ASPECTS OF THE PATHOGENESIS OF TRYPANOSOMIASIS

A Thesis Submitted for the Degree of Doctor of Philosophy

in the Faculty of Veterinary Medicine

of the University of Glasgow by

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GENERAL INTRODUCTION

Trypanosomes (Trypanosoma) are flagellated Protozoa belonging to the class Zoomastigophorea, order Kinetoplastida. They have the distinction of causing a wide variety of diseases in man and animals, thereby occupying a position of extreme parasitological importance. It is likely that phylogenetically they developed from trypanosomatid flagellates of the gut of non-haematophagous insects and adapted themselves to life in vetebrate hosts when some of these insects developed blood-sucking habits[1]. Apart from the odd exception, they are arthropod-borne, a cycle which is usually obligatory since they characteristically undergo a part of their life cycle in these hosts. The development pattern in the insect vector may be by posterior station or anterior station. Those which undergo the posterior station development belong to the section Stercoraria and the most important one of these is undoubtedly T. cruzi, the agent of Chagas' disease. In the salivarian trypanosomes, i.e., those which develop in the anterior station, the infective stages are found in the insect proboscis and salivary glands. These trypanosomes, the cause of the African trypanosomiases are transmitted by the tsetse fly (Glossina sp.) and the mammalian hosts are infected by the blood-sucking activities of such flies. The most important of the salivarian trypanosomes are T. gambiense and T. rhodesiense which cause sleeping sickness in man and those species which cause disease in domestic animals, i.e., T. vivax, T. congolense, T. brucei, T. simiae and T. suis.

Lumsden[2], discussing the economic importance of trypanosomiasis, details certain facts. The area in Africa virtually devoid of livestock from this cause is 10.4×10^6 sq km. It was estimated in 1963 that this would be capable of supporting 125×10^6 cattle which was considerably more than twice the total population in Africa at that time[3]. Paralleling the present world population explosion is

the need for an increased production of food and so, from an economic standpoint, the necessity to control trypanosomiasis is great. At the same time there is already a chronic protein shortage in Africa, which is magnified by the presence of drought conditions across the broad savannah belt of Northern Africa. Last year, widespread starvation was averted only by a massive international relief effort. The estimated need this year is for 715,000 tons of relief food to West Africa alone and metereologists predict that the situation will get worse.[4]

Trypanosomiasis is largely responsible for our inability to realise the enormous potential assets of savannah grazing and in consequence contributes greatly to the serious nutritional and economic position in these areas. On a lesser scale it also causes loss through morbidity and mortality of livestock in those areas which marginate fly belts and in the cost of providing prophylaxis for such animals.

Since the pre-colonial days of African exploration when men such as Richard Burton[5] and David Livingstone[6] first brought the tsetse fly problem to the attention of the world, attempts have been made to control or eliminate the disease; thus far, for various reasons, with only limited success. It was suggested by Philips[7] in 1959 that it would cost one thousand million pounds sterling, spent over 50 years to eradicate the fly. Currently these figures would have to be increased several fold. Although extensive control efforts have been conducted for several decades now, it is the opinion of Ford[8] that "the area infested with tsetse in Africa is larger than it was in 1900 after the passage of the rinderpest". It may have been that the effects of this particular disease were to reduce the availability of blood meals

to the fly at that time by killing vast numbers of ungulates, thereby controlling both fly numbers and trypanosomiasis. Nevertheless despite the deployment of vast intellectual and physical efforts over the past fifty years, and the accumulation of an immense body of knowledge on trypanosomiasis, the practical problem of animal trypanosomiasis would appear to be almost as great as ever.

The economic importance of trypanosomiasis has been recognised for years and great efforts have, and are, being made to eradicate the tsetse fly. For example, in Nigeria a scheme is being undertaken at the present to eradicate by aerial spraying with residual insecticides such as D.D.T. and Dieldrin[9]. Given political co-operation, technical help and enormous financial resources, trypanosomiasis could undoubtedly be eradicated from Africa by such techniques. There are, however, opinions which favour a less radical approach to the problem and consider that the maintenance of the present ecological structure might be more important than an all-out attack on the tsetse with the immediate objective of complete and speedy eradication. Apart from the possibility of causing sudden and irreversible changes in African ecology, one problem with such an approach is that people must be introduced to these cleared areas to practice agriculture. The alternative is a rapid reversal to the pre-control state of tsetse infestation. However, such large scale population manipulations might, in practice, be very difficult to achieve.

In any event, a gradual process of tsetse eradication seems to have gone on for generations in Africa by the simple process of bush clearance for cultivation. In The Gambia for example, the evidence suggests a progressive decrease in the occurrence of animal trypanosomiasis. For example, thirty years ago it was

impossible to keep horses there because of this disease[10]. Now, as a result of deforestation and recultivation, they are being maintained and bred successfully, albeit in a restricted area. Perhaps, instead of a blitz on the tsetse fly a more gradual and considered approach might pay greater dividends. Indeed the consequence of a radical and irreversible change in the extremely complex ecosystem of man, animals and the tsetse fly may be profound.

The situation with human trypanosomiasis seems to be relatively well in hand at the present, apart from some areas where, following political upheavals, the breakdown of surveillance has led to numerous human cases. Indeed Duggan[11] states "it is clear that the problem of trypanosomiasis in domesticated animals is now of far greater concern than human infection to the health and prosperity to Africa. Its biological complexity is vast, its physical extent is only vaguely defined and its significance is almost immeasurable. The burden of sleeping sickness in the 1930's was but thistledown in comparison to the leaden weight of this gigantic load".

Turning to the pathogenesis of trypanosomiasis, recent texts and review articles in this field state, perhaps overstate, that information on the pathology of trypanosomiasis is negligible and in consequence the process is poorly understood[12,13,14,15]. The reasons for this deficiency are to be found to some extent in an appraisal of the historical factors which have influenced the direction of trypanosomiasis research. With the colonisation of Africa around the turn of the century by European powers and the opening up of land and river routes, came extensive and severe outbreaks of human trypanosomiasis. This occurred mainly in Central and West Africa where old endemic foci of sleeping sickness flared up and extended. Because of the urgency of the situation in which many thousands of people were dying annually, the French

concentrated on the control of the disease initially by the isolation of suspects and subsequently by chemotherapy. In East Africa, on the other hand, the situation was different in that human trypanosomiasis tended to be more sporadic in occurrence and clinically much more acute. The British, not confronted by such an urgent situation as the French, therefore tended to favour the control of the disease in man and animals by selective bush clearing, i.e., a more epidemiological approach. As a result of these diverse approaches the emphasis on research on trypanosomiasis was for many years on chemotherapy and entomological control respectively. This has meant that studies on the pathogenesis of the disease have received relatively scant attention. Despite this dearth of information there are, in fact, some relevant and useful accounts of pathology in the early literature[16] and, more recently, considerable research has been devoted to the study of pathogenic mechanisms.

Perhaps one of the problems which has bedevilled our comprehension of the disease has been an understandable but regrettable tendency to subdivide the pathology into a wide variety of entities based on the host and the species of trypanosome. Although both the natural and experimental disease may present itself as a wide spectrum of syndromes, it would be scarcely surprising if the various species of trypanosome did not share a similar modus operandi with common pathogenic mechanisms. Thus, all trypanosomiases are characterised in varying degrees by lymph node enlargement, splenomegaly, anaemia and frequently by encephalitis, myocarditis and altered immunoglobulin synthesis[17]. It is likely, therefore, that many of the significant pathogenic effects of trypanosomes may be successfully elucidated in laboratory animals

using the variety of sophisticated research techniques currently available. Such an approach would perhaps define areas of potential significance in the natural disease.

Considered on this basis, three aspects of trypanosomiasis in laboratory rodents were selected for investigation. These were the pathology, the anaemia and the phenomenon of immunosuppression recently demonstrated in this infection. A chapter of the thesis is devoted to each of these aspects and in each is a review of the relevant literature. For ease of reference, the references cited are included in a bibliography at the end of each chapter. Details of results, too compendious to include in the text, may be found in the appendix together with a short account of a conjoint experiment with cattle in Ethiopia which was conducted as a result of the work described in Chapter III.

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GENERAL MATERIALS

AND METHODS

Experimental Animals

Mice

Mice were obtained from Carworth Europe, Alconbury, Huntingdon.

Random bred female CFLP (Carworth Farms Lane-Petter) mice were used in the majority of experiments. These mice were originally derived from the I.C.I. strain 1 mice (I.C.I., Alderley Park, Cheshire). Such mice were aged 6-8 weeks at the start of the experiment and weighed 27-32 g.

In experiments which involved cell transfers syngeneic N.I.H. mice were used. These inbred strains were obtained originally from the National Institute of Health, Bethesda, Maryland, U.S.A. At the start of each experiment these mice were aged 6-8 weeks and weighed 23-25 g.

Rats

Rats were obtained from Animal Suppliers (London) Ltd., Welwyn, Herts.

Random bred female hooded Lister rats were used in the majority of the experimental work which involved rats. The rats were aged 7-9 weeks at the start of each experiment and weighed 150-180 g.

In the experiment where ^{51}Cr-labelled syngeneic erythrocytes were transferred, syngeneic hooded Lister rats were used. These inbred strains were obtained from the Pathology Animal House, Veterinary Hospital, Glasgow, where they had been derived by brother-sister mating for at least 20 generations. These also were aged 7-9 weeks at the start of each experiment and weighed 120-150 g.

Feeding

Rats and mice were fed ad-lib on diet 41 (W. Shearer & Co., Glasgow).

Infection Procedures

The procedures used were similar in most respects to those described by Lumsden, Herbert and McNeillage[1].

Since continual serial passage of trypanosomes from animal to animal in the laboratory produces strains of the original material which vary in their biological characteristics, e.g., the antigenic nature of the trypanosome alters, or they vary in virulence. Efforts were therefore made to keep the infection technique standard.

Origin of Stabilate

A derivative of the stabilate TREU 667 (Trypanosomiasis Research Edinburgh University Stabilate Number 667) was used for all infections. The origin of this stabilate is unknown. However, it has the morphological characteristics of the subgenus Trypanozoon and produces a subacute infection in rats and mice (vide infra).

Cryopreservation of Derivative

A capillary tube of this stabilate was inoculated into two mice. After 6 days the mice were exsanguinated by cardiac puncture using heparin as anticoagulant. This blood was cryopreserved in glass capillary tubes after the addition of 10% glycerol by volume. These capillary stabilates were stored at -75°C in a Revco deep freeze (Revco Inc., Deerfield, Michigan, U.S.A.). Up to 150 tubes were obtained from the blood of one mouse.

Inoculations

For mouse inoculation, 1 tube of stabilate was diluted in 1 ml phosphate buffered saline pH 7.3 (PBS). Individual mice were given an intraperitoneal injection of 0.1 ml of this suspension.

For rat inoculation, 10 tubes of stabilate were diluted in 10 ml PBS. Each rat was given 0.5 ml of this suspension by intraperitoneal injection.

Although the infectivity of these trypanosomes was not determined, each 0.1 ml of the suspension contained approximately 1×10^4 trypanosomes and the inocula given invariably produced infection.

Course of Typical Infection

In the mouse, no trypanosomes were seen in wet-films of blood from the tail vein for five days after inoculation. Thereafter, they were visibly present, their numbers reaching a maximum by day 8 when about 200 trypanosomes per high power field were seen. At this point the numbers of trypanosomes declined rapidly but rose again within a few days. This cyclic variation in numbers continued throughout the course of the infection which persisted until the death of the host some 6-12 weeks after inoculation. During this period the mice remained lively and showed no signs of clinical disease other than moderate lymph node enlargement and abdominal distension associated with splenomegaly until a day or so before death when they became dull and lethargic; this terminal event was usually associated with a massive parasitaemia. The experiments described in this thesis, unless otherwise stated, were conducted on mice with an infection of 3 weeks duration.

In the rat, the course of events was similar to that described in the mouse. The infection was more acute however, and rats died 5-8 weeks after inoculation. One particular feature of the disease was a sudden terminal crisis, accompanied by haemoglobinuria, which occurred in a proportion of rats.

Trypanocidal Drug Treatment

Experimental animals were treated when required by the injection of diminazine aceturate (Berenil, Farbwerke Hoescht, Frankfurt, Germany). Berenil contains 44 per cent of the active substance, diminazine aceturate. Accordingly, mice were given 1.14 mg Berenil (0.5 mg diminazine aceturate) in 0.1 ml distilled water by intraperitoneal injection and rats received 6.82 mg Berenil (3.0 mg diminazine aceturate) in 0.5 ml distilled water by intraperitoneal injection.

Statistical Methods

Statistical methods employed were those described by Snedecor[2]. Regression analyses and comparison of data by means of the Students' t test were carried out in an "Olivetti Programma 101" desk computer (British Olivetti Ltd., Berkeley Square, London). Half-life values quoted were calculated by regression analysis. Unless otherwise stated correlation coefficients of activity against time were significant ($r = 0.95$) and significant P values (Students' t test) < 0.05 were obtained.

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CHAPTER 1

THE PATHOLOGY OF TRYPANOSOMA BRUCEI INFECTION IN THE RAT

INTRODUCTION

In the discussion of the pathology of animal trypanosomiasis, Lumsden[1] quotes Jubb and Kennedy (1963)[2] as saying "the pathology of trypanosomiasis, except for a few observations on Chagas disease in dogs has escaped better than cursory attention and regrettably little can be said of it". While this statement is rather unjust, it is true that the descriptive pathology of animal trypanosomiasis, particularly with regard to the sequential development of lesions, has been neglected. Lumsden[1] emphasised that mortality in trypanosomiasis occurs characteristically after a series of relapsing infections associated with progressive cachexia. Since during this period it is often difficult to demonstrate trypanosomes in the tissues and blood, he suggested that the pathogenic mechanisms of trypanosomiasis are likely to be akin to the mechanisms of immediate and delayed-type hypersensitivity.

Subsequently, in a review of the pathogenesis of animal trypanosomiasis, Fiennes[3], discussing the cellular pathology, noted that the most characteristic pathological change in chronic trypanosomiasis was the extensive involvement of the lymphoid-macrophage system. This occurred together with widespread lymphoid infiltration in many organs. However, he gives no further information on either of these points. Finally, while the work described below was in progress, a comprehensive historical review of the pathology of trypanosomiasis in domestic and laboratory animals was published by Losos and Ikede.[4] On the evidence available, these authors concluded that while anaemia played a major role in the morbidity of T. congolense and T. vivax infections, the lesions associated with T. brucei were primarily inflammatory, degenerative and necrotic changes in the tissues which they postulated

might be associated with a cell-mediated immune response. In contrast to the rather diffuse and unsatisfactory aspects of the descriptive pathology of animal trypanosomiasis, there are several papers which discuss specifically the possible role of vaso-active amines in the pathogenesis of the disease. Since this section is concerned with descriptive pathology these are not reviewed here but are included, where relevant, in the discussion.

The sequential development of gross and histological lesions in rats infected with T. brucei is described in this chapter. Since it is now recognised that trypanosome infections produce a range of immunological abnormalities, particular attention was paid to the changes in the lymph nodes, spleen and thymus.

MATERIALS AND METHODS

The rats used and the infection procedures were as described in General Materials and Methods.

Experimental Design

Thirty adult rats were inoculated with a derivative of T. brucei TREU 667 and groups of two were sacrificed at weekly intervals over a period of two months. In addition ten uninfected rats were sacrificed as controls at intervals during the course of this experiment.

Histology

At necropsy, samples of all tissues were taken and fixed in Carnoy's fluid for 24 to 48 hours, dehydrated and cleared in an alcohol-amyl acetate-chloroform series and embedded in paraffin wax. Sections were stained routinely with haematoxylin and eosin

and also with periodic acid-Schiff, picro-Mallory, methyl green-pyronin and Perls' Prussian blue[5].

RESULTS

Post-Mortem Findings

By 5 to 6 weeks after inoculation all rats were in poor condition and muscle wasting was obvious. Throughout the course of the infection the main findings were generalised lymph node enlargement, gross splenomegaly and hepatomegaly; some spleens weighed as much as 4.0g as compared with 0.5g in normal control rats (see Chapter 2). By 8 weeks these changes were less marked. Additional findings were increased amounts of fluid in the serosal cavities and slight subcutaneous oedema. A proportion of rats dying after five weeks had severe purulent bronchopneumonia.

Histological Findings

Immunological Apparatus including the Mononuclear phagocytic system (M.P.S.)

Throughout the course of this infection, a marked increase in the activity of the M.P.S. (as defined by Van Furth, Cohn, Hirsch, Humphrey, Spector and Langevoort)[6] was found in the liver, lymph nodes and spleen. In the liver, Kuppfer cells were prominent and some were found in mitosis. A range of mononuclear cells including monocytes, macrophages and lymphocytes were present in the vascular channels of the liver and in some locations were found marginating and migrating through the vessel wall into the extravascular spaces. The sinuses of the lymph nodes were packed with macrophages (Fig. 1) which were frequently found migrating between the medullary cords and sinuses. In the spleen, macrophages were numerous in the sinuses and cords of the red pulp and in areas surrounding the marginal zone of the white pulp. In all such areas, macrophages had a very active appearance

with abundant vacuolated cytoplasm; in the spleen they were frequently packed with red blood cells whilst in other areas they contained chromatin-like particles, possibly ingested trypanosomes.

Striking changes occurred in lymph nodes during the course of this infection. Shortly after inoculation, marked activity occurred in the cortex with lymphocytolysis and development of germinal centres. At the same time, the medullary cords became populated by large lymphoblasts which divided and progressively differentiated into plasma cells (Fig. 2). By fourteen days after inoculation, the lymph nodes were populated by a whole range of plasmablasts and plasmacells. As a result the lymph nodes were grossly enlarged. The medullary cords became thickened and tortuous because of plasma cell proliferation and plasma cells progressively encroached on the cortex, first replacing the paracortical thymic dependent zone and then by 3 to 4 weeks after inoculation the outer cortical area, leaving only a thin rim of cortex with a few follicles (Fig. 3). At this stage, large numbers of Russell-body containing plasma cells were present.

Throughout the period of infection, trypanosomes were found in the medullary cords, lymphatic channels and blood vessels of lymph nodes. In lymph node tissue, trypanosomes were easily located because of lack of cells, particularly plasma cells and lymphocytes, in their vicinity. This exposed the underlying framework of macrophages and reticulum cells which themselves were sometimes destroyed. In the first few weeks after inoculation these changes were only focal (Fig. 4) but as the infection progressed the areas of cellular depletion became more extensive and by 5 to 8 weeks after inoculation many lymph nodes were largely depleted of immunological cell types and only an underlying framework of

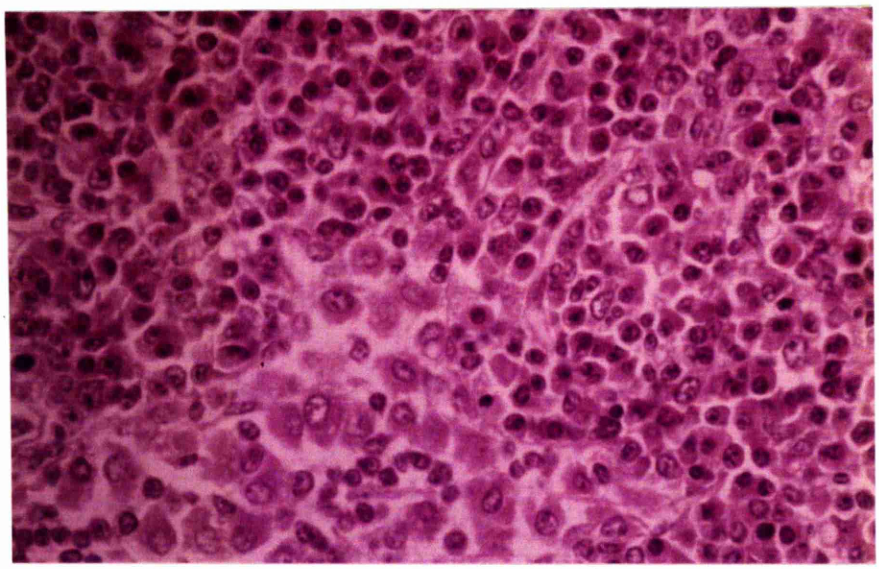

Figure 1. Lymph node medulla, 14 days after infection. The medullary sinuses are packed with macrophages and the surrounding medullary cords are distended by plasma cells.
Haematoxylin and eosin x 160.

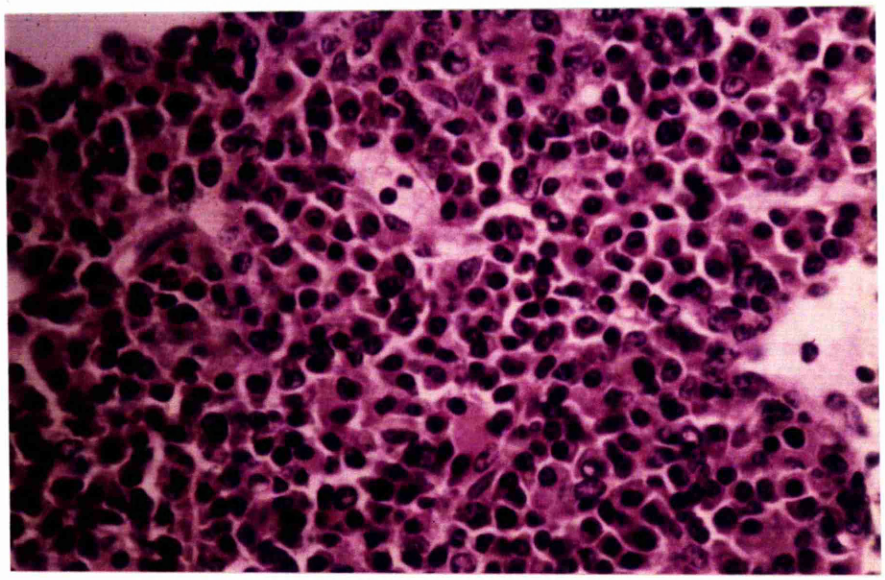

Figure 2. Juxtamedullary zone of a lymph node 28 days after infection. Numerous mature plasma cells are present.
Haematoxylin and eosin x 260

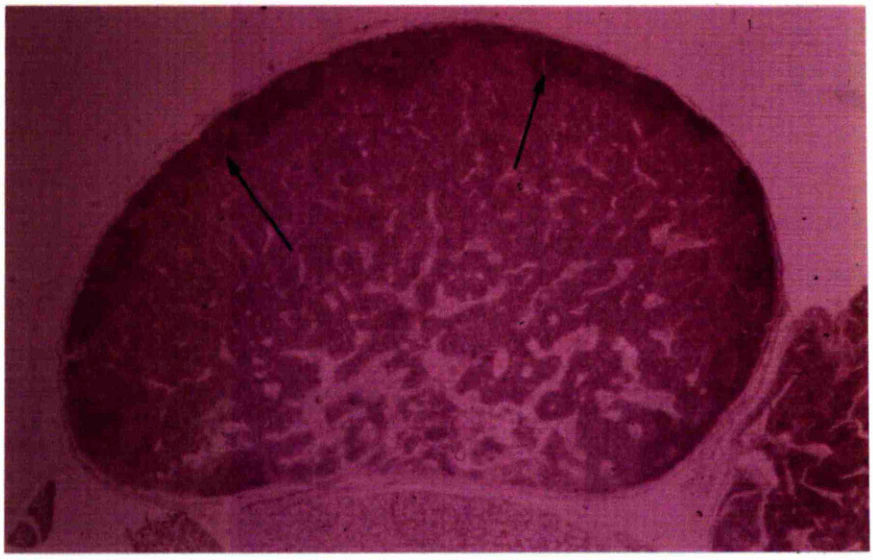

Figure 3. Lymph node 28 days after infection almost entirely replaced by plasma cells except for a narrow rim of lymphocytes in the outer cortex. Note the lack of germinal centres.
Haematoxylin and eosin x 10.

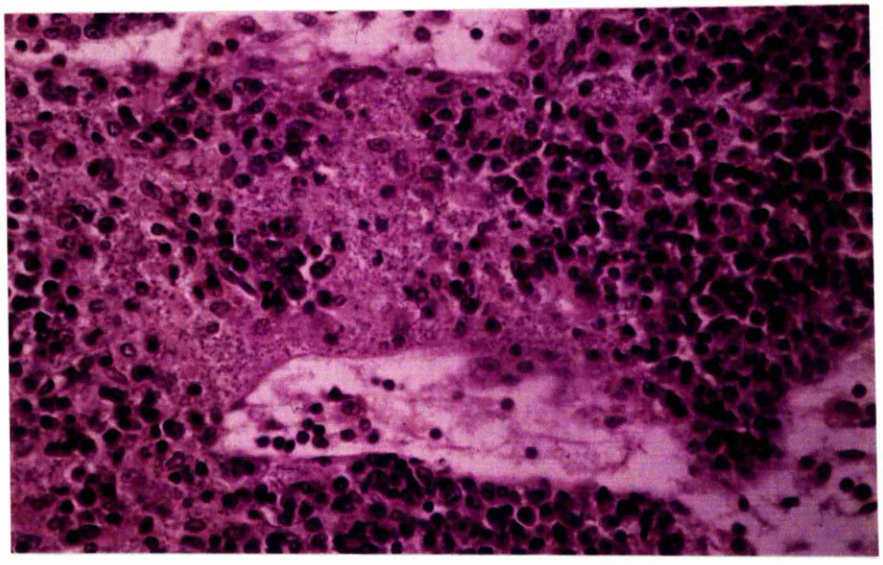

Figure 4. Lymph node medulla, 14 days after infection. Note the aggregates of trypanosomes in the sinuses and cords with the lack of cells in their vicinity.
Haematoxylin and eosin x 160.

reticulum cells and fibroblasts with a thin rim of cortex remained (Fig. 5).

The pattern of events seen in the spleen was similar to that described in the lymph nodes. Initially marked lymphocytolysis occurred in the white pulp particularly in the periarteriolar thymic dependent area. Aggregates of trypanosomes were often found in the white pulp in the vicinity of such changes. At this time the marginal zone became populated by large lymphoblasts which divided and progressively differentiated into plasma cells. By 3 weeks after inoculation, plasma cells were found encroaching on the white pulp and in some areas were encircling the follicular artery (Fig. 6). At the same time numerous plasmablasts and plasma cells were encountered in the red pulp where marked extramedullary haemopoiesis was also found (Fig. 7). As in the lymph nodes, numerous Russell-body containing plasma cells started to appear later in the infection. However, by five weeks, although still engorged with red blood cells, both the red and white pulp of the spleen were becoming depleted of immunological cells and this was marked by 8 weeks after infection (cf Figs. 8, 9, 10).

Apart from normal age involution, no changes were observed in the thymus gland except for occasional cellular infiltrates of macrophages and lymphocytes in the associated fascia, usually in the vicinity of trypanosomes.

Specific Organ Damage

Throughout the course of infection, trypanosomes were found in the blood vessels, lymphatic channels and in the tissues of most organs.

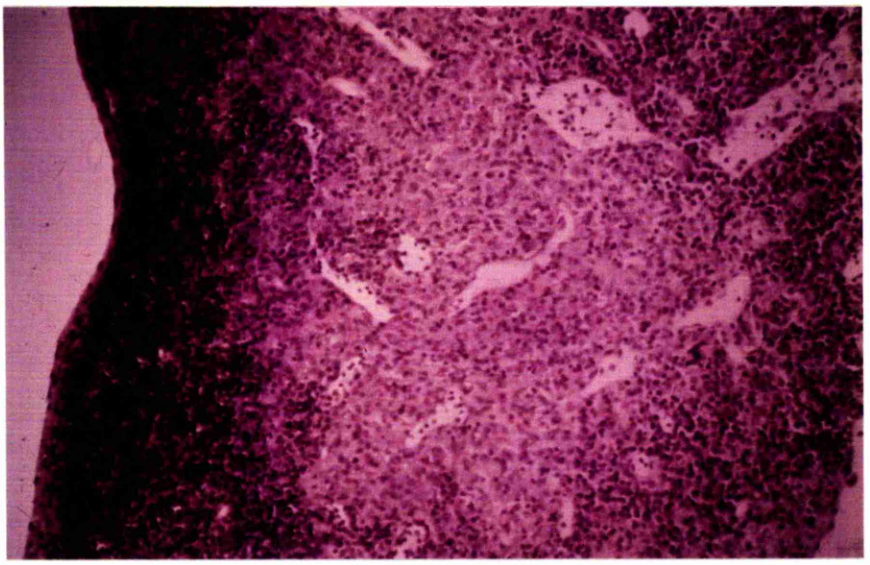

Figure 5. Lymph node medulla 42 days after infection. The node is largely depleted of immunological cell types except for a narrow rim of lymphocytes in the outer cortex and some plasma cells in the medullary cords.
Haematoxylin and eosin x 30.

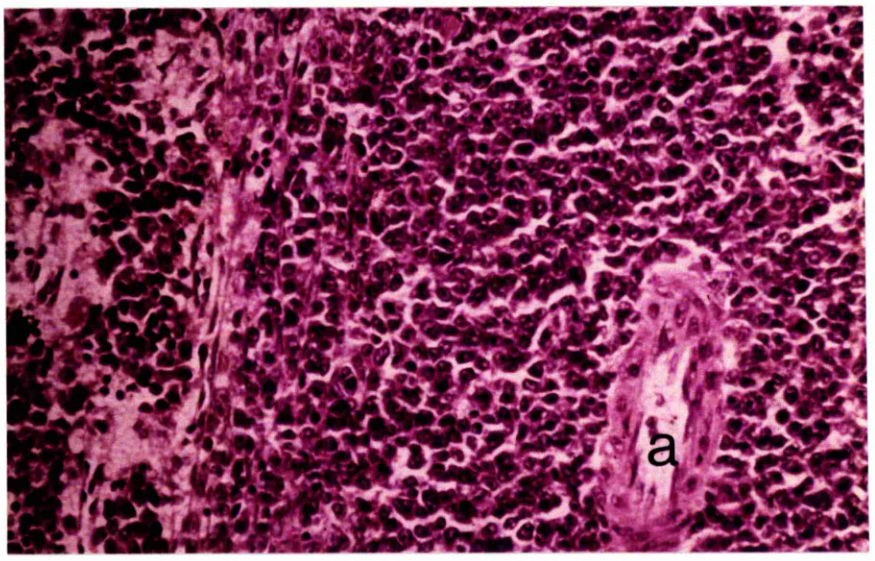

Figure 6. Lymphoblasts and plasma cells encircling a follicular artery (a) of a Malpighian corpuscle in a spleen 38 days after infection.
Haematoxylin and eosin x 160.

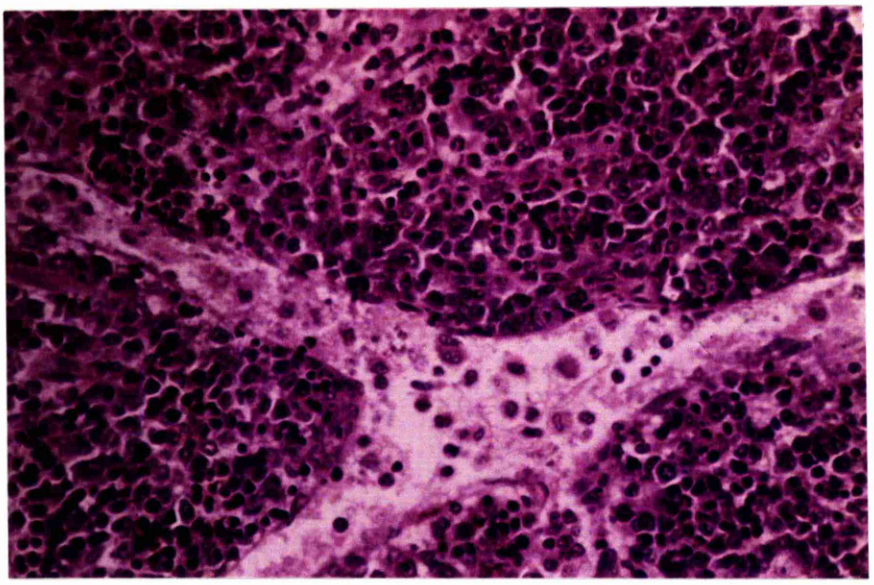

Figure 7. Splenic tissue 21 days after infection. Note the marked extramedullary erythropoiesis, the numerous plasma cells and the haemosiderin deposits in the medullary cords. Haematoxylin and eosin x 200.

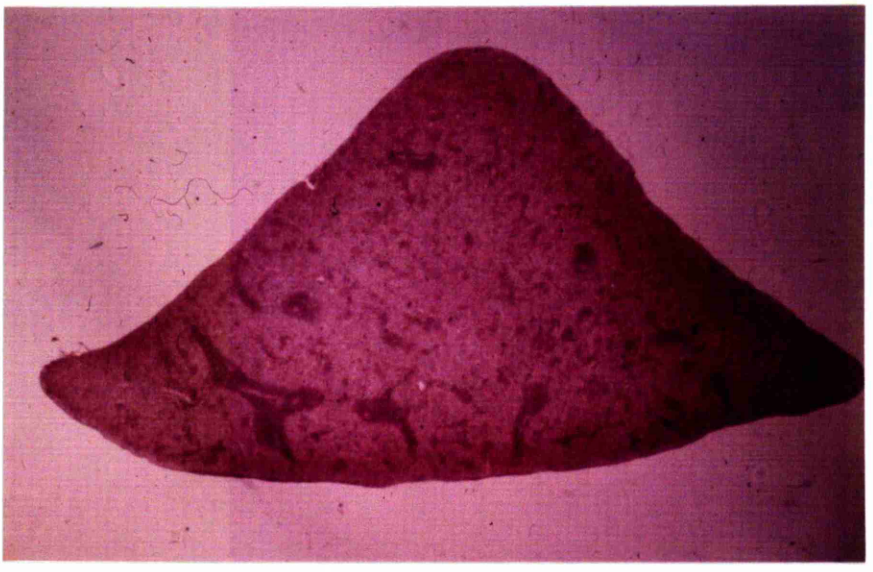

Figure 8. Spleen 56 days after infection. Although still engorged with red blood cells, both the red and white pulp are largely depleted of immunological cell types. Haematoxylin and eosin x 10.

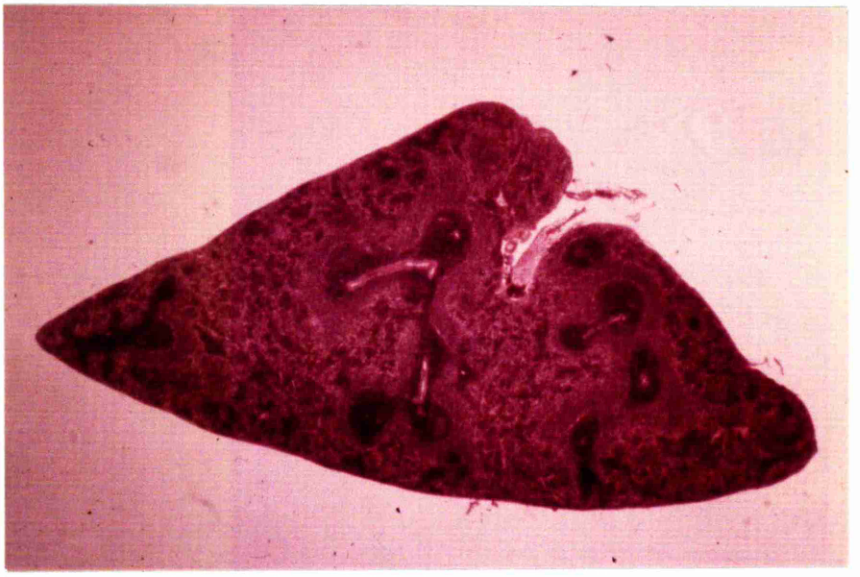

Figure 9. Spleen 21 days after infection. The spleen is distended by erythrocytes and a wide range of reactive cell types.
Haematoxylin and eosin x 350.

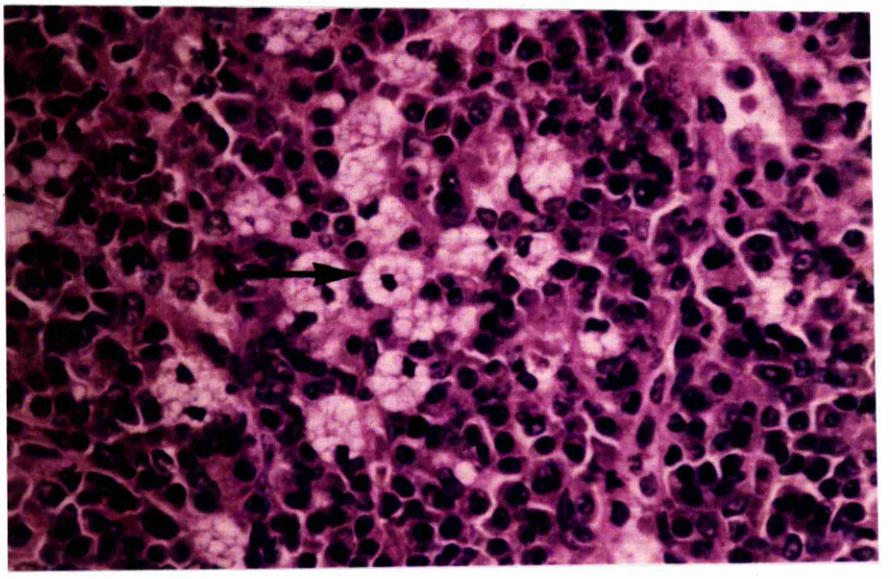

Figure 10. Splenic tissue showing macrophages packed with erythrocytes.
Haematoxylin and eosin x 350.

Heart

Of all organs the heart was most severely and consistently affected. Initially, trypanosomes were present in the heart chambers and in the surrounding pericardial cavity. Subsequently they were also found in the myocardium in blood vessels and lymphatic channels which they often appeared to occlude. Trypanosomes then became localised in the myocardial tissue where they caused marked damage. By 3 to 4 weeks after inoculation, severe myocarditis has developed (Fig. 11); cardiac muscle fibres were swollen and fragmented and they underwent hyaline degeneration and necrosis with sarcolemmal cell proliferation. These lesions were heavily infiltrated with mononuclear cells, mainly macrophages and also lymphocytes and plasma cells. Aggregations of trypanosomes were commonly found under the endothelium and mesothelium of the endocardium and the epicardium respectively. As a result, these areas were thickened by the presence of trypanosomes and the accumulation of macrophages.

Electrocardiography

The S-T segments of the electrocardiograms of the rats were isoelectric prior to infection (Fig. 12). As early as one week after inoculation, elevations were detected in some rats and these were very marked by three weeks. The degree of elevation varied between individual rats and from week to week.

Skeletal Muscle

At necropsy a major feature was muscle wasting and histological examination showed severe interstitial myositis which was often associated with the presence of trypanosomes.

Other Tissues and Organs

From time to time other tissues and organs showed mild degrees of damage and cellular infiltration. Occasionally some organs were

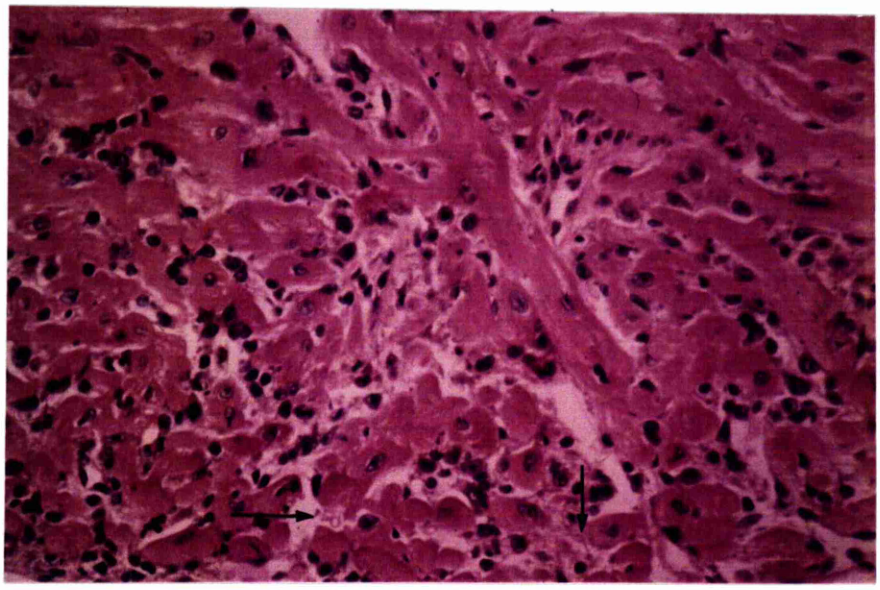

Figure 11. Myocardium 35 days after inoculation showing severe myocarditis. Note the presence of trypanosomes in the tissues.
Haematoxylin and eosin x 350.

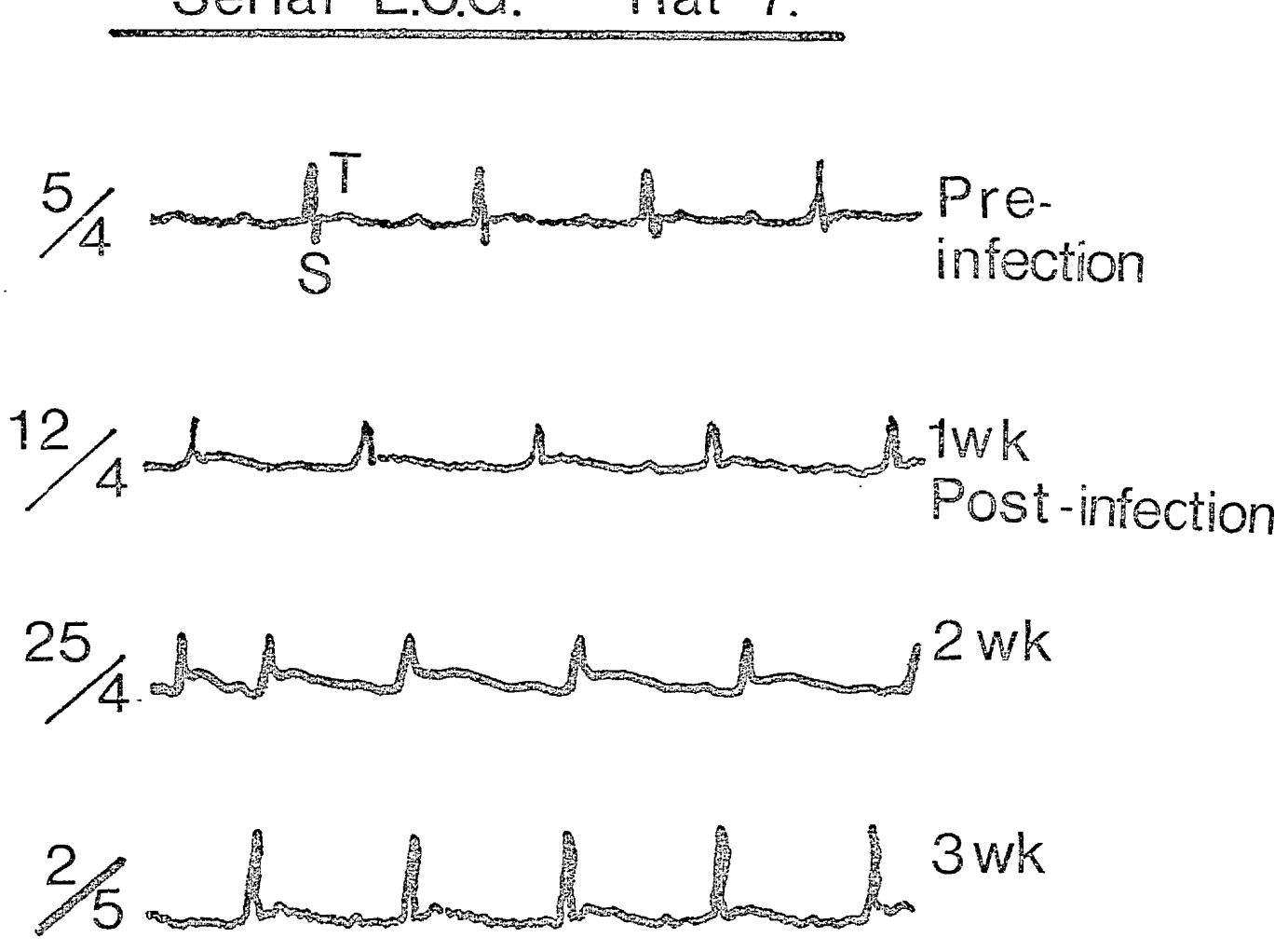

Figure 12. Electrocardiogram of a rat before and after infection. Note that prior to infection the S-T segments are isoelectric but that as early as seven days after infection these segments are elevated.

massively infiltrated with trypanosomes to the extent that serious malfunction must have occurred, e.g., in one rat two months after inoculation massive trypanosome invasion had occurred in the adrenal cortex and medulla, a change which may well have led to serious hormonal imbalance. Despite the fact that trypanosomes were found in many small blood vessels in the central nervous system, at no time was any lesion observed.

Between 5 and 8 weeks a proportion of infected rats died as a result of secondary bacterial infection. Most commonly this was severe purulent bronchopneumonia which in some cases was associated with generalised pyaemia. A wide range of bacteria were isolated from such cases.

Microcirculation

In many organs, perivascular oedema was found surrounding the blood vessels of the microcirculation, a change indicative of increased vascular permeability. In addition many small blood vessels were frequently plugged with trypanosomes and red blood cells.

Haemopoietic System

Within a few days of inoculation, all animals developed a characteristic anaemia with a 20% reduction in packed cell volume and this persisted throughout the course of infection (see Chapter 2). By fourteen days after inoculation, tissue sections indicated that there was increased erythropoietic activity in both bone marrow and spleen (Fig. 7). At necropsy there was massive splenomegaly largely due to red blood cells packing the sinuses and cords of the red pulp where there was also marked erythrophagocytosis. With time haemosiderin deposition became massive. During the terminal phase of the infection, a significant proportion of animals underwent a major haemolytic crisis with haemoglobinaemia and haemoglobinuria

and quickly died.

DISCUSSION

In hooded Lister rats infected with this derivative of T. brucei at least three major factors contributed to the disease process. First there was progressive alteration of the immunological apparatus of the lymph nodes and spleen. Initially, massive plasma cell hyperplasia replaced the normal cellular components of the immunological apparatus and these plasma cells in turn were destroyed as the lymph nodes and spleen became progressively depleted of cells. It would be of interest to determine what percentage of these plasma cells were producing specific antibody directed against trypanosomes and what was the nature of the immunoglobulin class. The reason for the subsequent cellular depletion is not known. It may be that the trypanosomes are producing some cytoxic factor directly. On the other hand, the cytotoxicity may be due to an immune reaction since trypanosomes frequently lay in close apposition to lymphocytes and plasma cells.

The progressive alteration of the immunological apparatus led to the development of a stage of immunosuppression to other infectious agents including bacteria and parasites (see Chapter 3). Between five and eight weeks after inoculation, a proportion of rats succumbed to secondary bacterial infections which resulted mainly in purulent bronchopneumonias. While it is easy to appreciate that animals with grossly depleted lymphoid systems may be immunosuppressed, the contemporaneous association of highly active lymphoid and mononuclear phagocytic systems with an immunosuppressed state to other antigens is more difficult to explain. The possible

mechanisms involved in the development of immunosuppression are discussed in Chapter 3.

Throughout the course of infection the M.P.S. in the liver, lymph nodes and spleen remained active and numerous monocytes and macrophages were encountered. These macrophages had a very active appearance with abundant vacuolated cytoplasm. In the spleen they frequently contained red blood cells and elsewhere cellular debris possibly trypanosomes. Whether or not these macrophages were activated by some immune process either of a cell mediated nature via soluble lymphokines or by cytophilic antibodies remains to be determined.

In a rarely quoted paper published in 1931, Hu[7] using a derivative of T. brucei which killed rats 2 weeks after inoculation, described a similar series of changes in the immunological apparatus, namely proliferation of the reticuloendothelial system, increased intramedullary and extramedullary haemopoiesis and plasma cell proliferation in lymph nodes and spleen followed, as early as day 6 after inoculation, by cellular depletion. It is remarkable that the series of changes described in this paper over a period of 8 weeks with one strain of T. brucei apparently occurred within 10 days with another.

The second major factor contributing to the disease process in T. brucei infected rats was specific organ damage and failure. Where trypanosomes became localised in tissues, they caused damage and necrosis with accumulation of macrophages, lymphocytes and plasma cells. This was most extensive in the myocardium where severe myocarditis developed in many rats and there is little doubt that a proportion of animals died in heart failure as a result. This was most severe in chronically-infected rats but

electrocardiograms showed distinct abnormalities of S-T segments, evidence of current episodes of myocardial injury, as early as 7 days after inoculation. Another major feature, although possibly not a lethal one, was severe muscle wasting with diffuse interstitial myositis. Jennings, Murray, Murray and Urquhart[8], using iodine labelled mouse serum proteins and a labelled globulin (IgG) fraction showed that in trypanosome infected mice there was increased protein catabolism, a factor that may have contributed to muscle wasting.

Whilst no obvious immunological lesions such as necrotising vasculitis were found, perivascular oedema occurred round many small blood vessels suggestive of increased vascular permeability. This may well have occurred as a result of the release of vasoactive kinins, shown by Boreham[9] and Goodwin[10] to occur in rabbit trypanosomiasis.

A limited amount of material from trypanosome infected domestic animals subsequently examined by Dr. M. Murray[11] has indicated that there might be pathogenic mechanisms similar to those described in the rat.

Four cattle, (aged from 1 year to aged) from the coastal area of Kenya and naturally infected with T. congolense and T. vivax showed on histological examination extensive depletion of the immunological apparatus of the lymph nodes and spleen, anaemia with marked erythrophagocytosis and haemosiderosis and focal myocarditis. In West Africa, MacLennan[12] also found heart lesions in acute T. vivax infections of cattle. At necropsy the most significant finding occurred in the heart which was pale and friable and was covered with endocardial and epicardial haemorrhages. Histological examination showed that there was myocardial damage so severe that it must have led to acute heart failure.

Three sheep, experimentally infected with T. vivax in Glasgow, showed demonstrably similar lesions to the cattle, i.e., focal areas of myocarditis together with anaemia and splenomegaly in which erythrophagocytosis and haemosiderosis were marked.

These preliminary findings suggest that in domestic animals, pathogenic mechanisms similar to those described in the rat may be operative. Thus, it might be appropriate to consider that the pathogenesis of the various animal trypanosomiases are probably similar than to accept the view of Losos and Ikede[4] that trypanosomiasis is "a group of diseases which are as many in number as there are species of trypanosomes".

Recently McKenzie, Boreham and Facer[13] have shown that the production of 3 naturally occurring auto-antibodies is enhanced during the course of T. brucei infection in rabbits and Goodwin[10] has associated the severe vascular lesions of this disease with the production of kinins possibly derived as a consequence of antigen-antibody interactions. Furthermore Boreham and Kimber[14] have demonstrated the presence of immune complexes in the kidneys of infected rabbits. Such observations tend to indicate that the pathogenesis of trypanosomiasis may have important immunological aspects and it is possible that the extensive organ damage seen in experimental infections might well be attributable to immunologically mediated cell damage. An interpretation of this nature would certainly explain the remarkably pathogenicity of T. congolense and T. vivax in cattle during a time when very few parasites can be seen in the blood[15,16]. It might, therefore, prove rewarding to determine the role of immuno-pathology in the production of the severe tissue and organ damage seen in experimental trypanosomiasis.

SUMMARY

In rats infected with T. brucei derived from the stabilate TREU 667, at least three pathogenetic mechanisms contribute to the disease process.

1. Progressive damage to the immunological apparatus of the lymph nodes and spleen leading to immunosuppression and susceptibility to secondary infection.
2. Specific organ damage particularly of the heart. This led to heart failure in a proportion of cases.
3. The development of a haemolytic anaemia with a proportion of animals dying as the result of a haemolytic crisis.

These findings in the rat are similar in certain respects to the pathology of the disease in domestic animals. Thus, it might be appropriate to consider that the pathogenesis of the various animal trypanosomiases are probably similar. The role of immuno-pathology in the development of these lesions remains to be determined although it may be of importance.

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CHAPTER 2

ANAEMIA IN TRYPANOSOME BRUCEI INFECTIONS OF RATS AND MICE.

INTRODUCTION

Clinically one of the cardinal signs of animal trypanosomiasis is the presence of anaemia[1] and in infected cattle marked falls in red cell indices, to levels which will not sustain life, are commonly observed. The morbidity and mortality seen in trypanosome infected animals is thus often intimately associated with the degree of anaemia[2]; indeed Hornby[3] in 1921 proposed that the clinical disease in cattle was entirely attributable to anaemia.

Despite these comments the aetiology of the anaemia associated with trypanosome infections in animals is inconclusive. In 1913, Boycott and Price-Jones[4] studying the anaemia in rabbits caused by T. brucei reported reduced packed cell volumes and haemoglobin concentrations, and on the basis of histological observations concluded that "active destruction of red blood cells occurred in lymph nodes and bone marrow to some degree". Fiennes[5] in a recent review of his own work carried out in the 1950's on T. vivax and T. congolense infections in cattle considered the anaemia to be of two types. First an acute form characterised by haemolysis; demonstration of the latter depended on positive indirect Van den Bergh reactions and an in vitro haemolytic test, devised by himself, in which normal bovine erythrocytes were haemolysed by plasma from infected and anaemic cattle. The second type of anaemia, was microcytic and attributed to "red cell failure".

Also in the 50's, Edwards, Judd and Squire[6] working with experimental infections of T. vivax, T. congolense and T. brucei in goats described haemosiderosis but no bilirubinaemia or reticulocytosis and from these findings concluded that the anaemia might be attributable to some failure in the mechanism of haemopoiesis.

More recently Naylor[7], on the basis of experimental infections of 10 cattle with T. congolense, concluded that the anaemia was normochromic and macrocytic and appeared to result from a combination of haemodilution, extravascular haemolysis and dyshaemopoiesis. Evidence of extravascular haemolysis was based on intermittent increases in serum bilirubin and dyshaemopoiesis on a failure to demonstrate any increase in reticulocytes. However, examination of the author's myelograms show no evidence of dyshaemopoiesis and in his own discussion he described the marrow as "hyperplastic" during the major part of the infection.

It is therefore apparent that views on the aetiology of the anaemia in animal trypanosomiasis are in a confused state, and haemolysis, dyshaemopoiesis and haemodilution have all been proposed as mechanisms operating either separately or in concert. It is also unfortunate that most of these conclusions are extrapolations based on inadequate evidence. The situation is further complicated by the increasing body of evidence that immunological mechanisms are responsible for at least part of the anaemias of other protozoal diseases.

While conventional haematological estimations such as PCV, red cell count, haemoglobin estimations, marrow examination, etc., are essential in a study of the severity and progression of anaemia, the additional use of radioisotopic labels in a pathophysiological investigation of red cell kinetics provide unique information on the quantitative aspects of erythrocyte production and destruction and on iron turnover. These techniques may also provide facts, unobtainable by other methods, which give clear evidence as to the actual aetiology. This chapter describes the experiments conducted in rats and mice infected with T. brucei, utilising both

conventional and radioisotopic methods, to elucidate the nature and aetiology of the anaemia in this infection.

MATERIALS AND METHODS

The rats and mice used and the infection procedures were as described in General Materials and Methods.

Rats were checked for the presence of latent Haemobartonella muris infection[8] by the prior splenectomy of a small number. Subsequently, stained blood films from these rats were examined daily. None were shown to be positive for H. muris.

Oxytetracycline Therapy

In the more protracted experiments, animals were maintained under oxytetracycline prophylaxis (Terramycin, Pfizer Ltd., Sandwich, Kent) to prevent intercurrent bacterial infections. The drug was added to their drinking water at a rate of 1.2 g/litre.

Splenectomy

Mice were anesthetised with Pentobarbitone Sodium (Nembutal, Abbott Laboratories Ltd., Queensborough, Kent) by giving 0.6 mg of the active principal/10g bodyweight. A small vertical incision was made through the left anterior abdominal wall and the spleen exteriorised. After ligating the splenic vessels with a single linen suture, the spleen was cut off, the pedicle returned to the abdomen and the abdominal wall sutured. Mice were allowed 2 weeks to recover before being used in an experiment.

Necropsy Procedures

For haematological investigations, 5 infected rats were sacrificed each week and 5 normal rats at fortnightly intervals. Rats were anesthetised with trichloroethylene (Trilene, I.C.I. Pharmaceuticals) and exsanguinated by cardiac puncture using

dipotassium ethylenediamine tetra-acetate (EDTA) at approximately 1 mg/ml blood as an anti-coagulant.

After death, the liver and spleen was removed together with the right femur for further examination.

Packed Cell Volume (PCV)

The packed cell volume percentage was determined by the capillary microhaematocrit method (Hawksley and Sons Ltd., Lancing, Sussex).

Erythrocyte (RBC) Counts

Erythrocyte counts were determined by an electronic counter (Coulter Counter Model D, Coulter Electronics Ltd., London).

Haemoglobin (Hb) Concentration

Haemoglobin concentrations were determined by the oxyhaemoglobin method[9]. 0.02 ml of blood was diluted in 2 ml 0.04% ammonia and the cells lysed by inverting several times. The absorption of this mixture was determined in an EEL colorimeter (Evans Electroselenium Ltd., Halstead, Essex) using an Ilford 625 green filter and the concentration of Hb obtained by reference to a standard plot.

This data was used to calculate the Mean Corpuscular Volume (MCV) and the Mean Corpuscular Haemoglobin Concentration (MCHC) according to the following formulae:

$$\text{MCV } (c\mu) = \frac{\text{PCV} \times 10}{\text{RBC count/cu.mm.}}$$

$$\text{MCHC } (\%) = \frac{\text{Hb (gm \%)} \times 100}{\text{PCV}}$$

Spleen Impression Smears

The spleen was cut transversely at mid-point and the cut end lightly touched on a clean glass slide in several places.

Bone Marrow Films

Both epiphyses were removed from the femur and the marrow in the medullary cavity forced out into 1.0 ml normal rat serum by expressing air from a 5 ml syringe through the cavity via a tight fitting plastic tube. The marrow plug so obtained was suspended by using a rotary mixer. After staining, 500 or more cells were counted in each marrow film and the cell types classified according to Ramsall and Yoffey[10] into erythroid, myeloid, lymphoid, blast and the remainder, including unidentified and damaged cells.

Blood Films

One blood smear was made from each rat. A visual assessment was made of the erythrocyte morphology and the numbers of normoblasts.

Staining

Slides were air dried, fixed in methanol for 10 minutes, stained by Lepehne's peroxidase reaction[11] to facilitate identification of the cells of the erythroid series and overstained with Giemsa.

Reticulocyte Counts

100 µl New Methylene Blue stain (1 g/100 ml PBS) was added to 200 µl blood and incubated at 37°C for 20 minutes. After this time films were made and air dried. The number of reticulocytes were counted by the method of Dacie and Lewis[9] using an oil immersion objective.

Erythrocyte Labelling with ^{51}Cr Sodium Chromate

1. In vivo

Approximately 10 microcuries (μCi) of ^{51}Cr sodium chromate was injected intravenously into each mouse via the tail vein.

2. In vitro

A sample of blood containing heparin as anti-coagulant was incubated with ^{51}Cr sodium chromate for 30 minutes at 37°C. After washing twice with phosphate buffered saline pH 7.3 (PBS) to remove the ^{51}Cr which was not bound to the red cells, the cells were reconstituted to a suitable volume in PBS for intravenous injection. In general each mouse received 10-20 μCi ^{51}Cr-labelled red cells while each rat received approximately 50 μCi of labelled cells.

^{59}Fe Ferrous Citrate Injections

Approximately 10 μCi ^{59}Fe as ferrous citrate was injected intravenously into each mouse selected for study with this technique.

Sampling

Mice were bled from the tail vein into heparinised micro-haematocrit tubes. After centrifugation for 5 minutes, that portion of the tube containing the packed red cells or plasma was weighed prior to and after expulsion of the contents into a counting vial containing 10 ml diluted NaOH.

Rats, maintained in individual metabolism cages, were bled daily by cardiac puncture. The total urine and faeces from each rat were collected daily and bulked in glass counting vials for radioactivity counting.

Radioactivity Measurements

Radioactivity determinations on blood and, in the rat experiment, on urine and faeces were carried out in an automatic

well-type gamma scintillation counter (Model 4230, Nuclear Chicago Corporation, Des Plaines, Illinois). Corrections for radioactive decay were based on the activity of appropriate ^{51}Cr and ^{59}Fe standard solutions. All samples were counted for a sufficient time to give a net count with less than 2% standard error. Counts were expressed as counts/min/mg packed red cells or plasma.

Whole body counting of mice was carried out using a large volume automatic gamma scintillation counter (Pannax "Gamma One-Sixty", Panax Equipment Ltd., Redhill, Surrey).

RESULTS

Body weight changes in the rat

Throughout the course of infection there was a general decrease in the body weights of infected rats despite a significant increase in their liver and spleen weights (Table 1). The spleen weights of infected animals were ten times that of controls six weeks after infection.

Of the pool of fifty infected rats, fourteen died or were killed in extremis from three weeks onwards. Generally it was found that these rats had bronchopneumonia occasionally with fibrinous pleurisy. By contrast the control rats showed occasional small areas of pulmonary consolidation but no signs of active pneumonia. This agreed with other reports of secondary bacterial infection consequent upon the immunosuppressed state of trypanosome infected animals (see Chapter 3). Three of the infected rats suffered a sudden and acute terminal illness with haemoglobinaemia and haemoglobinuria. The course of this severe intravascular haemolytic episode was extremely short in the order of 2 to 8 hours. Due to the small numbers affected in

Table 1 The effect of T. brucei infection in rats on the whole body, liver and spleen weights*

		Whole Body	Liver	Spleen
Normal Uninfected Rats	(12)	161.1 ± 3.8	6.3 ± 0.2++	0.46 ± 0.03°
Rats infected for 14 days	(5)	160.0 ± 5.8	7.2 ± 0.4	1.92 ± 0.12
Rats infected for 20 days	(5)	152.8 ± 3.1	7.3 ± 0.4	2.25 ± 0.19
Rats infected for 28 days	(5)	142.8 ± 9.0	7.7 ± 0.6	2.77 ± 0.51
Rats infected for 35 days	(4)	153.0 ± 3.8	8.5 ± 0.5	4.03 ± 0.71
Rats infected for 42 days	(3)	151.5 ± 9.7	8.9 ± 0.6++	4.10 ± 0.76°

*Numbers in parenthesis represent the number in each group.

Each value is the mean weight in grams ± standard error.

++ $P < 0.001$

° $P < 0.001$

this manner and the extreme rapidity of the terminal crisis it was difficult to obtain more detailed information on this aspect of the anaemia.

Haematological changes in the rat

As can be seen from Fig. 1 the PCV, RBC count and Hb concentration of infected rats had dropped within two weeks after infection and continued to decline to the end of the experiment. At this time, i.e., 6 weeks after infection, the mean PCV value of infected rats was 33.2 compared to 39.0 in the controls; the RBC counts had dropped from a mean of 5.3 x 10^6/cu mm to 3.4 x 10^6/cu mm and the haemoglobin concentration from 13.8g per cent to 8.9g per cent.

Fig. 2 shows the macrocytosis which occurred after three weeks of infection. The increase in MCV appeared to parallel very closely the appearance of large numbers of circulating reticulocytes; this was to be expected since immature erythroid cells are larger than mature erythrocytes[12,13]. The MCHC remained relatively constant throughout. A depression of the M.E. ratio accompanied the development of large numbers of normoblasts in the bone marrow (Figs. 3 and 4) and grossly the marrow changed from a soft pinkish plug of tissue to a deeper red, more fluid material. This normoblastic hyperplasia occurred throughout the experiment in infected rats and grossly, no evidence of bone marrow depression was seen.

An increase in erythropoietic activity was also evident in the splenic tissues. Although extra-medullary haemopoiesis occurs in normal rats, particularly young animals[8], a much greater number of nucleated normoblasts were seen in spleen impression smears from infected rats (Fig. 5). Not only was this increase in haemopoietic activity reflected in the circulation by the appearance of large

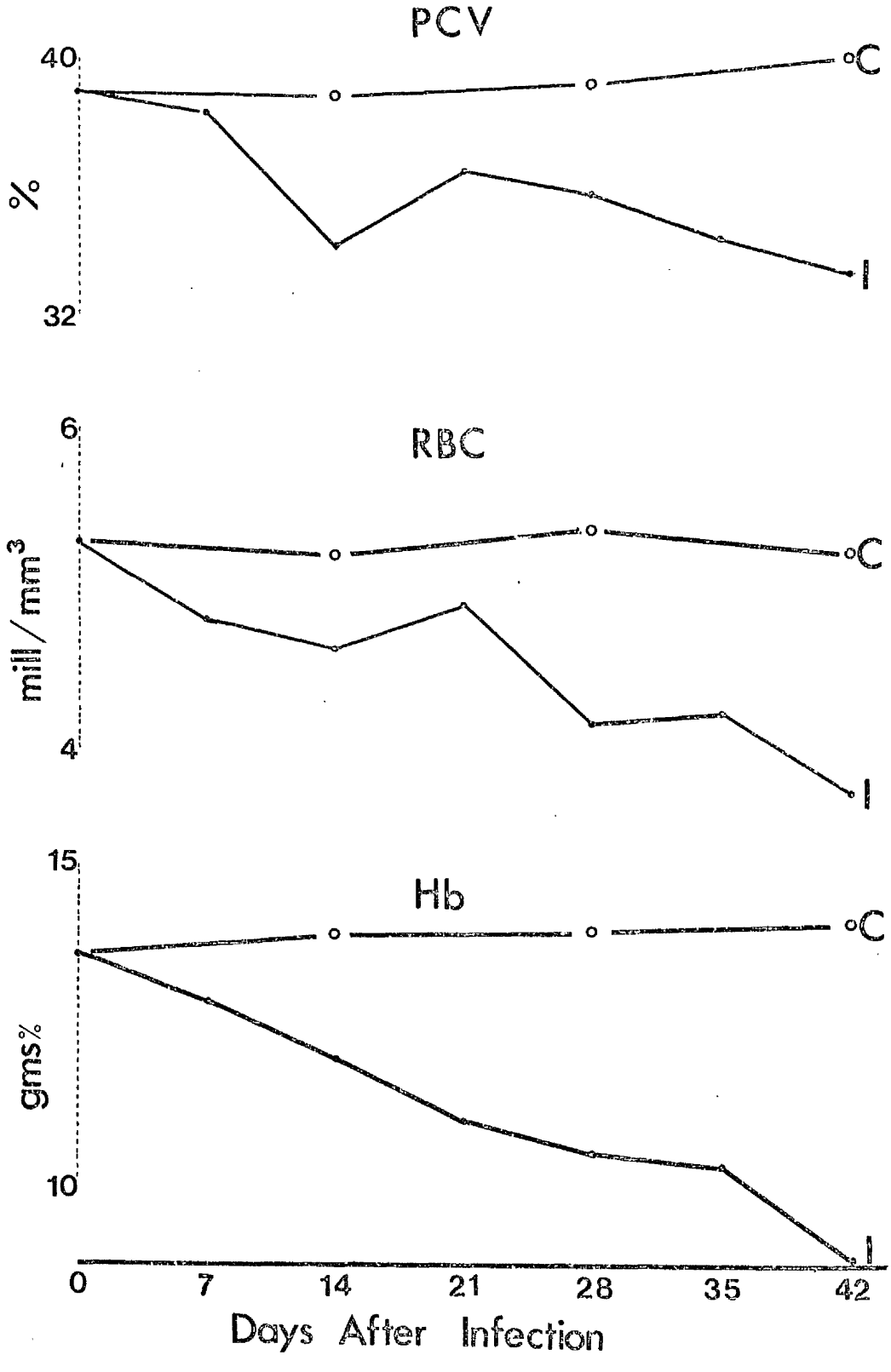

Figure 1. The haematological changes in rats infected with T. brucei TREU 667 (C = control rats and I = infected rats).

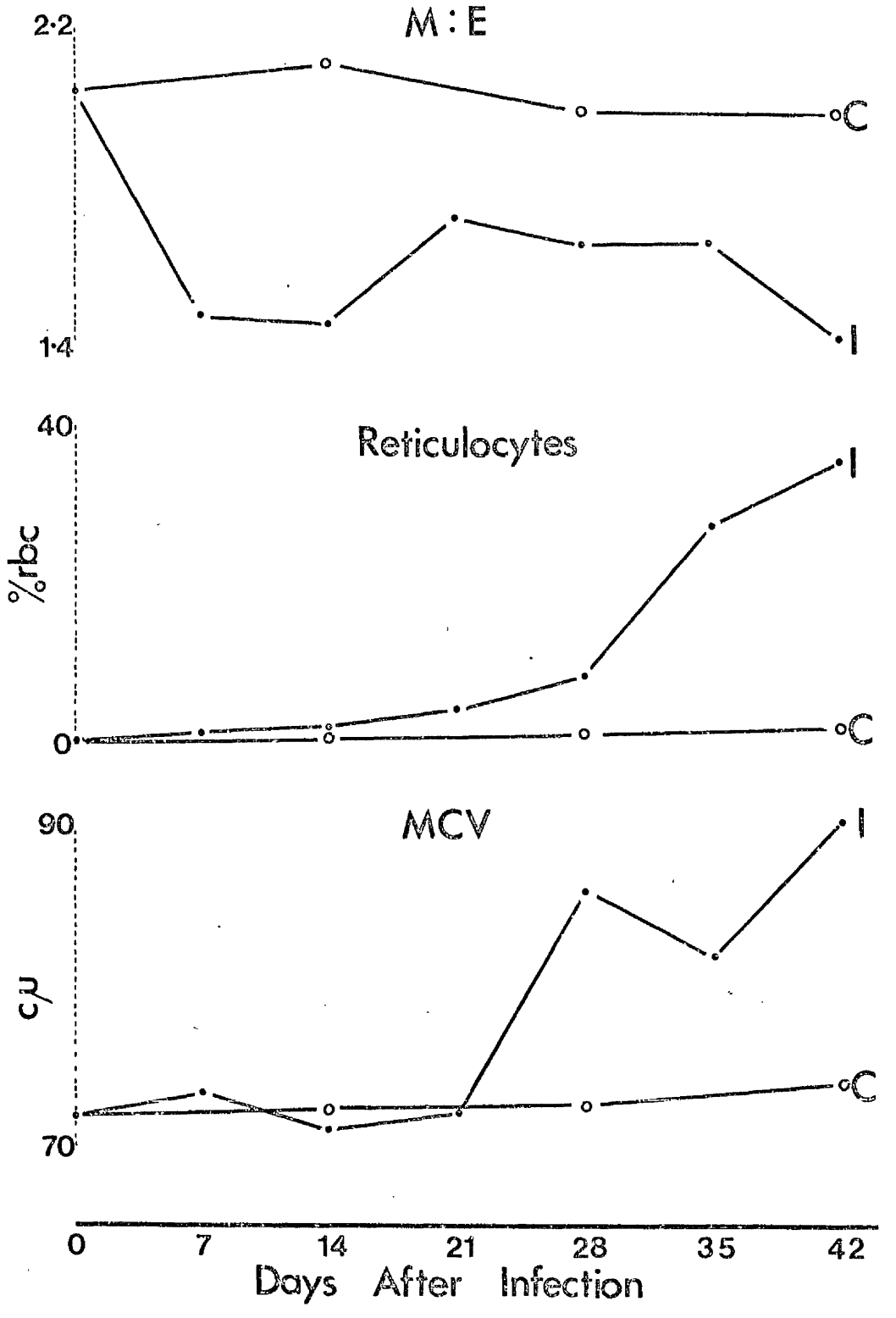

Figure 2. Alterations in the Myeloid:Erythroid (M:E) ratio (top); alterations in the numbers of circulating reticulocytes (middle); changes in the mean corpuscular volume (M.C.V.) (bottom) in rats infected with T. brucei TREU 667 (C = control rats and I = infected rats).

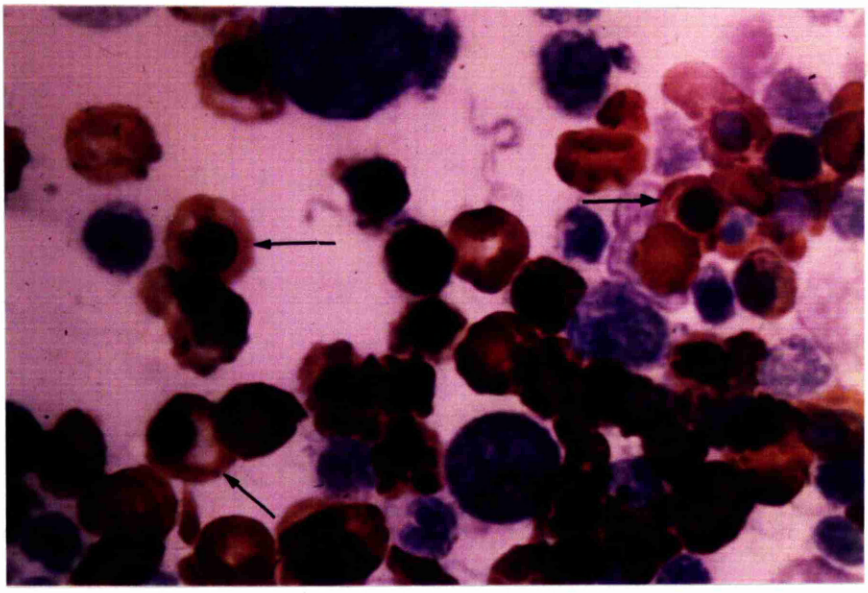

Figure 3. A marrow film from the femur of a rat infected for 3 weeks with T. brucei TREU 667. Note the presence of large numbers of normoblasts (Giemsa + Lepehne x 1,000).

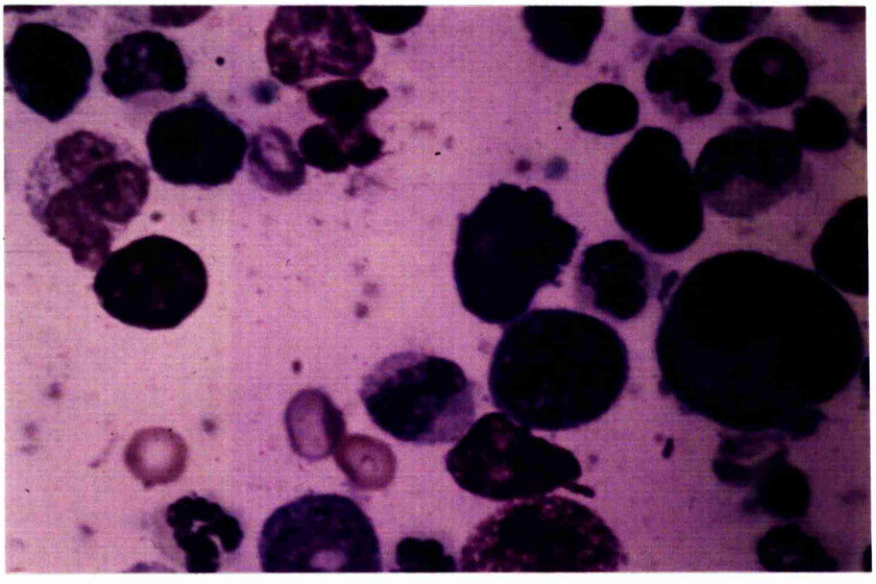

Figure 4. A marrow film from a normal rat (Giemsa + Lepehne x 1,000).

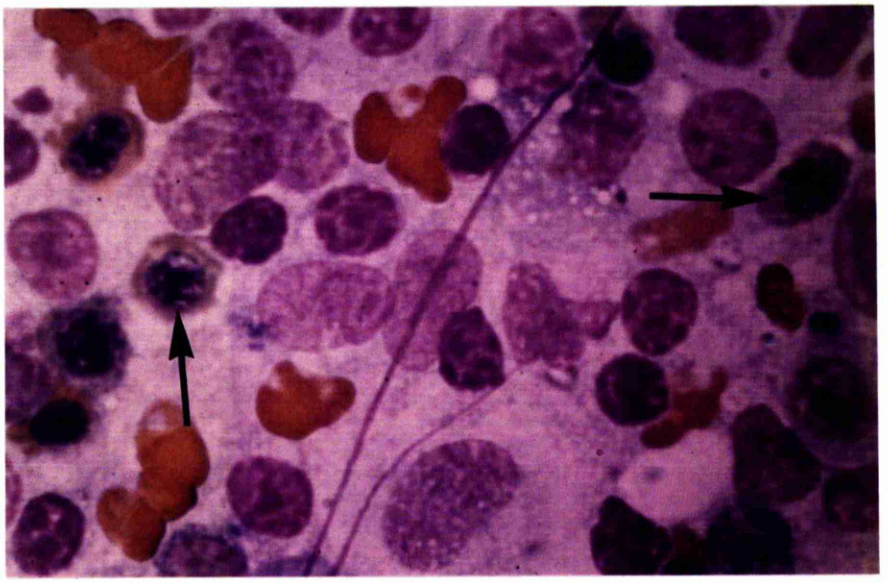

Figure 5. An impression smear from the spleen of a rat infected for 3 weeks with T. brucei 667. The presence of normoblasts (arrowed) indicates marked extra-medullary erythropoiesis (Giemsa + Lepehne x 1,000).

numbers of reticulocytes but also by the appearance of late and intermediate normoblasts which occurred in similar numbers to that of the total leukocyte count.

The erythrocyte morphology was altered after some weeks of infection in that polychromasia, poikilocytosis and anisocytosis were commonly found.

Haematological changes in the mouse

In mice from which serial blood samples were taken from the tail, a fall in PCV occurred within 4 days of inoculation and this remained at approximately 20% below normal values until the terminal stages of the infection (Fig. 6).

Experiments using ^{51}Cr

In preliminary experiments using ^{51}Cr-labelled red cells, the cells were labelled in vivo and the disappearance of the label was followed by whole body counting. The disappearance curves of a control and an infected group of mice are shown in Fig. 7. The cells were labelled prior to infection of the mice with T. brucei TREU 667 and the divergence of the curves was statistically significant within 7 days of infection. The half-life values of the labelled red cells which were calculated from the individual regression equations are given in Table 2.

Splenectomy had no apparent effect on these half-life values (Fig. 8 and Table 2). Thus, the half-life of ^{51}Cr-labelled cells in intact infected mice was 23.9 days and in splenectomised infected mice 24.1 days, compared to 31.1 days in normal mice. These infected splenectomised mice all showed a significant drop in PCV by 14 days; on that day the mean PCV of the 3 controls was 44.2 ± 0.8 and that of the 5 infected mice was 37.4 ± 1.8 ($P < 0.001$).

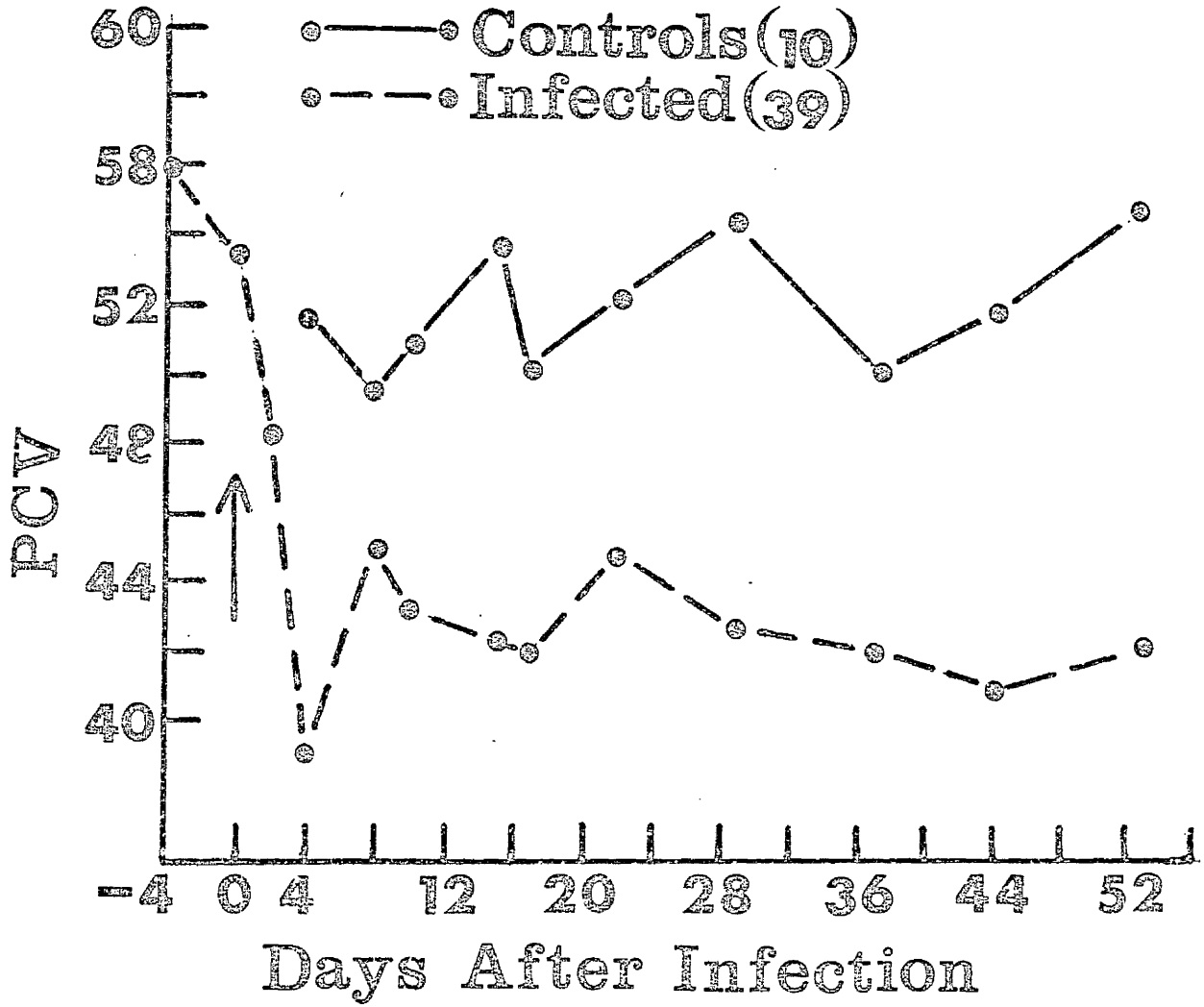

Figure 6. The packed red cell volume of normal mice and of mice infected with T. brucei TREU 667.

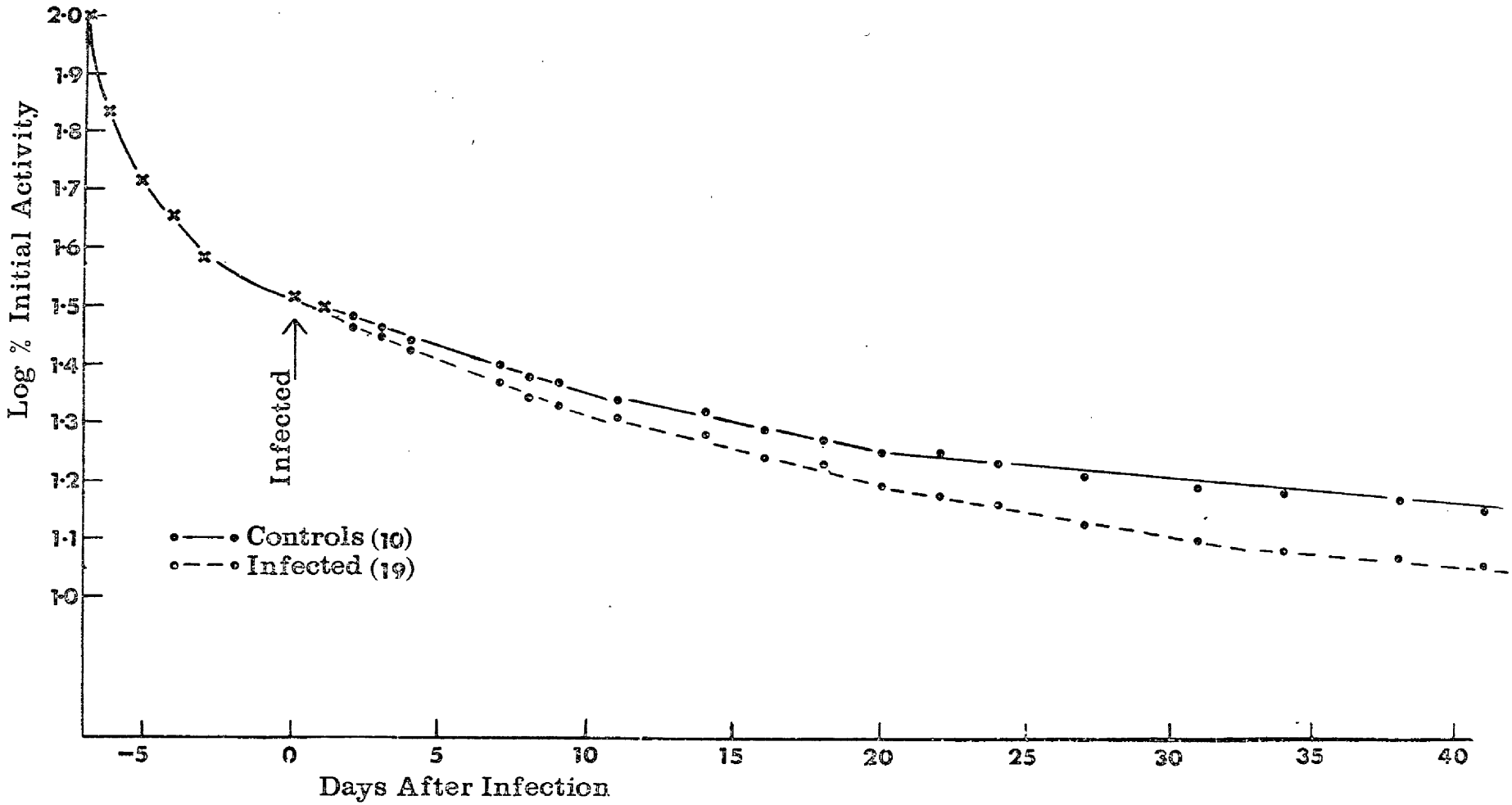

Figure 7. The disappearance of ^{51}Cr from the body of normal mice and mice infected with T. brucei TREU 667 after injection of ^{51}Cr-labelled red cells.

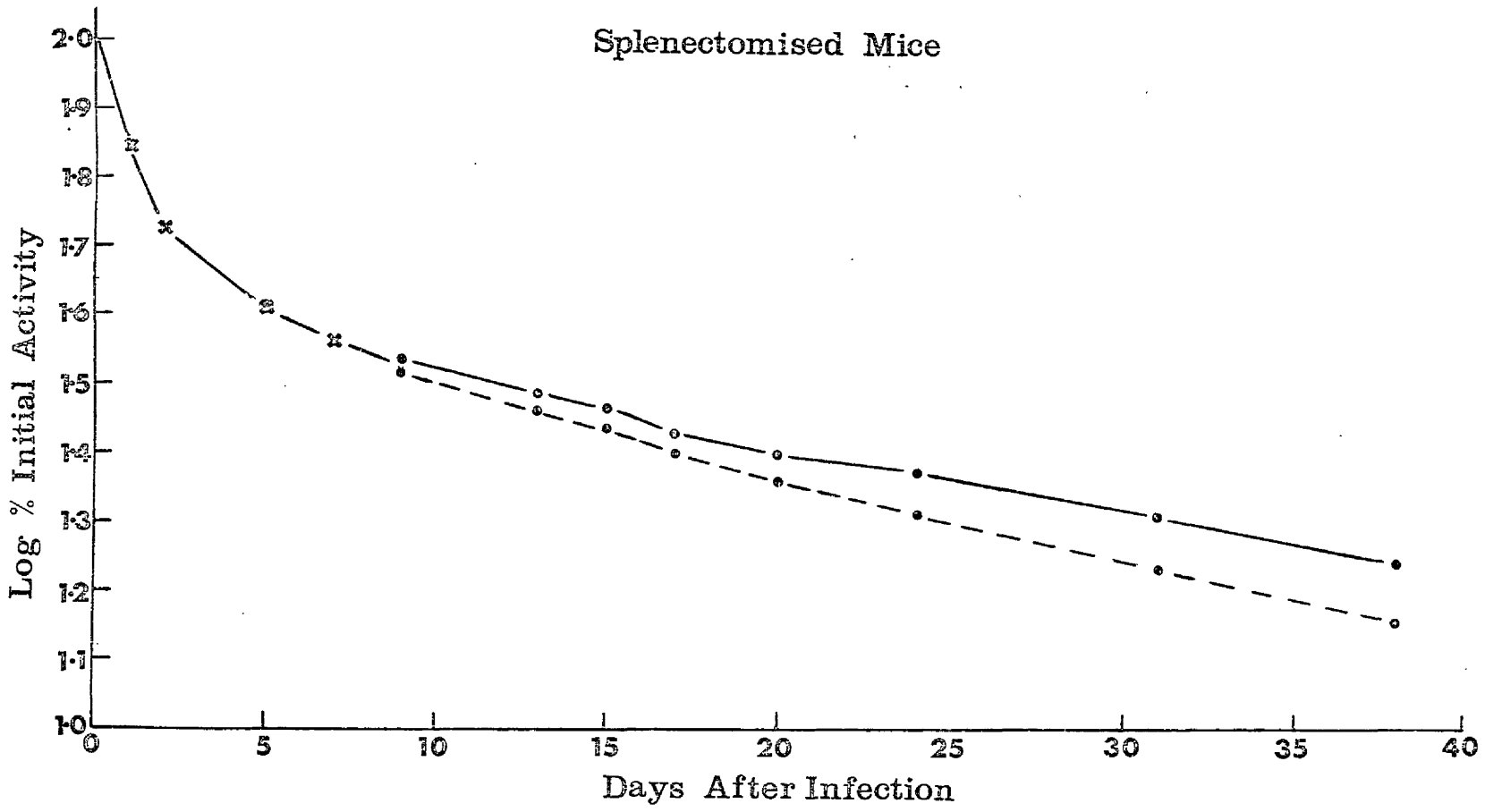

Figure 8. The disappearance of ^{51}Cr from the body of normal splenectomised mice and splenectomised mice infected with T. brucei TREU 667 after injection of ^{51}Cr-labelled red cells.

Table 2 Half-life (days) of ^{51}Cr *in vivo* labelled cells in intact and splenectomised mice as measured by whole body counting*

	Controls	Infected *T. brucei*	Significance
Intact Mice	31.1 ± 0.5 (10)	23.9 ± 0.04 (19)	P <.001
Splenectomised Mice	31.7 ± 0.5 (3)	24.1 ± 0.5 (5)	P <.001

*Values in parenthesis are the number of animals in the group ± standard error.

The limitation of this technique, i.e., whole body counting, is that it does not give a true estimate of the half-life of the circulating red cells, but is a measure of the retention of the ^{51}Cr within the body. In view of this a further experiment was undertaken involving unsplenectomised mice in which serial samples of blood were withdrawn daily and the radioactivity estimated. These showed that the disappearance of ^{51}Cr-labelled red cells from the circulation was very much faster than the disappearance of the ^{51}Cr from the body as a whole. Thus, the circulating half-life of the ^{51}Cr-labelled red cells was 8.3 ± 3.2 days in the control mice and 1.5 ± 0.9 days in the infected mice ($P < 0.02$). This should be compared with the whole body half-life values of 31 days and 24 days respectively (Table 2).

The results of these experiments indicated that ^{51}Cr-labelled red cells were being removed from the circulation at an accelerated rate and that the ^{51}Cr was being lost from the body more quickly in infected mice than in the uninfected control mice.

To obtain more information on the aetiology of this anaemia a group of infected rats received syngeneic ^{51}Cr-labelled red cells on the day they were infected with T. brucei and another group received the labelled cells 23 days after they had been infected with T. brucei. Appropriate uninfected control rats were included at both times. The disappearance of labelled red cells from the circulation of these rats is shown in Fig. 9. The half-life value of the circulating red cells in the control rats was constant at 12.2 days. This was also the case in the infected rats until 7 days after infection. By day 10 the red cell half-life had dropped to 2 days, i.e., 6 times faster than normal. Subsequently it remained at this level throughout the duration of the experiment.

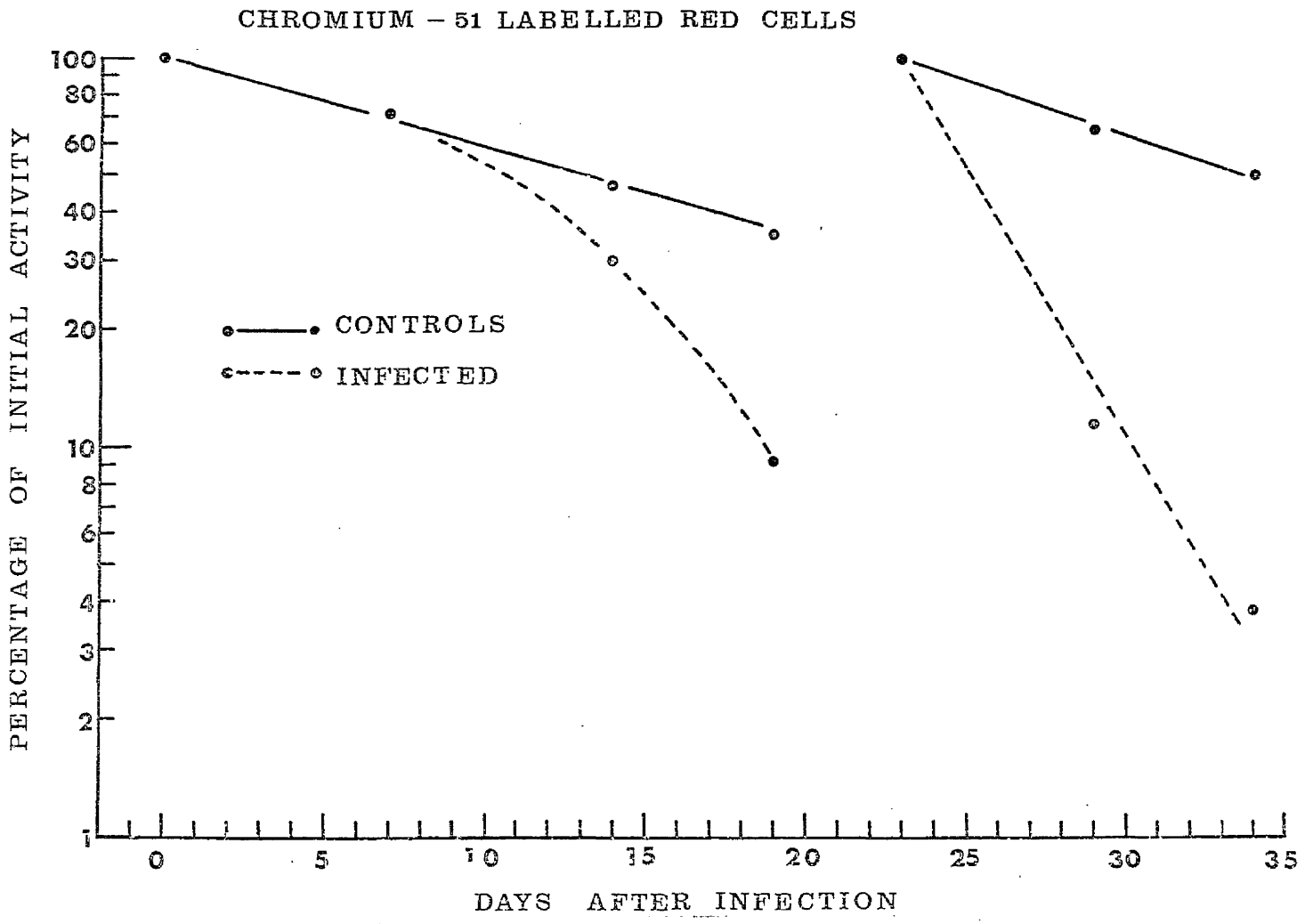

Figure 9. Disappearance of ^{51}Cr-labelled red cells from the circulation of normal rats and of rats infected with T. brucei.

The excreted activity appeared in the urine and not to any appreciable extent in the faeces. Urinary activity expressed as ml of packed red cells is shown in Fig. 10 (calculated using the activity of circulating red cells on the same day as the urinary collection). The results shown there indicated that up to 10 ml of packed red cells were being destroyed daily. This is obviously an overestimate of the amount of red cells being destroyed and the probable explanation is that the red cells, which had been removed from the circulation, were retained in the spleen for a number of days before the ^{51}Cr was excreted. Evidence that this is so is shown in Fig. 10, in which there is an apparent disparity in urinary excretion on day 24 in the 2 groups of rats. Presumably in the second group of rats the red cells were being removed at the same rate as in the first group but the excretion of the label was delayed for a period of days. An alternative explanation is that the labelled red cells, which were normal cells from syngeneic rats, introduced on the second occasion circulated for a period of days before becoming altered, i.e., coated with antibody or 'damaged'.

Experiments with ^{59}Fe

When ^{59}Fe in the form of ferrous citrate was injected intravenously into infected mice it disappeared from their plasma at a faster rate than from uninfected control mice (Fig. 11). The infected mice had plasma ^{59}Fe half-life value of 23.4 mins. compared to 89.4 mins. for the control mice. The activity of the circulating red cells (Fig. 12) after injection indicated that the iron was rapidly incorporated into the red cells. In addition, when ^{59}Fe as ferrous citrate was injected into mice 16 days prior to infection with T. brucei and the activity followed by whole body

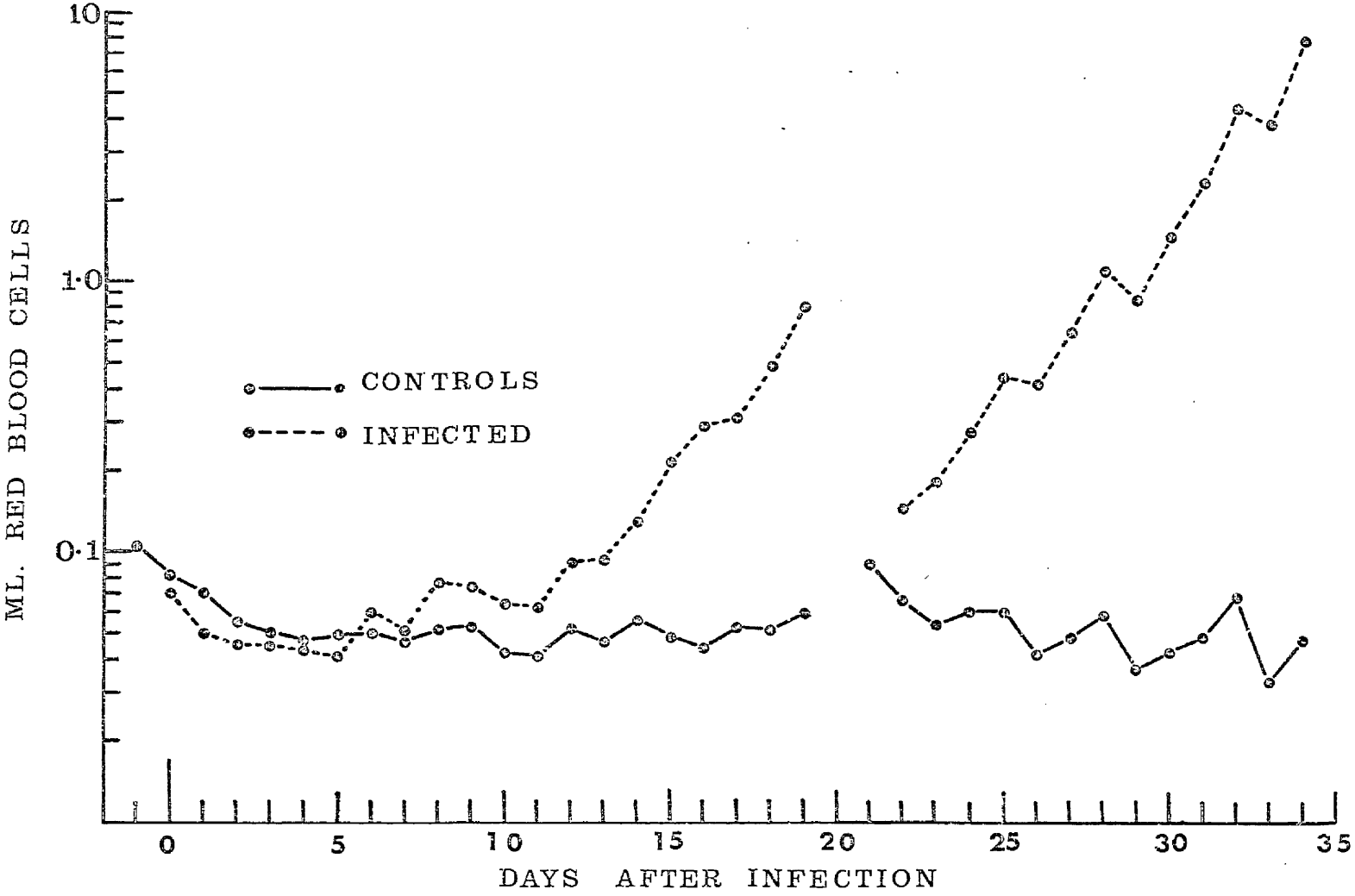

Figure 10. The excretion of ^{51}Cr in the urine of normal mice and of mice infected with T. brucei TREU 667 expressed as ml of red cells, calculated using the circulating red cell activity on the day of collection.

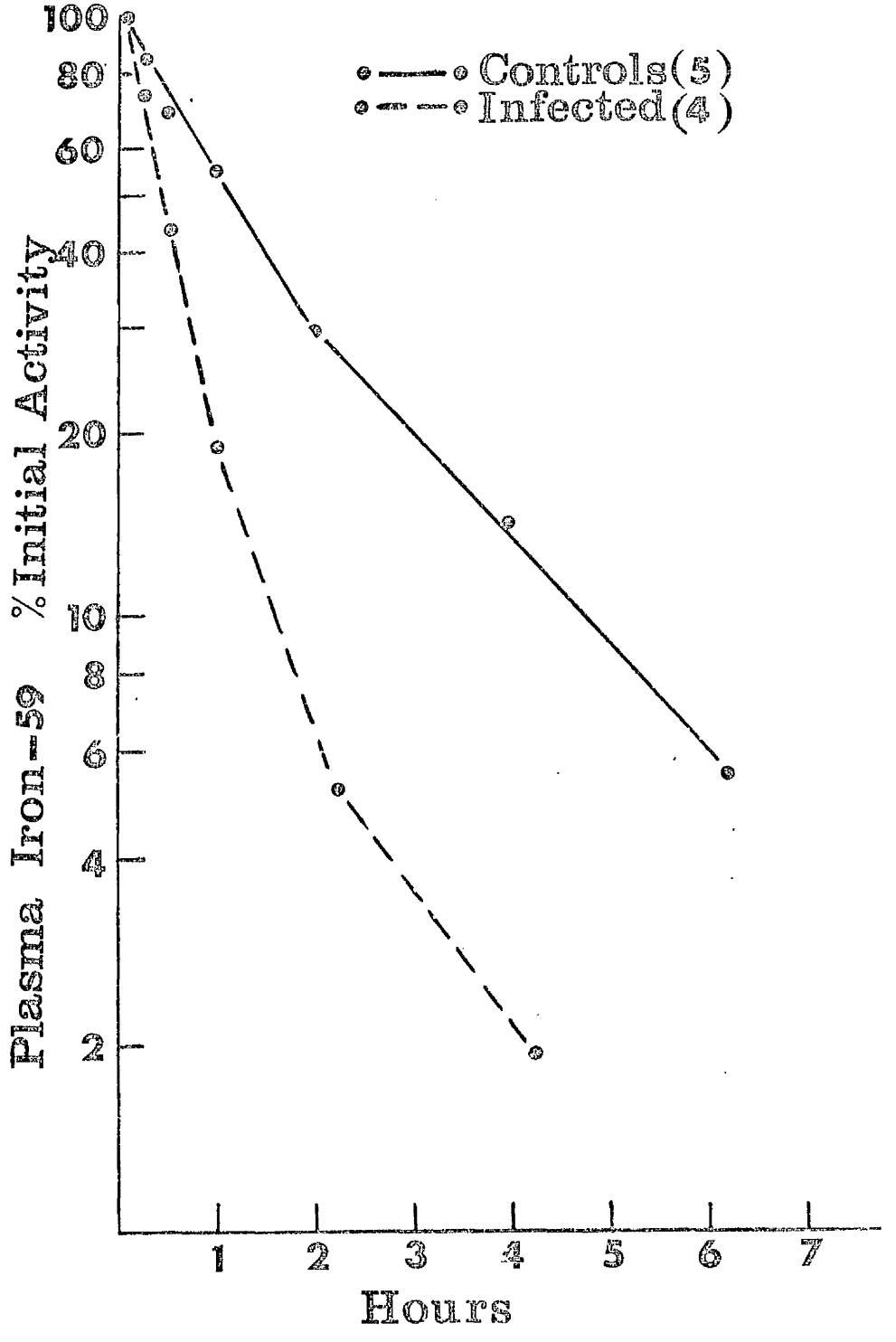

Figure 11. The disappearance of ^{59}Fe ferrous citrate from the plasma of normal mice and of mice infected with T. brucei TREU 667.

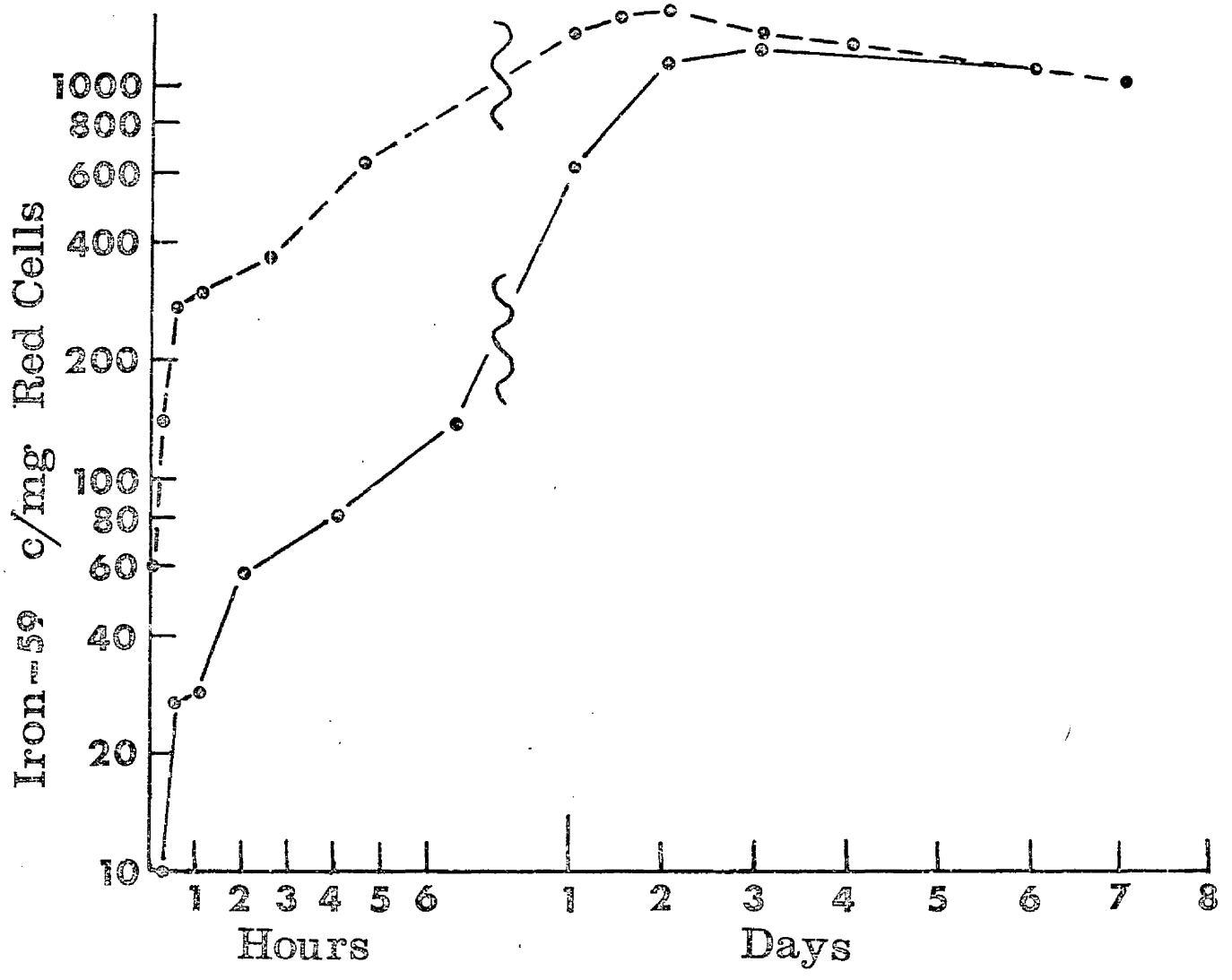

Figure 12. The incorporation of ^{59}Fe into the red cells of normal mice and mice infected with T. brucei TREU 667.

counting for a further period of 56 days, no statistical difference was found between the rates at which the ^{59}Fe was excreted from the body; the half-life value being 131 $\pm$ 3.84 days for the controls and 154.5 $\pm$ 10.95 for the infected mice.

DISCUSSION

The results of this study in rats and mice infected with a derivative of T. brucei TREU 667 all showed that the anaemia has a haemolytic origin. The anaemia was macrocytic, there was marked reticulocytosis and in the later stages circulating normoblasts were present. The bone marrow and the spleen showed normoblastic hyperplasia. All these findings, together with the fact that the ^{59}Fe was removed very rapidly from the serum, would indicate there is no loss of erythropoietic activity.

The experiment with ^{51}Cr-labelled red cells in the rats, which showed a very rapid removal of the labelled cells from the circulation and the appearance of this activity in the urine as distinct from the faeces, showed that there was little or no loss of red cells into the gastro-intestinal tract and that the red cells were being broken down within the body either intravascularly or extravascularly.

The findings with ^{59}Fe are also consistent with the haemolytic nature of the anaemia, i.e., the rapid disappearance of ^{59}Fe from the plasma, the rapid incorporation of this iron into the haemoglobin and the fact that iron was not lost from the body at an accelerated rate in the infected mice. These results together with the histological findings of extensive deposits of haemosiderin in the spleen, especially towards the latter stages of the disease, and the evidence of marked erythrophagocytosis are also indicative of a haemolytic anaemia.

Blood-borne protozoal infections are frequently characterised by splenomegaly and this has been advanced as a contributory or exclusive cause of the anaemia associated with malaria[14,15,16,17]. The spleens of our infected mice were invariably enlarged up to 15 times the normal size. However, our findings indicate that splenectomised mice also become anaemic and that if ^{51}Cr-labelled red cells were injected into splenectomised and infected mice they disappeared at the same rate as in intact infected mice. This would indicate that splenomegaly *per se* is not solely responsible for the anaemia although it may be that other organs and tissues take over the function of the spleen in such mice. In intact infected mice, the spleen undoubtedly plays a key role in the destruction of erythrocytes. Histologically (Chapter 1) erythrophagocytosis is marked and the red pulp is packed with erythrocytes. The haemostasis is such that, irrespective of the primary cause of the anaemia, it may lead to a degree of glucose depletion in the erythrocytes, probably augmented by the high metabolic activity of the trypanosomes themselves. This alteration, by decreasing the plasticity of the red cells[18], may render them susceptible to phagocytosis. Since macrophages proliferate in response to a work load[19], the spleen may become the site of a vicious cycle of erythrocyte destruction.

However, the situation may well be more complicated as has been shown to be the case in other protozoal infections. Schroeder and Ristic[20] provide evidence that in *Babesia*, *Anaplasma* and *Plasmodium* infections the numbers of infected erythrocytes are not always commensurate with the degree of anaemia, implying that although the parasite *per se* can destroy

erythrocytes immunogenic factors may also be involved. Opsonins which sensitised uninfected erythrocytes to phagocytosis have been demonstrated in the serum of P. berghei and B. rhodaini infected rats[20]. Similarly Woodruff, Ziegler, Hathaway and Gwata[21] have found both erythrocyte bound complement and immuno-conglutinin on the surface of red cells in some cases of human trypanosomiasis which suggests that the anaemia may have an immunological basis. Recently Woo and Kobayaski[22] found that addition of complement to a suspension of T. brucei infected rabbit erythrocytes caused marked haemolysis. This indicates that surface immunoglobulin was already present on these red cells and by fixing the complement had caused this haemolysis providing indirect evidence of an immunologically mediated component in the anaemia.

We have been unable to date to detect globulin on the surface of erythrocytes of mice infected with T. brucei TREU 667 using the globulin-antiglobulin technique of Coombs, Mourant and Race[23] but it is possible that other factors are present. One finding which argues against the anaemia having an immune mechanism however, is the presence of a marked anaemia in mice within four days of inoculation with this derivative T. brucei.

A further factor which may contribute to the drop in packed cell volume is that of haemodilution. This has been reported by a number of workers including Naylor[7] who described an increase of up to 50% in the plasma volume of cattle infected with Trypanosoma congolense and Jenkins, Forseberg, Brown and Boulton[24] in T. brucei infections in rabbits. The plasma volume changes in the anaemia induced by the derivative of T. brucei TREU 667 has not yet been studied; however, in another strain of

T. brucei, TREU 226, which invariably produces anaemia within 3 days, accompanied by massive parasitaemia, there is no detectable increase in plasma volume as measured by I^{125}-labelled albumin[25]. It is, therefore, unlikely in the T. brucei TREU 667-induced anaemia that plasma volume changes, if they do occur, play a significant part in the anaemia.

Goodwin[26] demonstrated that marked damage to the microcirculation occurred in rabbits infected with T. brucei. After 14 days of infection the extravascular tissues in the ear were heavily infiltrated with mononuclear cells and phagocytes collected on the endothelium of venules leading to obstruction, stasis and eventually to disintegration of the vasculature. Such lesions could undoubtedly be responsible for anaemia if microhaemorrhages occurred on a wide scale. However, in T. brucei infected mice the ^{51}Cr erythrocyte distribution in the carcass was not different from that of normal mice[27]. Leakage of blood from the capillaries does not therefore seem to be a feature of T. brucei TREU 667 infection of mice and so there is no evidence that this anaemia has a microangiopathic basis.

In conclusion, all these findings which we have discussed above, i.e., the macrocytosis, the reticulocytosis, the normoblastic hyperplasia of the bone marrow and spleen, the haemosiderin deposits and the increased erythrophagocytosis in the spleen, together with the shortened circulating half-life of labelled red cells, the accelerated urinary excretion of ^{51}Cr and the retention of ^{59}Fe in the body are all indicative of an anaemia of haemolytic origin. The spleen undoubtedly plays a major role in the phagocytosis of erythrocytes and may contribute to their

premature senescence. The contribution that immune mechanisms make to the anaemic stage is unknown but may, as in other protozoal infections, be found to be important.

SUMMARY

The anaemia associated with infections of T. brucei TREU 667 in rats and mice is described and discussed. It was characterised by macrocytosis, reticulocytosis and normoblastic hyperplasia of the bone marrow and spleen. Circulating half-life values of ^{51}Cr-labelled red cells were reduced in infected rats and the ^{51}Cr label was excreted in the urine. ^{59}Fe was rapidly removed from the serum of infected mice and incorporated into the circulating red cells. There was no increased loss of iron from the body.

These findings, all indicative of an anaemia of haemolytic origin, are discussed with regard to the aetiology.

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CHAPTER 3

IMMUNOSUPPRESSION IN TRYPANOSOMA BRUCEI INFECTIONS OF LABORATORY RODENTS.

INTRODUCTION

Immunosuppression is a term which has been used in a pharmacological context to describe the depressive effects of certain drugs on the immune response. In recent years it has been shown that this term can also be applied to the effect which any of a wide variety of diseases may have on the immune responsiveness of a host to certain antigens. Such interference with immune defences is likely to predispose to secondary infection and immunosuppression could therefore be a phenomenon of profound significance in the pathogenesis of such diseases. Immunosuppressive events have been shown to occur in a wide spectrum of diseases, e.g., bacterial infections of man such as leprosy[1] and syphilis[2], viral infections[3], mycoplasmosis[4], certain malnutrition states[5], helminth infection[6] and in a variety of protozoal infections[7,8]. In tropical and sub-tropical countries the widespread occurrence of both malnutrition and protozoal infections emphasises the need for a proper investigation of immunosuppression as a phenomenon of medical and veterinary importance in the epidemiology of disease.

Although circumstantial evidence of immunosuppression, for example the association of severe streptococcal infection and human sleeping sickness[9], has existed for many years now, the real importance of this factor in the epidemiology of disease has only recently been recognised. In 1962, McGregor and Barr[10], working in areas of endemic malaria in The Gambia found that the responsiveness of children to immunisation with tetanus toxoid was significantly reduced and suggested that there was a "possibility that malaria may interfere with the antibody

response to specific vaccines". These observations were confirmed and extended by Greenwood, Bradley-Moore, Palit and Bryceson[11], who showed that, in human malaria, immunosuppression was not a "blanket" phenomena since certain cellular immune responses, e.g., the response to the skin sensitising agent 2, 4 dinitrochlorbenzene, remained normal. The inter-relationship of malaria and other disease states has also been investigated to some extent and Greenwood[12] has suggested that the conspicuous absence of auto-immune disease in indigenous Nigerians is related to the occurrence of malaria. Some support for this theory has been obtained from experiments in which NZB mice, infected with malaria, failed to develop the auto-immune disease characteristic of this species[13]. Similarly the close geographical association of endemic malaria and Burkitt's lymphoma has led to the proposal that the immunosuppressive effect of the former predisposes to this neoplasia[14].

In view of these findings and the contemporaneous upsurge of interest in immunology, various workers have been prompted to investigate immunosuppression in laboratory rodents infected with malaria with a view to defining the nature of the immunological defect. The results of such work indicate that mice infected with the non-pathogenic Plasmodium berghei yoellii show a marked reduction in their antibody response to injections of heterologous erythrocytes[15,16]. A more extensive investigation into the mechanisms of immunosuppression was conducted by Greenwood, Playfair and Torrigiani[17]. By studying the humoral and cellular responses of malaria-infected mice to a variety of antigens they have suggested, on somewhat tenuous grounds, that

the immunological defect may be due to a disturbance of macrophage function. Further investigation into the role of the mononuclear phagocytic system of infected mice[18] did not however show any evidence of abnormality in this cell population, but did indicate that there may be a defect in the transport of antigen-antibody complexes by lymphocytes into the germinal centres of the spleens of infected mice. However, the macrophage was again incriminated as being the defective component in the immune response by Loose, Cook and DiLuzio[19] who concluded that there was a failure of "macrophage antigen processing" in mice infected with P. berghei yoellii based on the inability of macrophages from malaria-infected mice to transfer antigenic information to healthy recipient mice. It can be seen from such reports that the nature of immunosuppression is still poorly understood and that opinions as to the underlying cause vary considerably.

The earliest experimental evidence that immunosuppression was a feature of trypanosomiasis was reported by Goodwin in 1970[20] and Goodwin, Green, Guy and Voller in 1972[21]. Sheep erythrocytes were used as an antigen in these studies in Trypanosoma brucei infected rabbits and mice and it was found that after infection the haemagglutinin response was significantly reduced. Subsequently, Allt, Evans, Evans and Targett[22] have shown that rabbits infected with T. brucei failed to develop experimental allergic neuritis. Since then, investigations have been directed at characterising the immunological defect induced by trypanosome infections by using a broadly similar approach to those conducted in malaria.

The experiments reported here describe the responses of T. brucei infected mice to certain selected antigens and the analysis of these results provides the basis for discussions on the mechanisms of immunosuppression. The effects of trypanosomiasis on intercurrent disease is also one of considerable importance and in this connection some aspects of the inter-relationship between T. brucei infection and the nematode Nippostrongylus brasiliensis are reported and the findings discussed with regard to their possible extrapolation to the field situation. Finally the results of a conjoint experiment on immunosuppression in T. congolense infected Zebu cattle are presented in the appendix; these emphasise the possible importance of immunosuppression in areas of endemic trypanosomiasis.

SECTION 1.

THE ROLE OF THE MONONUCLEAR-PHAGOCYTIC SYSTEM IN IMMUNOSUPPRESSION

INTRODUCTION

Associated with the development of immunosuppression in experimental infections of rodents is the appearance of enlarged and reactive lymphoid organs. This is due largely to a marked cellular response in these tissues involving expansion of both the mononuclear phagocytic system, as defined by Van Furth, Cohn, Hirsch, Humphrey, Spector and Langevoort[23], and the plasma cell series (Chapter 1).

The results reported in this section are therefore concerned with possible alterations in the functional integrity of the expanded mononuclear phagocytic system of trypanosome-infected mice and their possible significance in the aetiology of immunosuppression. The role of other cellular components of the immune response is discussed in Section II of this chapter.

MATERIALS AND METHODS

The mice used, the infection procedures and the chemotherapy regimen were as described in General Materials and Methods.

Sheep Erythrocytes

Sheep red blood cells (SRBC) in Alsevers solution (Wellcome Reagents Ltd., Beckenham, Kent) were washed 3 times in phosphate buffered saline pH 7.3 (PBS)before use in the cell transfer experiments. Fresh SRBC were used in the experiment where isotopic labelling with radioactive sodium chromate (^{51}Cr) was required. Mice were immunised by the intraperitoneal injection of 5×10^8 SRBC in 1 ml PBS.

Haemagglutination

Six days after the SRBC immunisation procedure, the mice were exsanguinated by cardiac puncture. The sera were inactivated by

heating at 56°C for 30 minutes. Duplicate doubling dilutions of the antisera were prepared in 25 µl PBS in mocrotitre plates (Biocult Laboratories Ltd., Paisley, Scotland). To each well was added 25 µl of a 2% suspension of washed SRBC. The plates were covered and left overnight at room temperature before the haemagglutination titres were read. The antibody titres were read as being the highest dilution of antiserum giving complete agglutination.

^{51}Cr Labelling

Fresh sheep blood, containing heparin as anticoagulant, was incubated ^{51}Cr sodium chromate for 30 minutes at 37°C. This blood was then washed 3 times in PBS and the SRBC concentration adjusted after counting on a Coulter Counter (Coulter Electronics Ltd., Dunstable, Bedfordshire). 0.5 ml of the labelled cell suspension was injected intravenously. In the experiments where different doses of SRBC were required, unlabelled SRBC were added to the stock suspension containing the lowest numbers of ^{51}Cr SRBC; in this way the concentration was increased without any change in the total injected activity or the specific activity of each labelled cell.

Reticulo-endothelial Blockade

Carbon particles (C11/1431A Gunther Wagner, Pelikan Werke, Hannover) were suspended in a 1% gelatin solution in distilled water and mice were blockaded by a single intravenous injection of this suspension at a dose of 16 mg carbon per 100 g bodyweight[24] 6 hours before the injection of labelled SRBC.

Clearance and Organ Distribution of SRBC

Heparinised samples of blood were taken immediately before necropsy by cardiac puncture and centrifuged in microhaematocrit

tubes. That portion of each tube containing the packed red cells was then weighed prior to and after expulsion of the contents into a counting vial containing 10 ml diluted NaOH. All such samples were counted on an automatic well-type gamma scintillation counter (Nuclear Chicago, High Wycombe, Bucks.) for a sufficient time to give a net count with less than 2% standard error. All counts were expressed as counts/min/mg packed red cells.

Each mouse was separated into liver, spleen and the remainder of the carcass and these also were assayed for ^{51}Cr activity.

Macrophage Collection

Large numbers of macrophages were induced by the intraperitoneal (i.p.) injection of sterile thioglycollate medium[25] (Difco Laboratories, West Molesey, Surrey) 6 days prior to collection. 5×10^8 washed SRBC in 1 ml PBS were injected i.p. into the macrophage donor mice. Two hours later the peritoneal exudate (PE) cells were harvested by peritoneal lavage with 3 ml Medium 199 (Wellcome Reagents Ltd., Beckenham, Kent) containing 5 units/ml heparin, 10% foetal calf serum and 2% Hepes buffer under sterile conditions. The PE cells were kept on ice at all times and cell counts were carried out in a haemocytometer using 2% acetic acid with crystal violet as diluent. After the PE cells had been concentrated by centrifugation at 300 g for 5 minutes at $4^{\circ}C$, the extracellular SRBC were lysed by the method of Morita and Perkins[26] and the PE cells then washed in chilled Medium 199.

Macrophage Culture

1.0×10^7 PE cells in 4 ml medium were cultured in 65 mm tissue culture dishes for 40 minutes at $37^{\circ}C$ after which the non-adherent cells were removed by shaking the culture dish and discarding the medium. The adherent cells were washed once in

Medium 199 before being carefully scraped from the dish with a rubber-tipped spatula. The viability of the adherent cells was confirmed by their failure to stain with 0.5% trypan blue; their phagocytic ability as assessed by the ingestion of 0.1% neutral red was 85%

Macrophage Transfer

1.0×10^7 adherent cells were transferred to each recipient by i.p. injection in 1 ml Medium 199. Those mice receiving cells from T. brucei infected donors were treated 1 day before the transfer was effected by the i.p. injection of diminazene aceturate (see General Materials and Methods).

Histology

For histopathological examination, a group of mice were inoculated with T. brucei and killed in pairs at weekly intervals up to 10 weeks. Uninfected control mice were also killed at these intervals. Samples from all tissues and organs were taken at necropsy and processed according to the methods described in Chapter 1.

RESULTS

Onset and Course of Immunosuppression to SRBC

The results presented in Table 1 show that the immunosuppressive effect was present within 1 week of infection and persisted throughout the course of the disease. However, when SRBC were given 2 or 4 days before the T. brucei infection a normal haemagglutinin response was obtained.

Changes in the Mononuclear Phagocytic System (MPS)

Within a few days of inoculation and persisting throughout the infection there was a marked increase in numbers and activity of the cells of the MPS of the liver, lymph nodes, spleen, bone marrow and

Table 1 Suppression of the immune response to SRBC in mice infected with T. brucei (TREU 667) as shown by haemagglutinating titres of sera 6 days after immunisation with 5×10^8 SRBC.

Days between infection & immunisation	Haemagglutinin titres*	
	Infected	Normal
-4	7.4 ± 0.5	7.2 ± 0.6
-2	7.3 ± 0.5	-
+3	6.3 ± 0.5	8.5 ± 0.3
+7	Neg.	8.2 ± 0.4
+21	Neg.	7.6 ± 0.2
+70	Neg.	6.6 ± 0.2

* Each value is the mean $\log_2 \pm$ S.E. of 5 mice. The sera of infected and normal mice, not immunised with SRBC, were negative.

also of non-fixed macrophages of all tissues. In the liver, the Kuppfer cells were prominent, increased in number and were often found in mitosis. In addition, non-fixed macrophages and monocytes were common in the blood vessels and tissues of the liver. Lymph node sinuses were packed with macrophages (Fig. 1) and these cells were also prominent in the medullary cords and the paracortical area; in the spleen, macrophages were numerous. In infected mice, spleens were grossly enlarged and in some cases weighed as much as 30 times normal (Fig. 2). This was due not only to engorgement with erythrocytes but also to a marked increase in nucleated cells including macrophages; these cells were most commonly found in the sinuses and cords of the red pulp and in the marginal zone surrounding the white pulp. Macrophages were also numerous in bone marrow and throughout the organs and tissues of the body especially where trypanosomes were located. In all such areas macrophages had an active appearance with abundant vacuolated cytoplasm which often contained debris; many contained chromatin-like particles, possibly ingested trypanosomes, while in the spleen they were frequently packed with erythrocytes (Fig. 3).

Clearance of ^{51}Cr SRBC from the Circulation

SRBC are removed rapidly from the circulation of the trypanosome-infected mice (Table 2) indicating an expanded MPS and possibly an enhanced ability for erythrophagocytosis.

The Hepatic and Splenic Distribution of ^{51}Cr SRBC

Table 3 shows the distribution of radio-activity in the livers and spleens of infected and normal mice 24 hours after the intravenous injection of ^{51}Cr SRBC. From this it is apparent that the rapid removal of red cells from the circulation of the former

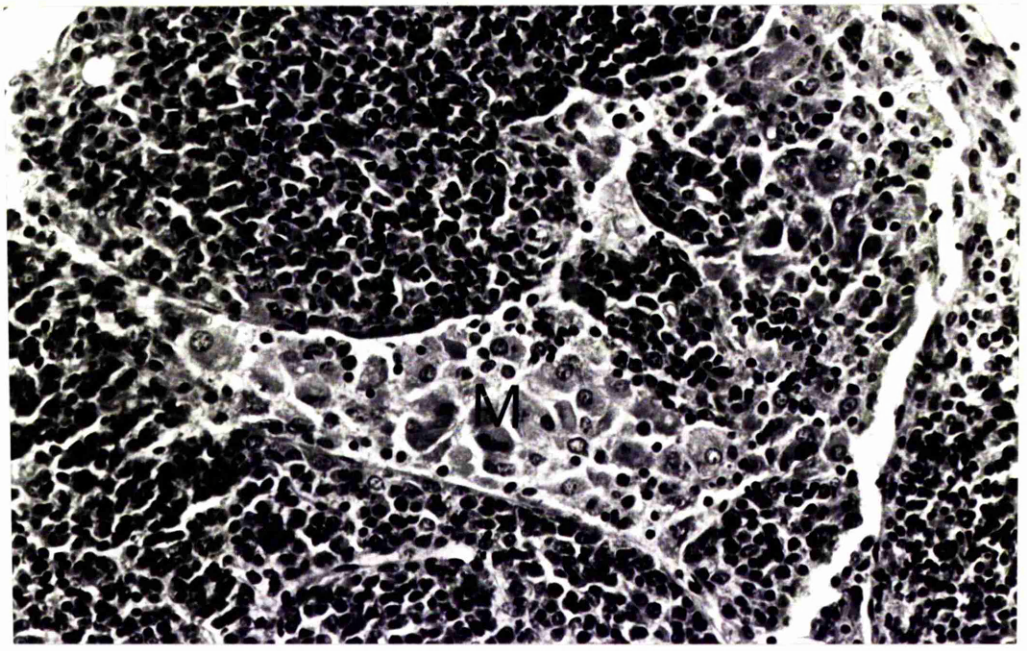

Figure 1. Lymph node medulla in mouse 21 days after inoculation with T. brucei. The medullary sinus (M) and medullary cord contain numerous macrophages. Note the plasma cells in the medullary cords. Haematoxylin and Eosin x 300.

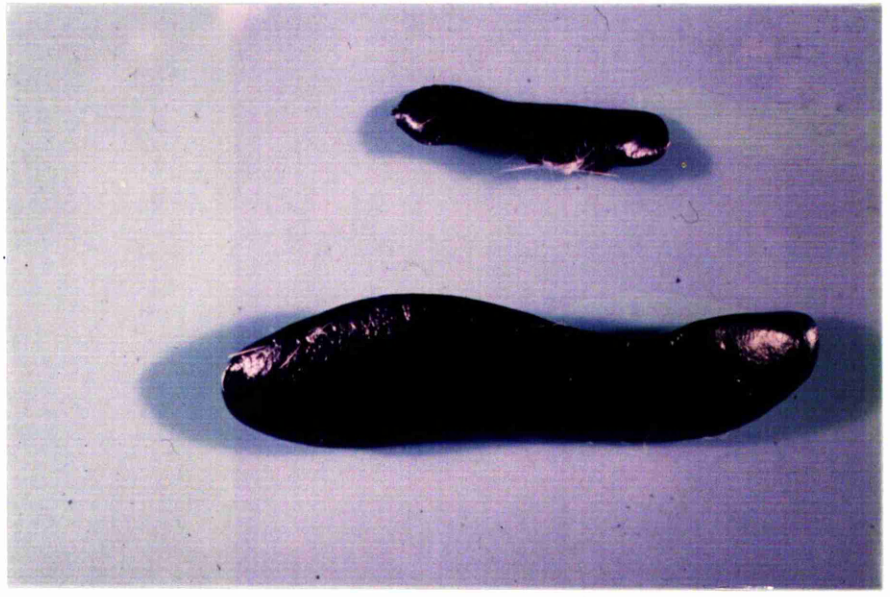

Figure 2. Spleen of a mouse infected for 28 days with T. brucei (below) and spleen of a normal mouse (x3). The spleens of infected mice can weigh up to 30 times normal.

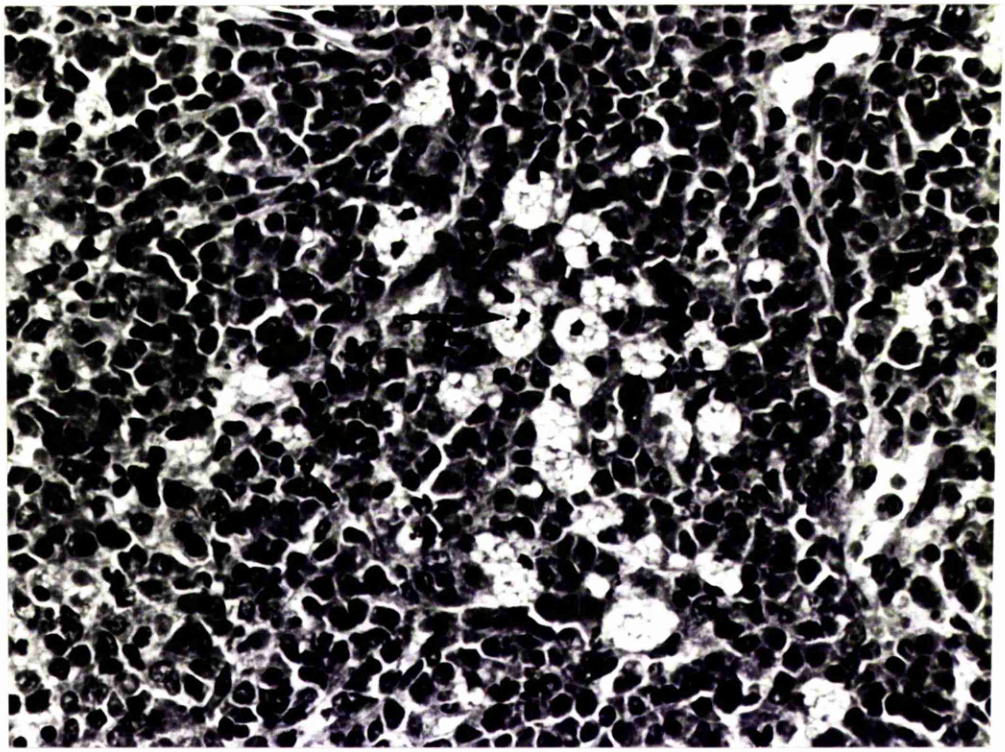

Figure 3. Splenic red pulp in mouse 28 days after inoculation with T. brucei. Macrophages are packed with red blood cells (arrow) and are surrounded by numerous plasma cells. Haematoxylin and Eosin x 350.

Table 2 The activity remaining in the circulation of mice 1 hour after the injection of ^{51}Cr SRBC.

No. of injected SRBC x 10^9	Activity (cpm/mg erythrocytes)*	
	Infected	Normal
5.0	1108 $\pm$ 262	2069 $\pm$ 162
2.0	330 $\pm$ 139	733 $\pm$ 131
0.9	42 $\pm$ 39	275 $\pm$ 34

* Each value is the AM $\pm$ SE of 8-10 mice.

Table 3 The activity in the livers and spleens of mice 24 hours after the intravenous injection of ^{51}Cr SRBC.*

No. of injected SRBC	Infected		Normal	
	Spleen	Liver	Spleen	Liver
2×10^9	15.8 ± 2.2	62.8 ± 8.9	18.9 ± 2.2	16.1 ± 0.7
2×10^8	3.0 ± 0.8	91.3 ± 1.5	4.8 ± 1.2	72.0 ± 3.7
2×10^7	2.8 ± 0.7	89.1 ± 3.0	1.2 ± 0.2	90.7 ± 1.1

* Each value is the AM $\pm$ SE of 3-8 mice expressed as a percentage of the total body activity at necropsy.

appeared to be largely due to the increased phagocytic ability of the liver. In contrast, the number of SRBC which localised in the spleen was the same in both normal and infected mice.

This was somewhat surprising in view of the marked splenomegaly and associated macrophage expansion and raised the possibility that the concentration of antigen in the spleen might be so low as to fail to stimulate production of antibody. In an effort to increase the amount of SRBC deposited in the spleen a prior injection of carbon was given before the administration of ^{51}Cr SRBC. In normal mice Souhami[24] has shown that this produces a blockade of the hepatic phagocytes and a diversion of SRBC to the spleen. While this was also observed in our normal mice (Table 4) it failed to occur in the trypanosome-infected mice, the expanded phagocytic systems of their livers apparently coping readily with both types of particulate material.

Macrophage Transfer Experiments

In addition to phagocytic ability it is likely that the presentation of processed antigen in an immunogenic configuration on the macrophage plasma membrane is a vital component for macrophage participation in the immune response[27]. To study this aspect, macrophage transfer experiments were performed as described below. Macrophages, allowed to ingest SRBC *in vivo*, were transferred by intraperitoneal injection to syngeneic recipient mice. Both infected and normal mice were used as macrophage donors and recipients. Six days after transfer, sera of these receipients was assayed for haemagglutinating antibody to SRBC. The results are shown in Table 5 and demonstrate that while macrophages containing SRBC from both normal and infected mice initiated an immune response when injected into normal mice, they failed to elicit a significant response in the presence of *T. brucei* infection in the recipients.

Table 4 The effect of prior carbon blockade on the activity in the livers and spleens of mice 2 and 24 hours after the injection of 2×10^8 ^{51}Cr SRBC.*

		Infected		Normal	
		No carbon	With carbon	No carbon	With carbon
2 hours	Spleen	2.3 ± 0.6	3.9 ± 0.6	3.0 ± 0.8	18.7 ± 3.3
	Liver	88.6 ± 1.9	88.9 ± 1.2	85.7 ± 1.5	59.9 ± 4.3
24 hours	Spleen	3.0 ± 0.8	1.7 ± 0.4	4.8 ± 1.2	24.7 ± 2.3
	Liver	91.3 ± 1.5	93.7 ± 1.0	72.0 ± 3.7	46.8 ± 3.6

* Each value is the AM $\pm$ SE of 3-8 mice expressed as a percentage of the total body activity.

Table 5 The immunogenicity of SRBC ingested by macrophages and transferred to syngeneic recipients.

Presentation of SRBC	Recipient Mice		Mean Log_2 Agglutination titre $\pm$ SE	Significance
In macrophages from normal mice	Normal	(6)*	3.3 ± 0.3	$P < 0.001$
	Infected	(5)	0.6 ± 0.4	
In macrophages from infected mice	Normal	(4)	3.3 ± 0.5	$P < 0.05$
	Infected	(3)	1.3 ± 0.3	
In P.B.S.	Normal	(6)	5.7 ± 0.9	$P < 0.001$
	Infected	(5)	1.0 ± 0.3	
Nil	Normal	(7)	Neg.	
	Infected	(5)	0.6 ± 0.4	

* Values in parentheses are the number of mice in each group.

DISCUSSION

The results reported in the first part of this section confirm that T. brucei infection of rodents is associated with a profound degree of immunosuppression to SRBC which becomes apparent between days 3 and 7 after infection and persists for up to 10 weeks at least. Similar results were obtained using a plaque-forming cell technique where, even as late as 8 days after SRBC immunisation, the spleen cells of infected mice showed no evidence of specific antibody production (see Section II). Thus the phenomenon appears to be one of immunosuppression rather than merely the delayed appearance of circulating antibody.

Since a defect in the macrophages may be responsible for a failure of the induction phase of the immune response, the second part of the section was therefore concerned with an examination of the functional integrity of the mononuclear phagocytic system in infected mice as determined by 3 parameters. First a histopathological appreciation of macrophage numbers and morphology in the organs of infected mice; secondly, the quantitative studies of their ability to remove injected SRBC from the circulation and their subsequent distribution in the liver and spleen; finally the ability of these cells to "process and transfer" antigenic information to the antibody-forming apparatus.

Histological examination showed a marked expansion of the MPS of the liver, spleen and bone marrow. Not only were the cells increased in number but also their morphological appearance indicated that they were active with abundant vacuolated cytoplasm often containing cellular debris. The cause of the expanded MPS is unknown but it may be that a mechanism similar to that proposed for tropical splenomegaly syndrome in man is operative. In this it has been

postulated that the expanded MPS particularly in the liver and spleen is produced by high levels of circulating macromolecular immune complexes[28].

In view of the vastly expanded MPS, it is perhaps not surprising that injected SRBC were removed very rapidly from the circulation. Thus, one hour after injection, 2 to 6 times more ^{51}Cr SRBC had disappeared from the circulation of infected, compared to uninfected, mice. This rapid clearance of SRBC raises the question of whether any of the immunosuppressive effects are attributable to the expanded MPS per se. Warr and Sljivic[29,30] have shown that stimulation of the reticulo-endothelial system by stilboestrol leads to an increased hepatic uptake of intravenously injected particulate materials such as SRBC, apparently the result of specific activation of the Kuppfer cells in the liver. This is associated with reduction in the splenic localisation of SRBC and a consequently diminished antibody response.

Our attempts to investigate the possibility that the expanded hepatic phagocytic system in trypanosome-infected mice is responsible for immunosuppression, have yielded some equivocal results. Thus, the percentage uptake of ^{51}Cr SRBC in the spleen and liver 2 hours after injection was 3% and 86% respectively in normal mice and 2% and 89% in infected mice. This indicated that a similar quantity of antigen was localised in the spleens in both cases. However, in view of the vastly increased splenic size in infected mice, i.e., up to 30 times normal, it is possible that the concentration of antigen may have been insufficient to initiate the production of antibody.

In an attempt to increase the quantity of antigen reaching the spleen, colloidal carbon was given intravenously to mice prior to the injection of ^{51}Cr SRBC. Souhami[24] has shown that this technique results in the reticulo-endothelial blockade of hepatic phagocytes and a consequent increase in splenic uptake of antigen. While this

occurred in our control mice (the splenic uptake of ^{51}Cr SRBC increased from 3 to 19% of the injected dose), the trypanosome-infected mice showed virtually the same relative distribution of ^{51}Cr SRBC both with and without the injection of carbon. It would thus appear that the hepatic phagocytic system in mice infected with trypanosomes was so expanded or activated that it readily coped with both carbon and red cells. This failure to obtain even a relative increase in splenic concentration of SRBC might be due to the fact that splenic engorgement leads to some degree of haemostasis and a consequently slow passage of the antigen through the spleen. Thus, over a given period of time it is likely that the liver would retain a disproportionate amount of antigen. Whatever the reason, the proportion of SRBC retained by the spleen in trypanosome-infected mice was nevertheless similar to that of normal mice and was associated with complete failure of antibody production in the former. Clearly the possibility that expansion of the MPS in mice with trypanosomiasis is responsible for the immunosuppression requires further investigation.

Apart from the rate of uptake and final distribution of injected antigen in trypanosome-infected mice, one other aspect of macrophage function was examined, i.e., the ability of the macrophage to co-operate with other cell types in the immune response by the suitable processing and presentation of SRBC antigens. This particular role was examined by the transfer of peritoneal macrophages containing SRBC from trypanosome-infected mice to the peritoneal cavities of normal mice. The results show that such macrophages were perfectly capable of initiating a significant antibody response. In contrast, when the same number of normal macrophages containing SRBC were transferred to trypanosome-infected mice, no antibody was produced. From this experiment it is apparent that the intrinsic

immunogenic potential of the macrophage in infected mice is unimpaired.

In conclusion, the only evidence presented here which might indicate that the MPS is at least a contributory factor in the development of the immunosuppressive phenomena associated with trypanosomiasis was the finding that the amount of injected SRBC which localised in the spleen was not increased despite an increased rate of removal of SRBC from the circulation and gross splenomegaly. Even a degree of hepatic blockade, normally sufficient to produce increased splenic localisation of subsequently injected SRBC, failed to increase uptake by this organ in infected mice.

It appears possible therefore that these two factors, i.e., increased hepatic uptake of particulate antigen and a possible selective failure of splenic uptake, might be responsible for a reduction in the concentration of antigen in the tissues of an enlarged spleen below the level necessary to initiate the formation of antibody. It is likely however that other factors, as well as the macrophage, are associated with immunosuppression in trypanosomiasis and some aspects of these are discussed in the next section.

SECTION II

THE ROLE OF THE T AND B LYMPHOCYTES IN IMMUNOSUPPRESSION

INTRODUCTION

The results presented in Section I of this chapter, together with the findings of other workers[20,21] clearly establish that Trypanosoma brucei infections in laboratory rodents have an immunosuppressive effect on their response to sheep red blood cells. Sufficient evidence is not available to indicate the basis of this immunosuppression although it is apparent that the macrophages may have a contributory role at least. In this section the results of a series of experiments are reported in which some aspects of the immunological competence of thymus-derived lymphocytes (T cells) and thymus-independent lymphocytes (B cells) were examined in mice infected with T. brucei to determine their possible contribution to the immune defect.

MATERIALS AND METHODS

The mice used, the infection procedures and the chemotherapy regimen were as described in General Materials and Methods.

Antigens

1. Sheep red blood cells

Sheep erythrocytes (SRBC) in Alsevers solution (Wellcome Reagents Ltd., Beckenham, Kent) were washed 3 times in phosphate buffered saline pH 7.3 (PBS). After washing, these cells were counted electronically (Coulter Electronics Ltd., Dunstable, Beds.) and adjusted to the required concentration in PBS. Each mouse was given SRBC in 1 ml PBS by intraperitoneal injection.

2. Lipopolysaccharide

Lipopolysaccharide (LPS) from the bacterium Escherichia coli 0111:B4 (Difco Laboratories, Detroit, Michigan, U.S.A.) extracted by the phenol-water method[31] was diluted in PBS to the required concentration. Each mouse was given 1 µg, 10 µg or 100 µg amounts

of LPS in 0.1 ml PBS by intravenous injection.

3. Oxazolone

A. Primary Responses.

Mice were sensitised to oxazolone (4-ethoxymethylene-2 phenyl oxazolone) by the application to each side of the thorax of a 10% solution of oxazolone in absolute alcohol[32].

B. Delayed Responses.

Contact hypersensitivity was induced in mice by the technique described by De Sousa and Parrott[33]. The ears of mice were sensitised by the application of 10% oxazolone in alcohol and challenged ten days later by a further application of 1% oxazolone in olive oil.

Responses to SRBC

The immunological response to SRBC was assayed by either of two methods, i.e., haemagglutination or localised haemolysis in gel.

1. Haemagglutination

Six days after the SRBC immunisation procedure, the mice were exsanguinated by cardiac puncture. Haemagglutinating antibody in the sera of such mice was estimated by the technique described in Section I of this chapter.

2. Localised haemolysis in gel

The production of 19s and 7s antibody to SRBC by spleen cells was assessed by the Jerne plaque technique[34] as modified by Dresser and Wortis[35] at various times after immunisation with SRBC. Dutton's balanced saline (DBS)[36] was the buffer used in all manipulations and agarose (L'Industrie Biologique, Gennevilliers, France), was dissolved and autoclaved simultaneously by boiling for 30 minutes in a container in a domestic pressure cooker. Single cell suspensions of whole uninfected mouse spleens were obtained in a loose fitting

ground glass homogeniser. Because infected mice show marked splenomegaly, their spleens weighing up to 30 times normal, only a portion (0.1-0.15 g) was suspended for use in the assay. Between manipulations, all spleen cell suspensions were kept in an iced water bath. 0.1 ml of a known number of these cells were added together with 0.1 ml of a 20% suspension of washed SRBC to a tube containing 2 ml 0.6% agarose in DBS at 46°C. This was then mixed carefully before being added to a 90 mm petri dish which contained 5 ml of 1.2% solidified agarose in DBS as a clear non-toxic base layer. Duplicate plates were produced in this manner from each lymphocyte suspension. These plates were then incubated for 2 hours in a humidified atmosphere at 37°C before the addition of complement. Fresh guinea pig serum, absorbed with washed SRBC for 25 minutes at 4°C (1 ml packed SRBC + 5 ml guinea pig serum) was used as a source of complement and 1 ml of a 10% solution of this serum in DBS was added to each plate. The plates were incubated for a further 45 minutes at 37°C before the addition of a few drops of 2% formalin as preservative. Spleen cells producing anti-SRBC IgM caused a clear circular zone of haemolysis of the SRBC in the surrounding agarose medium. Each area corresponded to one direct plaque forming cell (IgM PFC). Great care was taken to keep the surface of the agarose perfectly level while pouring the plates since a 10% sampling disc was used to facilitate counting when the numbers of PFC exceeded 300 per plate. Plaques were counted using darkground illumination. While it is generally accepted that IgM molecules are extremely efficient at fixing complement and therefore lysing SRBC, antibodies of other classes are much less so, unless complexed with another antiglobulin[37]. Indirect plaque forming cells were therefore obtained by using a developing serum (rabbit anti-mouse immunoglobulin, RAM Ig) to enhance the production of IgG PFC. Such serum also has a

tendency to inhibit IgM PFC and must therefore be used in an appropriate dilution in the top agarose layer containing the spleen cells and target SRBC to cause minimal inhibition of IgM PFC. Titrations of the RAM 1g in this system were conducted to elucidate its inhibitory potential and from these (Fig. 4) a satisfactory dilution of RAM 1g was obtained which would cause no inhibition i.e., the inhibition constant, KI = 1 at this dilution. Similarly the development potential was investigated by further titration (Fig. 5) and the development constant, KD, of the serum at this dilution was obtained. The techniques used for these titrations were as described in Weir's Handbook of Experimental Immunology[35]. It was found that at a 1:500 dilution of RAM 1g when KI = 1, KD = 2.9 and so the formula,

Developed PFC = (Total PFC - Direct PFC) x 2.9

was used to obtain the numbers of IgG PFC.

Responses to LPS

The serological response of mice to immunisation with LPS was assayed by the indirect haemolysis of LPS sensitised SRBC while the production of anti-LPS antibody by single cells was assayed by a modification of the technique of localised haemolysis in gel described earlier which utilised LPS sensitised SRBC.

1. Sensitisation of SRBC with LPS

SRBC were sensitised by the method of Andersson and Blomgren[38]. A volume of 3 ml PBS, pH 7.3 containing 3 mg LPS was boiled for 2 hours. Thereafter, 1 ml of packed SRBC was added and the mixture incubated for 45 minutes at 37°C. The SRBC were washed three times in PBS and diluted to a concentration of 1% before use.

2. Absorption of guinea pig serum

Fresh guinea pig serum was used as a source of complement and since it was found that this could cause a degree of haemolysis of

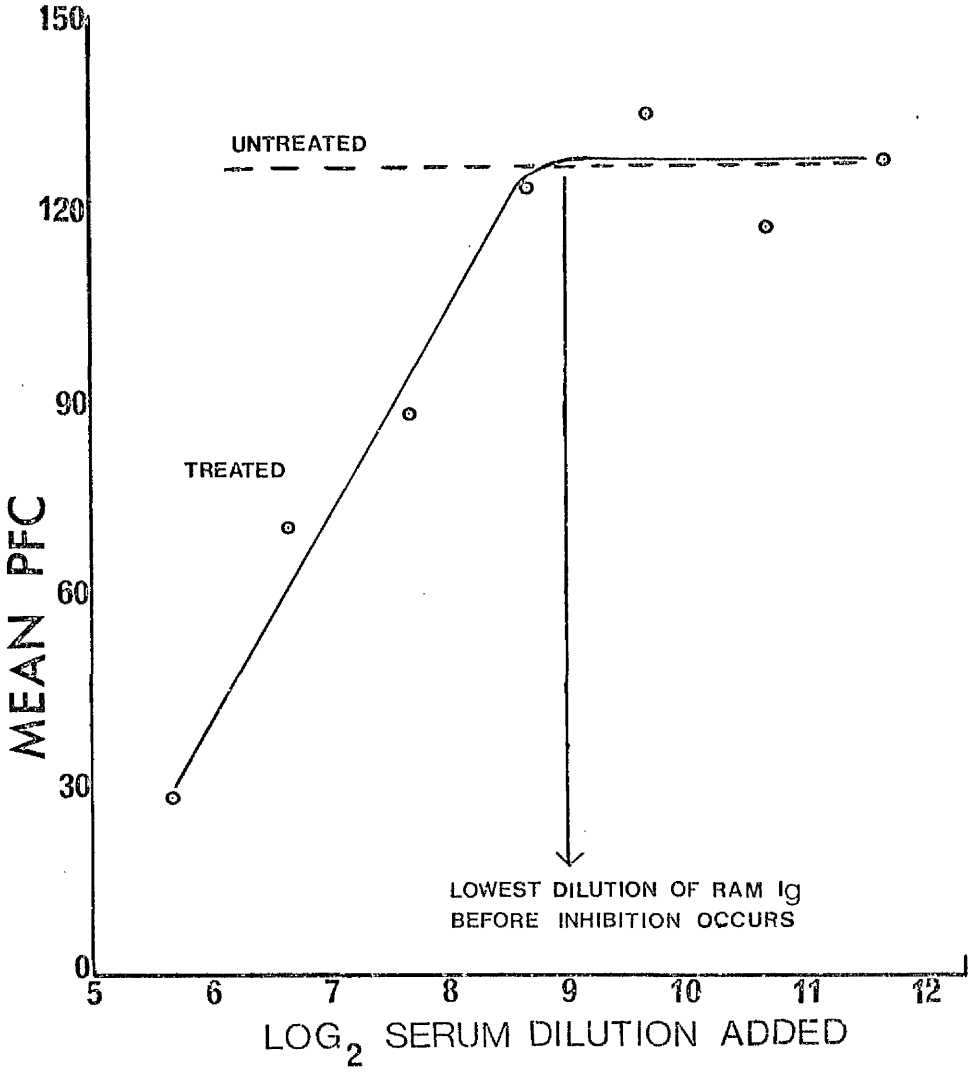

Figure 4. Plot of the inhibition of anti-SRBC PFC by rabbit anti-mouse globulin. Lymphocytes were obtained from the pooled spleen suspension of 3 mice immunised 3 days previously with 5×10^8 SRBC. Each point represents the arithmetic mean PFC from 3 plates to each of which was added 1.0×10^6 spleen cells. The untreated group represents the arithmetic mean PFC of 12 plates.

$$KI = \frac{\text{mean PFC with serum}}{\text{mean PFC without serum}}$$

$$KI \text{ at } \log_2 9 \text{ dilution (1:512)} = \frac{127}{126}$$

$$\underline{KI \ (0.002\%) = 1}$$

$$(KI \text{ at } \log_2 5.66 = 4.5)$$

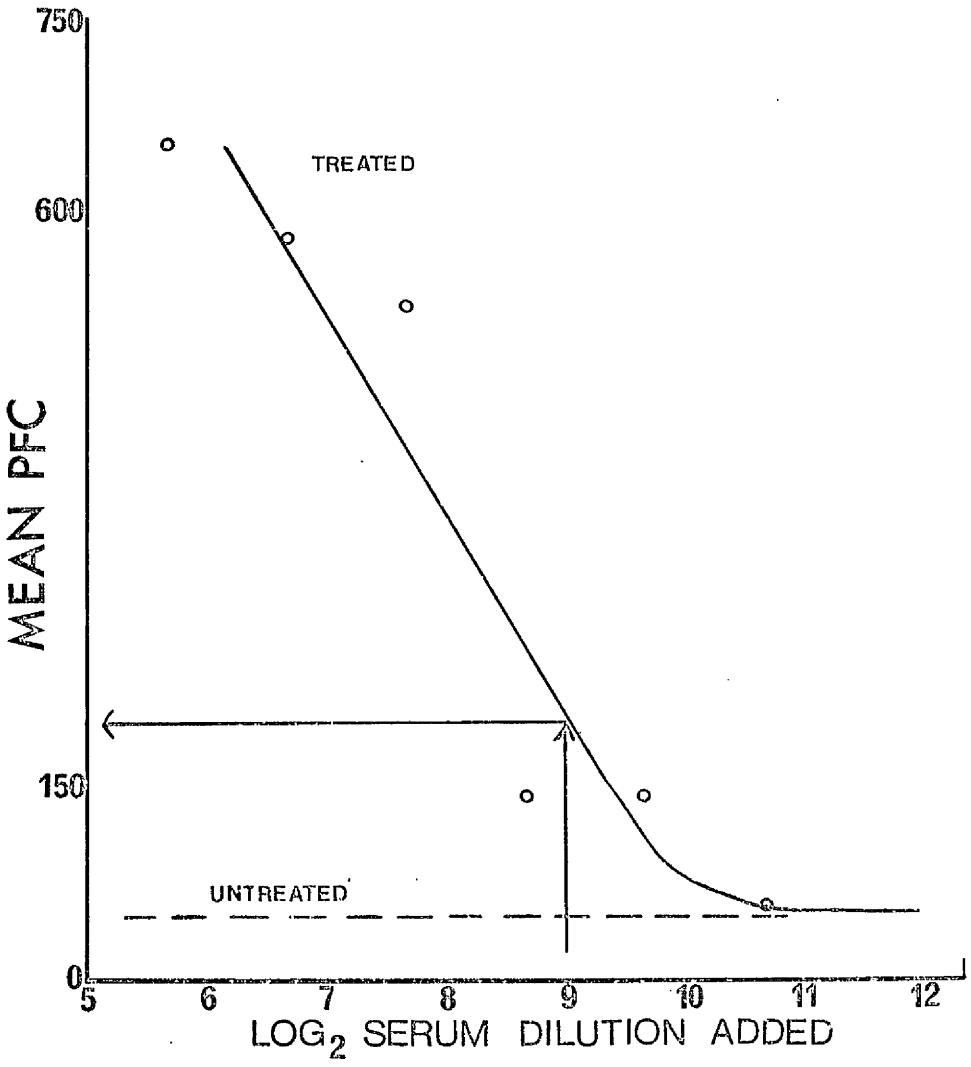

Figure 5. Plot of the development of anti-SRBC PFC by rabbit anti-mouse globulin. Lymphocytes were obtained from the pooled spleen suspension of 3 mice immunised 11 days previously with 5×10^8 SRBC. Each point represents the arithmetic mean PFC from 3 plates to each of which was added 1.0×10^6 spleen cells. The untreated group represents the arithmetic mean PFC of 12 plates.

$$\underset{(0.002\%)}{KD} = \frac{\text{Maximum PFC/plate obtained - direct PFC x KI at that conc.}}{\text{PFC at } \log_2\text{/serum dilution - direct PFC x KI at that conc.}}$$

$$= \frac{655 - (49 \times 4.5)}{195 - (49 \times 1)}$$

$$KD = \underline{\underline{2.9}}$$

the LPS sensitised SRBC on its own it was therefore absorbed before use with LPS sensitised SRBC for 30 minutes at 4°C (1 ml sensitised SRBC + 5 ml guinea pig serum).

3. Indirect haemolysis of LPS sensitised SRBC

Mice were bled by cardiac puncture and the sera inactivated by heating at 56°C for 30 minutes. Serial dilutions of this sera were prepared in 25 µl volumes PBS in microtitre plates. To each dilution was added 25 µl of a 1% suspension of sensitised erythrocytes followed by 25 µl of a 20% dilution of the absorbed guinea pig serum. After incubation for 45 minutes at 37°C the haemolytic antibody titre was read as being the highest dilution of serum giving complete haemolysis. E. coli O111:B4 antiserum (Difco Laboratories, Detroit, Michigan, U.S.A.) was used at a dilution of 1:200 as a positive control.

4. Estimation of anti-LPS PFC

The technique of localised haemolysis in gel as previously described was followed accurately except that SRBC sensitised with LPS were used as an indicator in place of normal SRBC and only direct plaques were counted.

Responses to Oxazolone

The primary response of lymph node cells to the antigen oxazolone was estimated by measuring their incorporation of an isotopically labelled amino-acid and the delayed type hypersensitivity response to oxazolone was estimated both on the basis of an increase in ear thickness following a challenge exposure to oxazolone and histological examination of these ears and their draining lymph nodes.

1. DNA synthetic response to oxazolone

Three days after oxazolone sensitisation, cell proliferation in the draining lymph nodes (axillary, brachial) was estimated by measuring the incorporation of ^{125}I iodo-deoxyuridine, ^{125}I UdR (Radiochemical Centre, Amersham, Berks.), after the technique

described by Pritchard and Micklem[32]. Mice received an intraperitoneal injection of 5 x 10^{-8} moles fluorodeoxyuridine in 0.2 ml distilled water followed after 10 minutes by 2 µCi ^{125}I UdR and the lymph nodes were excised and fixed in 10% formalin for 24 hours. The ^{125}I UdR which was not incorporated into newly formed DNA was extracted with 3 x 7 ml changes of 70% ethanol. The nodes were then assayed for activity in an automatic well-type gamma scintillation counter (Nuclear Chicago, High Wycombe, Bucks.) for a sufficient period of time to give a standard error of less than $\pm$ 2%. The ^{125}I UdR activity of the nodes was compared to an arbitrary standard of 500,000 counts per minute so that the results from different experiments were comparable.

2. Delayed-type hypersensitivity response to oxazolone

The thickness of a mouse's ears was ascertained immediately before a challenge application of oxazolone by measurement with a dial gauge micrometer and again 24 hours after challenge. Any increase in thickness was considered indicative of a cell mediated response and immediately after the challenge measurement the mice were necropsied and histological sections prepared from their ears and draining lymph nodes.

Histological Methods:

Source material and histological techniques were detailed in Materials and Methods, Section 1 of this chapter.

RESULTS

Histological Findings in the Immune System

In mice infected with T. brucei, the most significant findings at necropsy were generalised lymph node enlargement and marked splenomegaly with the spleens of some mice weighing up to 30 times

normal. Such changes developed soon after inoculation, persisted throughout the course of infection and were attributable largely to massive plasma cell hyperplasia.

In lymph nodes, within a few days, the medullary cords and juxtamedullary area became populated by large pyroninophilic lymphoid cells, many of which were found in mitosis. During the next 7 to 14 days these cells increased in number and progressively differentiated into immature and mature plasma cells. The result was that the medullary cords were thickened and tortuous, the paracortical area (thymus-dependent) was progressively replaced by large lymphoid cells and plasma cells, which were also present in the cortex surrounding lymphocytic follicles. Thus by 21 days the entire lymph node, apart from the lymphocytic follicles was occupied by large lymphoid cells, immature and mature plasma cells (Figs. 6 and 7). Throughout the infection (up to 70 days), lymphocytic follicles were found and many had active germinal centres with tingible body macrophages cupped by dividing large pyroninophilic lymphoid cells. Occasional foci of cellular lysis and necrosis occurred in the lymph nodes and these were invariably in association with tissue localisation of trypanosomes.

Similar changes occurred in the spleen where soon after inoculation the white pulp became populated by large pyroninophilic lymphoid cells which were frequently found in mitosis. These cells differentiated into immature and mature plasma cells which quickly came to occupy most of the white pulp causing it to become irregular and expand into the red pulp (Fig. 8). Thus,

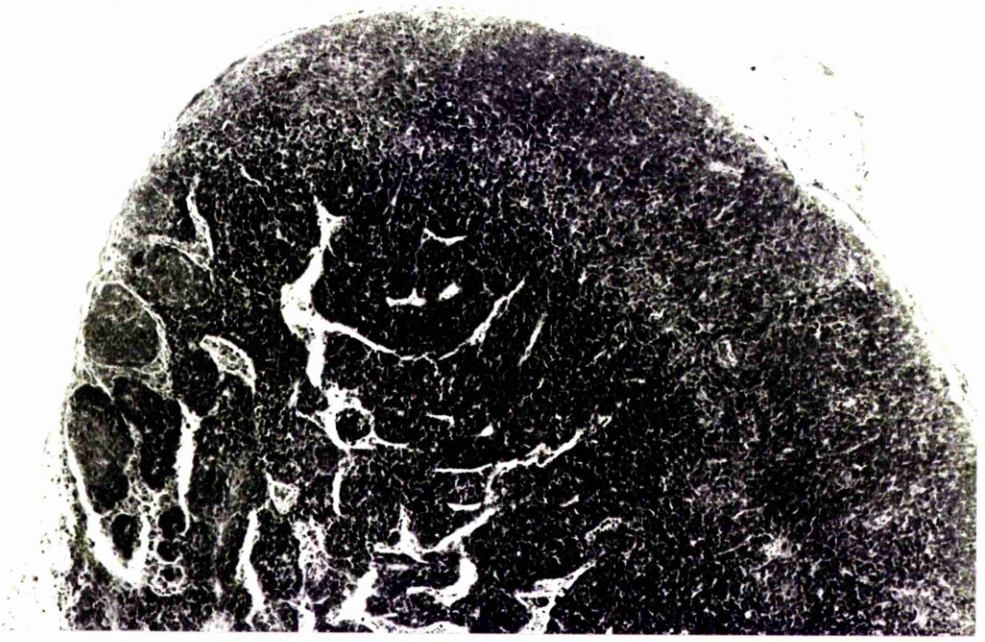

Figure 6. Lymph node of mouse infected with T. brucei for 14 days. The medullary cords are thickened by masses of pyroninophilic cells, mainly plasma cells. Note the paracortical area is becoming infiltrated by these cells.
Methyl green-pyronin x 120.

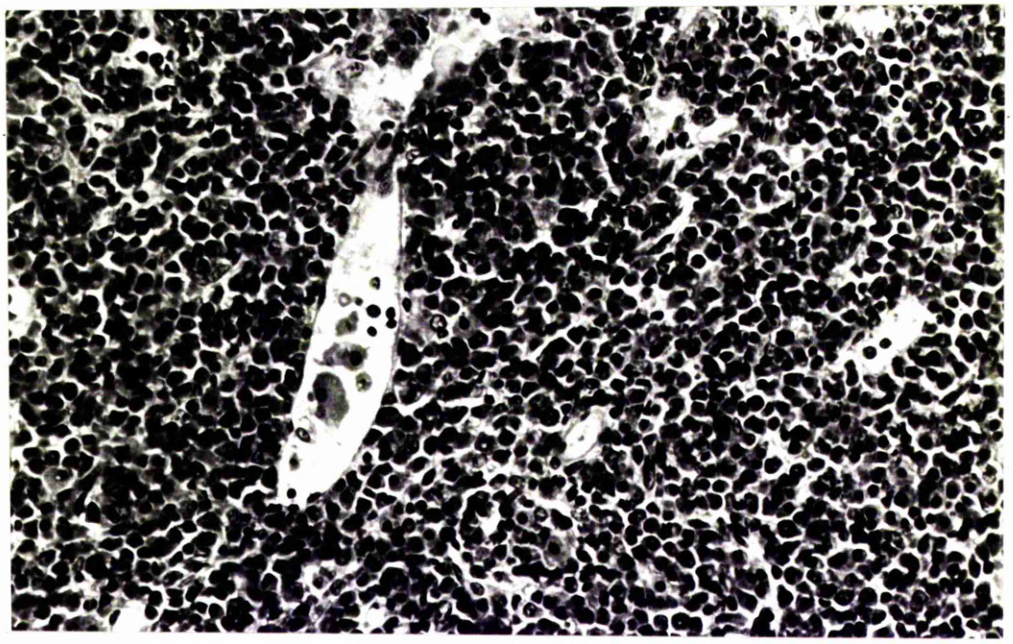

Figure 7. Lymph node medulla from same mouse showing the massive plasma cell response expanding the medullary cords.
Haematoxylin and Eosin x 300.

from the trabecular arterial sheath to the peri-arteriolar lymphocytic sheath (thymus-dependent area) the white pulp was populated by large lymphoid cells, immature and mature plasma cells and in time by Russell-body containing plasma cells. Lymphocytic follicles were present in the spleen throughout the infection and while some contained germinal centres, they usually had a disorganised appearance: at the same time in the cords of the red pulp there was a massive build up of cells of the plasma cell series. The above changes were marked by 14 to 21 days after inoculation and while they varied from week to week and between individual animals they were still extensive at 70 days.

Soon after inoculation marked changes developed in the thymus in that the cortex became narrow and irregular and sometimes the cortico-medullary junctions were difficult to delineate (Fig. 9). While such changes were morphologically dramatic, they were not progressive and at 70 days when the last mice were killed, the thymus was still present and its structure readily defined although altered as described above.

In addition to these changes in the immune system, marked cellular infiltration, sometimes associated with tissue localisation of trypanosomes, occurred in all tissues and organs of the body, in the connective tissue fascia and in the serous membranes. These infiltrates included large lymphoid cells and plasma cells as well as macrophages and often caused much structural disorganisation. For example, as the infection advanced, the cellular infiltrate encroached on the thymus via the connective tissue fascia and blood vessels of the interlobular septa and there expanded, distorting and compressing thymic tissue.

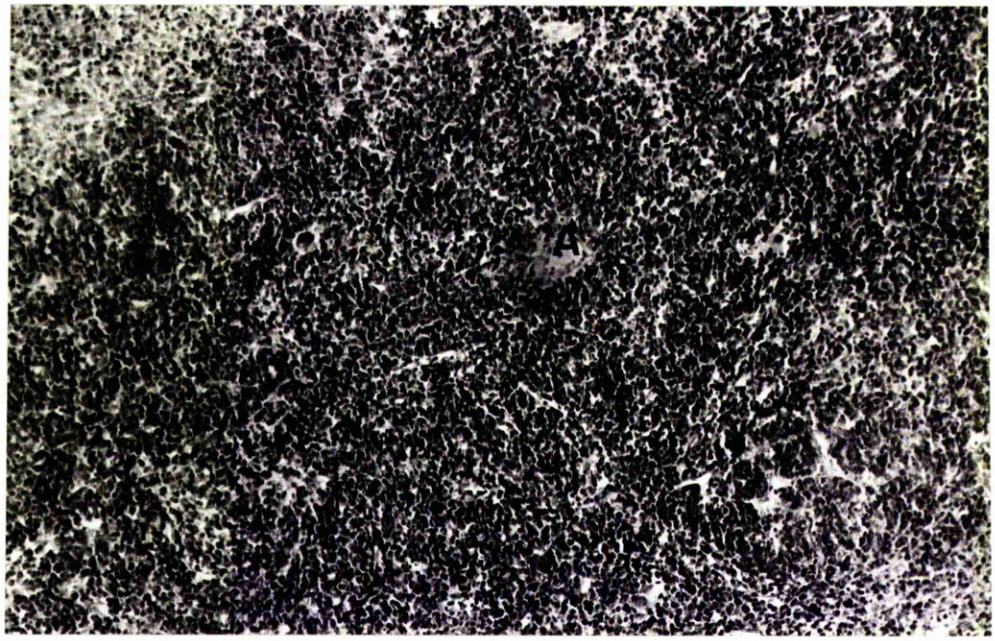

Figure 8. White pulp of mouse spleen 21 days after infection with T. brucei. The white pulp is expanded by pyroninophilic cells, mainly plasma cells.
A, central arterioie.
Methyl green-pyronin x 150.

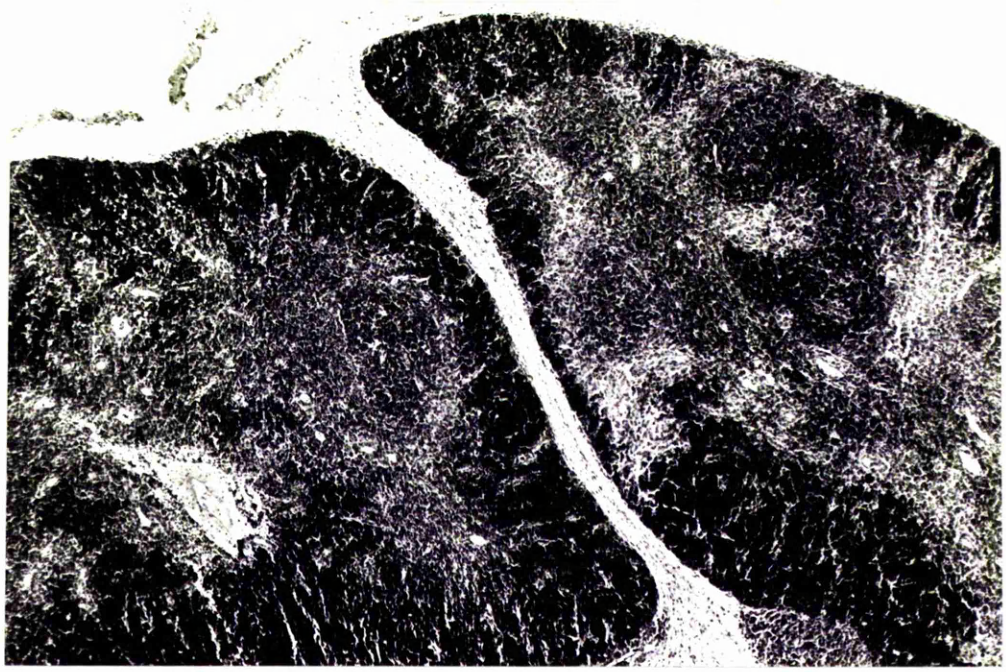

Figure 9. Thymus of mouse 21 days after infection with T. brucei. The thymic cortex is narrow and irregular while the medulla contains numerous macrophages, large lymphoid cells and plasma cells as well as small lymphocytes.
Haematoxylin and Eosin x 45.

It should also be noted, as described in the previous section, that throughout the course of infection there was a marked increase in the numbers of cells in the mononuclear phagocytic system not only in the liver, lymph nodes, spleen and bone marrow, but also in all tissues and organs of the body.

Plaque forming Cell (PFC) Responses to SRBC

Table 6 shows that 3 weeks after infection with T. brucei TREU 667, mice appeared to be unable to produce splenic PFC 3, 5 and 8 days after immunisation with 5×10^8 SRBC. There was a failure in the appearance of both direct PFC and developed PFC indicating the complete absence of anti-SRBC IgM and IgG production. The failure of infected mice to respond to SRBC was evident with a wide variety of SRBC immunising doses (Tables 7 and 8). Even at the highest dose used, i.e., 5×10^9 SRBC, neither the direct nor developed PFC responses occurred in the infected mice.

A comparison of the number of PFC found in the 2 groups of control mice, i.e., those which did not receive SRBC, showed that a non-specific haemolysin was present in the infected group. This is presumably due to the presence of cells producing heterophile antibody, a commonly encountered phenomenon in trypanosomiasis[39].

Responses to LPS

Using the immunising doses indicated in Table 9, infected mice were only able to produce insignificant antibody responses to LPS. Thus, with an immunising dose of 1 µg the infected mice did not respond at all and with 10 µg the mean response was $\log_2$ 3.1 compared to 7.1 in the uninfected mice. At the 100 µg dose level it is interesting that the response of normal mice 9 days

Table 6 The effect of T. brucei infection in mice on the PFC response to SRBC.

Days after Immunisation	PFC in Infected Mice IgM	PFC in Infected Mice IgG	PFC in Normal Mice IgM	PFC in Normal Mice IgG
3	389 (2.59 ± 0.87)	631 (2.80 ± 0.95)	17,780 (4.25 ± 0.14)	0
5	259 (2.41 ± 0.81)	7 (0.93 ± 0.93)	100,200 (5.00 ± 0.06)	7 (0.87 ± 0.87)
8	200 (2.30 ± 0.78)	295 (2.47 ± 0.26)	15,850 (4.20 ± 0.20)	38,020 (4.58 ± 0.22)
Unimmunised controls	997 (3.00 ± 0.96)	4 (0.6 ± 0.6)	3 (0.49 ± 0.48)	4 (0.55 ± 0.55)

The numbers of splenic plaque forming cells after immunisation i.p. with 5×10^8 SRBC. Each figure is the geometric mean of 4 mice. The figures in brackets represent the $\log_{10}$ GM ± SE.

Table 7 The direct PFC response of normal and T. brucei infected mice to various doses of SRBC.

Days after immunisation	Immunising Dose of SRBC 5×10^6		5×10^7		5×10^8		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal	Infected	Normal
3	468 (2.67 ± 0.91)	1380 (3.14 ± 0.17)	240 (2.38 ± 0.81)	7413 (3.87 ± 0.29)	389 (2.59 ± 0.87)	17780 (4.25 ± 0.14)	2630 (3.43 ± 0.22)	9120 (3.96 ± 0.10)
5	282 (2.45 ± 0.83)	3891 (3.60 ± 0.44)	25 (1.40 ± 0.83)	39810 (4.60 ± 0.14)	259 (2.41 ± 0.81)	100200 (5.00 ± 0.06)	646 (2.81 ± 0.94)	70790 (4.85 ± 0.22)
8	863 (2.92 ± 0.98)	1549 (3.19 ± 0.10)	32 (1.50 ± 0.87)	13800 (4.14 ± 0.17)	200 (2.30 ± 0.78)	15850 (4.20 ± 0.20)	32 (1.51 ± 0.90)	8511 (3.93 ± 0.17)

The numbers of direct splenic plaque forming cells (PFC) after immunisation with SRBC. Each figure is the geometric mean of 4 mice. The figures in brackets represent the $\log_{10}$ GM $\pm$ SE.

Table 8 The developed PFC response of normal and T. brucei infected mice to various doses of SRBC

Days after immunisation	Immunising Dose of SRBC							
	5×10^6		5×10^7		5×10^8		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal	Infected	Normal
3	692 (2.84 ± 0.96)	0 -	100 (2.00 ± 1.16)	8 (0.88 ± 0.87)	631 (2.80 ± 0.95)	0 -	36 (1.56 ± 0.9)	7 (0.86 ± 0.86)
5	55 (1.74 ± 1.01)	7 (0.82 ± 0.82)	333 (2.52 ± 0.88)	0 -	9 (0.93 ± 0.93)	7 (0.87 ± 0.87)	13 (1.12 ± 1.11)	0 -
8	15 (1.19 ± 0.69)	62 (1.79 ± 1.05)	14 (1.15 ± 0.67)	24,550 (4.39 ± 0.17)	295 (2.47 ± 0.26)	38,020 (4.58 ± 0.22)	0 -	10,720 (4.03 ± 0.14)

The numbers of developed splenic plaque forming cells (PFC) after immunisation with SRBC. Each figure is the geometric mean of 4 mice. The figures in brackets represent the $\log_{10}$ GM $\pm$ SE.

Table 9 The effect of T. brucei infection on the response of mice to lipopolysaccharide (LPS).

Days after immunisation	Immunising Dose of LPS 1 µg		10 µg		100 µg	
	Infected	Normal	Infected	Normal	Infected	Normal
3	0	0	0	2.1 ± 0.9	0	0
6	0	0	3.1 ± 1.6	7.1 ± 1.0	3.3 ± 1.9	6.9 ± 0.7
9	0	4.6 ± 1.0	0	11.4 ± 1.3	1.1 ± 1.1	5.4 ± 0.1

Each figure is the mean $\log_2 \pm$ SE indirect haemolytic antibody titre of 5 mice.

The sera of infected and normal mice, not immunised, were negative.

after immunisation was reduced (5.4 ± 0.1) in contrast to the response to 10 µg LPS (11.4 ± 1.3). Perhaps at this level more LPS had persisted in the circulation to cause a peripheral neutralisation of anti-LPS antibody in a similar manner to that which can follow pneumococcalpolysaccharide immunisation[40] and a consequent reduction in the circulating antibody level.

The results presented in Table 10 show that the absence of anti-LPS antibody in the serum of infected immunised animals is due to a failure of antibody production at the cellular level since the mean PFC response of infected mice immunised with both 10 and 100 µg was not above the immunised background level (813 and 156 as opposed to 470) while normal mice produced a marked response to these dose levels (3,443 and 13,130). Unfortunately, the significance of these results is somewhat limited by the small number of infected and immunised mice examined, a proportion succumbing to the endotoxic effect of LPS[41].

Responses to Oxazolone:

1. Contact hypersensitivity

The results, as measured by an increase in ear thickness 24 hours after challenge are shown in Table 11. It is apparent that the cell-mediated response of mice with a T. brucei infection of 3 weeks duration occurred to a significant degree but was not so marked as that of uninfected mice. Histological examination of the draining lymph node showed a marked expansion of the para-cortical thymus dependent areas.

2. DNA synthetic response to oxazolone

Normal and infected mice show a very similar response to oxazolone as measured by the synthesis of DNA after oxazolone stimulation (Fig. 10 and Table 12). However, when the ^{125}I UdR

Table 10 The response of normal and T. brucei infected mice to lipopolysaccharide (LPS) as measured by the numbers of direct PFC. The figures in brackets represent the $\log_{10}$ GM ± SE.

	Immunising Dose of LPS		
	0	10 µg	100 µg
Normal mice	0*	3,443 (3.54 ± 0.27)	13,130 (4.12 ± 0.26)
Infected mice	470* (2.67 ± 0.40)	813 (2.65 ± 0.59)	156* (1.19 ± 1.19)

* Each of these figures is based on 2 mice: otherwise each value is the geometric mean of 6 mice.

Table 11 The increase in ear thickness observed on challenge with 1% oxazolone of sensitised normal mice and of mice challenged on the 30th day of infection with T. brucei. Results expressed as the mean ear thickness ± SE 24 hours after challenge.

Group	Ear thickness	Significance
(1) Control - not sensitised (7)*	3.2 ± 0.15	
		(1) and (2) P <0.001
(2) Control - sensitised (7)	5.2 ± 0.16	
		(2) and (4) P <0.02
(3) Infected - not sensitised (15)	3.3 ± 0.08	
		(3) and (4) P <0.001
(4) Infected - sensitised (15)	4.5 ± 0.14	

*Values in parenthesis are the number of mice in each group.

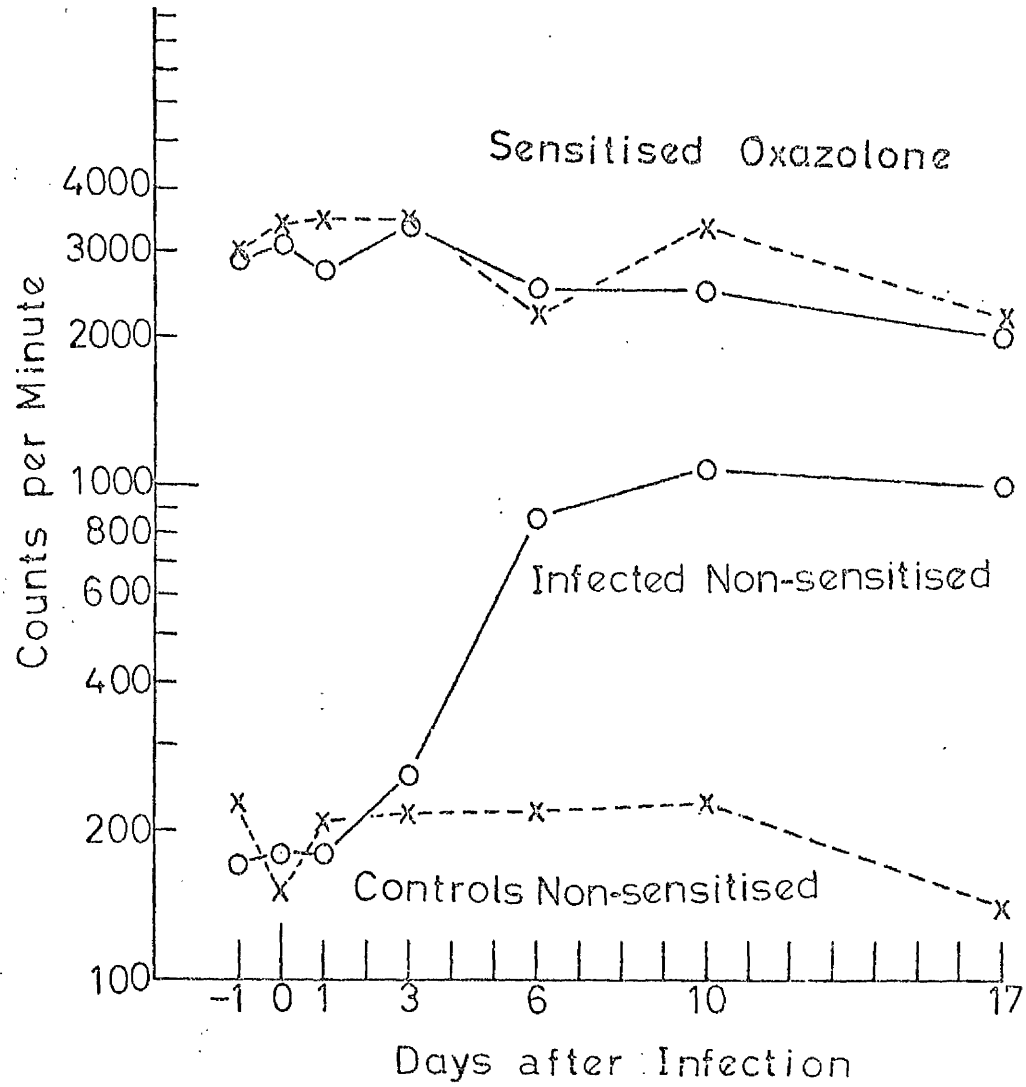

Figure 10. The incorporation of ^{125}I UdR into the regional lymph nodes of mice at intervals throughout a recent infection of T. brucei. Each point is the mean ^{125}I cpm of the nodes from 10 mice.

Table 12 The effect of T. brucei infection on the ^{125}I UdR incorporation in the regional lymph nodes of mice 3 days after sensitisation with oxazolone.*

Sensitised Oxazolone days after infection	Infected			Normal		
	Sensitised Oxazolone A	Non-Sensitised B	Ratio A/B	Sensitised Oxazolone C	Non-Sensitised D	Ratio C/D
- 1	2861 ± 116	174 ± 25	16.5	3031 ± 153	230 ± 33	13.2
0	3081 ± 219	181 ± 22	17.0	3410 ± 219	148 ± 13	23.0
+ 1	2730 ± 419	183 ± 18	14.9	3506 ± 268	214 ± 17	16.4
+ 3	3391 ± 182	263 ± 32	12.9	3473 ± 379	224 ± 22	15.5
+ 6	2532 ± 270	855 ± 137	3.0	2243 ± 174	220 ± 24	10.2
+ 10	2525 ± 204	1080 ± 193	2.3	3414 ± 237	233 ± 18	14.6
+ 17	2016 ± 178	1005 ± 118	2.0	2194 ± 240	144 ± 15	15.2
+ 24	2256 ± 473	666 ± 106	3.4	2553 ± 195	114 ± 15	22.3

*Each value is the arithmetic mean ^{125}I cpm ± SE of the nodes of 10 mice corrected to an arbitrary standard of 500,000 cpm.

incorporation of infected and sensitised mice is compared with that of infected and non-sensitised mice, it is apparent that the responsiveness of the former is, at least in part, attributable to the T. brucei infection per se and no solely to oxazolone. For example, 10 days after infection mice showed a five times greater incorporation of activity (1,000 cpm) in their lymph node than did their normal counterparts (200 cpm). This high background activity in the nodes of the infected mice presumably reduced the specific response to oxazolone stimulation to a factor of 2 (from 1,000 cpm to 2,000 cpm) since the normal and sensitised mice, showing a x10 specific increase in activity, also reached their "ceiling" at 2,000 cpm. However, despite this increased background incorporation of ^{125}I UdR in infected mice, the results indicate that the T cells of infected mice are able to mount a significant response to oxazolone.

This specific response was only found in mice infected for up to 5 weeks; mice tested 36 to 43 days after infected (Table 13) showed no significant increase in the ^{125}I UdR incorporation although a high "background" activity still persisted in the nodes in a majority of mice.

Restoration of the Immunological Response:

The restoration of immunological responsiveness in infected mice after trypanocidal therapy was investigated by using SRBC and oxazolone as antigens. In the previous section, it was shown that mice infected for 3 weeks with this strain of T. brucei failed to produce a significant antibody response when immunised with SRBC. Fig. 11 shows that treatment of such mice with diminazine aceturate rapidly restored immunological competence

Table 13 The effect of a prolonged *T. brucei* infection on the responsiveness of mice to oxazolone as measured by the incorporation of ^{125}I UdR. Each figure is the mean ^{125}I cpm $\pm$ SE of the regional nodes from 10 mice.

Days after Infection	Oxazolone Sensitised	Non-Sensitised
36	592.5 $\pm$ 120.0	790.5 $\pm$ 64.1
39	1395.8 $\pm$ 361.1	773.9 $\pm$ 129.9
43	1213.5 $\pm$ 244.5	803.4 $\pm$ 194.0

Differences between sensitised and non-sensitised groups are not significant.

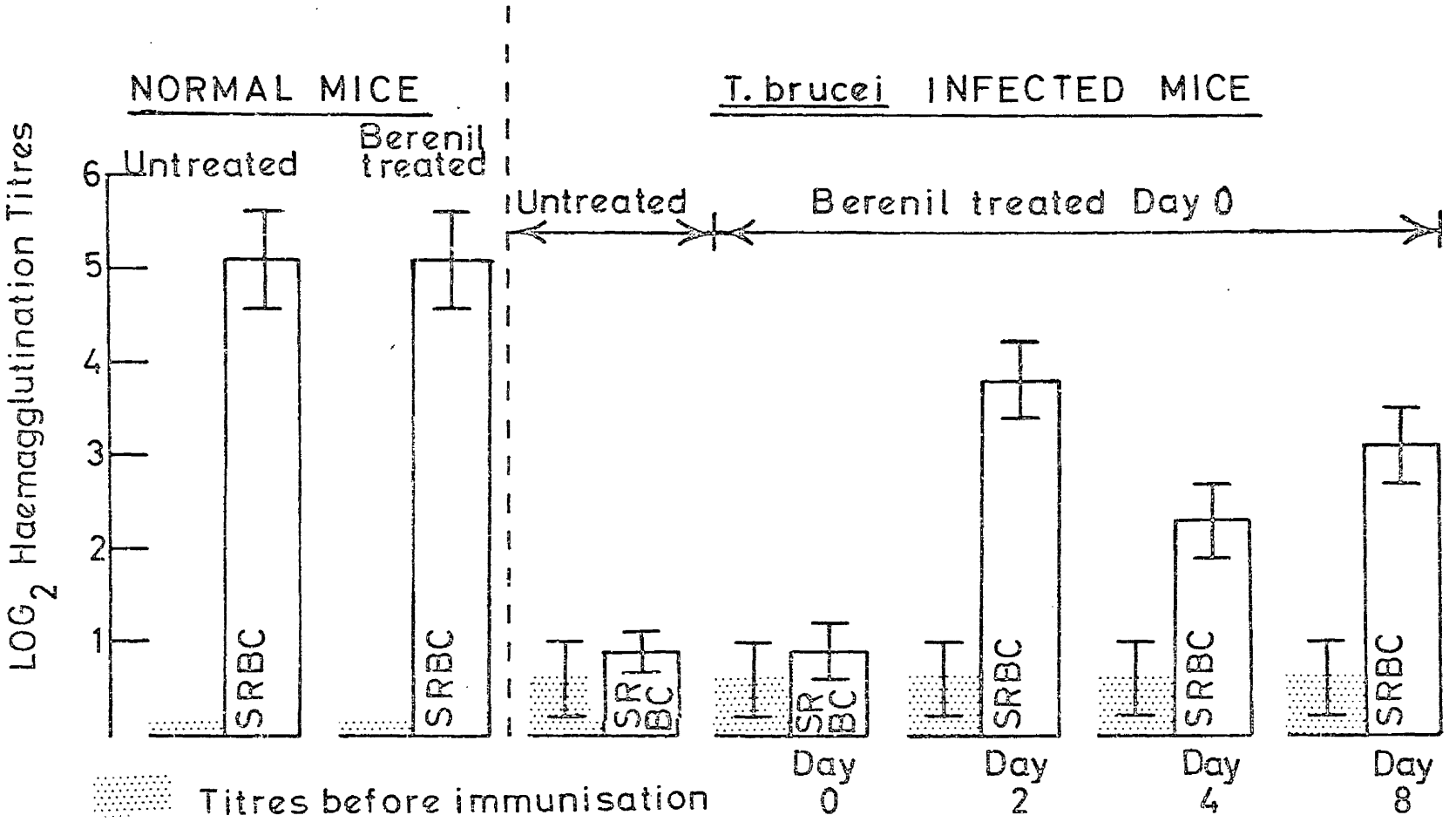

Figure 11 Restoration of the immune response of T. brucei infected mice after treatment with diminazine aceturate (berenil), as measured by their antibody response to 5×10^8 SRBC inoculated 0, 2, 4 and 8 days after treatment. Each column represents the mean haemagglutination titre $\pm$ S.E. of 10 mice.

in that SRBC immunisation as early as 2 days after treatment stimulated a significant serological response. The elevated haemagglutination titres in infected but unimmunised mice both before and after trypanocidal therapy are, as noted earlier, probably attributable to heterophile antibodies. Similarly the responsiveness to oxazolone was restored to normal soon after chemotherapy. Fig. 12 shows that treatment quickly caused a reduction to near normal levels in the "background" ^{125}I UdR incorporated in the lymph nodes of infected mice which were not stimulated with oxazolone. Thus, when the trypanocidal drug and oxazolone were administered at the same time the specific response to this sensitisation was statistically significant although the total incorporation of ^{125}I UdR was similar to that of the untreated sensitised mice. When oxazolone was applied 3 days after chemotherapy the responses had returned to normal, i.e., were similar to those of uninfected control mice.

DISCUSSION

The ability of an animal to elicit a primary immune response is normally dependent on the presence of a functional relationship between macrophages, thymus-dependent lymphocytes (T cells), and in the case of the humoral response, a population of thymus-independent lymphocytes (B cells). In section 1 of this chapter the functional integrity of the mononucelar phagocytic system of trypanosome-infected mice was examined with regard to its possible role in the immunosuppression associated with this disease. Clear evidence of macrophage dysfunction was not obtained, although there did appear to be a relative failure of splenic localisation of intravenously administered antigen (SRBC).

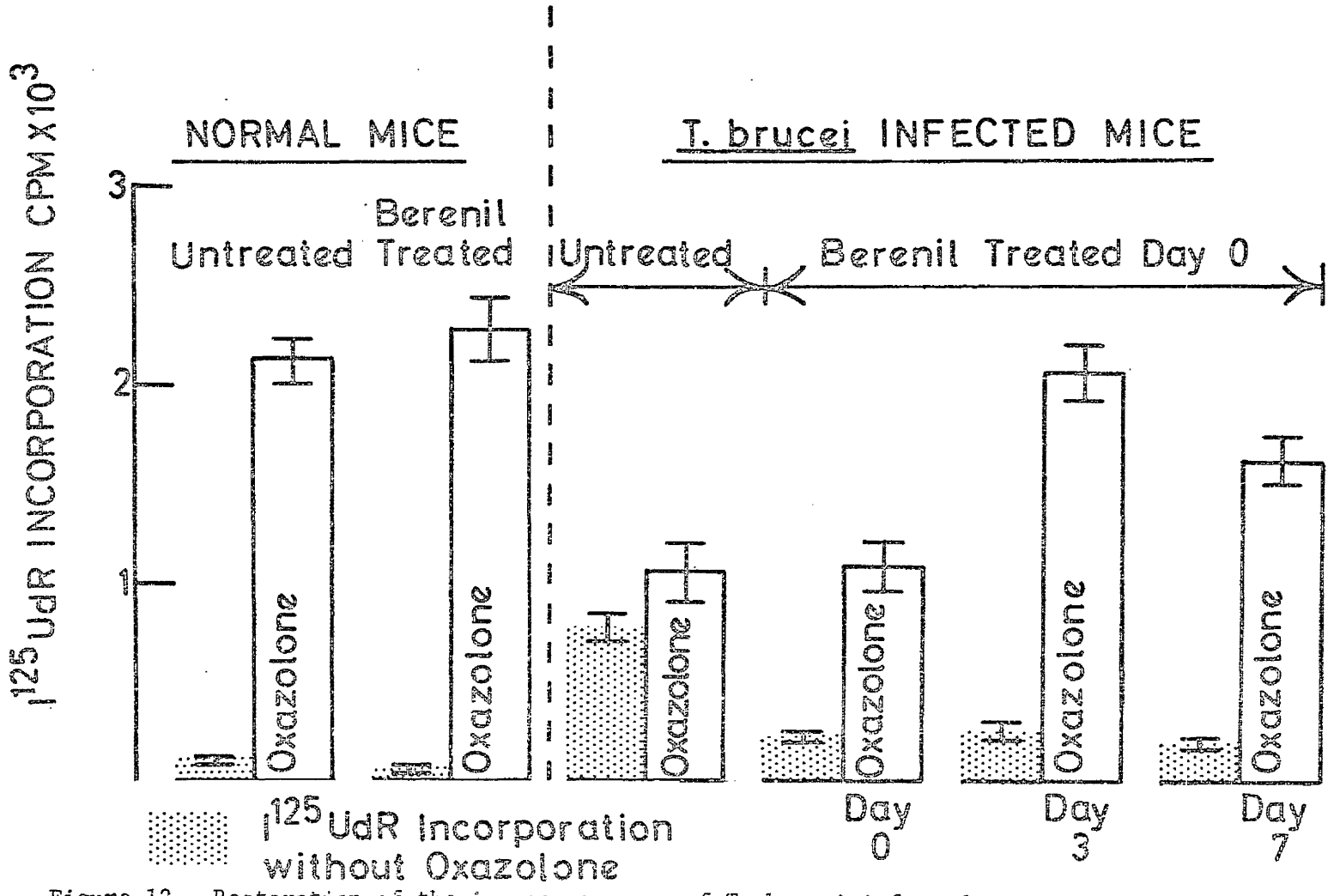

Figure 12 Restoration of the immune response of T. brucei infected mice after treatment with diminazine aceturate (berenil) as measured by the incorporation of ^{125}I UdR in the regional lymph nodes 3 days after sensitisation with oxazolone on days 0, 3 and 7 after treatment. Each column represents the mean ^{125}I cpm $\pm$ S.E. of 10 mice.

The experiments described in this section were carried out primarily in an attempt to establish if the immunosuppression associated with trypanosomiasis was caused by a defect in the function of either the T or B lymphocytes. On histological examination it was apparent that the latter cell population was grossly altered with a massive plasma cell response in lymph nodes, spleen and connective tissue fascia throughout the body. These changes were seen within 7 days of inoculation and persisted at least 70 days, the duration of the experiment. In contrast to this sustained plasma cell hyperplasia, it was found that the haemagglutination titres to SRBC were severely depressed and that this, as judged by the absence of PFC responses, was due to a failure of antibody production at the cellular level rather than solely to the increased catabolism of globulin[42]. These results are in agreement with those of Freeman, Hudson and Byner[43] who recently found that by 10 days of infection, "numbers of both IgM and IgG producing cells are down to below 2% of normal value". This failure involved both the IgM and IgG responses and could not be overcome by varying the dose of SRBC. If one assumes that antigen processing by the mononuclear phagocytic system is minimally competent, then the failure of IgM plaque production suggests the possible existence of a defect in the B lymphocyte population since it is generally agreed that a small, though significant, response to SRBC is produced by B cells in circumstances which apparently preclude the participation of T cells[44,45].

To obtain further evidence on the possible existence of a B cell defect, a group of T. brucei infected mice were immunised with bacterial lipopolysaccharide; the response to this antigen is generally regarded as being attributable to B cells alone[38,46].

The results based on indirect haemolysis and PFC responses showed that infected compared to normal mice failed to mount a significant antibody response, again suggesting the existence of a B cell dysfunction.

This conclusion does not, of course, preclude the co-existence of a defective T cell population. In this connection, marked histological changes were found in the thymus and in the thymus-dependent areas of the immune system. From a few days of inoculation to the end of the experiment, the thymic cortex was narrow and irregular while the thymus-dependent areas of the lymph nodes and spleen were replaced by expanding plasma cell populations. However, infected mice, sensitised by oxazolone, were able to mount a cell mediated response after challenge, as measured by an increase in ear thickness and by a re-appearance and expansion of the thymus-dependent areas of the draining lymph nodes, indicating the ability of their T cells to participate in delayed type reactions. The function of T cells was further assessed with regard to their ability to participate in a primary response by measuring the incorporation of ^{125}I UdR in the proliferating cells of the regional lymph nodes of the mice 3 days after sensitisation with oxazolone[32,47]. Davies[47] has shown that at this time the increased ^{125}I UdR incorporation is specifically associated with proliferation of thymus-derived cells in the paracortical areas of the lymph nodes.

It was found that although ^{125}I UdR incorporation after oxazolone sensitisation of mice infected with T. brucei for up to 3 weeks was apparently similar to that of sensitised, normal mice, examination of the response in unsensitised and infected mice revealed a very high "background" incorporation of ^{125}I UdR compared to unsensitised and normal mice; whether this elevated background

incorporation was caused by dividing trypanosomes in the lymph nodes or alternatively by the high mitotic activity of various cell types is unknown. Despite this reservation, the results indicated that the T cells of sensitised and infected mice were able to mount a significant response to oxazolone for at least 3 weeks after infection, providing further evidence of T cell competence at a time when humoral responses were shown to be reduced or absent. A point of some importance, however, is that subsequent studies showed that mice infected for longer periods, i.e., 5 to 6 weeks, failed completely to respond to oxazolone sensitisation despite the presence of a high background activity. This suggests that T cell failure may ultimately occur, probably in the terminal stages of the disease, and indicates that immunological unresponsiveness in murine trypanosomiasis may be attributable to more than one factor depending on the duration of infection. However, at this stage no new morphological changes were found to account for this development in contrast to the marked cellular depletion of the immune system which occurs in rats at this time (Chapter 1).

Despite the sustained morphological alterations in the immune system, one of the most interesting features of the unresponsiveness associated with trypanosomiasis was the rapidity with which competence was restored after treatment with a trypanocidal drug. Thus mice immunised with SRBC as soon as 2 days after treatment responded in a normal fashion. Similarly chemotherapy reduced the "background" incorporation of ^{125}I UdR within a few hours of treatment and within 3 days the specific response to oxazolone was normal. It is probable that a study of the histological changes following treatment would prove rewarding.

In summary, immunosuppression in sub-acute murine trypanosomiasis, i.e., 2 to 4 weeks post-infection, was characterised by the following features. First, despite the fact that the mononuclear phagocytic system of such animals possessed an enhanced ability to phagocytose SRBC and that their spleens were greatly enlarged, the total deposition of SRBC in the latter organ was not increased (Section I). While one might therefore tentatively conclude that an insufficient concentration of splenic antigen may play a role in immunosuppression it appears unlikely to be the sole or indeed the primary cause. Secondly, the T cells appeared relatively normal at this stage of infection as judged by their ability to proliferate following a primary stimulus with oxazolone and by the development of a cell-mediated response on challenge with oxazolone. In contrast, the function of the B cells appeared abnormal in that the response to antigens considered to be specific for B cells was almost completely suppressed despite the fact there was a remarkable plasma cell proliferation. Finally, perhaps one of the most significant findings is the speed with which immune competence was restored after trypanocidal therapy, even in infections of a longer duration than 4 weeks.

The rapid restoration of the immune response might suggest that the presence of living trypanosomes, rather than the development of a progressive lesion, initiated by the infection, is the basic cause of immunosuppression. It is possible that trypanosomiasis might be associated with the elaboration by the parasite of a substance akin to a plant mitogen which, while by aspecifically stimulating the multiplication

of B lymphocytes, prevents their subsequent participation in immune responses. Alternatively the mitogen might act by causing activation of a population of T suppressor cells in a manner analogous to that described by Rich and Pierce[48] who showed that thymus-derived lymphocytes, after activation by the plant mitogen concanavalin A, suppressed the development of PFC responses in cultures of mouse spleen cells stimulated with SRBC. Of the two, the former seems the more likely, since, if the latter were the case, one might not expect to see hyperplasia of the plasma cell series such a consistent feature of the infection.

Another possibility is that the fluctuating parasitaemia, presumably associated with the production of successive waves of variant antigens, produces a state of unresponsiveness to unrelated antigens by way of a type of antigenic competition[20]; the rapid return of competence after therapy neither supports nor refutes this theory.

The relative significance of these two factors as the possible causes of immunosuppression might be further clarified if two facts were established. First, the specificity to trypanosome antigens of the excessive amounts of immunoglobulins produced in this disease[39]. Secondly, to find if trypanosome products do, in fact, exert a mitogenic and immunosuppressive effect on lymphocyte cultures.

SECTION III

THE INTERACTION OF *TRYPANOSOMA BRUCEI* AND *NIPPOSTRONGYLUS BRASILIENSIS* INFECTIONS IN THE RAT

INTRODUCTION

The results reported in the two previous sections were primarily concerned with attempts to define and analyse the mechanism of the immunosuppression associated with T. brucei infection in laboratory rodents. Of necessity the antigens used were relatively defined and these studies gave no indication of the practical significance of the phenomenon in the face of challenge with pathogen-associated antigens.

It was therefore decided to investigate the effect of trypanosomiasis on the course of Nippostrongylus brasiliensis infection of rats. There were two reasons for the selection of this particular pathogen. First, it was readily available in the laboratory and, having been studied intensively by colleagues for several years, a great deal was known of the course of experimental infections. Secondly, the infection provided four separate parameters with which to measure the efficacy of the immune response to this nematode. These were:

1. The immune expulsion of adult worms during infection

In the normal course of events, rats with a single infection of N. brasiliensis undergo what has been termed "self-cure" in that from 10 to 16 days after the infection, the adult worms in the small intestine are expelled in an exponential fashion by a process known to be immunological[49]. The effect of T. brucei on this helminth infection is therefore readily assessed in terms of the numbers of N. brasiliensis remaining in the intestine after the period when immune expulsion is normally completed.

2. The development of so-called "protective" antibodies in the serum demonstrable by passive immunisation and challenge with N. brasiliensis larvae.

Evidence for the existance and characterisation of this IgG-type antibody, operating independently of the presence of homocytotropic antibody, has been provided by Jones, Edwards and Ogilvie[50].

3. The development of reaginic antibody to N. brasiliensis antigen, demonstrable by the passive cutaneous anaphylaxis (PCA) reaction.

In 1964 Ogilvie[51] reported that the serum of rats infected with N. brasiliensis contained a skin sensitising antibody from about the third week of a primary infection. Even in the absence of reinfection, this persisted for many months although challenge produced a sharp rise in titre. Subsequently, the biological characteristics and class of this antibody were shown to be analogous to human IgE [52,53,54].

4. Changes in the mast cell population of the intestinal mucosa

Studies on the dynamics of the intestinal sub-epithelial mast cell populations have shown that shortly after the young adult N. brasiliensis worms reach the intestine the mast cells, normally about 10-15 per villous crypt unit in the jejunum, become granulated and destroyed. A few days later, associated with the onset of worm expulsion, there is an exponential rise in the numbers of intestinal mast cells[55] reaching a peak of 35-50/V.C. unit at day 18. While the function of the mast cells in immune reactions is obscure it has been postulated that they are involved in the immune response at mucous surfaces by making the gut hyperpermeable for antibody translocation[56]. There is also evidence to indicate that the mast cells might be lymphoid in origin[57,58,59].

MATERIALS AND METHODS

The rats used, the infection procedures and chemotherapy regimen were as described in General Materials and Methods. A brief description of the normal course of *N. brasiliensis* infection in rats is included in the appendix.

Nippostrongylus brasiliensis Infection:

1. Culture of larvae

These were cultured essentially as described by Jennings, Mulligan and Urquhart[60]. Faeces were collected from rats infected with 4,000 larvae 7 days previously. The faeces were set up for culture in individual petri dishes on the centre of a moistened filter paper supported by a sponge. Approximately 2g of moistened faeces were used for each filter paper and the cultures were incubated at 27°C for at least 5 days, by which time the larvae had migrated to the edge of the filter papers and had reached the infective stage. The larvae were harvested by immersing the filter paper in water for 2 minutes then removing the filter paper. The filter paper, which retained the concentrated larvae, was then inverted on a fine sieve (300 mesh to the inch), placed in a Baerman funnel filled with water at 37°C and the larvae collected by sedimentation. This procedure gave a concentrated suspension of highly active larvae almost free from contaminating material.

2. Infection of rats

Larvae were diluted in an appropriate volume of water and then counted microscopically in 0.1 ml samples after spreading on a clean slide. By a suitable adjustment of larval concentration, infective doses were contained in 1 ml aliquots. Rats were infected by abdominal subcutaneous inoculation of these larvae. Care was taken to withdraw the needle slowly after such injections to avoid loss of infective material.

Assays of responses to N. brasiliensis:

1. Worm recovery

Recovery of adult worms from the intestine was as described by Mulligan, Urquhart, Jennings and Nielson[61]. At necropsy the small intestine was removed and opened longitudinally. This was suspended in a muslin bag in a beaker of physiological saline at 37°C. After 1 hour all the adult worms had collected in the bottom of the beaker. These were preserved by the addition of a small volume of 10% formalin and duplicate aliquots were counted and meaned to assess the total adult worm burden.

2. Assay of antibody protective against N. brasiliensis

Serum was collected from both T. brucei infected and control rats 18 days after infection with N. brasiliensis. The activity of protective antibody in the serum from these two sources was compared by intraperitoneal injection of 5 ml serum into each of 2 groups of 5 adult rats on days 1 and 3 of a larval infection with 1,000 N. brasiliensis; the rats were subsequently killed on day 9 and their worm burdens determined.

3. Reaginic antibody to N. brasiliensis

Serum was collected from both T. brucei infected and control rats 18 days after infection with N. brasiliensis, and the presence of reaginic antibody to N. brasiliensis was bioassayed by the technique of passive cutaneous anaphylaxis[51]. Appropriate dilutions of the test serum were made in saline. After shaving the backs of the test rats, 0.1 ml of each dilution was injected intradermally through a 26g 3/4" needle. A total of six injections were made in each rat; i.e., five dilutions of test serum and 1:5 dilution of normal rat serum as control. Forty-eight hours later a mixture of 0.5 ml N. brasiliensis antigen (prepared by homogenising 1,000 adult worms/ml saline, centrifuging and collecting

the supernatant; 0.5 ml of the latter contains 500 "worm equivalents") together with 0.5 ml 1% Evans blue in saline, was injected intravenously into each test animals. Twenty minutes later the rats were killed and skinned and the presence of reagins detected by the appearance of a deep blue area at the skin site where the serum was injected.

The reaginic titre was expressed as the reciprocal of the greatest dilution to give a clear positive reaction.

4. Counting of intestinal mast cells

The technique for the preparation and quantitation of sub-epithelial mast cells was that described by Jarrett, Jarrett, Miller and Urquhart[62]. At necropsy the small intestines were removed from the rats under investigation. A piece was excised from the jejunum at a point 20 cm from the pylorus. This was laid on a piece of dry filter paper and opened along its line of mesenteric attachment when it flattened out. Individual sections and papers were immersed in Carnoy's fluid. The specimens were trimmed longitudinally. Twenty-four to forty-eight hours later the tissues were dehydrated, cleared in an alcohol-amyl acetate-chloroform series and embedded in paraffin wax. Sections were stained by the Astra blue-Safranin technique at pH 0.3[63]. The number of mast cells were counted in 20 villous crypt units and expressed as the arithmetic mean number per villous crypt.

EXPERIMENTAL DESIGNS

Three separate experiments were conducted:

1. The effect of *T. brucei* infection on the primary immune response of rats to *N. brasiliensis*

50 rats were infected with *T. brucei*. 21 days later these and 50 control rats each received 1,000 *N. brasiliensis* larvae

subcutaneously. 7 and 14 days after the helminth infection 10 rats were necropsied from each of the 2 groups to establish their worm burdens and numbers of intestinal mast cells. The experiment was terminated 18 days after the helminth infection when, in addition to the determination of worm burdens and intestinal mast cell numbers, serum was collected for assay of "protective antibody" and reaginic antibody.

2. The effect of T. brucei on the immune response of rats to a challenge infection of N. brasiliensis

The experimental design is shown in Fig. 13. Ten rats in Group 1 were each given a primary infection of 4,000 N. brasiliensis larvae. Twenty-two days later, i.e., after completing the immune expulsion of the primary infection, each was infected with T. brucei. After a further 20 days each rat received a challenge dose of N. brasiliensis larvae. A second group (Group 2) of rats were given the primary and challenge N. brasiliensis infections on the same days as Group 1 but were not infected with T. brucei. The degree of immunity resulting from the primary N. brasiliensis infection in Groups 1 and 2 was assessed by comparison of the mean worm numbers after challenge, with those of a third group (Group 3) which received the challenge N. brasiliensis infection only.

3. The effect of trypanocidal therapy on the ability of rats to respond to a challenge infection of N. brasiliensis after infection with T. Brucei.

In this experiment, two groups of rats which had successfully undergone immune expulsion of a primary infection were infected with T. brucei. Twenty days later one group only was treated with a trypanocidal drug; both groups were subsequently challenged with N. brasiliensis. The experimental design is shown in Fig. 14.

Figure 13 The plan of an experiment in which rats, immune to *N. brasiliensis* and subsequently infected with *T. brucei*, were challenged with *N. brasiliensis* larvae.

Group Number	Number of rats	Primary *N. brasiliensis* Infection		*T. brucei* Infection		Challenge *N. brasiliensis* Infection		Necropsy
1	10	*N. brasiliensis*	22d	*T. brucei*	20d	*N. brasiliensis*	8d	Necropsy
2	8	*N. brasiliensis*		42d		*N. brasiliensis*	8d	Necropsy
3	10					*N. brasiliensis*	8d	Necropsy
4	10	*N. brasiliensis*	7d	Necropsy				
5	5	*N. brasiliensis*	22d	Necropsy				
6	5	*N. brasiliensis*	22d	*T. brucei*		28d		Necropsy

The interval between procedures is indicated in days.

Figure 14 The plan of an experiment in which rats, immune to *N. brasiliensis* and subsequently infected with *T. brucei*, were treated with a trypanocidal drug prior to challenge with *N. brasiliensis* larvae.

Group Number	Number of rats	Primary *N. brasiliensis* Infection		*T. brucei* Infection		Berenil Treatment		Challenge *N. brasiliensis* Infection		Necropsy
1	15	*N. brasiliensis*	21d	*T. brucei*		28d		*N. brasiliensis*	8d	Necropsy
1A	15	*N. brasiliensis*	21d	*T. brucei*	20d	Berenil	8d	*N. brasiliensis*	8d	Necropsy
2	12	*N. brasiliensis*			49d			*N. brasiliensis*	8d	Necropsy
2A	12	*N. brasiliensis*			41d	Berenil	8d	*N. brasiliensis*	8d	Necropsy
3	10			*T. brucei*	20d	Berenil				
4	10	*N. brasiliensis*	9d	Necropsy						

Control groups were included to confirm the efficacy of trypanocidal therapy and to demonstrate that the drug had no anthelmintic effect on the challenge infection of N. brasiliensis.

RESULTS.

1. The effect of T. brucei on the primary immune response of rats to N. brasiliensis.

Worm Burdens

These are shown in Table 14. In the control group immune expulsion of the adult worm burden occurred normally, whereas in the rats with T. brucei infection, expulsion did not occur. Thus, on day 18 there was no significant reduction of the adult N. brasiliensis worm burden in the trypanosome infected group (i.e., a mean of 218 $\pm$ 45) while in the control rats, immune expulsion was complete (i.e., a mean of only 5 $\pm$ 5 worms).

Assay of protective antibody to N. brasiliensis (Table 15)

The rats passively immunised with serum obtained from the control group on day 18 were found to be protected to a significant degree against challenge ($P<0.01$) whereas the rats immunised with serum from the T. brucei infected rats had similar worm burdens to those which received no serum.

Reaginic antibody to N. brasiliensis

The results are shown in Table 16 from which it appeared that reaginic antibody was normally present in the serum of the control rats and absent from the serum of T. brucei infected rats.

Table 14 The effect of a T. brucei infection on the development of immunity to N. brasiliensis infection in rats.

Duration of N.brasiliensis infection in days	Arithmetic mean ± SE of N.brasiliensis	
	T.brucei infected rats	Control rats
7	253 ± 67	248 ± 84
14	199 ± 71	9 ± 2
18*	218 ± 45**	5 ± 5***

*P <0.001 **n = 16 ***n = 27

Table 15 The effect of a *T. brucei* infection on the development of protective antibody to *N. brasiliensis* as measured by passive immunisation.

Source of Serum	Arithmetic mean ± SE of *N. brasiliensis*
Rats with *N. brasiliensis* infection only	357 ± 42*
Rats with *T. brucei* and subsequent *N. brasiliensis* infection	661 ± 72*
No serum	591 ± 51

*$P < 0.01$

Table 16 The effect of a T. brucei infection of the development of reaginic antibody to N. brasiliensis as measured by PCA titres 18 days after infection.

Group	PCA titres			
	Bulk sera	Individual sera		
		1	2	3
Control rats	$\frac{1}{5}$	$\frac{1}{625}$	$\frac{1}{625}$	Neg.
T. brucei rats	Neg.	Neg.	Neg.	Neg.

Kinetics of intestinal mast cells

These, presented in Table 17 show that while a 6 or 7 fold increase in numbers of mast cells occurred during the period of immune expulsion of adult worms in the control rats, no such increase was found in the T. brucei infected rats. The almost complete absence of mast cells in the presence of trypanosome infection is shown histologically in Figs. 15 and 16.

2. The effect of T. brucei on the response of rats to a challenge infection of N. brasiliensis

Worm Burdens

These are shown in Table 18. A comparison of Groups 4 and 5 shows that the primary N. brasiliensis infection had established successfully and that immune expulsion occurred. Subsequently the rats were highly immune to reinfection, i.e., a mean of 20 worms developed in the N. brasiliensis reinfection group (Group 2) compared with 397 in the group given the challenge infection only (Group 3). In contrast the rats infected with T. brucei after completion of the immune expulsion of the primary N. brasiliensis infection (Group 1) were completely susceptible to the challenge nematode infection, having a mean of 395 worms.

Reaginic antibody to N. brasiliensis

The presence of reaginic antibody to N. brasiliensis as measured by the PCA test in bulked samples of the sera is shown in Table 19. The sera of rats which were given only the challenge infection and killed 8 days later was, as expected, negative. Of the two groups of rats which received the challenge infections, the sera from the trypanosome-free group showed a titre of 1:2000 while that of the T. brucei infected group failed to rise above 1:25.

Table 17 The effect of a *T. brucei* infection on the kinetics of intestinal mast cells during the course of a *N. brasiliensis* infection in rats.

Duration of *N. brasiliensis* infection in days	Arithmetic mean ± SE of mast cells/villous crypt		Significance
	T. brucei infected rats	Control rats	
7	6.9 ± 4.6	4.7 ± 1.0	NS
14	5.0 ± 2.4	30.2 ± 5.2	$P < 0.01$
18	8.5 ± 3.6	37.1 ± 3.1	$P < 0.001$

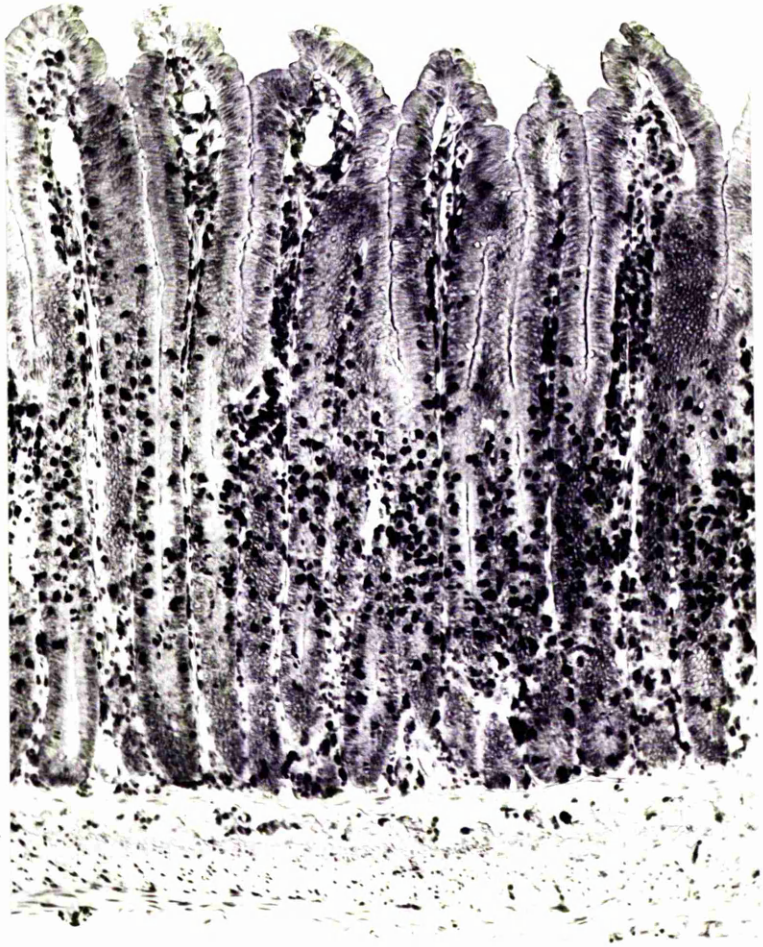

Figure 15. Small intestine of rat 18 days after infection with 1,000 N. brasiliensis larvae. Note large numbers of mast cells and absence of worms.
Astra blue-safranin x 130.

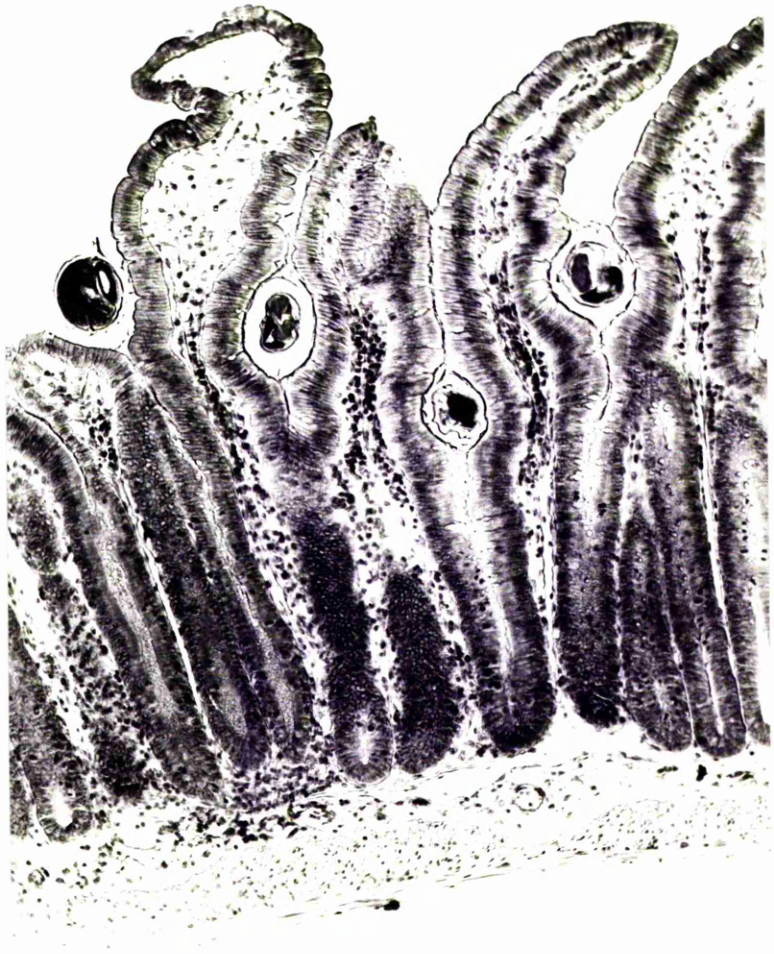

Figure 16. Small intestine of T. brucei infected rat 18 days after infection with 1,000 N. brasiliensis larvae. Note absence of mast cells and presence of adult worms.
Astra blue-safranin x 130.

Table 18 The effect of *T. brucei* infection in rats on the immune expulsion of a challenge infection of *N. brasiliensis*.

Group		Numbers of *N. brasiliensis* recovered from the intestine	Significance
1. *N. brasiliensis* reinfection + *T. brucei*	(10)*	395 ± 45	1-2 P <0.001 2-3 P <0.001 1-3 NS
2. *N. brasiliensis* reinfection	(8)	20 ± 9	1-6 P <0.001
3. *N. brasiliensis* challenge infection only	(10)	397 ± 37	3-6 P <0.001 4-5 P <0.001
4. *N. brasiliensis* primary infection at 7 days	(10)	1502 ± 34	2-6 NS
5. *N. brasiliensis* primary infection at 22 days	(5)	98 ± 32	
6. *N. brasiliensis* primary infection and *T. brucei*	(5)	64 ± 21	

* The figures in parenthesis represent the numbers of rats in each group. Each value is the arithmetic mean ± SE of the worm burdens.

Table 19 The effect of *T. brucei* on the development of reaginic antibody eight days after a challenge infection of *N. brasiliensis* as measured by PCA titres.

Group	PCA titres of bulk sera - duplicate tests	
1. *N. brasiliensis* reinfection + *T. brucei*	1:25	1:25
2. *N. brasiliensis* reinfection	1:2,000	1:800
3. *N. brasiliensis* challenge infection only	Negative	Negative

Intestinal mast cell numbers

The mean numbers of mast cells per villous crypt unit are shown in Table 20. Before reinfection the mean numbers of mast cells in the rats which had received a sensitising infection of N. brasiliensis was 20 per villous crypt unit; this was the case in both trypanosome-free and trypanosome infected groups. However, 8 days after challenge, the unit mast cell numbers in the trypanosome-free group had increased to a mean of 36 while the numbers in the T. brucei infected group remained static.

3. The effect of trypanocidal therapy on the ability of rats to respond to a challenge infection of N. brasiliensis after infection with T. brucei

Worms Burdens

The results presented in Table 21 show that in rats in which the T. brucei infection was treated with diminazine aceturate before their challenge with N. brasiliensis, the ability to mount a secondary response to the helminth infection was almost completely restored. This is shown by a comparison of Groups 1 and 1A. In the former, where the T. brucei infection was untreated, 299 adult worms, i.e., 30% of the challenge larvae, developed to maturity. In the latter group, treated with diminazine aceturate only 28 worms, i.e., 3% of the larvae, developed. A comparison of groups 2 and 2A showed that treatment with berenil did not alter the vigour of the secondary immune response to N. brasiliensis infection.

The inclusion of Groups 3 and 4 provided evidence of the trypanocidal efficacy of diminazine aceturate and of the infectivity of the primary N. brasiliensis infection.

Table 20 The effect of a *T. brucei* infection on the numbers of intestinal mast cells eight days after challenge infection of *N. brasiliensis*.

Group		Numbers of mast cells/villous crypt unit	Significance
1. *N. brasiliensis* reinfection + *T. brucei*	(10)	20.68 ± 1.41	1u2 P <0.001 1u3 P <0.001
2. *N. brasiliensis* reinfection	(8)	34.29 ± 1.81	2u3 P <0.001
3. *N. brasiliensis* challenge infection only	(10)	4.44 ± 1.05	
4. *N. brasiliensis* primary infection at 7 days	(10)	0	

Table 21 The effect of trypanocidal therapy on the response of rats to a challenge infection of *N. brasiliensis* after an infection of *T. brucei*.

Group		Numbers of *N. brasiliensis* recovered from the intestine (Arithmetic mean ± Standard Error)	Significance
1. *N. brasiliensis* reinfection + *T. brucei*	(10)	299 ± 30	1 -1A P <0.001 1A-2A P <0.005
1A. *N. brasiliensis* reinfection + *T. brucei*	(11)	28 ± 9	
BERENIL TREATED			
2. *N. brasiliensis* reinfection	(11)	1	
2A. *N. brasiliensis* reinfection	(11)	8 ± 3	
BERENIL TREATED			
4. *N. brasiliensis* primary infection at 9 days	(11)	1756 ± 57	

DISCUSSION

The results of the first experiment provide further evidence that infection with T. brucei may induce a significant degree of immunosuppression of the host. Thus in rats in which a N. brasiliensis infection was superimposed on a previously existing T. brucei infection of 3 weeks' duration, the normal process of immune expulsion of adult worms did not occur, the production of circulating protective antibody (IgG) and of reaginic antibody (IgE) was grossly impaired and there was no increase in the numbers of mast cells in the intestinal villi.

In addition to suppressing the primary response to N. brasiliensis infection, the results of the second experiment demonstrated that the immunity which rats acquire after a primary infection is completely ablated if they are infected with T. brucei. Thus, rats infected with trypanosomes 3 weeks after completion of a primary infection were as susceptible to challenge with 1,000 larvae as were control rats in that 40% of the larvae developed to adult worms. In contrast, only 2% of the challenge infection developed in immune rats not infected with T. brucei.

Perhaps one of the most interesting and important aspects of this suppression of secondary responses was the further finding that responsiveness was completely restored after the administration of a trypanocidal drug. This was demonstrated in the third experiment where, after treatment of the trypanosome infection, only 3% of the challenge infection of N. brasiliensis larvae developed to maturity compared to 30% in untreated and previously immune rats. This implies that immunological memory to previously encountered antigens is not destroyed but is in some way suppressed;

when the trypanosome infection is terminated, immunological memory appears to re-assert itself.

The results reported in this section show that immunosuppression during trypanosomiasis, so readily demonstrated with simple and defined antigens, also occurs when infected animals are exposed to a pathogen. The significance of these findings might be reasonably extrapolated to three separate situations of practical importance. First, the susceptibility of naturally infected animals to other infections. Secondly, the possibility of commensal organisms in these individuals becoming pathogenic; the necropsy findings of bronchopneumonia in rats (Chapter 1) could be ascribed to such an effect. Finally, the possibility that such immunosuppression might have considerable veterinary importance in Africa where vaccination campaigns are vital to the development of the livestock industry.

As a result of such findings an experiment was designed in conjunction with Dr. P.H. Holmes, International Atomic Energy Adviser in Ethiopia to demonstrate the possible existence of immunosuppression in cattle infected with T. congolense. At the time of writing, the results of this work are still being evaluated. However, it is apparent that immunosuppression is a prominent feature of cattle trypanosomiasis and an initial short communication on the results of this experiment is included in the appendix.

SUMMARY

In section I, investigations of the functional integrity of the mononuclear-phagocytic system (MPS) in mice infected with Trypanosoma brucei were reported and discussed with regard to its possible significance in the aetiology of the immunosuppression characteristic of this disease.

In infected mice the spleens and lymph nodes were grossly enlarged and on histological examination it was shown that the mononuclear phagocytic system of the liver, lymph nodes, spleen and bone marrow was markedly expanded. Macrophages presented an active appearance and often contained cellular debris. Clearance of intravenously injected SRBC was increased, in that one hour after injection, 2 to 6 times more ^{51}Cr SRBC had disappeared from the circulation of infected, compared to uninfected mice; this was due largely to an increased uptake by the expanded phagocytic system of the liver.

The intrinsic immunogenic potential of individual macrophages appeared to be unimpaired as judged by the ability of SRBC-containing macrophages from infected mice to elicit a response in syngeneic normal recipient mice.

It was concluded that the only evidence that immunosuppression might be associated with an altered activity of the MPS was an increased hepatic uptake of particulate antigen with a relative failure of splenic uptake. Together these might be responsible for a reduction in the concentration of antigen in the tissues of an enlarged spleen below the level necessary to initiate the formation of antibody.

Subsequent investigations, reported in section 2, on the role of the thymus-derived lymphocytes (T cells) and the thymus-

independent lymphocytes (B cells) showed that:

1. there was a massive plasma cell response in lymph nodes and spleen which replaced the thymus-dependent areas.
2. a failure of antibody production at the cellular level occurred as shown by the absence of IgM PFC responses to SRBC and lipopolysaccharide.
3. T cells appeared relatively normal as judged both by their ability to proliferate following a primary stimulus with oxazolone (unless measured during the terminal stages of the disease) and by their ability to mount a characteristic contact hypersensitivity response on challenge with oxazolone.
4. immune competence was rapidly restored after treatment with a trypanocidal drug.

It appeared that immunosuppression was closely associated with the presence of living trypanosomes, possibly mediated through a B cell defect. The mechanism whereby this might occur is discussed.

These conclusions on the role of the various immunological cell types in immunosuppression were based largely on the analysis of the responses of infected mice to certain defined antigens. In section III, an attempt to evaluate the importance of such immunosuppression on the interaction of T. brucei infection with other pathogens is reported. The nematode Nippostrongylus brasiliensis infection in the rats was used as the pathogen in these experiments.

It was found that in rats in which N. brasiliensis infection was superimposed on a previously existing T. brucei infection of 3 weeks' duration, the normal process of immune expulsion of adult worms did not occur, the production of circulating protective antibody (IgG) and of reaginic antibody (IgE) was

grossly impaired and there was no increase in the numbers of mast cells in the intestinal villi. The results of a later experiment showed that rats, which had successfully undergone immune expulsion of a primary N. brasiliensis infection before being inoculated with T. brucei, subsequently lost their ability to mount an immunological response to a challenge infection of the nematode. When the trypanosome infection was cured however, there was a rapid return to normality in the response of such rats to the challenge N. brasiliensis infection. These findings show that immunological memory is not destroyed during a bout of trypanosomiasis but in some way suppressed and that when the trypanosomes are removed, this memory can manifest itself in the secondary response. These results are discussed with particular reference to the part which such immunosuppression may play in the pathogenisis of disease in areas of endemic trypanosomiasis.

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APPENDIX

RAT WHOLE BODY, LIVER AND SPLEEN WEIGHTS* - INDIVIDUAL RESULTS

(Infected groups inoculated with *T. brucei* on 2/12/71)

	Body Weight		Liver Weight		Spleen Weight	
Date	Normal	Infected	Normal	Infected	Normal	Infected
16/12/71	176.5	139.0	6.70	5.95	0.40	1.75
	170.0	159.0	6.35	7.10	0.50	1.90
	156.0	173.0	6.20	8.20	0.45	1.80
	155.0	161.0	5.95	7.60	0.30	2.40
	173.0	168.0	7.10	7.10	0.65	1.75
Mean±SE	166.1±4.5	160.0±5.8	6.46±0.20	7.19±0.37	0.46±0.05	1.92±0.12
23/12/71	-	142.5	-	6.40	-	1.95
	-	160.0	-	6.70	-	2.20
	-	156.0	-	8.10	-	3.00
	-	149.5	-	7.00	-	2.00
	-	156.0	-	8.40	-	2.10
Mean±SE	-	152.8±3.1	-	7.32±0.39	-	2.25±0.19
30/12/71	173.0	136.0	6.70	7.50	0.40	1.60
	164.8	159.0	6.20	8.80	0.50	2.70
	143.3	159.4	5.60	8.90	0.60	4.00
	161.5	148.3	5.50	8.10	0.50	3.85
	131.4	111.3	5.20	5.50	0.50	1.70
Mean±SE	154.8±7.6	142.8±9.0	5.84±0.27	7.76±0.62	0.50±0.03	2.77±0.51
6/1/72	-	152.5	-	7.90	-	3.00
	-	163.5	-	9.30	-	4.70
	-	145.3	-	7.50	-	2.70
	-	150.6	-	9.40	-	5.70
Mean±SE	-	153.0±3.8	-	8.53±0.48	-	4.03±0.71
14/1/72	163.5	132.6	6.40	7.70	0.35	2.60
		157.5		9.20		4.60
		164.5		9.70		5.10
Mean±SE		151.5±9.7		8.87±0.60		4.10±0.76

*Each value is the organ weight in grammes.

RAT HAEMATOLOGY EXPERIMENT - INDIVIDUAL RESULTS

(Infected groups inoculated with T. brucei TREU 667 on 2/12/71)

	Haematocrits %		RBC Count x 10^6/cu mm.		Hb concentration gms %	
Date	Normal	Infected	Normal	Infected	Normal	Infected
2/12/71	42.0	-	5.21	-	15.6	-
	37.0	-	5.33	-	12.8	-
	38.5	-	5.29	-	13.2	-
	38.0	-	5.37	-	12.8	-
	39.0	-	5.23	-	13.5	-
Mean±SE	38.9±0.8	-	5.29±0.00	-	13.58±0.52	-
9/12/71	-	40.0	-	5.23	-	14.1
	-	38.5	-	4.82	-	12.6
	-	37.0	-	5.09	-	14.5
	-	36.0	-	4.93	-	11.9
	-	40.0	-	4.82	-	11.0
Mean±SE	-	38.3±0.8	-	4.98±0.08	-	12.82±0.66
16/12/71	40.0	35.0	5.40	4.50	13.8	11.3
	38.5	35.0	4.99	4.86	14.2	12.5
	40.0	34.5	5.08	4.79	14.4	11.9
	37.5	32.0	5.06	4.69	12.5	11.9
	37.5	34.0	5.44	4.90	14.4	11.3
Mean±SE	38.7±0.6	34.1±0.6	5.19±0.09	4.75±0.07	13.9±0.36	11.80±0.22
23/12/71	-	38.0	-	5.71	-	11.9
	-	36.0	-	4.93	-	10.7
	-	38.0	-	5.04	-	10.7
	-	34.0	-	4.33	-	11.3
	-	35.0	-	4.54	-	10.3
Mean±SE	-	36.2±0.8	-	4.91±0.24	-	10.75±0.20
30/12/71	38.0	41.0	5.58	4.94	14.4	11.9
	39.0	35.0	5.17	3.77	13.8	9.9
	38.0	31.0	5.12	3.78	13.3	9.9
	40.0	36.0	5.45	4.27	13.5	10.5
	40.0	35.0	5.55	3.99	14.1	10.2
Mean±SE	39.0±0.5	35.6±1.6	5.37±0.09	4.15±0.22	13.82±0.2	10.48±0.37
6/1/72	-	36.5	-	4.69	-	10.0
	-	34.0	-	4.02	-	10.7
	-	31.5	-	4.06	-	9.4
	-	35.0	-	4.09	-	9.9
Mean±SE	-	34.25±1.1	-	4.22±0.20	-	10.00±0.27
14/1/72	42.0	33.0	5.24	4.03	14.1	9.1
	-	33.0	-	3.17	-	7.8
	-	33.5	-	3.99	-	9.7
Mean±SE		33.17±0.2		3.73±0.28		8.87±0.56

RAT HAEMATOLOGY EXPERIMENT - INDIVIDUAL RESULTS (CONT'D).

	Blood Reticulocytes (% RBC)		MCHC (%)		MCV (cμ)	
Date	Normal	Infected	Normal	Infected	Normal	Infected
2/12/71	<1	-	29.9	-	80.6	-
	<1	-	24.0	-	69.4	-
	<1	-	24.9	-	72.8	-
	<1	-	23.8	-	70.8	-
	<1	-	25.8	-	74.6	-
Mean±SE		-	25.7±1.10	-	73.6±1.95	-
9/12/71	-	2.5	-	27.0	-	76.5
	-	1.5	-	26.1	-	79.9
	-	1.8	-	28.5	-	72.7
	-	1.6	-	24.1	-	73.0
	-	1.0	-	22.8	-	83.0
Mean±SE	-	1.68±0.24	-	25.7±1.01	-	77.0±2.00
16/12/71	1.0	1.2	25.6	25.1	74.1	77.8
	1.3	1.5	28.5	25.7	77.2	72.0
	0.2	3.0	28.4	24.8	78.7	72.0
	1.3	2.4	24.7	25.3	74.1	68.2
	1.3	1.2	26.5	23.1	68.9	69.4
Mean±SE	1.02±0.21	1.86±0.36	26.7±0.75	24.8±0.40	74.6±1.68	71.9 ±1.65
23/12/71	-	4.0	-	20.8	-	66.5
	-	4.0	-	21.7	-	73.0
	-	3.6	-	21.2	-	75.4
	-	3.1	-	26.1	-	78.5
	-	7.0	-	22.7	-	77.1
Mean±SE	-	4.34±0.68	-	22.5±0.95	-	74.1±2.10
30/12/71	1.3	12.5	25.8	24.1	68.1	83.0
	<1.0	-	26.7	26.3	75.4	92.8
	2.5	11.8	26.0	26.2	74.2	82.0
	<1.0	12.0	24.8	24.6	73.4	84.3
	1.8	4.7	25.4	25.6	72.1	87.7
Mean±SE	1.52±0.28	10.25±1.86	22.3±3.46	25.4 ±0.44	72.6±1.26	86.0±1.96
6/1/72	-	21.0	-	21.3	-	77.8
	-	35.0	-	26.2	-	84.6
	-	17.0	-	23.2	-	77.6
	-	35.0	-	24.2	-	85.6
Mean±SE	-	27.0±4.69	-	23.7±1.02	-	81.4±2.12
14/1/72	1.0	8.5	26.9	22.6	80.2	81.9
	-	70.0	-	24.6	-	104.1
	-	25.0	-	24.3	-	84.0
Mean±SE		34.5±18.4		23.8±0.62		90.0±7.08

RAT HAEMATOLOGY EXPERIMENT - INDIVIDUAL RESULTS (CONT'D.)

BONE MARROW DIFFERENTIAL COUNTS

Date	Erythroid Cells		Myeloid Cells		Others		Myeloid/Erythroid Ratios	
	Normal	Infected	Normal	Infected	Normal	Infected	Normal	Infected
2/12/71	23.0	-	49.0	-	27.9	-	2.13	-
	23.2	-	41.4	-	35.3	-	1.78	-
	19.3	-	35.3	-	35.4	-	1.83	-
	24.2	-	43.9	-	31.5	-	1.81	-
	16.0	-	41.2	-	42.8	-	2.60	-
Mean ± SE	21.1±1.53	-	42.2 ±2.22	-	34.6±2.50	-	2.03±0.15	-
9/12/71	-	31.7	-	27.4	-	47.8	-	1.10
	-	28.0	-	39.1	-	41.2	-	1.95
	-	31.0	-	42.5	-	33.4	-	1.85
	-	38.2	-	37.6	-	34.2	-	1.33
	-	47.6	-	34.6	-	33.2	-	1.10
Mean ± SE	-	35.3±3.50	-	36.2±2.60	-	38.0±2.87	-	1.47±0.18
16/12/71	19.7	30.5	53.7	38.5	26.9	31.2	2.73	1.26
	16.1	21.0	50.6	44.4	34.1	34.7	-	-
	29.1	29.4	55.6	37.5	28.7	32.2	1.91	1.24
	21.4	29.0	46.6	34.5	32.0	36.0	2.18	1.19
	24.6	27.6	43.9	39.8	31.6	32.7	1.53	1.45
Mean ± SE	22.2±2.21	27.5±1.69	50.1±2.17	38.9±1.62	30.7±1.27	33.4±0.87	2.09±0.25	1.29±0.05

BONE MARROW DIFFERENTIAL COUNTS (CONT'D.)

Date	Erythroid Cells		Myeloid Cells		Others		Myeloid/Erythroid Ratios	
	Normal	Infected	Normal	Infected	Normal	Infected	Normal	Infected
23/12/71	-	27.2	-	44.6	-	28.8	-	1.63
	-	29.9	-	40.7	-	29.5	-	1.36
	-	21.1	-	42.0	-	36.9	-	2.04
	-	21.1	-	40.9	-	37.9	-	2.03
	-	27.9	-	52.4	-	33.2	-	1.88
Mean ± SE	-	25.4±1.83	-	44.1±2.18	-	33.3±1.85	-	1.79±0.13
30/12/71	25.2	30.0	50.0	41.6	24.8	30.0	1.99	1.40
	20.0	34.7	54.3	40.3	25.0	25.0	-	1.16
	21.1	26.7	41.8	52.3	25.1	21.3	1.98	1.94
	13.4	35.6	54.9	47.2	30.8	17.3	-	1.33
	15.1	21.5	47.3	51.7	37.1	26.8	-	2.40
Mean ± SE	19.0±2.17	29.7±2.61	49.7±2.41	46.6±2.49	28.6±2.42	24.1±2.20	1.99±0.00	1.65±0.23
6/1/72	-	28.6	-	43.7	-	27.6	-	1.53
	-	30.7	-	35.3	-	34.0	-	1.15
	-	21.8	-	43.1	-	35.1	-	1.98
	-	26.5	-	51.3	-	22.1	-	1.93
Mean ± SE	-	26.9±1.90	-	43.4±3.27	-	29.7±3.03	-	1.65±0.19
14/1/72	26.8	35.2	44.7	40.0	28.6	24.8	1.97	1.14
	-	30.4	-	48.8	-	20.4	-	1.61
	-	39.7	-	43.5	-	23.52	-	1.46
Mean ± SE		31.8±1.73		44.1±2.56		22.9±1.30		1.40±0.14

RADIOISOTOPE EXPERIMENTS - INDIVIDUAL RESULTS

HALF-LIVES OF ^{51}Cr *IN VIVO* LABELLED ERYTHROCYTES IN MICE ON WHOLE BODY COUNTING.

	Intact Mice		Splenectomised Mice	
	Controls	Infected	Controls	Infected
	32.36	23.51	32.71	22.29
	32.36	28.12	31.02	24.64
	27.36	23.70	31.34	23.51
	31.35	26.40	-	26.17
	29.50	26.17	-	23.88
	33.07	24.66	-	24.06
	30.70	22.13	-	-
	31.35	22.13	-	-
	32.36	22.46	-	-
	31.02	25.08	-	-
	-	21.81	-	-
	-	23.70	-	-
	-	24.46	-	-
	-	19.80	-	-
	-	23.70	-	-
	-	25.08	-	-
	-	25.08	-	-
	-	23.51	-	-
	-	22.97	-	-
Mean $\pm$ SE	31.1 ± 0.5	23.9 ± 0.04	31.7 ± 0.5	24.1 ± 0.5

Each value is the ^{51}Cr half-life of one mouse in days.

RADIOISOTOPE EXPERIMENTS - INDIVIDUAL RESULTS (CONT'D).

HALF-LIVES OF ^{51}Cr-LABELLED ERYTHROCYTES IN THE CIRCULATION OF MICE.

	Normal	Infected
	12.54	1.16
	7.38	0.84
	10.45	2.51
	5.02	-
	5.97	-
Mean $\pm$ SE	8.3 $\pm$ 1.4	1.5 $\pm$ 0.5

Each value is the circulating half-life of ^{51}Cr of one mouse in days.

RADIOISOTOPE EXPERIMENTS - INDIVIDUAL RESULTS (CONT'D).

HALF-LIVES OF ^{51}Cr-LABELLED ERYTHROCYTES IN THE CIRCULATION OF RATS*.

	Phase I 0-21 days of infection**	Normal	Phase II > 21 days of infection**	Normal
	2.0	12.28	1.9	10.31
	1.8	12.75	1.8	10.06
	1.8	12.80	1.8	11.80
	2.5	12.43	1.8	12.64
	2.8	11.31	-	13.68
	3.2	-	-	14.33
Mean±SE	2.35±0.23	12.31±0.27	1.83±0.00	12.14±0.71

* Each value is the circulating ^{51}Cr half-life of one mouse in days.

** Values obtained from semi-logarithmic plots.

RADIOISOTOPE EXPERIMENTS - INDIVIDUAL RESULTS (CONT'D).

HALF-LIVES OF ^{59}Fe IN THE PLASMA OF MICE*.

	Infected	Normal
	0.33	1.57
	0.44	1.74
	0.28	1.72
	0.52	1.43
	-	0.98
Mean ± SE	0.39 ± 0.04	1.49 ± 0.14

* Each value is the serum ^{59}Fe half-life of one mouse in hours.

HALF-LIVES OF ^{59}Fe IN MICE ON WHOLE BODY COUNTING*.

	Infected	Normal
	196.67	133.73
	180.18	123.32
	182.36	150.45
	98.01	74.11
	129.14	114.41
	120.84	128.59
	181.27	125.38
	109.82	138.03
	125.90	134.93
	164.43	-
Mean ± SE	154.50 ± 10.95	131.10 ± 3.84

* Each value is the whole body ^{59}Fe half-life of one mouse in days.

CHAPTER 3

SUPPRESSION OF THE IMMUNE RESPONSE TO SRBC IN MICE INFECTED WITH T. BRUCEI*

	4 d between immunisation & infection	Normal	2 d between immunisation & infection	Normal	3 d between infection & immunisation	Normal
	6.0	7.0	8.0	-	7.0	9.0
	9.0	6.0	7.0	-	6.0	9.0
	8.0	6.0	6.0	-	5.0	8.0
	7.0	9.0	8.0	-	7.0	8.0
	7.0	8.0	-	-	-	-
Mean±SE	7.4±0.5	7.2±0.6	7.3±0.5	-	6.3±0.5	8.5±0.3

	7 d between infection & immunisation	Normal	21 d between infection & immunisation	Normal	70 d between infection & immunisation	Normal
	0	9.0	0	7.0	0	7.0
	0	7.0	0	8.0	0	7.0
	0	8.0	0	8.0	0	6.0
	0	8.0	0	8.0	0	6.0
	0	9.0	0	7.0	0	7.0
	0	-	0	-	-	-
Mean±SE	0	8.2±0.4	0	7.6±0.2	0	6.6±0.2

* Each value is the $\log_2$ haemagglutinating antibody titre of the serum of an individual mouse 6 days after immunisation with 5×10^8 SRBC.

CIRCULATING ^{51}Cr ACTIVITY 1 HOUR AFTER INJECTION OF ^{51}Cr SRBC*

No.of injected SRBC	Infected	Normal
5×10^9	916.487	1496.045
	2029.577	1519.381
	141.271	2112.200
	2070.557	1788.394
	1405.907	2613.345
	1390.178	2705.356
	399.627	2295.250
	723.843	2020.942
	601.849	-
Mean $\pm$ SE	1108 $\pm$ 262	2069 $\pm$ 162
2×10^9	52.929	1290.786
	6.435	1398.013
	1054.924	281.140
	334.490	811.072
	0	288.136
	0	-
	694.858	-
	497.150	-
Mean $\pm$ SE	330 $\pm$ 139	733 $\pm$ 131
0.9×10^9	12.387	192.44
	0	100.463
	0	281.448
	0	385.133
	397.003	250.358
	0	341.333
	0	264.047
	0	383.345
	0	-
	14.819	-
Mean $\pm$ SE	42 $\pm$ 39	275 $\pm$ 34

* Each value is the mean number of counts/minute/mg packed erythrocytes.

24 HOUR LIVER/SPLEEN DISTRIBUTION OF INTRAVENOUS ^{51}Cr SRBC*

No.of injected SRBC	Infected		Normal	
	Spleen	Liver	Spleen	Liver
2×10^9	20.24	53.15	21.12	16.00
	14.15	54.80	12.03	14.22
	13.28	80.57	21.65	15.11
	-	-	24.12	17.72
	-	-	15.54	17.36
Mean$\pm$SE	15.8$\pm$2.2	62.8$\pm$8.9	18.9$\pm$2.2	16.1$\pm$0.7
2×10^8	5.25	90.06	8.28	74.84
	2.12	92.99	3.56	79.53
	2.21	87.80	1.15	57.89
	2.30	94.33	5.37	75.89
	-	-	5.84	71.95
Mean$\pm$SE	3.0$\pm$0.8	91.3$\pm$1.5	4.8$\pm$1.2	72.0$\pm$3.7
2×10^7	1.95	90.54	2.00	89.60
	5.48	72.27	1.05	93.86
	2.17	94.03	1.04	92.29
	1.61	92.34	1.36	87.23
	3.01	91.18	0.73	90.34
Mean$\pm$SE	2.8$\pm$0.7	89.1$\pm$3.0	1.2$\pm$0.2	90.7$\pm$1.1

* Each value is the percentage of the total body activity at necropsy.

EFFECT OF CARBON BLOCKADE ON ^{51}Cr SRBC LIVER/SPLEEN DISTRIBUTION*

Time after SRBC injection		Infected		Normal	
		No Carbon	With Carbon	No Carbon	With Carbon
2 hours	Spleen	1.74	1.71	2.01	19.16
		2.49	3.77	2.22	16.54
		0.94	4.90	6.17	17.69
		4.47	5.08	2.41	33.94
		1.83	3.84	2.10	12.36
		-	-	-	12.20
Mean±SE		2.3±0.6	3.9±0.6	3.0±0.8	18.7±3.3
	Liver	90.70	91.45	87.63	62.53
		81.17	88.64	82.71	65.86
		91.45	84.97	81.85	60.61
		88.87	88.23	86.18	38.73
		90.70	91.29	90.11	66.78
		-	-	-	64.86
Mean±SE		88.6±1.9	88.9±1.2	85.7±1.5	59.9±4.3
24 hours	Spleen	5.25	2.48	8.28	34.21
		2.12	1.28	3.56	27.60
		2.21	1.46	1.15	32.30
		2.30	-	5.37	14.73
		-	-	5.84	22.96
		-	-	-	23.80
		-	-	-	18.00
		-	-	-	24.12
Mean±SE		3.0±2.8	1.7±0.4	4.8±1.2	24.7±2.3
	Liver	90.06	92.51	74.84	42.85
		92.99	95.49	79.53	34.44
		87.80	42.26	57.89	39.61
		94.33	-	75.89	66.27
		-	-	71.95	45.93
		-	-	-	45.93
		-	-	-	57.20
		-	-	-	42.24
Mean±SE		91.3±1.5	93.7±1.0	72.0±3.7	46.8±3.6

* Each value is the percentage of the total body activity at necropsy.

MACROPHAGE/SHEEP ERYTHROCYTE TRANSFER EXPERIMENT

Presentation of SRBC	Log_2 SRBC agglutinin titres of the sera of recipient mice	
	Normal	Infected
A In macrophages from normal mice	4.0	2.0
	4.0	0
	3.0	0
	2.0	0
	4.0	1.0
	3.0	-
Mean ± SE	3.3±0.3	0.6±0.4
B In macrophages from infected mice	4.0	1.0
	3.0	1.0
	4.0	2.0
	2.0	-
Mean ± SE	3.3±0.5	1.3±0.3
C 5×10^8 SRBC in 1.0 ml PBS	7.0	2.0
	7.0	0
	8.0	1.0
	5.0	1.0
	5.0	1.0
	2.0	-
Mean ± SE	5.7±0.9	1.0±0.3
D Nil	0	1.0
	0	0
	0	0
	0	0
	0	2.0
	0	-
	0	-
Mean ± SE	0	0.6±0.4

SPLENIC PLAQUE-FORMING CELL RESPONSE AT DIFFERENT TIMES AFTER IMMUNISATION WITH 5×10^8 SRBC*

Days after immunisation	Infected Mice IgM PFC	Infected Mice IgG PFC	Normal Mice IgM PFC	Normal Mice IgG PFC
3	3.2201	4.0161	4.6406	0.0000
	3.8617	4.0107	4.2181	0.0000
	3.3493	3.1911	4.0342	0.0000
	0.0000	0.0000	4.1459	0.0000
Mean ± SE	2.59±0.87	2.80±0.95	4.25±0.14	0.0000
5	3.2949	0.0000	4.8717	0.0000
	0.0000	3.7281	4.9414	3.5027
	3.2300	0.0000	5.1599	0.0000
	3.1284	0.0000	5.0302	0.0000
Mean ± SE	2.41± 0.80	0.93±0.93	5.00±0.06	0.87±0.87
8	2.4518	3.2180	3.7826	4.0697
	3.6224	2.1584	4.6571	4.8332
	0.0000	2.3856	4.0051	4.5839
	0.0000	2.1553	4.3720	4.8591
Mean ± SE	1.51±0.90	2.47±0.26	4.20±0.20	4.58±0.22
Unimmunised Controls	4.9293	0.0000	1.9685	0.0000
	4.6852	0.0000	0.0000	0.0000
	4.2377	0.0000	0.0000	0.0000
	0.0000	3.6006	0.0000	2.2279
	4.1412	0.0000	-	-
	0.0000	0.0000	-	-
Mean ± SE	2.99±0.96	0.60±0.60	0.49±0.48	0.55±0.55

* Each value is the $\log_{10}$ total splenic PFC from one mouse.

THE DIRECT PLAQUE-FORMING CELL RESPONSE TO VARIOUS DOSES OF SRBC*

Days after immunis.	Immunising Dose 5×10^6		5×10^7		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal
3	3.9302	3.6355	3.1430	3.6993	3.2584	4.0429
	2.9315	2.9939	2.8016	3.8581	2.9465	3.9073
	3.8293	3.0367	0.0000	4.3387	3.5894	3.7871
	0.0000	2.9180	3.6123	3.6180	3.9205	4.1415
Mean $\pm$ SE	2.67 ± 0.91	3.14 ± 0.17	2.38 ± 0.81	3.87 ± 0.20	3.42 ± 0.22	3.96 ± 0.10
5	2.9058	4.4440	2.3385	4.3736	3.5284	5.0697
	0.0000	2.6618	0.0000	4.7500	3.9729	5.1759
	3.6304	3.0630	0.0000	4.7874	0.0000	4.8569
	3.2806	4.2695	3.2727	4.5275	3.7433	4.3446
Mean $\pm$ SE	2.45 ± 0.83	3.60 ± 0.44	1.40 ± 0.83	4.60 ± 0.14	2.81 ± 0.94	4.85 ± 0.22
8	4.2168	3.1473	2.6972	4.2754	2.4518	4.3678
	3.8042	3.4009	3.3416	4.2790	3.6224	3.8327
	3.6839	3.2148	0.0000	4.2191	0.0000	3.7447
	0.0000	3.0320	0.0000	3.8468	0.0000	3.7927
Mean $\pm$ SE	2.92 ± 0.98	3.19 ± 0.10	1.50 ± 0.87	4.14 ± 0.17	1.51 ± 0.90	3.93 ± 0.17

* Each value is the $\log_{10}$ total direct splenic PFC from one mouse. The response to 5×10^8 SRBC can be seen in the table headed "Splenic plaque-forming cell response at various times after immunisation with 5×10^8 SRBC".

THE DEVELOPED PLAQUE-FORMING CELL RESPONSE TO VARIOUS DOSES OF SRBC*

Days after immunis.	Immunising Dose 5×10^6		5×10^7		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal
3	4.2495	0.0000	0.0000	3.5245	0.0000	3.4707
	3.5515	0.0000	3.8988	0.0000	3.2653	0.0000
	0.0000	0.0000	4.1399	0.0000	3.0051	0.0000
	3.5877	0.0000	0.0000	0.0000	0.0000	0.0000
Mean $\pm$ SE	2.84 ± 0.96	0.0000	2.00 ± 1.16	0.88 ± 0.87	1.56 ± 0.90	0.86 ± 0.86
5	3.2068	3.3092	2.6580	0.0000	4.4843	0.0000
	0.0000	0.0000	4.0712	0.0000	0.0000	0.0000
	3.7735	0.0000	3.3809	0.0000	0.0000	0.0000
	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mean $\pm$ SE	1.74 ± 1.01	0.82 ± 0.82	2.52 ± 0.88	0.0000	1.12 ± 1.11	0.0000
8	2.1987	4.1093	0.0000	4.7041	0.0000	4.0697
	2.5922	3.0916	2.4871	4.0580	0.0000	4.8332
	0.0000	0.0000	2.1553	4.4448	0.0000	4.5839
	0.0000	0.0000	0.0000	4.3799	0.0000	4.8591
Mean $\pm$ SE	1.19 ± 0.69	1.79 ± 1.05	1.15 ± 0.67	4.39 ± 0.17	0.0000	4.58 ± 0.22

* Each value is the $\log_{10}$ total developed splenic PFC from one mouse. The response to 5×10^8 SRBC can be seen in the table headed "Splenic plaque-forming cell response at various times after immunisation with 5×10^8 SRBC".

THE PASSIVE HAEMOLYTIC ANTIBODY RESPONSE TO LIPOPOLYSACCHARIDE (LPS)

Days after immunis.	Immunising dose of LPS 1 µg		10 µg		100 µg	
	Infected	Normal	Infected	Normal	Infected	Normal
3	0	0	0	0.00	0	0
	0	0	0	4.59	0	0
	0	0	0	0.00	0	0
	0	0	0	4.59	0	0
	0	0	0	3.59	0	0
	0	0	0	0.00	0	0
Mean ± SE	0	0	0	2.12±0.95	0	0
6	0	0	8.59	7.59	6.59	7.59
	0	0	2.59	7.59	6.59	8.59
	0	0	0.00	4.59	0.00	8.59
	0	0	0.00	3.59	0.00	7.59
	0	0	4.59	8.59	-	4.59
	0	0	-	10.59	-	4.59
Mean ± SE	0	0	3.15±1.60	7.09±1.05	3.29±1.10	6.92±0.75
9	0	5.59	0	13.59	0.00	5.59
	0	6.59	0	13.59	0.00	4.59
	0	6.59	0	8.59	0.00	5.59
	0	4.59	0	7.59	4.59	5.59
	0	0.00	0	13.59	-	5.59
	0	4.59	0	-	-	5.59
Mean ± SE	0	4.65±1.00	0	11.39±1.35	1.14±1.14	5.42±0.14

Each value is the $\log_2$ indirect haemolytic antibody titre of one mouse. LPS sensitised SRBC were used as an indicator.

THE DIRECT PLAQUE-FORMING CELL RESPONSE TO LIPOPOLYSACCHARIDE (LPS)

	Immunising dose of LPS		
	0	10 µg	100 µg
Normal mice	0	3.0656	5.1914
	0	3.3284	3.7226
	-	3.4362	4.1959
	-	4.3181	3.4133
	-	-	4.4354
	-	-	3.7513
Mean ± SE	0	3.54±0.27	4.12±0.26
Infected mice	3.0755	4.0367	2.3856
	2.2695	3.8828	0.0000
	-	2.5263	-
	-	2.5658	-
	-	0.0000	-
	-	2.8871	-
Mean ± SE	2.67±0.40	2.65±0.59	1.19±1.19

Each value is the $\log_{10}$ total plaque-forming cells from the spleen of one mouse.

LPS sensitised SRBC were used as an indicator.

CHANGES IN EAR THICKNESS ON EXPOSURE TO OXAZOLONE

Control	Sensitised & challenged	Challenged only	Infected	Sensitised & challenged	Challenged only	
2.8	4.8	2.8	3.3	4.6	3.3	
2.3	5.3	3.3	2.8	5.0	2.8	
2.7	5.3	2.8	3.2	3.3	3.2	
2.5	4.8	3.1	2.7	5.0	3.0	
-	6.0	3.6	2.3	4.5	3.0	
-	5.2	3.0	2.8	4.1	3.0	
-	4.8	3.8	3.3	4.5	3.3	
-	-	-	-	3.8	3.5	
-	-	-	-	5.0	3.3	
-	-	-	-	4.5	3.2	
-	-	-	-	4.8	3.5	
-	-	-	-	4.5	3.5	
-	-	-	-	4.5	3.7	
-	-	-	-	3.8	3.3	
-	-	-	-	5.5	4.0	
-	-	-	-	-	-	
2.6 ± 0.1	5.2 ± 0.16	3.2 ± 0.14	2.9 ± 0.14	4.5 ± 0.14	3.3 ± 0.08	Mean $\pm$ SE

Each value represents the mean thickness in mm of readings taken from the anterior margin, middle and bottom margin of the ear.

^{125}I UdR INCORPORATION IN THE REGIONAL LYMPH NODES AFTER SENSITISATION WITH OXAZOLONE

Days after infection	Infected		Normal	
	Sensitised	Non-sensitised	Sensitised	Non-sensitised
-1	2290.2	162.8	2417.6	87.9
	3155.8	301.4	3137.6	382.3
	3399.3	75.9	3132.0	243.4
	2426.9	245.9	3454.9	363.5
	3017.3	225.1	2417.0	69.7
	2488.6	62.2	2919.4	325.1
	3128.2	82.2	3136.8	229.4
	3130.7	165.5	2859.7	179.9
	2683.3	186.6	2773.7	201.8
	-	229.4	4060.0	219.5
Mean$^{+}_{-}$SE	2861$^{+}_{-}$116	174$^{+}_{-}$25	3031$^{+}_{-}$153	230$^{+}_{-}$33
0	3414.9	160.1	2721.0	162.4
	3127.8	206.5	2723.3	155.1
	1696.4	106.0	2460.3	119.2
	2984.5	151.7	2958.2	215.1
	2829.7	280.2	4308.0	215.4
	4157.9	232.9	3255.0	96.7
	3457.2	101.0	4220.0	116.4
	2860.6	84.2	3648.5	141.1
	3203.3	272.7	3583.5	158.5
	-	216.7	4232.5	106.2
Mean$^{+}_{-}$SE	3081$^{+}_{-}$219	181$^{+}_{-}$22	3410$^{+}_{-}$219	148$^{+}_{-}$13
+1	2654.5	91.2	2762.2	200.2
	3442.0	246.8	4130.9	180.3
	3205.8	237.4	4311.8	252.1
	776.0	180.1	3665.1	205.7
	865.7	218.2	2058.1	289.8
	3994.8	192.4	2901.2	280.2
	1031.1	211.2	3869.2	147.2
	3767.9	199.5	3374.2	139.9
	3637.7	73.8	4484.5	186.2
	3923.3	179.8	-	262.5
Mean$^{+}_{-}$SE	2730$^{+}_{-}$419	183$^{+}_{-}$18	3508$^{+}_{-}$268	214$^{+}_{-}$17

* Each value is the ^{125}I cpm of the nodes of one mouse, corrected to an arbitrary standard of 500,000 cpm.

^{125}I UdR INCORPORATION IN THE REGIONAL LYMPH NODES AFTER SENSITISATION WITH OXAZOLONE (CONT'D).

Days after infection	Infected		Normal	
	Sensitised	Non-sensitised	Sensitised	Non-sensitised
+3	3212.4	413.5	3821.3	364.8
	2786.4	227.2	3712.2	278.1
	2680.0	213.4	3112.8	178.6
	3309.4	330.6	3343.0	208.3
	3854.9	164.5	2554.0	267.6
	3841.3	366.1	2915.7	197.7
	2956.6	229.9	3220.2	104.3
	2960.2	360.0	3724.8	257.6
	3893.6	92.7	6441.5	206.1
	4418.2	230.2	1883.6	180.3
Mean ± SE	3391±182	263±32	3473±379	224±22
+6	2671.9	390.4	2354.9	205.1
	1750.6	224.2	1735.5	200.8
	2814.9	814.2	2320.2	177.7
	2229.3	1307.3	1932.5	188.8
	4111.7	838.1	1724.9	349.0
	3073.2	1358.2	1905.7	157.4
	2878.7	1292.3	2904.6	356.1
	1162.8	1288.2	1611.3	153.9
	2992.9	466.4	3166.4	254.2
	1638.9	566.9	2776.4	157.3
Mean ± SE	2532±270	855±137	2243±174	220±24
+10	2699.8	625.3	5709.7	288.1
	2759.2	1669.5	3452.0	270.0
	1775.7	1909.2	2535.4	242.4
	1970.1	2164.3	3020.0	184.9
	3015.7	588.9	3893.7	190.2
	2814.6	682.0	3271.8	314.3
	2477.9	503.7	3125.3	226.5
	1624.4	622.9	3989.2	116.8
	3795.3	996.0	2280.8	223.8
	2321.6	1038.3	4860.2	274.9
Mean ± SE	2525±204	1080±193	3413±237	233±18

Each value is the ^{125}I cpm of the nodes of one mouse corrected to an arbitrary standard of 500,000 cpm.

^{125}I UdR INCORPORATION IN THE REGIONAL LYMPH NODES AFTER SENSITISATION WITH OXAZOLONE (CONT'D).

Days after infection	Infected		Normal	
	Sensitised	Non-sensitised	Sensitised	Non-sensitised
+17	2173.4	1485.4	2317.0	88.3
	2086.2	799.6	1335.0	105.7
	2886.3	835.0	2175.8	116.1
	2882.8	1339.4	2604.9	173.4
	1274.6	1192.2	2595.6	129.1
	1623.9	602.3	1526.6	217.8
	1710.6	884.6	2426.2	169.0
	2141.8	1496.8	2716.6	208.6
	1308.0	380.0	829.5	144.5
	2074.6	1042.0	3409.3	87.3
Mean $\pm$ SE	2016$\pm$178	1005$\pm$118	2194$\pm$240	144$\pm$15
+24	2480.6	455.3	2308.8	140.4
	1754.1	823.5	2170.6	133.7
	1588.0	512.5	3094.5	74.2
	4013.4	871.5	2237.2	109.3
	1448.0	-	2954.1	-
Mean $\pm$ SE	2256$\pm$473	666$\pm$106	2553$\pm$195	114$\pm$15

Each value is the ^{125}I cpm of the nodes of one mouse, corrected to an arbitrary standard of 500,000 cpm.

THE RESTORATION OF ^{125}I UdR INCORPORATION IN THE REGIONAL LYMPH NODES AFTER SENSITISATION WITH OXAZOLONE ON TREATMENT OF A 36 DAY T. BRUCEI INFECTION.

	Infected Mice					
	Sensitised day 0	Sensitised day 3	Sensitised day 7	Not sensitised with oxazolone day 0	day 3	day 7
Berenil treated day 0*	855.9	2503.5	1388.6	185.7	324.1	113.4
	1569.7	2009.2	1207.6	150.2	182.8	148.6
	1312.5	2862.7	2500.7	223.9	145.8	242.6
	1138.5	2010.8	1339.1	291.9	259.6	150.3
	482.0	2241.9	1727.9	226.0	229.5	185.8
	1443.5	1841.7	1397.5	178.9	160.5	291.5
	1265.1	2609.4	1448.9	48.3	544.5	185.8
	1086.5	1565.5	1995.9	385.3	84.0	241.4
	1372.9	1593.5	1637.6	399.2	249.7	180.8
	364.3	1514.0	1507.0	154.6	288.8	203.1
Mean $\pm$ SE	1089.0$\pm$127.7	2075.3$\pm$148.6	1615.1$\pm$121.2	224.4$\pm$34.3	246.9$\pm$40.2	194.3$\pm$16.7
Not treated with Berenil	907.1	812.8	863.9	949.2	574.4	297.4
	946.5	33.9	1142.3	421.4	1258.9	553.4
	539.7	1914.1	594.2	935.5	685.2	427.4
	1422.4	2773.3	1152.6	817.5	1368.6	662.7
	309.8	1429.7	795.6	964.0	117.1	587.8
	461.3	66.0	220.0	697.4	487.1	2177.5
	376.3	1383.7	2635.6	819.5	1269.2	435.7
	244.7	3154.9	1684.2	1014.6	810.1	1194.4
	309.3	993.4	1833.5	479.8	740.3	894.0
	407.9	-	-	806.1	427.6	-
Mean $\pm$ SE	592.5$\pm$120.0	1395.8$\pm$361.1	1213.5$\pm$244.5	790.5$\pm$64.1	773.9$\pm$129.9	803.4$\pm$ 194.0

* Day 0 of berenil treatment corresponds to the thirty sixth day of infection. Each value is the ^{125}I cpm of the nodes of one mouse, corrected to an arbitrary standard of 500,000 cpm.

THE RESTORATION OF ^{125}I UdR INCORPORATION IN THE REGIONAL LYMPH NODES AFTER SENSITISATION WITH OXAZOLONE ON TREATMENT OF A 36 DAY T. BRUCEI INFECTION (CONT'D).

	Control Mice					
	Sensitised day 0	Sensitised day 3	Sensitised day 7	Not sensitised with oxazolone day 0	day 3	day 7
Berenil treated day 0	2219.4	-	-	72.9	-	-
	2232.5	-	-	35.5	-	-
	1684.0	-	-	47.8	-	-
	3028.4	-	-	70.8	-	-
	2210.3	-	-	27.7	-	-
	2468.0	-	-	54.6	-	-
	2891.0	-	-	90.0	-	-
	1814.8	-	-	61.6	-	-
	2103.9	-	-	42.5	-	-
Mean ± SE	2294.7±148.1	-	-	55.9±6.6	-	-
Not treated with Berenil	2357.0	2029.3	1881.4	224.5	61.3	82.7
	1233.8	2200.8	2282.9	233.4	82.6	74.1
	1616.9	2100.4	2378.1	101.0	87.0	91.1
	2306.7	1587.6	1425.1	82.2	79.3	115.5
	2045.8	2200.8	2178.1	201.3	136.8	105.7
	2390.5	3833.6	1495.9	92.6	87.5	43.5
	2248.6	1406.5	1669.0	254.4	34.3	88.7
	2570.9	3660.9	1860.5	35.5	39.3	59.5
	2459.3	3498.4	2169.6	86.4	101.6	79.6
	2008.7	1815.3	1514.0	62.3	80.8	63.1
Mean ± SE	2123.8±131.5	2433.4±281.5	1855.5±111.4	137.4±25.7	79.1±9.4	80.4±6.8

Each value is the ^{125}I cpm of the nodes of one mouse, corrected to an arbitrary standard of 500,000 cpm.

COURSE OF A TYPICAL NIPPOSTRONGYLUS BRASILIENSIS INFECTION

Following subcutaneous inoculation of N. brasiliensis into adult rats, larvae travel via the lungs to the small intestine where by day 3-4 they establish a stable adult worm population. At this time the majority of the parasites, which lie intertwined between intestinal villi, are concentrated in a 4-5 cm length of the jejunum about 20 cm from the pylorus. The host's immune response to these worms can be assessed by the worm loss which occurs in two phases. The initial phase begins around day 10 of inoculation when there is a slow loss of worms; the subsequent phase starts at day 12 or 13, is exponential, and proceeds at a faster rate. By day 18 virtually all the worms have been lost from the intestine.

IMMUNOSUPPRESSION IN BOVINE TRYPANOSOMIASIS

By

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The association of immunosuppression to foreign antigens by hosts concurrently infected with protozoal diseases has recently received considerable attention. This phenomenon was first described in human malaria and its practical significance is such that it is now recommended that vaccination campaigns against the common childhood diseases should not be carried out during periods of the year when the incidence of malaria is high (Greenwood et al., 1972).

Goodwin (1970) and Goodwin et al., (1972) first reported the immunosuppressive effect of trypanosome infections in rabbits and mice. In this work sheep erythrocytes were used as the antigen and it was found that after infection the haemagglutinin response was significantly reduced. Several other workers have now confirmed and extended these findings (Urquhart et al., 1973 and Freeman et al., 1974).

Unfortunately to date all the reported studies on immunosuppression in trypanosomiasis have been confined to small laboratory animals, and whilst these species are undoubtedly of considerable value in studying underlying mechanisms, the practical relevance of the phenomenon can only be determined in large domestic ruminants. Recently we were able to examine the immune responsiveness of Zebu cattle to a polyvalent clostridial

vaccine* after experimental infection with T. congolense and this is a preliminary report of some of our findings.

Sixteen healthy male yearling Zebu cattle were purchased from a trypanosome-free area at an altitude of 7,000'-8,000' on the Ethiopian plateau adjacent to Addis Ababa. They were initially all treated with anthelmintics to remove gastro-intestinal nematodes** and liver fluke+ and then divided into two groups; one of six animals (Group A) and one of ten animals (Group B). Both groups were then inoculated with a polyvalent clostridial vaccine and three weeks later Group A was infected with a recently isolated field strain of T. congolense. After a further three weeks both groups received a second inoculation of the clostridial vaccine. Regular serum samples were taken throughout the study until two weeks after the second vaccination. All the infected animals developed a positive parasitaemia, a degree of anaemia, and an unthrifty appearance although weight losses were not significant and their appetites remained good.

The sera were returned to the U.K. for examination of the antitoxin responses to the various components of the vaccine. A dramatic immunosuppressive effect after infection with T. congolense was found to all three vaccine components (Tetani, Septicum and Oedematiens alpha). The mean response of the two groups to the Oedematiens alpha component are given in Figure 1. It is readily apparent that the primary immune response of both groups, prior to infection of Group A, were identical, but that following infection of this group there was a marked difference

* Tribovax - Wellcome Research Laboratories, Beckenham.
** Thiabenzole - Merck, Sharp & Dohme.
\+ Zanil - I.C.I.

in the secondary response between the two groups ($P < 0.001$) clearly illustrating the suppressive effect of the trypanosome infection on the immune response to a second vaccination. These findings thus confirm those of earlier studies on small laboratory animals.

The exact mechanisms involved in trypanosome-induced immunosuppression are still the subject of considerable debate although it has been suggested from recent findings in small laboratory animals that it is possibly due to the specific effect of trypanosomes on the B lymphocyte population (Murray et al., 1974).

However, regardless of the exact mechanisms involved, the demonstration of immunosuppression in bovine trypanosmiasis could clearly have considerable veterinary importance especially in Africa, where vaccination campaigns are vital to the successful development of the livestock industry. In Africa trypanosomiasis is already recognised as a cause of massive economic loss and the results of the present study suggest that immunosuppression is another aspect of trypanosomiasis which may contribute to these serious economic effects through interference with immunisation and a predisposition to other infections.

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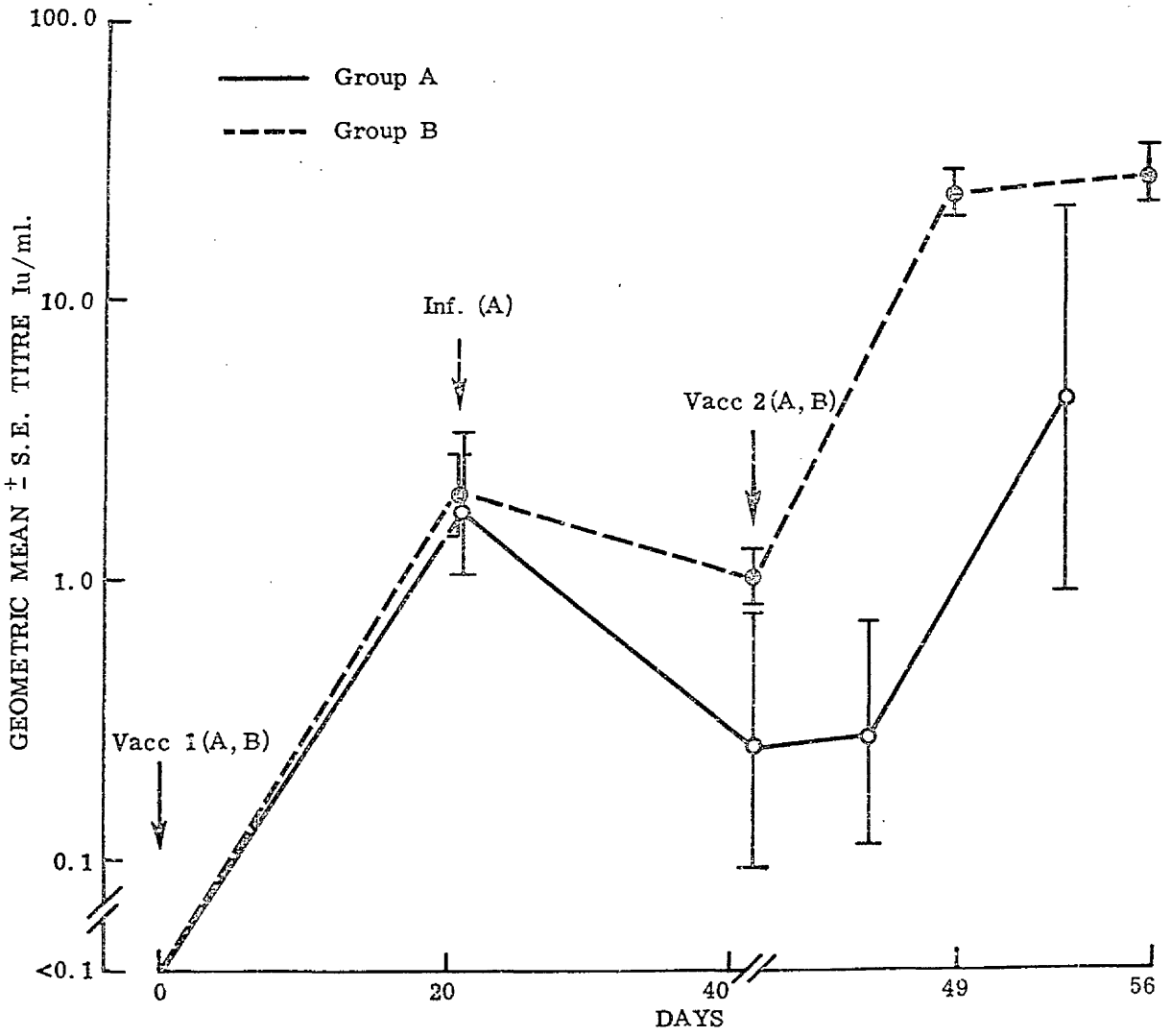

The antitoxin response of cattle experimentally infected with T. congolense (Group A) and normal cattle (Group B) to a booster inoculation of Clostridium oedamatiens alpha vaccine.

THE EFFECT OF T. BRUCEI INFECTION ON THE MAST CELL RESPONSE DURING N. BRASILIENSIS INFECTION*

	Duration of N. brasiliensis infection (days)					
	7		14		18	
	Infected	Control	Infected	Control	Infected	Control
	25.10	5.45	9.40	28.50	12.20	45.25
	2.00	2.65	00	44.40	3.00	40.45
	3.80	4.25	11.85	35.95	3.40	39.95
	1.35	2.90	00	29.37	3.00	32.20
	2.15	8.30	4.05	13.00	21.10	27.50
Mean±SE	6.9±4.6	4.7±1.0	5.0±2.4	30.2±5.2	8.5±3.6	37.1±3.1

* Each value is the mean number of mast cells seen per villous crypt unit from an individual rat.

THE EFFECT OF T. BRUCEI INFECTION ON A CHALLENGE INFECTION OF 1,000 N. BRASILIENSIS LARVAE*

Group No.	1	2	3	4	5	6
	484	10	392	1478	9	80
	243	32	510	1418	69	35
	586	3	395	1604	91	3
	349	44	440	1448	113	72
	424	5	298	1403	207	130
	191	3	340	1307	-	-
	552	0	285	1570	-	-
	437	54	666	1633	-	-
	476	-	340	1560	-	-
	206	-	320	1603	-	-
Mean±SE	395±45	20±9	397±37	1502±34	98±32	64±21

* Each value is the total number of adult N. brasiliensis obtained from the small intestine of the rats at necropsy.

THE EFFECT OF A T. BRUCEI INFECTION ON THE MAST CELL RESPONSE 8 DAYS AFTER A CHALLENGE N. BRASILIENSIS INFECTION*

Group No.	1	2	3
	23.85	37.75	3.60
	21.45	38.90	2.35
	15.90	29.85	8.45
	19.30	30.65	3.80
	22.90	34.30	4.00
Mean ± SE	20.7 ±1.4	34.3 ±1.8	4.4 ±1.0

* Each value is the mean number of mast cells seen per villous crypt unit from an individual rat.

THE EFFECT OF BERENIL ON THE RESPONSE OF RATS TO A CHALLENGE N. BRASILIENSIS INFECTION AFTER A T. BRUCEI INFECTION*

Group No.	1	1A	2	2A	4
	305	8	0	8	1747
	431	7	0	33	1693
	205	10	0	8	2122
	331	12	0	4	1700
	227	18	0	20	1822
	475	47	0	0	1644
	240	32	9	5	1717
	349	46	0	2	1604
	213	100	0	0	-
	217	0	0	6	-
	-	25	-	7	-
Mean±SE	299±30	28±9	1±1	8±3	1756±57

* Each value is the total number of adult N. brasiliensis obtained from the small intestine of the rats at necropsy.