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THE PATHOPHYSIOLOGY OF OVINE TRYPANOSOMIASIS CAUSED BY *TRYPANOSOMA CONGOLENSE*

by

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A thesis submitted for the degree of Doctor of Philosophy
in the Faculty of Veterinary Medicine,
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August, 1992.

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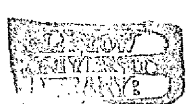

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the Association of Commonwealth Universities, British Commonwealth for their financial support and to Makerere University Administration for granting me study leave.

I would like to thank Professors Peter H. Holmes and Professor Max Murray, my supervisors, for their patient guidance, ready assistance and helpful criticisms and advice. I am also grateful to Dr J.J. Parkins, G. Fishwick and P.F. Winstanley for their helpful discussions and to Professor G. Gettinby for guiding me through statistical techniques.

I am grateful to the friendship and willing assistance afforded me in the Departments of Veterinary Physiology and Veterinary Medicine and acknowledge with particular thanks the assistance of Mrs D. McGruer with computer word processing, the technical contributions of Miss E. Dunlop, Miss D. McKechnie and Mr G. Jackson and the general help of Mrs I. Gilmour and Mr R. McFadyen.

I also thank members of the Veterinary library, the technical staff of the Departments of Veterinary Animal Husbandry, Veterinary Medicine, Veterinary Pathology (Haematology section), and Veterinary Biochemistry for their conscientious assistance whenever required.

I wish to acknowledge with great appreciation the prayers and support from my mother Mrs Agrace Rwakishaya, the family of Mr Eldard Katakari, my brothers, sisters and relatives.

Finally, I wish to thank my wife Mrs Ketrah Katunguka, and my children Alexandra Yvonne Akantorana, Ralph Eli Akorabirungi and Lisa Catriona Atukunda for their support, company and encouragement.

To all these people who contributed to my successful completion of this work, I say a big Thank You.

DECLARATION

I hereby declare that the work presented in this thesis is original and was conducted by the author with the exception of some of the work described in Chapter 9, which was carried out with Dr P. Winstanley, Institute of Biochemistry, Glasgow Royal Infirmary.

I also hereby certify that no part of this thesis has been submitted previously in any form to any university for the award of a degree, but has been published in part as scientific abstracts or as papers:

1. **Katunguka-Rwakishaya, E., Murray, M. and Holmes, P.H. (1991).** The pathophysiology of *Trypanosoma congolense* in sheep. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 841.
2. **Katunguka-Rwakishaya, E., Murray, M. and Holmes, P.H. (1991).** Haematological, erythrokinetic and blood lipid changes in sheep infected with *Trypanosoma congolense*. In: Proceedings of the 21st Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Yamoussoukro, Cote d'Ivoire, 21-25th October 1991.
3. **Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M. and Holmes, P.H. (1991).** The influence of dietary protein on the pathophysiology of *Trypanosoma congolense* infection in sheep. In: Proceedings of the 21st Meeting of the ISCTRC, Yamoussoukro, Cote d'Ivoire, 21-25th October 1991.
4. **Winstanley, F.P., Holmes, P.H., Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G. and Murray, M. (1992).** Tumor necrosis factor alpha-receptor activity in ovine trypanosomiasis. In: Proceedings of the British Society of Immunology, Sheffield, 3rd-4th April 1992.
5. **Katunguka-Rwakishaya, E., Murray, Max and Holmes, P.H. (1992).**The pathophysiology of ovine trypanosomiasis: ferrokinetics and erythrocyte

DEDICATION

TO

MY FATHER

(The late BLASIO RUKUMBIRA RWAKISHAYA)

AND

MY MOTHER

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ACRONYMS

%SAT	Percentage saturation
BV	Blood volume
BW	Body weight
CA	Intravascular pool of albumin
C	Cholesterol
^oC	Degrees celsius
CE	Cholesterol ester(s)
CF	Crude fibre
CNS	Central nervous system
cpm	Counts per minute
Cr	Chromium
d	Day(s)
DAFS	Department of Agriculture and Fisheries for Scotland
DAI	Days after infection
DANI	Department of Agriculture for Northern Ireland
DIC	Disseminated intravascular coagulation
DM	Dry matter
EDTA	Ethylene diamine tetra acetic acid
EE	Ether extract
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agricultural Organisation of the United Nations
FD	Finn Dorset
FDP	Fibrin degradation products
Fe	Iron
FFA	Free fatty acids

GE	Gross energy
g	Gram(s)
Hb	Haemoglobin
HDL	High density lipoproteins
h	Hour(s)
IDRC	International Development and Research Council
IFN	Interferon
IL	Interleukin
ILRAD	International Laboratory for Research in Animal Diseases
I.M	Intramuscular
I.V	Intravenous
KBq	Kilobecquerel
kg	Kilogram(s)
KI	Potassium iodide
LCAT	Lecitin-cholestryl acyltransferase
LDL	Low density lipoproteins
LHDL	Low high density lipoproteins
l	Litre(s)
LPC	Lysophosphatidylcholine
LPL	Lipoprotein lipase
MAFF	Ministry of Agriculture, Food and Fisheries
Mbq	Megabecquerel
MCHC	Mean corpuscular haemoglobin concentration
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
ME	Metabolisable energy
mg	Milligram(s)

MJ	Megajoules
ml	Millilitre(s)
mmol	Millimole(s)
umol	Micromole(s)
MPS	Mononuclear phagocytic system
NEFA	Non-esterified fatty acids
OM	Organic matter
PAO	Polyamine oxidase
PBS	Phosphate buffered saline
PC	Phosphatidylcholine
PCV	Packed cell volume
PE	Phosphatidylethanolamine
PITR	Plasma iron turnover rate
PL	Phospholipids
PUFA	Polyunsaturated fatty acids
PV	Plasma volume
RBC	Red blood cell count
RCIR	Red cell iron renewal
RCI	Total red cell iron
RCIT	Red cell iron turnover rate
RCU	Red cell utilisation of iron
RCV	Red cell volume
RDP	Rumen degradable protein
SBF	Scottish Blackface
sem	Standard error of the mean
SI	Serum iron
TER_{alb}	Transcapillary escape rate of albumin

TIBC	Total iron binding capacity
TL	Total lipids
TNF	Tumor necrosis factor
TP	Total protein
UIBC	Unsaturated iron binding capacity
UDP	Undegradable protein
VHDL	Very high density lipoproteins
VLDL	Very low density lipoproteins
WBC	Total white blood cell count
WHO	World Health Organisation

SUMMARY

This thesis concerns the pathophysiology of ovine trypanosomiasis caused by *Trypanosoma congolense*, and includes a review of relevant literature and description of a series of experiments conducted to investigate various aspects of this subject area, including pathogenesis, genetic and nutritional influences of susceptibility, and TNF-α receptor expression on peripheral blood leucocytes.

Chapter 1 comprises an introduction and review of previously published work on the subject of African animal trypanosomiasis with particular reference to pathogenesis of, and genetic resistance/host susceptibility to animal trypanosomiasis. In Chapter 2, general materials and analytical techniques used in experimental work are described.

Chapter 3 describes the haematological and blood biochemical changes in Scottish Blackface sheep infected experimentally with *T. congolense*. It was observed that infected sheep developed a macrocytic anaemia, the onset of which coincided with the appearance of detectable parasites in the circulation. Biochemical analyses revealed that infection leads to a decrease in the concentrations of plasma albumin, plasma cholesterol and serum phospholipids with a resultant reduction in serum total lipid concentration. These lipid changes occur before patent parasitaemia, suggesting that the trypanosomes or trypanosomal products may have a profound effect on lipid metabolism of the host. Such alterations could play an important role in the pathogenesis of the disease process.

In the following study (Chapter 4), the underlying causes of the anaemia observed in infected animals were investigated after 11 weeks of infection. In addition to conventional haematological techniques, radioisotopic tracers were employed. With ^{51}Cr-labelled erythrocytes, alterations in red cell volumes and rates of disappearance of erythrocytes from the circulation in infected and control groups were measured. The rate and efficiency of erythropoiesis were assessed with ^{59}Fe as ferric citrate, while plasma volumes and changes in albumin metabolism were

monitored by the use of ^{125}I-labelled albumin. It was found that infected sheep had significantly lower mean circulating red cell volumes, but higher plasma and blood volumes than control sheep. Infected animals also had enhanced erythropoietic activity as judged by significantly higher plasma iron turnover rates, faster disappearance of radiolabelled iron, higher iron incorporation rates and faster rate of disappearance of ^{51}Cr-labelled red cells than control sheep. It was concluded that the anaemia observed at this stage of infection was due toincreasedrate of loss of red cells from the circulation coupled with haemodilution, with no evidence of dyshaemopoiesis.

Chapter 5 describes a study of comparative susceptibility of Scottish Blackface and Finn Dorset sheep to experimental infection with *T. congolense*. It was observed that infected Scottish Blackface sheep developed a higher level of parasitaemia, more pronounced pyrexia, higher degree of anaemia, more severe thrombocytopaenia and hypoalbuminaemia than infected Finn Dorset sheep. At the same time, the Scottish Blackface sheep had higher initial cholesterol levels, and following infection the concentrations of plasma cholesterol, serum phospholipids and total lipids decreased significantly in Scottish Blackface sheep but not in Finn Dorset sheep. It was proposed that, as trypanosomes are dependent on host cholesterol for growth and multiplication, the availability of high cholesterol levels in Scottish Blackface sheep might have permitted the maintenance of higher levels of parasitaemia and resulted in a more severe anaemia.

A comparative study of the pathogenicity of three clones of *T. congolense* (GRVPS 57/6, GRVPS 3/2 and GRVPS 92/3) is described in Chapter 6. It was found that GRVPS 93/3 was associated with higher level of parasitaemia, and more severe anaemia and lymphocytosis than the other two clones. All clones caused similar biochemical changes. It was concluded that different clones of T. congolense may cause varying pathophysiological responses; however, the pathogenic effects of the three clones in this study were not markedly different.

Nutritional influences of the pathophysiology of *T. congolense* infection in sheep were evaluated in experiments described in Chapters 7 and 8. In Chapter 7, experimental animals were divided into two groups and given either a high protein (crude protein 176 gkg^{-1} dry matter) or a low protein (crude protein 81 gkg^{-1} dry matter) diet. These diets were isocaloric. Infected animals on a high protein diet tended to develop higher intensity of parasitaemia than those on a low protein diet. Both infected groups exhibited similar degrees of anaemia, but the erythropoietic activity, as judged by the increases in mean corpuscular volumes and appearance of normoblasts in the circulation, was significantly greater in the animals on the high protein diet. The infected animals on the high protein diet gained weight at similar rates as their uninfected controls, while those on the low protein diet gained significantly less than their controls between 0 and 70 days after infection. Following treatment with a trypanocidal drug, isometamidium chloride, at 70 days after infection, both infected groups recovered from the anaemia; however, the rate of recovery was faster in the animals on the high protein diet. Dissection of the indicator joints and carcase chemical analysis revealed few differences between groups of animals.

Chapter 8 describes the influence of energy intake on the pathophysiology of *T. congolense* infection in Scottish Blackface sheep. Animals were divided into two groups and allowed either a high energy intake (9.9 MJ of metabolisable energy per day) or a low energy intake (6.1 MJ of metabolisable energy per day). The diets were formulated so as to provide similar levels of crude protein at the levels of fresh matter intake given to the high energy group (1.2 kg per day) and low energy group (0.71 kg per day). It was observed that animals on the low energy intake had a longer prepatent period, but following patency, they developed a higher level of parasitaemia, greater degree of anaemia and greater retardation of growth than those on a high energy intake. Both infected groups exhibited significant decreases in the

concentrations of serum total lipids, phospholipids, plasma cholesterol and albumin. However, these changes were more severe in the animals on a low energy intake than in those on a high energy intake.
These nutritional studies indicate that adequate protein and energy nutrition enhance the ability of trypanosome-infected animals to withstand the adverse effects of infection by promoting better body weight gains and reducing the degree of anaemia.

In Chapter 9, the expression of tumor necrosis factor alpha (TNF-α) receptors on peripheral blood leucocytes of sheep infected with *T. congolense* and allowed either a high or a low energy intake was investigated. This was conducted by using labelled recombinant human tumor necrosis factor alpha and cytofluorimetric methods. It was found that tumor necrosis factor-alpha receptor expression changed throughout a course of trypanosome infection. The greatest increases in the percentage of cells expressing these receptors were observed in the granulocyte populations of infected animals, and these changes appeared to fluctuate with development of waves of parasitaemia. The monocytes of infected animals displayed a relative decrease in expression of tumor necrosis factor-alpha receptors compared to their uninfected controls while little variation in receptor activity was found in the lymphocytes. It was further observed that infected animals on high energy intake showed greater tumor necrosis factor-alpha receptor activity, and this was associated with greater resistance of these animals to the disease, as judged by lower intensity of parasitaemia, less severe anaemia and better weight gains, than the animals on low energy intake. These observations indicate that adequate energy intake may enhance the ability of trypanosome-infected animals to mobilise effective non-specific defence mechanisms against the parasite.

CHAPTER 1

INTRODUCTION AND GENERAL REVIEW OF LITERATURE

INTRODUCTION

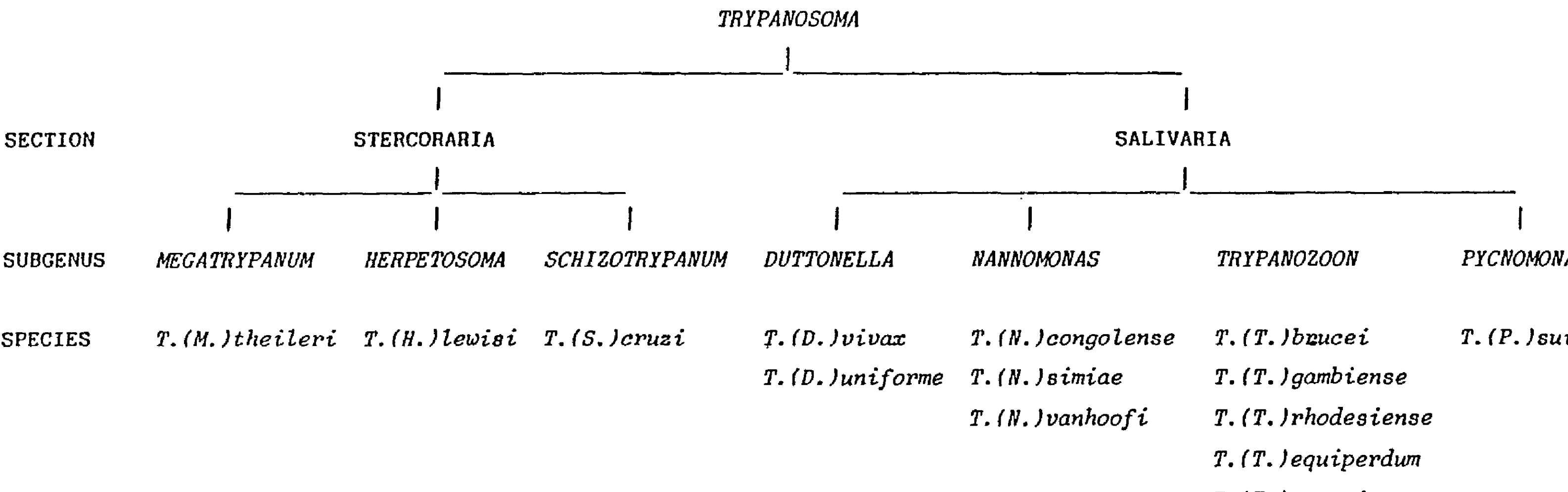
Parasitic organisms of the family Trypanosomatidae cause disease in man and animals throughout the tropical areas of the world and in some temperate areas, but they exert their greatest economic and social effects in Africa south of the Sahara desert. This region provides climatic and geographical factors which favour the existence of the flies of the genus *Glossina* that are responsible for the cyclical transmission of the organisms of the genus *Trypanosoma*. The presence of tsetse flies and trypanosomes are major constraints to the development of livestock in these areas which are among the best watered and most fertile parts of Africa (Wilson *et al.*, 1963). It has been estimated that 40% of tropical Africa suffers from tsetse infestation, and that livestock production losses, in meat alone, in Africa owing to trypanosomiasis amount to US$5 billion annually (FAO-WHO-OIE, 1982). Despite the control measures directed against the parasite and the vector, the situation appears to have been deteriorating over recent years.

Classification of Trypanosomes

The causative organisms of trypanosomiasis are flagellate protozoa of the Class Mastigophora, Family Trypanosomatidae and the genus *Trypanosoma* (Hoare, 1964, 1972). The genus *Trypanosoma* is further sub-divided into two main groups, namely, the Stercorarian and Salivarian groups of trypanosomes (Table 1.1). The Stercorarian group comprises a large number of non-pathogenic trypanosomes and their development in the vector is completed in the posterior section of the gut. As such, their transmission is contaminative. One important species in this group is *Trypanosoma cruzi*, the causative agent of Chagas' disease in man in South and Central America. Other members of this group include, *T. lewisi*, *T. musculi*, and *T. theileri*.

Table 1.1

Classification of trypanosomes (adapted from Stephen, 1986)



The salivarian group consists of trypanosome species that are of major medical and veterinary importance. This group is divided into four sub-genera namely, Nannomonas, Duttonella, Trypanozoon and Pycnomonas (Hoare, 1972). The Nannomonas subgenus contains *T. congolense* and *T. simiae* and the Duttonella subgenus contains *T. vivax* and *T. uniforme*. The Trypanozoon subgenus consists of *T. gambiense*, *T. rhodesiense*, *T. brucei*, *T. equiperdum* and *T. evansi.*

T. gambiense and *T. rhodesiense* infect man and cause African human trypanosomiasis (sleeping sickness) while *T. brucei*, together with *T. congolense* and *T. vivax* cause African animal trypanosomiasis (nagana) in cattle and other domestic animals.

It was thought that *T. congolense* produces the most important form of animal trypanosomiasis in East Africa while in West Africa, animals were also infected by this species but the incidence appeared to be overshadowed by the prevalence of *T. vivax*. However this distribution appears to have changed over the years and there are increasing reports of both species of trypanosomes from East and West Africa.

Distribution of Trypanosomes Within the Host Tissues

The location of trypanosomes within affected animals varies according to the species involved. Thus trypanosomes of the *brucei* group (*T. brucei, T. gambiense* and *T. rhodesiense)* are capable of penetrating the capillary endothelium (Losos and Ikede, 1970; Goodwin, 1971) and of multiplying outside the blood vascular system. These organisms may therefore be widely distributed and can be encountered in connective tissue fluid, lymph, cerebrospinal fluid and acqueous humour (Fernhead *et al.*, 1974).

Trypanosoma vivax and *T. congolense* have generally been regarded as being confined to the vasculature of the infected host because extensive tissue lesions were rarely seen (Losos and Ikede, 1972; Maxie *et al.*, 1979). It was therefore thought that the pathogenic effects of these two organisms were similar in ruminant hosts (Fiennes, 1970). With the advent of modern electron microscopic techniques, it is now known that, like *T. brucei*, *T. vivax* also has the capacity to invade tissues in domestic ruminants (van den Ingh *et al.*, 1976a, b; Emery *et al.*, 1980; Murray *et al.*, 1980; Dwinger *et al.*, 1988). There are reports of *T. vivax* being found in lymphatic vessels (Emery *et al.*, 1980), in cerebro-spinal fluid, aqueous humour (Whitelaw *et al.*, 1988) and in cardiac muscle (Kimeto *et al.*, 1990) following intravenous inoculation with bloodstream forms of the parasite.

The ability of *T. vivax* and *T. brucei* to develop in extravascular locations is of considerable importance in the context of chemotherapy. It has now been well established that relapse of *T. brucei* infections after chemotherapy is associated with extravascular sequestration of the trypanosomes in the central nervous system of the host where it is inaccessible to drug action (Jennings *et al.*, 1977; Morrison *et al.*, 1983; Whitelaw *et al.*, 1985a). A similar situation may occur in *T. vivax* infections (MacLennan, 1971; Whitelaw *et al.*, 1988). Apart from an extravascular phase in the skin (Luckins and Gray, 1978; Akol and Murray, 1986) and occasional presence in cerebro-spinal fluid (Masake *et al.*, 1984) *T. congolense* parasites are confined to the circulation (Tizard *et al.*, 1978d; Murray and Dexter, 1988; Murray, 1989) where they may remain free or attach themselves by their anterior end to red cells or endothelial cells lining blood vessels (Bungener and Muller, 1976; Banks, 1979). Because of binding to the endothelium, the concentration of these organisms may be five to ten times greater in capillary beds than in large vessels such as the jugular vein (Tizard *et al.*, 1978c)

Diseases Caused by Tsetse-transmitted Trypanosomes

Tsetse-transmitted trypanosomiases form a very important group of diseases of man and animals in tropical Africa. In man *T. gambiense* and *T. rhodesiense* cause sleeping sickness which is characterised in the initial stages by fever, polyadenitis, anaemia and parasitaemia and in the later stages, the involvement of the central nervous system. *T. gambiense* is associated with the chronic form while *T. rhodesiense* is associated with the acute form of the disease. Sleeping sickness is restricted to tropical Africa roughly between 15°N and 20°S of the equator.

T. congolense, T. vivax and *T. brucei* cause African animal trypanosomiasis and the greatest economic effects are associated with *T. congolense* and *T. vivax* infection in cattle, sheep and goats. The pathological changes subsequent to trypanosome infections and the underlying causes have not been fully elucidated and many aspects remain controversial (Losos and Ikede, 1972). One reason for the controversy is possibly the wide variety of diseases caused by different trypanosomes. The precise nature of each disease depends not only upon the species of the host but also upon the particular strain of trypanosome involved (Losos and Ikede, 1972) and in each case there may be acute, subacute and chronic forms. This is well illustrated by the differences in virulence for susceptible hosts which are found with different strains of the same parasite (Fairbairn, 1953; Stephen, 1970).

Incidence and significance of trypanosmiasis in sheep

In spite of the fact that trypanosomiasis has been recognised for decades as thegreatestobstacle to increased productivity of livestock in Africa, until recently very little research has been conducted on the disease in sheep. Most of the studies on natural and experimental trypanosome infections have been carried out in cattle and in laboratory animals (Jennings et al., 1974; Murray and Dexter, 1988). While Stephen (1986) suggested that sheep are not often infected with trypanosomiasis

under natural conditions and Kramer (1966) and MacLennan (1970) thought that the disease was not of significance in small ruminants, recent epidemiological surveys indicate that it is more important than it was previously believed (Robson and Ashkar, 1972; Zwart *et al.*, 1973; Makumyaviri *et al.*, 1989; Kalu, 1991). Studies of natural infections have revealed that incidences of trypanosmiasis vary with the seasons, for example, Griffin and Allonby (1979a) observed that tsetse numbers increase following the rains leading to high infection rates in sheep.

Such high incidences may have significant economic effects accruing from reduced weight gains, poor reproductive performance and death of infected animals (Griffin and Allonby, 1979b; Kanyari *et al.*, 1983). Another important aspect of trypanosomiasis in small ruminants lies in the possibility of their passing on infection to other animals (Mahmoud and Elmalik, 1977) and man (Rickman, 1974; Kageruka *et al.*, 1991; Okuna and Mayende, 1983). In Zaire, Kageruka *et al.* (1991) have isolated *T. brucei* spp from sheep and goats which were similar in type to human *T. gambiense* isolates from the same area. They suggested that sheep play an important role in maintaining the infections during inter-epidemic periods especially in situations where villagers live in close proximity to their livestock and in close contact with vectors.

The disease syndrome in sheep

In a discussion of the course and outcome of trypanosome infection in sheep, it is important to distinguish between a natural and an experimentally-induced infection. This is largely because most natural fly challenges induce mixed infections (MacKenzie and Cruickshank, 1973). Most accounts of naturally-occurring trypanosomiasis come from East Africa and concern infections with *T. congolense*, although *T. vivax* has been detected intermittently.

Sheep have often been used as convenient hosts in studies of experimental trypanosomiasis, as models of the disease in cattle (Bouitelle *et al.*, 1988a, b) rather than because they may be important hosts of the parasite. However, this has changed over recent years and there is an increasing volume of literature on ovine trypanosomiasis, especially as it relates to trypanotolerance. Most reports of experimental trypanosomiasis concern *T. congolense* and *T. vivax*, as these are thought to be more pathogenic than *T. brucei* subgroup (Losos and Ikede, 1972).

The course of experimental trypanosome infection in sheep has been divided into three stages, namely, subacute, acute and chronic depending on the period of detectable parasitaemia and the outcome of the infection (Griffin and Allonby 1979c). The subacute form has been described as lasting up to twelve weeks and is characterised by a slow but steady decline in packed red cell volume (PCV) and weight, with fluctuating temperatures. The acute form is thought to last up to six weeks and is characterised by a rapid decrease in PCV and widely fluctuating temperature during the parasitaemic period, with the outcome as death or in some cases as apparent self cure. In the chronic form, the host appears to make complete recovery but maintains moderate anaemia, normal temperature and parasites may not be demonstrated in blood using standard trypanosome detection methods. It is generally believed that sheep can withstand both natural and experimental infection for a long time and death has been recorded on a few occasions (Griffin, 1978; Griffin and Allonby, 1979a, b). However, it is possible that death in infected sheep may not be attributed to trypanosomiasis alone, but to secondary infections as a result of trypanosme-induced immunosuppresion (Griffin *et al.*, 1980; MacKenzie *et al.*, 1975). The most common secondary complication of ovine trypanosomiasis has been suggested to be bacterial pneumonia (Department of Veterinary Services, Rhodesia, 1974).

Other factors that may affect the outcome of trypanosme infection in sheep include, the virulence of the infecting trypanosome species or strain, the age and breed of the animal, and plane of nutrition (Murray and Dexter, 1988).

PATHOGENESIS OF AFRICAN ANIMAL TRYPANOSOMIASIS

The principal manifestations of trypanosome infection in domestic animals are the development of anaemia and cachexia. As the infection progresses in trypanosome-infected animals, the kinetics of parasitaemia and the underlying mechanisms of anaemia change (Murray and Dexter, 1988). The initial or acute phase of infection is characterised by a rapidly developing anaemia which is attended by high levels of parasitaemia (Murray, 1979; Dargie *et al.*, 1979a; Maxie *et al.*, 1979; Morrison *et al.*, 1981). During this stage of infection the anaemia is usually normochromic and normocytic (occasionally macrocytic) in type (Valli *et al.*, 1978; Murray, 1979; Maxie and Valli, 1979; Valli and Mills, 1980). However, Roberts and Gray (1973) observed that the anaemia in mixed *T. vivax* and *T. congolense* infections in cattle was hypochromic and macrocytic. Parkin (1935) reported the occurrence of anisocytosis, polychromasia, basophilia and normoblasts in the blood of trypanosome-infected sheep.

In this phase, the trypanosome is important in maintaining the progress of the development of anaemia, and the elimination of the parasites either by spontaneous recovery or treatment with a trypanocidal drug results in the return of haematological values to normal (Holmes and Jennings, 1976). In animals which remain infected for several months, a chronic form of anaemia may develop. This form has not been adequately investigated under experimental conditions but is reported to be associated with absence or presence of very few parasites in the blood. An interesting aspect of this form of trypanosomal anaemia is that the role of

the trypanosome in the maintenance of this anaemia is less important, hence anaemia persists in the apparent absence of detectable parasites in the blood and the affected animals usually respond poorly to trypanocidal therapy (Morrison *et al.*, 1981; Murray and Dexter, 1988).

In N'Dama and Zebu cattle infected with *T. congolense*, Dargie *et al.* (1979a) observed that the degree of anaemia that developed following infection was related to the intensity of parasitaemia. However, in goats infected with *T. vivax*, Saror (1980) observed that the anaemia that developed was not correlated with the intensity of parasitaemia. Paling *et al.* (1987) have suggested that in the absence of other anaemia-causing factors, the intensity and duration of parasitaemia, on one hand and (PCV) on the other, are not quantitatively linked to each other although both of them may be genetically controlled. It appears that such a correlation may also be influenced by the the species or strain of trypanosomes and the hosts.

The factors underlying the anaemia have still to be properly delineated, although three mechanisms, acting singly or in concert have been implicated. These are: haemodilution (Fiennes, 1954; Naylor, 1971a,b; Holmes, 1976), dyshaemopoiesis (Fiennes, 1954, 1970) and haemolysis (Mamo and Holmes, 1975; Holmes, 1976; Preston and Wellde, 1976; Dargie *et al.*, 1979a). Recently, cytokines and particularly tumor necrosis factor (TNF), which is produced in response to the presence of trypanosomes as part of non-specific defence mechanism, have been incriminated in the aetiology of anaemia. Investigations of trypanosomal anaemia have been largely conducted in the bovine and laboratory animals and there are scanty reports of *T. congolense* infection in sheep. The current understanding of this anaemia is therefore derived from the large volume of literature on bovine trypanosomiasis which will form a central reference in this discussion.

Aetiology and Pathogenesis of Anaemia

Haemodilution

The role of haemodilution in the development of anaemia in animal trypanosomiasis has not been totally resolved. While some authors have suggested that it is important (Clarkson, 1968; Fiennes, 1970; Naylor, 1971a, Holmes, 1976; Valli *et al.*, 1978), others have disagreed (Dargie *et al.*, 1979a). Blood volumes in sheep infected with trypanosomes have been measured on a few occasions, and only in *T. vivax* infections. An increase in plasma volume has been reported in *T. vivax*-infected sheep and goats (Clarkson, 1968; Anosa and Isoun, 1976) and in *T. vivax*-infected calves (Clarkson *et al.*, 1975). In addition to an increase in plasma volume, Anosa and Isoun (1976) observed an increase in total blood volume.

Using radio-isotopes in N'Dama and Zebu cattle infected with *T. congolense* and *T. brucei*, Dargie *et al.* (1979a, b) found that total blood volumes were unaltered by infection. They however found that plasma volumes were higher in infected cattle than in control ones. It was suggested that the increase in plasma volume is a normal homeostatic response for the maintenance of blood volume and pressure and not a pathological accumulation of fluid within the circulation (Dargie *et al.*, 1979a).

Dyshaemopoiesis

It has been reported that in the early stages of trypanosome infection, the bone marrow responds to the haemolytic crisis by a hyperplastic reaction (Dargie *et al.*, 1979a; Anosa and Isoun, 1980) but it becomes dysfunctional in the chronic phase (Preston and Wellde, 1976). The anaemia then becomes microcytic (Fiennes, 1954) or normocytic and normochromic (Losos and Ikede, 1972) with evidence of hypoferraemia (Tartour and Idris, 1973, Wellde *et al.*, 1989c), low plasma iron turnover rates (Preston and Wellde, 1976) and absence of immature red blood cells in circulation (Naylor, 1971a; Losos and Ikede, 1972). In *T. congolense* infection in

goats, Kaaya *et al.* (1977) found that bone marrow inhibition was characterised by an apparent maturation arrest at the prorubricyte or rubricyte stages, a drop in the maturation ratio and a terminal drop in the marrow red cell precursors. In animals necropsied, longitudinal sections of the femur showed the marrow to be yellow and gelatinous, indicating almost total unresponsiveness

Dyshaemopoiesis at this stage of infection has been attributed to reticulo-endothelial iron blockage (Dargie *et al.*, 1979a) resulting in impaired reutilisation of iron from degraded red blood cells. This would explain the poor clinical response to trypanocidal drug therapy in animals with long-standing infections (Wellde *et al.*, 1989c). A similar situation has been observed in chronic infections in man (Cartwright and Lee, 1975) and in experimentally-induced inflammation in laboratory rodents (Hershko *et al.*, 1974). The fact that many of these anaemias are resolved by testosterone or erythropoietin (Haurani and Green, 1967; Zucker *et al.*, 1974) suggests that in addition to their direct action on haemopoietic progenitor cells, such hormones are capable of directly or indirectly releasing iron from the reticulo-endothelial system. This offers the interesting possibility that their administration may have therapeutic value in trypanosomiasis.

Haemolysis

Earlier studies suggested that anaemia in animal trypanosomiasis was of the simple fever type (Fiennes *et al.*, 1946) and not haemolytic but there is now general agreement that increased erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) occurs (Jennings *et al.*, 1974; Mamo and Holmes, 1975; Holmes, 1976; Preston and Wellde, 1976); few studies have indicated that haemolysis may be intravascular also (Esievo *et al.*, 1984) particularly in the acute phase of infection. Haemolysis is accompanied by an increase in haemosiderin deposition (Veenendaal *et al.*, 1976; van den Ingh *et al.*, 1976b; Maxie

et al., 1979) particularly in the liver (Krampitz, 1970; Tartour and Idris, 1973). Factors which have been postulated to induce haemolysis include, a) immunologic factors, b) haemolytic factors produced by trypanosomes, c) activated mononuclear phagocytic system (MPS), d) disseminated intravascular coagulation (DIC), e) direct trauma to the red cells, and f) pyrexia (reviewed by Murray and Dexter, 1988).

a) Immunological factors

It has been known for a long time that immunological mechanisms may be involved in the pathogenesis of anaemia in African trypanosomiasis. In cattle infected with *T. congolense* (Kobayashi *et al.*, 1976) and with *T. vivax* (Facer *et al.*, 1982; Assoku and Gardiner, 1989), IgM and IgG were detected on the surface of red blood cells by the direct haemagglutination test, immunodiffusion and immunofluorescence techniques. It was found that the red blood cells from *T. congolense*-infected cattle had immunoglobulins with antibody activity against *T. congolense* (Kobayashi *et al.*, 1976), while red cells from *T. vivax*-infected cattle had immunoglobulins with antibody activity against *T. vivax* (Facer *et al.*, 1982). However, Assoku and Gardiner (1989) reported that antibodies which were adsorbed on the red cells could be eluted and the eluted antibodies did not react with the infecting *T. vivax*. Herbert and Inglis (1973) and Woo and Kobayashi (1975) have suggested that soluble trypanosome antigen, released from dying trypanosomes, is adsorbed on the red cells with subsequent opsonisation of antibody and complement. Using the indirect fluorescent antibody test, MacKenzie *et al.* (1978) demonstrated trypanosomal antigen on the surface of red cells of sheep infected with *T. congolense*. The reaction was evident after the first peak of parasitaemia and was thought to be a result of trypanolysis.

Autoantibodies to a variety of hosts cells or their products have been reported in man and in laboratory animals infected with trypanosomes (MacKenzie and Boreham, 1974; Kobayakawa *et al.*, 1979). It has been suggested that parasite-derived factors may be the primary cause of red cell damage and that exposing hidden epitopes on the red cell membrane induces the production of autoantibodies which then play a key role in the maintenance and progression of the anaemia (Murray and Dexter, 1988). Sensitisation of red blood cells may result from passive attachment of trypanosome antigen-antibody complexes to the membrane of red cells. In *T. congolense* infections in rabbits, Banks (1980) demonstrated, in an *in vitro* system, that the binding of anti-trypanosomal antibody and complement activation, with ensuing red cell damage only occurred when the organisms had attached to the red cells. In contrast, Assoku and Gardiner, (1989) found that IgM and IgG on the surface of the red cells from *T. vivax*-infected cattle was specific for red cells. The enzyme-linked immunosorbent assay demonstrated antibodies to normal erythrocytes in the plasma of infected animals just after the first peak of parasitaemia.

b) Haemolytic factors produced by trypanosomes

The development of anaemia becomes obvious after the first peak of parasitaemia when as a result of antibody responses, a major trypanolytic crisis occurs. This crisis and subsequent ones may lead to release of biologically active factors known to be present in trypanosomes (Tizard *et al.*, 1978a-c). Fiennes (1954) demonstrated the presence of a factor in *T. congolense*-infected cattle that was capable of lysing normal red cells.

Studies in mice infected with *T. congolense, T. vivax* and *T. brucei* (Murray *et al.*, 1979) and in rats infected with *T. brucei* (Murray, 1979) also indicated the presence of haemolysins in plasma. Haemolytic activity has also been demonstrated in lysates of *T. brucei* (Huan *et al.*, 1975), *T. congolense* and *T. vivax* (Murray *et al.*, 1979).

The haemolytic factors have been identified as either small proteins (Huan *et al.*, 1975) or lipids (Tizard and Holmes, 1976). It has been observed that the lipid material is composed of a mixture of free fatty acids (FFA) (Tizard and Holmes, 1976; Tizard *et al.*, 1977) the most potent of which is linoleic acid (Tizard *et al.*, 1978b,c; Meade and Mertin, 1976, Lawrence et al., 1974). The mechanism of this lysis is probably due to its detergent activity or due to the activity of its oxidised products (Tizard *et al.*, 1978b,c).

It has been proposed that the source of these free fatty acids is a potent phospholipase A which is found in high levels in pathogenic trypanosomes (Hambrey *et al.*, 1980, Hambrey et al., 1984). It hydrolyses exogenous lysophospholipids to yield glycerophosphocholine and free fatty acids, including, palmitic, stearic and linoleic acids with lesser amounts of arachidonic and oleic acids (Tizard *et al.*, 1977; Mellors, 1985). Some free fatty acids may also originate from the unesterified free fatty acid pool, for example, free stearate, palmitate and oleate are present in *T. rhodesiense* (Dixon and Williamson, 1970). In addition, trypanosomal phospholipases could have a direct pathological effect on the red cell membranes leading to early clearance from circulation.

Entry of unbound free fatty acids into the erythrocytic membrane causes massive crenation (Kamada *et al.*, 1987) and this may contribute to an early removal of erythrocytes since normal morphology is necessary for proper dynamics of blood in the microcirculation (Braasch, 1971). Free fatty acids do not usually exist free in the bloodstream but are rapidly bound to serum albumin (Goodman, 1958; Spector

et al., 1969) which renders them non-haemolytic (Starinsky and Shafrir, 1970). However, *T. congolense* tends to congregate in dense clusters in capillary beds attaching to red cells or vascular endothelium and it is possible that FFA generated in such locations may reach sufficiently high concentrations to induce red cell damage. Similarly, damage to the red cells could result from large quantities of FFA released during trypanolytic crises and although serum albumin binds fatty acids and largely neutralises their membrane activity both *in vivo* and *in vitro*, it is possible that this is of reduced significance in trypanosomiasis, because animals and patients with trypanosomiasis are usually severely hypoalbuminaemic (Gall, 1956; Valli *et al.*, 1980).

Sialic acid, the acylated neuraminic acid (Blix *et al.*, 1957) occurs in appreciable quantities on the erythrocyte surface in several animal species (Eylar *et al.*, 1962; Seaman and Uhlenbrock, 1963) and a reduction in the erythrocyte surface sialic acid content is thought to be a mechanism of erythrocyte senescence and destruction (Durocher *et al.*, 1975). It has been shown that *T. congolense* binds to the bovine erythrocyte *in vitro*, through the neuraminic acid receptors on the erythrocytes (Banks, 1979) leading to erythrocyte damage (Banks, 1980). It has been observed that *T. vivax* produces neuramidase, also called sialic acid cleaving enzyme (Esievo, 1979; Esievo and Saror, 1983) which removes sialic acid, rendering the erythrocyte more prone to phagocytosis by the reticulo-endothelial system (Esievo *et al.*, 1982).

Red cell damage might also result from proteases released from trypanosomes (Rautenberg *et al.*, 1981) especially following trypanolytic crises. A peptidase, apparently of trypanosomal origin has been identified in the plasma of cattle infected with *T. congolense* and of mice infected with *T. brucei* (ILRAD, 1986; Knowles *et al.*, 1987).

c)The role of the mononuclear phagocytic system (MPS).

Splenomegaly, hepatomegally and lymphadenopathy are often seen, especially during acute trypanosome infection in man and animals (Losos and Ikede, 1972; Woodruff, 1973). The most striking pathology in the enlarged spleen, liver and lymph nodes is the hyperactivity of the mononuclear phagocytic cells (Anosa, 1988; Anosa and Kaneko, 1989; Murray, 1974).

It has been observed that the MPS expanded by repeated stimulation remains active long after the stimulant has been withdrawn (Jandl *et al.*, 1965), thereby, causing anaemia by hypersequestration and erythrophagocytosis (Dargie *et al.*, 1979a, b). The phagocytosis of erythrocytes has also been reported in sheep infected with *T. congolense* (MacKenzie and Cruickshank, 1973), in cattle infected with *T. vivax* (Isoun and Esuruoso, 1972) and *T. congolense* (Mamo and Holmes, 1975) and in rats infected with *T. brucei* (Murray *et al.*, 1974; Jennings *et al.*, 1974). The accelerated removal of red cells leads to shortening of the erythrocyte life span (Mamo and Holmes, 1975; Valli and Forsberg, 1977; Ikede *et al.*, 1977; Preston et al., 1979) and development of anaemia.

The slow mixing and stagnation of red cells in the large extrasinusoidal compartments of enlarged spleens may have an injurious effect on red cells (Anosa and Kaneko, 1989; McCrorie *et al.*, 1980) and may slow down the rate of exchange between the spleen and the body circulation. This may deprive the body circulation of a large volume of red cells. However, the contribution of this effect to the anaemia in *T. brucei*-infected rabbits was found to be small (Goodwin, 1970).

Splenomegaly, by extending travel through a lengthened vascular network and leading to more protracted contact with numerous and active macrophages lining the channels, is likely to contribute to red cell destruction (Jenkins and Facer, 1985), including removal by phagocytosis even of normal cells. Removal of mature and immature erythroid cells associated with activated MPS in the bone marrow was

also observed in calves infected with *T. vivax* (Logan *et al.*, 1989) using transmission electron microscopy. They observed extensive phagocytosis of erythroid and myeloid cells by macrophages in the rib and femur marrows, as early as 11 - 12 days after infection.

d) Disseminated intravascular coagulation (DIC)

The involvement of the coagulation system in the pathogenesis of animal trypanosomiasis has not been studied extensively. The formation of microthrombi in acute trypanosomiasis has been reported in cattle (Fiennes, 1970; Isoun and Esuruoso, 1972), in goats (Losos and Ikede, 1972; van den Ingh *et al.*, 1976b), while fibrin degradation products (FDPs) were demonstrated in rabbits experimentally-infected with *T. brucei* (Boreham and Facer, 1974; Boulton *et al.*, 1974) and in man with an active form of sleeping sickness caused by *T. rhodesiense* (Barrett-Connor *et al.*, 1973). DIC can lead to microangiopathic haemolytic anaemia which is characterised by widespread fibrin deposition in the microvasculature and various forms of abnormal red cells, including, schisocytes, burr cells, poikilocytes, acanthocytes and helmet cells (Assoku and Gardiner, 1989). The damaged erythrocytes are rapidly phagocytosed.

e) Direct trauma to red cells

The destruction and abnormalities of the red cells observed in trypanosomiasis have been considered to be caused, at least partially, by direct mechanical injury to the erythrocytes. This injury might be due to the lashing action of trypanosomes especially during peaks of parasitaemia (Anosa and Kaneko, 1983; Valli *et al.*, 1978). In addition, mature red blood cells, reticulocytes and some platelets were observed to adhere firmly to trypanosomes (Banks, 1980; Bungener and Muller, 1976). However, the role of adhesion in precipitating increased red cell

destruction and other abnormalities is not clear, though Anosa and Kaneko (1989) suggested that adhesion of trypanosomes to red cell surface promotes haemolysis and speculated that, as the parasitaemia increases, the predisposition to adhesion would increase.

f) Pyrexia

Undulating fever is a well recognised feature of African trypanosomiasis and pyrexia is often associated with trypanolytic crises (Fiennes, 1954). Studies with human and rabbit red cells have shown that small elevation in temperature for a few hours causes increased osmotic fragility, increased permeability and reduced plasticity with the result that *in vivo* red cell survival time is shortened due to increased destruction (Karle, 1974). These findings indicate a possible role for fever in the anaemia of African trypanosomiasis.

The role of cytokines

Cytokines are polypeptide mediators produced by macrophages and lymphocytes as a consequence of immune response to invading organisms. They transmit signals from one cell to another and together with other substances they constitute the molecular language of inflammation and immunity (Old, 1988). The commonly studied cytokines include interferon, interleukins and tumor necrosis factor (TNF). TNF was discovered because of its cancer killing activity and later Beutler *et al.*, (1985b) found that it was similar to a factor they had called cachectin because of its involvement in a state of cachexia observed in many chronic diseases. Since then the two have been use synonymously, i.e, cachectin/TNF.

Hotez *et al.* (1984) observed that lysates of *T. brucei* and *Plasmodium berghei* stimulated murine peritoneal exudate cells (primary macrophages) to release a factor which suppresses lipoprotein lipase activity in preadipocytes. A reduction in

lipoprotein lipase activity was also observed in sheep infected with *T. congolense* and *T. vivax* (Roberts, 1973) but it was not clear whether this was due to production of cachectin/TNF or was secondary to a reduction in total serum lipids.

The role of cachectin/TNF in the anaemia of trypanosome infections is not clear. It has been reported that cachectin/TNF blocks the release of iron from the macrophages (Alvarez-Hernandez *et al.*, 1989). Hyperactivity of the MPS is known to be a common feature of trypanosome infections (Anosa and Kaneko, 1983, 1989; Murray, 1974) and it is associated with excessive hamosiderosis in the spleen, liver and lymph nodes of infected animals (Fiennes, 1954; Jennings *et al.*, 1974). It is possible that cachectin/TNF blocks the transfer of iron from the macrophages to the bone marrow leading to inadequate erythropoietic response. In addition, cachectin/TNF has been shown to cause dyserythropoiesis by its direct effect on the bone marrow by inhibiting the growth and differentiation of erythroid progenitor cells (Sassa *et al.*, 1983; Tracey *et al.*, 1988). Despite the apparent significance of cachectin/TNF in the pathogenesis of anaemia, it has not been investigated extensively in trypanosome infections.

Leucocytic Changes

The drop in total white cell count in bovine trypanosomiasis has been well documented (Naylor, 1971a; Wellde *et al.*, 1974; Anosa, 1983) and there is evidence that this coincides with the onset of anaemia in the acute phase of infection (Valli *et al.*, 1979). The leucopaenia is associated with neutropaenia, eosinopaenia and lymphopaenia (Anosa and Kaneko, 1983) but with chronicity, a lymphocytosis develops with a total white cell count returning to normal or even exceeding pre-infection levels (Valli *et al.*, 1979). It has been suggested that leucopaenia in the pathogenic trypanosome infections could be due to increased erythropoiesis at the expense of leucopoiesis (Valli *et al.*, 1979), due to massive leucophagocytosis in the

liver after coating of the leucocytes with trypanosomal antigen (McKenzie *et al.*, 1978) or the result of depression of stem cells caused by a circulating toxic factor released from disrupted trypanosomes (Kaaya *et al.*, 1979).

Esievo and Saror (1983) have suggested that leucopaenia could also result from plasma neuramidase released by the trypanosomes (Esievo, 1979) which cleaves sialic acid from the leucocytes and thus diverts them from the lymph nodes and spleen to the liver for destruction as has been noted to occur with influenza virus neuramidase (Woodruff and Woodruff, 1976).

Thrombocytopaenia

Thrombocytopaenia is a constant feature of human and animal trypanosomiasis (Robbins-Browne *et al.*, 1975; Davis, 1982; Wellde *et al.*, 1983). It has been reported in *T. congolense* infection (Wellde *et al.*, 1978; Forsberg *et al.*, 1979) and in *T. vivax* infection (Olubayo and Mugera, 1985) of cattle. The cause of thrombocytopaenia appears to be multifactorial. Assoku and Gardiner (1989) detected antibodies to platelets in the plasma of cattle infected with *T. vivax* using enzyme-linked immunosorbent assay (ELISA). The antibodies were detected following the first peak of parasitaemia which may suggest its association with trypanosomal-derived products (Syndercombe-Court, 1985).

Thrombocytopaenia may also result from ineffective thrombopoiesis (Forsberg *et al.*, 1979), platelet aggregation (Anosa and Kaneko, 1989) or direct injury to platelets by the trypanosomes (Davis *et al.*, 1974) which predisposes them to phagocytosis in the spleen and haemal nodes. While thrombocytopaenia in the early stages of infection has been associated with trypanosome products, its maintenance in the late stages of infection has been associated with antiplatelet antibody and the expanded mononuclear phagocytic system (Syndercombe-Court, 1985). Anosa (1988) has suggested that thickening of the marginal zone of the

megakaryocyte may interfere with platelet release and this may contribute to the maintenance of thrombocytopaenia. Severe thrombocytopaenia has been associated with widespread haemorrhages observed in some strains of *T. vivax* infections in cattle in Kenya (Gardiner et al., 1989; Mwongela *et al.*, 1981; Olubayo and Mugera, 1985; Wellde *et al.*, 1983; Wellde *et al.*, 1989b) and this contributes to the development of anaemia.

Biochemical Changes in Animal Trypanosomiasis

Lipid metabolism

African animal and human trypanosomiasis is associated with profound blood biochemical changes, particularly with regard to lipid, protein and carbohydrate metabolism. The observations on the lipid metabolism of trypanosomes (Godfrey, 1967) and the chemical composition of plasma and sera from man and animals exposed to trypanosomiasis have led many to believe that lipids may have a central role to play in the pathogenesis of trypanosomiasis (Assoku *et al.*, 1977; Tizard *et al.*, 1978a,c; Roberts, 1975a,b; Roberts and Clarkson, 1977), in trypanotolerance (Murray *et al.*, 1982) and in refractoriness to the disease (Rifkin, 1978a, b, 1984). In order to understand how lipids may influence the outcome of trypanosome infection in the ruminant, it is necessary to review briefly the normal lipid metabolism in the trypanosome and in the ruminant host.

Lipid metabolism in trypanosomes

Studies on the lipid composition of trypanosomes have largely been confined to the *brucei* group because of their ability to grow and multiply in culture media. However, recent investigations by Bastin *et al.* (1991) have shown that lipid metabolism in *T. congolense*, and *T. vivax* is similar to that in the *brucei* group. It has been observed that trypanosomes contain large amounts of lipid material

constituting 15 - 20% of the dry weight of the parasite (Williamson and Brown, 1964; Venkatesan and Ormerod, 1976), and that phospholipids form about 80% of trypanosomal total lipid (Godfrey, 1967; Venkatesan and Ormerod, 1976). Phospholipids are essential components of the plasma membrane and the major phospholipids in trypanosomes are the same as those in the mammalian host.

There is evidence to suggest that trypanosomes are able to regulate their lipid composition (Godfrey, 1967; Dixon *et al.*, 1971; Brenton *et al.*, 1988; Mellors and Samad, 1989), for example, *T. rhodesiense* possesses a higher proportion of long chain polyunsaturated fatty acids (PUFA) and linoleate and lower levels of oleate than is contained in the plasma of the human host, and *T. brucei* contains higher concentrations of cholesterol esters, free cholesterol and free fatty acids than is contained in the plasma of their rodent host (Carrol and McCrorie, 1986). The fatty acids of trypanosomes are mainly esterified as phospholipids or cholesterol esters, though they also exist as free fatty acids (Dixon *et al.*, 1972), and triglycerides constitute a minor part of trypanosomal lipid (Venkatesan and Ormerod, 1976).

It has been suggested that African trypanosomes take up lipid molecules or lipid components from the plasma of the host for their own metabolic requirements (Threfall *et al.*, 1965; Coppens *et al.*, 1987; Mellors and Samad, 1989). There is evidence to suggest that metacyclic African trypanosomes use exogenous lysophospholipds as a major source of choline, ethanolamine and other nutrients including fatty acids (Mellors and Samad, 1989). It has been observed that the main sterol in the *brucei* group of trypanosomes is cholesterol (Venkatesan and Ormerod, 1976; Threfall *et al.*, 1965) and since these trypanosomes can not synthesise cholesterol *de novo* (Dixon *et al.*, 1972; Carrol and McCrorie, 1986), they must obtain it from the host. Studies using *T. brucei* in culture have shown that *T. brucei* can take up low density lipoproteins (LDL) or high density lipoproteins

(HDL) (Coppens *et al.*, 1987; Gillet and Owen, 1987; Black and Vanderweed, 1989) and these lipoproteins serve as a source of cholesterol, phospholipids and fatty acids which the trypanosomes require for growth and multiplication.

The mechanism of uptake of these lipoproteins has been a subject of intense study. By using gold-labelled LDL, Coppens *et al.* (1987) were able to show that *T. brucei* takes up LDL particles by endocytosis involving a receptor. Later, Coppens *et al.*, (1988) isolated the receptor, purified it and obtained monospecific polyclonal antibodies against it. They suggested that the receptor is localised on the flagella pocket membrane and the flagella membrane, and is completely absent from the rest of the pellicular surface. However, Vanderweed and Black (1989) do not agree with the endocytotic method of uptake. In their studies of the interaction between *T. brucei* and lipoproteins, they found no evidence of binding of lipoproteins to *T. brucei* and the interaction did not require calcium. They suggested that these differences could be related either to the *T. brucei* populations used or to differences in experimental protocols.

The demonstration that the surface of the bloodstream-form trypanosome has receptors that are both essential for optimal growth and are accessible to such large particles as LDL and HDL may be of great practical importance. The identification, on the plasma membrane of trypanosomes, of distinct and stable proteins such as receptors, could provide better targets for immunologic and chemotherapeutic interventions against trypanosome infections.

Lipid metabolism in the ruminant host

In the adult ruminant animal, the ingested food first passes into the reticulo-rumen where it is subjected to microbial fermentation. The lipid content of fresh and dried forages consumed by cattle and sheep is relatively low and is rich in polyunsaturated fatty acids (PUFA) especially linoleic acid (Vernom and Flint,

1988). In the rumen, lipids are hydrolysed and the released fatty acids are extensively biohydrogenated by the microbial population, the net result being the formation of large amounts of stearic acid and small amounts of linoleic and linolenic acids. The unesterified fatty acids are adsorbed onto particulate matter within the rumen and pass in this form through to the abomasum. There is some *de novo* synthesis of lipids by rumen micro-organisms which also pass into the abomasum, hence the amount of lipid and fatty acids entering the abomasum exceeds that ingested (Moore and Christie, 1984).

Microbial lipid released from killed micro-organisms and dietary fatty acids pass through into the duodenum where they mix with pancreatic secretions (containing lipases and phospholipases), and bile which contains lipid (particularly phosphatidylcholine) and bile acids (Noble, 1981; Moore and Christie, 1984). In cattle and sheep, most of the fatty acids are absorbed in the unesterified form in the small intestines. Within the enterocytes, the absorbed fatty acids are esterified principally by the glycerol-3-phosphate pathway with little being esterified by the monoacylglycerol pathway (Noble, 1981). Triglycerides, phospholipids and cholesterol esters are produced and used for the production of chylomicrons which are secreted into lymph and they reach the blood through the thoracic duct. As ruminant diets are low in cholesterol, the enterocyte is an important site for cholesterol synthesis.

The lipids in the blood may arise from intestinal absorption of ingested lipids, mobilisation of lipids from storage or synthetic processes especially in the liver. Garton and Duncan (1964) and Leat (1966) observed that the major lipid components of sheep plasma are cholesterol esters and phospholipids which account for 75-80% of the total plasma lipids. They reported that triglycerides and free fatty

acids are relatively minor components. Most of the blood lipids are present as lipoproteins and the non esterified fatty acids are transported bound to albumin (Goodman, 1958; Spector *et al.*, 1969).

Plasma lipoproteins are complex high molecular weight particles composed primarily of esterified and non-esterified cholesterol, triglycerides and phospholipids (Table 1.2) and several specific proteins that, in their free state, are called apolipoproteins. Lipoproteins have lower hydrated densities than other plasma proteins, a property that has been used as a basis for their separation from the plasma and the separation of lipoproteins from each other (Bachorik, 1982). The four major classes of lipoproteins; the chylomicrons, very low density lipoproteins (VLDL), LDL and HDL (Havel, 1980) are defined in terms of their densities and differ in lipid and apolipoprotein composition, molecular mass, particle size, electrophoretic mobility and physiologic function.

Results from studies involving non-ruminants clearly indicate that the metabolic fate of a lipoprotein is determined by its apolipoprotein composition which catalyses enzymatic reactions important in its metabolism or serve as ligands or receptors involved in its cellular uptake (Grummer *et al.*, 1987).

Lipoprotein lipase (LPL) is the rate limiting enzyme in the hydrolysis of triglycerides and therefore has a key role to play in the metabolism of triglyceride-rich particles, the chylomicrons and VLDL. Lipoprotein lipase activity is present in many tissues, including, adipose tissue, skeletal and cardiac muscle, lungs, mammary gland (Eckel and Robbins, 1984; Etienne, 1984), macrophages and smooth muscle cells (Vance *et al.*, 1982). LPL does not circulate freely in plasma but is attached to the surface of the endothelial cells where its action occurs (Pedersen *et al.*, 1983). The physiological substrates of LPL are long chain triglycerides in chylomicrons and VLDL and in the process of triglyceride removal LDL is formed (Palmquist, 1976). It has been demonstrated that LPL is involved in

Table 1.2

Plasma lipids: Composition of lipoprotein fractions of plasma of cattle and sheep (Adapted from Christie, 1981)

Species	Lipoprotein fraction	Density gradient (P)	Proportion of lipid (wt %)	Lipid composition (% wt of total)				
				Triglycerides	CE	Cholesterol	FFA	Phospholipids
Cattle	VLDL and chylomicrons	< 1.006	1.5	58	19	6.4		17
	LDL	1.006-1.040	5.9	12	47	10		31
	HDL_1	1.040-1.063	35.3	-	55	6		41
	HDL_2	1.063-1.210	57.3	-	54	40		4.6
Sheep	VLDL and chylomicrons	< 1.006	0.2	54	20.5	24.1	0.5	<1
	LDL	1.006-1.063	33.6	2.7	48.8	11.8	<1	36.7
	LHDL	1.063-1.075	4.5	4.3	54.7	8.0	1.3	26.4
	HDL	1.075-1.20	33.2	1.0	53.2	5.5	3.8	36.5
	VHDL	> 1.20	28.6	<0.5	13.2	9.8	43.1	33.4

VLDL = very low density lipoproteins, LDL = low density lipoproteins, LHDL = low high density lipoproteins, HDL = high density lipoproteins, VHDL= very high density lipoproteins, CE = Cholesterol esters, FFA = Free fatty acids

the interconversions of different lipoproteins particularly in the formation of HDL (Taskinen and Nikilla, 1981). The inverse association between VLDL and HDL has been documented and suggests a precursor-product relationship.

It has been observed that the concentration of total lipids in sheep plasma and serum is low when compared to other species (Nelson, 1973; Mills and Taylaur, 1971) and Nelson (1973) reported that VLDL and chylomicrons were absent from sheep plasma. They suggested that VLDL are not essential for fat transport and metabolism at least in these species. However, the failure of these authors to detect VLDL and chylomicrons in these species may be associated with the rapid clearance of these lipoprotein fractions from plasma (Palmquist, 1976).

Effect of trypanosome infection on blood lipids

The effect of infection with trypanosomes on the blood and tissue lipids of laboratory animals has been studied extensively. There is, however, little agreement in the results obtained and increased, normal or decreased levels of blood lipids have been reported (Von Brand, 1973; Roberts, 1975b). Recent work suggests that trypanosome infection in laboratory animals usually results in hyperlipidaemia. Increased triglyceride concentration has been reported in rats infected with *T. rhodesiense* (Dixon, 1967a, b) and in rabbits infected with *T. brucei* (Guy, 1975; Rouzer and Cerami, 1980). Goodwin and Guy (1973) found greatly increased serum cholesterol levels in rabbits infected with *T. brucei* but humans infected with *T. gambiense* showed slightly reduced serum cholesterol particularly in the early stages of infection (Monnet and Baylet, 1951; Evens, 1963). This change in the concentration of serum cholesterol was attributed to a redistribution of cholesterol into other tissues or to plasma dilution (Hoffman, 1970).

Measurement of blood lipid levels in ruminants infected with trypanosomes have been made on few occasions. Fiennes *et al.* (1946) recorded that blood cholesterol levels in cattle infected with *T. congolense* decreased with the development of anaemia and Valli *et al.* (1980) recorded a decrease in total serum lipids, cholesterol and an increase in triglycerides in cattle infected with *T. congolense*. Sheep infected with either *T. vivax*, *T. congolense* or *T. brucei* showed a large decrease in cholesterol and phospholipids leading to a marked fall in total serum lipids to half or less than half of the pre-infection values (Roberts, 1974; 1975a, b) and similar decreases in cholesterol were recorded in cattle infected with *T. congolense* (Traore-Leroux *et al.*, 1987a). Roberts (1975a) suggested that the decline in serum phospholipids was mainly due to a reduction in phosphatidylcholine and he suggested that this could be associated with hepatic pathology since the liver is the main site of phosphatidylcholine synthesis.

Decreases in the serum concentration of lysophosphatidylcholine from a normal concentration of 7-10% of total phospholipid concentration to 0-3% were recorded in sheep infected with *T. congolense* (Roberts, 1975a, b; Roberts *et al.*, 1977). This may be explained in part by the activity of phospholipases and lysophospholipases released by disrupted trypanosomes. Similar enzyme activities have been identified in tissue fluids of rabbits infected with *T. brucei* (Hambrey *et al.*, 1980). These enzymes act on endogenous phospholipids leading to the release of fatty acids and a subsequent reduction in the concentration of serum phospholipids, interference with formation of HDL and a reduction in lipoprotein lipase activity (Roberts, 1974). Studies involving *T. brucei* have demonstrated that trypanosomes contain significant amounts of phospholipids and cholesterol which they obtain from their hosts (Coppens *et al.*, 1987, 1988). The rise in parasitaemia in the early stages of infection may lead to uptake of large amounts of these substances to the extent that their concentration in serum may decline.

It has been suggested that lysophosphatidylcholine is essential for utilisation of cholesterol and possibly other lipids (Roberts *et al.*, 1977) and its significant reduction may result in blocking of lipid utilisation and subsequent mobilisation of body fats.

A state of cachexia characterised by severe weight loss and anaemia occurs in cattle and rabbits infected with trypanosomes. Rabbits infected with *T. brucei* develop a hypertriglyceridaemia associated with an increase in VLDL (Rouzer and Cerami, 1980). The increase in triglyceride concentration in plasma appears to result from a defect in its degradation due to loss of lipoprotein lipase activity (Rouzer and Cerami, 1980) which is responsible for uptake of fatty acids by adipocytes and peripheral tissues. The reduction in lipoprotein lipase has also been reported in sheep infected with *T. vivax* and *T. congolense* (Roberts, 1974; 1975b) but it was not associated with hypertriglyceridaemia in these animals. Rouzer and Cerami (1980) have suggested that the deficiency in activity may be due to an actual decrease in the amount of enzyme protein, a loss in the specific activity of the protein or to the presence of an inhibitor of the enzyme.

Experiments in rabbits and mice infected with *T. brucei* have demonstrated a serum factor called cachectin which suppresses lipoprotein lipase and other key lipogenic enzymes in the adipocytes (Beutler *et al.*, 1985a, b; Cerami and Beutler, 1988; Cerami *et al.*,1985; Kawakami *et al.*, 1982). It has been observed that lysates of *T. brucei* induce peritoneal exudate cells to produce cachectin/TNF which may be largely responsible for reduced lipid utilisation by the infected host (Hotez *al.*, 1984). While cachectin/TNF appears to be important in the pathogenesis of hyperlipidaemia and cachexia in *T. brucei* infections in mice, rabbits and man, its significance in the pathogenesis of *T. congolense* and *T. vivax* infections of domestic ruminants has not been elucidated.

Glucose metabolism

There are conflicting reports on the levels of glucose in the blood of animals infected with trypanosomes. Low levels have been recorded in buffalo calves infected with *T. evansi* (Kathiria and Avsatthi, 1985), in rabbits (Omole and Onawunmi, 1979) and in rats (Sanchez and Dusanic, 1968) infected with *T. brucei.* Similar observations have been reported in cattle infected with *T. rhodesiense* (Wellde *et al.*, 1989a), but Grant and Fulton (1957) and Goodwin and Guy (1973) found increased blood glucose and pyruvate levels in rats infected with *T.brucei.* On the other hand, Edwards *et al.* (1956b) observed that plasma glucose levels in sheep and goats infected with *T. vivax*, *T. congolense* or *T. brucei* remained within the normal range.

Hypoglycaemia was thought to be important in the pathogenesis of trypanosomiasis particularly in hyperacute infections such as *T. vivax* in cattle and *T. simiae* in pigs (Hudson, 1944) where there are enormous numbers of trypanosomes in the blood. This has been attributed to high sugar consumption by the trypanosomes (Sanchez and Dusonic, 1968; Simaren and Awopetu, 1973). It is possible that disturbances in carbohydrate metabolism observed in some cases of human and animal trypanosomiasis may be associated with hepatic lesions. Lesions recorded in cattle infected with *T. congolense* have included periportal round cell infiltration, degeneration and necrosis of parenchymatous cells and hypertrophy of kupffer cells (Fiennes, 1970; Losos *et al.*, 1973; Valli *et al.*, 1980). However, Edwards *et al.* (1956b) found that the bilrubin level and icterus index of plasma of sheep infected with *T. congolense, T. vivax* or *T. brucei* remained within normal limits.

There is controversy in the interpretation of enzymatic changes in the blood which may indicate liver damage. Lippi and Sebastiani (1958) attributed the changes in the concentration of serum aminotransferases during *T. brucei* infections

in guinea pigs to lesions of the liver and other organs but Gray (1963) stated that increased levels of serum aminotransferase activity in cattle and sheep infected with *T. vivax* resulted from the release of trypanosomal enzyme following destruction of the trypanosomes. Reynolds *et al.* (1973) similarly found no evidence for changes in the activity of liver specific enzymes in the serum of cattle infected with *T. vivax* and Moon *et al.* (1968) reported that some enzyme changes occurring in the serum of mice infected with *T. rhodesiense* appeared to be directly related to the degree of parasitaemia.

Blood proteins

Studies on total blood proteins in trypanosome-infected animals have yielded contradictory results. Significant decreases in total proteins have been observed in cattle infected with *T. congolense* (Tabel *et al.*, 1980; Valli *et al.*, 1980), *T. vivax* (Sekoni *et al.*, 1990) and in sheep infected with either *T. congolense* or *T. vivax* (Edwards *et al.*, 1956b; Clarkson, 1968; Anosa and Isoun, 1976). However, increases have been reported in sheep (Bouteille *et al.*, 1988b) infected with *T. brucei* and in cattle infected with *T. rhodesiense* (Wellde *et al.*, 1989a).

Hypoalbuminaemia is a consistent finding in trypanosome-infected animals (Valli *et al.*, 1980; Anosa and Isoun, 1976; Kalu *et al.*, 1989; Wellde *et al.*, 1989b). It is commonly associated with a marked increase in immunoglobulins as has been observed in cattle experimentally infected with *T. vivax* (Clarkson *et al.*, 1975), in cattle exposed to natural (Luckins, 1972) and experimental challenge (Wellde *et al.*, 1989b), leading to a decrease in the albumin to globulin ratio. The decline in total plasma protein and albumin may be attributed to haemodilution (Clarkson, 1968), consumption by trypanosomes or increased utilisation by the infected host (Vickerman and Tetley, 1979).

Blood inorganic constituents

Changes in the concentrations of inorganic constituents in ruminant animals infected with trypanosomes have been investigated on a few occassions. Many of the blood inorganic constituents showed considerable fluctuation (Fiennes *et al.*, 1946). Kalu *et al.* (1989) observed an increase in the concentration of serum chloride and calcium in goats infected with *T. vivax*. In cattle infected with *T. congolense,* Hudson (1944) recorded a gradual fall in the concentration of inorganic phsphate. However, French (1938) did not observe any significant changes in blood inorganic phosphate. It is possible that altered metabolism of the thyroid and the adrenocortical glands during a course of trypanosome infection may account for most of the electrolyte disturbances.

Low serum iron has been reported in cattle with acute trypanosomiasis (Valli *et al.*, 1978) as well as in more chronic infections (Tartour and Idris, 1973. Using Zebu calves, Tartour and Idris (1973) recorded a hypoferraemia in the early stages of infection and a hyperferraemia in the terminal stages of infection. The hypoferraemia could be attributed to either less retention of iron in the body or to rapid removal of plasma iron by the reticulo-endothelial system (Underwood, 1971).

GENETIC RESISTANCE/HOST SUSCEPTIBILITY TO ANIMAL TRYPANOSOMIASIS

It is now well known that some animals are able to survive and be productive in areas infested by tsetse flies while others succumb to infection and die. These variations occur between and within breeds of animals and they may be attributed to either a) genetic influences and/or b) host factors such as age, sex, breed and nutritional status (Murray *et al.*, 1982; Murray, 1979).

Genetic Influences

It has been established that host genetics is a key factor in regulating the distribution of parasites in an outbred host population. The heritable nature of the host responsiveness raises the possibility of selective breeding of animals which are less susceptible to infection, thus limiting parasite establishment and / or survival. The crucial prerequisite of the progress towards practical implications of this approach is a readily identifiable marker of the host which is linked to the gene(s) regulating the responsiveness of the host.

The resistance of man to infection by *T. brucei* and his susceptibility to the morphologically identical *T. gambiense* and *T. rhodesiense* is one of the most well documented examples of host-parasite specificity. It has been observed that *T. brucei* is lysed on exposure to human plasma (Hawking, 1979) by a non-immune killing factor which has been identified as the human HDL (Rifkin, 1978a, b). This factor is non-toxic to the African sleeping sickness trypanosomes, *T. rhodesiense* and *T. gambiense* (Hawking, 1976) and is thought to be the key to the refractoriness of man to *T. brucei* infection. Trypanolytic activity is thought to reside in the major portion of the human HDL (Rifkin, 1978a) but disagreement with this suggestion exists (Hajduk *et al.*, 1989). Seed and Sechelski (1989) have suggested that the trypanocidal factor is not one of the major apolipoproteins but appears to be a minor portion of HDL and its chemical nature is still uncertain. These studies suggest that the lytic pathway may involve uptake of trypanosomal subspecies of HDL by endocytosis (Hajduk *et al.*, 1989, Hajduk *et al.*, 1992) causing massive accumulation of cholesterol esters and release of autolytic enzymes. However, Gillet and Owen (1987) reported that the binding of HDL is accompanied by slight degradation and inhibition of esterification and they suggested that the lethal action of this lipoprotein occurs directly at the trypanosomal surface. This lytic factor was not found in the bovine, rabbit and rat sera (Rifkin, 1978b).

It has been demonstrated that certain cattle can thrive with little or no parasitaemia in areas heavily infested with trypanosome-infected *Glossina* and are thus considered trypanotolerant while other individuals of the same race appear to be trypanosensitive in that they become heavily parasitised and soon die of trypanosomiasis. Trypanotolerance has been attributed to the taurine breeds of cattle in West and Central Africa, namely the N'Dama and West African Shorthorn (Murray and Trail, 1984). Using animals that had not been previously exposed to trypanosomiasis, it was found that N'Dama cattle were significantly more resistant than Zebu cattle to experimental challenge with wild caught tsetse (Roberts and Gray, 1973), natural field exposure (Toure *et al.*, 1978; Murray *et al.*, 1982; Paling *et al.*, 1991a, b) and to trypanosomes inoculated by syringe (Murray *et al.*, 1979; Saror *et al.*, 1981).

It is only recently that studies have been conducted to determine comparative susceptibility of various breeds of small ruminants to *T. congolense* infection (Griffin and Allonby, 1979d, Whitelaw and Murray, 1982, Toure *et al.*, 1983; Whitelaw *et al.*, 1985b) and *T. vivax* (Maikaje *et al.*, 1989). Griffin and Allonby (1979d) found that indigenous East African goats (Galla) were more tolerant to *T. congolense* infection than Saanen goats and their crossbreeds showed intermediate susceptibility. However, Whitelaw *et al.* (1985b) failed to demonstrate any superiority in resistance of the indigenous small East African goats over other breeds in Kenya following experimental challenge with *T. congolense*-infected tsetse. They found that all the five breeds (East African, Galla and crossbreeds between East African and Galla, Nubian or Toggenberg) were equally highly susceptible and suffered a severe disease. Work by Griffin and Allonby (1979d) suggested that trypanotolerance also occurs in sheep which have been reported to be less susceptible to *T. congolense* infection than cattle. This may explain why they are able to survive, in areas infested with tsetse flies, without the aid of trypanocidal

agents. Various mechanisms have been suggested to explain trypanotolerance which appears to depend upon the inherent capacity to control the level of parasitaemia and/or to limit the degree of anaemia. In cattle, Desowitz (1959) suggested that the N'Dama were able to mount a superior secondary immune response to trypanosomes and this was later confirmed by Shapiro and Murray (1982) using *T. brucei* infections in N'Dama and Zebu cattle. Another possibility is that resistant animals are less susceptible to immunodepression, a common feature of trypanosome infections (Holmes *et al.*, 1974; Scott *et al.*, 1977).

Studies involving the Red Maasai sheep suggest that resistance is primarily a result of its ability to mount an effective erythropoietic response (Whitelaw and Murray, 1982) as opposed to the ability to control the level of parasitaemia. The survival of sheep may not be attributed to genetic resistance to trypanosomiasis alone but tsetse host preference may be a contributory factor. Boyt (1971) has suggested that *Glossina* find sheep unpalatable compared to cattle and feed on them only when they are hungry, and there is no alternative. Also the presence of hair or wool in different breeds may affect the feeding behaviour of *Glossina* and, in turn, the infection rate under natural conditions.

The mechanism of resistance in animals is unclear and it has been suggested that this could be due, in part, to non-immune factors which affect trypanosome growth and differentiation (Murray *et al.*, 1982). Since the identification of non-immune factors in man that affect *T. brucei*, many workers have tried to search for similar factors or markers that could be used to identify trypanotolerant animals.

Traore-Leroux *et al.* (1987a) estimated cholesterol levels in the serum of trypanosensitive and trypanoresistant cattle during *T. congolense* infection and they observed that cholesterol levels were significantly higher in Zebu cattle (trypanosensitive) than in Baoule cattle (trypanotolerant). Since Zebu cattle are generally considered to be more susceptible to trypanosomiasis than West African

taurine breeds, the observations of Traore-Leroux *et al* (1987a), tend to suggest that animals with higher plasma cholesterol concentrations would be more susceptible than those with low concentrations. It has been demonstrated that trypanosomes require cholesterol and phospholipids for growth and multiplication and it is possible that high lipid levels in plasma support higher parasite numbers which may in turn lead to a more severe disease. However, this possibility needs further investigation.

It has been demonstrated that *T. vivax* (Esievo, 1979) and *T. cruzi* (Pereira, 1983) produce sialidase (neuramidase) whose activity increases linearly with increasing numbers of parasites (Esievo *et al.*, 1982). This enzyme releases sialic acid from both the substrate *in vitro* (Esievo and Saror, 1983) and bovine erythrocytes during trypanosome infection (Esievo *et al.*, 1982; Esievo et al., 1986). Jancik *et al.* (1978) have suggested that desialylated erythrocytes are rapidly opsonised by immunoglobulin and complement molecules for degradation by phagocytic cells of the liver and spleen, hence contributing to the development of anaemia.

There are reports that resistant N'Dama cattle have higher erythrocyte surface sialic acid concentrations than the sensitive Zebu breed of cattle (Esievo *et al.*, 1982; Esievo *et al.*, 1986). Esievo *et al.* (1990) have identified a band of sialic acid of an estimated molecular weight of 30 kDa in N'Dama cattle and not in Zebu cattle and they have suggested that this may account for high sialic acid levels in the former breed. The high erythrocyte sialic acid content is thought to confer on the erythrocyte membrane a barrier that controls or regulates the transcellular movement of fluids making the red cell more resistant to lysis (McKnight, 1969; Esievo et al., 1981).

There is evidence that trypanotolerant cattle have higher levels of polyamine oxidase (PAO) than sensitive ones (Traore-Leroux *et al.*, 1987b). Polyamine oxidase oxidises spermine and spermidine which are present in African trypanosomes (Bacchi *et al.*, 1979) leading to the release of unstable aldehydes, acrolein, ammonia and hydrogen peroxide (Ganem, 1982; Pegg and McCann, 1982; Matsui *et al.*, 1982). These amino aldehydes undergo B-elimination to yield acrolein which has been implicated in intraerythrocyte killing of *Babesia* and *Plasmodium* species (Morgan and Christensen, 1983). It has been shown that bovine serum contains appreciable quantities of polyamine oxidase (Morgan, 1980) but trypanosomes even at the peak parasitaemia would not release sufficient quantities of spermidine for the generation of enough toxic degradation products. Additional polyamines would be derived from tissues damaged as a result of infection. Traore-Leroux *et al.* (1987b) suggest that levels of PAO are positively correlated with resistance to trypanosomiasis and should be used as a parameter for selection, at least in West Africa.

Some work has been done to investigate whether haemoglobin typing can be used as a tool to identify responsiveness to parasite infections. Most of the studies relating to the influence of haemoglobin polymorphism on parasitism has been conducted in sheep infected with helminths and it has been observed that sheep of Hb type AA are more resistant to *Haemonchus contortus* infection than those of Hb type AB or BB (Allonby and Urquhart, 1976; Altaif and Dargie, 1978; Preston and Allonby, 1979a). Allonby and Urquhart (1976) also observed that in Merino sheep in Kenya, sheep of Hb type AA had the higher PCV and Hb concentration values than those of Hb type BB and those of Hb type AB were intermediate.

Petit (1976) and Queval and Petit (1982) conducted studies on trypanotolerant (N'Dama and Baoule) and trypanosensitive (Zebu) cattle of West Africa and reported predominance of HbA in the former and of HbB in the latter.

From these observations, they suggested that HbA was associated with resistance or tolerance to trypanosome infections. Later, Queval *et al.* (1989) compared osmotic resistance of erythrocytes of Zebu with those of Baoule cattle. They observed that the erythrocytes of Baoule cattle were more resistant to haemolysis than those of Zebu, and Zebu x Baoule cattle and they suggested that this could explain lower severity of anaemia in Baoule cattle suffering from trypanosomiasis. However, certain exotic breeds of cattle such as the Friesian are also predominantly Hb AA (Bangham and Blumberg, 1958) and are as highly susceptible to trypanosomiasis as the Zebu with Hb AA.

Queval (1989) recorded a significant difference in albumin phenotype between trypanoresistant and sensitive cattle. He observed that N'Dama and Boule cattle had a high frequency of Albumin (Alb) FF phenotype comapred to Zebu cattle, and within the Baoule breed alone, animals with Alb FF phenotype have six times more chances of being trypanoresistant than those with Alb FS or SS phenotypes. From haemoglobin and albumin typing studies, Queval (1989) concluded that Hb AA and Alb FF can be used as criteria to select trypanoresistant cattle in West Africa. However, further investigations are needed to ascertain whether haemoglobin and albumin polymorphism, in cattle and small ruminants, can be used to predict trypanotolerance.

It has been suggested that levels of plasma zinc may also be an influential factor in determining susceptibility to trypanosomiasis (Traore-Leroux *et al.* 1985). There are reports that zinc deprivation in rodents impairs antibody response against T-dependent antigens and the generation of T cells (Fernandes *et al.*, 1979; Bach, 1981) and that mice given a zinc free diet are more sensitive to *T. musculi* infection than mice given a normal diet (Lee *et al.*, 1983). Zinc, on the other hand, inhibits motility and phagocytic activity of neutrophils and macrophages and interferes with antigen presentation (Kiremidjian-Schumacher *et al.*, 1981; Chandra and Tejpar,

1983). Traore-Leroux *et al.* (1985) have suggested that zinc acts on the immune system of sensitive animals by interfering with antigen handling by macrophage-like cells which are known to play an important role in trypanosomiasis (Grosskinsky *et al.*, 1983).

It has been reported that after the onset of an infectious process, zinc is immediately withdrawn from plasma and sequestered in the liver. Pekarek and Engelhardt (1981) suggest that this serves a useful purpose in host defence by depressing zinc concentrations in plasma to a range favourable for phagocytic cells to function most effectively.

Host Factors

Several factors have been shown to have a major effect on the susceptibility of ruminant hosts to trypanosome infection. The most significant of which are age and nutritional status (Murray *et al.*, 1982, Murray and Dexter, 1988; Murray 1989). Previous exposure to trypanosme infections (Paling *et al.*, 1991a), stress (Agyemang *et al.*, 1992), and the sex of the animal may also affect the duration and severity of trypanosome infections, however, these factors have not been adequately investigated.

a) Age

A number of workers have confirmed that calves of less than one year old are more resistant to the effects of trypanosomiasis than adults (Fiennes, 1970; Fimmen *et al.*, 1983; Maxie and Valli, 1979; Mehlitz *et al.*, 1983; Wellde *et al.*, 1981). Moreover, the observation that the longer a cow stays in a trypanosome endemic area the longer it takes for its calf to develop a trypanosome infection (Whiteside, 1962), suggests some level of resistance is being acquired.

There is evidence that ingestion of colostrum containing trypanosome specific antibodies by kids suppressed infection with *T. brucei* for about 4 weeks (Whitelaw and Jordt, 1985). In another experiment in which does were immunised with the surface coat of a clone of *T. congolense*, Mwamachi *et al.* (1991) observed that kids which received colostrum from immunised dams developed lower parasitaemia, lower degree of anaemia, had superior weight gains, and survived the experimental infection longer than kids that received colostrum from non-immunised dams. These observations suggest that colostral antibodies from dams under repeated trypanosome challenge in endemic areas could confer protection to the offsprings, particularly in the early stages of life, resulting in longer survival times.

b) Nutritional status

The interaction between the plane of nutrition and the presence of haemoprotozoan infectious agents has not received much attention although it is generally believed that the quality and quantity of feed influences the course and the outcome of bacterial, viral and parasitic infections. Most of the work on the interaction between nutrition and disease has been carried out in laboratory animals infected with bacterial and viral agents and results suggest that malnutrition lowers resistance to bacterial diseases but not necessarily viral diseases (Newberne and Williams, 1970).

The interaction between nutrition and parasitism in domestic ruminants has been studied using experimental nematode infections (Berry and Dargie, 1976; Abbott *et al.*, 1985). Berry and Dargie (1976) observed that sheep infected with *Fasciola hepatica* and fed a low protein ration developed anaemia, hypoalbuminaemia and weight loss much faster than their better fed counterparts.

Studies in sheep infected with *Haemonchus contortus* (Abbott *et al.*, 1985) showed that Finn Dorset sheep fed a low protein diet were more susceptible to infection than those maintained on a high protein diet.

There is little information on the interaction between the presence of trypanosomes in the blood and quantified nutritional status in cattle and small ruminants. In the tropics, parasitic diseases are usually aggravated by malnutrition particularly during the months of the dry season when feeds and feedstuffs are in short supply (Akerejola, 1980; ILCA, 1980). It is a common observation that animals frequently lose weight and develop more severe trypanosomiasis during the dry periods in spite of apparently good appetite, and it is possible that nutritional deficiencies, especially of energy and protein, are responsible.

Information on quantified feed intake in trypanosome infections is scanty. Anorexia has been observed in experimental infection of sheep with *T. brucei* (Ikede and Losos, 1975) and in cattle experimentally infected with either *T. brucei* (Losos and Ikede, 1972) or *T. rhodesiense* (Wellde *et al.*, 1989a). In pigs infected with *T. simiae*, Ilemobade and Balogun (1981) concluded that a reduction in weight gain was due to reduced feed intake and impaired feed conversion efficiency. Similar observations were made by Omole and Onawunmi (1979) in rabbits infected with *T. brucei*.

A number of theories have been advanced to explain the cause of reduced feed intake in parasitised hosts. Wellde *et al.* (1989a) observed that cattle infected with *T. rhodesiense* experienced ineffective feeding which was caused by inability to masticate properly and to swallow food and water. These authors attributed these defects to the central nervous system (CNS) involvement observed in infected cattle.

In ruminants, tissue damage, inflammation and invasion by pathogenic micro-organisms induces systemic changes collectively known as the "acute phase response" (van Miert, 1985) among which are fever, inappetence and gastric

hypomotility. Inappetence and hypomotility appear to be marked in bacterial infections and not in protozoan infections, for example, moderate hypomotility was observed in febrile goats suffering from *T. vivax* infection (Veenendal *et al.*, 1976) and tick-borne fever (Van Miert *et al.*, 1984).

Considerable attention has been paid to elucidation of the roles of pyrogenic cytokines derived from reticulo-endothelial cells and macrophages in the control of feed intake in infected hosts (Dinarello, 1985, Tracey *et al.*, 1988). The cytokines involved are interleukins (IL-1), interferons (IFN-α, IFN-β) and tumor necrosis factor (TNF-α). There is evidence that increased amounts of tumor necrosis factor are produced by laboratory animals in response to experimental *T. brucei* infection (Beutler *et al.*, 1985a; Beutler and Cerami, 1986; Rouzer and Cerami, 1980). However, there are no such reports in trypanosome infections in ruminants. Experiments in which rats and mice were injected with some pyrogenic cytokines (Tracey *et al.*, 1988, Soccher *et al.*, 1988) have indicated that these endogenous peptides inhibit food consumption. The relationship between pyrogenic cytokines and feed intake in trypanosome-infected ruminants remains to be investigated.

Studies in man and rodents (Jose *et al.*, 1973; Faulk *et al.*, 1974; Good *et al.*, 1976) have indicated that protein deprivation results in decreased antibody production. Abdullahi *et al.* (1986a) reported that ewes on a low crude protein intake of 4% had lower gamma globulin concentration compared to those on protein intake of 7.5% and 15% following administration of salmonella vaccine. In the tropics, livestock rearing depends primarily on extensive grazing of poor, sparse natural grasslands with practically no concentrate supplementation. It is possible that under conditions of severe protein deprivation, as occurs in many trypanosome endemic areas, animals are unable to mount effective immunological responses to control the intensity of parasitaemia. As a result, these animals may suffer severe forms of the disease. Studies on the relationship between protein and energy

nutrition, and susceptibility to trypanosome infection in domestic ruminants are lacking and apart from the general belief that poor fed animals are more prone to infection than better fed ones, it is not clear how the level of nutrition influences the pathogenesis of trypanosome infections.

INTRODUCTION TO EXPERIMENTAL WORK

The experiments described in this thesis were conducted to study the pathophysiology of ovine trypanosomiasis caused by *Trypanosoma congolense*. The work described in Chapter 3 investigated the haematological and blood biochemical changes, with particular emphasis on lipid and protein metabolism, in sheep infected with *T. congolense*. Chapter 4 deals with ferrokinetics and erythrocyte survival in sheep after 11 weeks of infection .

A study of comparative pathophysiology of *T. congolense* infection in Scottish Blackface and Finn Dorset sheep is reported in Chapter 5, and the experiment described in Chapter 6 investigated comparative pathogenicity of three clones of *T. congolense*.

Nutritional studies described in Chapter 7 were conducted to examine the influence of dietary protein on the pathophysiology of ovine trypanosomiasis, and on the rate of recovery following administration of a trypanocide, isometamidium chloride. The studies reported in Chapter 8 investigated the effect of energy intake on the pathophysiology of *T. congolense* infection. Chapter 9 deals with the expression of tumor necrosis factor-α receptors on the surface of peripheral blood leucocytes, and the influence of energy intake on the percentage of individual leucocytes expressing these receptors.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Experimental Animals

The animals in these experiments were Scottish Blackface male castrate lambs except in the study described in chapter 4 in which male castrate Finn Dorset lambs were used in a comparative breed study. The origin and details of the lambs are described in the relevant chapters.

Management Before the Experiments

Upon arrival from the local farms, the animals were housed and ear tagged. In cases where these animals had not been vaccinated, they were immunised against pasteurella and clostridial infections using Ovivac-P (Hoechst Animal Health, Milton Keynes). Faecal samples were checked for nematode eggs and other parasites. The sheep were given a 2.5% suspension of fenbendazole (Panacur^{R}) (Hoechst, Milton Keynes) at a dose rate of 5 $mgkg^{-1}$. They were checked again two to three weeks later to confirm their worm-free status.

The feet of these animals were trimmed and regularly dipped in a 10% solution of zinc sulphate (Gold Hoop, Sheep Fair Products Ltd., Brecon) to prevent the development of foot rot. At regular intervals before the start of the experiments, blood samples were collected for routine haematological and blood biochemical analyses. The animals were also weighed once a week, using a sheep weighing scale, (Poldenvale Ltd., Williton, Somerset) before and during the experiments. These procedures helped the sheep to become used to handling and they were no longer excitable when the experiments started.

Management During the Experiments

The management of experimental animals varied from one experiment to another and the details are given in the relevant chapters.

Clinical Observations

Experimental animals were examined each day for any abnormal behaviour during the course of the experiment and body temperatures were recorded three times a week using centigrade thermometers (except in experiments described in Chapters 7 and 8).

Experimental Infections

The trypanosomes used in infections were clones of *Trypanosoma congolense*. These trypanosomes had been maintained in liquid nitrogen through serial passages in mice. Before infection, irradiated mice were infected with the trypanosomes by the intraperitoneal route. At the peak of the first rising parasitaemia, the mice were anaesthetised with chloroform and bled by cardiac puncture. An estimate of the intensity of parasitaemia of pooled blood was made using a haemocytometer. The blood was then diluted with phosphate buffered saline (PBS)(pH 8.0) containing 1.5% glucose to give $1x10^5$ trypanosomes in about 3-4 ml of inoculum. The description of trypanosomes used and details of preparation of inocula are described in the relevant chapters. As a routine, four irradiated mice were injected with 0.2 ml of the inoculum each, by the intraperitoneal route, to confirm the viability and infectivity of the trypanosomes.

Parasitological technique

The intensities of parasitaemia were estimated three times a week, on jugular blood collected into EDTA-containing tubes. Trypanosomes were detected by the dark ground/buffy coat method described by Murray *et al.* (1977). The entire coverslip was examined. To ensure consistency in the interpretation of results,

observations were restricted to two practised observers only. The intensities of parasitaemia were graded from 0 to 5 using a modification of the scoring system suggested by Paris *et al.* (1982) (Table 2.1).

Haematological Techniques

Collection of blood samples

Samples were obtained from experimental animals in a quiet environment with minimum excitement during restraint. They were collected from the jugular vein on three occasions each week, between 9.00 and 11.00 a.m, before the morning feed by using evacuated tubes and 19 gauge needles. Five ml of blood were collected into tubes containing ethylene diamine tetra acetic acid (EDTA) for estimation of parasitaemia, determination of packed cell volume (PCV) and for general haematological investigation.

Examination of blood

The packed cell volume (PCV) was determined three times a week. Two microhaematocrit tubes were filled with blood from each sample and the tubes were spun in a Hawksley microhaematocrit centrifuge for 8 minutes. The tubes were read with a microhaematocrit reader and the mean reading of the two capillary tubes, rounded off to the nearest half percentage, was recorded. In the majority of cases the two readings were identical.

The red cell counts and mean corpuscular volumes (MCV) were provided by an automated blood cell counter (ABX-Minos, Roche Diagnostics). Haemoglobin concentration was determined colorimetrically by a haemoglobinometer (ABX-Minos, Roche Diagnostics) after its conversion to cyanomethaemoglobin.

Table 2.1

Darkground/phase contrast buffy coat parasitaemia scoring system

Score	Trypanosomes per field*	Estimated parasitaemia (trypanosomes per ml)
0	None in film	$< 10^2$
1	1 - 3 per film	$10^2 - 10^3$
2	4 - 19 per film	$10^3 - 5 \times 10^4$
3	1 per field	$5 \times 10^3 - 5 \times 10^4$
4	2 - 10 per field	$10^4 - 5 \times 10^5$
5	>10 per field	$> 5 \times 10^5$

* magnification = x 400

Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the formulae of Dacie and Lewis (1984):

Mean corpuscular volume (MCV) (provided by automated blood cell counter)

$$\text{MCV (femtolitres)(fl)} = \frac{\text{PCV } (l\ l^{-1}) \times 1000}{\text{RBC } (\times 10^{12}\ l^{-1})}$$

Mean corpuscular haemoglobin (MCH)

$$\text{MCH (picograms)(pg)} = \frac{\text{Hb } (g\ dl^{-1}) \times 10}{\text{RBC } (\times 10^{12}\ l^{-1})}$$

Mean corpuscular haemoglobin concentration (MCHC)

$$\text{MCHC } (g\ dl^{-1}) = \ = \frac{\text{Hb } (g\ dl^{-1})}{\text{PCV } (l\ l^{-1})}$$

Total white blood cell count (WBC) was estimated by the automated cell counter (ABX Minos, Roche Diagnostica) and the differential cell counts were performed manually on blood smears stained with May and Grunwald's stain. Two hundred cells were counted and counts were expressed as absolute values.

Biochemical Techniques

Collection of samples

Fifteen millilitres of blood were obtained from the jugular vein for biochemical analyses as follows: 5 ml were collected into tubes containing EDTA for measurement of plasma total cholesterol, triglycerides, non-esterified fatty acids (NEFA) and β-hydroxybutyrate; 5 ml into tubes containing lithium heparin for measurement of plasma urea, magnesium, inorganic phosphate, total protein and albumin and 5ml into tubes containing citrate oxalate for measurement of plasma glucose concentration. In addition, blood was collected into 10 ml tubes containing lithium heparin as an anticoagulant for measurement of plasma zinc and copper.

Ten ml of blood was also drawn into EDTA containing tubes for plasma and into iron-free tubes for serum which were stored and analysed later. The samples for serum were left at room temperature for 24 h after which they were centrifuged at 700 g for 25 min. Serum was then harvested and stored in small aliquots at -20 ^{o}C until required.

Analytical methods

A number of biochemical parameters were measured, including, serum total lipids and phospholipids, plasma cholesterol, triglycerides, free fatty acids, β-hydroxybutyrate, total plasma protein and albumin, urea, glucose, magnesium, zinc, copper, serum iron and total iron-binding capacity.

Blood lipid assays

The concentrations of serum total lipids and phospholipids were determined by enzymatic colorimetric methods using commercially available kits (Boehringer Mannheim Diagnostica). The absorbances were read on a spectophotometer (Pye

Unicam Ltd., Cambridge, England). The method used to assay phospholipids measures the concentration of choline-containing phospholipids, and since they constitute more than 80% of total phospholipids (Nelson, 1967), their measurement gives a good indication of the changes in total phospholipid concentrations.

Total cholesterol, triglyceride and non-esterified fatty acid (NEFA) concentrations were measured by reagent kits using a multichannel autoanalyser (Cobas Mira, Roche Diagnostica). The kits for total cholesterol and triglycerides were supplied by Boehringer Mannheim UK Ltd. and the kit for NEFA was supplied by Wako Chemicals GmbH (The Wako NEFA C, West Germany).

Plasma proteins and urea

Plasma total protein, albumin and urea were measured by continuous flow analysis (Standard Technicon Auto-Analyser II method). Plasma globulin concentration was recorded as the difference between total plasma protein and albumin.

Plasma glucose, β-hydroxybutyrate and inorganic phosphate

Commercial kits were used for the assays of plasma glucose, inorganic phosphate (Roche Diagnostica) and B-hydroxybutyrate concentrations (Randox Laboratories Ltd., Co. Antrim, Ireland) using a multichannel autoanalyser (Cobas Mira, Roche Diagnostica).

Plasma zinc and copper

Plasma zinc and copper were measured using an atomic absorption spectrophotometer (Perkin Elmer Ltd., Post office lane, Beaconfield, Bucks HP9 1QA, England) and an HGA programmer (2380 AAS, Perkin Elmer Ltd).

Serum iron, total iron-binding capacity (TIBC) and percentage saturation of transferrin

Serum iron and TIBC were determined spectophotometrically using commercial test kits (Roche Diagnostica). Samples were analysed in duplicate and the mean value was recorded. Control sera of known serum iron and TIBC concentrations were used in each assay.

The unbound-iron binding capacity (UIBC) was recorded as the difference between the serum TIBC and serum iron concentrations and the percentage saturation of transferrin with iron was calculated as follows:

$$\%\text{saturation} = \frac{\text{serum iron } (\mu\text{mol l}^{-1})}{\text{TIBC } (\mu\text{mol l}^{-1})}$$

Haemoglobin Typing

The haemoglobin types were identified at the beginning of each study by electrophoresis of haemolysed red blood cells on cellulose acetate membranes using the method described by Queval and Petit (1982).

Analysis of Experimental Diets

Some diets used in various studies were commercial concentrate feeds and others were formulated and mixed by hand to give the desired nutrient composition. At periodic intervals, samples of these diets were examined. All the analytical methods used were officially established procedures (MAFF *et al.*, 1981).

Dry matter (DM)

The dry matter in diets was determined by heating known quantities (400 - 500 g) in a forced hot-air oven at 80 ^{o}C for 48 - 72 h until a constant weight was attained.

Gross energy (GE)

The energy content of dried feed samples was measured in an automatic adiabatic bomb calorimeter (Gallenkamp autobomb).

Crude fibre (CF)

The crude fibre content of dried feed was determined using the semi-automated fibertec system (Tecator Fibertec 1010 Heat Extractor).

Ether extract (EE)

The oil content was measured by extraction with petroleum spirit under controlled conditions.

Ash

Ash content was the residue remaining after the feed sample was heated at 500 ^{o}C in a muffle furnace overnight.

Organic matter (OM)

Organic matter was calculated as the ash-free dry matter.

The metabolisable energy (ME) and crude protein (CP) contents were calculated from the energy and protein contents of individual feed components (MAFF *et al.*, 1981)

Statistical Methods

Results are presented as individual values, or as group means plus or minus the standard error of the mean (sem). Comparisons between two groups were made using one way analysis of variance. In experiments involving more than two treatment groups, comparisons were achieved by the two way analysis of variance using the least square method. Results from the analysis of variance were then used in the Newman-Keuls multiple range test to identify treatment differences.

Intensities of parasitaemia were evaluated by the nonparametric Mann Whitney test. These statistical analyses were conducted using MINITAB (Ryan, Penn State University) and ANIMAL DESIGNS 1, V 1.21 5/6 (Data International Services, Glasgow) programmes on an IBM computer (Personal system/2, Model 30). Significance was considered where $p < 0.05$.

CHAPTER 3

THE PATHOPHYSIOLOGY OF *TRYPANOSOMA CONGOLENSE* INFECTION IN SCOTTISH BLACKFACE SHEEP: HAEMATOLOGICAL AND BLOOD BIOCHEMICAL STUDIES

INTRODUCTION

To date most studies on African trypanosomiasis have been carried out in cattle or in laboratory rodents with little attention being paid to small ruminants. This is partly because trypanosomiasis was not considered an important disease of sheep and goats (MacLennan, 1970). Kramer (1966) reported that the disease in Nigeria was of little importance in these hosts and Finelle (1974) stated that sheep were seldom infected with trypanosomes under natural conditions. However, recent epidemiological surveys in tsetse-infested areas indicate that the disease is more important than was previously thought. In a Sleeping Sickness focus in Bas-Zaire, Makumyaviri *et al.* (1989) observed that 31.3% of the sheep sampled were infected with *Trypanosoma congolense*, as determined by the miniature anion-exchange centrifugation technique. In addition to *T. congolense* infections, they reported that 6.2% of the sheep were infected with the *brucei* subgroup trypanosomes. In tsetse-endemic areas of Kenya, Griffin and Allonby (1979a) observed seasonal and breed variations in infection rates of sheep. In one rainy season, they reported an infection rate of 50-85% in Karakul sheep. These findings confirm that sheep can be important hosts of African trypanosomes and their infection may lead to severe economic losses through death and debility (Griffin and Allonby, 1979b; Kanyari *et al.*, 1983; Dirie *et al.*, 1988) and reduced reproductive efficiency (Adeyemo *et al.*, 1990).

It has been suggested that sheep may be important reservoirs of infection which may later be passed onto other livestock (Mahmoud and Elmalik, 1977) and man (Okuna and Mayende, 1983; Kageruka *et al.*, 1991; Truc *et al.*, 1991), especially in circumstances where these animals live in close proximity to man and the vectors of trypanosomes. Characterisation of stocks of *brucei* species isolated from animals in a Sleeping Sickness area of Zaire proved that sheep harboured *T. gambiense* type comparable to the human *T. gambiense* (Kageruka *et al.*, 1991).

It was therefore proposed that sheep and other domestic animals play an important role in maintaining the infection during inter-epidemic periods. As a result of the appreciation that sheep and goats may act as a reservoir for human infections, proposals have been put forward to include the treatment of these animals in national and international trypanosomiasis control programmes (Mahmoud *et al.*, 1991).

The pathogenesis of *T. congolense* infection in sheep has been studied only on a few occasions and anaemia has been reported as the most important pathological feature (MacKenzie and Cruickshank, 1973; Griffin, 1978; Griffin and Allonby, 1979c). It has been proposed that lipids may have a central role to play in the pathogenesis of trypanosomiasis (Tizard *et al.*, 1978a, b). There is evidence to suggest that lipids may be involved in the development of anaemia (Tizard *et al.*, 1978b), in non-specific resistance of man to *T. brucei* infection (Rifkin 1978a), in trypanotolerance in cattle (Traore-Leroux *et al.*, 1987a), in immunosuppression (Tizard *et al.*, 1978b) and cachexia (Rouzer and Cerami, 1980).

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 triglycerides, cholesterol and total lipids occurred. Similar observations were made in rats infected with *T. rhodesiense* (Dixon, 1967a) and in dogs infected with *T. brucei* (Ndung'u *et al.*, 1989; Ndung'u, 1990). However the picture in ruminants infected with trypanosomes is less clear. Roberts (1974, 1975a, b) observed a marked fall in total lipid, cholesterol and phospholipids in sheep infected with either *T. congolense, T. brucei or T. vivax* while Valli *et al.* (1980) observed an increase in plasma triglycerides in calves infected with *T. congolense*. These contrasting observations suggest that there are possibly profound differences in lipid metabolism of ruminant

and non-ruminant animals infected with trypanosomes. The present study was intended to monitor the progress of *T. congolense* infection in sheep using parasitological, haematological and blood biochemical methods.

Considering the available control measures against trypanosomiasis and realising the fact that there is no possibility of developing a vaccine against trypanosomiasis in the near future, chemotherapy and chemoprophylaxis appear to be the only practical methods of controlling the disease in endemic areas. It is therefore of major importance to assess the response of infected animals to treatment, and this was investigated in sheep infected with *T. congolense* and treated with a trypanocidal drug, diminazene aceturate after 12 weeks of infection.

MATERIALS AND METHODS

Experimental Animals

The four month old, male castrate sheep were bought in from Cashel farm (Balmaha, Stirlingshire, Scotland) and were kept in pens in animal houses for about five months before they were infected. The management of the experimental animals before the experiment has been described (Chapter 2).

Experimental Design.

Eleven Scottish Blackface lambs were divided into two groups depending on their live body weights, packed cell volumes (PCV) and haemoglobin types. One group of six animals was infected with 1×10^5 *T. congolense* intravenously (infected group) while the other group of five animals served as uninfected controls (Control group). The livebody weights, PCV values and haemoglobin types of the experimental sheep are shown in Table 3.1.

Table 3.1

The liveweight, PCV and haemoglobin types of experimental animals before infection with *Trypanosoma congolense*

Group	Sheep No.	live weight (kg)	PCV (ll^{-1})	Haemoglobin type
	34	37.0	0.31	AB
	36	37.0	0.33	AB
Infected*	79	33.0	0.29	A
	81	37.5	0.34	B
	84	34.0	0.34	B
	Mean ± sem	**35.7 ± 0.9**	**0.32 ± 0.01**	
	37	39.0	0.34	AB
	80	34.0	0.30	AB
Control	82	37.0	0.28	A
	83	32.5	0.28	A
	86	42.0	0.32	B
	Mean ± sem	**36.9 ± 1.7**	**0.30 ± 0.01**	

*Initially six animals were infected but following the death of sheep No. 35, only five animals were involved in the study.

Seventy five days after infection, all animals were injected with radiolabelled materials (^{51}Cr, ^{59}Fe, ^{125}I) to measure their blood volumes and the rates of red cell breakdown and synthesis. (The results of this study are reported in Chapter 4)

Eighty four days after infection all the experimental animals were treated with the trypanocidal drug, diminazene aceturate (Berenil^{R}, Hoechst)) at a dose rate of 3.5 $mgkg^{-1}$ intramuscularly and the animals were monitored for a further 12 days.

Housing and Feeding

Experimental animals were moved into a flyproof isolation unit two weeks before infection and were kept together in one large pen. Each lamb received 500g of concentrate feed (Lamb finisher pellets, BOCM Silcock Ltd., Barringstone, Hampshire) per day, and hay and water were available *ad libitum*. Wood shavings were used as bedding. The nutrient composition of the concentrate feed is shown in Table 3.2.

Experimental Infection

Six lambs were infected with *T. congolense* 1180 (GRVPS 61/4) which is a cloned derivative of an isolate made from the Serengeti, Tanzania (Nantulya *et al.*, 1984). This clone of *T. congolense* was originally imported from ILRAD (Kenya) and was maintained in liquid nitrogen after serial passages in mice.

Table 3.2

**Proximate analysis of the commercial concentrate feed *
(lamb finisher pellets)**

Nutrient	**Composition (gkg^{-1} DM)**
Dry matter (gkg^{-1} fresh matter)	864
Crude protein	147
Ether extract	45
Crude fibre	130
Ash	94
Gross energy ($MJkg^{-1}$ DM)	17.4

* Commercial concentrate feed used to supplement hay

Preparation of inoculum

The stabilated trypanosomes in frozen capillary tubes were emptied into small bottles of PBS (containing 1.5% glucose)(pH 8.0). The trypanosomes were then injected intraperitoneally into four irradiated mice. On rising parasitaemia, the mice were bled, under terminal anaesthesia, by cardiac puncture. The parasitaemia of pooled blood was estimated using a haemocytometer. Two ml of pooled blood containing 5×10^5 trypanosomes/ml was made up to 40ml with PBS. Each lamb received 4 ml of inoculum, by the jugular route, containing 1×10^5 trypanosomes.

To confirm the infectivity of the trypanosomes, 0.2 ml of a similar inoculum was injected intraperitoneally into four irradiated mice. The mice were then checked for parasitaemia daily until they developed a high parasitaemia and were sacrificed.

The history of the *T. congolense* clone used in this study is shown in Figure 3.1

Parasitological, Haematological and Biochemical Methods

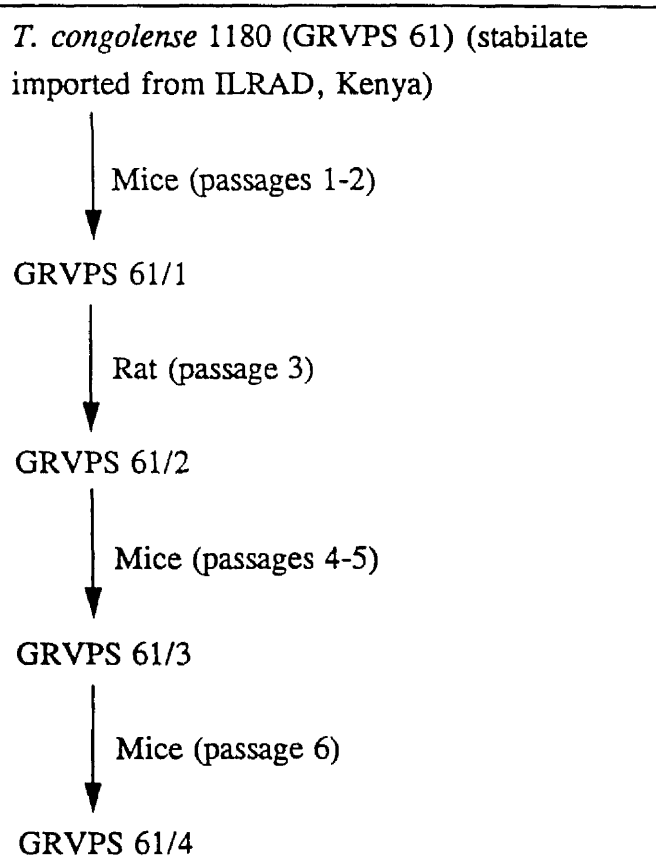
These methods have been described in detail in general materials and methods (Chapter 2)

RESULTS

Clinical Observations

All infected animals apparently maintained good appetites except sheep No. 35 which died suddenly after 34 days of infection. Examination of a blood sample obtained before death revealed that it had a PCV value of $0.25\ ll^{-1}$, temperature of $>42\ ^oC$ and a parasitaemia score of 1. A post-mortem examination was performed immediately and the findings included, subendocardial ecchymotic haemorrhages, grossly enlarged and oedematous lymphnodes, marked prominence of haemonodes

Figure 3.1 The history of the *Trypanosoma congolense* clone 1180 (GRVPS 61/4)



and a markedly enlarged spleen. Samples were obtained from the spleen, liver, kidney, brain and heart, and preserved in jars containing formal saline for histological examination. Part of the liver was sent in ice for immediate bacteriological examination. *Pasteurella haemolytica* type T was isolated from the liver and it was concluded that the animal died of acute pasteurellosis.

The clinical, body weight, parasitological, haematological and blood biochemical measurements that had been carried out on samples from sheep 35 were deleted from statistical analyses.

Body Weight Changes

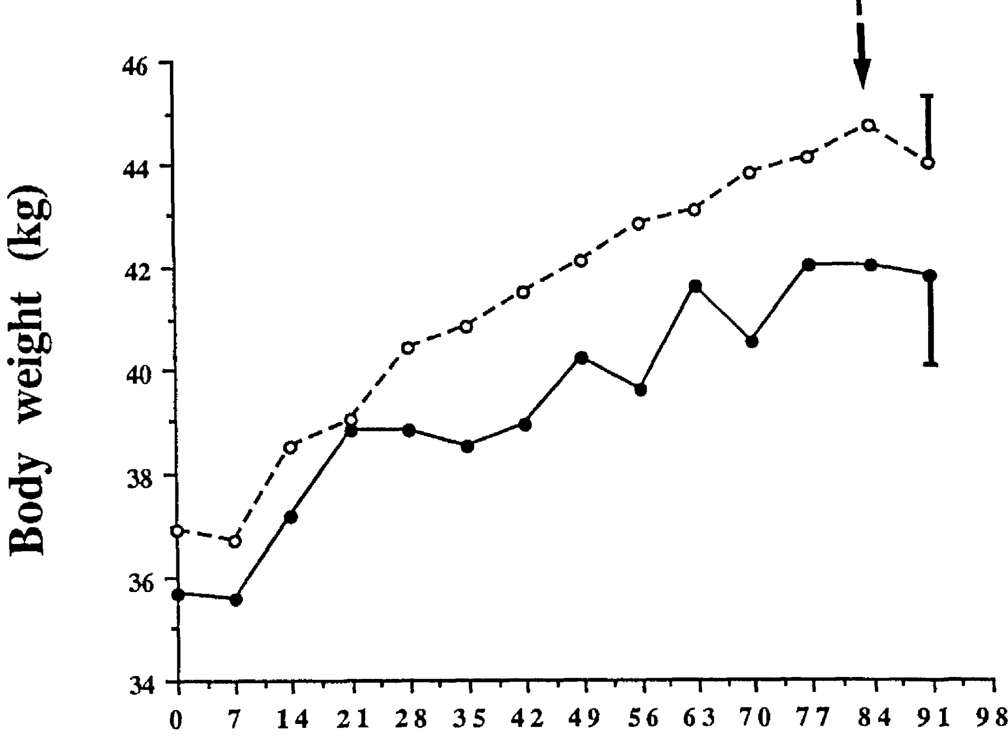
All experimental animals continued to grow but the infected animals showed a moderate retardation of growth between 21 and 77 days after infection (DAI) (Figure 3.2). In the infected lambs, the mean body weight increased from 35.7 $\pm$ 0.9 at 0 DAI to 42.0 $\pm$ 1.4 kg at 84 DAI. The mean weight in control animals increased from 36.9 $\pm$ 1.7 kg at day 0 to 44.7 $\pm$ 1.4 kg at 84 DAI. However, the changes in body weights of infected animals were not significantly different from those of the control animals.

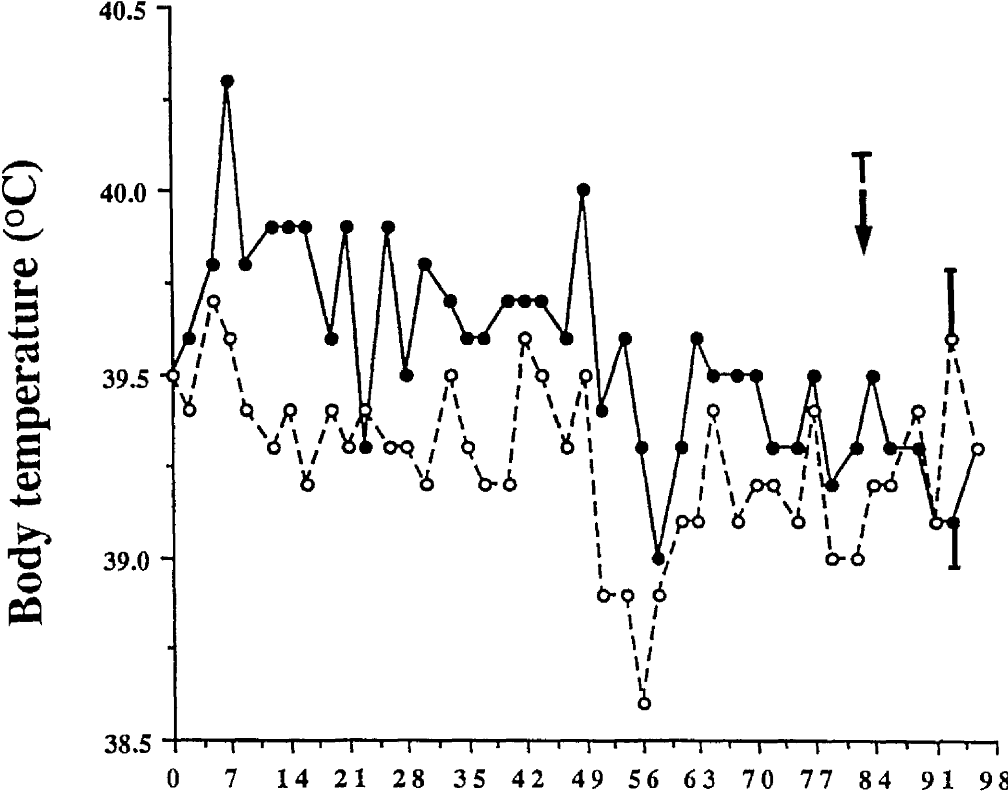
Body Temperature Changes.

Following infection, the mean temperatures in infected lambs increased steadily from 39.5 $\pm$ 0.4 ^{O}C to reach the first peak of 40.3 $\pm$ 0.3 ^{O}C by 7 DAI (Figure 3.3). Thereafter, it tended to decrease but peaked again to 40.0 $\pm$ 0.1 ^{O}C at 49 DAI, after which it fluctuated. After treatment at 84 DAI, the mean body temperatures of infected animals decreased. The temperatures in control sheep fluctuated between 38.6 $\pm$ 0.2 and 39.7 $\pm$ 0.1 ^{O}C throughout the experimental period. The drop in temperature in infected and control sheep at 54 DAI could be attributed to shearing.

Figure 3.2 Body weights of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.3 Body temperatures of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.


Body weight (kg)
46
44
42
40
38
36
34
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98


Body temperature (°C)
40.5
40.0
39.5
39.0
38.5
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Time after infection (days)

Parasitological Findings

The mean prepatent period was 7.8 days (range 7-9 days) (Figure 3.4). The mean parasitaemia score increased steadily to reach the first peak of 4.0 $\pm$ 0.3 by 16 DAI after which it decreased to 1.0 $\pm$ 0.4 by 26 DAI and then increased to reach another peak of 4.2 $\pm$ 0.2 by 49 DAI. After this time, the parasitaemia fluctuated but the parasitaemia peaks showed a progressive decrease. Aparasitaemic periods ranging from 3 - 4 days were often observed in individual sheep.

After treatment, the parasites disappeared from the circulation and could not be detected for the following 12 days. The control sheep remained aparasitaemic for the whole period of observation.

Haematological Changes

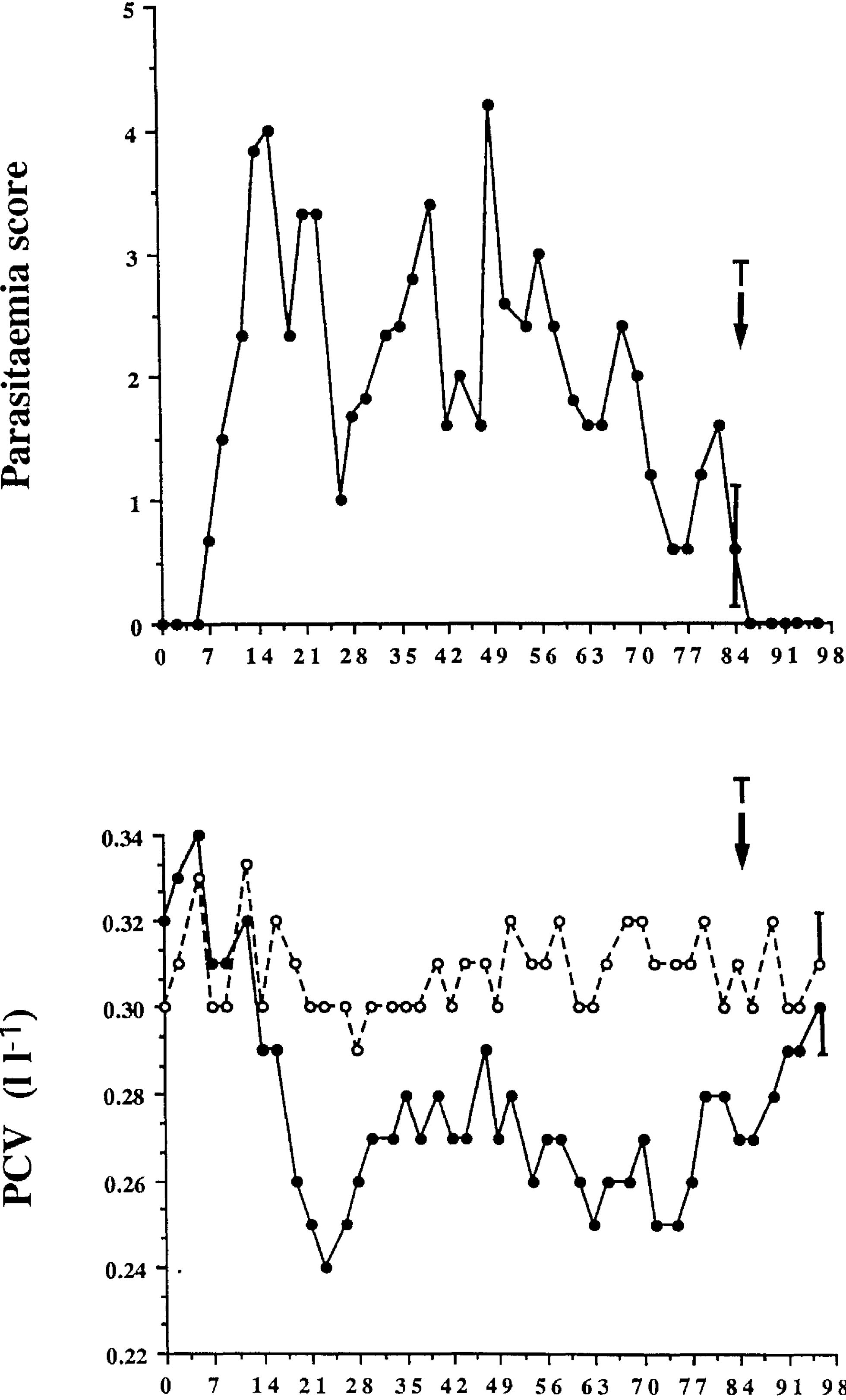
Packed cell volume (PCV)

Infection caused a significant decrease in mean PCV values between 0 and 84 DAI. The mean values decreased from 0.32 $\pm$ 0.01 ll^{-1} at day 0 to 0.24 $\pm$ 0.01 ll^{-1} at 23 DAI (Figure 3.5). The values then tended to increase but showed another decline from 0.29 $\pm$ 0.01 ll^{-1} at 47 DAI to 0.25 $\pm$ 0.01 ll^{-1} at 63 DAI. Following treatment at 84 DAI, the values increased from 0.27 $\pm$ 0.01 to 0.30 $\pm$ 0.01 ll^{-1} at 96 DAI.

Apart from an initial fluctuating decrease between 0 and 21 DAI, the mean values in control animals varied from 0.29 $\pm$ 0.02 to 0.32 $\pm$ 0.01 ll^{-1}.

Figure 3.4 Parasitaemia scores of sheep infected with *T. congolense* (—●—). T denotes treatment.

Figure 3.5 Packed cell volumes (PCV) of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.


Parasitaemia score
5
4
3
2
1
0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
T
PCV (l l-1)
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
T
Time after infection (days)

Red blood cell count (RBC)

The mean RBC in infected sheep decreased significantly from 11.34 $\pm$ 0.34 on 0 DAI to 7.73 $\pm$ 0.43 x$10^{12}l^{-1}$ at 26 DAI (Figure 3.6) after which the values tended to increase but showed another downward trend between 47 and 75 DAI, decreasing from 8.31 $\pm$ 0.43 to 7.46 $\pm$ 0.72 x $10^{12}l^{-1}$. The values increased moderately after treatment. The mean values in the control group showed an initial downward trend up to 19 DAI after which they stabilised to fluctuate between 9.16 $\pm$ 0.44 and 10.40 $\pm$ 0.47 x $10^{12}l^{-1}$.

Haemoglobin (Hb) concentration

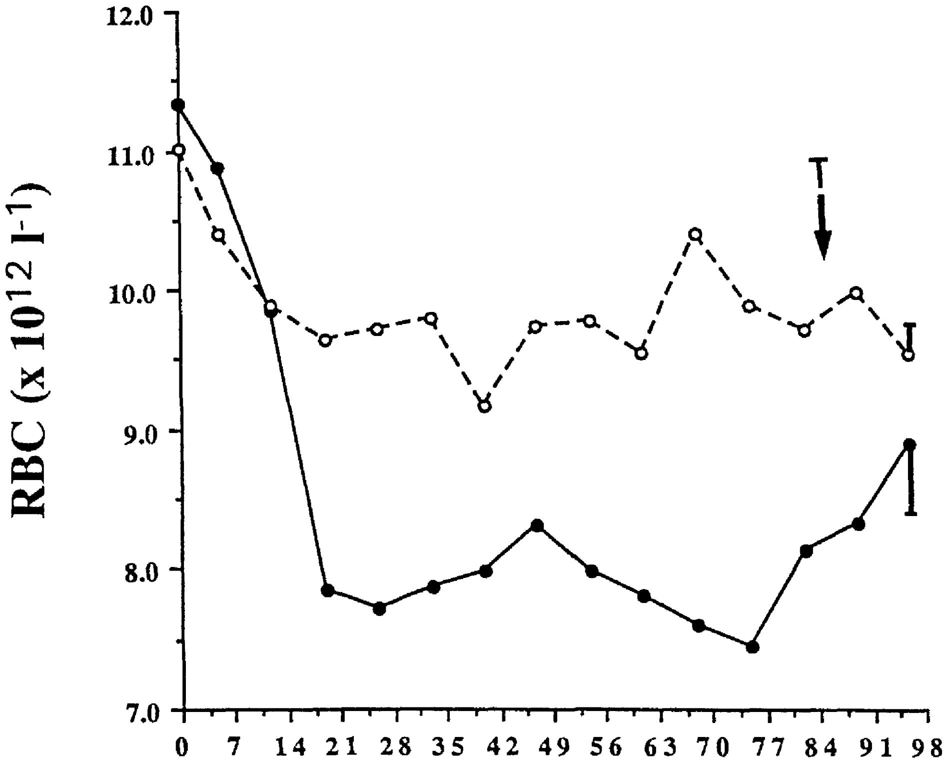
The mean Hb concentration in the infected group decreased significantly from 12.8 $\pm$ 0.2 gdl^{-1} at 0 DAI to 9.2 $\pm$ 0.2 at 19 DAI (Figure 3.7). Thereafter, it increased moderately to 10.1 $\pm$ 0.4 at 47 DAI but decreased again to 8.8 $\pm$ 0.7 at day 75 DAI. After treatment the concentration increased to reach 10.12 $\pm$ 0.21 gdl^{-1} by 96 DAI. The values for the control sheep decreased moderately between 0 and 19 DAI from 12.4 $\pm$ 0.5 to 11.1 $\pm$ 0.3 gdl-1 respectively, but later fluctuated between 10.1 + 0.4 and 11.4 $\pm$ 0.4 gdl^{-1}.

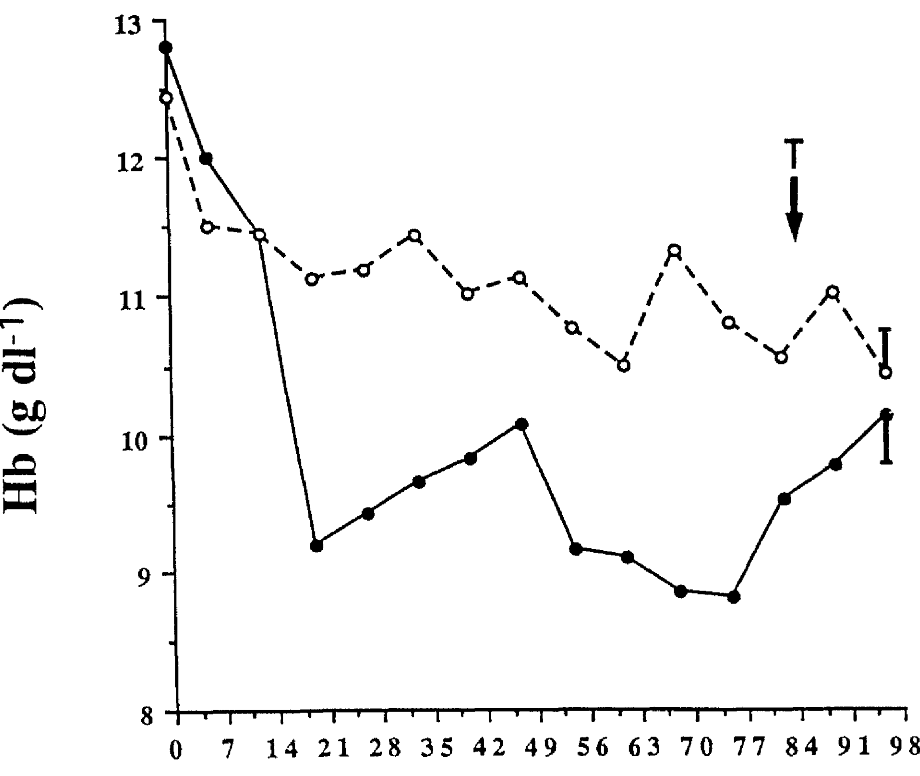
Mean corpuscular volume (MCV)

Infection caused a significant increase in MCV values from 31.0 $\pm$ 0.5 fl at 0 DAI to 37.2 $\pm$ 1.4 fl at 75 DAI (Figure 3.8). It declined slightly to 36.4 $\pm$ 1.2 fl at 82 DAI, and the values declined further after treatment at 84 DAI. The control values increased moderately from 30.4 $\pm$ 0.6 fl at day 0 to 32.4 $\pm$ 0.6 at 26 DAI, and thereafter fluctuated between 31.8 $\pm$ 0.6 and 32.4 $\pm$ 0.7 fl.

Figure 3.6 Red blood cell counts (RBC) of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (--○--). T denotes treatment.

Figure 3.7 Haemoglobin concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (--○--). T denotes treatment.





Time after infection (days)

Mean corpuscular haemoglobin concentration (MCHC)

The mean MCHC of infected animals decreased significantly from 36.8 ± 0.2 at 12 DAI to 32.1 ± 0.7 gdl^{-1} at 75 DAI (Figure 3.9). The mean values increased moderately after treatment. In the control group, the mean values varied between 34.1 ± 0.7 and 37.1 ± 0.3 gdl^{-1}.

Mean corpuscular haemoglobin (MCH)

The mean MHC values of infected animals increased from 11.2 ± 0.2 pg at 0 DAI to 12.3 ± 0.5 pg at 40 DAI after which they decreased to fluctuate between 11.4 ± 0.4 and 11.8 ± 0.4 pg, however, these changes were not significant. The mean values in control animals fluctuated between 10.9 ± 0.2 and 11.6 ± 0.1 pg.

Total white cell count (WBC)

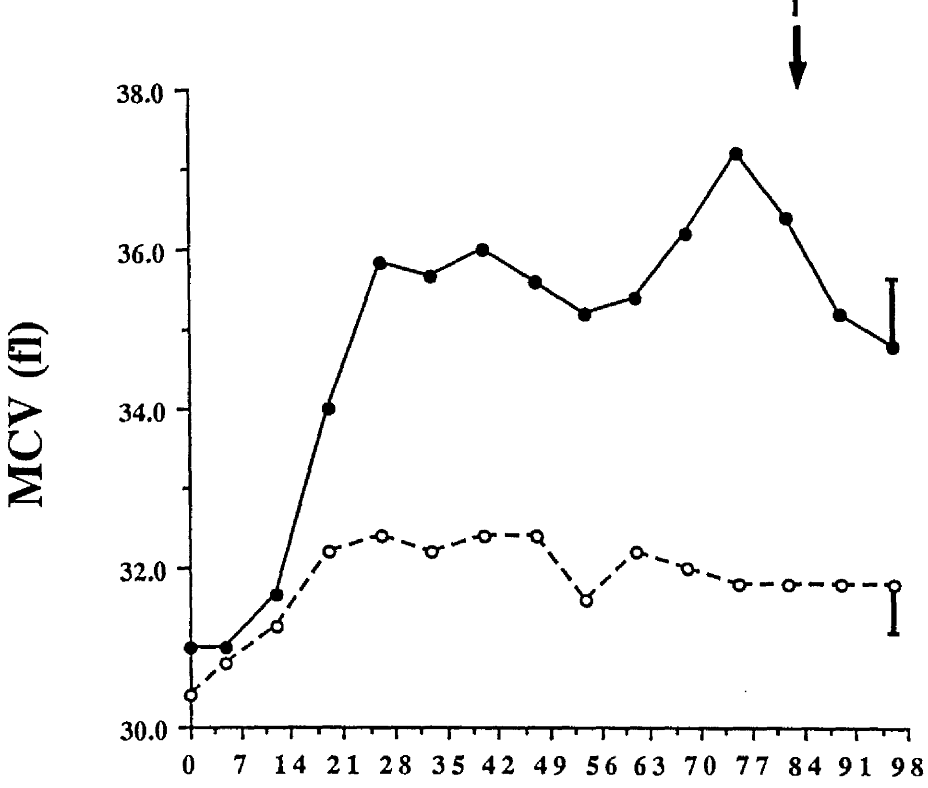
The mean WBC of the infected group showed a significant fluctuating increase from 8.70 ± 0.22 at 0 DAI to 14.44 ± 1.37 $x10^9l^{-1}$ at 40 DAI (Figure 3.10). The mean values then decreased moderately to 13.00 ± 1.29 at 68 DAI but increased again to 14.24 ± 1.25 x 10^9l^{-1} at 82 DAI. Treatment at 84 DAI caused a marked drop in WBC values. The mean WBC values in the control group fluctuated between 8.12 ± 0.43 and 8.88 ± 0.39 $x10^9l^{-1}$.

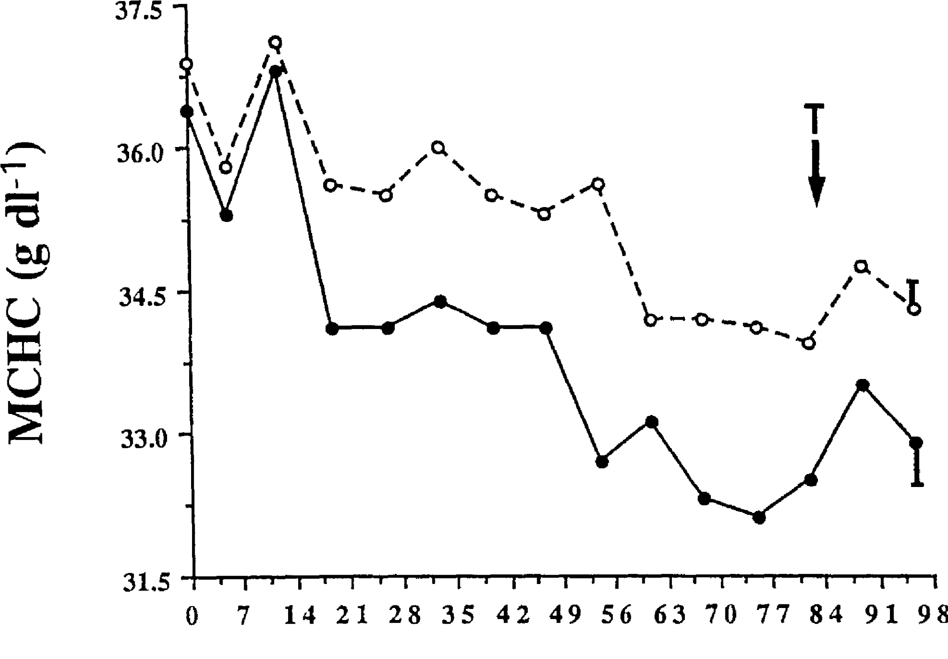
Absolute Lymphocyte count

The mean lymphocyte counts showed a similar trend as that of WBC. The values of infected group increased significantly from 7.45 ± 0.27 at 0 DAI to 12.86 ± 1.38 $x10^9l^{-1}$ at 40 DAI (Figure 3.11). The values tended to decrease but showed another increase to 12.70 ± 1.40 at 82 DAI. Following treatment, the mean values decreased to 10.46 ± 0.63 $x10^9l^{-1}$ at 96 DAI. The counts in the control group fluctuated between 5.93 ± 0.56 and 7.69 ± 0.45 $x10^9l^{-1}$.

Figure 3.8 Mean corpuscular volumes (MCV) of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.9 Mean corpuscular haemoglobin concentrations (MCHC) of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

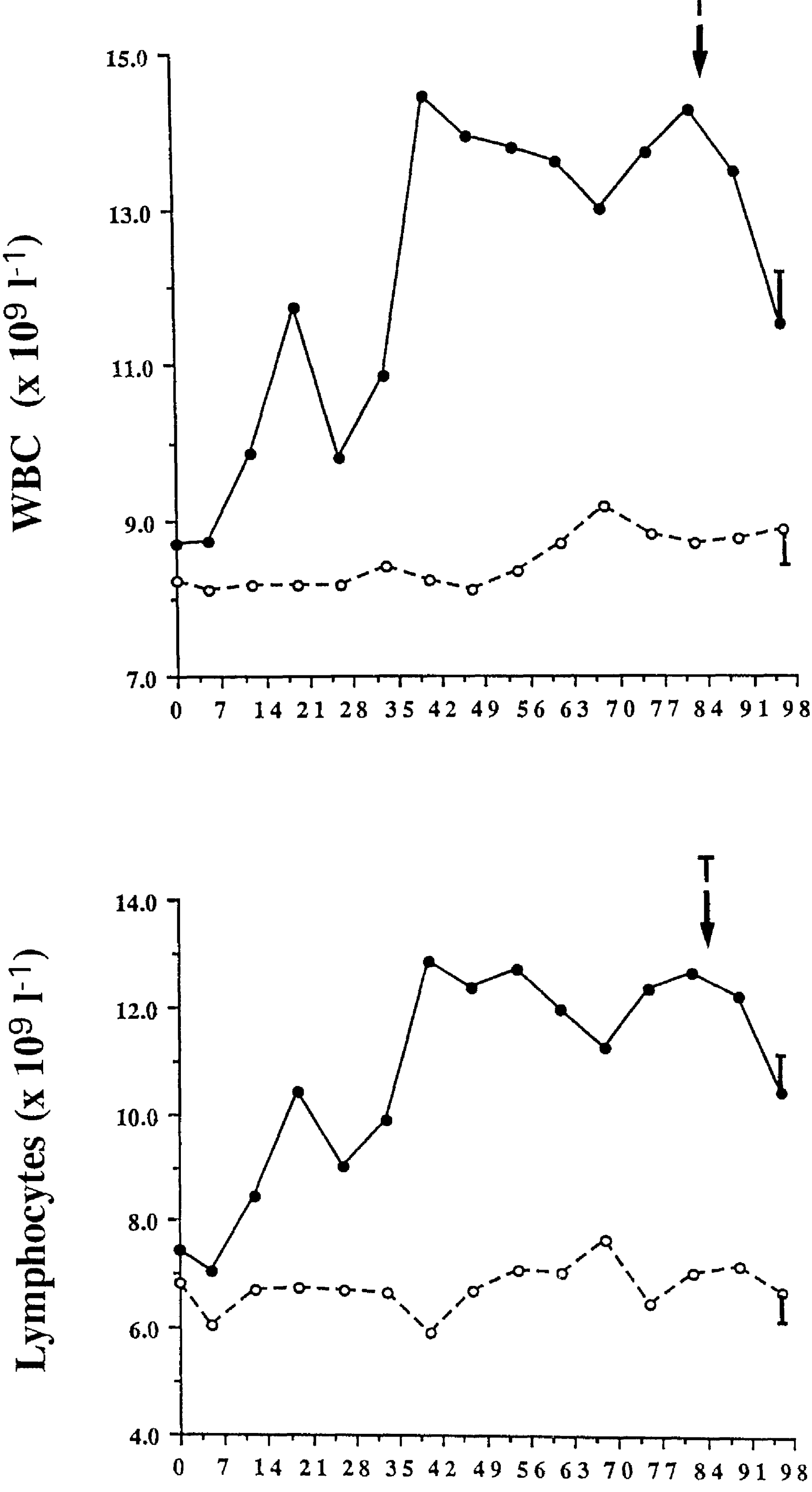




Time after infection (days)

Figure 3.10 White cell counts (WBC) of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.11 Total lymphocyte counts of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.



WBC (x 10^{9} l^{-1})
15.0
13.0
11.0
9.0
7.0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
T
Lymphocytes (x 10^{9} l^{-1})
14.0
12.0
10.0
8.0
6.0
4.0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
T
Time after infection (days)

Monocyte, Neutrophil, Eosinophil and Basophil counts

The mean monocyte counts fluctuated considerably in both infected and control groups, however, the values in infected group showed a sharp increase at 61 and 75 DAI.

The neutrophil numbers of infected animals showed an initial decline from 1.07 $\pm$ 0.07 at 0 DAI to 0.62 $\pm$ 0.17 x $10^9 l^{-1}$ at 26 DAI after which they showed a fluctuating increase, however these changes were not significant. Treatment at 84 DAI did not affect neutrophil numbers. The values in the control group fluctuated between 1.20 $\pm$ 0.21 and 2.16 $\pm$ 0.89 x $10^9 l^{-1}$.

The mean eosinophil (0.07 - 0.15 x 10^9 l^{-1}) and basophil (0 - 0.06 x 10^9 l^{-1}) counts were unaffected by infection and treatment.

Blood Biochemical Changes

Serum total lipids

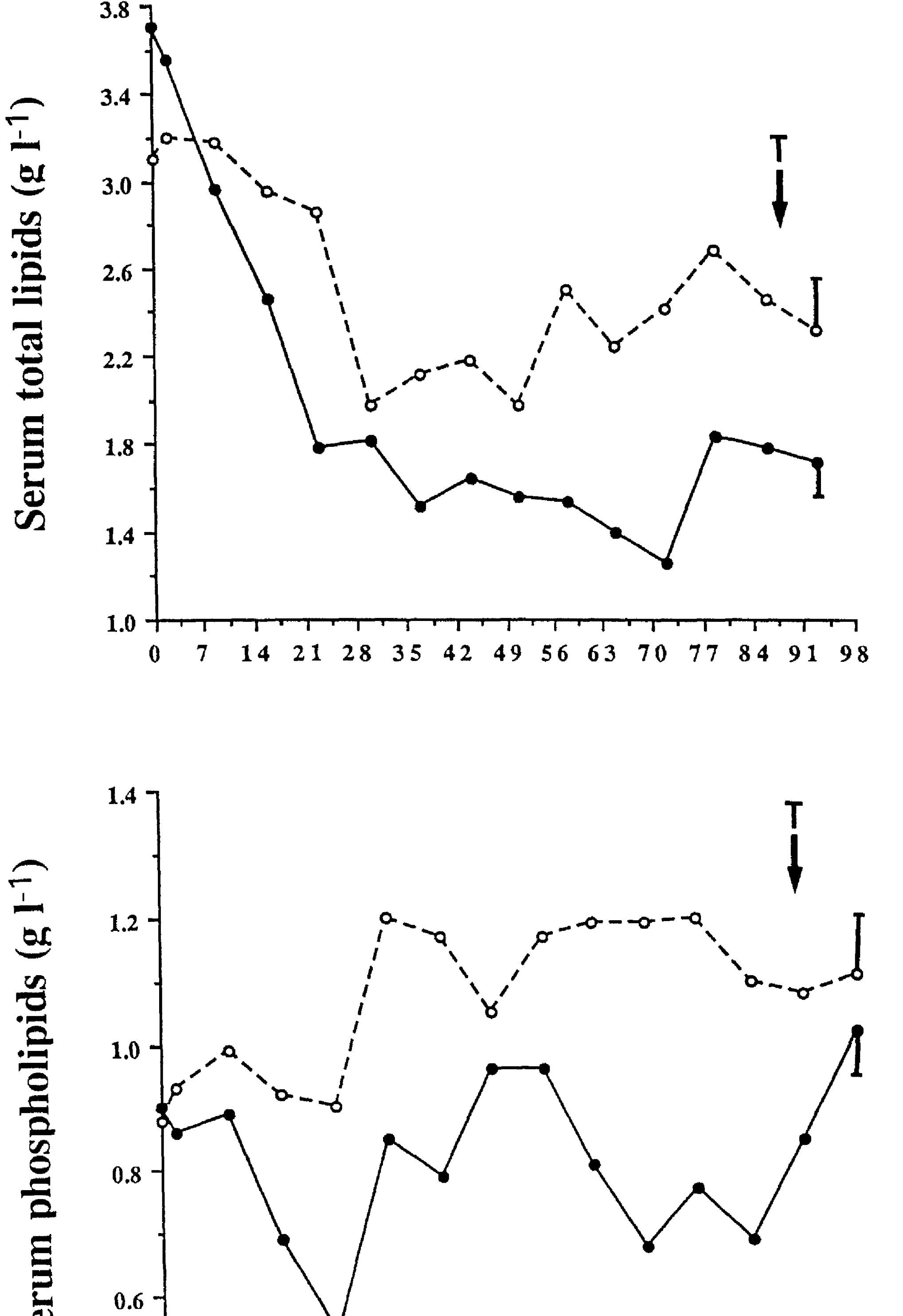
There was a significant decrease in mean serum total lipid concentration of infected sheep from 3.70 $\pm$ 0.39 gl^{-1} at 0 DAI to 1.26 $\pm$ 0.17 gl^{-1} at 72 DAI (Figure 3.12). The serum lipid concentration of control animals also decreased from 3.10 $\pm$ 0.26 at 0 DAI to 1.98 $\pm$ 0.19 gl^{-1} at 30 DAI after which the values tended to increase. Treatment with Berenil^R at 84 DAI had little effect on the serum lipid concentration.

Serum phospholipids

Infection caused a significant decrease in serum phospholipid concentrations from 0.90 $\pm$ 0.06 gl^{-1} at 0 DAI to 0.54 $\pm$ 0.08 gl^{-1} at 23 DAI (Figure 3.13). The concentration then tended to increase but showed another decline from 0.81 $\pm$ 0.12 at 58 DAI to 0.68 $\pm$ 0.08 at day 65. Following treatment, the concentration of

Figure 3.12 Serum total lipid concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.13 Serum phospholipid concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.



Serum total lipids (g l-1)
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Serum phospholipids (g l-1)
1.4
1.2
1.0
0.8
0.6
0.4
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Time after infection (days)

serum phospholipids increased significantly from 0.69 + 0.09 at 79 DAI to 1.02 $\pm$ 0.06 gl^{-1} at day 93. The mean values in the control sheep fluctuated between 0.88 $\pm$ 0.11 and 1.20 $\pm$ 0.12 gl^{-1}.

Plasma cholesterol

The mean plasma cholesterol concentration in the infected sheep showed an apparent decrease from 1.61 $\pm$ 0.10 at 0 DAI to 1.03 $\pm$ 0.09 $mmoll^{-1}$ at 21 DAI after which it tended to recover (Figure 3.14). The mean concentration decreased again between 42 and 49 DAI from 1.29 $\pm$ 0.02 to 1.01 $\pm$ 0.09 $mmoll^{-1}$ respectively. Treatment caused a moderate increase in the concentrations of infected animals. The mean values in the control animals showed a moderate decline between 7 and 28 DAI from 1.68 $\pm$ 0.18 to 1.30 $\pm$ 0.19 $mmoll^{-1}$, respectively after which they fluctuated between 1.50 $\pm$ 0.20 and 1.83 $\pm$ 0.20 $mmoll^{-1}$.

Plasma triglycerides

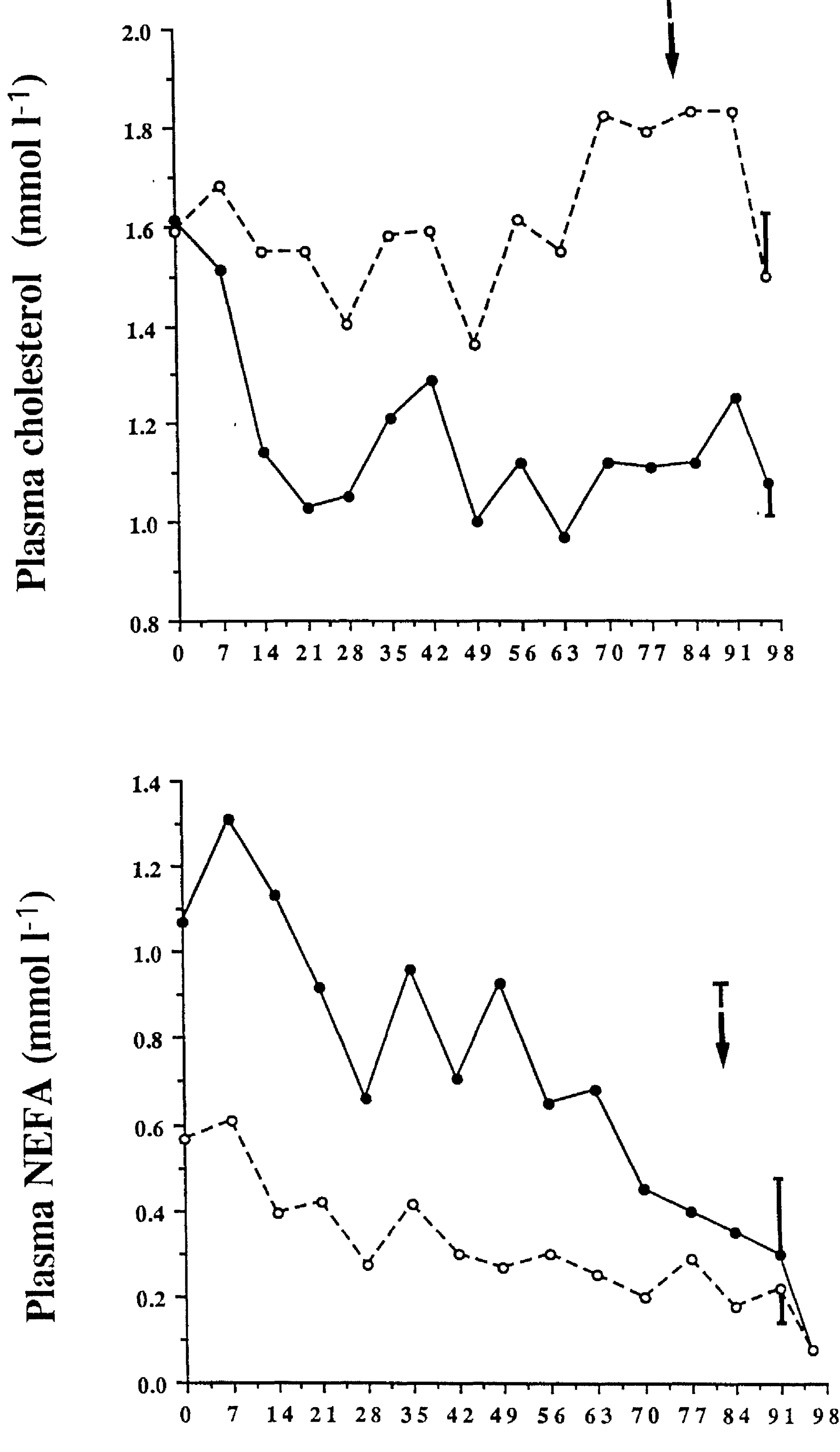
The concentration of plasma triglycerides fluctuated a great deal in both infected and control animals but tended to be lower in the former (0.01 - 0.36 $mmoll^{-1}$) than in the latter (0.09 - 0.82 $mmoll^{-1}$).

Non-esterified fatty acids (NEFA)

The concentration of NEFA in infected sheep decreased from 1.07 $\pm$ 0.23 at 0 DAI to 0.35 $\pm$ 0.14 $mmoll^{-1}$ at 84 DAI (Figure 3.15). The mean values of control animals also showed a downward trend which was more gradual than that displayed by infected animals. The mean concentrations were lower in control animals than in infected ones before infection and throughout the course of infection, however these differences were not significant.

Figure 3.14 Plasma cholesterol concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.15 Plasma NEFA concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.



Plasma cholesterol (mmol l^{-1})
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Plasma NEFA (mmol l^{-1})
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Time after infection (days)

Total plasma protein

Total plasma protein concentration in infected sheep decreased from 64.7 $\pm$ 0.9 gl^{-1} at 0 DAI to 59.8 $\pm$ 2.2 gl^{-1} at 21 DAI (Figure 3.16). It then increased steadily to 67.4 $\pm$ 3.1 gl^{-1} at 42 DAI and later showed a moderate decline, however these changes were not significant. The values in control animals fluctuated between 60.4 $\pm$ 1.0 and 65.4 $\pm$ 1.3 gl^{-1}.

Plasma albumin

Plasma albumin concentration in infected sheep decreased significantly from 37.2 $\pm$ 0.6 at 0 DAI to 33.0 $\pm$ 1.1 gl^{-1} at 21 DAI (Figure 3.17). The mean albumin concentration then tended to increase to 34.8 $\pm$ 0.9 gl^{-1} at 42 DAI but decreased again to 32.0 $\pm$ 1.0 gl^{-1} at 63 DAI. The mean values in control animals fluctuated between 35.8 $\pm$ 0.8 and 38.4 $\pm$ 0.7 gl^{-1}.

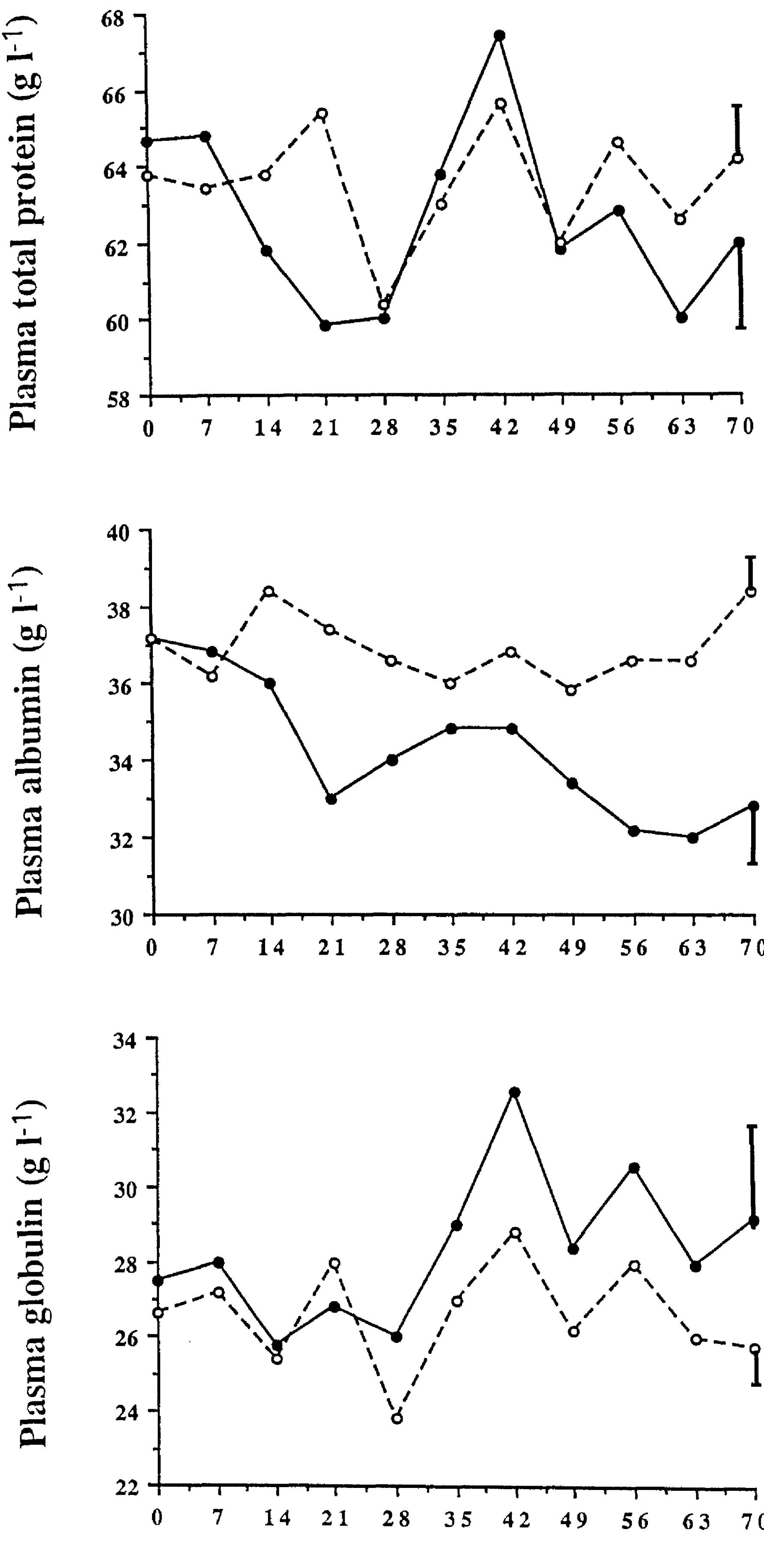
Plasma globulin concentration

Infected animals showed a non significant increase in plasma globulin concentration from 27.5 $\pm$ 0.8 at 0 DAI to 32.6 $\pm$ 2.3 gl^{-1} at 42 DAI (Figure 3.18). Thereafter, it declined to 29.0 $\pm$ 1.4 at 63 DAI. The values in control sheep varied between 25.8 $\pm$ 0.9 and 28.8 $\pm$ 0.6 gl^{-1}.

Figure 3.16 Plasma total protein concentration of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (– –○– –).

Figure 3.17 Plasma albumin concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (– –○– –).

Figure 3.18 Plasma globulin concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (– –○– –).


Plasma total protein (g l-1)
68
66
64
62
60
58
0 7 14 21 28 35 42 49 56 63 70
Plasma albumin (g l-1)
40
38
36
34
32
30
0 7 14 21 28 35 42 49 56 63 70
Plasma globulin (g l-1)
34
32
30
28
26
24
22
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

Serum iron, total iron binding capacity (TIBC) and percentage saturation of transferrin

The concentration of serum iron of infected animals decreased moderately from 37.0 $\pm$ 1.5 $\mu moll^{-1}$ at 0 DAI to 32.2 $\pm$ 2.1 $\mu moll^{-1}$ at 16 DAI (Figure 3.19) after which it fluctuated a great deal but showed sharp increases at 23 and 51 DAI. The mean values in control animals showed a downward trend between 0 and 65 DAI but were not significantly different from those of infected animals.

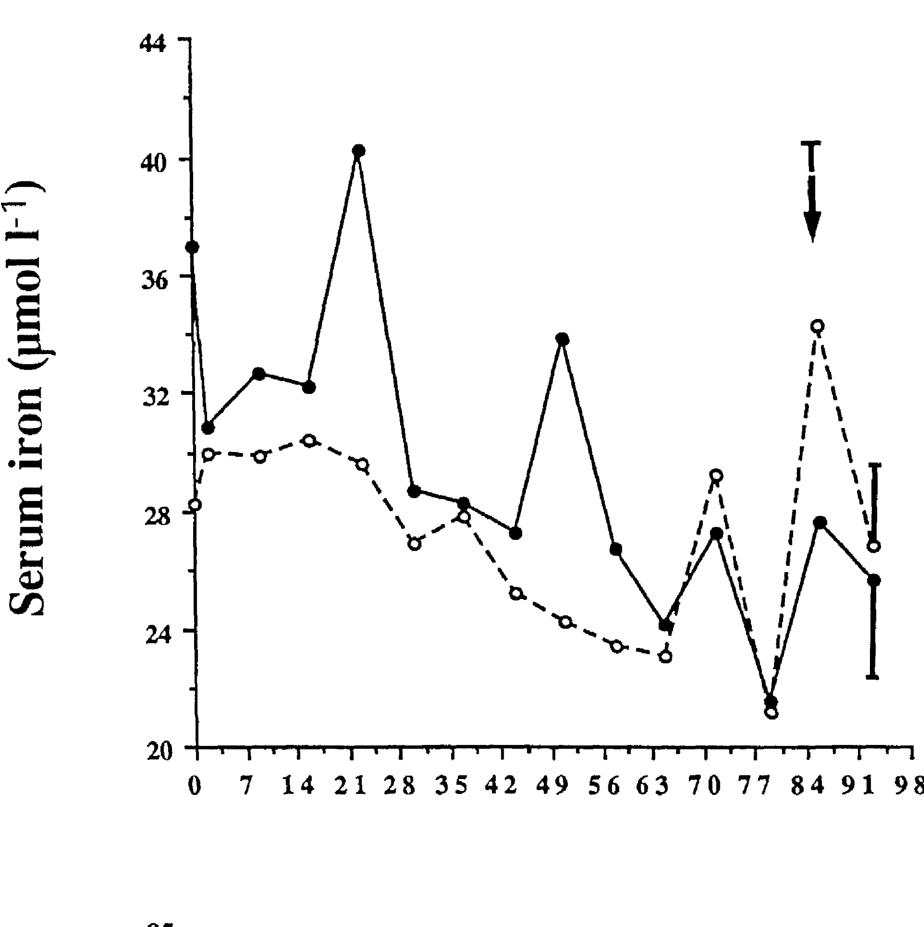
There were no significant differences in the levels of serum TIBC (Figure 3.20) UIBC (Infected 23.5-48.5, Control 28.5-49.5 $\mu moll^{-1}$) and percentage saturation of transferrin (Infected 32.5-61.2%, Control 31.1-55.2%) between infected and control animals.

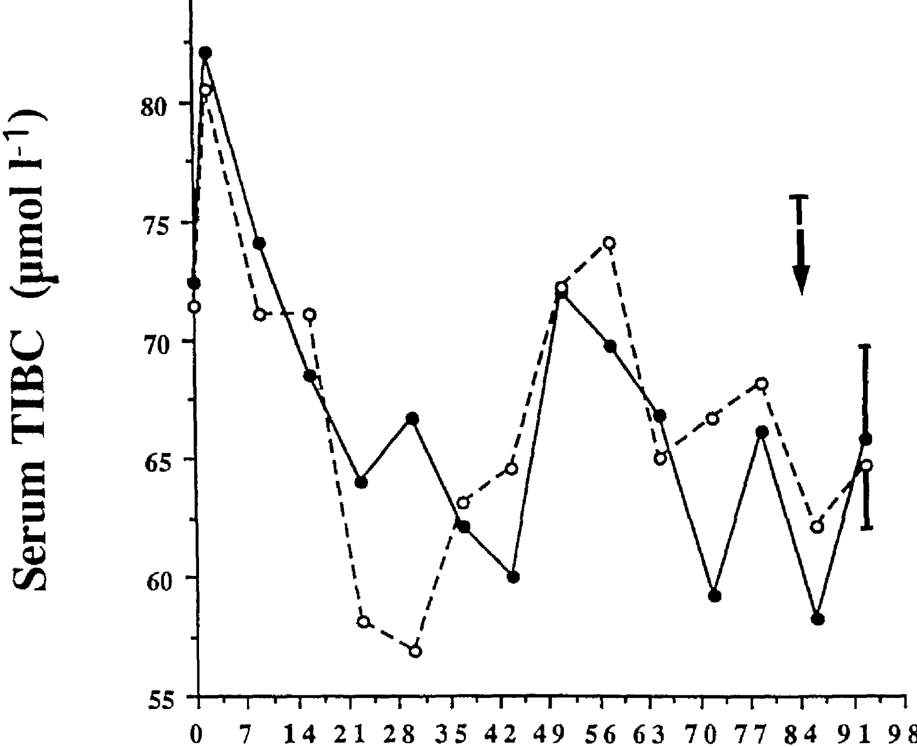
Plasma zinc, copper, magnesium, inorganic phosphate, calcium, urea, glucose and β-hydroxybutyrate

There were no significant differences observed in the plasma concentrations of zinc, copper (Table 3.3), urea, calcium, magnesium (Table 3.4), glucose, inorganic phosphate and β-hydroxybutyrate (Table 3.5) between infected and control animals throughout the experimental period.

Figure 3.19 Serum iron concentrations of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.

Figure 3.20 Serum TIBC of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (- -○- -). T denotes treatment.





Time after infection (days)

Table 3.3

Plasma zinc and copper concentrations of sheep infected with *Trypanosoma congolense* and of uninfected control sheep

Days after infection	Zinc (mmol l^{-1})		Copper (mmol l^{-1})	
	Infected	Control	Infected	Control
0	0.76 ± 0.12	0.94 ± 0.15	14.26 ± 1.15	13.14 ± 1.42
5	0.75 ± 0.07	0.80 ± 0.09	14.00 ± 0.71	12.47 ± 0.99
12	1.09 ± 0.09	1.26 ± 0.13	13.29 ± 0.99	10.94 ± 0.97
19	1.30 ± 0.18	0.95 ± 0.04	15.37 ± 0.81	10.96 ± 0.71
26	1.19 ± 0.10	1.04 ± 0.08	9.64 ± 0.54	7.01 ± 0.44
33	0.87 ± 0.08	0.86 ± 0.11	9.58 ± 0.52	7.29 ± 0.53
40	0.89 ± 0.05	0.75 ± 0.05	8.18 ± 0.66	6.27 ± 0.64
47	0.81 ± 0.04	0.94 ± 0.15	9.81 ± 0.39	8.86 ± 1.04
54	0.87 ± 0.06	0.82 ± 0.06	11.35 ± 0.18	8.98 ± 0.76
61	0.83 ± 0.07	1.04 ± 0.09	13.98 ± 0.73	10.95 ± 0.78
68	0.86 ± 0.06	0.98 ± 0.07	12.75 ± 0.69	9.89 ± 0.65

Table 3.4

Plasma urea, calcium and magnesium concentrations in sheep infected with *Trypanosoma congolense* and in uninfected control sheep

Days after infection	Urea ($mmoll^{-1}$)		Calcium ($mmoll^{-1}$)		Magnesium ($mmoll^{-1}$)	
	Infected	Control	Infected	Control	Infected	Control
00	2.97 ± 0.30	3.56 ± 0.47	2.59 ± 0.08	2.58 ± 0.03	0.75 ± 0.02	0.73 ± 0.02
07	3.60 ± 0.33	3.66 ± 0.43	2.72 ± 0.03	2.74 ± 0.03	0.74 ± 0.03	0.73 ± 0.03
14	4.80 ± 0.36	4.22 ± 0.28	2.58 ± 0.04	2.61 ± 0.03	0.79 ± 0.02	0.82 ± 0.03
21	5.72 ± 0.13	4.92 ± 0.27	2.70 ± 0.06	2.73 ± 0.02	0.82 ± 0.02	0.85 ± 0.02
28	6.18 ± 0.30	6.24 ± 0.32	2.49 ± 0.04	2.36 ± 0.03	0.89 ± 0.03	0.88 ± 0.03
35	5.64 ± 0.41	5.68 ± 0.26	2.72 ± 0.40	2.75 ± 0.85	0.85 ± 0.02	0.85 ± 0.01
42	6.42 ± 0.51	5.90 ± 0.23	2.55 ± 0.02	2.57 ± 0.02	0.85 ± 0.03	0.84 ± 0.02
49	5.74 ± 0.41	5.28 ± 0.21	2.51 ± 0.05	2.52 ± 0.04	0.84 ± 0.03	0.87 ± 0.02
56	5.94 ± 0.25	5.82 ± 0.16	2.57 ± 0.02	2.64 ± 0.04	0.87 ± 0.04	0.90 ± 0.02
63	6.52 ± 0.69	5.36 ± 0.21	2.45 ± 0.07	2.50 ± 0.04	0.81 ± 0.03	0.82 ± 0.02
70	5.96 ± 0.61	5.56 ± 0.34	2.44 ± 0.02	2.50 ± 0.03	0.70 ± 0.03	0.68 ± 0.01

Table 3.5

Plasma glucose, inorganic phosphate and β-hydroxybutyrate concentrations in sheep infected with *Trypanosoma congolense and in uninfected control sheep**

Days after infection	Glucose ($mmoll^{-1}$)		Inorganic phosphate ($mmoll^{-1}$)		β-hydroxybutyrate ($mmoll^{-1}$)	
	Infected	Control	Infected	Control	Infected	Control
0	3.97 ± 0.20	3.64 ± 0.23	2.56 ± 0.08	2.37 ± 0.08	0.25 ± 0.04	0.24 ± 0.05
7	4.28 ± 0.46	3.42 ± 0.05	2.50 ± 0.16	2.36 ± 0.14	0.18 ± 0.05	0.18 ± 0.02
14	3.58 ± 0.12	3.46 ± 0.11	2.11 ± 0.12	2.13 ± 0.11	0.18 ± 0.05	0.16 ± 0.04
21	3.82 ± 0.18	3.10 ± 0.08	2.11 ± 0.16	2.04 ± 0.16	0.27 ± 0.02	0.14 ± 0.03
28	3.48 ± 0.09	3.16 ± 0.10	2.70 ± 0.23	2.99 ± 0.10	0.25 ± 0.03	0.20 ± 0.02
35	3.72 ± 0.11	3.36 ± 0.07	2.46 ± 0.90	2.43 ± 0.14	0.26 ± 0.04	0.20 ± 0.04
42	3.52 ± 0.20	2.98 ± 0.14	2.25 ± 0.22	2.17 ± 0.25	0.24 ± 0.04	0.20 ± 0.02
49	3.32 ± 0.21	2.98 ± 0.10	2.25 ± 0.21	2.40 ± 0.14	0.22 ± 0.04	0.24 ± 0.07
56	3.20 ± 0.19	2.94 ± 0.10	2.33 ± 0.18	2.33 ± 0.23	0.20 ± 0.03	0.10 ± 0.03
63	3.56 ± 0.13	2.90 ± 0.08	2.12 ± 0.14	2.21 ± 0.18	0.28 ± 0.06	0.28 ± 0.04
70	3.54 ± 0.20	3.12 ± 0.11	2.33 ± 0.16	2.37 ± 0.08	0.28 ± 0.05	0.20 ± 0.04
77	-	-	-	-	0.18 ± 0.02	0.14 ± 0.02
84	-	-	-	-	0.20 ± 0.05	0.20 ± 0.01
92	-	-	-	-	0.14 ± 0.02	0.16 ± 0.02
96	-	-	-	-	0.38 ± 0.04	0.36 ± 0.04

*Infected animals were treated with diminazene aceturate at 84 days after infection

DISCUSSION

The disease pattern observed in infected animals in this study could be divided into two phases. Phase one, lasting 6 - 8 weeks, was characterised by high intensities of parasitaemia, pyrexia and rapid decreases of PCV, red blood cell counts and haemoglobin concentrations. Phase two occurred after 8 weeks and was characterised by a fluctuating low intensity of parasitaemia, and in some animals aparasitaemic periods ranging from 3 to 4 days were recorded. The packed cell volume in this phase was correlated closely with the intensity of parasitaemia in that a decrease in intensity was invariably associated with a rise in PCV. Some animals attained PCV values comparable to those of control animals in the presence of very few parasites in circulation. These patterns described here conform closely to the acute and chronic phases described by MacKenzie and Cruickshank (1973) and Griffin and Allonby (1979c) in sheep infected with *T. congolense*, and by Murray (1979) and Murray and Dexter (1988) in their reviews of bovine trypanosomiasis.

After a prepatent period of 7-9 days, infected animals developed fluctuating parasitaemia reaching the first peak of +4 by 16 DAI. However, after 49 days of infection, peaks of parasitaemia showed a progressive decrease until treatment at 84 DAI. Waves of parasitaemia are known to arise from antigenic variation. This is a biological phenomenon, whereby, trypanosomes sequentially express a series of antigens on their surface coat. Infected animals respond immunologically to the current antigenic combination but the response appears too late to affect that proportion of trypanosomes that have already changed their antigenic identity. Therefore a population of parasites persist and parasitaemia rises and falls, displaying peaks of varying heights and widths. The ability of infected animals to limit the intensity of parasitaemia with progress of infection, as observed in this study, is largely attributed to an efficient immune response. Some species and certain breeds of animals (trypanotolerant) are able to reduce intensities of

parasitaemia to such an extent that they continue to grow and reproduce despite presence of very few parasites; certain animals even effect self cure in that they eliminate trypanosomes completely (MacKenzie and Cruickshank, 1973; Griffin and Allonby, 1979c; Murray *et a*l., 1982).

The observation of significant decreases in PCV, red cell counts and haemoglobin concentration in infected animals is in support of previous reports that anaemia is an important feature of ovine trypanosomiasis (MacKenzie and Cruickshank, 1973; Griffin and Allonby, 1979c). A number of factors have been incriminated in the aetiology of this anaemia, and these include, haemolysis as a result of immunological factors, biologically active products derived from trypanosome, haemodilution and dyshaemopoiesis. The first trypanolytic crisis in this study occurred on day 14 and was followed by a decrease in PCV, RBC and Hb concentration which reached their lowest values 23 days after infection. This observation suggests that the trypanosomes or trypanosome products following their immunological destruction may be responsible for the initiation of the anaemia.

There is general agreement that haemolysis is the dominant factor during the acute stages of the disease although the causes of haemolysis are debatable. It has been suggested that trypanolytic crises may lead to release of biologically active factors known to be present in trypanosomes (Tizard *et al.*, 1978c). Some of these haemolytic factors have been identified as proteins (Murray *et al.*, 1979) or lipids (Tizard and Holmes, 1976; Tizard *et al.*, 1978a, b). Damaged erythrocytes undergo extravascular haemolysis by an activated mononuclear phagocytic system (MacKenzie and Cruickshank, 1973; MacKenzie *et al.*, 1978)).

The anaemia in infected animals was macrocytic and normochromic in type and this is in agreement with reports of Anosa and Isoun (1980) in sheep infected with *T. vivax*. In addition to macrocytosis, Parkin (1935) observed polychromasia, basophilia and the presence of normoblasts in sheep infected with trypanosomes

while MacKenzie and Cruickshank (1973) recorded a marked increase in reticulocyte count in *T. congolense*-infected sheep that experienced a marked drop in PCV values during the acute phase of infection. These observations are consistent with enhanced erythropoietic activity during the early stages of trypanosomiasis (Murray and Dexter, 1988).

Griffin and Allonby (1979c) observed that some sheep with naturally acquired infection underwent self cure in that their PCV values and body temperatures reverted to normal after the disappearance of trypanosomes from the circulation. In this study, trypanosomes could not be detected in some animals for a variable period after which they reappeared. These animals showed recovery of PCV values and a very low level of intermittent parasitaemia. It is possible that the apparent self cure reported by Griffin and Allonby (1979c) was associated with failure to detect trypanosomes by the thick blood smear method in those animals that showed normal PCV values. It is now accepted that the thick smear method is less sensitive for detection of very low levels of parasitaemia compared to concentration methods like the dark ground/phase buffy coat method (Murray *et al.*, 1977, Paris *et al.*, 1982) employed in this study.

The PCV values of infected animals in this study showed a rapid recovery following treatment at 84 DAI. It has been reported that cattle in chronic stages of infection respond poorly to trypanocidal treatment (Murray and Dexter, 1988; Wellde *et al.*, 1989c) indicating poor responsiveness of the bone marrow. The rapid increase of PCV values after treatment suggests the bone marrow activity of the infected sheep was not impaired and confirms the observations of Mackenzie and Cruickshank (1973) that the erythropoietic tissue of the bone marrow remains hyperplastic during the acute and chronic stages of trypanosomiasis in sheep.

Infected animals showed a marked increase in total white cell counts, the increase beginning from 7 DAI up to 84 DAI when the animals were treated. The leucocytosis was mainly due to a lymphocytosis and apart from an initial moderate neutropaenia, the changes in eosinophil, basophil and monocyte counts were not significant. This observation is in contrast to reports of leucopaenia, associated with severe lymphopaenia and neutropaenia in the early stages of trypanosome infections in sheep (MacKenzie and Cruickshank, 1973; Igbokwe and Anosa, 1989) and cattle (Naylor, 1971b; Maxie *et al.*, 1979; Kaaya *et al.*., 1980; Valli and Mills, 1980; Ellis *et al.*, 1987). Leucopaenia has been attributed to leucophagocytosis (Mackenzie and Cruickshank, 1973) as a result of trypanosomal antigen coating of leucocytes (MacKenzie *et al.*, 1978; Maxie *et al.*, 1979), or their damage following neuramidase production (Esievo, 1979), or depression of leucocyte production as a result of hyperplasia of erythropoietic cells (Valli *et al.*, 1979; Kaaya *et al.*, 1980). In these cases recovery of infected animals is associated with a return to normal of leucocyte counts or a leucocytosis.

It is possible that the lymphocytic response observed in this study could be associated with the ability of these sheep to mount an effective immunological response to limit the intensity of parasitaemia. Morrison *et al.* (1978) reported that *T. congolense* infection in mice results in non-specific polyclonal activation of B-lypmhocytes and in comparative studies of the susceptibility of N'Dama and Boran cattle to *T. congolense* infection, Paling *et al.* (1991a) observed that the trypanotolerant N'Dama developed a leucocytosis and lymphocytosis while the susceptible Boran showed a leucopaenia and lymphopaenia. The sheep in this study were able to withstand the effects of trypanosome infection and they demonstrated an ability to control the intensities of parasitaemia with the progress of infection. They also continued to grow at a rate comparable to that of uninfected controls and they showed a gradual improvement of their PCV values during the chronic stage of

infection. At the same time, none of the infected animals became severely anaemic to warrant chemotherapeutic intervention. It is possible that animals which are less susceptible to trypanosome infection and which have no previous exposure to trypanosomes develop a marked lymphocytosis which may be associated with the ability to produce a more effective immune response.

It was observed , in the present investigation, that the serum concentration of total lipids decreased as a result of reduction in the concentrations of serum phospholipids, plasma cholesterol and free fatty acids. The plasma triglyceride concentration showed an initial fall between 7 and 35 DAI after which it tended to increase but remained lower that the values in control animals. These observations are in contrast to those recorded in rabbits infected with *T. brucei* (Guy, 1975; Rouzer and Cerami, 1980) or *T. gambiense* (Diehl and Risby, 1974), in rats infected with with *T. rhodesiense* (Dixon, 1967a) and in dogs infected with *T. brucei* (Ndung'u *et al.*, 1989; Ndung'u, 1990). These studies in laboratory animals and dogs reported occurrence of hyperlipidaemia associated with hypertriglyceridaemia and hypercholesterolaemia.

Hypolipidaemia has been recorded in sheep infected with either *T. congolense, T. brucei* or *T. vivax* (Roberts, 1973, 1974, 1975a, b; Roberts and Clarkson, 1977), in cattle infected with *T. congolense* (Traore-Leroux *et al.*, 1987a), *T. vivax* (van den Ingh *et al.*, 1976b) and *T. rhodesiense* (Wellde *et al.*, 1989a). These contrasting observations suggest that there are possibly profound differences in lipid metabolism between ruminant and non-ruminant trypanosome hosts.

The decrease in serum phospholipids was observed to begin even before trypanosomes appeared in circulation and continued with rising parasitaemia. Following treatment at 84 DAI, the concentration of serum phospholipids increased. These observations suggest that trypanosomes or their metabolic products exert a direct effect on the hosts phospholipid metabolism. Roberts (1975b) observed that

the decline in phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were largely responsible for the decrease in phospholipids. He observed that LPC decreased from a normal concentration of 7-10% of total phospholipid to 0-3% in infected sheep. This decrease was attributed to hepatic pathology since the liver is the main site of phospholipid synthesis. However, it has been reported on many occasions that unlike *T. brucei* infections, *T. congolense* infections are not associated with major histopathological changes, including in organs like the liver (Losos and Ikede, 1972). It is therefore unlikely that hepatic pathology may account for a significant decrease in serum phospholipids.

Hypophospholipidaemia may be due to release of phospholipases and lysophospholipases by dying trypanosomes (Tizard *et al.*, 1977; Tizard *et al.*, 1978a, b; Hambrey *et al.*, 1984). These enzymes act on host phosphatidylcholine leading to release of glycerophosphorycholine and free fatty acids (FFA). Phospholipases themselves may exert a direct effect on the infected animals since they can destroy cell membrane phospholipids, particularly so when they act in conjunction with detergent like molecules, such as FFA. As a result they may be both cytotoxic and haemolytic (Colley *et al.*, 1973). It is possible that the combination of phospholipases and FFA account for a large portion of haemolytic activity observed in trypanosome infections (Huan *et al.*, 1975; Murray, 1979; Murray *et al.*, 1979).

Most studies of lipid metabolism in trypanosomes deal with the *brucei* group of trypanosomes. However, Bastin *et al.* (1991) have provided evidence that lipid metabolism among members of the genus Trypanosoma is similar. There is evidence that trypanosomes contain large amounts of lipid material constituting 15-20% of the dry weight of trypanosomes (Venkatessan and Ormerod, 1976) and that phospholipids form 80% of trypanosomal total lipid (Godfrey, 1967). It has been reported that trypanosomes take up low and high density lipoproteins from the host through receptors located on the flagellar pocket (Coppens *et al.*, 1987; Black

and Vanderweed, 1989; Bastin *et al.*, 1991). The degradation of these lipoproteins provides phospholipid residues such as choline, ethanolamine, inositol and FFA which the trypanosomes need for synthesis of membrane phosphoglycerides and membrane protein anchors (Mellors and Samad, 1989). It is therefore possible that the rise in parasitaemia in the early stages of infection may lead to uptake of large amounts of phospholipids to the extent that the concentration in serum may decline. The observation of a decrease in serum concentrations with rising parasitaemia and the increase after treatment confirm this suggestion.

Infected animals in the present study also developed hypocholesterolaemia. The decrease started before appearance of trypanosomes in the circulation and the concentrations remained lower in infected animals than in control ones. Treatment led to a moderate increase in plasma cholesterol concentrations. Observation of hypocholesterolaemia is in agreement with the reports of Roberts (1975b) in sheep infected with either *T. congolense, T. vivax* or *T. brucei.* Similar results have also been recorded in cattle infected with *T. congolense* (Traore-Leroux *et al.*, 1987a) and *T. rhodesiense* (Wellde *et al.*, 1989a). Experiments using the *Trypanozoon* group of trypanosomes have provided evidence that the main sterol in trypanosomes is cholesterol and that trypanosomes are incapable of synthesising it *de novo* (Dixon *et al.*, 1972; Carrol and McCrorie, 1986). Further studies have indicated that trypanosomes obtain cholesterol by uptake and degradation of low and high density lipoproteins (Coppens *et al.*, 1987; Gillet and Owen, 1987; Black and Vanderweed, 1989; Bastin *et al.*, 1991). From these lipoproteins, trypanosomes derive cholesterol, phospholipids and fatty acids which are essential for growth and multiplication. It has been suggested that phospholipids and cholesterol are major components of the trypanosomal membrane and that fatty acids are used as sources of energy.

Observation of hypocholesterolaemia with rising parasitaemia may suggest that trypanosomal uptake of cholesterol may account for a large part of the decrease in plasma cholesterol concentrations.

In sheep virtually all the plasma cholesterol esters originate from the plasma lecithin-cholesteryl acyltransferase (LCAT) system and a small portion is derived from the small intestine and the liver (Noble *et al.*, 1975a, b). LCAT system catalyses the formation of lysophosphatidylcholine and cholesterol esters in plasma and Noble *et al.* (1971) observed that cholesterol esters in sheep account for 47% of total lipid in plasma. There is evidence that the LCAT system has preference of phospholipid substrates circulating as high density lipoproteins (HDL) Glomset, 1972). Consequently, a reduction in phospholipid and cholesterol concentrations, as observed in this study, might result in reduction in LCAT activity with a subsequent decrease in production of LPC and cholesterol esters. The observation of a reduction in the ratio of cholesterol:cholesterol esters (C:CE) from 2.1 to 1.7 in sheep infected with *T. congolense* (Roberts, 1975b) supports this suggestion. One possible outcome of the hypocholesterolaemia might be to damage the integrity of the erythrocyte plasma membrane which could be a causative factor in the development of anaemia of trypanosome infections. Association of anaemia and hypocholesterolaemia has been observed in man (Westerman, 1975), and in cattle during a course of *Babesia bovis* infection (Ellisdale *et al.*, 1983).

O'Kelly (1973a, b), Noble *et al.*, (1973) and Noble *et al.* (1976) have reported that lipid metabolism in ruminant animals is affected by exposure to high temperatures. They observed that the concentrations of plasma cholesterol, FFA and phospholipids were lower in heat stressed animals than in animals in a normal environment. Infected animals in this study developed pyrexia which continued until treatment. It is possible that part of the decrease in serum lipids could have been due to hyperthermia. Failure to record a dramatic increase in the concentrations of

plasma cholesterol and FFA after treatment, in this study, is in agreement with the observations of Noble *et al.* (1976) and O'Kelly 1973a, b). These authors reported that when animals are exposed to high temperatures over a long period of time, the concentrations of total fatty acids and cholesterol take a long time to recover after return to a normal environment.

Weight loss is known to be accompanied by a decrease in cholesterol concentration (O'Kelly 1974). However in this study, the decrease in plasma cholesterol concentration started before trypanosomes appeared in the circulation and long before infected animals began to show retarded growth. Lack of correlation between weight loss and cholesterol concentration was also reported by Traore-Leroux *et al.* (1987a) in cattle infected with *T. congolense.*

It was observed that the concentration of plasma FFA decreased with progress of infection and no significant changes occurred in plasma triglyceride concentration. Similar observations were made by Roberts (1975a, b) in *T. congolense*-infected sheep and by Wellde *et al.* (1989a) in *T. rhodesiense*-infected cattle. It has been reported that FFA are released from trypanosomes after autolysis and following the action of phospholipases on host phospholipids (Tizard *et al.*, 1978b, c). No increase in plasma FFA was recorded following massive destruction of trypanosomes at the end of parasitaemia waves in this study. This could be partly due to rapid binding to plasma albumin, or rapid uptake by trypanosomes or the host tissues for energy metabolism. Failure to record an increase in plasma FFA does not rule out their potential toxic and haemolytic effects in the microcirculation. It has been suggested that *T. congolense* organisms may be five to ten times more numerous in capillary beds than in large vessels (Tizard *et al.*, 1978c) and FFA may reach high concentrations in this environment leading to red cell damage. On erythrocytes, FFA cause crenation and decreased deformability (Kamada *et al.*, 1987). As a result, blood flow in the microcirculation

is impaired leading to pooling of blood in splenic sinusoids, as is observed in trypanosome-infected animals (Murray and Dexter, 1988) and increased removal of erythrocytes by the activated mononuclear phagocytic system (MPS).

The net effect of the lipid changes observed in this study was a decrease in serum total lipids in infected animals. The reduction of total lipids in infected animals could deprive the host tissues of essential energy and lead to breakdown of body fat, as recorded by Losos and Ikede (1972). This could in turn lead to debility and poor rates of weight gains that occurs in trypanosome-infected animals.

Infected animals also developed hypoalbuminaemia and hyperglobulinaemia, and the changes in plasma total protein concentration were not significant. These observations are in agreement with the previous findings of Edwards *et al.* (1956b) and Bouteille *et al.* (1988b) in ovine trypanosomiasis. The cause of a decline in plasma albumin concentration could be the result of haemodilution, decreased synthesis by the liver, trypanosomal uptake of albumin-bound fatty acids and lipoproteins or increased catabolism by the host (Vickerman and Tetley, 1979). Coppens *et al.* (1987) reported that albumin is required by trypanosomes for growth and multiplication. Since infected animals developed high parasitaemia particularly in the early stages of infection, it is possible that increased utilisation by the parasites might have contributed to the decrease in plasma albumin concentration.

An increase in plasma globulin concentration was observed after 4 weeks of infection. Hyperglobulinaemia has previously been recorded in sheep and cattle infected with trypanosomes (Luckins, 1972; Clarkson, 1976; Clarkson *et al.*, 1975). The increase in globulin concentration has been attributed mainly to an increase of IgM (Clarkson, 1976) and, to a lesser extent, of IgG in sheep (Mackenzie *et al.*, 1979) . It has been suggested that an increase in these immunoglobulins may be important in the pathogenesis of trypanosomiasis (Boreham and Facer, 1974; Holmes, 1976). MacKenzie and Boreham (1974) have suggested that a marked

increase in IgM may lead to increased erythrocyte sedimentation rate, increased viscosity of plasma causing circulatory embarrassment, and release of pharmacologically active substances following formation of immune complexes. These changes may contribute to pathogenic effects observed in trypanosomiasis. Dargie (1980) has also suggested that increased synthesis of immunoglobulins occurs at the expense of muscle protein and would result in loss of weight.

Infected animals showed a fluctuating decrease in serum iron concentration with time but displayed sharp rises at 23 and 51 DAI. These days corresponded to periods of marked decreases in RBC and PCV values after parasitaemic waves. Since most of the iron in the body is in the haemoglobin of red blood cells, such an increase in serum iron may be associated with intravascular haemolysis. That intravascular destruction of red cells may occur in trypanosomiasis has been observed in cattle infected with *T. congolense* (Dargie *et al.* (1979a) and *T. vivax* (Esievo *et al.*, 1984).

Investigations of blood sugar levels in animals infected with trypanosomes have yielded equivocal results. In this study, infected animals maintained normal glucose levels. This observation is in agreement with those of Edwards *et al.* (1956b) in sheep infected with trypanosomes. However, in cattle infected with *T. rhodesiense* (Wellde *et al.*, 1989a), a reduction of plasma glucose was recorded. It has been proposed that hypoglycaemia may be an important factor in the pathogenesis of animal trypanosomiasis particularly in hyperacute infections associated with *T. vivax* in cattle and *T. simiae* in pigs (Hudson, 1944). These infections are characterised by enormous numbers of trypanosomes in the blood. This study has indicated that hypoglycaemia does not occur in *T. congolense* infections in sheep which are characterised by low transient parasitaemia especially in the chronic stages.

The present investigation has shown that Scottish Blackface sheep infected with *T. congolense* develop macrocytic normochromic anaemia, the onset of which coincides with the appearance of trypanosomes in the circulation. Anaemia was accompanied by a leucocytosis which was principally a lymphocytosis. Biochemical determinations of blood lipids and proteins showed that infection leads to marked decreases in the concentrations of plasma albumin, and plasma cholesterol and serum phospholipids with a resultant reduction in serum total lipid concentration. These lipid changes start to occur before the appearance of detectable levels of parasitaemia, suggesting that the trypanosomes or their products have a profound effect on host lipid metabolism. It is possible that such alterations in host lipid metabolism could play a significant role in the pathogenesis of the disease process.

CHAPTER 4

THE PATHOPHYSIOLOGY OF OVINE TRYPANOSOMIASIS: FERROKINETICS AND ERYTHROCYTE SURVIVAL STUDIES

INTRODUCTION

Anaemia is recognised as the most important pathological feature of animal and human trypanosomiasis (Hornby, 1921; Murray, 1979) and has been the subject of considerable study particularly in laboratory animals (Murray *et al.*, 1974; Jennings *et al.*, 1974) and cattle (Naylor, 1971a, b; Murray and Dexter, 1988). Few studies have been reported in sheep (MacKenzie and Cruickshank, 1973; Edwards *et al.*, 1956a; Anosa and Isoun, 1976). Many of the results from studies of the aetiology of anaemia are equivocal. It has been proposed that haemodilution (Fiennes, 1954; Holmes, 1976; Maxie and Valli, 1979), increased red cell destruction (Mamo and Holmes, 1975; Dargie *et al.*, 1979a, b) and dyshaemopoiesis (Preston *et al.*, 1979, Wellde *et al.*, 1989c) or a combination of these factors are responsible for the anaemia. It is possible that the contributions of these mechanisms vary with the stage of the disease process and the host-parasite system being studied.

While it is known that sheep infected either naturally or experimentally with trypanosomes develop anaemia (Griffin and Allonby, 1979c; Dirie *et al.*, 1988), its causes have been investigated only on a few occasions. Clarkson (1968) used Evan's blue to measure plasma volumes in sheep infected with *T. vivax*. He recorded an increase of 16-18% in plasma volumes of infected animals but blood volumes were unchanged. An increase in plasma volume was also reported by Anosa and Isoun (1976) using ^{131}I-labelled albumin and they also observed an increase in blood volumes of *T. vivax* infected sheep. In sheep infected with *T. vivax*, Anosa and Isoun (1976) also observed that infected animals had a shorter red cell survival time as measured by the ^{51}Cr-rbc half-life. The controversy in the results reported on blood volumes, could be due to differences in the methods employed or differences in the stages of disease processes when these measurements were made.

In the previous study (Chapter 3), it was observed that *T. congolense* infected sheep developed macrocytic normochromic anaemia which persisted until treatment, despite a gradual decrease in intensities of parasitaemia. While there is general agreement that anaemia is haemolytic, particularly in the early stages of infection, the contribution of haemodilution and dyshaemopoiesis in ovine trypanosomiasis has not been resolved. The present study using radio-isotopes provides unique information on the aetiology of the anaemia by permitting measurements of the dynamic aspects of red cell production, distribution and breakdown in sheep experimentally infected with *T. congolense.*

The application of radio-isotopic tracer techniques in the study of the pathogenesis of trypanosomiasis has been reviewed by Holmes (1984). The three radio-isotopes used in this experiment were ^{51}Chromium-labelled erythrocytes, ^{59}Fe as ferric citrate and ^{125}Iodine-labelled albumin. Erythrocytes are labelled with ^{51}Cr by incubation with anionic hexavalent sodium chromate ($Na_2{}^{51}CrO_4$) (Gray and Sterling, 1950). The labelled cells are then returned to the donor by intravenous injection, and by the dilution principle, the circulating red cell volume is calculated. The rate of disappearance of the labelled cells from the circulation, expressed as the apparent half-life ($T_{1/2}$), provides information on the apparent red cell lifespan. The term "apparent" is used because a proportion of ^{51}Cr is lost from the red cells by elution before they are destroyed, and the calculated value is therefore an underestimate of the true half-life of the red cells. Following breakdown of erythrocytes and their removal from the circulation, the isotope is not re-utilised but is excreted in urine (Holmes *et al.*, 1968). This character makes it suitable for measuring red cell survival and quantifying loss by haemolysis.

^{59}Fe as ferric citrate can be used to provide information on the erythropoietic activity. Injected intravenously, it labels transferrin and allows measurement of iron removal from plasma ($T_{1/2}$) and plasma iron turnover rates

(PITR). Most of the circulating iron is transported to the bone marrow for incorporation into haemoglobin of new red cells. The rate of disappearance of ^{59}Fe from plasma and the speed of its appearance in circulating erythrocytes, serve as indices of erythropoietic activity (Wasserman *et al.*, 1952). Dargie *et al.* (1979a) have suggested that because ^{59}Fe is rapidly removed from the circulation, the rate of removal depending on the degree of erythropoiesis, it does not equilibrate well with plasma and is therefore unsuitable for measurement of plasma volume. This suggestion was investigated in the present study, by calculating plasma volumes using ^{59}Fe, and comparing them with those obtained by using ^{125}I-albumin.

^{125}I has been used on many occasions to measure plasma volumes and to study albumin metabolism in parasitic diseases. An advantage of this label is that it is not re-utilised after degradation of labelled albumin, but is quantitatively excreted if uptake of iodine by the thyroid gland has been blocked by previous administration of inactive iodine. Injection of ^{125}Iodine-labelled albumin allows measurement of the intravascular pool of albumin and transcapillary escape rate of albumin (TER_{alb}), which gives some information on the involvement of capillary permeability in the possible development of haemodilution. Using the above mentioned techniques, the present experiment was conducted to investigate the underlying causes of anaemia observed in sheep after 75 days of infection. These studies have not been conducted before in sheep infected with trypanosomes.

MATERIALS AND METHODS

Experimental Animals, Housing and Feeding

The experimental animals, their housing and feeding have been described (Chapter 3)

Radioisotopic Techniques

1. Daily routine

The sheep were dosed orally with 10 ml of 0.75% potassium iodide (KI) to prevent uptake of radioactive iodine by the thyroid gland. The dosing started five days before injection of radio-isotopes and continued until the end of the experiment. Blood samples from each animal were collected regularly for the measurement of radioactivity.

2. Labelling techniques

^{51}Cr-labelled erythrocytes

Twenty ml of blood were collected, from each animal, into heparinised tubes on the day of injection. After centrifugation for 10 minutes at 500g, the plasma was removed and cells were suspended in physiological saline. An appropriate amount of $Na_2{}^{51}CrO_4$ in isotonic saline (specific activity 37 MBq/ml, chromium content 5.4 ug/ml: Amersham International plc, U.K) was divided amongst the blood samples, each sample receiving approximately 37 MBq of ^{51}Cr.
The isotope and cell suspension were mixed gently, then incubated at 37 ^{o}C for 30 minutes to allow labelling to occur. After this, the samples were centrifuged at 500 g for 10 minutes and the supernatant removed and discarded. The unbound isotope was removed by washing cells several times (four washes) with physiological saline, and the cells were finally resuspended in plasma, each animal receiving its own cells in its own plasma. The final volume for injection was about 15 ml.

^{125}I-labelled albumin

Commercial sheep albumin was trace-labelled with radioiodine by the method of McFarlane (1958). The protein in slightly alkaline medium was treated with iodine monochloride to which radioactive iodine had been added as carrier-free iodine.

Materials

1. Albumin

Commercial sheep albumin was used (Sigma Chemical Co. Ltd., U.K.) and a 2% solution prepared by dissolving 600 mg of freeze-dried protein in 30 ml of isotonic saline.

2. Stock iodine monochloride solution

This was prepared by dissolving 5.0 g of potassium iodate in 37.5 ml of distilled water. 37.5 ml of concentrated hydrochloric acid (Hcl) and 5 ml of carbon tetrachloride were added to give a faint pink colour to the solution. This solution was diluted 1:350 with distilled water to give a solution containing 0.42 mg iodine per ml as iodine monochloride which was then used for labelling procedure.

3. Glycine buffers (A and B)

Buffer A (pH 8.5) was prepared by adding 9 ml molar glycine in N/4 NaCl solution (Aqupharm, Animalcare Ltd., U.K.) to 1 ml NaOH. This was used to convert iodine monochloride to the hypoiodite.

Buffer B (pH 9.0) was prepared by adding 8 ml molar glycine in N/4 NaCl solution to 2 ml NaOH. This provided the alkaline medium necessary for the reaction to occur.

Procedure

Fifteen ml of buffer A was added to 6 ml of the diluted stock iodine monochloride (ICl) solution. The radioactive iodine (approximately 74 MBq) was added to the solution and was immediately transferred to the buffered protein solution, 30 ml of 2% sheep albumin + 15 ml of buffer B. This brought the specific activity of the solution to 123 KBq\mg of protein. The solution was then transferred to a dialysis sack. The unbound isotope was removed by dialysis for 48 h against two changes of 20 l of isotonic saline.

^{59}Fe citrate

^{59}Fe citrate was diluted with physiological saline and each animal received about 1.85 MBq in about 5 ml.

Preparation of standards

A known weight (approximately 1 ml) of each of the isotopically labelled preparations was emptied into a 100 ml volumetric flask. The contents were made up to the mark with 0.02N NaOH. One ml of this was dispensed into counting vials and made up to 10 ml with 0.02N NaOH. The ^{51}Cr standards were made up for each individual sheep but the ^{125}I and ^{59}Fe standards were made up singly. The standards served as corrections against decay, changes in the sensitivity of the counting equipment and for calculation of the injected dose.

Injection of isotopes

Syringes containing each isotope were weighed before and after injection of each isotope and the difference gave the volume weight of the injected isotope. Each isotope was injected using a jugular catheter and timing was started halfway during the injection process.

Collection and preparation of blood samples for counting

Blood samples (5 ml) were collected from the opposite jugular into heparinised evacuated tubes, 10, 30, 60, 90 and 120 minutes after injection of the isotopes. Thereafter, samples were collected once daily for the next ten days and once every two to three days for the duration of the experiment.

Estimation of PCV was carried out on all the blood samples, then 1 ml of whole blood and 1 ml of plasma were pipetted into counting vials and made up to 10 ml with 0.02 N NaOH for counting.

Radioactivity measurements

Count rates of the three isotopes in the blood and plasma were determined in an autogamma scintillation counter (Packard Instruments Co., Inc, U.S.A.). The calculation of crossover factors was based on the relative count rates of the standard solutions of each isotope at each photo peak. After correction for cross-over, the radioactivity for each radioisotope was referred to as the 'corrected count'.

Calculations and presentation of results

^{51}Cr-labelled RBC

The radioactivity of each blood sample was corrected for background radioactivity and physical decay and expressed as counts/minute (cpm)/ml of red cells using the PCV of each sample.

$$\text{cpm/ml RBC} = \frac{\text{cpm/ml of blood x100}}{\text{PCV (\%)}}$$

"Apparent" half-life ($T_{1/2}$)

When autologous ^{51}Cr-labelled red cells are injected into an animal, there is rapid loss of activity over the first 48 hours due to elution. Once this rapid phase is complete, a slower exponential phase occurs and the red cell half-life is calculated after extrapolation of this second curve (Holmes and Maclean, 1969). Since a population of red cells of all ages were labelled (random labelling) and because of the elution of the isotope, the value for the half-life of the cells is an under-estimate of the true value and is thus referred to as the "apparent" half-life. The apparent half-life of the cells is the time taken in hours for the radioactivity to fall by 50%.

Calculation of circulating volumes

Red cell volume (RCV)

This was calculated using the dilution principle and was expressed as $mlkg^{-1}$ BW.

$$\text{RCV } (mlkg^{-1}) = \frac{\text{Total injected } ^{51}\text{Cr activity (cpm).}}{\text{"Corrected" radioactivity of 1ml RBC at } T_0 \times \text{BW (kg)}}$$

The corrected radioactivity (cpm/ml) of red cells or plasma samples taken at 10, 20, 30, 60 and 120 minutes after injection was plotted against time on a semilogarithmic paper. The best straight line through the points was drawn and the radioactivity at T_0 was obtained by extrapolation to time zero.

Plasma volume (PV)

This was calculated from the radioactivity counts of ^{125}I-albumin using the dilution principle and was expressed as $ml kg^{-1}$ BW.

$$PV\ (ml kg^{-1}) = \frac{\text{Total injected } ^{125}I \text{ activity (cpm)}}{\text{Corrected activity of 1 ml of plasma at } T_0 \times BW\ (kg)}$$

Blood volume (BV) was obtained as the sum of circulating red cell volume and plasma volume.

Plasma volumes were also calculated using corrected ^{125}I counts at T_{10}, and these were compared with plasma volumes obtained by using corrected ^{59}Fe counts in plasma at T_0 and T_{10}.

Red cell synthesis and life-span

Red cell synthesis was assessed on the basis of the rates of plasma iron turnover (PITR) and red cell iron utilisation. The PITR was determined by expressing the ^{59}Fe radioactivity of each plasma sample taken during the first two hours as a percentage of the 10-min post-injection activity and making a semi-log plot of activity against time. The rate of disappearance of isotope was expressed as a half-life ($T_{1/2}$) value. The half-life value together with the serum iron concentration and plasma volume enabled the calculation of PITR from the formula of Bothwell *et al.*, (1957).

$$\text{PITR (mg/kg/day)} = \frac{\text{serum iron (mg/ml)} \times PV\ (ml) \times 0.693 \times 1440}{T_{1/2}\ (min) \times BW\ (kg)}$$

where 0.693 is the natural log of 2

1440 is the number of minutes per day

Red cell utilisation (%) of ^{59}Fe (RCU)

The percentage utilisation of the injected iron by newly formed red blood cells was calculated every day up to 9 days after injection, and at intervals of either 2 or 3 days thereafter until the end of the isotopic study using the following formula:

$$\% \text{ RCU} = \frac{\text{RCV (ml) x 100 x cpm/ml RBC}}{\text{Total injected } ^{59}\text{Fe activity}}$$

The red cell iron incorporation rate was obtained as the product of the percentage utilisation and PITR. For calculation of red cell lifespan it was assumed that the rates of red cell iron incorporation and red cell iron turnover (RCIT) were approximately equal during the isotopic study. Hence the red cell renewed daily (RCIR) could be determined from:

$$\text{RCIR} = \frac{\text{RCIT (mg/day)}}{\text{RCI (mg)}}$$

where RCI equals the total red cell iron, obtained as the product of total blood volume, blood haemoglobin concentration and 3.4. 3.4 is the iron content per g of haemoglobin. The red cell life-span (days) was then calculated as the reciprocal of RCIR.

125Iodine-labelled albumin

The half-life ($T_{1/2}$) of ^{125}Iodine-labelled albumin was calculated from the plasma disappearance curve as for other radio-isotopes.

Intravascular pool of albumin (CA)

This was obtained by multiplying the plasma volume (ml) by the plasma albumin concentration (gl^{-1}) and dividing the result by the body weight (kg). This value was expressed as $g[kgBW]^{-1}$.

$$CA\ (g[kg\ BW]\text{-}1) = \frac{\text{Plasma volume (l) x plasma albumin } (gl^{-1})}{\text{BW (kg)}}$$

Transcapillary escape rate of albumin (TER_{alb})

Transcapillary escape rate of albumin (TER_{alb}) was calculated by the formula of Parving and Gyntelberg (1973)(cited by Holmes, 1976):

$$TER_{alb}\ (\%/h) = \frac{0.693}{T_{1/2}\ (h)}$$

Experimental Design

Seventy five days after infection with *T. congolense* (IL 1180), both infected and control animals were injected with radio-isotopes (^{125}I-labelled albumin, ^{59}Fe as ferric citrate and ^{51}Cr-labelled autologous red cells) to investigate the underlying causes of anaemia in infected animals. The animals were sampled regularly for radioactive measurements for the following 21 days.

RESULTS

Haematological and Parasitological Findings

At 75 DAI, some animals (No 34 and 84) had attained PCV values comparable to those of control animals (Table 4.1). However, the mean PCV, Hb concentration and MCHC of infected animals were significantly lower, and MCV and MCH were significantly higher than those of control animals. At this stage of infection, some animals (Nos 34, 79 and 81) had undetectable parasites in the circulation while sheep No 36 and 84 had parasitaemia scores of 1 and 2, respectively. The mean parasitaemia score was at its lowest at this stage of infection (Figure 4.1).

Serum Iron and Iron-Binding Capacities

There was considerable variation in the concentrations of serum iron, unbound iron-binding capacity (UIBC), total iron-binding capacity (TIBC) and percentage saturation of transferrin among infected and control animals (Table 4.2). However, the mean values of infected sheep were not significantly different from those of control ones.

Blood Volumes

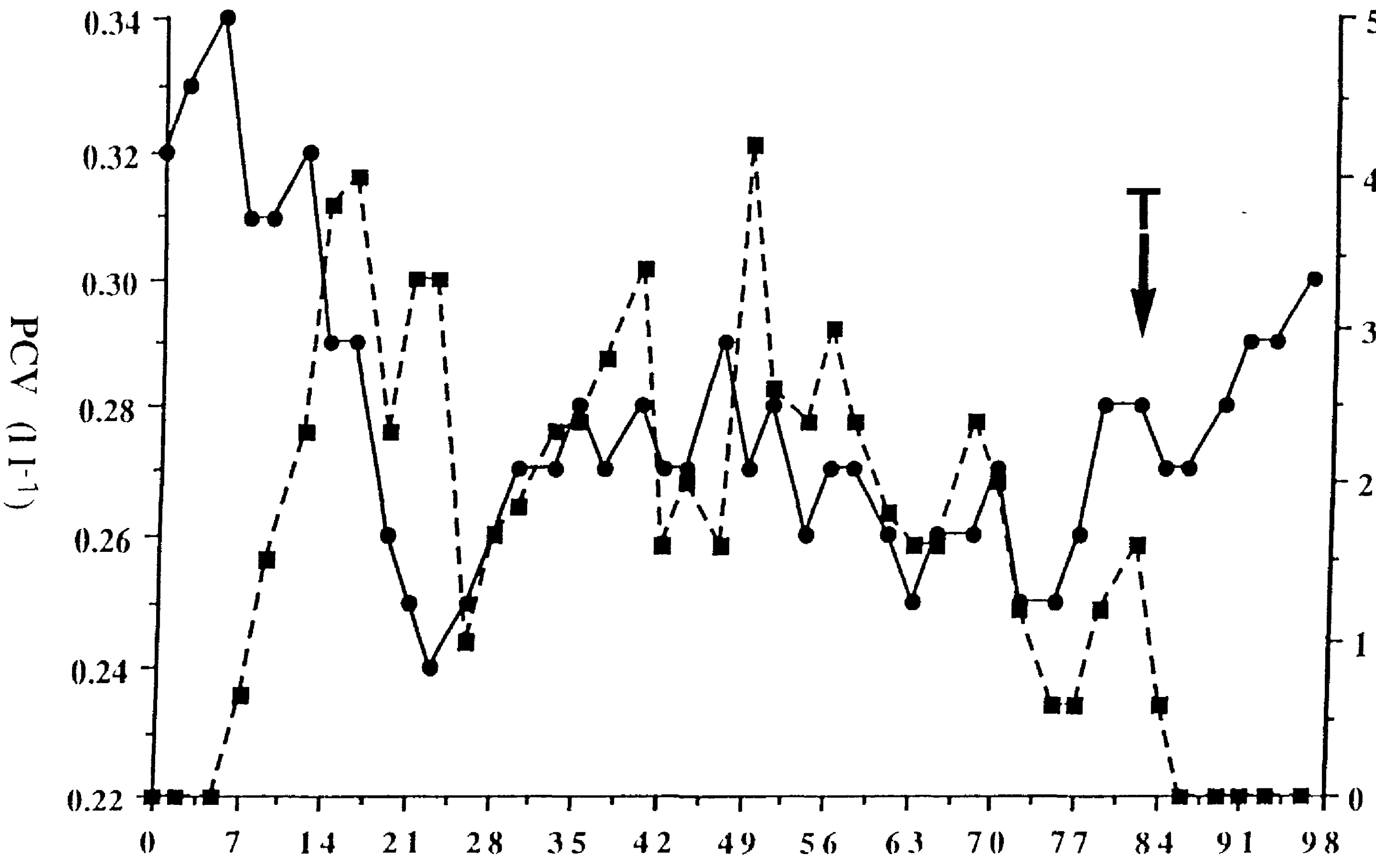
The results of the estimations of circulating blood volumes using ^{125}I-albumin demonstrate that there was marked variation in the circulating red cell volumes of infected animals (Table 4.3) and a significant correlation was recorded between red cell volumes and PCV ($r = 0.946$). The mean circulating red cell volume was significantly lower while the plasma and blood volumes were significantly higher in the infected group than in the control group.

Table 4.1

Red cell values of sheep infected with *Trypanosoma congolense* and of uninfected control sheep, 75 days after infection

Group	Sheep No.	RBC ($x10^{12}l^{-1}$)	Hb (gdl^{-1})	PCV (ll^{-1})	MCV (fl)	MCH (pg)	MCHC (gdl^{-1})
	34	9.51	10.9	0.32	34	11.4	33.8
	36	7.40	8.3	0.27	36	11.2	31.2
Infected	79	6.14	8.1	0.24	40	13.1	33.3
	81	5.67	6.9	0.23	41	12.1	30.0
	84	8.57	9.7	0.30	35	11.3	
	Mean$\pm$sem	**7.46 $\pm$ 0.32**	**8.8 $\pm$ 0.31**	**0.27 $\pm$ 0.02**	**37.2 $\pm$ 0.6**	**11.8 $\pm$ 0.2**	**32.1 $\pm$ 0.3**
	37	10.06	11.4	0.33	33	11.3	34.6
	80	9.41	10.7	0.32	33	11.3	33.9
Control	82	9.83	10.0	0.30	30	10.1	33.4
	83	9.51	10.2	0.29	31	10.7	34.9
	86	10.65	11.7	0.35	32	10.9	35.9
	Mean$\pm$sem	**9.89 $\pm$ 0.10**	**10.8 $\pm$ 0.2**	**0.33 $\pm$ 0.01**	**31.8 $\pm$ 0.3**	**10.9 $\pm$ 0.1**	**34.1 $\pm$ 0.1**
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Figure 4.1. Packed cell volumes (—●—) and parasitaemia scores (- -■- -) of sheep infected with *T. congolense*. T denotes time of treatment.



PCV (l l-1)
0.34
0.32
0.30
0.28
0.26
0.24
0.22
Parasitaemia score
5
4
3
2
1
0
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98
Time after infection (days)

Table 4.2

Serum iron (SI), iron-binding capacities and percentage saturation (% SAT) of transferrin of sheep infected with *Trypanosoma congolense* and of uninfected control sheep

Group	Sheep No.	SI ($\mu moll^{-1}$)	UIBC ($\mu moll^{-1}$)	TIBC ($\mu moll^{-1}$)	% SAT.
	34	19.6	48.3	67.9	28.9
	36	24.2	39.9	64.1	37.8
Infected	79	24.6	34.3	58.9	41.8
	81	21.5	50.1	71.6	30.0
	84	15.7	52.9	68.6	22.9
	Mean ± sem	**21.1 ± 1.6**	**45.1 ± 2.9**	**66.2 ± 2.2**	**32.3 ± 3.4**
	37	25.1	39.8	64.9	38.7
	80	19.2	49.7	68.9	27.9
Control	82	17.9	52.2	70.1	25.5
	83	19.7	48.2	67.9	29.0
	86	22.4	47.0	69.4	32.3
	Mean ± sem	**20.9 ± 1.3**	**47.4 ± 2.1**	**68.2 ± 0.9**	**30.7 ± 2.3**
	P	NS	NS	NS	NS

UIBC = unsaturated iron-binding capacity, TIBC = Total iron-binding capacity, NS = Not significant.

Table 4.3

Blood volumes of sheep infected with *Trypanosoma congolense* and of uninfected control sheep, 75 days after infection

Group	Sheep No.	Red cell volume ($mlkg^{-1}$)	Plasma volume ($mlkg^{-1}$)	Blood volume ($mlkg^{-1}$)
	34	14.6	40.9	55.5
	36	11.9	45.2	57.1
Infected	79	11.9	44.0	55.7
	81	10.8	50.4	61.2
	84	14.8	45.1	59.9
	Mean ± sem	**12.8 ± 0.8**	**45.1 ± 1.5**	**57.9 ± 1.1**
	37	17.1	33.2	54.3
	80	16.5	38.9	55.4
Control	82	14.4	35.1	49.5
	83	14.7	37.9	52.6
	86	17.0	35.7	52.7
	Mean ± sem	**15.9 ± 1.3**	**36.2 ± 1.0**	**52.9 ± 2.2**
	P	< 0.05	< 0.01	< 0.01

Plasma volumes were also calculated using corrected ^{125}I and ^{59}Fe counts at 0 and 10 minutes after injection of radioisotopes. It was observed that plasma volumes of infected animals calculated with either isotope at T_0 were similar, however plasma volumes obtained with ^{59}Fe at T_{10} were significantly higher than the values at T_0 in both infected and control animals. (Table 4.4). Considering volumes obtained with counts at T_{10} compared to those at T_0, it was evident that ^{59}Fe significantly overestimates plasma volumes of both infected and control animals, with the greatest effect being in infected animals. The plasma volumes obtained with ^{125}I at T_0 were similar to those obtained at T_{10} in both infected and control animals.

Erythrokinetics and Ferrokinetics

The rates of red cell breakdown and synthesis were measured in individual infected and control animals to examine the basis of anaemia at 75 DAI. The rates of disappearance of ^{51}Cr-rbc and ^{125}I-albumin from blood and plasma, between 92 and 212 h after injection, are shown in Figure 4.2 and Figure 4.3, respectively, and the values for ^{51}Cr-rbc half-life, plasma ^{59}half-life, and PITR are listed in Table 4.5. Infected animals had shorter half-lives of ^{51}Cr-rbc and ^{59}Fe, and higher PITR than control animals.

Infected animals also showed a more rapid utilisation of labelled iron reaching a mean maximum by 7.2 $\pm$ 0.8 days after injection (Table 4.6). In contrast, control animals utilised labelled iron more slowly and had not reached a maximum by 20 days after injection (Figure 4.4). The mean rates of iron incorporation into new red cells was significantly higher, and calculated red cell lifespan significantly lower in infected animals than in control animals (Table 4.6). Among the infected group, animals with the lowest calculated red cell lifespans had the lowest PCV values while those with higher lifespans had near normal PCV values.

Table 4.4

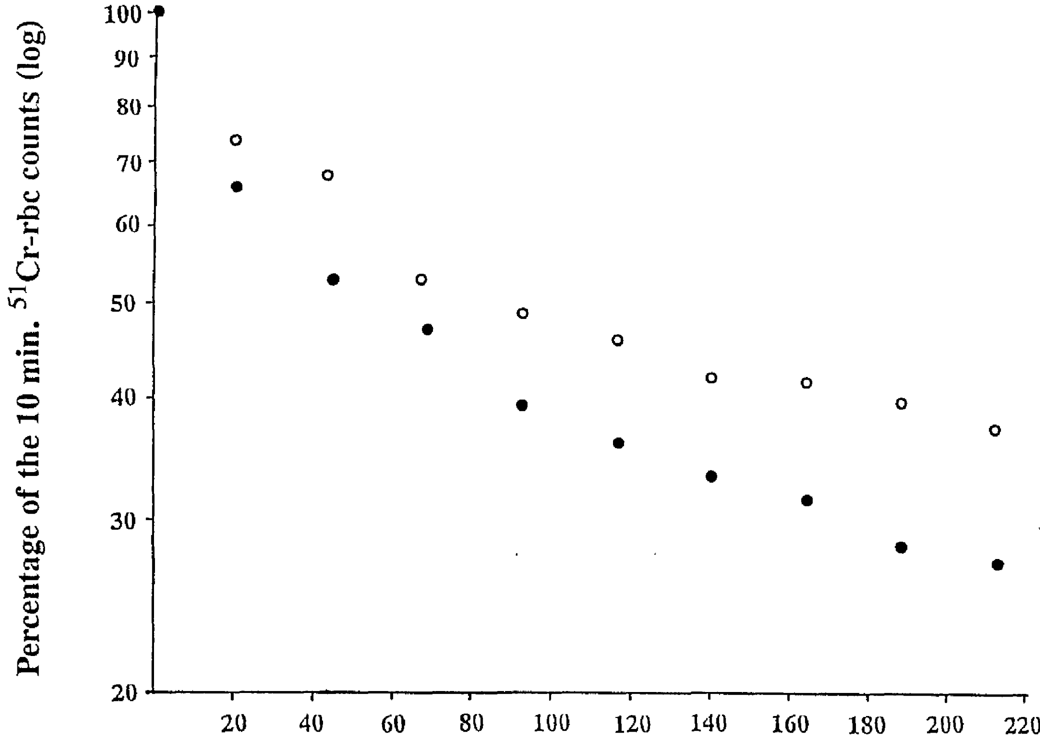
Plasma volumes of sheep infected with *Trypanosoma congolense* and of uninfected control sheep, measured with corrected ^{59}Fe and ^{125}I-albumin counts at 0 and 10 minutes after injection of radioisotopes

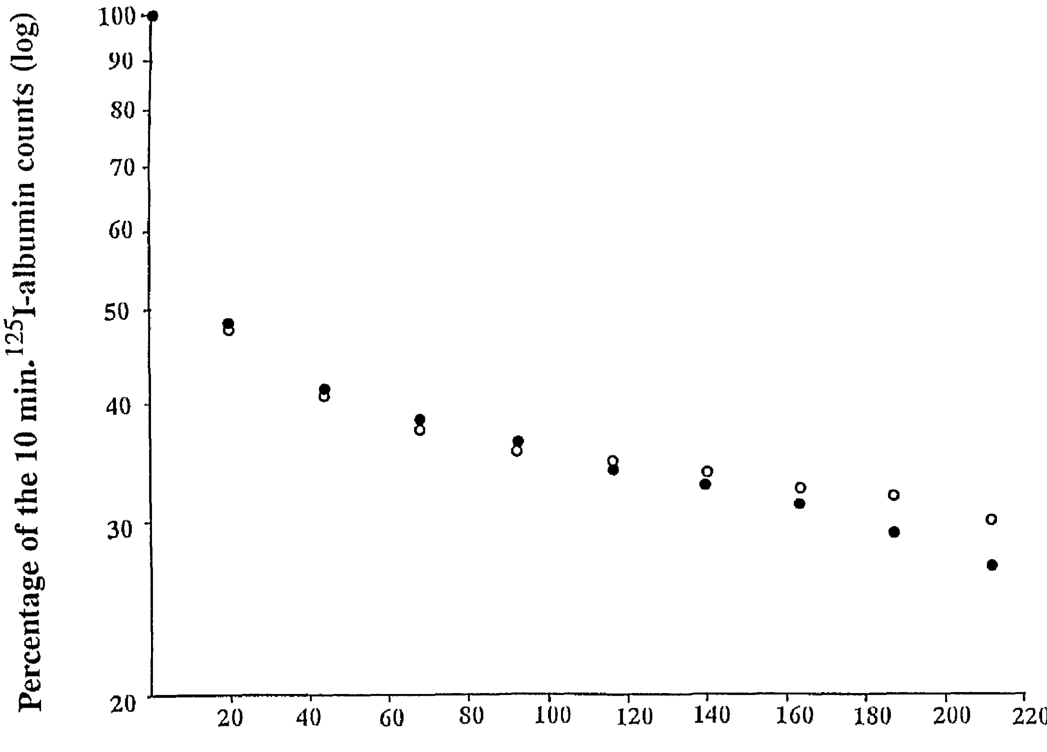
Group	No.	^{59}Fe		^{125}I-albumin	
		T_0	T_{10}	T_0	T_{10}
Infected	34	42.3	47.3	40.9	41.7
	36	45.7	50.4	45.2	46.7
	79	45.7	50.4	44.0	44.1
	81	50.1	55.1	50.4	51.1
	84	46.2	49.7	45.1	45.2
	Mean±sem	**46.0 ± 1.1**	**50.6 ± 1.3[a]**	**45.1 ± 1.5**	**45.8 ± 1.6[c]**
Control	37	37.5	39.6	33.2	33.3
	80	41.6	44.0	38.9	39.3
	82	38.3	41.0	35.1	36.3
	83	40.7	43.4	37.9	37.9
	86	37.8	39.2	35.7	35.8
	Mean±sem	**39.2 ± 0.8**	**41.4 ± 1.0[a]**	**36.2 ± 1.0[b]**	**36.5 ± 1.0[c]**

[a] Significantly different from the corresponding values at T_0, [b] Significantly different from the ^{59}Fe value at T_0,
[c] Significantly different from the ^{59}Fe value at T_{10}

Figure 4.2 Rate of disappearance of ^{51}Cr-rbc in sheep infected with *T. congolense* (—●—) and in uninfected control sheep (--○--) between 20 and 212 h after injection.

Figure 4.3 Rate of disappearance of ^{125}I-albumin in sheep infected with *T. congolense* (—●—) and in uninfected control sheep (--○--) between 20 and 212 h after injection





Time after injection of radioisotope (h)

Table 4.5

Red cell survival and plasma iron kinetics of sheep infected with *Trypanosoma congolense* and of uninfected control sheep, 75 days after infection

Group	Sheep No.	^{51}Cr red cell half-life (d)	Plasma ^{59}Fe half-life (min)	PITR ($mgkg^{-1}d^{-1}$)
Infected	34	10.3	115	0.39
	36	7.9	74	0.82
	79	10.5	84	0.72
	81	8.5	85	0.71
	84	8.8	90	0.44
	Mean ± sem	**9.2 ± 0.5**	**89.6 ± 6.8**	**0.62 ± 0.08**
Control	37	13.0	137	0.34
	80	13.0	115	0.36
	82	13.5	124	0.28
	83	14.7	131	0.32
	86	12.3	144	0.31
	Mean ± sem	**13.3 ± 0.4**	**130.2 ± 5.1**	**0.32 ± 0.01**
	P	< 0.001	< 0.01	< 0.01

Table 4.6

Red cell kinetics and estimated lifespan of sheep infected with *Trypanosoma congolense* and of uninfected control sheep, 75 days after infection.

Group	Sheep No.	^{59}Fe utilization (%)	Maximum utilization (days)	RBC iron incorporation ($mgkg^{-1}d^{-1}$)	RBC lifespan (days)
Infected	34	61.4	8.8	0.24	85.8
	36	75.5	4.8	0.62	26.0
	79	69.3	5.8	0.50	30.7
	81	68.3	8.8	0.48	28.8
	84	70.7	7.8	0.31	63.7
	Mean ±sem	**69.0 ± 2.3**	**7.2 ± 0.8**	**0.43 ± 0.07**	**47.2 ± 11.8**
Control	37	59.1	8.8	0.20	97.6
	80	50.8	20.8	0.18	111.9
	82	45.4	20.8	0.13	129.4
	83	49.8	20.8	0.16	114.0
	86	52.8	20.8	0.16	130.9
	Mean ±sem	**51.6 ± 2.2**	**18.4 ± 2.4**	**0.17 ± 0.01**	**116 ± 6.2**
	P	< 0.001	< 0.01	< 0.01	< 0.001

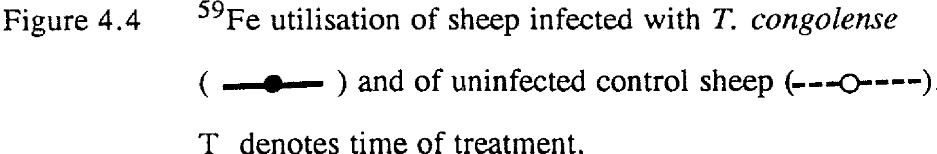
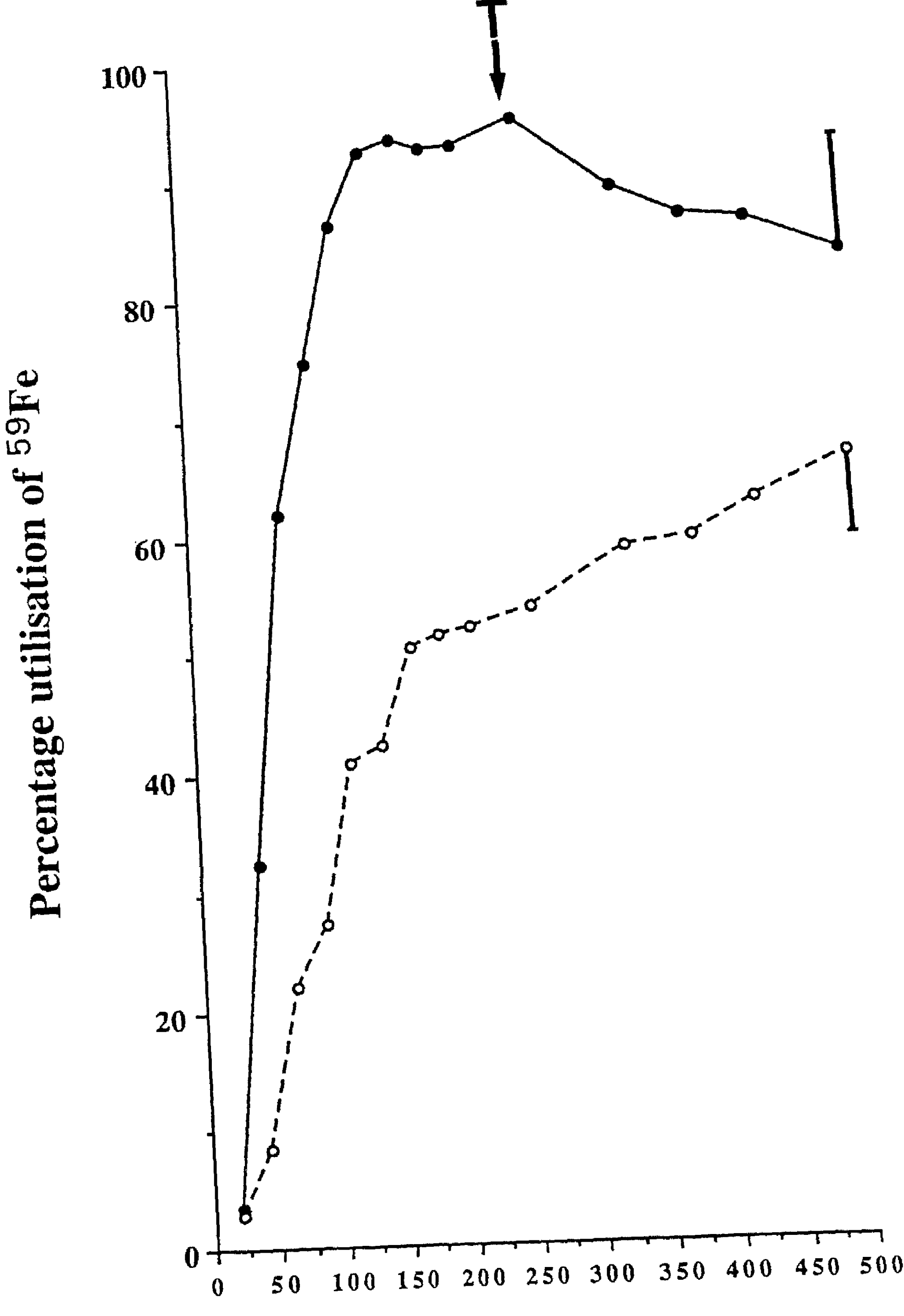


Figure 4.4 ^{59}Fe utilisation of sheep infected with *T. congolense* (—●—) and of uninfected control sheep (---○----).
T denotes time of treatment.



100
80
60
40
20
0
Percentage utilisation of 59Fe
0
50
100
150
200
250
300
350
400
450
500
Time after injection of 59Fe (h)

Albumin Metabolism Studies

Infected animals had significantly lower ^{125}I-albumin half-life and plasma albumin concentration than control animals (Table 4.7). However, the intravascular pool of albumin (CA) of infected animals was the same as that of control animals, and the TER_{alb} was higher in infected than in control animals. However, this difference was not significant.

DISCUSSION

Haematological values recorded in the present study indicate that sheep infected with *T. congolense* developed macrocytic normochromic anaemia which persisted up to the time of treatment at 84 DAI (Chapter 3). It is well recognised that anaemia is an important feature of animal trypanosomiasis and despite numerous investigations, several aspects of the aetiology of trypanosomal anaemia remain unclear. Studies of the causes of the anaemia have been largely conducted in laboratory animals (Jennings *et al.*, 1974) and cattle (Holmes, 1976; Murray, 1979). To date only a few studies have been conducted in sheep infected with *T. vivax* (Clarkson, 1968; Anosa and Isoun, 1976), and in these ferrokinetic, erythrokinetic and blood volumes were not measured at the same time in the same animals. The information provided by the present study, therefore, allows for the first time a direct assessment of these parameters in sheep chronically infected with *T. congolense*.

Red cell survival studies with ^{51}Cr-rbc revealed that infected animals had a significantly lower circulating red cell volume and a much shorter ^{51}Cr-rbc half life than control animals. This shows that infected animals were experiencing faster removal of erythrocytes from the circulation than control animals. This was confirmed by a finding of a much shorter calculated red blood cell lifespan, derived

Table 4.7

Albumin metabolism studies in sheep infected with *Trypanosoma congolense*, and in uninfected control sheep, 75 days after infection

Group	Sheep No.	^{125}I-albumin $t_{1/2}$ (days)	Plasma albumin (gl^{-1})	CA (gkg^{-1})	TER_{alb} (%/h)
	34	12.2	37	1.51	5.68
	36	13.7	32	1.45	5.06
Infected	79	14.1	29	1.28	4.91
	81	12.4	34	1.70	5.59
	84	14.5	32	1.44	4.78
	Mean±sem	**13.4 ± 0.5**	**32.8 ± 1.3**	**1.48 ± 0.07**	**5.20 ± 0.18**
	37	21.7	41	1.35	3.19
	80	18.9	36	1.40	3.67
Control	82	18.0	39	1.37	3.85
	83	17.9	38	1.44	3.87
	86	17.7	38	1.36	3.92
	Mean±sem	**18.8 ± 0.8**	**38.4 ± 0.8**	**1.38 ± 0.02**	**3.70 ± 0.30**
P	P	<0.001	<0.001	>0.05	< 0.01

from ^{59}Fe data, of infected animals (47.2 $\pm$ 11.8 days) compared to that of control ones (116.8 $\pm$ 13.8 days). The values recorded in control animals fall within the range (70 - 153 days) recorded by Tucker (1963) using ^{59}Fe in sheep of the same age as those used in the present study. Accelerated removal of erythrocytes from the circulation has also been recorded in cattle infected with *T. congolense* (Mamo and Holmes, 1975; Holmes, 1976; Dargie *et al.*, 1979a).

It is now generally accepted that anaemia during the acute stages of infection is haemolytic and largely extravascular (MacKenzie and Cruickshank, 1973; MacKenzie *et al.*, 1978; Murray and Dexter, 1988; ILRAD, 1990). MacKenzie and Cruickshank (1973) provided evidence of erythrocyte and leucocyte phagocytosis on a large scale throughout the reticulo-endothelial tissues in sheep infected with *T. congolense*. There is evidence that the mononuclear phagocytic system becomes very active during trypanosome infections (Murray, 1974; Anosa and Kaneko, 1989) and its continued destruction of erythrocytes is largely responsible for maintenance of anaemia, at least during the early stages of infection. There is, however, no widely accepted view concerning the exact mechanism(s) which predispose erythrocytes to phagocytosis. Many theories have been advanced and these include coating of red cells with antibody, damage of erythocytes by haemolytic factors produced by trypanosomes, fever, and disseminated intravascular coagulation (reviewed by Murray and Dexter, 1988). It has been observed that mature as well as immature red cells are phagocytosed by the active MPS in the bone marrow (ILRAD, 1990).

Recent studies in cattle infected with *T. vivax* have indicated that infected animals are stimulated to produce antibodies against normal red cells and platelets (Assoku and Gardiner, 1989) and this may contribute to persistent low PCV values that occur in cattle despite very low or undetectable levels of parasitaemia. What induces infected animals to produce autoantibodies is still unknown.

Infected animals in the present study had significantly higher plasma volumes and blood volumes, and lower circulating red cell volumes than control animals. There are few studies where blood volumes have been measured in sheep infected with trypanosomes, and those available had been conducted in sheep infected with *T. vivax*. Clarkson (1968) reported an increase of 16-18% in plasma volumes of sheep infected with *T. vivax* based on Evan's blue dilution. Using radioisotopic methods, Anosa and Isoun (1976) measured plasma volumes and red cell volumes in sheep infected with *T. vivax*. They recorded significant decreases in circulating red cell volumes and significant increases in plasma and total blood volumes. Increases in plasma volume of 24.5% and total blood volume of 9.5% recorded in the present study support their findings.

When the suitability of either ^{59}Fe or ^{125}I to measure plasma volumes was investigated, it was observed that both radioisotopes gave similar results in infected animals when the corrected counts extrapolated to time zero were used. However, when the counts at T_{10} were used to calculate the plasma volumes, it was evident the ^{59}Fe overestimates plasma volumes particularly of infected animals. In a comparable study, Dargie *et al.* (1979a) measured plasma volumes of cattle infected with *T. congolense*. However, they calculated plasma volumes based on radioactivity counts obtained 15 minutes after injection. Such a long time lag would definitely overestimate plasma volumes especially of infected animals, bearing in mind that the half-life of ^{59}Fe is significantly shorter, in infected than in control animals. However, such a defect created by a shortened half-life would be corrected by using counts extrapolated to T_0. Plasma volumes obtained by ^{125}I at T_0 and T_{10} were similar and this could be attributed to the fact that the rate of removal of ^{125}I-albumin is much slower than that of ^{59}Fe.

Increases in plasma volume have also been observed in cattle (Naylor, 1971a; Holmes, 1976; Maxie and Valli, 1979; Valli *et al.*, 1978; Dargie et al., 1979a, b) and in rabbits (Holmes, 1976) infected with *T. congolense*. All these studies reported marked decreases in circulating red cell volumes of infected animals. Naylor (1971a) observed that the plasma volume in cattle infected with *T. congolense* increased progressively from one week to six weeks post infection. The actual causes of an expansion in plasma volume have not been investigated and remain unknown. It has been suggested that it may be a response to a reduction in circulating red cell volume as an attempt to prevent circulatory collapse (Dargie *et al.*, 1979a; Mamo and Holmes, 1975) but such a response may be disproportionate leading to an increase in total blood volume (Naylor, 1971a). Clarkson (1968) has proposed that the increase in plasma volume may be attributed to a marked increase in the concentration of gamma globulins recorded in animal trypanosomiasis where they may act as plasma expanders by increasing the plasma colloid osmotic pressure. It is generally believed that globulins due to their large particle size are not highly osmotic, their role in the causation of haemodilution remains unresolved.

Some workers have suggested that high results for plasma volumes may be obtained in trypanosome infected animals because of increased vascular permeability associated with elevated kinins in tissues (Goodwin, 1976; Boreham, 1979; Zwart *et al.*, 1979). This suggestion arose from studies conducted in *T. brucei* infections in cattle and rabbits (van den Ingh, 1977; Boreham, 1968), and in *T. vivax* infections in goats (Veenendaal *et al.*, 1976). These authors reported a peak increase in blood kinin concentration coinciding with or immediately subsequent to the first major wave of parasitaemia. One of the effects of an increase in blood kinin concentration is an increase in capillary permeability, a feature that may explain the

development of oedema observed in *T. brucei* infection in rabbits (van den Ingh, 1977). However, there is no information on the changes in blood kinin levels, and no report of development of oedema in *T. congolense* infections.

The possibility that capillary permeability may be altered in *T. congolense* infection was investigated in the present study, by measuring the transcapillary escape rate of labelled albumin (TER_{alb}). The formula used to calculate the TER_{alb} is based on the half-life of ^{125}I-albumin. It is important to note that while increased capillary permeability may contribute to the shortened half-life of labelled albumin, other factors such as haemodilution may also play a part. The results showed that the TER_{alb} was elevated in infected animals, but there was no evidence that it accounted for any significant changes in the concentration of plasma albumin or the half-life of ^{125}I-albumin. The changes in these variables could be attributed to an increase in plasma volume and this suggestion was confirmed by the observation that the intravascular pool of albumin in infected animals was the same as that of control animals. These observations support the views of Clarkson (1968) and Holmes (1976) that the changes in plasma albumin concentrations and the shortened half-life of ^{125}I-albumin are mainly due to the dilution effect of an increased plasma volume. Holmes (1976) suggested that there was no excessive breakdown of albumin in infected animals . He based this suggestion on failure to demonstrate higher radioactivity of ^{125}I-albumin in urine of animals infected with *T. congolense* compared with the uninfected controls. Failure to record an increase in plasma urea in infected animals compared to their control counterparts, in the previous study, would also suggest that there was no excessive protein breakdown in infected animals.

Measurements of PITR have been made on several occasions during the course of trypanosome infections in cattle (Mamo and Holmes, 1975; Holmes, 1976; Dargie *et al.*, 1979a, b; Wellde *et al.*, 1989c) and it has been suggested that it is a

reliable indicator of erythropoietic activity (Bothwell *et al.*, 1957). Since not all the iron in plasma goes to the bone marrow for haemoglobin synthesis, measurement of the rate of iron incorporation into new red cells would appear to be a better indicator of erythropoietic activity than the PITR. Ferrokinetic studies indicated that infected sheep had a significantly higher PITR, iron incorporation rates and percentage utilisation of labelled iron than control animals. These findings agree with those of Mamo (1974) in Ethiopian cattle infected with *T. congolense* and they are consistent with enhanced erythropoiesis at this stage of infection. Ferrokinetic studies have not been conducted in sheep infected with trypanosomes before but similar results, as those reported here, have been recorded in cattle infected with *T. congolense* (Mamo, 1974; Mamo and Holmes, 1975; Holmes, 1976).

Despite recording two and three fold increases in PITR of infected N'Dama and Zebu cattle compared to their uninfected controls, Dargie *et al.* (1979a) suggested that this increase in erythropoietic activity was less than was expected from the haematocrit values of infected animals. They therefore suggested that dyshaemopoiesis played a part in the development of anaemia. They however based their suggestion on the previous report by Bush *et al.* (1956) that sheep and swine can increase their erythropoietic response four to six times during parasitic or chemically induced anaemia. In a study of sheep infected with *H. contortus*, Altaif and Dargie, (1978) also observed a six fold increase in PITR in animals with low PCV values (0.10-0.12 ll^{-1}) compared with those with normal PCV values (0.30-0.31 ll^{-1}). While this degree of response in sheep is known, there are no studies that have evaluated the maximum erythropoietic response in cattle subjected to different degrees of anaemia, and it may be erroneous to expect cattle to behave like sheep in this respect.

Convincing evidence of dyshaemopoiesis has been presented by Wellde *et al.* (1989c) in cattle infected with *T. congolense*. These authors observed that PITR of infected animals at 8 weeks post infection was three times greater than that of control animals, but it fell to less than that of control animals by 28 weeks after infection. Dyshaemopoiesis in chronic bovine trypanosomiasis has been attributed to reticulo-endothelial iron blockage resulting in impaired reutilisation of iron from degraded red cells (Dargie *et al.*, 1979a). The observation of excessive haemosiderosis in the spleen and lymph nodes of cattle infected with *T. congolense* (Fiennes, 1954) lends support to this hypothesis.

In the current study, the mean PITR and ^{59}Fe utilisation were two fold greater than those of control animals. Considering that the mean PCV of infected animals was 0.27 ll^{-1} compared to 0.33 ll^{-1} of control animals, ferrokinetic observations indicate that infected sheep were able to moderate the degree of anaemia by an increased erythropoietic response. In fact some infected animals (No 34 and 84) had attained near normal values by 75 DAI. These observations are in agreement with those of MacKenzie and Cruickshank (1973) that enhanced erythropoietic activity persists in chronic infections in sheep. However, further studies are needed to confirm this suggestion, in animals exposed to trypanosome infection for a long period of time.

In conclusion, the present study has shown that the anaemia in sheep chronically-infected with *T. congolense* is associated with accelerated red cell loss from the circulation and haemodilution. Ferrokinetic studies have confirmed that erythropoiesis is enhanced but does not fully compensate for the accelerated red cell destruction, hence anaemia persists even in the absence of, or presence of very few parasites in the circulation.

CHAPTER 5

COMPARATIVE SUSCEPTIBILITY OF SCOTTISH BLACKFACE AND FINN DORSET SHEEP TO EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CONGOLENSE*

INTRODUCTION

Increasing consideration is being given to the propagation of breeds of livestock that can withstand the effects of trypanosome infections in regions of Africa infested with tsetse flies. It has been observed in the field and confirmed under experimental conditions that certain breeds of cattle particularly the N'Dama and West African Shorthorn have an inherent degree of trypanotolerance or natural resistance to trypanosomiasis (Roberts and Gray, 1973; Murray, 1979; Williams *et al.*, 1991; Paling *et al.*, 1991a, b). This trait has been associated with the ability to control trypanosome numbers and resist the effects of disease, mainly anaemia (Murray *et al.*, 1982; Trail *et al.*, 1990, 1991a, b).

In contrast to the increasing amount of information that has become available on cattle, the status of sheep is much less clear. However, the fact that indigenous sheep throughout, West, Central and East Africa are apparently able to survive in tsetse infested areas without the aid of trypanocidal agents and show little or no signs of disease is considered an indication of their trypanotolerance nature. It is only recently that studies have been carried out to investigate susceptibility of different breeds of sheep to experimental and natural trypanosome challenge. In West Africa, the Djallonke sheep were found to be more resistant to experimental *T. congolense* infection than the Sahelian Fulani sheep, in that they sustained less intensity of parasitaemia and developed less severe anaemia (Toure *et al.*, 1983). In East Africa, Griffin and Allonby (1979d) observed that the indigenous sheep (Red Maasai and Blackhead Persian) were more resistant than imported breeds (Merino sheep) to syringe passaged *T. congolense* and also to field challenge. There are also reports that the Red Maasai are resistant to *Haemonchus contortus* infection (Preston and Allonby, 1978, 1979b). At the same time, studies in Scottish Blackface and Finn Dorset lambs infected experimentally with *H. contortus* revealed that the former developed less anaemia than the latter (Abbott *et al.*, 1985). As anaemia is a

common feature of both haemonchosis and trypanosomiasis in sheep and the ability to control the degree of anaemia is considered to be a reliable indicator of resistance to these diseases, part of the present study was undertaken to compare the responses of Scottish Blackface and Finn Dorset lambs to experimental infection with *T. congolense*.

Breed differences in lipid metabolism have only been reported in cattle. In the tropics, Zebu cattle (*Bos indicus*) were found to have higher plasma cholesterol, phospholipid, triglyceride and total lipid concentrations than *Bos taurus* breeds of cattle (O'Kelly, 1968, 1972, 1974, 1977). Similar results were reported by Traore-Leroux *et al.* (1987a) in their comparative studies of Zebu cattle and Baoule (West African Shorthorn) in West Africa. In addition, these authors observed that the Zebu cattle developed a higher intensity of parasitaemia and more severe anaemia following experimental challenge with *T. congolense*. It has been demonstrated that trypanosomes require HDL and LDL for their survival (Coppens *et al.*, 1987, Coppens *et al.*, 1988; Black and Vanderweed, 1989; Bastin *et al.*, 1991). HDL transports 90% of plasma cholesterol and 80% of phospholipids in cattle and sheep (Christie, 1981; Traore-Leroux *et al.*, 1987a). These lipoproteins by acting as vehicles for cholesterol and phospholipid transport favour increased survival and multiplication of trypanosomes. It is possible that animals with higher lipid concentrations therefore maintain higher intensities of parasitaemia which in turn lead to development of more severe anaemia than those with low lipid concentrations.

The present study was undertaken to compare the susceptibility of Scottish Blackface and Finn Dorset lambs to experimental infection with *T. congolense*, and to investigate whether a correlation exists between the blood lipid and protein levels, and intensity of parasitaemia and degree of anaemia in the two breeds of sheep.

MATERIALS AND METHODS

Experimental Animals

The animals used in this study were purchased from two local farms in the west of Scotland. The management of experimental animals before and during the experiment, housing and feeding were similar to those described in Chapter 3.

Parasitological, Haematological and Blood Biochemical Methods

These techniques have already been described (Chapter 2).

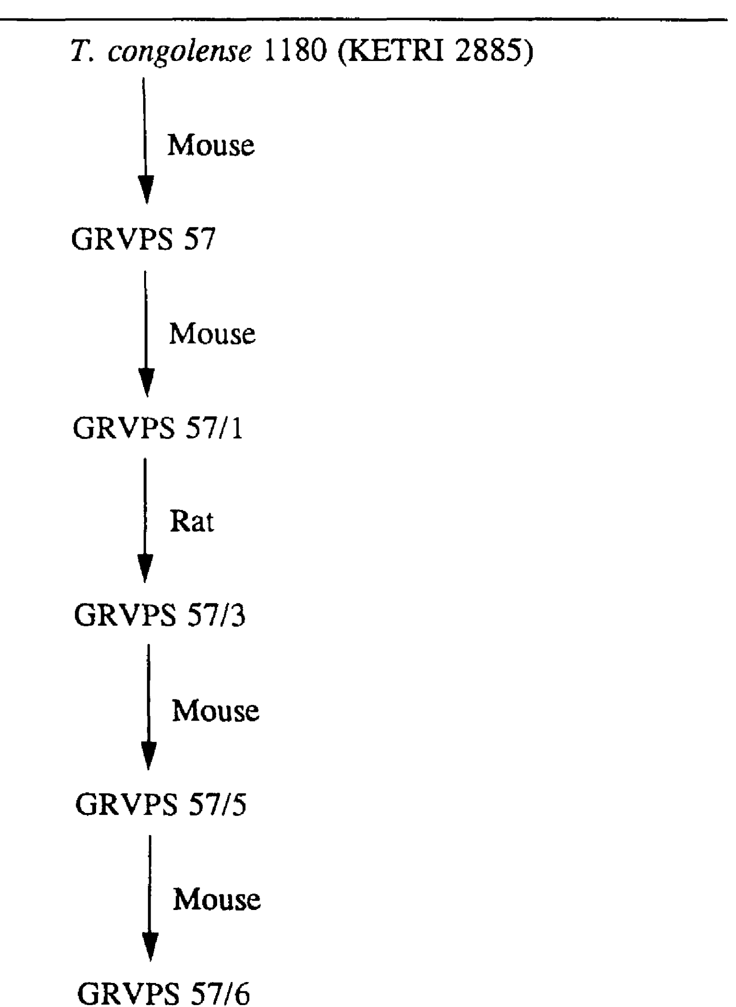
Trypanosomes

Trypanosoma congolense 1180 (GRVPS 57/6) was used to infect the sheep. This clone was derived from an isolate made in the Serengeti, Tanzania as described by Nantulya *et al.* (1984) and was maintained in liquid nitrogen. The history of GRVPS 57/6 is shown in Figure 5.1. The trypanosomes were harvested from irradiated mice on the first rising parasitaemia and the inoculum was prepared by diluting the pooled mice blood with phosphate buffered saline (containing 1.5% glucose)(pH 8.0) to give 1 x 10^5 trypanosomes in 3 ml of inoculum. Each lamb received 3ml of inoculum via the jugular vein.

Experimental Design

Ten Scottish Blackface (SB) and 10 Finn Dorset (FD) male castrate lambs, about one year old were divided into two groups depending on their body weights and PCV values. Five SB and five FD lambs were infected with *T. congolense* by the jugular route, while five animals of each breed acted as uninfected controls. At 56 DAI, infected animals were treated with diminazene aceturate (Berenil^R, Hoechst, West Germany) at a dose rate of 3.5 $mgkg^{-1}$ intramuscularly (I.M.).

Figure 5.1 The history of the *Trypanosoma congolense* clone 1180 (GRVPS 57/6)



Following a relapse of infection in some animals of both breeds, a repeat treatment at a dose rate of 7.0 $mgkg^{-1}$ was given at 67 DAI and animals were monitored for a further 17 days.

RESULTS

Parasitological Findings

Apart from one FD lamb showing detectable parasitaemia 5 DAI, the prepatent period in infected groups of animals was 9-10 days. Following patency of parasitaemia, the infected SB developed a significantly higher intensity of parasitaemia than infected FD lambs (Figure 5.2). The mean parasitaemia score in infected SB increased steadily to reach the first peak of 4 ± 0.7 by 17 DAI, whereas in infected FD, the first peak of 3.6 ± 1.5 was reached 33 DAI. Thereafter, the intensity of parasitaemia fluctuated a great deal but tended to be higher in infected SB than in FD lambs especially between 35 and 49 DAI. Following treatment at 56 DAI, the parasites could not be detected in jugular blood for 7 days after which a relapse occurred in two animals of each breed. When animals were given a second treatment at 67 DAI, relapse occurred 10 days later in one animal of each group. The uninfected control animals remained aparasitaemic throughout the period of observation.

Live Body Weight

All experimental animals appeared to maintain good appetites throughout the period of observation. The mean liveweight of infected SB lambs increased from 39.5 ± 2.5 kg at 0 DAI to 43.6 ± 2.4 kg at 54 DAI (Figure 5.3). Infected FD also gained weight from 36.3 ± 1.9 kg at 0 DAI to 40.9 ± 1.2 kg at 54 DAI. Treatments at 56 and 67 DAI did not lead to an improvement in body weights. Control SB lambs increased in liveweight from 40.1 ± 3.3 kg to 46.2 ± 2.0 kg, while control FD

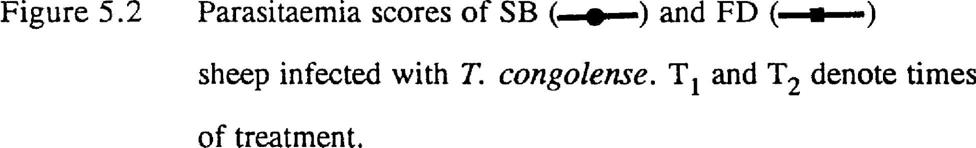
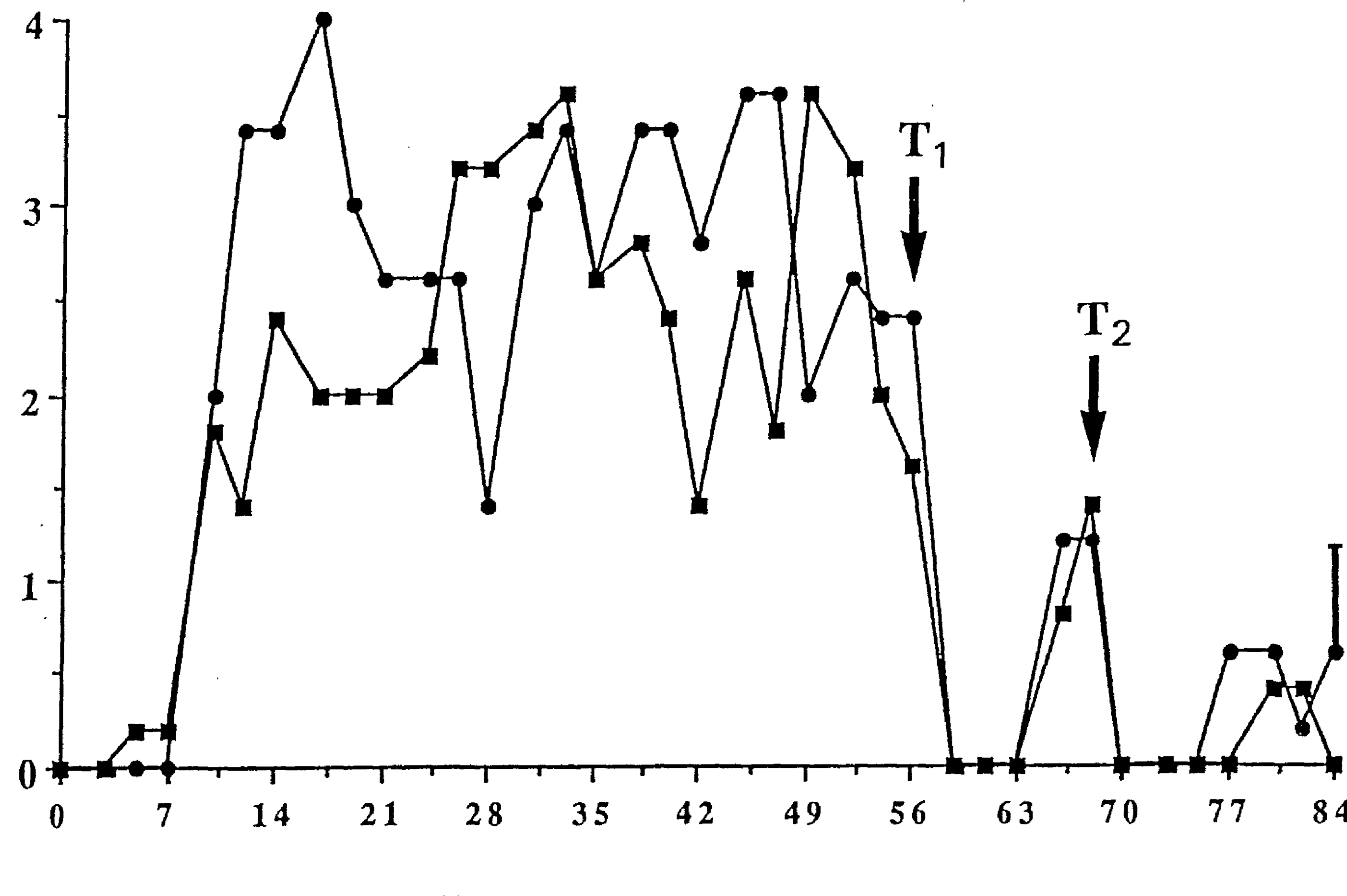


Figure 5.2 Parasitaemia scores of SB (—●—) and FD (—■—) sheep infected with *T. congolense*. T_1 and T_2 denote times of treatment.


Parasitaemia score
4
3
2
1
0
0
7
14
21
28
35
42
49
56
63
70
77
84
T1
T2
Time after infection (days)

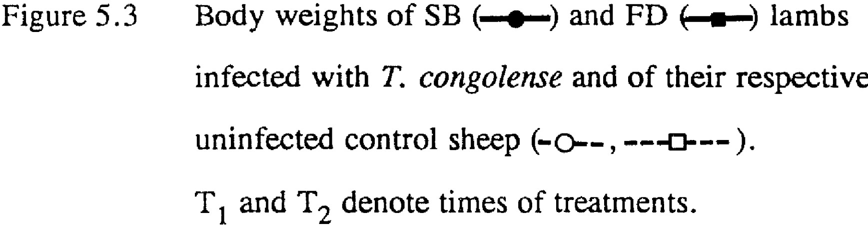
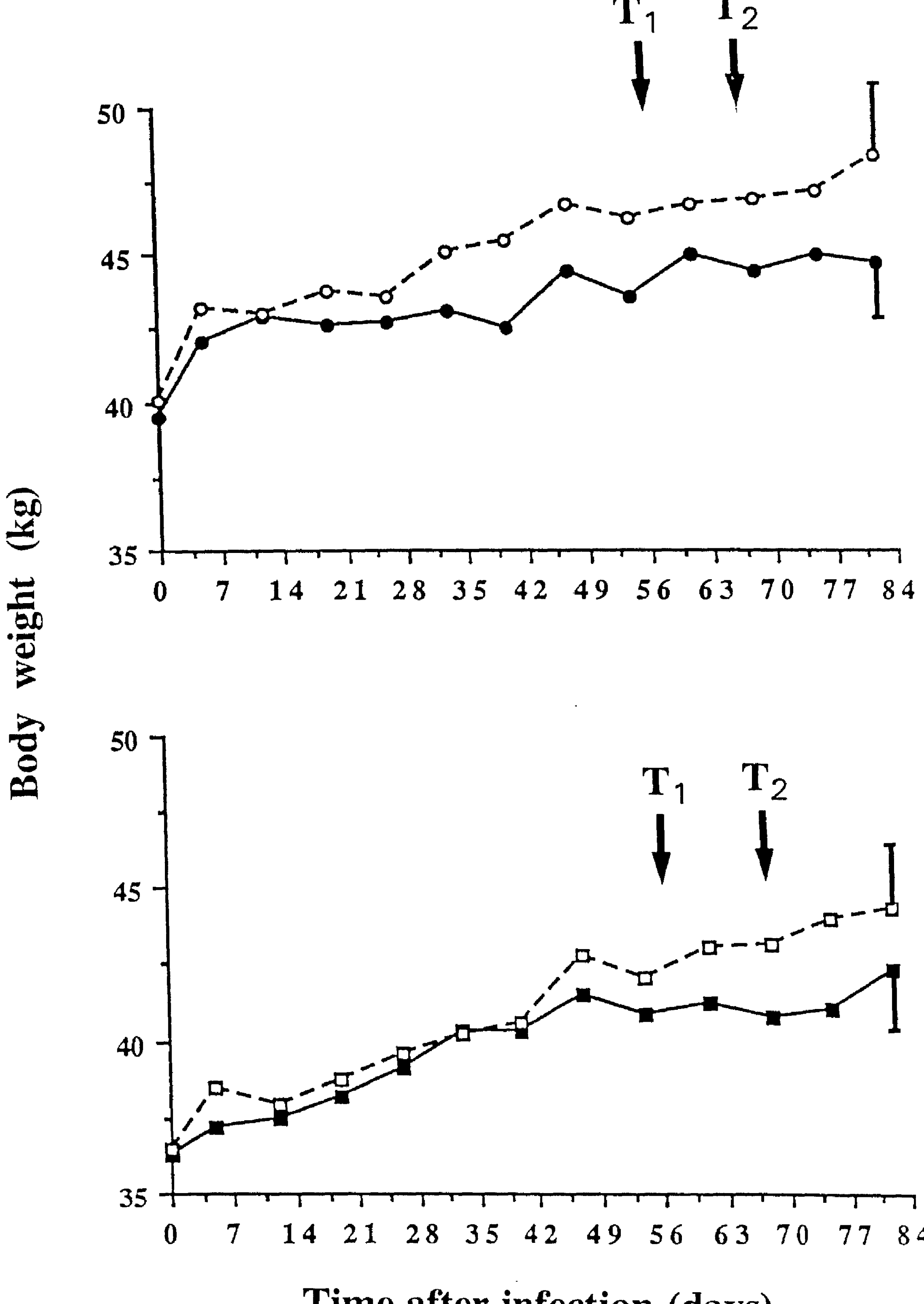


Figure 5.3 Body weights of SB (—●—) and FD (—■—) lambs infected with *T. congolense* and of their respective uninfected control sheep (-○--, ---□---). T_1 and T_2 denote times of treatments.


T1
T2
50
45
40
35
0 7 14 21 28 35 42 49 56 63 70 77 84
Body weight (kg)
T1
T2
50
45
40
35
0 7 14 21 28 35 42 49 56 63 70 77 84
Time after infection (days)

increased from 36.5 ± 2.2 kg to 42.8 ± 0.9 kg between 0 and 54 DAI. These differences in body weights between and within breeds did not reach a level of significance.

Body Temperature

Infected animals of both breeds developed significant increases in body temperature between 0 and 56 DAI. The mean rectal temperature of infected SB increased from 39.1 ± 0.2 ^{o}C at 0 DAI to 40.0 ± 0.1 ^{o}C by 5 DAI (Figure 5.4). In the infected FD, the mean temperatures increased from 39.4 ± 0.2 ^{o}C at 0 DAI to reach 40.0 ± 0.1 at 17 DAI, after which they fluctuated considerably but rose again to 40.4 ± 0.4 ^{o}C in infected SB and to 40.0 ± 0.3 ^{o}C in infected FD at 52 DAI. After treatment, the mean temperatures decreased to fluctuate between 39.2 ± 0.1 and 39.6 ± 0.2 ^{o}C. The mean body temperatures in control groups varied between 39.1 ± 0.1 and 39.7 ± 0.1 ^{o}C throughout the period of observation.

Haematological Findings

Packed cell volume (PCV).

Infection caused a significant decrease in mean PCV values of SB but not of FD lambs. The mean PCV values of infected SB decreased from 0.31 ± 0.01 ll^{-1} at 0 DAI to 0.23 ± 0.01 ll^{-1} at 26 DAI (Figure 5.5). The mean values then tended to increase but showed another decline to 0.23 ± 0.01 ll^{-1} at 45 DAI. In infected FD, the mean values decreased from 0.29 ± 0.01 at 0 DAI to 0.24 ± 0.01 ll^{-1} at 33 DAI after which they tended to recover. Following treatments at 56 and 67 DAI, the mean PCV values of both infected groups increased to near control values. In the control groups, the mean PCV values fluctuated between 0.29 ± 0.01 and 0.33 ± 0.01 ll^{-1}.

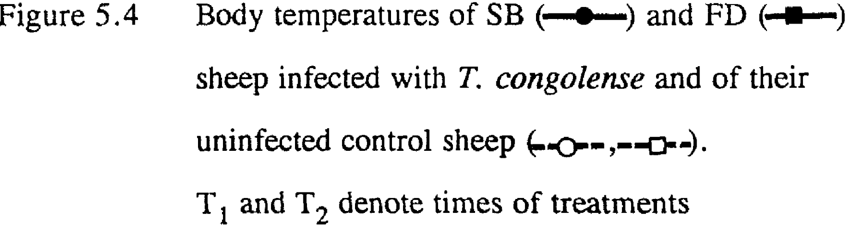
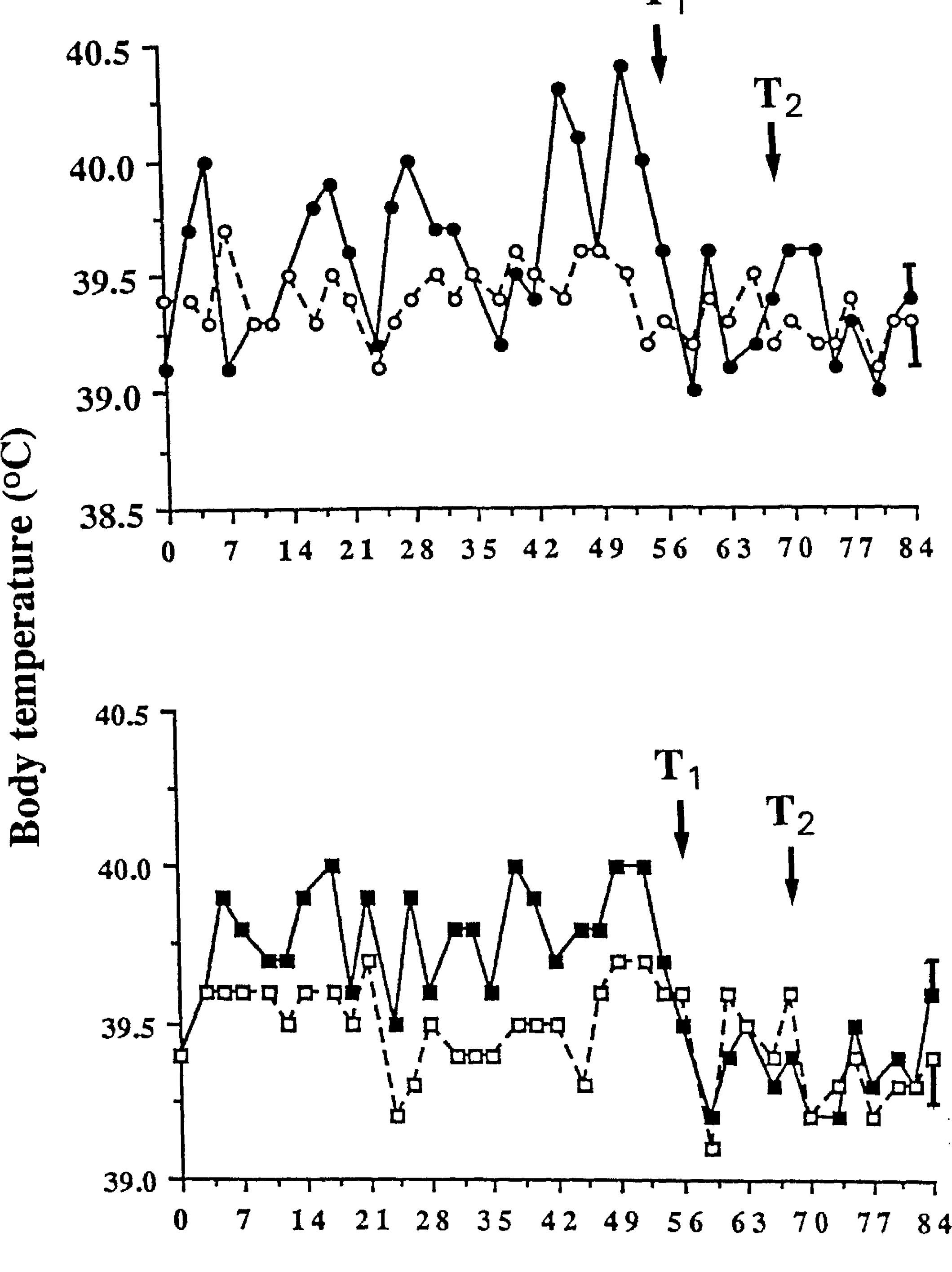


Figure 5.4 Body temperatures of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their uninfected control sheep (--○--, --□--). T_1 and T_2 denote times of treatments


T1
T2
40.5
40.0
39.5
39.0
38.5
0 7 14 21 28 35 42 49 56 63 70 77 84
Body temperature (oC)
T1
T2
40.5
40.0
39.5
39.0
0 7 14 21 28 35 42 49 56 63 70 77 84
Time after infection (days)

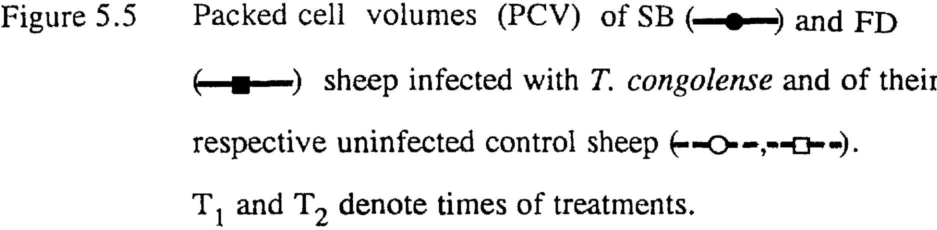
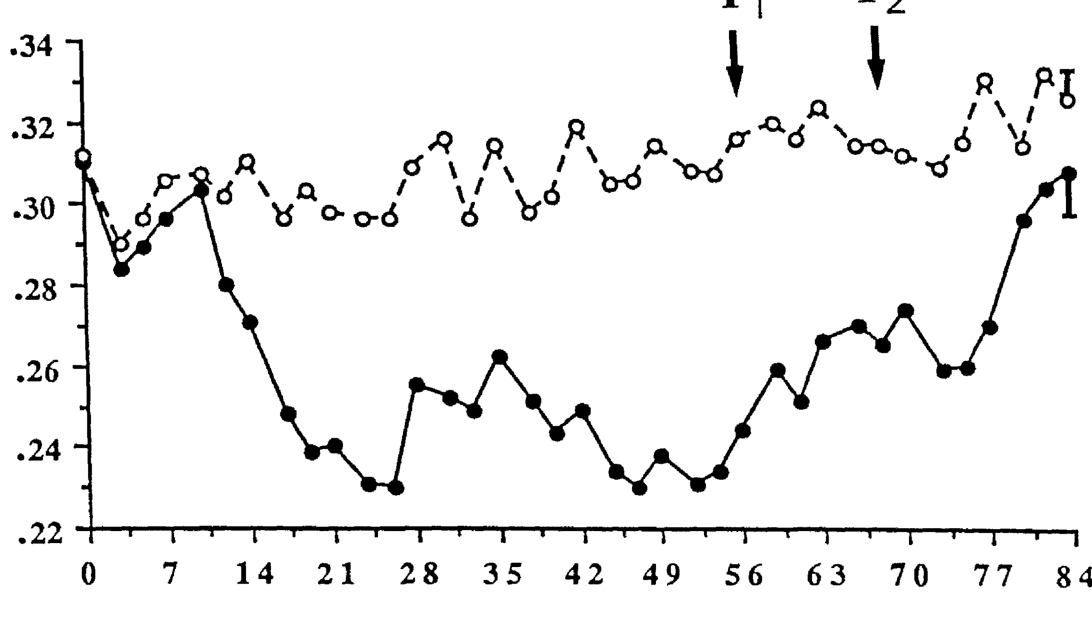
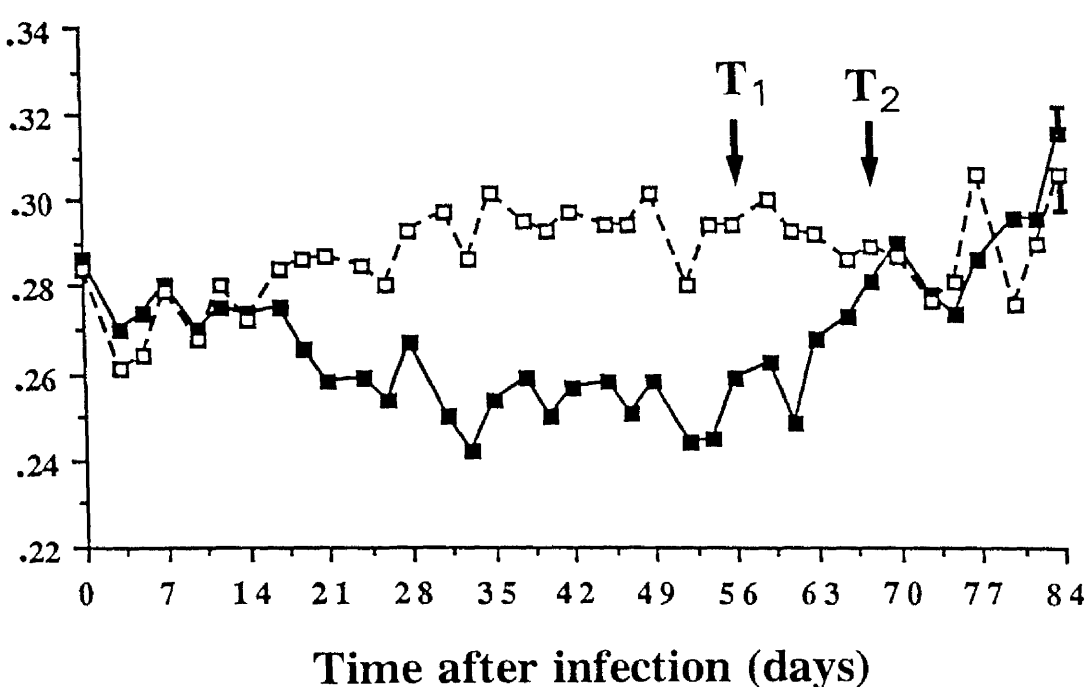


Figure 5.5 Packed cell volumes (PCV) of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (- -○- -, - -□- -). T_1 and T_2 denote times of treatments.



PCV ($l\,l^{-1}$)



Red blood cell count (RBC)

Infection caused an apparent decrease in mean RBC of both breeds. The mean RBC in infected SB decreased from $10.02 \pm 0.34 \times 10^{12}l^{-1}$ at 0 DAI to $7.42 \pm 0.27 \times 10^{12}l^{-1}$ at 49 DAI (Figure 5.6). In the infected FD, the mean values decreased from 9.60 ± 0.28 at 0 DAI to $8.08 \pm 0.53 \times 10^{12}l^{-1}$ at 56 DAI. However, these changes did not reach statistically significant values in either breed. Following treatments at 56 and 67 DAI, the mean RBC of infected animals increased to near those of control animals. Normoblasts were occasionally observed in thin smears from some SB lambs between 14 and 42 DAI. The mean RBC in control animals fluctuated between 9.19 ± 0.35 and $9.85 \pm 0.37 \times 10^{12}l^{-1}$.

Haemoglobin (Hb) concentration

There was a significant decrease in Hb concentration of SB but not of FD lambs. The mean Hb concentration of infected SB decreased from 10.3 ± 0.3 at 0 DAI to 8.1 ± 0.4 gdl^{-1} at 21 DAI after which it tended to increase but fell again to 8.3 ± 0.3 gdl^{-1} at 49 DAI (Figure 5.7). In the infected FD, the mean Hb concentration decreased from 9.9 ± 0.3 at 0 DAI to 8.9 ± 0.4 gdl^{-1} at 35 DAI after which it tended to increase. Following treatment, the mean values increased, the increase being more marked in infected SB than in FD lambs. The mean Hb values of control animals varied between 9.4 ± 0.5 and 11.4 ± 0.8 gdl^{-1}.

Mean corpuscular volume (MCV)

The mean MCV values of infected animals showed a moderate, non-significant increase compared to the values in uninfected controls. In infected SB, it increased from 31.2 ± 0.6 fl at 0 DAI to 34.4 ± 0.5 fl at 49 DAI. During the same period of time, the values of infected FD increased from 30.4 ± 0.5 to 34.6 ± 1.3 fl.

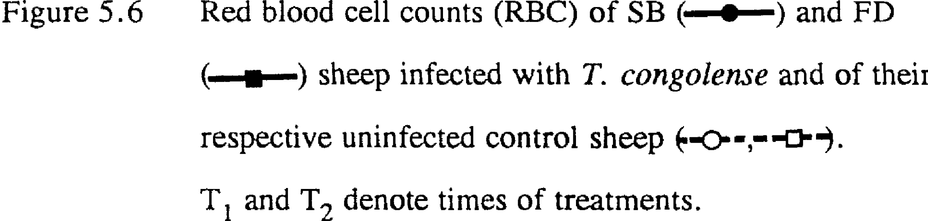
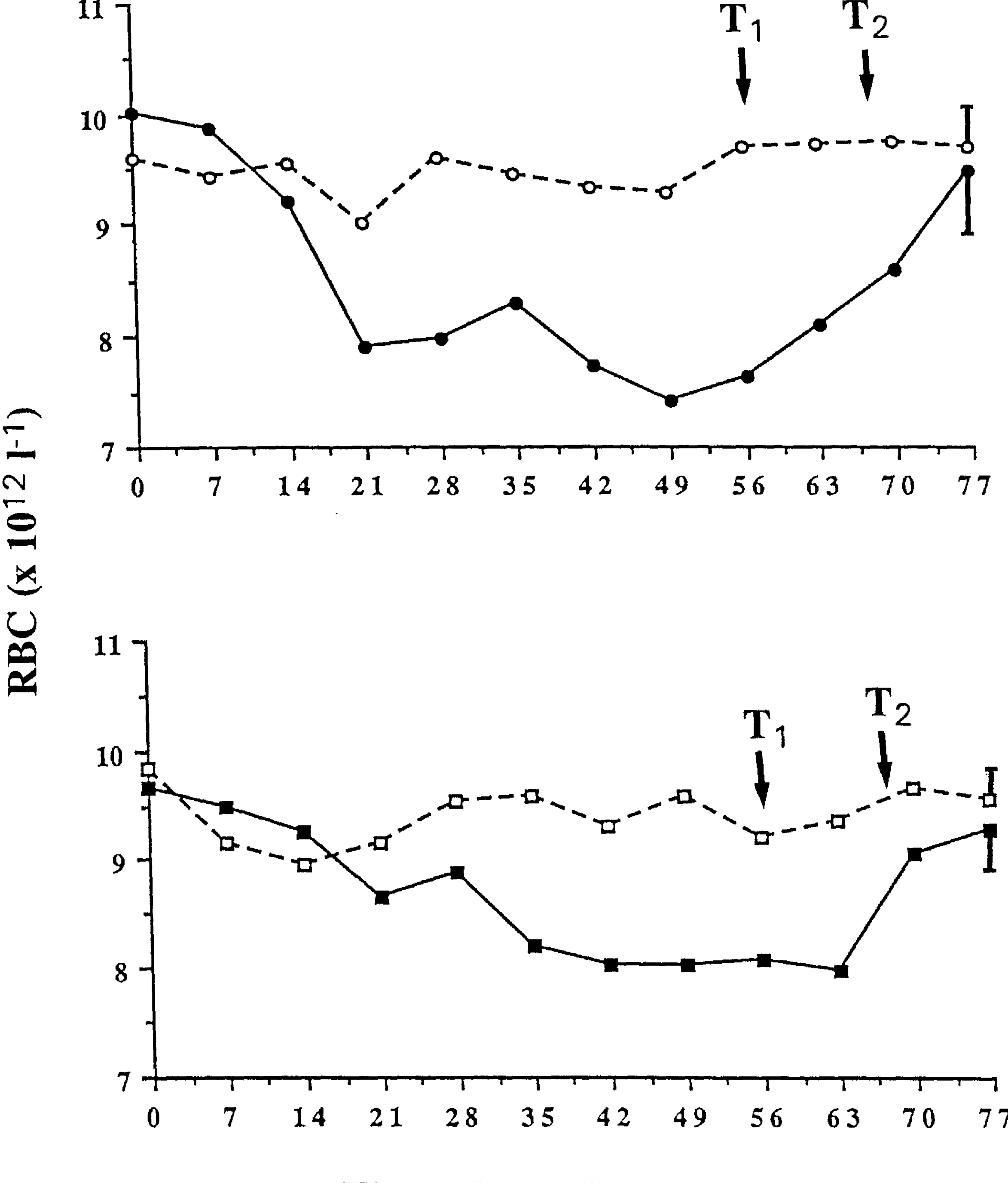


Figure 5.6 Red blood cell counts (RBC) of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (--○--,--□--). T_1 and T_2 denote times of treatments.



RBC (x 10^12 l^-1)
T1
T2
11
10
9
8
7
0
7
14
21
28
35
42
49
56
63
70
77
Time after infection (days)

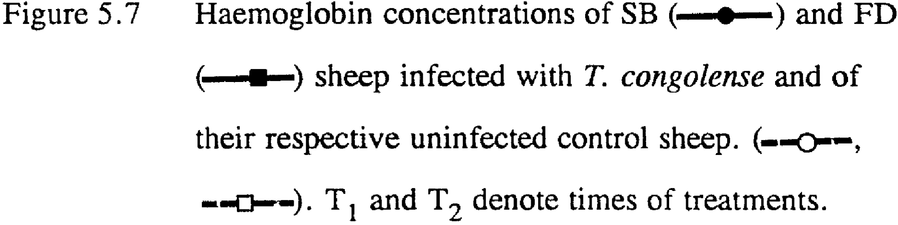
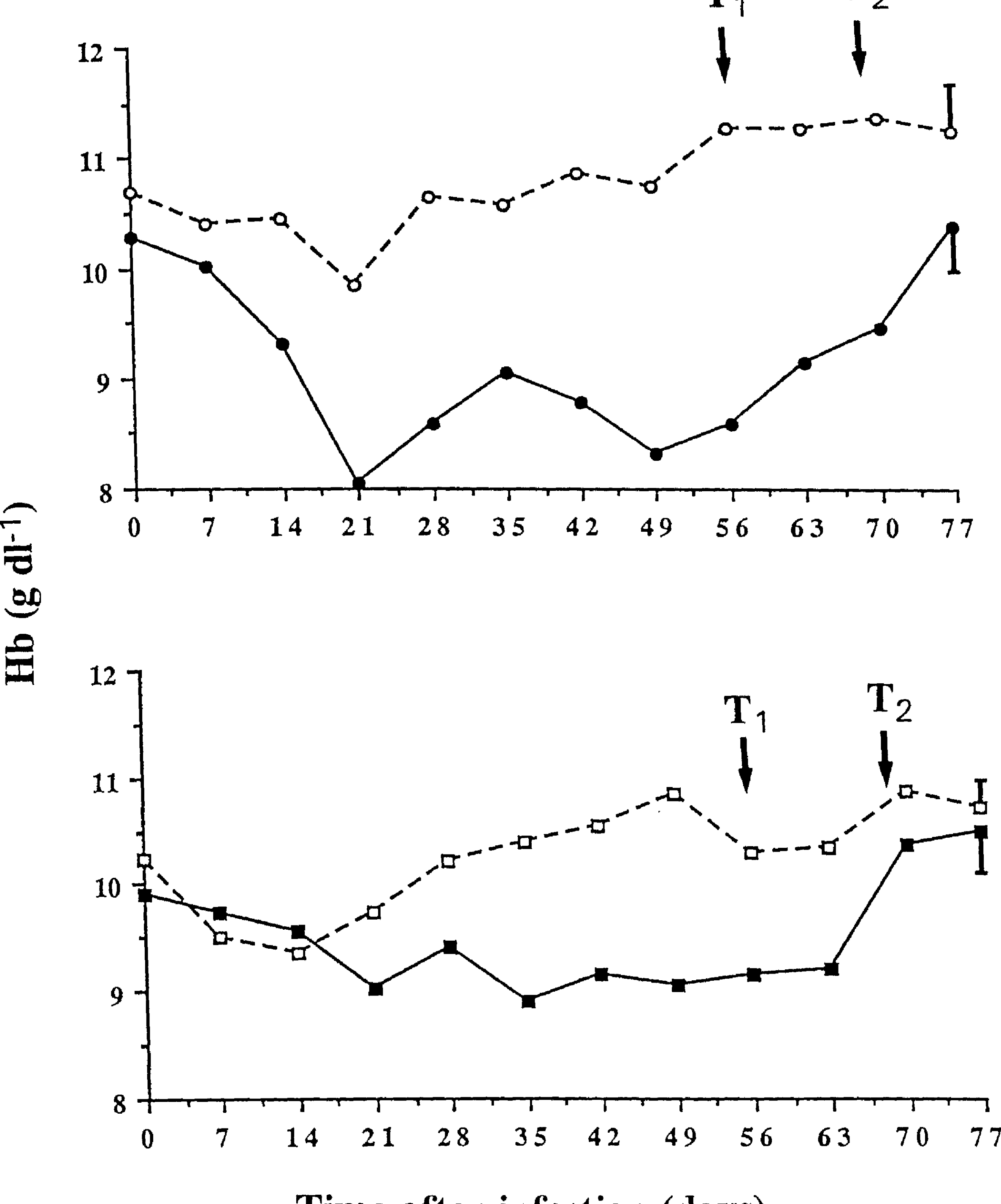


Figure 5.7 Haemoglobin concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep. (--○--, --□--). T_1 and T_2 denote times of treatments.



T1
T2
12
11
10
9
8
0
7
14
21
28
35
42
49
56
63
70
77
Hb (g dl-1)
Time after infection (days)

The values continued to increase after initial treatment at 56 DAI but started to decline after the second treatment at 67 DAI. The mean values in control animals fluctuated between 30.8 $\pm$ 0.4 and 35.0 $\pm$ 0.8 fl.

Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

The mean values of MCH and MCHC of infected animals tended to be lower than those of control animals in each breed, however, the differences were not significant.

Total white cell count (WBC)

In infected SB, the mean WBC showed an initial decrease from 8.1 $\pm$ 0.5 at 0 DAI to 6.5 $\pm$ 0.6 x $10^9 l^{-1}$ at 28 DAI after which it tended to increase (Figure 5.8). In infected FD, the mean WBC increased from 10.2 $\pm$ 0.9 at 0 DAI to 14.3 $\pm$ 1.3 x $10^9 l^{-1}$ at 42 DAI after which the values tended to decrease. Treatment at 67 DAI caused a downward trend in the WBC values of infected animals. In control animals, the mean values fluctuated between 7.2 $\pm$ 0.8 and 9.8 $\pm$ 0.7 x $10^9 l^{-1}$.

Lymphocyte count

The absolute lymphocyte counts in infected SB decreased from 6.0 $\pm$ 0.3 at 0 DAI to 5.4 $\pm$ 0.9 x $10^9 l^{-1}$ at 28 DAI (Figure 5.9). In infected FD, the mean values increased from 7.2 $\pm$ 0.7 at 0 DAI to 11.5 $\pm$ 1.6 x $10^9 l^{-1}$ at 42 DAI. Treatment did not have a significant effect on lymphocyte counts. The mean lymphocyte counts in control animals ranged from 5.2 $\pm$ 0.6 to 6.7 $\pm$ 0.9 x $109 l^{-1}$.

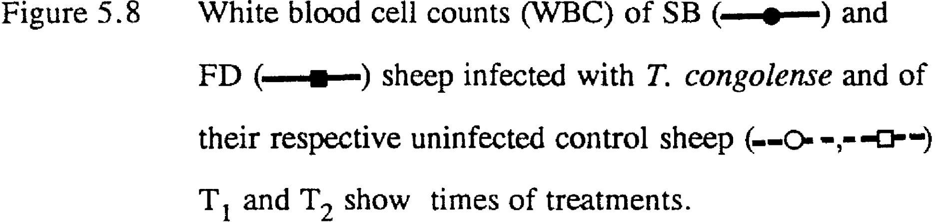
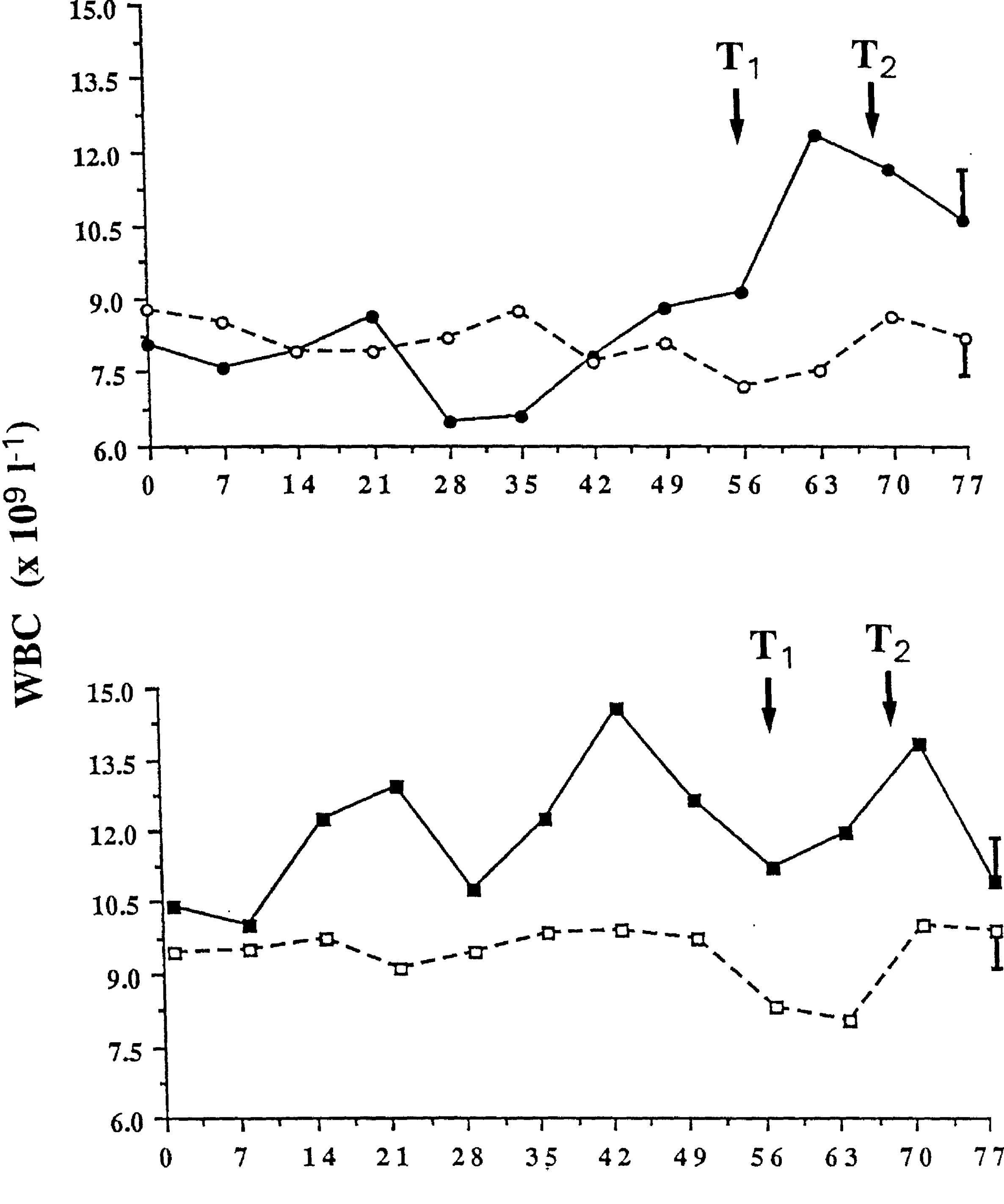


Figure 5.8 White blood cell counts (WBC) of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (--○--,--□--). T_1 and T_2 show times of treatments.



15.0
13.5
12.0
10.5
9.0
7.5
6.0
T1
T2
0
7
14
21
28
35
42
49
56
63
70
77
WBC (x 10⁹ l⁻¹)
15.0
13.5
12.0
10.5
9.0
7.5
6.0
T1
T2
0
7
14
21
28
35
42
49
56
63
70
77
Time after infection (days)

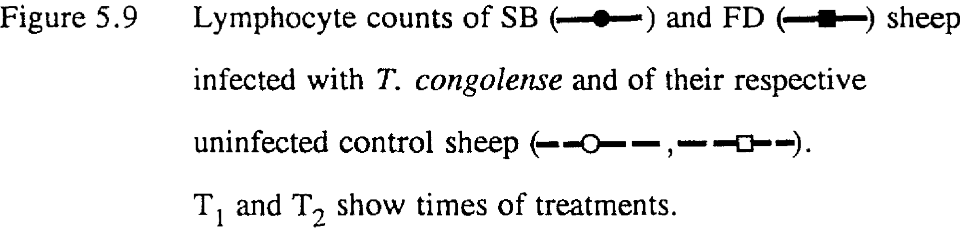
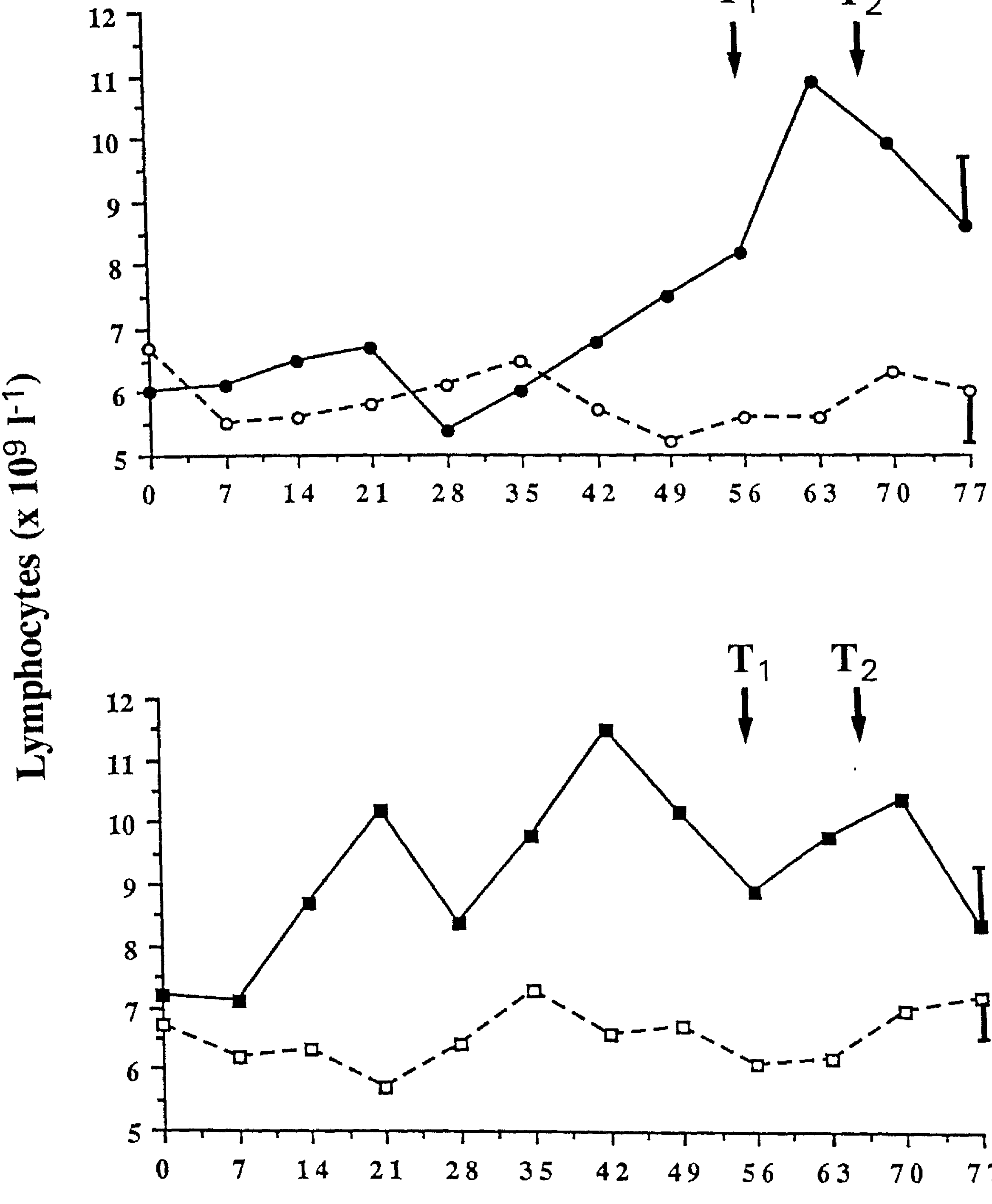


Figure 5.9 Lymphocyte counts of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (– –○– –, – –□– –). T_1 and T_2 show times of treatments.



T1
T2
12
11
10
9
8
7
6
5
0
7
14
21
28
35
42
49
56
63
70
77
Lymphocytes (x 10⁹ l⁻¹)
T1
T2
12
11
10
9
8
7
6
5
0
7
14
21
28
35
42
49
56
63
70
77
Time after infection (days)

Neutrophil count

In infected FD, the absolute neutrophil count increased from 2.70 $\pm$ 0.40 at 0 DAI to 3.06 $\pm$ 0.59 x 10^9l^{-1} at 14 DAI after which they showed a fluctuating decrease to 1.78 $\pm$ 0.31 x 10^9l^{-1} at 56 DAI. In infected SB, the mean neutrophil counts decreased from 1.79 $\pm$ 0.17 at 0 DAI to 0.61 $\pm$ 0.15 x 10^9l^{-1} at 35 DAI after which they tended to increase, but fell again to 0.69 $\pm$ 0.23 x 10^9l^{-1} at 56 DAI. (Figure 5.10). Treatment caused a marked increase in the mean values of SB lambs but had no effect on FD values. In control animals, the mean neutrophil count fluctuated between 1.18 $\pm$ 0.12 and 2.89 $\pm$ 0.62 x 10^9l^{-1}.

Monocyte count

The mean absolute monocyte counts fluctuated considerably in both infected and control groups of animals, however, the values tended to be higher in infected than in control animals. Treatment did not affect the trends of monocyte counts.

Platelet count

The platelet counts underwent a significant decrease in infected groups of both breeds. The mean counts in infected SB decreased from 230 $\pm$ 33 at 0 DAI to 68 $\pm$ 29 x 10^9l^{-1} at 35 DAI after which they tended to increase (Figure 5.11). In infected FD the mean values decreased from 169 $\pm$ 15 at 0 DAI to 88 $\pm$ 37 x 10^9l^{-1} at 35 DAI. Following treatment and subsequent reduction in levels of parasitaemia, the mean counts increased but decreased again with relapse of infection in both groups of infected sheep. The mean counts in control sheep fluctuated between 177 $\pm$ 24 and 289 $\pm$ 34 x 10^9l^{-1} throughout the experimental period, and there were no significant differences between breeds.

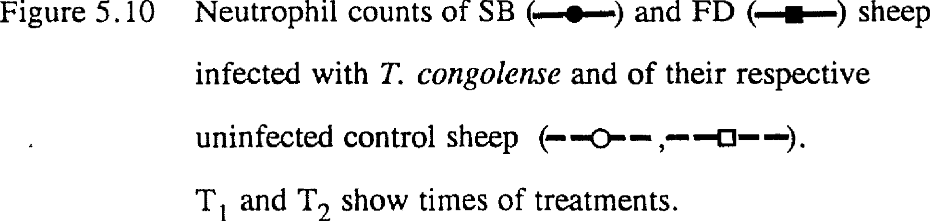
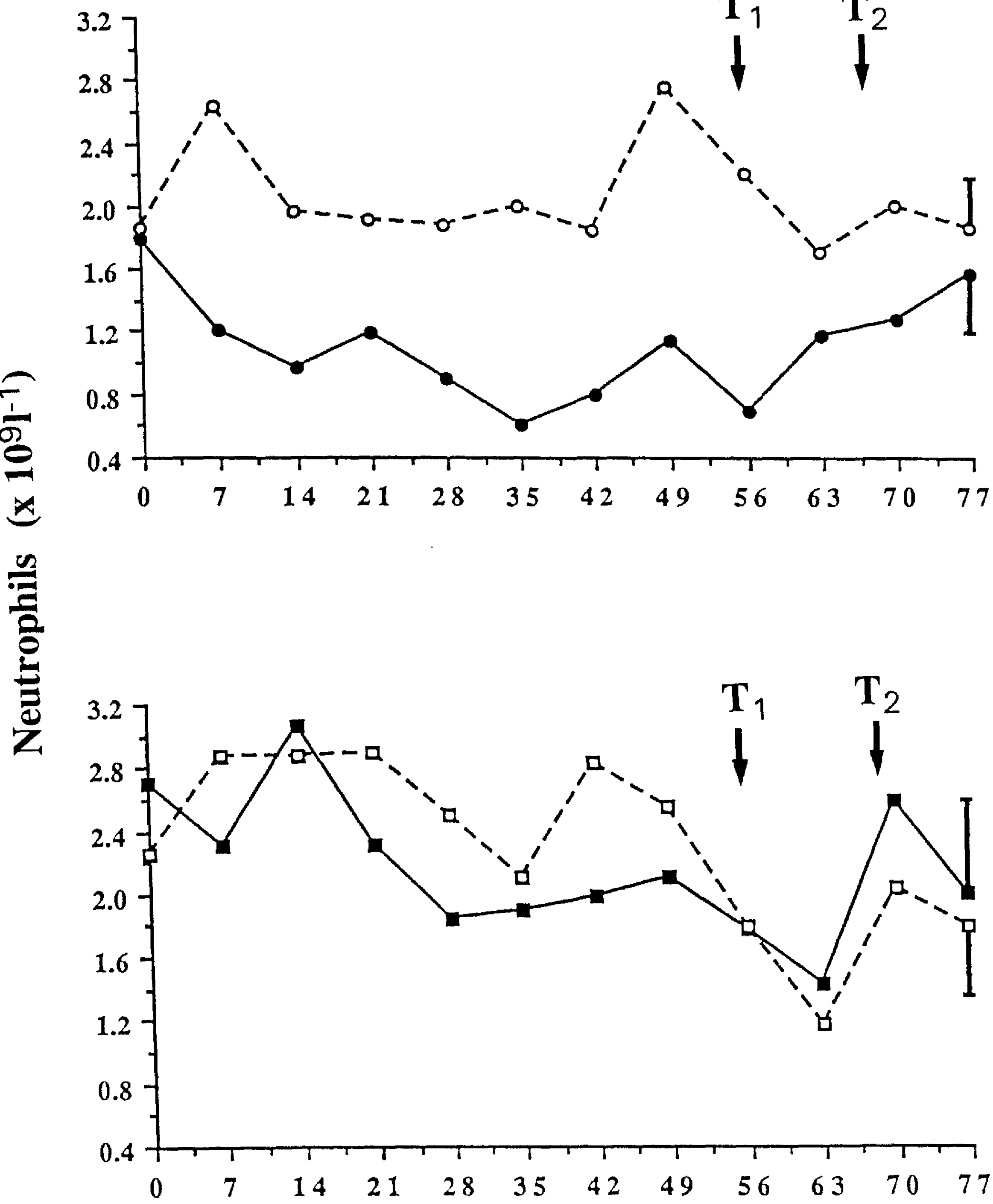


Figure 5.10 Neutrophil counts of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (– –○– –, – –□– –).
T_1 and T_2 show times of treatments.


T1
T2
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
7
14
21
28
35
42
49
56
63
70
77
Neutrophils (x 10⁹l⁻¹)
Time after infection (days)

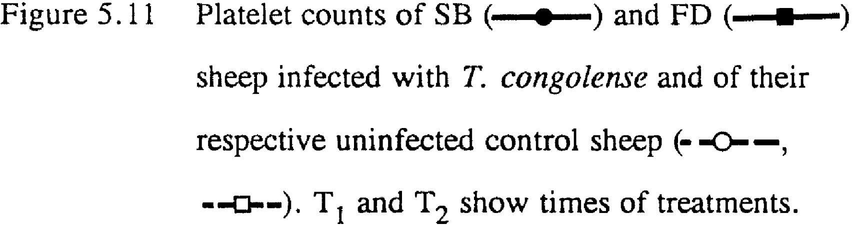
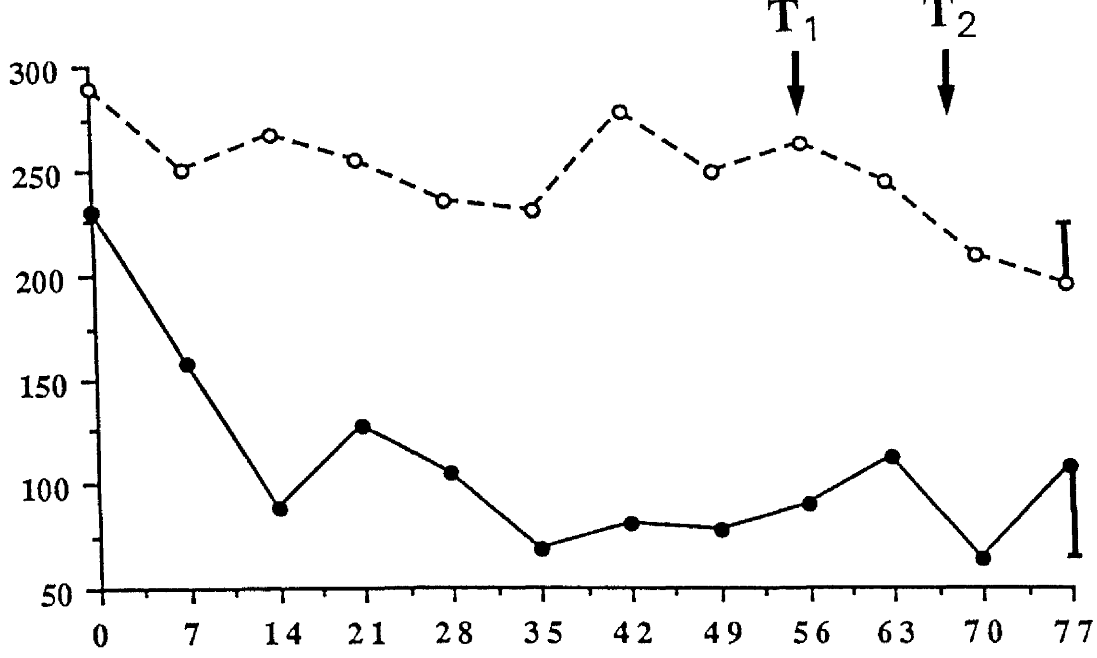
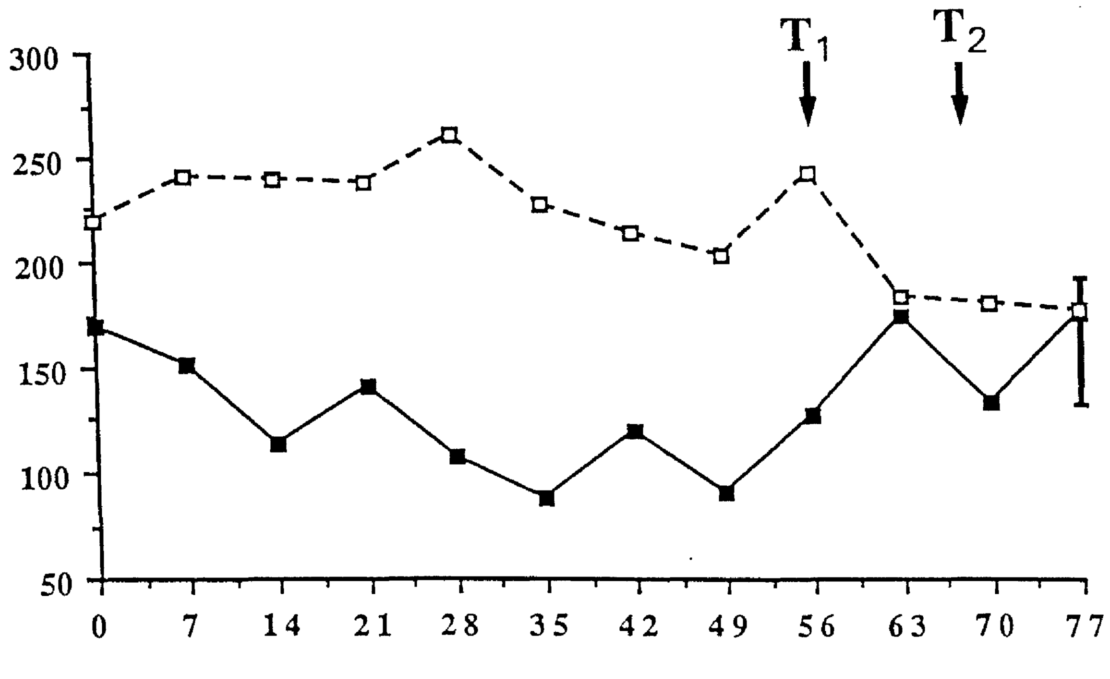


Figure 5.11 Platelet counts of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (- -○- -, - -□- -). T_1 and T_2 show times of treatments.


T1
T2
300
250
200
150
100
50
0
7
14
21
28
35
42
49
56
63
70
77


T1
T2
300
250
200
150
100
50
0
7
14
21
28
35
42
49
56
63
70
77
Platelets (x 10^9 l^-1)
Time after infection (days)

Blood Biochemical Observations

Serum total lipid concentration

Infection caused a significant decrease in serum total lipid concentration of SB but not of FD lambs. In the SB, the mean total lipid concentrations decreased from 2.58 $\pm$ 0.27 gl^{-1} at 0 DAI to 1.45 $\pm$ 0.16 gl^{-1} at 38 DAI (Figure 5.12). Thereafter, the mean values increased moderately. In infected FD, the mean values declined from 2.10 $\pm$ 0.21 gl^{-1} at 0 DAI to 1.62 $\pm$ 0.14 gl^{-1} at 38 DAI after which the values also tended to increase. The values continued to increase after initial treatment at 56 DAI. In control groups, the mean total lipid concentrations varied from 1.86 $\pm$ 0.05 to 2.65 $\pm$ 0.14 gl^{-1}.

Serum phospholipid concentration

The mean concentrations of serum phospholipids showed consistent and significant decreases in both groups of infected animals between 0 and 38 DAI. In the infected SB, the mean phospholipid concentration decreased from 1.02 $\pm$ 0.08 to 0.66 $\pm$ 0.05 gl^{-1} while in infected FD, the mean values decreased from 0.96 $\pm$ 0.05 to 0.59 $\pm$ 0.05 gl^{-1} between 0 and 38 DAI (Figure 5.13). After initial treatment, the mean phospholipid concentrations of infected animals increased markedly but decreased again with relapse of infection. These changes were more marked in infected SB than in FD lambs. In control animals, the mean values fluctuated between 0.94 $\pm$ 0.06 and 1.18 $\pm$ 0.08 gl^{-1}.

Plasma cholesterol concentration

The initial plasma cholesterol concentration in the infected SB (2.33 $\pm$ 0.18 $mmoll^{-1}$) was significantly higher than that of infected FD (1.70 $\pm$ 0.17 $mmoll^{-1}$). Following infection, plasma cholesterol concentrations decreased but did not reach significance. The mean values decreased in infected SB from 2.33 $\pm$ 0.18 $mmoll^{-1}$ at

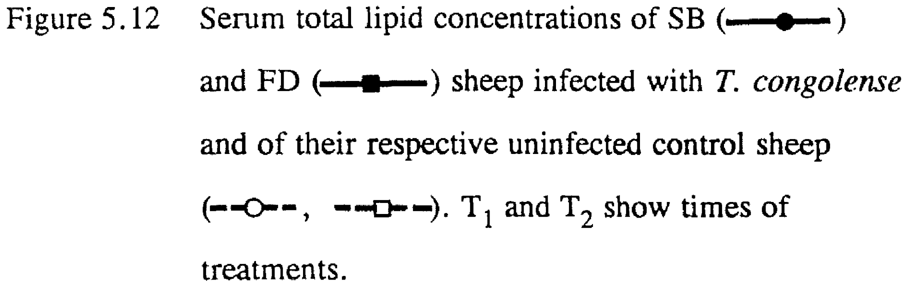
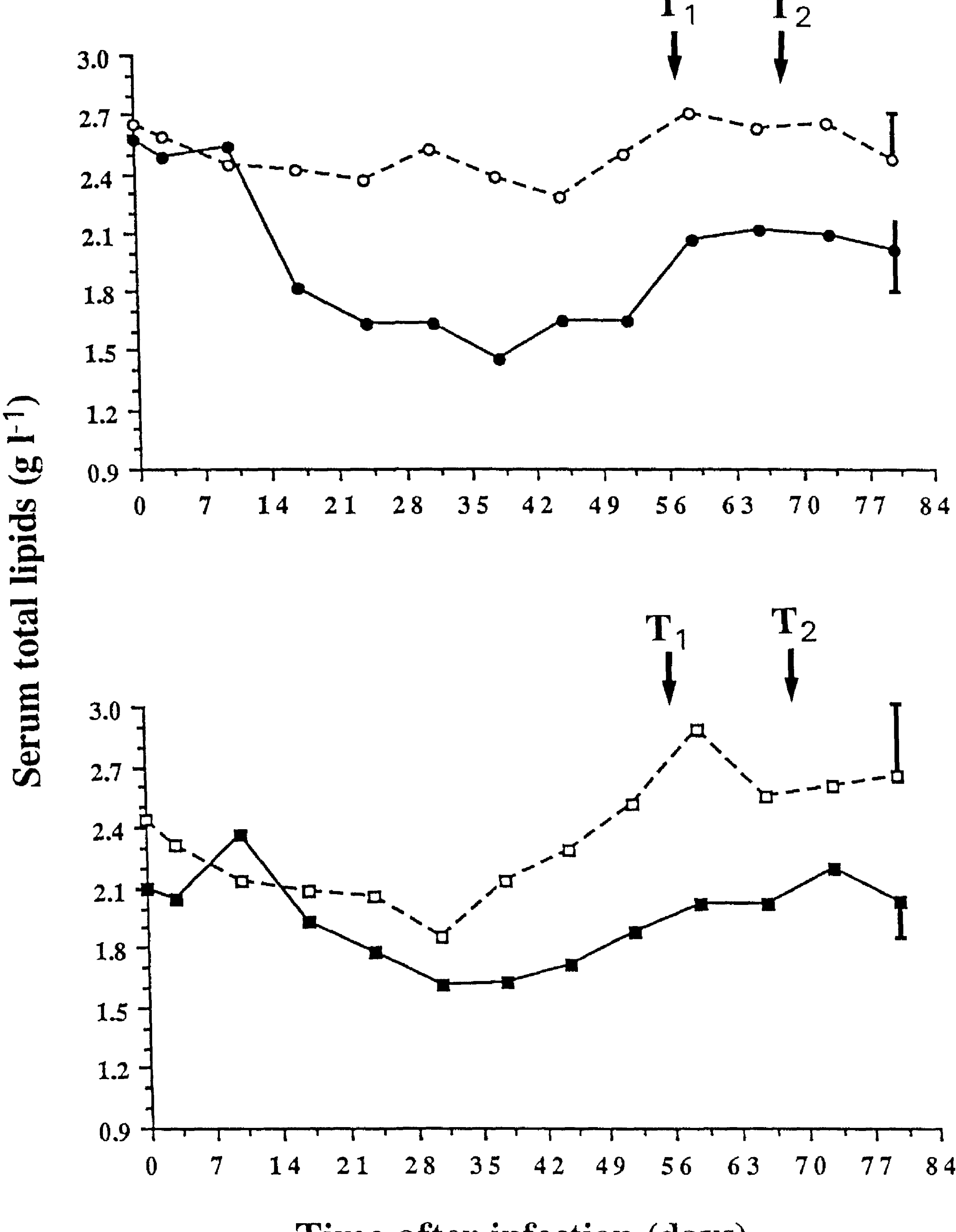


Figure 5.12 Serum total lipid concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (--○--, --□--). T_1 and T_2 show times of treatments.


T1
T2
3.0
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0 7 14 21 28 35 42 49 56 63 70 77 84
Serum total lipids (g l-1)
T1
T2
3.0
2.7
2.4
2.1
1.8
1.5
1.2
0.9
0 7 14 21 28 35 42 49 56 63 70 77 84
Time after infection (days)

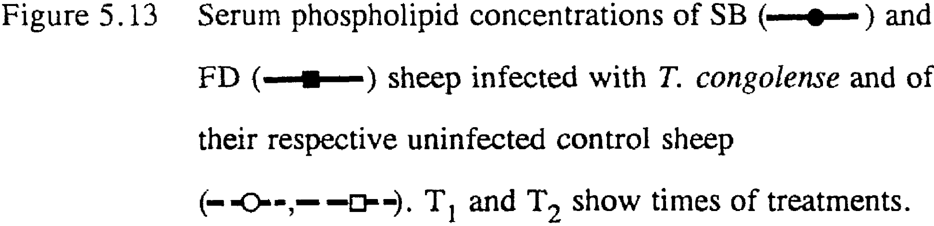
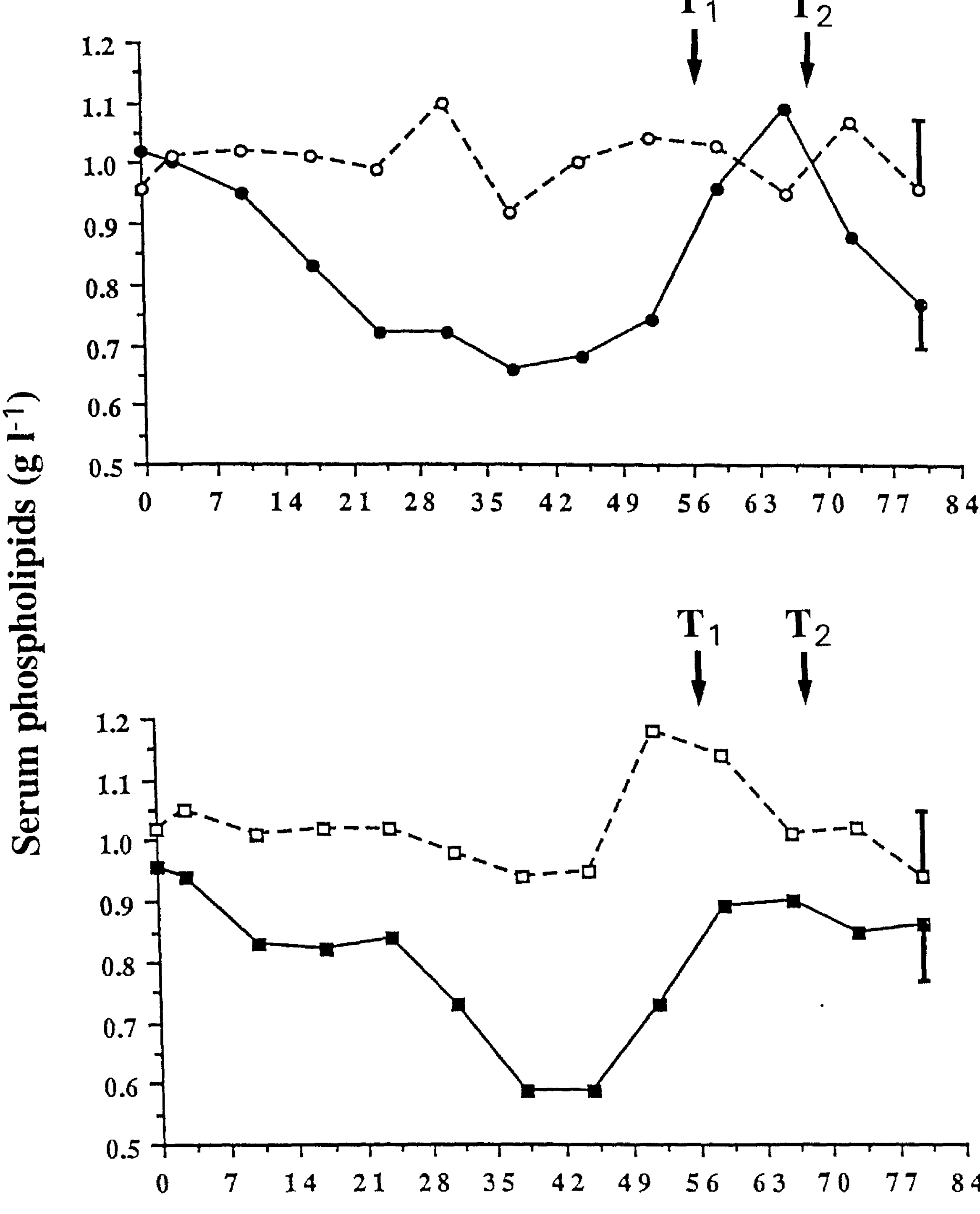


Figure 5.13 Serum phospholipid concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (- -○- -, - -□- -). T_1 and T_2 show times of treatments.


T1
T2
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0
7
14
21
28
35
42
49
56
63
70
77
84
Serum phospholipids (g l-1)
Time after infection (days)

0 DAI to 1.06 $\pm$ 0.16 $mmoll^{-1}$ at 33 DAI after which they showed a moderate increase (Figure 5.14). In infected FD, the mean cholesterol concentrations decreased from 1.70 $\pm$ 0.17 at 0 DAI to 1.06 $\pm$ 0.12 $mmoll^{-1}$ at 47 DAI. Following initial treatment, the mean values in infected animals increased but showed a downward trend with relapse of infection. The mean plasma cholesterol concentration in control animals fluctuated between 1.40 $\pm$ 0.13 and 2.02 $\pm$ 0.18 $mmoll^{-1}$, with no significant between breed differences.

Plasma triglycerides and non-esterified fatty acids (NEFA)

The mean concentrations of plasma triglycerides and NEFA fluctuated considerably in both infected and control animals of both breeds. However, the mean concentrations tended to be higher in infected than in control animals. These differences were not significant and treatment did not influence their plasma concentrations.

Plasma total protein (TP) concentration

Infection had no significant effect on the concentrations of plasma total protein in both breeds of sheep. In infected SB, the mean TP showed an initial moderate decrease from 68.0 $\pm$ 1.1 gl^{-1} to 65.8 $\pm$ 1.0 gl^{-1} between 0 and 20 DAI. Thereafter, the concentrations increased and were higher than in control SB between 33 and 54 DAI. The mean TP concentrations in infected FD decreased progressively from 68.6 $\pm$ 0.7 gl^{-1} at 0 DAI to 63.2 $\pm$ 2.2 gl^{-1} at 40 DAI after which the values tended to increase. Treatments tended to cause a moderate increase in TP concentrations of infected animals. The mean TP values in control animals fluctuated between 63.0 $\pm$ 1.2 and 67.8 $\pm$ 1.2 gl^{-1}.

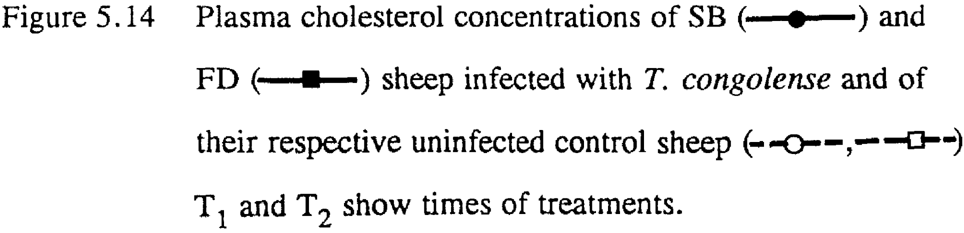
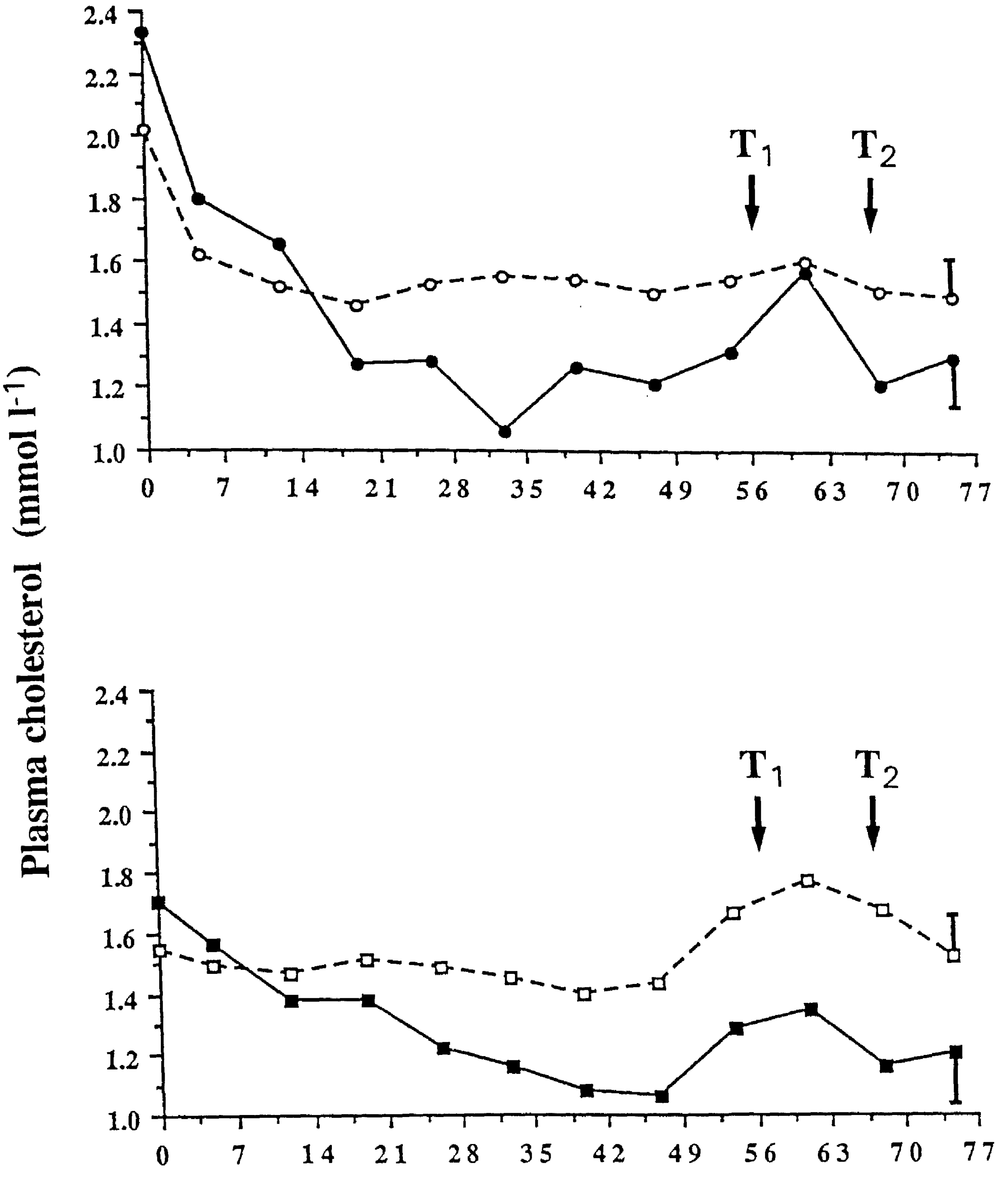


Figure 5.14 Plasma cholesterol concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (- -○- -, - -□- -). T_1 and T_2 show times of treatments.



Plasma cholesterol (mmol l-1)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
T1
T2
0
7
14
21
28
35
42
49
56
63
70
77
Time after infection (days)

Plasma albumin concentration

Infection caused a significant decrease in plasma albumin concentration of infected SB and not FD lambs. In infected SB, the mean values decreased from 30.8 $\pm$ 0.4 at 0 DAI to 26.1 $\pm$ 0.8 gl^{-1} at 47 DAI and tended to increase thereafter (Figure 5.15). In the infected FD, they decreased from 32.8 $\pm$ 0.4 at 0 DAI to 29.0 $\pm$ 0.5 gl^{-1} at 40 DAI. Treatment tended to cause an increase in plasma albumin concentrations of SB but had little effect on those of FD lambs. The mean albumin concentrations in control animals fluctuated between 31.2 $\pm$ 0.5 and 33.8 $\pm$ 0.4 gl^{-1}.

Plasma globulin concentration

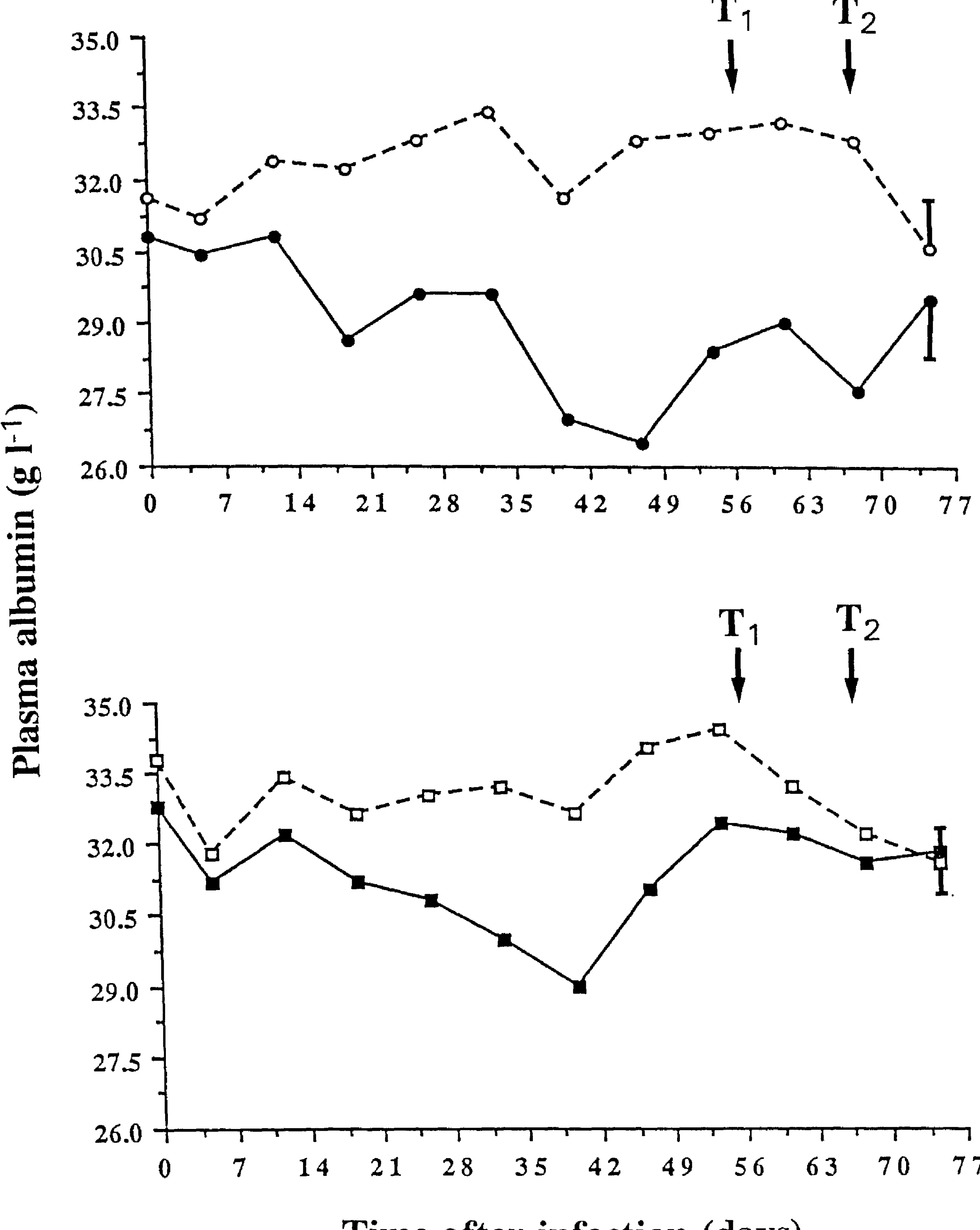
The mean globulin concentration showed a significant increase in SB but not in FD. In infected SB, the mean values increased from 36.2 $\pm$ 0.8 gl^{-1} at 0 DAI to 43.7 $\pm$ 3.1 gl^{-1} at 47 DAI (Figure 5.16). In infected FD, the globulin concentration decreased from 37.2 $\pm$ 1.1 gl^{-1} at 12 DAI to 31.0 $\pm$ 1.8 gl^{-1} at 54 DAI. Treatment did not have significant effects on the globulin concentrations of infected animals. The mean values in control animals fluctuated between 28.6 $\pm$ 0.9 and 35.4 $\pm$ 0.9 gl^{-1} throughout the period of observation.

Serum iron and TIBC concentrations

The mean serum iron concentration of infected and control animals showed an initial decrease between 0 and 24 DAI (Figure 5.17). Thereafter the mean values fluctuated considerably but tended to be higher in infected than in control animals.

The mean TIBC values of infected animals showed a fluctuating increase between 0 and 54 DAI and the values in control SB tended to be higher, while they were lower in control FD than in infected animals. Treatments tended to cause a reduction in plasma TIBC concentrations.

Figure 5.15 Plasma albumin concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (--○--, —-□--). T_1 and T_2 show the times of treatment.



T1
T2
35.0
33.5
32.0
30.5
29.0
27.5
26.0
0
7
14
21
28
35
42
49
56
63
70
77
Plasma albumin (g l-1)
Time after infection (days)

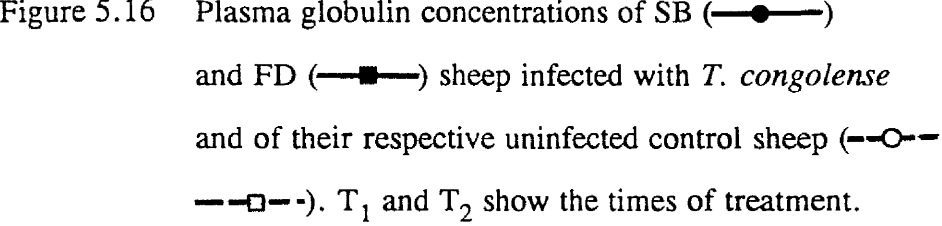


Figure 5.16 Plasma globulin concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (--○--, --□--). T_1 and T_2 show the times of treatment.

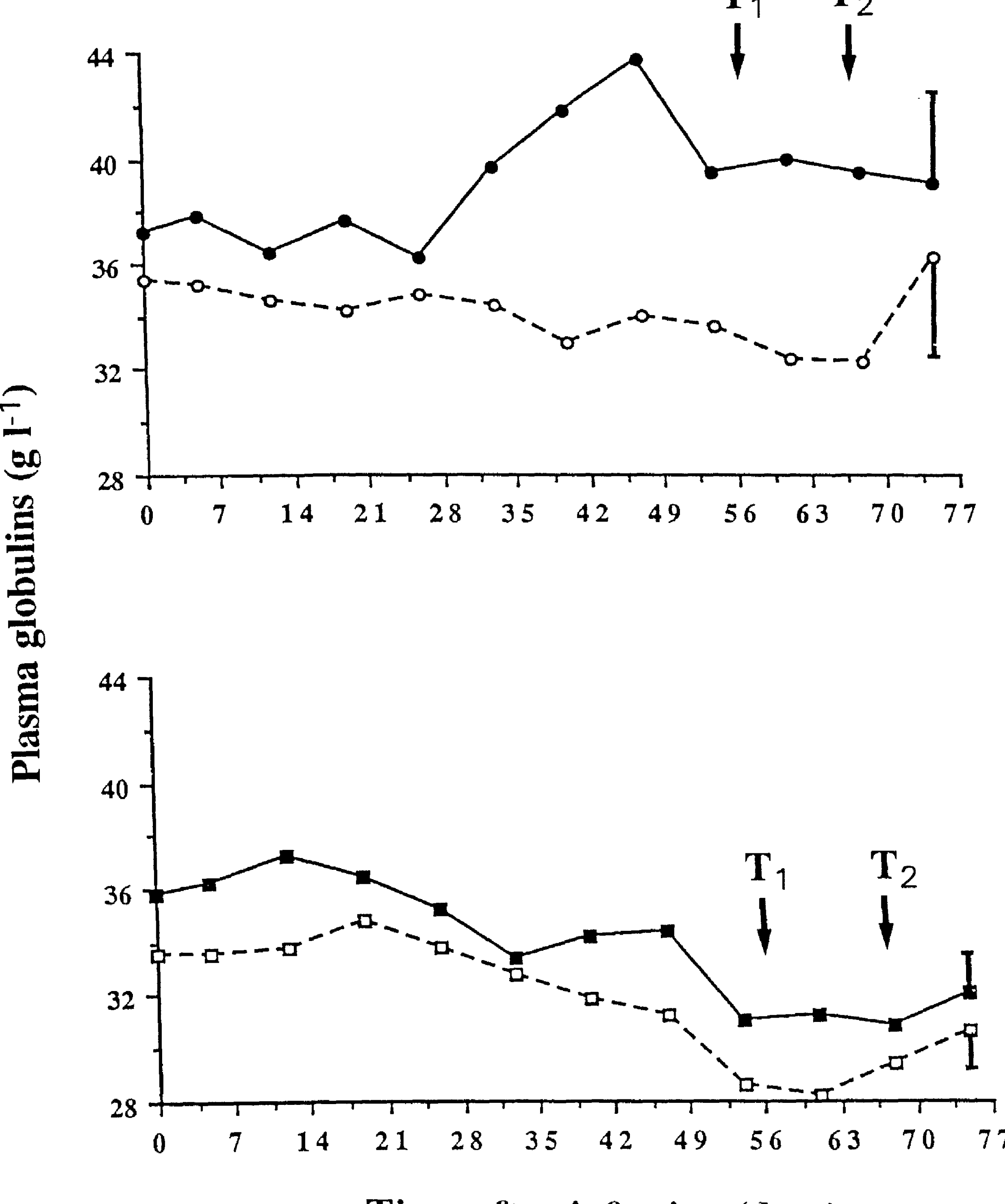
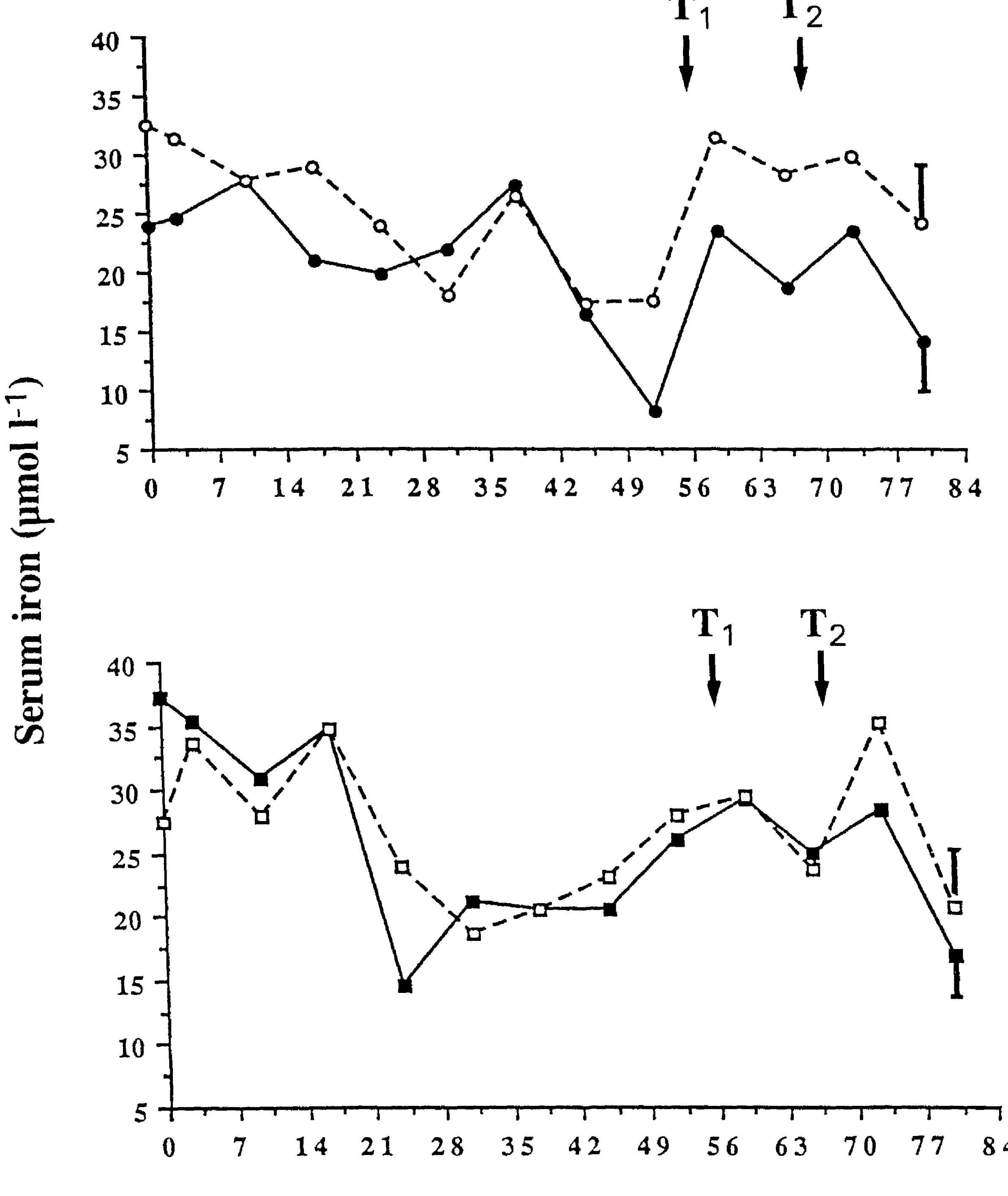

T1
T2
44
40
36
32
28
0
7
14
21
28
35
42
49
56
63
70
77
Plasma globulins (g l-1)
Time after infection (days)

Figure 5.17 Serum iron concentrations of SB (—●—) and FD (—■—) sheep infected with *T. congolense* and of their respective uninfected control sheep (- -○- -, - -□- -). T_1 and T_2 show the times of treatment.


T1
T2
40
35
30
25
20
15
10
5
0
7
14
21
28
35
42
49
56
63
70
77
84
Serum iron (μmol l-1)
T1
T2
Time after infection (days)

Plasma glucose, β-hydroxybutyrate and urea

There were no infection or breed effects in the levels of plasma glucose (SB 2.5-3.2, FD 2.7-3.7 $mmoll^{-1}$), β-hydroxybutyrate (SB 0.12-0.30, FD 0.14-0.34 $mmoll^{-1}$) or urea (SB 3.2-8.3, FD 3.1-7.1 $mmoll^{-1}$) throughout the period of observation.

DISCUSSION

In the present study, the infected SB lambs developed higher pyrexia, greater intensity of parasitaemia and more severe degree of anaemia than infected FD lambs, and both groups of infected animals gained less weight compared to their uninfected controls between 0 and 56 DAI. Breed differences in susceptibility of sheep to trypanosome infections have been reported from West Africa by Toure *et al.* (1983). These authors observed that Djallonke sheep developed less intensity of parasitaemia and less severe anaemia than the Sahelian Fulani sheep following experimental infection with *T. congolense*. While Whitelaw and Murray (1982) did not record differences in parasitaemia patterns between Red Maasai and Merino sheep infected with *T. congolense*, they reported that the rate of development of anaemia was slower in the Red Maasai. Maikanje *et al.* (1989) observed that the West African Dwarf sheep developed more severe anaemia and lost more weight than the Balami breed after experimental infection with *T. vivax*, despite both breeds having similar intensities of parasitaemia.

In another study from Kenya, Griffin and Allonby (1979d) infected Merino, Blackhead Persian and Red Maasai sheep with *T. congolense*. They reported that all Merino and Blackhead Persian sheep developed parasitaemia and remained positive for a period of 14 weeks while only five out of 10 Red Maasai developed any detectable parasitaemia, and two of the five parasitaemic sheep showed transient parasitaemia which disappeared rapidly. This is the only report where susceptible

animals have remained aparasitaemic after intravenous inoculation with a large number (10^5) of trypanosomes. It is, however, possible that all Maasai sheep in the study reported by Griffin and Allonby (1979d) became parasitaemic but this was not detected by the thin and thick smear methods employed in their study. It has been observed that these two methods are less sensitive for detection of trypanosomes than, for example, the dark ground/buffy coat method, described by Murray *et al.* (1977) and used in the present study.

The mean PCV of infected SB lambs decreased significantly between 0 and 56 DAI while the decrease was not significant in FD lambs. These changes were accompanied by reductions in red cell counts and Hb concentrations, which were also more severe in infected SB. Furthermore a significant correlation was observed between mean PCV and parasitaemia in both groups of infected animals ($r = -0.656$ for SB and $r = -0.684$ for FD) between 0 and 56 DAI. These observations suggest that the FD developed less severe anaemia as a result of their ability to control the intensity and duration of parasitaemia.

There was a significant increase in WBC associated with a lymphocytosis in infected FD in the present study. The WBC in infected SB showed an initial decline followed by a tendency to increase after 35 DAI. The moderate leucopaenia in infected SB between 21 and 35 DAI could be attributed to neutropaenia which was more pronounced than in FD lambs. These observations in infected FD are in contrast to reports of severe leucopaenia in the initial stages of *T. congolense* infection in sheep (MacKenzie and Cruickshank, 1973). Studies of peripheral blood leucocytes in experimental bovine trypanosomiasis have reported an early leucopaenia (Fiennes *et al.*, 1954; Naylor, 1971a; Wellde *et al.*, 1974) which may be followed by either a lymphocytosis (Valli *et al.*, 1979) or a continuing leucopaenia (Wellde *et al.*, 1974). The relationship between leucocyte responses observed in infected SB and FD observed in the present study, and the ability of these animals to

limit the levels of parasitaemia is not clear. Morrison *et al.* (1978) observed that *T. congolense* infection in mice results in non-specific polyclonal activation of B-lymphocytes and Assoku and Tizard (1978) demonstrated that autolysates of *T. congolense* were potentially mitogenic for mouse spleen cells. Later Assoku *et al.* (1979) attributed this mitogenic activity to free fatty acids particularly stearic and palmitic acids, released by autolysing trypanosomes. In comparative studies of N'Dama (trypanotolerant) and Boran cattle (trypanosensitive), Ellis *et al.* (1987) and Paling *et al.* (1991a) reported that during a course of *T. congolense* infection, the N'Dama had significantly higher numbers of B-cells and null cells than the Boran cattle. It was suggested that these cells may be important in the ability of N'Dama cattle to mount an effective immune response to trypanosome infection. In this study, infected FD developed a lymphocytosis and although both infected groups became neutropaenic, this was less severe in infected FD. Although peripheral blood leucocyte subpopulations were not analysed in this study, it is possible that lymphocytic proliferation as observed in infected FD may be a reflection of enhanced immunocompetence, which may play a part in limiting intensity and duration of parasitaemia.

It is not clear what impact neutropaenia may have had on the ability of infected SB to control intensities of parasitaemia. In cattle infected with *T. congolense*, Kissling *et al.* (1982) observed that neutrophils were involved in the clearance of opsonised trypanosomes, and the ability of trypanotolerant cattle to control the level and duration of parasitaemia has been attributed to a superior capacity to eliminate the parasites by antibody-mediated phagocytosis (Rurangirwa *et al.*, 1986; Kamanga-Sollo *et al.*, 1991). It is possible that neutropaenia in infected SB may have contributed to their inability to limit parasitaemia to a significant

degree. However, further studies are needed to define the precise role of lymphocytes and neutrophils in the immune response of trypanosome-infected animals.

Thrombocytopaenia developed in both groups of infected sheep but the decline in thrombocyte counts was much faster in infected SB than in infected FD. Thrombocytopaenia has been reported in *T. congolense* infections in cattle (Wellde *et al.*, 1974; Forsberg *et al.*, 1979; Davis, 1982) and in *T. vivax* infections in goats (van den Ingh *et al.*, 1976a; Anosa and Kaneko, 1989). The increase in platelet counts following initial treatment at 56 DAI and the subsequent decrease after relapse of infection suggests a close relationship between the intensity of parasitaemia and degree of thrombocytopaenia. This observation is in agreement with the report of Wellde *et al.* (1978) in their studies of *T. congolense* infection in Hereford cattle. The causes of thrombocytopaenia appear to be mutlifactorial and they may include ineffective thrombopoiesis (Forsberg *et al.*, 1979), platelet aggregation (Anosa and Kaneko, 1989), direct injury to platelets by trypanosomes (Davis *et al*, 1974) and production of antibodies against normal platelets (Assoku and Gardiner, 1989). In the present study, severe clumping of platelets on thin smears of some infected animals was observed 35 to 42 DAI and this could have contributed to the development of thrombocytopaenia. It has been proposed that persistent destruction of platelets by the expanded mononuclear phagocytic system is responsible for maintaining low thrombocyte counts particularly during chronic stages of infection (Syndercombe-Court, 1985).

Following infection, the SB developed hypocholesterolaemia and hypophospholipidaemia leading to a significant decrease in serum total lipid concentration. Similar changes of a lesser degree were observed in infected FD compared to their uninfected controls. The initial cholesterol concentration in infected SB was significantly higher than in infected FD and the concentrations

decreased with time in both groups of infected animals. The decrease in lipid constituents in sheep infected with *T. congolense*, recorded in the current study, confirms previous observations (Chapter 3) and those of Roberts (1974, 1975a, b). There are no breed lipid comparison studies in sheep but those in cattle have indicated that Zebu cattle, which are generally regarded as trypanosensitive, have higher cholesterol levels than West African Shorthorn cattle, which are known to be trypanotolerant (Traore-Leroux *et al.*, 1987a). Traore-Leroux *et al* (1987a) reported that Zebu cattle developed higher intensity of parasitaemia and more severe anaemia than Baoule cattle following *T. congolense* infection. In the current study, the SB developed a higher intensity of parasitaemia and greater degree of anaemia and pyrexia than the infected FD. These observations suggest a possible role for cholesterol in susceptibility of sheep to trypanosomiasis as has been proposed for cattle.

Trypanotolerance has been related to the ability of the host to regulate parasite growth, a capacity that has been largely attributed to the immune system. However, Murray *et al.* (1982) have suggested that non-immune factors may also affect trypanosome growth and differentiation in trypanotolerant cattle. It has been demonstrated that trypanosomes require cholesterol for growth and multiplication (Vanderweed and Black, 1989; Black and Vanderweed, 1989) and previous studies using *T. brucei* showed that these trypanosomes obtain it by internalisation of low and high density lipoproteins (Gillet and Owen, 1987) through receptors located on the flagellar pocket (Coppens *et al.*, 1987; Coppens *et al.*, 1992). More recent studies have indicated that *T. congolense* and *T. vivax* also possess these receptors (Bastin *et al.*, 1991), suggesting that the methods of uptake of lipoprotein fractions is the same as that of *T. brucei*. Further studies revealed that trypanosome growth *in*

vitro can be inhibited by either removal of low and high density lipoproteins from the culture media or by antibodies against the purified receptors (Coppens et al.,1988; Black and Vanderweed, 1989).

It was observed in the present study that the initial cholesterol concentration in SB was significantly higher than that of FD lambs and that the intensity of parasitaemia and the fall in plasma cholesterol following infection were greater in SB than in FD. This suggests that cholesterol could have been taken up in large quantities to support trypanosome growth. Traore-Leroux *et al.* (1987a) reported that the Zebu cattle had higher plasma cholesterol concentrations and experienced a greater fall in these concentrations with subsequent development of higher parasitaemia. These observations suggest that availability of higher levels of plasma cholesterol may support greater trypanosome numbers in the circulation with subsequent more pathogenic effects.

The reduction in plasma cholesterol was also accompanied by a fall in serum phospholipid concentrations leading to a more severe decrease in total lipids of infected SB. Since the SB developed more severe anaemia and pyrexia than FD, these observations suggest that the decrease in blood lipids may be related to the severity of infection. This is in agreement with the report of Roberts (1973).

O'Kelly (1973a, b) and Noble *et al.* (1976) have provided evidence that lipid metabolism in ruminants is affected by exposure to high temperatures. They observed that the concentrations of cholesterol, free fatty acids and phospholipids were lower in heat stressed animals than in those in a normal environment. Infected sheep in the present study developed pyrexia which was more severe in SB than in FD. It is possible that this contributed to the differences in the decreases in blood lipids.

Infected animals, in the present study, also became hypoalbuminaemic and hyperglobulinaemic which were more pronounced in infected SB than in FD. These changes resulted into an increase in total protein concentration of infected SB and a moderate decline in the levels of infected FD. Hypoalbuminaemia has been recorded on many occasions of trypanosome infections (Vickerman and Tetley, 1979; Tabel *et al.*, 1980) and the present observation suggests that the degree of hypoalbuminaemia is related to the severity of trypanosome infection in sheep.

In conclusion, this study has shown that, on the basis of intensities of parasitaemia, degree of anaemia, thrombocytopaenia and pyrexia, the SB lambs were more susceptible to the effects of *T. congolense* infection than FD lambs. Blood biochemical analyses revealed that the SB underwent more pronounced decreases in plasma cholesterol, serum phospholipids and plasma albumin than the FD. These observations suggest that the changes in blood lipids and protein in sheep infected with *T. congolense* are closely related to the severity of infection and may play a role in host susceptibility, by regulating parasite growth.

CHAPTER 6

A COMPARATIVE STUDY OF THE PATHOGENICITY OF THREE CLONES OF *TRYPANOSOMA CONGOLENSE* IN SCOTTISH BLACKFACE SHEEP.

INTRODUCTION

Several host and parasite factors have been shown to have major influences on the susceptibility of hosts to trypanosome infections. The most important host factors are age, breed, and nutritional status (Murray, 1989; Murray *et al.*, 1982). At the same time the anaemia that follows infection may be affected by differences in virulence that exist among different species of trypanosomes and among the large number of strains belonging to each species (Stephen, 1986; Murray and Dexter, 1988). African trypanosomes exhibit a wide range of virulence in vertebrate hosts and antigenic variation has dominated most considerations of pathogenicity of African trypanosomiasis (Barry *et al.*, 1979; Barry and Turner, 1991).

In rabbits infected with either *T. brucei* S42 or *T. brucei* 427, Jenkins *et al.* (1980) observed that, while anaemia developed within 7 days, it was more severe in those infected with S42 stock. The possibility that different strains of *T. congolense* can give rise to varying pathogenicity in a given host has only been investigated in rabbits using anaemia as the indicator of degree of infection (Jenkins *et al.*, cited by Seed and Hall, 1985). These authors infected rabbits with three stocks of *T. congolense*, either GAMB 19, TSW 99/77 or S104. They observed that GAMB 19 and TSW 99/77 produced a chronic infection lasting more than 150 days while survival was between 39 and 100 days with S104. The patterns of parasitaemia, in animals given the same inoculum, were different and were reflected in the degrees of anaemia. There are no reports of pathogenicity studies of different stocks of *T. congolense* in ruminant animals.

Studies in mice have revealed that two (GRVPS 57/6 and GRVPS 92/3) of the three clones used in the present study, show varying pathogenic effects. GRVPS 57/6 shows a longer prepatent period and following patency, parasitaemia rises and falls leading to death of infected mice within two weeks.

Clone GRVPS 92/3, on the other hand, has a shorter prepatent period, maintains high intensities of parasitaemia and does not lead to death of infected mice, until after 30 days (unpublished personal observations).

The present study was therefore intended to investigate the pathological responses in sheep infected with one of three clones of *T. congolense*. The main limitation of this study, however, was that only two animals were used in each infection group and this complicates any statistical comparisons.

MATERIALS AND METHODS

Experimental Animals

Scottish Blackface lambs of the same stock as those described in the previous experiment (Chapter 5) were used in this study. The management, feeding and housing of animals, parasitological, haematological and blood biochemical methods employed have been described (Chapter 2).

Trypanosomes

Three clones of *T. congolense*, GRVPS 57/6, GRVPS 3/2, and GRVPS 92/3 were used to infect the sheep. The origin of GRVPS 57/6 has been described (Chapter 5). GRVPS 3/2 and GRVPS 92/3 were derived from TREU 1627 and TREU 1457, respectively, which were cloned stocks of *T. congolense* originally obtained from West Africa. TREU 1627 was obtained from the Gambian isolates Kantongo Kunda/77/LUMP/1794 and TREU from Nigerian isolates Zaria/67/LUMP/69 (Ross *et al.*, 1985).

Preparation of the inoculum

The stabilated trypanosomes in a microhaematocrit tube were transferred into a tube containing phosphate buffered saline (PBS) (containing 1.5% glucose)(pH 8.0). Each clone of trypanosomes was then injected intraperitoneally into irradiated mice at one day intervals starting with GRVPS 3/2, then GRVPS 57/6 and lastly GRVPS 92/3. Nine days after the initial injection, all positive mice were anaesthetised and bled, and the blood from each group was pooled together. Counts were performed on the pooled blood samples using a haemocytometer, and the blood samples were diluted with PBS to give $1x\ 10^5$ trypanosomes in 3 ml of inoculum. Each animal then received 3 ml of the appropriate inoculum.

Experimental Design

Six male castrate Scottish Blackface lambs about one year old, were divided into three groups depending on their live body weights and PCV. Each group of two animals was then injected with one of the three *T. congolense* clones, GRVPS 3/2, GRVPS 57/6 or GRVPS 92/3 (1×10^5 trypanosomes) by the jugular route. The animals were monitored for 56 days.

Statistical Methods

No statistical methods were employed in this study because there were too few animals in each infection group. Results of individual animals in each infection group have therefore been presented.

RESULTS

Parasitological Findings

There was considerable variation in patterns of parasitaemia in individual animals. Following patency, intensities of parasitaemia increased to reach the first peaks 12-14 days after infection (Figure 6.1). Thereafter, the parasitaemia fluctuated considerably but GRVPS 92/3 appeared to maintain high levels of parasitaemia particularly between 12 and 26 DAI. After 33 DAI, aparasitaemic periods, ranging from three to four days were observed in the sheep infected with GRVPS 92/3, while the rest of the animals were positive throughout the period of observation.

Clinical Condition and Body Weight Changes

Five of the six infected animals appeared to maintain good appetites and showed fluctuating increase in body weight with time. One animal, No. 126 infected with GRVPS 3/2, died after 37 days of infection. Ante-mortem examination of this animal revealed a high temperature of >42 ^{o}C, parasitaemia score of 1 and a PCV of 0.20 ll^{-1}. Post-Mortem examination revealed a grossly enlarged spleen, prominent haemal nodes and general palor of abdominal organs. On submission of post-mortem samples, no organisms that could be incriminated were isolated.

There were no apparent differences in liveweights, however sheep No. 103 infected with GRVPS 92/3 appeared to show the lowest weight gain particularly between 0 and 28 DAI (Figure 6.2).

Body Temperature

All infected animals developed fluctuating pyrexia (Figure 6.3), and there were no clear differences among the three clones of *T. congolense*.

Figure 6.1. Parasitaemia scores of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.

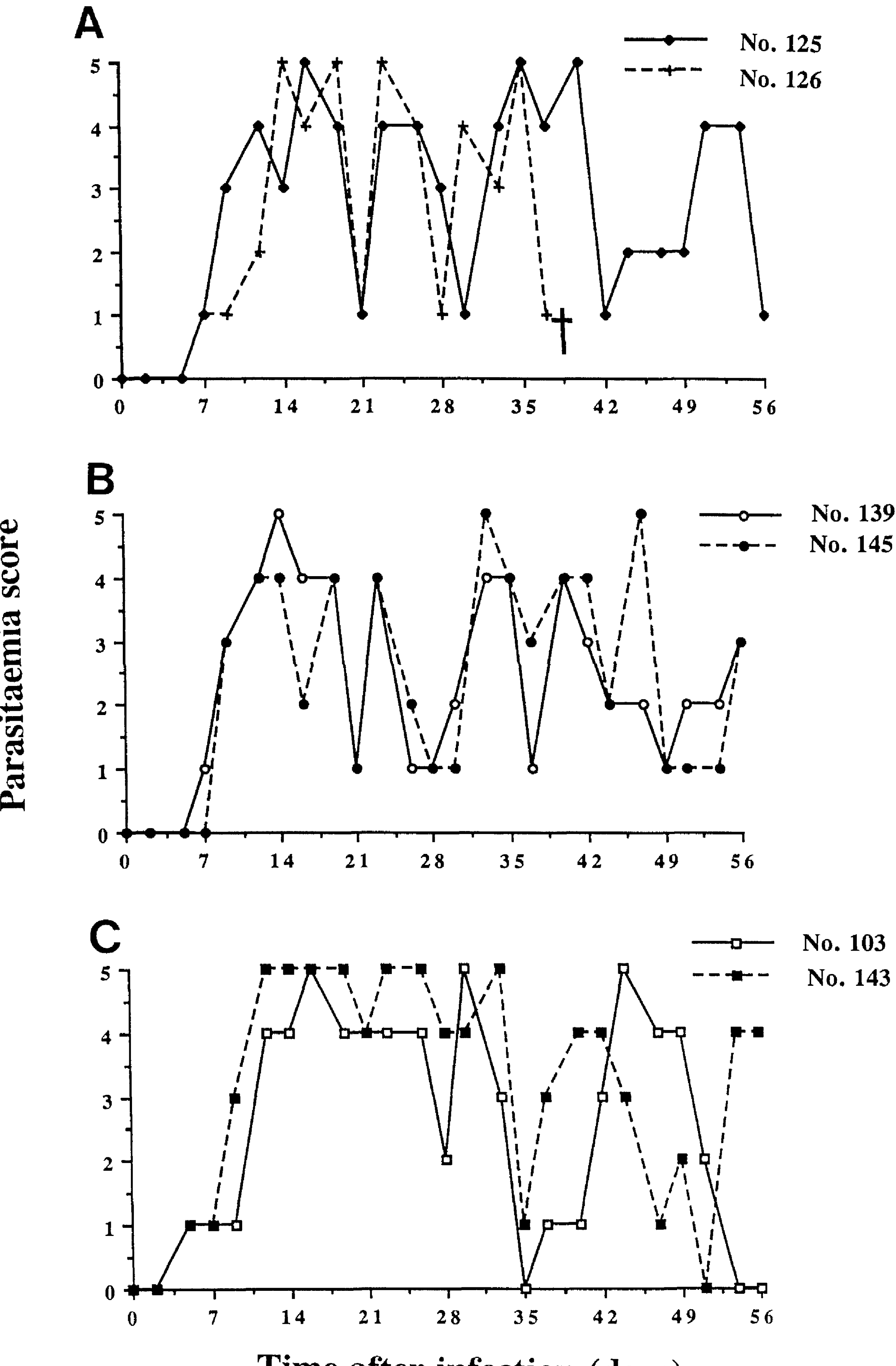

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Parasitaemia score
0
1
2
3
4
5
0
7
14
21
28
35
42
49
56
Time after infection (days)

Figure 6.2 Body weights of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 B) or GRVPS 92/3 (C).

† denotes death.

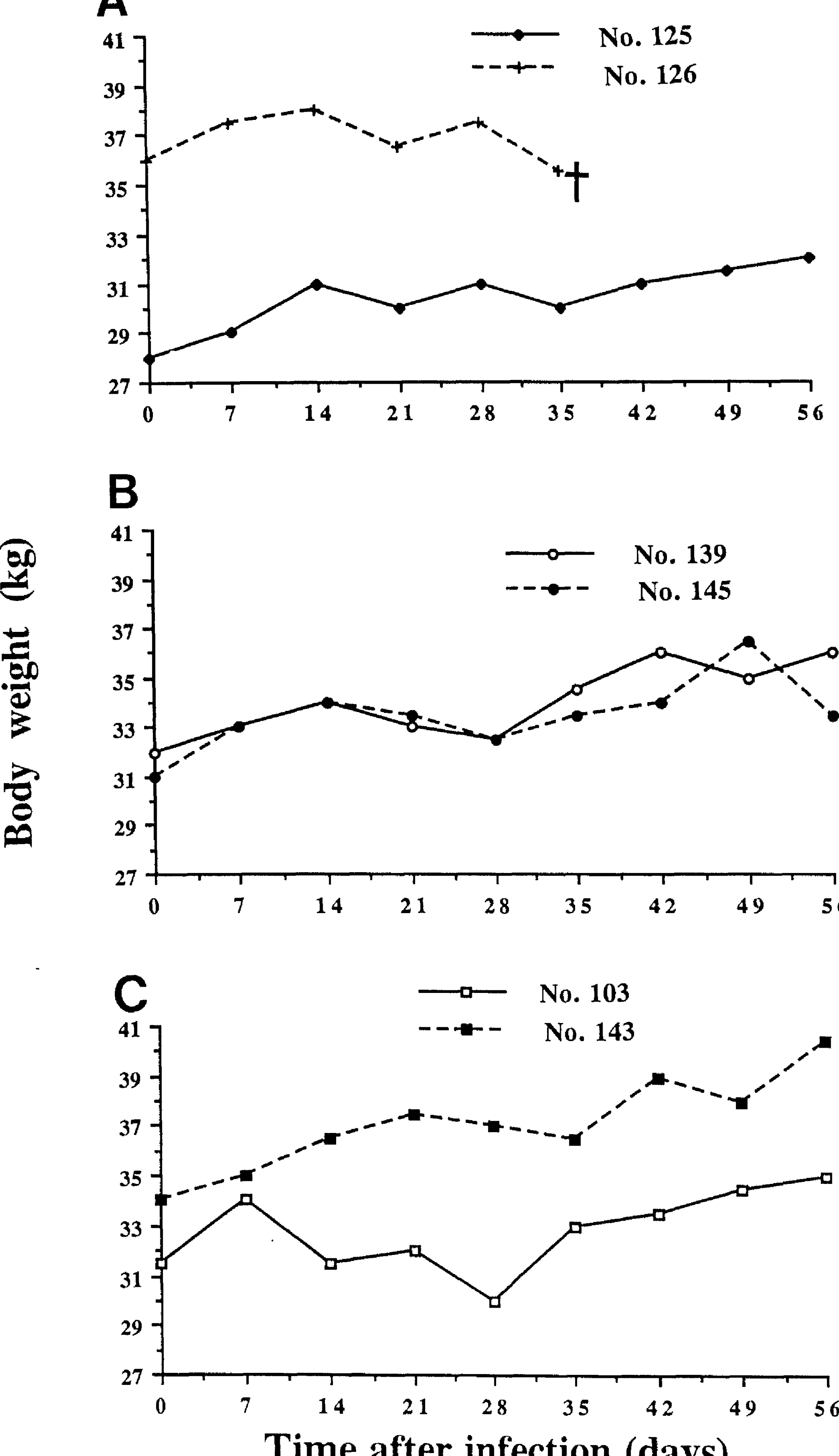
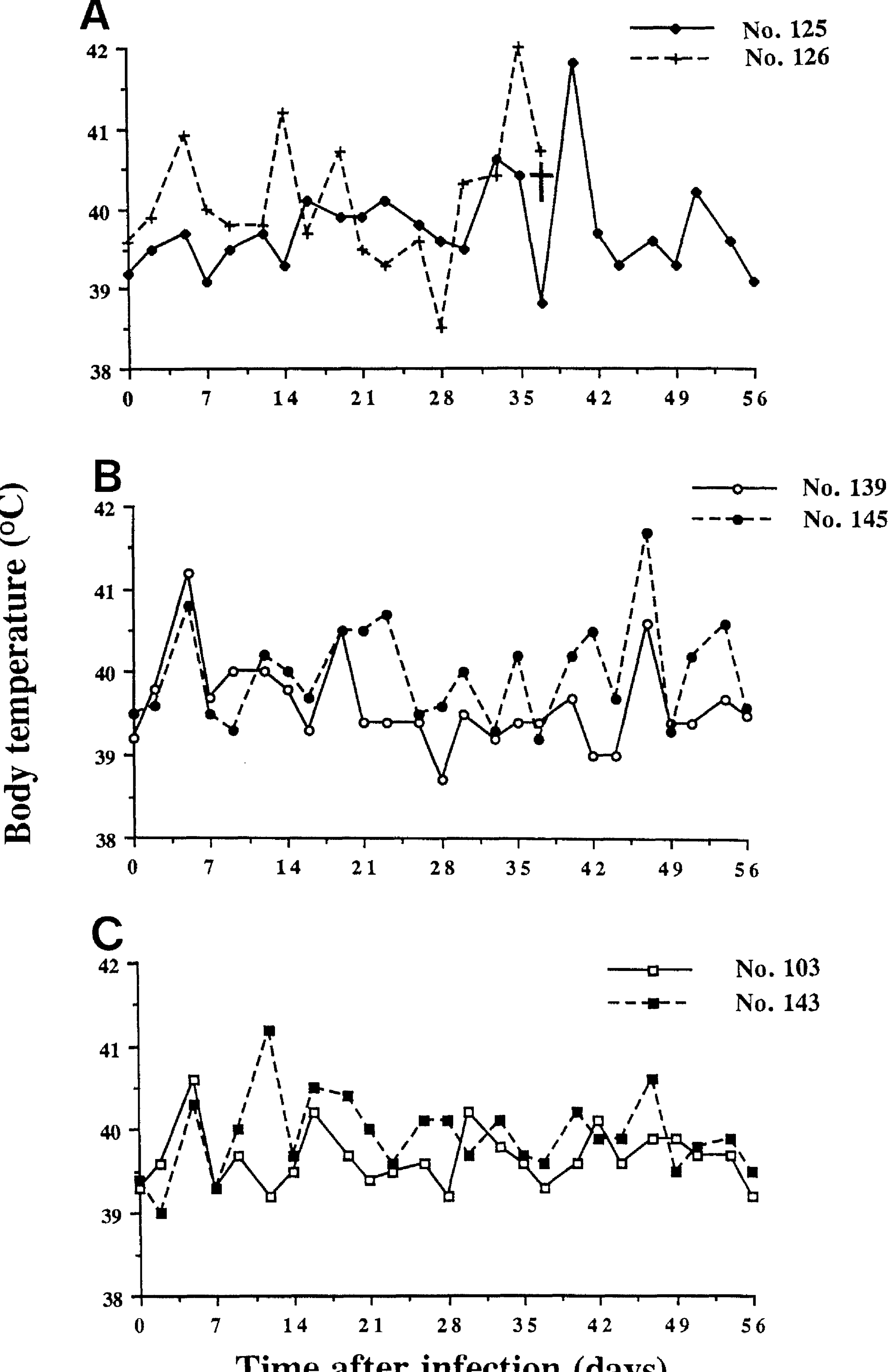

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Body weight (kg)
Time after infection (days)
41
39
37
35
33
31
29
27
0
7
14
21
28
35
42
49
56

Figure 6.3 Body temperatures of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 or GRVPS 92/3 (C). † denotes death.


A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
42
41
40
39
38
0
7
14
21
28
35
42
49
56
Body temperature (°C)
Time after infection (days)

Haematological Observations

Packed cell volume (PCV)

All infected animals showed a decrease in PCV with time of infection, the decline starting with the appearance of trypanosomes in the circulation. Animal 126 infected with GRVPS 3/2 developed the most rapid anaemia with the PCV decreasing from 0.29 ll^{-1} at 0 DAI to 0.20 ll^{-1} at 37 DAI when death occurred (Figure 6.4). Sheep No. 103 (infected with GRVPS 92/3) also reached a low PCV of 0.20 ll^{-1} at 49 DAI. Considering trypanosome clones, it appeared that GRVPS 92/3 caused more severe anaemia than the other two clones.

Red blood cell count (RBC) and Hb concentration

All clones caused decreases in RBC (Figure 6.5) and Hb concentrations (Figure 6.6) of infected animals. However GRVPS 92/3 appeared to cause a more severe and protracted decrease in RBC and Hb concentration, particularly of sheep No. 103.

Total white cell count (WBC) and lymphocytes

The changes in WBC and lymphocytes varied within individual animals and with the type of infecting clone of trypanosomes. Sheep No. 126 (infected with GRVPS 3/2) showed a fluctuating decrease in WBC from 13.1 x $10^9 l^{-1}$ at 5 DAI to 5.8 x $10^9 l^{-1}$ at 33 DAI (Figure 6.7). Little variation in these variables occurred in other animals infected with GRVPS 3/2 and GRVPS 57/6. However, animals infected with GRVPS 92/3 showed a fluctuating leucocytosis and lymphocytosis starting with patency of parasitaemia. The greatest lymphocytic response was observed in sheep No. 143. The monocyte, neutrophil and eosinophil counts were unaffected by infection, irrespective of the infecting stock.

Figure 6.4 Packed cell volumes (PCV) of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.

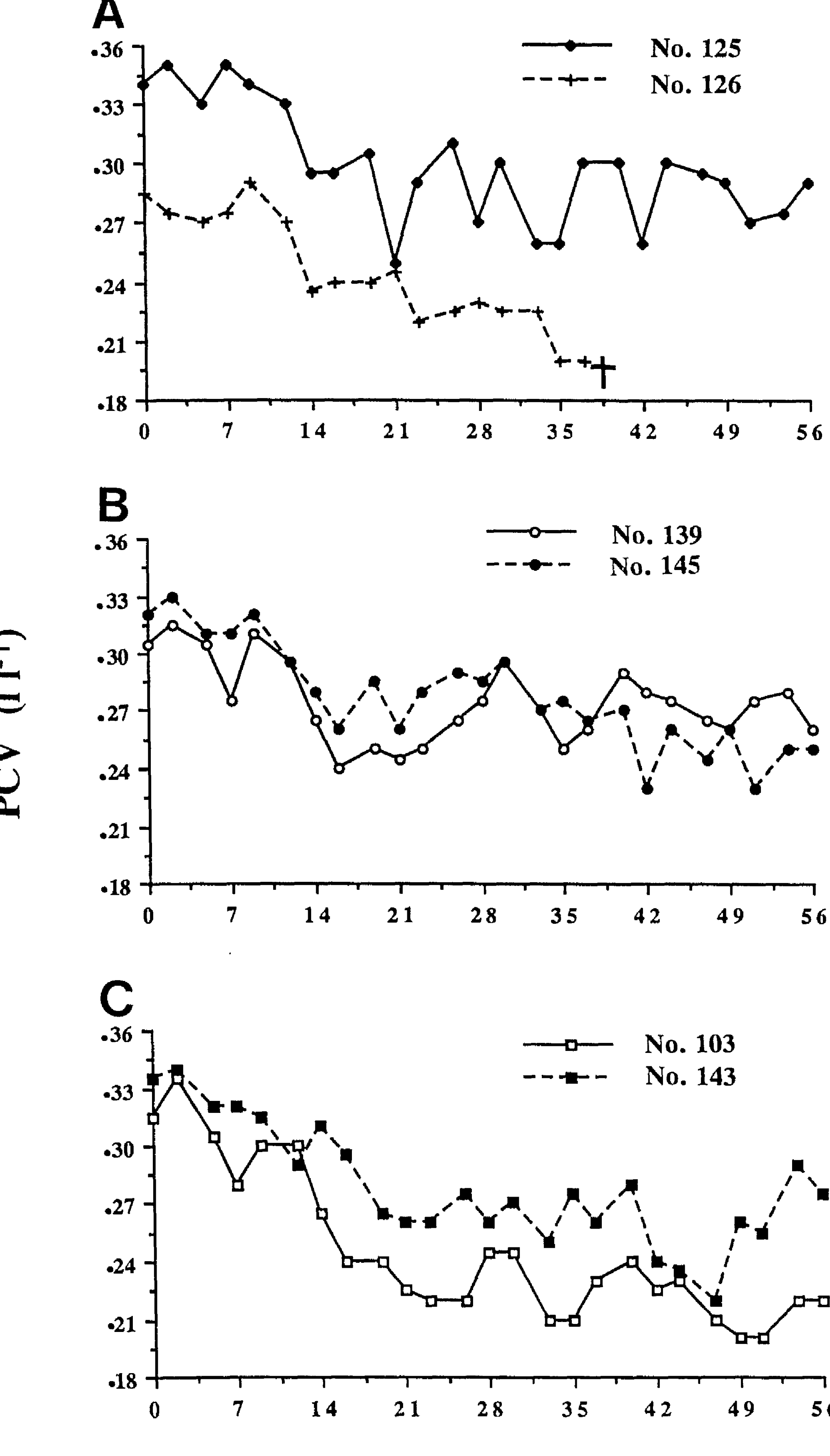
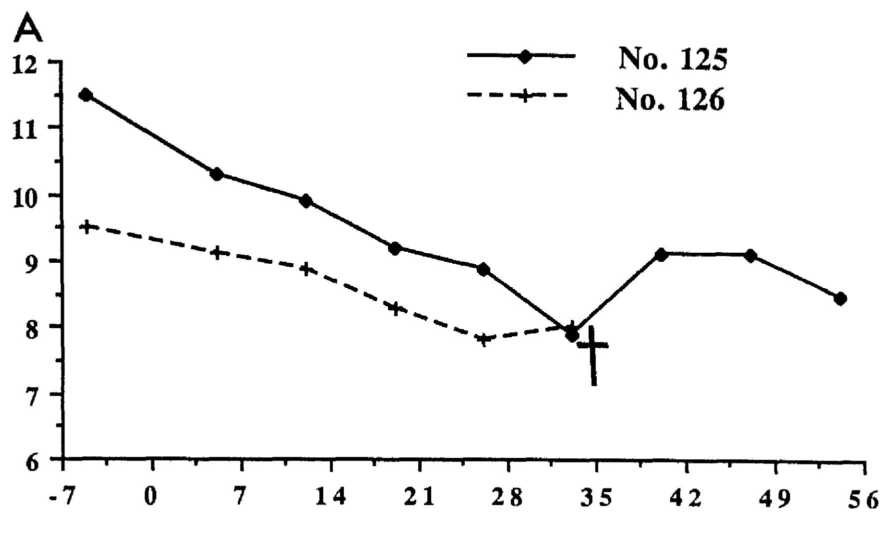
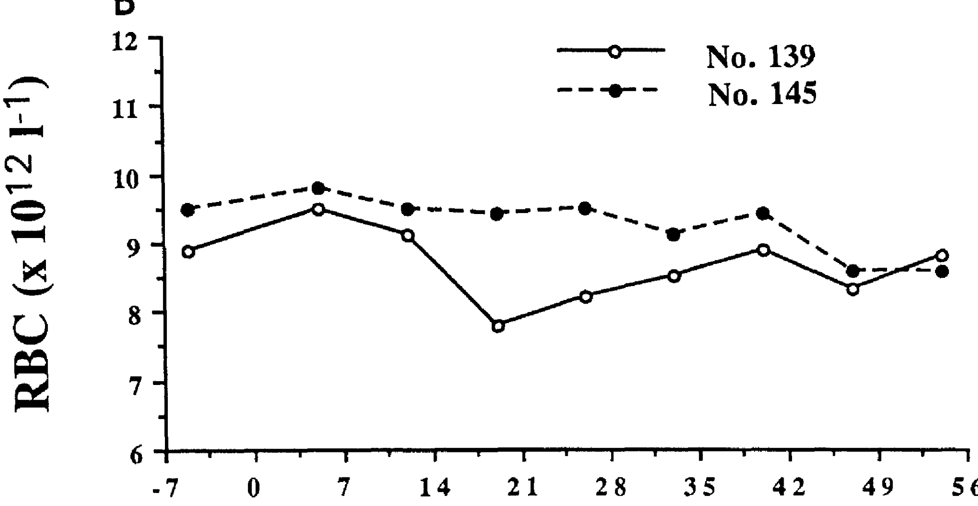
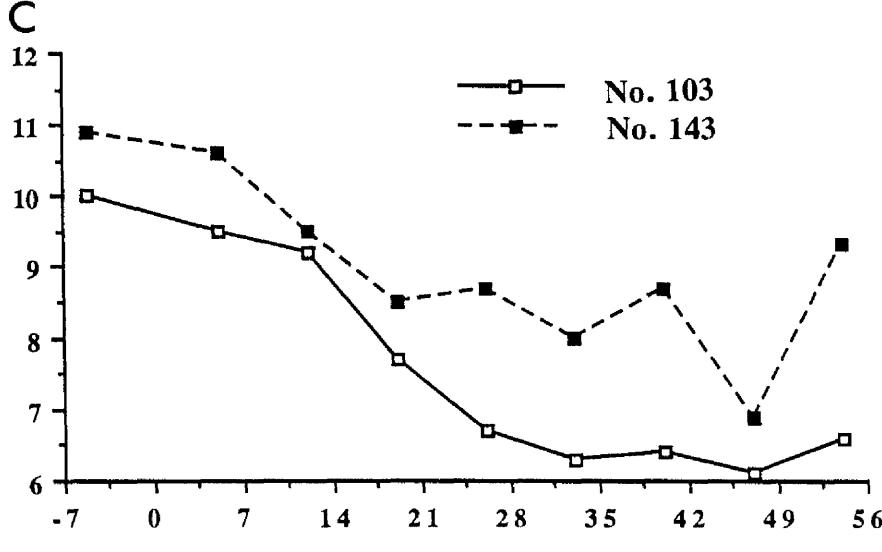

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
PCV (l l-1)
.36
.33
.30
.27
.24
.21
.18
0
7
14
21
28
35
42
49
56
Time after infection (days)

Figure 6.5 Red blood cell counts (RBC) of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.







Time after infection (days)

Figure 6.6 Haemoglobin (Hb) concentrations of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (B). † denotes death.

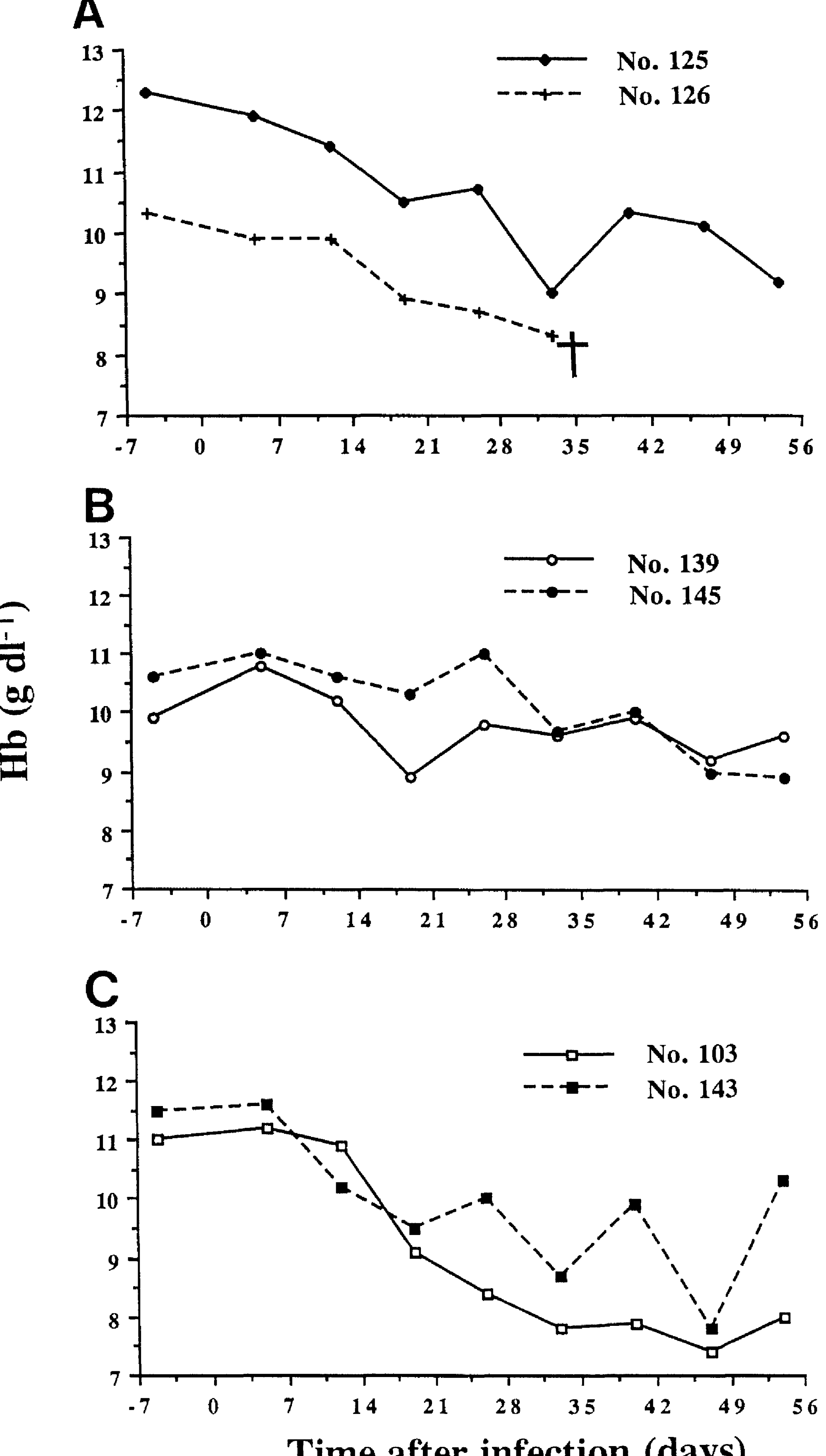
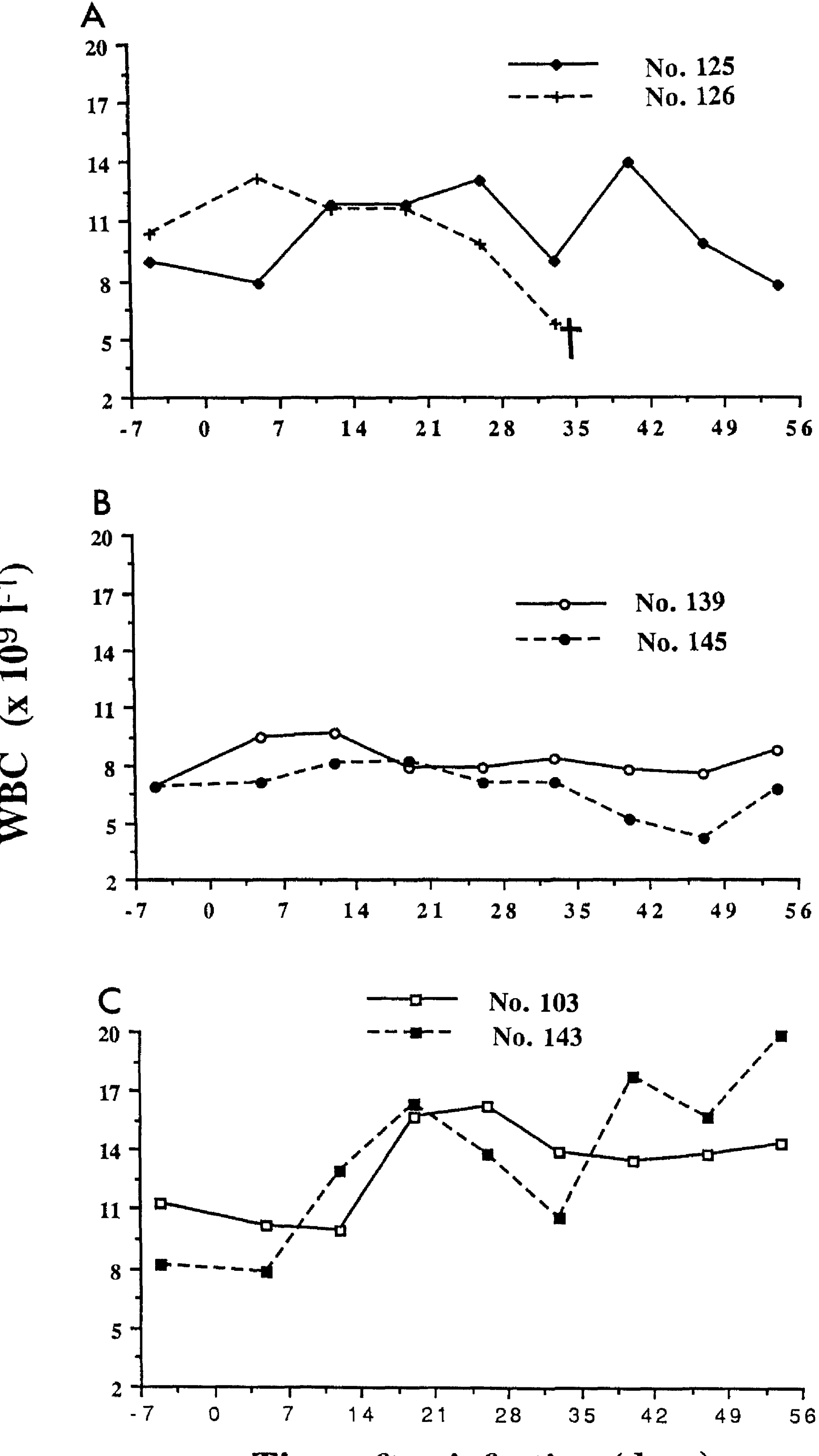

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Hb (g dl-1)
Time after infection (days)
13
12
11
10
9
8
7
-7
0
7
14
21
28
35
42
49
56

Figure 6.7 White blood cell counts (WBC) of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.



A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
WBC (x 10^9 l^-1)
20
17
14
11
8
5
2
-7
0
7
14
21
28
35
42
49
56
Time after infection (days)

Biochemical Findings

Serum total lipids

The serum total lipids concentrations of individual animals fluctuated considerably but showed a tendency to decrease with time of infection (Figure 6.8). The decreases appeared to be pronounced in animals infected with GRVPS 57/6 and sheep No. 126, infected with GRVPS 3/2.

Plasma cholesterol and serum phospholipids

Plasma cholesterol and serum phospholipid concentrations displayed marked variations in individual animals, but tended to decrease following patency of parasitaemia. The decrease continued up to 21 days after which the concentrations varied with the peaks of parasitaemia.

Plasma NEFA and triglycerides

There was a fluctuating decrease in the concentration of plasma NEFA in all animals (Figure 6.9), however the greatest consistent decrease occurred in the group infected with GRVPS 92/3. The concentration of plasma triglycerides fluctuated a great deal and displayed no particular trend.

Plasma total protein (TP) and albumin

All animals initially developed hypoproteinaemia between 0 and 21 DAI after which the concentration of TP tended to increase (Figure 6.10). Plasma albumin concentration showed little variation in animals infected with GRVPS 3/2 (Figure 6.11). Animals infected with either GRVPS 57/6 or GRVPS 92/3 showed a fluctuating decrease in plasma albumin up to 42-49 DAI when the values increased.

Figure 6.8 Serum total lipid concentrations of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B), or GRVPS 92/3 (C). † denotes death.

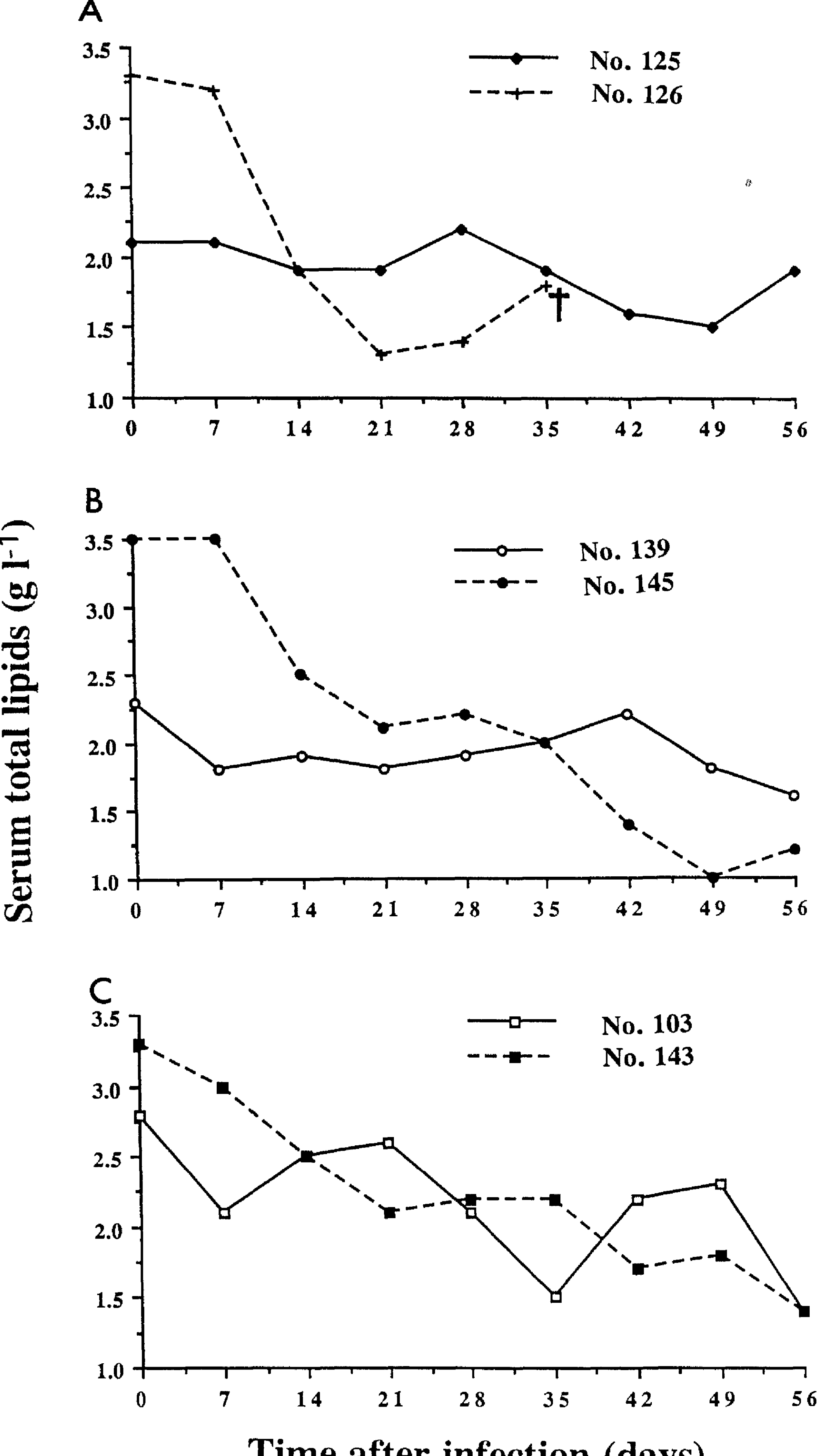
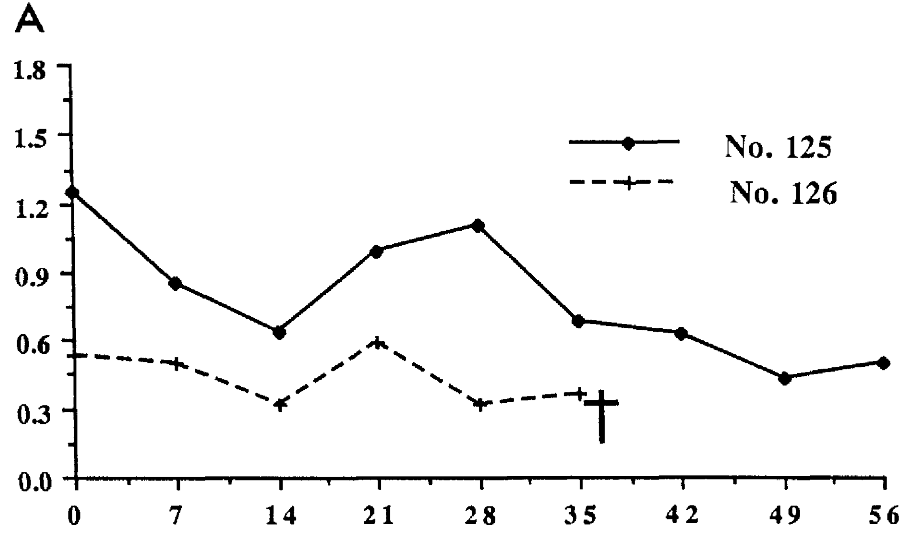
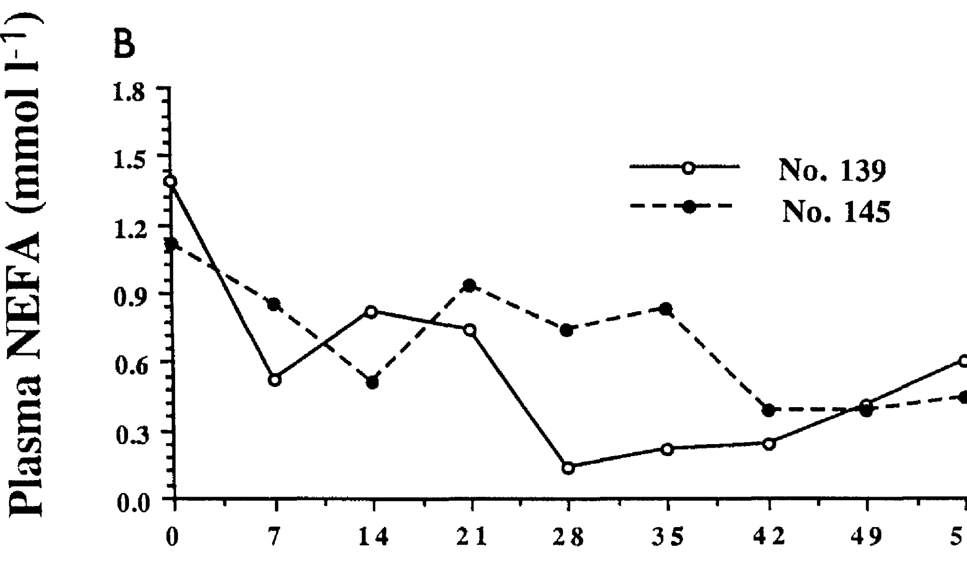

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Serum total lipids (g l-1)
Time after infection (days)
3.5
3.0
2.5
2.0
1.5
1.0
0
7
14
21
28
35
42
49
56

Figure 6.9 Plasma NEFA concentrations of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.


A
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0
7
14
21
28
35
42
49
56
No. 125
No. 126


Plasma NEFA (mmol l-1)
B
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0
7
14
21
28
35
42
49
56
No. 139
No. 145

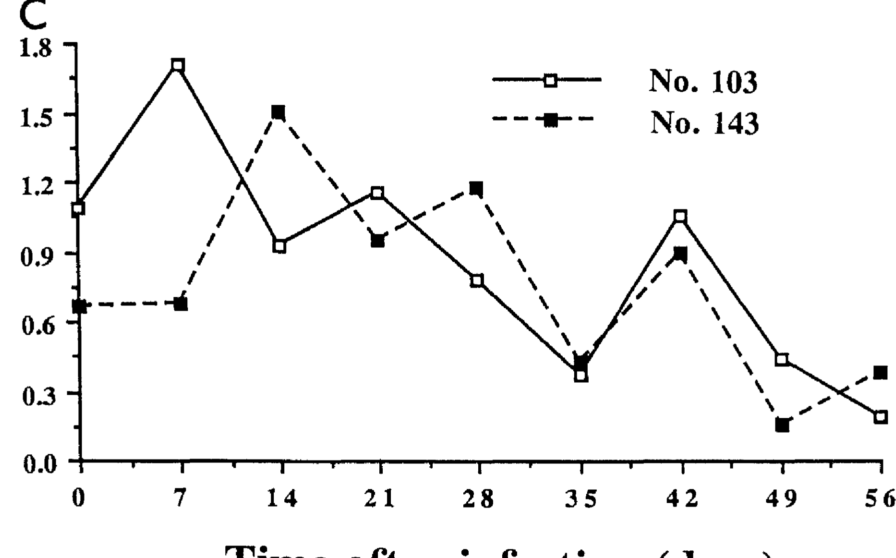

C
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0
7
14
21
28
35
42
49
56
No. 103
No. 143
Time after infection (days)

Figure 6.10 Plasma total protein concentrations of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.

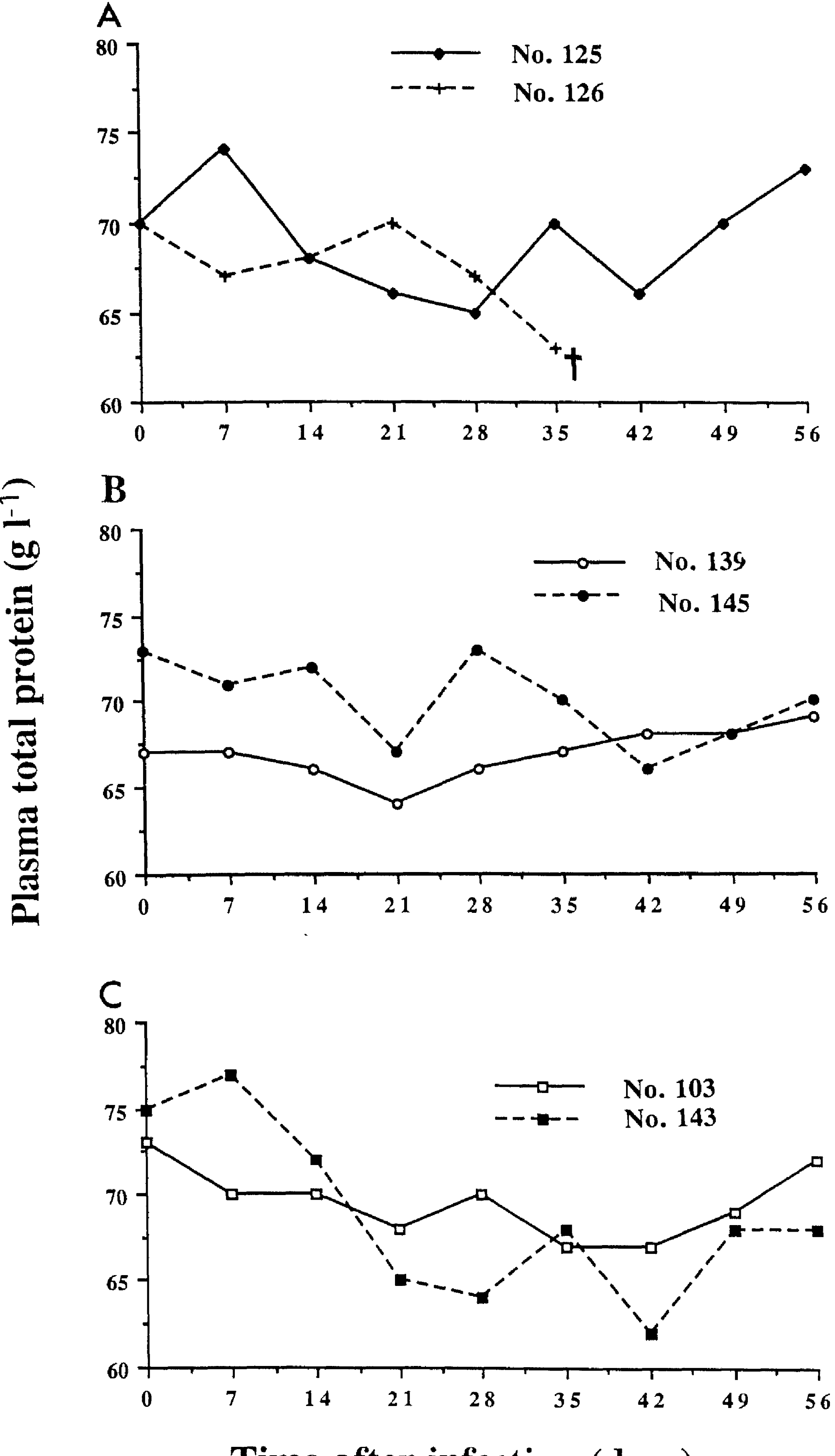
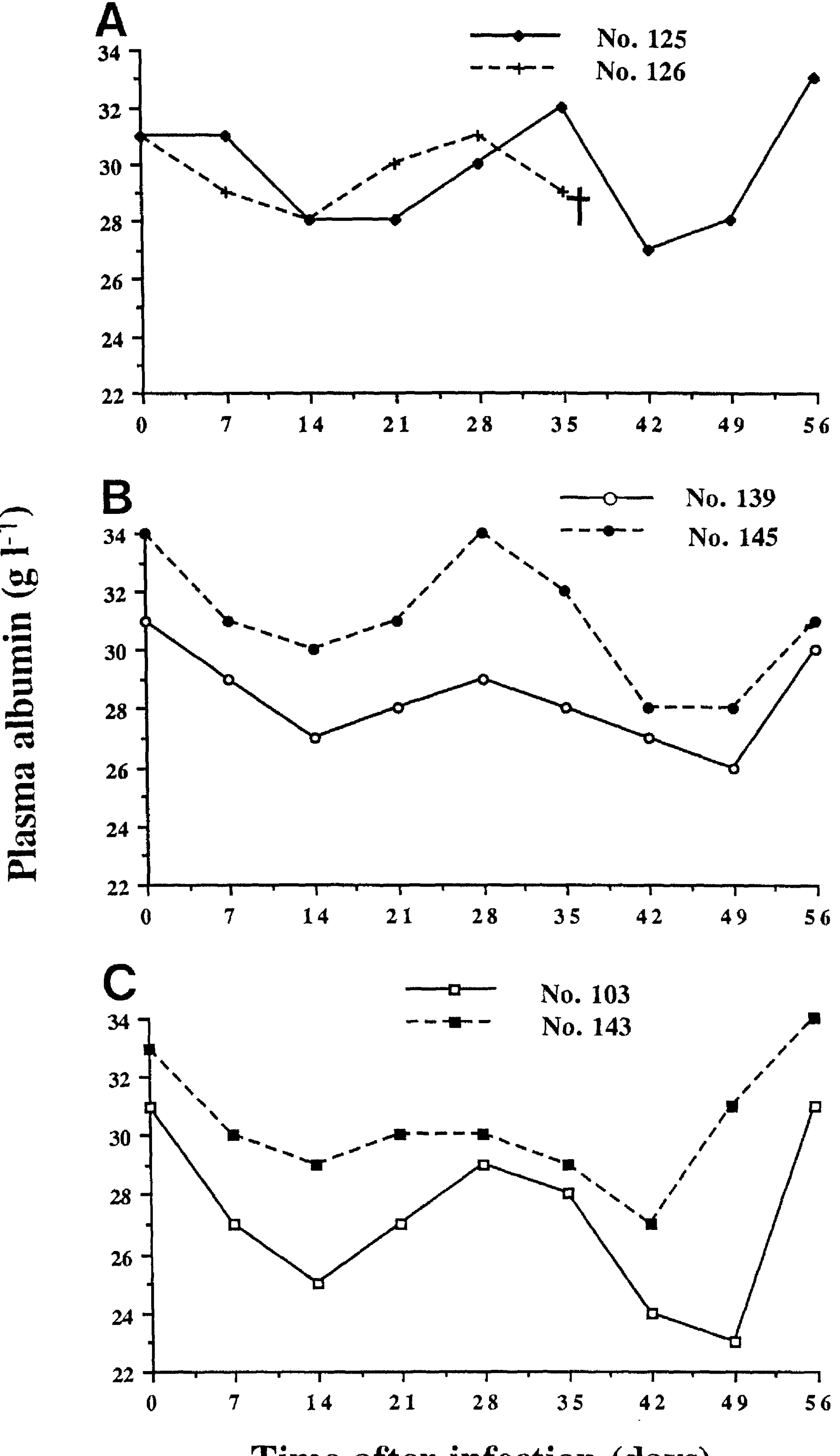

A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Plasma total protein (g l-1)
Time after infection (days)
80
75
70
65
60
0
7
14
21
28
35
42
49
56

Figure 6.11 Plasma albumin concentrations of sheep infected with either *T. congolense* GRVPS 3/2 (A), GRVPS 57/6 (B) or GRVPS 92/3 (C). † denotes death.


A
No. 125
No. 126
B
No. 139
No. 145
C
No. 103
No. 143
Plasma albumin (g l-1)
Time after infection (days)
34
32
30
28
26
24
22
0
7
14
21
28
35
42
49
56

Plasma urea, glucose, β-hydroxybutyrate, serum iron and TIBC

The values of these variables fluctuated in individual animals and there were no differences associated with the type of infecting clone of trypanosomes. The values remained within the suggested normal ranges (results not presented).

DISCUSSION

In the present study, all infected animals developed fluctuating pyrexia, parasitaemia, and anaemia, however the degree of anaemia appeared to be more severe in the animals infected with GRVPS 92/3. This clone of *T. congolense* tended to be more infective in that it was able to establish itself faster and to maintain moderately higher intensities of parasitaemia than either GRVPS 3/2 or GRVPS 57/6. It therefore appeared that this stock of *T. congolense* was more virulent for sheep.

One sheep infected with GRVPS 3/2 died after 37 days of infection. At the time of death, its PCV was 0.20 ll^{-1} and parasitaemia score was 1 with a high temperature of 40.7 ^{o}C. Studies reported in previous chapters demonstrated that sheep can withstand *T. congolense* infection for at least 14 weeks. Stephen (1986) proposed that this period may vary from weeks to months depending on the strain and morphology of infecting trypanosomes. He was basing his suggestion on the observations of Godfrey (1961) who showed that the long forms or *dimorphon* type *T. congolense* is more pathogenic for cattle than the short type *T. congolense* referred to as the *congolense* type. While post-mortem examination failed to reveal the actual cause of death, it is possible that secondary infection, which was not detected, was the cause of death of sheep No. 126. Stephen (1986) has stated that mortalities in sheep infected with *T. congolense* should be viewed with caution and should be reported only when intercurrent infection can be ruled out.

In the annual report of the Department of Veterinary Services, Rhodesia, (1974) it was observed that sheep suffered high incidences of *T. congolense* infections and that death in these animals was frequently complicated by secondary infections, of which bacterial pneumonia was most common. These observations tend to confirm the suggestion that *T. congolense* infections alone, may not have been the cause death of sheep No. 126.

Clone GRVPS 92/3 tended to be more infective and virulent than GRVPS 3/2 and GRVPS 57/6, in the present study. However, this is stated cautiously bearing in mind that only two animals were used in each infection group. The observation of differences in virulence among stocks of *T. congolense* is in agreement with the results of Jenkins *et al.*(cited by Seed and Hall, 1985) in rabbits infected with *T. congolense* stocks. These authors observed that GAMB 19 and TSW 99/77 produced a chronic infection while S104 produced an acute infection with shorter survival periods. They also observed that stocks associated with higher parasitaemia caused higher degree of anaemia. In the present investigation, GRVPS 92/3 appeared to produce more intense parasitaemia during the first 4 weeks of infection while parasitaemia rose and fell many times with GRVPS 3/2 and GRVPS 57/6. The resulting anaemia appeared to be related to the intensity of parasitaemia, however the differences were not marked.

Virulence has been largely attributed to the capacity of different strains of the parasite to generate biologically active molecules, to activate complement or to undergo antigenic variation (Murray *et al.*, 1982). It has been observed that anaemia that follows trypanosome infection is related to the intensity of parasitaemia which is controlled by the growth capacity of the parasite and its ability to stimulate antibody response (Roelants and Pinder, 1984). These immune reactions control the height of parasitaemia and cause wave remissions. Another possible aspect of trypanosome virulence was proposed by Sacks and

Askonas (1980) who found that differences in virulence of *T. brucei* strains were directly associated with the capacity to induce immunosuppression. They reported that the severity of trypanosome-induced suppression of anti-trypanosome response and Ig M response in particular, forms the basis of parasite virulence and determines to a large extent, the course and outcome of infection in the parasitised host. This implies that those trypanosomes which are able to induce severe immunosuppression would impair the ability of the host to limit parasite growth and multiplication, with consequent high levels of parasitaemia and severe anaemia.

The present study has demonstrated that different stocks of *T. congolense* induce different leucocyte responses in sheep. While GRVPS 3/2 and GRVPS 57/6 had little effect on leucocyte and lymphocyte values, except in one sheep that died in which a leucopaenia was observed, GRVPS 92/3 caused a marked leucocytosis and lymphocytosis. It has been suggested that *T. congolense* infections in mice are associated with non specific polyclonal activation of splenic lymphocytes (Morrison *et al.*, 1978) and that relatively large quantities of non-esterified fatty acids (mostly stearic, linoleic, palmitic and oleic acids) which are mitogenic to spleen lymphocytes, are generated by autolysing trypanosomes (Assoku *et al.*, 1977; Tizard *et al.*, 1978b, c). The differences in leucocyte responses observed in the three clones of *T. congolense*, used in this study, may be related to differences in their capacities to generate potent B-cell mitogens to which the host spleen responds.

The observation of decreases in blood lipids of infected animals, particularly cholesterol and phospholipids supports the results obtained in previous studies (Chapter 3 and 5). However, changes in plasma cholesterol and phospholipid concentrations in the animals infected with the three clones of *T. congolense* were similar. Roberts *et al.* (1977) suggested that changes in blood

lipids of sheep infected with *T. congolense* were associated with the species and strain of infecting trypanosome. This could not be ascertained, in the present study, because of the small number of animals used in each infection group and because the pathogenicities of the three clones of trypanosomes were not markedly different.

Plasma albumin concentrations decreased in all infected animals, tending to be more severe in those infected with GRVPS 92/3. This observation is in agreement with reports of a close association between parasitaemia and severity of infection, and the degree of hypoalbuminaemia.

In conclusion, this study has demonstrated that different clones of *T. congolense* can produce different pathophysiological responses in sheep, however the pathogenicities of the three clones used were not markedly different.

CHAPTER 7

THE INFLUENCE OF DIETARY PROTEIN ON THE PATHOPHYSIOLOGY OF *TRYPANOSOMA CONGOLENSE* INFECTION IN SCOTTISH BLACKFACE SHEEP

INTRODUCTION

Ruminant animals show considerable variation in their susceptibility to parasitic infections and nutritional status of the host has been suggested as one of the possible causes of this variation (Murray and Dexter, 1988). Much of the work on the interaction between nutrition and pathogenicity of parasite infections in ruminant animals has been conducted in sheep experimentally infected with helminths. For example, Abbott *et al.* (1985) observed that lambs given a low protein diet, during *Haemonchus contortus* infection, developed a higher degree of anaemia, gained less weight and experienced a higher mortality rate than lambs given a high protein diet. Similar observations were made by Berry and Dargie (1976) in sheep infected with *Fasciola hepatica.*

In most tsetse-infested areas, livestock rearing depends largely on extensive grazing of poor, sparse natural grasslands with no concentrate supplementation and it is often observed that trypanosomiasis becomes more severe in cattle at times of the year when they are under nutritional stress, for example, during the months of the dry season (Agyemang *et al.*, 1992). Under such conditions, deficiencies of protein are common and it is possible that reduced protein intake may have a part to play in the increased severity of infection.

It has been demonstrated that members of the family Trypanosomatidae, including African trypanosomes, are capable of metabolising the aromatic amino acids, especially tryptophan, tyrosine and phenylalanine (Seed and Hall, 1985). The consequence of this, is the production of potentially toxic compounds and a marked decrease in the serum concentrations of these amino acids especially tyrosine and tryptophan (Newport *et al.*, 1977). It is therefore possible that specific nutritional deficiencies of these essential amino acids can have deleterious effects on the host.

Apart from the general belief that poor-fed animals are more prone to parasitic infections than well-fed ones, there are no reports of properly controlled experiments to investigate the interaction between protein intake and pathogenicity of trypanosomiasis. The first part of this study therefore aimed at providing this information.

Considering the available control methods, chemoprophylaxis and chemotherapy appear to be the main methods of controlling animal trypanosomiasis in endemic areas (Trail *et al.*, 1985) and the effect of host nutrition on response to chemotherapy needs to be evaluated. Such information would be helpful if benefits from these control programmes were to be enhanced, for example, by strategic chemotherapy during periods when animals are not under nutritional stress. The second part of this study investigated the response of infected sheep on differing dietary protein levels to treatment with a trypanocidal drug, isometamidium chloride.

MATERIALS AND METHODS

Experimental Animals

Male castrate, six month old Scottish Blackface lambs were used in this study. They were purchased from a local hill farm in the west of Scotland.

Feeding and Housing

The basic diet consisted of a mixture of shredded sugar beet pulp (SBP), barley siftings and a mineral/vitamin/sheep trace element mixture. Soya bean meal (SBM) was added to the high protein diet only. The proportions of the components of both diets are presented in Table 7.1 and the mean proximate analyses of the two

Table 7.1

Composition of the high protein (HP) and low protein (LP) experimental diets (gkg^{-1} fresh matter)

	HP	LP
Sugar beet pulp	435	652
Barley siftings	326	326
Soya bean meal (SBM)	217	-
Vitamin/mineral/trace elements mixture	22	22

diets are shown in Table 7.2. The diets were made up by hand in bulk each fortnight and were fed ready-mixed. Samples of the diet components were taken each time the feed was mixed, for the appropriate analyses.

The lambs were housed in individual pens with a concrete floor and wood shavings were used as bedding. The daily allowance of 1 kg fresh matter (FM) was offered in two feeds at 9.00 and 16.00 h. The residue was collected the following morning, and weighed to determine the actual amount of feed consumed.

Five litres of water were offered each morning and the left over was also measured the following morning to give the amount of water consumed.

Requirements for a 25 kg lamb growing at about 120 gd^{-1} given a complete diet with an M/D ratio (number of MJ of metabolisable energy [ME] per kg dry matter [DM]) of about 10 are 9 MJME (MAFF *et al.* 1984) and 65 g digestible crude protein (DCP) (MAFF, 1980). A daily allowance of 1 kg fresh matter high protein diet supplied 9.8 MJME and 116 g DCP while the same quantity of LP diet supplied 10.1 MJME but only 51.5 g DCP. Therefore, 1 kg fresh matter per day of low protein diet supplied adequate energy but suboptimal amount of protein for growth of about 120 gd^{-1} while the high protein diet supplied more protein than is required for the daily weight gain.

Experimental Infection

Trypanosomes

After 4 weeks on experimental diets, six sheep from the low protein group and six from the high protein group were infected with *T. congolense* 1180 (GRVPS 57/6). The origin of this clone has been described (Chapter 2).

Table 7.2

Mean proximate analyses and calculated metabolisable energy of the high protein (HP) and low protein (LP)experimental diets (gkg^{-1} DM)

	HP	LP
Dry matter (gkg^{-1})	880	880
Crude protein	176	81
Crude fibre	169	192
Ether extract	5	4
Ash	77	80
Organic matter	923	920
ME ($MJkg^{-1}$ DM)	9.8	10.1

Preparation of inoculum and infection of animals

Four irradiated female CD 1 mice were infected intraperitoneally with *T. congolense* (GRVPS 57/6) derived from a stabilate maintained in liquid nitrogen. On the first rising parasitaemia, the mice were anaesthetised and bled by cardiac puncture and the inoculum was prepared as previously described (Chapter 2).

Parasitological, Haematological and Biochemical Techniques

The techniques used to collect and analyse samples for parasitaemia, haematological and blood biochemical parameters have been described (Chapter 2).

Treatment of Infected Sheep

After 70 days of infection, three infected animals from the LP group and three animals from the HP group were injected with isometamidium chloride. 0.4 g of Samorin (Lot PA 0043) was dissolved in 20 ml of distilled water to give a 2% solution w/v. The sheep received 1 $mgkg^{-1}$ by the deep intramuscular injection in the hind leg.

Carcase Evaluation

Slaughter and preparation of samples

Lambs were killed by a captive bolt followed by immediate exsanguination via the large vessels in the neck. The carcases were dressed in the normal way and the viscera were discarded. Wet dressed carcases were weighed and allowed to set for 48 h at 4 ^{o}C. They were then halved longitudinally through the vertebrae and sternum and each half was sealed in polythene bags and stored at -40 ^{o}C for subsequent dissection and mincing for body composition analyses.

Dissection of the indicator joint

The best end neck (i.e 7th to the 10th rib) joint was selected for dissection. This was a modification of the method of Kempster *et al.* (1976) who showed that the composition of the shoulder (i.e 7th to the 12th rib) joint was a reliable indicator of carcase composition. One half of the carcase was allowed to thaw and the joint was removed and weighed. It was dissected into muscle, fat and bone immediately to avoid excessive loss of moisture by evaporation. The bones, muscle and fat were then weighed to the nearest gram and the composition expressed as relative percentages.

Preparation of minced samples

One half of each carcase previously stored at -20 ^{O}C was allowed to thaw overnight. It was then weighed and sawed into small portions not exceeding 15x10x10 cm. An industrial machine (Model F46: Karl Scnell GMBH & Co., Maschinefabrik, 7065 Winterbach, Austria) was used to reduce the portions to a uniform mince. The samples were minced twice, first through a plate with 15 mm perforations and then through one with 5mm perforations. The machine was cleaned between samples to ensure that there was no carry-over between samples.

The minced samples were mixed well and subsamples were collected. Duplicate aliquots of about 500 g each were obtained and stored at -20 ^{O}C for subsequent freeze drying and chemical analysis.

Chemical analyses of minced samples

About 200 g of frozen carcase mince from each sheep were dried to a constant weight in a high vacuum freeze drier (Edwards EFO3 Freeze dryer: Edwards High Vacuum, Crawley, England). The dried samples were finely chopped in a liquidiser (ATO-MIX, M.S.E.) and subsamples of this were analysed for

protein, energy, ether extract and ash using methods previously described (Chapter 2). The samples were analysed in duplicate and the mean result was expressed as g (or MJ) kg^{-1} of the original sample.

Calculation of total carcase composition

The quantities of each fraction in the original carcase (c) were calculated. For example total carcase gross energy (TCGE) was calculated as follows:

$$TCGE\ (MJ) = GE_c\ (MJ[kgDM]^{-1} \times DM_c \times \text{carcase weight (kg)}$$

The total carcase protein (TCP), fat (TCEE), and ash were calculated in a similar way.

Chemical Analyses of Feed

Representative samples of the experimental diets were analysed periodically using standard procedures (MAFF *et al.*, 1981). The details of the analytical procedures have been described (Chapter 2).

Experimental Design

Twenty four animals were involved in this study. They were divided into two groups on the basis of their liveweights and PCV, and introduced to either a low protein (LP) or a high protein (HP) diet. After 4 weeks on respective diets, three animals from each group were killed and their carcases frozen for future analysis of body composition to give baseline data. At the same time six animals from the low protein group (LPI group) and six animals from the high protein group (HPI) were

infected with *T. congolense* while three animals from each dietary group acted as uninfected controls (LPC and HPC). The findings reported in results I relate to the observations made between 0 and 70 DAI.

Seventy days after infection three animals from the HPI group and three animals from the LPI group were treated with isometamidium chloride, and the animals were monitored for a further 21 days. The observations made after 70 DAI are described in results II.

RESULTS I

Parasitological Findings

Changes in mean parasitaemia of infected groups of animals are shown in Figure 7.1. Apart from one sheep in the HPI group which showed detectable parasitaemia 5 DAI, the prepatent period ranged from 7 to 9 days. In the HPI group, the parasitaemia score reached the first peak of 3.7 ± 0.3 at 14 DAI. In the LPI group, the first peak of 3.3 ± 0.4 was reached at 16 DAI. Thereafter, the mean parasitaemia fluctuated considerably in both groups of sheep but the intensity of parasitaemia was significantly higher in the HPI group than in the LPI group. The overall mean parasitaemia score between 0 and 70 DAI was 2.5 ± 0.2 in the HPI group and 1.9 ± 0.1 in the LPI group. Although these differences were small, they were significant. The uninfected control sheep remained aparasitaemic throughout the period of the investigation.

Feed Intake

Animals were introduced to experimental diets four weeks before infection with *T. congolense* to give them time to adapt to the diets. During the course of the experiment, it was observed that one infected sheep in the high protein group and three infected sheep in the low protein group did not consume all the feed given to

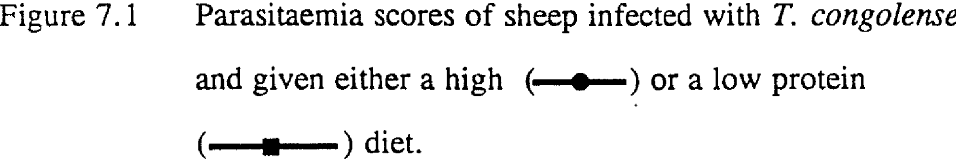
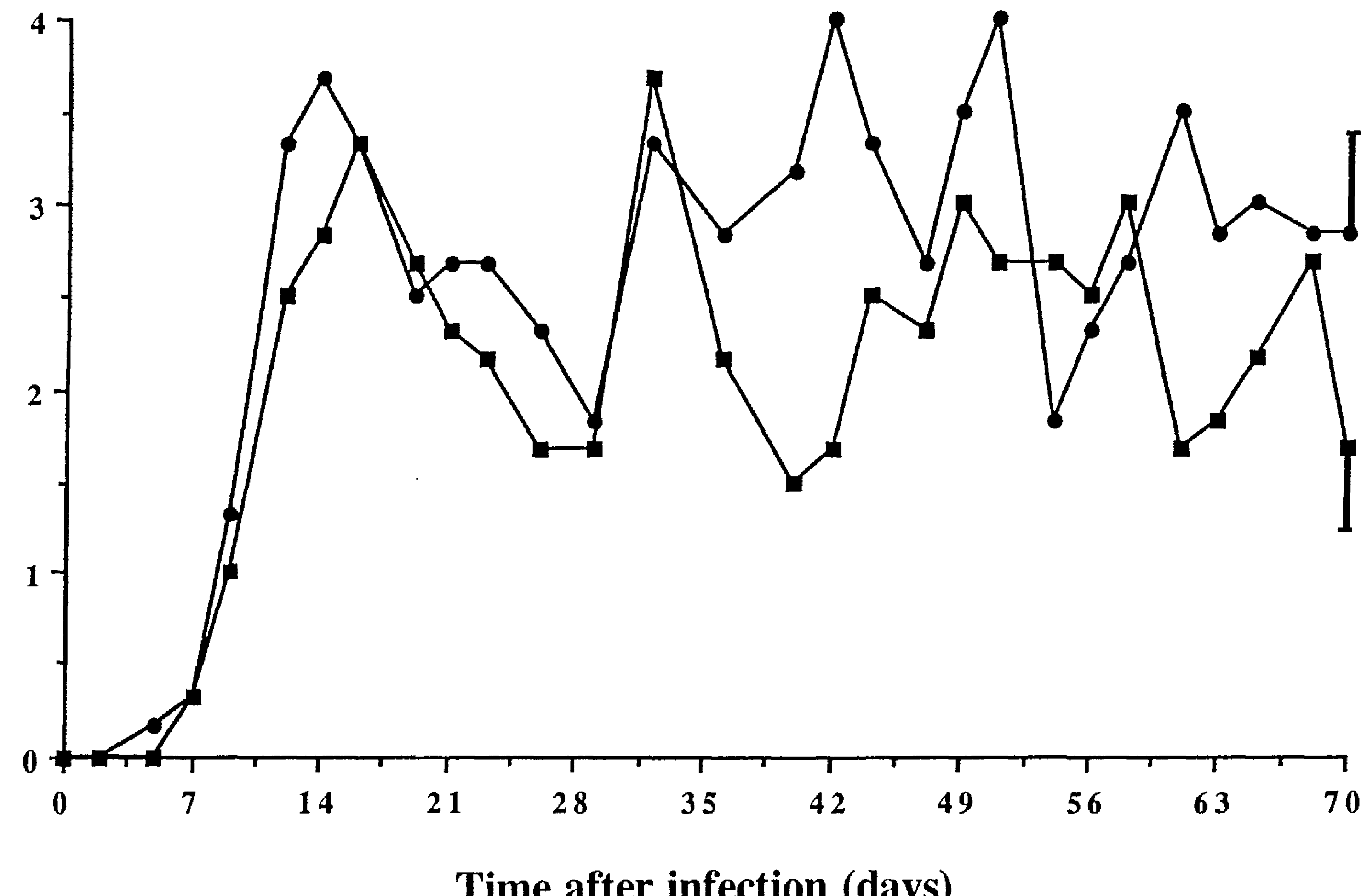


Figure 7.1 Parasitaemia scores of sheep infected with *T. congolense* and given either a high (—●—) or a low protein (—■—) diet.


Parasitaemia score
0
1
2
3
4
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

them for most of the period of observation. As a result, the mean feed intake of infected sheep receiving low protein diet (0.93 ± 0.03 kgd^{-1}) was lower than that of infected sheep on a high protein diet (0.98 ± 0.02 kgd^{-1}) between 0 and 70 DAI (Table 7.3). However, this difference did not reach a level of significance. Control animals on both dietary treatments consumed all the feed offered to them.

It was also observed that, between 0 and 70 DAI, the mean water intake of infected sheep on a low protein diet (2.10 ± 0.07 ld^{-1}) was significantly lower than that of infected sheep on a high protein diet (2.51 ± 0.10 ld^{-1}). Mean water consumption in control animals was the same, i.e, 2.31 ± 0.10 ld^{-1} for animals on a low protein diet and 2.30 ± 0.11 ld^{-1} for those on a high protein diet

Body Weight Changes

Infection caused a significant retardation of growth in the low protein infected (LPI) group but not in the high protein infected (HPI) group. In the HPI group, live body weight increased from 29.1 ± 0.8 at 0 DAI to 37.7 ± 1.2 kg at 70 DAI (Figure 7.2). In the LPI group, the mean body weight increased from 28.2 ± 0.6 at 0 DAI to 33.0 ± 1.1 kg at 70 DAI. These changes in body weight were significant between the HPI and LPI groups and between the LPC and LPI groups. In the low protein control (LPC) group, the mean weight increased from 28.5 ± 0.6 to 36.0 ± 1.3 and in the high protein control (HPC) group it increased from 28.3 ± 0.5 to 38.0 ± 1.2 between 0 and 70 DAI. There were no significant differences between the HPC and the LPC groups.

Table 7.3

Mean (± sem) feed and water intake of sheep infected with *Trypanosoma congolense* and of uninfected control sheep given either a low or a high protein diet between 0 and 70 days after infection

	Low protein			High protein		
Group	**Sheep no.**	**Feed intake (kgd^{-1})**	**Water intake (ld^{-1})**	**Sheep no.**	**Feed intake (kgd^{-1})**	**Water intake (ld^{-1})**
Infected	124	0.79 ± 0.02	1.66 ± 0.09	127	0.89 ± 0.02	1.99 ± 0.03
	130	0.88 ± 0.02	1.77 ± 0.07	131	1.00 ± -	2.71 ± 0.09
	138	0.92 ± 0.01	2.04 ± 0.09	133	1.00 ± -	2.61 ± 0.07
	144	1.00 ± -	2.29 ± 0.07	140	1.00 ± -	2.89 ± 0.09
	146	1.00 ± -	2.29 ± 0.07	149	1.00 ± -	2.65 ± 0.09
	148	1.00 ± -	2.57 ± 0.07	151	1.00 ± -	2.51 ± 0.10
	Mean ± sem	**0.93 ± 0.03**	**2.10 ± 0.07**		**0.98 ± 0.02**	**2.51 ± 0.10**
Control	123	1.00 ± -	2.10 ± 0.11	132	1.00 ± -	2.38 ± 0.08
	150	1.00 ± -	2.39 ± 0.10	142	1.00 ± -	2.08 ± 0.11
	152	1.00 ± -	2.45 ± 0.09	147	1.00 ± -	2.30 ± 0.11
	Mean ± sem	**1.00 ± -**	**2.31 ± 0.10**		**1.00 ± -**	**2.30 ± 0.11**

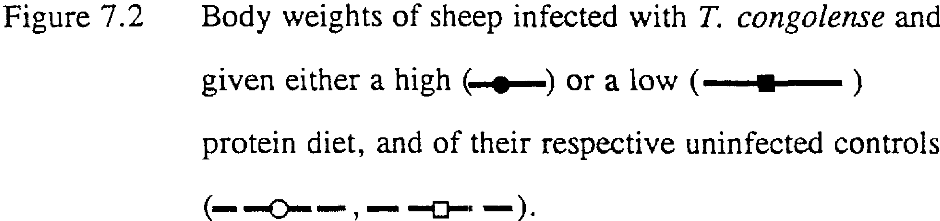
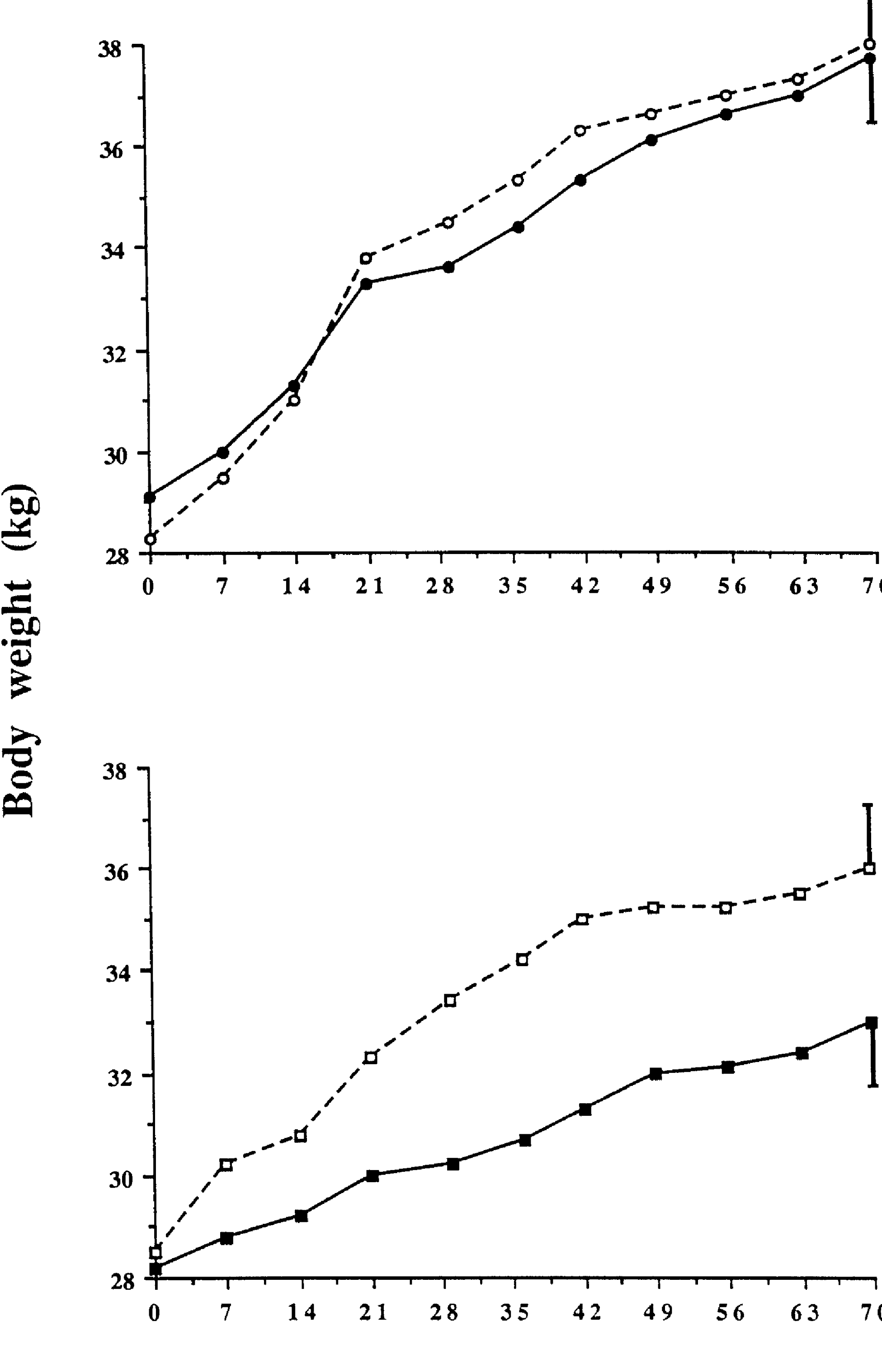


Figure 7.2 Body weights of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (– –○– –, – –□– –).


Body weight (kg)
38
36
34
32
30
28
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Haematological Findings

Packed cell volume (PCV)

Infection caused significant decreases in PCV of both infected groups compared to their controls. In the HPI group, the mean PCV decreased from 0.37 $\pm$ 0.01 at 0 DAI to 0.28 $\pm$ 0.01 ll^{-1} at 23 DAI (Figure 7.3). It then fluctuated but decreased to its lowest level of 0.26 $\pm$ 0.02 at 70 DAI. The mean PCV in the LPI group decreased from 0.35 $\pm$ 0.01 ll^{-1} at day 0 to 0.27 $\pm$ 0.01 ll^{-1} at 21 DAI (Figure 7.3). It then increased moderately to 0.30 $\pm$ 0.01 at 29 DAI after which it decreased to its lowest value of 0.24 $\pm$ 0.01 ll^{-1} at 70 DAI. The PCV values in the control groups (LPC and HPC) fluctuated between 0.32 + 0.01 and 0.40 $\pm$ 0.01 ll^{-1}. These values are within the suggested normal ranges for lambs and there was no detectable effect of dietary protein on the recorded PCV values.

Red blood cell count (RBC)

Following the development of patent parasitaemia, the mean RBC decreased significantly in both infected groups compared to their uninfected controls. In the HPI group, the mean RBC values decreased from 12.4 $\pm$ 0.1 at 0 DAI to 7.5 $\pm$ 1.6 x $10^{12}l^{-1}$ at 68 DAI (Figure 7.4). Normoblasts were frequently observed in stained blood smears from two infected sheep in the HP group (Sheep No. 127 and 140). These sheep had the lowest RBC values in their group. The mean RBC, in the LPI group, decreased steadily from 12.3 $\pm$ 0.2 x$10^{12}l^{-1}$ at 0 DAI to 7.9 $\pm$ 0.4 x$10^{12}l^{-1}$ at 61 DAI. These values were significantly different from those of the LPC group but not the HPI group. In the control animals, the RBC values fluctuated between 11.2 $\pm$ 0.4 and 12.9 $\pm$ 0.5 x $10^{12}l^{-1}$ displaying no significant dietary effects.

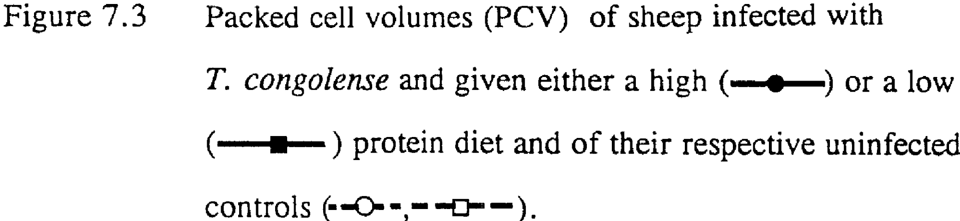
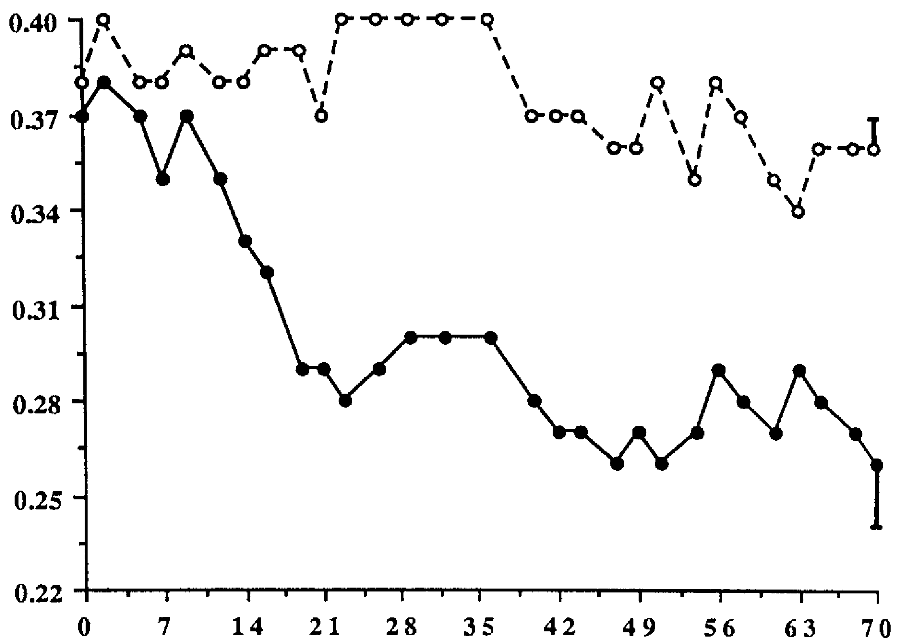
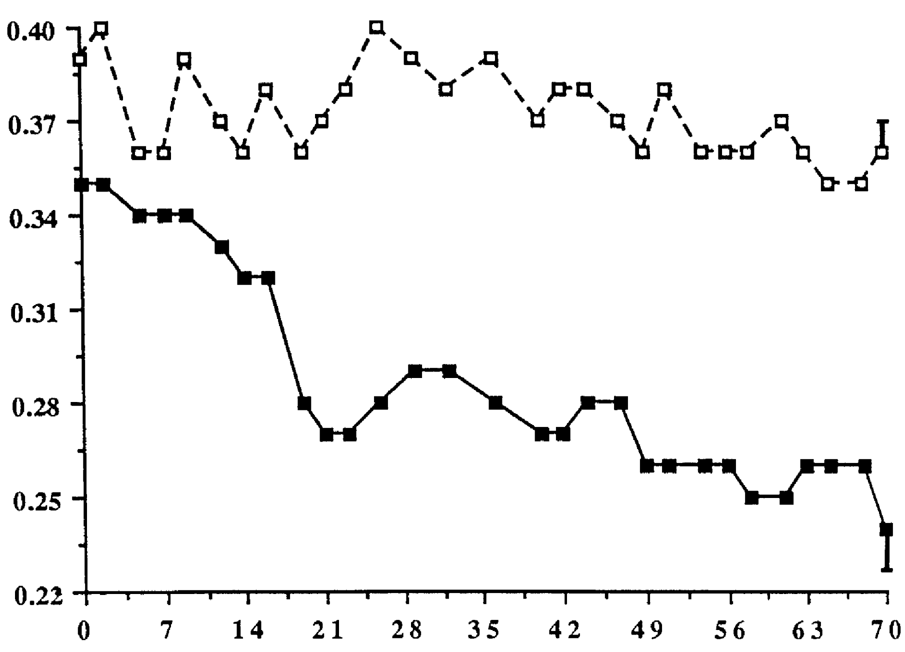


Figure 7.3 Packed cell volumes (PCV) of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (- -○- -, - -□- -).

PCV ($l\,l^{-1}$)





Time after infection (days)

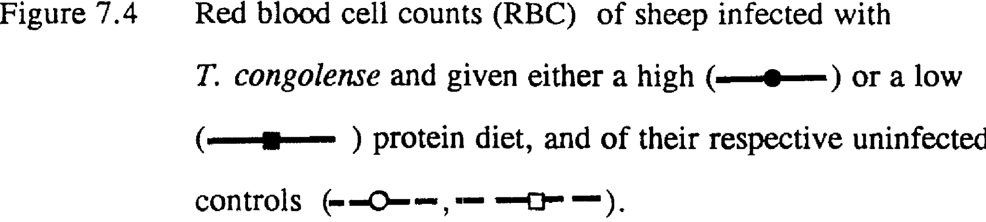
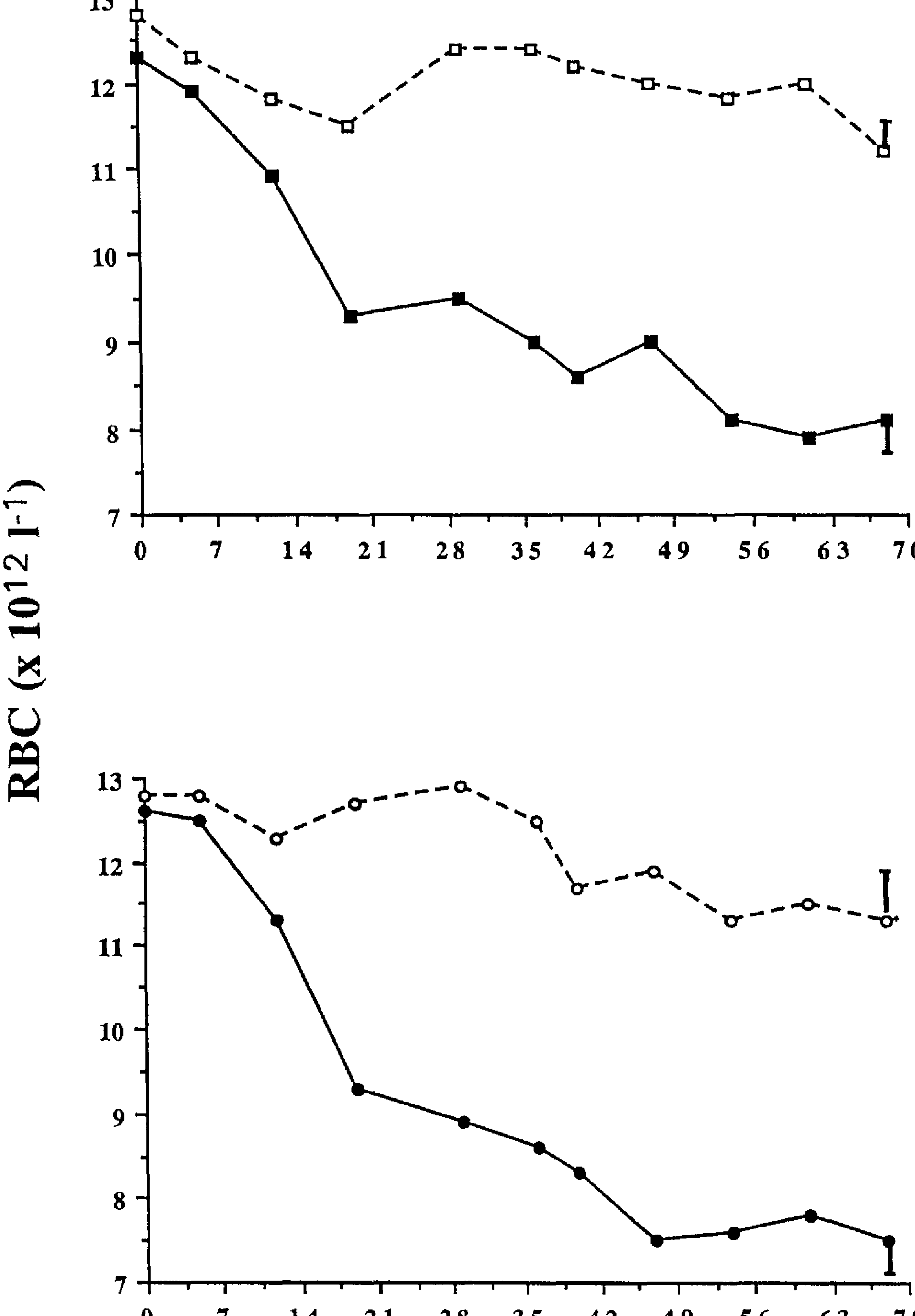


Figure 7.4 Red blood cell counts (RBC) of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (- -○- -, - -□- -).



RBC (x 10¹² l⁻¹)
13
12
11
10
9
8
7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Haemoglobin (Hb) concentration

The mean haemoglobin concentration showed a significant decrease in LPI and HPI groups compared to their uninfected control groups, and there were no significant differences between the infected groups. In the HPI group, the mean values decreased from 13.1 $\pm$ 0.3 gdl^{-1} at 0 DAI to 9.0 $\pm$ 1.2 gdl^{-1} at 47 DAI (Figure 7.5). It decreased from 12.5 $\pm$ 0.2 gdl^{-1} at 0 DAI to 8.7 $\pm$ 0.4 gdl^{-1} at 61 DAI in the LPI group. The mean values in the control groups fluctuated between 12.3 $\pm$ 0.6 and 13.8 $\pm$ 0.8 gdl^{-1}. The values in the HPC group were not significantly different from those of the LPC group.

Mean corpuscular volume (MCV)

Following infection both groups showed significant increases in MCV compared to their uninfected controls, but the increase was significantly greater in the HPI group in relation to the LPI group. In the HPI group, the mean MCV increased from 31.2 $\pm$ 0.3 fl at 0 DAI to 38.5 $\pm$ 1.2 at 29 DAI (Figure 7.6). The values then decreased moderately to 35.8 $\pm$ 1.2 at 47 DAI before increasing again to 36.8 $\pm$ 2.5 fl at 68 DAI. In the LPI group, the mean MCV was 31.0 $\pm$ 0.7 at the time of infection. The values then increased gradually to 35.0 $\pm$ 0.6 at 36 DAI before decreasing to 32.3 $\pm$ 0.7 at 68 DAI. The mean MCV values in control groups fluctuated between 30.0 $\pm$ 0.6 and 33.0 $\pm$ 0.6 fl. with no significant differences between the LPC and HPC groups.

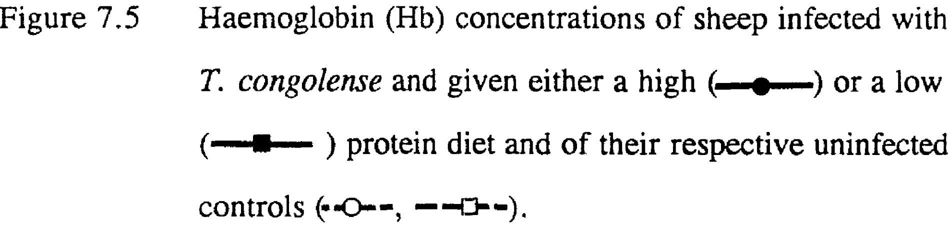
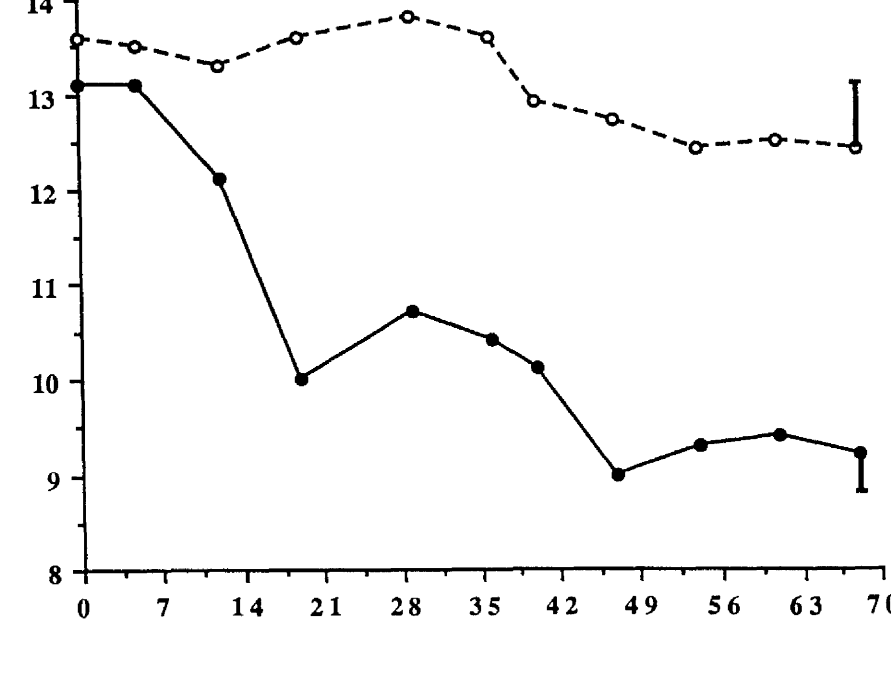
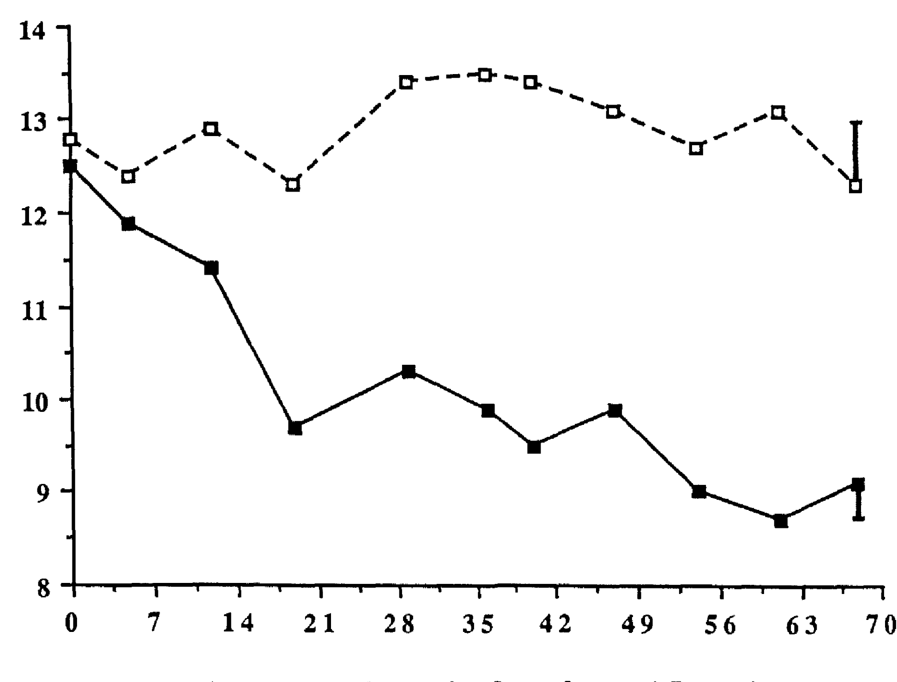


Figure 7.5 Haemoglobin (Hb) concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (--○--, --□--).



Hb (g dl^{-1})



Time after infection (days)

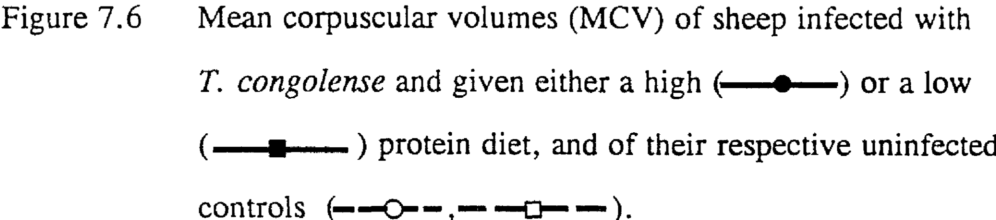
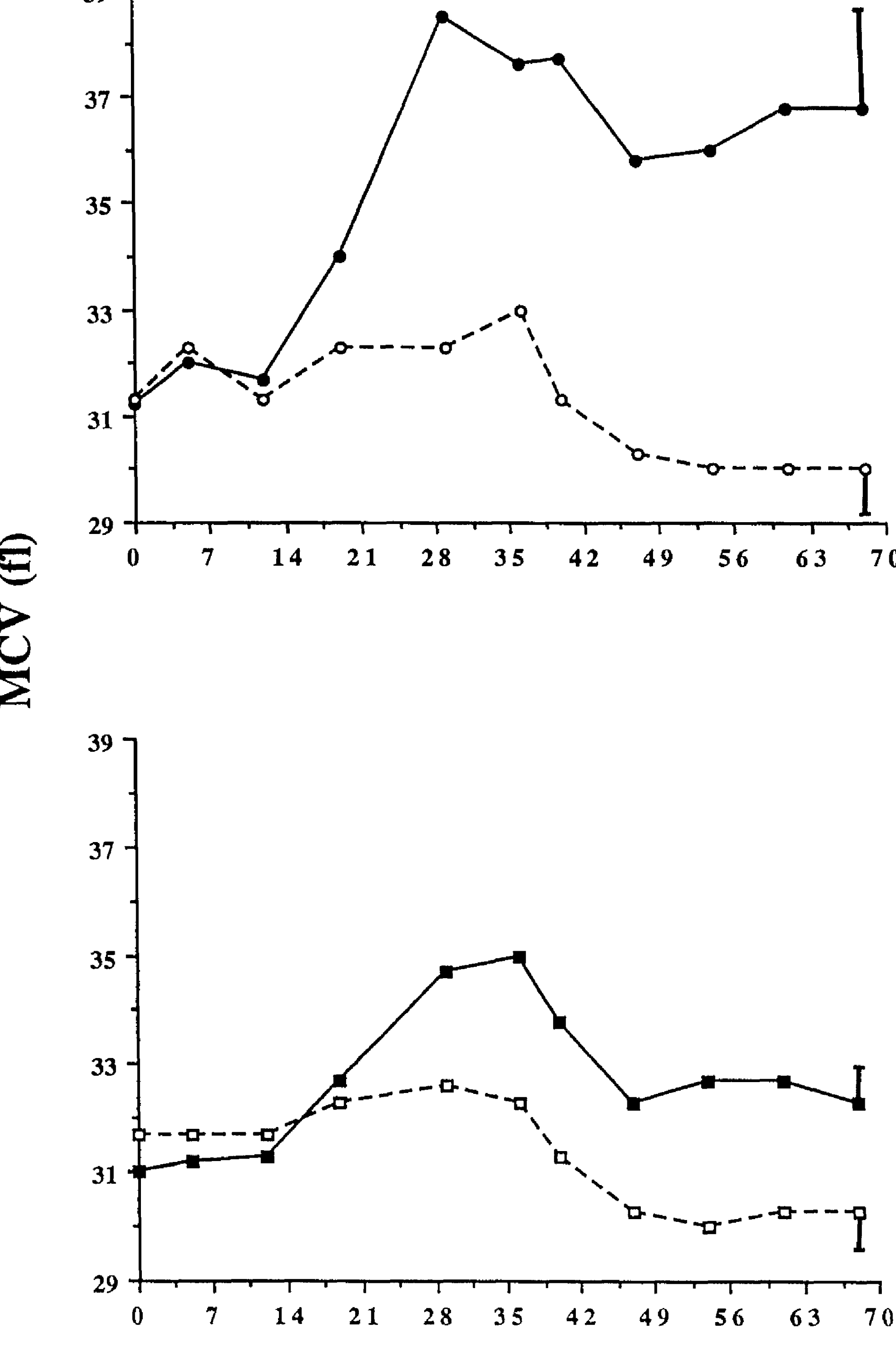


Figure 7.6 Mean corpuscular volumes (MCV) of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (--○--, --□--).



MCV (fl)
39
37
35
33
31
29
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Mean corpuscular haemoglobin (MCH)

The mean MCH showed a significant increase in the HPI group and not in the LPI group. In the HPI group, the values increased from 10.4 $\pm$ 0.2 at 0 DAI to 12.5 $\pm$ 0.7 at 68 DAI (Figure 7.7). In the LPI group, the MCH showed a fluctuating upward trend, from 10.2 $\pm$ 0.2 at 0 DAI to 11.3 $\pm$ 0.2 at 68 DAI. The values in the control groups remained within the normal ranges.

Mean corpuscular haemoglobin concentration (MCHC)

The mean MCHC values showed a fluctuating increase in all groups of animals (Figure 7.8). However, the values of infected groups were significantly lower than the values of control animals.

Total white blood cell count (WBC)

The mean WBC showed a significant increase in the LPI group, but tended to decrease in the HPI group. In the HPI group, the values increased initially from 9.0 $\pm$ 0.5 at 0 DAI to 10.1 $\pm$ 1.0 $\times 10^9 l^{-1}$ at 19 DAI before decreasing gradually to 6.5 $\pm$ 1.0 $\times 10^9 l^{-1}$ at 54 DAI (Figure 7.9). The values then tended to increase but remained much lower than those in the HPC group. The mean WBC values increased from 9.9 $\pm$ 0.7 at 0 DAI to 11.7 $\pm$ 1.3 $\times 10^9 l^{-1}$ at 47 DAI. Thereafter, the values fluctuated but remained much higher than the values in the LPC group. In the control groups, the mean WBC values fluctuated between 7.1 $\pm$ 0.8 and 10.9 $\pm$ 1.3 $\times 10^9 l^{-1}$, with a tendency to be higher in the HPC than in the LPC group.

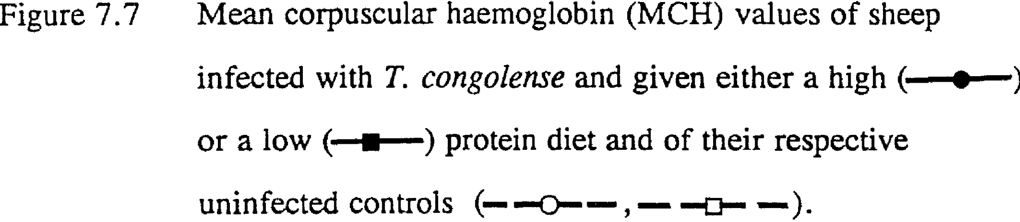
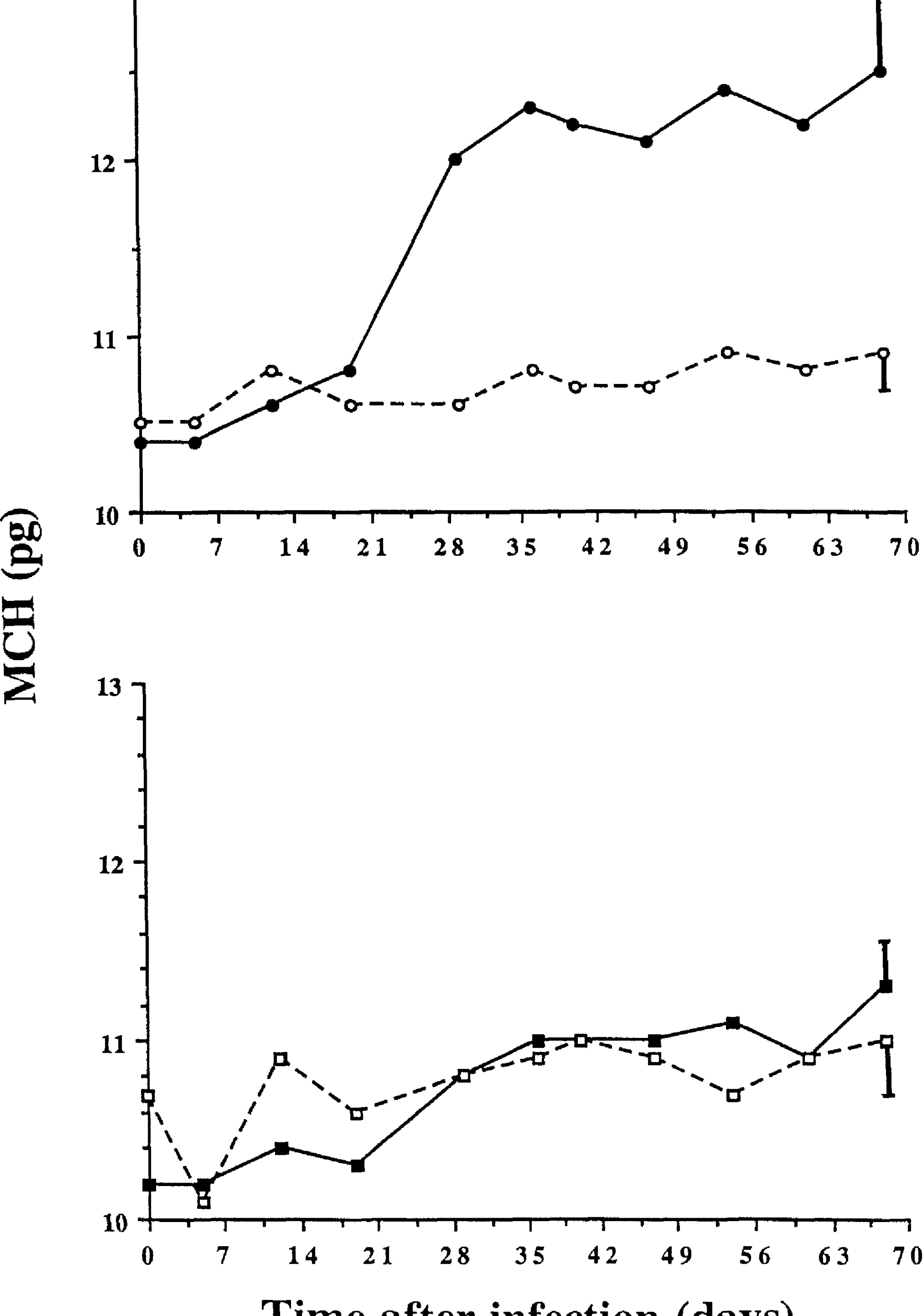


Figure 7.7 Mean corpuscular haemoglobin (MCH) values of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (– –○– –, – –□– –).


MCH (pg)
13
12
11
10
0
7
14
21
28
35
42
49
56
63
70
13
12
11
10
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

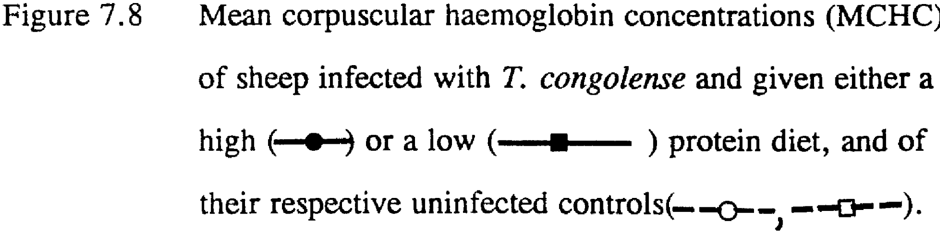
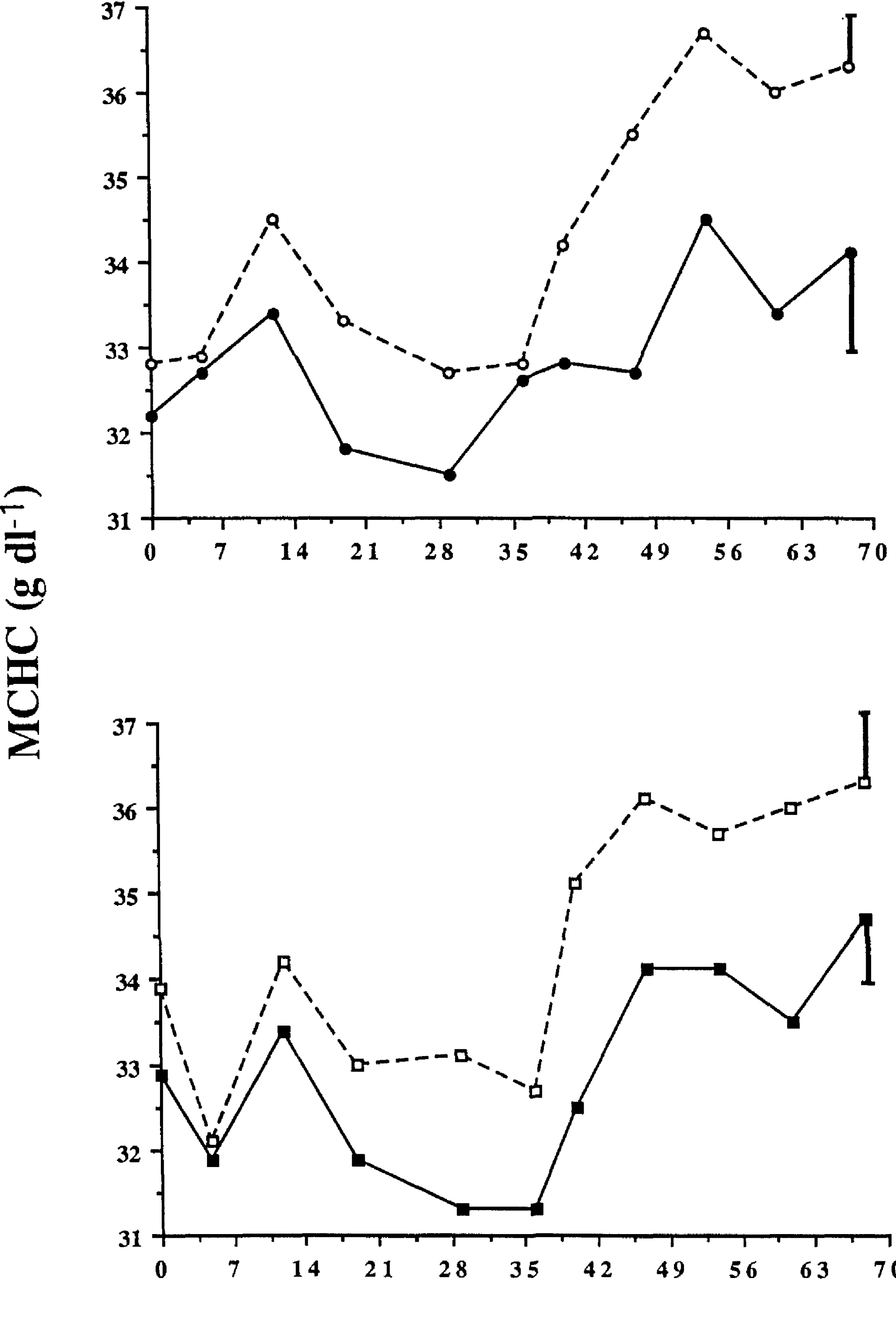


Figure 7.8 Mean corpuscular haemoglobin concentrations (MCHC) of sheep infected with *T. congolense* and given either a high (—●—) or a low (——■——) protein diet, and of their respective uninfected controls(– –○– –, – –□– –).


MCHC (g dl-1)
37
36
35
34
33
32
31
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

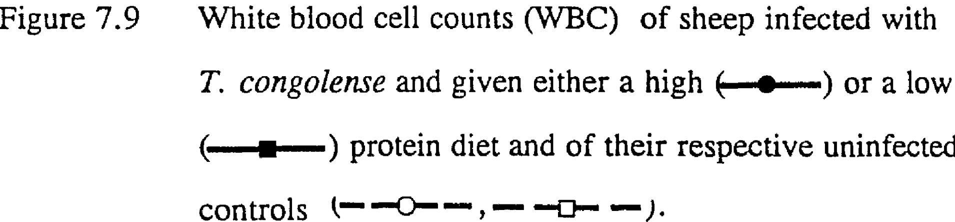
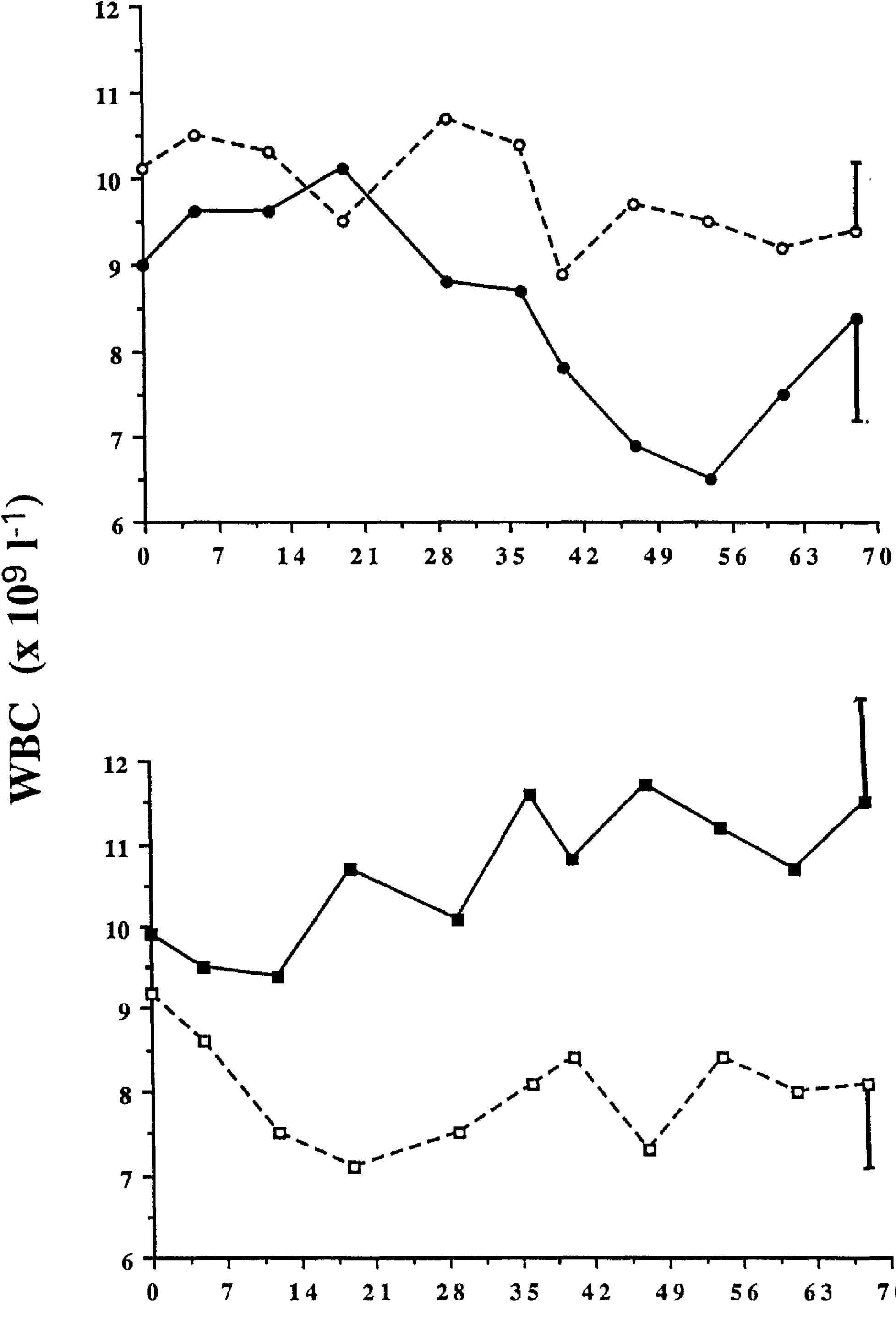


Figure 7.9 White blood cell counts (WBC) of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (– –○– –, – –□– –).


WBC (x 10^9 l^{-1})
12
11
10
9
8
7
6
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

Lymphocyte counts

The mean absolute lymphocyte counts followed almost a similar trend as that described for WBC. In the HPI group, the mean values increased from 6.8 $\pm$ 0.4 at 0 DAI to 8.3 $\pm$ 0.9 $x10^9l^{-1}$ at 19 DAI before decreasing to 5.1 $\pm$ 0.8 $x10^9l^{-1}$ at 47 DAI (Figure 7.10). The values then tended to increase. The mean values in the LPI group increased moderately from 7.2 $\pm$ 0.7 at 0 DAI to 10.1 $\pm$ 0.1 $x10^9l^{-1}$ at 47 DAI after which they showed a moderate decline. In control groups, lymphocyte counts fluctuated between 5.3 $\pm$ 0.4 and 6.8 $\pm$ 0.7 x 10^9l^{-1} in the LPC group, and between 7.1 $\pm$ 0.9 and 9.0 $\pm$ 1.6 x 10^9l^{-1} in the HPC group. These differences were not significant.

Platelet counts

Infection caused a significant decrease in platelet counts of both infected groups compared to their controls, but the values between infected groups were not significantly different. The mean values in the HPI group decreased from 249.0 $\pm$ 37.6 at 0 DAI to 105.5 $\pm$ 25.0 x 10^9l^{-1} at 12 DAI after which the values tended to increase (Figure 7.11). However there was a further decrease between 54 and 68 DAI. In the LPI group, the mean counts decreased from 282.3 $\pm$ 46.1 at 0 DAI to 84.2 $\pm$ 13.0 $X10^9l^{-1}$ at 19 DAI. The values fluctuated considerably thereafter, but showed a tendency to increase with a decline in the intensity of parasitaemia. The mean values in control groups varied from 218.7 $\pm$ 53.6 and 299.0 $\pm$ 31.5 x 10^9l^{-1} throughout the period of observation, and there were no significant dietary effects.

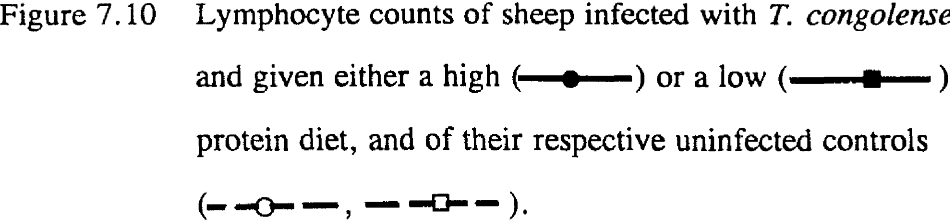
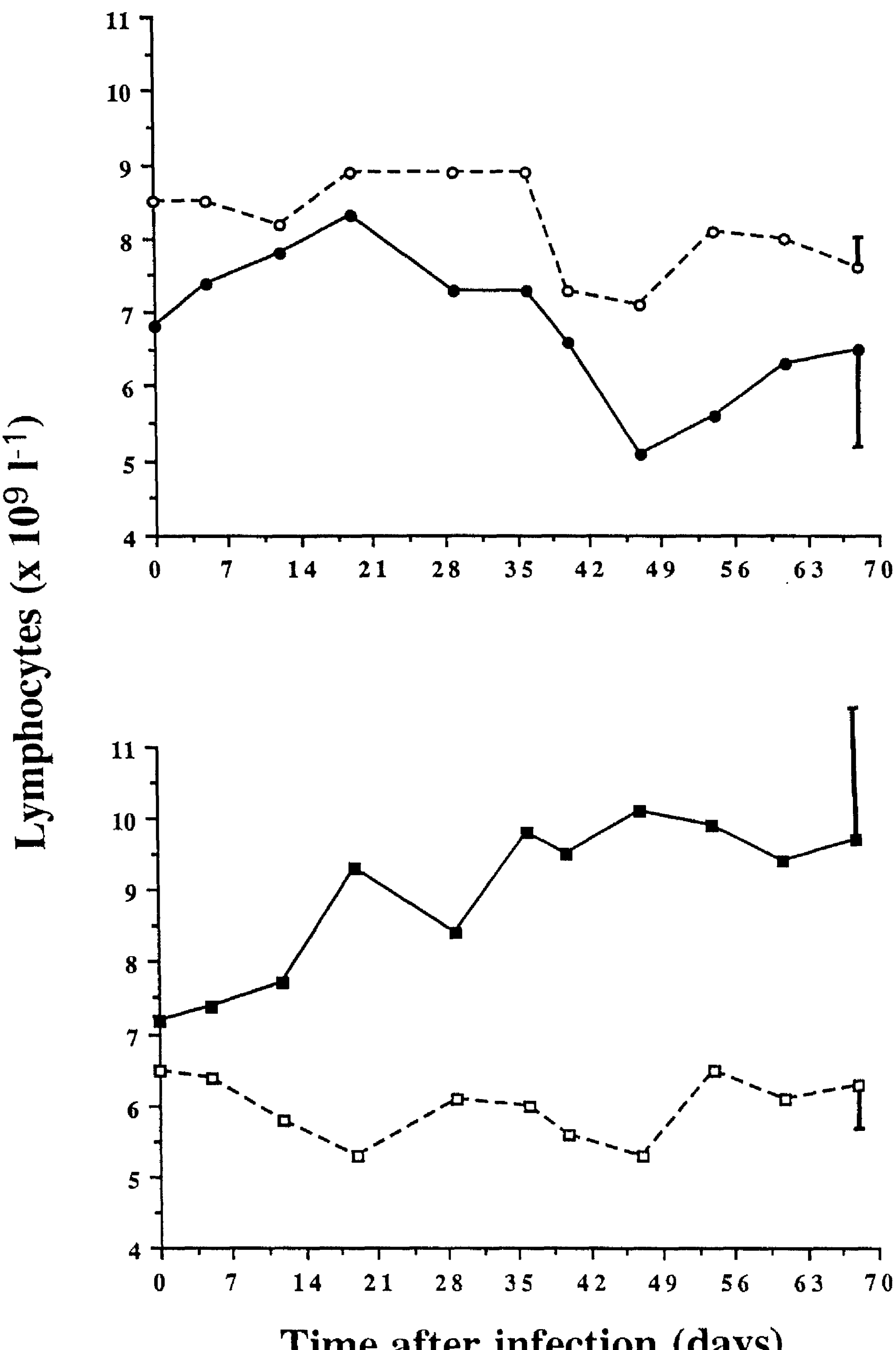


Figure 7.10 Lymphocyte counts of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (- -○- -, - -□- -).


Lymphocytes (x 10⁹ l⁻¹)
11
10
9
8
7
6
5
4
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

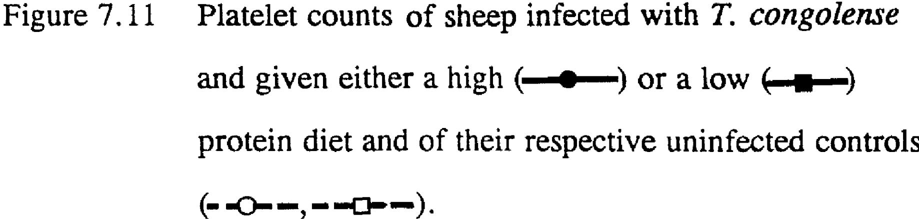
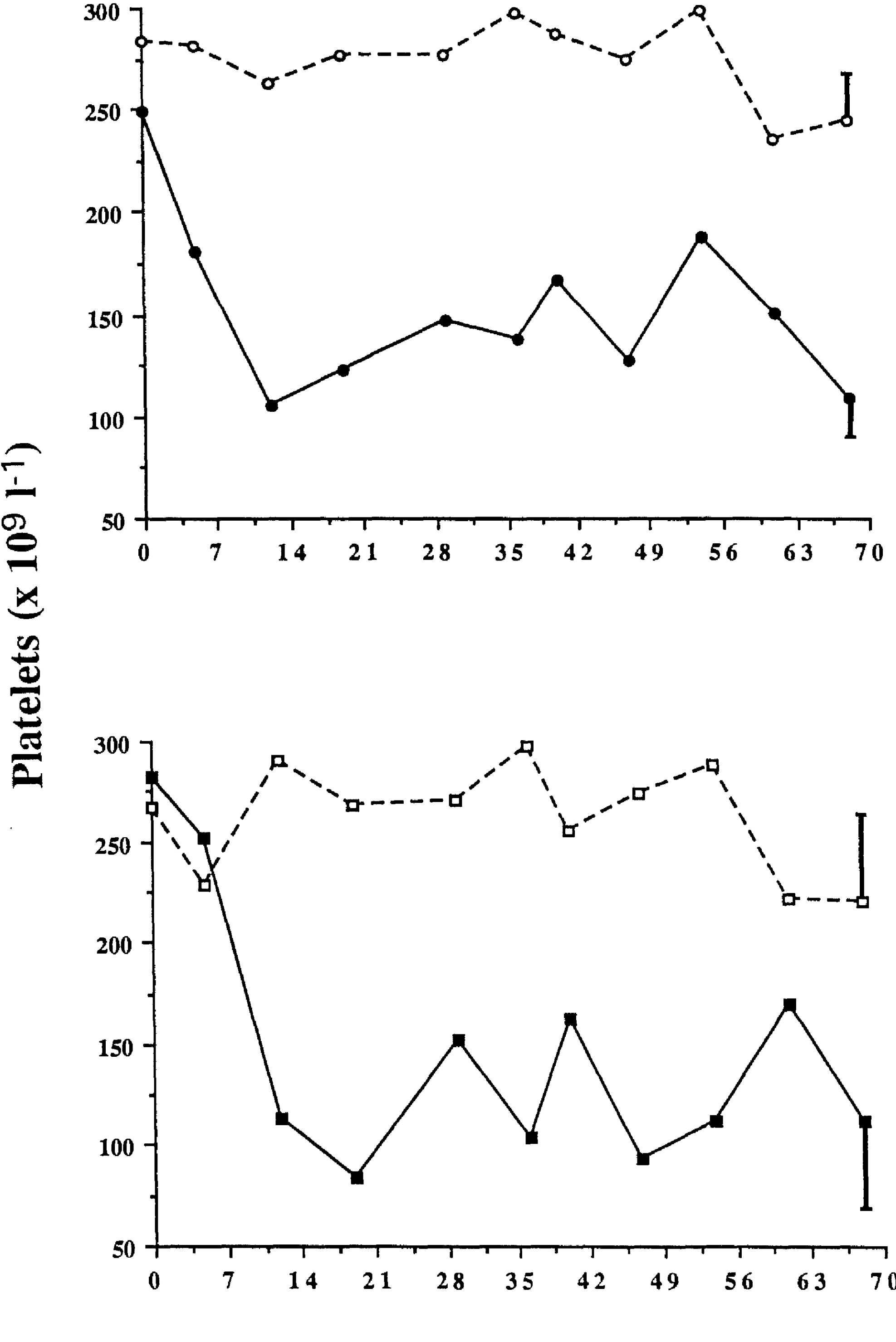


Figure 7.11 Platelet counts of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (- -○- -, - -□- -).



Platelets (x 10^9 l^-1)
300
250
200
150
100
50
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Biochemical Findings

Serum total lipid concentration

Infection and diet had significant effects on the concentrations of serum total lipids. In the HPI group, the mean serum total lipid concentration decreased from 2.77 ± 0.09 gl^{-1} at 5 days before infection to 1.02 ± 0.07 gl^{-1} at 21 DAI (Figure 7.12). The values then increased to 1.62 ± 0.22 gl^{-1} at 35 DAI before gradually decreasing to $1.00 + 0.17$ gl^{-1} at 70 DAI. In the LPI group, the mean values showed a downward trend decreasing from 2.41 ± 0.24 gl^{-1} at 5 days before infection to 0.92 ± 0.01 gl^{-1} at 21 DAI. The values then tended to increase but showed another decline between 56 and 70 DAI. The values in the LPI group were not significantly different from those in the HPI group. The concentrations of serum total lipids in the control animals showed a moderate fluctuation but over time, they were significantly higher in the HPC than in the LPC group.

Serum phospholipid concentration

Infection caused a significant decrease in mean phospholipid concentrations of both LPI and HPI groups compared to their controls, but the values in the LPI group were not significantly different from those of the HPI group. In the HPI group, the mean values decreased from 0.95 ± 0.02 gl^{-1} at 5 days before infection to 0.41 ± 0.02 gl^{-1} at 21 DAI (Figure 7.13). Thereafter, the values tended to recover but remained much lower than the values in the HPC group. In the LPI group, the serum phospholipid concentration decreased from 0.84 ± 0.06 gl^{-1} at 5 days before infection to 0.46 ± 0.04 gl^{-1} at 21 DAI after which the values tended to recover. The mean values in the control groups showed an initial decline up to 21 DAI after which they stabilised. There was no noticeable effect of dietary protein on serum phospholipid concentrations in control groups.

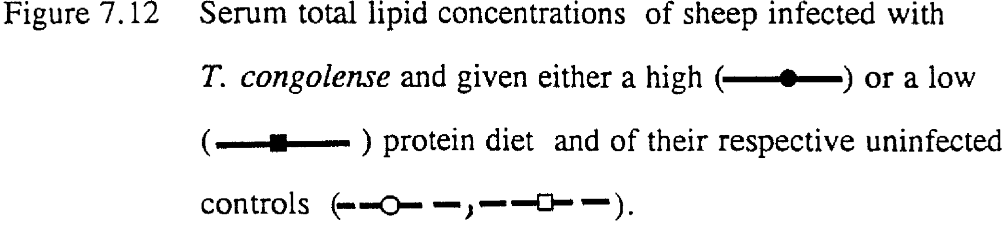
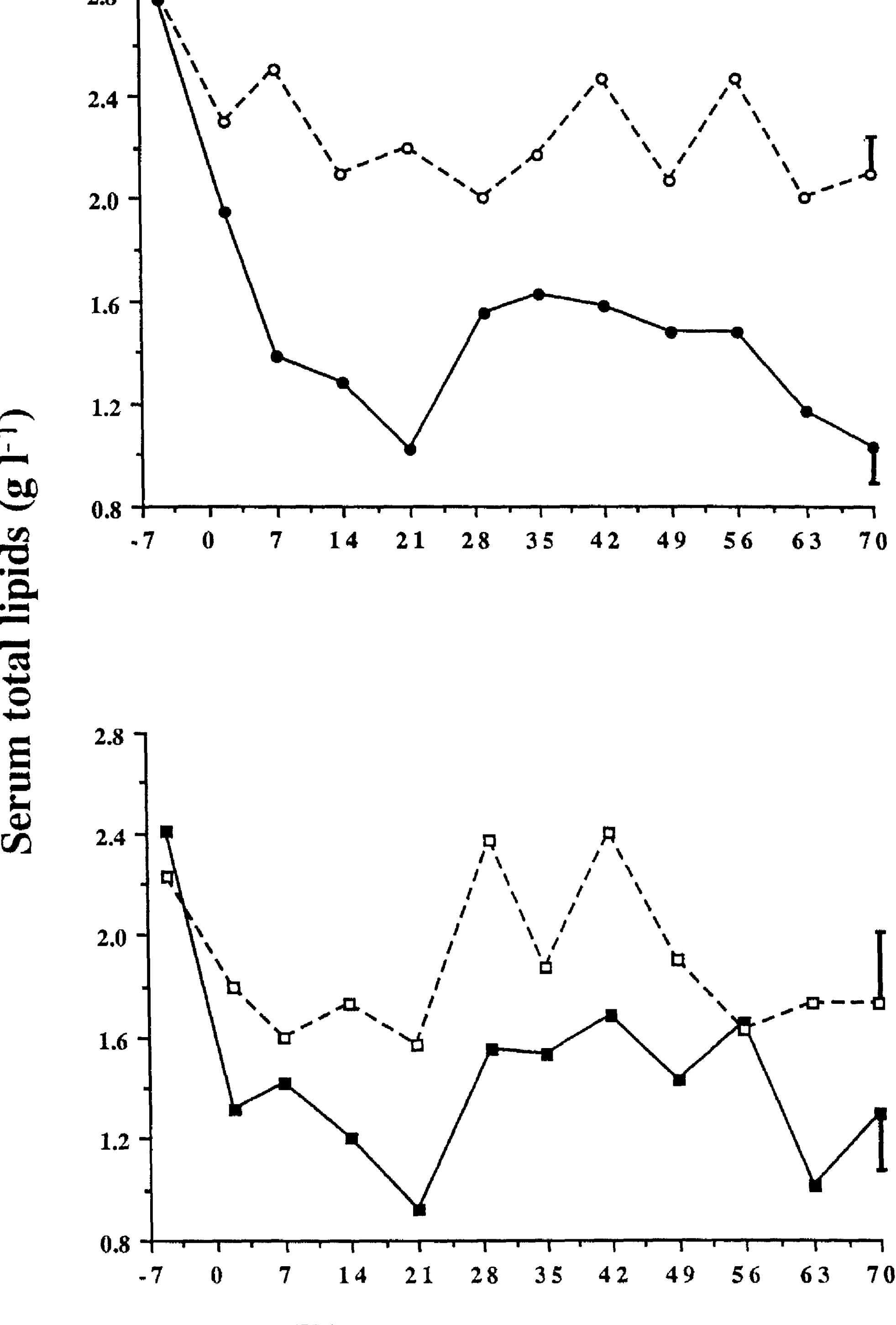


Figure 7.12 Serum total lipid concentrations of sheep infected with *T. congolense* and given either a high (——●——) or a low (——■——) protein diet and of their respective uninfected controls (– –○– –, – –□– –).



Serum total lipids (g l-1)
2.8
2.4
2.0
1.6
1.2
0.8
-7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

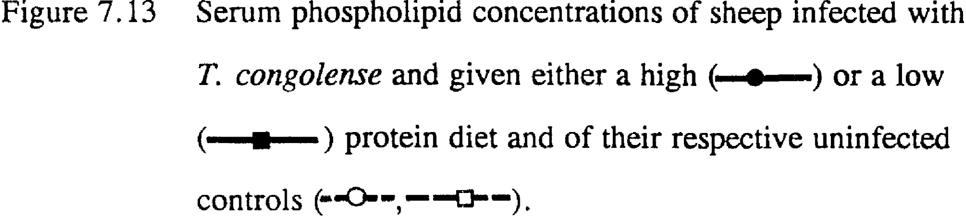
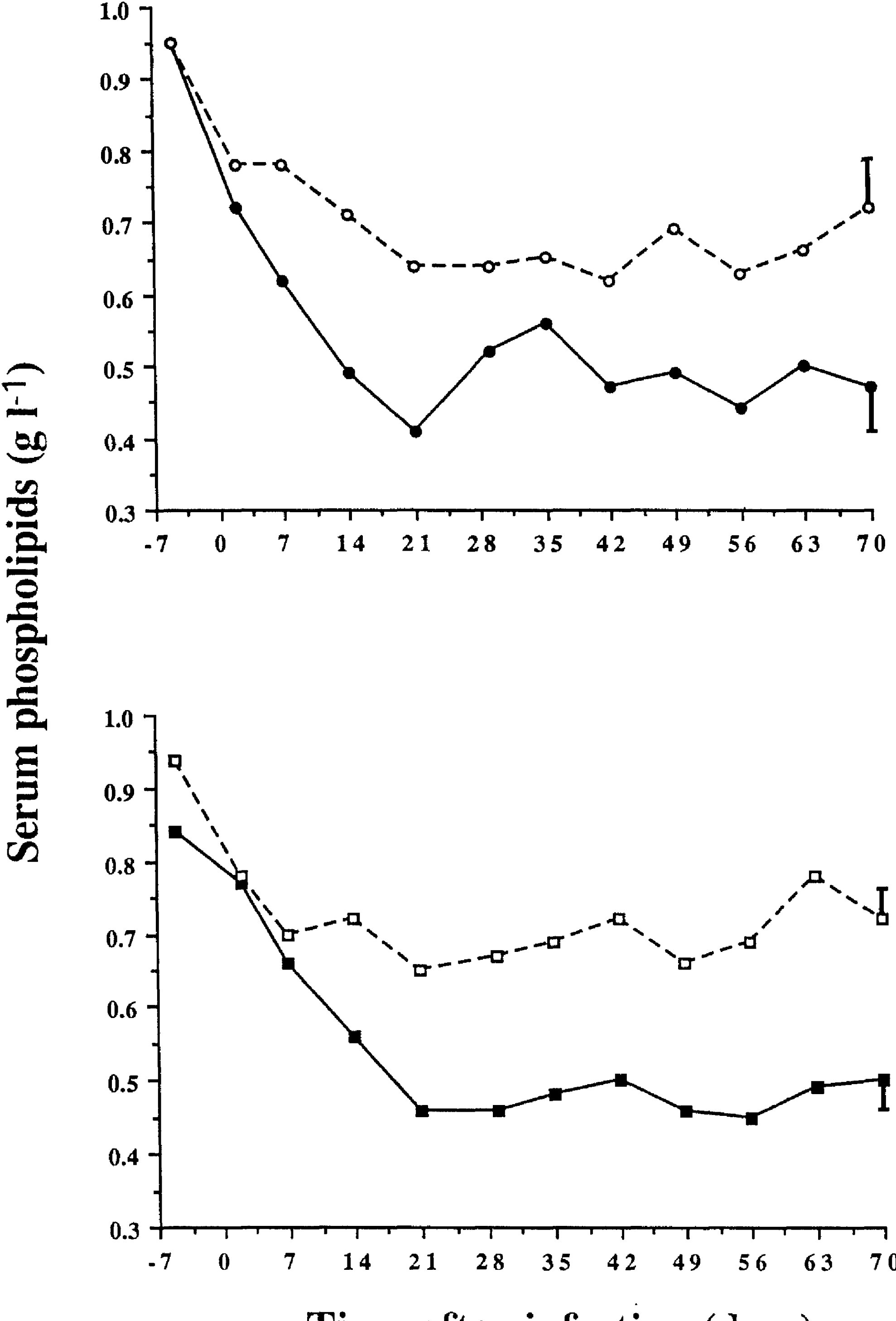


Figure 7.13 Serum phospholipid concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (--○--, --□--).



1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
-7 0 7 14 21 28 35 42 49 56 63 70
Serum phospholipids (g l-1)
Time after infection (days)

Plasma cholesterol concentration

Following infection, the plasma cholesterol concentrations of both infected groups showed a significant decline, and there were no differences between the infected groups. In the HPI group the values decreased from 0.73 $\pm$ 0.06 $mmoll^{-1}$ at 0 DAI to 0.53 $\pm$ 0.02 at 21 DAI (Figure 7.14). The values then increased moderately to 0.77 $\pm$ 0.11 at 36 DAI before decreasing to 0.56 $\pm$ 0.08 $mmoll^{-1}$ at 42 DAI.

In the LPI group, it decreased from 0.68 $\pm$ 0.08 $mmoll^{-1}$ at 0 DAI to 0.49 $\pm$ 0.05 $mmoll^{-1}$ at 21 DAI. It then increased to 0.65 $\pm$ 0.07 at 29 DAI before decreasing steadily to 0.44 $\pm$ 0.08 $mmoll^{-1}$ at 49 DAI. The values then showed a moderate improvement between 49 and 70 DAI. The mean cholesterol concentrations in HPC group showed a slight increase with time and remained higher than the values of the LPC group, particularly after 35 DAI. However, this difference was not significant.

Plasma non-esterified fatty acids (NEFA)

Infection and diet had significant effects on the plasma NEFA concentrations of the animals in the HP group. In the HPI group, the mean NEFA values fluctuated initially before increasing moderately from 0.26 $\pm$ 0.06 $mmoll^{-1}$ at 36 DAI to 0.80 $\pm$ 0.15 $mmoll^{-1}$ at 63 DAI (Figure 7.15). The values in the HPI group were significantly lower than the values in the HPC group. The mean NEFA concentration in the LPI group fluctuated considerably but were not significantly different from the values in the control animals (Figure 7.14). In the control groups, the mean values tended to increase with time and the values of the HPC group were significantly higher than those of the LPC group.

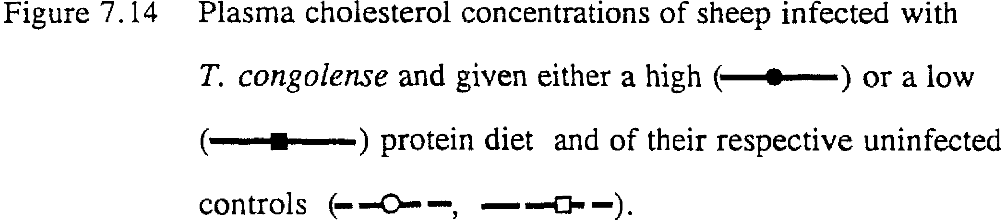
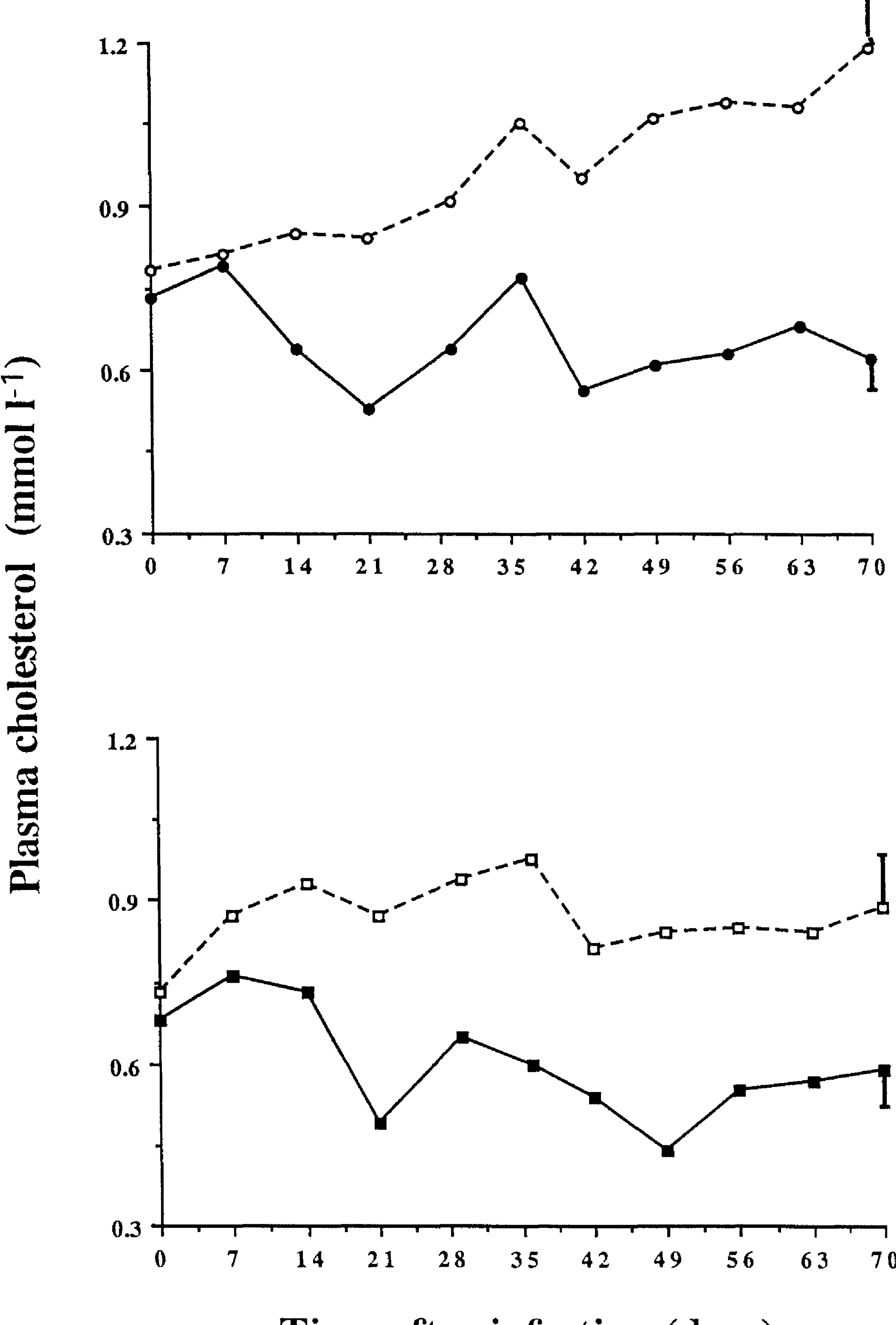


Figure 7.14 Plasma cholesterol concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (- -○- -, - -□- -).



1.2
0.9
0.6
0.3
0
7
14
21
28
35
42
49
56
63
70
Plasma cholesterol (mmol l-1)
Time after infection (days)

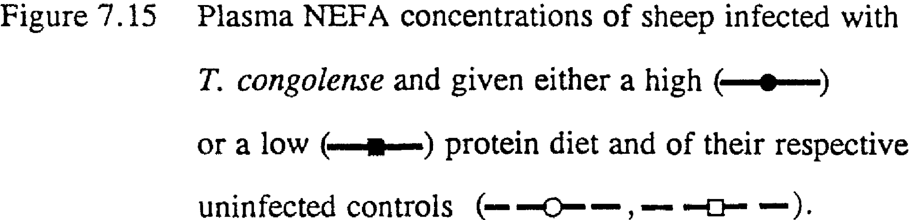
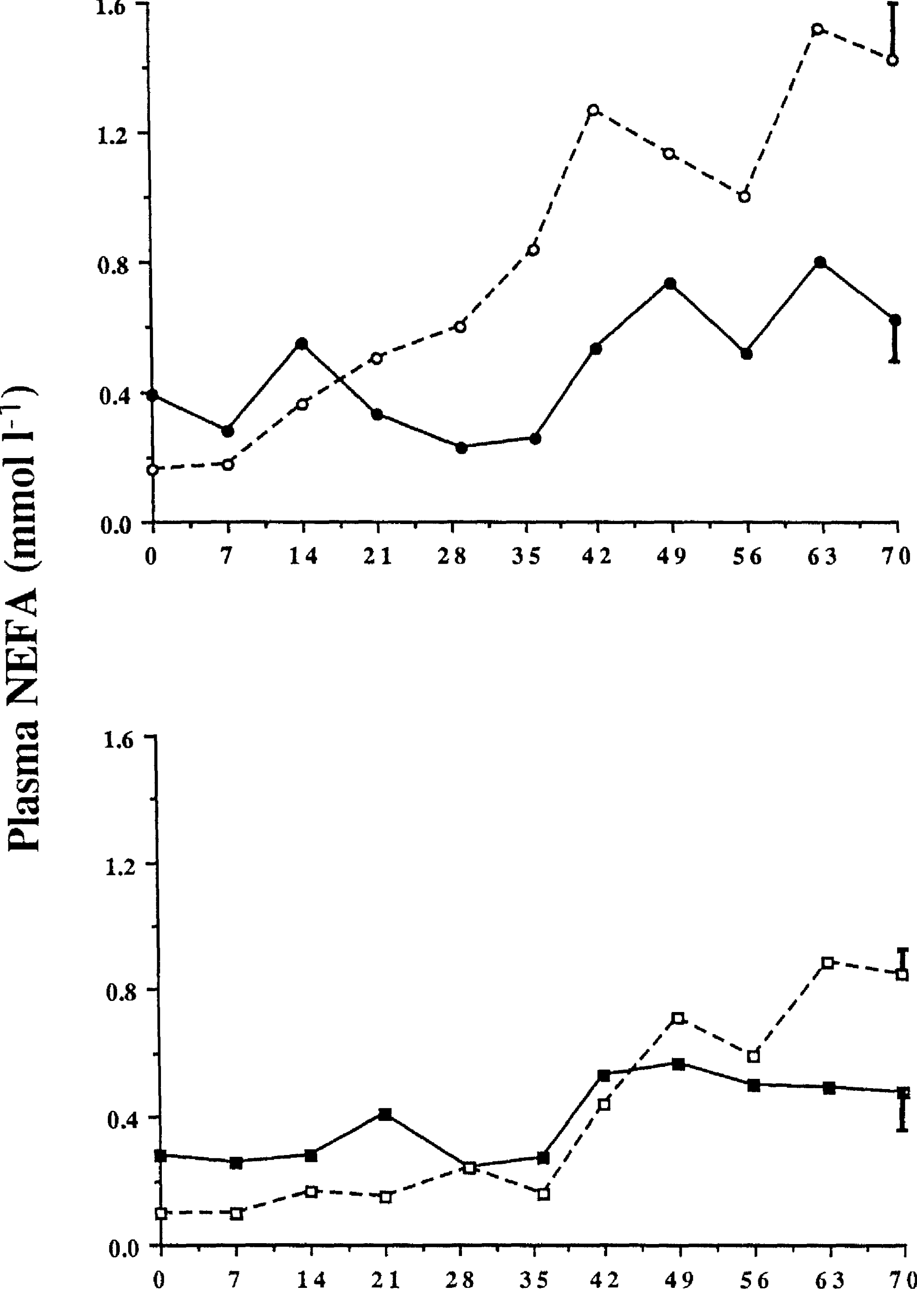


Figure 7.15 Plasma NEFA concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (– –○– –, – –□– –).



Plasma NEFA (mmol l^{-1})
1.6
1.2
0.8
0.4
0.0
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Plasma total protein (TP) concentration

The mean TP concentration decreased after infection, and remained lower than preinfection levels in both groups of infected sheep, however these differences were not significant. In the HPI group, the values decreased from 68.5 $\pm$ 0.8 at 0 DAI to 56.2 $\pm$ 1.5 gl^{-1} at 21 DAI after which they tended to recover (Figure 7.16). In the LPI group the TP concentration decreased from 67.0 $\pm$ 1.9 at 0 DAI to 53.5 $\pm$ 1.9 gl^{-1} at 49 DAI.

The mean TP values in the control animals fluctuated between 62.0 $\pm$ 1.0 and 73.0 $\pm$ 1.2 gl^{-1}. There was a tendency for the values to be higher in the HPC than in the LPC group, but the difference was not significant.

Plasma albumin concentration

The mean plasma albumin concentration decreased significantly in both groups of infected sheep and also showed dietary influences. In the HPI group, plasma albumin decreased from 36.2 $\pm$ 0.4 gl^{-1} at 0 DAI to 28.7 $\pm$ 0.9 gl^{-1} at 21 DAI (Figure 7.17). The values then fluctuated but showed a tendency to recover. In the LPI group, it decreased from 30.8 $\pm$ 0.7 gl^{-1} at 0 DAI to 26.3 $\pm$ 0.6 gl^{-1} at 21 DAI and tended to recover thereafter, but remained much lower than the values in the LPC group. The plasma albumin concentration in the LPI group was significantly lower than the values in the HPI group. In the control groups, plasma albumin fluctuated between 28.3 $\pm$ 1.8 and 32.0 $\pm$ 2.0 gl^{-1} in the LPC group, and between 32.3 $\pm$ 0.9 and 36.3 $\pm$ 0.7 gl^{-1} in the HPC group. The values in the HPC group were significantly higher than the values in the LPC group.

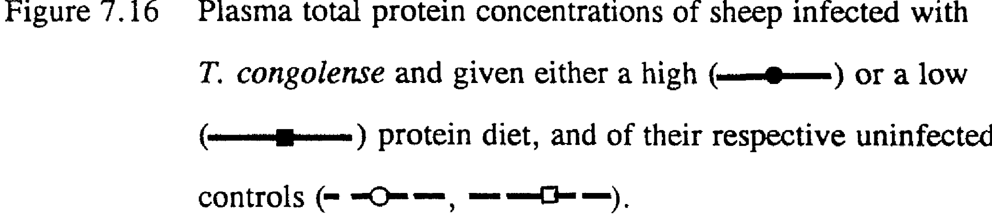
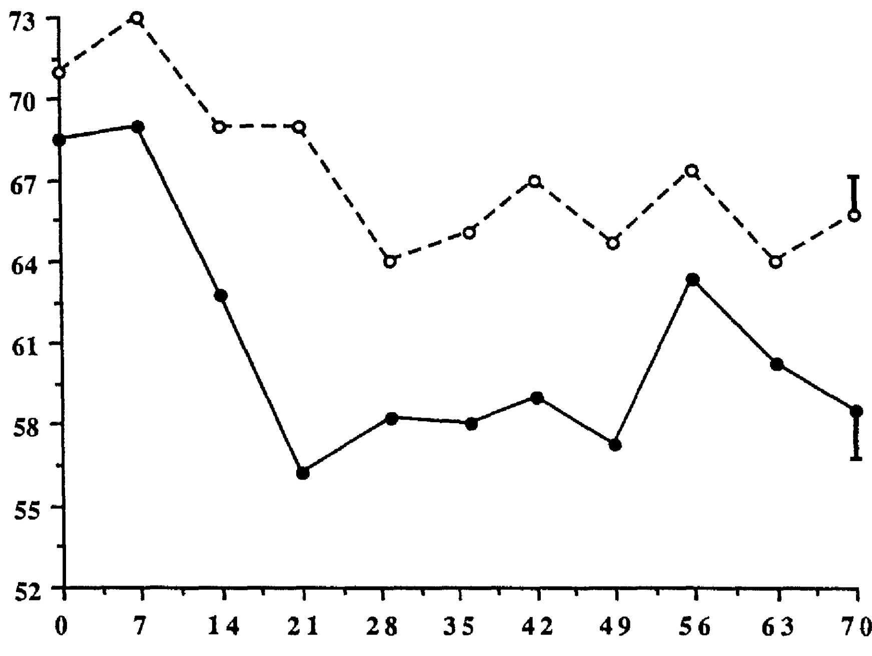
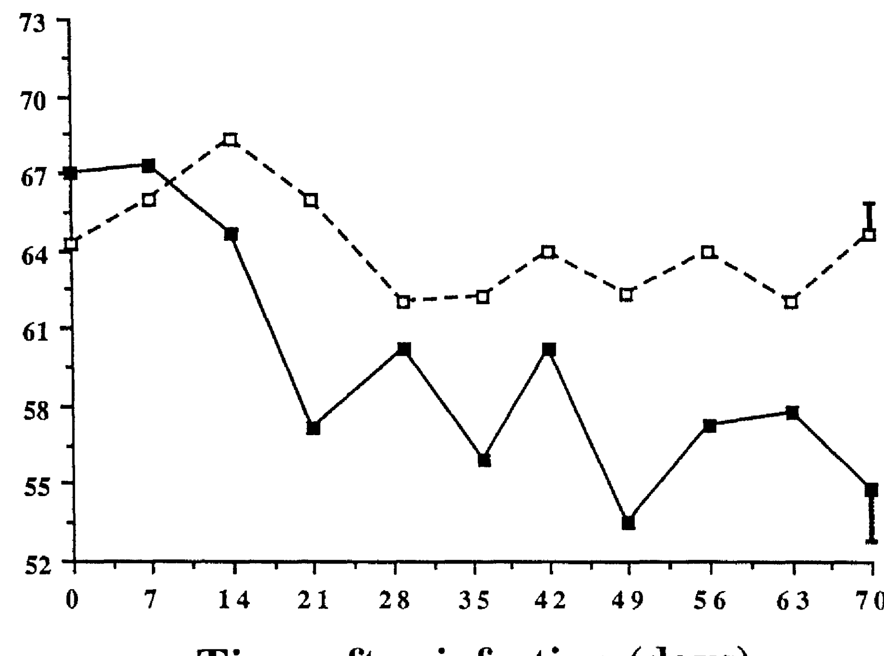


Figure 7.16 Plasma total protein concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (- -○- -, - -□- -).

Plasma total protein (g l-1)


73
70
67
64
61
58
55
52
0
7
14
21
28
35
42
49
56
63
70


73
70
67
64
61
58
55
52
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

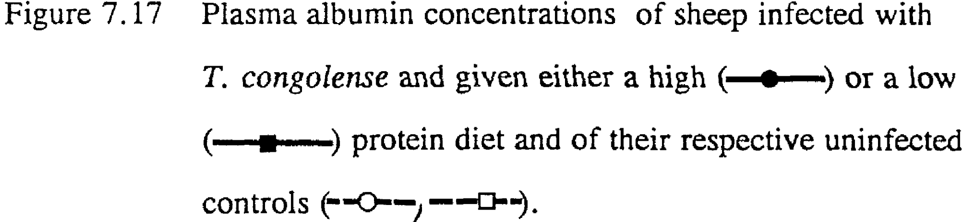
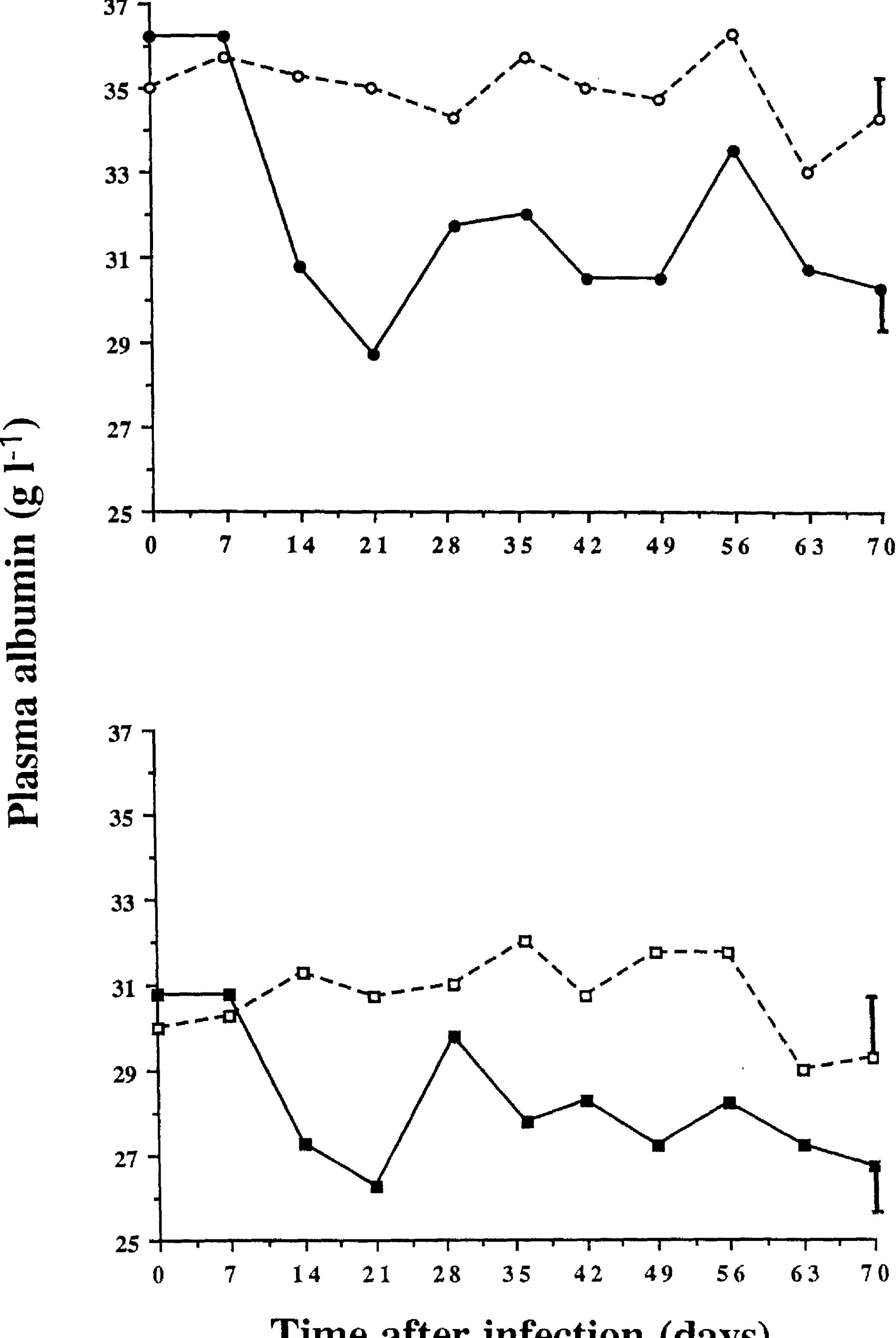


Figure 7.17 Plasma albumin concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (--○--, --□--).


Plasma albumin (g l-1)
37
35
33
31
29
27
25
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Plasma globulin concentration

The mean plasma globulin concentration showed an initial decline in both groups of infected sheep up to 36 DAI after which it increased moderately. The mean globulin concentration in the HPI group decreased from 35.3 $\pm$ 0.9 at 0 DAI to 26.0 $\pm$ 1.3 at 36 DAI after which it increased. In the LPI group, the mean values decreased from 38.8 $\pm$ 1.4 gl^{-1} at 0 DAI to 26.3 $\pm$ 2.0 at 49 DAI. The values in the HPI group were not significantly different from those of either the LPI or the HPC groups. The mean values in the control groups fluctuated between 29.3 $\pm$ 0.9 and 37.3 $\pm$ 1.2 gl^{-1} and there were no dietary differences.

Plasma albumin : globulin (A:G) ratio

The A:G ratios fluctuated considerably in all groups of sheep. They showed an initial moderate increase up to 36 - 49 DAI after which they moderately decreased. The mean values were higher in animals receiving a high protein diet (HPI and HPC groups) than in those receiving a low protein diet (LPI and LPC groups). There was no observable effect of infection on the A:G ratios.

Plasma urea concentration

There was no influence of infection on the concentrations of plasma urea, however, the urea concentrations in groups of sheep on a high protein diet were significantly higher than those on a low protein diet (Figure 7.18). The values fluctuated between 5.4 $\pm$ 0.1 and 9.2 $\pm$ 0.6 $mmoll^{-1}$ in animals on the HP diet and between 1.5 $\pm$ 0.1 and 3.3 $\pm$ 0.5 $mmoll^{-1}$ in the animals on the LP diet.

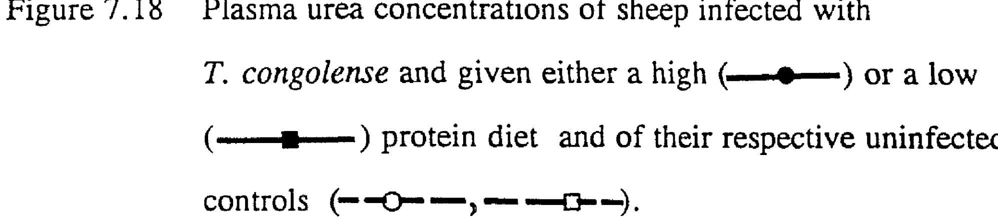
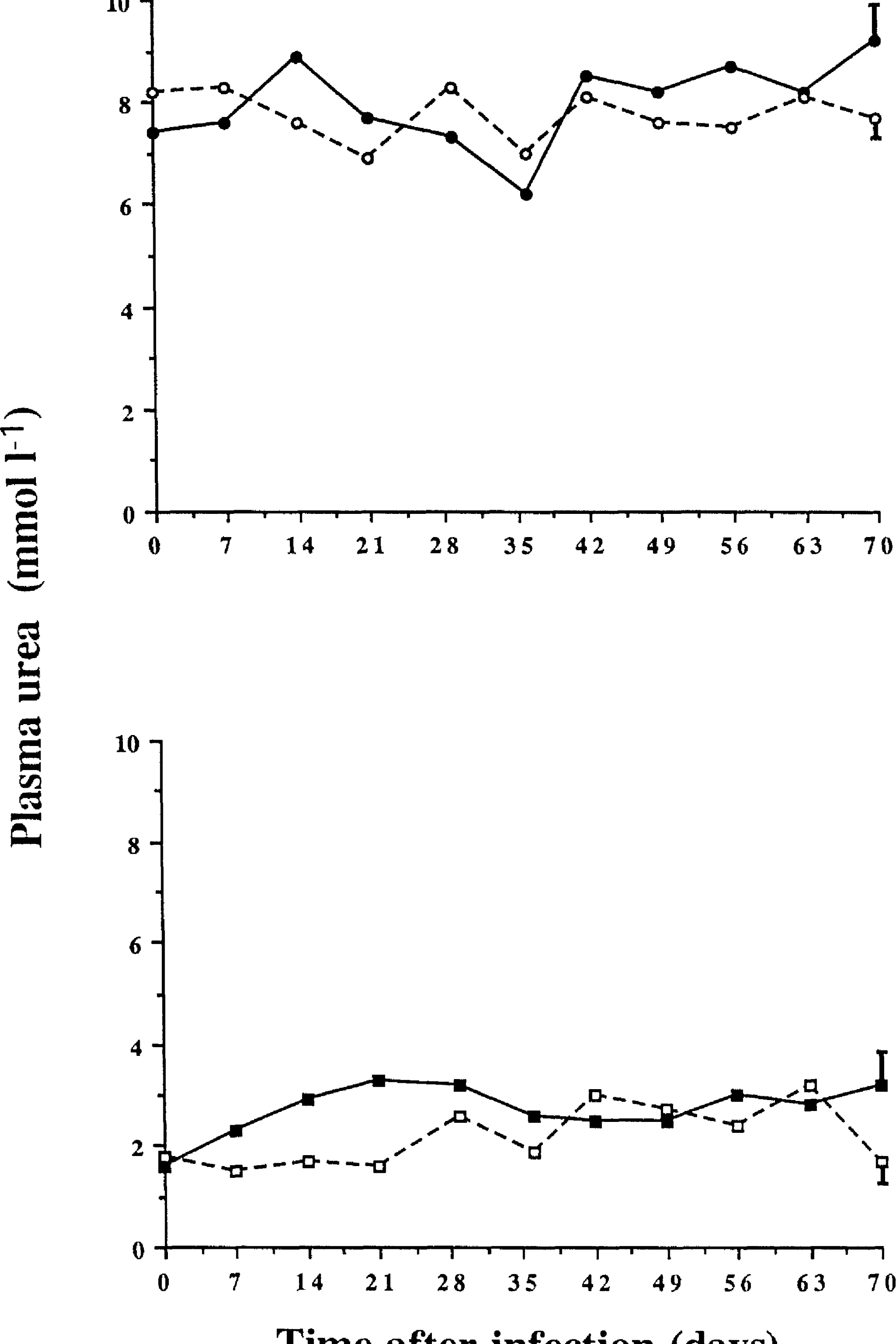


Figure 7.18 Plasma urea concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet and of their respective uninfected controls (- -○- -, - -□- -).



Plasma urea (mmol l-1)
10
8
6
4
2
0
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Serum iron (SI) and TIBC concentrations

The concentrations of serum iron in both infected groups, showed a fluctuating increase between 0 and 29 DAI after which they tended to decrease (Figure 7.19). The values were lower in the LPI group than in the LPC group between 29 and 70 DAI.

Infection caused a significant decrease in the concentrations of TIBC of the LPI group compared to the LPC group, but the changes in the HPI and HPC groups were similar (Figure 7.20). There were also dietary effects in that animals on the HP diet tended to have higher TIBC values than those on the LP diet.

Plasma glucose and β-hydroxybutyrate concentrations

The mean values of plasma glucose(HP 3.2-3.8, LP 3.0-3.7 $mmol^{l-1}$) and β-hydroxybutyrate (HP 0.13-0.47, LP 0.13-0.53 $mmoll^{-1}$) fluctuated but remained within the normal ranges and there were no infection or nutritional effects.

RESULTS 11

The parasitological, body weight, haematological and blood biochemical observations described in results 11 relate to the effect of dietary protein on the response of infected animals to treatment with a trypanocide, isometamidium chloride, at 70 DAI.

Parasitological Findings

After 70 days of infection, three animals from each dietary group were injected with 2% isometamidium chloride at a dose rate of 1 $mgkg^{-1}$ by the deep intramuscular route. In all animals, trypanosomes disappeared from the circulation following treatment.

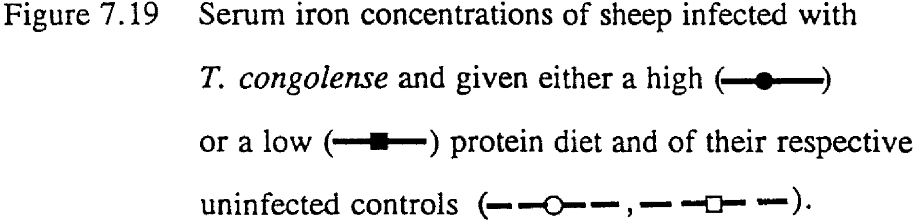
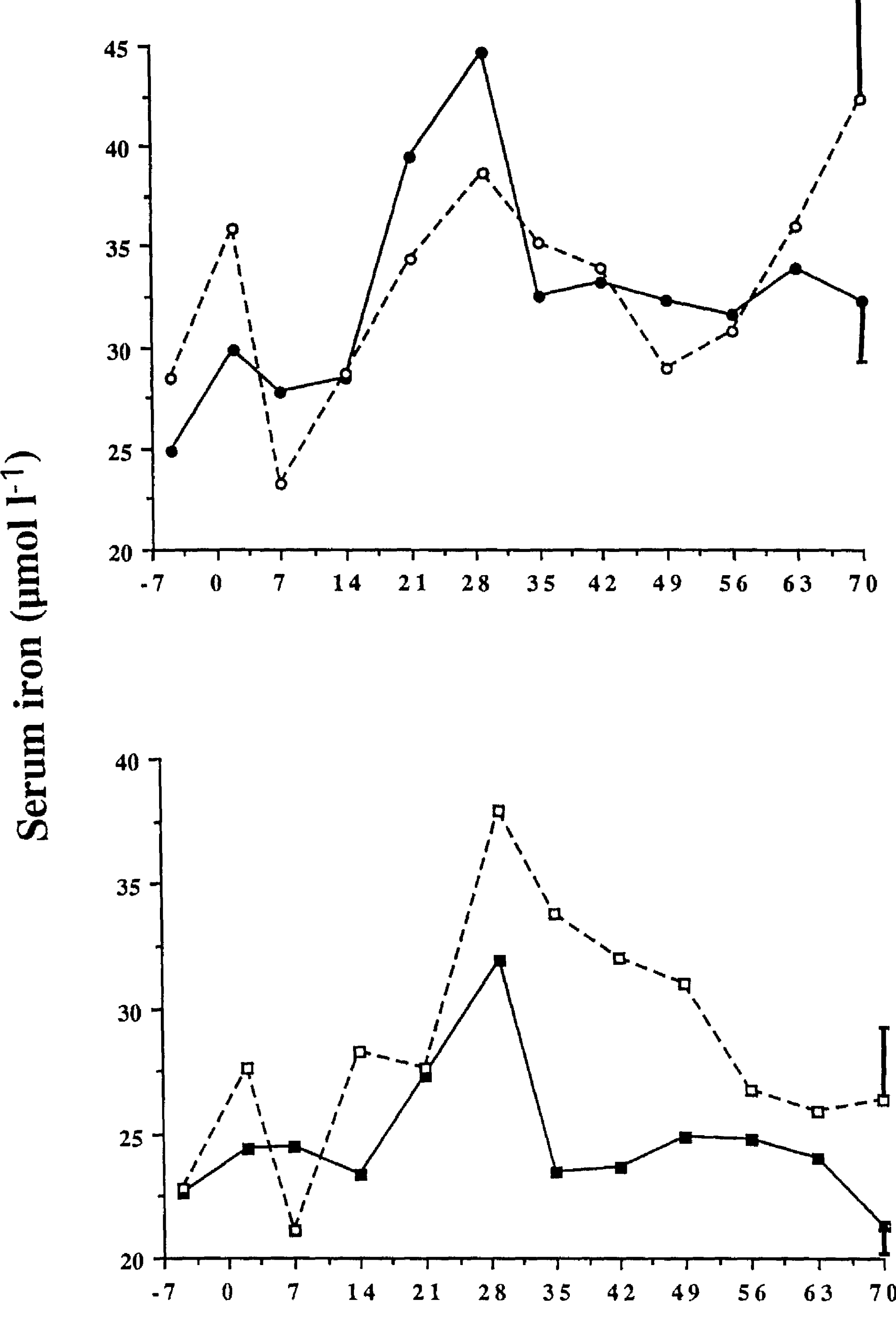


Figure 7.19 Serum iron concentrations of sheep infected with *T. congolense* and given either a high ( ) or a low ( ) protein diet and of their respective uninfected controls (,).


Serum iron (μmol l-1)
45
40
35
30
25
20
-7 0 7 14 21 28 35 42 49 56 63 70
40
35
30
25
20
-7 0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

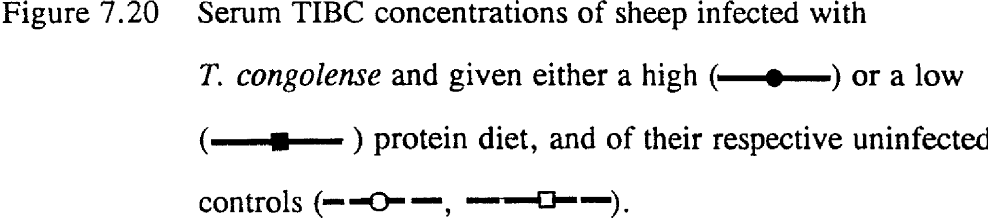
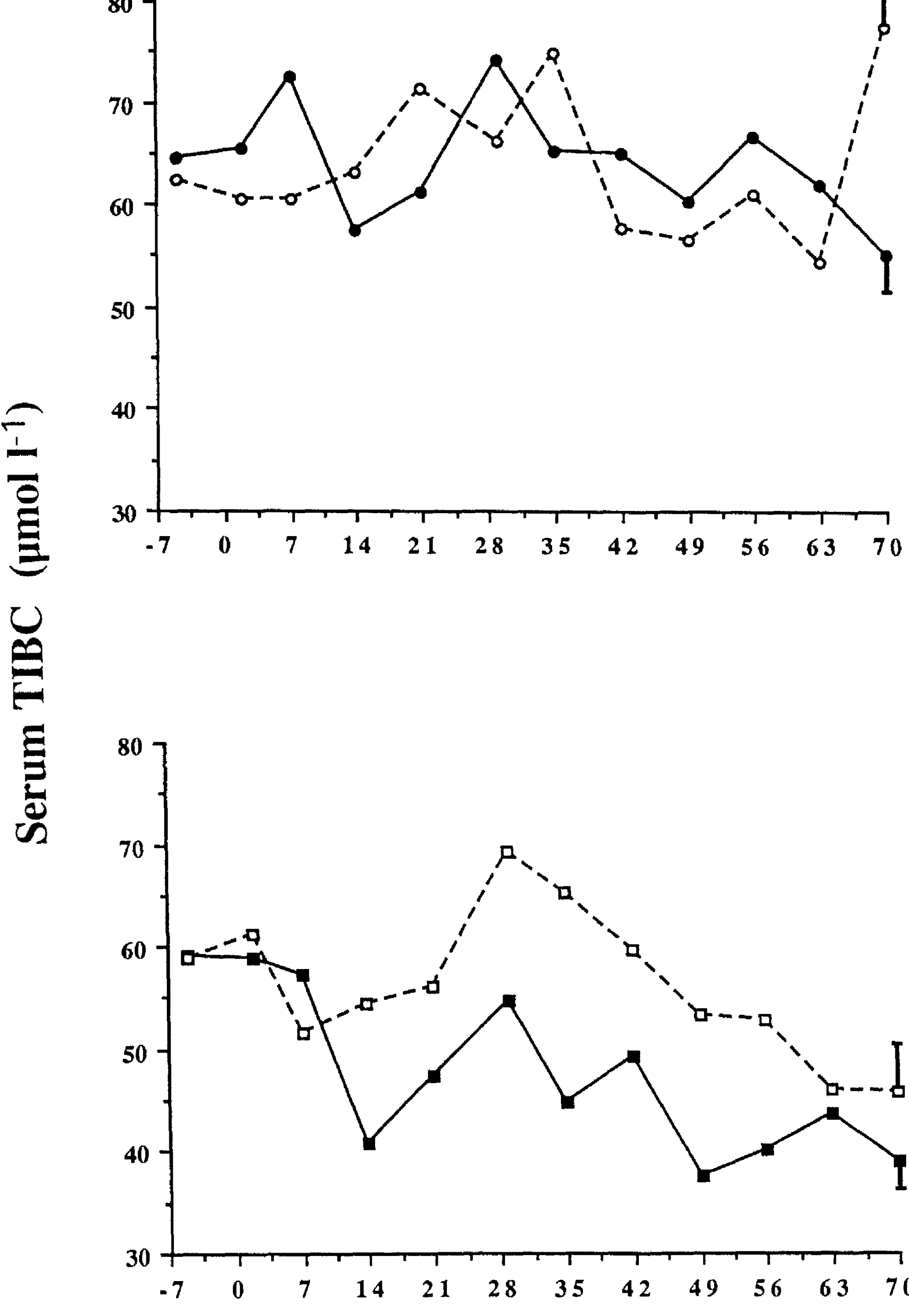


Figure 7.20 Serum TIBC concentrations of sheep infected with *T. congolense* and given either a high (—●—) or a low (—■—) protein diet, and of their respective uninfected controls (- -○- -, —□—).



Serum TIBC (µmol l-1)
80
70
60
50
40
30
-7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Changes in Body Weight

Following treatment both groups increased in body weight. The treated group on the LP diet (LPIT) gained 0.7 kg while that on HP diet (HPIT) gained 1.8 kg during 20 days after treatment. The weights in the LPC and HPC groups increased by 0.8 and 1.8 kg respectively in the same period of time.

Haematological Changes

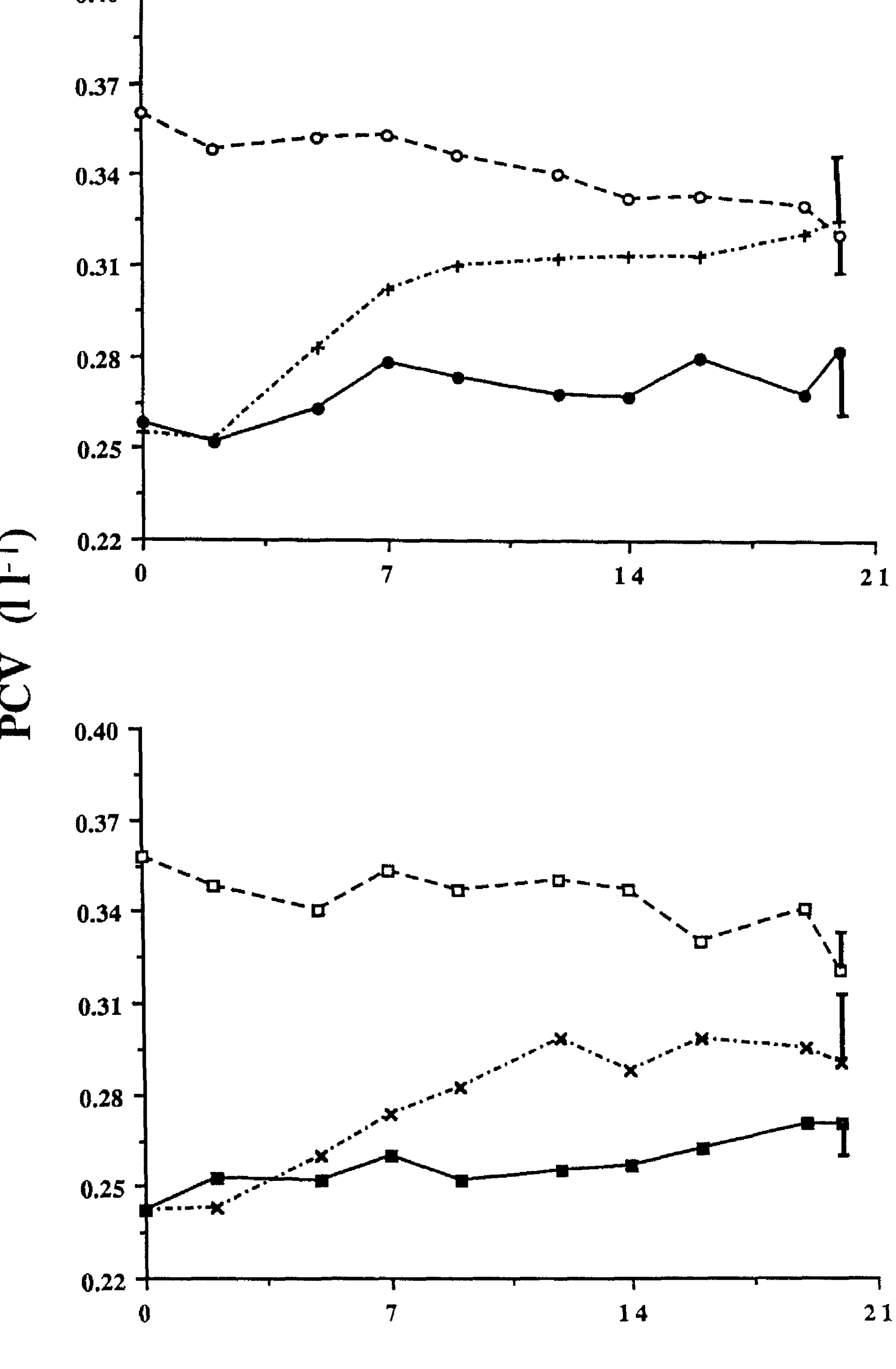
Packed cell volume (PCV)

Both groups of treated animals showed an improvement in their mean PCV values following treatment, however, the rate of recovery appeared to be moderately faster in the animals on a HP diet than in those on a low protein diet (Figure 7.21). The mean values in animals on a high protein diet increased from 0.26 ± 0.02 to 0.32 ± 0.01 ll^{-1} by 14 days after treatment while in the LPI group, the values increased from 0.24 ± 0.01 to 0.30 ± 0.01 ll^{-1} between 0 and 16 days after treatment. By 20 days after treatment, the PCV values in the treated animals on a high protein diet were level (0.33 ± 0.01 ll^{-1}) with those of their uninfected controls (0.32 ± 0.01 ll^{-1}), while the values in treated animals on a low protein diet (0.29 ± 0.02 ll^{-1}) were lower than the values of their uninfected controls (0.32 ± 0.01 ll^{-1}).

Red cell count (RBC) and Hb concentration

Both groups of treated animals showed a recovery of RBC and Hb concentration following treatment (Table 7.4). In the treated HPI group (HPIT), the mean values increased from 7.9 ± 1.1 at 2 days before treatment (DBT) to 9.6 ± 0.5 x $10^{12}l^{-1}$ at 19 days after treatment (DAT). The mean RBC in the treated LPI group (LPIT) increased from 8.0 ± 0.5 x $10^{12}l^{-1}$ at 2 days before treatment (DBT) to 9.3 ±

Figure 7.21 Packed cell volumes of *T. congolense*-infected sheep treated with isometamidium chloride at 70 DAI, of untreated infected and uninfected control sheep given either a high or low protein diet.
(——●——) HPI, (- · - - ·+· - - · - ·-) HPIT, (— —○— —) HPC,
(——■——) LPI, (- · - - ·*· - · - - ·-) LPIT, (— —□— —) LPC.



PCV (l l⁻¹)
0.40
0.37
0.34
0.31
0.28
0.25
0.22
0
7
14
21
Time after treatment (days)

Table 7.4

RBC, Hb concentration and MCV values of sheep after treatment with isometamidium chloride at 70 days after infection with *Trypanosoma congolense*, and of untreated infected, and uninfected control sheep given either a high or a low protein diet

Variable	Group	Days after treatment -2	5	12	19
RBC ($x10^{12}l^{-1}$)	HPI	7.21±1.50	7.20±1.62	7.40±1.83	7.37±1.61
	HPIT	7.85±1.10	7.85±0.59	8.82±0.53	9.55±0.51
	HPC	11.34±0.32	11.46±0.18	10.67±0.44	10.85±0.35
	LPI	8.19±0.77	8.13±0.83	7.83±0.61	8.43±0.78
	LPIT	7.98±0.51	8.07±0.41	9.20±0.66	9.27±0.49
	LPC	11.16±0.37	11.14±0.56	11.07±0.31	10.98±0.67
Hb ($g\ dl^{-1}$)	HPI	8.90±1.10	8.93±1.29	9.10±1.42	9.13±1.08
	HPIT	9.50±1.30	9.60±0.55	10.67±0.54	11.07±0.66
	HPC	12.40±0.10	12.59±0.38	11.38±0.59	11.83±0.57
	LPI	9.07±0.55	8.73±0.58	8.60±0.35	9.10±0.44
	LPIT	9.17±0.38	9.09±0.34	10.30±0.69	10.30±0.61
	LPC	12.30±0.60	12.20±1.01	12.47±0.52	12.07±1.10
MCV (fl)	HPI	38.70±5.70	39.70±6.69	39.30±7.40	38.67±6.65
	HPIT	35.00±0.58	35.70±1.20	34.67±0.67	34.00±0.58
	HPC	30.00±0.58	31.00±0.58	30.30±0.33	30.30±0.33
	LPI	32.00±1.20	32.70±1.40	32.00±1.76	32.30±1.80
	LPIT	32.70±0.88	33.30±1.20	32.30±1.20	32.00±0.58
	LPC	30.30±0.88	31.00±0.58	30.69±0.33	31.00±0.24

LP & HP = Low and high protein respectively, I = Infected, T = Treated, C = Control.

0.5 x $10^{12}l^{-1}$ at 19 days after treatment (DAT). The values of LPC and HPC groups at 90 DAI were 11.0 ± 0.7 and 10.9 ± 0.4 x $10^{12}l^{-1}$, respectively. The values in the HPI and LPI groups varied from 7.20 - 7.40 and 7.83 and 8.4 x $10^{12}l^{-1}$, respectively.

Haemoglobin concentrations also improved in treated animals of both groups, and remained lower in the infected groups than in the control groups (Table 7.4)

White cell counts and platelet counts

Following treatment, there was an increase in WBC, lymphocyte and platelet counts (Table 7.5). Both groups of treated animals showed a dramatic increase in platelet counts within the first week of treatment (Table 7.5). The mean platelet counts in treated animals on a HP diet (HPIT group) increased from 128.8 ± 13.8 to 416.3 ± 41.0 x$10^{9}l^{-1}$ between -2 and 5 DAI. The mean counts in the LPIT group increased from 102.6 ± 39.1 x $10^{9}l^{-1}$ to 501.3 ± 76.6 x$10^{9}l^{-1}$ between the same period of time. The values in infected animals remained significantly lower than those in treated and control groups.

Biochemical Observations

Total serum lipids

The serum total lipid concentrations increased markedly in both groups of infected animals following treatment (Table 7.6). In animals on a LP diet, the mean concentration of serum total lipids increased from 1.32 ± 0.03 to 1.93 ± 0.39 gl^{-1} between 0 and 20 days after treatment. In animals on a high protein diet the mean values doubled, increasing from 0.87 ± 0.03 to 1.83 ± 0.12 gl^{-1}, in the same period of time. Infected groups of animals maintained significantly lower concentrations than treated and control groups.

Table 7.5

Total white cell count, lymphyocyte and platelet counts of sheep after treatment with isometamidium chloride at 70 days after infection with *Trypanosoma congolense*, and of infected untreated, and uninfected control sheep given either a high or a low protein diet

		Days after treatment			
Variable	Group	-2	5	12	19
	HPI	7.07±1.60	7.97±2.10	6.03±1.20	7.40±1.37
	HPIT	9.73±2.03	8.87±1.53	10.97±1.07	10.83±1.26
	HPC	9.40±0.85	9.90±0.90	10.97±2.37	9.13±0.77
WBC ($x\ 10^9 l^{-1}$)	LPI	11.33±2.20	11.23±2.50	10.47±1.67	11.30±1.95
	LPIT	11.60±3.55	10.87±1.94	11.87±1.79	11.87±0.80
	LPC	9.07±0.85	9.07±0.59	9.07±0.24	8.40±0.31
	HPI	5.64±1.90	6.54±1.85	9.46±1.80	6.51±1.63
	HPIT	7.34±2.06	7.20±1.81	9.78±1.66	7.60±1.14
	HPC	7.63±0.44	8.30±0.49	6.14±0.88	7.71±0.43
Lymphocytes ($x\ 10^9 l^{-1}$)	LPI	9.48±1.87	9.36±2.60	4.87±1.34	9.48±2.14
	LPIT	9.88±0.54	9.67±1.86	8.94±2.04	9.95±0.59
	LPC	6.26±0.54	6.84±0.70	8.95±1.62	6.11±0.46
	HPI	142.0±27.5	156.7±65.1	161.0±32.1	172.7±11.6
	HPIT	114.5±15.1	416.3±41.0	432.0±38.1	428.0±22.7
	HPC	245.2±24.6	205.0±22.6	226.3±32.3	244.7±10.3
Platelets ($x\ 10^9 l^{-1}$)	LPI	116.0±43.0	150.0±45.9	165.1±11.7	173.3±37.3
	LPIT	102.6±39.1	501.3±76.6	500.0±80.2	490.2±59.1
	LPC	220.0±46.2	219.0±38.0	245.6±50.1	218.7±53.6

LP & HP = low and high protein respectively, I = Infected, T = Treated and C = Control

Serum phospholipids

The concentration of serum phospholipids showed a similar trend to that described for serum total lipid concentrations. In animals on a LP diet, the mean values increased from 0.49 ± 0.05 to 0.66 ± 0.02 $mmoll^{-1}$ between 0 and 20 days after treatment (Table 7.6). In animals on the HP diet, the values doubled, increasing significantly from 0.44 ± 0.07 to 0.87 ± 0.03 $mmoll^{-1}$ within 20 days after treatment.The values in infected groups remained significantly lower than those in treated and control groups.

Plasma cholesterol and NEFA concentrations

The concentration of plasma cholesterol increased in both treated groups of animals (Table 7.6). In animals on a LP diet the mean concentration increased from 0.61 ± 0.05 to 0.97 ± 0.05 $mmoll^{-1}$ within 20 days after treatment. In animals receiving a HP diet, the mean values increased from 0.63 ± 0.07 to 1.05 ± 0.08 $mmoll^{-1}$ within 14 days after treatment. In infected untreated groups, plasma concentrations remained lower (LPI 0.41-0.59, HPI 0.61-0.76 $mmoll^{-1}$) than the values in treated groups and control groups (LPC 0.80-0.91, HPC 1.01-1.19 $mmoll^{-1}$)

The plasma concentration of NEFA fluctuated considerably in treated animals on a LP diet while in those receiving a HP diet, they decreased from 0.66 ± 0.10 $mmoll^{-1}$ at the time of treatment to 0.25 ± 0.04 $mmoll^{-1}$ by 7 days after treatment (Table 7.7). The mean values then tended to increase. These values were lower than control values of both groups.

Table 7.6

The concentrations of serum total lipids, phospholipids and cholesterol of sheep following treatment with isometamidium chloride at 70 days after infection with *Trypanosoma congolense*, of untreated infected, and uninfected control sheep given either a high or a low protein diet

Variable	Group	Days after treatment 0	7	14	20
Total lipids (gl^{-1})	HPI	1.03±0.08	1.00±0.17	1.23±0.24	1.33±0.19
	HPIT	0.87±0.03	1.33±0.09	1.60±0.21	1.83±0.12
	HPC	2.10±0.10	1.77±0.08	1.83±0.24	1.89±0.03
	LPI	1.30±0.18	0.90±0.12	0.77±0.12	1.20±0.20
	LPIT	1.32±0.03	1.67±0.38	1.57±0.21	1.93±0.39
	LPC	1.73±0.21	1.53±0.12	2.07±0.20	1.63±0.12
Phospholipids (gl^{-1})	HPI	0.47±0.04	0.50±0.05	0.50±0.06	0.58±0.03
	HPIT	0.44±0.01	0.62±0.01	0.76±0.07	0.87±0.03
	HPC	0.72±0.04	0.71±0.04	0.64±0.01	0.70±0.01
	LPI	0.50±0.04	0.46±0.04	0.46±0.08	0.44±0.05
	LPIT	0.49±0.05	0.58±0.01	0.70±0.07	0.66±0.02
	LPC	0.72±0.04	0.75±0.10	0.75±0.06	0.67±0.06
Cholesterol ($mmoll^{-1}$)	HPI	0.62±0.04	0.61±0.06	0.62±0.07	0.76±0.05
	HPIT	0.63±0.07	0.74±0.08	1.05±0.08	0.90±0.05
	HPC	1.19±0.09	1.01±0.02	1.11±0.05	1.04±0.05
	LPI	0.59±0.05	0.41±0.08	0.53±0.14	0.44±0.11
	LPIT	0.61±0.05	0.68±0.04	0.95±0.03	0.97±0.05
	LPC	0.89±0.10	0.80±0.06	0.91±0.03	0.91±0.07

LP & HP = low and high protein respectively, I = Infected, T = Treated, and C = Control

Plasma proteins

Both groups of treated animals showed marked increases in plasma total protein, albumin and globulin while the A:G ratio decreased with time after treatment (Table 7.7). In treated animals on a LP diet, the total protein increased from 54.4 $\pm$ 1.9 to 65.3 $\pm$ 2.4 gl^{-1} between 0 and 20 days after treatment. In treated animals on a HP diet, the mean TP values increased from 59.6 $\pm$ 1.7 to 72.0 $\pm$ 3.5 gl^{-1} within the same period of time. The TP concentrations in infected groups remained lower than in treated and control groups.

Mean plasma albumin in the LPIT group increased from 27.6 $\pm$ 0.8 to 28.7 $\pm$ 0.9 between 0 and 20 days after treatment while, in the HPIT group, it increased from 29.4 $\pm$ 0.7 to 36.3 $\pm$ 1.9 gl^{-1} in the same period of time (Table 7.7). Recovery from hypoalbuminaemia was significant in the HPIT group, while there was little effect of treatment on albumin levels of the LPIT group. Plasma albumin concentrations in untreated infected groups were lower than in treated and uninfected control groups for the 20 days after treatment.

Carcase Evaluation

Killing out (KO) percentages

At 90 DAI, the liveweight, carcase weight and killing out percentages (Table 7.8) were much lower in both groups of infected animals compared to their uninfected controls, but this was only significant in the animals on the HP group. In animals on a low protein diet, the mean carcase weight in infected and control groups were 13.5 $\pm$ 1.0 and 15.5 $\pm$ 0.8 kg respectively, and in the HP group, they were 15.8 $\pm$ 0.6 and 18.6 $\pm$ 0.3 kg, respectively. The killing out percentage in the LPI and HPI groups were 40.4 $\pm$ 0.6% and 42.0 $\pm$ 0.5% respectively, while it was 41.9 $\pm$ 1.9% in the LPC group and 47.8 $\pm$ 1.4% in the HPC group. After treatment, the KO percentage of the HPIT group was significantly greater than that

Table 7.7

Plasma non-esterified fatty acids, total protein and albumin concentrations of sheep treated with isometamidium chloride at 70 days after infection with *Trypanosoma congolense* and of untreated infected, and uninfected control sheep given either a high or a low protein diet.

Variable	Group	Days after treatment 0	7	14	20
	HPI	0.62±0.10	0.65±0.26	0.54±0.11	0.60±0.27
	HPIT	0.66±0.10	0.25±0.04	0.37±0.03	0.39±0.04
	HPC	1.42±0.14	1.45±0.13	1.45±0.08	1.10±0.22
NEFA ($mmoll^{-1}$)					
	LPI	0.48±0.12	0.38±0.10	0.27±0.08	0.44±0.19
	LPIT	0.53±0.20	0.42±0.11	0.32±0.04	0.48±0.05
	LPC	0.85±0.05	0.91±0.09	0.56±0.18	0.95±0.23
	HPI	58.5±1.50	56.3±4.10	62.0±4.00	62.3±3.90
	HPIT	59.6±1.70	61.7±1.50	70.3±1.70	72.0±3.50
	HPC	65.7±1.30	62.7±2.30	65.7±1.50	63.7±1.30
Total protein					
(gl^{-1})	LPI	54.8±2.10	54.0±1.70	59.0±2.70	57.0±1.50
	LPIT	54.4±1.90	60.0±1.50	69.0±3.00	65.3±2.40
	LPC	64.7±1.30	62.0±1.20	65.0±1.70	62.3±0.90
	HPI	30.3±0.80	29.0±2.30	31.0±2.50	31.3±0.70
	HPIT	29.4±0.70	33.3±0.90	34.3±0.20	36.3±1.90
	HPC	34.3±0.90	33.3±0.90	33.0±0.60	33.0±0.60
Albumin					
(gl^{-1})	LPI	26.7±0.90	27.0±0.60	28.7±1.20	28.0±0.60
	LPIT	27.6±0.80	28.3±1.50	29.7±0.70	28.7±0.90
	LPC	29.3±1.20	28.3±1.80	30.3±0.90	29.3±1.2

LP & HP = low and high protein respectively, I = Infected, T = Treated and C = Control

Table 7.8

Carcase evaluation: liveweight, carcase weight and killing out percentage of sheep infected with *Trypanosoma congolense* and of uninfected control and treated sheep, given either a low or a high protein diet.

Group	liveweight (kg)	Carcase weight (kg)	Killing out percentage (%)
LPB	25.0 ± 0	11.1 ± 1.0	44.3 ± 2.5
LPI	33.4 ± 2.9[a]	13.5 ± 1.0	40.4 ± 0.6
LPIT*	34.2 ± 1.0[a]	13.8 ± 0.3	40.5 ± 0.5
LPC	36.8 ± 1.1[a]	15.5 ± 0.8[a]	41.9 ± 1.9
HPB	25.0 ± 0.5	11.5 ± 1.0	46.0 ± 2.5
HPI	37.7 ± 0.7[a]	15.8 ± 0.6[a]	42.0 ± 0.5
HPIT*	39.3 ± 1.8[a]	17.8 ± 0.7[ab]	45.2 ± 0.9[b]
HPC	39.8 ± 0.9[a]	18.6 ± 0.3[ab]	47.8 ± 1.4[b]

* Sheep were treated with isometamidium chloride 70 days after infection and were killed 90 days after infection.
LPB and HPB were killed at the start of the experiment, and uninfected control, infected and treated groups were slaughtered at 90 DAI. [a] Sigificantly different from the corresponding baseline values (LPB, HPB), [b] Significantly different from the HPI group.

of the HPI group, while the values of the LPIT and LPI groups were the same. Infection therefore led to a reduction in the killing out percentage, and a HP diet was associated with a high KO percentage and greater response to treatment compared to the LP diet.

Indicator joint dissection

The mean joint weights of the sheep killed at the beginning of the experiment (LPB and HPB), of infected sheep (LPI and HPI), control sheep (LPC and HPC) and of infected and treated sheep (LPIT and HPIT) killed at 90 DAI are shown in Table 7.9. The mean weights of the dissected joints in the LPC, LPI and LPIT groups were 599.6 g, 490.0 g and 499.9 g, respectively, and they were 654.5 g, 580.6 g and 636.6 g in the HPC, HPI and HPIT groups. Infection therefore resulted in a more severe reduction in the indicator joint weight in the animals on a low protein group than in the animals on a HP diet, and following treatment, the improvement was better in the animals on a HP diet than in those on a LP diet. There were no significant differences in the percentage composition of muscle, fat and bone among the infected, control and treated groups of animals.

Chemical analysis

There was marked variation in individual animals within groups and there were no significant differences in the mean percentage composition of crude protein, energy, ether extract and ash among infected, control and treated groups of animals (Table 7.10). However, considering total carcase composition, infection caused a marked decrease in total carcase protein, energy, fat and ash (Table 7.11). Following treatment, these variables improved, the increase being greater in animals on a high protein diet than in those on a low protein diet.

Table 7.9

Mean (± sem) weight and composition of the best end neck joint of sheep infected with *Trypanosoma congolense*, of treated and of uninfected control sheep given either a low or a high protein diet.

Group	Weight of joint (g)	% Bone	% Fat	% Muscle	Muscle/Bone
LPB*	462.8 ± 43.4	23.3 ± 2.1	21.6 ± 8.4	55.1 ± 8.6	2.4 ± 0.5
LPI	490.0 ± 75.0	24.1 ± 3.5	25.8 ± 11.3	50.1 ± 8.1	2.1 ± 0.2
LPIT	499.9 ± 77.5	23.8 ± 4.1	21.6 ± 1.7	54.6 ± 3.0	2.3 ± 0.5
LPC	599.6 ± 63.9	25.4 ± 7.4	26.2 ± 2.6	48.5 ± 4.8	2.1 ± 0.9
HPB*	524.9 ± 68.8	23.0 ± 2.5	20.0 ± 2.3	56.9 ± 4.7	2.5 ± 0.5
HPI	580.6 ± 66.8	25.4 ± 2.7	26.7 ± 8.0	47.9 ± 5.8	1.9 ± 0.2
HPIT	636.6 ± 77.5	19.8 ± 5.3	27.3 ± 8.7	52.8 ± 6.9	2.8 ± 0.8
HPC	654.5 ± 70.9	21.4 ± 7.0	27.1 ± 4.3	51.5 ± 7.9	2.6 ± 1.1

*LPB & HPB = Baseline values of low and high protein groups obtained at the time of infection, and other groups were killed at 90 DAI.

Table 7.10

Mean ± (sem) carcase composition of lambs infected with *Trypanosoma congolense*, of treated, and of uninfected control lambs given either a low or a high protein diet.

Group	Dry matter (gkg^{-1})	Crude protein (gkg^{-1} DM)	Energy ($MJkg^{-1}$ DM]	Ether extract (gkg^{-1}DM)	Ash (gkg^{-1} DM)
LPB*	435.5 ± 12.5	366.1 ± 15.2	30.5 ± 0.7	531.1 ± 15.4	79.6 ± 1.4
LPI	436.0 ± 15.7	381.7 ± 24.6	28.1 ± 1.9	469.0 ± 36.4	121.6 ± 14.6
LPIT	422.7 ± 2.7	406.3 ± 16.5	27.0 ± 1.7	417.9 ± 27.9	137.0 ± 11.8
LPC	452.0 ± 11.5	370.6 ± 16.8	29.5 ± 0.5	505.3 ± 21.5	123.7 ± 12.7
HPB*	421.0 ± 2.3	377.8 ± 24.3	29.1 ± 0.4	478.1 ± 16.0	98.8 ± 7.7
HPI	427.3 ± 19.0	390.8 ± 28.1	28.4 ± 0.6	457.9 ± 30.4	97.4 ± 4.0
HPIT	468.0 ± 12.5	371.9 ± 17.3	29.8 ± 1.1	516.1 ± 40.5	92.0 ± 16.5
HPC	431.3 ± 5.5	383.0 ± 7.5	28.0 ± 0.4	485.5 ± 21.4	111.1 ± 10.1

* LPB & HPB = Baeline values of low and high protein groups obtained at the time of infection. Other groups were killed at 90 DAI

Table 7.11

Mean (±sem) total carcase composition of sheep infected with *Trypanosoma congolense*, of treated and of uninfected control sheep given either a high or a low protein diet

Group	Carcase weight (kg)	Carcase protein (kg)	Carcase energy (MJ)	Carcase fat (kg)	Carcase ash (kg)
LPB*	11.8 ± 0.8	1.87 ± 0.14	155.7 ± 2.0	2.71 ± 0.01	0.41 ± 0.01
LPI	13.5 ± 0.5	2.23 ± 0.12	166.2 ± 19.6	2.78 ± 0.39	0.71 ± 0.08
LPIT	13.8 ± 0.2	2.37 ± 0.06	158.4 ± 11.9	2.45 ± 0.20	0.80 ± 0.06
LPC	15.5 ± 0.5	2.60 ± 0.12	206.7 ± 5.0	3.54 ± 0.15	0.87 ± 0.09
HPB*	11.4 ± 0.7	1.81 ± 0.16	139.6 ± 11.1	2.30 ± 0.02	0.47 ± 0.04
HPI	15.8 ± 0.3	2.63 ± 0.12	191.7 ± 9.6	3.11 ± 0.30	0.66 ± 0.01
HPIT	17.8 ± 0.7	3.09 ± 0.07	249.0 ± 15.1	4.32 ± 0.44	0.76 ± 0.01
HPC	18.6 ± 0.3	3.07 ± 0.04	224.2 ± 5.2	3.90 ± 0.26	0.89 ± 0.07

* LPB & HPB = Baseline values of low and high protein groups obtained at the time of infection. Other groups were killed at 90 DAI.

DISCUSSION

In the present study, it was shown that dietary protein had a marked influence on growth rates, intensity of parasitaemia, blood biochemical changes and rate of recovery from anaemia following administration of a trypanocidal drug.

The prepatent periods, in both infected groups of animals, were similar. However, following patency, animals on a high protein diet tended to develop a higher intensity of parasitaemia than those on a low protein diet. Observation of a similar prepatent period in both infected groups is in agreement with the report of Little *et al.* (1990) in N'Dama cows experimentally infected with *T. congolense* and given either a low or a high plane of nutrition. However, in a comparable study in sheep, Hecker *et al.* (1991) observed that animals supplemented with cotton seed cake and maize bran, and exposed to high natural tsetse challenge in the field, showed a delayed onset of parasitaemia compared with non-supplemented animals.

The main difficulty associated with such field trials is that the protein intakes of the animals are not easy to establish and although it is assumed that all animals are subjected to equal infection challenge from the tsetse flies, this may not be absolutely true in real terms. While levels of protein intake may not be easily quantified under field conditions, it is possible that unsupplemented animals receive a protein level far below maintenance requirements for the animals, and much below the protein levels used in the present study. It needs to be emphasised that the protein levels used in the present investigation (i.e 17.6% for HP and 8.1% for LP) are much higher than what is usually available to animals in the fields of tsetse endemic areas of Africa (Sawadogo *et al.*, 1991). These protein levels fall even further during the dry seasons (Topps, 1977) and the feed is so scarce to the extent that animals may die of starvation (Personal observation). It has been proposed that there is a minimum dietary protein level that is necessary for immunological defence against infection (Newberne and Williams, 1970), and studies by Axelrod (1958)

showed that adequate nutrition is required for both primary and secondary responses to an antigen. Newberne and Williams (1970) have indicated that protein deficiency can affect not only antibody production but also phagocytosis both qualitatively and quantitatively. These studies have been confirmed by Abdullahi *et* al. (1986b) who showed that sheep fed on protein deficient diets (4% crude protein) produced less immunoglobulins than animals fed on a ration containing either 7.5% or 15% crude protein, which elaborated similar quantities of immunoglobulins. It is therefore conceivable that animals which are under severe protein deficiency in the field would not be able to resist establishment of trypanosome infection. In confirmation of this suggestion, Agyemang *et* al. (1992) have observed that animals under severe physiological stress have higher incidences of trypanosome infections.

The development of moderately higher parasitaemia by animals on a high protein diet is not easy to explain. However, it is possible that in a situation where the parasite derives all its nutrients from the host, provision of excess nutrients might benefit the parasite as well, allowing it to grow and multiply. This has been partly confirmed by the studies of Black and Vanderweed (1989) who observed that provision of lipoproteins in culture to *T. brucei* enhanced its growth and withdrawal of the lipoproteins suppressed growth.

Following the establishment of infection, animals on a low protein diet experienced greater retardation of growth than the LPC group while infected and control animals on a high protein diet grew at the same rate. These observations are in agreement with those of Hecker *et al.* (1991) and Agyemang *et al.* (1990). The observation of more pronounced retardation of growth in infected animals on a low protein diet can not be attributed to decrease in feed intake as this was not significantly different from that of control animals. This observation is in contrast with that of reduced feed intake in goats infected with *T. brucei* (van Miert *et al.*, 1990) or *T. vivax* (Zwart *et al.*, 1991). Experiments in goats infected with *T. vivax*

(Verstegen *et al.*, 1991) have demonstrated that development of fever during a course of trypanosome infection is associated with increased heat production and increased metabolisable energy for maintenance. The consequence of this, is that the proportion of feed that can be used for growth is reduced leading to a poor feed conversion efficiency (Omole and Onawunmi, 1979; Ilemobade and Balogun, 1981). This effect would be more pronounced for the animals receiving just above maintenance requirements, e.g the LPI group, than for the animals receiving well above maintenance and growth requirements, e.g the HPI group. This study has shown that a high protein diet ameliorates the effect of trypanosome infection on growth rate.

Infected animals on a low (LPI) or a high (HPI) protein diet developed similar degrees of anaemia, as judged by PCV, RBC, and haemoglobin concentration, following patency of parasitaemia; however macrocytosis and an increase in MCH were significant only in the HPI group compared to the HPC group.

Observation of similar degrees of anaemia in the LPI and HPI groups is in agreement with the findings of Agyemang *et al.* (1990) in N'Dama cattle maintained on two planes of nutrition and exposed to natural trypanosome challenge. However, the present study may not be directly compared with those of Agyemang *et al.* (1990), Little *et al.* (1990) and Hecker *et al.* (1991) largely because their studies were conducted in animals placed on high and low planes of nutrition, rather than on high and low dietary protein levels. In addition, the studies reported by Agyemang *et al.* (1990) and Hecker *et al.* (1991) exposed cattle and sheep, respectively, to natural fly challenge and under these circumstances, the nutrient intakes and trypanosome challenge are not easy to quantify. Little *et al.* (1990) observed that the rate of development of anaemia in N'Dama cattle supplemented with extra groundnut cake,

was slower than in unsupplemented cattle. The present study has shown that the rate of development of anaemia in trypanosome infected sheep is not influenced by dietary protein.

From the haematological data, it is apparent that dietary protein had a major influence on both the morphological characteristics and rate of recovery from the anaemia. The anaemia in HPI group was macrocytic and normochromic while it was normocytic and normochromic in LPI group. After treatment at 70 DAI, both infected groups recovered from the anaemia, but the rate of recovery tended to be faster in animals on a HP diet than in those on a LP diet. This observation is in agreement with that of Agyemang *et al.* (1990) who reported that N'Dama cattle on a high plane of nutrition recovered from anaemia, without treatment, much faster than those on a low plane of nutrition.

The recorded PCV values in trypanosome-infected animals show the balance between red cell production and destruction and Woodliff and Hermann (1973) and Anosa and Isoun (1976) suggested that macrocytosis is indicative of the presence of large numbers of reticulocytes in the circulation. The observation of macrocytosis and increased MCH in the HPI group therefore suggests enhanced erythropoietic activity and may account for rapid recovery from anaemia following treatment.

While it has been suggested that protein deficiency *per se* does not impair erythropoiesis directly, studies in sheep (Abdullahi *et al.*, 1986a) and cattle (Oyedipe *et al.*, 1984) indicated low PCV and Hb concentrations in protein deprived animals. It is therefore possible that severe protein deficiency deprives the haemopoietic organs of amino acids necessary for red cell and haemoglobin production. Reissmann (1964) has suggested that this could be associated with lack of the stimulating agent, erythropoietin. Such a mechanism could account for poor erythropoietic response, of animals fed on LP diet, following infection and treatment. However, failure to record low PCV in control animals on a LP diet

indicates that animals in the present study were not severely protein deficient compared to the sheep used by Abdullahi *et al.* (1986a) where a protein level of 4% was used. The sheep fed on a protein level of 7.5% had similar haematological values as those fed on 15%, suggesting that protein deficiency needs to be very severe before major haematological effects are observed.

Infection and level of dietary protein had no significant effects on total and differential white cell counts; however following treatment, animals on a HP diet showed faster increases in WBC, lymphocyte and monocyte counts. The finding that diet had no influence on total or differential white cell counts is in agreement with the findings of Oyedipe *et al.* (1984) and Abdullahi *et al.* (1986a).

Both infected groups of animals developed similar degrees of thrombocytopaenia, and following treatment, thrombocyte counts rapidly returned to normal. The possible causes of thrombocytopaenia during a trypanosome infection have been discussed earlier (Chapter 5). The rebound thrombocytosis is an interesting observation. It is possible that it is due to rapid maturation of bone marrow megakaryocytes leading to release of large numbers of platelets into circulation. There is evidence that in splenomegaly, a consistent feature of animal trypanosomiasis, the exchangeable pool of platelets increases and may represent 90 per cent of marrow output (Jenkins and Facer, 1985). Following chemotherapy and elimination of trypanosomes from the circulation, the large numbers of platelets which are sequestered in the spleen may be released suddenly.

Infected animals of both dietary groups showed significant decreases in plasma cholesterol and serum phospholipid concentrations with non-consistent changes in plasma NEFA and triglycerides, with a resultant decrease in serum total lipids. After treatment, total lipid, cholesterol and phospholipid concentrations increased, the increase being greater in animals on the HP diet. In addition, there were nutritional effects, with the HP group having higher concentrations of serum

total lipids, plasma cholesterol and NEFA than those on LP diet. Observation of a decrease in plasma cholesterol and phospholipids with infection also confirms findings in previous studies (Chapters 3 and 5). The observation that the HP diet group had higher concentrations of serum total lipids, cholesterol and NEFA is not easy to elucidate. However, it may partly explain why the HPI group was able to sustain higher intensity of parasitaemia. It has been proposed that cholesterol is essential for trypanosome growth (Vanderweed and Black, 1989, Black and Vanderweed, 1989) and results obtained in Chapter 5 indicated that animals with higher cholesterol concentration support higher parasite numbers. There is evidence also that trypanosomes need fatty acids which they use for energy metabolism (Tizard *et al.*, 1978c). In addition to fatty acids circulating freely in plasma, trypanosomes obtain more fatty acids by uptake of albumin-bound fatty acids (Vickerman and Tetley, 1979) and lipoproteins, especially low and high density lipoproteins. From the present study, it appears that provision of high protein in the diet has a sparing effect on lipid metabolism of the host making these nutrients available for the trypanosomes.

Both infected groups in the present study developed hypoproteinaemia which was associated with hypoalbuminaemia, and plasma urea concentrations were not affected by infection. However, dietary effects were observed, in that animals on the HP had higher total protein, plasma albumin and urea concentrations than those on a LP diet. Observation of hypoalbuminaemia in infected animals has been reported and discussed (Chapter 3). The finding of higher albumin concentration which was associated with higher parasitaemia confirms the results recorded in Chapter 5 and indicates that availability of albumin, like cholesterol may enhance parasite growth and multiplication. The association of high plasma albumin and urea concentrations with increased protein intake has previously been recorded (Katunguka-Rwakishaya *et al.*, 1987; Rewkot *et al.*, 1989; Sawadogo *et al.*, 1991).

There is evidence that high protein diets may result in an excess of rumen ammonia, due to microbial degradation, which will be metabolised in the liver to urea and excreted (Hibbit, 1988). The amount of urea produced, depends to a large extent on the quality of the dietary protein. Diets with a high proportion of rumen degradable protein (RDP) are associated with higher plasma urea concentrations than those with high undegradable protein (UDP)(Twigge and Van Gils, 1988). It has been proposed that to increase the efficiency of protein utilisation, diets with a high UDP composition should be selected. The effect of such diets on the pathogenesis of animal trypanosomiasis needs to be evaluated at a later date.

Infection caused an apparent decrease in the concentrations of SI and TIBC of animals on a LP diet and not of those on a HP diet. Dietary effects were observed with HP group having higher TIBC than the LP group. This is in support of the findings of Stevenson (1989) in sheep of about the same age, as those in the present study, and given similar protein levels. Total iron-binding capacity (TIBC) is an indirect measurement of transferrin, a B-globulin protein that transports iron to the bone marrow for incorporation into haemoglobin (Lewis, 1977). A reduction in the concentration of TIBC, as observed in animals on a LP diet may therefore be part of a general decrease in protein availability.

Dissection of the indicator joints revealed few differences between groups. However, it was observed that animals on HP diet had heavier joint weights than those on LP diet. This method of studying body composition was not without problems. First, an attempt was made to divide the carcases accurately along the midline using a hand saw. This was not achieved in all cases as a result of which some carcase halves had more bone than others. The second problem may be associated with dissection, in that it was not easy to separate all muscle from bone and from fat. These errors may affect composition in individual animals, particularly of bone.

Carcase chemical analysis also did not reveal significant differences in the relative composition of protein, energy, fat and ash. Failure to observe any significant changes may be associated with the fact that differences in protein intake were not large enough to impart severe changes in carcase composition. However, in consideration of the whole carcase, infection caused a reduction in total protein, energy, fat and ash and following treatment, animals on the HP diet showed an improvement in these variables. These results need to be interpreted with caution because they were derived from few animals and again, small samples of mince which were analysed, may not be totally representative of the whole carcase.

In conclusion, the results obtained in the present study suggest that dietary protein can play an important role in the pathogenesis of animal trypanosomiasis, and can also influence the rate of recovery following chemotherapy.

CHAPTER 8

THE INFLUENCE OF ENERGY INTAKE ON THE PATHOPHYSIOLOGY OF *TRYPANOSOMA CONGOLENSE* INFECTION IN SCOTTISH BLACKFACE SHEEP

INTRODUCTION

Trypanosome infections are often modulated by interacting factors within and without the host (Murray *et al.*, 1982; Murray and Dexter, 1988). Under field conditions, particularly during the dry season, feedstuffs become scanty and poor in both protein and energy content and this imparts stressful conditions on animals in trypanosomiasis endemic areas. The ability of infected animals to withstand infection is impaired and this has largely been attributed to failure to mount effective immune responses (Abdullahi *et al.*, 1986a; Sanders *et al.*, 1990). Most work investigating the influence of nutrition on the pathogenesis of trypanosome infections have used either a low or a high plane of nutrition with either experimental infection (Little *et al.*, 1990) or natural tsetse fly challenge (Agyemang *et al.*, 1990; Hecker *et al.*, 1991). The respective role of protein and energy have not been evaluated.

In a previous study (Chapter 7), an investigation of the influence of dietary protein on the pathogenesis of *T. congolense* infection was conducted. It was observed that dietary protein influenced both the pathogenesis of trypanosome infection and rate of recovery following chemotherapy. The present experiment was carried out to investigate the influence of energy intake on the course of *T. congolense* infection in sheep.

MATERIALS AND METHODS

Experimental Animals

Ten pairs of male castrate, twin Scottish Blackface lambs, about four months of age were used in the present study. They were bought from a local hill farm in the west of Scotland.

Feeding and Housing

The diets consisted of a mixture of sugar beet pulp, barley siftings, soyabean meal and vitamin/mineral/ sheep trace elements in varying proportions. The proportion of these individual ingredients is shown in Table 8.1 and the mean proximate analyses of experimental diets is given in Table 8.2. The diets were mixed by hand as explained in the previous experiment (Chapter 7).

All experimental animals were housed in individual pens. Infected animals were housed in a flyproof isolation unit while control uninfected animals were housed in an open shed at Cochno experimental unit.

Feeding strategy

Initially, the animals on a high energy (HE) intake were given 1.1 kg of fresh matter (FM) and those on low energy (LE) intake were given 0.64 kg FM in two feeds, at 10.00 and 16.00 h. However, after two weeks of infection, it was found necessary to increase the intake of these animals because they now weighed heavier than 20 kg, the weight for which this diet had been designed. The intake of the HE group was increased to 1.2 kg FM and that of the LE group to 0.71 kg FM. These amounts of fresh matter provided the high energy (HE) group with 109.2 g of crude protein (CP) and 9.9 MJME per day and the low energy (LE) group with 109.6 g CP and 6.1 MJME per day. At these levels of energy and protein intakes, animals on the HE intake were expected to gain 131 gd^{-1} while those on LE would gain 40 gd^{-1} (MAFF *et al.*, 1984).

Table 8.1

Composition of experimental diets given to the high energy (HE) and low energy (LE) intake groups (gkg^{-1} FM*)

	HE	LE
Sugar beet pulp	434.5	519.1
Barley siftings	325.9	389.4
Soyabean meal	217.3	64.9
Vitamin/mineral/sheep element mixture	22.3	26.6

*FM = Fresh matter

Table 8.2

Mean proximate analysis of experimental high energy (HE) and low enrgy (LE) diets*

		HE	LE
DM	(gkg^{-1} FM)	878.0	877.7
CP	(gkg^{-1} DM)	104.9	176.7
CF		237.2	205.5
Ash		71.1	71.1
EE		6.92	6.86
ME	$MJkg^{-1}$ DM	9.4	9.8

*The energy contents of the diets were similar but the energy intakes of the HE and LE groups of animals were different

Trypanosomes and Preparation of the Inoculum

Trypanosoma congolense (GRVPS 57/6) was used to infect the sheep. The details of this clone of *T. congolense* has been described (Chapter 5) and the method used to prepare the inoculum was the same as that described in the previous study (Chapter 7).

Parasitological, Haematological, Blood Biochemical and Feed Analytical Methods

Methods used to collect and examine blood samples for intensity of parasitaemia, haematology and blood biochemistry, and feed analytical techniques have been described in general materials and methods (Chapter 2).

Experimental Design

The animals were divided into two groups of five twin pairs each and one group was allowed a high energy (HE) intake while the other was allowed a low energy (LE) intake. It should be noted here that energy composition of the two diets were similar but the amount of fresh matter given to the HE and LE groups were different.

After two weeks on the experimental diets, one member of each pair was infected with *T. congolense* by the jugular route, while the other member served as the uninfected control. The choice of which one to infect was random. All animals were then monitored for 70 days.

RESULTS

Parasitological Findings

The prepatent periods in the HEI and LEI groups were 14.8 ± 0.8 days (range 12-16 days) and 17.8 ± 2.5 days (range 12-26 days), respectively. Following patency, the parasitaemia increased in both infected groups to reach the first peak by 23 DAI in the HEI group and by 34 DAI in the LEI group (Figure 8.1). Thereafter, the parasitaemia fluctuated considerably with a tendency to be higher in the LEI group than in the HEI group, however, none of these differences were significant.

Body Weight Changes

Infection and energy intake had significant effects on body weight gain. The mean live body weight of the HEI group increased from 25.9 ± 3.1 at 0 DAI to 30.9 ± 1.5 kg at 70 DAI. In the HEC group (Figure 8.2), it increased from 26.5 ± 1.8 kg at 0 DAI to 34.9 ± 1.1 kg at 70 DAI. Between 0 and 70 DAI, the weights of LEI increased from 24.8 ± 1.2 to 27.4 ± 1.5 kg while that of the LEC group increased from 25.0 ± 0.7 to 29.2 ± 0.7 kg. The body weight of HEI group was significantly greater than that of the HEC and LEI groups. In addition, there were nutritional effects in that the HEC group grew at a significantly higher rate than the LEC group. The gains in body weight between 0 and 70 DAI were 2.6, 4.2, 5.0 and 8.4 kg in LEI, LEC, HEI and HEC groups, respectively.

Haematological Observations

Packed cell volume (PCV)

Infection caused a significant drop in mean PCV values of both groups. In both infected groups, the PCV started to decrease after patency of parasitaemia and continued to reach the lowest value of 0.28 ± 0.02 ll^{-1} by 51 DAI in the HEI group (Figure 8.3). The lowest value in the LEI group was 0.27 ± 0.02 and it was

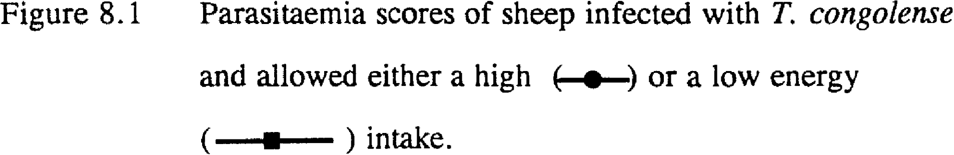
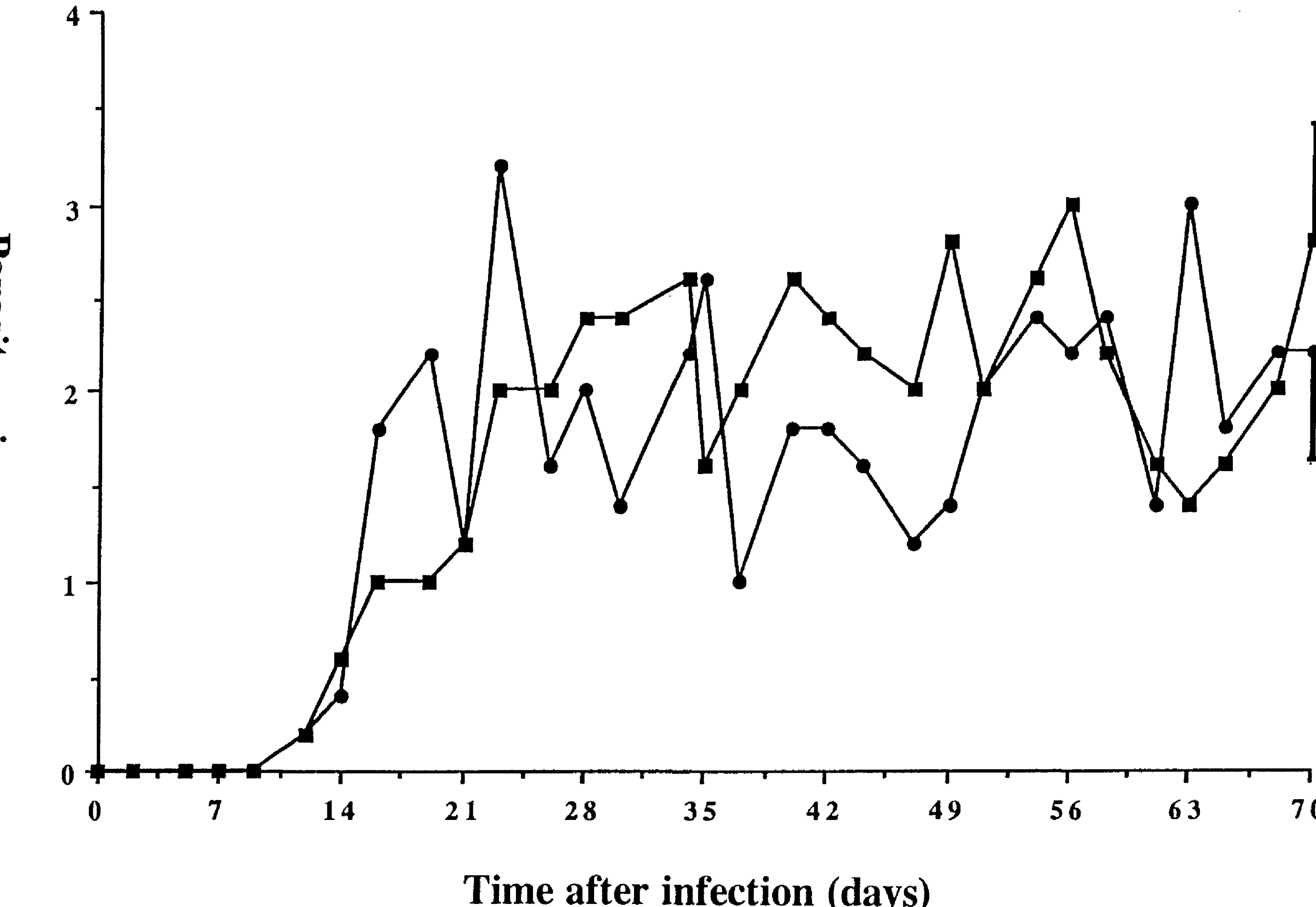


Figure 8.1 Parasitaemia scores of sheep infected with *T. congolense* and allowed either a high (—●—) or a low energy (——■——) intake.


Parasitaemia score
0
1
2
3
4
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

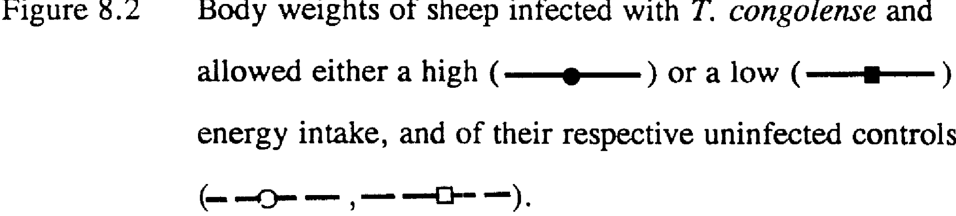
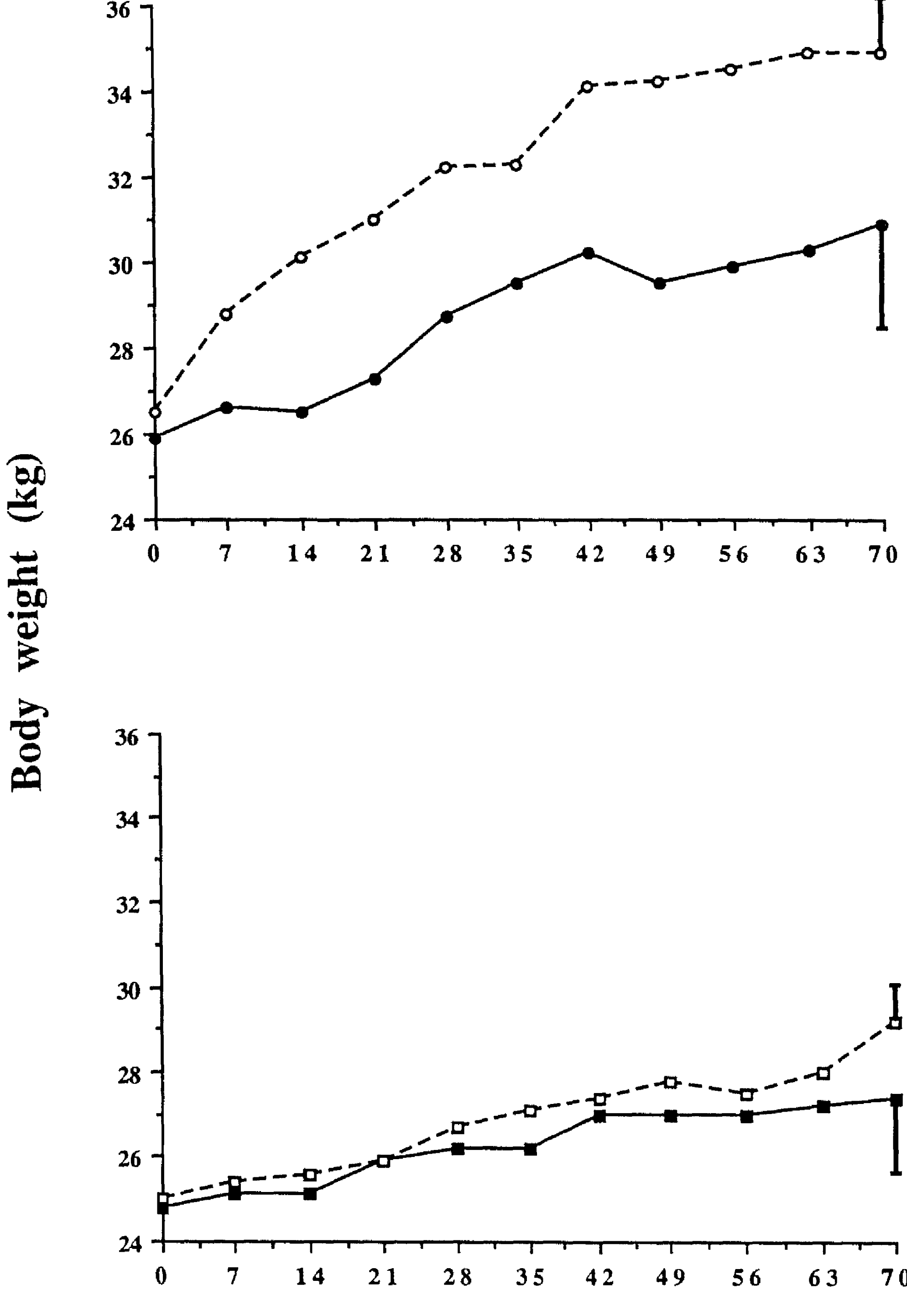


Figure 8.2 Body weights of sheep infected with *T. congolense* and allowed either a high (——●——) or a low (——■——) energy intake, and of their respective uninfected controls (– –○– –, – –□– –).


36
34
32
30
28
26
24
0
7
14
21
28
35
42
49
56
63
70
Body weight (kg)
36
34
32
30
28
26
24
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

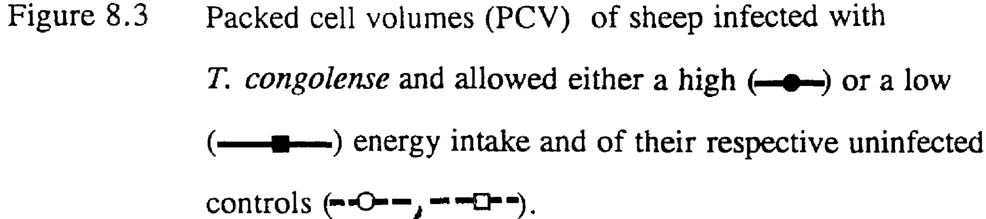
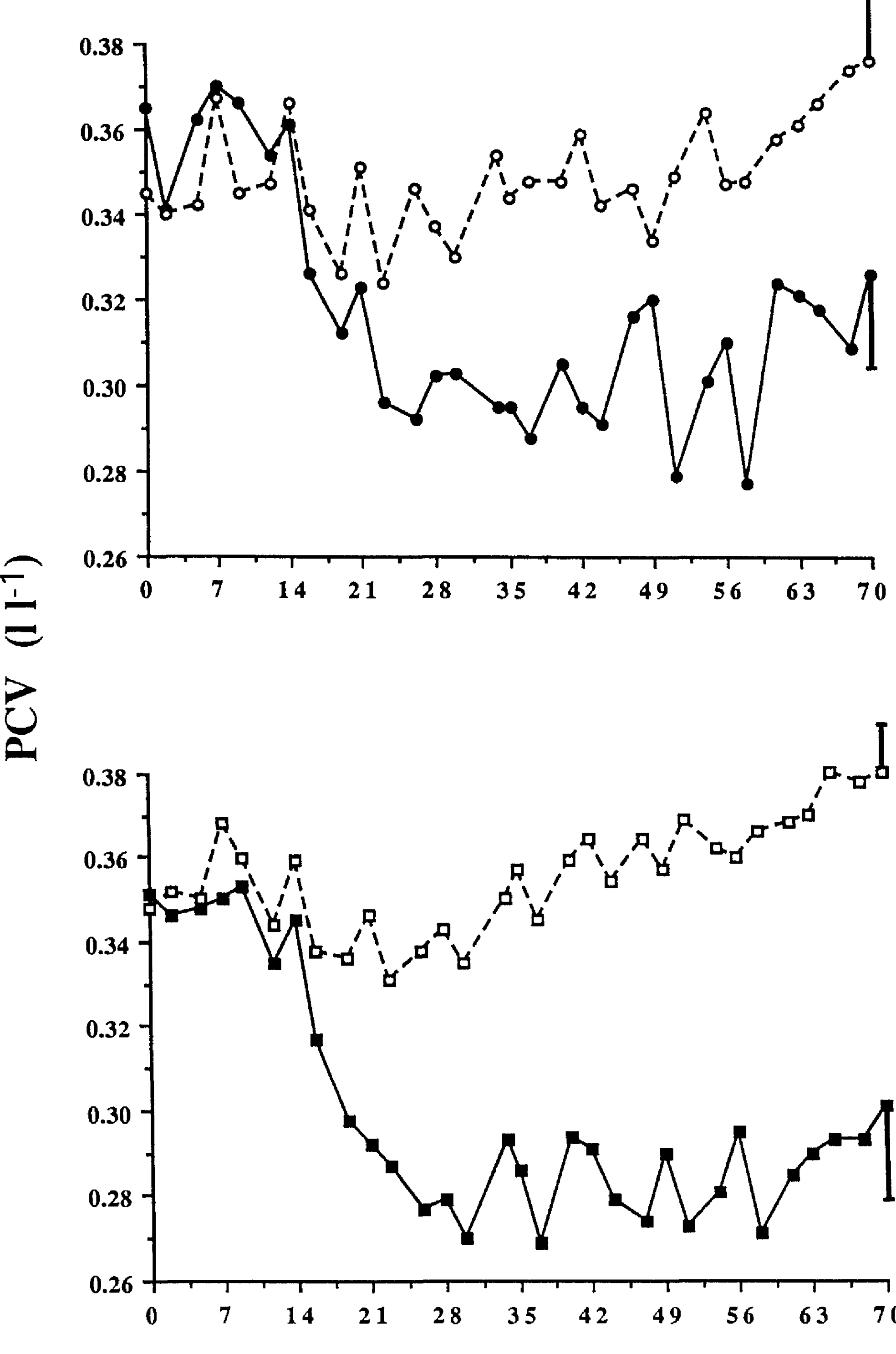


Figure 8.3 Packed cell volumes (PCV) of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (——■——) energy intake and of their respective uninfected controls (--○--, --□--).



PCV (l l-1)
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0 7 14 21 28 35 42 49 56 63 70
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

reached by 37 DAI. The PCV in the LEI group was significantly lower than that of the HEI group. In control animals, the PCV fluctuated between 0.33 $\pm$ 0.01 and 0.38 $\pm$ 0.02 ll^{-1}, and showed no significant energy effects

Red blood cell count (RBC)

The RBC decreased significantly in both infected groups. The decline was more pronounced in the LEI group compared with the HEI group. In the HEI group, the mean RBC decreased from 13.52 $\pm$ 0.48 at 2 days before infection (DBI) to 8.84 $\pm$ 0.70 x $10^{12}l^{-1}$ at 61 DAI (Figure 8.4). In the LEI group, it decreased from 12.93 $\pm$ 0.40 at 2 DBI to 8.56 $\pm$ 0.20 x $10^{12}l^{-1}$ by 54 DAI. The RBC values in control animals fluctuated between 11.66 $\pm$ 0.24 and 13.07 $\pm$ 0.41 x $10^{12}l^{-1}$.

Haemoglobin (Hb) concentration

Infection caused a significant decrease in Hb concentration. However, the Hb concentration in the LEI group was lower than that in the HEI group, but this difference was not significant. The Hb concentration in the HEI group decreased from 13.2 $\pm$ 0.3 gdl^{-1} at 2 DBI to 10.0 $\pm$ 0.8 gdl^{-1} by 61 DAI (Figure 8.5). In the LEI group, the Hb concentration decreased from 12.9 $\pm$ 0.5 at 2 DBI to 9.3 $\pm$ 1.0 gdl^{-1} at 34 DAI. The values in control animals ranged from 11.7 $\pm$ 0.3 to 13.4 $\pm$ 0.5 gdl^{-1}.

Mean corpuscular volume (MCV)

The MCV increased significantly in both infected groups compared with their respective uninfected controls. It increased, in the HEI group, from 28.3 $\pm$ 0.9 fl at 19 DAI to 33.0 $\pm$ 0.8 fl at 61 DAI (Figure 8.6). In the LEI group, it increased from 28.8 $\pm$ 0.4 at 26 DAI to 34.2 $\pm$ 1.5 fl at 61 DAI. In control animals, the values fluctuated between 27.8 $\pm$ 0.5 and 29.8 $\pm$ 0.6 fl.

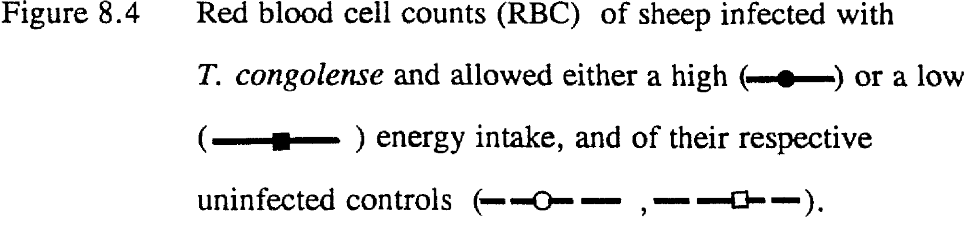
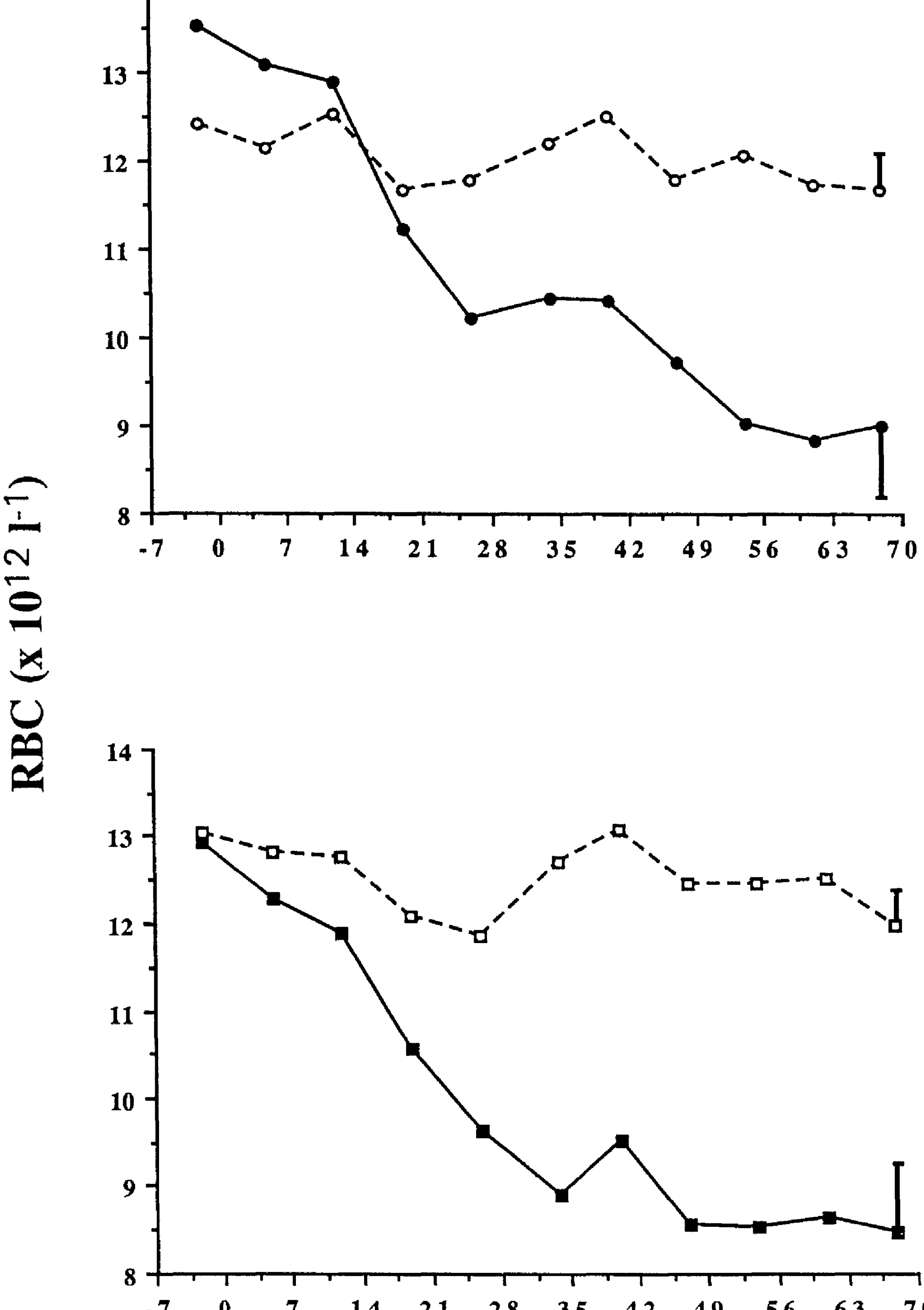


Figure 8.4 Red blood cell counts (RBC) of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake, and of their respective uninfected controls (—○—, —□—).



RBC (x 10^12 l^-1)
Time after infection (days)
14
13
12
11
10
9
8
-7
0
7
14
21
28
35
42
49
56
63
70

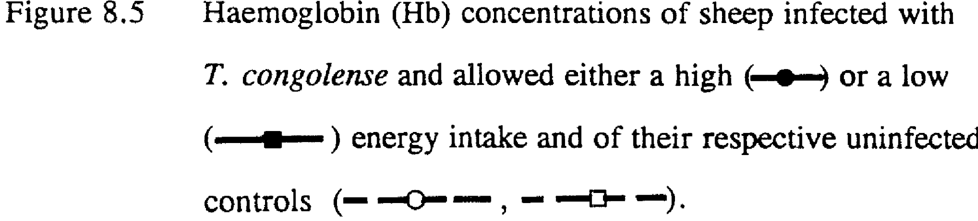
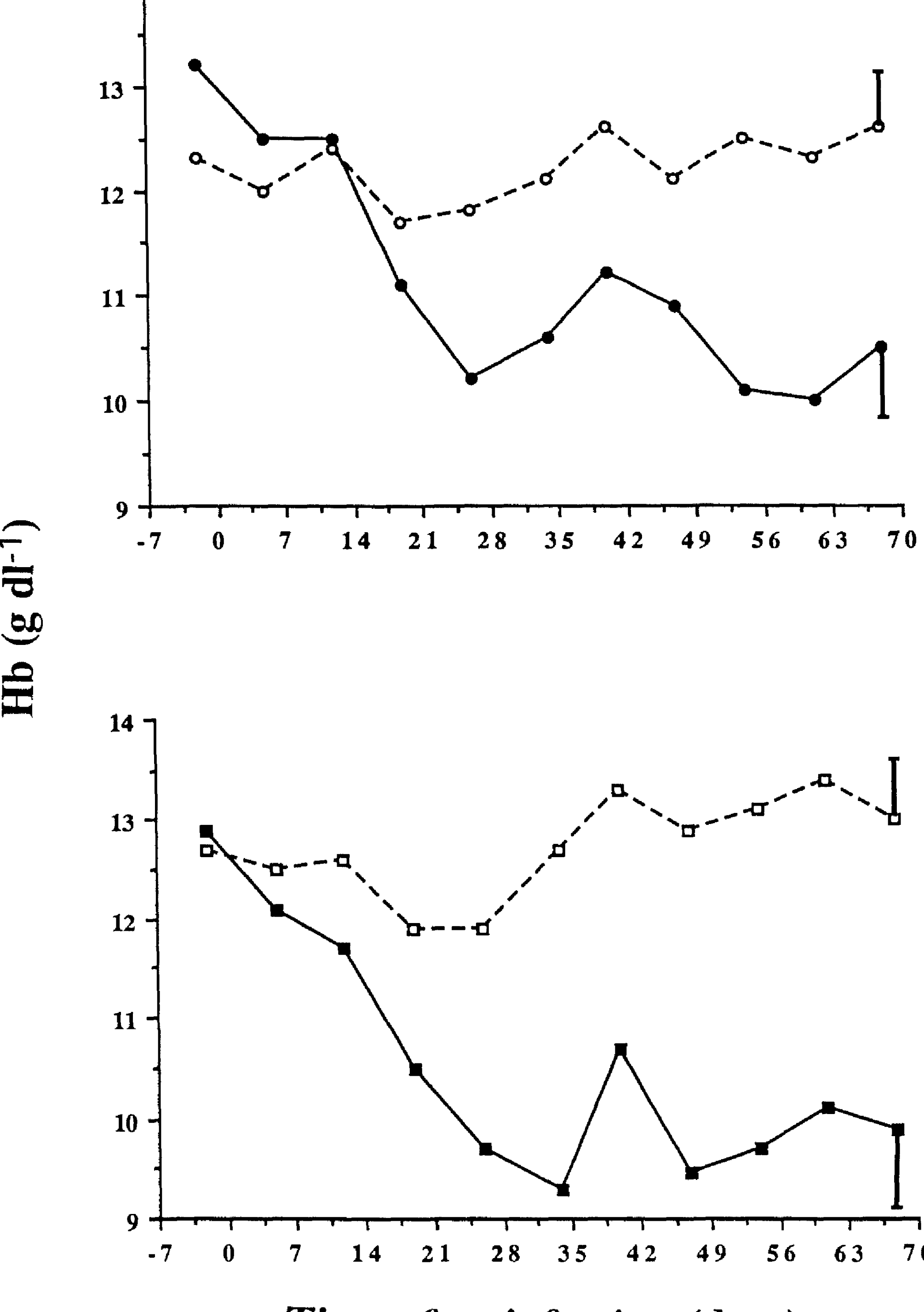


Figure 8.5 Haemoglobin (Hb) concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (– –○– –, – –□– –).



Hb (g dl-1)
14
13
12
11
10
9
-7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

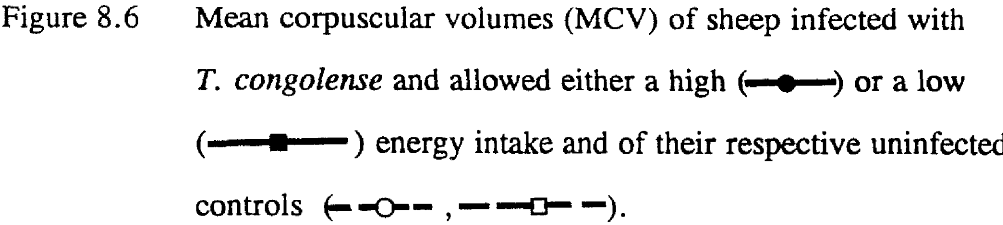
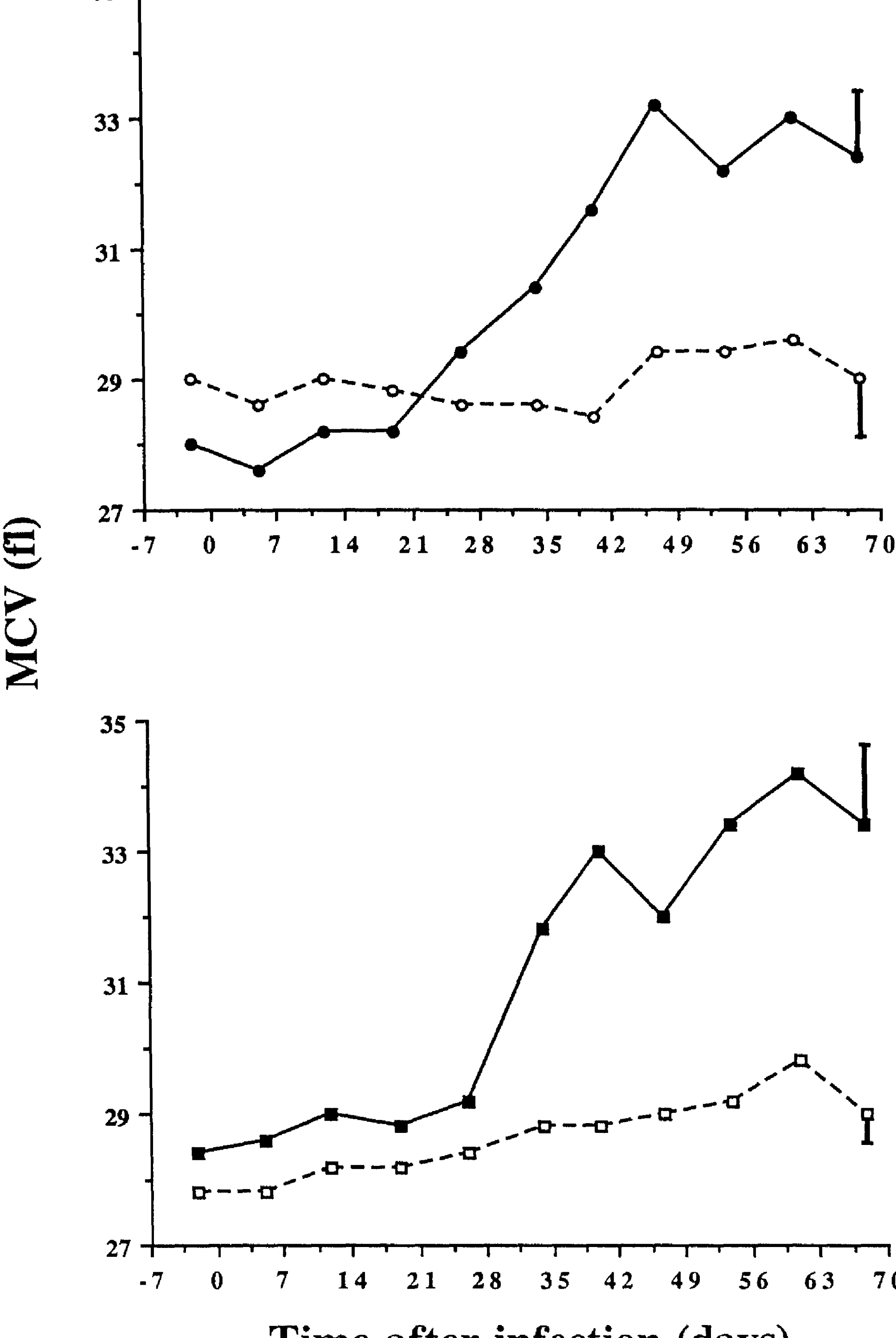


Figure 8.6 Mean corpuscular volumes (MCV) of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (- -○- -, - -□- -).


MCV (fl)
35
33
31
29
27
-7 0 7 14 21 28 35 42 49 56 63 70
35
33
31
29
27
-7 0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

Mean corpuscular haemoglobin (MCH) and MCHC

Infection caused a moderate increase in MCH and a decrease in MCHC. These changes were not significant. The values in control animals were within the normal ranges.

White cell count (WBC), lymphocytes and neutrophils

Both infected groups showed a fluctuating increase in WBC (Figure 8.7) and lymphocyte counts with time. In addition, the neutrophil counts in the HEI group were significantly greater than the counts in the LEI group (Figure 8.8). There were also nutritional effects with the HEC group having significantly higher WBC, and neutrophil values than the LEC group.

Platelet counts

Infection caused a significant drop in platelet counts, and the values in the LEI group were significantly lower than the values in the HEI group. The platelets decreased in the HEI group from 340.6 ± 13.9 at 5 DAI to $96.6 \pm 27.6 \times 10^9 l^{-1}$ at 54 DAI (Figure 8.9). In the LEI group, it decreased from 240.2 ± 7.9 at 5 DAI to $38.6 \pm 25.8 \times 10^9 l^{-1}$ at 54 DAI. The values in control animals did not show any nutritional influences, and they fluctuated between 195.6 ± 25.1 and $331.8 \pm 53.7 \times 10^9 l^{-1}$.

Blood Biochemical Observations

Serum total lipids

Infected groups of animals showed significant decreases in serum total lipid concentrations. The decline appeared to be greater in the LEI group than in the HEI group. In the HEI group, the mean serum total lipid concentration decreased from

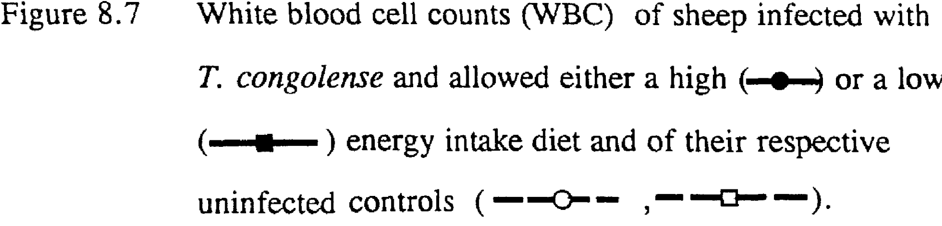
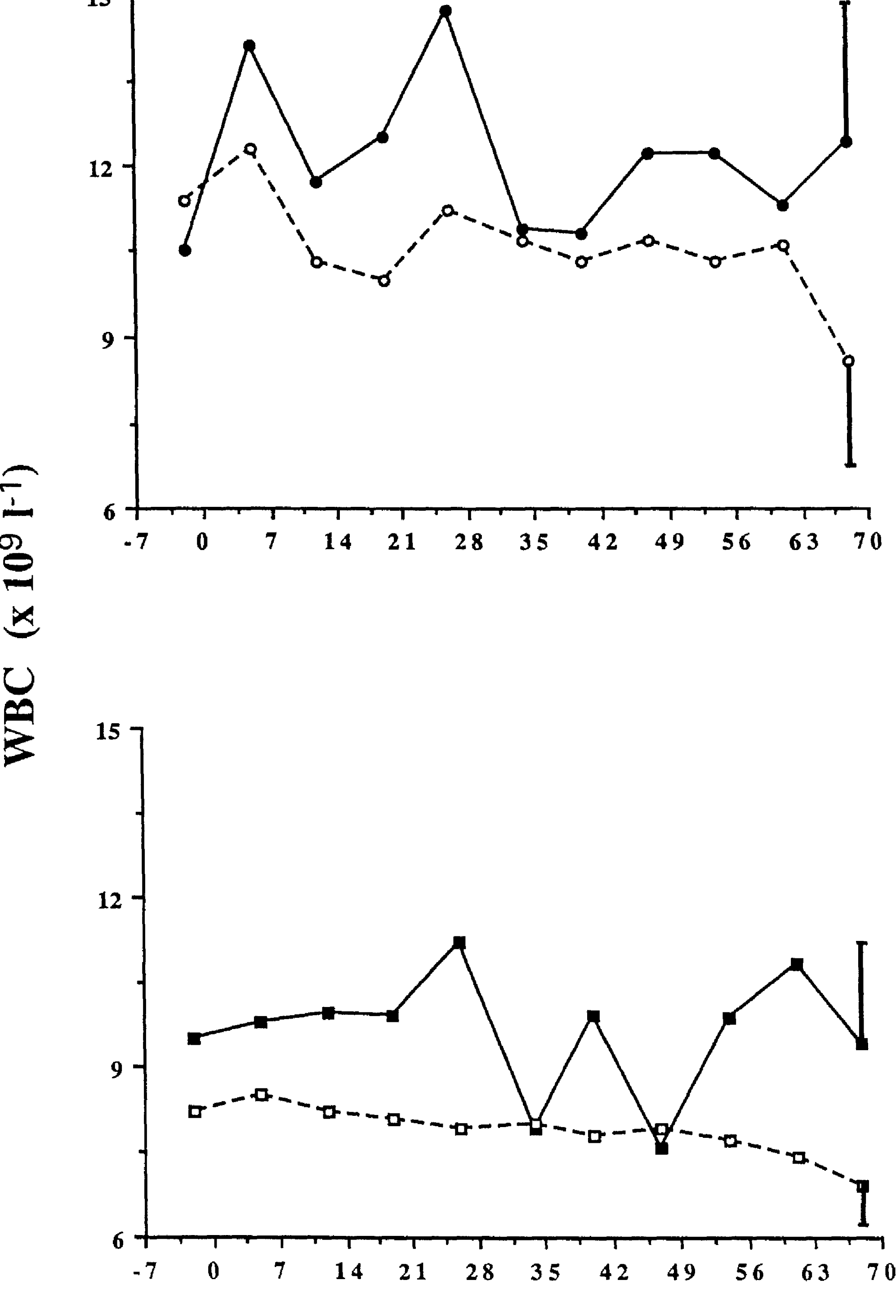


Figure 8.7 White blood cell counts (WBC) of sheep infected with *T. congolense* and allowed either a high ( ) or a low ( ) energy intake diet and of their respective uninfected controls (,).


15
12
9
6
-7
0
7
14
21
28
35
42
49
56
63
70
WBC (x 10^9 l^{-1})
15
12
9
6
-7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

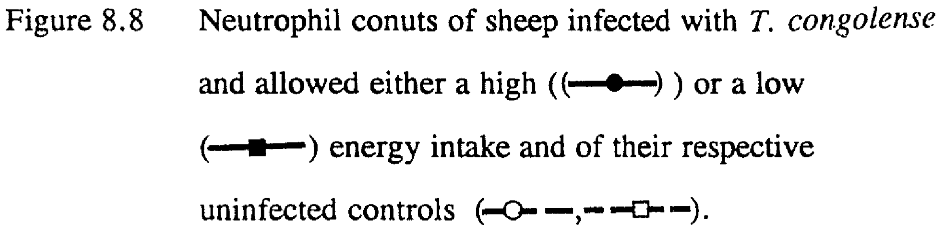
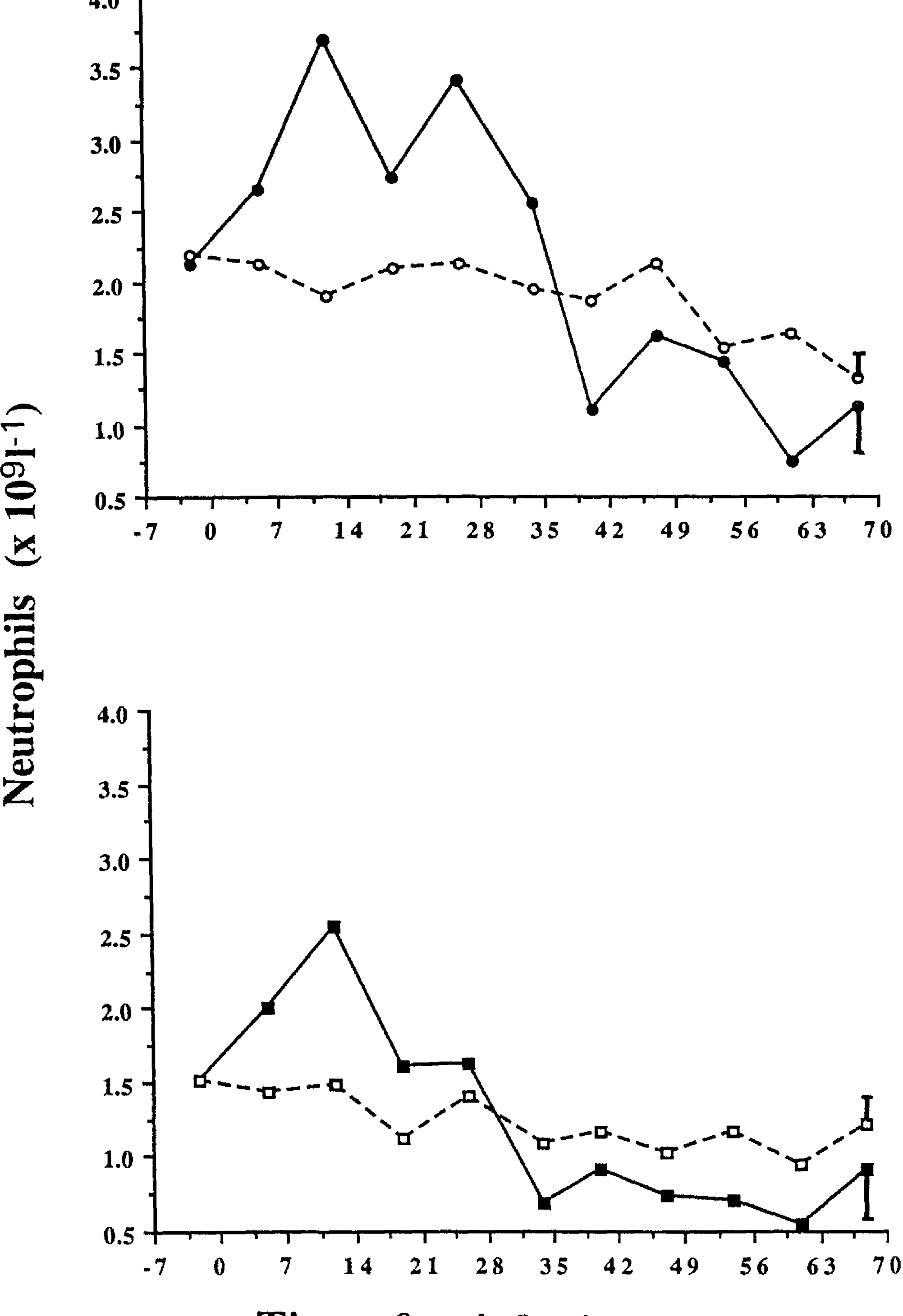


Figure 8.8 Neutrophil conuts of sheep infected with *T. congolense* and allowed either a high ((—●—)) or a low (—■—) energy intake and of their respective uninfected controls (–○– –,– –□– –).



Neutrophils (x 10^9l^{-1})
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-7
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

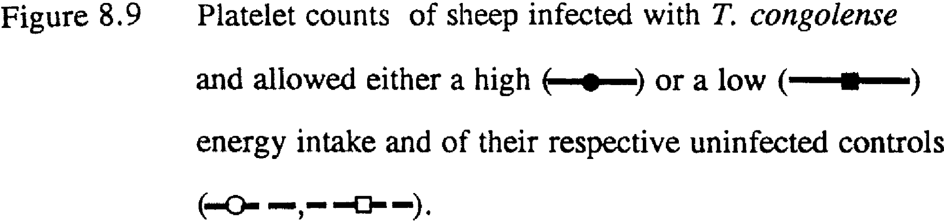
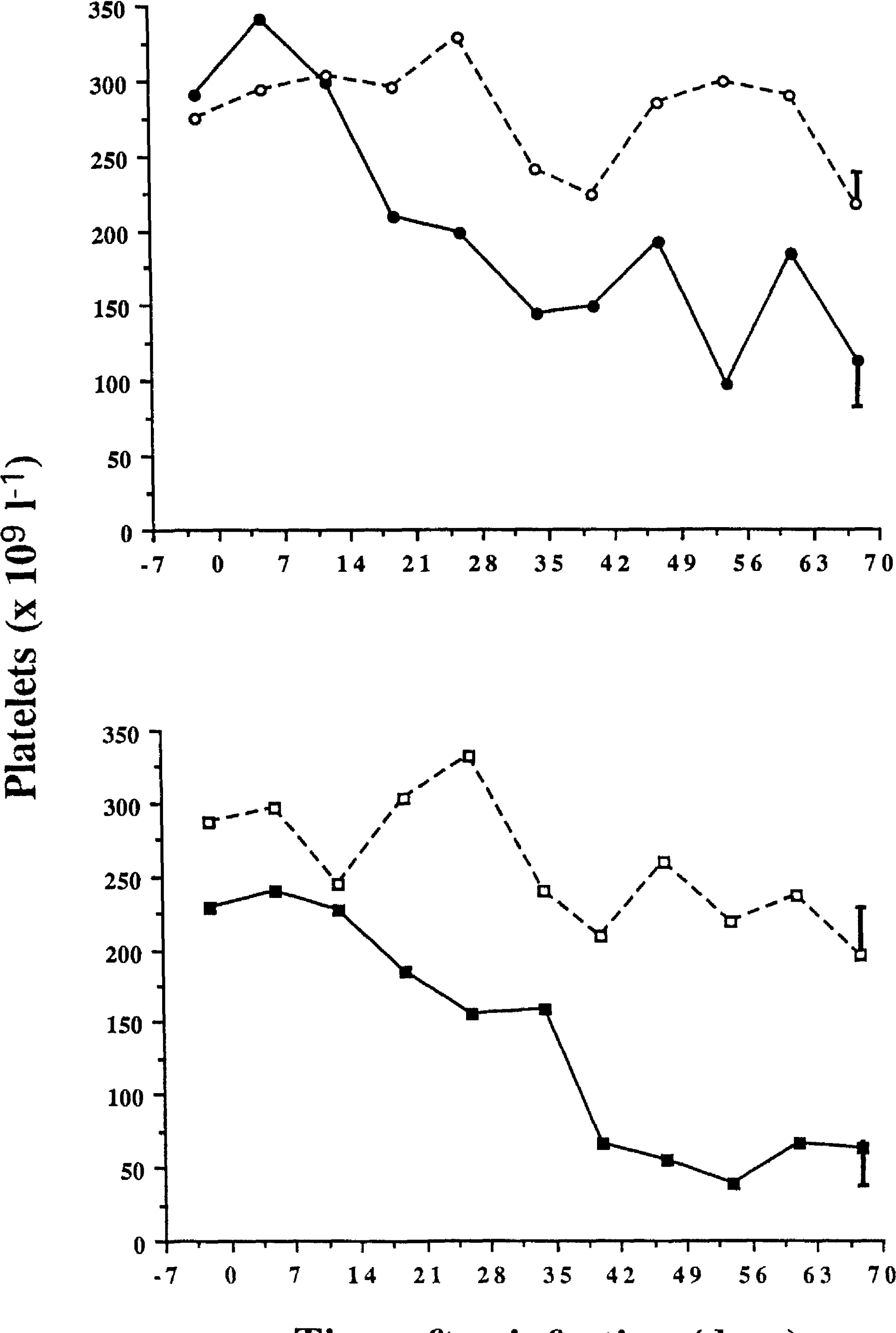


Figure 8.9 Platelet counts of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (–○–,–□–).



350
300
250
200
150
100
50
0
-7 0 7 14 21 28 35 42 49 56 63 70
Platelets (x 10^9 l^-1)
Time after infection (days)

2.14 ± 0.12 at 0 DAI to 1.20 ± 0.18 gl^{-1} at 56 DAI (Figure 8.10). In the LEI group, it decreased from 2.38 ± 0.49 at 0 DAI to 1.16 ± 0.10 gl^{-1} at 63 DAI. In control animals, the values fluctuated between 1.68 ± 0.13 and 2.40 ± 0.37 gl^{-1}.

Serum phospholipids

Following infection, the serum phospholipid concentration in both groups (HEI and LEI) decreased significantly. It decreased from 0.72 ± 0.04 gl^{-1} at 0 DAI in the HEI group to 0.38 ± 0.03 gl^{-1} at 42 DAI (Figure 8.11). In the LEI group, it decreased from 0.80 ± 0.04 to 0.35 ± 0.08 gl^{-1} between 0 and 56 DAI. The serum phospholipid values in the HEI group were not significantly different from those in the LEI group. There was a tendency for the HEC to have higher serum phospholipid concentrations than the LEC group, however, this was not significant.

Plasma cholesterol

The initial cholesterol concentration of the LE group was significantly higher than that of HE group. Following infection, the concentrations decreased significantly in both energy groups. In the HEI group, the plasma cholesterol concentration decreased from 0.84 ± 0.08 $mmoll^{-1}$ at 0 DAI to 0.51 ± 0.04 $mmoll^{-1}$ at 28 DAI after which it showed a moderate improvement (Figure 8.12). In the LEI group, it decreased from 1.22 ± 0.04 at 0 DAI to 0.44 ± 0.02 $mmoll^{-1}$ at 28 DAI. It tended to increase thereafter but showed a further decrease between 42 and 70 DAI. The concentration of plasma cholesterol in the HEI group was not significantly different from that in the LEI group. In control groups, the values in the LEC group (0.87-1.13 $mmoll^{-1}$) tended to be higher than those in the HEC group (0.79-1.03 $mmoll^{-1}$), but these differences were not significant.

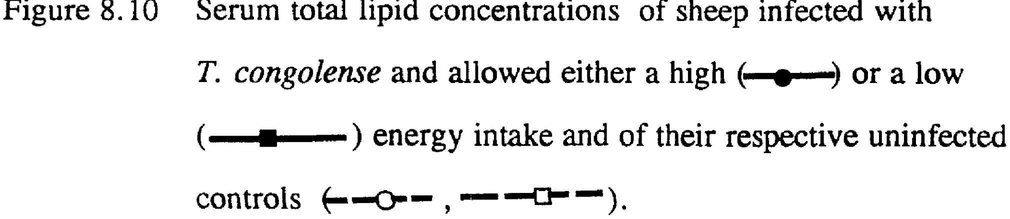
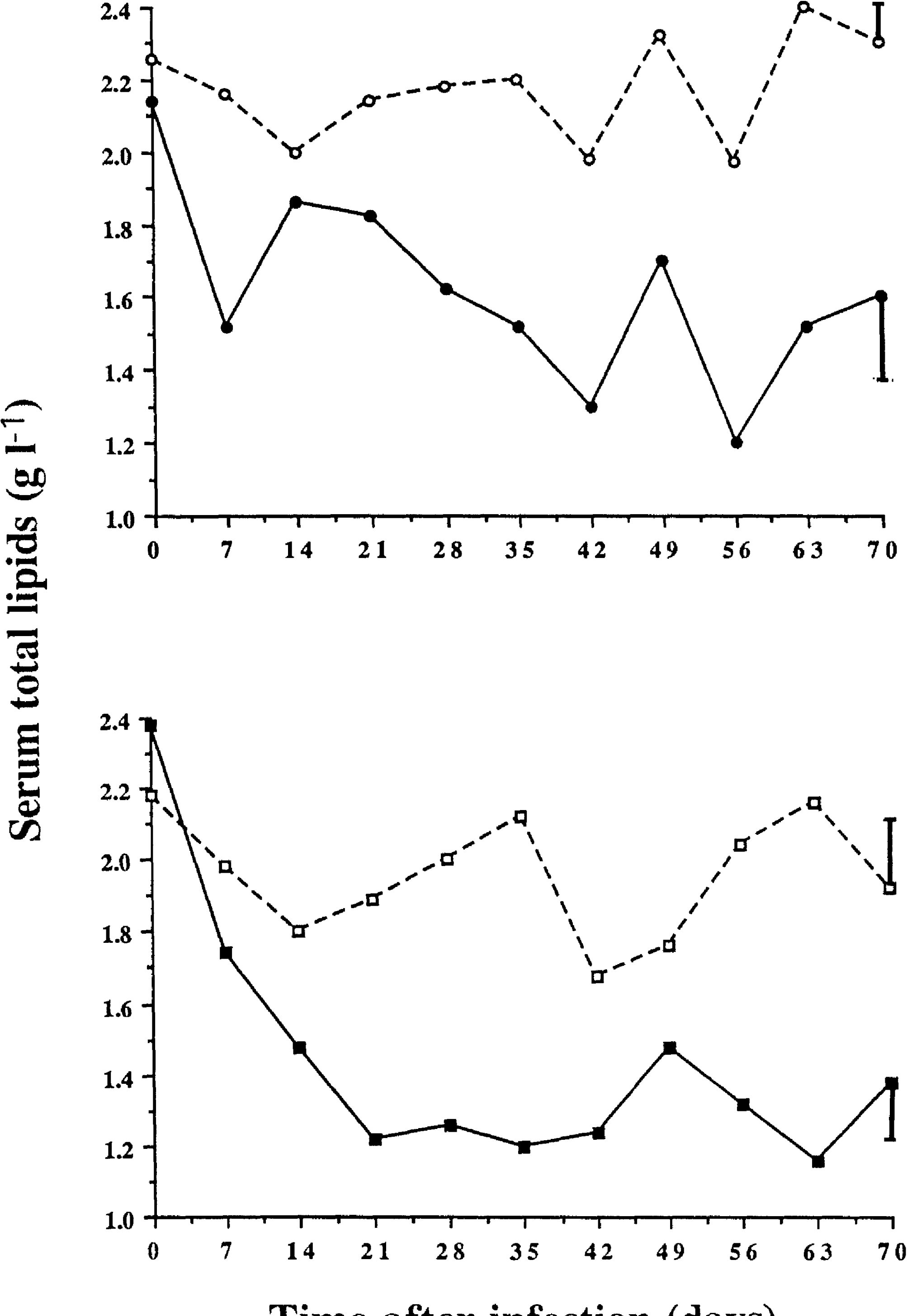


Figure 8.10 Serum total lipid concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (--○--, --□--).


Serum total lipids (g l-1)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0 7 14 21 28 35 42 49 56 63 70
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

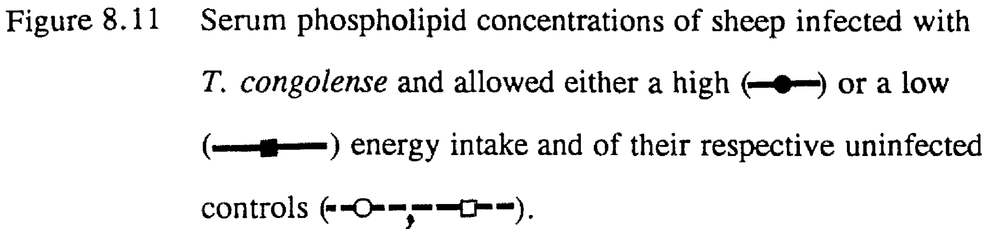
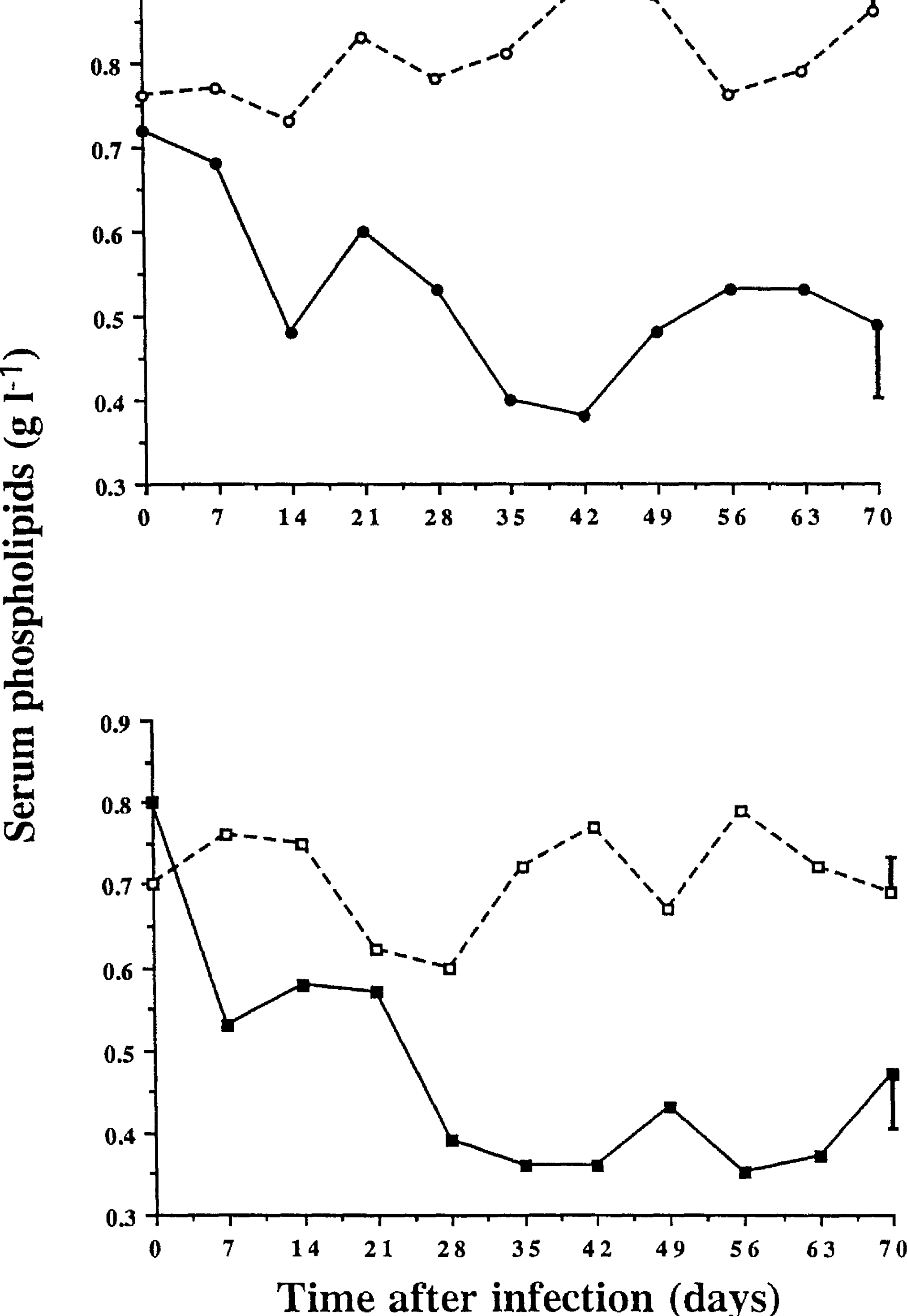
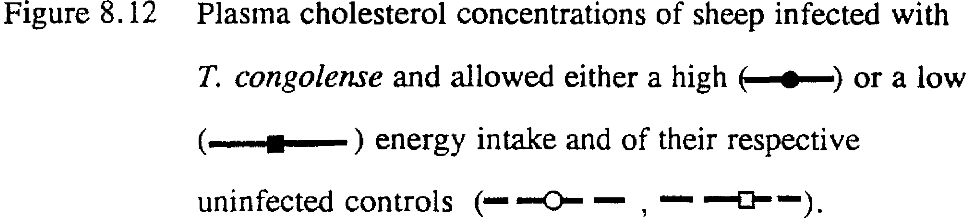


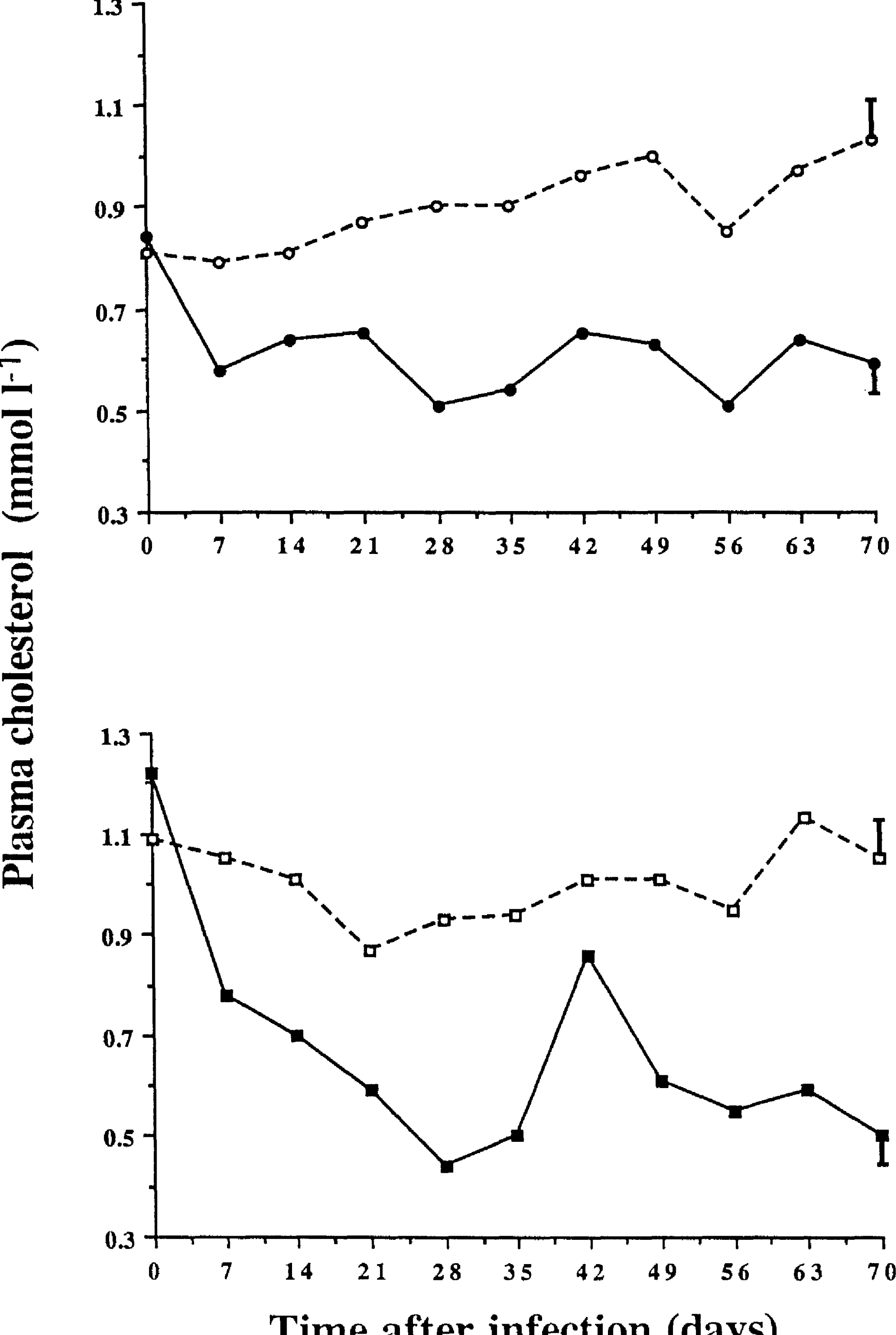
Figure 8.11 Serum phospholipid concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (——■——) energy intake and of their respective uninfected controls (- -○- -, - -□- -).



Serum phospholipids (g l-1)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

Figure 8.12 Plasma cholesterol concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (– –○– –, – –□– –).





Plasma cholesterol (mmol l-1)
1.3
1.1
0.9
0.7
0.5
0.3
0 7 14 21 28 35 42 49 56 63 70
1.3
1.1
0.9
0.7
0.5
0.3
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

Plasma triglycerides and NEFA

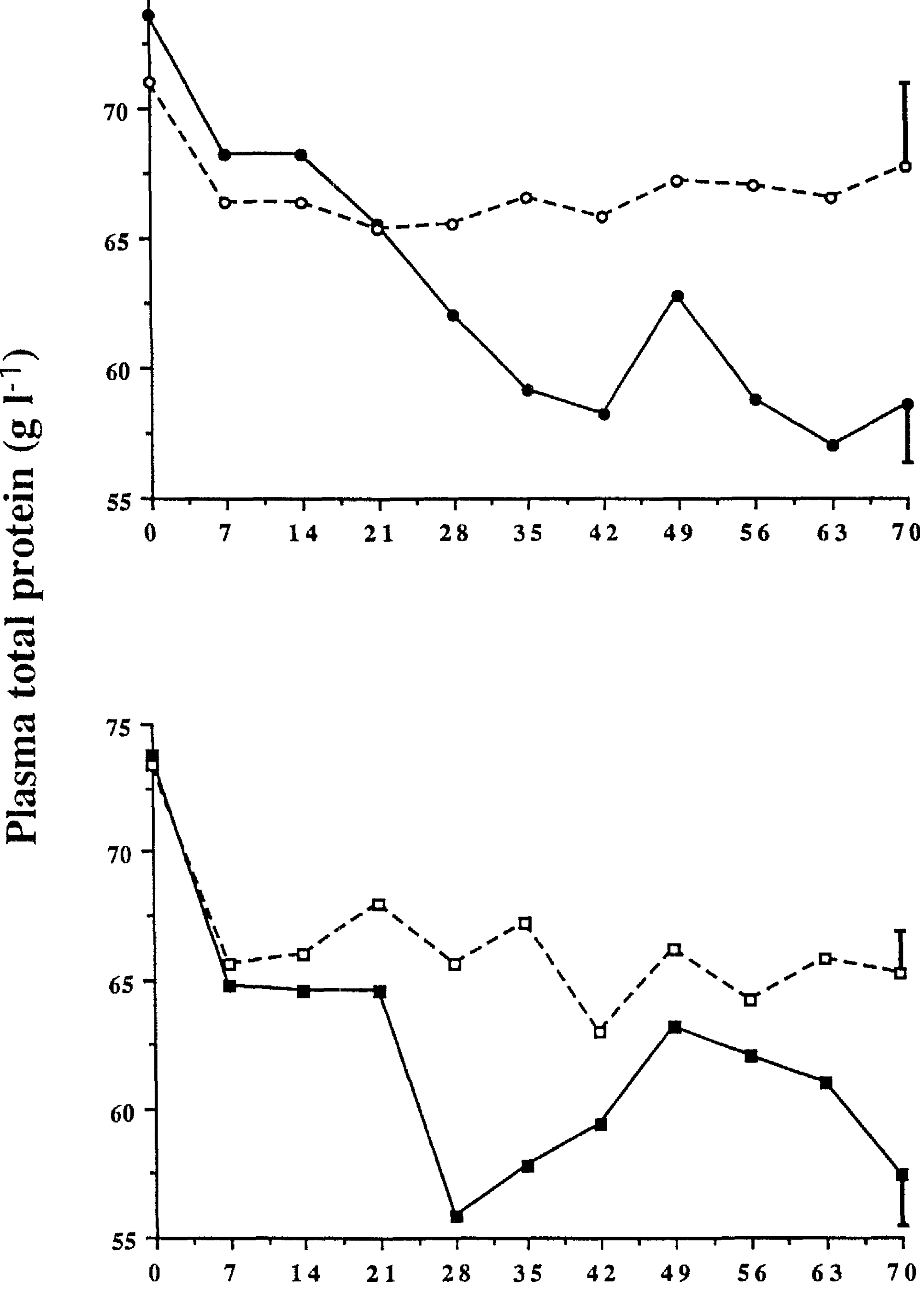
Plasma triglyceride concentrations fluctuated considerably in both infected groups. However, they tended to be higher in the HEI (0.14-0.23 $mmoll^{-1}$) than in the HEC group (0.10-0.19 $mmoll^{-1}$). Plasma NEFA also fluctuated a great deal with a tendency to be higher in infected (0.20-0.90 $mmoll^{-1}$) than in control (0.07-0.20 $mmoll^{-1}$) groups of animals.

Plasma total protein (TP)

Both infected groups developed hypoproteinaemia but this was significant only in the LE group. In the HEI group, the plasma TP concentration decreased from 73.6 ± 1.9 gl^{-1} at 0 DAI to 58.2 ± 4.3 at 42 DAI (Figure 8.13). Thereafter it tended to recover but decreased again between 49 and 63 DAI. In the LEI group, the TP concentration decreased from 73.8 ± 1.1 at 0 DAI to 55.8 ± 2.7 gl^{-1} at 28 DAI after which it showed a moderate increase. The values in the HEI group were not significantly different from those in the LEI group. In control animals the values fluctuated between 63.0 ± 2.4 and 73.4 ± 1.0 gl^{-1}, with no significant energy effects.

Plasma albumin and globulin

Infection caused a significant decline in the concentration of plasma albumin in both energy groups, the decrease starting at 14 DAI. The decline was greater in the LEI group than in the HEI group, but this was not significant. Plasma albumin concentration decreased from 36.2 ± 0.4 gl^{-1} at 0 DAI in the HEI group to 29.2 ± 1.2 gl^{-1} at 28 DAI (Figure 8.14). In the LEI group, it decreased from 39.0 ± 0.7 to 28.2 ± 0.8 gl^{-1} between 0 and 28 DAI after which it tended to recover. In control animals, plasma albumin fluctuated between 34.0 ± 0.5 and 38.6 ± 0.7 gl^{-1}, with no significant energy effects.



Plasma total protein (g l-1)
75
70
65
60
55
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

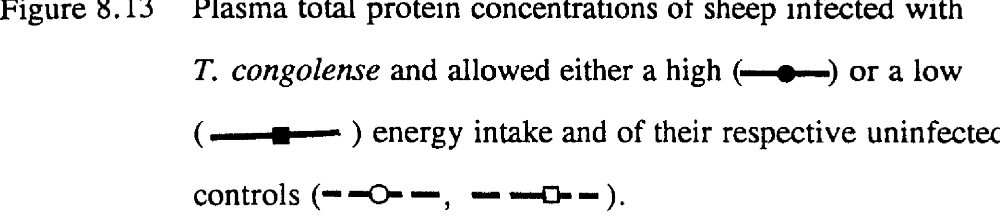


Figure 8.13 Plasma total protein concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (– –○– –, – –□– –).

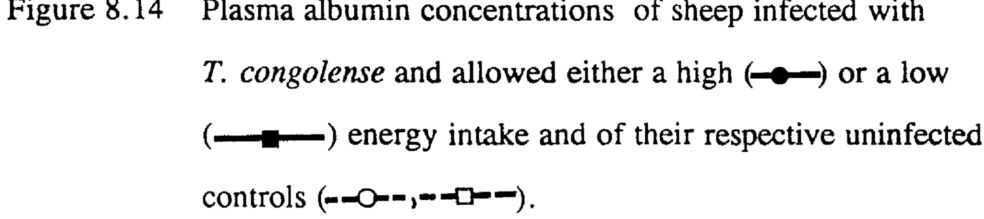
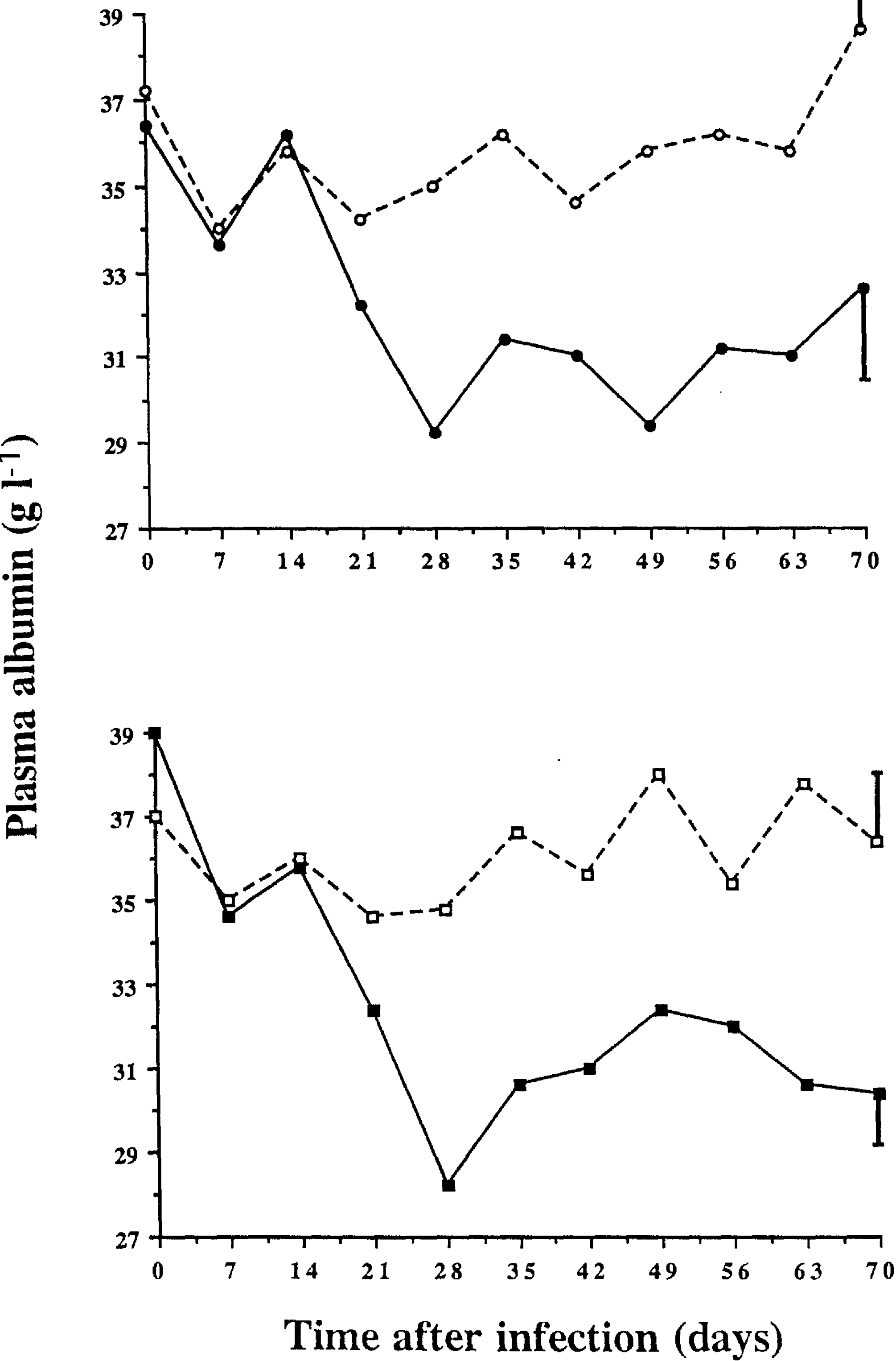


Figure 8.14 Plasma albumin concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (——■——) energy intake and of their respective uninfected controls (--○--, --□--).



Plasma albumin (g l-1)
39
37
35
33
31
29
27
0 7 14 21 28 35 42 49 56 63 70
Time after infection (days)

Plasma globulin concentrations fluctuated a great deal in all groups of animals with a tendency to decrease with time.

Plasma urea

Infection had no significant effect on the concentrations of plasma urea in both energy groups. However, there were nutritional effects with the LE groups having significantly higher plasma urea levels than the HE groups (Figure 8.15).

Plasma β-hydroxybutyrate

Plasma β-hydroxybutyrate concentrations were significantly lower in infected animals (HEI 0.30 - 0.46, LEI 0.34 - 0.50 $mmoll^{-1}$) than in control ones (HEC 0.46 - 0.66, LEC 0.46 - 0.64 $mmoll^{-1}$) of both energy intakes and there was no influence of energy intake.

Serum iron, TIBC, plasma glucose concentrations

There were no observable effects due to infection or energy intake on the concentrations of serum iron (HE 20.4 - 33.6, LE 21.8 - 35.0 $\mu moll^{-1}$), TIBC (HE 43.9 - 81.6, LE 39.4 - 71.3 $\mu moll^{-1}$), and plasma glucose (HE 2.7 - 3.9, LE 2.7 - 3.6 $mmoll^{-1}$).

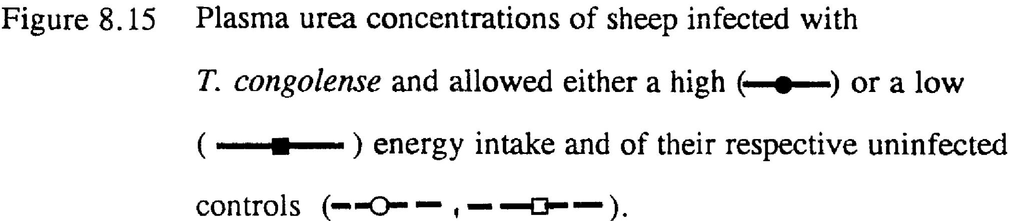
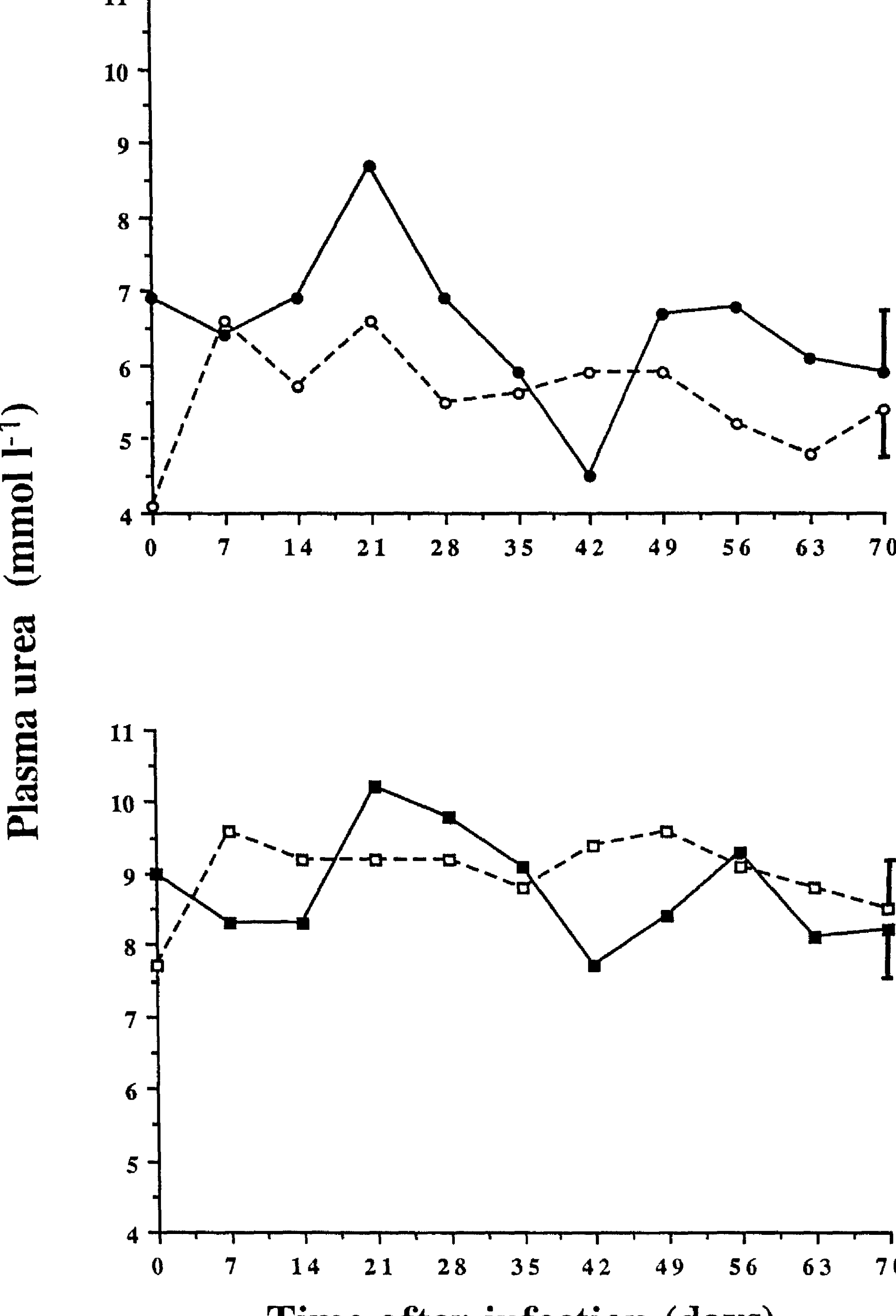


Figure 8.15 Plasma urea concentrations of sheep infected with *T. congolense* and allowed either a high (—●—) or a low (—■—) energy intake and of their respective uninfected controls (– –○– –, – –□– –).



Plasma urea (mmol l-1)
11
10
9
8
7
6
5
4
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)

DISCUSSION

The results obtained in the current study indicated that the infected animals on high energy intake developed less intense parasitaemia, gained more weight and exhibited less severe anaemia than the infected animals on a low energy intake.

It was observed that infected animals on a high energy intake (HEI) had a shorter prepatent period than infected animals on a low energy intake (LEI). After patency, the parasitaemia fluctuated in both infected groups but tended to be higher in the LEI group than in the HEI group. This observation is in agreement with the findings of Fagbemi *et al.* (1990) in pigs infected with *T. brucei* but is in contrast with those of Hecker *et al.* (1991). Hecker *et al.* (1991) observed that supplementation of sheep exposed to natural trypanosome challenge delayed the onset of parasitaemia. This was based on the assumption that animals on the high and low planes of nutrition were inoculated with similar numbers of trypanosomes which is difficult to ascertain. However, development of earlier patent parasitaemia and maintenance of higher parasitaemia during the first 4 weeks of infection by the HEI group, tends to lend support to the previous observations (Chapter 7) that availability of extra nutrients may be beneficial to the parasite.

The HEI group gained 5.0 kg between 0 and 70 DAI while the LEI group gained 2.6 kg. The weight gains in control groups were 4.2 and 8.4 kg for the LEC and HEC groups, respectively. The observations suggest that high energy intake allowed infected sheep to gain weight despite the infection, but not at the same rate as their uninfected controls. Infected animals on low energy intake showed greater retardation of growth compared to the infected animals on the HE intake. This observation agrees with the report of Fagbemi *et al.* (1990) in pigs infected with *T. brucei* and placed on either high or low energy ration. The weight gains in infected pigs on LE and HE intake, between 0 and 56 days after infection, were 3.0 and 14.0 kg, respectively, while their respective controls gained 7.3 and 27 kg.

Recent studies in goats infected with *T. vivax* (Verstegen *et al.*, 1991) have indicated that, due to fever, trypanosome-infected animals increase their heat production by 15%, with the result that their maintenance energy requirement increases by 25% compared to the uninfected control animals. The consequence of this, is that the net energy stored as gain by infected animals is markedly reduced leading to a reduction in weight gain, especially for the animals on the low energy intake.

Both infected groups developed a significant drop in PCV, RBC and Hb concentrations compared to their uninfected controls, however, the decline in these variables was greater in animals with a low energy intake. In contrast to the current observation, Agyemang *et al.* (1990) reported that animals on either low or high plane of nutrition developed similar degrees of anaemia. The present observation agrees with the report of Little *et al.* (1990) in N'Dama cattle experimentally infected with *T. congolense*, and given either a high or a low plane of nutrition and that of Fagbemi *et al.* (1990) in pigs infected with *T. brucei* and given either a high or a low energy intake. The present study indicates that while high energy intake may not prevent rapid establishment of trypanosomes following experimental inoculation, the ensuing anaemia is less severe than the anaemia in animals receiving low energy intake.

It has been reported that poor pastures cause a decrease in the haematocrit (Sawadogo *et al.*, 1991) and failure to observe lower erythrocyte values in the LEC group compared to the HEC group suggests that the energy and protein levels used in the present experiment may be higher than the levels available to the animals in trypanosomiasis endemic areas, and especially so during the dry seasons.

Both infected groups developed macrocytosis and an increase in MCH. It has been proposed that an increase in MCV is a reflection of the increasing number of reticulocytes in circulation which is also an indicator of erythropoietic activity

(Anosa and Isoun, 1976). Failure to record differences in MCV between LEI and HEI is in agreement with the observations of Makinde *et al.* (1991) and suggests that energy intake, unlike protein, may not have a significant influence on erythropoietic activity of sheep.

Infected animals on LE and HE intakes developed leucocytosis associated with lymphocytosis. In addition there were nutritional influences with the HEC having higher WBC, lymphocytes and neutrophils than the LEC. A leucocytosis in Scottish Blackface lambs infected with *T. congolense* has been recorded in the previous study (Chapter 3). Nutritional influences on leucocyte numbers have been recorded on a few occasions in calves (Stufflebeam *et al.*, 1969; Katunguka-Rwakishaya *et al.*, 1987). These authors recorded higher leucocyte numbers in animals on a higher energy intake. It is not clear whether high leucocyte numbers, associated with high energy intake, had any influence on the ability of HEI group to limit the intensity of parasitaemia, and to withstand the effects of infection, in the current study.

Infection caused a significant drop in the concentrations of serum total lipids, phospholipids and plasma cholesterol. The decline appeared to be greater in the LEI group than in the HEI group. Also, animals on the LE intake had higher initial cholesterol concentrations than the HE intake. The observation of hypolipidaemia, hypophospholipidaemia and hypocholesterolaemia in infected animals confirms previous observations (Chapter 3). Also the finding that the group with higher initial cholesterol (LE) tended to have higher parasite numbers and develop more severe anaemia tends to support the observations made in a comparative breed study (Chapter 5).

Infected animals in the present study developed hypoalbuminaemia and hypoproteinaemia, which were greater in the LEI group than in the HEI group. This finding is in support of the report of Otesile *et al.* (1991) in boars placed on different

dietary energy levels and infected with *T. brucei.* Measurement of intravascular pool of albumin has revealed that hypoalbuminaemia may be largely due to haemodilution (Katunguka-Rwakishaya *et al.*, 1992a). However, Wellde *et al.* (1974) have suggested that it may be due to excessive protein catabolism.

Under normal circumstances excessive protein breakdown is associated with an increase in plasma urea concentration (Ehoche *et al.*, 1990). Failure to record higher plasma urea levels in infected animals compared to their uninfected controls tends to suggest that, in these animals, trypanosome infection was not associated with excessive protein breakdown. However, it was observed that animals on the HE intake had lower plasma urea levels than those on LE intake. This is in agreement with the report of Blowey *et al.* (1973) and Al-Rabbat *et al.* (1971) in cattle. These authors suggested that high energy intake facilitates microbial protein synthesis in the rumen and thus reduces ammonia concentration and blood urea levels. On the other hand, low energy intake tends to increase protein degradation in the rumen resulting in production of large quantities of ammonia which is converted into urea by the liver and excreted (Hibbitt, 1988). These observations suggest that sufficient energy is required for efficient utilisation of dietary protein by the ruminant animal.

On the basis of the changes in liveweight, intensity of parasitaemia and degree of anaemia that developed following experimental infection of sheep with *T. congolense*, it was concluded that animals on a low energy intake were more susceptible to the pathogenic effects of infection. However, comparing these observations with the previous ones (Chapter 7), high protein intake was more was associated with better weight gains in infected animals compared to their uninfected controls than high energy intake.

In conclusion, the results obtained in the previous (Chapter 7) and the present investigations suggest that adequate protein and energy nutrition enhance the ability of trypanosome infected animals to withstand infection, by promoting body weight gains and development of less severe anaemia.

CHAPTER 9

TUMOR NECROSIS FACTOR ALPHA-RECEPTOR EXPRESSION ON BLOOD PERIPHERAL LEUCOCYTES OF SHEEP INFECTED WITH *TRYPANOSOMA CONGOLENSE*

INTRODUCTION

Infectious diseases result in dramatic metabolic, physiological and immunological changes in the host. Recent studies suggest that many of these metabolic abnormalities associated with haemoprotozoan diseases are mediated by cytokines of immunologic origin, particularly macrophage derived products such as interleukin-1 (Dinarello, 1985) and tumor necrosis factor (TNF) or cachectin (Beutler and Cerami, 1986). In addition to its tumoricidal activity (Old, 1988), TNF-α has been implicated in the development of cachexia during chronic parasitic infections (Rouzer and Cerami, 1980; Hotez *et al.*, 1984; Beutler *et al.*, 1985b). There is evidence that TNF-α impairs the activity of cell surface lipoprotein lipase (Cerami *et al.*, 1985; Grunfeld *et al.*, 1981) leading to inhibition of fatty acid uptake by the adipocytes. It also inhibits the biosynthesis of key *de novo* fatty acid synthesis enzymes (Pekala *et al.*, 1984) and causes an increase in hepatic lipid synthesis (Grunfeld *et al.*, 1988; Feingold and Grunfeld, 1987). The net result of these abnormalities is a state of hyperlipidaemia and depletion of total body fat during chronic infection. It is interesting to note that cachexia in ruminant animals infected with trypanosomes is associated with hypolipidaemia (Roberts , 1975b; Wellde *et al.*, 1989a; Katunguka-Rwakishaya *et al.*, 1992b) and not a hyperlipidaemia as reported in rabbits (Rouzer and Cerami, 1980) and dogs (Ndung'u *et al.*, 1989).

Tumor necrosis factor has a wide range of biological activities, some of which are protective and some of which are detrimental to the host depending on the level of TNF produced. Some metabolic effects of TNF-α include: (a) immunological activities such as regulation of production of IL-1 (Dinarello, *et al.*, 1986) and prostaglandins (Dayer *et al.*, 1985), (b) influence on inflammatory responses such as induction of fever (Dinarello *et al.*, 1986), (c) inhibition of

erythropoiesis *in vitro* and *in vivo* (Akahane *et al.*, 1987; Roodman, 1987), and (d) activation of polymorphonuclear leucocytes (Chang *et al.*, 1991; Gamble *et al.*, 1986; Silberstein and David, 1986; Steinbeck and Roth, 1989).

To exert these effects, TNF interacts with sensitive cells by binding to cell surface receptors (Ruggiero *et al.*, 1987). Two distinct TNF receptors, which differ immunologically and in molecular weight, have been identified and they are expressed to varying degrees in different cell types. These are termed TNF receptor alpha (TNF-α, 75 kD) and TNF receptor beta (TNF-β, 55 kD) (Brockhaus *et al.*, 1990; Loetscher *et al.*, 1991). Studies with radiolabelled TNF have revealed that receptor density differs between cell types, for example, human peripheral blood granulocytes have three times more receptors than unstimulated lymphocytes but about a third of receptors on myeloid cell lines (Munker *et al.*, 1987). Winstanley (1992) reported that the density of receptors per cell and the proportion of cells expressing TNF-α receptor activity in sheep was of the rank order: monocytes > granulocytes > lymphocytes.

Following the observation that there is cross reactivity between recombinant human TNF (rh TNF) and ovine effector systems (Johnson *et al.*, 1989; Wheeler *et al.*, 1990), it was possible to identify TNF receptors on ovine peripheral blood leucocytes using commercially available fluorescent rh TNF and flow cytofluorimetry (Winstanley, 1992). This approach has been used in the present study to investigate the changes in expression of vacant TNF receptors on circulating leucocytes in sheep throughout an experimental infection with *Trypanosoma congolense*. Any changes in expression of TNF binding receptors could be related to parasite burden and other haematological indicators of severity of infection, for example, PCV. This experiment was conducted on a group of animals

in which the influence of dietary energy intake upon the pathophysiology of ovine trypanosomiasis was being investigated. Consequently, this study will also compare TNF-α receptor activity in groups of animals on different energy intakes.

MATERIALS AND METHODS

Experimental Animals, Feeding and Housing

Eight animals (four twin pairs) were randomly chosen from a group of 10 twin pairs which were involved in the energy experiment (Chapter 8). The feeding and housing of experimental animals has been described in the previous study (Chapter 8).

Parasitological and Haematological Methods

The techniques used to estimate the intensity of parasitaemia and PCV have been reported (Chapter 2).

Collection of Blood Samples

Jugular blood was collected into EDTA-tubes twice each week, between 9.00 and 10.00 h for estimation of parasitaemia and PCV. At the same time samples for TNF-x assay were taken into tubes containing sodium azide as described below.

TNF-α Receptor Assay

Three ml of blood were taken into EDTA anticoagulant containing sodium azide to give a final concentration of 0.1% and was stored on ice for up to 3 hours before processing. The erythrocytes were lysed by addition of 2 ml of blood to 10 ml of hypotonic ammonium chloride buffer (0.155M NH_4Cl, 0.01M $KHCO_3$, 0.1mM EDTA, pH 7.4). Erythrocyte ghosts and stroma were removed by centrifugation at 150 g for 10 minutes. The leucocyte pellet was washed twice in

ice-cold saline and all subsequent procedures were conducted in a cold room at 4 ^{o}C. The leucocyte pellet was washed twice in the "fluorokine" assay buffer and the washed cells were resuspended in the buffer at approximately 10^7 cells ml^{-1}. Twenty five microlitre volumes of cell suspension were placed in polypropylene microfuge tubes and 10 ul of phycoerythrin-conjugated recombinant human TNF alpha (PE-TNF) ("Fluorokine" R & D Systems) at a concentration of 0.33 $\mu g\ ml^{-1}$ was added to each tube. After gentle mixing the tubes were left to equilibrate for 1 h. An aliquot of unlabelled cells was also processed through the washes to provide cells for the determination of the background signal.

The cell suspensions were analysed using a flow cytofluorimeter (FACScan, Becton Dickinson). Ten thousand events were recorded using live gating to exclude any debris. The fluorescence detection was in logarithmic mode. The granulocyte, monocyte and lymphocyte populations were identified by the characteristic positions in plots of forward angle scatter(an index of cell diameter) against side angle scatter (an index of cell granularity) (Carter and Mayer, 1990). Each cell population was gated in turn and a histogram of red fluorescence was made. The mean and mode fluorescence was determined. To determine the proportion of gated cells expressing receptor activity, a threshold was designated for each cell population using unlabelled cell suspensions. The percentage of cells with fluorescence exceeding this threshold was calculated.

Data Analysis

The difference in percentage TNF-α positive cells between the infected and the corresponding uninfected control twin lamb was calculated for each data point. The area under the curve of percentage positive cells, on each cell type, against time was also calculated. This measure of the area gives an indication of the fluorescent signal.

Experimental Design

Four twin pairs, two on high energy intake (Nos. 305-306, 315-316) and two on low energy intake (312-311, 314-313) were involved in this study. As previously described one member of each pair was infected while the other was uninfected control. The animals were monitored for 70 days.

RESULTS

TNF-α Receptor Activity on Granulocytes

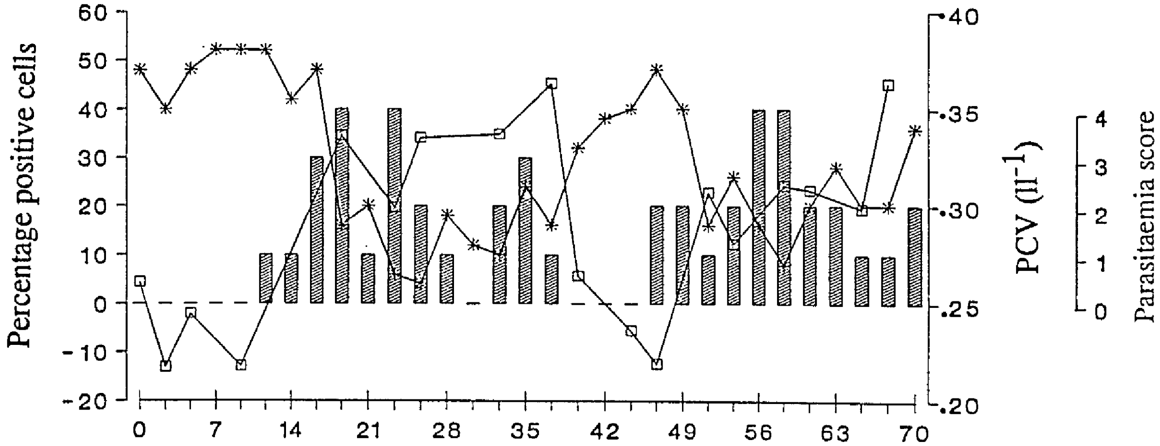
Infection caused an increase in TNF-α receptor expression on ovine circulating granulocytes. It was observed that the first peak of activity occurred immediately after the first major peak of parasitaemia (Figures 9.1-9.4). However, some nutritional effects were apparent. (1) The first peak of receptor activity tended to be higher in animals on high energy intake than in animals on a low energy intake. (2) The receptor activity peaks following the subsequent parasitaemic waves were of the same magnitude as the first peak in animals on HE intake but were diminished in those on LE intake. There was an apparent correlation between receptor activity and intensity of parasitaemia in the animals on HE intake but not in animals on a LE intake.

TNF-α Receptor Activity on Monocytes

There was a relative fluctuating decrease in TNF-α receptor activity on monocytes (Figure 9.5-9.8). The decrease was more apparent in animals on HE intake than in those on LE diet. The decrease in receptor activity on monocytes was associated with an increase in percentage positive cells of uninfected control

Figure 9.1 Percentage TNF-α receptor positive cells on the granulocytes (twin pair 305-306) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 305 allowed a high energy intake

Figure 9.2 Percentage TNF-α receptor positive cells on the granulocytes (twin pair 315-316) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 315 allowed a high energy intake


Percentage positive cells
PCV (ll-1)
Parasitaemia score
60
50
40
30
20
10
0
-10
-20
0
7
14
21
28
35
42
49
56
63
70
.40
.35
.30
.25
.20
4
3
2
1
0

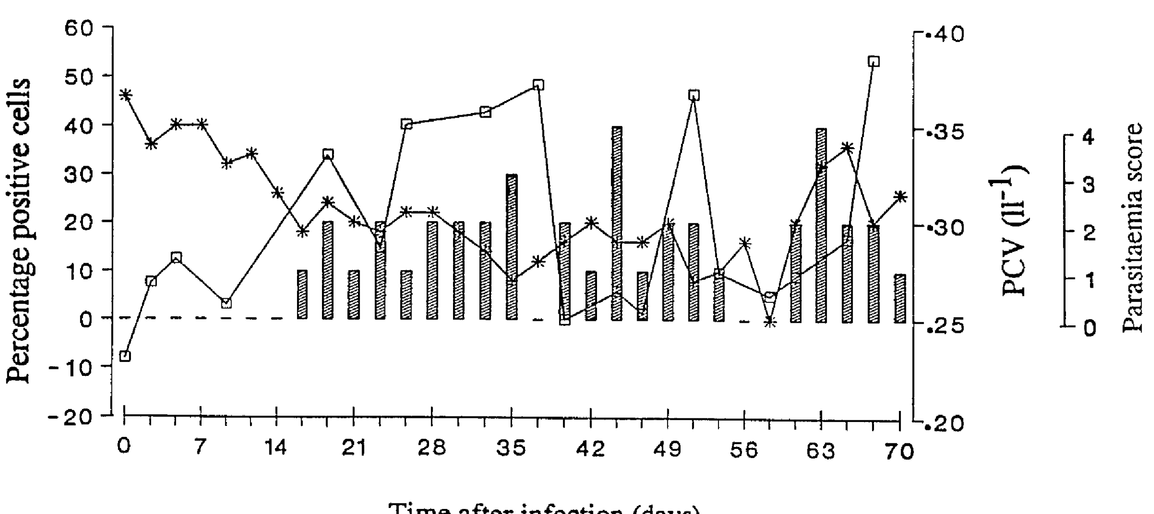
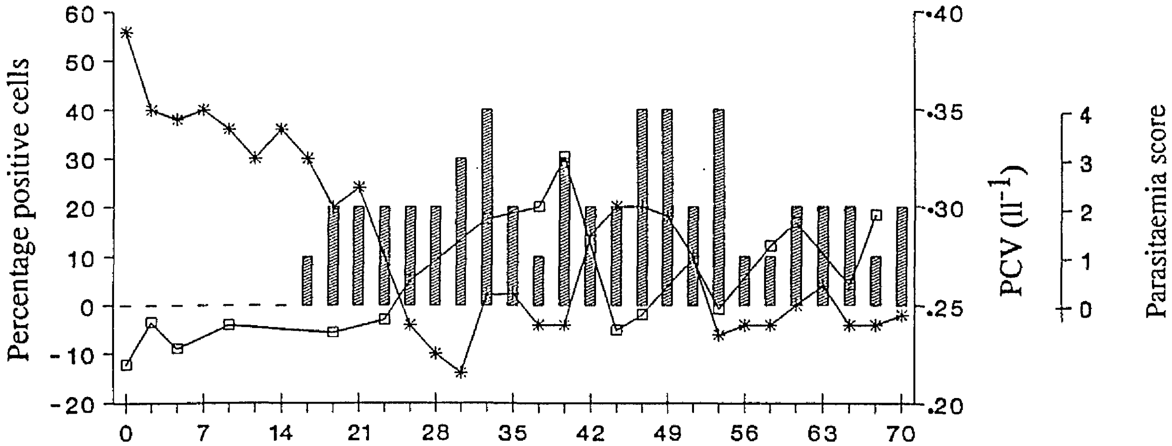
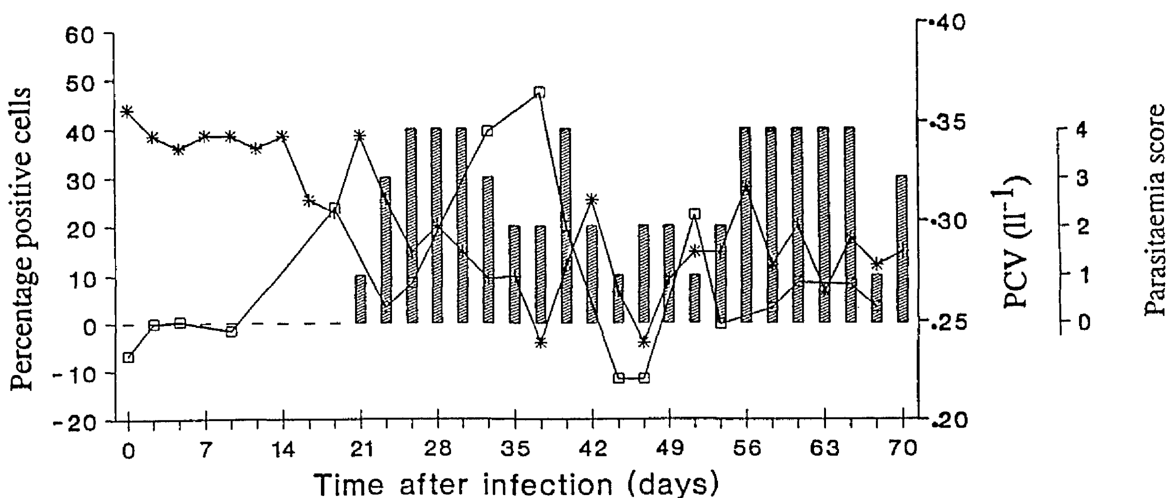

Percentage positive cells
PCV (ll-1)
Parasitaemia score
60
50
40
30
20
10
0
-10
-20
0
7
14
21
28
35
42
49
56
63
70
.40
.35
.30
.25
.20
4
3
2
1
0
Time after infection (days)

Figure 9.3 Percentage TNF-α receptor positive cells on the granulocytes (twin pair 312-311) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 312 allowed a low energy intake

Figure 9.4 Percentage TNF-α receptor positive cells on the granulocytes (twin pair 314-313) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 314 allowed a low energy intake



Percentage positive cells
PCV (ll^{-1})
Parasitaemia score
60
50
40
30
20
10
0
-10
-20
0
7
14
21
28
35
42
49
56
63
70
.40
.35
.30
.25
.20
4
3
2
1
0



Percentage positive cells
PCV (ll^{-1})
Parasitaemia score
Time after infection (days)
60
50
40
30
20
10
0
-10
-20
0
7
14
21
28
35
42
49
56
63
70
.40
.35
.30
.25
.20
4
3
2
1
0

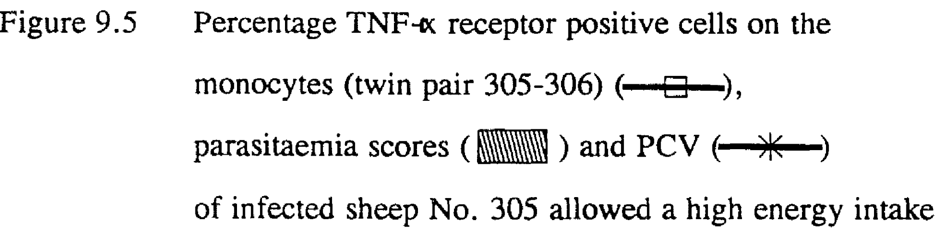
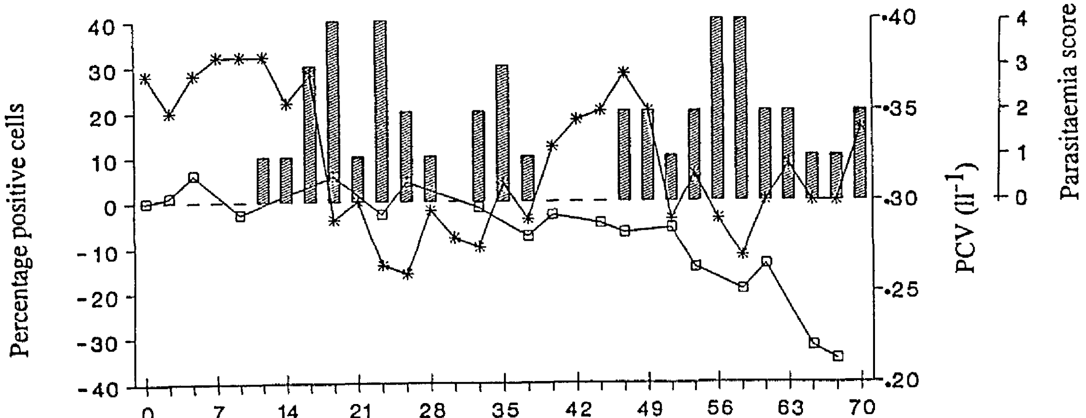
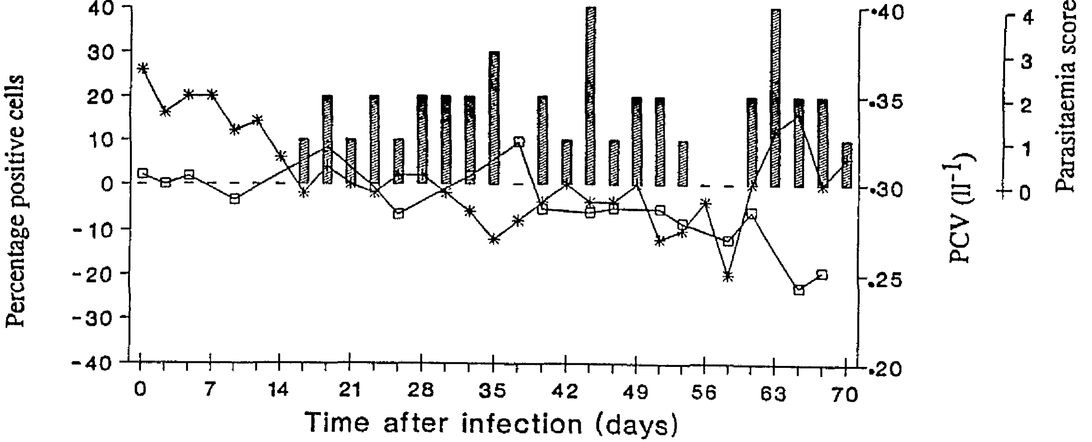


Figure 9.5 Percentage TNF-α receptor positive cells on the monocytes (twin pair 305-306) (—⊟—), parasitaemia scores (▒) and PCV (—✳—) of infected sheep No. 305 allowed a high energy intake

Figure 9.6 Percentage TNF-α receptor positive cells on the monocytes (twin pair 315-316) (—⊟—), parasitaemia scores (▒) and PCV (—✳—) of infected sheep No. 315 allowed a high energy intake



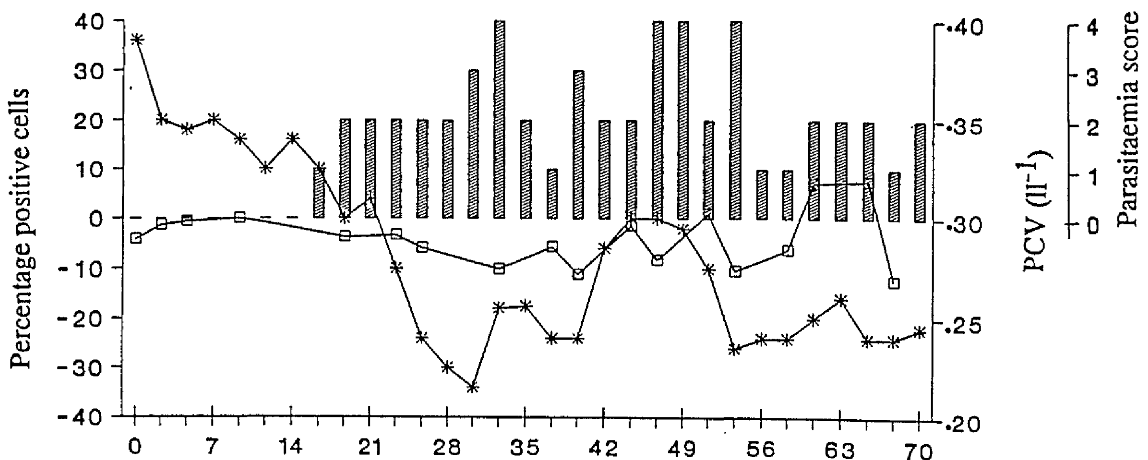
Percentage positive cells
40
30
20
10
0
-10
-20
-30
-40
0
7
14
21
28
35
42
49
56
63
70
•40
•35
•30
•25
•20
PCV (ll-1)
4
3
2
1
0
Parasitaemia score

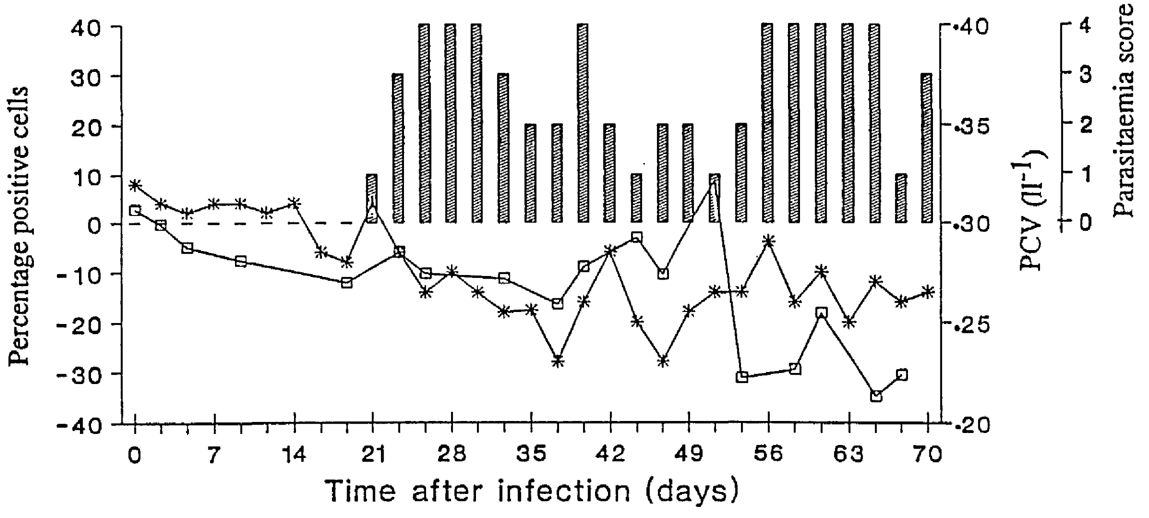


Percentage positive cells
40
30
20
10
0
-10
-20
-30
-40
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)
•40
•35
•30
•25
•20
PCV (ll-1)
4
3
2
1
0
Parasitaemia score

Figure 9.7 Percentage TNF-α receptor positive cells on the monocytes (twin pair 312-311) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 312 allowed a low energy intake

Figure 9.8 Percentage TNF-α receptor positive cells on the monocytes (twin pair 314-313) (—⊟—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 314 allowed a low energy intake


Percentage positive cells
PCV (ll⁻¹)
Parasitaemia score
40
30
20
10
0
-10
-20
-30
-40
0
7
14
21
28
35
42
49
56
63
70
•40
•35
•30
•25
•20
4
3
2
1
0


Percentage positive cells
PCV (ll⁻¹)
Parasitaemia score
Time after infection (days)
40
30
20
10
0
-10
-20
-30
-40
0
7
14
21
28
35
42
49
56
63
70
•40
•35
•30
•25
•20
4
3
2
1
0

animals with time. The activity on infected animals showed little variation and there was no clear correlation between intensity of parasitaemia and expression of TNF-α receptors on blood monocytes.

TNF-α Receptor Expression on Lymphocytes

TNF-α receptor activity on lymphocytes fluctuated considerably in all twin pairs, with a tendency to increase following the first wave of parasitaemia (Figures 9.9-9.12). There were minimal responses to subsequent parasitaemia waves and no nutritional influences were observed.

Parasitaemia, PCV and Area Under the Curve

The mean parasitaemia scores, PCV and area under the curve (AUC) of percentage positive cells against time are shown in Table 9.1. Infected animals receiving high energy intake developed less intensity of parasitaemia and less severe anaemia than animals allowed a low energy intake. The area under the curve shows that infected animals on high energy intake showed a greater TNF-α expression on granulocytes than those on low energy intake. The monocyte and lymphocyte responses were not directly related to the intensities of parasitaemia and showed no dietary influences.

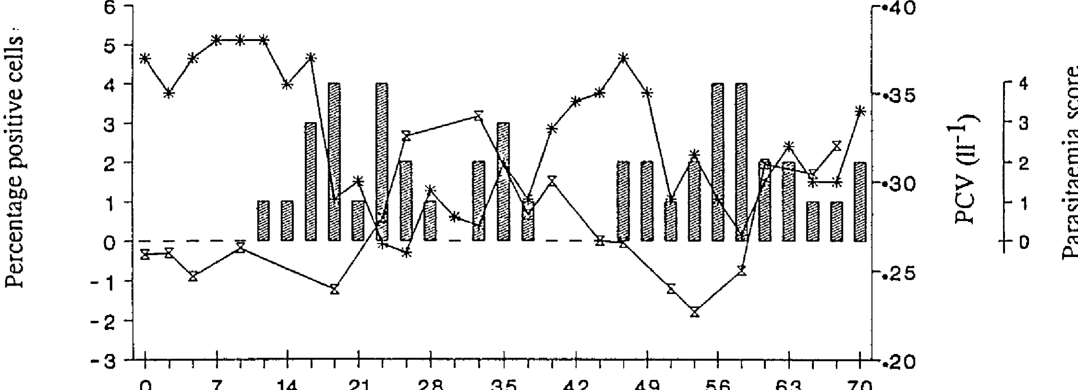
DISCUSSION

The present study has indicated that expression of TNF-alpha receptors on peripheral blood leucocytes of sheep infected with *T. congolense* changed throughout the course of infection and is influenced by the level of energy intake.

Macrophage derived TNF is increasingly recognised as a central mediator in a wide spectrum of physiologic and immune functions. Studies of cachectin/TNF in African trypanosomiasis have been conducted mainly in laboratory animals infected

Figure 9.9 Percentage TNF-α receptor positive cells on the lymphocytes (twin pair 305-306) (—⧖—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 305 allowed a high energy intake

Figure 9.10 Percentage TNF-α receptor positive cells on the lymphocytes (twin pair 315-316) (—⧖—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 315 allowed a high energy intake


Percentage positive cells
PCV (ll⁻¹)
Parasitaemia score

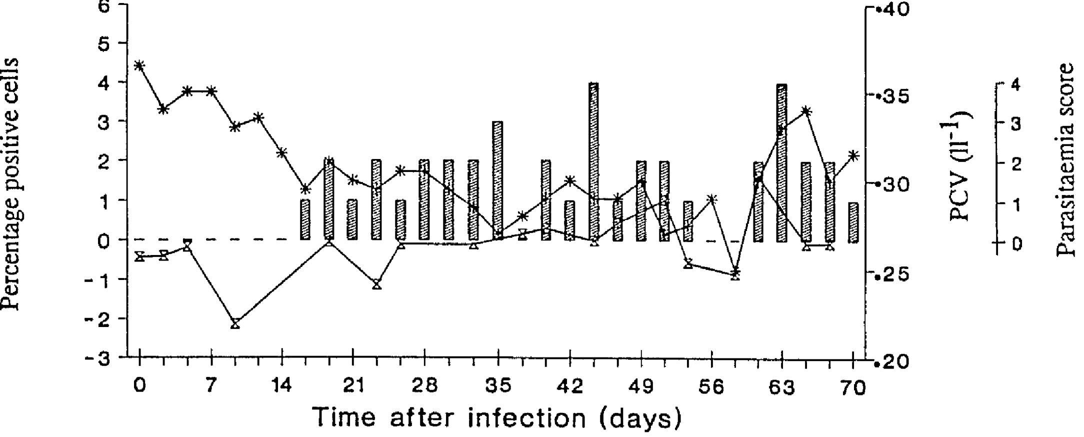
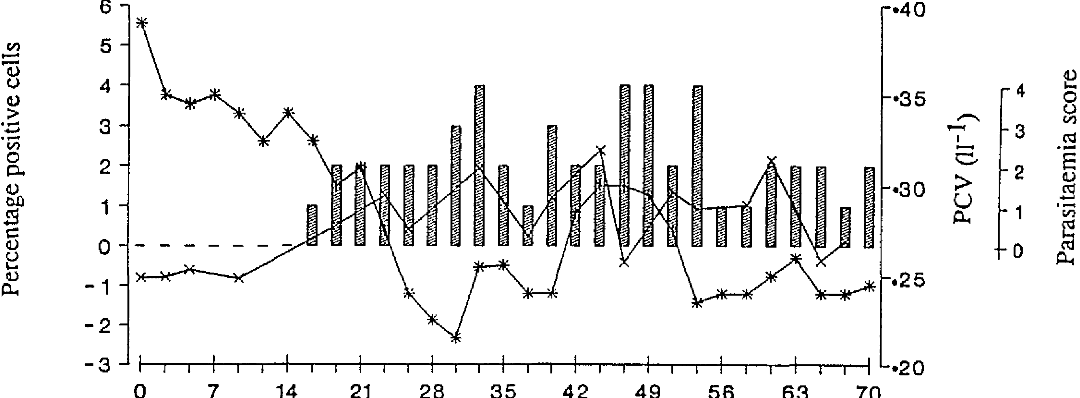

Percentage positive cells
PCV (ll⁻¹)
Parasitaemia score
Time after infection (days)

Figure 9.11 Percentage TNF-α receptor positive cells on the lymphocytes (twin pair 312-311) (—⧖—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 312 allowed a low energy intake

Figure 9.12 Percentage TNF-α receptor positive cells on the granulocytes (twin pair 314-313) (—⧖—), parasitaemia scores (▨) and PCV (—✳—) of infected sheep No. 314 allowed a low energy intake


Percentage positive cells
6
5
4
3
2
1
0
-1
-2
-3
0
7
14
21
28
35
42
49
56
63
70
•40
•35
•30
•25
•20
PCV (ll-1)
Parasitaemia score
4
3
2
1
0

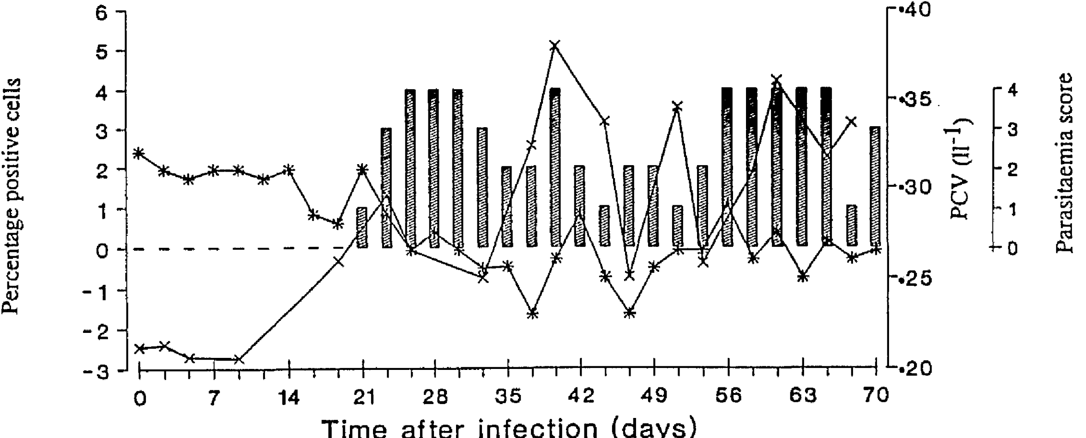

Percentage positive cells
6
5
4
3
2
1
0
-1
-2
-3
0
7
14
21
28
35
42
49
56
63
70
Time after infection (days)
•40
•35
•30
•25
•20
PCV (ll-1)
Parasitaemia score
4
3
2
1
0

Table 9.1

Parasitaemia, PCV and area under the curve (AUC) of percentage of peripheral leucocytes expressing TNF-α receptors in sheep allowed either a high or a low energy intake.

Group	Sheep No.	Mean Parasitaemia	Mean PCV	Area under the curve		
				Granulocytes	Monocytes	Lymphocytes
HEI	305	1.5 ± 0.2	0.31 ± 0.01	2608	115	283
HEC	306	-	0.36 ± 0	1463	1672	321
HEI	315	1.3 ± 0.2	0.30 ± 0.01	2043	1401	316
HEC	316	-	0.33 ± 0	525	1711	304
LEI	312	1.7 ± 0.2	0.28 ± 0.01	1165	1285	247
LEC	311	-	0.32 ± 0	838	1569	205
LEI	314	2.0 ± 0.3	0.28 ± 0.04	1157	1235	345
LEC	313	-	0.32 ± 0	868	2167	288

HEI & LEI = High and low energy infected respectively, HEC & LEC = High and low energy control respectively

with the *brucei* subgroup of trypanosomes. These investigations have revealed that live trypanosomes or trypanosomal lysates stimulate peritoneal macrophages to secrete TNF/cachectin, a cytokine that has been implicated in the development of cachexia. An increase in TNF production has also been observed in malaria and visceral leishmaniasis (Peyron *et al.*, 1990; Scuderi *et al.*, 1986).

There is agreement that TNF is involved in the recruitment of both immune and nonimmune cells to participate more effectively in the hosts response to an invasive agent (Tracey *et al.*, 1988). To effect these functions, TNF binds to specific surface cellular receptors. In the present study, it was observed that infection with *T. congolense* was associated with an increase in the percentage of cells expressing TNF-α receptors on the surface of circulating granulocytes. This increase was more marked in animals on HE intake than in those on LE intake and in each dietary group, it appeared to be correlated with the level of parasite burden. There is evidence that the interaction of TNF with neutrophils results in neutrophil activation (Tennenberg and Solomkin, 1990) and in an increase in expression of surface adherence glycoproteins which promote adherence to endothelial cells and to parasites leading to increased phagocytosis (Klebanoff *et al.*, 1986). In cattle infected with *T. congolense*, Kissling *et al.* (1982) observed that neutrophils play a leading role in the clearance of opsonised trypanosomes. Recently, it was found that mature neutrophils can also synthesise and secrete TNF-α , and that polymorphonuclear oxidative metabolism and killing of pathogens is markedly enhanced after priming with TNF-α (Dubravec *et al.*, 1990). An increase in the number of cells expressing TNF-α may be an indication of an attempt to compete more effectively for TNF circulating in plasma, and to amplify the immunological effects of granulocytes.

Other proteins capable of binding TNF-α have been identified in plasma and have been called TNF-binding proteins I and II (Loetscher *et al.*, 1991) to correspond to TNF-α and TNF-β. These proteins act as physiologic TNF antagonists or as buffering systems. Of further interest was the observation that animals on HE intake expressed a higher percentage of positive granulocytes than those on LE intake, and in each dietary group the increase was correlated with the intensity of parasitaemia. This suggests that the increase in the percentage of granulocytes with TNF-α receptors may be associated with the intensity of parasitaemia and the ability to respond to this challenge may be modulated by the nutritional status of the host.

The percentage of monocytes expressing TNF-α receptors fluctuated in both HE and LE groups, with no clear nutritional effects. However, it was interesting to observe that TNF-α receptor activity on control animals increased with time in the experiment. This is not easy to explain. Since control animals were kept in a different environment, this increased TNF-α activity may be associated with environmental influences. Lack of TNF-α activity on monocytes was unexpected. It has been reported that TNF can induce the production of itself, of other cytokines such as IL-1 and IL-6, and that IL-1 can downregulate the function of monocytes (Titus *et al.*, 1991). On the other hand, the trypanosomes may have induced TNF production which could have occupied most of the receptors on the monocytes leading to a reduction in the number of vacant receptors. The interaction of TNF and monocytes or other macrophages results in production of TNF and other cytokines and an increase in phagocytic activity (Titus *et al.*, 1991).

There were minor changes in receptor activity on lymphocytes of infected sheep and no nutritional influences were recorded in the present study. The effects of TNF-α on B and T cells are not clearly understood. However, it has been reported that TNF supports the differentiation and proliferation of B lymphocytes independently of IL-1, increases the generation of specific antibody secreting cells

in mitogen-treated B cell cultures and stimulates T-cell growth (DeForge *et al.*, 1989). It is possible that the amalgamation of lymphocyte subsets could have obscured changes of TNF-α receptor expression on, say T-cells or B-cells.

The present study has shown that TNF-α expression on peripheral blood leucocytes changes throughout a course of *T. congolense* infection. The greatest changes were observed in the granulocyte population and these changes followed the development of parasitaemic waves. The monocytes displayed a relative decrease in expression of TNF-α receptors compared to the uninfected controls and little variation in receptor activity was observed in the lymphocytes. In conclusion, this study has shown that *T. congolense* infection in sheep induces blood leucocytes to express greater TNF-α receptors, as a means of enhancing their non specific defence mechanisms. The pattern of high TNF-α activity on granulocytes and low TNF-α activity on monocytes was observed in the infected animals on HE intake. This was associated with greater resistance of these animals to the disease compared to the animals on LE intake.

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