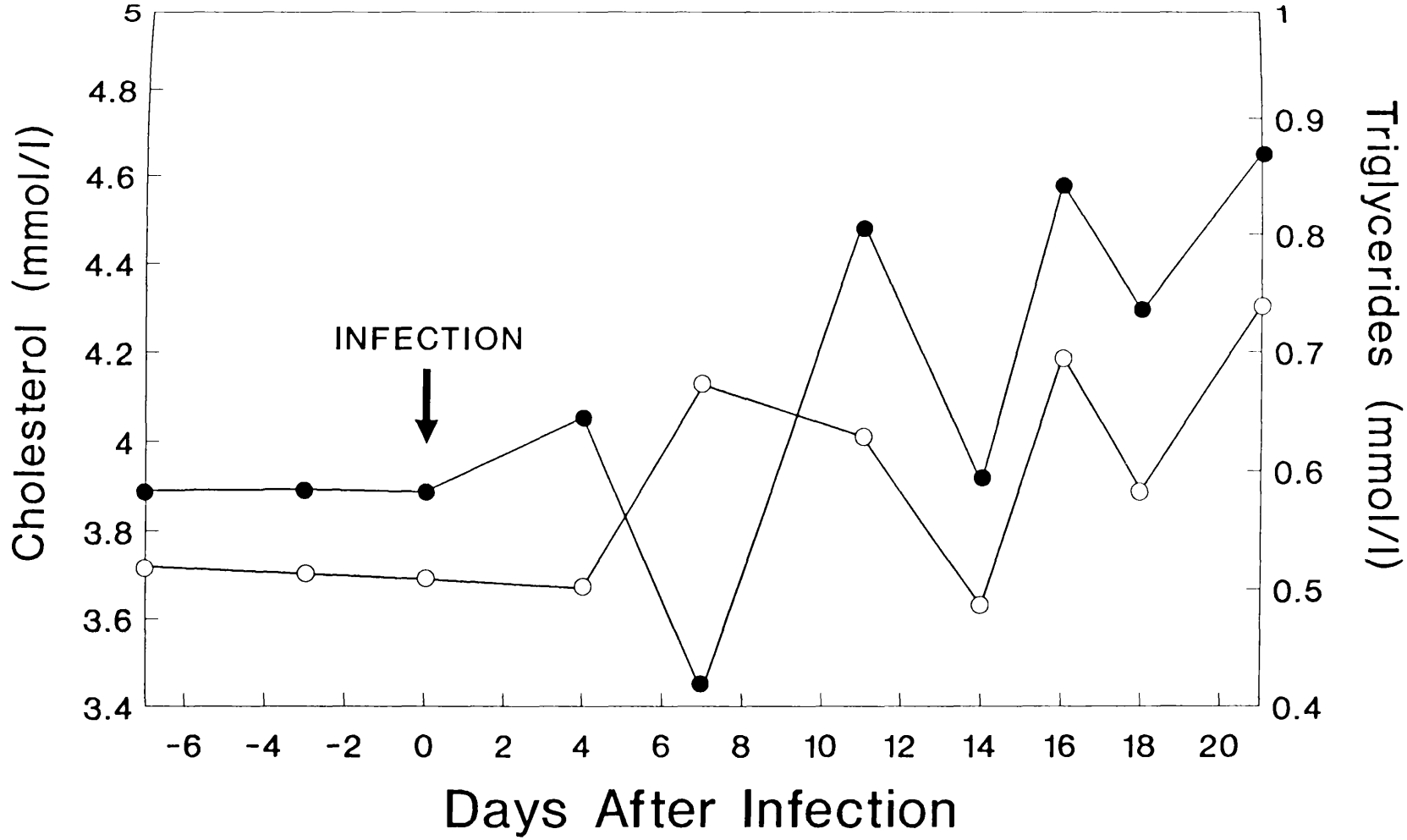
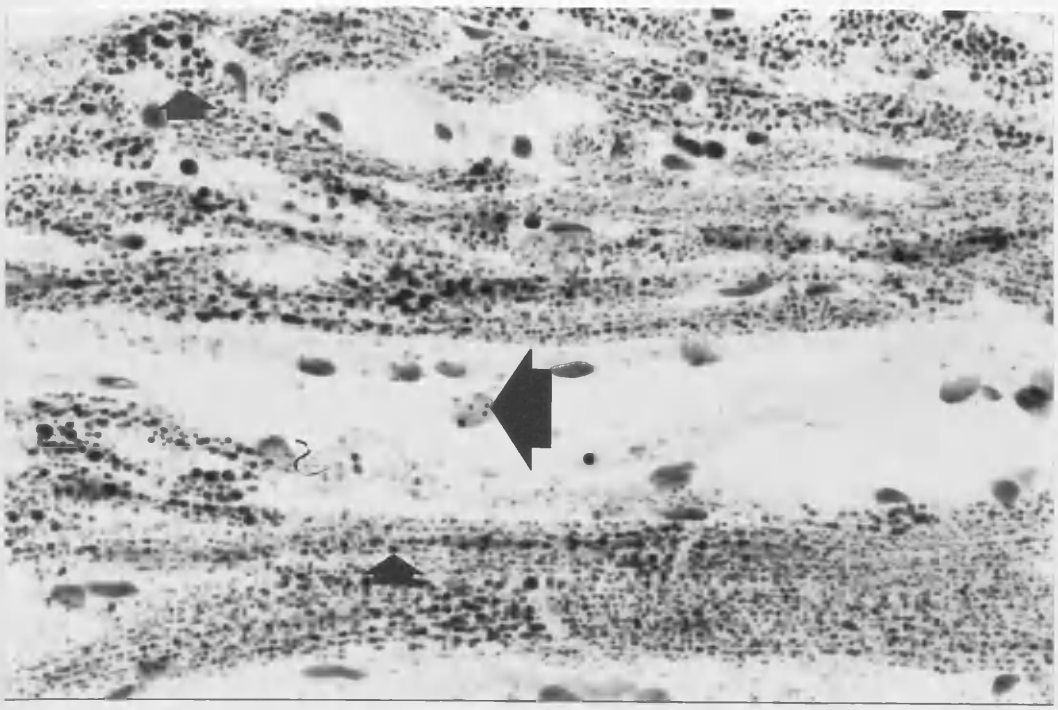

Cholesterol (mmol/l)
Triglycerides (mmol/l)
INFECTION
Days After Infection
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
1
0.9
0.8
0.7
0.6
0.5
0.4
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20

Figure 6.5. The left ventricular myocardium of a dog infected with T.brucei for 22 days. There is diffuse lipid deposition (small arrows) within myocytes, and in macrophages in the interstitial spaces (large arrow). Oil red O. x250.

Figure 6.6. A thick section of the subepicardium of the left atrium of the dog in Figure 6.5. There is intracellular lipid deposition in the infiltrating macrophages (arrows). Toluidine blue. x320.



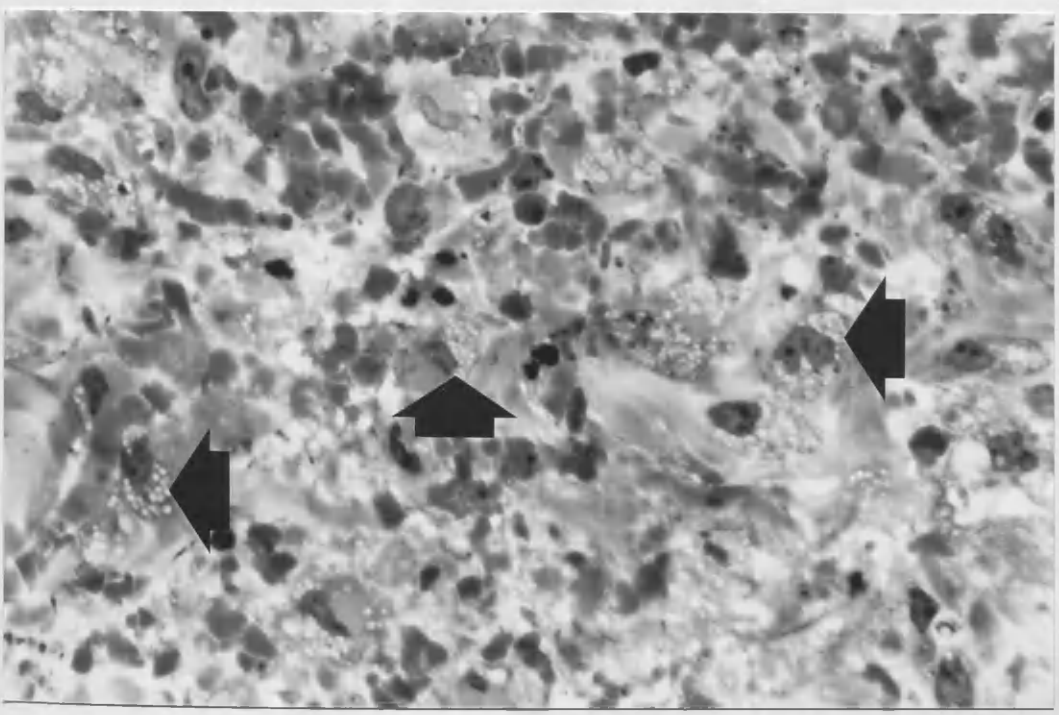
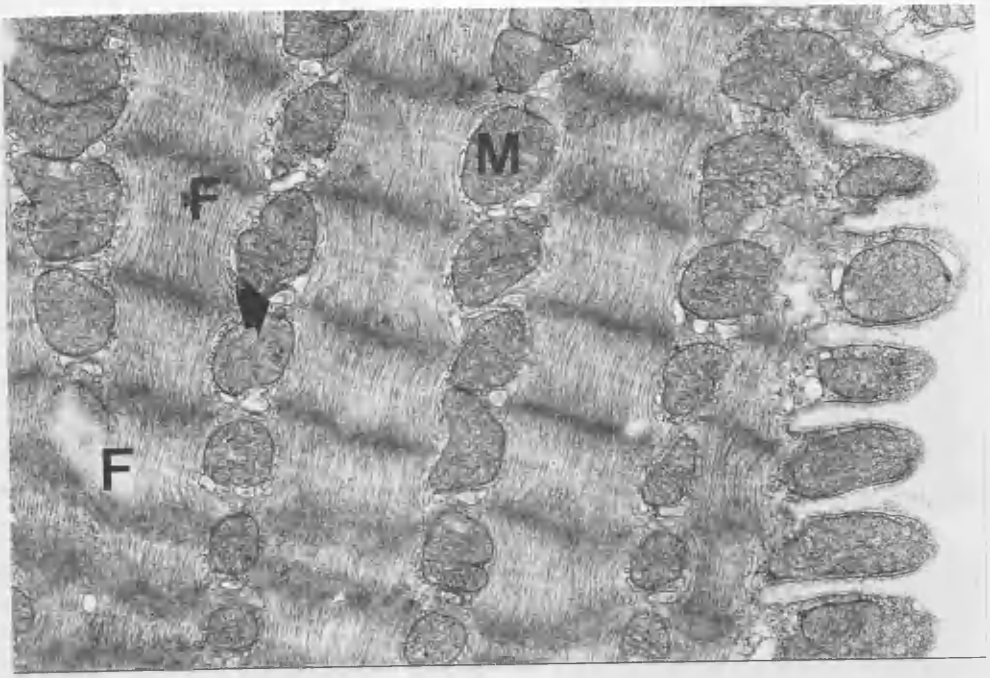


Figure 6.7. A section of a myocyte in the right ventricle of the heart of an uninfected dog. M - Mitochondrion. F - Myofibrils. Arrow - T tubule. TEM. x20,000.

Figure 6.8. A section of a myocyte in the right ventricle of a dog infected with T.brucei for 22 days. There is intense lipid deposition (L). TEM x 20,000.


M
F
F

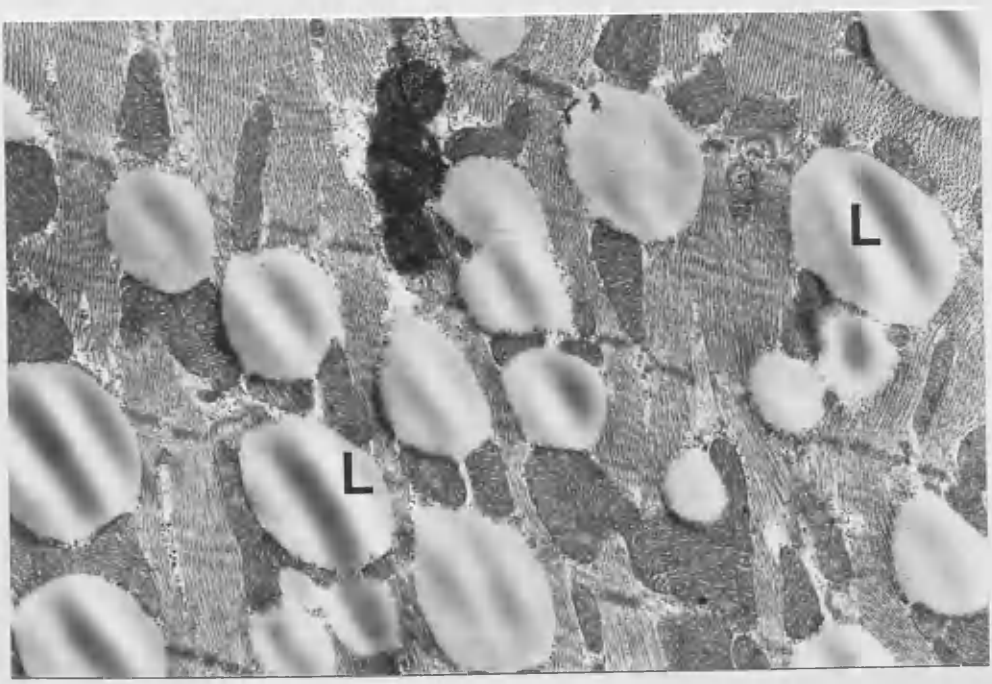

L
L

Figure 6.9. A macrophage in the interstitium of the left ventricle of a dog infected with T.brucei for 21 days. There are large lipid droplets in the cytoplasm (L). F - Fibrin. TEM. x20,000.

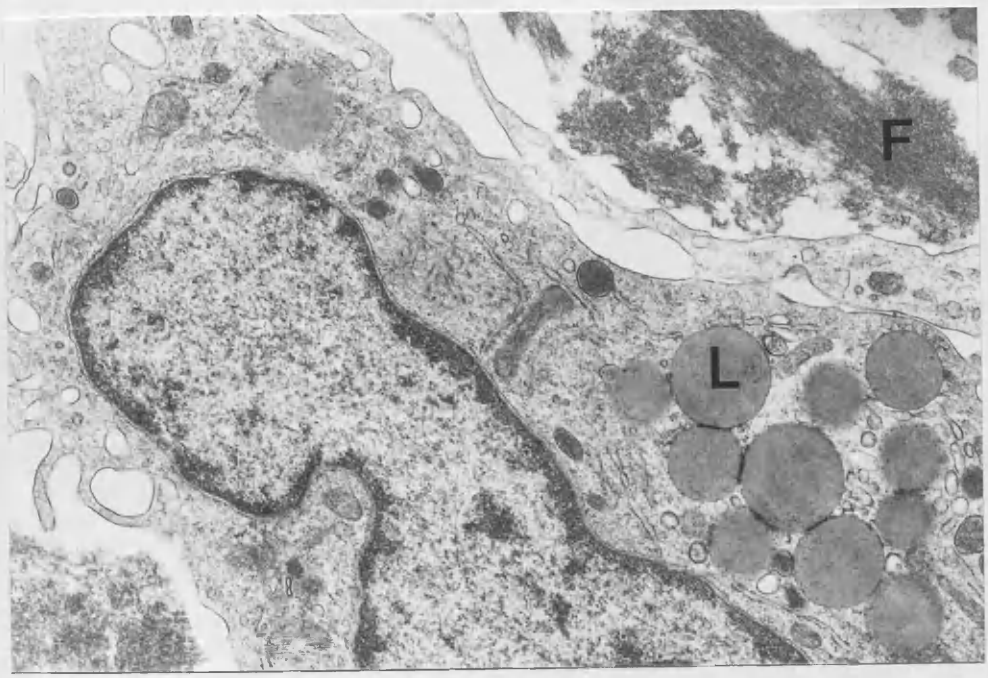
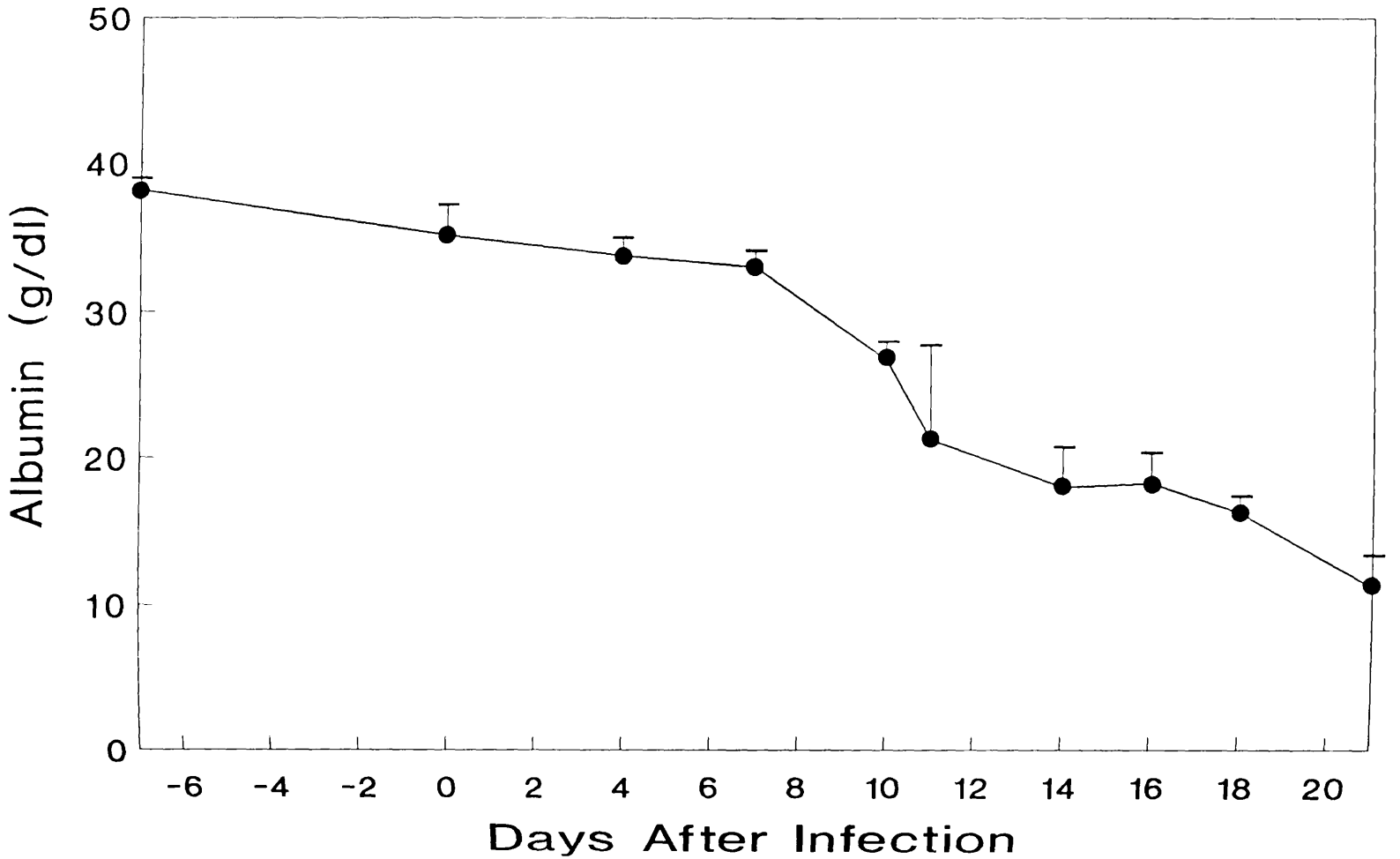

F
L

Figure 6.10. Changes in plasma albumin in dogs infected with T.brucei. There was rapid decrease in albumin during week 2 of infection, after which the decrease became more gradual.


50
40
30
20
10
0
Albumin (g/dl)
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Days After Infection

CHAPTER 7.

ATRIAL NATRIURETIC FACTOR AND PLASMA RENIN ACTIVITY IN DOGS INFECTED WITH T.brucei.

7.1. INTRODUCTION.

In addition to its role as a vascular pump, the mammalian heart is an important endocrine organ that produces a recently discovered peptide hormone, atrial natriuretic factor (ANF), which has powerful diuretic, natriuretic and hypotensive actions (Atlas and Laragh, 1986; Ballermann and Brenner, 1986; Genest and Cantin, 1987; Harris et al., 1987).

ANF is stored in secretory granules in atrial myocytes as pro-ANF (Ballermann and Brenner, 1986; Crozier et al., 1987). The accepted major stimulus for release of ANF is mechanical distention of atria (Dietz, 1984; Lang et al., 1985; Ledsome et al., 1985; Raine et al., 1986; Crozier et al., 1987; Cantin et al., 1988). Secretion of ANF can also be stimulated by tachycardia, without the additional stimulus of stretch (Ballermann and Brenner, 1986; Goetz et al., 1986; Crozier et al., 1987), and sometimes by acute hypoxaemia (Baertschi et al., 1988).

Increased concentration of ANF in plasma is observed in response to acute or chronic volume expansion (Lang et al., 1985; Salazar et al., 1986), rapid atrial tachyarrhythmias (Walsh et al., 1987), systemic hypertension (Ballermann and Brenner, 1986), congestive heart failure (Burnett et al., 1986; Baertschi et al, 1988; Cantin et al., 1988) and renal insufficiency (Ballermann and Brenner, 1986). In human patients with congestive heart failure (Cody et al., 1986; Raine et al., 1986), cardiomyopathic hamsters (Genest and Cantin, 1987; Cantin et al., 1988), and in rats with myocardial infarction (Mendez et al., 1987), the atrial

stores of ANF were found to be markedly reduced. The total ANF secreted from the heart into the circulation was however increased, due to atrial hypertrophy (Mendez et al., 1987) and recruitment of ventricular cardiocytes to secrete ANF (Cantin et al., 1988). This led to increased plasma ANF concentration.

In the circulation, ANF exerts a vasorelaxant action by inhibiting the vasoconstrictor effect of angiotensin II (AII) (Harris et al., 1987; Bache et al., 1988). In the kidneys, it increases glomerular filtration rate (Huang et al., 1985; Harris et al., 1987) by causing relaxation of the afferent but not the efferent arterioles, and by inhibiting the contractile effects of AII on mesangial cells (Leckie, 1987).

By its inhibitory effect on renin secretion from juxtaglomerular cells (Maack et al., 1984; Kurtz et al., 1986; Walsh et al., 1987; Williams et al., 1988), ANF causes an indirect reduction in plasma AII concentration.

ANF decreases reabsorption of the glomerular filtrate from the proximal tubules by inhibiting AII-stimulated sodium and water transport (Harris et al., 1987). In the adrenal glomerulosa cells, ANF inhibits AII-, potassium and adrenocorticotropic hormone (ACTH)-stimulated aldosterone secretion (Maack et al., 1984; Harris et al., 1987; McMurray et al., 1988; Schiebinger et al., 1988), in addition to having a direct inhibitory action on aldosterone release (Schiebinger et al., 1988).

In addition, ANF is a potent inhibitor of arginine vasopressin (AVP) secretion from the paraventricular

nucleus in the hypothalamus (Standaert et al., 1987; Williams et al., 1988). Through these effects, ANF exerts its powerful diuretic, natriuretic and hypotensive actions.

In a another part of this work, T.brucei infection of dogs was characterised by pantropic tissue damage, including severe pancarditis (Ch. 8). The present part of the study was designed to investigate the effects of infection on plasma and atrial concentrations of ANF and plasma renin activity (PRA), and to determine the contribution that such changes might make to the heart failure syndrome observed.

7.2. MATERIALS AND METHODS.

7.2.1. ANIMALS.

The dogs, their management, and the stabilate of T.brucei used to infect them have previously been described (Ch. 2). Briefly, 10 dogs were intravenously infected with T.brucei GVR 35/c.1. Before and during the course of the disease, daily clinical examinations were performed. Two dogs were euthanised on each of days 10, 15, 21, 22 and 26, and tissue blocks taken from the heart for histopathological studies. Four dogs served as uninfected controls and were euthanised after the last pair of infected ones.

7.2.2. PLASMA ATRIAL NATRIURETIC FACTOR.

Before and during the infection, 5 ml blood samples were collected from the jugular vein, at least twice a week, in chilled tubes containing EDTA as anticoagulant and sufficient Trasylol (Bayer, U.K.) to give a final

concentration of 50 kallikrein inhibitor units per ml of blood. The samples were centrifuged at 3000g for 10 minutes at 4°C. The plasma obtained was then stored at -20°C. Assay of plasma ANF was performed using the radioimmunoassay method described by Richards et al. (1987).

7.2.3. PLASMA RENIN ACTIVITY.

1 ml blood samples were collected in chilled tubes containing EDTA as anticoagulant and maintained at 3°C, and centrifuged at 3000g for 10 minutes at room temperature. Plasma was stored at -20°C and PRA estimated using the method of Millar et al. (1980).

7.2.4. HISTOLOGY AND TRANSMISSION ELECTRON MICROSCOPY.

Following euthanasia, tissue blocks were taken from the atria and ventricles for histopathological studies (Ch. 8).

For TEM studies, small blocks of cardiac muscle, less than 1 mm thick were obtained from the walls of both atria and ventricles immediately after the dogs were euthanised. The tissues were processed as described in Chapter 6. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JOEL CX100II transmission electron microscope.

7.3. RESULTS.

7.3.1. CLINICAL FINDINGS.

The detailed clinical findings in these dogs have been described elsewhere (Ch. 3). In brief, after a prepatent period of between 5 and 6 days, all 8 infected dogs developed signs of disease. This was characterised by high

parasitaemia, persistent fever of up to 40.6°C, tachycardia, lymph node and splenic enlargement, severe anaemia, wasting and weight loss. In weeks 3 and 4, clinical signs of cardiac damage developed. These included bradycardia, weak heart beats, increased capillary refill times, dyspnoea and coughing, poor left ventricular function (LVF), valvular incompetence, heart blocks (HB), sinus arrest, S-T segment elevation, and accumulation of pericardial effusion (PE) (Ch. 4).

7.3.2. PLASMA **ATRIAL NATRIURETIC FACTOR.**

In control dogs, the concentration of ANF remained within the range 18.75 ± 3.55 pg/ml (mean ± 1SEM). The changes in plasma ANF in infected dogs are shown in Figure 7.1. Slight increases in ANF were observed on days 3 and 15 of infection. These were followed during week 3 by a consistent drop in concentration in all dogs, persisting up to the terminal stages of the infection in week 4 (Fig. 7.1).

7.3.3. PLASMA RENIN ACTIVITY.

The level of PRA remained low during the first 2 weeks of infection (Fig. 7.1). In weeks 3 and 4, there was a rapid rise in PRA, coinciding with the decrease in ANF. The level of PRA in control dogs remained within the range 8.44 ± 1.8 mU/ml (mean ± 1SEM) during the study period.

7.3.4. HISTOLOGICAL AND ULTRASTRUCTURAL FINDINGS.

The detailed histopathological findings in these dogs have been described elsewhere (Ch. 8). In brief, after 2 weeks following infection, there was severe myocardial

damage in the form of myocyte degeneration and necrosis, including increased lipid deposition, a feature in both atrial and myocardial cells. The atria appeared to be more severely affected than the ventricles.

TEM examination of the atrial myocardium of uninfected dogs demonstrated the presence of many electron-dense granules in the myocytes. The granules were present in both right and left atrial myocytes. Most of the granules were preferentially located at the perinuclear region, close to the Golgi complex (Fig. 7.2) and had a uniform size and density (Fig. 7.3). Occasional less dense granules were also seen. A few of the granules were scattered within the myofibrils and occasionally located in the subsarcolemmal region (Fig. 7.4).

In atrial myocytes from dogs infected for 21, 22 and 26 days, there was marked reduction in the number, size and density of atrial granules (Fig. 7.5). In most myocytes the Golgi complexes were prominent and distended (Fig. 7.6). No significant changes in the number and density of atrial granules were seen in dogs euthanised on or before day 15 of infection. Similar granules or changes in Golgi complexes were not found in ventricular myocardial cells of either infected or control dogs.

7.4. DISCUSSION.

In the present investigation, infection of dogs with T.brucei resulted in severe pancarditis (described in detail in Ch. 8), reduction in plasma and atrial ANF, and increased PRA, with death invariably occurring in week 4 of

infection, as has been described in previous studies (Sayer et al., 1979; Morrison et al., 1981a; Mwambu et al., 1979). While death most probably resulted from failure of the heart as a pump, the present findings indicated that the severity of the disease might well be influenced by impaired homeostatic regulation of hormones that control blood volume and blood flow dynamics.

On day 15 of infection, the plasma concentration of ANF increased slightly. A similar observation has been made in dogs following rapid atrial pacing (Walsh et al., 1987), in human patients with tachycardia (Crozier et al., 1987), and in acute and chronic volume expansion (Lang et al., 1985); the increase in plasm ANF occurred as a result of increased intra-atrial pressure. In a previous part of this work, dogs infected with T.brucei developed tachycardia in the early stages of the disease between days 6 and 15 of infection, followed thereafter by bradycardia (Ch. 4). At the same time, from day 10 of infection, progressive valvular incompetence and deterioration of LVF were demonstrated by echocardiography. In the present part of the work, significant increase in plasma ANF only occurred after day 12 of infection. This indicated that progressive valvular incompetence, and not tachycardia, caused increased secretion of ANF from atrial myocytes during that period as a result of increased intra-atrial pressure.

The concentration of ANF decreased progressively after day 15 of infection, persisting up to termination of the study on day 22. The drop in plasma ANF was associated with reduction in the number, size and density of the storage

granules in the atria, together with distension of Golgi complexes. In contrast in human patients with congestive heart failure (Cody et al., 1986; Raine et al., 1986), and in cardiomyopathic hamsters (Cantin and Genest, 1988), the plasma levels of ANF were found to be elevated, while the atrial storage sites were depleted of ANF; it appeared that atrial hypertrophy (Mendez et al., 1987) and recruitment of ventricles to secrete ANF (Cantin et al., 1988) were responsible for maintaining the increased concentration of plasma ANF.

In the current study, decreased plasma and atrial ANF may have been caused by two factors, either acting individually or in concert. Firstly, as a result of atrial stretch, it is possible that secretion of ANF was occurring faster than synthesis, leading to secretory exhaustion. In contrast to congestive heart failure in human patients and cardiomyopathic hamsters, where failure results after a protracted period of time, the acute nature of the disease in T.brucei-infected dogs does not allow for atrial hypertrophy or recruitment of ventricles to secrete ANF and thus maintenance of elevated plasma levels. Hence the observed decrease in plasma ANF in terminal stages.

Secondly, decreased synthesis, secondary to myocyte damage, might have triggered the reduction in both ANF granules and plasma ANF. In another section of this work, dogs infected with T.brucei did indeed show severe myocardial damage (Ch. 8). Poor LVF and valvular incompetence probably caused increased intra-atrial pressure, leading to oversecretion of ANF. At the same time

increased damage to atrial cardiocytes resulted in decreased synthesis of ANF. The presence of distended Golgi complexes in the damaged atrial myocytes suggested that synthesis of ANF was still taking place but could not keep up with the rate of secretion.

In the terminal stages of the infection in week 4, the fall in ANF was accompanied by marked increase in PRA. A similar inverse relationship between ANF and PRA has already been demonstrated in *in vitro* studies in sodium-deprived human patients in whom infusion of ANF decreased PRA and plasma aldosterone in a dose-dependent manner (Oelkers *et al.*, 1988). Likewise, endogenous ANF released by rapid atrial pacing in dogs caused decreased PRA, followed by a marked rebound increase at 60 minutes post-pacing (Walsh *et al.*, 1987). *In vitro* studies have also demonstrated inhibition of renin secretion from rat juxtaglomerular cells (Kurtz *et al.*, 1986). In the present study, the observed increase in PRA may have been the result of removal of the inhibitory effect of ANF on juxtaglomerular cells, hence the inverse relationship between ANF and PRA.

At the same time, it is possible that the reduction in renal arterial blood pressure, secondary to poor LVF, contributed to the increase in PRA. The renal baroreceptors have a threshold pressure slightly below resting systemic pressure. A decrease in blood pressure by as little as 1.5 to 2.5 mmHg below threshold pressure in dogs increases resting renin release by 100 % of control (Kirchheim *et al.*, 1988). Poor LVF was demonstrated in dogs infected with

T.brucei (Ch. 4).

The decreased plasma ANF and increased PRA could be operating through various mechanisms to exacerbate the heart failure syndrome in T.brucei-infected dogs. Under normal physiological conditions, renin would stimulate the angiotensin converting enzyme, leading to increased production of AII from angiotensin I. Due to its dual activities of vasoconstrictor action and enhancement of sodium retention, AII can increase blood pressure, thereby having a negative feedback action on the pressure-dependent release of renin (Kirchheim et al., 1988). In the present study, however, the failing heart was probably unable to sustain arterial blood pressure, leading to continued secretion of renin and retention of sodium and water. In this respect, reduction of blood pressure by constriction of the pulmonary artery or thoracic caudal vena cava in conscious dogs is known to result in increased PRA, aldosterone and plasma volume (Watkins et al., 1976). In animals in which blood pressure was not restored, PRA and plasma aldosterone remained elevated throughout the period of constriction. For this reason, human patients suffering from congestive heart failure are given ACEI. It is possible that the use of ACEI at the time of trypanocidal treatment in parasitised dogs might decrease volume load in the heart and reduce the risk of development of congestive heart failure. In dogs, single injections of ACEI decreases blood pressure when PRA is elevated (Watkins et al., 1976).

The changes in ANF and PRA in this study suggest that both might be playing a significant role in the

pathogenesis of the heart failure syndrome. It is possible that an increase in PRA and decrease in ANF caused sodium and water retention, and increased water intake, leading to plasma volume expansion and increased venous return to an already failing heart. In the absence of ANF, the dogs were then unable to control the increased volume load, thereby exacerbating the severity of heart failure. The availability of an ANF infusion to replenish the plasma levels would be the ideal treatment to counter these changes.

Figure 7.1. Plasma concentration of ANF (pg/ml) (□) and PRA (mU/l) (▨) in dogs infected with T.brucei. Slight increases in ANF occurred on days 3 and 15, after which the concentration dropped up to termination of the study. PRA increased markedly after day 15. Values represent the mean ± 1SEM.

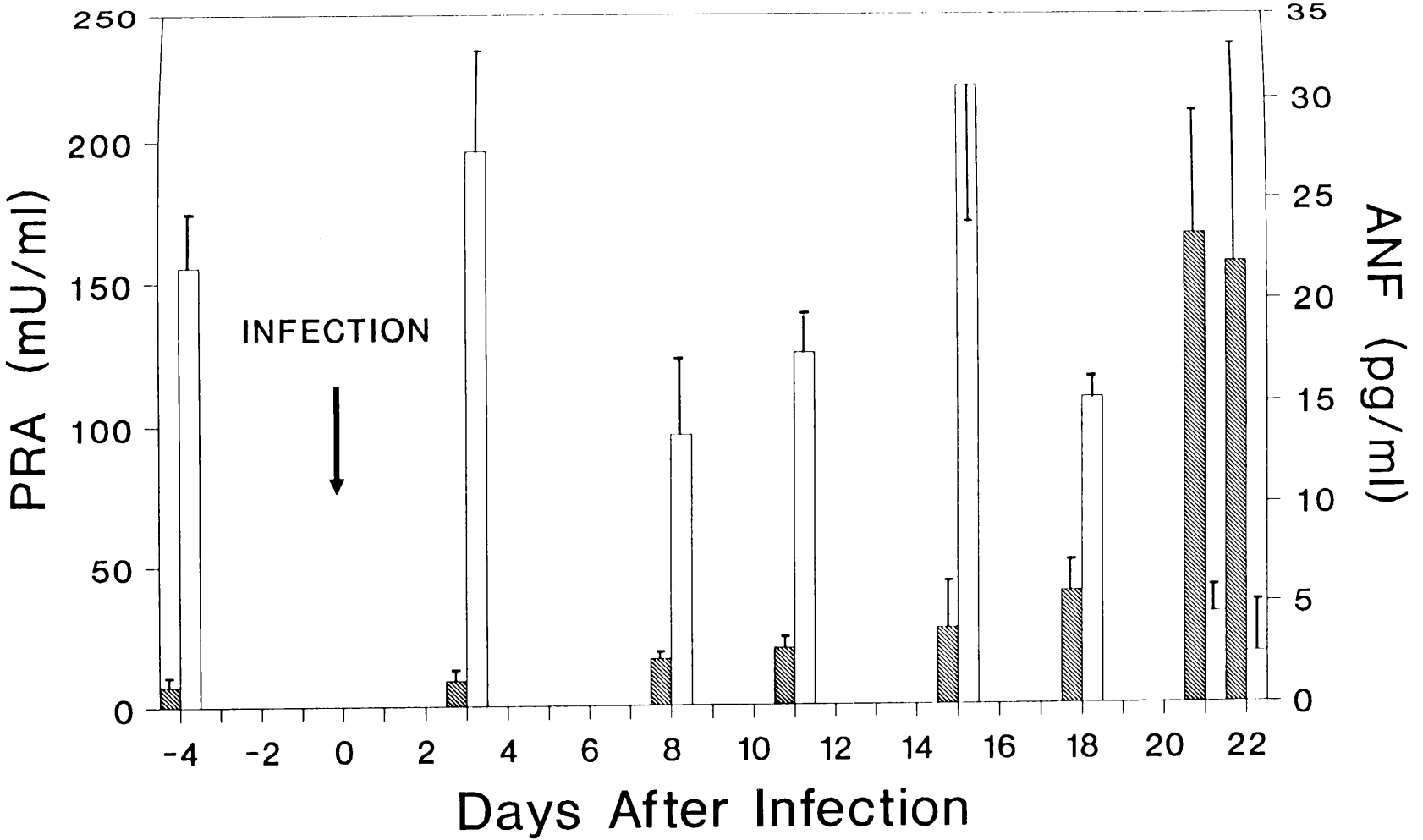
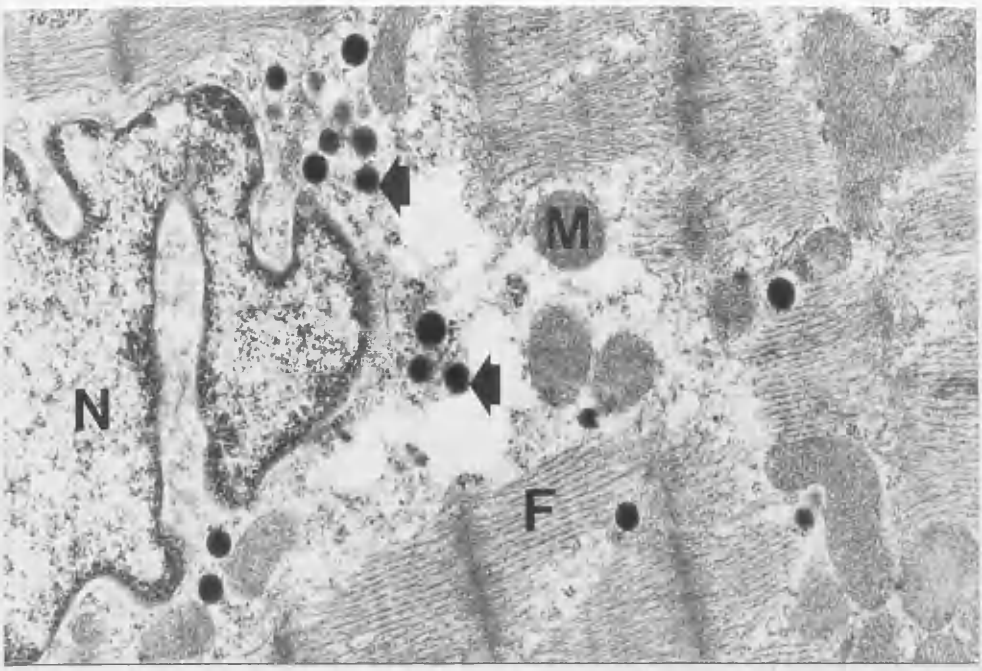

250
200
150
100
50
0
PRA (mU/ml)
INFECTION
35
30
25
20
15
10
5
0
ANF (pg/ml)
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
Days After Infection

Figure 7.2. A myocyte from the left atrium of a normal dog. Atrial granules (arrows) are located in the perinuclear region. N - Nucleus. M - Mitochondrion. F - Myofibrils. TEM. x28,000.

Figure 7.3. Atrial granules in the perinuclear region of an atrial myocyte from a normal dog. Most of the granules are highly electron-dense and are of uniform size. The Golgi complex (G) is small. TEM. x40,000.


M
N
F

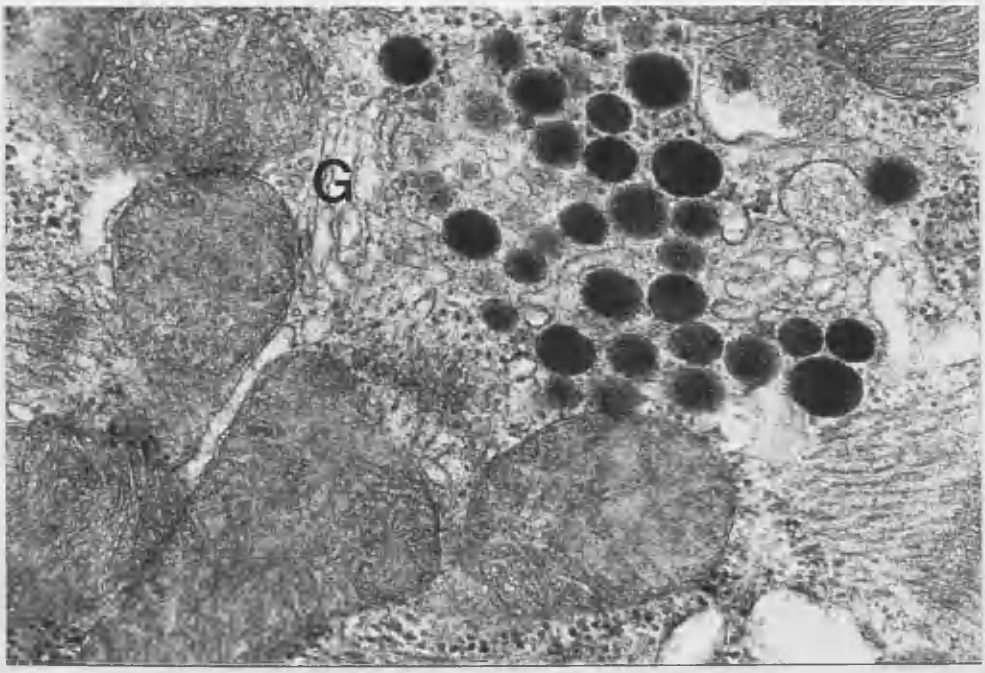
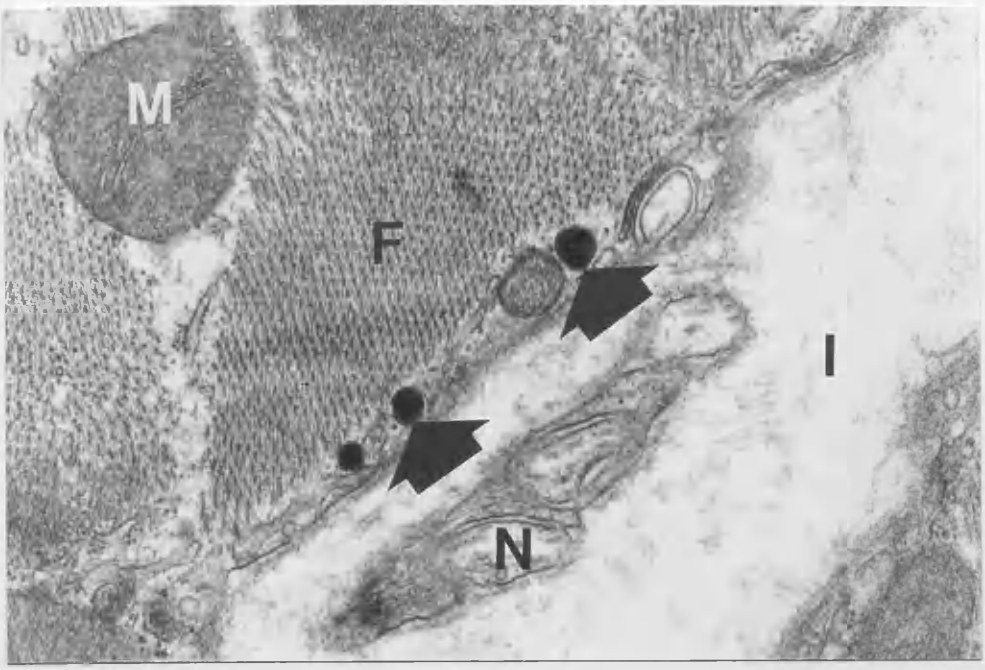

G

Figure 7.4. Atrial granules (arrows) in a subsarcolemmal location in a myocyte from a normal dog. M - Mitochondrion. F - Myofibrils. I - Intercellular space. N - Nerve processes. TEM. x40,000.

Figure 7.5. Atrial granules in a myocyte from a dog infected with T.brucei for 22 days. The granules are smaller and less electron dense than in uninfected dogs. The Golgi complex (G) is prominent. N - Myocyte nucleus. M - Mitochondrion. L - Lipid. TEM. x40,000.


M
F
I
N

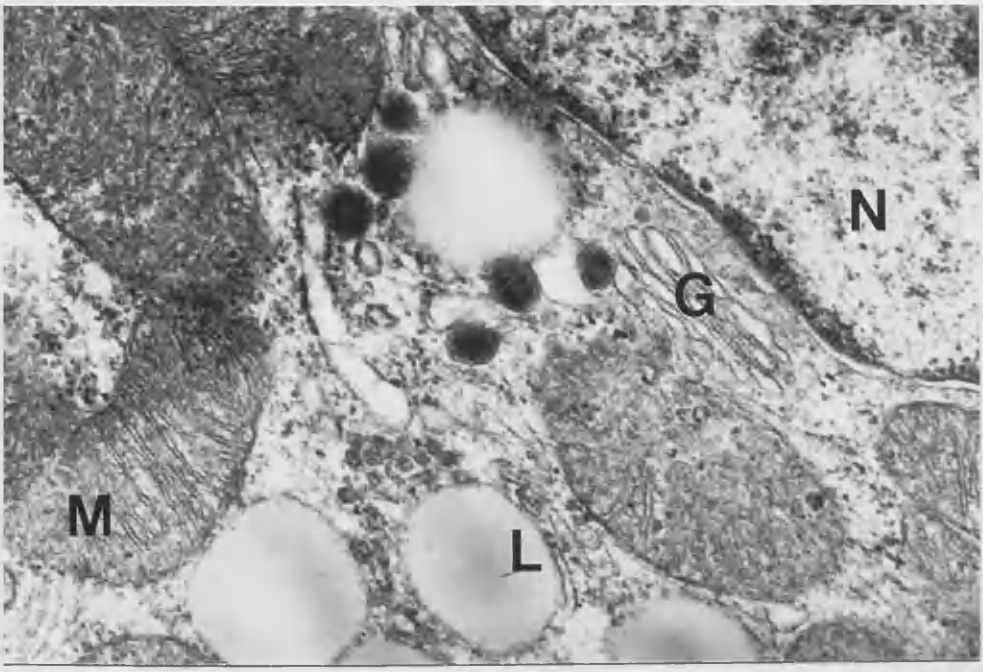
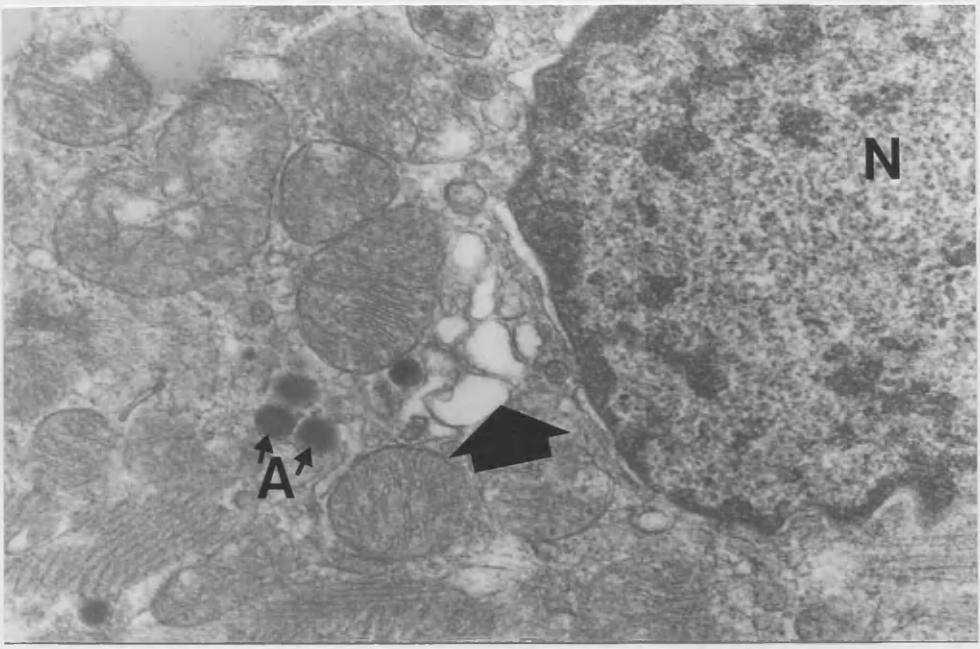

N
G
M
L

Figure 7.6. The perinuclear region of an atrial myocyte from a dog infected with T.brucei for 22 days. The Golgi complex (arrow) is distended. A few poorly electron-dense granules are visible (A). N - Myocyte nucleus.
TEM. x40,000.


N
A

CHAPTER 8.

THE PATHOLOGY OF CARDIAC DAMAGE IN DOGS INFECTED WITH *T.brucei*.

8.1. INTRODUCTION.

The pathology of the disease in dogs infected with T.brucei is associated with pantropic tissue damage, including severe pancarditis (Sayer et al., 1979; Morrison et al., 1981a; Kaggwa et al., 1983). Cardiac damage in African trypanosomiasis has been reported in human patients in both T.rhodesiense (Hawking and Greenfield, 1941; Manson-Bahr and Charters, 1963; De Raadt and Koten, 1968; Poltera et al., 1976; Poltera and Cox, 1977) and T.gambiense cases (Lavier and Leroux, 1939; Schyns and Janssen, 1955; Bertrand et al., 1971; Bertrand, 1987; Mbala et al., 1988).

Experimental infections with T.brucei in dogs (Morrison et al., 1981a; Kaggwa et al., 1983), mice (Galvao-Castro et al., 1978; Poltera et al., 1980), rats (Murray et al., 1974), goats (Whitelaw et al., 1985), sheep (Ikede and Losos, 1972b), cattle (Moulton and Sollod, 1976; Morrison et al., 1983) and vervet monkeys (Poltera and Sayer, 1983; Poltera et al., 1985), and with T.rhodesiense in dogs (Losos and Ikede, 1972) and vervet monkeys (Schmidt and Sayer, 1982; Poltera and Sayer, 1983; Poltera et al., 1985) indicate that the basic cardiac lesion is similar to that in man.

The mechanisms involved in the generation of heart lesions in African trypanosomiasis are not known. The severe pathology appears to be related to the invasive capacity of trypanosomes, and to their ability to generate biologically-active mediators (Tizard et al., 1978; reviewed by Mellors, 1985), or through

immunologically-mediated reactions (Galvao-Castro et al., 1978; Poltera et al., 1980; Greenwood and Whittle, 1980; Lambert et al., 1981; Kaggwa et al., 1983). The present part of the study was designed to investigate the possible mechanisms of cardiac damage in dogs infected with T.brucei.

8.2. MATERIALS AND METHODS.

8.2.1. ANIMALS.

The dogs, their management, and the stabilate used to infect them have been described before (Ch. 2). Briefly, 10 dogs were infected by intravenous inoculation with 5 x 10^3 T.brucei GVR35/c.1. Before and during the course of the disease, detailed clinical examinations were performed at daily intervals. Two dogs were euthanised on days 10, 15, 21, 22 and 26, and tissue blocks taken from the heart for histopathological studies. Four uninfected dogs served as controls and were euthanised after the last pair of infected animals.

8.2.2. HISTOLOGICAL TECHNIQUES.

Representative transmural blocks of tissue were taken from the walls of both ventricles and atria, and from the interventricular septum. Tissue samples were also selected from the cardiac valves. The method of sampling was such that part of the anterior papillary muscle and the mural leaflet of the mitral valve were included in the same section. This ensured that the whole length of the valve from the base to the tip was taken on the same plane. The same was done for the right ventricle, to include the

tricuspid valve. Sections taken from the interventricular septum inluded the aortic and pulmonary valves. The tissues were fixed in 10% neutral buffered formalin and post-fixed in mercuric chloride formal. Tissue blocks were embedded in paraffin wax and sections cut at 3 um, then stained with Mayer's haematoxylin and eosin (H&E). Selected sections were stained with Martius scarlet blue (MSB) and Massons trichrome stain as described by Lendrum et al. (1962).

8.2.3. TRANSMISSION ELECTRON MICROSCOPY.

Tissue blocks were taken from both ventricles and atria immediately after euthanasia of the dogs. The tissues were processed as previously described (Ch. 6), and examined with a JOEL CX100II transmission electron microscope.

8.2.4. IMMUNOFLUORESCENCE TECHNIQUES.

Representative tissue blocks were taken from the atria and ventricles and snap frozen at -70^{o}C. Three um sections were then cut using a Reichert freezing microtome, and following fixation in acetone, were stained with fluorescene icethiocyanate-conjugated rabbit anti-dog IgG, IgM, IgA, C3 and fibrinogen. The stained sections were then viewed with a Leitz fluorescence microscope equipped for incident light and the fields photographically recorded.

8.3. RESULTS.

8.3.1. CLINICAL FINDINGS.

The detailed clinical and haematological findings in these dogs have already been described (Ch. 3). Briefly, the dogs developed signs of disease after a prepatent

period of 5 to 6 days. The dogs developed high parasitaemia, persistent fever, lymphadenopathy, splenomegaly, severe anaemia, and weight loss.

In weeks 2 and 3, signs of cardiac damage appeared, in the form of tachycardia, valvular incompetence and heart blocks. From day 15, the severity of cardiac damage increased, and in the terminal stages in week 4, clinical signs of heart failure were pronounced. These included bradycardia, weak heart beats, increased capillary refill time, dyspnoea and coughing, poor left ventricular function (LVF), sinus arrest, S-T segment elevation, and accumulation of pericardial effusion (PE).

8.3.2. POST MORTEM FINDINGS.

Days 10 and 15: The carcasses were in good condition. At this time, there were no gross abnormalities indicating cardiac damage. Generalised oedema of subcutaneous tissues, lymph node and splenic enlargement were observed. In the two dogs euthanised on day 15, the pyloric region of the stomach and the cranial part of the duodenum were hyperaemic and oedematous. A few petechial haemorrhages were observed in the urinary bladder of one of them.

Days 21 to 26: All the carcasses were in poor condition, with loss of body fat and marked wasting of skeletal muscles. Marked changes were observed in the heart, being most severe in dogs euthanised on day 26. The pericardial and epicardial fat was gelatinous. The pericardial sac was distended with 20 to 50 ml blood-stained fluid (Fig. 8.1). The myocardium was pale, mottled and flabby, with

widespread petechial and ecchymotic epicardial haemorrhages on the atria and ventricles (Fig. 8.2). The haemorrhagic areas extended into the myocardium. A few petechial haemorrhages were observed at the base of the papillary muscles of the ventricles and on the endocardial surfaces of the atria. Other features related to cardiac damage were hydrothorax, pulmonary congestion and oedema, and ascites.

In most dogs, the liver was pale and large. The spleen was more than twice normal in size, and on cut section, the lymphoid follicles were very prominent. In addition, the pyloric and duodenal lesions had reached a more advanced stage, with haemorrhagic foci and small ulcers 2 to 10 mm in diameter. In some dogs, there were ulcers and linear haemorrhages in the colon. All lymph nodes were generally enlarged and there was oedema of subcutaneous tissues.

These changes were accompanied by corneal clouding and marked body wasting. The femoral bone marrow was red, and in the kidneys, there were focal haemorrhages in the medulla. A few petechial haemorrhages were found in the lungs.

8.3.3. HISTOLOGICAL FINDINGS.

The histological changes in the heart were similar in all the dogs examined at the intervals recorded. The lesions found in the heart varied with the region examined, and the duration of the infection before the dogs were euthanised. Generally, lesions in both atria were always higher than in the ventricles. The intensity of trypanosome and cellular infiltration, and the severity of myocardial damage in infected dogs are shown in Tables 8.1

and 8.2. From these, it can be seen that the severity of damage increased the longer the dogs survived. The histological findings are described in relation to the myocardium, the conducting system, valvular and vascular changes.

8.3.3.1. The myocardium.

Day 10: The subepicardial connective tissue was oedematous and contained a sparse cellular infiltrate consisting of mainly plasma cells, lymphocytes and macrophages. A few trypanosomes were found scattered within the interstitium.

The myocardium of both ventricles and the atria was normal, except for mild interstitial oedema, and a few infiltrating plasma cells and lymphocytes. A small number of trypanosomes were seen in the interstitium. In the lumen of most capillaries and small venules, there were aggregates of large lymphoid cells which were adherent to the vessel walls (Fig. 8.3). In some vessels, the lumen appeared to be occluded by lymphoid cells. Subendocardial oedema caused connective tissue separation and stretching of endocardial endothelial cells (Fig. 8.4).

Day 15: The epicardial fat was oedematous and some adipocytes were undergoing liquefactive necrosis. Cellular infiltration into the adipose tissue had occurred, and consisted of a small number of small lymphocytes and plasma cells. The cellular reaction was greater in the region of adipose tissue adjacent to the myocardium. The subepicardial connective tissue was oedematous and was infiltrated with many trypanosomes, plasma cells and

macrophages, together with a few lymphocytes and neutrophils.

In the ventricular myocardium, there was interstitial oedema with small numbers of plasma cells, macrophages, lymphocytes and trypanosomes (Table 8.1). In the atrial myocardium however, oedema and diffuse infiltration with trypanosomes, macrophages, plasma cells and lymphocytes had taken place at this time. Interstitial oedema caused marked separation of myocardial fibres.

The subendocardial cellular reaction was similar to, but less intense than that observed in the subepicardial myocardium. In several areas in the subendocardium, however, there were focal cellular infiltrates of macrophages, plasma cells and lymphocytes.

Day 21 to 26: Marked atrial and ventricular myocardial changes were observed at this time. There was oedema, cellular infiltration and necrotic changes in the subepicardial adipose tissue. Cellular infiltration into the adipose tissue consisted of large numbers of macrophages, neutrophils and plasma cells, and to a lesser extent lymphocytes. Many trypanosomes were present (Fig. 8.5). Macrophages were active and their cytoplasm contained necrotic debris, trypanosomes and large clear vacuoles. In another section of this work, the clear vacuoles in macrophages were shown to contain lipid (Ch. 6).

Some areas of the epicardium were denuded of cells, while others were covered with variable amounts of fibrin. Fibrin deposition and a cellular reaction similar to that

in the adipose tissue was observed in the subepicardial myocardium, accompanied by focal haemorrhages.

In the myocardium, there was widespread interstitial and perivascular oedema, causing separation of myocardial fibres. Diffuse infiltration with large numbers of foamy macrophages, neutrophils, trypanosomes, and to a lesser extent plasma cells and lymphocytes, had occurred (Figs. 8.6 and 8.7). Random haemorrhages were occasionally seen in the myocardium of the right ventricle and interventricular septum.

Multifocal areas of myocardial degeneration and fragmentation were observed (Fig. 8.8). In other regions of the myocardium, swollen and highly eosinophilic myocytes with pyknotic nuclei occupied circumscribed foci, and were usually associated with interstitial oedema and congested capillaries (Fig. 8.9).

A rare but prominent finding in the two dogs euthanised on day 26 were bands of muscle fibres thrown into regular wavy patterns in localised areas of the myocardium (Fig. 8.10). Myocytes at such sites were thin, with stretched nuclei, and arranged parallel to each other. In most cases the wavy myocardial folds appeared in locations close to areas undergoing ischaemic necrosis. These changes indicated acute myocardial infarction.

Changes in the septal myocardium were like those in the ventricular free wall. The papillary muscles were also involved, to the same extent as the myocardium.

In the subendocardium, there was marked cellular infiltration consisting of macrophages, neutrophils, plasma

cells, occasional lymphocytes and neutrophils. The cellular infiltration caused distention of the subendocardium (Fig. 8.11). In some sites, the endothelial lining was broken down and the aggregations of cells appeared to be on the endocardial surface (Fig. 8.12).

As stated earlier, the atria were more severely damaged than the ventricles, with focal subepicardial, myocardial and subendocardial haemorrhages (Fig. 8.13). There were foci of myocyte degeneration and fragmentation, associated with large numbers of macrophages, neutrophils and trypanosomes.

8.3.3.2. The conducting system.

In the present study, only changes in the subendocardial Purkinje fibres were determined. The changes appeared early in the infection and increased in severity with progress of the disease. Thus in the two dogs euthanised on day 10, swelling and vacuolation of Purkinje fibres had occurred (Fig. 8.14). In addition there was marked interstitial oedema. With progress of the disease, necrosis of Purkinje fibres was accompanied by infiltration of inflammatory cells, similar to those in the surrounding subendocardium, and with trypanosomes (Fig. 8.15).

8.3.3.3. The vasculature.

Blood vessels: In the early stages of the disease on day 10, there was swelling of capillary endothelial cells, in addition to accumulation of large lymphoid cells in the lumen (Fig. 8.3). Later, on day 15 of infection, oedema and infiltration of trypanosomes, macrophages, plasma cells

and a few lymphocytes was observed in perivascular areas of coronary arteries and veins.

Marked vascular changes were observed in the heart of dogs euthanised on day 26 of infection. At this time, there was a severe necrotising vasculitis of some medium-sized coronary arteries, characterised by swelling and disruption of endothelial and smooth muscle cells of the tunica media (Fig. 8.16). Subintimal and adventitial oedema caused almost total occlusion of the vessel lumen. A few trypanosomes were observed in damaged parts of the tunica media. Accompanying the necrotising vasculitis was a perivascular cellular infiltration around arteries and veins. The cellular reaction was similar to that in the surrounding myocardium. Variable amounts of fibrin were also deposited in the perivascular areas.

Lymphatic vessels: Changes in the lymphatic vessels varied with the stage of infection. On day 10, distention of subepicardial vessels with lymph was observed. Later, on day 15, the subepicardial, myocardial and subendocardial lymphatic vessels were markedly distended. A few lymphocytes, plasma cells, macrophages and many trypanosomes were suspended in the lymph fluid (Fig. 8.17). In dogs euthanised between days 21 and 26, the number and composition of cells in the lymphatic vessels had increased, sometimes causing total occlusion of the vessels. In some lymphatic vessels, foamy macrophages were predominant (Fig. 8.18), while in others, macrophages, neutrophils, lymphocytes, plasma cells and trypanosomes appeared together (Fig. 8.19). In most cases, these cells

appeared to be trapped in a fibrin clot. The endothelial lining of some vessels had disintegrated (Fig. 8.20).

8.3.3.4. The valves.

All cardiac valves were involved, the atrioventricular more than the aortic and pulmonary valves.

Day 10: There was generalised oedema at the valve base and cusp tissue, causing connective tissue separation and focal fibroblast degeneration. Endothelial cells on the valves were attenuated, and there was infiltration by occasional lymphocytes and plasma cells; no trypanosomes were identified (Fig. 8.21).

Day 15: The degree of fibroblast degeneration and necrosis was more obvious than on day 10. At this time, cellular infiltration was more intense and diffuse. There were extensive foci of connective tissue separation due to interstitial oedema. Only a few trypanosomes were present in the interstitium.

Days 21 to 26: Oedema, fibroblast degeneration and necrosis, and trypanosome and cellular infiltration were greater. Accumulation of cells and trypanosomes had occurred predominantly under the endothelium on the flow side of the valves. The cells consisted mainly of macrophages, plasma cells and to a lesser extent, lymphocytes and neutrophils (Fig. 8.22). In some cases, trypanosomes had penetrated deep into the collagenous tissue of the valve leaflets; cellular infiltration did not occur in this location.

Changes in other organs that were probably secondary to cardiac damage included pulmonary congestion, interstitial and alveolar oedema in the lungs, and venous congestion of the liver. These changes occurred in dogs euthanised from day 21 of infection.

8.3.4. ULTRASTRUCTURAL FINDINGS.

As well as confirming the histological findings, TEM provided additional findings in the heart muscle, in the cell types, in the cardiac blood vessels, and in the autonomic nerve tissue. The changes varied with the stage of the disease.

8.3.4.1. The heart muscle.

Day 10: No abnormal changes had occurred in myocytes and interstitium of either the ventricles or the atria, apart from a few lipid droplets in some myocytes. Such droplets were also found in myocytes from the hearts of some of the uninfected dogs and were therefore not regarded as significant.

Day 15: Myocytes were swollen, the glycogen content was reduced, and lipid deposition increased. At the same time, interstitial oedema had increased.

Days 21 to 26: Marked changes were observed in the cardiac myocytes. Most myocytes in the atria and ventricles were distended with fluid, had little glycogen, and contained many lipid droplets (Fig. 8.23). The mitochondria in such myocytes were swollen and contained matrical electron-dense deposits close to the cristal membranes. In other myocytes,

the mitochondria were swollen and the cristae broken down (Fig. 8.24). In addition, fragmentation and disorganisation of myofibrils was observed, while the sarcoplasmic membrane was breached in some myocytes. Intercalated discs between adjacent myocytes were separated to varying degrees, especially in dogs euthanised on day 26 of infection.

8.3.4.2. Cellular changes.

Days 10 and 15: A few macrophages, lymphocytes and plasma cells were observed in the interstitium by day 10 of infection. Trypanosomes were only demonstrated in the lumen of intramyocardial capillaries. On day 15 of infection, trypanosomes could be seen migrating between myocytes (Fig. 8.25) and never through capillary walls.

Days 21 to 26: Cellular changes in the myocardium were more intense. Trypanosomes, accompanied by plasma cells, macrophages, lymphocytes, neutrophils and free erythrocytes were present in large numbers in the interstitium (Fig. 8.26). In addition to increased numbers, the cells were at various stages of activity. Plasma cells were particularly active, with many showing markedly distended cisternae of the granular endoplasmic reticulum (Fig. 8.27). Macrophages were distended with phagocytosed material, which included cellular organelles, leucocytes, lipid, fibrin, erythrocytes, platelets, and sometimes whole trypanosomes (Figs. 8.28, 8.29, 8.30, 8.31). Neutrophils were at advanced stages of degranulation (Figs. 8.32, 8.33). Trypanosomes were present in large numbers in the

myocardial interstitium; some appeared to be dead and at different stages of disintegration. In other areas, necrosed inflammatory cells were trapped by large quantities of fibrin (Fig. 8.34).

8.3.4.3. The vasculature.

Occlusion of capillary lumina by lymphocytes and monocytes was observed from day 10 of infection, confirming the histological findings (Fig. 8.35). On day 15 of infection, swelling of capillary endothelial cells had occurred, causing reduction in lumen size.

In dogs euthanised from day 21, capillary endothelial cells were swollen, accompanied by cytoplasmic vacuolation and nuclear degeneration. As a result, some of the capillary lumina were occluded (Fig. 8.36). In other capillaries, the basal lamina was disrupted and the plasma membranes of endothelial cells formed pockets projecting towards the perivascular space, even when the junctional attachments between the endothelial cells remained intact (Fig. 8.37). At this time too, some of the capillaries were occluded by erythrocytes, (Fig. 8.38). Junctional attachments between smooth muscle cells in the walls of arterioles were disrupted and distended with fluid (Fig. 8.39).

8.3.4.4. The autonomic nerve ganglia.

Figure 8.40 shows the appearance of an autonomic nerve ganglion from an uninfected dog. Up to day 15 of infection, there were no observable changes in the appearance of nerve ganglia. Thereafter, marked vacuolation and necrosis of

Schwann cells and nerve axons had occurred (Fig. 8.41). There was interstitial oedema surrounding the nerve ganglia, and a cellular infiltrate consisting of neutrophils, macrophages and plasma cells. Variable amounts of fibrin were also deposited in the interstitial spaces surrounding the ganglia.

8.3.5. IMMUNOFLUORESCENCE FINDINGS.

From day 15 of infection, fibrinogen was demonstrated in increasing concentration in the perivascular areas and interstitium between myocytes in both the atria and the ventricles (Fig. 8.42). Small quantities of IgM and IgG were also demonstrated in similar areas. These were considered to be the result of vascular damage and subsequent leakage of plasma proteins.

8.3.6. THE KIDNEYS.

Immunofluorescence staining of kidney sections from infected dogs demonstrated the presence of variable quantities of IgG, IgM, C3, fibrinogen and trypanosome antigen from day 10, increasing with progress of the disease. The staining was most intense in the glomeruli (Fig. 8.43). TEM confirmed the presence of immune complex deposits in the mesangium (Fig. 8.44).

8.4. DISCUSSION.

In the present study, T.brucei infection in dogs resulted in generalised tissue damage involving all body organs, including severe pancarditis. At necropsy, the finding of a mottled, flabby heart, in addition to

hydrothorax, hepatic and splenic enlargement and ascites, and pulmonary congestion and oedema on histology, indicated that congestive heart failure might be the main cause of death in infected dogs.

Histological evidence of cardiac damage appeared early in the disease, and consisted of subendocardial oedema, valve damage and occlusion of small capillaries, all indicative of the severe inflammatory nature of T.brucei infection in dogs. These changes were consistent with the demonstration of increased acute phase proteins (APP) in the plasma of infected dogs at this time (Ch. 5). The presence of valvular damage was also consistent with echocardiographic demonstration of valvular incompetence reported in Chapter 4. Subendocardial and valvular oedema was probably part of the generalised oedema observed clinically in subcutaneous tissues in different parts of the body. Inflammatory responses to infection have previously been shown to occur early in both dogs (Mwambu et al., 1979; Sayer et al., 1979; Morrison et al., 1981a) and rabbits (van den Ingh and van Dijk, 1975) infected with T.brucei. This was considered to be associated with release of biologically active substances by trypanosomes, or by the host in response to infection, which cause increased vascular permeability. A permeability increasing factor has been isolated from extracts from T.gambiense (Seed, 1969). Trypanosomal infection also leads to the release of kinins, associated with antigen-antibody reactions, which may also be responsible for changes in vascular permeability and oedema (Boreham, 1968; Goodwin, 1971).

Adhesion to the endothelium of small blood vessels by lymphoid cells occurred at an early stage of the disease. In inflamed areas in general, the adhesive capacity of endothelial cells to leucocytes is increased. Leucocytes coming into contact with such cells attach to them, and thus interfere with blood flow in the small capillaries (Schmid-Schonbein and Engler, 1986). Following attachment to the endothelium, leucocytes then migrate across the vessel walls into the interstitium, as part of the inflammatory reaction.

Myocardial infiltration with plasma cells and macrophages early in the disease indicated rapid activation of humoral immune mechanisms. Increased plasma cell infiltration has been demonstrated in dogs (Morrison et al., 1981a), sheep (Ikede and Losos, 1972b), mice (Poltera et al., 1980) and rats (Murray et al., 1974) infected with T.brucei, accompanied by an activated mononuclear phagocytic system (MPS) (Murray et al., 1974). The process of lymphocyte activation by trypanosome antigens could be taking place directly. When trypanosome antigens bind to Class II major histocompatibility complex (MHC) (Ia) receptors on B lymphocytes, they are processed rapidly and presented to CD4 receptors on helper/inducer T lymphocytes (Th) (Abbas, 1987). Proliferation of Th cells and B cell stimulation takes place, causing a large increase in the numbers of plasma cells early in the infection.

Some types of macrophages are highly potent antigen-presenting cells too, and in the presence of interleukin-1, stimulate marked Th cell proliferation and B

cell stimulation (Kurt-Jones et al., 1987). The result is increased antibody synthesis by the activated B cells and high levels of immunoglobulins and immune complexes. Increased levels of immunoglobulins and immune complexes have been observed in the plasma of dogs (Kaggwa et al., 1984) and intercellular spaces in the hearts of mice (Galvao-Castro et al., 1978) and dogs (Kaggwa et al., 1983) infected with T.brucei, and in the plasma of human patients infected with T.rhodesiense (Lambert et al., 1981). Similarly in the present study, the presence of circulating immune complexes was confirmed by their presence in the kidneys.

In dogs infected for 21 days or more, the granular endoplasmic reticulum of plasma cells was shown by TEM to be highly distended with colloid. Distention of plasma cells, eventually leading to formation of Russell body containing cells (frequently referred to as Mott cells or morula cells) is a common finding in African trypanosomiasis (Poltera et al., 1976; Morrison et al., 1981b). In autoimmune diseases, Mott cells are found in large numbers in lymphoid organs as well as in other tissues (Alanen et al., 1985). They are also found in chronic inflammatory cell infiltrates and in spleens of chickens which have been immunosuppressed by treatment with cyclosporin A (Nowak et al., 1982), and have been shown to be plasma cells that are defective in immunoglobulin secretion (Alanen et al., 1985). The distended plasma cells observed in the current study may well have later developed into Russell body containing

plasma cells.

In addition to a marked increase in cellular and trypanosome infiltration into the myocardium, the composition of cells changed rapidly during and after the third week of infection. As a result, highly activated macrophages, and neutrophils at various stages of degranulation became the major cell types. The degree of tissue damage increased at this time too. This indicates that neutrophils and macrophages, in the presence of trypanosomes in extravascular sites, might be playing a major role in the pathogenesis of cardiac damage in particular, and tissue damage in general in dogs. While this has been recognised with regard to macrophages, the importance of neutrophils in causing tissue damage in African trypanosomiasis has been underestimated, despite their continued presence in tissues of animals and human patients infected with trypanosomes of the Trypanozoon subspecies (Yorke, 1911; Hawking and Greenfield, 1941; Manuelidis et al., 1965; Koten and De Raadt, 1969; Ikede and Losos, 1972b; van den Ingh and van Dijk, 1975; Facer et al., 1978; Galvao-Castro et al., 1978; Poltera and Sayer, 1983). The finding that neutrophils are particularly predominant in some acute trypanosome infections indicates that they play a central role in mediating tissue damage, especially in dogs.

There are many causes of neutrophil chemotaxis and degranulation, including complement-derived factors (Wierusz-Wysocka et al., 1987), macrophages and lymphocytes reacting with microbial products and antigens (Nathan,

1987), cachectin/TNF (Ji Ming et al., 1987; Nathan, 1987) and reaction of lipids or vascular endothelial cells with reactive oxygen metabolites (ROM) (Petrone et al., 1980). ROM are released by several systems, some of which are trypanosomes themselves (Turrens, 1987) and endothelial cells following direct or ischaemic damage (Hernandez et al., 1987). At sites of accumulation, neutrophils undergo 'respiratory bursts', leading to rapid utilization of glucose (through the hexose monophosphate shunt), degranulation, and release of highly potent oxidants that are toxic to tissues (Wierusz-Wysocka et al., 1987). The release of more ROM by neutrophils further exacerbates their chemotaxis and degranulation (Baboir, 1984). In the current study, activated macrophages, lipid deposition, vascular damage, focal ischaemia and trypanosomes were demonstrated together with degranulate neutrophils in regions of extensive tissue damage.

When neutrophils encounter large targets, they deliver their oxidants into spaces formed between them and the target. This way, leakage of oxidants into the surroundings takes place, leading to increased tissue damage (Petrone et al., 1980). It is possible that this is the only way that neutrophils can effectively deal with large targets such as trypanosomes. Eventually the neutrophils are themselves overwhelmed and undergo autolysis, releasing all their toxic enzymes into the interstitium, and thus contributing to further tissue damage. It is therefore possible that neutrophils, in life and in death, play a dual role in the pathogenesis of T.brucei-induced pancarditis in the dog.

Vascular damage has previously been reported in other dogs (Morrison et al., 1981a), sheep (Ikede and Losos, 1972b) and rabbits (Goodwin, 1971) infected with T.brucei, and in human African trypanosomiasis (Lavier and Leroux, 1939). However, the presence of almost completely occluded medium-sized intramyocardial arteries in the present study indicates that vascular occlusion contributed significantly to the pathogenesis of cardiac damage, especially the development of myocardial ischaemia. In addition, the severe anaemia which occurred in T.brucei-infected dogs (Ch. 3), coupled with vascular damage and extensive interstitial oedema, might have increased the resistance of intracardiac vessels to blood flow, hence tissue hypoxia and ischaemia. Myocardial ischaemia develops when cellular oxygen demand exceeds oxygen supply (Harken et al., 1981). Tissue hypoxia probably contributed to the poor LVF that was demonstrated in the infected dogs (Ch. 4).

In terminally ill dogs, in addition to focal ischaemic necrosis, bands of wavy muscle fibres were demonstrated in the myocardium. Formation of wavy bands is an indication of acute myocardial infarction (Bouchardy and Majno, 1977). Waviness results when excessive tension is applied on akinetic muscle fibres in the infarcted zone by the normal myocardium during contraction, together with outward bulging of the infarcted area during systole (Davies, 1985). This was consistent with the observation of S-T segment elevation and depression on ECG at this time (Ch. 4).

The cause of myocardial infarction in infected dogs was

difficult to determine, especially because no vascular thrombosis was demonstrated. Thrombosis has been observed in other organs in dogs infected with T.brucei (Morrison, et al., 1981a). Poltera and Sayer (1983) observed large infarct-like zones in the hearts of T.rhodesiense-infected vervet monkeys without any evidence of vascular obstruction. In the current study, obliteration of blood supply to certain regions of the heart was probably secondary to the occlusive vasculitis observed in some coronary arteries. In this respect, it is worth noting that in man, hypoxaemia of the myocardium without total coronary occlusion can cause myocardial infarction (Rubio and Berne, 1975).

TEM demonstrated mitochondrial damage, together with deposition of electron-dense granules on the cristal membranes of mitochondria in severely damaged myocytes. In several models of cell injury, amorphous mitochondrial densities have been considered a reliable sign of irreversible injury, and are frequently encountered in cases of ischaemic injury (Kloner et al., 1974). The presence of matrical electron-dense granules in mitochondria was an indication that severe electrolyte imbalances were occurring within the myocardium of T.brucei infected dogs.

Abnormalities in the conducting system of the heart were apparent by day 10 of infection, in the form of swollen Purkinje fibres, extensive interstitial oedema around them, and later cellular infiltration. In terminally infected dogs, necrosis of Schwann cells and axons of

autonomic nerve ganglia was demonstrated by TEM. In another part of this work, abnormalities in generation and conduction of electrical impulses through the hearts of infected dogs were recorded on ECG (Ch. 4). Damage to the conducting system has been observed histologically in mice (Poltera et al., 1980) and dogs (Morrison et al., 1981a) infected with T.brucei, in human patients infected with T.rhodesiense (Poltera et al., 1975; Poltera et al., 1976) and in vervet monkeys infected with T.rhodesiense and T.brucei (Poltera and Sayer, 1983). In the current study, the inflammatory reactions and direct damage to autonomic neurones may have contributed to the ECG abnormalities. In addition, the observation that atria were more severely damaged than the ventricles indicated that generation of electrical impulses by the sinuatrial node (SAN), and conduction through the atrial ventricular node (AVN), was affected. This is in agreement with clinical demonstration of sinus arrest and HB in infected dogs (Ch. 3).

Both intracellular and extracellular lipid deposition in the myocardium of trypanosome-infected but not control dogs has been demonstrated (Ch. 6). At necropsy, the adipose tissue of infected dogs was gelatinous, while there was histological evidence of adipocyte necrosis. This was accompanied by generalised body wasting. Gelatinisation of adipose tissue has previously been reported in other dogs infected with T.brucei (Morrison et al., 1981a; Kaggwa et al., 1983), cattle dying of trypanosomiasis (Fiennes, 1970) and sheep infected with T.brucei (Losos and Ikede, 1972b). This is further evidence that infection with African

trypanososmes leads to extensive breakdown of body fat.

In infected dogs, lymphatic vessels became distended with lymph. Later, accumulation and obstruction by inflammatory cells and fibrin, with evidence of necrotic changes in the vessel walls, were observed. Lymphatic obstruction has previously been demonstrated in mice (Poltera et al., 1980), rats (Murray et al., 1974), sheep (Ikede and Losos, 1972b) and dogs (Morrison et al., 1981a; Kaggwa et al., 1983) infected with T.brucei, human patients (Hawking and Greenfield, 1941; Poltera and Cox, 1977) and vervet monkeys infected with T.rhodesiense (Poltera and Sayer, 1983). Following lymphatic obstruction, removal of metabolic wastes, excess inflammatory fluids, and toxic and biologically active agents released from dying trypanosomes and inflammatory cells must have been impaired, resulting in their accumulation, and hence increased myocardial damage. It is known that in dogs, ligation of myocardial lymphatic vessels causes subendocardial oedema and haemorrhage within 3 hours. This is accompanied by ischaemic myocardial injury, including lymphangiectasis, myofibrillar degeneration, disruption of Z bands and intercalated discs, and various mitochondrial derangements (Sun and Lie, 1977). Similar observations were made in the current study. Moreover, accumulation of PE would also have been enhanced by the impaired lymphatic drainage.

In addition to myocarditis, valve damage increased in severity with progress of the disease, and was associated with interstitial oedema, cellular and trypanosome infiltration. This was consitent with the echocardiographic

demonstration of valvular incompetence beginning from day 10 of infection (Ch. 4). The presence of trypanosomes deep in the collagenous part of valves has previously been reported in vervet monkeys infected with T.rhodesiense (Poltera et al., 1985). This would make the trypanosomes in deeper areas poorly accessible by trypanocidal drugs due to the avascular nature of the collagenous part of the valve, raising the possibility that cardiac valves could serve as cryptic sites from which relapse infections might result.

Immunofluorescence and TEM studies demonstrated deposition of fibrin in large quantities in perivascular and interstitial sites. In addition, by immunofluorescence, small amounts of IgG and IgM were observed. TEM however failed to demonstrate immune complex deposition in the heart. Though immune complexes have previously been observed in African trypanosomiasis (Lambert and Houba, 1974; Murray, 1974; Galvao-Castro et al., 1978; Poltera et al., 1980; Lambert et al., 1981; Kaggwa et al., 1983), their direct role in mediating tissue damage in the current study could not be conclusively established. The presence of immunoglobulins and trypanosome antigens in extravascular sites in the heart was probably the result of leakage across damaged blood vessels. However, immune complex deposition was demonstrated in the kidneys of these dogs, indicating that immune complex-mediated tissue damage contributes to general tissue damage directly, and possibly cardiac damage indirectly, in dogs infected with T.brucei.

From the foregoing, it would appear that cardiac damage

in dogs infected with T.brucei is initiated by acute inflammatory reactions by the host in response to circulating trypanosomes and substances generated by them, resulting in production of vasoactive substances that increase vascular permeability and interstitial oedema. Several factors later contribute to progressive tissue damage, including immunological responses to the highly antigenic trypanosomes in tissue, leading to cellular infiltration. Inflammatory cells and dying trypanosomes release highly toxic substances into the interstitium, and because lymphatic drainage is impaired, the toxic substances accumulate, causing electrolyte imbalances and myocyte damage. At the same time anaemia and vascular obstruction lead to tissue hypoxia and ischaemic necrosis of the myocardium. Presence of lipid deposits in the myocardium would further interfere with electrolytes and tissue oxygenation, exacerbating the degree of damage. These changes, in addition to valvular incompetence, would affect myocardial contractility and precipitate heart failure.

TABLE 8.1.

INTENSITY OF TRYPANOSOME AND CELLULAR INFILTRATION IN THE HEARTS OF DOGS INFECTED WITH T.brucei.

DAY OF EUTHANASIA	EPICARDIUM					MYOCARDIUM					ENDOCARDIUM				
	P	M	L	N	T	P	M	L	N	T	P	M	L	N	T
10	1+	1±	1+	-	1±	1±	-	1±	-	1±	1+	1±	1+	-	1±
15	2+	2+	2+	1+	2+	2+	2+	1+	1±	2+	3+	2+	1+	1±	3+
21-26	2+	3+	2+	3+	3+	2+	2+	2+	2+	3+	2+	3+	2+	3+	3+

P - Plasma cells.
M - Macrophages.
L - Lymphocytes.
N - Neutrophils.
T - Trypanosomes.

\- Absent.
1± Minimal in some dogs, absent in others.
1+ Minimal.
2+ Moderate.
3+ Marked.

TABLE 8.2.

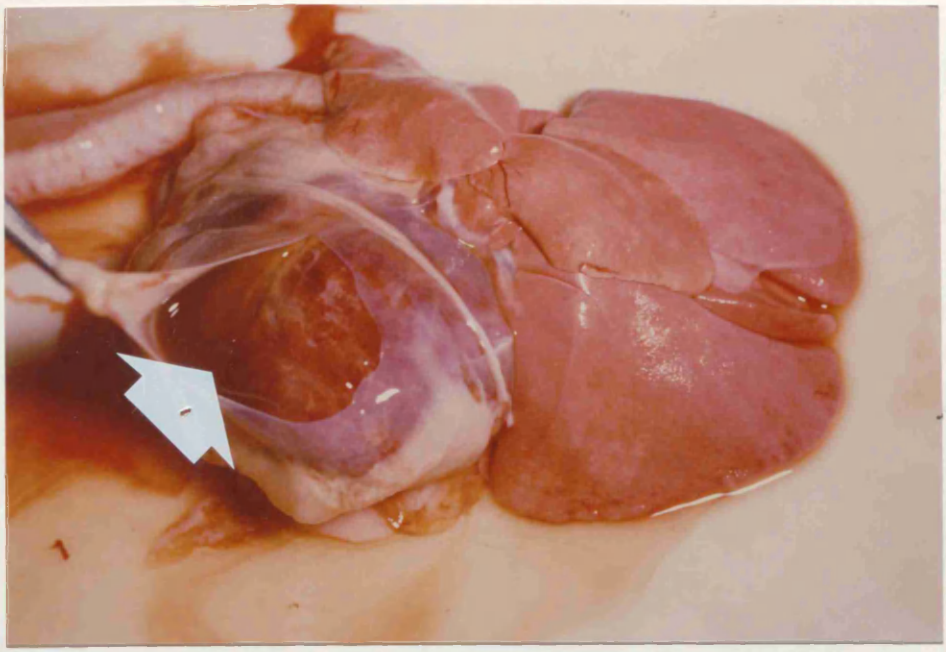
GENERAL SEVERITY OF TISSUE DAMAGE WITH PROGRESS OF THE DISEASE IN DOGS INFECTED WITH *T.brucei*.

DAY OF EUTHANASIA	EPICARDIUM	MYOCARDIUM	ENDOCARDIUM
10	1+	-	1+
15	2+	1+	2+
21-26	3+	2+	3+

- Absent.
1+ Minimal.
2+ Moderate.
3+ Marked.

Figure 8.1. The heart and lungs from a dog infected with T.brucei for 26 days. The pericardial sac has been opened to display a large volume of bloodstained pericardial effusion (arrow). There is marked gelatinisation of the pericardial fat.

Figure 8.2. The pericardial sac in Figure 8.1 has been removed to expose a globular shaped heart with widespread petechial and ecchymotic haemorrhages (arrow).



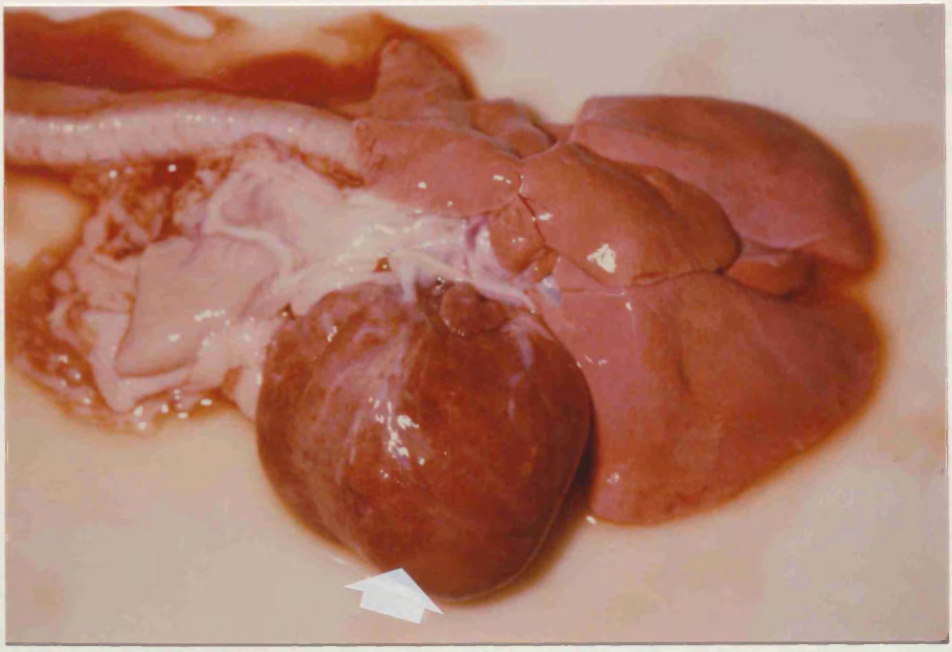
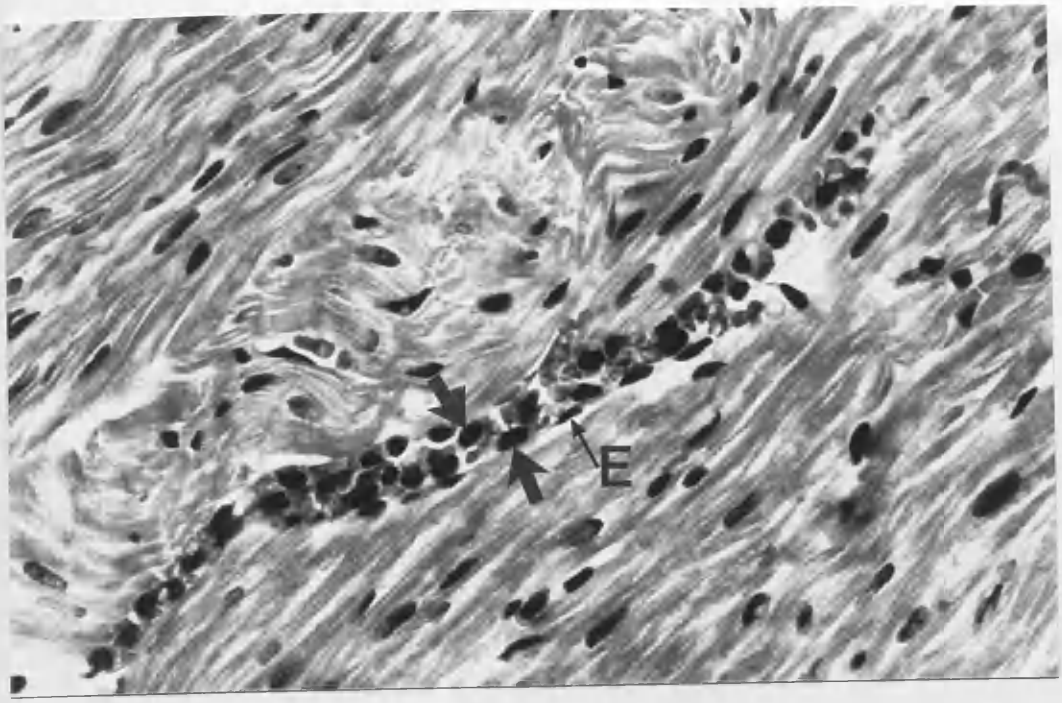


Figure 8.3. Adhesion of large lymphoid cells (arrows) to the endothelium of a small blood vessel in the right ventricle of a dog infected with T.brucei for 10 days. The myocardium is normal. E - Endothelial cell. H&E. x320.

Figure 8.4. Subendocardial oedema in the interventricular septum of a dog infected with T.brucei for 10 days. There is attenuation of fibrous tissue in the subendocardium (arrows). There is no trypanosome or cellular infiltration and the underlying myocardium is normal. E - Endocardium. H&E. x260.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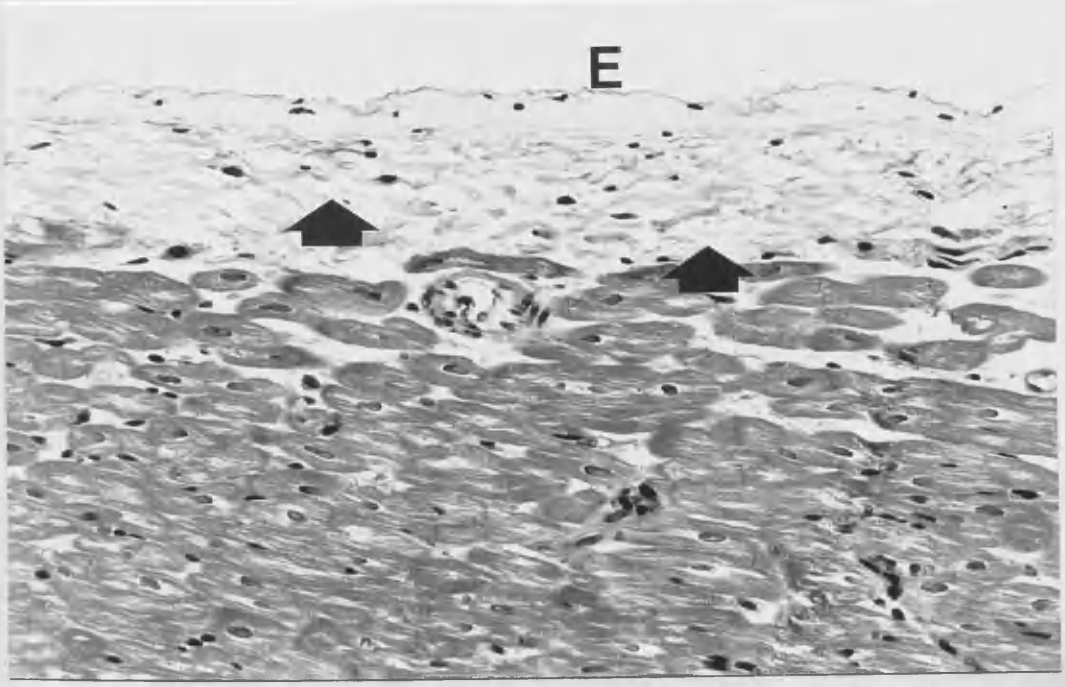
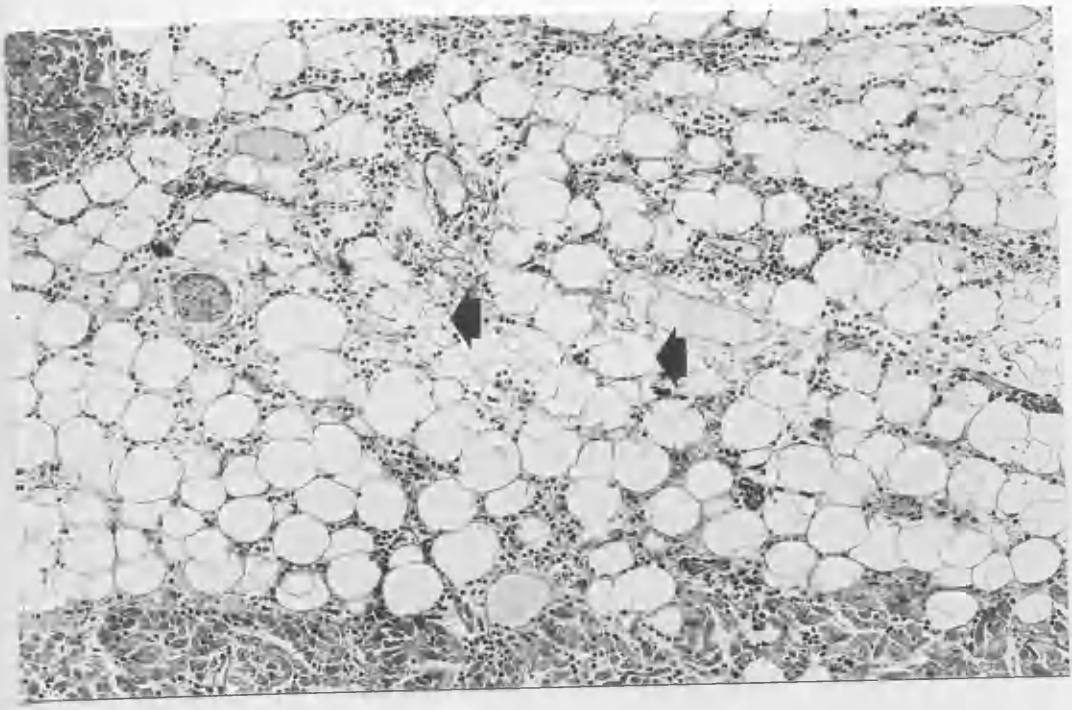

E

Figure 8.5. Necrosis of adipocytes (arrows) in the subepicardial adipose tissue at the base of the heart in a dog infected with T.brucei for 21 days. There is diffuse trypanosome, polymorphonuclear and mononuclear cellular infiltration in the adipose tissue and adjacent myocardium. H&E. x100.

Figure 8.6. Severe myocarditis of the left ventricle in a dog infected with T.brucei for 26 days. There is myocytolysis (large arrows) and infiltration with trypanosomes, vacuolated macrophages (small arrows), neutrophils, plasma cells and a few lymphocytes. H&E. x320.



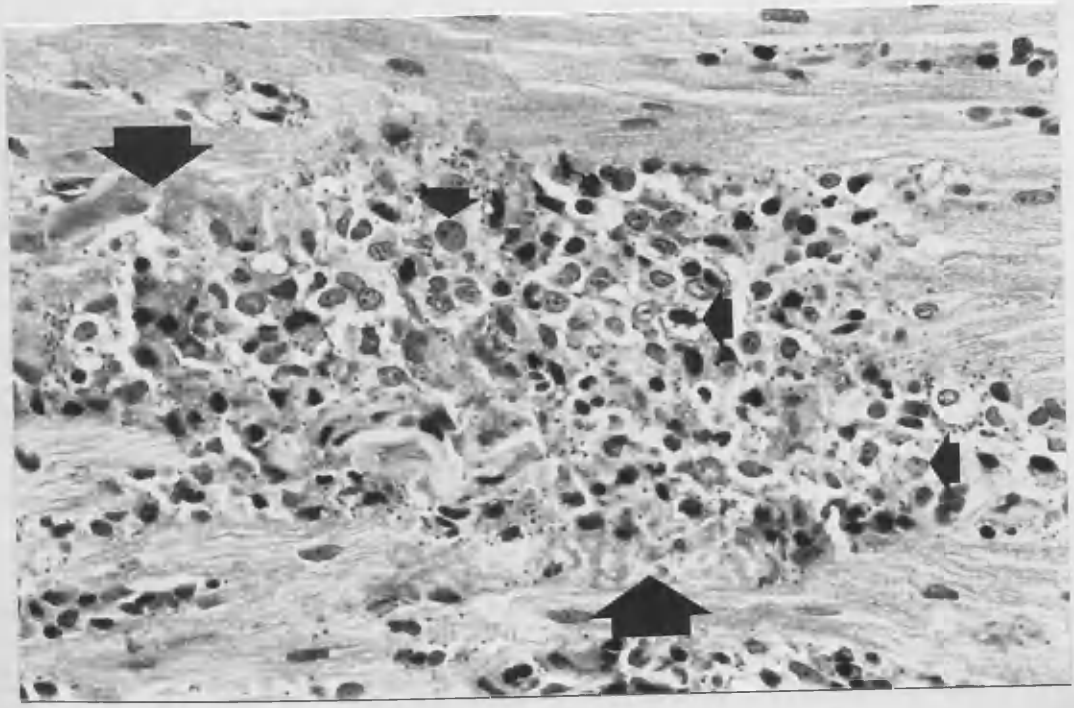
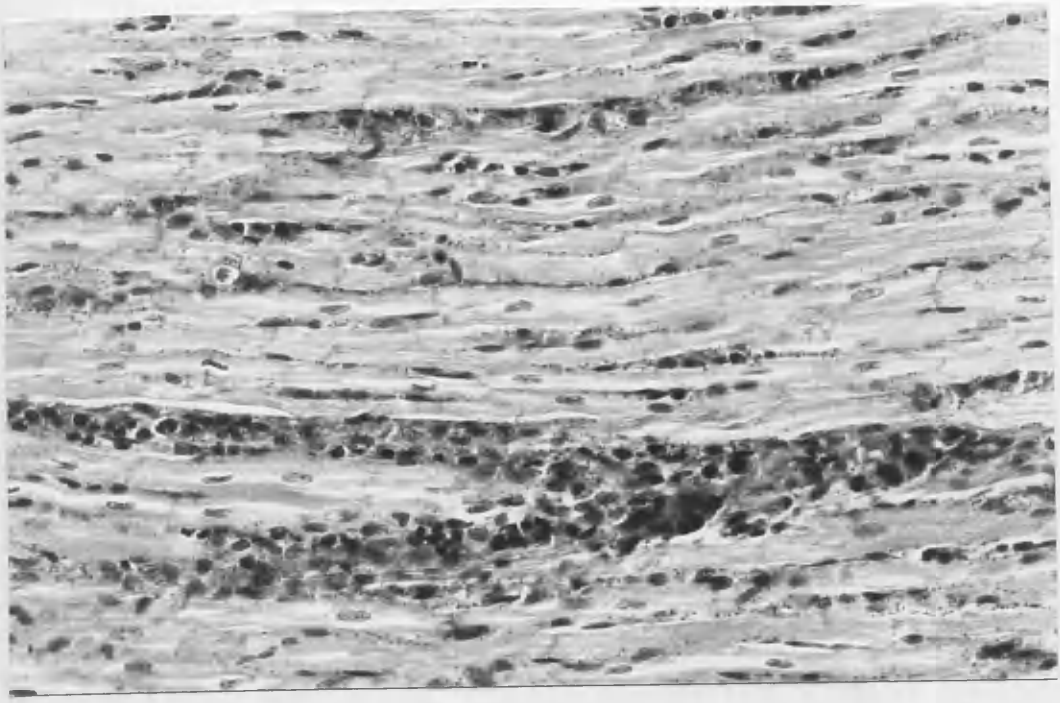


Figure 8.7. Severe myocarditis of the interventricular septum of the dog in Figure 8.6. There is diffuse trypanosome and cellular infiltration. H&E. x260.

Figure 8.8. Focal myocytolysis (arrow) in the right ventricle of a dog infected with *T.brucei* for 26 days. There is diffuse myocardial cellular and trypanosome infiltration. H&E. x300.



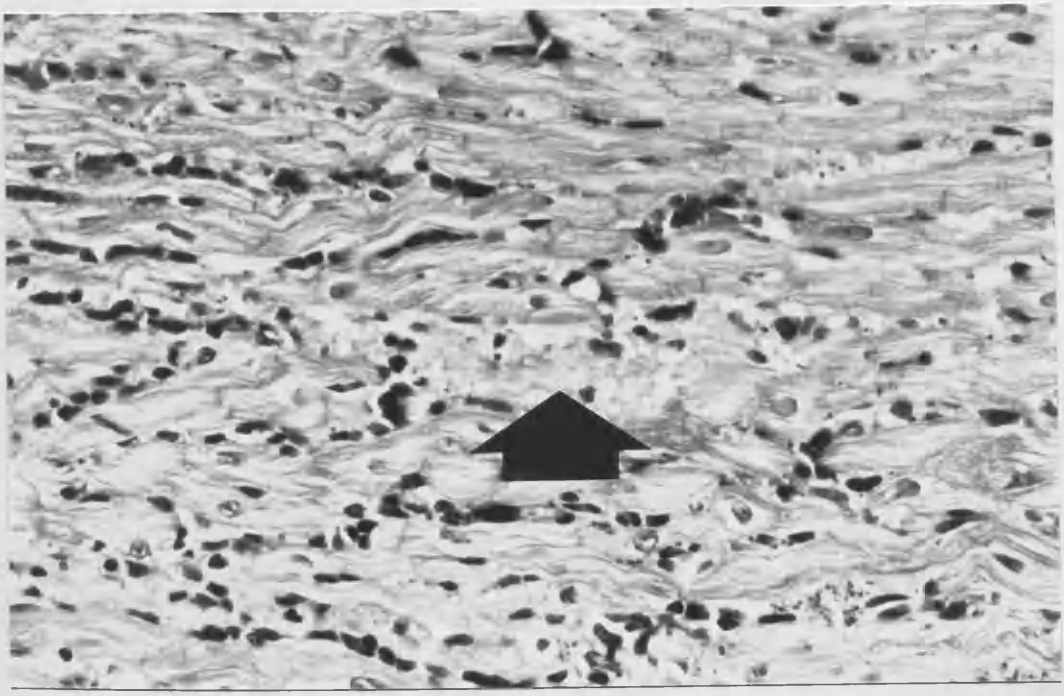
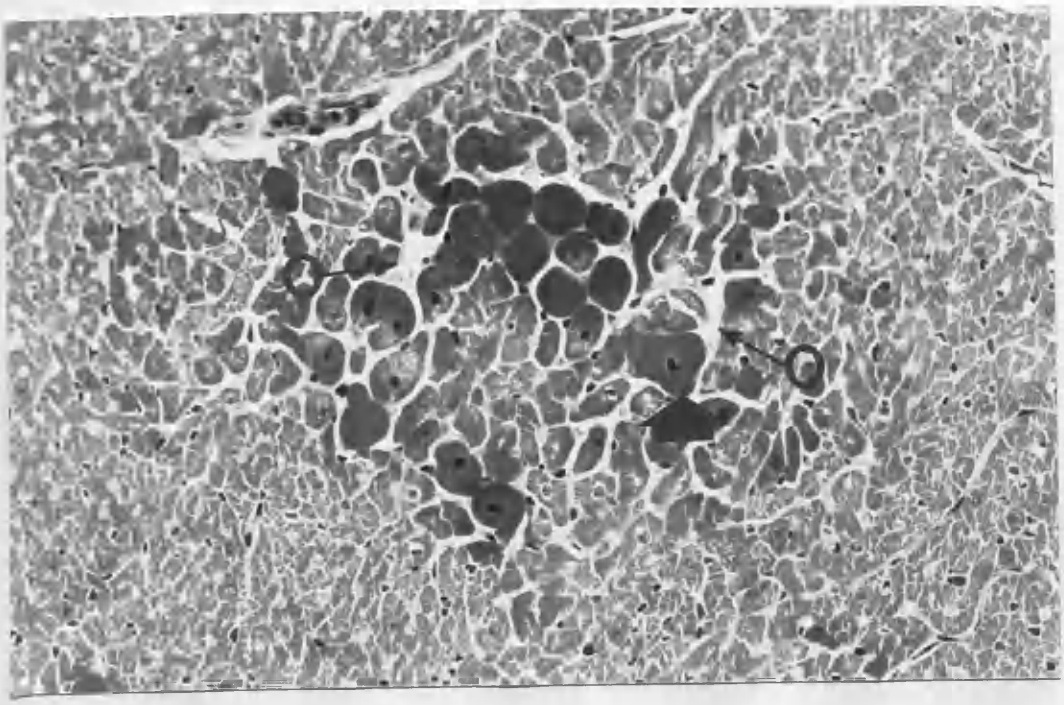


Figure 8.9. Focal ischaemic necrosis of the right ventricular myocardium in a dog infected with T.brucei for 21 days. There is oedema of the affected area (O →) and the myocytes are undergoing coagulative necrosis. They are swollen and the nuclei are pyknotic (large arrow). H&E. x260.

Figure 8.10. A band of wavy muscle fibres in the left ventricle of a dog infected with T.brucei for 26 days. The myocardial fibres are thin (arrows) and the nuclei are stretched. H&E. x100.



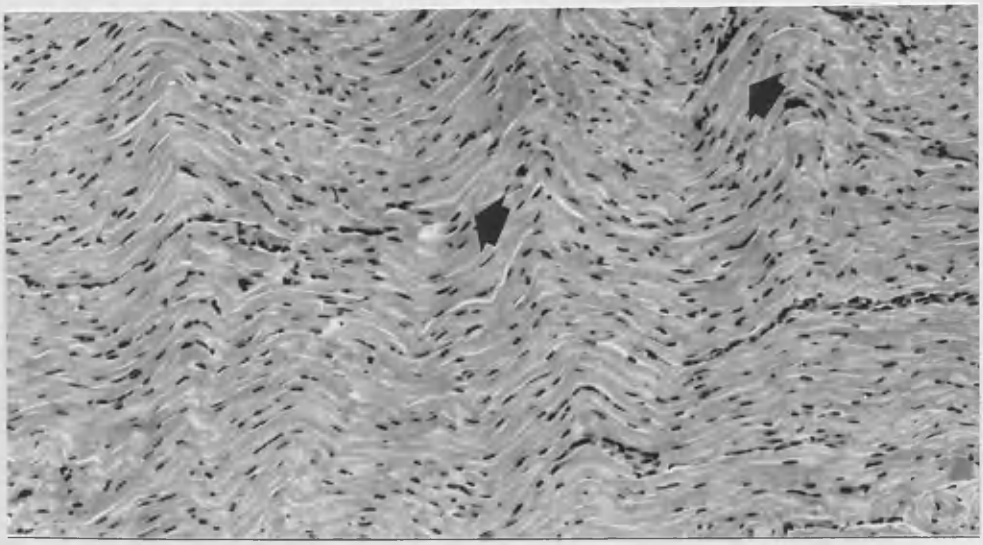
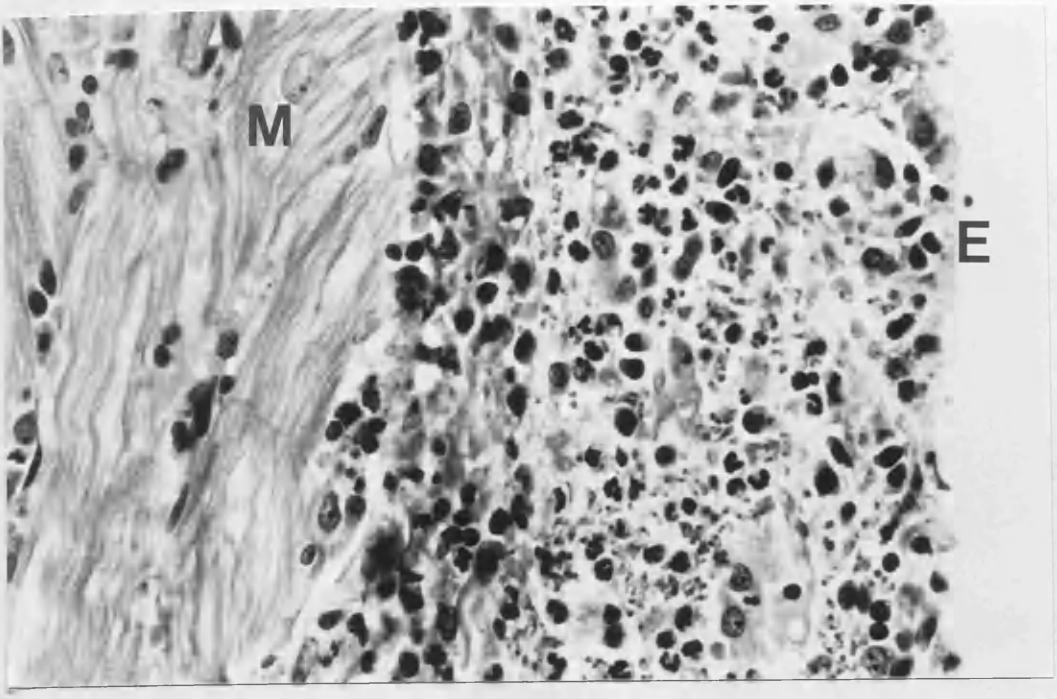


Figure 8.11. Subendocardial cellular and trypanosome infiltration in the right ventricle of a dog infected with T.brucei for 22 days. Large numbers of neutrophils, macrophages, trypanosomes, and fewer lymphocytes and plasma cells are trapped by fibrin. E - Endocardium. M - Myocardium. H&E. x320.

Figure 8.12. Subendocardial and myocardial cellular and trypanosome infiltration in the right ventricle of the dog in Figure 8.11. The endothelial lining of the endocardium is broken down (arrows). H&E. x320.


M
E

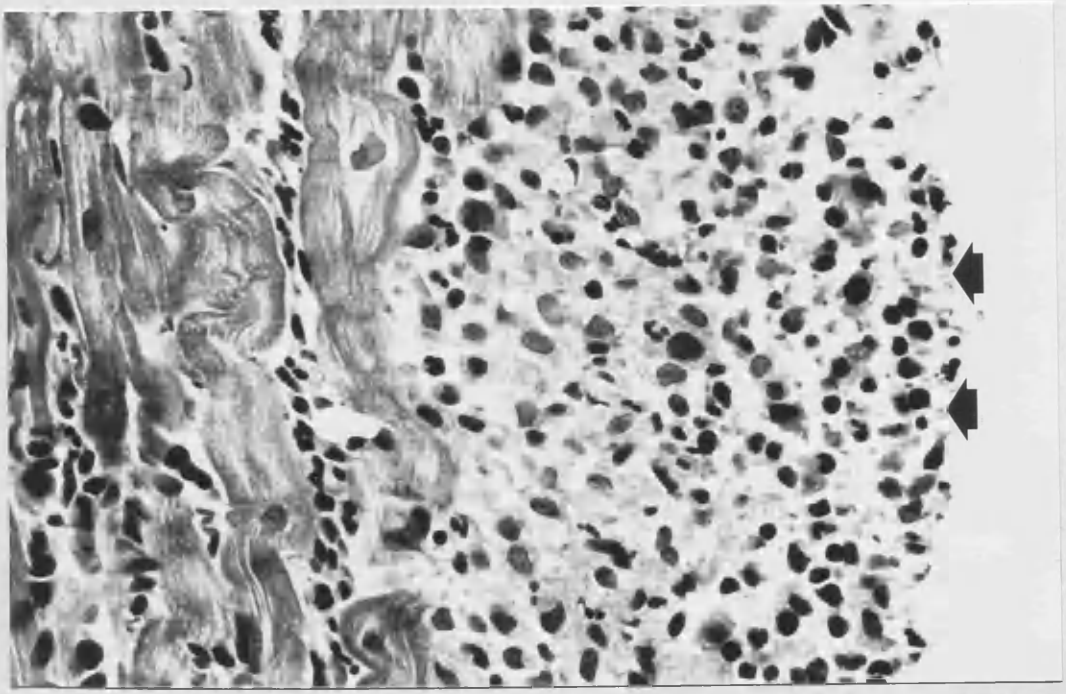
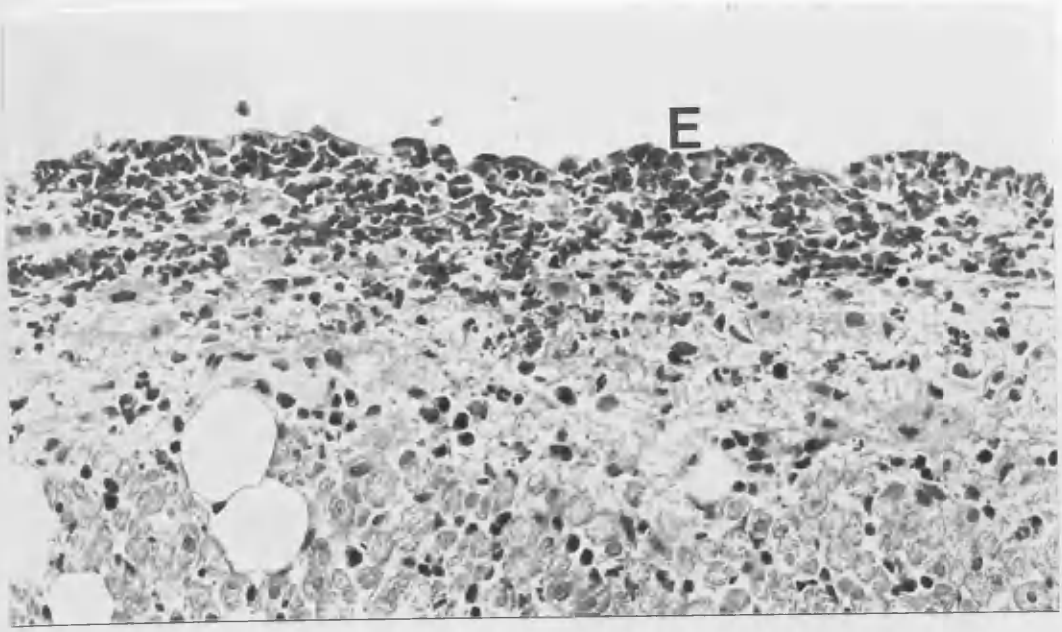


Figure 8.13. Subendocardial haemorrhage and cellular infiltration in the right atrium of a dog infected with T.brucei for 22 days. There is diffuse macrophage, plasma cell, lymphocyte and trypanosome infiltration. The endocardium (E) is intact. H&E. x260.

Figure 8.14. Subendocardial oedema, swelling and vacuolation of Purkinje fibres (arrow) in the left ventricle of a dog infected with T.brucei for 10 days. There is no cellular infiltration in the myocardium (M) or the subendocardium (S). H&E. x200.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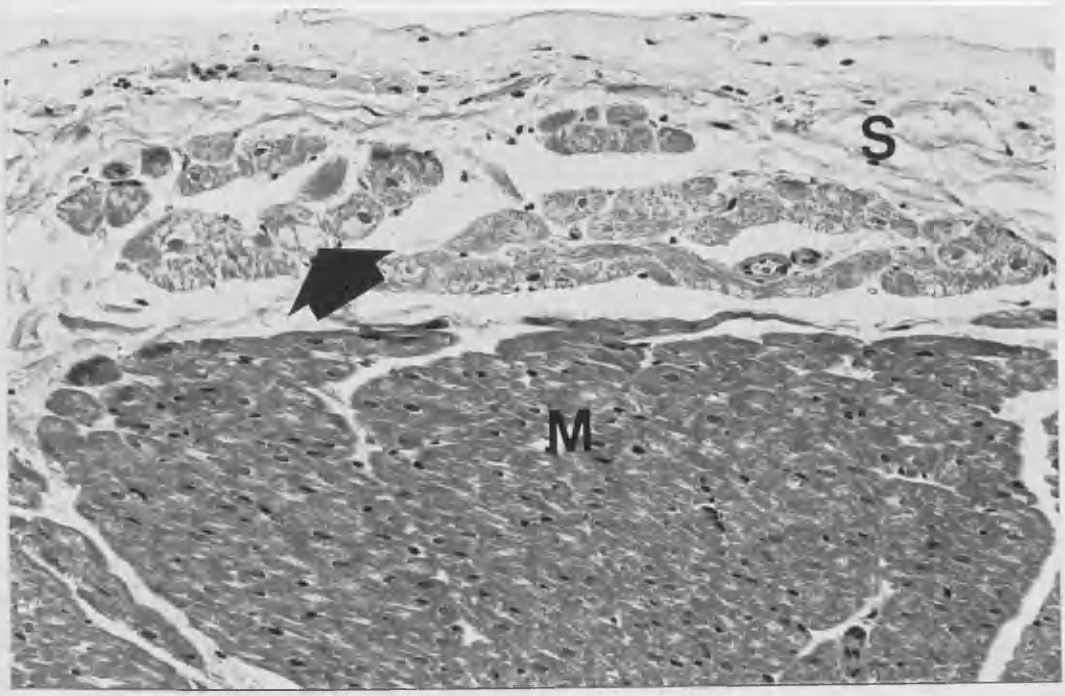
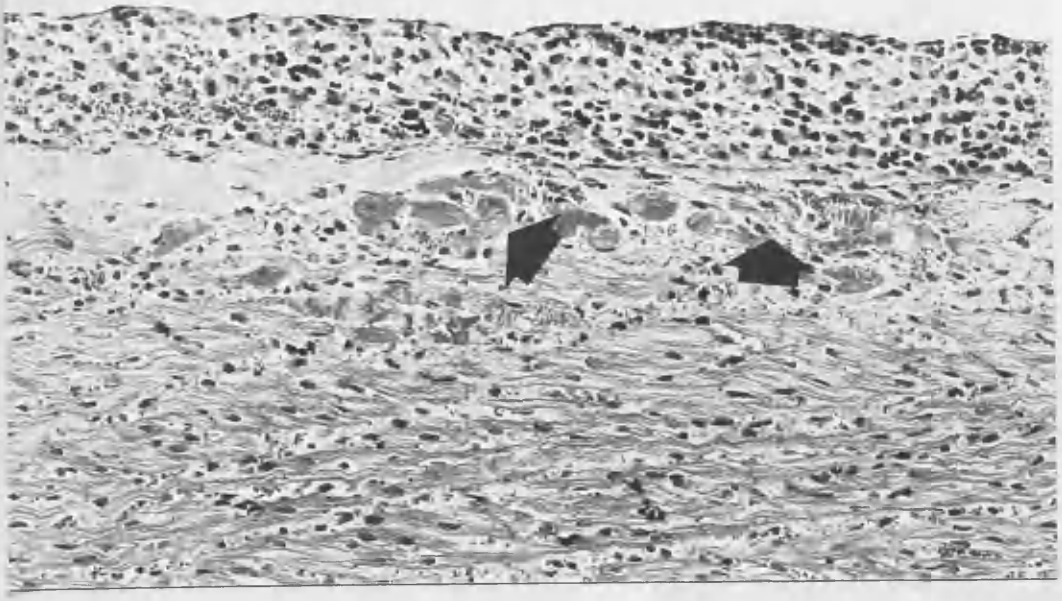

S
M

Figure 8.15. Necrosis of Purkinje fibres (arrows) in the subendocardium of the left ventricle in a dog infected with T.brucei for 22 days. There is diffuse infiltration of the subendocardium and the myocardium with cells and trypanosomes. The endocardium is intact. H&E. x200.

Figure 8.16. Vasculitis of a coronary artery in the left ventricle of a dog infected with T.brucei for 26 days. There is necrosis of smooth muscle cells in the tunica media (large arrow) and almost complete occlusion of the vessel lumen. A few trypanosomes are present in the media (small arrows). O - Perivascular oedema. H&E. x320.



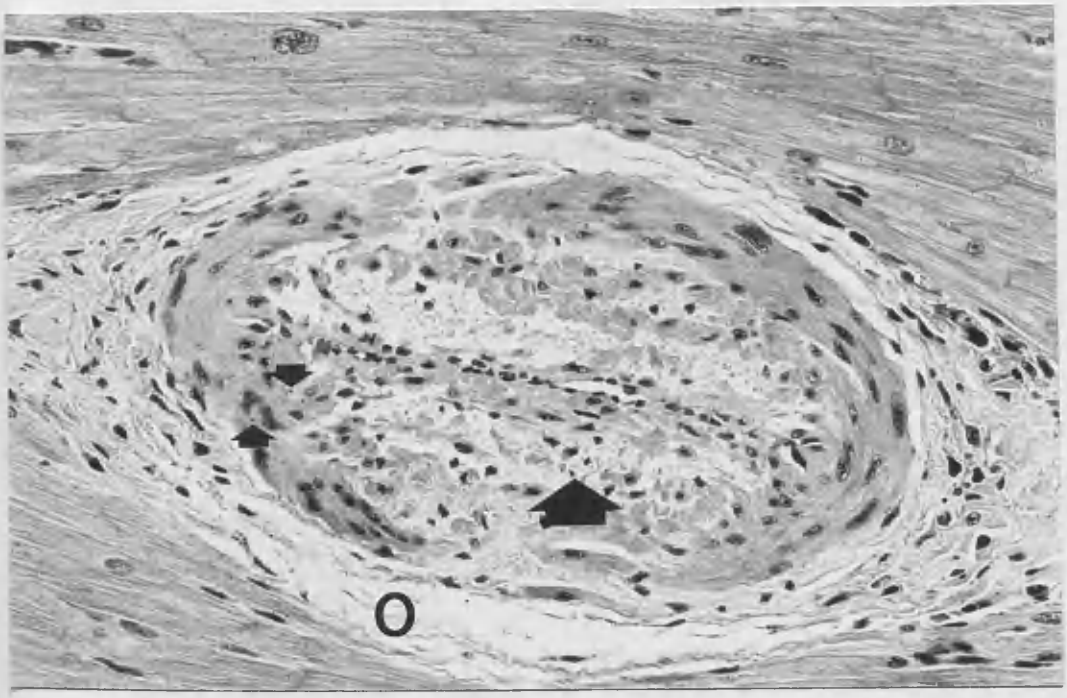
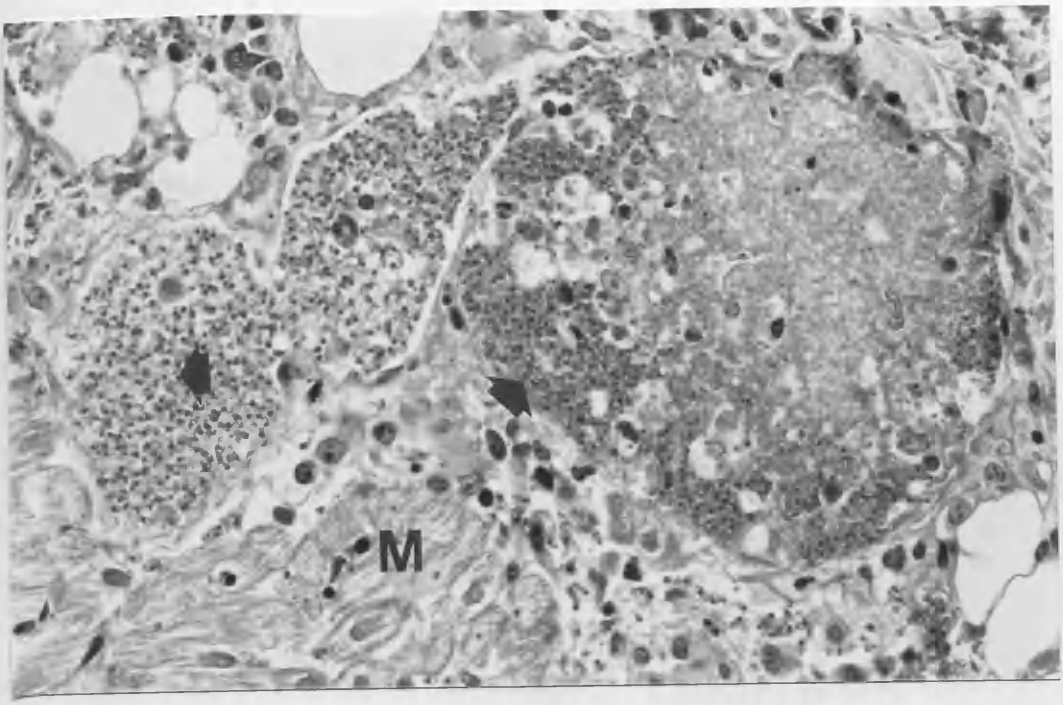

O

Figure 8.17. Lymphatic vessels in the subepicardium of the left ventricle in a dog infected with T.brucei for 21 days. Numerous trypanosomes (arrows) and a few inflammatory cells are suspended in the distended lymphatic vessels. M - Myocardium. H&E. x320.

Figure 8.18. A lymphatic vessel in the subepicardium of the left ventricle in a dog infected with T.brucei for 26 days. The vessel is blocked by a mixture of cells, consisting mainly of vacuolated macrophages (arrows) and a few other lymphoid cells. H&E. x260.


M

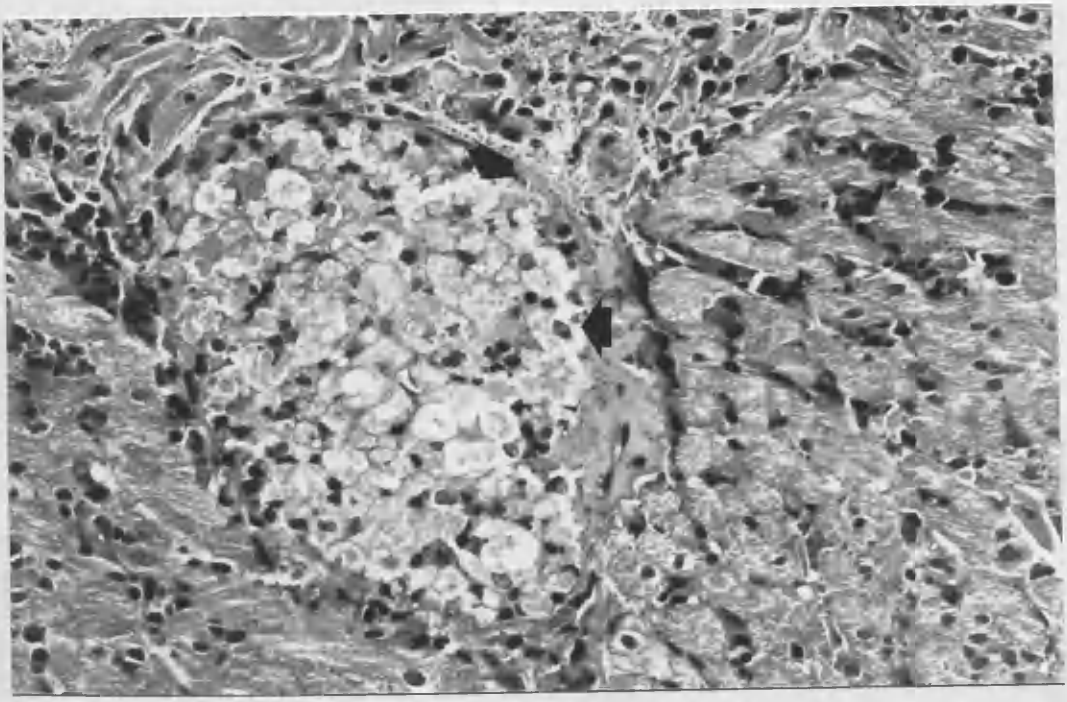
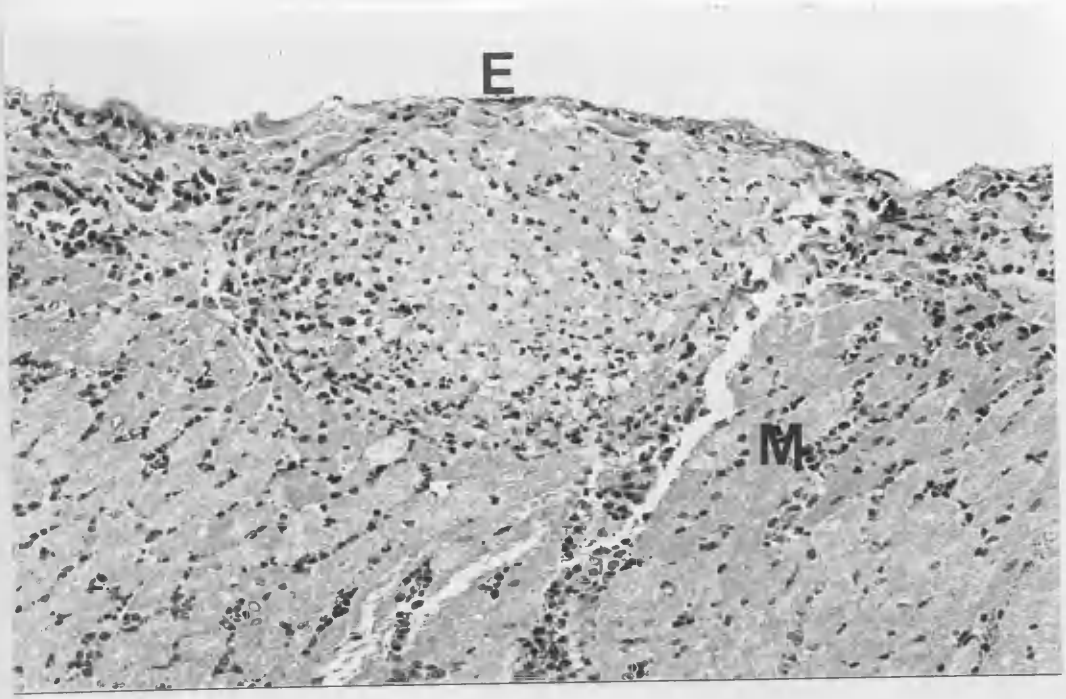


Figure 8.19. A lymphatic vessel in the subepicardium of the left ventricle in a dog infected with T.brucei for 26 days. The lymphatic vessel is distended with a mixture of cells, trypanosomes and lymph. Nuclei of necrosed cells have undergone karyorrhexis. M - Myocardium. E - Epicardium. H&E. x200.

Figure 8.20. An intramyocardial lymphatic vessel in the interventricular septum of the heart in a dog infected with T.brucei for 26 days. The lymphatic vessel is distended with a mixture of inflammatory cells, trypanosomes and fibrin, forming a plug. In some areas, the walls of the lymphatic vessel are broken down (arrows). V - Vein. A - Artery. H&E. x100.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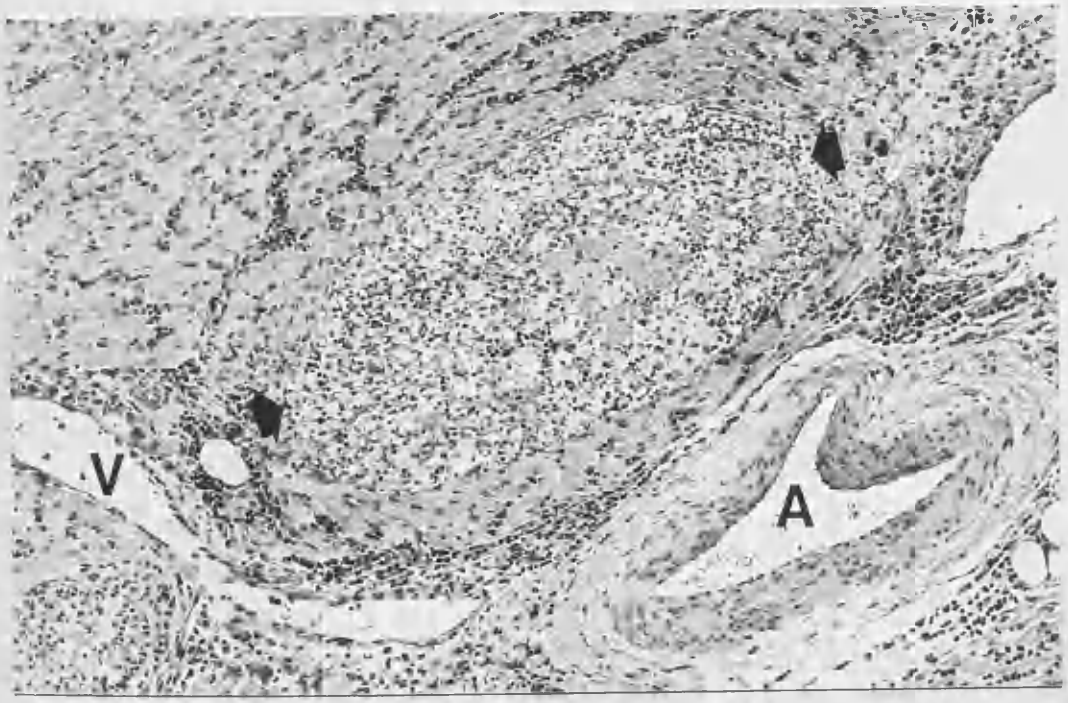
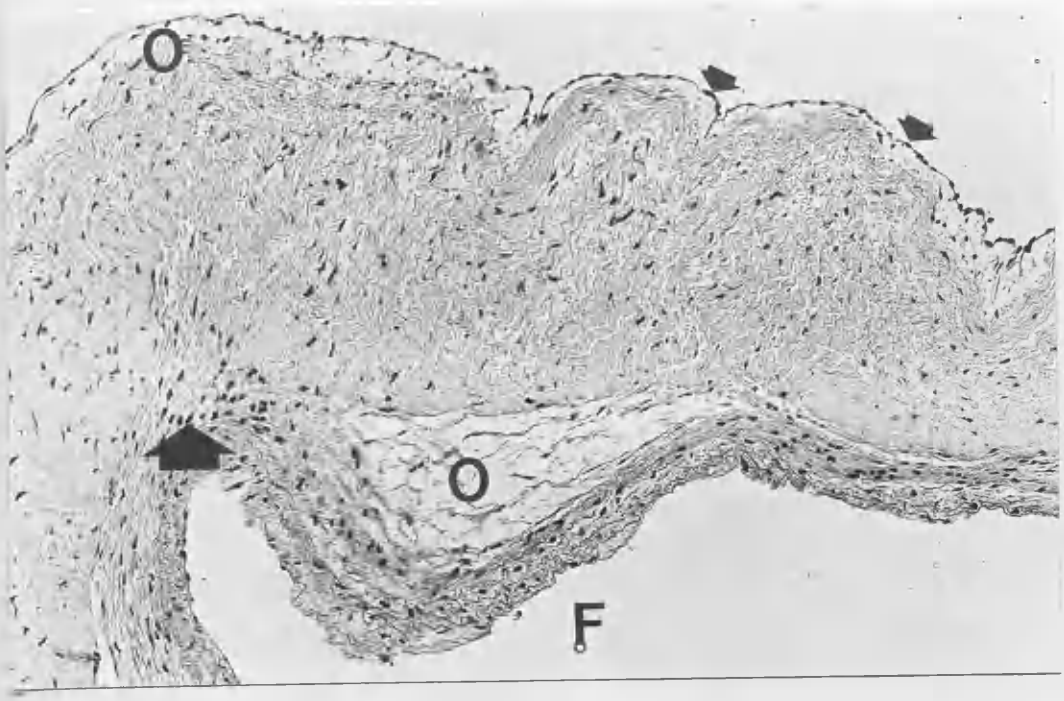

V
A

Figure 8.21. An aortic valve cusp from the heart of a dog infected with T.brucei for 10 days. There is subendothelial and interstitial oedema (O), causing stretching of endothelial cells (small arrows). A few inflammatory cells are present at the base of the valve cusp (large arrow) on the flow side of the valve (F). H&E. x132.

Figure 8.22. A mitral valve leaflet from the heart of a dog infected with T.brucei for 26 days. There is marked subendothelial and interstitial cellular infiltration on the flow side of the valve (F). Oedema has caused stretching of endothelial cells on the mural side of the valve leaflet (arrow). H&E. x132.


O
O
F

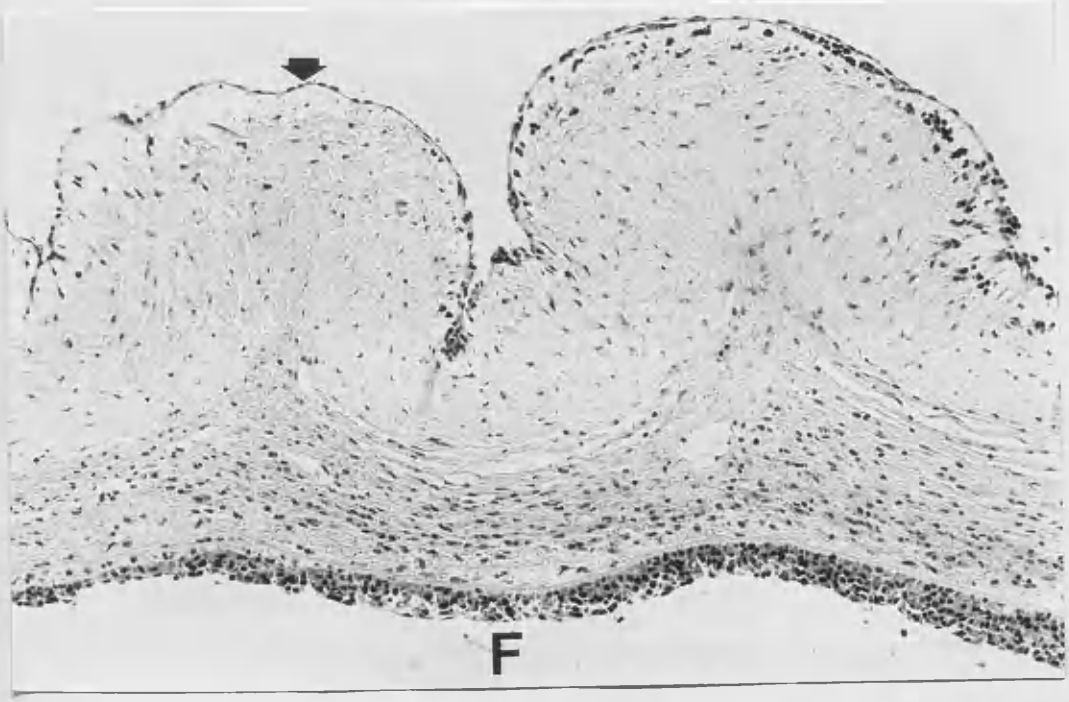
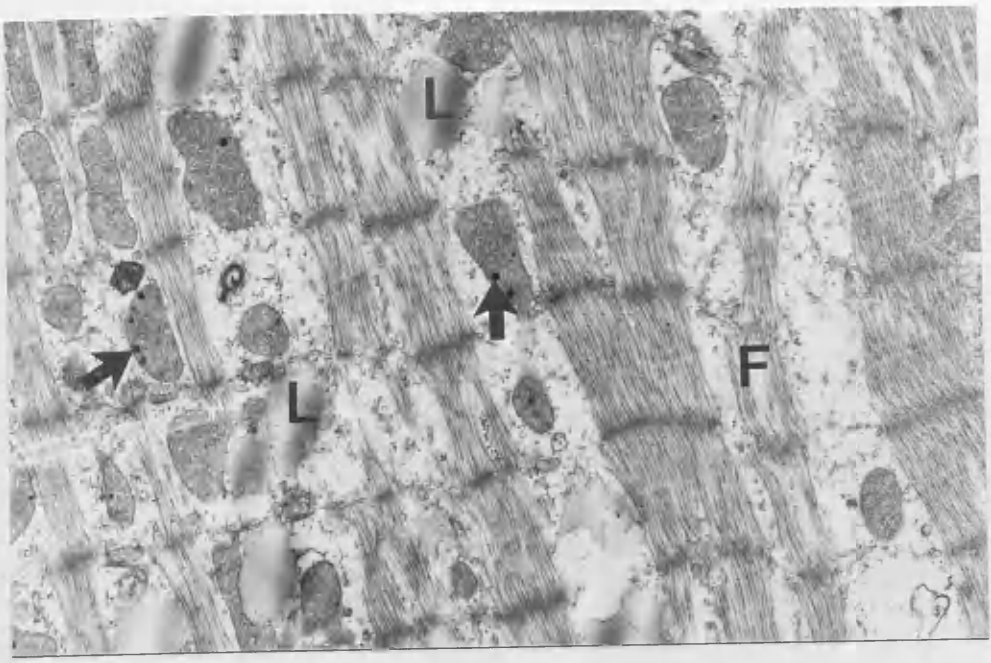

F

Figure 8.23. The right ventricle of a dog infected with T.brucei for 26 days. There is severe myocytolysis with fluid accumulation, lipid deposition (L) and calcium-like electron dense granules in the mitochondria (arrows). There is breakdown of myofibrils (F). TEM. x16,000.

Figure 8.24. The left ventricle of a dog infected with T.brucei for 26 days. The mitochondria in the myocytes are swollen and their cristal membranes are disrupted (arrows). L - Lipid. I - Intercalated disc.
F - Myofibrils. G - Glycogen granules. TEM. x16,000.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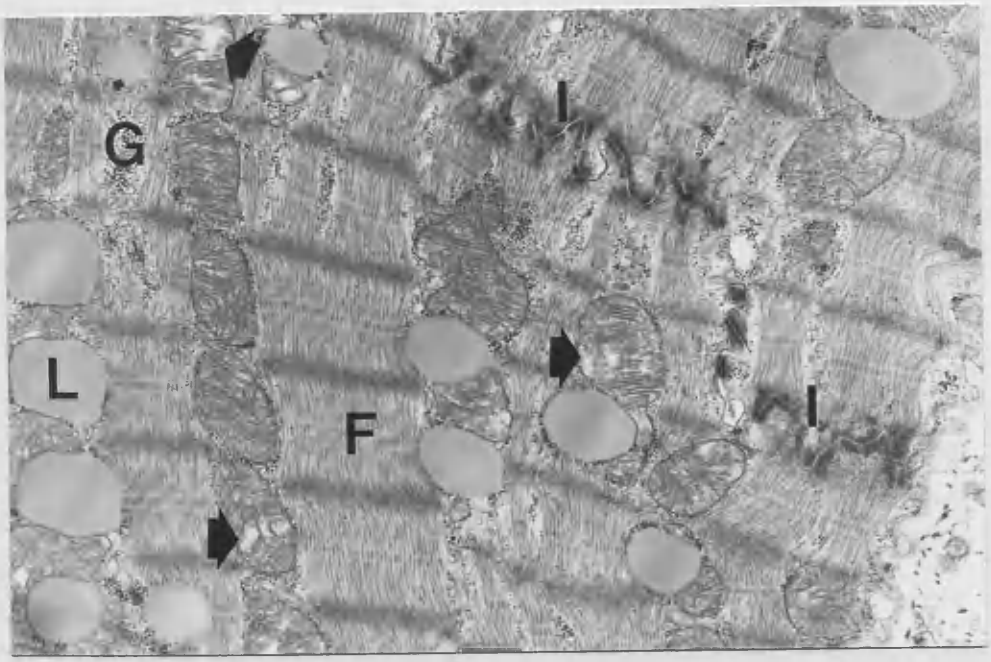
G
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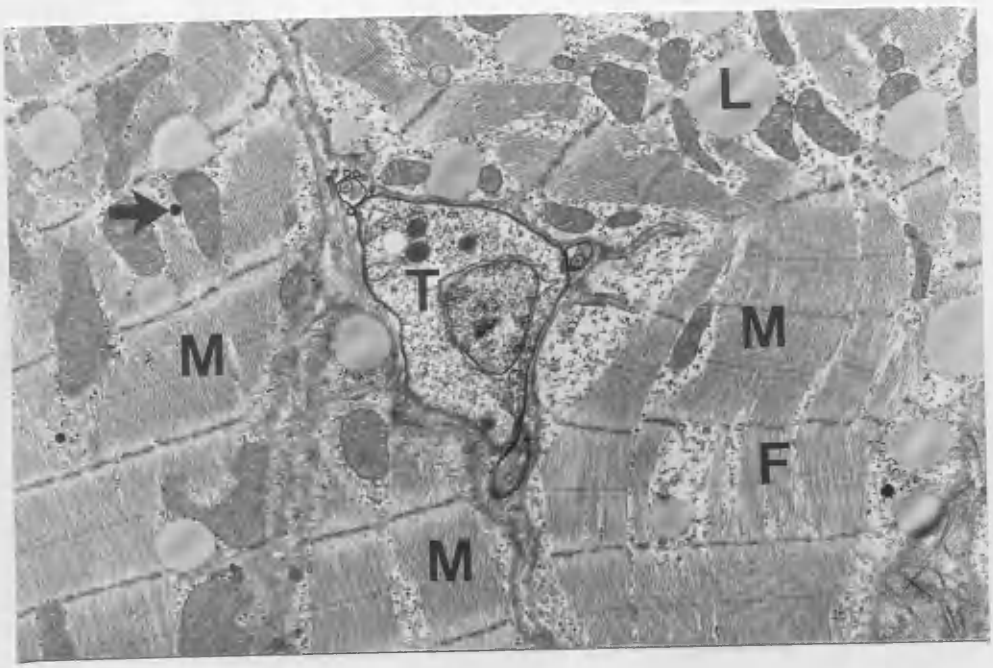
Figure 8.25. Cross section of a trypanosome (T) migrating between three myocytes (M) in the right atrial myocardium of a dog infected with T.brucei for 26 days. F - Myofibrils. L - Lipid. Arrow - Atrial natriuretic granule. TEM. x10,000.

Figure 8.26. Trypanosome (T), macrophage (M) and plasma cell (P) infiltration in the right atrial myocardium of a dog infected with T.brucei for 26 days. One of the myocytes (F) is undergoing myocytolysis. E - Erythrophagocytosis. C - A capillary with a red blood cell in the lumen. TEM. x5,400.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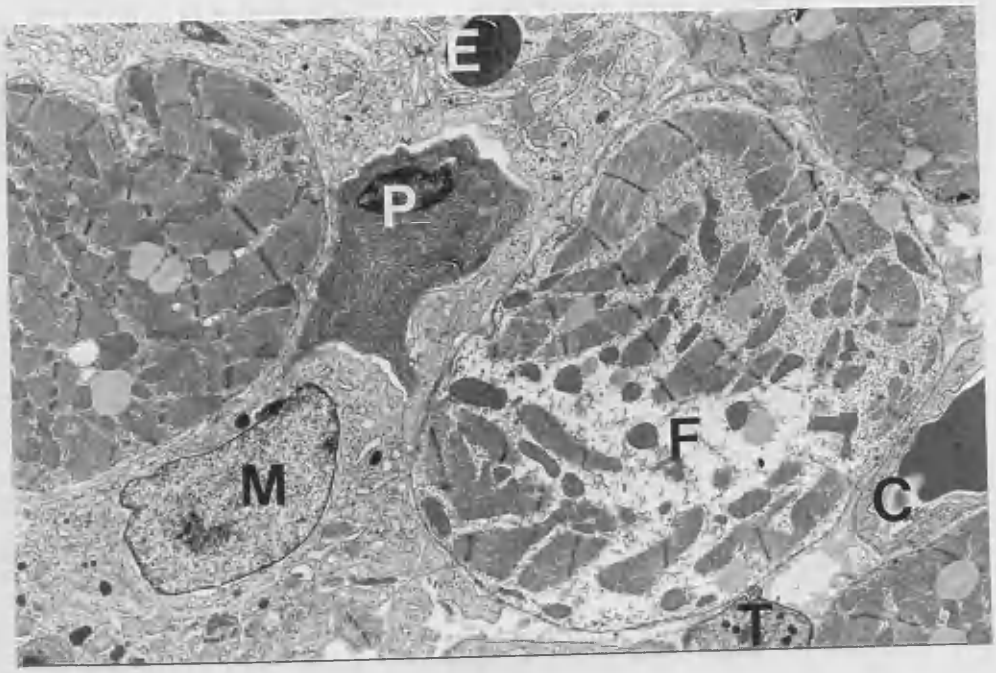
E
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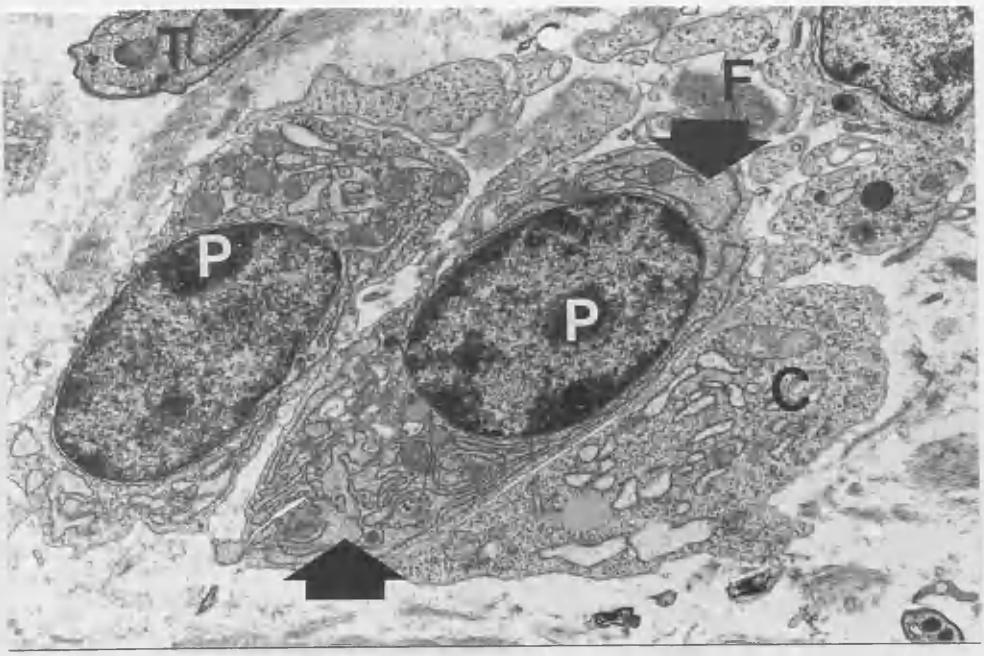
Figure 8.27. Plasma cells (P) in the interstitium of the right atrium of a dog infected with T.brucei for 26 days. The cisternae of the rough endoplasmic reticulum are distended with colloid (arrows). T - Trypanosome. F - Fibrin. C - Cytoplasmic processes of a macrophage. TEM. x8,000.

Figure 8.28. An activated macrophage in the right ventricle of a dog infected with T.brucei for 21 days. The phagolysosomes are filled with necrotic debris (arrows). C - Collagen. TEM. x10,000.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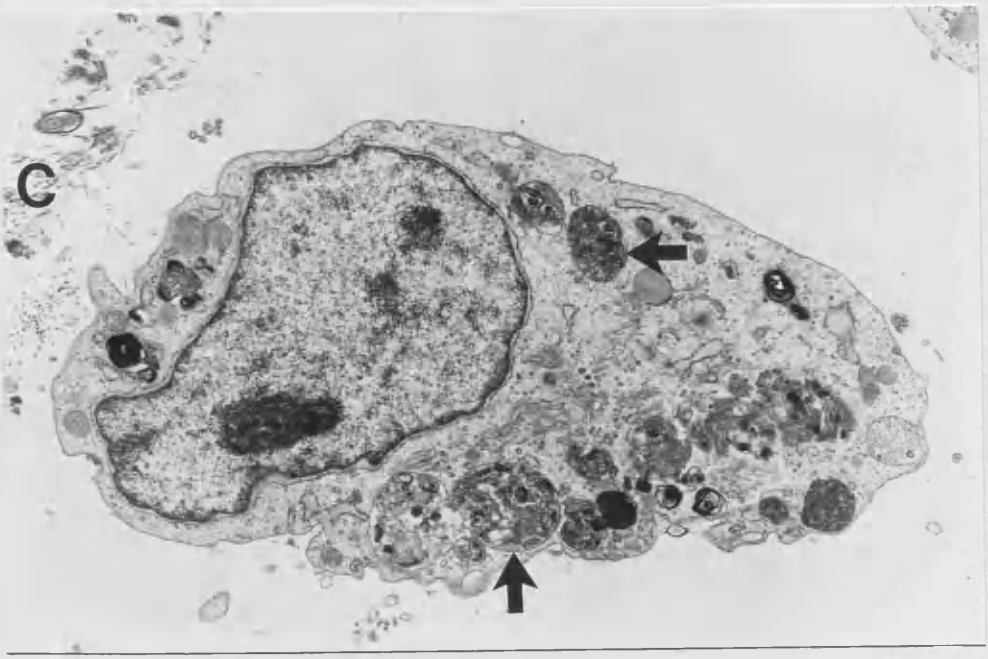
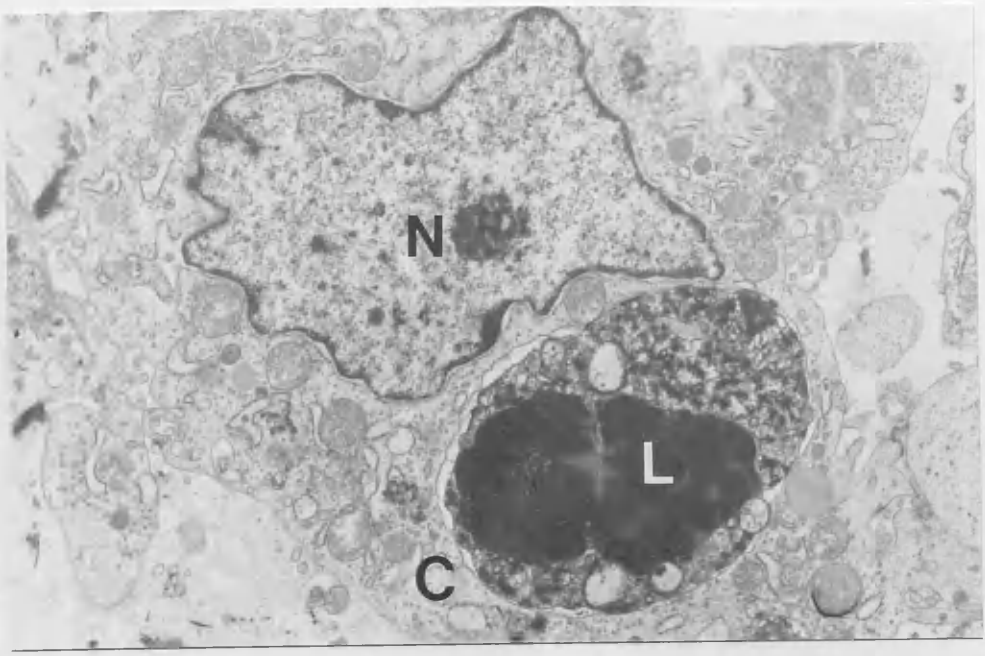

C

Figure 8.29. Leucophagocytosis in the right atrium of a dog infected with T.brucei for 22 days. A leucocyte (L) is engulfed by the macrophage cytoplasmic processes (C). N - Macrophage nucleus. TEM. x10,000.

Figure 8.30. Erythophagocytosis in a perivascular site in the right atrium of a dog infected with T.brucei for 22 days. E - Red blood cell. L - Lipid droplet. M - Macrophage cytoplasm. C - Capillary. P - Necrotic debris in a phagolysosome. TEM. x20,000.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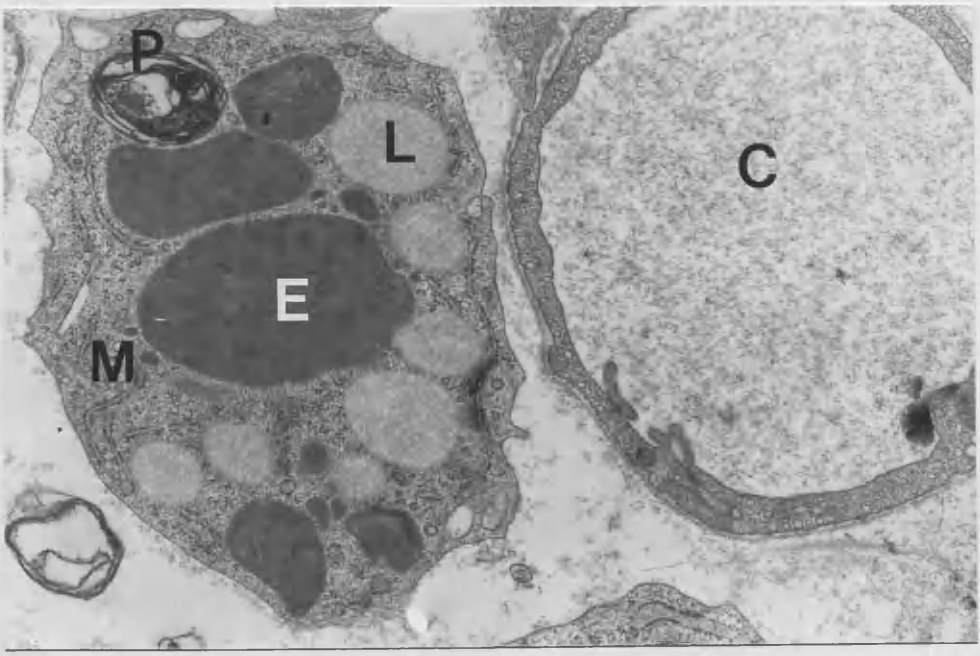
P
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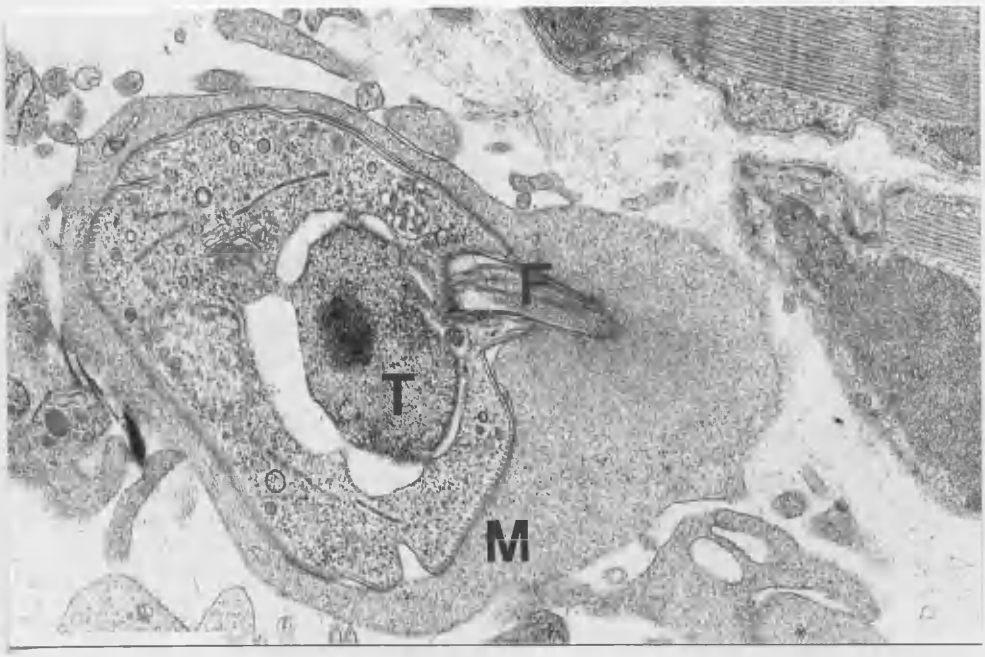
M

Figure 8.31. Phagocytosis of a trypanosome (T) in the right atrium of a dog infected with T.brucei for 22 days. The trypanosome is surrounded by macrophage cytoplasm (M). F - Trypanosome flagellum. TEM. x16,000.

Figure 8.32. The normal appearance of a neutrophil. Most of the neutrophil granules are electron dense (arrows). Glycogen granules (g) are suspended in the neutrophil cytoplasm. N - Nucleus. G - Golgi apparatus.
TEM. x16,000.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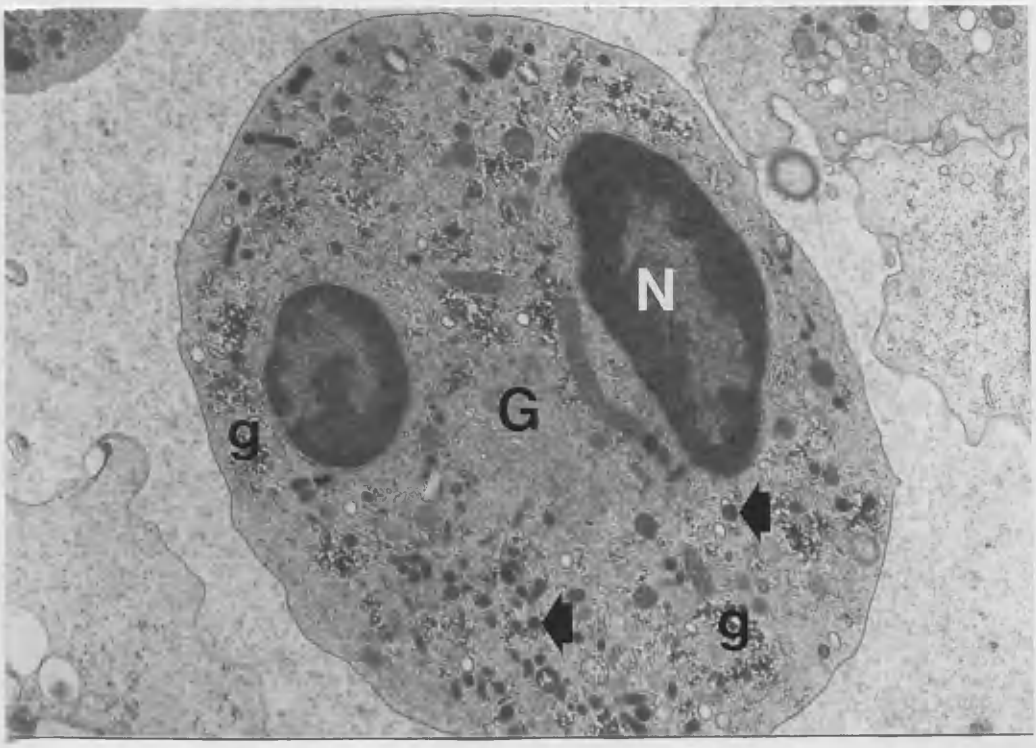
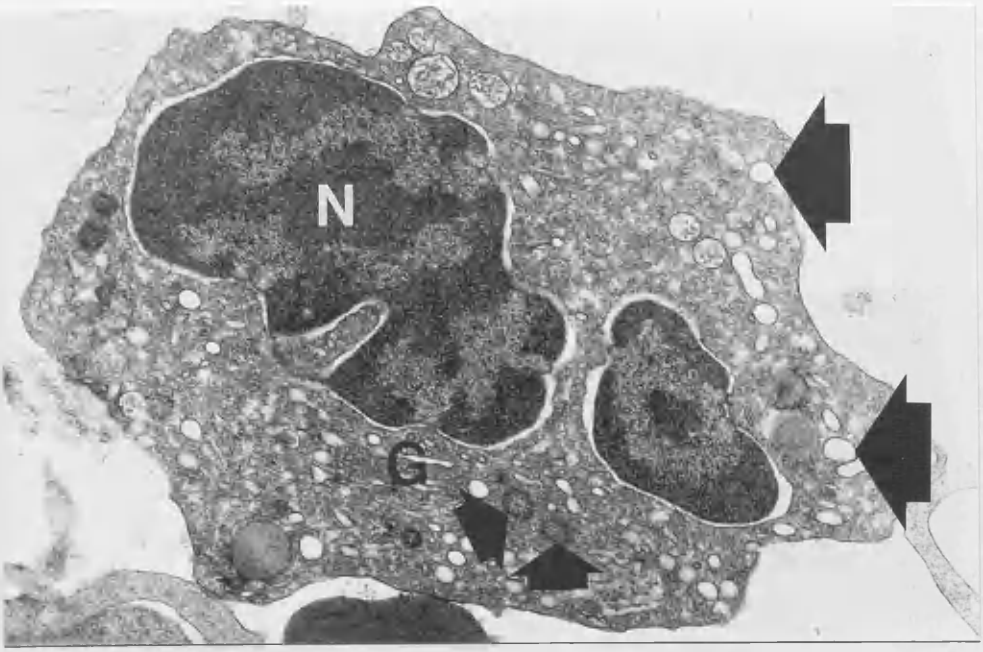

N
G
g
g

Figure 8.33. A degranulated neutrophil in the interstitium of the left atrium of a dog infected with T.brucei for 22 days. The neutrophil granules are electron lucent (large arrows) because they have discharged their enzymes. N - Nucleus. G - Golgi apparatus. Small arrows - Centrioles. TEM. x20,000.

Figure 8.34. A focus of necrosis in the ventricular myocardium of a dog infected with T.brucei for 26 days. There is marked fibrin deposition (F) and necrosis of inflammatory cells. N - Nuclei of necrosed cells. TEM. x5,400.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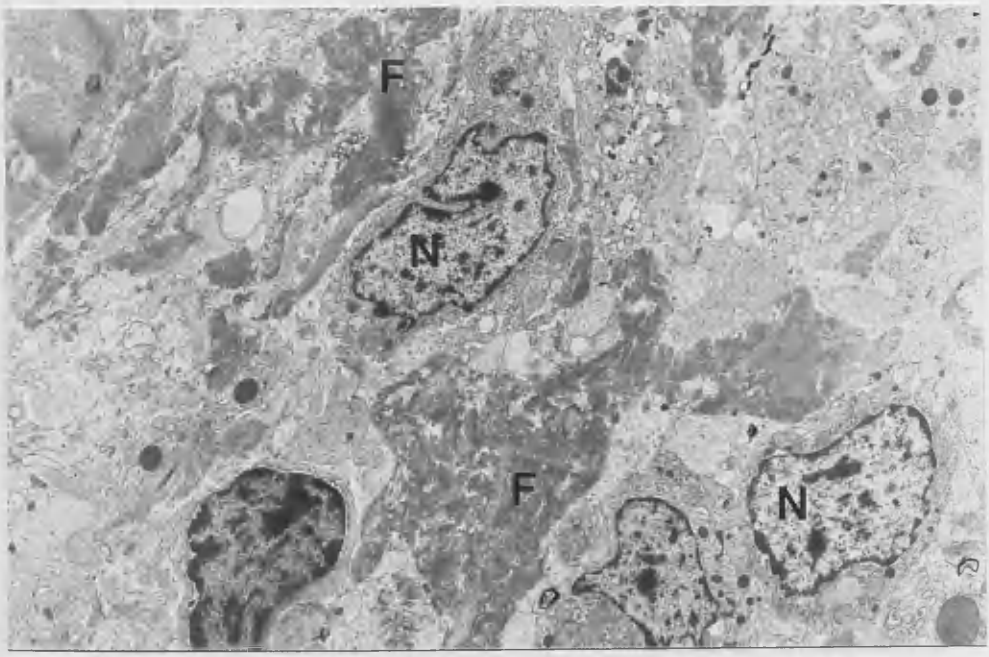
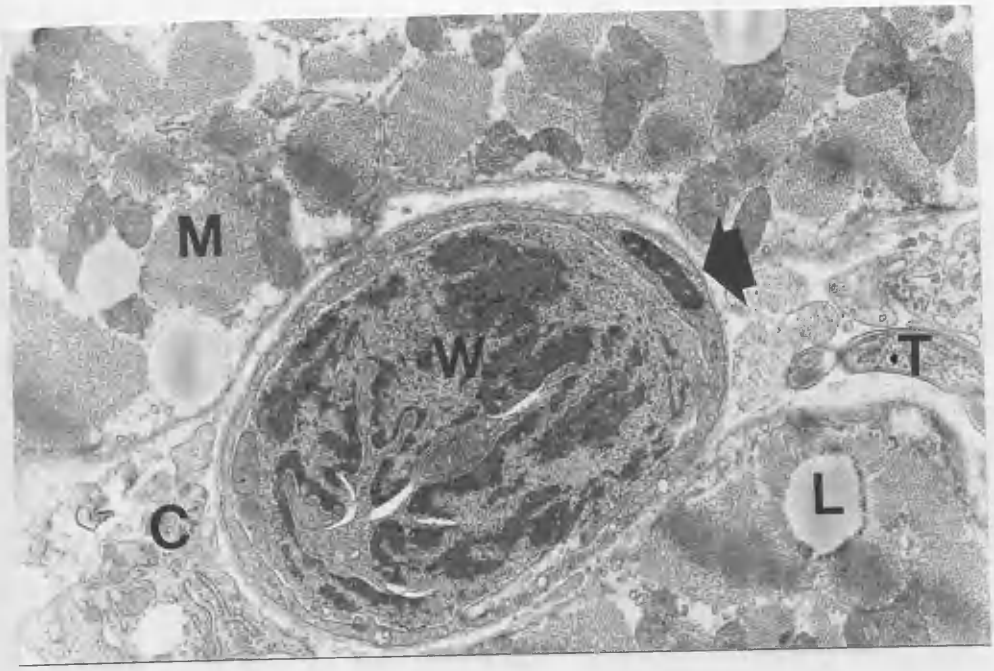

F
N
F
N

Figure 8.35. Occlusion of a capillary by a white blood cell (W) in the left ventricle of a dog infected with T.brucei for 15 days. The basal lamina of the capillary is intact (arrows). T - Trypanosome. M - Myocyte. C - Macrophage cytoplasm. L - Lipid. TEM. x13,400.

Figure 8.36. Occlusive vasculitis of a capillary in the left ventricle of a dog infected with T.brucei for 21 days. One endothelial cell (E) is swollen, causing occlusion of the vessel lumen (large arrow). O - Oedema. Small arrow - Trypanosome flagellum. L - Lipid. TEM. x13,400.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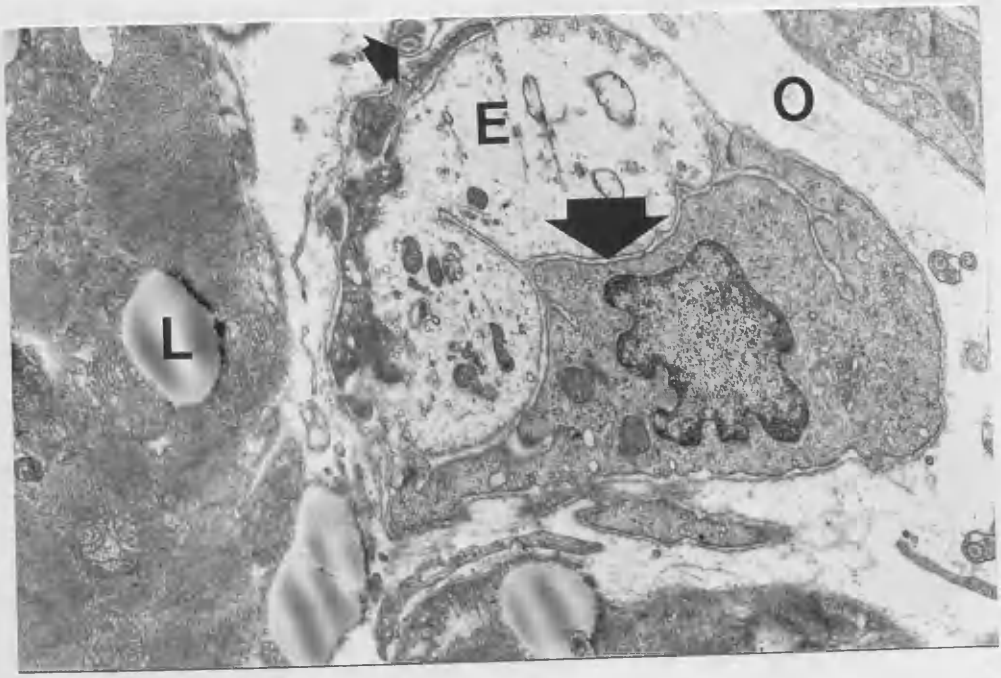
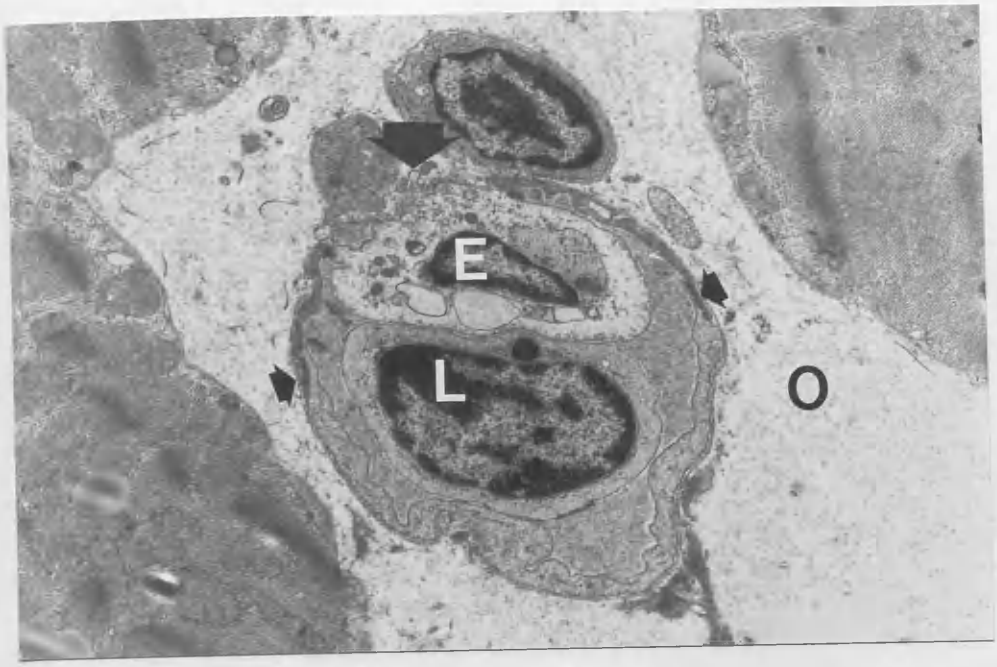

E
O
L

Figure 8.37. Vasculitis of a capillary in the right ventricle of a dog infected with T.brucei for 22 days. The endothelial cell (E) is necrosed. The basal lamina is broken down and a cytoplasmic extension of the endothelial cell projects towards the perivascular space (large arrow). The junctional attachments between the endothelial cells are intact (small arrows). There is marked oedema (O). L - Lymphocyte. TEM. x10,000.

Figure 8.38. Necrotising vasculitis and occlusion with red blood cells (R) in the right ventricle of a dog infected with T.brucei for 26 days. The endothelial cell (E) is necrosed. There is perivascular infiltration of activated macrophages (M). N - Necrotic debris in a phagolysosome. F - Myocyte. TEM. x8,000.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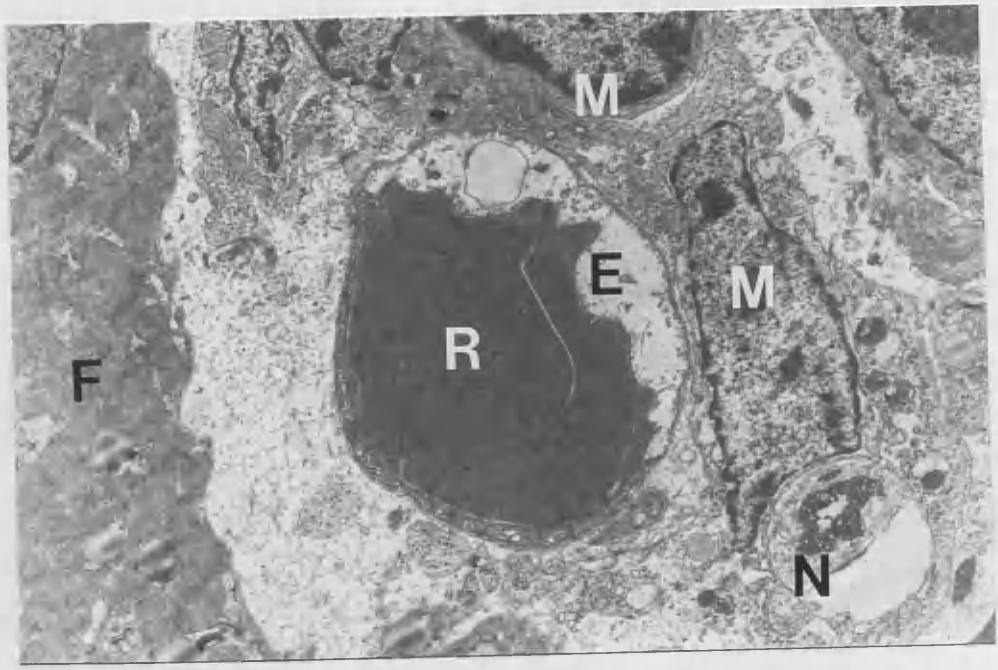
M
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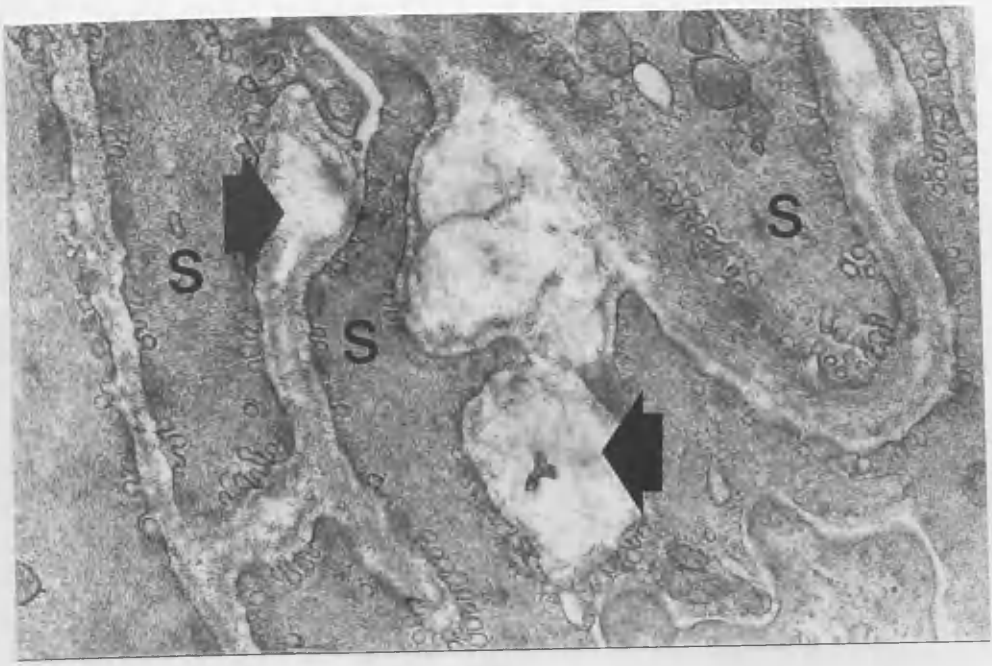
Figure 8.39. Separation of junctional attachments between smooth muscle cells (S) in the wall of an arteriole in the left ventricle of a dog infected with T.brucei for 21 days. There is accumulation of fluid in the spaces formed (arrows). TEM. x40,000.

Figure 8.40. An autonomic nerve ganglion in the left atrium of an uninfected dog. N - Schwann cell nucleus. The nerve axons (A) are surrounded by Schwann cell cytoplasmic processes (C →). TEM. x8,000.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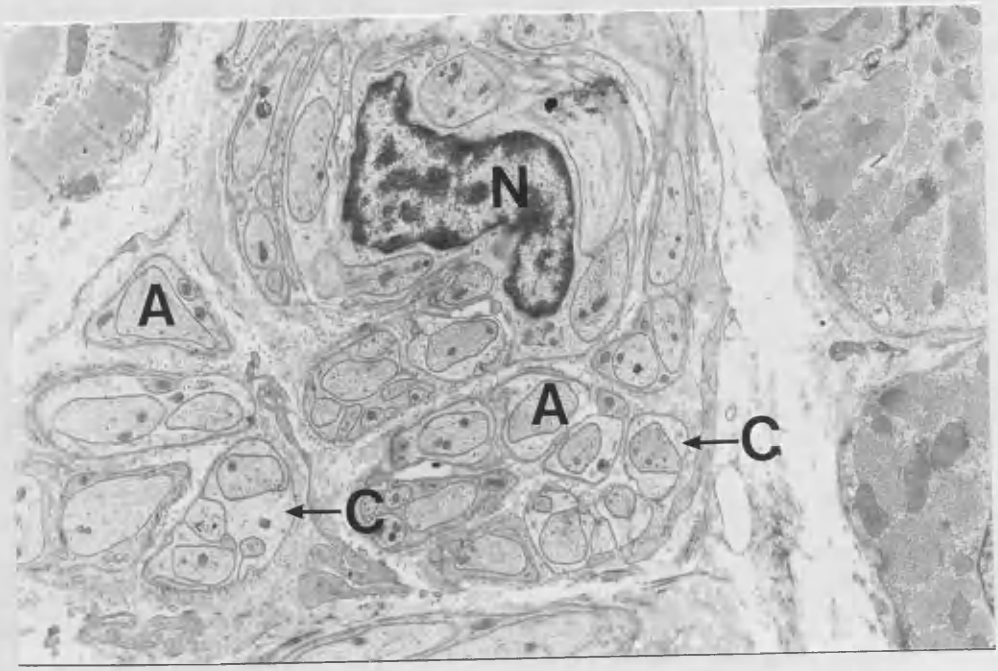
N
A
A
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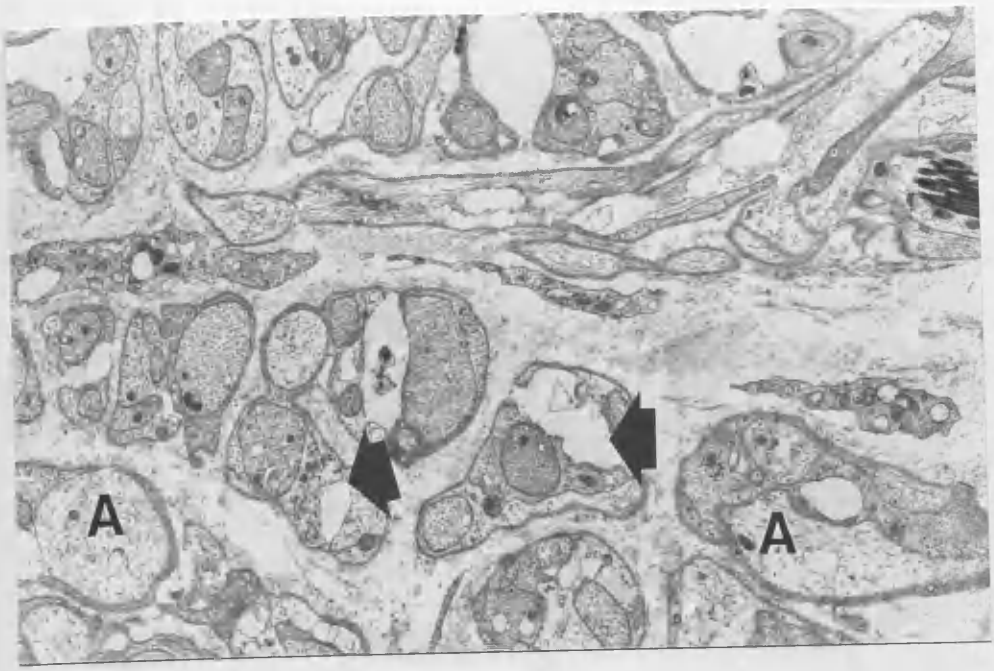
Figure 8.41. An autonomic nerve ganglion in the left ventricle of a dog infected with T.brucei for 26 days. Some of the nerve axons are undergoing liquefactive necrosis (A). The Schwann cell cytoplasmic processes are necrosed (arrows). TEM. x8,000.

Figure 8.42. Immunofluorescence staining of fibrinogen in the left ventricular myocardium of a dog infected with T.brucei for 22 days. There is widespread deposition of fibrinogen in the interstitium (arrows). x320.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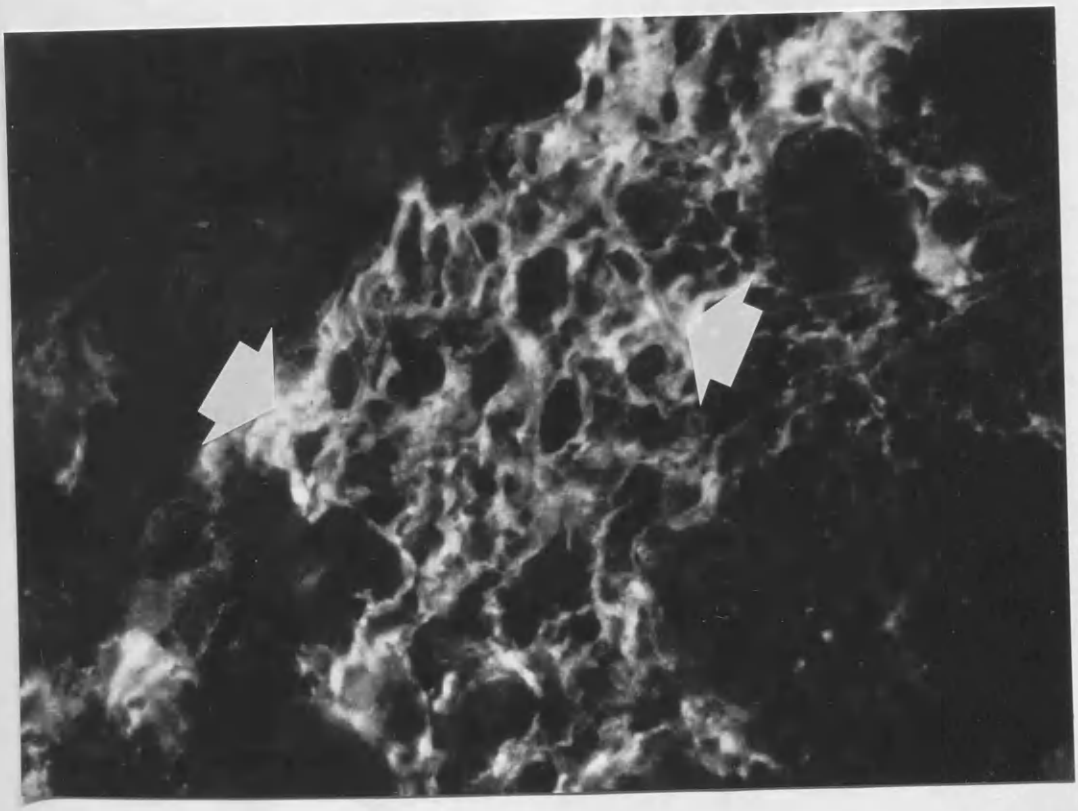
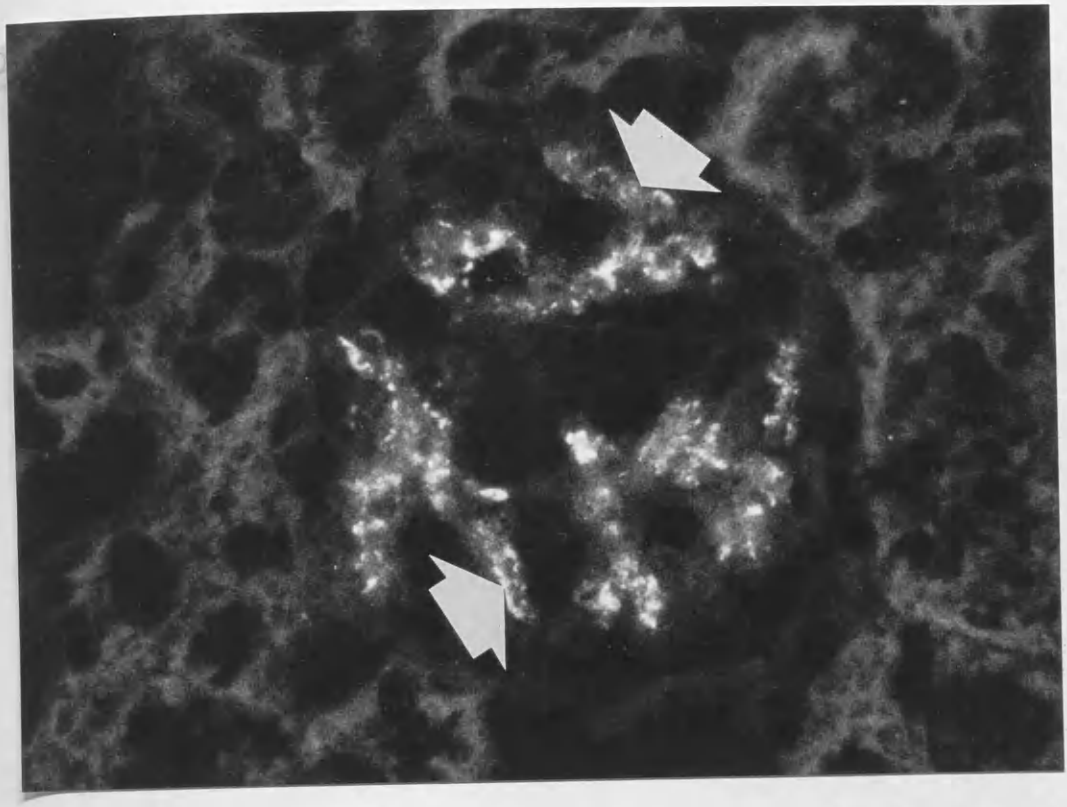
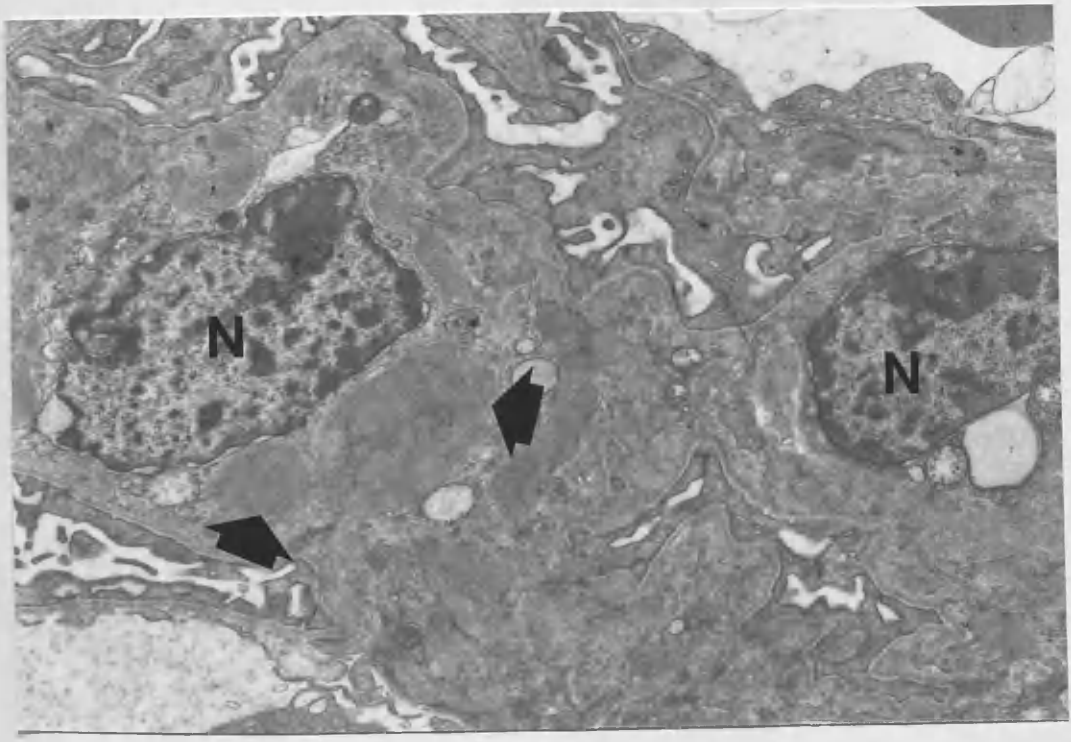


Figure 8.43. Immunofluorescence staining of IgM deposits (arrows) in the left kidney of a dog infected with T.brucei for 22 days. x520.

Figure 8.44. Mesangial deposition of immune complexes (arrows) in the kidney of a dog infected with T.brucei for 22 days. N - Mesangial cell nucleus. TEM. x20,000.

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PART III.

TREATMENT AND MANAGEMENT.

CHAPTER 9.

EFFECTS OF TRYPANOCIDAL DRUG TREATMENT ON THE PATHOGENESIS OF CARDIAC DAMAGE IN DOGS INFECTED WITH T.brucei.

9.1. INTRODUCTION.

In Part II of these studies, the pathogenesis of cardiac damage induced in dogs by T.brucei was investigated. Infected dogs developed an acute disease syndrome, with severe pancarditis, resulting during the fourth week in heart failure. The syndrome was remarkably similar to that caused by T.rhodesiense and T.brucei in vervet monkeys. However, it was more acute than human African trypanosomiasis caused by T.rhodesiense or T.gambiense, although occasionally, T.rhodesiense infection in man can have fatal consequences after only 6 weeks duration (Manuelidis et al., 1965; Apted, 1970), with clinical and histopathological features of cardiac damage that resemble those observed in dogs infected with T.brucei.

One of the trypanocidal drugs commonly used for treatment of acute T.rhodesiense infection in man is suramin (Moranyl^{R} - Specia), an organic polyanion which is negatively charged under physiological conditions (Fairlamb and Bowman, 1980). Suramin is important in the treatment of early stage (Apted, 1970; Molyneux et al., 1984) and, in combination with other trypanocides, late stage encephalitic (Jennings et al., 1983; Clarkson et al., 1984; Bales, 1987) and drug-resistant (Arroz and Djedje, 1988; Nieuwenhove, 1988) T.rhodesiense infections in man. Treatment of human patients with trypanocidal drugs can have severe side effects: e.g., treatment can result in shock-like Jarisch-Herxheimer reactions (Ormerod, 1970; Molyneux et al., 1984) similar to those occasionally

observed during treatment of syphilis. It is believed that in these reactions, antigen to which the host is already sensitised is suddenly liberated from dying parasites, with resulting tissue destruction due to anaphylaxis (Ormerod, 1970; Murray, 1974; Lambert and Houba, 1974).

Diminazene aceturate (Berenil^{R} - Hoechst, Germany) is the drug commonly used for treating dogs infected with T.brucei and T.gambiense (Omamegbe et al., 1984; Kaggwa et al., 1988; Ch. 2). Nevertheless, relapse infections do occur, even when treatment is carried out on day 8 of infection (Kaggwa et al., 1988), a time when trypanosomes are not expected to have entered sites inaccessible to the drug. In order to offer alternative forms of treatment in such situations, it is necessary that the chemotherapeutic potential of other trypanocidal compounds on T.brucei infected dogs be determined.

At the same time, if dogs infected with T.brucei are treated with subcurative doses of trypanocidal drugs, the rate of development of cardiac lesions may be reduced and the survival time prolonged, leading to a chronic form of cardiac damage similar to that observed in human patients suffering from T.rhodesiense or T.gambiense infections. Such an approach might provide a large monogastric laboratory animal model ideal for investigating cardiac disease in human African trypanosomiasis.

The current study was designed to investigate the possible presence of post-treatment adverse reactions, the effectiveness of suramin as a trypanocidal compound for treating dogs infected with T.brucei, and the possibility

of developing a canine chronic model of cardiac damage, in dogs, that mimicked the disease in man.

9.2. MATERIALS AND METHODS.

9.2.1. ANIMALS.

The breed, sex, age and management of the dogs, and the stabilate of T.brucei used to infect them, have been described (Ch. 2), as have the clinical, parasitological, haematological and histopathological features of the disease in untreated dogs (Part II).

9.2.2. TRYPANOCIDAL DRUG TREATMENT.

Four dogs were infected intravenously with T.brucei. At intervals, terminally sick dogs were treated by intravenous injection with various doses of suramin (Table 9.1). The criteria for determining terminal sickness were: i) persistent recumbency for at least 24 hours, ii) development of diffuse corneal opacity and blindness, and iii) inappetence for at least 36 hours.

Dogs 1, 2 and 4 were treated with suramin at a dose of 10 mg/kg body weight (Table 9.1). Further treatments were carried out whenever a dog's condition deteriorated again. The dogs were euthanised when treatment failed to cause any improvement. Dog 3 was treated with suramin at a dose of 20 mg/kg on day 28, followed by 40 mg/kg for 3 days from day 30 to 32 (Table 9.1).

9.2.3. CLINICAL STUDIES.

Daily clinical examination was carried out before and following treatment. Electrocardiography (ECG) was performed twice every week and limb-lead II ECG tracings

used to determine the electrical activity of the heart (described in detail in Ch. 4). Four ml of venous blood samples were collected for parasitological, haematological and biochemical studies.

9.2.4. PATHOLOGY.

After euthanasia, tissue blocks were taken from the heart and processed for histology, ultrastructural and immunofluorescence studies (as described in Ch. 8).

9.3. RESULTS.

9.3.1. CLINICAL FINDINGS.

9.3.1.1. Pre-treatment changes.

The pre-treatment clinical findings in these dogs have been described in detail in Part II (Ch. 4,5,6,7). Briefly, the dogs developed signs of disease after a prepatent period of 5 to 6 days. These were associated with high parasitaemia, persistent fever, lymphadenopathy, splenomegaly, severe anaemia, wasting and weight loss. In addition, there was marked increase in the acute phase proteins (APP), C-reactive protein (CRP) and haptoglobin (Hp). Clinical evidence of cardiac damage appeared in weeks 2 and 3, as tachycardia, valvular incompetence and heart blocks (HB). In the terminal stages in week 4, clinical signs of heart failure appeared, as bradycardia, weak heart beats, increased capillary refill time, dyspnoea and coughing, poor left ventricular function (LVF), sinus arrest, S-T segment elevation on ECG, and accumulation of pericardial effusion (PE).

9.3.1.2. Post-treatment changes.

General body condition.

Following treatment, the condition of dogs 1 and 2 deteriorated during the succeeding few hours, then improved with time. In dog 1 for example, tachycardia of 160 BPM had developed 24 hours after treatment on day 24. Dog 2 showed an arrhythmia, with occasional missed beats. Relapse infections were associated with periods of bradycardia and irregular heart rhythm. Ventricular premature beats (VPBs) were occasionally heard. The audibility of the heart beat varied from time to time, and both mitral incompetence (MI) and tricuspid incompetence (TI) persisted throughout the post-treatment period.

After successful treatment of dog 3, clinical signs of heart damage gradually disappeared. MI and TI decreased. The heart beat became loud and femoral pulses of normal strength were felt. In addition the dog's body condition improved gradually. From day 43 however, signs of kidney damage developed, including depression, increased BUN and plasma creatinine. In addition, the dog had diarrhoea and dehydration. The dog was euthanised on day 50 when its condition deteriorated. At that time, however, there were no clinical features indicating cardiac damage.

Within 12 hours of treatment, dog 4 developed severe bradycardia (40 $\pm$ 4 BPM: mean $\pm$ 1SD). Femoral pulses weakened and the capillary refill time increased to 4 seconds. The dog died 24 hours after treatment.

Electrocardiographic findings.

Unsuccessful treatment of dogs 1 and 2 resulted in a variety of ECG changes. Twenty four hours after the first treatment of dog 1 on day 24, S-T segment elevation of up to 0.4 mV was recorded on ECG (Fig. 9.1). This was followed by increased height and width of T waves. On day 32, for example, the T waves were 0.6 mV and Q waves were deep. With time, however, P-R intervals returned to normal but S-T segment, T and Q wave abnormalities persisted.

Treatment of dog 2 on day 24 resulted in marked prolongation of P-R intervals and second degree heart block (IIHB). R wave amplitudes, which were very low before treatment, increased rapidly after temporary clearance of trypanosomes from the blood (Fig. 9.2), indicating a reduction in the amount of PE. The IIHB observed soon after treatment disappeared gradually, but when relapses occurred, S-T segment depression and severe bradycardia (60 ± 10 BPM) appeared. T waves became tall and wide (Fig. 9.3). An arrhythmia developed, and VPBs were recorded (Fig. 9.4). R wave voltages then decreased to low levels again (Fig. 9.2). This indicated that the dog was going into severe heart failure when it was euthanised on day 61.

After clearance of trypanosomes from the blood of dog 3 by treatment, the ECG abnormalities present before treatment decreased rapidly. The voltage of R waves increased (Fig. 9.5), and 10 days post-treatment, R-wave notching which had developed before treatment was absent. P-R intervals became shorter and first degree heart block

(IHB) disappeared.

Dog 4 died 24 hours after suramin treatment. An ECG recorded a few hours before death revealed IHB, with P-R intervals of 0.16 seconds, as compared to 0.1 seconds on day 18 of infection.

9.3.2. PARASITOLOGICAL FINDINGS.

Treatment of dogs 1 and 2 with suramin at 10 mg/kg on day 24 of infection cleared trypanosomes from the blood temporarily (Fig. 9.6). Relapses occurred a week later, and the parasitaemia increased to pre-treatment levels again. Subsequent treatments with up to 40 mg/kg of suramin had minimal effect on the parasitaemia.

When dog 3 was treated with suramin at a dose of 20 mg/kg on day 28, a slight drop in the parasitaemia occurred (Fig. 9.6). Repeated treatment with 40 mg/kg for 3 days beginning on day 30 effectively cleared trypanosomes from the blood. The dog was euthanised while still aparasitaemic on day 50. Dog 4 died 24 hours after treatment with suramin at 10 mg/kg (Table 9.1). No post-treatment parasitological study was carried out on the dog.

9.3.3. THE HAEMOPOIETIC SYSTEM.

Haematological findings in untreated dogs have been described in Chapter 3. Briefly, the dogs developed severe anaemia, characterised by reduced total red blood cell (RBC) mass, thrombocytopaenia and leucocytopaenia. The latter was due to a decrease in the number of both lymphocytes and neutrophils.

9.3.3.1. Changes in red cell parameters.

Following treatment of dogs 1 and 2 with suramin on day 24, there was a transient recovery in all the haematological parameters estimated, including the packed red cell volume (PCV), total red cells (RBC), haemoglobin (Hb) concentration, and the mean corpuscular volume (MCV) (Figs 9.7-9.10). At the same time, a reticulocytosis developed (Fig. 9.11). Following relapses on days 31 and 32, the PCV, RBC and Hb concentration decreased again. The rate of decrease was, however, markedly reduced. Indeed in dog 2, PCV, RBC and Hb concentration did not fall until day 39, and had started to recover by the third treatment on day 46. Unlike dog 2, dog 1 was unable to control RBC mass for long. Nevertheless, on day 45, the PCV, RBC and Hb concentration were still higher than at first treatment on day 24 (Figs. 9.7-9.9). The MCV, after recovery, remained within normal range during the rest of the survival period for both dogs (Fig. 9.10). Moderate reticulocytosis was observed in the two dogs, and only decreased during the final week in dog 2 (Fig. 11).

At the time of first treatment of dog 3 on day 28, the PCV was 0.175 l/l (pre-infection mean - 0.49 l/l) (Fig. 9.7). This was accompanied by a similar decrease in RBC (from a pre-infection mean of 7.0×10^{12}/l to 2.58×10^{12}/l) (Fig. 9.8) and Hb concentration (from a pre-infection mean of 17 g/dl to 6.1 g/dl) (Fig. 9.9). After elimination of trypanosomes from the blood by treatment, the PCV, RBC and Hb concentration gradually increased up to day 43, then rapidly towards the time of

euthanasia on day 50 (Figs. 9.7-9.9). The MCV increased up to day 35 (Fig. 9.10), accompanied by a reticulocytosis (Fig. 9.11). Thereafter, the MCV dropped steadily until the dog was euthanised on day 50 (Fig. 9.10). Similarly, the number of reticulocytes decreased, and were absent on day 50 (Fig. 9.11). This terminal deterioration of haematological parameters occurred during the time when the dog was uraemic and had severe diarrhoea.

9.3.3.2. Changes in platelets.

Approximately 72 hours after treatment of dogs 1 and 2 on day 24, at which time trypanosomes had been cleared from the circulation, a rapid rise in platelet numbers occurred (Fig. 9.12). The elevated levels persisted for as long as the dogs remained aparasitaemic, dropping again when trypanosomes reappeared. Where treatment failed to clear trypanosomes from the blood, the number of platelets remained low. In dog 2 for example, treatment with suramin at 40 mg/kg on day 52 caused a transient decrease in trypanosome numbers, resulting in a rebound increase in platelets (Fig. 9.12).

On day 28 of infection of dog 3, the number of platelets in the blood were 16 x 10^9/l (pre-infection mean - 280 x 10^9/l). Treatment effectively cleared trypanosomes from the blood, resulting in a marked increase in platelets, which reached levels higher than at pre-infection on day 37. After that, no significant changes occurred until the dog was euthanised on day 50 (Fig. 9.13).

9.3.3.3. Changes in white cell parameters.

Leucocytosis, mainly due to increased neutrophils, developed rapidly after the first treatment of dogs 1 and 2 on day 24. Subsequently, the dogs became leucocytopaenic (Fig. 9.14). Small peaks of leucocytosis, also associated with increased neutrophils, were occasionally observed. In contrast, the number of lymphocytes remained relatively low (Fig. 9.14). Occasional small peaks of monocytosis were observed at various stages of the disease.

As in dogs 1 and 2, a rapid increase in white blood cells (WBC) took place in dog 3 after treatment. On day 32 for example, the number of WBC had gone up to 19.1 x 10^9/l (from 9.9 x 10^9/l on day 25). The increase in WBC was also due to a neutrophilia that developed soon after treatment. An accompanying increase in monocytes was observed. Subsequently, the neutrophilia receded, resulting in a decrease in total WBC. Leucocytopaenia then persisted up to day 50.

9.3.4. CHANGES IN ACUTE PHASE PROTEINS.

In dogs 1 and 2, the concentration of CRP fell rapidly soon after the first treatment. With relapses, however, CRP increased again (Fig 9.15). Subsequent treatments were followed by notable decreases in CRP, but with failure to clear trypanosomes from the blood, CRP increased to high levels again. On the other hand in dog 3, following clearance of trypanosomes from the circulation by treatment, the concentration of CRP dropped rapidly, reaching pre-infection values in 10 days (Fig. 9.16).

The changes in plasma concentration of Hp following

treatment of dogs 1, 2 and 3 with various doses of suramin are indicated in Table 9.2. Successful clearance of trypanosomes from the circulation (Dog 3) caused a gradual but slow decrease in Hp. However, at the time of termination of the experiment on day 50, the concentration of Hp was still more than double that at pre-infection. In dogs 1 and 2, Hp remained elevated up to the end of the study period.

9.3.5. POST MORTEM FINDINGS.

The carcasses of all the dogs were in poor condition, with markad wasting of skeletal muscles and gelatinisation of the perirenal and omental fat.

The heart of dog 1 was pale and flabby, with focal haemorrhages in both atria. The spleen was moderately enlarged but lymphoid follicles were not prominent. All lymph nodes were moderately enlarged and there was corneal opacity of the left eye.

The pericardial sac of dog 2 was distended with approximately 60 ml of straw coloured fluid and contained creamy fibrin deposits which were attached to the epicardial wall. The heart was firm to cut. The ventricular myocardium was extremely pale, and on section, it had pale demarcated zones extending from the epicardium into the deeper areas. The kidneys were pale and a single infarct, about 1 cm in diameter, was found in the left one. In addition, all lymph nodes were enlarged.

The heart of dog 3 was macroscopically normal. However, the kidneys were very pale and friable, and in the lungs, a haemorrhagic area approximately 1cm in diameter and

extending approximately 2 cm into the stroma was found in the right caudal lobe.

The pericardial sac of dog 4 was distended with 15 ml of straw coloured fluid. The ventricular myocardium was flabby and pale beige in colour. Petechial haemorrhages were found in the myocardium around the posterior papillary muscle and in the septum. Similar haemorrhages were present in the cranial lobe of the left lung. The spleen was enlarged, with prominent lymphoid follicles. The kidneys had focal haemorrhages approximately 0.5 cm in diameter (Fig. 9.17), extending up to 1cm into the cortex (Fig. 9.18), giving the appearance of infarcts. In addition, there was bilateral corneal opacity.

9.3.6. HISTOLOGICAL FINDINGS.

9.3.6.1. DOGS 1, 2 AND 3.

All three dogs were found to be suffering from chronic myocarditis which was particularly active in dogs 1 and 2, but appeared to be healing in dog 3. In all 3 dogs, lesions in the left ventricle displayed a more chronic appearance when compared with those in the right ventricle and in both atria.

The subepicardium:

Subepicardial fibrosis occurred in all cases but was more marked in dog 2 (Fig. 9.19). In dogs 1 and 2 there was oedema and myocytolysis. The subepicardial interstitium contained many vacuolated macrophages and lymphocytes, a few plasma cells, neutrophils and trypanosomes (Fig. 9.20). A similar cellular infiltration was present in

the subepicardial fat. The lymphatic vessels were moderately distended with lymph and inflammatory cells, and occasional areas of lymphatic obstruction were seen. In dog 3, subepicardial fibrosis was the most prominent feature (Fig. 9.21). Cellular infiltration in this dog was sparce, and consisted of macrophages and a few lymphocytes and plasma cells. No neutrophils were seen.

The myocardium:

Extensive atrial and ventricular myocardial fibrosis was observed in all 3 dogs, particularly in the left ventricle, and was more marked in dogs 2 and 3. Though generalised, fibrosis was encountered as small scattered foci (Fig. 9.22) or, in ventricles, as part of diffuse lesions, which were sometimes transmural. The diffuse lesions constituted sections of healing infarcts. Whenever infarction involved the subendocardial myocardium, a thin layer of normal muscle fibres was found bordering the endocardial side (Fig. 9.23). In the myocardium adjacent to areas of infarction, the myocytes were hypertrophied, with a few macrophages and lymphocytes interspersed between the myofibrils (Fig. 9.24).

In dogs 1 and 2, there was extensive myocardial degeneration and fragmentation, and diffuse infiltration with numerous macrophages, lymphocytes and plasma cells (Fig. 9.25). In addition, focal areas of the ventricular myocardium were hypereosinophilic due to coagulative necrosis, and the accompanying congestion and oedema indicated ischaemia. Fibrin deposition occurred in perivascular sites and in areas undergoing myocytolysis.

In dog 3, while there was marked myocardial fibrosis, trypanosomes were not seen, nor were myocardial degeneration or necrosis found. Small numbers of macrophages and lymphocytes, insinuating between myocardial fibres, were observed. There were hypertrophied myocytes around areas of fibrosis.

The subendocardium:

Histological findings in the atrial and ventricular subendocardium of dogs 1 and 2 were similar, though more marked in the latter dog. Subendocardial fibrosis was present with, in places, dense collagenous scars. The latter was accompanied by a cellular reaction similar to that observed in the subepicardial myocardium.

The conducting system:

In dogs 1 and 2, swelling and vacuolation of Purkinje fibres in the subendocardial myocardium, with cellular and trypanosome infiltration, was observed. In some areas, extensive fibrotic scars surrounded the conducting system (Fig. 9.26). Autonomic nerve ganglia in the subepicardial connective tissue at the base of the heart were involved, with necrosis of neurones and infiltration of trypanosomes, macrophages and plasma cells (Fig. 9.27). In dog 3, there was an increase in fibrous tissue around Purkinje fibres.

The vasculature:

Vascular changes were confined to dogs 1 and 2. Marked perivascular cellular infiltration around coronary arteries and veins was found. In addition, in some of the blood vessels, there was endothelial cell swelling and

surrounding perivascular fibrosis.

The valves:

Involvement of all cardiac valves was observed in the three dogs. In dogs 1 and 2, fibroblast necrosis, interstitial oedema and infiltration with macrophages, plasma cells, lymphocytes, neutrophils and trypanosomes occurred. Some foci of fibrosis were also seen. In dog 3, the degree of valvular fibrosis was more advanced and involved both the valve base and the cusp tissue.

In addition to changes in the heart, severe proximal tubular necrosis was observed in the cortex of the kidneys of dog 3, probably the result of suramin toxicity.

9.3.6.2. DOG 4:

The heart was severely involved. Most lesions were either similar or more prominent than those previously recorded in infected untreated dogs euthanised on day 24 (Ch. 8). The histological changes included severe pancarditis affecting the myocardium, valves, conducting system and vasculature. The lesions in this dog were, however, complicated by presence of large areas of haemorrhage, fibrin deposition and ventricular myocardial necrosis. In other regions of the ventricles, ischaemic myocardial necrosis, with wavy muscle fibres and contraction band necrosis indicated the presence of acute myocardial infarction (Fig. 9.28). In the atria, extensive areas of haemorrhage were found.

9.3.7. ULTRASTRUCTURAL FINDINGS.

Together with providing additional information,

transmission electron microscopy (TEM) confirmed most of the histological findings described above.

9.3.7.1. DOGS 1, 2 AND 3.

Changes in myocytes:

In the atria and ventricles of dogs 1 and 2, changes in myocytes varied with the region examined. Necrotic myocytes were found adjacent others that were in the process of myolysis (Fig. 9.29). Variable quantities of lipid deposits were observed in degenerating myocytes (Fig. 9.30). In some myocytes, the mitochondria were swollen and the cristae were disintegrating (Fig. 9.31). In other areas, there was distinct separation of myocytes along intercalated discs (Fig. 9.32). Separation was present even in intercalated discs joining relatively normal myocytes. The spaces thus formed contained material which resembled myelin configurations. In dog 3, however, there was very little evidence of myocyte degeneration, with only occasional cytoplasmic lipid droplets. In the atria of this dog, natriuretic granules were numerous, large, and more electron-dense than those found in terminally infected untreated dogs (Ch. 7). Fewer granules and of smaller size were seen in the atria of dogs 1 and 2.

In all 3 dogs, there were varying degrees of interstitial fibrosis. In dogs 1 and 2, collagen deposition occurred mainly in areas undergoing degenerative changes, while in dog 3, fibrosis was more diffuse (Fig. 9.33). Around autonomic nerve ganglia in dogs 1 and 2, macrophages, lymphocytes, plasma cells and neutrophils were found, and there was marked fibrin deposition.

Cellular changes:

The cellular reaction varied from dog to dog and the region of the myocardium examined. In some areas, large numbers of highly active macrophages were found together with a few plasma cells and lymphocytes (Fig. 9.34). The amount of lipid in macrophages was less than had been noted earlier in infected untreated dogs (Ch. 6). In other areas, cellular infiltration was dominated by aggregates of very active plasma cells (Fig. 9.35). On occasion, plasma cells were seen together with lymphocytes in equal numbers (Fig. 9.36). Variable quantities of fibrin deposits were observed between myocytes and at perivascular locations.

The vasculature:

A vasculitis of capillaries, arteries and venules of dogs 1 and 2 and not dog 3 was observed, and was most marked in areas where myocytolysis was taking place. Endothelial cells of capillaries were necrosed and proteinaceous fluid had leaked into the interstitium (Fig. 9.37). In other places, perivascular accumulation by inflammatory cells had caused occlusion of capillaries (Fig. 9.34).

9.3.7.2. DOG 4.

Extensive myocytolysis was observed in the atrial and ventricular myocardium. As a result, profiles of organelles could be seen lying free in the interstitium. There was marked lipid deposition, both within myocytes (Fig. 9.38) and in the interstitium (Fig. 9.39). The composition of infiltrating cells was similar to that

observed in terminally infected untreated dogs (Ch. 8), and consisted mainly of macrophages, neutrophils, plasma cells and a few lymphocytes. Most of these cells were necrosed and undergoing fragmentation. Surviving macrophages were very active, ingesting the necrotic debris, including erythrocytes and large quantities of fibrin (Fig. 9.40). There were numerous trypanosomes; most were dead, trapped in the deposits of fibrin, and showing varying degrees of disintegration (Fig. 9.41). Others were being phagocytosed by macrophages. At several perivascular sites, irregular electron-dense material resembling calcium were deposited (Fig. 9.42).

In the atrial myocytes, natriuretic granules were few, small and poorly electron-dense.

9.3.8. IMMUNOFLUORESCENCE FINDINGS.

In both the ventricular and atrial myocardium of dogs 1, 2 and 4, deposition of large quantities of fibrinogen in perivascular and interstitial spaces was confirmed by immunofluorescence. In addition, small quantities of IgM and IgG were found in areas where extensive tissue damage and vascular leakage was taking place. Such deposits were not found in the heart of dog 3. On the other hand in the kidneys of all dogs, mesangial deposits of IgG, IgM and C3 were demonstrated in the glomeruli by immunofluorescence, and by TEM, dense mesangial deposits were also present.

9.4. DISCUSSION.

Treatment of terminally ill T.brucei-infected dogs with various doses of suramin resulted in a variety of

responses. While a single dose of 10 mg/kg on day 24 of infection cleared trypanosomes from the blood for at least one week, subsequent doses of up to 40 mg/kg had only minimal effect on the parasitaemia. Nevertheless, the survival time was markedly prolonged. Effective clearance of trypanosomes was achieved only when repeated high doses of suramin were used, a treatment that proved to be nephrotoxic.

Trypanocidal drug treatment caused a decrease in both CRP and Hp, with CRP dropping at a faster rate than Hp. Relapse infections resulted in a quick return of high CRP levels. This observation, and those of other workers (Thomasson et al., 1973; Pepys et al., 1985), confirmed the value of CRP assays in estimating the severity of active tissue damage and treatment response in disease states. Normal clearance of Hp from the blood is very slow, increasing when intravascular haemolysis occurs (Ch. 5; Esievo et al., 1984). The slow decrease in Hp following elimination of trypanosomes in the present study thus indicated that there was no significant intravascular haemolysis.

Curative treatment resulted in rapid improvement of the haematological parameters. A similar observation has been made in humans infected with T.rhodesiense (Barrett-Connor et al., 1973) and cattle infected with T.brucei, T.vivax and T.congolense (Murray and Dexter, 1988), indicating that the anaemia in the early stages of the disease is highly dependent on the presence of the parasite.

Subcurative treatment prolonged the survival period,

despite the persistence of high parasitaemia. This was more so in dog 2, which managed to control anaemia for a long period, indicating that a form of treatment that controls the rate of development of anaemia might be best suited for inducing a chronic relapsing model of trypanosomiasis in dogs. In cattle infected with T.brucei and T.congolense, the longest survival time has been observed in animals that are capable of controlling anaemia (Murray et al., 1979).

Soon after suramin treatment, abnormal ECG changes were recorded, including IIHB, S-T segment elevation and tall, wide T waves. This would indicate that after treatment, an uncontrolled inflammatory reaction took place, resulting in electrolyte imbalances and ischaemic myocardial damage. Increased cardiac damage has been demonstrated by ECG following treatment of T.rhodesiense-infected human patients with suramin (Jones et al., 1975). It is possible that after treatment, the large number of killed trypanosomes in the myocardial interstitium induces massive localised antigen-antibody reactions with resultant inflammation and increased myocardial damage, an observation that was confirmed in the present study by TEM.

Extensive tissue damage, involving the heart, kidneys and lungs, was demonstrated in dog 4, which died 24 hours after treatment with suramin at 10 mg/kg. Several factors could have contributed to death. Firstly, the disease was probably too advanced at the time of treatment for suramin to have any beneficial effect, death occurring due to the severity of the disease per se. The extent of myocardial,

renal and lung involvement, however, was higher than was demonstrated in infected untreated dogs euthanised in terminal infection on day 26 (Ch. 8), or even in dogs dying after infection with T.brucei (Meirvenne et al., 1972; Losos and Ikede, 1972; Moloo et al., 1973; Mwambu, 1979; Kaggwa et al., 1983), indicating that the severity of the disease per se could not have been the main cause of death in the current study. Secondly, death could have been exacerbated by the direct toxic action of suramin. However, the extensive use of suramin in experimental studies with T.brucei (Jennings et al., 1983) and T.rhodesiense (Ndung'u and Akol, 1988) in mice, T.rhodesiense in vervet monkeys (Sayer et al., 1987), and for treating human beings infected with T.rhodesiense (Apted, 1970; Molyneux et al., 1984; Harries and Wirima, 1988), indicates that suramin, used at recommended therapeutic doses, is relatively non-toxic on its own. This suggests that suramin toxicity is unlikely to have been the direct cause of death. Thirdly, substances released by trypanosomes killed by suramin could have led to increased tissue damage. In this respect, in vitro studies have shown that dying trypanosomes generate a range of biologically active factors, including proteases, phospholipases, free fatty acids (FFA) and lipopolysaccharides (Mellors, 1985; Murray and Dexter, 1988). Indeed as large numbers of dead trypanosmes were found together with inflammatory cells undergoing fragmentation, it is possible that treatment caused death of the trypanosomes, which in turn released large quantities of toxic substances that destroyed the

inflammatory cells, thus exacerbating the degree of inflammation. In addition, tissue hypoxia increased the extent of myocardial ischaemia, leading to even further damage. Wavy bands of myocardial fibres and contraction-band necrosis were observed in infarcted zones. Such changes have been observed in human patients dying suddenly of acute myocardial infarction, and are generally considered to be an indication of a fairly acute type of injury (Bouchardy and Majno, 1974).

Treatment of dog 3 with high, repeated doses of suramin caused kidney damage. This was demonstrated clinically 14 days after treatment and was associated with the development of diarrhoea, dehydration, depression, increased plasma creatinine and blood urea nitrogen (BUN), and confirmed by histology and TEM. The increase in BUN was an indication that the dog was uraemic, and this most likely caused the deterioration of clinical and haematological parameters terminally, a common sequel of uraemia in dogs (Osborne et al., 1983). Renal damage is the most common toxic side effect of suramin treatment in human beings if large doses are used, leading to albuminuria (Apted, 1970).

Treatment of infected dogs resulted in transient leucocytosis, which was mainly due to a neutrophilia. The fact that neutrophilia and ECG abnormalities occurred almost at the same time following treatment suggested a possible relationship between the two findings. It may be possible that factors produced in the myocardium stimulated increased production of neutrophils. Several factors are

chemotactic for neutrophils; including the reaction of lipids or vascular endothelial cells with reactive oxygen metabolites (ROM) (Petrone et al., 1980). ROM can be released by trypanosomes (Turrens, 1987) and by endothelial cells following ischaemic damage (Hernandez et al., 1987); the latter mechanism is the most potent. Myocardial infarction also leads to chemotactic mobilization of neutrophils from the bone marrow, resulting in a leucocytosis that does not last for more than a week (Govan et al., 1986). In the present study, it would appear that excessive inflammatory reactions took place after treatment, causing myocardial infarction and hence the neutrophilia. This would explain the finding, at histology, of ischaemic foci and extensive myocardial scars at several stages of development, especially in dog 2, which survived for 61 days. Similar infarct-like zones have been observed in the heart of vervet monkeys infected with T.rhodesiense (Poltera and Sayer, 1983).

Treatment of infected dogs was associated with return of normal platelet numbers in the blood, and recurrence of thrombocytopaenia immediately after relapse infection. Similar observations have been made in T.rhodesiense-infected human patients following treatment with suramin (Barrett-Connor et al., 1973) and in cattle infected with T.congolense after treatment with diminazene aceturate (Wellde et al., 1978). In rats and rabbits infected with T.rhodesiense, thrombocytopaenia has been noted to persist for as long as trypanosomes are present in the blood, and the severity directly related to the height

of parasitaemia (Davis et al., 1974). Similar observations were made in the present work. In the pre-treatment study (Ch. 3), it has already been demonstrated that severe thrombocytopaenia developed immediately trypanosomes appeared in the blood, indicating that the presence of trypanosomes in the circulation was crucial for the development of thrombocytopaenia.

The possible causes of thrombocytopaenia in infected dogs have been reviewed elsewhere in this work (Ch. 3), and these include excessive pooling of platelets in the spleen, decreased production of platelets by the bone marrow, and shortened platelet lifespan, the result of activation of the coagulation system. The finding of large deposits of fibrin in the myocardium of infected dogs, and rapid return to normal platelet numbers in the blood after treatment is further indication that platelet production by the bone marrow was taking place normally, or even at an increased rate, but could not cope with excessive utilization. It is possible that living and dead trypanosomes, through production of biologically active products (Davis et al., 1974; reviewed by Mellors, 1985; Murray and Dexter, 1988) stimulate increased platelet utilization in the coagulation system, and possibly removal by a stimulated MPS.

Treatment of dogs with suramin at 10 mg/kg on day 24 of infection cleared trypanosomes temporarily from the blood. Nevertheless, when treatment was carried out at a later stage of the disease, single doses of up to 40 mg/kg had minimal effect on the level of parasitaemia. This might have been caused by several factors, operating separately

or in concert. Underdosage, resistance by the trypanosomes, and decreased availability of suramin to the trypanosome target may all be causative factors. Treatment with an inadequate dose of trypanocide kills only a certain percentage of trypanosomes, leaving those that are more resistant to repopulate the blood. Further treatments therefore require higher doses of the drug in order to achieve cure.

An already drug-resistant trypanosome strain can also cause treatment failure. In the present study, however, the T.brucei stabilate used had already been shown to be sensitive to conventional trypanocides, including suramin (Jennings and Gray, 1983), and the first treatment of dogs with a low dose of suramin caused aparasitaemia, indicating that the infecting stabilate was drug-sensitive.

It could be urgued that poor availability of suramin to the trypanosomes might be a factor. Firstly, the trypanosomes may be located in inaccessible sites where suramin cannot penetrate. Treatment would therefore clear trypanosomes from the blood and other sites where it can penetrate; release of trypanosomes from the cryptic sites would cause relapses. This form of relapses has been demonstrated using diminazene aceturate and isometamidium chloride in dogs (Kaggwa et al., 1988) and mice infected with T.brucei (Jennings et al., 1977), using suramin alone or in combination with other trypanocides in vervet monkeys infected with T.rhodesiense (Sayer et al., 1987), and in human patients infected with T.rhodesiense after treatment with suramin (Arroz and Djedje, 1988). In those reports

however, trypanosomes did not reappear in the blood in less than three weeks, and subinoculation of blood from treated hosts into mice during the period of aparasitaemia did not cause infection (Jennings et al., 1977). In the current study, trypanosomes reappeared in the blood after only one week, and further treatment had minimal effect. This indicated that treatment had only reduced parasite numbers to undetectable levels in the blood, and when the drug concentration went down, parasitaemia was re-established.

Secondly, in order for suramin to kill trypanosomes, it has to be bound to plasma albumin, then be taken up by the trypanosome by endocytosis (Muller and Wollert, 1976). It has already been shown in another part of this work that infected dogs develop severe hypoalbuminaemia (see Ch. 6). It is possible that the hypoalbuminaemic state decreased the amount of albumin-bound suramin available to trypanosomes, causing reduction in effectiveness of treatment. At the same time, dogs infected with T.brucei developed marked hyperlipidaemia, the concentration of lipids in the blood increasing with the length of infection (Ch. 6). Suramin, being a polyanion (Fairlamb and Bowman, 1980), might then bind to plasma lipoproteins in a disproportionate manner, thus decreasing the amount of drug available to bind to albumin. As a result, the total concentration of suramin in the blood, while still relatively high, would not be sufficient to kill trypanosomes. This might therefore have caused the decrease in efficacy of suramin with prolongation of the survival time of the dogs. As such, in about 10% of human patients

with African trypanosomiasis, a form of infection relapse similar to the one in the present study has been observed within a few days after the last course of suramin injections (Hawking, 1940).

Treatment had a distinct effect on the survival periods of dogs, but in so doing lead to chronic pancarditis. Ventricular myocardial damage was confirmed on ECG by the presence of VPBs and S-T segment changes. Similar ECG abnormalities are frequently seen in human patients infected with T.rhodesiense (Jones et al., 1975) and T.gambiense (Bertrand et al., 1971; Francis, 1972; Bertrand et al., 1974; Bertrand, 1987). Chronic pancarditis, with PE, multiple foci of fibrosis and extensive healing infarcts in ventricles, and valvular damage, were demonstrated in the current study. The post mortem and histological lesions observed were similar to those caused by T.rhodesiense infection in man (Koten and De Raadt, 1969; Poltera et al., 1975; Poltera and Cox, 1977) and in vervet monkeys after subcurative treatment with trypanocidal compounds (Schmidt and Sayer, 1982), by T.gambiense infection in human beings (Bertrand et al., 1967; Bertrand et al., 1971), T.brucei infection in mice (Poltera et al., 1980) and cattle (Morrison et al., 1983), and some forms of cardiomyopathies in man (Poltera and Cox, 1977).

Lymphatic obstruction was demonstrated in the hearts of infected dogs and dogs subjected to subcurative treatment. Chronic lymphatic obstruction in general can lead to endomyocardial fibrosis in man and in dogs (Miller, 1976).

Thus lymphatic obstruction might have contributed to some of the fibrotic changes observed in the present study. Moreover, endomyocardial fibrosis has been found in the hearts of humans from trypanosomiasis-endemic regions of East Africa (Poltera and Cox, 1977). It is possible that the patients had been exposed to trypanosomiasis and, after being cured of the disease, persistent lymphatic obstruction led to endomyocardial fibrosis. This form of cardiomyopathy can also occur in association with a pancarditis and chronic cellular infiltration (Farrer-Brown and Tarbit, 1972). Certain forms of cardiomyopathy observed in Africans probably result from previous exposure to trypanosomes. In this connection, high serological titres of antibodies for trypanosomiasis have been demonstrated in congestive cardiomyopathies of unknown origin in Cameroon (Blackett and Ngu, 1976). The degree of heart failure that occurred in the dog that survived longest in the present study, and the severity of cardiac damage observed, indicated that if treatment against the trypanosomes had become successful at that time, the dog would probably have survived but remained in congestive heart failure.

From the results of the present study, it was demonstrated that treatment of dogs with suramin can sometimes result in adverse reactions, and even death. The severity of the post-treatment reactions did not appear to be related to the dose of suramin used, but rather to uncontrolled inflammatory reactions in the myocardium that took place soon after treatment. The presence of many dead trypanosomes and necrotic inflammatory cells in the

myocardial interstitium indicated the sudden release of large quantities of toxic substances. The latter caused direct damage to myocardial cells and, as a result of severe inflammation, ischaemic myocarditis. At the same time, the inability of suramin to clear trypanosomes from the circulation the longer the dogs survived was probably due to reduced availability of the drug to the trypanosomes, despite high concentrations in the blood. This was because either suramin selectively bound to lipoproteins or, due to hypoalbuminaemia or both, the amount of albumin-bound drug was too low to kill trypanosomes.

Even though the dogs became refractory to treatment with suramin, their survival time was prolonged. This was more so when the dogs were able to control anaemia. As a result, a chronic form of cardiac damage, which eventually resulted in heart failure, was precipitated. This was an indication that, if effective clearance of trypanosomes had been achieved, at day 50 for example, when chronic myocardial damage had already occurred, the dogs might have survived, myocardial healing taking place by scar tissue formation. In the long-term, such hearts would have been incapable of coping with the pumping action demanded of normal heart muscle, resulting in chronic congestive heart failure. As such, this might be a good model for studying the mechanisms that lead to congestive heart failure, and other forms of cardiomyopathy, in both dogs and man.

TABLE 9.1.

SURAMIN TREATMENT REGIMENS AND DAYS OF EUTHANASIA IN DOGS INFECTED WITH T.brucei.

DOG NUMBER	1	2	3	4
DOSE OF SURAMIN USED AND DAY OF TREATMENT (D)	D24-10mg/kg D45-20mg/kg D46-20mg/kg	D24-10mg/kg D41-10mg/kg D46-20mg/kg D52-40mg/kg	D28-20mg/kg D30-40mg/kg D31-40mg/kg D32-40mg/kg	D20-10mg/kg
DAY OF EUTHANASIA	47	61	50	21

TABLE 9.2.

PLASMA CONCENTRATION OF HAPTOGLOBIN (MG OF HAEMOGLOBIN BOUND COMPLEX/100ML) IN DOGS INFECTED WITH T.BRUCEI.

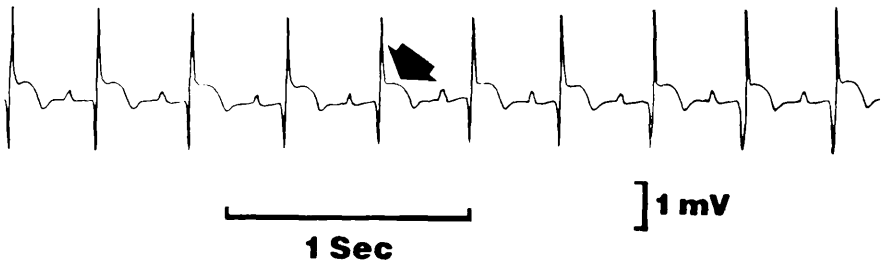
DAY OF INFECTION	DOG 1	DOG 2	DOG 3
24	303*	183*	182
26	600	546	182
28	350	454	102*
31	372	396	275
33	539	360	264
35	600	420	222
38	491	480	212
40	327	458	189
42	314	294	ND
45	327	385	178

* - Last sample taken before treatment begun.

ND - Not done.

Figure 9.1. S-T segment elevation (arrow) in a limb-lead II ECG tracing of dog 1, 24 hours after treatment with suramin on day 24 of infection.

Figure 9.2. Changes in the voltage of R waves (●) in limb-lead II ECGs and the parasitaemia (□) in dog 2 during the course of T.brucei infection and treatment with suramin (arrows). Infection was associated with a rapid drop in R wave voltage from day 17. The first treatment caused recovery, but with relapses, R wave voltages decreased again.


1 mV
1 Sec

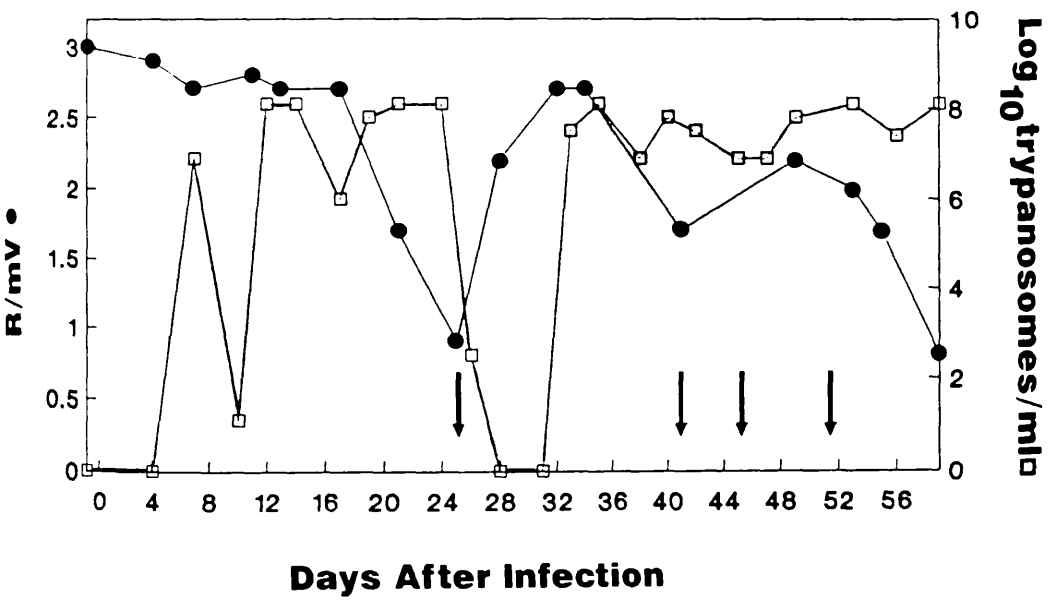
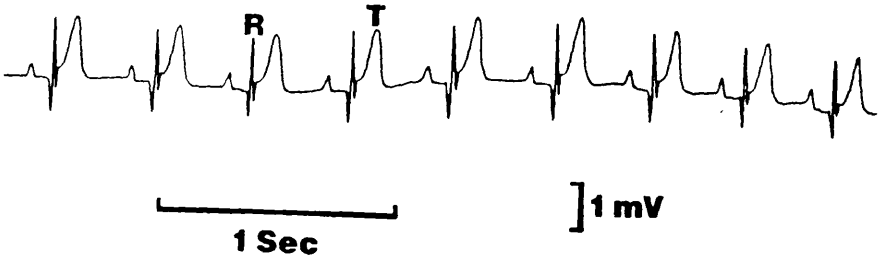

R/mV •
Log10 trypanosomes/ml□
Days After Infection
0
0.5
1
1.5
2
2.5
3
0
2
4
6
8
10
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56

Figure 9.3. Limb-lead II ECG tracing of dog 2, 41 days after infection with T.brucei, following unsuccessful treatment with suramin on day 24. T waves are tall and wide and the voltage of R waves low.

Figure 9.4. Limb-lead II ECG tracing of dog 2, 34 days after infection. There are several ventricular premature beats, indicated by bizarre complexes (arrows). PQRST waves of a normal cardiac cycle are indicated.


R
T
1 mV
1 Sec

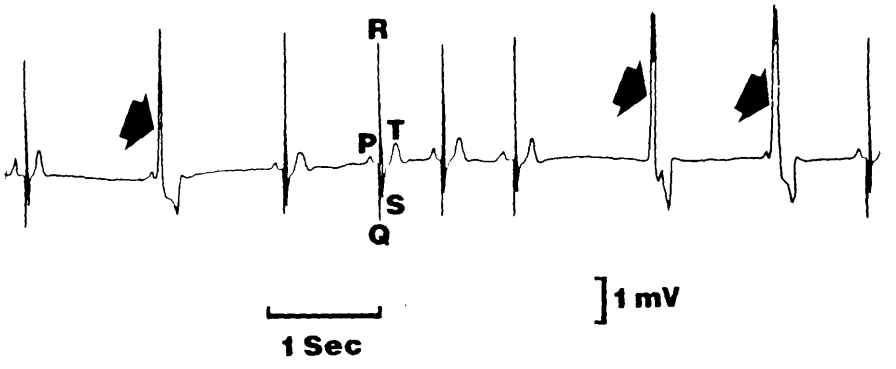

R
P
T
S
Q
1 mV
1 Sec

Figure 9.5. Changes in the voltage of R waves (●) in limb-lead II ECGs and the parasitaemia (□) in dog 3 during the course of *T.brucei* infection and following curative treatment with suramin from day 28 of infection (arrow). The decrease in R wave voltage was due to accumulation of pericardial effusion. After treatment the pericardial effusion decreased.

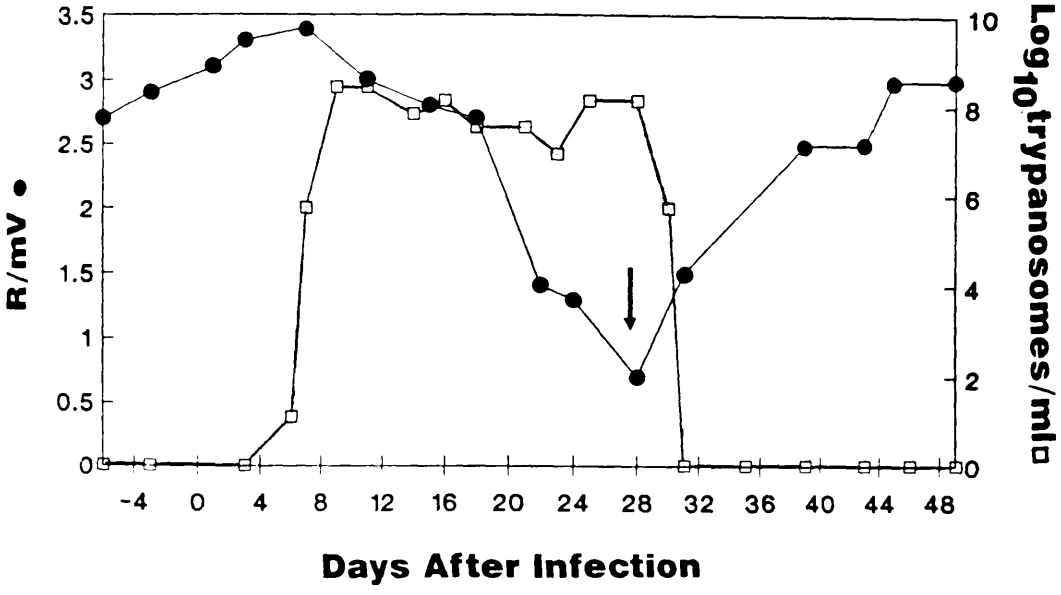

3.5
3
2.5
2
1.5
1
0.5
0
R/mV ●
10
8
6
4
2
0
Log10trypanosomes/ml□
-4
0
4
8
12
16
20
24
28
32
36
40
44
48
Days After Infection

Figure 9.6. Effect of suramin treatment on the parasitaemia in dogs 1 (■), 2 (●) and 3 (O). The parasitaemia decreased rapidly after the first treatment (✱). Relapses occurred in dogs 1 and 2 but not in dog 3.

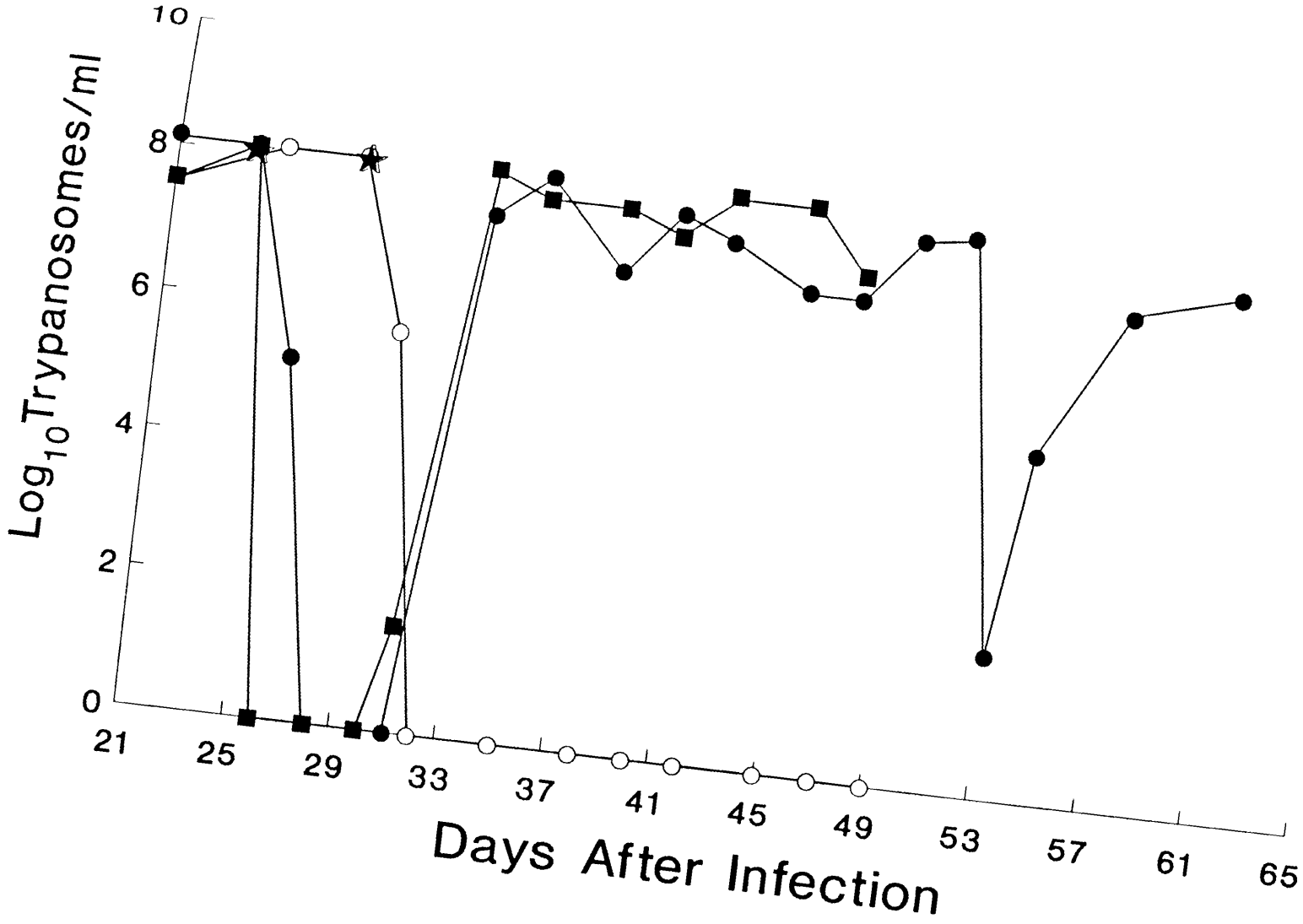

Log10Trypanosomes/ml
10
8
6
4
2
0
21
25
29
33
37
41
45
49
53
57
61
65
Days After Infection

Figure 9.7. Packed red cell volume (PCV) (l/l) in T.brucei-infected dogs following treatment with suramin. In dog 3 (O), there was gradual improvement after curative treatment. The decrease in PCV in dogs 1 (■) and 2 (●) was reduced after subcurative treatment. Dog 2 was able to control the PCV despite the presence of parasites in the blood. ★ - Day of first treatment.

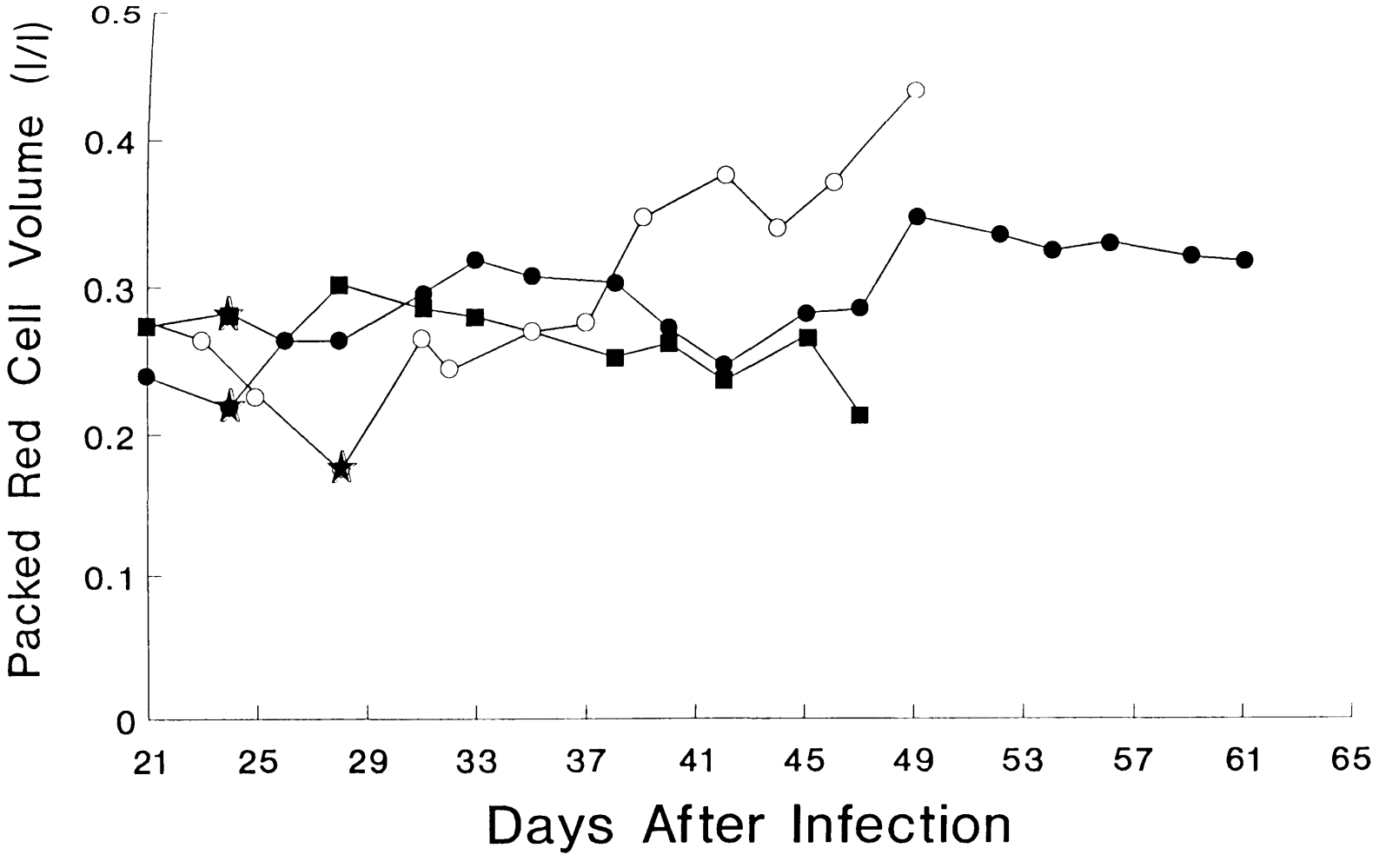

Packed Red Cell Volume (l/l)
0.5
0.4
0.3
0.2
0.1
0
21
25
29
33
37
41
45
49
53
57
61
65
Days After Infection

Figure 9.8. Total red blood cells (RBC) ($x10^{12}/l$) in T.brucei-infected dogs following treatment with suramin. There was gradual improvement in dog 3 (O) following successful treatment. While dog 1 (■) succumbed on day 47, dog 2 (●) was able to control anaemia despite the presence of trypanosomes in the blood, hence the prolonged survival time. ★ - Day of first treatment.

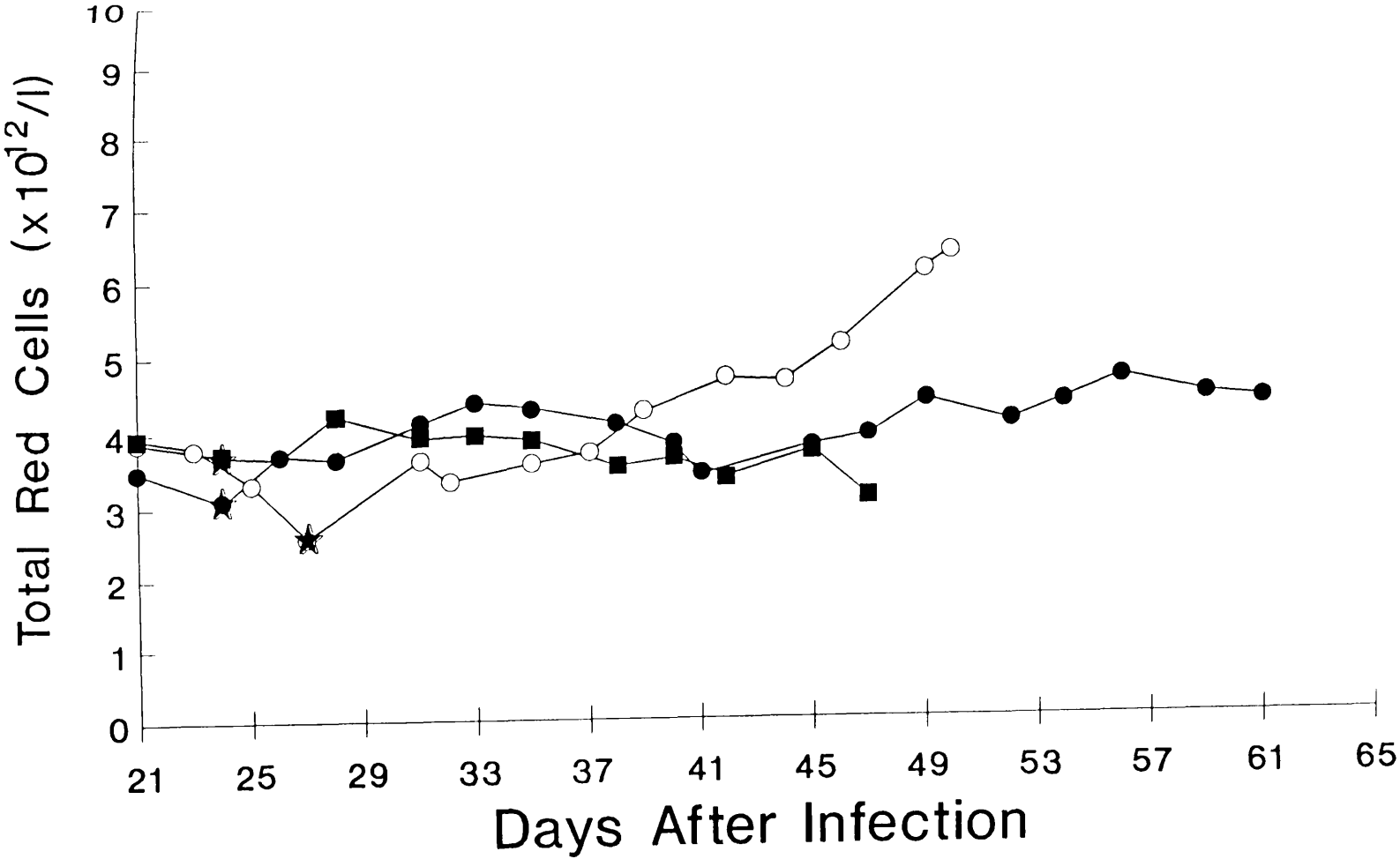

Total Red Cells (x10^12/l)
10
9
8
7
6
5
4
3
2
1
0
21
25
29
33
37
41
45
49
53
57
61
65
Days After Infection

Figure 9.9. Haemoglobin concentration (Hb) (g/dl) in dogs infected with T.brucei, following treatment with suramin. Dog 3 (O) was treated successfully, hence better improvement. Dogs 1 (■) and 2 (●) were treated unsuccessfully. The rapid increase in Hb in dog 3 from day 45 was probably due to haemoconcentration. ★ - Day of first treatment.

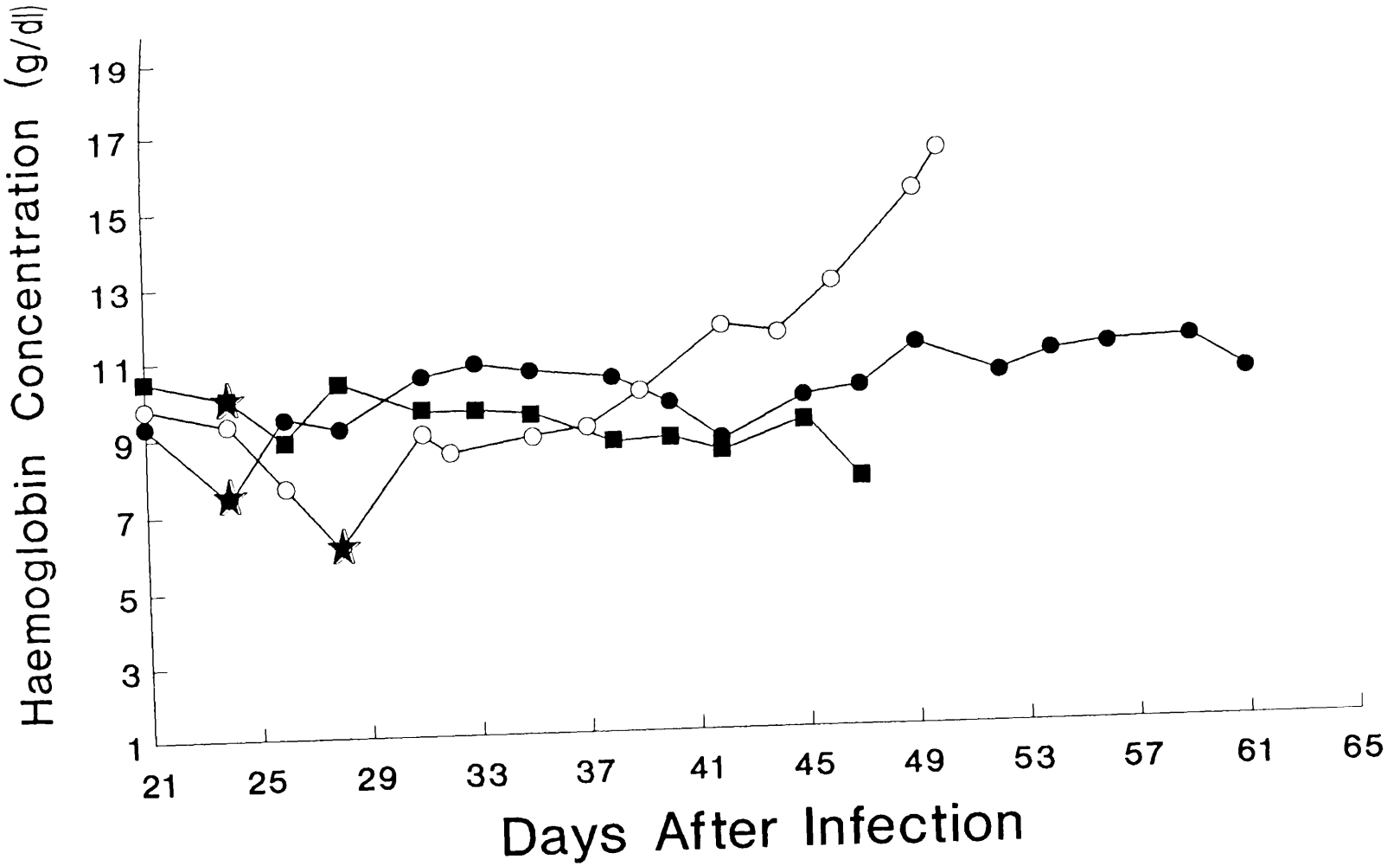

Haemoglobin Concentration (g/dl)
19
17
15
13
11
9
7
5
3
1
21
25
29
33
37
41
45
49
53
57
61
65
Days After Infection

Figure 9.10. Mean corpuscular volume (MCV) (fl) changes in dogs infected with T.brucei following treatment with suramin (as per Table 9.1). There was no significant changes in MCV in dogs 1 (■) and 2 (●), while a gradual decrease in MCV in dog 3 (O) took place from day 35. ★- Day of first treatment.

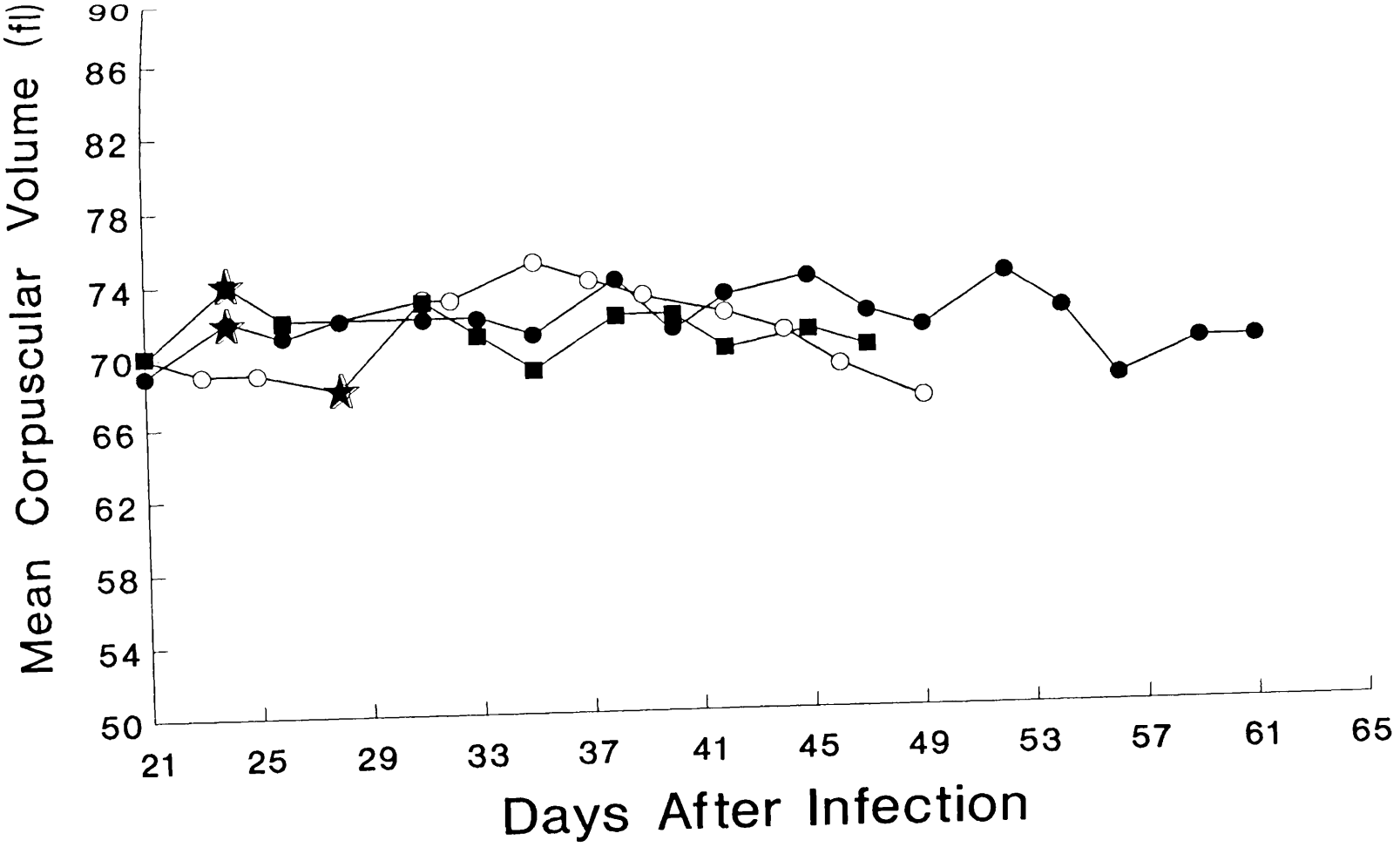

Mean Corpuscular Volume (fl)
90
86
82
78
74
70
66
62
58
54
50
21
25
29
33
37
41
45
49
53
57
61
65
Days After Infection

Figure 9.11. Reticulocyte changes ($x10^{12}/l$) in dogs infected T.brucei following treatment with suramin (as per Table 9.1). Treatment caused a reticulocytosis in the three dogs, which decreased rapidly after day 30 in dog 3 (O). The reticulocytosis persisted in dogs 1 (■) and 2 (●). ★- Day of first treatment.

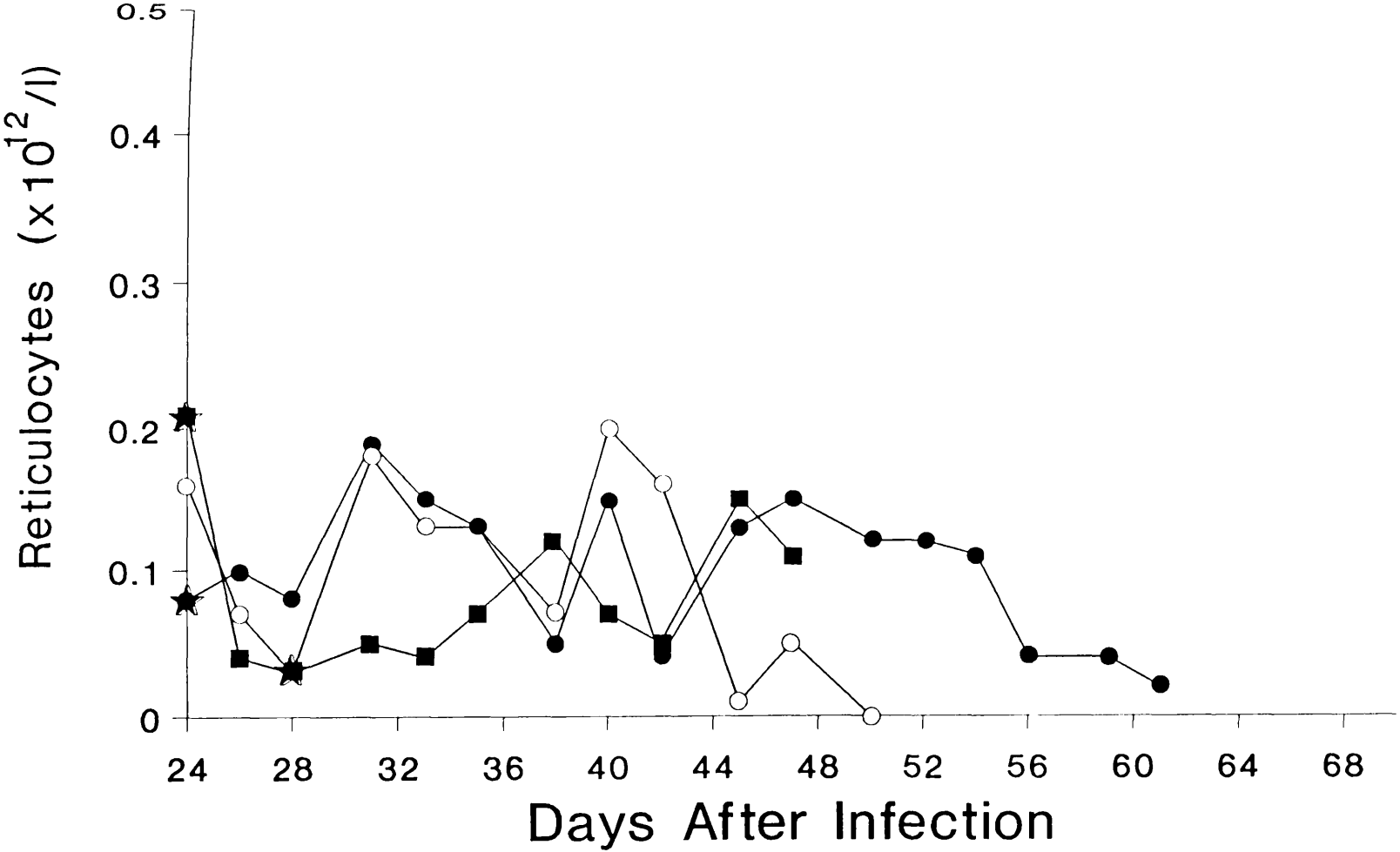

Reticulocytes (x10^12/l)
0.5
0.4
0.3
0.2
0.1
0
24
28
32
36
40
44
48
52
56
60
64
68
Days After Infection

Figure 9.12. Platelet numbers (●) ($x10^9/l$) and the parasitaemia (O) (log_{10}trypanosomes/ml) in dog 2, during a course of suramin treatments (★). The first treatment cleared trypanosomes from the blood, causing platelets to increase. After relapses, thrombocytopaenia developed again. Subsequent treatments had minimal or no effect on parasitaemia and platelet numbers. There was an indirect relationship between the parasitaemia and the number of platelets.

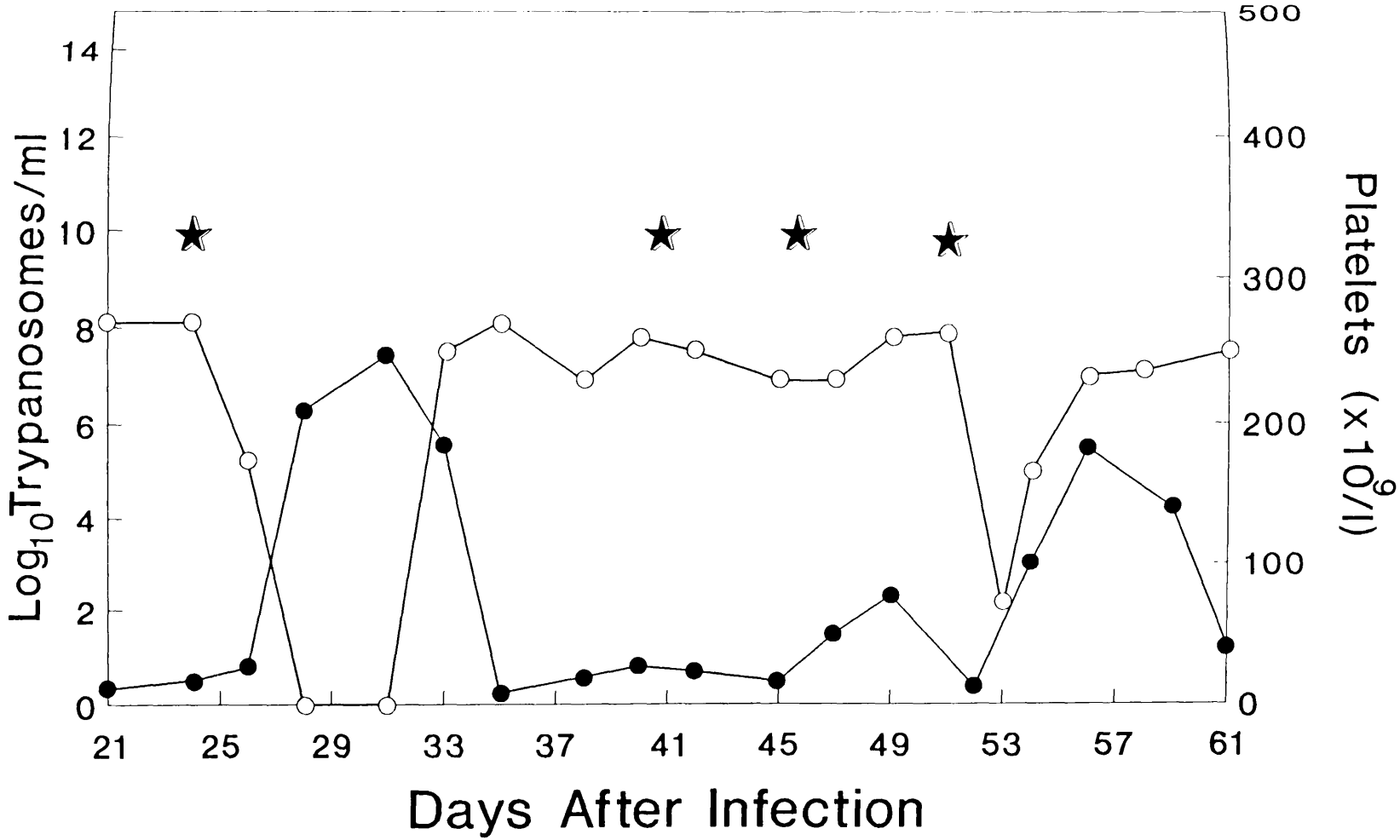

Log10Trypanosomes/ml
Platelets (x10^9/l)
Days After Infection
0
2
4
6
8
10
12
14
0
100
200
300
400
500
21
25
29
33
37
41
45
49
53
57
61

Figure 9.13. Platelet numbers (●) ($x10^9/l$) and parasitaemia (log_{10}trypanosomes/ml) (O) in dog 3. Treatment with suramin from day 28 to 32 cleared trypanosomes from the blood, resulting in rapid increase in platelets.

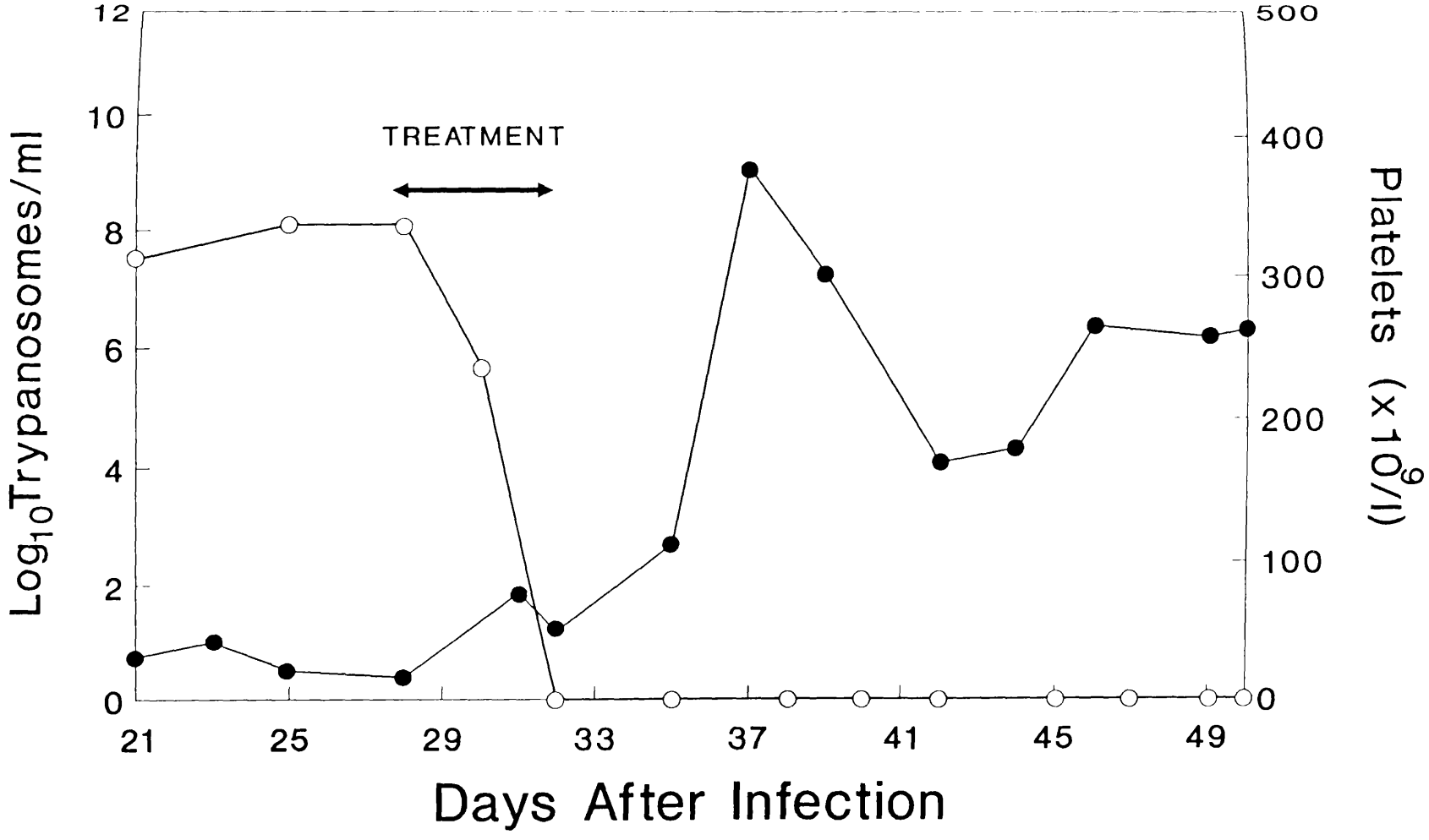

12
10
8
6
4
2
0
500
400
300
200
100
0
TREATMENT
Log10Trypanosomes/ml
Platelets (x10^9/l)
21
25
29
33
37
41
45
49
Days After Infection

Figure 9.14. Leucocyte changes in dog 1 during the course of suramin treatments (★). The first treatment was followed by rapid increase in total white blood cells (WBC) (●). The rise in WBC took place at the same time as increases in neutrophils (■), with minimal changes in lymphocyte numbers (○).

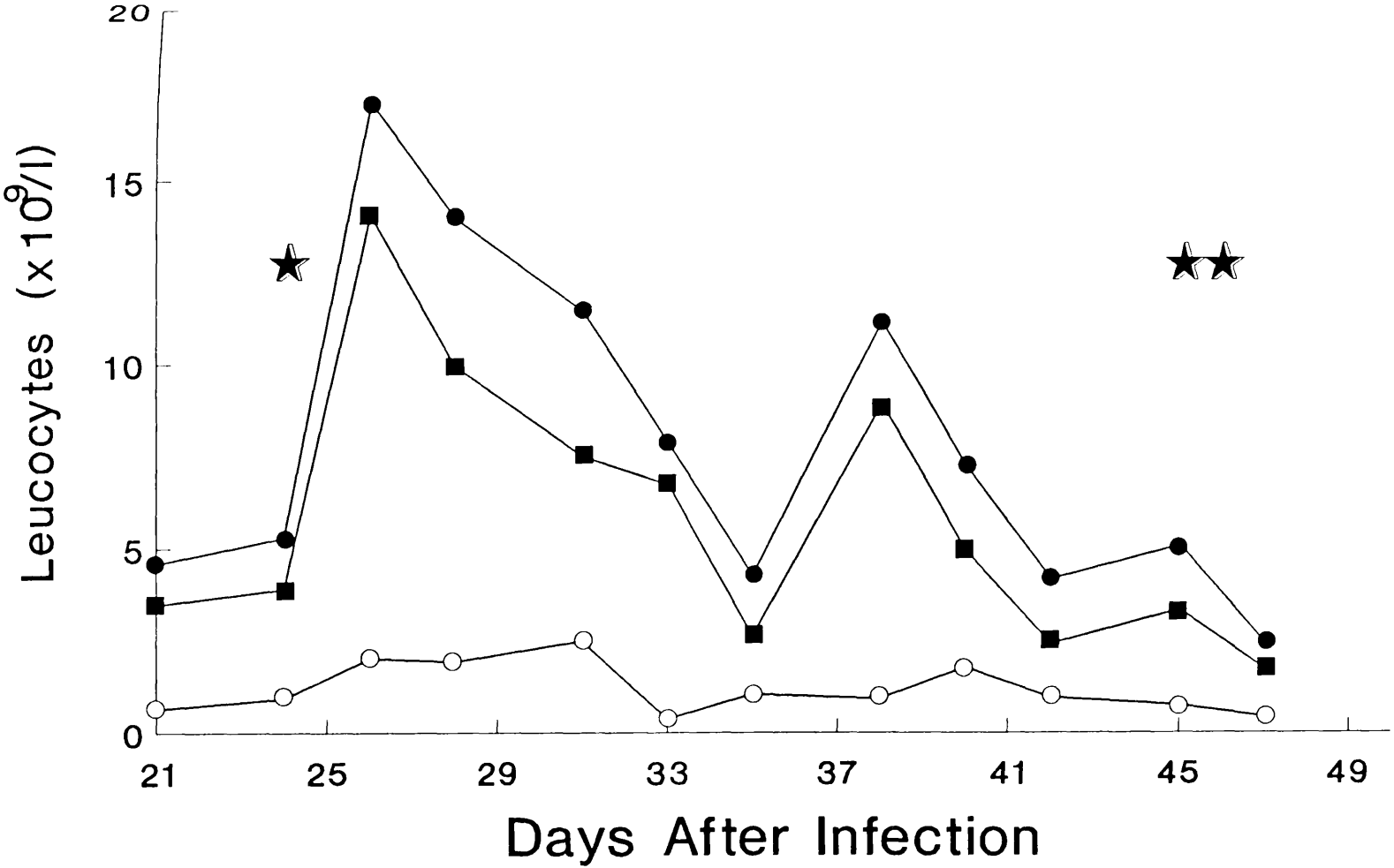

20
15
10
5
0
Leucocytes (x10⁹/l)
21
25
29
33
37
41
45
49
Days After Infection

Figure 9.15. Plasma concentration of CRP (●) and the parasitaemia (O) in dog 4 during the course of T.brucei infection and following subcurative treatment with suramin (arrows). CRP followed closely the changes in parasitaemia. 200 mg/l of CRP was the highest concentration that could be estimated acurately with the method employed, hence the two plateaus at X.

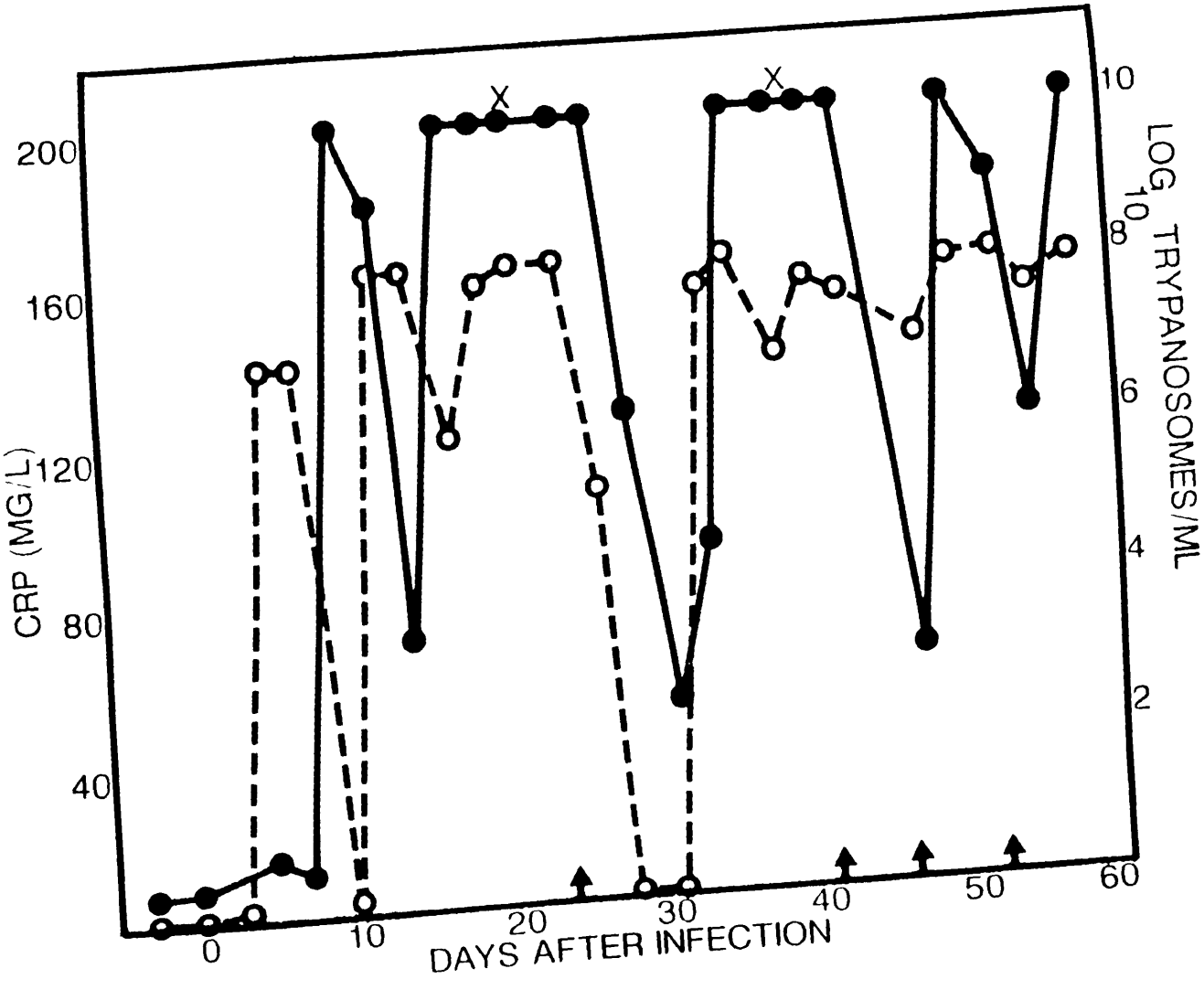

CRP (MG/L)
200
160
120
80
40
LOG10 TRYPANOSOMES/ML
10
8
6
4
2
X
X
0
10
20
30
40
50
60
DAYS AFTER INFECTION

Figure 9.16. Plasma concentration of CRP (●) and the parasitaemia (O) in dog 3 during the course of T.brucei infection and following curative treatment with suramin (arrows). After successful treatment, there was a rapid fall in CRP.

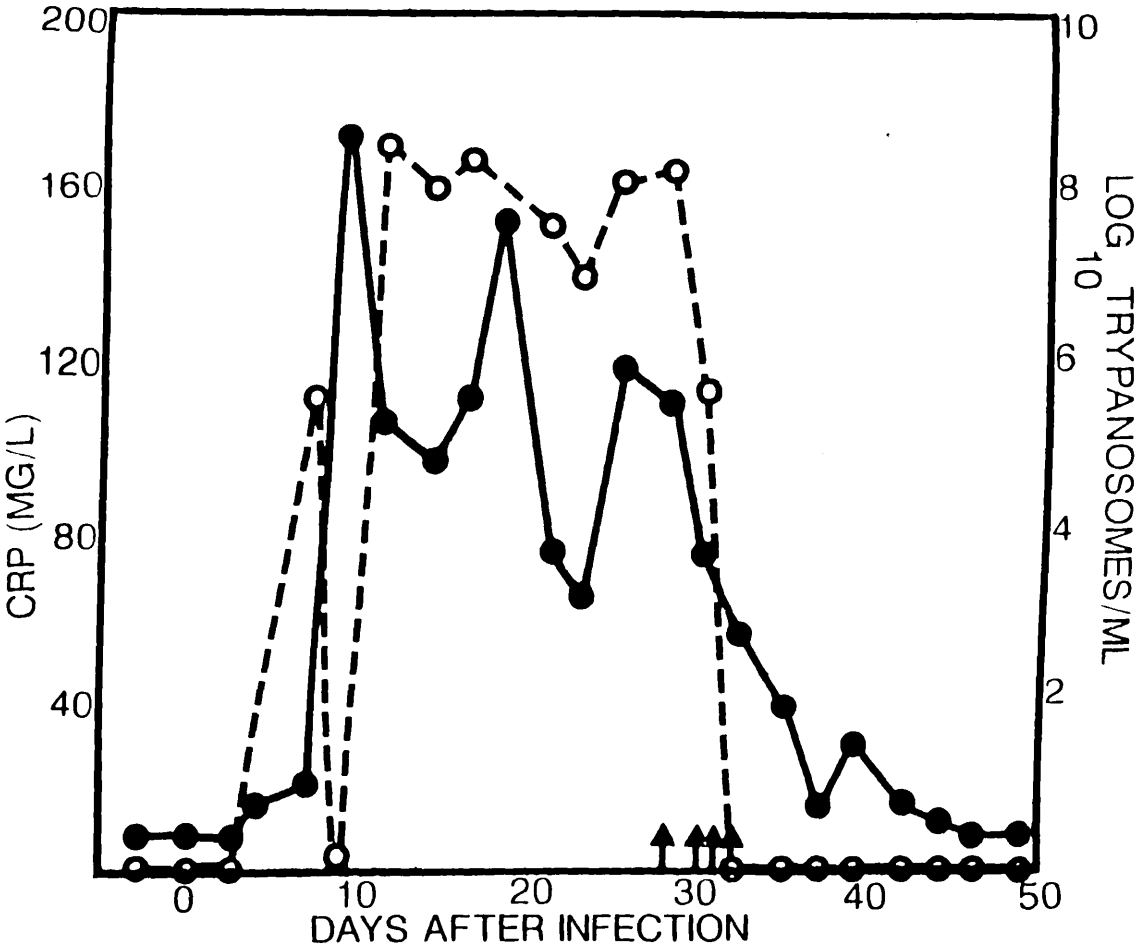

200
160
120
80
40
CRP (MG/L)
10
8
6
4
2
LOG 10 TRYPANOSOMES/ML
0
10
20
30
40
50
DAYS AFTER INFECTION

Figure 9.17. The left kidney of dog 4, 24 hours after treatments with suramin at 20 mg/kg on day 20 of infection. Focal circular haemorrhages are present at several locations (arrows).

Figure 9.18. A cut section from the left kidney of dog 4, 24 hours after treatment with suramin at 10 mg/kg on day 20 of infection. Haemorrhagic foci extend into the cortex of the kidney (arrows).

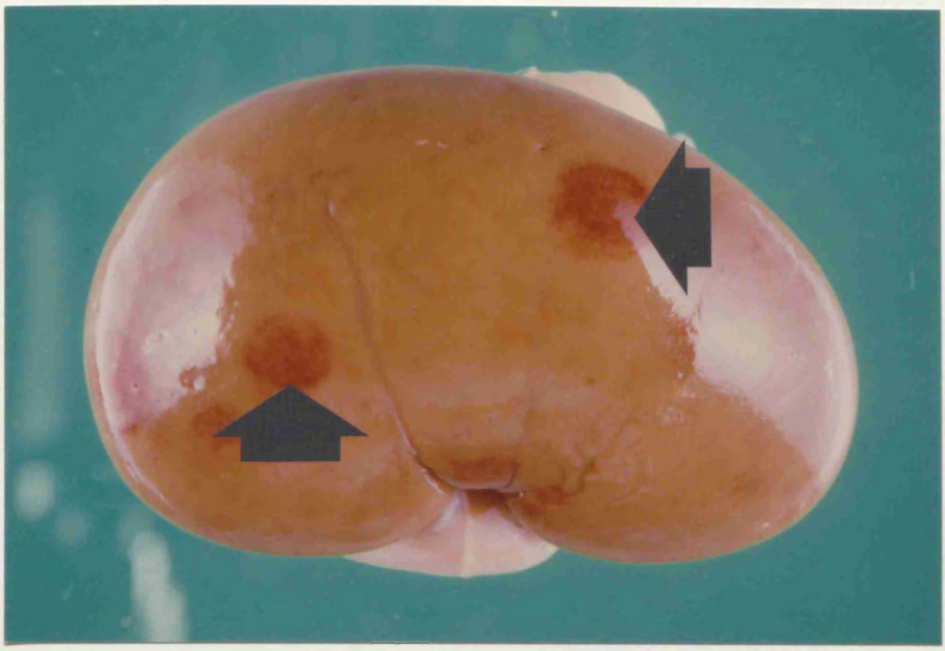


Figure 9.19. Subepicardial fibrosis in the left ventricle of dog 2, 61 days after infection with *T.brucei*. The dog had been unsuccessfully treated with suramin. There is chronic inflammation of the subepicardial myocardium. Trypanosomes are scattered throughout the myocardium (small arrows). Necrosed myocardial cells have been replaced by fibrous tissue (large arrow). MSB. x200.

Figure 9.20. Chronic subepicardial myocarditis in the right ventricle of dog 2, 61 days after infection. There is extensive myocytolysis and diffuse infiltration of vacuolated macrophages (large arrows), lymphocytes and plasma cells. A few trypanosomes are present in the myocardium (small arrows). H&E. x530.

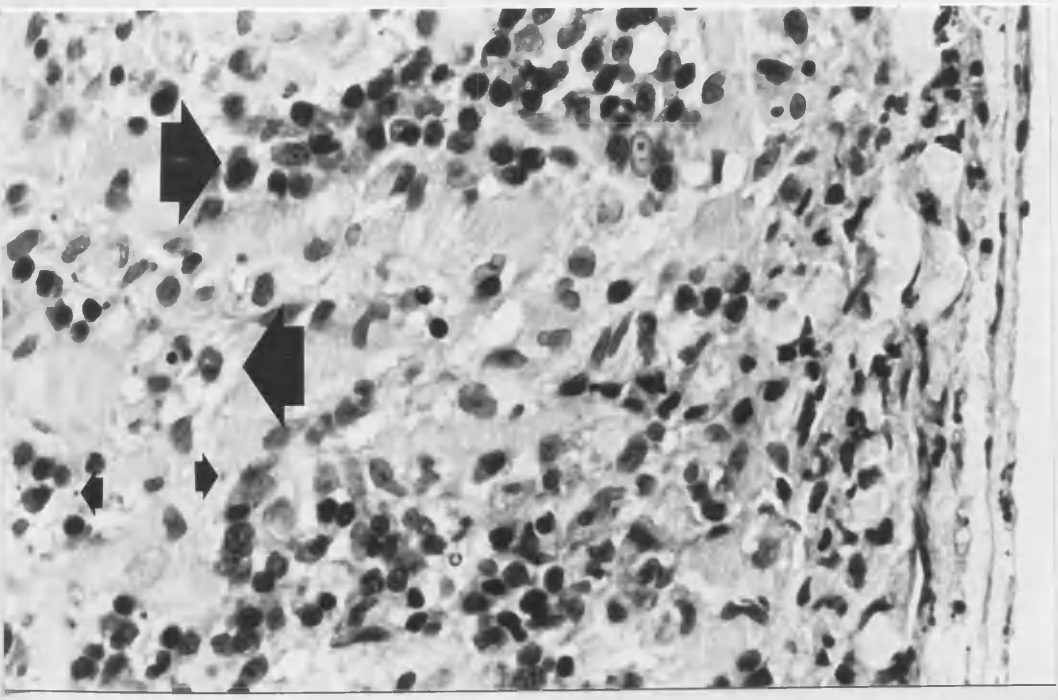
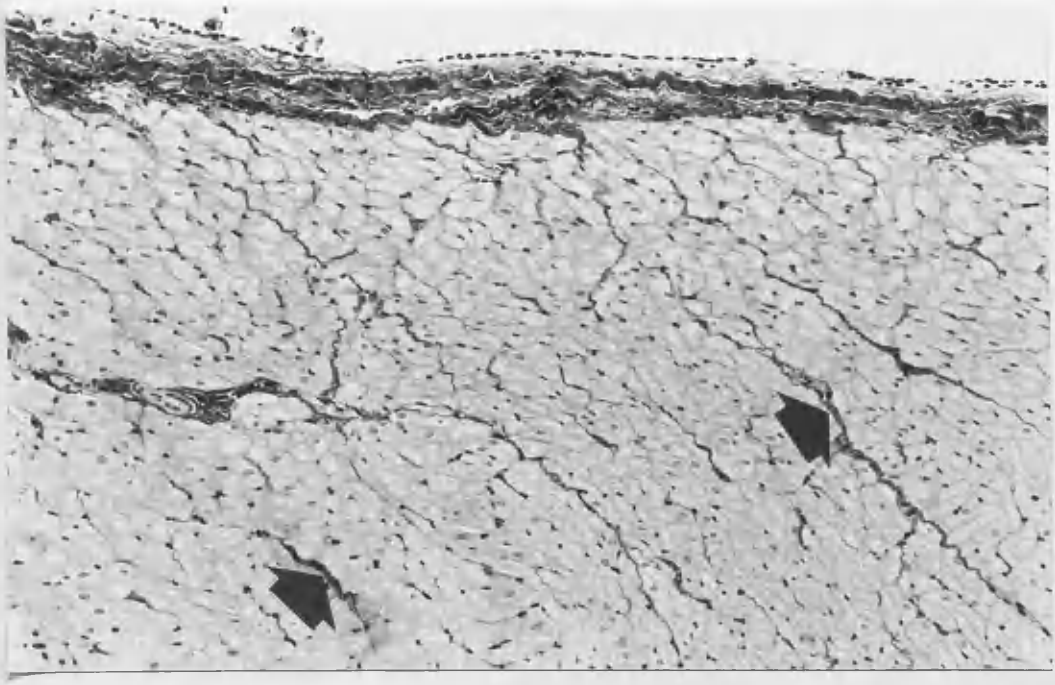


Figure 9.21. Subepicardial and myocardial fibrosis in the left ventricle of dog 3, 50 days after infection. The dog was treated with suramin from day 28 to 32, resulting in rapid recovery. There is increased fibrous tissue in the subepicardium and between the muscle fibres (arrows). MSB. x140.

Figure 9.22. Myocardial fibrosis in the left ventricle of dog 3, 50 days after infection. Fibrosis appears as scattered foci (arrows). Myocytes adjacent to the fibrotic areas are hypertrophied. MSB. x200.



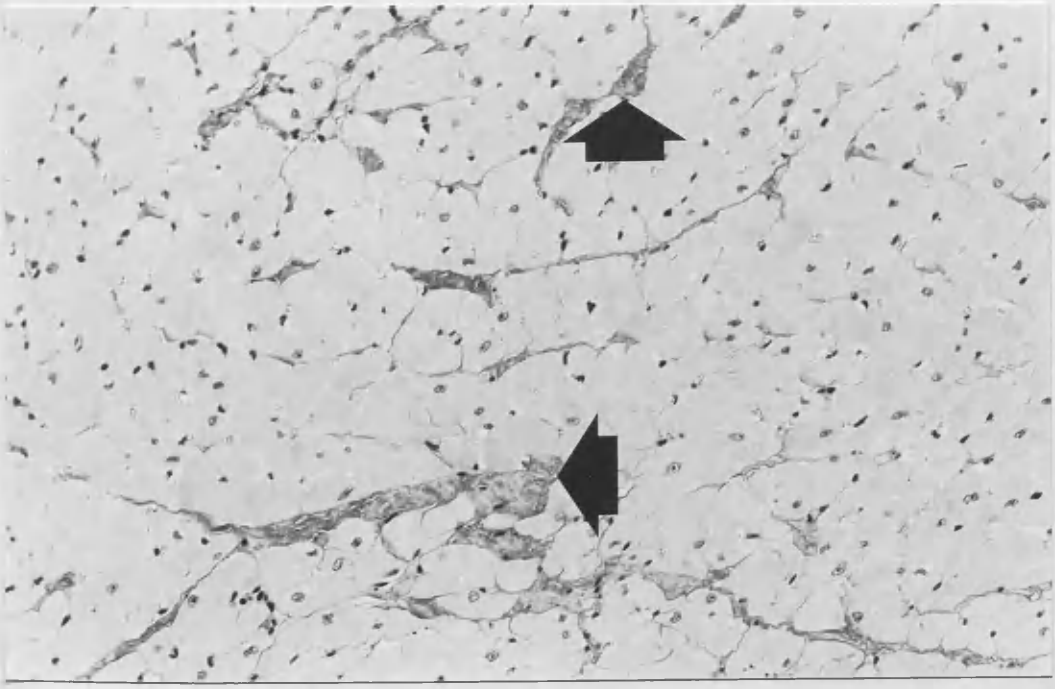
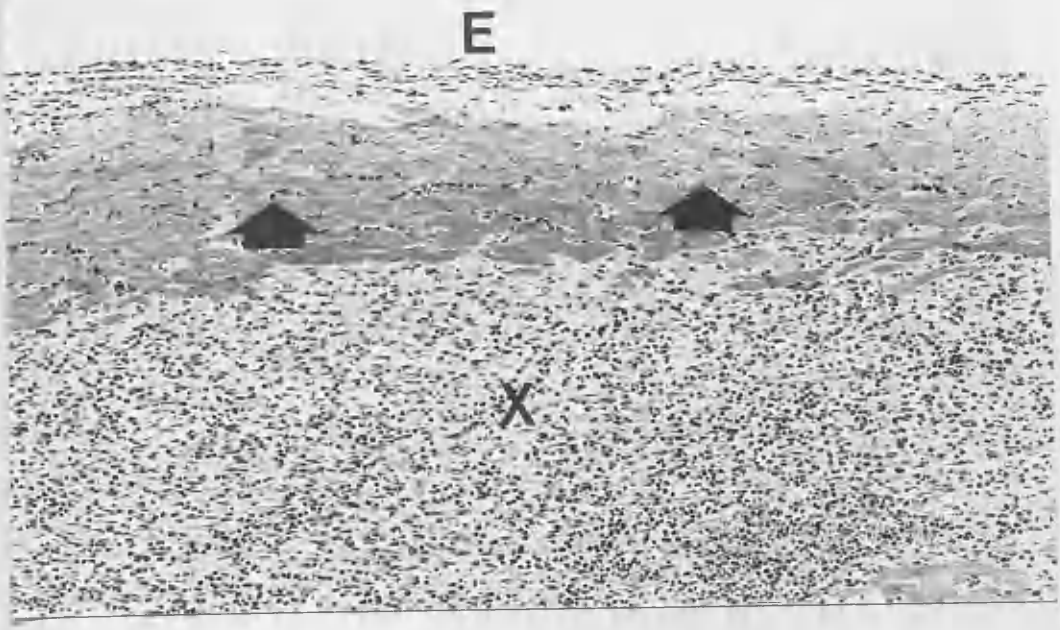


Figure 9.23. Chronic myocardial infarction in the interventricular septum of dog 1, 47 days after infection. The dog had been unsuccessfully treated with suramin. There is preservation of a band of muscle fibres (arrows) on the endocardial side (E) of the infarcted myocardium. Myocardial cells within the infarct (X) have been dissolved. H&E. x100.

Figure 9.24. The edge of an area of chronic myocardial infarction in the left ventricle of dog 1, 47 days after infection. There is necrosis and dissolution of myocytes in the infarcted region (large arrows). Most of the myocytes in the adjacent myocardium are hypertrophied (small arrows), with a few macrophages and lymphocytes interspersed between them. H&E. x300.


E
X

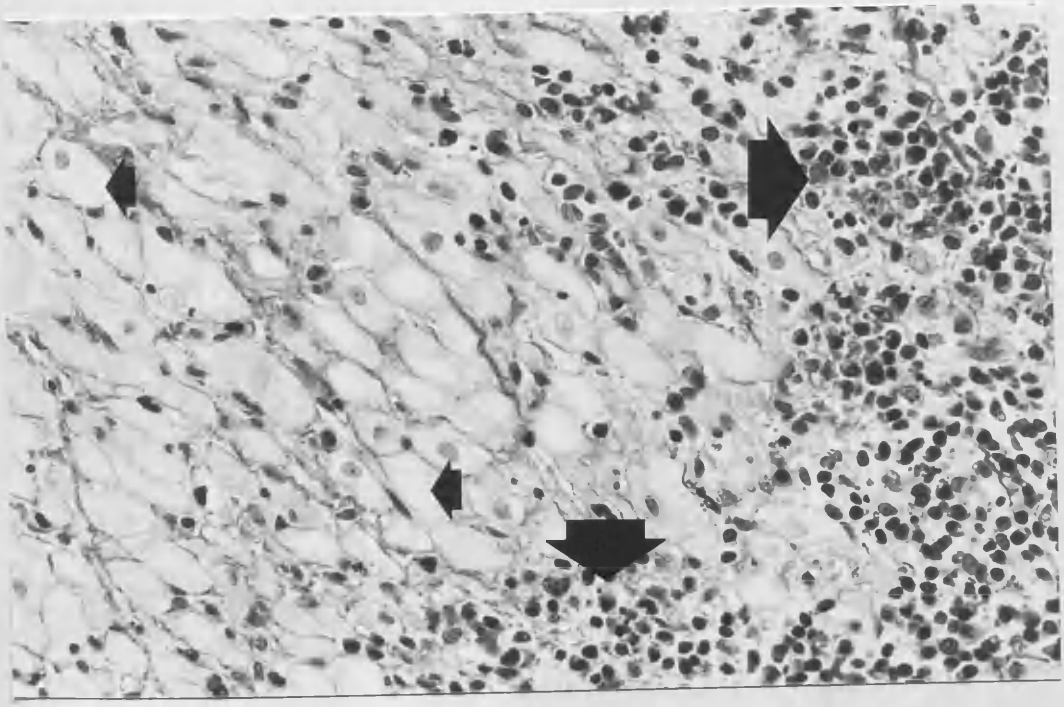
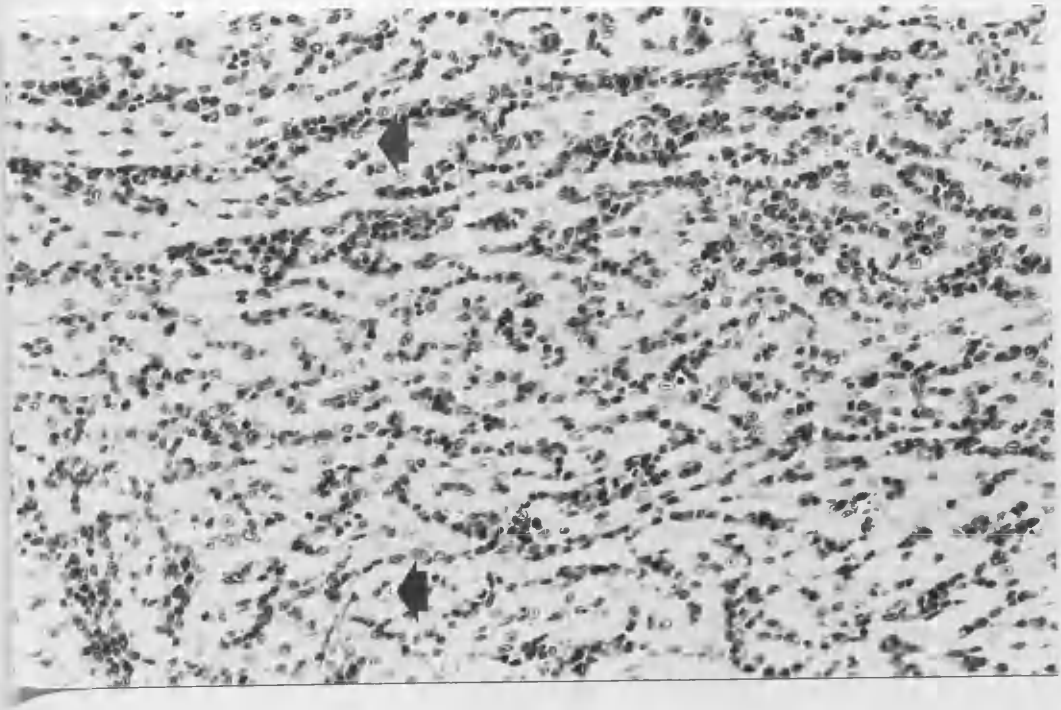


Figure 9.25. Diffuse chronic myocarditis in the interventricular septum of dog 2, 61 days after infection. There is intense infiltration of macrophages, lymphocytes and plasma cells. A few trypanosomes are also visible (arrows). H&E x200.

Figure 9.26. Subendocardial fibrosis around the conducting system in the left ventricle of dog 1, 47 days after infection. Most of the Purkinje fibres (P) have been preserved. Another one is undergoing myolysis (arrow). There is a necrotising myocarditis of the subendocardial myocardium with infiltration of macrophages, lymphocytes, plasma cells and trypanosomes. MSB. x400.



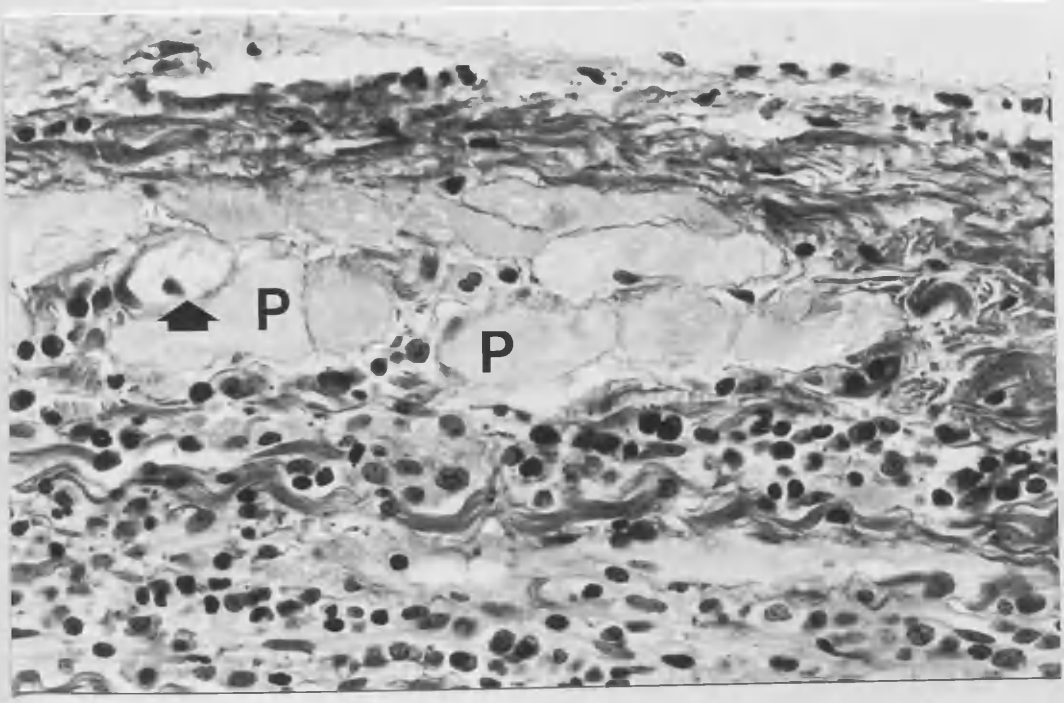
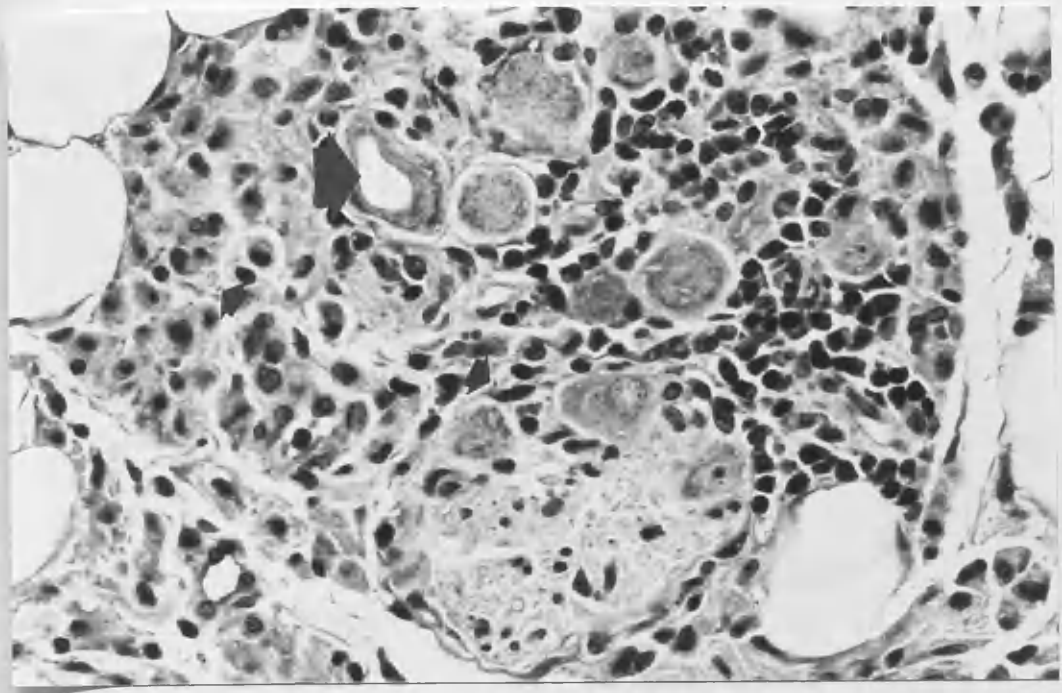

P
P

Figure 9.27. Neuritis of an autonomic nerve ganglion in the subepicardial adipose tissue at the base of the heart of dog 1, 47 days after infection. One nerve cell is undergoing liquefactive necrosis (large arrow). There is diffuse cellular infiltration in the ganglion, consisting of macrophages, lymphocytes and plasma cells. A few trypanosomes are also visible (small arrows). H&E. x400.

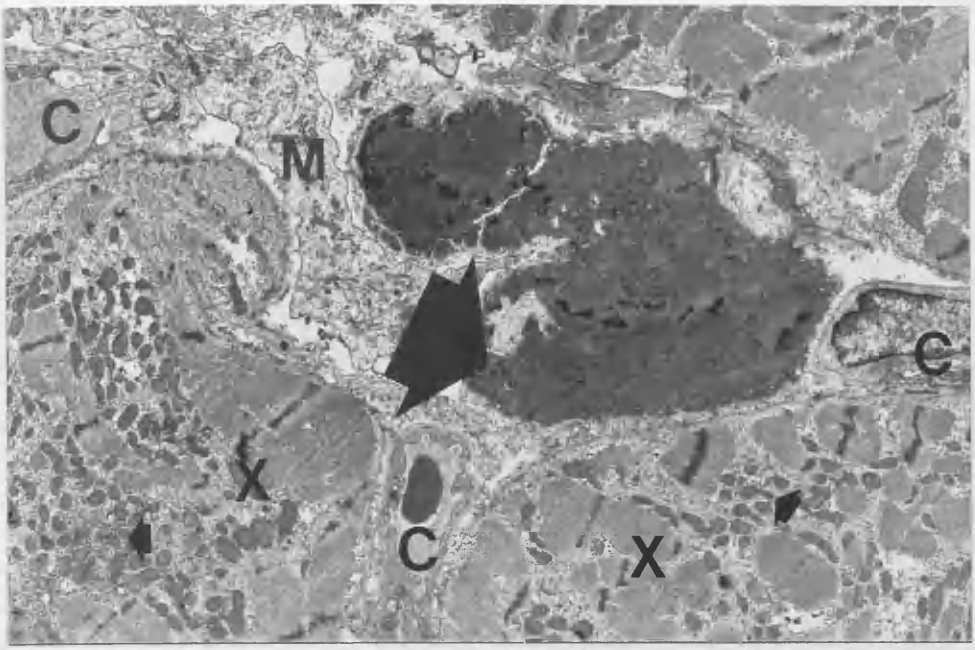
Figure 9.28. Acute myocardial infarction in the left ventricle of dog 4, 21 days after infection. The dog had been treated with suramin on day 20. There are extensive areas of coagulative necrosis (large arrows), contraction bands (C→) and haemorrhage (H→). Neutrophils (N→) are scattered at the periphery of the infarct. H&E. x200.




C
C
H
N

Figure 9.29. The left ventricle of dog 1, 47 days after infection. A necrotic myocyte (large arrow) is seen adjacent two others (X) that are in the process of myolysis. There is dissolution of the myofibrillar apparatus in the two myocytes (small arrows). A few capillaries are visible (C). M - Cytoplasmic process of a macrophage. TEM. x5,400.

Figure 9.30. The right ventricle of dog 1, 47 days after infection. There is lipid deposition in the myocyte (L). Macrophages (M) and a plasma cell (P) are present in the interstitium. The cisternae of the granular endoplasmic reticulum of the plasma cell are distended with colloid (R). TEM. x8,000.


C
M
C
X
C
X

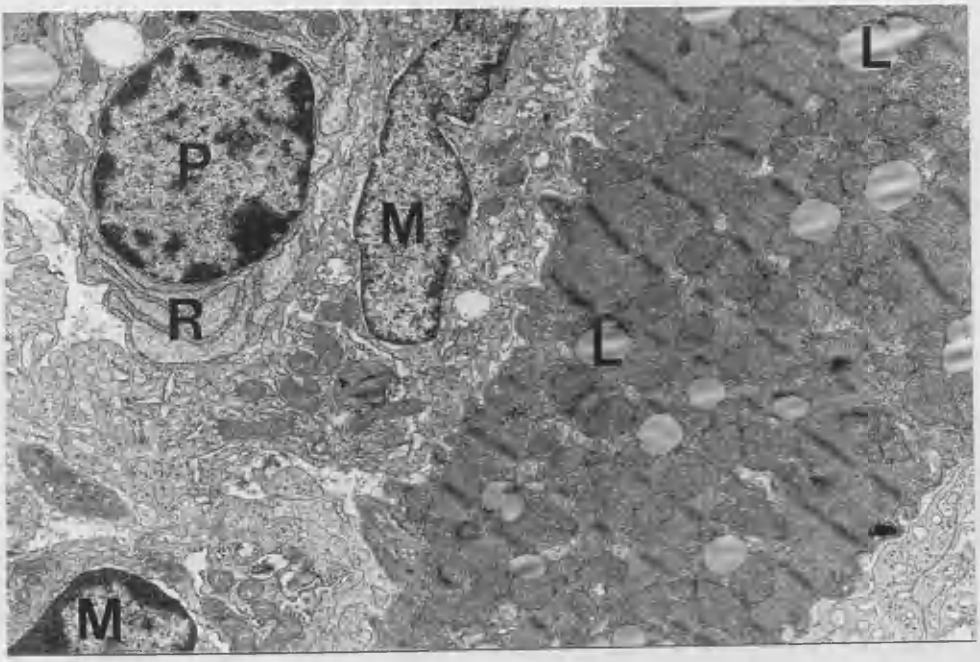
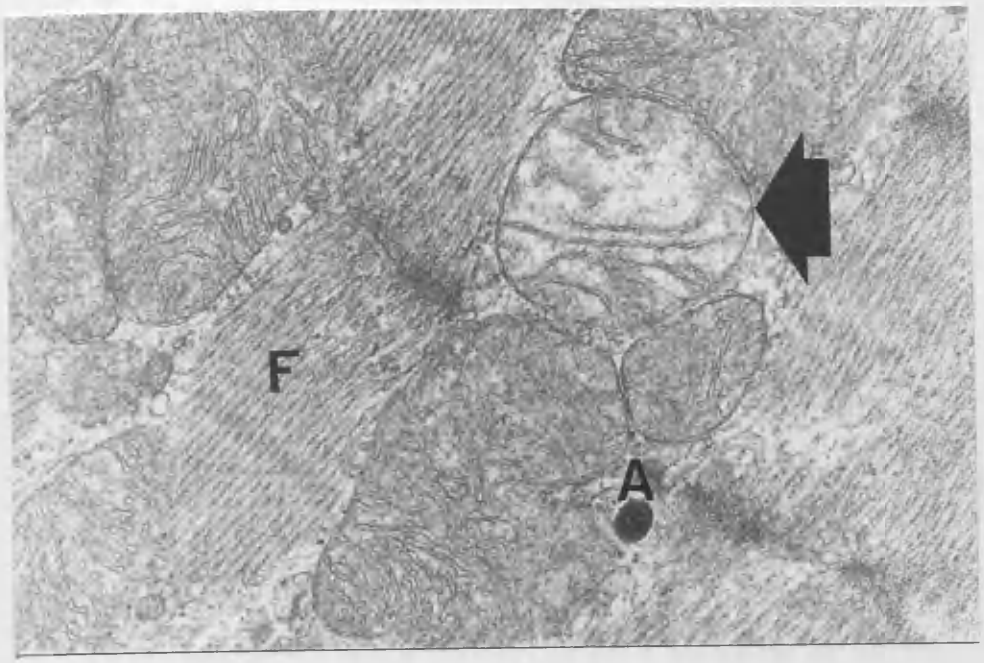

L
P
M
R
L
M

Figure 9.31. The right atrium of dog 2, 61 days after infection, showing degenerative changes in mitochondria. One mitochondrion is swollen (arrow) and most of the cristae have disintegrated. F - Myofibrils. A - An atrial natriuretic granule. TEM. x40,000.

Figure 9.32. The left ventricle of dog 2, 61 days after infection. There is separation of the intercalated disc between two myocytes (M). Material resembling myelin configurations is present in the space formed by the separated intercalated disc (arrow). A normal intercalated disc (X) joins two other myocytes. TEM. x8,000.


F
A

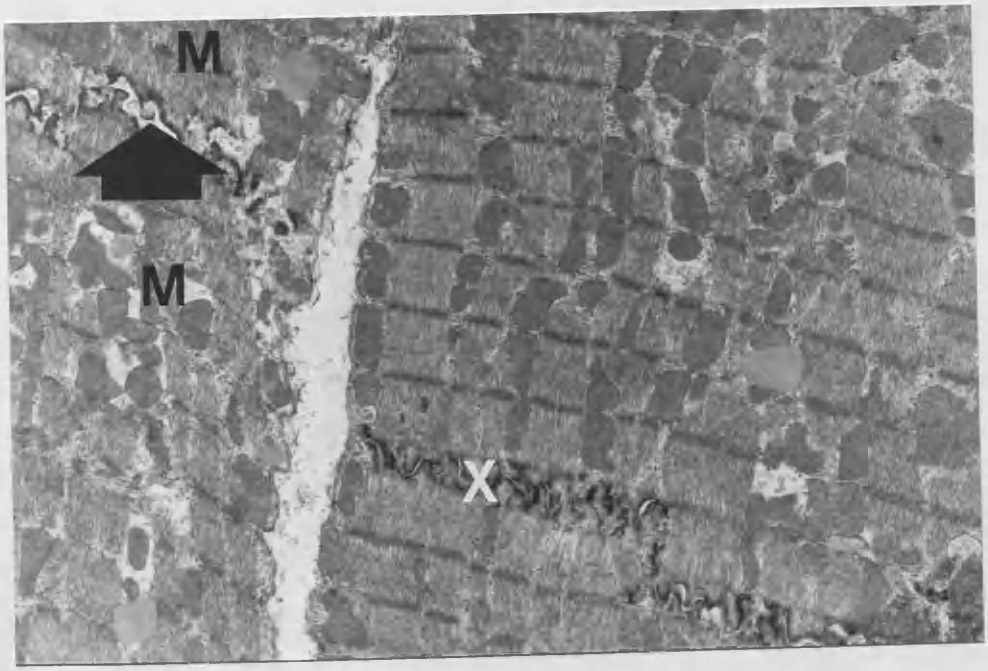
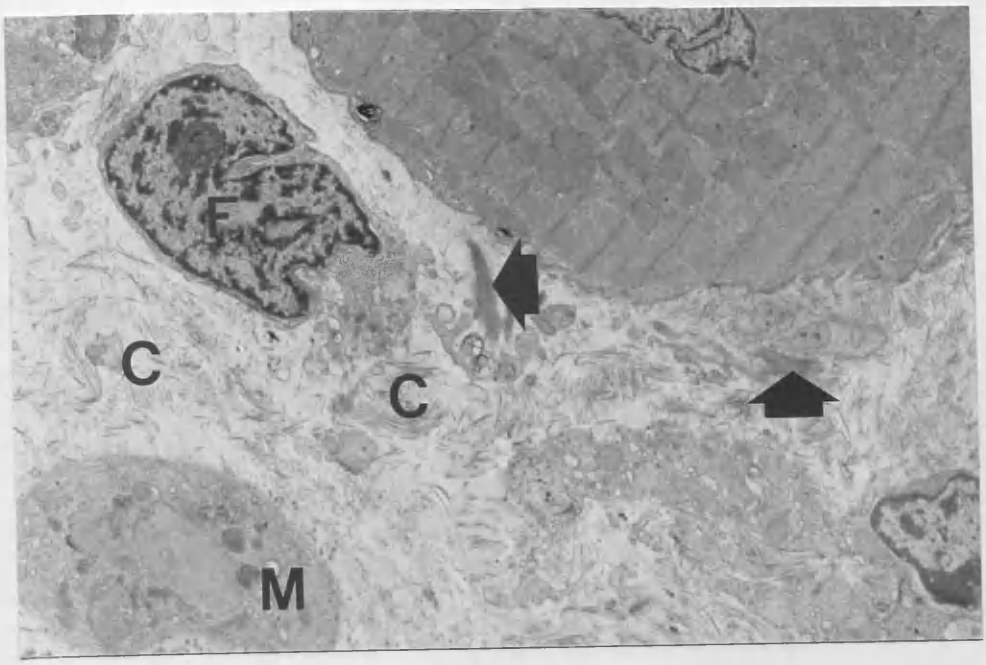

M
M
X

Figure 9.33. The right atrium of dog 3, 50 days after infection. There is increased collagen deposition (C). Some fibrin deposits are also present (arrow). F - Fibroblast. M - Macrophage cytoplasm. TEM. x5,400.

Figure 9.34. The right ventricle of dog 1, 47 days after infection. Macrophages (M), a plasma cell (P) and a lymphocyte (W) are present in the interstitium. The macrophages have engulfed necrotic debris (N). Pressure from the infiltrating cells has caused collapse of a capillary (arrow). L - Lipid in a myocyte.
TEM. x5,400.


F
C
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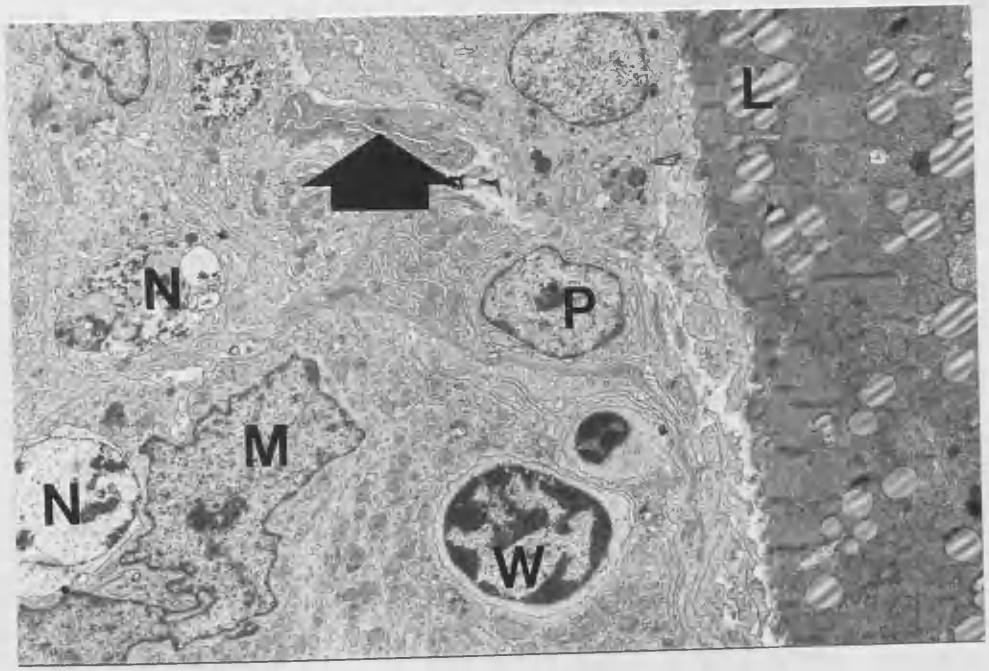
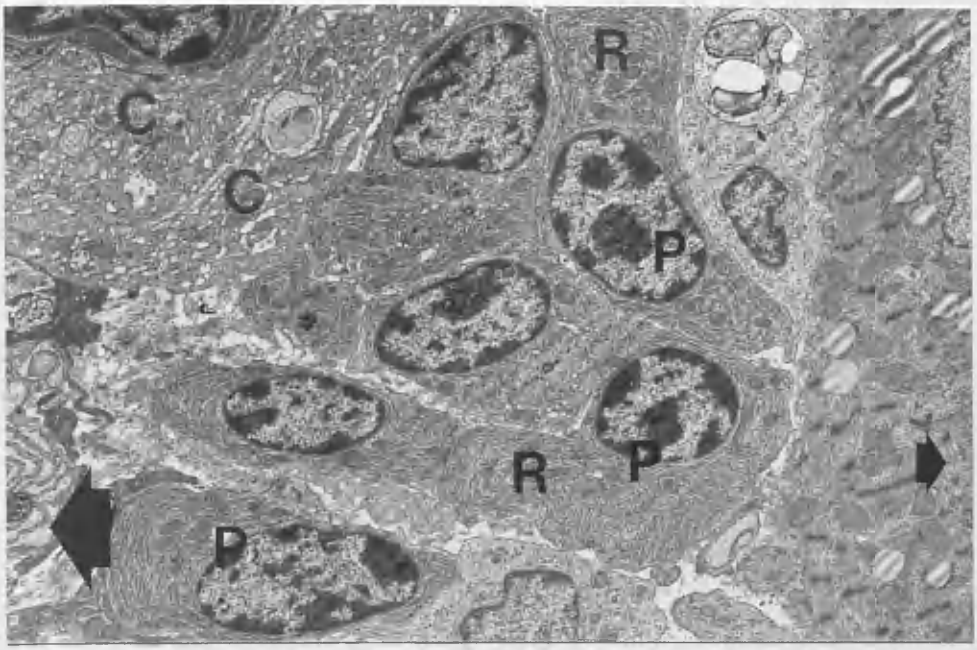

L
N
P
M
N
W

Figure 9.35. The right ventricle of dog 1, 47 days after infection. The interstitium is filled with plasma cells (P) with extensive granular endoplasmic reticulum (R). The cisternae of the granular endoplasmic reticulum of one of the plasma cells is distended with colloid (large arrow). Sarcomeres in the myocyte are undergoing dissolution (small arrow). C - Macrophage cytoplasm. TEM. x5,400.

Figure 9.36. The left atrium of dog 1, 47 days after infection. There are several plasma cells (P) and lymphocytes (W) in an interstitial location. The cisternae of the granular endoplasmic reticulum of one of the plasma cells are distended with colloid (E). C - Capillary lumen. R - Red blood cell. F - Fibroblast. TEM. x5,400.


C
C
R
P
R
P
P

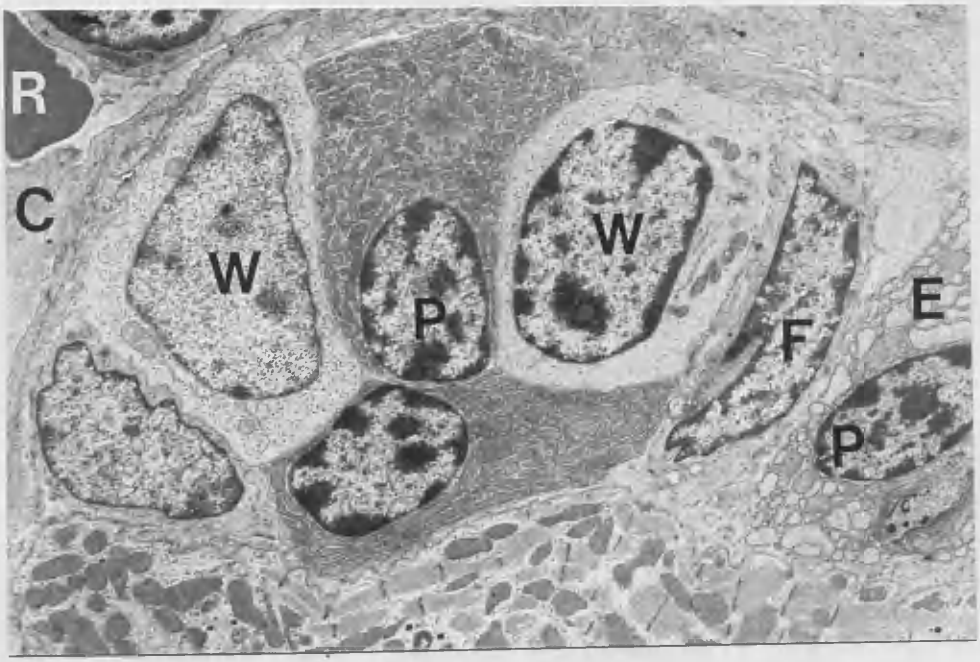
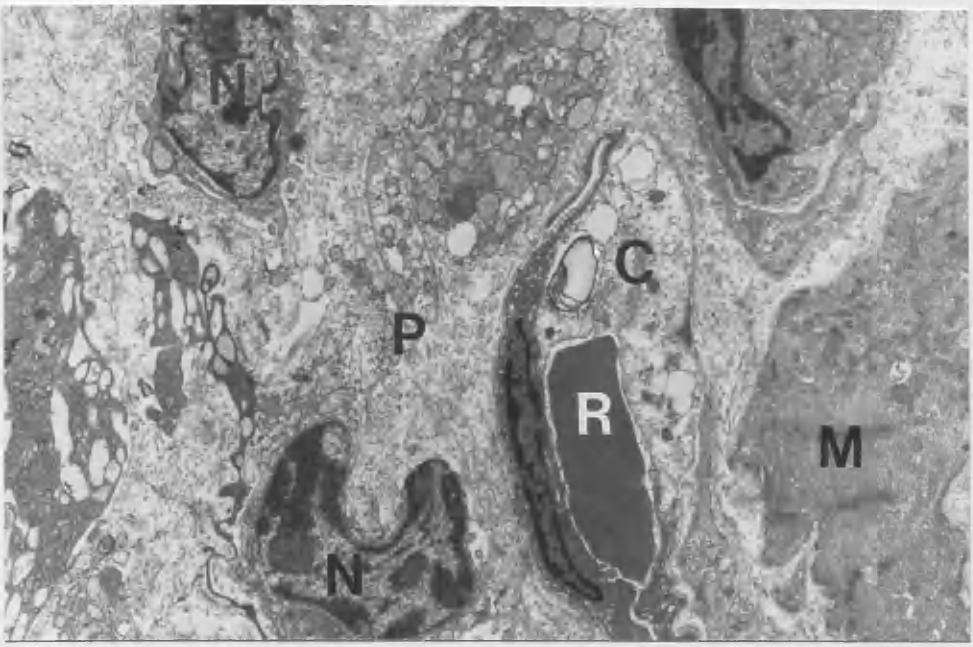

R
C
W
P
W
F
E
P

Figure 9.37. The left ventricle of dog 1, 47 days after infection. A focus of severe myocardial necrosis, involving a capillary (C), a myocyte (M) and inflammatory cells, whose nuclei (N) are bathed in the interstitial fluid containing a high protein content (P), hence the granular appearance. R - Red blood cell. TEM. x8,000.

Figure 9.38. The right atrium of dog 4, 21 days after infection. There in myocyte necrosis (N), marked lipid deposition in myocytes and in the interstitium (small arrows), oedema and extensive fibrin deposition (F). Pressure on a capillary has caused occlusion of its lumen (large arrow). C - Collagen. M - Macrophage. TEM. x5,400.


N
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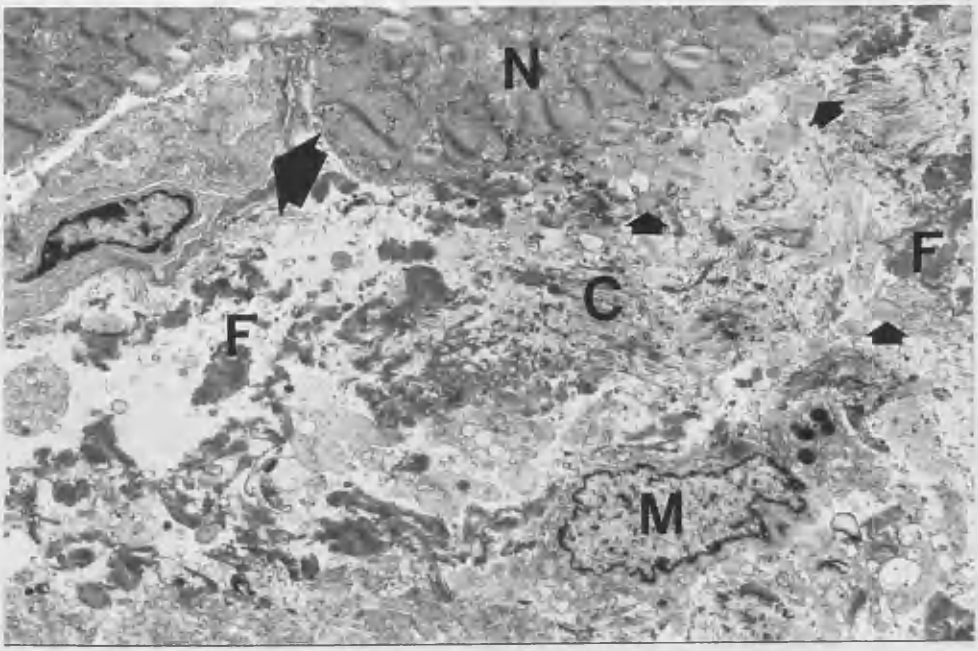
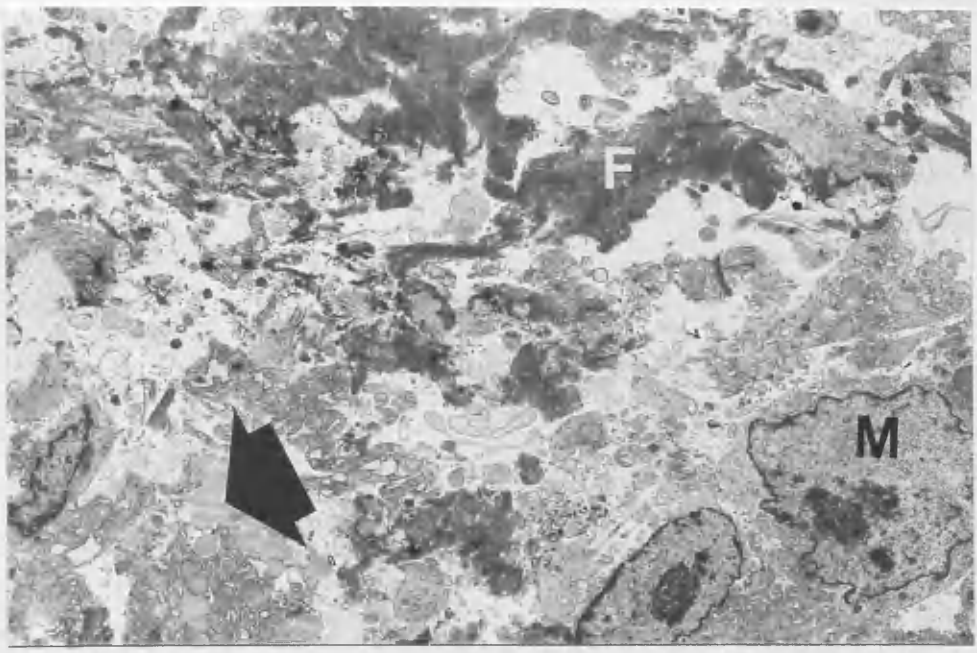

N
F
C
F
F
M

Figure 9.39. The right atrium of dog 4, 21 days after infection. The dog had been treated with suramin on day 20 of infection. There is marked fibrin (F) and lipid (arrow) deposition in the interstitium. M - Macrophage. TEM. x5,400.

Figure 9.40. An interstitial space in the right atrium of dog 4, 21 days after infection. A macrophage (M) is in the process of engulfing large quantities of fibrin (F) deposited in the interstitium. Lipid droplets are present in the macrophage cytoplasm (arrows). TEM. x8,000.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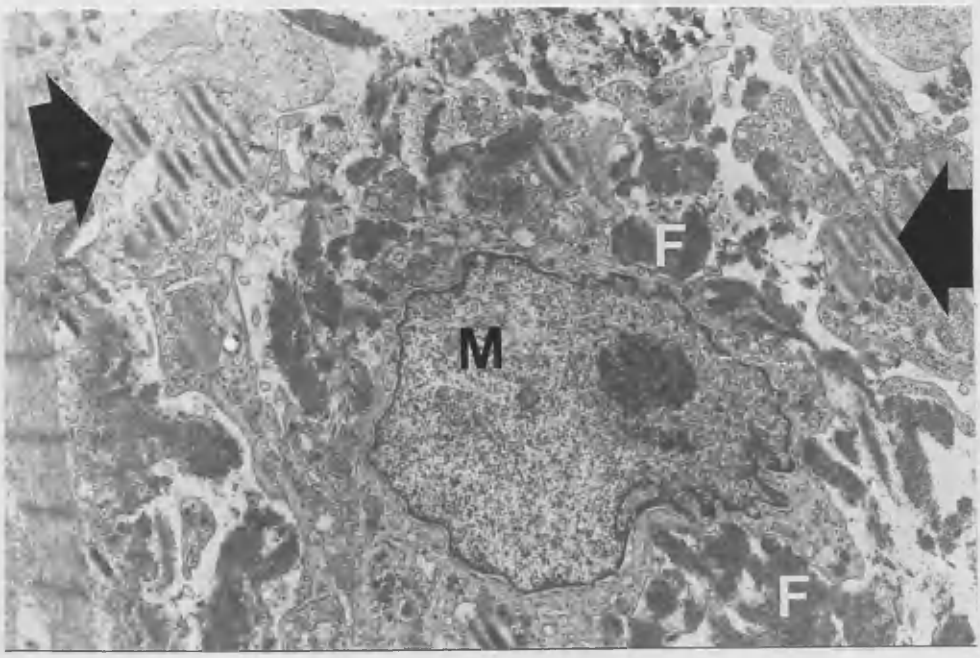
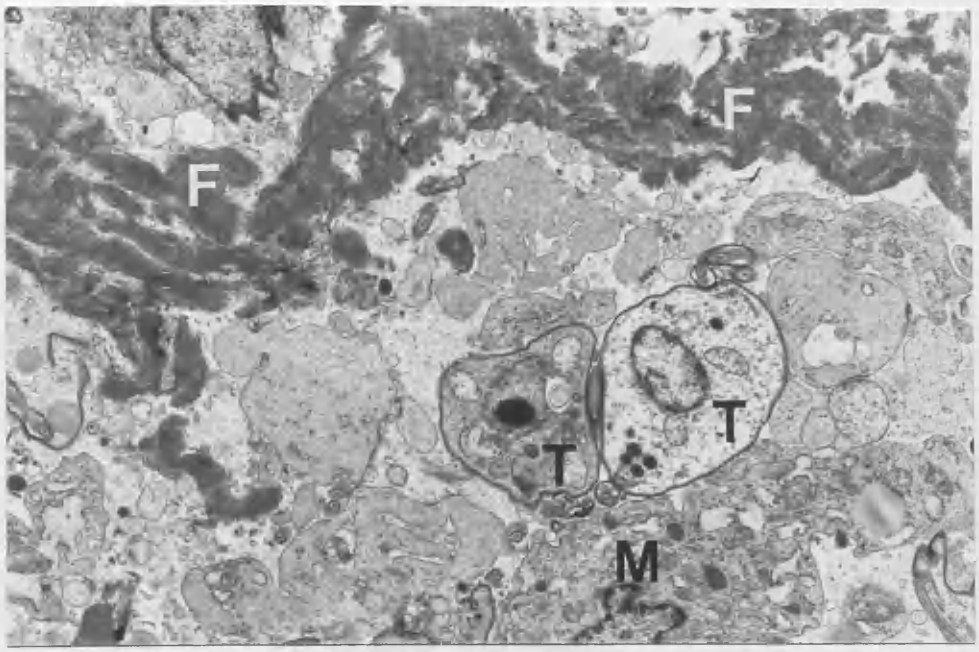

F
M
F

Figure 9.41. The right atrium of dog 4, 21 days after infection. There is marked fibrin deposition (F). Dead trypanosomes (T), at different stages of disintegration, are trapped in the necrotic debris. M - Macrophage. TEM. x8,000.

Figure 9.42. The left ventricle of dog 4, 21 days after infection. There are calcium-like deposits in a perivascular site (arrows). TEM. x10,000.


F
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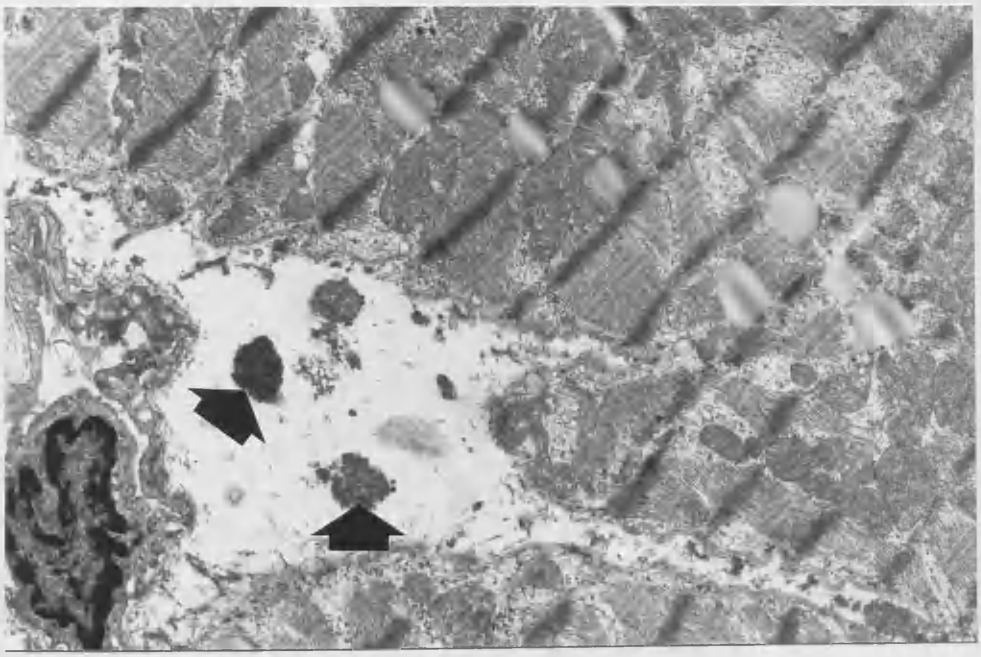


CHAPTER 10.

EFFECTS OF STEROIDAL AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON THE PATHOGENESIS OF CARDIAC DAMAGE INDUCED BY *T.brucei* IN DOGS.

10.1. INTRODUCTION.

In Part II of this work, it was found that dogs infected with T.brucei developed an acute disease syndrome characterised by high persistent parasitaemia, severe anaemia, leucocytopaenia, wasting and weight loss. The disease was accompanied by hypoalbuminaemia and a gradually developing hyperlipidaemic state. Increased plasma concentration of acute phase proteins (APP) and generalised oedema of subcutaneous tissues and organs confirmed the inflammatory nature of the disease. Splenomegaly, generalised lymph node enlargement, and infiltration of the heart by inflammatory cells indicated massive immunological responses by the host to this highly invasive parasite, including the formation of immune complexes, as demonstrated by their deposition in the kidneys.

Due to obstruction of draining lymphatic vessels, it is likely that the severity of cardiac damage was increased by accumulation of toxic substances released from dying trypanosomes, phagocytic cells, and from injured muscle tissue. Poor tissue perfusion, resulting from damage to small blood vessels, anaemia and interstitial oedema probably resulted in hypoxia and ischaemic myocardial damage. The severity of myocardial ischaemia was compounded by the marked lipid deposition that accompanied the infection. The pumping action of the heart was impaired further by valvular incompetence and damage to the conducting system. Decreased plasma atrial natriuretic factor (ANF) and increased plasma renin activity (PRA) in week 4 of the disease meant that the dogs were unable to

control blood volume, leading to congestive heart failure. These findings indicated that cardiac damage in dogs infected with T.brucei was initiated by an overwhelming immunological response by the host to the parasite. The severity of subsequent cardiac damage was influenced by a complex of other mechanisms operating together.

The inflammatory and toxic nature of cardiac damage was further demonstrated after treatment of infected dogs with the trypanocidal drug suramin (Ch. 9). In that study, a post-treatment reaction was observed in all dogs, and possibly was the direct cause of death of one of them. Similar post-treatment reactions have been observed in humans infected with T.rhodesiense (Jones et al., 1975; Harries and Wirima, 1988) and T.gambiense (Bertrand et al., 1971).

From the foregoing findings, it would appear that if the immunological and subsequent inflammatory responses by the host to the parasite are able to be controlled by immunosuppressive and anti-inflammatory drugs, the severity of the resultant cardiac damage might be reduced. If at the same time the invading trypanosomes are killed by trypanocidal drug treatment, further tissue damage might well be prevented and consequently the chances of recovery increased. In this context, various steroidal compounds have previously been used during treatment of humans infected with T.gambiense (Bertrand et al., 1971; Bertrand, 1987; Arroz, 1987; Junyent et al., 1988; Pepin et al., 1989) and T.rhodesiense (Fulkes, 1975; Harries and Wirima, 1988) but the usefulness of such treatment has been

difficult to analyse, especially in T.rhodesiense-infected patients.

Non-steroidal anti-inflammatory drugs (NSAIDs) are currently being used in increasing frequency in animals (Putnam et al., 1975; Ridgway, 1984; Ihrke et al., 1985) and man (De Groen et al., 1987; De Groen, 1988; Tegzess et al., 1989) for the treatment of a variety of autoimmune diseases and prevention of graft versus host reactions. Cyclosporin A is a powerful anti-inflammatory drug, and like other NSAIDS, reduces inflammation by inhibiting cyclooxygenase, an enzyme responsible for prostaglandin synthesis from arachidonic acid (Hardie, 1986). Prostaglandins are mediators of inflammation. Cyclosporin A also acts as a powerful immunosuppressant by inhibiting the activation of T cells. It does so by preventing both the production of interleukin-1 by antigen presenting cells, and generation of interleukin-2 (IL-2), which causes proliferation of T cells that have IL-2 receptors (Thomson et al., 1986). Azathioprine on the other hand modulates immunological responses by inhibiting division and differentiation of B lymphocytes to plasma cells, and T lymphocytes to T memory cells (Schwartz, 1965). It has a selective toxicity on cells undergoing mitosis (Ogilvie et al., 1988). Steroids are known to potentiate the immunosuppressive effects of azathioprine beacause, like azathioprine, they prevent the generation of prostaglandins in response to tissue damage (Flower and Blackwell, 1979).

The current study was designed to determine the effects of steroidal and NSAIDs on the pathogenesis of cardiac

damage in dogs infected with T.brucei.

10.2. MATERIALS AND METHODS.

10.2.1. ANIMALS.

The dogs, their management, and the stabilate used to infect them have been described in Chapter 2. In the current study, 14 dogs were infected by intravenous inoculation with $5x10^3$ T.brucei GVR35/c.1. Before and during the course of the disease, detailed clinical examinations were performed at daily intervals. Blood samples were collected from the jugular vein at least twice a week for parasitological, haematological and biochemical investigations. The dogs were euthanised on day 15 and tissue samples taken from the heart for histopathological investigations. Four dogs served as uninfected controls and were euthanised after the infected dogs.

10.2.2. EXPERIMENTAL DESIGN.

Two dogs, 1 and 2, were treated intravenously with the NSAID cyclosporin A (Sandimmun^R, Sandoz) at a dose of 5mg/kg daily for 3 days after the first peak of parasitaemia (day 8 for dog 1 and day 9 for dog 2 - Table 10.1). A further 8 dogs, 3 to 10, were divided into four groups of 2 and treated orally with the NSAID azathioprine (Imuran^R, Wellcome). The doses of azathioprine and the treatment regimes employed are shown in Table 10.1. Treatment was carried out either from day 3, i.e., prior to detection of parasitaemia, or after the first peak of parasitaemia on day 8. Dogs 11 and 12 were treated with a

combination of azathioprine and oral prednisolone (Evans Medical Ltd., England) from day 3 to 14 of infection. Dogs 13 and 14 were not treated, and served as infected untreated controls.

10.3. RESULTS.

10.3.1. EFFECTS OF CYCLOSPORIN A ON T.brucei INFECTED DOGS.

GENERAL BODY CONDITION.

Approximately 5 minutes the first injection with cyclosporin A, signs attributable to drug toxicity were observed. They included violent shaking of the head, hyperaemia of mucous membranes and tachypnoea. These signs disappeared within an hour of drug administration. With subsequent treatments (Table 10.1), signs of toxicity returned and lasted for up to 3 hours.

During the treatment period the dogs showed some recovery from the infection. Rectal temperatures, although elevated, remained less than 39.5°C. The superficial lymph nodes, which were enlarged at the beginning of treatment became smaller. Subcutaneous oedema remained mild and the spleen did not become enlarged. The dogs were dull during the period of treatment. After termination of treatment, signs of sickness became more pronounced. Superficial lymph nodes became enlarged and there was marked subcutaneous oedema of the face and periorbital space. Dog 2 became profoundly dull and anorexic. Rectal temperatures rose again, such that at the end of the study on day 15, the rectal temperature of dog 2 was 40.2°C. That of dog 1 was

39.2°C.

THE CARDIOVASCULAR SYSTEM.

Apart from an initial increase in pulse and respiratory rates, no major cardiovascular changes were recorded during the period of treatment. 24 hours after the last treatment, the heart rate became elevated (160 ± 6 BPM). Femoral pulses weakened and there was pallor of the mucous membranes. No murmurs were heard on auscultation of the thoracic cavity, unlike the infected untreated dogs in which systolic murmurs were evident by day 10 of infection. By ECG, mild S-T segment elevation was revealed (mean 0.25mV), and this only after cessation of treatment. S-T segment elevation persisted up to termination of the study on day 15 of infection. This was in contrast with infected untreated dogs in which second degree heart block (IIHB) and marked sinus arrhythmia were recorded but no S-T segment changes.

Doppler echocardiography demonstrated mitral incompetence (MI) and tricuspid incompetence (TI) beginning on day 10 of infection. MI and TI were mild, as they were only detected close to the valve orifices. At the same time, thickening of the atrioventricular septum and the left ventricular wall was shown by two-dimensional (2D) and M-mode echocardiography (Fig. 10.1). The echocardiographic changes persisted throughout the treatment period and up to day 15 of infection. In contrast, in infected untreated dogs, in addition to MI and TI, aortic incompetence (AI) also developed, and increased in severity as the disease

progressed.

PARASITOLOGICAL FINDINGS.

As in infected untreated dogs, parasitaemia developed on day 5 of infection, simultaneous with the onset of fever, and persisted up to termination of the study. For both dogs, peak parasitaemia occurred on day 11 (7.65 $\log_{10}$ trypanosomes/ml) and was not affected by treatment.

THE HAEMOPOIETIC SYSTEM.

Treatment had no significant effect on the haematological parameters. As in untreated dogs, a normocytic and normochromic anaemia, severe thrombocytopaenia and leucocytopaenia developed and progressed up to the end of the study. In both treated dogs, while a leucocytopaenia developed, the total white blood cells (WBC) after treatment were higher than in infected untreated dogs, due to a relative increase in neutrophils (Table 10.2).

PLASMA BIOCHEMISTRY.

The concentration of the APP, C-reactive protein (CRP) and haptoglobin (Hp), increased from day 5 of infection, coinciding with the first parasitaemia. As in infected untreated dogs, these increases were sustained up to termination of the study.

Plasma albumin concentration decreased following the onset of parasitaemia. During the period of treatment, however, the rate of drop in albumin was less (Table 10.3).

In dog 1 for example, there was no decrease in albumin between days 8 and 11, in contrast with the infected untreated dogs in which albumin dropped from a mean of 33g/l to 21g/l during the same period (Table 10.3). After treatment was withdrawn, hypoalbuminaemia worsened.

Following infection and during the treatment period, a gradual fall in the plasma concentration of CPK was observed. Soon after the last treatment, creatinine phosphokinase (CPK) increased rapidly in both dogs (Table 10.4). In dog 2, CPK increased from 70U/l on day 11 to 142U/l on day 15 of infection while in untreated dogs CPK dropped from a mean of 100U/l to 87U/l during the same period (Table 10.4). No major plasma changes in other tissue enzymes were observed during the study period.

Lipoprotein electrophoresis of EDTA plasma demonstrated gradual increases in the relative percentages of very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and decreases in high density lipoproteins (HDL), which were similar in both treated and untreated dogs. Similarly, changes in the plasma concentration of triglycerides (TG), cholesterol (CH) and non-esterified fatty acids (NEFA) were as in infected untreated dogs, only marginally increased.

PATHOLOGY.

At post mortem, the carcasses of both dogs were in good condition. Generalised lymph node enlargement was found in both treated and untreated dogs. The heart of dog 1 was relatively normal while in that of dog 2, the right atrium

had a large diffuse haemorrhagic lesion approximately 10mm in diameter, a finding not seen in untreated dogs. The spleen was moderately enlarged. In dog 1, severe diffuse haemorrhages were seen throughout the whole length of the duodenum. In dog 2, the haemorrhagic lesion was not as widely spread, consisting of ecchymotic foci 5mm in diameter confined to the proximal quarter of the duodenum. Similar but less severe lesions were seen in infected untreated dogs.

The histological findings in the heart were variable, depending on the region of the heart examined, but generally were more severe than in infected untreated dogs. Lesions in the atria were more marked than in the ventricles.

In both ventricles and the atria, there was moderate subepicardial and subendocardial oedema, associated with marked cellular infiltration consisting mainly of neutrophils, macrophages, and a few plasma cells and lymphocytes. A similar, more intense cellular reaction occurred in the subepicardial adipose tissue at the base of the heart. A few subepicardial lymphatic vessels were distended, mainly with lymph, but others contained macrophages, trypanosomes, lymphocytes, neutrophils and plasma cells suspended in the lymph. In untreated dogs however, the predominant cell types in the subendocardium and subepicardium were plasma cells and macrophages, and fewer lymphocytes and neutrophils.

In the myocardium, the cellular reaction was similar but milder than in the subepicardium. Neutrophils were

only seen in perivascular areas. Likewise, oedema and cellular infiltration in the subendocardial myocardium was less than in the subepicardium, but higher than in the myocardium. Trypanosomes were found scattered in all layers of the heart.

Multiple foci of ischaemic necrosis were found in the right ventricle of dog 2. These were associated with interstitial oedema, swollen and hypereosinophilic myocytes with pyknotic nuclei, and sometimes haemorrhage (Fig. 10.2). Some foci were at a more advanced stage and macrophage, lymphocyte and plasma cell infiltration had taken place (Figs. 10.3 and 10.4). No ischaemic foci were seen in the left ventricular myocardium, and in the hearts of dog 1 and untreated dogs.

In addition to cellular and trypanosome infiltration, diffuse haemorrhage occurred in the atria of both treated dogs, involving all the layers (Fig. 10.5). There were no significant histological changes in the conducting system.

Myocardial tissue samples stained with oil red O demonstrated intense lipid deposition in myocytes, and was higher than in untreated dogs. Very little lipid was present in infiltrating macrophages.

All cardiac valves were affected. There was moderate oedema involving the valve base and cusp tissue. A few macrophages, plasma cells and lymphocytes were found, mainly on the flow side of the valve. Focal fibroblast necrosis was seen in the mitral valve cusp of dog 2.

Other significant histological findings were in the lungs, urinary bladder and duodenum, where haemorrhage,

oedema and infiltration with large numbers of neutrophils, macrophages, trypanosomes, plasma cells and a few lymphocytes was found.

In untreated dogs, the duodenal lesion was milder and no significant changes occurred in the urinary bladder and the lungs.

The histological findings were confirmed by transmission electron microscopy (TEM). In addition, occasional evidence of vascular damage, including swelling and necrosis of capillary endothelial cells, was found. This was similar to findings in infected untreated dogs. Separation of the intercalated discs between adjacent myocytes occurred in both the right and left ventricular myocardium (Fig. 10.6), a feature not present in untreated dogs. In both atria, atrial natriuretic granules were not affected. As in infected untreated dogs, the granules were many, electron-dense, and large (Fig. 10.7). The autonomic nerve ganglia were not affected.

IMMUNOFLUORESCENCE FINDINGS.

The immunofluorescence findings in the heart and kidneys of the dogs in the present study are indicated in Table 10.5. In the hearts of both dogs, no evidence of IgM, IgG or C3 deposition was found. This was similar to findings in infected untreated dogs. Small quantities of fibrinogen were deposited, mainly in perivascular areas. In dog 1, small quantities of plasma cell IgG were found. In the kidneys, large quantities of IgG, and less IgM and C3 were found.

10.3.2. EFFECTS OF AZATHIOPRINE ON *T.brucei* INFECTED DOGS.

10.3.2.1. ORAL TREATMENT WITH 10mg/kg DAILY FOR 5 DAYS.

Dogs 3 and 4 were treated with azathioprine at 10mg/kg *per os* beginning on day 8 of infection, i.e., at the first peak of parasitaemia (Table 10.1).

GERERAL BODY CONDITION.

At the time of first treatment on day 8, there was mild subcutaneous oedema of the face and the periorbital space in both dogs. All superficial lymph nodes were slightly enlarged and the spleen was just palpable. Rectal temperatures were high (Table 10.6). In dog 3, the stool was slightly dark. By the third treatment on day 10, superficial lymph nodes were still moderately enlarged and the spleen palpable. Subsequent treatments resulted in reversal of this state, in that at the last treatment on day 12, the superficial lymph nodes were almost back to normal sizes and the spleen was not palpable. In untreated dogs, subcutaneous oedema, splenomegaly and lymph node enlargement were persistent.

During the course of treatment however, dogs 3 and 4 became dull, were moderately anorexic and for dog 3, the stool became mucoid and the dog had tenesmus. Though rectal temperatures dropped slightly during treatment, they were not significantly different from infected untreated dogs (Table 10.6).

After cessation of treatment, superficial lymph nodes again became enlarged and subcutaneous oedema reappeared.

The dogs remained anorexic up to termination of the study. Dogs that did not receive treatment remained bright and active, and with a good appetite.

THE CARDIOVASCULAR SYSTEM.

For dog 3, apart from an increase in heart rate, no major cardiac changes were found on auscultation of the thoracic cavity during the study period. By pulsed-wave (PW) Doppler echocardiography, mild MI and AI were demonstrated from day 13 to 15, at which time treatment had been withdrawn. No ECG abnormalities were recorded. On the other hand, dog 4 had mild MI (grade 1/6) on day 9 of infection. Occasional missed beats were also heard, and were shown by ECG to be due to IIHB. With continued treatment, the IIHB disappeared but MI persisted. Echocardiography confirmed the MI and revealed AI. Both MI and AI persisted up to termination of the study on day 15 of infection, but the severity was reduced. In infected untreated dogs, the IIHB, sinus arrhythmia and valvular incompetence that developed after day 9 of infection deteriorated with progress of the disease (see also Ch. 4).

PARASITOLOGICAL FINDINGS.

Trypanosomes appeared in the blood on day 5 of infection. Apart from a transient drop on days 9 to 11, high parasitaemia was maintained up to termination of the study; this was similar to untreated dogs.

THE HAEMOPOIETIC SYSTEM.

As in cyclosporin A treated dogs, treatment with

azathioprine at 10mg/kg for 5 days from day 8 had no significant effect on the anaemia induced by infection. Likewise, the dogs developed a thrombocytopaenia and leucocytopaenia. In the course of treatment, the decrease in WBC was less in dog 3, due to a relative increase in neutrophils (Table 10.7). The same happened for dog 4 after treatment was withdrawn on day 12. As a result the total WBC in the blood of azathioprine treated dogs was higher than in control untreated dogs infected for a similar number of days (Table 10.7). Treatment did not affect the number of monocytes.

PLASMA BIOCHEMISTRY.

As in infected untreated dogs, the concentration of CRP and Hp increased from day 5 of infection, coinciding with the first parasitaemia. The elevation in CRP and Hp persisted up to the end of the study.

Plasma albumin concentration dropped rapidly following the onset of parasitaemia. During the treatment period however, albumin concentration in dog 4 did not fall any further, and in dog 3 it only decreased from 31g/l to 29g/l from day 8 to 11 of infection (Table 10.8). In contrast in infected untreated dogs, albumin dropped from 33g/l to 21g/l during the same period. After withdrawal of treatment in dogs 3 and 4, albumin again decreased (Table 10.8).

During the course of treatment, rapid increase in plasma alanine aminotransferase (ALT) and alkaline phosphatase (AP) occurred (Table 10.9). ALT increased more than fivefold in both dogs between day 8 and 11 of

infection. Aspartate aminotransferase (AST) and CPK did not change significantly in the same period.

After treatment was withdrawn, ALT decreased, while AP continued to rise. In addition at this time, CPK and AST were elevated, with CPK showing the greater rise (Table 10.9). In contrast, in infected untreated dogs, only mild increases in ALT and AP, and moderate increase in AST occurred during the infection period (Table 10.9).

No major changes in the plasma levels of TG and CH were seen in treated dogs. Nevertheless at the end of the study on day 15, TG and CH levels in treated dogs were falling, while those of infected untreated dogs were rising (Table 10.10). Likewise, percentage VLDL and LDL increased relative to HDL, but not as dramatically as in infected untreated dogs. The changes in NEFA were not significant.

PATHOLOGY.

The carcasses of both dogs were in good condition. The hearts were macroscopically normal. In dog 4, there was generalised lymph node enlargement with associated interstitial oedema. No intestinal lesions were seen.

The histological lesions in the hearts were generally less severe than those found in infected untreated dogs. Most regions of the heart were involved, the atria more so than the ventricles. In the latter, there was moderate subepicardial and subendocardial oedema, and infiltration with many macrophages and a few lymphocytes and plasma cells. In the myocardium, small numbers of lymphocytes and macrophages were present. Trypanosomes were scattered in

large numbers throughout the myocardium.

In the left ventricle of dog 3, there were a few focal areas of myocyte swelling, hypereosinophilia and lysis but not associated with a cellular reaction. Subepicardial adipose tissue was involved, with marked adipocyte necrosis and macrophage, neutrophil and trypanosome infiltration. Distended lymphatic vessels were occasionally seen. Intense lipid deposition in myocytes was demonstrated by oil red O staining, a finding similar to that in infected untreated dogs.

Moderate interstitial oedema and fibroblast necrosis occurred at the bases and cusp tissues of all the valves. Trypanosomes, macrophages, a few lymphocytes and plasma cells were scattered at the valve base and on the flow side of the valve cusp.

In addition to cardiac lesions, there was multifocal hepatocellular necrosis with neutrophil infiltration (Fig. 10.8), and marked interstitial pneumonia with haemorrhage, neutrophil and macrophage infiltration.

The histological findings were confirmed by TEM. Lipid deposition in myocytes continued to occur (Fig. 10.9). Occasionally in some myocytes, the mitochondria were swollen, their cristae damaged, and clear areas were present between the cristal membranes (Fig. 10.10). Such mitochondrial changes were encountered mainly in distended myocytes. Necrosis of capillary endothelial cells was rare. Instead, most of the endothelial cells were swollen, resulting in narrowing of the capillary lumen (Fig. 10.11). Schwann cells and axons of autonomic nerve

ganglia were not affected, apart from increased interstitial fluid and cellular infiltration around the axons (Fig. 10.12). The latter consisted mainly of macrophages and a few lymphocytes. Plasma cells were rarely found.

IMMUNOFLUORESCENCE FINDINGS.

As in infected untreated dogs, and in dogs treated with cyclosporin A, there was no evidence of IgG, IgM or C3 deposition in the hearts of both dogs (Table 10.5). Likewise, small quantities of fibrinogen were deposited in perivascular areas but no plasma cell IgG. In the kidneys, IgG was present in large quantities, with very little IgM and C3, unlike in untreated dogs where higher quantities were found.

10.3.2.2. REDUCED DOSE REGIMEN: TREATMENT WITH 5mg/kg FOR 5 DAYS.

Dogs 5 and 6 were treated with azathioprine at 5 mg/kg daily for 5 days beginning on day 8 of infection (Table 10.1). The reduction in drug dosage was an attempt to prevent the acute liver damage that was observed in dogs treated with twice the dose of azathioprine.

GENERAL BODY CONDITION.

At the time of first treatment on day 8, a fever of 40.2°C and 39.7°C was recorded for both dogs 5 and 6, respectively. All superficial lymph nodes were moderately enlarged and the spleen palpable. As in dogs treated with azathioprine at 10mg/kg for 5 days, subcutaneous oedema

decreased with treatment, such that at the time of the last treatment on day 12, oedema could only be detected around the eyes. The lymph node and splenic sizes were constant during the treatment period. Both dogs remained bright, active and with a good appetite. No significant changes in body weight were recorded, in contrast to infected untreated dogs where considerable loss in body weight had occurred by day 15. Though rectal temperatures decreased during the course of treatment, temperature appeared to be related more to the parasitaemia than to treatment (Fig. 10.13).

Cessation of treatment on day 12 saw a dramatic change in the condition of the dogs. Subcutaneous oedema increased, and the spleen and all superficial lymph nodes became enlarged. A soft nonproductive cough was noticed on both dogs on day 13 of infection. On day 15, expiratory and inspiratory dyspnoea developed. At that time too, the dogs were rather dull. There was no change in the character of the stool throughout the study. In infected untreated dogs, respiratory changes and dullness were not observed at this time.

THE CARDIOVASCULAR SYSTEM.

At the time of first treatment on day 8, both dogs had a tachycardia of 160 BPM. On day 9 (day 2 of treatment), first degree heart block (IHB) was recorded in both dogs on ECG. This was followed by IIHB on day 10. At this time, the frequency of missed beats for dog 6 was approximately 10 per minute. Echocardiography demonstrated mild MI, AI and

TI.

With continued treatment, marked improvement occurred, such that on day 12, ECG only revealed IHB in dog 5 and by ultrasound, MI, TI and AI could hardly be detected. Upon withdrawal of treatment on day 12, the condition of the heart deteriorated, the heart rate increased again, femoral pulses became weak and the capillary refill time increased to three seconds. IIHB reappeared in dog 6 and the severity of MI and AI increased in both dogs.

PARASITOLOGICAL FINDINGS.

Both dogs became parasitaemic on day 5 of infection, corresponding to the first peak of fever (eg. Fig. 10.13). Parasitaemia was not significantly affected by treatment and, as in untreated dogs, a transient drop in parasite numbers occurred between days 8 to 10, before returning to high levels (Fig. 10.13).

THE HAEMOPOIETIC SYSTEM.

As in the dogs treated with azathioprine at a dose rate of 10 mg/kg daily for 5 days, reduction to half the dose had no significant effect on anaemia and thrombocytopaenia. The dogs developed a leucocytopaenia, associated with low numbers of neutrophils and lymphocytes. The fall in neutrophil numbers was not significantly affected by treatment (Table 10.11). This was in contrast with dogs treated with twice the dose of azathioprine, in which neutrophils had increased (Table 10.7).

PLASMA BIOCHEMISTRY.

Treatment did not affect the changes in Hp, which increased with progress of the disease. On the other hand the concentration of CRP in dog 6 fell from 114mg/l on day 9 to 32mg/l on day 10 (day 3 of treatment). After drug withdrawal on day 12 however, CRP increased, to 337mg/l on day 14 of infection.

As with dogs treated with azathioprine at the higher dose rate, plasma albumin content was markedly affected by treatment. In dog 5, albumin concentration remained constant at 26g/l, while in dog 6, the drop was only from 27g/l to 25g/l during the course of treatment. In contrast, albumin in untreated infected dogs dropped from 33g/l to 21g/l in a similar period (see also Ch.6).

No significant changes in tissue enzymes occurred during the treatment period. In dog 5 for example, the concentration of AP on day 7 was 207U/l. With treatment, AP dropped to 163U/l on day 11. Similarly AST, ALT and CPK were 60, 55 and 144U/l, respectively, on day 7, and went down to 25, 48 and 73U/l, respectively, on day 11 of infection. In the same period, the relative percentages of VLDL, LDL and HDL gradually returned to normal (Fig. 10.14). This change was more noticeable with respect to HDL. At the same time the concentration of TG and CH gradually returned to normal. There were no significant changes in the levels of NEFA.

PATHOLOGY.

As in untreated dogs, the carcasses were in good condition. The hearts were moderately pale with no other

obvious changes. Superficial lymph nodes and the spleen were moderately enlarged and the liver pale. A few haemorrhagic foci, approximately 3mm in diameter, were seen in the proximal part of the duodenum.

Unlike the two dogs treated with the higher dose of azathioprine, the severity of tissue damage in the heart and other organs was less than untreated infected controls. The most significant lesion was moderate interstitial oedema, the severity of which was greatest in the subepicardial and subendocardial regions (Fig 10.15). All the valves were similarly involved. There were no necrotic foci in the myocardium.

Moderate swelling and vacuolation of Purkinje fibres in the subendocardium was seen. In addition in the subendocardium and the subepicardium of the ventricles, there was moderate infiltration with macrophages together with a few lymphocytes and plasma cells. Very few of these cells were present in the myocardium. In the subepicardial adipose tissue, adipocyte necrosis and cellular infiltration was similar to that in dogs treated with azathioprine at 10 mg/kg for 5 days.

Cellular infiltration in the atria was similar to, but more intense than in the ventricles. Marked trypanosome infiltration occurred throughout the heart. Oil red O staining demonstrated lipid deposition, the intensity of which was not different from that in untreated dogs infected for a similar number of days.

By TEM, most of the histological findings were confirmed, including myocardial lipid deposition, oedema

and trypanosome infiltration (Fig. 10.16). Swelling and disruption of capillary endothelial cells was occasionally seen. In some areas, endothelial cell swelling had caused total occlusion of the capillary lumen. In addition to trypanosomes, infiltrating cells consisted mainly of macrophages, a few lymphocytes and, rarely, plasma cells. The macrophages were active but had ingested very little necrotic debris (Fig. 10.17). Changes in autonomic nerve ganglia were similar to those in dogs treated with twice the dose of azathioprine, consisting of oedema and cellular infiltration around the nerve axons. In the atria, atrial natriuretic granules appeared to be unaffected, as was the case in untreated dogs infected for 15 days.

IMMUNOFLUORESCENCE FINDINGS.

As in infected untreated dogs, and in dogs treated with twice the dose of azathioprine, immunofluorescence staining of the hearts for IgG, IgM and C3 was negative (Table 10.5). Sparse deposits of fibrinogen were found in perivascular areas and between myofibrils. In the kidneys, intense IgM, IgG and C3 deposition was found. This was significantly higher than in dogs treated with twice the dose of azathioprine, infected untreated dogs, and dogs treated with cyclosporin A.

10.3.2.3. TREATMENT WITH 5mg/kg FROM DAY 3 OF INFECTION.

Dogs 7 and 8 were treated with azathioprine at 5mg/kg from day 3 to 12 of infection, then euthanised on day 15, i.e., treatment was started prior to detection of parasitaemia. For dogs 9 and 10, treatment with a similar

dose was started on day 3 and continued up to day 14, 24 hours before the dogs were euthanised (Table 10.1).

GENERAL BODY CONDITION.

In all four dogs, treatment started before any clinical changes were detected. A slight fever peak was noted on day 4 of infection (day 2 of treatment) (Table 10.12). On day 7, a low grade subcutaneous oedema developed on the face and around the eyes. This was accompanied by moderate enlargement of the superficial cervical and retropharyngeal lymph nodes. On day 10, the spleen was just palpable, there was no change in subcutaneous oedema and the dogs were bright and active. By day 12, while all the superficial lymph nodes remained moderately enlarged, the spleen was not palpable.

The condition of dog 8 deteriorated from day 9 of infection (day 7 of treatment). The dog vomited after eating and had diarrhoea. This was followed by a soft non-productive cough. At the time treatment was stopped on day 12, the dog had become dull, inactive and anorexic. This state did not change up to the end of the study on day 15.

In dog 7, subcutaneous and periorbital oedema increased after treatment was stopped on day 12. The spleen became palpable and all superficial lymph nodes were enlarged. The dog also developed a fever.

Dogs 9 and 10 remained clinically stable up to day 14 of infection (day of last treatment) when a slight degree of dullness was noted. Then dog 9 became very weak and had

difficulty walking. Both dogs exhibited occasional vomiting and became anorexic. On day 15, dog 9 had lost 1kg and dog 10 only 0.6kg in body weight. This was less than in infected untreated dogs, which had lost up to 2kg (Ch. 3, Fig. 3.2).

10.3.2.3.2. THE CARDIOVASCULAR SYSTEM.

In both dogs 9 and 10, apart from intermittent tachycardia, no significant cardiovascular changes were detected during the treatment period. ECGs were normal, and by ultrasonography, MI, TI and AI were not found. A similar situation was observed for dog 7, up to the last treatment on day 12, after which it developed a tachycardia and mild MI, TI and AI.

Dog 8, which had an unusual course, developed marked MI and TI without any AI. The left ventricle became dilated (Figs. 10.18 and 10.19) and left ventricular function (LVF) deteriorated.

PARASITOLOGICAL FINDINGS.

The dogs became parasitaemic between day 4 and 5 of infection, at least 24 hours after treatment had begun. Subsequently, the parasite numbers increased, to peak levels on day 7 and 8, then remained elevated throughout the study (Table 10.13). In contrast, in infected untreated dogs, trypanosomes could only be detected between day 9 and 11 by examination of the buffy coat, after which high parasitaemias were re-established. A comparison of rectal temperatures with parasitaemia showed that during the treatment period, significant increases in temperature did

not occur (Fig. 10.20). This was in contrast to untreated dogs, in which temperature was directly related to the level of parasitaemia during the first 2 weeks of infection (Ch.3, Fig.3.1).

THE HAEMOPOIETIC SYSTEM.

As in infected untreated dogs, all four dogs developed a normochromic and normocytic anaemia. In spite of the severe anaemia, reticulocytes were never found in the blood, unlike in untreated dogs in which reticulocytes were present most of the time (Ch.3, Fig.3.6). Thrombocytopaenia and leucocytopaenia occurred, and was similar to infected untreated dogs.

PLASMA BIOCHEMISTRY.

Studies on CRP and Hp demonstrated gradual increases in both APP beginning on day 4 of infection. Unlike infected untreated dogs, the plasma levels of CRP and Hp were unrelated to the parasitaemia. As such, peak CRP occurred a few days after peak parasitaemia, and remained elevated up to the end of the study (eg. Fig. 10.21).

Treatment with azathioprine from day 3 caused a rapid increase in AP, to peak on day 7 of infection (mean 181U/l on day 4 to 325U/l on day 7), then the levels dropped (mean 208U/l on day 11). Discontinuation of treatment in dogs 7 and 8 resulted in return of AP to pre-infection levels. Continued treatment of dog 10 caused further increase in AP (from 219U/l on day 10 to 432U/l on day 15). Gradual elevation in ALT occurred, beginning soon after treatment was initiated. After day 11 of treatment, the

increase in ALT became more pronounced, especially in the dogs for which treatment was continued up to day 14. As such, ALT in dog 9 rose from 111U/l on day 11 to 600U/l on day 15. These tissue enzyme changes were higher than in dogs treated with the same dose of azathioprine from day 8 to 12, and less than in dogs treated with twice the dose from day 8 to 12. No significant changes in other tissue enzymes occurred.

As in dogs treated with azathioprine from day 8, albumin concentration was markedly affected by treatment with azathioprine from day 3 of infection. The effect was not obvious until after day 7 when albumin dropped from 32g/l to 26.75g/l in the treated dogs, while in the untreated ones, it fell from 33.0g/l to 21.3g/l. The prolonged treatment had less effect than treatment from day 8 with the same dose, where minimal changes in albumin had occurred in a similar period.

Changes in plasma TG and CH were unaffected by azathioprine treatment. An increase in the relative percentage of VLDL, and to a lesser extent LDL, and a decrease in HDL, occurred from day 3 of infection (Fig. 10.22). This continued, despite treatment, up to day 9, after which no further change took place.

PATHOLOGY.

At post mortem examination, the carcasses were in good condition. Moderate generalised lymph node enlargement was observed in most dogs, except dog 10, in which lymph nodes were of normal size. In all cases, the heart was moderately

pale but with no other obvious changes. This was in contrast to infected untreated dogs where pallor of the heart was not a feature at this time. The liver in dog 9 was very pale, and in the same dog, a few ulcerative lesions were found in the duodenum and jejunum. Unlike in treated dogs, pallor of the liver was not seen in untreated infected dogs.

Degenerative changes and cellular infiltration in the heart were less severe than in dogs treated from day 8. This finding was more obvious in dogs treated up to day 14. The major lesion was moderate interstitial oedema throughout the heart, and intense trypanosome infiltration into the myocardium (Fig. 10.23). Oedema was more severe than in untreated dogs infected for a similar number of days. There was moderate subepicardial and subendocardial infiltration with macrophages, fewer lymphocytes, and occasional plasma cells. Subepicardial lymphatic vessels were distended with fluid and occasionally, a mixture of activate macrophages, trypanosomes and lymphocytes. Plasma cells were few. No necrotic foci were seen in the myocardium.

In the myocardium of dog 8, which ran an unusual course, there was marked myocyte oedema and fragmentation, especially in the right ventricle. Trypanosomes and macrophages were in large numbers. As in infected untreated dogs, lipid deposition was demonstrated by oil red O staining.

In all the animals, the liver was found to be involved, with marked fatty degeneration and occasional hepatocyte

necrosis (Fig. 10.24). In addition, there was generalised neutrophil infiltration. Discrete foci of hepatic necrosis, as seen in dogs treated with azathioprine at 10mg/kg for 5 days, were not found.

By TEM, myocardial lipid deposition was confirmed, and the distribution was similar in dogs treated up to day 12 or day 14. In dogs treated from day 3 to 12 however, there was marked interstitial oedema with high protein content. This was accompanied in most cases by swelling of myocytes (Fig. 10.25). The latter was more severe than in dogs treated for 5 days and also untreated dogs infected for a similar number of days. Trypanosomes were found in large numbers, especially in the subepicardium and subendocardium (Fig. 10.26). In addition, vascular damage was greater than in untreated dogs (Fig. 10.27), and some capillaries were blocked by lymphoid cells (Fig. 10.28). Haemorrhage, unlike in untreated dogs infected for 15 days, was consistently observed (Fig. 10.29). The cellular infiltrate consisted mainly of macrophages and occasionally, lymphocytes. Plasma cells were rarely encountered. Most of the macrophages were very active, with long cytoplasmic extensions (Fig. 10.30). Phagocytosis of RBC and trypanosomes was taking place (Fig. 10.31) and some macrophages had lipid in the cytoplasm. In contrast, in untreated dogs infected for 15 days, trypanosomes were few in number, and the macrophages had neither lipid, nor necrotic debris. In the heart of dogs treated up to day 14, though interstitial oedema was present, there was no haemorrhage (Fig. 10.32).

IMMUNOFLUORESCENCE FINDINGS.

As in infected untreated dogs, no evidence of IgG, IgM or C3 deposition was observed in the hearts of all 4 dogs (Table 10.5). Fibrinogen deposition in dogs 9 and 10 was significantly less than in dogs 7 and 8, and in infected untreated dogs. Likewise in the kidneys, while high quantities of IgG, IgM and C3 were found in dogs 7 and 8, very little was present in dogs 9 and 10.

10.3.3. COMBINATION TREATMENT WITH AZATHIOPRINE AND PREDNISOLONE.

Dogs 11 and 12 were treated with azathioprine and oral prednisolone from day 3 to 14 of infection. The doses and treatment regimens used are shown in Table 10.1.

GENERAL BODY CONDITION.

Both dogs developed a fever from day 4 to 6, coinciding with the first parasitaemia. Subsequently, rectal temperature dropped below that of infected untreated dogs, but remained above the pre-infection normal (Fig. 10.33). This was despite the fact that the parasitaemia was much higher than in the untreated dogs (Fig. 10.34). From day 7 to 11, there was moderate enlargement of the superficial cervical and retropharyngeal lymph nodes. Subcutaneous oedema and ocular changes were not recorded at this time. By day 11, the spleen was just palpable. This was followed by moderate enlargement of all superficial lymph nodes.

From day 13 to 15, dog 11 became rather dull and inactive. It started vomiting and a watery diarrhoea

developed. Vomiting and diarrhoea were not observed in dog 12, which remained bright, active, and with normal appetite.

THE CARDIOVASCULAR SYSTEM.

Throughout the period of study, apart from intermittent tachycardia, no cardiac abnormalities were detected by auscultation of the thoracic cavity or by ECG. By PW Doppler echocardiography, mild MI and AI were demonstrated in dog 11 on days 14 and 15 of infection.

PARASITOLOGICAL FINDINGS.

The dogs developed massive parasitaemias on day 4 of infection (day 2 of treatment), reaching peak levels on day 5 (Fig. 10.33). The parasite numbers remained much higher than in both untreated dogs and those treated with azathioprine alone.

THE HAEMOPOIETIC SYSTEM.

The mean changes in haematological parameters in dogs 11 and 12 are shown in Table 10.14. As in all azathioprine treated and untreated dogs, dogs 11 and 12 became rapidly anaemic, with reduction in PCV, RBC and Hb concentration. No reticulocytes were found in the blood, and this was similar to dogs treated with azathioprine from day 3. As a result the MCV only increased slightly, and a decrease in the MCH occurred (Table 10.14). This was in contrast with infected untreated dogs, in which a mild reticulocytosis developed, associated with slightly elevated MCV and MCH (Ch.3, Fig.3.6). In addition, the dogs developed a

thrombocytopaenia and leucocytopaenia, changes similar to the ones seen in dogs treated with azathioprine for a similar number of days.

PLASMA BIOCHEMISTRY.

As in dogs treated with azathioprine from day 3 to 14, the plasma concentration of CRP and Hp increased gradually from day 4 of infection (day 2 of treatment), reaching a peak on day 7. The peak levels of both APP was higher than in dogs treated with azathioprine alone and in infected untreated ones (Figs. 10.35 and 10.36). The changes in APP were accompanied by marked increases in AP, and later ALT and AST. The rise in tissue enzymes was higher than in dogs treated with azathioprine alone (Table 10.15), but less than in dogs treated with azathioprine at 10mg/kg for 5 days (Table 10.9). No significant changes in CPK occurred (Table 10.15).

A fall in plasma albumin concentration in dogs treated with both azathioprine and prednisolone occurred only on day 15, 24 hours after treatment was stopped. This was in contrast to dogs treated with azathioprine alone (where a gradual decrease occurred) and in infected untreated dogs in which severe hypoalbuminaemia developed (Fig. 10.37). There were no significant changes in TG and CH during the period of study. Relative percentages of lipoproteins were similar to dogs treated with azathioprine alone. VLDL and LDL increased until day 10 of infection, then stabilised up to day 15.

PATHOLOGY.

The carcasses of all dogs were in good condition. The heart of dog 11 was very pale, while that of dog 12 was less so; there were no other visible abnormalities. There was marked pallor of the liver and moderate generalised lymph node enlargement. Multiple petechial haemorrhages were present in the pyloro-duodenal junction, and from midway down the duodenum to the ileo-cecal junction in dog 12. The spleen was normal in size.

The histological changes in the heart of dog 11 were more obvious than in dog 12. Generally the lesions were similar to those in dogs treated with azathioprine alone for a similar number of days. The atria were more severely affected than the ventricles. There was marked trypanosome infiltration throughout the heart, with very few infiltrating cells (Fig. 10.38). Moderate myocardial interstitial oedema occurred, being higher in dog 11. In addition in the left ventricle of dog 11, occasional foci of myocytolysis, without an accompanying cellular infiltration, were found. Such foci were not present in the hearts of either dog 12, dogs treated with azathioprine at 3 or 5mg/kg, or untreated dogs infected for 15 days. In the subepicardial and subendocardial myocardium of the ventricles, moderate macrophage and lymphocyte infiltration was observed in both dogs. Plasma cells were rare. Purkinje fibres in the subendocardial myocardium were swollen. Myocardial lipid deposition was similar to dogs treated with azathioprine alone for a similar period.

Marked fatty degeneration was observed in the liver of dog 11, and less so in dog 12. In the lungs, interstitial

oedema with macrophage, lymphocyte and neutrophil infiltration was occasionally observed.

By TEM, the histological findings were confirmed, and were similar to those in dogs treated with azathioprine from day 3 to 14, with a few exceptions. In dog 11 for example, myocyte lipid deposition was higher, and was similar to that seen in dogs euthanised from day 21 to 26 of infection (Fig. 10.39; Ch. 8). Swollen myocytes, interstitial oedema, many trypanosomes, activated macrophages and lymphocytes were consistently seen in the ventricles and atria.

IMMUNOFLUORESCENCE FINDINGS.

As in other dogs in this study, no evidence of IgM, IgG or C3 deposition was found in the hearts of dogs 11 and 12 (Table 10.5). Fibrinogen deposition was less than in untreated dogs and dogs treated with azathioprine from day 8. Likewise in the kidneys, the extent of IgG, IgM and C3 deposition was less. In dog 11, fibrinogen deposition in the heart, and IgG in the kidneys was higher than in dogs treated with azathioprine from day 3 to 14 (Table 10.5).

10.4. DISCUSSION.

The current study demonstrated that when dogs infected with T.brucei were treated with various NSAIDS, alone or in combination with the steroidal compound prednisolone, the course of the disease was modified, depending on the drug used, the dosage, the period of infection before treatment was initiated, and the duration of treatment.

Intravenous treatment with cyclosporin A at the peak of first parasitaemia resulted in signs of acute drug toxicity, including head-shaking and injection of mucous membranes. Since such signs abated a short period after treatment, they were probably caused by the presence of high plasma concentration of the drug. In humans undergoing immunosuppressive therapy with cyclosporin A after liver transplantation, a syndrome of central nervous system involvement consisting of confusion, flushing, cortical blindness, quadriplegia, seizures and coma has been observed soon after drug administration. The side effects are reversed when treatment is discontinued or the dose of cyclosporin A reduced (De Groen et al., 1987). It is possible that if, in the present study, the drug had been divided into small doses and administered several times in a day, the high plasma levels could have been avoided and acute toxicity prevented.

During the period of treatment with cyclosporin A, the clinical condition of the dogs remained better than that of infected untreated dogs. The signs of improvement included decreased subcutaneous oedema and reduced cardiac abnormalities such as IHB, IIHB, sinus arrest and valvular incompetence. This outcome supported the conclusions from studies in untreated infected dogs, which indicated that cardiac damage was initiated by acute inflammatory reactions that followed immunological response by the host to the parasite (Ch. 8).

In the present study, cyclosporin A was administered when an immunological response had already taken place, and

response to drug therapy was observed within 24 hours of treatment. This initial response was too quick to be attributed to a primary immunosuppressive effect of cyclosporin A, but rather to its powerful anti-inflammatory component. The massive inflammatory reaction that followed withdrawal of cyclosporin A would also indicate that the dogs were still immunologically competent at the time treatment was withdrawn. In mice infected with T.brucei, meningoencephalitis could not be prevented by administration of cyclosporin A for a short period of time before Berenil treatment (Jennings et al., 1989), indicating that immunosuppression had not been fully achieved by the time Berenil was administered. On the other hand, the fact that splenic and lymph node sizes in dogs infected with T.brucei did not increase further after treatment suggested that the immunological response by the dogs to the parasite had been reduced by treatment.

In a previous section of this work (Ch. 9), it was found that treatment of T.brucei infected dogs with suramin was followed within 24 hours by increased cardiac damage, mainly due to increased inflammation following antigen-antibody reactions and release of large quantities of toxic substances by dying trypanosomes. From the current experiments, it would appear that if cyclosporin A had been administered in conjunction with the trypanocidal drug, subsequent adverse reactions in response to sudden release of toxic substances might have been avoided, thereby reducing the extent of the cardiac damage.

When cyclosporin A was withdrawn, the severity of the

general disease increased, more so than in untreated infected dogs. This was demonstrated clinically by raised plasma CPK and S-T segment elevation on ECG. Histologically, there was myocardial ischaemia, accompanied by lesions in the gastrointestinal tract, lungs and the urinary bladder. These changes could not be attributed to drug toxicity per se, especially because the cytotoxic action of cyclosporin A involves mainly the kidneys and no other organ systems (Tegzess et al., 1989). As such, it appears that tissue invasion by the parasite was improved during the period of immunosuppressive therapy. When the drug was later withdrawn, a more dramatic inflammatory reaction occurred.

Studies in humans have shown that cyclosporin A is highly bound to lipoproteins in plasma (Sgoutas et al., 1986; De Groen, 1988). Lipid binding interferes with the immunosuppressive capacity of cyclosporin A. Indeed, a relative absence of immunosuppression with high doses of cyclosporin A in the blood of patients with hypertriglyceridaemia has been reported (De Groen, 1988). In the current work, it was found that dogs infected with T.brucei became hyperlipidaemic during weeks 3 and 4 of infection (Ch. 6). In cyclosporin A-treated dogs, in spite of an absence of hyperlipidaemia, cardiac lipid deposition was demonstrated. Although it is not possible at the present time to determine to what extent abnormal lipid metabolism interfered with the immunosuppressive capacity of cyclosporin A, this consideration would be important if therapy was to be carried out in animals that were already

hyperlipidaemic.

It would appear that for cyclosporin A to be used in the management of cardiac damage during trypanocidal drug treatment, the dose of the drug would have to be reduced or the daily dose divided, to prevent acute toxicity. In addition, the plasma and tissue lipid content would have to be taken into account in order to improve therapeutic efficiency.

When azathioprine was administered to dogs infected with T.brucei, a range of effects were achieved, and were affected by the dose, treatment regime and duration of treatment. Treatment with 10mg/kg for 5 days from day 8 caused rapid regression of subcutaneous oedema, lymph node and splenic sizes. ECG and echocardiographic abnormalities were less than in untreated dogs infected for a similar number of days. During the course of treatment however, liver damage occurred, associated with increased ALT and AP. The dogs also became dull and anorexic, a feature not observed in untreated dogs. Toxicity studies with azathioprine in dogs have previously demonstrated raised ALT and AP (Starzl et al., 1965; Haxhe et al., 1967). Increased AP has also been observed in humans following azathioprine therapy (Weinhelman et al., 1966). ALT and AP increase due to both hepatocellular necrosis and biliary stasis (Hardy, 1983). Toxicity to azathioprine appears to be a species characteristic, as doses of up to 100mg/kg in mice are not toxic (Jennings et al., 1989).

That liver damage was directly related to azathioprine was demonstrated by a drop in ALT after the drug was

withdrawn. The plasma half-life of ALT is only 2 to 5 hours (Hardy, 1983); a decrease in ALT in the current study after treatment was withdrawn indicated that hepatocyte damage had stopped.

When treatment was withdrawn on day 12, the general clinical state of the dogs deteriorated, and was accompanied by increased AST and CPK. Such increases in tissue enzymes indicated that in addition to the liver, other body organs were also affected. High CRP levels further showed that inflammation was severe, again consistent with the presence of a haemorrhagic pneumonia, duodenal haemorrhages and cardiac damage. Although these final changes were observed after azathioprine had been withdrawn for at least 2 days, it is difficult to determine whether tissue damage at that time was the result of increased virulence by trypanosomes on the already immunosuppressed animal, or the combined effect of the trypanosome and the drug.

When the dose of azathioprine was reduced by half, the problem of drug toxicity was overcome, without loss of the anti-inflammatory and immunosuppressive potential of azathioprine. This was demonstrated by a drop in CRP, decreased subcutaneous oedema and lymph node sizes, improved general demeanour of the dogs, and less cardiac damage than in untreated dogs infected for a similar number of days. In addition, there were minimal changes in the dogs' body weights and tissue enzymes. Diarrhoea was also absent in the treated dogs. The parasitaemia was however not affected, indicating that at that time, like

cyclosporin A, azathioprine was acting more as an anti-inflammatory drug than an immunosuppressive agent, otherwise if immunosuppression had been achieved to an appreciable degree, parasitaemia would have been higher. This is consistent with the observation by Whittington (1970) that the immune induction process of adjuvant arthritis was more vulnerable to the drug than the established disease.

The immunosuppressive potential of azathioprine was further demonstrated when treatment was initiated on day 3. The dogs rapidly developed high parasitaemias without any remission. In addition, the spleen and superficial lymph nodes were only slightly enlarged, unlike in untreated dogs where marked enlargement had occurred. This was in agreement with the observation that the effect of azathioprine is most marked when the drug is present during the first four days of exposure to antigen (Rollinghoff et al., 1973). Immunological incompetence was further demonstrated by the presence of very few plasma cells in the hearts of treated dogs, despite the presence of numerous trypanosomes in the myocardium. Since azathioprine also modulates the immunological response by inhibiting division and differentiation of B lymphocytes to plasma cells, and T lymphocytes to T memory cells (Schwartz, 1965), this would explain the presence of so few plasma cells in the heart. Further, by reducing the temperature reaction, the severity of subcutaneous oedema and cardiac abnormalities, azathioprine also acted as an anti-inflammatory drug. The most useful chemotherapeutic

effect of azathioprine appears to be when the drug is administered on a more chronic basis (Elion and Hitchings, 1965). It would seem that if an adverse immunological response by the host to the sudden release of large quantities of antigen by trypanocidal drug treatment was to be avoided, the dogs would have to be under azathioprine treatment for a few days before the trypanocidal compound is administered. In mice infected with T.brucei, the severity of the meningoencephalitis that results after Berenil treatment is reduced when the mice have previously been under azathioprine treatment (Jennings et al., 1989).

On the other hand, the number of macrophages found in the heart was unaffected by treatment. In previous studies, no significant alteration in macrophage activity was achieved by azathioprine treatment (Gotjamanos, 1971). In these studies, the hyperlipidaemic state and widespread myocardial deposition of lipids in dogs infected with T.brucei was associated with cachectin/TNF, secreted by an activated mononuclear phagocytic system (MPS) (Ch. 6). Myocardial lipid deposition continued to occur in immunosuppressed dogs. The lack of effect by azathioprine on macrophages would suggest that their ability to secrete cachectin/TNF was not impaired, hence continued lipid deposition.

Despite reduced drug dosage, signs of hepatotoxicity developed when the duration of azathioprine treatment was increased from 5 to 10 and 12 days. The toxic effects consisted of anorexia, vomiting, diarrhoea, weakness, increased plasma ALT and AP, and elevated APP that was

unrelated to parasitaemia. An extremely pale liver associated with fatty degeneration and hepatocellular necrosis confirmed the clinical observations. This is consistent with previous observations that when dogs are treated with azathioprine at 2 to 4mg/kg/day for prolonged periods, pallor of the liver and centrolobular necrosis results (Starzl et al., 1965).

A significant haematological finding in treated dogs was the absence of reticulocytes in the blood, despite an accompanying anaemia. This was not unexpected, especially because azathioprine is selectively toxic to cells undergoing mitosis (Ogilvie et al., 1988). However, one of the toxic side effects of azathioprine is inhibition of haematopoiesis, with depression of production of most cellular elements in the bone marrow (Elion et al., 1961; Hunstein et al., 1967).

When infected dogs were treated with a combination of azathioprine and prednisolone, clinical signs associated with trypanosomiasis were reduced further. Cardiac abnormalities and inflammatory reactions were almost totally inhibited. In addition, only slight lymph node and splenic enlargement occurred, despite persistently high parasitaemias. One dog remained bright and active throughout the study period. In line with this, there is evidence that steroids potentiate the immunosuppressive effects of azathioprine on antibody production and skin graft survival in mice (Friedman et al., 1971), and skin allograft survival in rabbits (Friedman et al., 1973). The synergy occurs because, like azathioprine,

anti-inflammatory steroids also prevent the generation of prostaglandins in response to tissue damage (Flower and Blackwell, 1979). The mechanism of action involves inhibition of the enzyme phospholipase A_2, which generates arachidonic acid from cell membrane phospholipids. Arachidonic acid is a precursor of prostaglandins and leucotrienes, both of which are inflammatory mediators (Flower and Blackwell, 1979; Keen, 1987). In dogs, chronic colitis (Ridgway, 1984) and pemphigus foliaceus (Ihrke et al., 1985) have been shown to respond favourably to the combined administration of azathioprine and corticosteroids.

While corticosteroids have been used extensively during treatment of humans infected with T.rhodesiense (Fulkes, 1975; Harries and Wirima, 1988) and T.gambiense (Fouchet and Gateff, 1968; Bertrand et al., 1971; Arroz, 1987; Junyent, et al., 1988; Pepin et al., 1989), their importance as an adjunct to therapy has always been unclear. Experimental studies in mice infected with T.brucei (Petana, 1964) or T.cruzi (Meckert et al., 1988), and sheep infected with T.brucei (Dwinger et al., 1984) demonstrated increased parasitaemia following treatment with various corticosteroids, further indicating that the immunological competence of trypanosomiasis infected animals was reduced.

Combination treatment with azathioprine and prednisolone for prolonged periods resulted in severe liver, lung and intestinal damage, more than when azathioprine was used alone. This was reflected by higher

levels of APP, ALT, AP, and AST. That CPK did not increase meant that the heart was not significantly affected by treatment with both drugs. The liver and the heart in one of the dogs were extremely pale, probably due to increased fat deposition. In addition, in the liver, there was hepatocellular necrosis and intense fatty degeneration. In beagle dogs and Sprague Dawley rats, steroids have been shown to potentiate the toxicity of azathioprine (Hovland and Ellis, 1967).

The increased lipid deposition in the liver and heart of dogs treated with both drugs may have been exacerbated by the steroid. In this respect, steroids have been known to increase peripheral fatty acid mobilization and to inhibit hepatic TG reesterification (Rogers and Ruebner, 1977), both of which could lead to abnormal accumulation of lipids. It is possible that in the present study, toxic side effects of combination treatment could have been avoided by reducing the doses of the two drugs, while at the same time maintaining their therapeutic effectiveness. During management of chronic colitis in the dog (Ridgway, 1984) and in canine renal transplant recipients (Putnam et al., 1975), the doses of either drug have been reduced without affecting their efficacy.

An interesting observation in this study was the prevention of hypoalbuminaemia by all the anti-inflammatory agents used. In a previous part of this work, the possible causes of hypoalbuminaemia in dogs infected with T.brucei were outlined, including decreased hepatic synthesis of albumin in the acute phase response, increased utilization

by trypanosomes, and excessive loss through the gastrointestinal tract (Ch. 6). In the current study, reduced albumin drop in the presence of high parasitaemias was an indication that excessive utilization by trypanosomes could not have caused the hypoalbuminaemia observed in untreated dogs. Since immunosuppressive treatment led to a decrease in both the acute inflammatory reactions and gastrointestinal lesions, it is possible that the acute phase response and loss through the gastrointestinal tract contributed additively to the hypoalbuminaemia in untreated dogs.

This study demonstrated that, when dogs are infected with T.brucei, clinical features associated with trypanosomiasis, and more specifically cardiac damage, can be delayed or reduced when the dogs are treated with NSAIDS, alone or in combination with prednisolone. Cyclosporin A produced signs of acute toxicity, but it is possible that toxicity could be overcome by splitting the daily dose and giving it at intervals spread over the whole day. Azathioprine, alone or in combination with prednisolone, produced promising results. However, high drug dosages and treatment for prolonged periods of time caused hepatic damage. Toxicity might have been avoided by reducing the doses of both drugs and then using them together. The immunosuppressive and anti-inflammatory properties shown by these drugs indicated that the increased cardiac damage that follows trypanocidal drug treatment (Ch. 9) might be avoided if the NSAIDs or prednisolone are administered at the time of trypanocidal

treatment. The drugs could then be withdrawn after the dogs had gone over the acute trypanolytic crisis. Since increased cardiac damage following trypanocidal drug treatment has also been observed in humans infected with T.gambiense (Bertrand et al., 1971) and T.rhodesiense (Jones et al., 1975; Harries et al., 1988) the results of the current study have important implications for man. At the present time, it is difficult to predict the treatment regime that would be best suited for this, but pre-treatment with an immunosuppressant, followed by a trypanocidal compound, would seem to be the most promising approach.

TABLE 10.1.

DRUG DOSAGES AND DAYS OF TREATMENT IN DOGS INFECTED WITH *T.brucei*.

DOG NUMBER	DRUG USED	mg/kg x DAILY DOSES	DAYS OF TREATMENT
1	Cy	5x3 iv	8, 9, 10
2	Cy	5x3 iv	9, 10, 11
3, 4	Aza	10x5 po	8 - 12
5, 6	Aza	5x5 po	8 - 12
7, 8	Aza	5x10 po	3 - 12
9, 10	Aza	5x12 po	3 - 14
11,12	Aza	5x12 po	3 - 14
	Pred	2x7 po	3 - 9
	Pred	1x5 po	10 - 14
13,14	No treatment		

Cy - Cyclosporin A.
Aza - Azathioprine.
Pred - Prednisolone.
iv - Intravenous.
po - *per os*.

TABLE 10.2.

LEUCOCYTE NUMBERS ($x10^9/l$) IN DOGS INFECTED WITH T.brucei AND TREATED WITH CYCLOSPORIN A.

DAY OF INFECTION	TOTAL WHITE CELLS			NEUTROPHILS			LYMPHOCYTES		
	DOG 1	DOG 2	CONT	DOG 1	DOG 2	CONT	DOG 1	DOG 2	CONT
0	22.4	15.7	19.2	17.92	12.09	11.53	3.58	3.14	5.86
8	8.2	8.1	8.7	5.33	4.62	5.42	2.38	3.40	3.12
11	6.9	8.3	4.43	4.83	4.81	3.19	1.35	2.32	2.49
15	8.3	8.7	6.53	7.06	6.00	3.37	1.16	2.70	1.60

CONT - Infected Control (mean of dogs 13 and 14).

TABLE 10.3.

ALBUMIN CONCENTRATION (g/l) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH CYCLOSPORIN A.

DAY OF INFECTION	DOG 1	DOG 2	INFECTED CONTROL*
0	38	42	36
8	26	30	33
11	27	27	21
15	22	22	18

* - Mean of dogs 13 and 14.

TABLE 10.4.

CONCENTRATION OF TISSUE ENZYMES (U/l) IN PLASMA OF DOGS INFECTED WITH T.brucei AND TREATED WITH CYCLOSPORIN A.

DAY OF INFECTION	CPK			ALT			AP			AST		
	DOG 1	DOG 2	CONT	DOG 1	DOG 2	CONT	DOG 1	DOG 2	CONT	DOG 1	DOG 2	CONT
0	130	97	164	92	60	73	202	171	124	25	16	44
8	92	89	124	89	69	80	203	282	156	23	14	49
11	72	70	100	73	71	65	231	205	162	47	33	39
15	106	142	87	50	70	105	136	154	179	22	37	70

CPK - Creatinine phosphokinase.
ALT - Alanine aminotransferase.
AP - Alkaline phosphatase.
AST - Aspartate aminotransferase.
CONT - Infected control (mean of dogs 13 and 14).

TABLE 10.5.

IMMUNOFLUORESCENCE STAINING IN THE HEARTS AND KIDNEYS OF DOGS INFECTED WITH T.brucei AND TREATED WITH VARIOUS DOSES OF CYCLOSPORIN A, AZATHIOPRINE AND PREDNISOLONE (*).

DOG NUMBER	HEART					KIDNEY		
	IgG	IgM	C3	PLASMA CELL IgG	FIBRINOGEN	IgG	IgM	C3
1	-	-	-	2+	2+	3+	1+	2+
2	-	-	-	-	1+	4+	2+	2+
3	-	-	-	-	1+	3+	1+	1+
4	-	-	-	-	2+	3+	2+	1+
5	-	-	-	1+	2+	4+	3+	3+
6	-	-	-	-	2+	4+	3+	3+
7	-	-	-	-	2+	4+	3+	2+
8	-	-	-	2+	3+	4+	3+	2+
9	-	-	-	1+	1+	3+	ND	1+
10	-	-	-	1+	1+	1+	ND	1+
11	-	-	-	2+	3+	3+	1+	1+
12	-	-	-	1+	1+	2+	ND	1+
13	-	-	-	2+	2+	1+	ND	1+
14	-	-	-	2+	1+	1+	ND	1+

- - Negative.
1+ - Trace amounts in some sections.
2+ - Minimal deposits in all sections.
3+ - Moderate deposits in all sections.
4+ - Marked deposition in all sections.

* - Treated as indicated in Table 10.1.
ND - Not done.
Dogs 13 and 14 were infected untreated controls.

TABLE 10.6.

RECTAL TEMPERATURES (^{O}C) IN DOGS INFECTED WITH T.brucei AND TREATED WITH AZATHIOPRINE FROM DAY 8 TO 12.

DAY OF INFECTION	10mg/kg x 5		5mg/kg x 5		INFECTED CONTROL[M]
	DOG 3	DOG 4	DOG 5	DOG 6	
0	38.3	39.1	38.6	38.6	38.9
1	38.5	39.0	38.6	38.4	38.9
2	38.6	38.8	38.5	38.5	38.8
3	38.0	39.0	38.6	38.6	38.9
4	38.6	39.1	38.8	38.7	39.0
5	39.3	39.4	40.0	40.4	39.3
6	38.7	39.9	39.6	40.6	39.8
7	39.3	39.4	39.2	39.1	40.1
8	39.4[+]	39.6[+]	39.6[+]	39.7[+]	39.9
9	38.2	38.8	38.6	39.6	39.8
10	39.2	39.2	38.7	38.8	39.7
11	39.8	39.4	39.3	39.2	39.0
12	39.2*	39.1*	39.7*	40.2*	39.3
13	38.8	38.8	39.8	39.4	39.6
14	38.4	39.4	39.7	39.6	39.7
15	38.9	39.4	39.8	39.2	39.8

\+ - Day of first treatment.
* - Day of last treatment.
M - Mean of dogs 13 and 14.

TABLE 10.7.

LEUCOCYTE NUMBERS ($x10^9/l$) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH AZATHIOPRINE AT 10mg/kg x 5 FROM DAY 8 TO 12.

DAY OF INFECTION	TOTAL WHITE CELLS			NEUTROPHILS			LYMPHOCYTES			MONOCYTES		
	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT
0	14.5	13.90	19.20	11.70	11.68	11.53	2.90	1.95	5.86	0	0	0.4
8	9.00	10.00	8.70	5.13	8.50	5.42	2.88	1.20	3.12	0.45	0	0.01
11	10.00	7.10	4.43	8.10	6.04	3.19	1.15	0.32	2.49	0.35	0.11	0.12
15	8.60	14.00	6.53	6.54	13.44	3.37	1.38	0.56	1.60	0.25	0	0.08

CONT - Infected control (mean of dogs 13 and 14).

TABLE 10.8.

ALBUMIN CONCENTRATION (g/l) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH AZATHIOPRINE AT 10mg/kg x 5 ON DAYS 8 TO 12.

DAY OF INFECTION	DOG 3	DOG 4	INFECTED CONTROL[M]
0	42	37	36
8	31	28	33
11	29	28	21
15	24	22	18

M - Mean of dogs 13 and 14.

TABLE 10.9.

CONCENTRATION OF TISSUE ENZYMES (U/l) IN PLASMA OF DOGS INFECTED WITH T.brucei AND TREATED WITH AZATHIOPRINE AT 10mg/kg FROM DAY 8 TO 12.

DAY OF INFECTION	CPK			ALT			AP			AST		
	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT
0	117	100	164	51	211	73	188	58	124	21	35	44
8	85	96	124	107	54	80	285	254	156	17	27	49
11	76	106	100	859	275	65	883	725	162	37	48	39
15	156	174	87	469	150	105	1316	2087	179	49	73	70

CPK - Creatinine phosphokinase.
ALT - Alanine aminotransferase.
AP - Alkaline phosphatase.
AST - Aspartate aminotransferase.
CONT - Infected control (mean of dogs 13 and 14).

TABLE 10.10.

TOTAL PLASMA CHOLESTEROL AND TRIGLYCERIDES (mmol/l) IN DOGS INFECTED WITH T.brucei AND TREATED WITH AZATHIOPRINE AT 10mg/kg x 5 FROM DAY 8 TO 12.

DAY OF INFECTION	CHOLESTEROL			TRIGLYCERIDES		
	DOG 3	DOG 4	CONT	DOG 3	DOG 4	CONT
0	4.20	3.19	3.70	0.64	0.51	0.58
8	5.45	4.00	4.01	0.84	0.40	0.41
11	5.38	4.02	3.63	0.63	0.31	0.81
15	5.00	3.91	4.12	0.61	0.37	0.81

CONT - Infected control (mean of dogs 13 and 14).

TABLE 10.11.

LEUCOCYTE NUMBERS ($x10^9/l$) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH AZATHIOPRINE AT 5mg/kg x 5 FROM DAY 8 TO 12.

DAY OF INFECTION	TOTAL WHITE CELLS			NEUTROPHILS			LYMPHOCYTES			MONOCYTES		
	DOG 5	DOG 6	CONT	DOG 5	DOG 6	CONT	DOG 5	DOG 6	CONT	DOG 5	DOG 6	CONT
0	15.10	10.20	19.20	8.23	6.12	11.56	5.13	2.55	5.86	0.60	0.82	0.47
8	3.20	3.20	8.70	1.92	2.18	5.42	1.02	0.96	3.12	0.03	0.03	0.01
11	2.60	3.30	4.43	1.56	2.18	3.18	0.83	0.86	2.49	0.16	0.13	0.12
15	4.60	3.80	6.53	3.50	2.89	3.37	0.64	0.65	1.60	0	0.08	0.08

CONT - Infected control (mean of dogs 13 and 14).

TABLE 10.12.

RECTAL TEMPERATURES (°C) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH AZATHIOPRINE AT 5mg/kg FOR 10 AND 12 DAYS.

DAY OF INFECTION	DOG 7	DOG 8	DOG 9	DOG 10	INFECTED CONTROL[M]
0	38.5	38.6	39.1	39.1	38.9
1	38.5	38.5	38.9	39.1	38.9
2	38.4	38.5	39.1	38.9	38.8
3	38.6[+]	38.8[+]	38.9[+]	39.1[+]	38.9
4	39.4	39.3	39.1	39.1	39.0
5	39.4	39.1	40.1	39.7	39.3
6	39.3	39.2	39.9	39.7	39.8
7	38.8	38.9	39.3	38.8	40.1
8	38.8	39.2	38.9	38.9	39.9
9	39.4	38.9	39.3	38.9	39.8
10	38.8	39.2	39.4	39.3	39.7
11	38.9	39.0	39.4	39.2	39.0
12	38.9[*]	38.8[*]	39.0	39.0	39.3
13	39.6	38.9	38.3	38.3	39.6
14	39.8	39.2	38.5[*]	39.0[*]	39.7
15	38.6	38.0	38.6	38.6	39.8

\+ - Day of first treatment.
* - Day of last treatment.
M - Mean of dogs 13 and 14.

TABLE 10.13.

TRYPANOSOME NUMBERS (log_{10}/ml) IN DOGS INFECTED WITH *T.brucei* AND TREATED WITH AZATHIOPRINE*.

DAY OF INFECTION	DOG 7	DOG 8	DOG 9	DOG 10	INFECTED CONTROL[M]
0	0	0	0	O	0
4	0	0	6.6	6.6	0
5	6.0	5.7	8.1	8.1	1
6	5.4	5.4	ND	ND	5.6
7	7.8	8.1	8.1	8.4	7.1
8	8.1	7.8	7.8	8.1	ND
9	8.1	7.5	ND	ND	8.0
10	7.8	7.5	8.4	7.5	ND
11	7.2	7.8	7.8	7.5	4.0
14	7.5	7.5	ND	ND	7.0
15	7.8	8.1	8.1	7.8	7.0

ND - Not done.
* - Dogs 7 and 8 were treated on days 3 to 12 and dogs 9 and 10 on days 3 to 14.
M - Mean of dogs 13 and 14.

TABLE 10.14.

HAEMATOLOGICAL CHANGES IN DOGS INFECTED WITH T.brucei AND TREATED WITH AZATHIOPRINE AND PREDNISOLONE FROM DAY 3 TO 14.

DAY OF INFECTION	PCV (l/l)		RBC ($x10^{12}$/l)		HB (g/dl)		MCV (fl)		MCH (pg)		MCHC (g/dl)	
	A+P	CONT	A+P	CONT	A+P	CONT	A+P	CONT	A+P	CONT	A+P	CONT
0	0.362	0.416	5.7	6.4	13.3	14.9	63.5	64.5	23.4	23.2	36.8	35.9
3	0.389	0.386	6.1	6.2	14.1	14.5	63.8	62.5	23.2	23.5	36.3	37.6
7	0.347	0.393	5.4	5.5	12.2	13.8	64.3	72.0	22.6	25.2	35.2	35.5
11	0.293	0.312	4.6	4.5	10.2	10.8	64.3	69.0	22.3	23.9	34.7	34.6
15	0.260	0.250	4.0	4.5	8.8	8.7	64.8	70.0	22.1	23.6	34.0	38.8

A - Azathioprine.
P - Prednisolone.
PCV - Packed red cell volume.
RBC - Red blood cells.
HB - Haemoglobin.
CONT - Infected control (mean of dogs 13 and 14).
MCV - Mean corpuscular volume.
MCH - Mean corpuscular haemoglobin.
MCHC - Mean corpuscular haemoglobin concentration.

TABLE 10.15.

CONCENTRATION OF TISSUE ENZYMES (U/l) IN PLASMA OF DOGS INFECTED WITH T.brucei AND TREATED WITH AZATHIOPRINE AND PREDNISOLONE FROM DAY 3 TO 14.

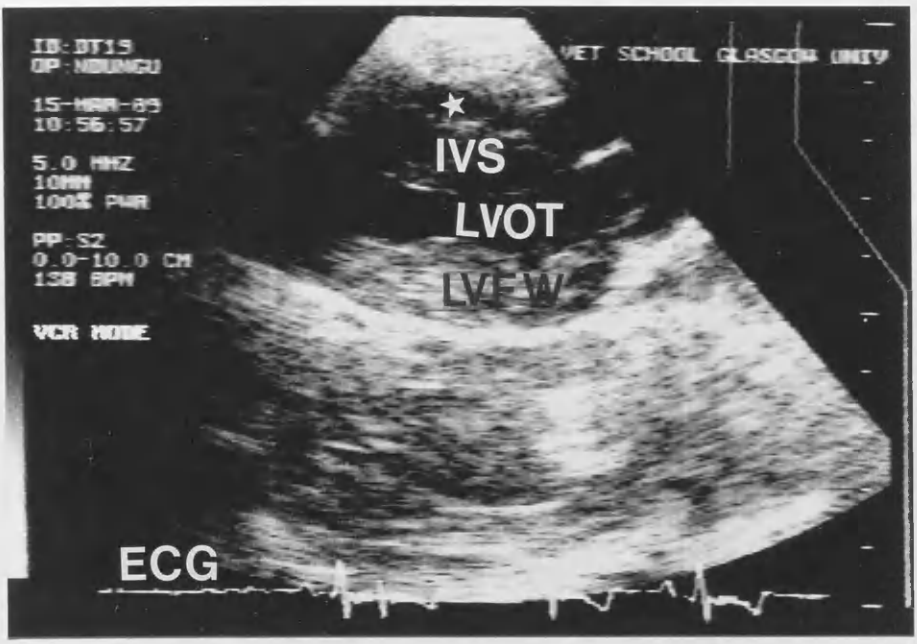
DAY OF INFECTION	CPK			ALT			AP			AST		
	A	A+P	CONT	A	A+P	CONT	A	A+P	CONT	A	A+P	CONT
0	260	224.5	119	42.75	28.5	44	205	227	197	31.25	26.0	21.0
3	194.3	231.5	184	36.75	33.5	32	181	212	179	24.75	26.0	33.0
7	113.5	119	144	77	44	55	325	504	207	44.5	23.0	60.0
10	93.75	74.5	165	99.3	69	76	208	534	186	41.25	37.5	35.0
15	96.3	110.5	87	343	587	35	323	488	255	88.5	87.0	45.0

CPK - Creatinine phosphokinase.
ALT - Alanine aminotransferase.
AP - Alkaline phosphatase.
AST - Aspartate aminotransferase.
CONT - Infected control (mean of dogs 13 and 14).

A - Azathioprine.
P - Prednisolone.

Figure 10.1. A right parasternal two-dimensional long axis view of the heart of a dog infected with *T.brucei* and treated with cyclosporin A at a dose of 5mg/kg intravenously on days 8, 9 and 10. The echocardiogram was taken on day 11 of infection, 24 hours after the last treatment. There is marked thickening of the interventricular septum (IVS) (13 ± 2mm: mean ± 1SD) and the left ventricular free wall (LVFW) (11 ± 1mm). LVOT - Left ventricular outflow tract. * - Right ventricle.

Figure 10.2. A focus of myocardial ischaemia in the right ventricle of a dog infected with *T.brucei* and treated with cyclosporin A at a dose of 5mg/kg on days 9, 10 and 11, then euthanised on day 15. The affected myocytes (dark stained) have undergone coagulative necrosis. There is myolysis (arrows) and diffuse trypanosome infiltration. H&E. x400.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IVS
LVOT
LVFW
ECG

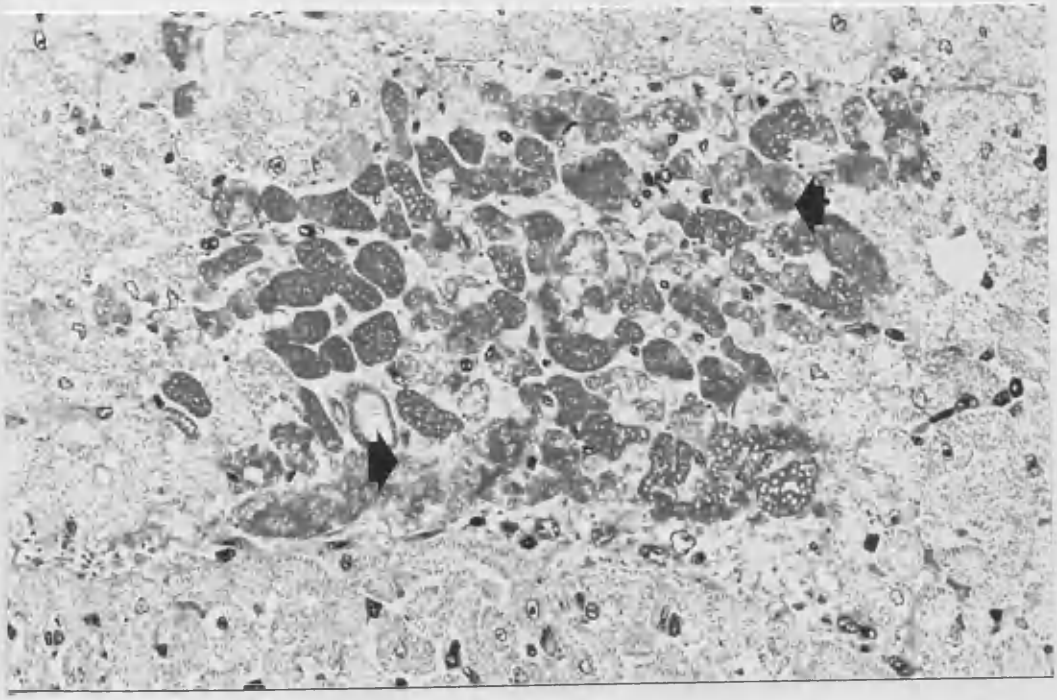
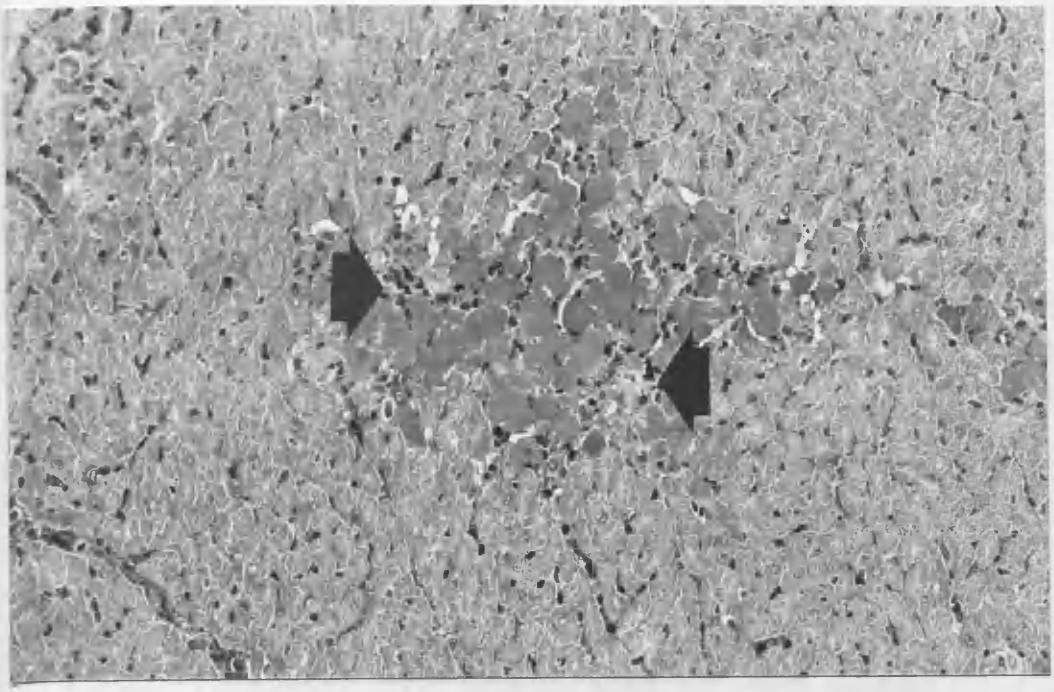


Figure 10.3. A focus of myocardial ischaemia in the right ventricle of the dog in Figure 10.2. There is an increase in inflammatory cells in the ischaemic region (arrows). H&E. x200.

Figure 10.4. A focus of previous myocardial ischaemia in the right ventricle of the dog in Figures 10.2 and 10.3. Most of the necrotic myocytes have been replaced by mononuclear cells, consisting mainly of macrophages (large arrows) and a few lymphocytes. Some myocytes are in the process of myolysis (small arrow). H&E. x320.



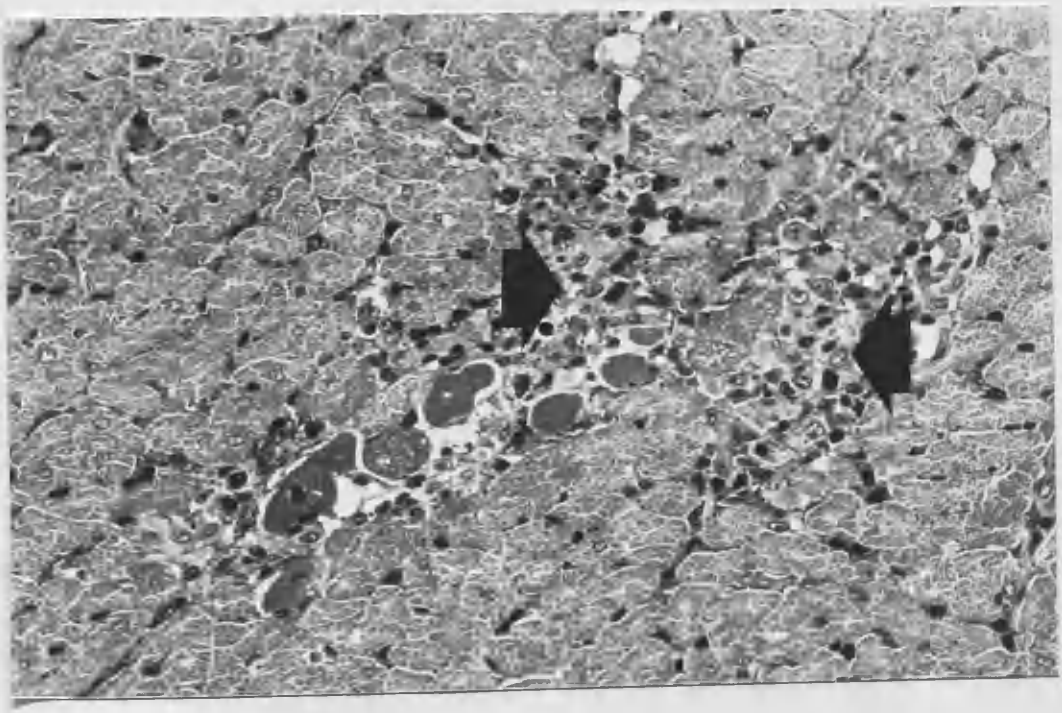
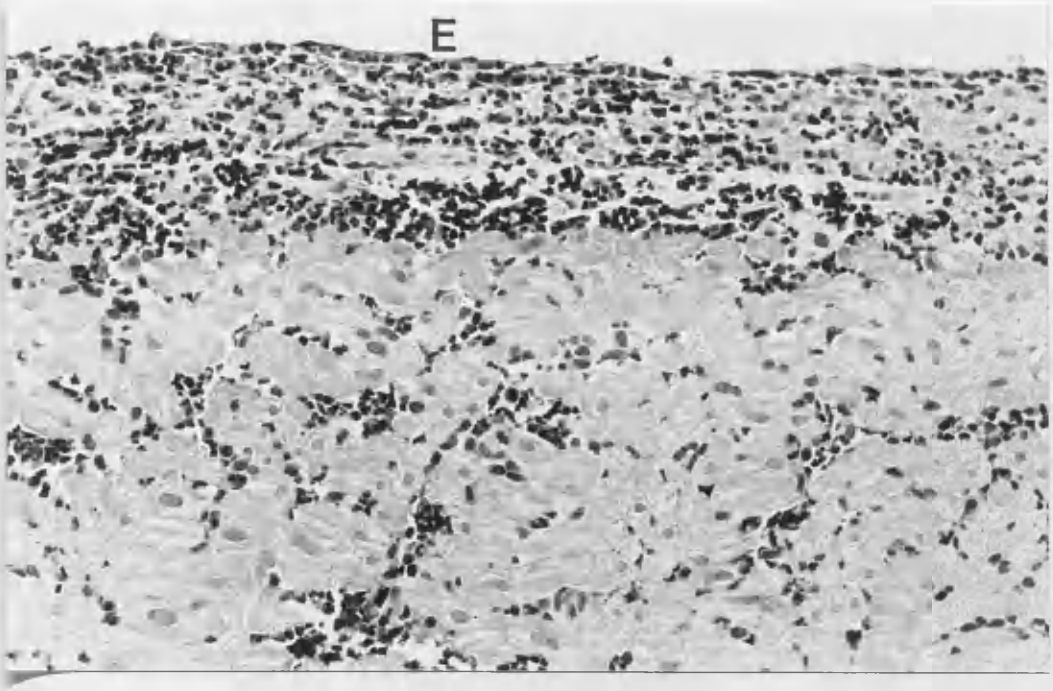


Figure 10.5. Subendocardial and myocardial haemorrhage in the right atrium of a dog infected with T.brucei and treated with cyclosporin A at 5mg/kg intravenously on days 9, 10 and 11, then euthanised on day 15. The endocardium (E) is intact. H&E. x260.

Figure 10.6. Separation of the intercalated disc (arrow) between two myocytes (M) in the left ventricular myocardium of a dog infected with T.brucei and treated with cyclosporin A at a dose of 5mg/kg intravenously on days 8, 9 and 10, then euthanised on day 15.
TEM. x13,400.


E

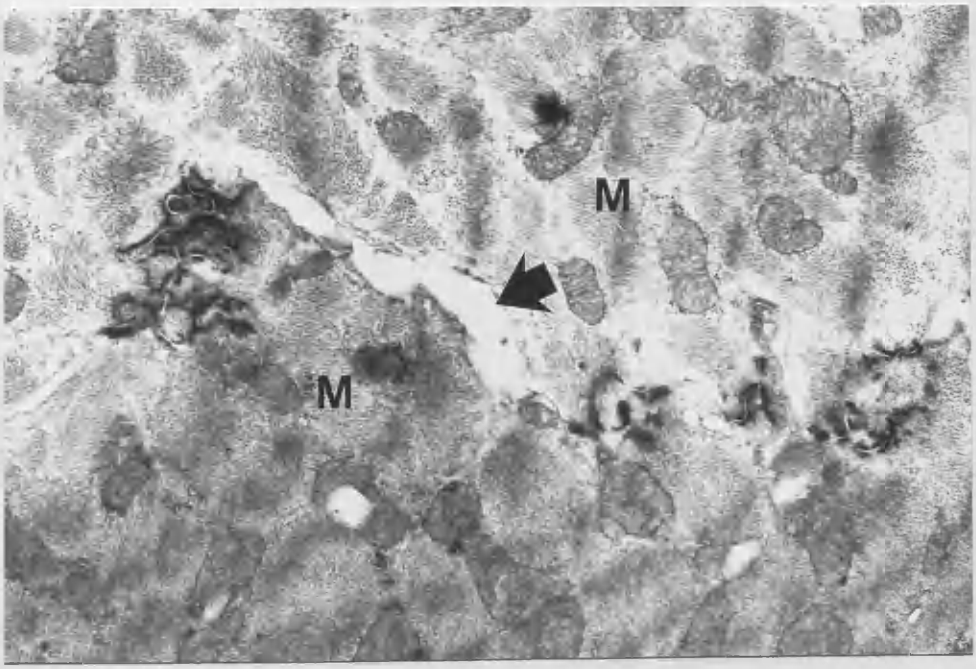
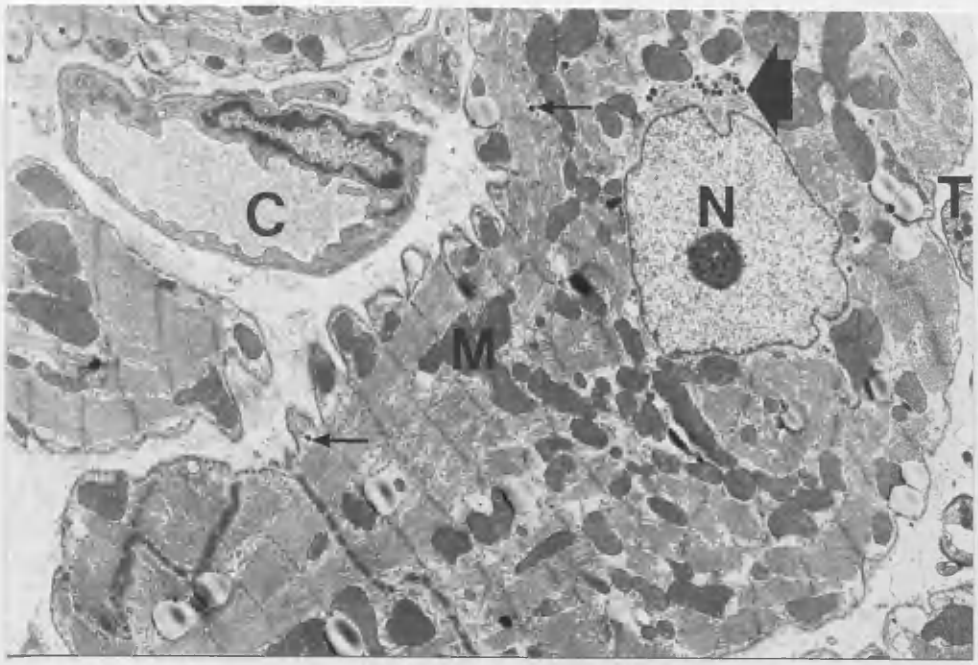

M
M

Figure 10.7. The left atrium of a dog infected with T.brucei and treated with cyclosporin A at a dose of 5mg/kg intravenously on days 8, 9 and 10, then euthanised on day 15. Electron dense atrial natriuretic granules are present in the perinuclear region (large arrow). A few others are scattered in the sarcoplasm (small arrows). N - Myocyte nucleus. C - Capillary. M - Mitochondria.
T - Trypanosome. TEM. x5,400.

Figure 10.8. Focal hepatic necrosis in a dog infected with T.brucei and treated with azathioprine at 10mg/kg orally from day 8 to 12, then euthanised on day 15. There is neutrophil infiltration in the necrotic focus (arrows). H&E. x400.


C
N
T
M

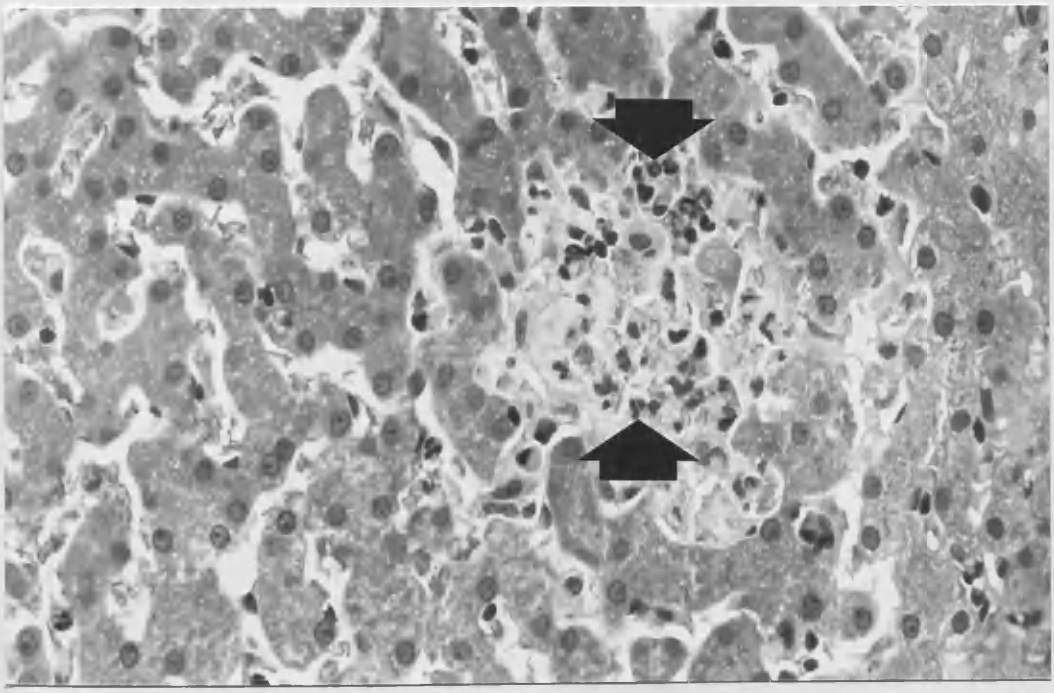
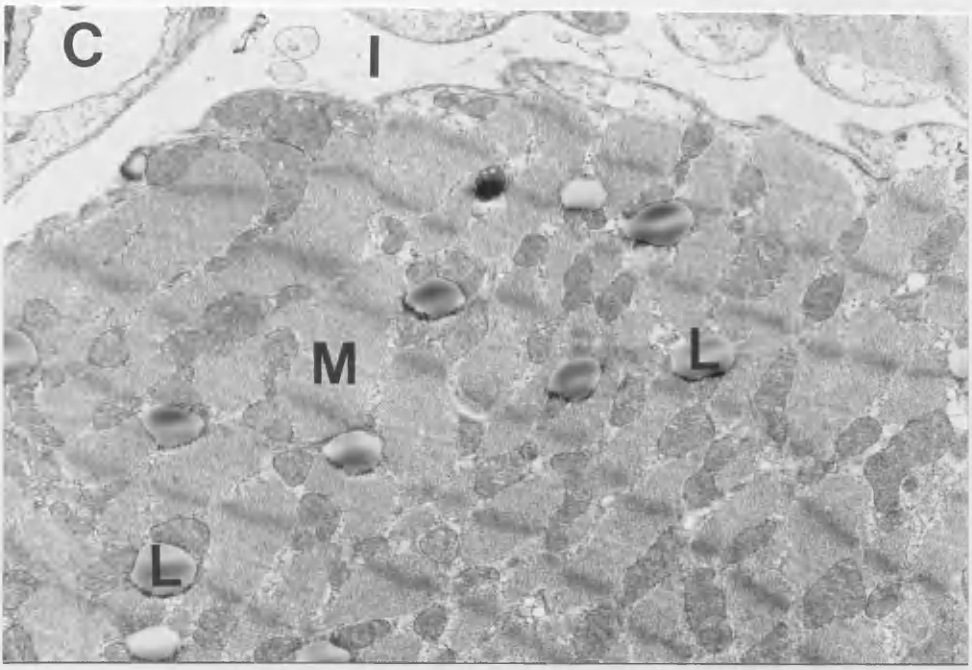


Figure 10.9. The right atrium of a dog infected with T.brucei and treated with azathioprine at 10mg/kg orally from day 8 to 12, then euthanised on day 15. A few lipid droplets (L) are present in the myocyte (M). C - Capillary. I - Intercellular space. TEM. x10,000.

Figure 10.10. Mitochondrial degeneration in the left ventricular myocardium of a dog infected with T.brucei and treated with azathioprine at 10mg/kg orally from day 8 to 12, then euthanised on day 15. The mitochondrion (arrow) is swollen and there are electron lucent areas between disintegrating cristal membranes. There is increased sarcoplasm (S), causing separation of myofibrils (F). R - T tubule. TEM. x40,000.


C
I
M
L
L

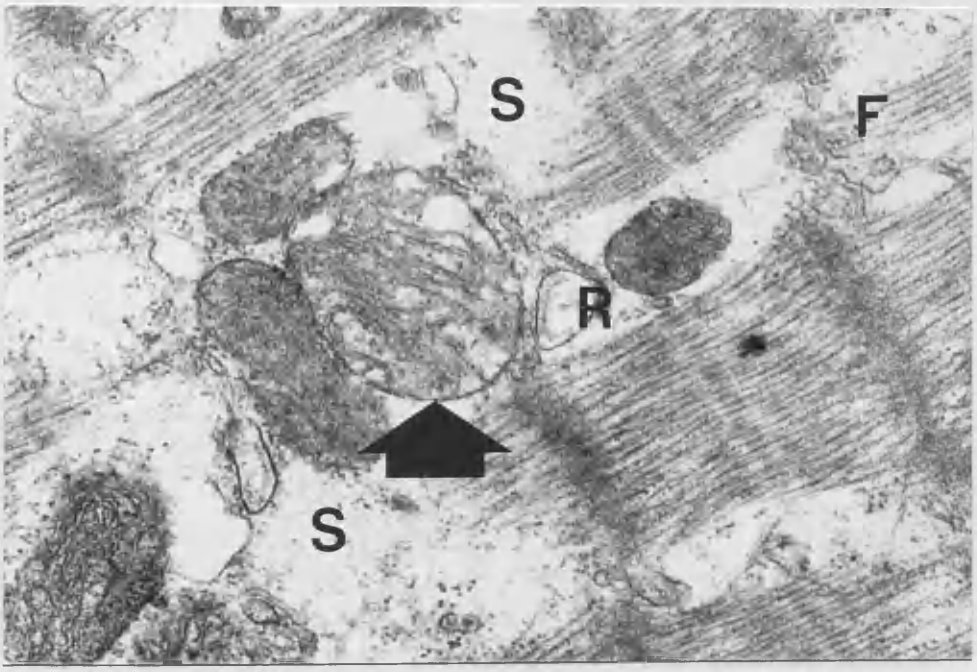
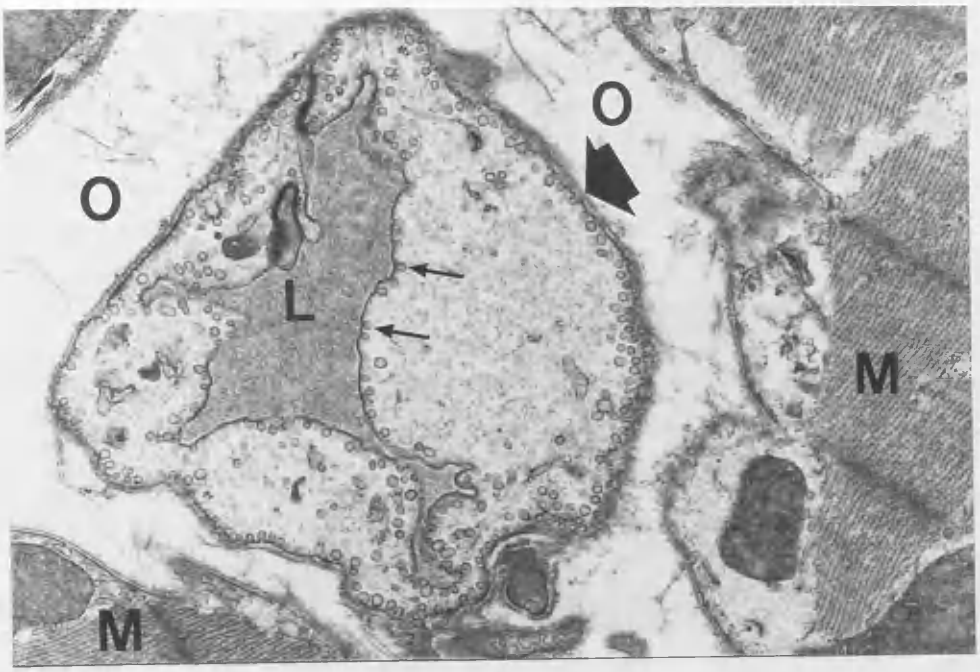

S
F
R
S

Figure 10.11. Swelling of an endothelial cell in the right atrial myocardium of a dog infected with T.brucei and treated with azathioprine at 10mg/kg orally from day 8 to 12, then euthanised on day 15. Fluid accumulation in the endothelial cell has caused stretching and bulging of the plasma membrane on the luminal side, hence the fewer numbers of plasmalemmal vesicles on that side (small arrows). Swelling of the endothelial cell has caused narrowing of the capillary lumen (L). The basal lamina (large arrow) is intact and no protein leakage has taken place. As a result the increased perivascular fluid (O) is electron lucent, in contrast to the more electron-dense protein-rich plasma in the capillary. M - Myocyte. TEM. x20,000.

Figure 10.12. An autonomic nerve ganglion in the right atrial myocardium of a dog infected with T.brucei and treated with azathioprine at 10mg/kg orally from day 8 to 12, then euthanised on day 15. There is increased interstitial fluid (I) in the ganglion. The Schwann cell (S) and nerve axons (A) are not damaged. Schwann cell cytoplasm surrounds the axons uniformly (arrows). L - Lymphocyte. TEM. x8,000.


O
O
L
M
M

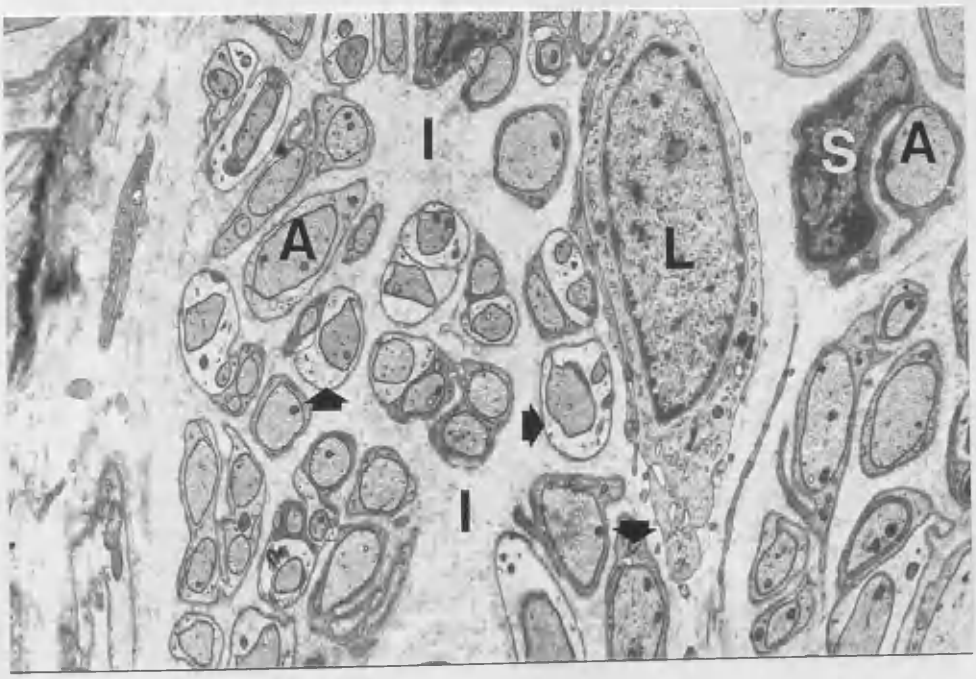

I
S
A
A
L
I

Figure 10.13. Rectal temperatures (●) and parasitaemia (O) in dog 6 following infection with T.brucei and treatment with azathioprine at 5mg/kg daily for 5 days. There was a direct relationship between temperature and parasitaemia, this was unaffected by treatment.

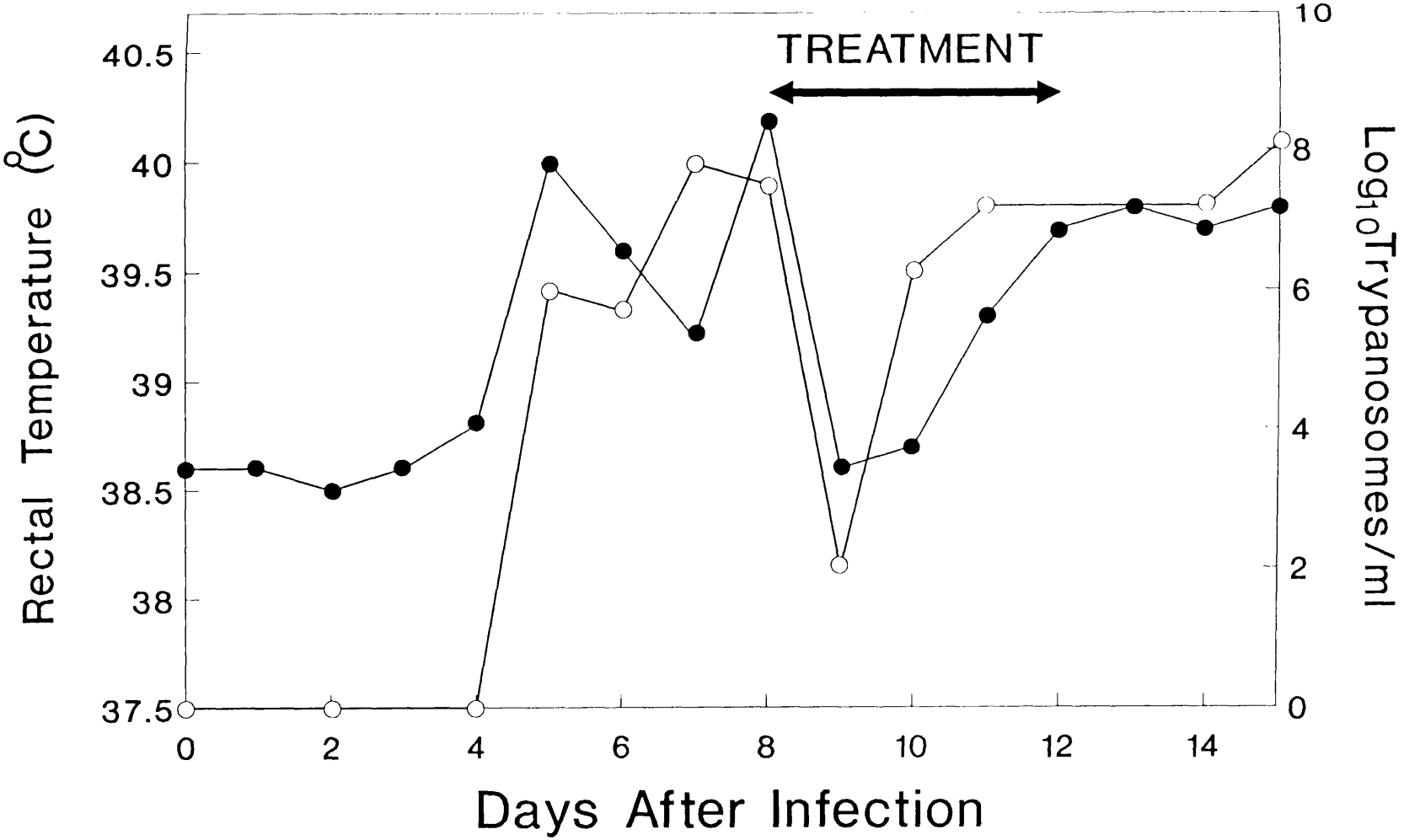

TREATMENT
Rectal Temperature (°C)
40.5
40
39.5
39
38.5
38
37.5
Log10Trypanosomes/ml
10
8
6
4
2
0
0
2
4
6
8
10
12
14
Days After Infection

Figure 10.14. Mean relative percentages of lipoproteins and parasitaemia (O) in dogs infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 8 to 12. There was an increase in very low density lipoproteins (VLDL) (●) and low density lipoproteins (LDL) (□) and decreased high density lipoproteins (HDL) (■) from day 4 of infection. During and after treatment, a return towards normal values of VLDL, LDL and HDL occurred. Chylomicrons (+) were not affected by infection or treatment.

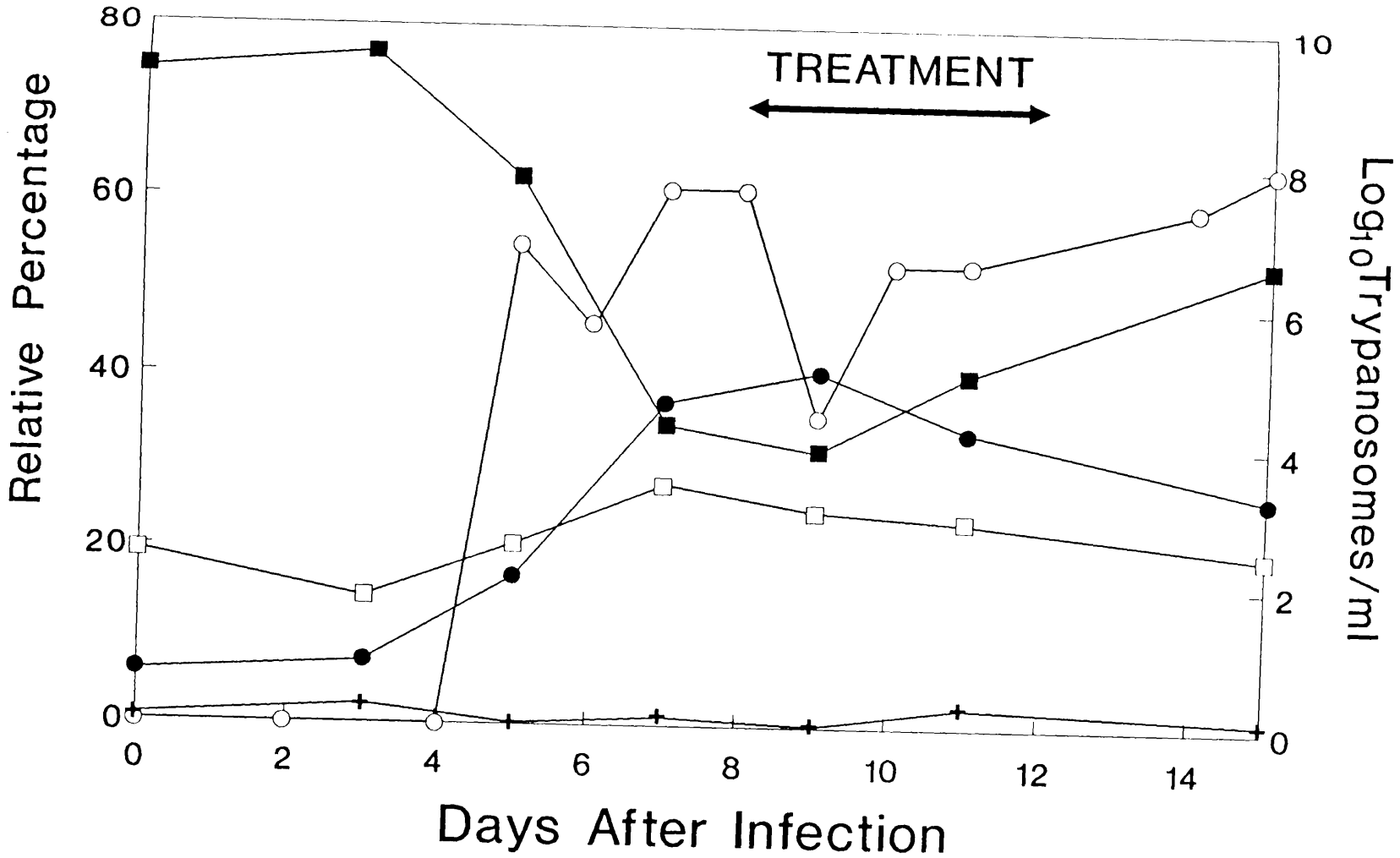
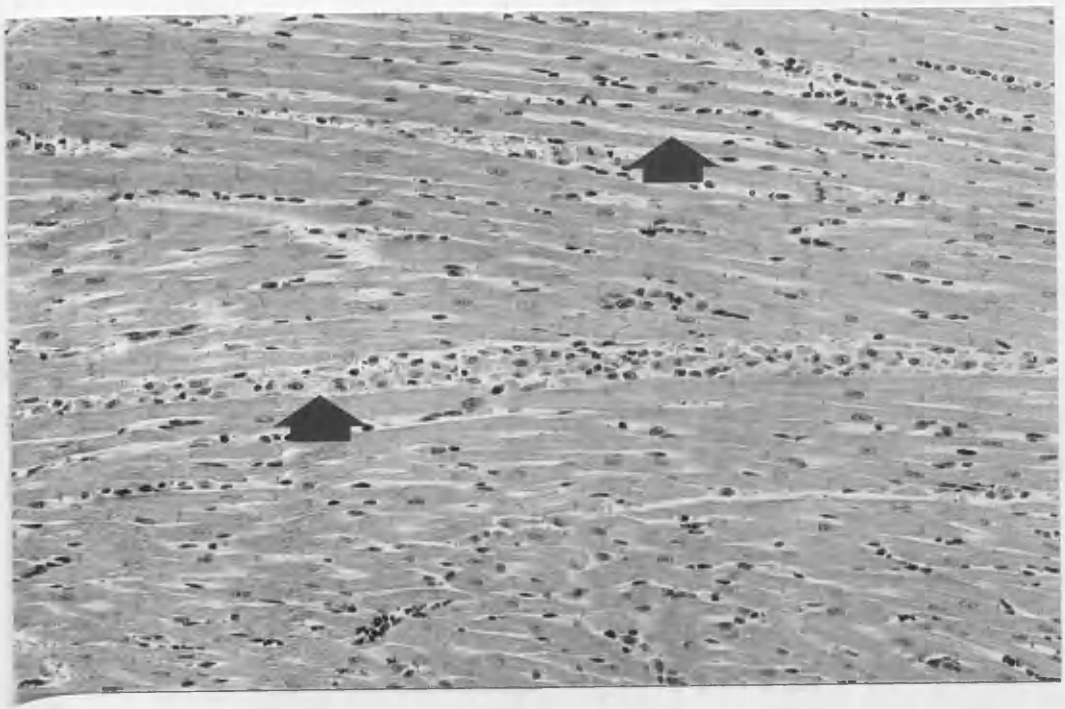

TREATMENT
Relative Percentage
Log10Trypanosomes/ml
Days After Infection
0
20
40
60
80
0
2
4
6
8
10
0
2
4
6
8
10
12
14

Figure 10.15. The left ventricular myocardium of a dog infected with T.brucei and treated with azathioprine at 5 mg/kg orally from day 8 to 12, then euthanised on day 15. There is moderate interstitial oedema (arrows) and cellular infiltration, consisting mainly of macrophages, a few lymphocytes and plasma cells. Trypanosomes are scattered uniformly in the myocardium. H&E. x120.

Figure 10.16. The left ventricular myocardium of a dog infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 8 to 12, then euthanised on day 15. There is oedema (O), increased lipid deposition in myocytes (L) and trypanosome infiltration (T).

C - Capillary. TEM. x5,400.



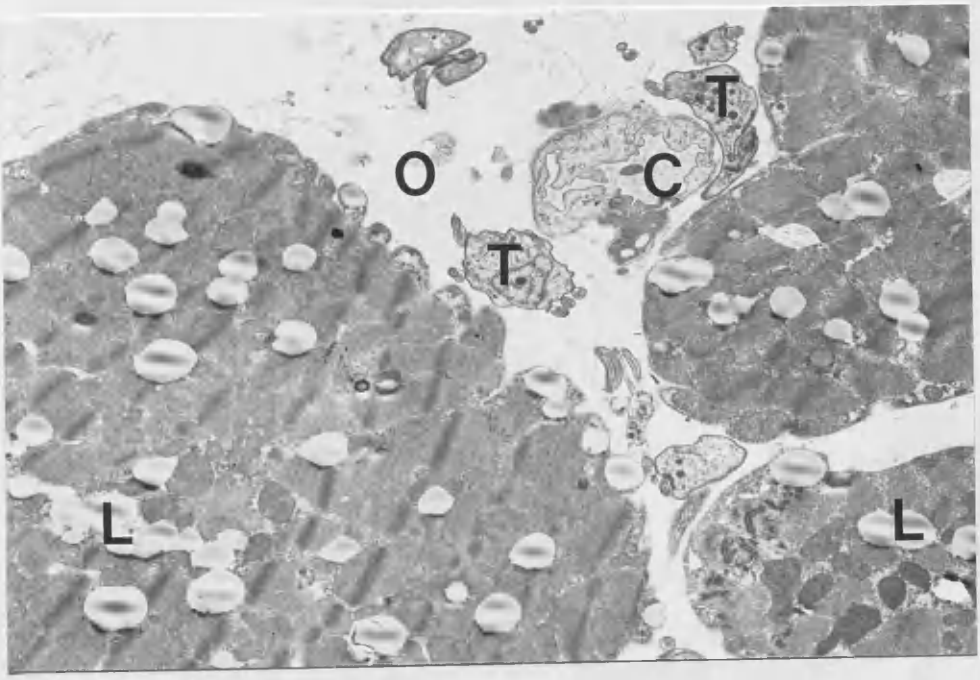
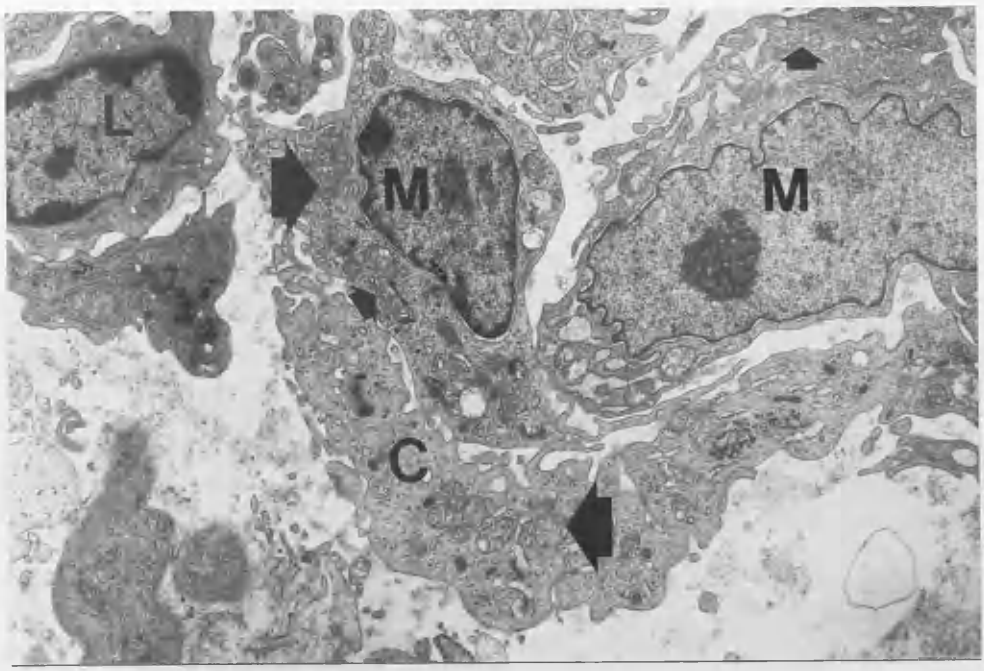

O
C
T
T
L
L

Figure 10.17. Active macrophages (M) in the interstitium of the left ventricle of a dog infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 8 to 12, then euthanised on day 15. The macrophages have many mitochondria (large arrows) and prominent Golgi apparatus (small arrows), indicating increased activity. There are no ingested material in the extended cytoplasmic processes (C). L - Lymphocyte. TEM. x8,000.

Figure 10.18. A two-dimensional short axis view of the heart of dog 8, infected with T.brucei and treated with azathioprine at 5 mg/kg orally from day 3 to 12. The echocardiogram was taken on day 15. The right ventricle (RV) is markedly dilated. LV - Left ventricle.


L
M
M
C

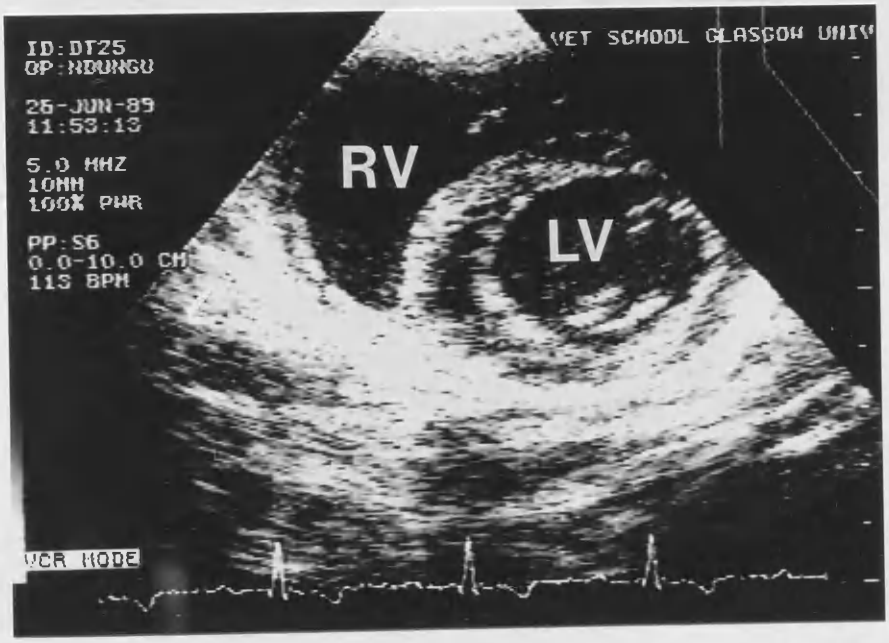
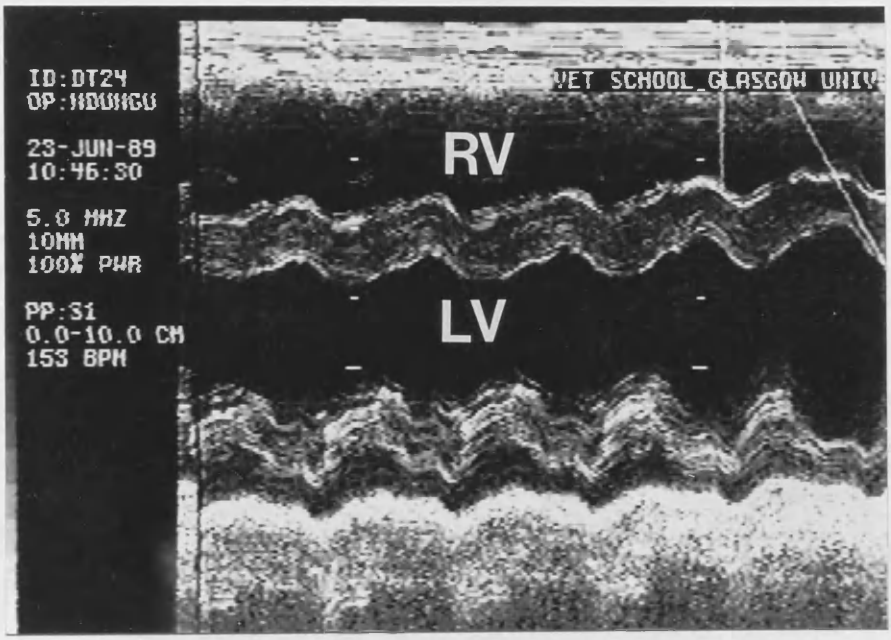

ID:DT25
OP:NDUNGU
26-JUN-89
11:53:13
5.0 MHZ
10MM
100% PWR
PP:S6
0.0-10.0 CM
113 BPM
VET SCHOOL GLASGOW UNIV
RV
LV
VCR MODE

Figure 10.19. An M-mode echocardiogram taken through the heart of the dog in Figure 10.18, showing a dilated right ventricle (RV). LV - Left ventricle.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OP:MDUNGU
23-JUN-89
10:46:30
5.0 MHZ
10MM
100% PWR
PP:31
0.0-10.0 CM
153 BPM
VET SCHOOL GLASGOW UNIV
RV
LV

Figure 10.20. Rectal temperatures (●) and parasitaemia (O) in dog 7 following infection with T.brucei and treatment with azathioprine at 5mg/kg orally from day 3 to 12. While the parasitaemia was persistently high, rectal temperature remained less than 39.5°C during the treatment period in contrast to the infected untreated dogs where rectal temperatures in excess of 40.6°C were recorded.

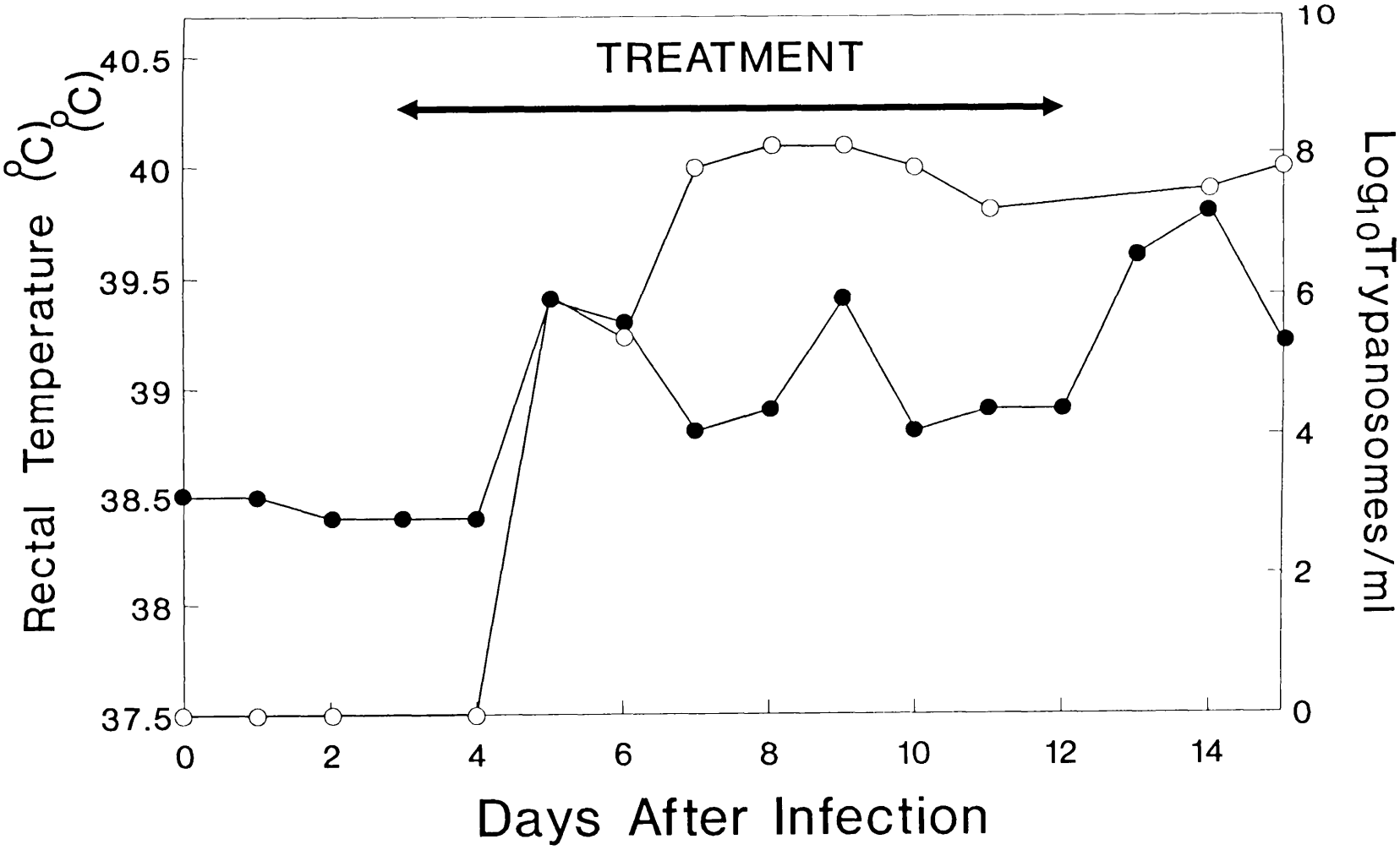

TREATMENT
Rectal Temperature (°C)
(°C)
40.5
40
39.5
39
38.5
38
37.5
0
2
4
6
8
10
12
14
Days After Infection
Log10Trypanosomes/ml
10
8
6
4
2
0

Figure 10.21. Plasma C-reactive protein (CRP) (●) and the parasitaemia (o) in dog 8 following infection with T.brucei and treatment with azathioprine at 5mg/kg orally from day 3 to 12. There was a gradual increase in CRP from day 4. The increase in CRP was not related to the parasitaemia.

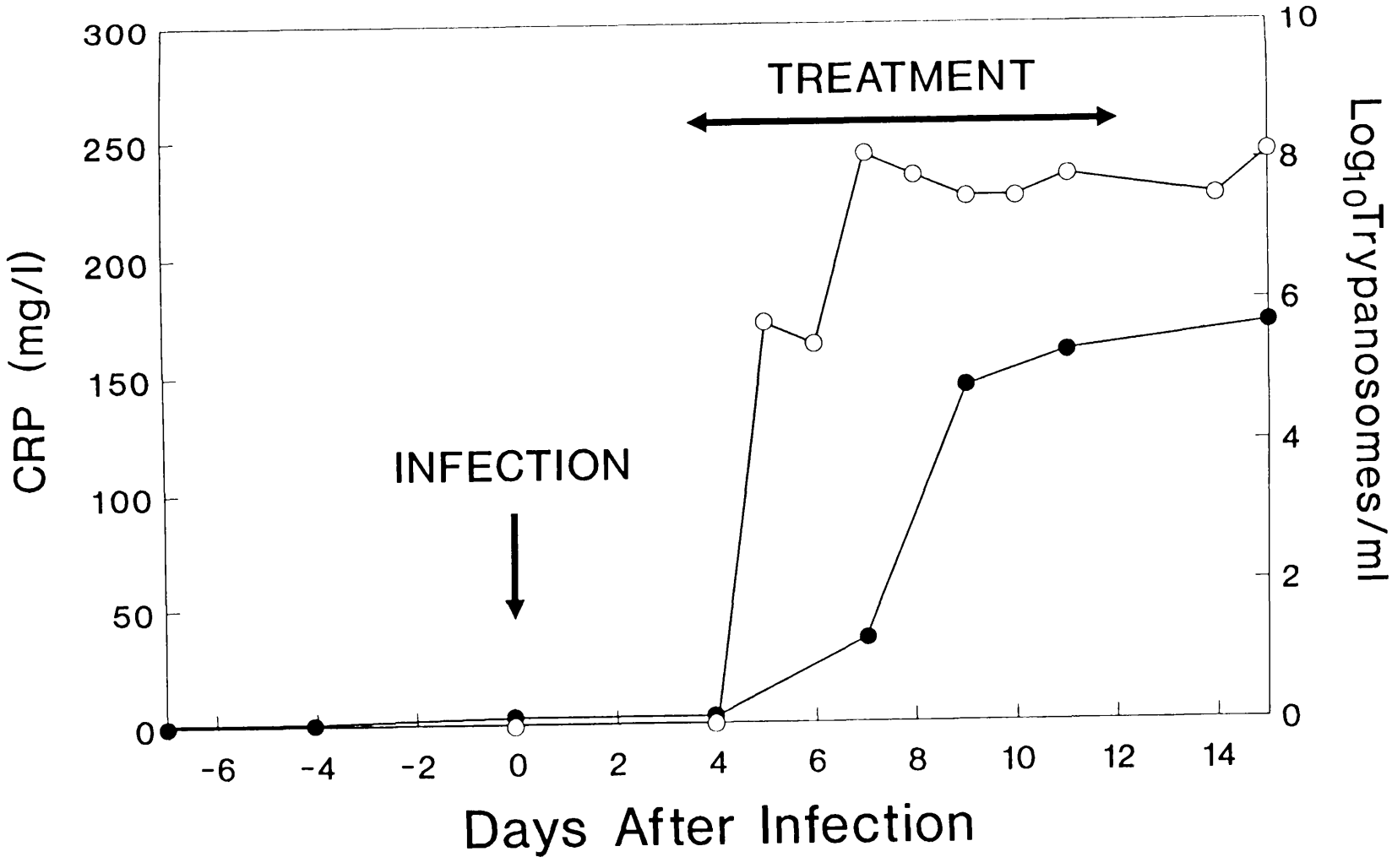

TREATMENT
INFECTION
CRP (mg/l)
Log10Trypanosomes/ml
300
250
200
150
100
50
0
10
8
6
4
2
0
-6
-4
-2
0
2
4
6
8
10
12
14
Days After Infection

Figure 10.22. Mean relative percentages of lipoproteins and parasitaemia (o) in dogs infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 3 to 12. There was an increase in very low density lipoproteins (VLDL) (●) and low density lipoproteins (LDL) (□) and decreased high density lipoproteins (HDL) (■) from day 4 of infection. These changes continued, up to day 9, after which no further changes took place. Chylomicrons (+) were not affected by infection or treatment.

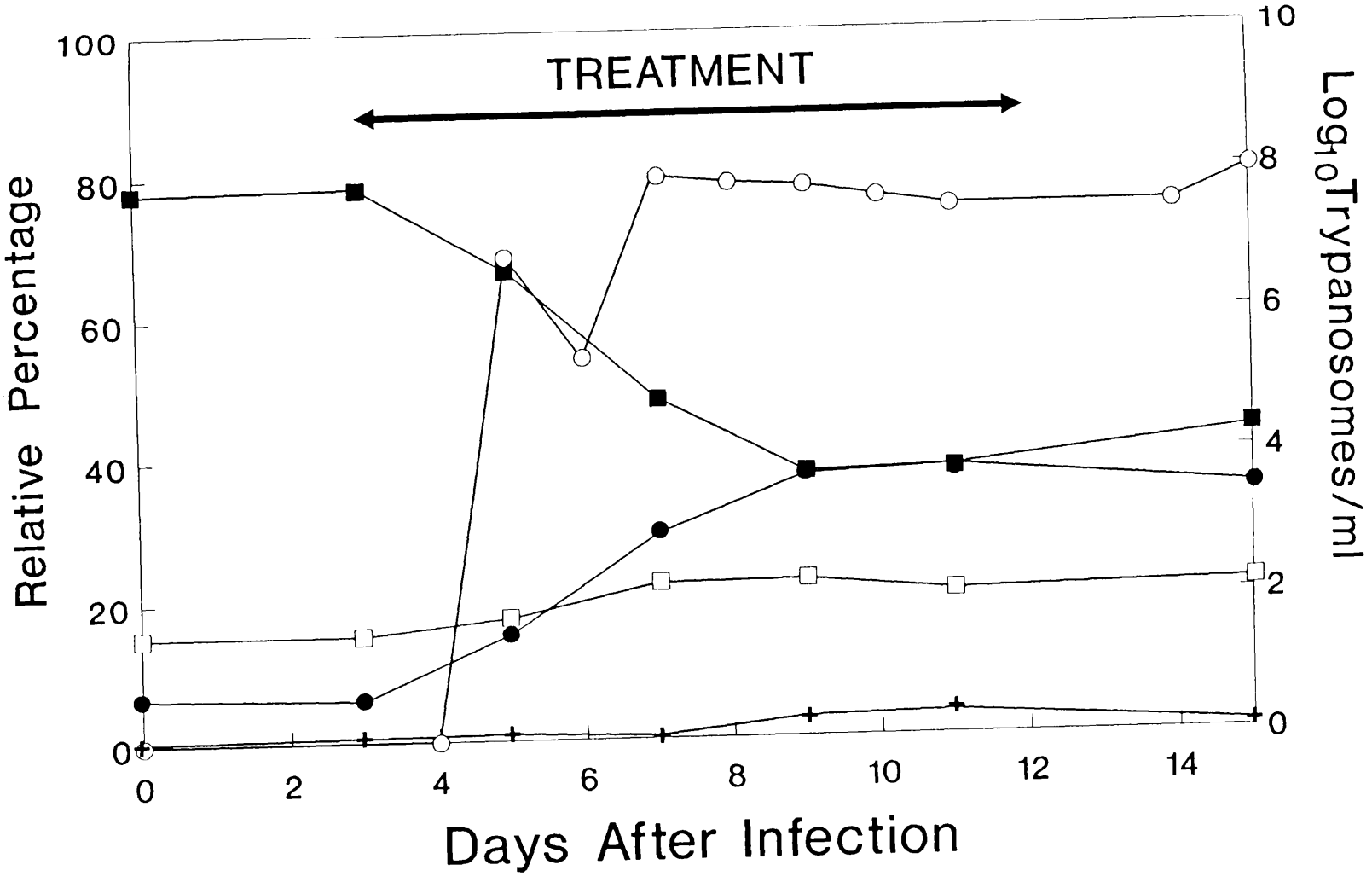

TREATMENT
Relative Percentage
Log10Trypanosomes/ml
Days After Infection
100
80
60
40
20
0
10
8
6
4
2
0
0
2
4
6
8
10
12
14

Figure 10.23. Diffuse fatty degeneration in the liver of a dog infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 3 to 14, then euthanised on day 15. There is minimal hepatocyte necrosis. C - Central vein. H&E. x200.

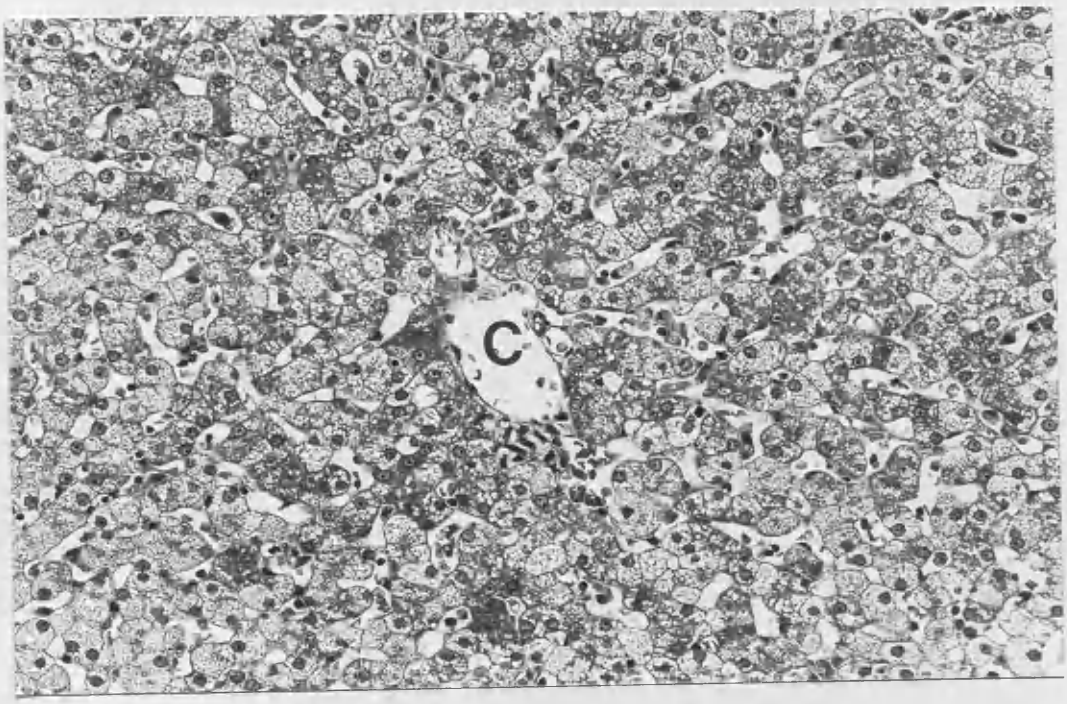
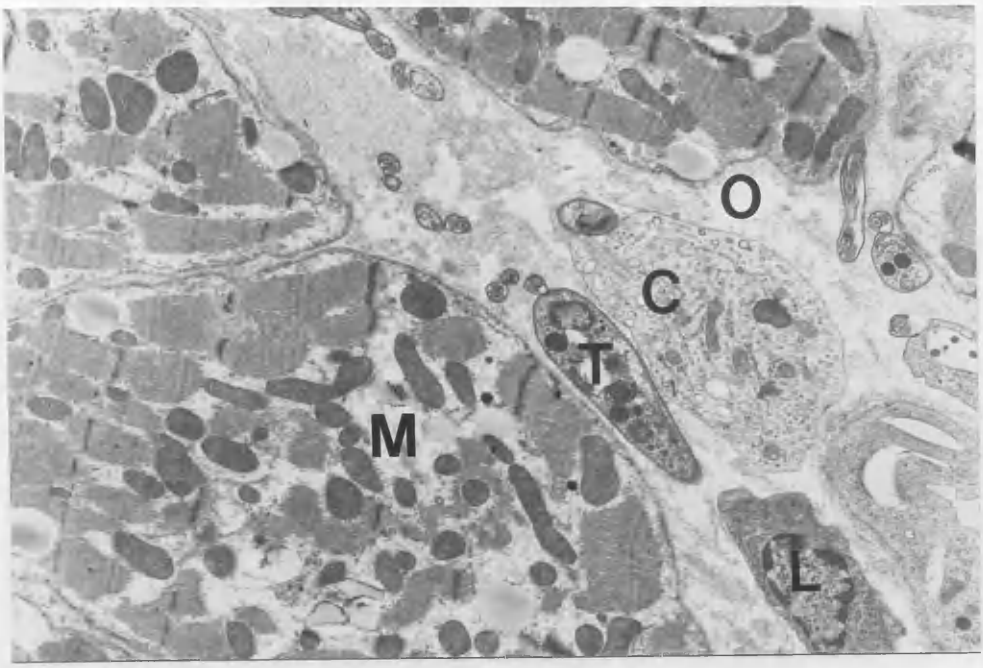

C

Figure 10.24. The right atrial myocardium of a dog infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 3 to 12, then euthanised on day 15. There is swelling of myocytes (M) , oedema (O), macrophage (C), trypanosome (T) and lymphocyte (L) infiltration.
TEM. x8,000.

Figure 10.25. Intense subendocardial trypanosome infiltration (T) in the right atrium of a dog infected with T.brucei and treated with azathioprine at 5mg/kg orally from day 3 to 12, then euthanised on day 15. Some of the trypanosomes are dead (D) and trapped in necrotic debris. TEM. X13,400.


O
C
T
M
L

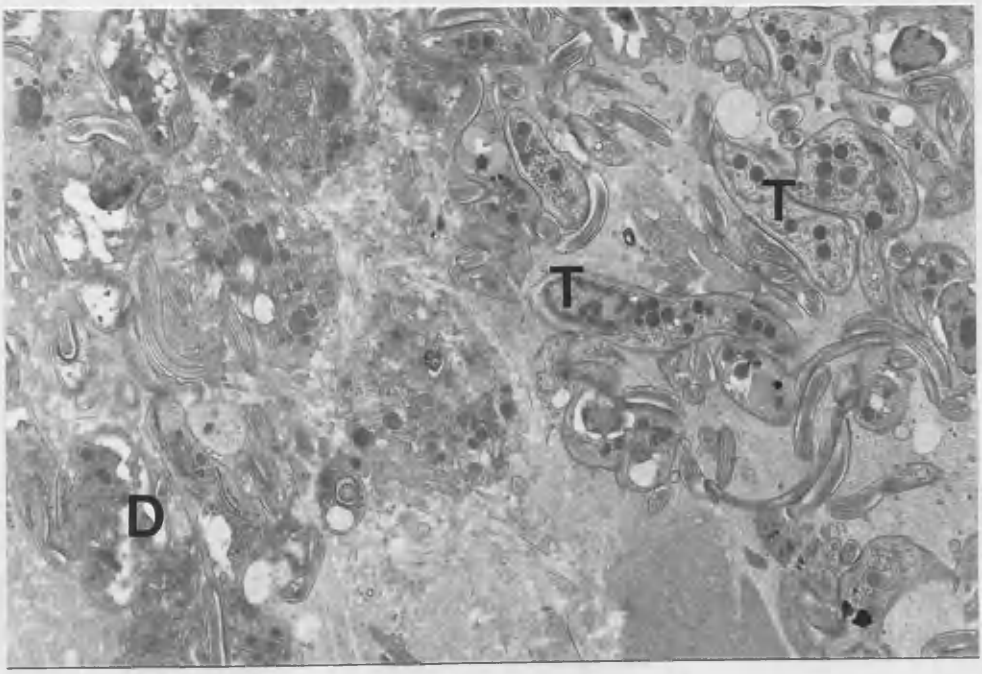
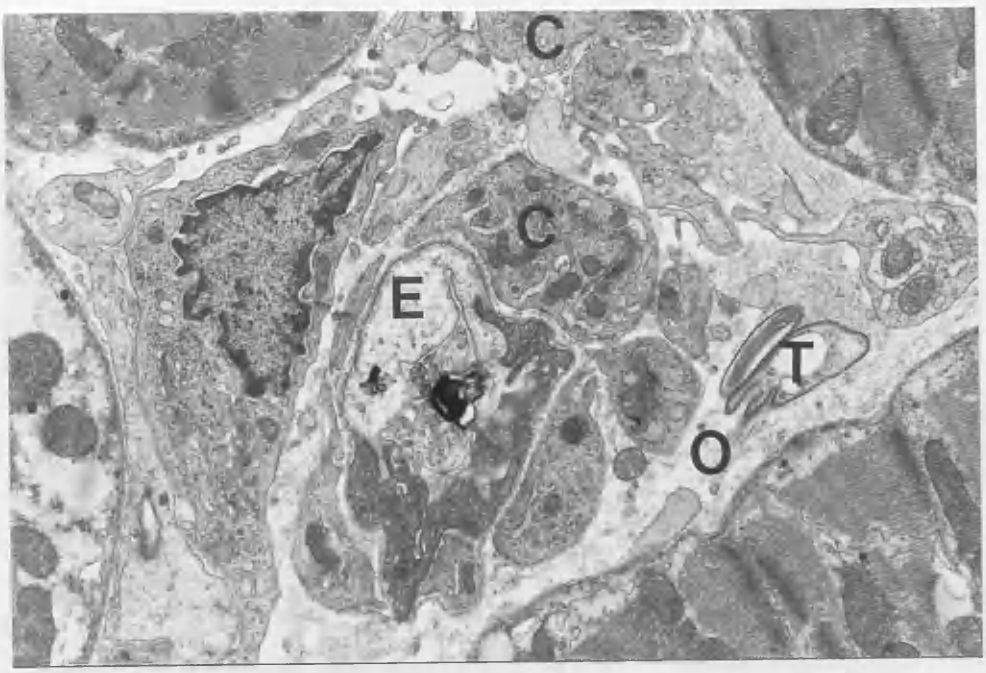

T
T
D

Figure 10.26. Vascular damage in the right atrium of the dog in Figure 10.25. The capillary endothelial cell (E) is undergoing degeneration. Perivascular macrophage (M) and trypanosome (T) infiltration, and oedema (O) has increased external pressure on the capillary, resulting in reduced lumen size. C - Macrophage cytoplasmic processes. TEM. x10,000.

Figure 10.27. The left ventricular myocardium of a dog infected with *T.brucei* and treated with azathioprine at 5mg/kg orally from day 3 to 12, then euthanised on day 15. Two capillaries (C) have been blocked by mononuclear cells (W). There is marked oedema (O) with protein leakage, hence the granular appearance. T - Trypanosome. P - Plasma cell cytoplasm. TEM. x13,400.


C
C
E
T
O

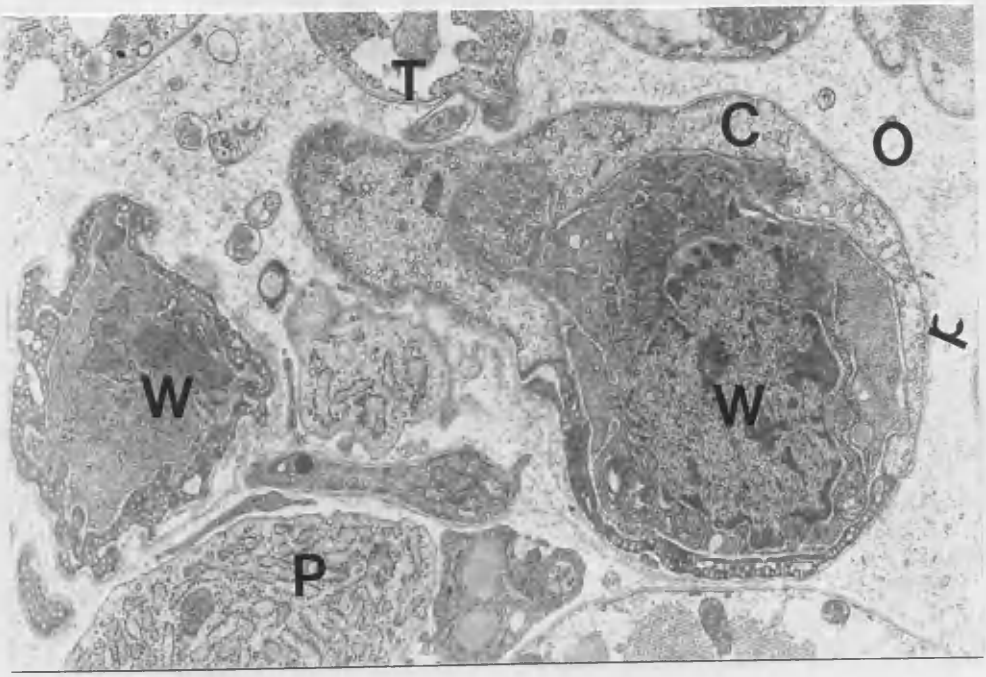
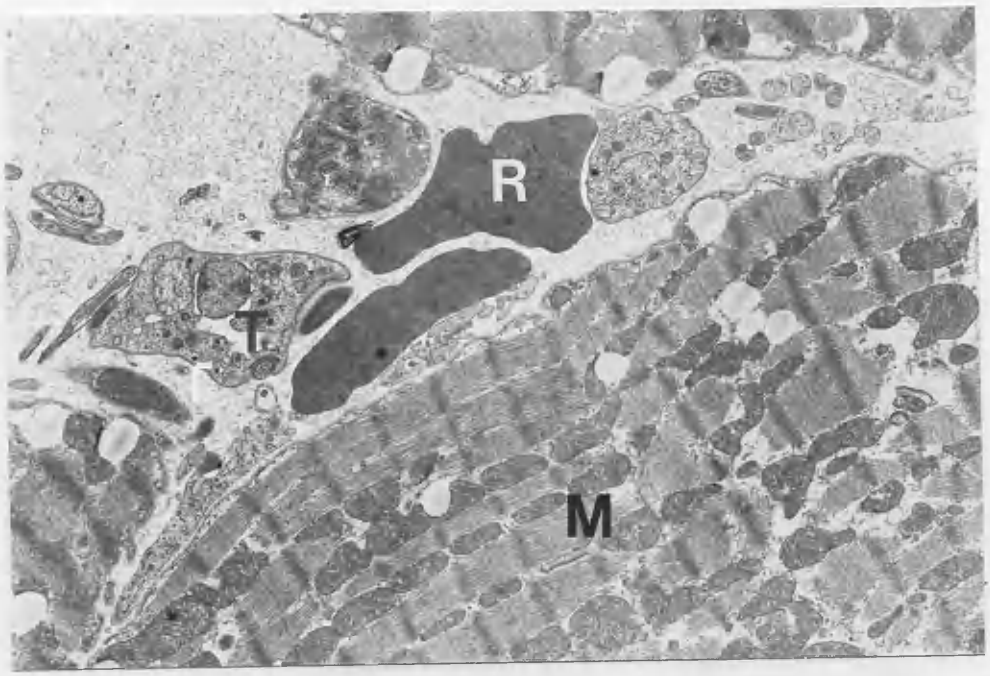

T
C
O
W
W
P

Figure 10.28. The left ventricular myocardium of the dog in Figure 10.27. There is haemorrhage and trypanosome infiltration. The interstitial fluid has a granular appearance due to the high protein content resulting from vascular leakage. R - Red blood cell. M - Myocyte.
TEM. x8,000.

Figure 10.29. The right atrial myocardium of the dog in Figure 10.27, with macrophage cytoplasmic processes in the interstitium . The macrophages are very active, with many, large mitochondria (small arrows), abundant lysosomes (P →), Golgi apparatus (G →) and granular endoplasmic reticulum (R →). Some necrotic debris (N) have been ingested and there is lipid in the cytoplasm (L→) and in the myocytes. One myocyte (large arrow) is swollen, with separation of myofibrils. T - Trypanosome. C - Capillary.
TEM. x5,400.


R
T
M

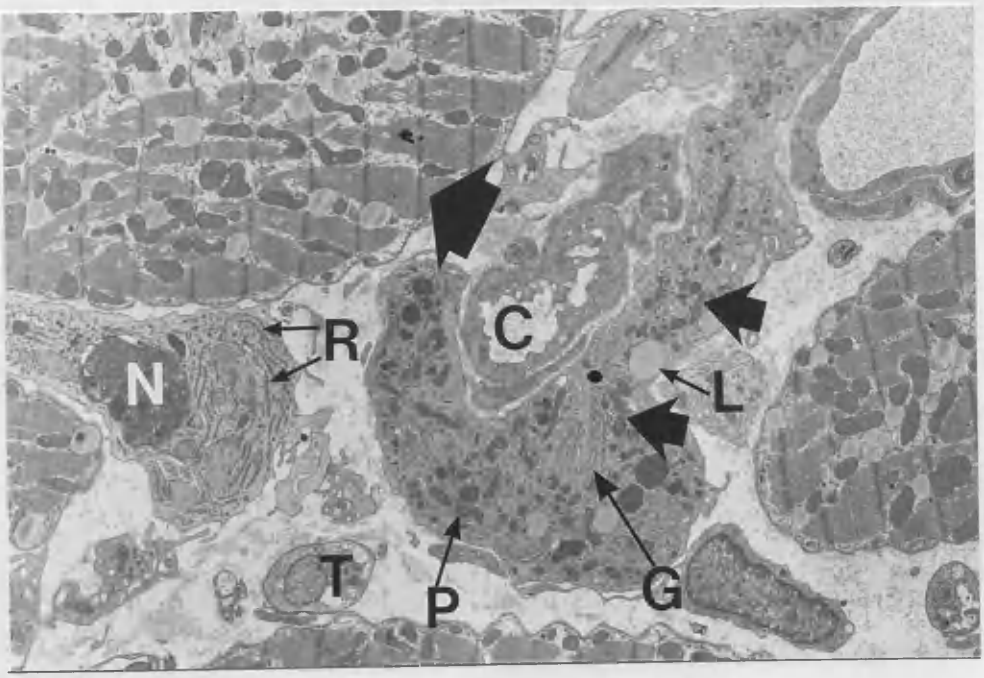
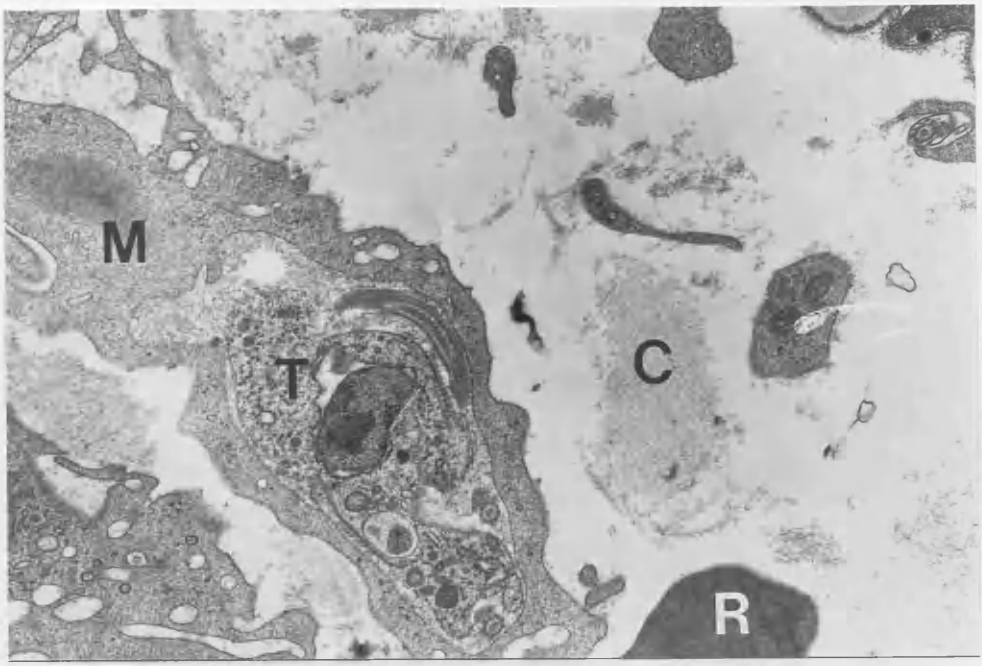

R
C
N
L
T
P
G

Figure 10.30. Phagocytosis of a trypanosome (T) in the right atrial myocardium of the dog in Figure 10.27. The trypanosome is surrounded by macrophage cytoplasm (M). C - Collagen. R - Red blood cell. TEM. x16,000.

Figure 10.31. The left ventricular myocardium of a dog infected with *T.brucei* and treated with azathioprine at 5mg/kg orally from day 3 to 14, then euthanised on day 15. There is marked interstitial oedema (O) and lipid deposition (L) in the myocytes. The interstitial fluid is electron lucent because it has a low protein content. N - Myocyte nucleus. R - Red blood cell in a capillary. TEM. x5,400.


M
T
C
R

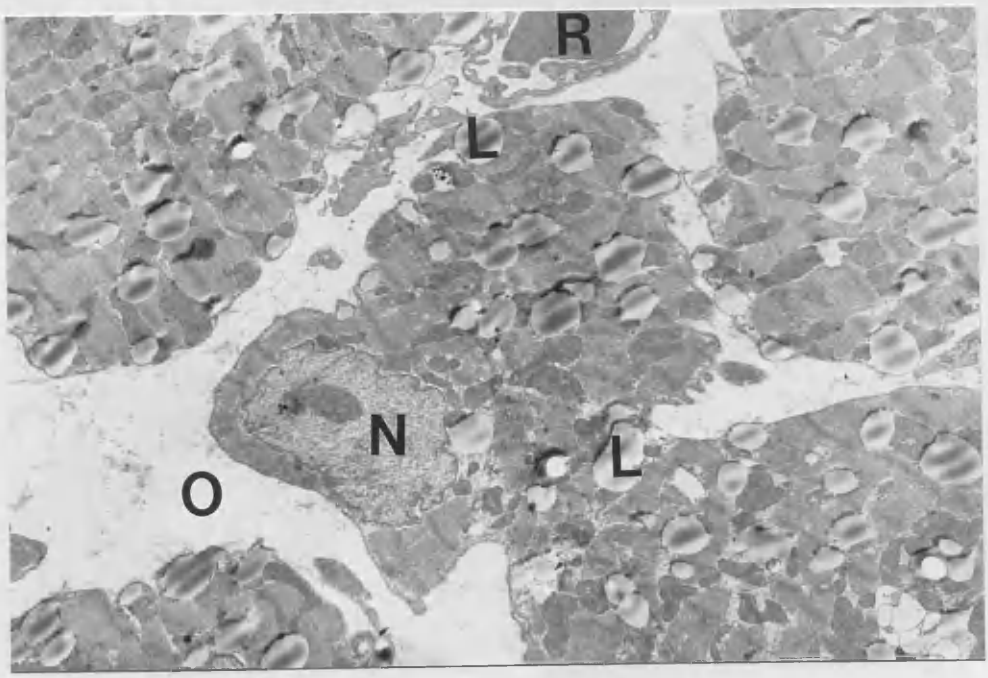

R
L
N
L
O

Figure 10.32. Mean rectal temperatures in dogs infected with T.brucei and treated with azathioprine (5mg/kg from day 3 to 14) and prednisolone (2mg/kg from day 3 to 9, then 1mg/kg from day 10 to 14) (●), and in infected untreated dogs (O). In the treated dogs, after reaching peak levels on day 5 of infection, temperature dropped to pre-infection values.

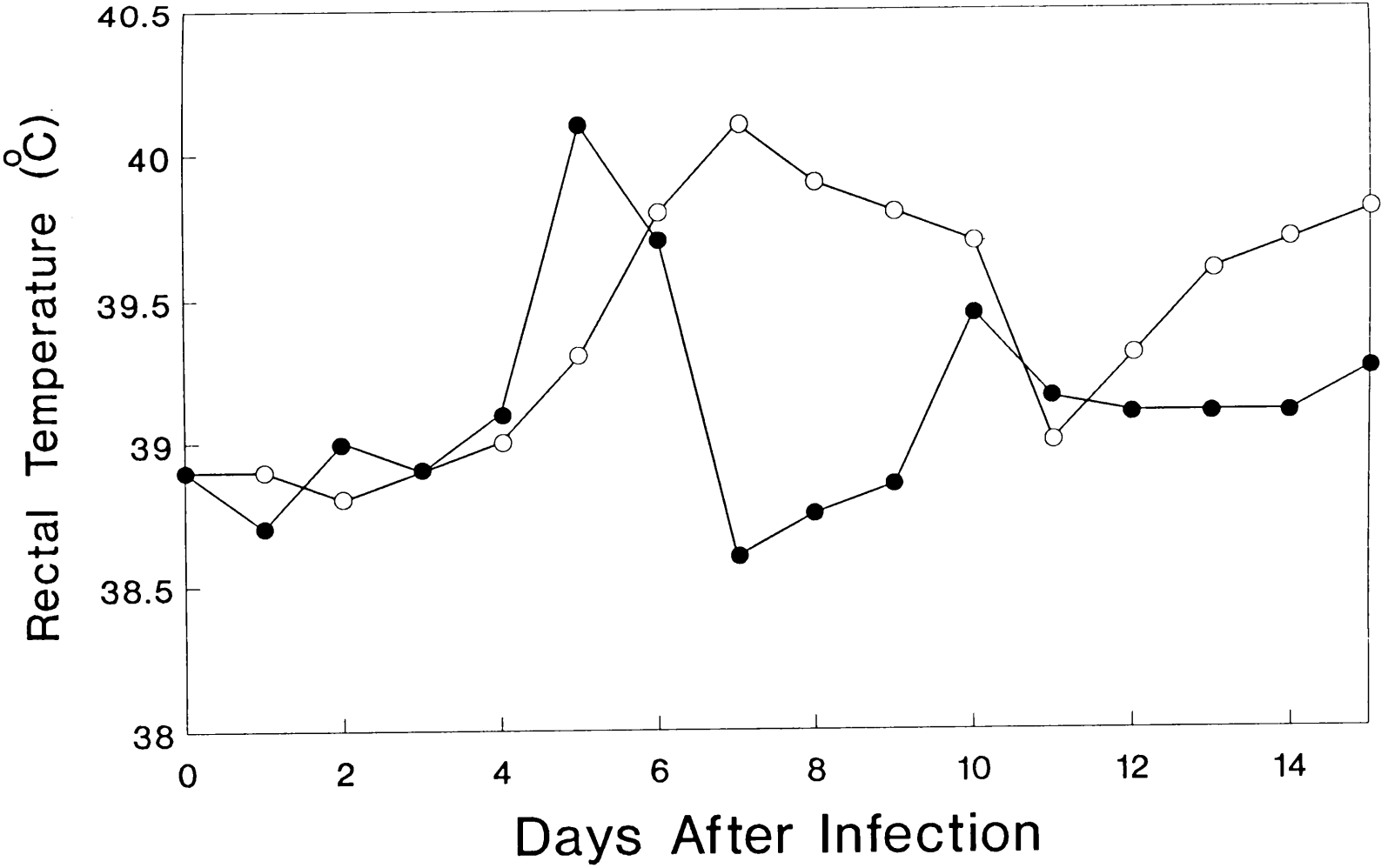

Rectal Temperature (°C)
40.5
40
39.5
39
38.5
38
0
2
4
6
8
10
12
14
Days After Infection

Figure 10.33. Parasitaemia in dogs infected with T.brucei and treated with azathioprine (5mg/kg from day 3 to 14) and prednisolone (2mg/kg from day 3 to 9, then 1mg/kg from day 10 to 14) (●), and in infected untreated dogs (O). The onset of parasitaemia was earlier and higher in the treated than in untreated dogs.

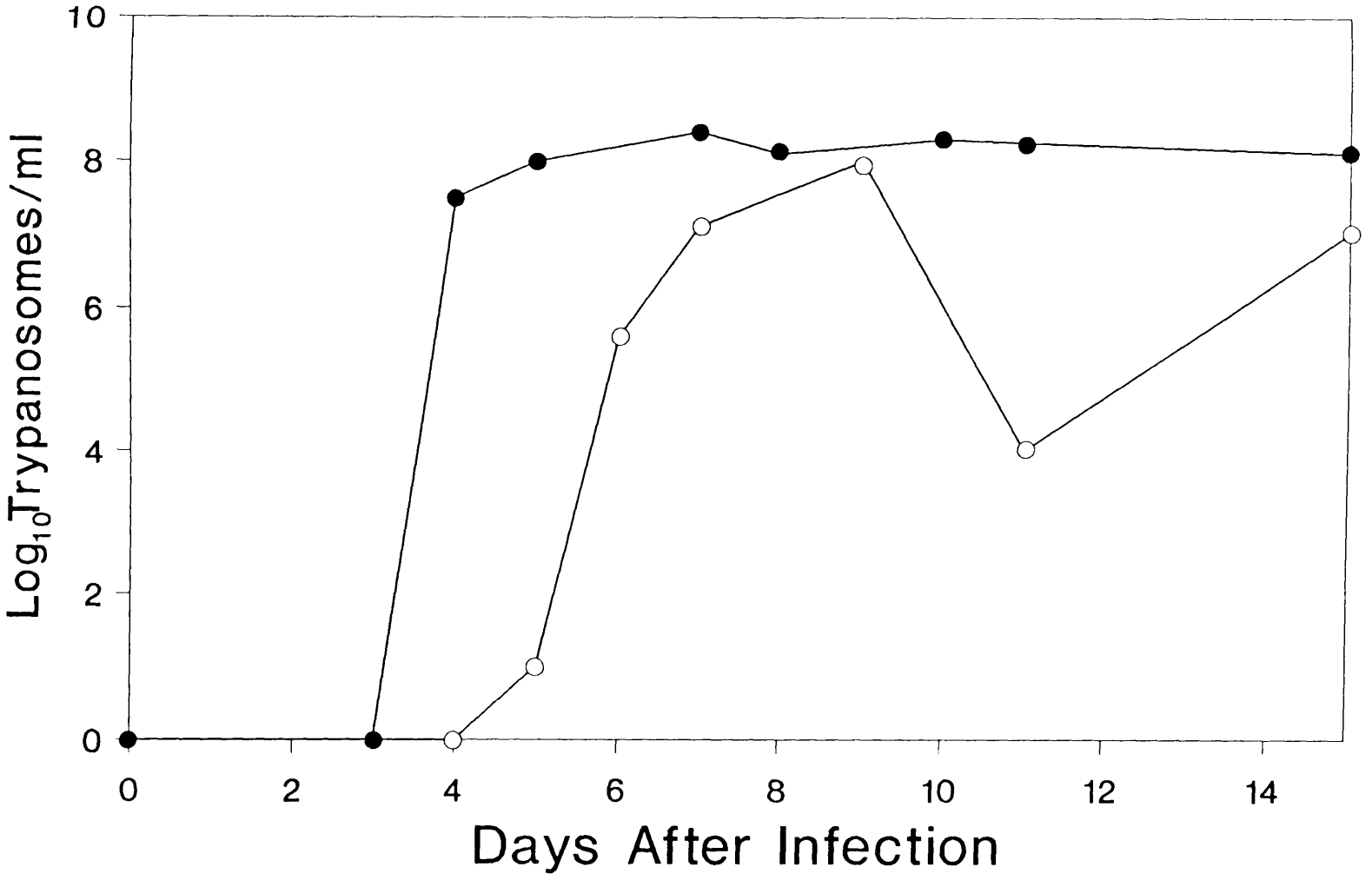

10
8
6
4
2
0
Log10Trypanosomes/ml
0
2
4
6
8
10
12
14
Days After Infection

Figure 10.34. Plasma C-reactive protein (CRP) in untreated dogs (▩), dogs treated with azathioprine from day 3 to 14 (■), and dogs treated with a combination of azathioprine and prednisolone from day 3 to 14 (▨) after infection with T.brucei. CRP in dogs treated with both drugs was higher than in dogs treated with azathioprine alone, and in untreated dogs.

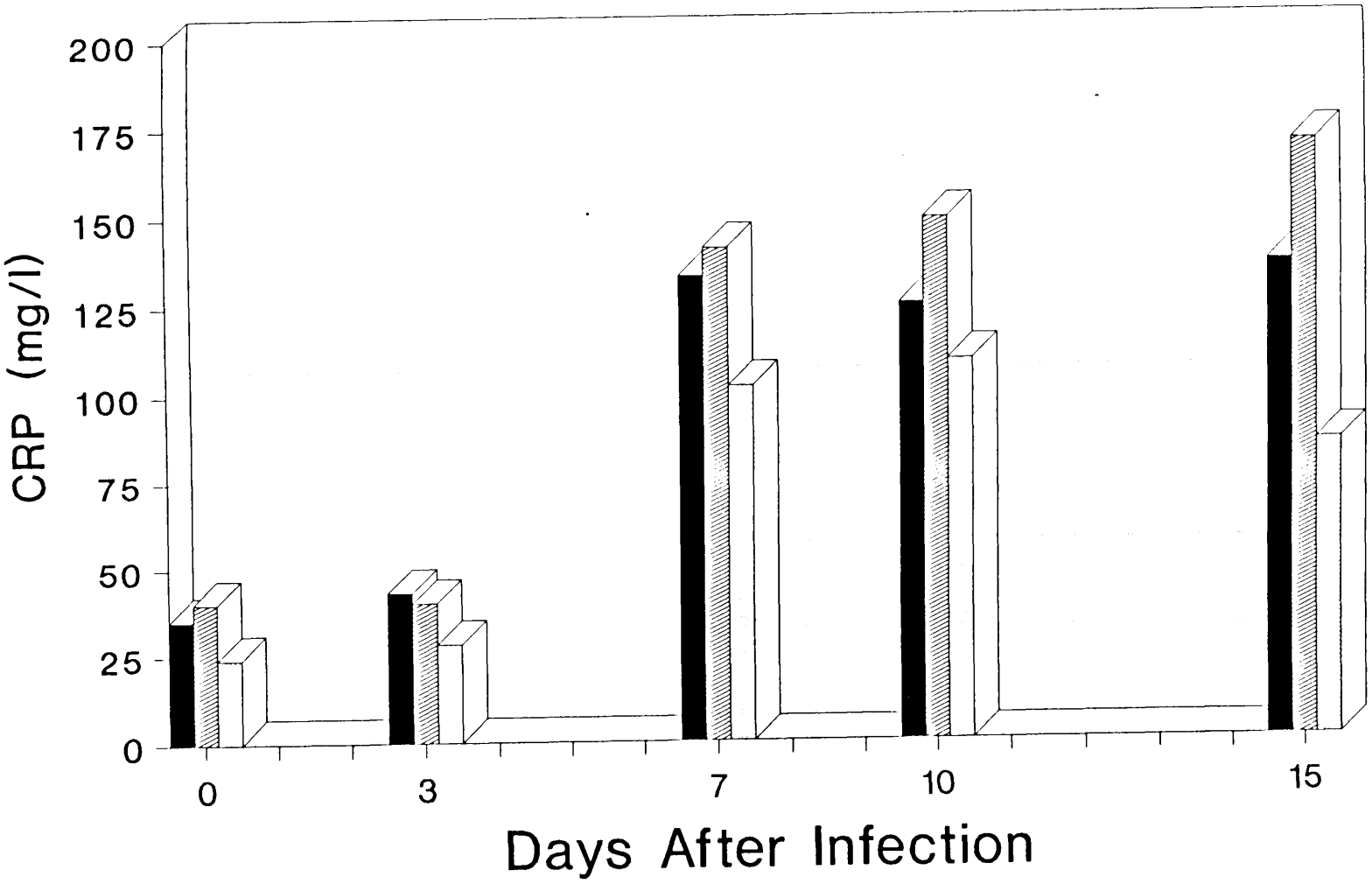

CRP (mg/l)
200
175
150
125
100
75
50
25
0
0
3
7
10
15
Days After Infection

Figure 10.35. Plasma haptoglobin (Hp) in untreated dogs (▩), dogs treated with azathioprine from day 3 to 14 (■), and dogs treated with a combination of azathioprine and prednisolone from day 3 to 14 (▨) after infection with T.brucei. Hp in dogs treated with both drugs was higher than in dogs treated with azathioprine alone, and in untreated dogs.

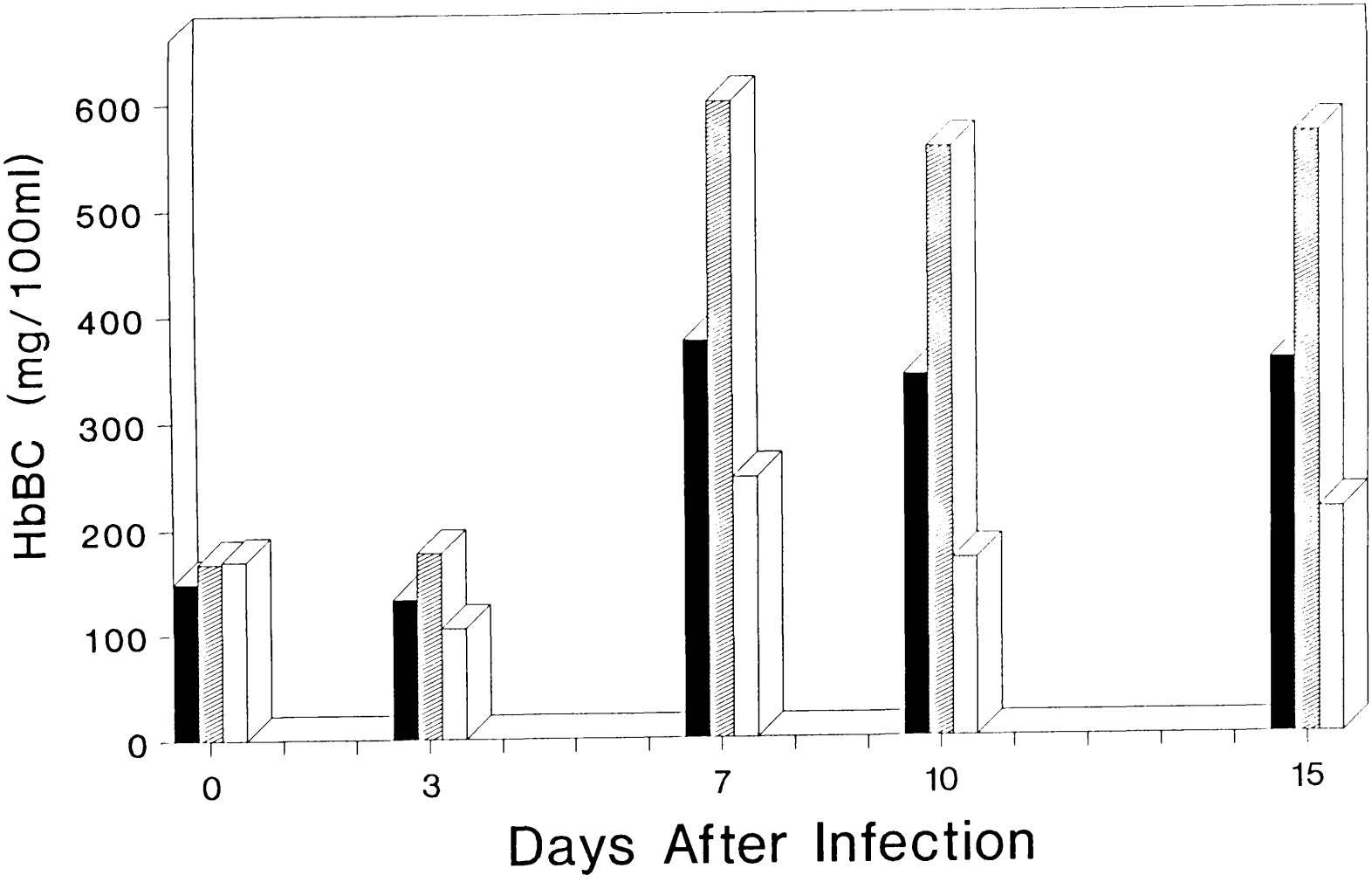

HbBC (mg/100ml)
600
500
400
300
200
100
0
0
3
7
10
15
Days After Infection

Figure 10.36. Plasma albumin concentration in untreated dogs (▩), dogs treated with azathioprine from day 3 to 14 (■), and dogs treated with a combination of azathioprine and prednisolone from day 3 to 14 (▨) after infection with T.brucei. The decline in albumin concentration in dogs treated with both drugs was less than in dogs treated with azathioprine alone, and in infected untreated dogs.

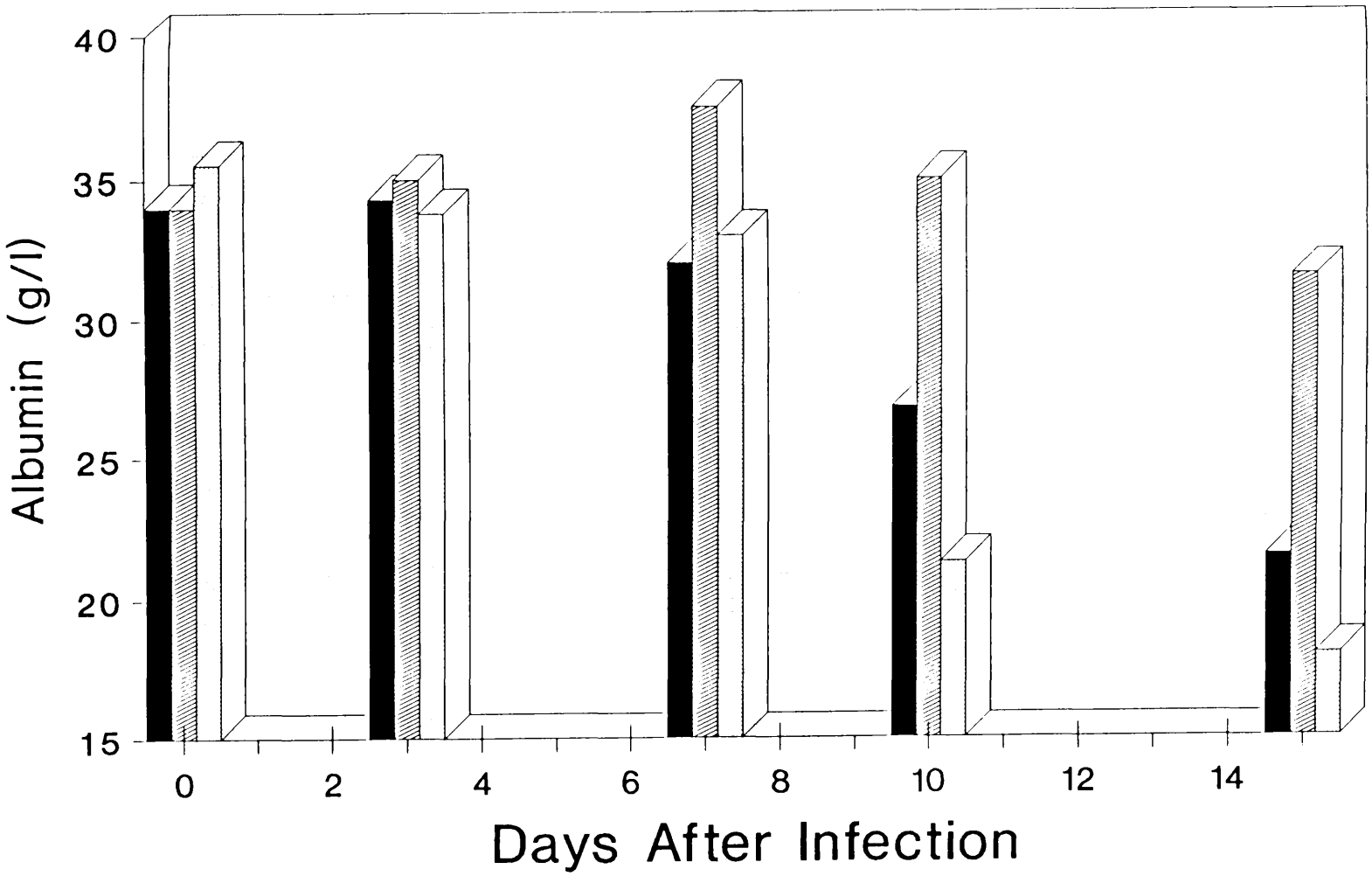
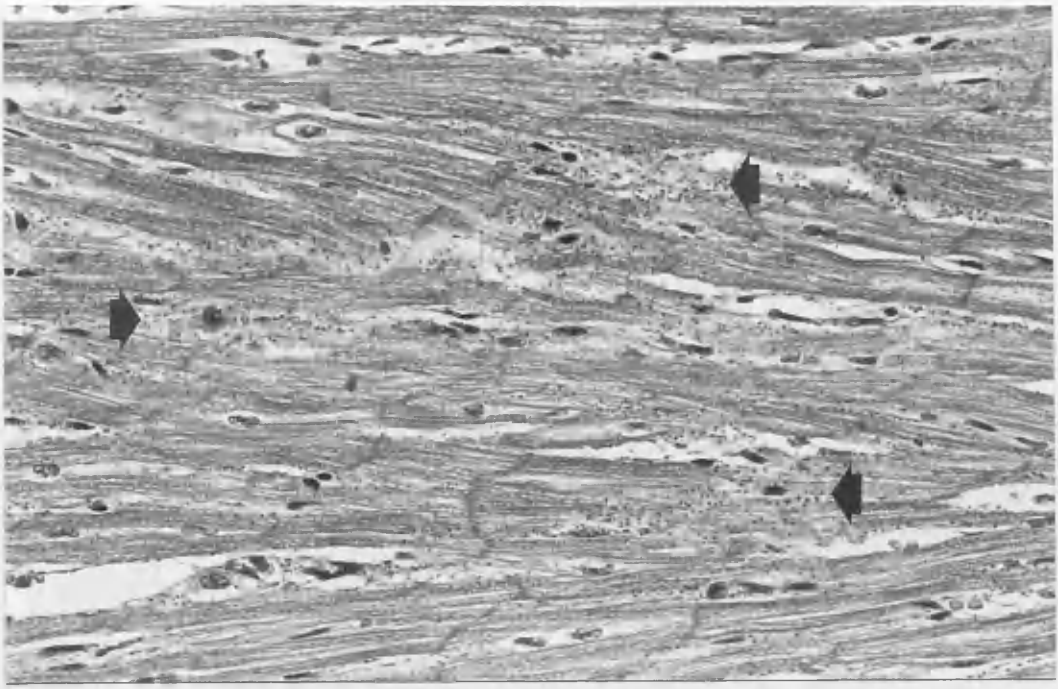
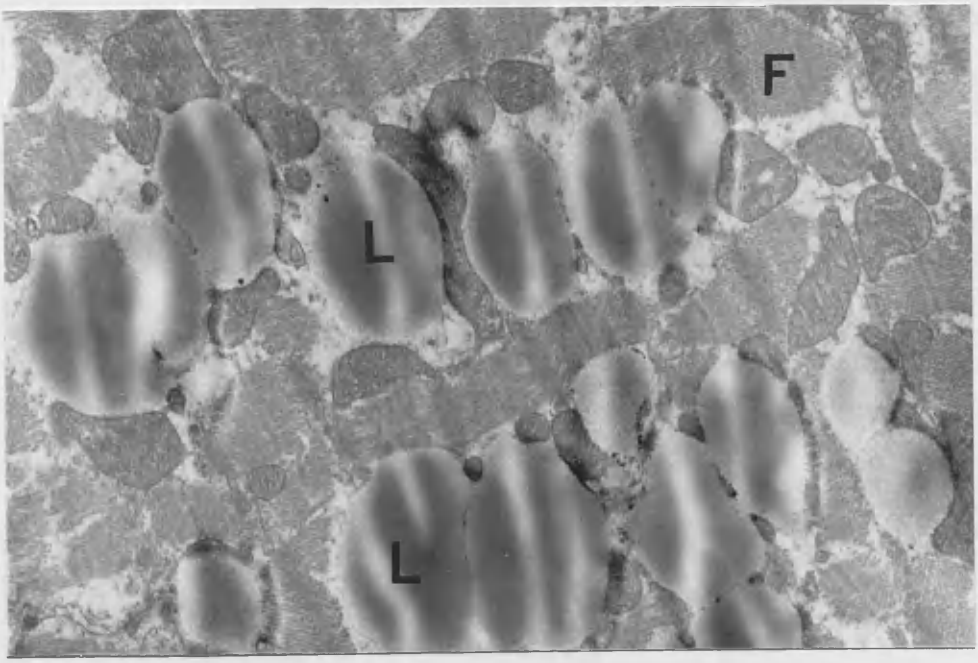

Albumin (g/l)
40
35
30
25
20
15
0
2
4
6
8
10
12
14
Days After Infection

Figure 10.37. The left ventricular myocardium of a dog infected with T.brucei and treated with azathioprine (5mg/kg daily from day 3 to 14) and prednisolone (2mg/kg from day 3 to 9; 1mg/kg from day 10 to 14) orally, then euthanised on day 15. There is diffuse trypanosome infiltration throughout the myocardium (arrows). There is no cellular infiltration. H&E. x260.

Figure 10.38. Myocyte lipid deposition in the left ventricular myocardium of the dog in Figure 10.37. There are large lipid droplets (L) in the myocyte.
F - Myofibrils. TEM. x16,000.




F
L
L

PART IV.

GENERAL SUMMARY AND CONCLUSIONS.

A review of the cardiac disease in dogs caused by T.brucei, and that in humans infected with T.rhodesiense and T.gambiense revealed marked similarities in their clinical and pathological manifestations. Although the severity of myocardial damage in dogs is greater than that in humans, it would appear that the underlying mechanisms of tissue damage are similar. Cardiac damage and death in African trypanosomiasis appears to be mediated through biologically active substances, which are released by the host in immunologically mediated hypersensitivity reactions, or generated by dying trypanosomes. In order to improve the clinical management of both canine and human African trypanosomiasis, it was necessary that the mechanisms of tissue damage be fully understood, and parameters that would allow for objective assessment of clinical progress and prognosis be identified. The large size of the dog, its ease of adaptation to a laboratory environment and handling by man, and the similarity of the cardiac disease to that in man made it ideal for this work.

The advent of Doppler echocardiography has added a new dimension to non-invasive diagnostic veterinary cardiology. Previously, this was limited to palpation, auscultation, electrocardiography (ECG) and radiography. The present work improved, among others, the understanding and application of pulsed-wave (PW) and continuous-wave (CW) Doppler echocardiography as a diagnostic tool in dogs. The ideal acoustic window is one that would allow for linear measurements of cardiac structures, and Doppler studies for both valvular incompetence and blood flow velocities across

all the cardiac valves, to be made with accuracy. This would reduce the number of sites from which hair has to be clipped. As it is impossible to get one such window, it is necessary to minimise the number of acoustic windows without affecting diagnostic accuracy. In the present work, therefore, in addition to using the right parasternal window for linear measurements, the window was also used to investigate valvular function successfully.

Doppler echocardiography requires that, for blood flow velocities to be determined accurately, the ultrasound signals should be in line with the direction of blood flow. In the present work, this was successfully achieved for aortic and mitral blood flow using the subcostal window. CW Doppler measurement of aortic blood flow from the suprasternal window further increased diagnostic accuracy. The availability of a large number of dogs, of the same breed, age, sex, and of a narrow weight margin, was ideal for establishing normal Doppler values of mitral and aortic blood flow, and indices of diastolic function.

The importance of ultrasonography as a diagnostic tool in cardiology was demonstrated in dogs infected with T.brucei. Echocardiography revealed functional abnormalities in the heart, some of which it would have been difficult to identify using other conventional non-invasive techniques such as radiology or ECG. The ability to detect regurgitant blood from particular cardiac valves as early as day 10 of infection was further indication of the sensitivity and specificity of this technique. When complemented by ECG, it was possible to investigate cardiac performance conclusively.

Intravenous infection of dogs with T.brucei GVR35/c.1 resulted an acute disease syndrome, which if untreated culminated in congestive heart failure during week 4 of infection. The disease was characterised by severe anaemia, lymph node and splenic enlargement, weight loss, and clinical and histopathological features of severe pancarditis.

Anaemia developed rapidly after the onset of parasitaemia. The net effect was reduced red cell (RBC) mass. Erythrophagocytosis by an activated mononuclear phagocytic system (MPS) appeared to be the main mechanism by which damaged erythrocytes were removed from the circulation. The mechanisms that led to the RBC damage were probably immunological. RBC damage was also probably caused by substances generated from living or dead trypanosomes. Following the onset of parasitaemia, it is possible that massive antigen-antibody reactions took place, leading to increased trypanolysis and release of biologically active substances. Anti-trypanosome antibodies, immune complexes or autoantibodies attached to erythocyte surfaces, resulting in their recognition by the MPS as foreign. The presence of circulating immune complexes was confirmed by their demonstration in the kidneys of infected dogs, indicating that immune complexes probably played a significant role in the pathogenesis of anaemia. Other factors that probably contributed to the severity of anaemia included fever, and elevated plasma free fatty acid (FFA) concentration. In addition, dyshaemopoiesis, resulting from blocked transportation of iron from the MPS back to the bone marrow for reutilization, or from direct inhibition of the bone

marrow, probably increased the severity of anaemia. In this respect, cachectin/tumour necrosis factor (TNF) is known to inhibit iron release from macrophages (Alvarez-Hernandez et al., 1989), and to cause dyserythropoiesis by its direct effect on the bone marrow (Tracey et al., 1988). The demonstration of cachectin/TNF secretion by monocytes from infected dogs suggested that such an inhibitory effect was taking place.

The net reduction in RBC mass probably led to poor oxygenation of the myocardium, and therefore affected cardiac function directly. Tissue oxygenation was further affected by increased resistance of damaged vessels to blood flow. In the heart, the massive inflammatory reactions that took place were accompanied by severe interstitial oedema. This made gaseous exchange difficult, increasing the incidence of ischaemic myocardial damage.

Thrombocytopaenia was a prominent finding in dogs infected with T.brucei. Its severity was related to the presence and number of trypanosomes in the circulation. As such the appearance of trypanosomes in the blood was accompanied by a rapid fall in platelet numbers. Any treatment that cleared trypanosomes caused a rebound increase in platelets within 2 days; the numbers falling again with relapses. That thrombocytopaenia was directly related to the presence of trypanosomes was further demonstrated when immunosuppressive treatment failed to alter the fall in platelet numbers. It is possible that substances from trypanosomes, thromboplastins released from injured endothelial cells and tissues, phospholipases, or endotoxin,

caused activation of the clotting mechanism. Platelet activation could also have been caused by direct or complement-mediated attachment of trypanosome antigen on the platelet membrane.

The plasma concentration of the acute phase protein (APP), C-reactive protein (CRP), increased rapidly at the same time as detection of trypanosomes in the circulation. The degree of inflammation was directly related to the concentration of CRP. As such, curative treatment of dogs with suramin resulted in a return of CRP to normal levels within a few days. Some anti-inflammatory treatment regimes caused more extensive tissue damage than was seen in infected untreated dogs, and thus higher CRP levels. Generally, CRP is not affected by homeostatic control mechanisms that work to maintain a normal value, and therefore the concentration at any one time reflected, closely, the extent of the underlying tissue damage, confirming its prognostic value.

Dogs infected with T.brucei developed a hyperlipidaemia, associated with increased triglycerides (TG), cholesterol (CH), FFA, very low density lipoproteins (VLDL) and low density lipoproteins (LDL), and decreased high density lipoproteins (HDL). In addition to increased FFA, the concentration of albumin fell dramatically, leading to a net increase in total unbound FFA. The cause of the hyperlipidaemic state was not conclusively determined. Hyperlipidaemia probably resulted from reduced plasma lipid degradation for energy metabolism or for storage, increased breakdown of tissue lipids, or increased lipogenesis in the liver. The finding that monocytes from infected dogs were

primed to produce cachectin/TNF, a potent inhibitor of the lipogenic enzyme lipoprotein lipase (LPL) (Rouzer and Cerami, 1980) that is also capable of stimulating hepatic lipogenesis in disease states (Feingold and Grunfeld, 1987), indicated that cachectin/TNF played a central role in the pathogenesis of hyperlipidaemia. The situation was probably aggravated by increased fat breakdown following stimulation of the adipose tissue enzyme triglyceride lipase (TGL), either by catecholamines released due to the stress of infection, or by cachectin/TNF. The fact that lipoproteins play a central role in CH transport for trypanosome metabolism, and that they are also capable of binding trypanocidal compounds, indicated that abnormal lipid metabolism had an effect on trypanosome survival and therapeutic efficacy. This fact was underlined by the inability of suramin, at doses that are known to effect cure, to eliminate trypanosomes from the blood of infected dogs. It is possible that the use of anti-lipolytic drugs at the time of trypanocidal treatment could have increased therapeutic efficacy.

Progressive increase in myocardial lipid deposition occurred in infected dogs. While this was probably the result of a net flux of FFA from plasma, either due to increased total FFA, or an altered equilibrium between the FFA and albumin, lipid deposition was exacerbated by defective myocardial metabolism. The presence of lipid deposits in myocardium undergoing ischaemic insults probably resulted in altered lipid peroxidation, release of reactive oxygen metabolites (ROM), and hence more severe damage.

The hypoalbuminaemia that developed apeared to be

related to the severity of gastroenteritis and acute inflammatory reactions, and less so the parasitaemia. Anti-inflammatory treatment therefore markedly reduced the fall in albumin concentration, in spite of the presence of massive parasitaemia.

While death in T.brucei infected dogs most probably resulted from failure of the heart as a pump, endocrinological studies indicated that the severity of the disease was influenced by impaired regulation of blood volume and blood flow dynamics. As such, in terminally infected dogs, there was a marked reduction in plasma and atrial concentration of atrial natriuretic factor (ANF), accompanied by an inverse increase in plasma renin activity (PRA). The terminal decrease in plasma ANF was probably the result of secretory exhaustion of the atrial stores, after the preceding tachycardia, or decreased synthesis of ANF in atria secondary to myocyte damage. While PRA probably increased in response to a fall in renal arterial blood pressure, secondary to poor left ventricular function (LVF), PRA could also have increased after the removal of the inhibitory effect of ANF on juxtaglomerular cells. It is possible that a fall in plasma ANF and increased PRA caused sodium and water retention, and increased water intake, leading to plasma volume expansion and increased venous return to an already failing heart. In the absence of ANF, the dogs were unable to regulate the increased volume load, thereby exacerbating the severity of heart failure. It is possible that if angiotensin-converting enzyme inhibitors (ACEI) were used at the time of trypanocidal treatment, the severity of heart

failure might be reduced.

Histopathological and histochemical studies revealed a severe, progressive pancarditis involving the myocardium, the valves and the vasculature. The myocardial changes that occurred indicated that massive antigen-antibody reactions were taking place. In would appear that dying trypanosomes and inflammatory cells undergoing autolysis released toxic and biologically active substances into the interstitium. Lymphatic obstruction and inadequate perfusion of tissues led to the accumulation of toxic substances and further tissue damage. Mesangial deposits of immune complexes in the kidneys confirmed their presence in the circulation, and they too, most likely contributed to the severity of myocardial damage.

Unsuccessful treatment of dogs with suramin prolonged their survival period and precipitated chronic myocardial damage. Dogs that were best able to control anaemia survived longest. In most dogs, treatment was followed by increased ECG abnormalities, and death to one of them. The severity of post-treatment reactions did not appear to be related to the dose of suramin used, but rather to uncontrolled inflammatory reactions in the myocardium that took place soon after treatment. The inability of suramin to clear trypanosomes from the blood the longer the dogs survived was probably due to reduced availability of the drug to the trypanosomes, despite high concentrations in the blood i.e., suramin either selectively bound to lipoproteins or, due to hypoalbuminaemia or both, the amount of albumin-bound suramin was too low to kill the trypanosomes.

That overwhelming inflammatory reactions following

immunological response by the host to the parasite were the major cause of damage was confirmed when the extent of damage was reduced by immunosuppressive and anti-inflammatory treatment, in spite of the presence of large numbers of trypanosomes in the myocardium. Although drug toxicity occurred in dogs treated with cyclosporin A, clinical reduction in the severity of cardiac damage was achieved. It is possible that if the drug had been divided into smaller doses and administered several times a day, acute toxicity might have been avoided. On the other hand, azathioprine was well tolerated, and although clinical improvement in cardiac performance occurred, the higher dosage regimes were hepatotoxic. Therapeutic effect was most pronounced when treatment was carried out for prolonged periods of time with lower doses. When a combination of azathioprine and prednisolone was used, cardiac damage was reduced even further, at the expense of increased liver damage. It appears that the doses of both drugs could have been reduced without affecting therapeutic efficacy, as has been done in chronic collitis in the dog (Ridgway, 1984) and in canine renal transplant recipients (Putnam et al., 1975). The immunosuppressive and anti-inflammatory properties shown by these drugs indicated that post-treatment adverse reactions, as were seen in suramin-treated dogs, might be avoided if non-steroidal anti-inflammatory drugs or prednisolone are administered at the time of trypanocidal treatment. The drugs could then be withdrawn after the dogs had gone over the acute trypanolytic crisis.

The present work, in addition to providing an

opportunity to improve diagnostic canine cardiology, revealed exciting information on cardiac damage in dogs infected with T.brucei, and increased the understanding of the mechanisms by which tissue damage occurs. The identification of endocrinological abnormalities and defective lipid metabolism in infected dogs revealed areas where supportive therapeutic intervention might prove to be of value. Increased ECG abnormalities and death after trypanocidal treatment confirmed that adverse reactions do occur. That such reactions could be prevented by treatment with trypanocidal compounds together with anti-inflammatory and immunosuppressive drugs was supported by reduced cardiac damage in infected dogs after they were treated with NSAIDs and prednisolone. The fact that prolongation of survival by treatment with suramin resulted in chronic cardiac damage indicated that such dogs might in future serve as good models of cardiomyopathy.

THE WAY AHEAD.

The present work, while achieving most of the initial objectives, opened up new areas, both in diagnostic cardiology, and in the pathogenesis of cardiac damage in canine African trypanosomiasis, that might have major implications in small animal cardiology and in African trypanosomiasis in general. In the first place, the Doppler echocardiographic data presented for normal dogs were the first to ever be reported. This technique needs to be developed further, in order to enable for accurate

measurements of cardiac output, diastolic and left ventricular function, to be carried out, and to determine whether the values presented in this work are affected by breed or age.

The abnormalities in lipid metabolism revealed most likely played a major role in the pathogenesis of T.brucei infection in dogs. It is necessary that the role be conclusively defined. The actual composition of the plasma and tissue lipids needs to be established. The contribution by cachectin/TNF in the development of hyperlipidaemia should be determined, and the possibility of using anti-cachectin/TNF serum to prevent the hyperlipidaemic state be established. Further, whether elevated plasma and tissue lipid levels affect therapeutic efficacy and trypanosome suvival needs to be established. It is necessary to determine whether pre-treatment with anti-lipolytic drugs before trypanocidal treatment could have any beneficial effects. Such investigations should be extended to include other monogastric animals such as monkeys, and possibly even humans suffering from sleeping sickness.

That heart failure in dogs infected with T.brucei was accompanied by reduced ANF and increased PRA indicated a possible area of therapeutic intervention in the management of cardiac damage during trypanocidal treatment. It is necessary to determine whether ACEI might have any beneficial effects in such cases.

Immunosuppressive and anti-inflammatory treatment reduced the severity of cardiac damage in dogs infected with T.brucei. However, the most appropriate treatment regimes and

treatment combinations were not conclusively established. Treatment with prednisolone alone, and treatment with both anti-inflammatory and trypanocidal compounds was never done, leaving a wide area for further study. Finally, there is the possibility that the dog can be development as a model of cardiomyopathy by subcurative treatment with trypanocidal compounds.

PART V.

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