THIS THESIS IS DEDICATED

TO

THE MEMORY OF MY PARENTS.

MORPHOLOGICAL AND ULTRASTRUCTURAL CHANGES

IN SMALL BLOOD VESSELS OF RABBITS INFECTED

WITH *TRYPANOSOMA BRUCEI*

by

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ADDENDUM

All measurements given in Angström ($\overset{o}{A}$)

units should be divided by 10, to obtain

the nanometre (nm) equivalent

ABSTRACT

A combination of light and electron microscopic techniques was used to investigate changes in small blood vessels of rabbits infected with *Trypanosoma* (*Trypanozoon*) *brucei*; with a view to elucidating the mechanisms involved in the damage.

About 21 days after infection, rabbits showed gross oedema and scabby necrotic lesions which increased with time. Histopathological studies showed features of inflammation manifested by infiltration of tissues by leucocytes including the lining of venules and capillaries, oedema and tissue necrosis.

The mesentery was used as a model to study changes in the micro-vessels. In-vivo microscopy showed disturbance in venular flow, indicating possible haemodynamic defects. Ultrastructural studies were unsuccessful, but light microscopic results conformed to the histopathological observations.

The cremaster muscle was chosen as an alternative to the mesentery, for further studies on the small blood vessels.

Light microscopic and ultrastructural results showed damage to the endothelium of venules and capillaries.

Gaps formed between venular endothelial cells allowed the escape of amorphous material (probably plasma proteins) and other endogenous elements; indicating increase in vascular permeability.

Leucocyte margination, emigration (through the endothelial gaps) and infiltration of extravascular tissues occurred largely in the venules. Leucocytes marginated on vascular endothelium, either by means of cytoplasmic processes on both cells or by cell to cell contact.

Erythrocytes and their fragments extravasated through the gaps were phagocytosed by macrophages. It is suggested that erythrophagocytosis and fragmentation of the erythrocytes could contribute to the haemolytic anaemia.

Stasis and microthrombi occurred predominantly in the venules 14 days after infection. These events support the changes known to occur in the rheological properties of blood in rabbits infected with *T. brucei* and provide further evidence of vascular damage.

The degenerative changes seen in the muscle fibres and other tissues are probably due to ischaemia and anoxia resulting from the vascular damage observed.

These observations are consistent with known vascular events in inflammation caused by chemical mediators, such as histamine and kinins and give the ultrastructural basis for the pathological changes in the blood during trypanosome infection of rabbits.

PART 1.   INTRODUCTION

1.1   TRYPANOSOMES AND TRYPANOSOMIASES

1.1.1. ECONOMIC IMPORTANCE

African trypanosomiases constitutes a group of diseases in humans and domestic animals caused by members of the genus *Trypanosoma* spp. These parasites cause sleeping sickness in humans and nagana in cattle. About 35 million people in Africa are at risk from sleeping sickness. During epidemics, mortality from the human disease approaches 100%. Typical examples are the epidemics in Zaire and on the Northern shore of Lake Victoria at the beginning of the century, when thousands of the inhabitants died. Many villagers are driven away from their fertile lands during such outbreaks, with the result that food production is diminished and the population suffer from malnutrition.

Although about 10,000 new cases of sleeping sickness are reported annually (WHO, 1976), many cases go unnoticed either out of ignorance, poverty or inadequate medical facilities. The disease however continues to pose a serious threat to the population at large, especially in endemic areas (e.g. Gboko in Benue State of Nigeria). Social and political situations in many parts of the continent negate control programmes, as people from endemic areas are forced to move to other parts and thus establish new areas of endemicity.

The disease in animals is a big obstacle to the economic development of Africa, as it is impossible to keep most breeds of domestic animals in about four million square miles of the Continent, because of trypanosomiasis (Goodwin 1974).

The current trend of trypanosomiasis in the Continent is summarized by Tizard *et al* (1978) in the statement "Inspite of the rapid development of medicine in recent years, the pathogenic trypanosomes continue to

maintain their lethal grip on both humans and domestic animals over much of tropical Africa".

However, there is hope for the future, as many African governments are now aware of the threat posed by trypanosomiasis and with the help of the dedicated workers in the developed countries, the knowledge being accumulated could lead to effective methods of treatment and control of the disease in humans and his domestic animals.

1.1.2. CAUSATIVE ORGANISMS

(1) TAXONOMY

The classification of trypanosomes according to Hoare(1966, 1972) will be adopted. In this classification, the subgenus *Trypanozoon* and three other subgenera are grouped into section B (Salivaria) of the genus *Trypanosoma*. The Salivarian trypanosomes are cyclically trans- mitted through inoculation and develop in the anterior parts of their vectors' alimentary system (cf the Stercoraria). It is intended to place emphasis on three species of the subgenus *Trypanozoon*, viz; *Trypanosoma (Trypanozoon) brucei brucei* Plimmer and Bradford, 1899, which cause disease only in animals; *T. (T.) gambiense* Dutton, 1902 and *T. (T.) rhodesiense* Stephen and Fantham, 1910, which are pathogenic to man and cause sleeping sickness.

The organisms belonging to the *Trypanozoon* subgenus are pleomorphic and morphologically similar to the type species *T. brucei*.

Other trypanosomes of veterinary importance that would be referred to are *T. (Nannomonas) congolense* Broden, 1904 and *T. (Duttonella) vivax* Ziemann, 1905 which cause disease in domestic animals.

(II) DEVELOPMENT IN THE MAMMALIAN HOST

The developmental cycle of the subspecies of *T. brucei* in mammals, follows the same general pattern. When an infected tsetse fly (*Glossina* spp) takes a blood meal from a susceptible host, metatrypanosomes are inoculated

into the subcutaneous tissues.

The metatrypanosomes are transformed into long, slender and stumpy forms at the site of inoculation. These forms occur in the blood and tissue fluids of the host, where they multiply throughout the infection. Since pleomorphism is a feature of these organisms, intermediate forms between the slender and stumpy types sometimes occur. During rising parasitaemias, the slender forms predominate and may revert to the stumpy forms under the influence of the host's immune response (see Hoare 1972).

Several workers have described amastigotes and other forms in mammalian hosts infected with *T. brucei* subgroup organisms (see Ormerod and Venkatesan 1971a, b), but their role in the life cycle of these parasites remain controversial.

(III) DEVELOPMENT IN THE VECTOR

Trypanosomes of the *brucei* subgroup are transmitted by tsetse flies of the genus *Glossina* spp. *T. rhodesiense* organisms are transmitted by tsetse flies of the *G. morsitans* group while *G. palpalis* group act as vectors of *T. gambiense*.

The life cycle of the parasite in the vector was elucidated by Kleine (1909) while Robertson (1913) described the morphological changes the trypanosomes undergo in the tsetse fly.

When an uninfected tsetse fly obtains a blood meal from an infected mammalian host, it picks up trypomastigotes. These trypomastigotes and the ingested blood pass through the oesophagus into the mid-gut. About the third or fourth day, they are transformed into a new trypomastigote form in the endoperitrophic space of the mid-gut. They undergo intense multiplication in the lumen of the gut. On reaching the open end of the peritrophic membrane, they enter the ectoperitrophic space and gradually migrate forwards to the cardia (proventriculus). In the

cardia, the flagellates become more slender and elongated. These proventricular forms penetrate the endoperitrophic space of the cardia and later pass through the oesophagus and pharynx into the mouth parts. They enter the hypopharynx at its open anterior end and eventually get to the salivary glands through the salivary ducts. In the salivary glands, they attach themselves to the walls and metamorphose into broad epimastigotes, which multiply and eventually give rise to metatrypanosomes. The metatrypanosomes are the infective forms to susceptible mammalian hosts.

For many years, it was thought that the parasites developed only in the digestive tract and salivary glands of the vectors, until try- panosomes were located in the haemocoel (Mshelbwala, 1972; Otieno 1973). These trypanosomes probably got to the haemocoel by penetrating the gut wall as demonstrated by Evans and Ellis (1975). However, this observation does not exclude the classical life cycle described above and may occur in parallel.

1.2. PATHOLOGY AND PATHOGENESIS OF TRYPANOSOMIASIS

After the detailed histopathological observations carried out on the diseases caused by trypanosomes, especially of the *T. brucei* subgroup by earlier workers (e.g. Mott, 1910; Peruzzi, 1928), it is surprising that there are still gaps in our knowledge of the disease processes. A major drawback is that, in the human disease it is difficult to obtain necropsy materials for detailed histopathological studies with the modern techniques available. However, there are several reviews in the literature on the pathology and pathogenesis of trypanosomiasis in humans and domestic animals (Fiennes 1970; Goodwin 1970, 1974; Ormerod 1970; Hutt and Wilks 1971; Losos and Ikede 1972; Boreham 1979a) Most of these authors stressed the need for further studies to be undertaken, particularly on

the mechanisms underlying the lesions caused by these parasites.

A thorough knowledge of the mechanisms involved in the changes that occur in the host after infection with trypanosomes, could lead to better methods of treatment or at least allow alleviation of some of the symptoms observed in the human disease. Furthermore, an understanding of host-parasite-drug interactions may help to prevent some of the side effects of specific trypanocidal drugs (Boreham 1979a).

African pathogenic trypanosomes are divided into two groups (viz the haematic and humoral) on the basis of their distribution in the mammalian host and the type of lesions they cause during infection (Losos and Ikede 1972). The haematic group (*T. congolense* and *T. vivax*) are plasma parasites i.e. they are confined to the blood whereas the humoral group, the *T. brucei* subgroup organisms are widely distributed in the body and tissue fluids. This division is not absolute since recently, Luckins and Gray (1978) provided light and electron microscopic evidence of *T. congolense* developing and multiplying outside the blood vessels. Van den Ingh and de Neijs-Bakker (1979) have also demonstrated *T. vivax* in the extravascular tissue.

It is however necessary to retain this division in terms of patho-genesis, as the humoral organisms are known to cause extensive inflammatory, degenerative and necrotic lesions as they invade the hosts' connective tissues, while severe anaemia is the major pathological effect of the haematic group.

The review following will concentrate on infections due to *T. brucei* subgroup organisms; however reference will be made to *T. congolense* and *T. vivax* infections where pertinent.

1.2.1. CLINICAL SIGNS AND SYMPTOMS

The clinical signs and symptoms of the diseases caused by *T. gambiense*

and *T. rhodesiense* in humans are similar, except that the latter causes

an acute and often fatal infection, while the former produces a chronic

illness. However, there are intermediate forms between these two types

of human trypanosomiasis (see Apted, 1970). The important clinical

features are, the initial lesion at the site of the tsetse bite followed

by the blood stage with febrile responses and later central nervous

system (CNS) involvement.

(a) Initial stage

At the site of the tsetse bite, there is a local inflammatory

reaction, manifested by itching, erythema, swelling, pain and local

heat. The initial lesion occurs within 5 to 15 days after infection,

in the form of a large 'boil' i.e. the trypanosomal chancre.

In *T. gambiense* infections, this lesion is less conspicuous whereas

in *T. rhodesiense* it is found on the head, face and legs depending on

the species of *Glossina* that transmitted the trypanosomes.

Histologically, the lesion shows an inflammatory infiltrate, made up

of leucocytes (mononuclear and polymorphs) and oedema (Fairbairn and

Godfrey 1957). Luckins and Gray (1978) found similar local skin re-

actions in rabbits bitten by *Glossina morsitans;* which were experiment-

ally infected with *T. congolense.*

The invasion of the blood stream by the parasites follows this

initial reaction.

(b) Blood stage

The trypanosomes are found in the peripheral blood about 5 - 21

days after infection. In *T. gambiense* infection, parasites are scanty

in the blood right from the onset, whereas varying peaks of parasitaemia

occur in *T. rhodesiense* infections. The features associated with the

appearance of the parasite within the blood include, irregular fever which

is sometimes mild, severe or hyperpyrexial, anaemia, headaches, pains

in the joints and limbs and a general feeling of illness and discomfort.

There is a generalized enlargement of the lymph nodes, spleen and some-

times the liver. The enlarged lymph nodes of the posterior cervical

triangle (Winterbottom's Sign) are visible and characteristic of

T. gambiense infections (Apted 1970; Ormerod 1970). The swollen glands

never suppurate, unless there is a secondary infection. There is also

a local oedema particularly in the face and eyelids. In laboratory and

domestic animals experimentally infected with *T. brucei*, similar swellings

have been observed (Goodwin and Hook, 1968; Ikede and Losos 1975; Van

den Ingh 1976).

Other unusual symptoms include hyperesthesia (Kerandel's sign), persistent

tachycardia, serous effusions, pancarditis and electrocardiographic

abnormalities (see Manson-Bahr and Charters, 1963; Ormerod 1970; Hutt

and Wilks 1971). The disease may terminate at this stage, especially

in *T. rhodesiense* infections or progress to the cerebral stage which

involves the CNS.

(c) Cerebral stage

This is the advanced stage of the disease, depending on the in-

vasion of the CNS by the parasites. In *T.rhodesiense* infections, CNS

involvement is not prominent as in the disease caused by *T. gambiense*.

However, when the CNS is involved, the clinical signs are similar to those

of *T. gambiense* (Hutt and Wilks 1971).

The involvement of the CNS is characterized by cerebrospinal

fluid abnormalities and a perivascular microscopic inflammation, which

gradually develops into a chronic meningoencephalitis, with severe

cerebral oedema and punctate petechiae in the brain tissues (Hutt and

Wilks 1971; Poltera *et al* 1977). Neurological signs have been reported

in animals infected with *T. brucei* (Ikede and Losos 1974; Ikede and Losos

1975). The early clinical features of the CNS involvement in human

trypanosomiasis include high irritability, insomnia and changes in personality. At a later stage, there is a gradual depression and disintegration of CNS function, reflected by delayed and deep hyperesthesia, inability to coordinate muscular movements, convulsive and somnolent tendencies and in the absence of chemotherapy coma and death.

The clinical manifestations of the diseases caused by *T. congolense* and *T. vivax* in animals resemble those of the human disease, except that neurological signs are rare (see Stephen 1970; Losos and Ikede 1972).

1.2.2. CHANGES IN THE BLOOD

(a) ANAEMIA

Although anaemia is a consistent feature of trypanosomiasis, it is more pronounced in infections caused by *T. congolense* and *T. vivax* in animals (see Losos and Ikede 1972; Goodwin 1974).
There is a consensus on the anaemia in experimental *T. brucei* infections of animals. It is characterized by decreases in erythrocyte count, haemoglobin concentration, packed cell volume and life-span of circulating erythrocytes (Boreham 1967; Jennings *et al* 1972, 1974; Holmes and Jennings 1976). Morphological changes in the erythrocytes include polychromasia, anisocytosis and basophilic stippling; together with circulating normoblasts and spherocytes (Boreham 1967; Boreham and Goodwin 1967; Jenkins *et al* 1974a).

In rabbits infected with *T. brucei*, the erythrocytes show no increase in fragility (Boreham 1967; MacKenzie and Boreham 1974a) contrary to observations in mice infected with *T. congolense* (Ikede *et al* 1977).

There are conflicting descriptions for the anaemia in bovine trypanosomiasis caused by *T. congolense* and *T. vivax*. For example, some workers

have described it as normocytic and normochromic (Losos *et al* 1973;

Mamo and Holmes 1975) while others describe the anaemia as macrocytic

in the acute phase and microcytic or normocytic in the chronic phase

of the disease (Fiennes 1970; Naylor 1971; Maxie *et al* 1976; Valli

et al 1978).

The aetiology of the anaemia in trypanosomiasis has been a subject

of much controversy. The anaemia in bovine trypanosomiasis has been

attributed to dyshaemopoiesis by several workers (e.g. Fiennes 1954, 1970;

Naylor 1971). However, inhibition of haemopoeisis is not a likely cause

of the anaemia, since erythropoietic response is increased in most in-

fected animals (Holmes and Mamo 1975; Mamo and Holmes 1975; Maxie *et al*

1976; Valli *et al* 1978). Although Dargie *et al* (1979) found increased

red cell synthesis in cattle infected with *T. congolense*, they suggested

that in long-standing infections, the bone marrow may be starved of

iron, such that the anaemia could be complicated by dyshaemopoeisis.

It is well documented by several workers that in infected animals,

there is an increased removal of erythrocytes from the circulation

(Holmes and Mamo 1975; Holmes and Jennings 1976; Ikede *et al* 1977; Valli

*et al*1978; Dargie *et al* 1979). Furthermore marked haemosiderin deposits

and erythrophagocytosis occur in various organs, such as the spleen and

liver (Boreham and Goodwin 1967; Jennings *et al* 1974; Murray *et al* 1974;

Valli and Forsberg 1979). These observations are consistent with an

anaemia of haemolytic origin (Jennings 1976). In fact, there is a con-

sensus that the anaemia in trypanosomiasis is essentially haemolytic and

that the mechanism underlying the increased removal or lysis of erythro-

cytes may be partly immunological (see Woodruff 1973; Jennings *et al* 1974;

Holmes and Jennings 1976; Kobayashi *et al* 1976; Valli *et al* 1978).

It is likely that trypanosome antigen/antibody complexes are formed

on the surface of erythrocytes which render them susceptible to phagocytosis or lysis if complement is fixed to the immune complex. This hypothesis is supported by several findings reported in the literature. It has been demonstrated that *T. brucei* antigen is readily absorbed onto erythrocytes of infected and normal animals (Herbert and Inglis 1973; Woo and Kobayashi 1975). Woodruff and Co-workers (Woodruff 1973; Woodruff *et al*, 1973) showed that erythrocytes from patients infected with *T. rhodesiense* have complement on their surface. They also reported high levels of immunoconglutinins i.e. antibodies to bound complement. Kobayashi *et al* (1976) demonstrated the presence of immunoglobulins on the surface of erythrocytes from cattle infected with *T. congolense*. They found that out of the 74 eluates prepared from the erythrocytes, 16 had IgG and IgM and that the positive eluates exhibited antibody activity against *T. congolense*. Maxie *et al* (1976) also showed that erythrocytes from cattle infected with *T. vivax* were coated with immunoglobulins. More recently, Tabel *et al* (1979) detected IgM, IgG_1, IgG_2 and C_3 on the surface of erythrocytes in some cattle (19 out of 382 samples) infected with *T. congolense* and *T. vivax* and concluded that attachment of immune complexes to erythrocytes contributed to the anaemia in trypanosomiasis.

Balber (1974) was able to reduce the degree of anaemia in mice infected with *T. brucei* , by using immunosuppressive drugs; indicating a relationship to antibody response. Further support for the immune haemolytic mechanism is provided by Assoku (1975). He gave rats multiple injections of soluble *T. evansi* antigen and produced a moderately severe anaemia with antibody response. Assoku (1975) suggested that antigen/ antibody complexes probably formed in the blood stream are absorbed onto erythrocytes which are then removed extravascularly. However, it has not been shown conclusively, whether the antigen and antibody are

absorbed onto the erythrocyte surface as a complex or separately.

In addition to the immunological mechanisms postulated, there are evidences which suggest that the trypanosomes themselves produce haemolytic substances which can cause lysis of erythrocytes directly. Fiennes (1954) showed that when erythrocytes from normal cattle were incubated with plasma from infected animals, the erythrocytes were haemolysed. Nguyen Huan Chi *et al* (1975) isolated a haemolytic factor from *T. brucei* which caused lysis of normal erythrocytes. They detected this factor as early as the second day of infection and concluded that immune mechanisms were not important in the acute phase of the anaemia. Several workers have shown that trypanosomes during autolysis generate phospholipase A activity and free fatty acids that can destroy erythrocytes (reviewed by Tizard *et al* 1978).

A second mechanism, in addition to immune haemolysis has been suggested for the anaemia in trypanosomiasis, particularly in rabbits infected with *T. brucei* (Facer 1974; Jenkins *et al* 1974a).
Brain and coworkers (reviewed by Brain 1970) adapted the term micro-angiopathic haemolytic anaemia (MHA) to describe conditions where erythrocytes are damaged, secondary to various disorders of small blood vessels; most of which are associated with disseminated intravascular coagulation (DIC). They suggested that the microthrombi are the cause of haemolysis; the erythrocytes being fragmented as they pass through the fibrin clots. MHA is characterized by small red cell fragments or schistocytes, helmet cells, Burr Cells and polychromasia, in peripheral blood smears (Huntsman and Jenkins 1974). There are evidences for DIC in trypanosome infections (see Boreham 1974; Section 1.2.2.b.). The morphological alterations in erythrocytes from rabbits infected with *T. brucei* described by Jenkins *et al* (1974a) are consistent with MHA.

Valli *et al* (1978) also observed a significant number of highly distorted

erythrocytes in cattle infected with *T. congolense.*

Another factor that could contribute to the anaemia in trypan-

osomiasis, is the increase in plasma volume reported in ruminants in-

fected with *T. congolense* and *T. vivax* (Clarkson 1968; Fiennes 1970;

Naylor 1971; Holmes and Mamo 1975; Valli *et al* 1978) and laboratory

animals infected with *T. brucei* (Boreham 1967; Boreham and Goodwin 1967;

Jennings *et al* 1974; Holmes and Jennings 1976).

(b) FIBRINOLYTIC AND COAGULATION SYSTEMS

Platelets play a vital role in the maintenance of haemostasis

and restoration of vascular integrity when there is damage to the blood

vessel wall. Thrombocytopenia has been reported in humans infected

with *T. brucei* subgroup trypanosomes (Barrett-Connor *et al* 1973; Robins-

Browne *et al* 1975; Greenwood and Whittle, 1976a), rhesus monkeys, rats

and rabbits experimentally infected with *T. rhodesiense* (Sadun *et al* 1973;

Davis *et al* 1974), in *T. brucei* infected rabbits (Jenkins *et al* 1974a)

and in cattle trypanosomiasis (Maxie *et al* 1976; Wellde *et al* 1978;

Forsberg *et al* 1979).

The mechanisms suggested for the thrombocytopenia, include excessive

removal or pooling of normal or immunologically damaged platelets from

the circulation by the reticuloendothelial system and consumption of

platelets in disseminated intravascular coagulation (D.I.C.) The

former mechanism is supported by the observation that in rabbits in-

fected with *T. brucei,* the thrombocytopenia was prevented by splenectomy

(Jenkins *et al* 1974b) and the fact that splenomegaly with increased

cellularity of the splenic sinusoids is a prominent feature of try-

panosomiasis (Ormerod 1970; Losos and Ikede 1972). D.I.C. has been

recorded in humans infected with trypanosomes (Barrett-Connor *et al* 1973;

Robins-Browne and Schneider, 1977).

Davis *et al* (1974) suggested that the thrombocytopenia could result
from direct destruction of the platelets by trypanosomes, without con-
current immunological damage or D.I.C. They showed that concentrated
trypanosomes and trypanosome-free supernates of disrupted *T. rhodesiense*
aggregated platelets *in vitro*, when added to normal rat, rabbit and
human blood. Greenwood and Whittle (1976a) unable to repeat these
experiments with *T. gambiense*, concluded that the thrombocytopenia
was due to enhanced splenic trapping of the platelets rather than their
aggregation by trypanosomes.

The fibrinolytic system involves the dissolution and removal of
fibrin deposits while the coagulation system lays down fibrin to repair
any damage to the blood vessel wall. Both processes are complex and
involve several factors (activators and inhibitors) in the blood and
tissues. In the normal living organism, there exists an equilibrium
between the two systems and any shift in this balance leads to pathological
complications. There are increases in fibrinogen/fibrin degradation
products (FDP) as a result of plasminogen activation, in rabbits in-
fected with *T. brucei* (Boreham and Facer 1974a; Facer 1974); suggesting
either primary fibrinolysis (fibrinogenolysis) or secondary fibrinolysis
as a result of D.I.C. Facer (1974) also found qualitative changes in
plasma fibrinogen from infected rabbits. She obtained positive protamine
paracoagulation tests which indicated the presence of heparin pre-
cipitable fibrinogen, fibrinogen B and cryofibrinogen; suggesting low
grade D.I.C. and microthrombi formation. FDP have also been reported
in humans infected with *T. gambiense* (Greenwood and Whittle, 1976a)
and *T. rhodesiense* (Robins-Browne and Schneider 1977).

There are very few reports in the literature on changes in the
coagulation system during trypanosome infections. The partial thrombo-
plastin time is prolonged in humans and animals infected with trypanosomes;

suggesting defects in the coagulation pathway (Barrett-Connor *et al* 1973; Essien and Ikede 1976; Wellde *et al* 1978). Boulton *et al* (1974) showed an increase in the levels of many of the clotting factors, particularly factors VIII and XII in rabbits infected with *T. brucei*.

Recently, a detailed investigation was carried out on five patients infected with *T. rhodesiense* by Robins-Browne and Schneider (1977). They found that four patients showed clinical evidence of coagulation defects, which they attributed to thrombocytopenia. Three of their patients showed varying degrees of alterations in coagulation factors, in particular elevated concentrations of factors V, VIII and fibrinogen, indicating rebound hypercoagulability. It was also found that thrombocytopenia was marked in four patients treated with suramin. The authors suggested that the trypanocidal therapy probably led to the release of large amounts of soluble antigen with subsequent immune complex formation, which damage the platelets and/or endothelial cells and thus aggravate the coagulation defects.

(c) CHANGES IN PLASMA CONSTITUENTS

It has been well documented by several workers that during trypanosome infections of man and animals, there is a decrease in serum albumin and an increase in gamma-globulins (Jenkins and Robertson, 1959; Smithers and Terry, 1959; Woodruff, 1959; Clarkson, 1968; Goodwin and Guy, 1973; Wellde *et al* 1974; Van den Ingh, 1976; Boreham *et al* 1977). The rise in globulins is characterized by an increase in the production of large molecular weight immunoglobulin M (IgM), with an accompanying but lesser increase in immunoglobulin G (IgG) (Mattern *et al* 1961; Masseyeff and Lamy, 1966; Seed *et al* 1969; Onyango *et al* 1972; Luckins, 1976; Nielsen *et al* 1978a). Several workers have shown that not all of the IgM produced is directed against the trypanosomes (see Houba *et al* 1969; Mackenzie *et al* 1972).

In addition, heterophile and autoantibodies have been found in clinical

and experimental trypanosomiasis (Houba and Allison, 1966; Seed and

Gam, 1967; Houba *et al* 1969; Boreham and Facer 1974b; Mackenzie and

Boreham, 1974b). It is also known that low molecular weight IgM and

even free light chains are produced (Frommel *et al* 1970; Greenwood and

Whittle, 1975). There is also an increase in protein catabolism in

animals and humans infected with trypanosomes (Jennings *et al* 1973;

Nielsen *et al* 1978b; Robins-Browne *et al* 1975).

The elevated concentrations of immunoglobulins is further com-

plicated by the increase in plasma fibrinogen (Boreham and Facer 1974a;

Facer 1974; Greenwood and Whittle 1976a; Boreham *et al* 1977) and lip-

oproteins (Diehl and Risby 1974). A combination of the increases in

macroglobulins and plasma fibrinogen could affect the rheology of blood,

particularly in the microcirculation.

The changes in the flow properties of blood in rabbits infected with

T. brucei have been investigated by Facer (1976) and Boreham *et al* (1977).

These authors found significant increases in the viscosity of all three

compartments of blood, i.e. whole blood, serum and plasma; with maximum

values three to four weeks after infection.

The elevated viscosity was attributed to increased concentrations of

macroglobulins and plasma fibrinogen.

It was suggested that the observed hyperviscosity could have im-

portant pathological effects, manifested by sludging of red blood cells

and decrease in blood flow, with subsequent microthrombi formation (Boreham

1974; Facer 1976). There are histopathological evidences of sludging

of red blood cells in rabbits (Goodwin 1971), mice (Murray *et al* 1974)

and cattle (Losos and Ikede 1972) infected with trypanosomes.

There is an increase in the concentration of serum total lipids

in rabbits infected with *T. brucei* subgroup organisms·Facer (1974) found

that this was due almost entirely to the elevated concentration of neutral fats (triglycerides), with slight increases in cholesterol. However, marked increases in cholesterol levels have been shown at the terminal stages of infections in rabbits (Goodwin and Guy 1973; Diehl and Risby 1974). In contrast to the findings reported for rabbits, Roberts (1975) found a marked decrease in the concentrations of all the serum lipids in ruminants infected with *T. congolense* or *T. vivax*.

Some biochemical changes in the blood of rabbits infected with *T. brucei* have been studied by Goodwin and Guy (1973). It was found that the concentrations of serum urea, creatinine and phosphate increased while bicarbonate decreased, suggesting renal damage. They also reported increases in aspartate transaminase and creatinine phosphokinase activities, which are indicative of cellular necrosis and muscle cell damage. In addition, there was an increase in pyruvate levels in the blood; a finding which coincided with the peaks of parasitaemia. Goodwin and Guy (1973) provided evidence to show that renal insufficiency was a contributory factor in the events leading to death in rabbits infected with *T. brucei*. The biochemical changes in the blood of cattle infected with *T. congolense* are given by Fiennes (1970) and Wellde *et al* (1974).

Several investigators have shown that there is a decrease in some components (e.g. C1, C3) of the complement system, in clinical and experimental trypanosomiasis (Nagle *et al* 1974; Greenwood and Whittle, 1976b; Kobayashi and Tizard, 1976; Nielsen *et al* 1979) suggesting activation of both the classical and alternate pathways. The mechanisms suggested for this hypocomplementaemia include, direct activation of the system through materials derived from the trypanosomes themselves (Musoke and Barbet, 1977; Nielsen and Sheppard, 1977; Tizard *et al* 1978) and consumption of the components by immune complexes formed

during the disease (Lambert and Houba, 1974; Nagle *et al* 1974).

(d) THE HEART

Cardiac lesions have been described in naturally acquired and experimental trypanosomiasis (Peruzzi, 1928; Hawking and Greenfield, 1941; Koten and de Raadt, 1969; Hutt and Wilks, 1971; Losos and Gwamaka, 1973; Murray *et al*, 1974; Poltera *et al*, 1976, 1977). Manson-Bahr and Charters (1963) suggested the presence of a myocarditis on clinical observation of two patients infected with *T. rhodesiense*.

The myocarditis may be mild, moderate or severe and the histo-pathological features include, focal or diffuse inflammatory infiltration consisting of plasma cells, mononuclear cells, lymphocytes and histio-cytes. Morular cells have also been observed, either isolated or within the cellular infiltrate (Koten and de Raadt, 1969; Poltera *et al*, 1976, 1977). The cardiac muscle fibres are separated by oedema and the cellular infiltrate and in chronic cases, there is a marked atrophy and degeneration of the fibres. Koten and de Raadt (1969) also observed interstitial haemorrhage in all six patients infected with *T. rhodesiense*. Trypanosomes have been found in the interstitium i.e. between muscle fibres by several workers. For example, Peruzzi (1928) described the presence of trypanosomes including leishmanoid forms in cardiac lesions in monkeys infected with trypanosomes of human origin.

It was thought that the heart valves were not involved in human trypanosomiasis (Hutt and Wilks, 1971) until Poltera *et al* (1976, 1977) observed inflammation of the valves in 3 cases and the conducting system (in 2 cases) in necropsy materials from humans infected with *T. rhodesiense* in Uganda.

Electrocardiographic abnormalities have also be reported in murine (Murray *et al*, 1974) and human trypanosomiasis (e.g. Betrand *et al*, 1967;

Jones *et al*, 1975).

Rabbits infected with *T. brucei* have been shown to be hypotensive
(Boreham and Wright, 1976; Boreham *et al* 1977; Yates, 1978). In most
cases there were no significant changes in the heart rate of these
animals. Boreham and Wright (1976) in a series of experiments provided
evidence to show that the hypotension was caused by immune complex
formation of trypanosomes with antibody which activated plasma Kallikrein
with the subsequent release of hypotensive plasma Kinins.

In human and cattle trypanosomiasis hypotension is not a striking feature,
although Sice (1937) and Buyst (1975) recorded hypotension in some of
their patients infected with *T. gambiense* and *T. rhodesiense* respectively.

The changes in the blood vessels during trypanosomiasis will be
considered in Section 1.3.3.

1.3. VASCULAR SYSTEM

The vascular system consists of arteries, the microvascular bed,
veins and the lymphatic vessels. It has long been recognised that these
channels are responsible for the transport and subsequent exchange of
metabolites between the blood and the tissues or organs they supply.
The description following will concentrate on the microvascular bed which
is made up of small blood vessels, which are ramifications of arteries and
veins. It is through the walls of these microvessels that the major
interchange of materials between the blood and tissue take place.

1.3.1. MICROVASCULAR BED

It is well known that there is no stereotyped microvascular
pattern; each organ or tissue within the mammalian body has a micro-
vascular architecture which is intimately related to its' structure and
function (see Majno 1965).

Terminologies and Classifications.

The criteria used in the designation of the various segments of
the microvascular bed include luminal diameter and the number, arrangement
and continuity of the cellular and non-cellular components making up the
vessel wall (Bennett *et al* 1959; Majno 1965; Rhodin 1967, 1968).
The various segments of the microvascular bed with their characteristics,
based on ultrastructural studies by several workers (e.g. Fernando and
Movat 1964b; Rhodin 1967, 1968; Mohammed *et al* 1973; Simionescu M *et al*
1975) are shown in the table below:

Vessel Type	Luminal diameter	Pericytes	Primitive smooth muscle cells	Smooth muscle cells
Capillaries	up to 8µm	Occasional	Absent	Absent
Post capillary venules	8-30µm	Increasing number	Absent	Absent
Collecting venules	30-50µm	Complete layer	Present	Absent
Muscular venules	50-100µm	Absent	Absent	1-2 layers
Small collecting veins	100-300µm	Absent	Absent	Several layers
Arterioles	50-100µm	Absent	Absent	1-2 layers
Terminal arterioles	less than 50µm	Absent	Absent	1 layer
Metarteriole	about 10µm	Absent	Absent	a few cells

Some segments of the microvascular bed from rabbit mesentery
observed by *in vivo* microscopy are shown in fig. 1. For the sake of
convenience, the terms arterioles, venules and capillaries are used.

STRUCTURE

In conventional histological sections examined with the light
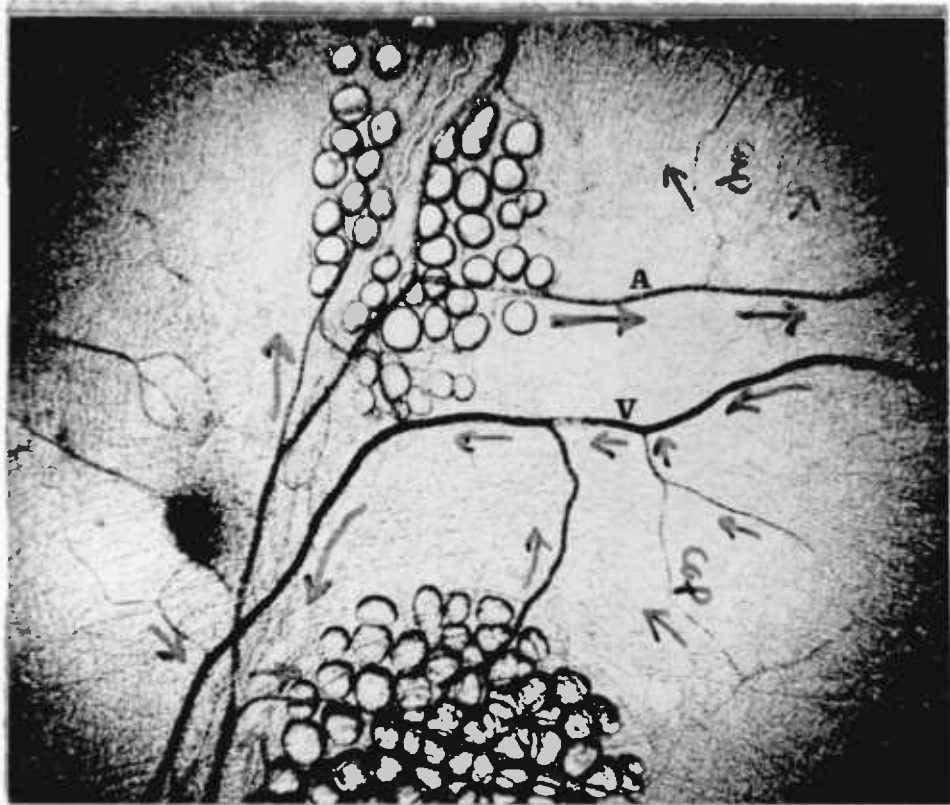
Figure 1. *In vivo* microscopy of rabbit mesenteric microvascular
system showing Arteriole (A),

Venule (V) and

Capillaries (Cap)

Arrows indicate direction of blood flow. X 100

microscope, venules are usually recognised by their thin walls, larger

vascular lumina and irregular outlines, in contrast to arterioles which

have thicker walls and smaller lumina.

It is intended to describe the components of the vessel wall

individually, with emphasis on those structures that have been shown

to be involved in transcapillary exchange and in the reaction of the

blood vessel to injury. Of necessity, these descriptions are based

mainly on observations made with the electron microscope. Attempts

will be made to indicate variations among the segments of the micro-

vascular bed.

(a) Endothelium

All blood vessels are lined on their inner surfaces by a vascular

endothelium; which constitutes the main barrier between the blood and

tissues. There are three main types of vascular endothelia (see Bennett

et al 1959; Majno 1965; Florey 1966) viz continuous, discontinuous and

fenestrated endothelium. The continuous endothelium, which by definition

has no discontinuities or channels across the cytoplasm, is found in

capillary blood vessels of the lung, connective tissue, brain and muscle

(cardiac, skeletal and smooth). It is the commonest type of endothelium

in the mammalian vascular system; also lining all arteries and veins.

Since the tissues used in the present study are known to have blood vessels

with the continuous-type of vascular endothelial lining, the structural

survey following will be limited to this type of endothelium.

The endothelium is made up of flattened (squamous) epithelial cells,

0.1 to 0.4μm thick, except at the nuclear region where there is a bulge

1 to 5μm thick. The number of endothelial cells seen in any vascular

cross section varies from one blood vessel to another. For example,

capillaries are lined by one or two endothelial cells, whereas more cells

are needed to line larger vessels such as venules and arterioles (Fernando and Movat 1964a, b; Movat and Fernando 1964).

The endothelial cell has a single nucleus and its cytoplasm contains the usual complement of cell organelles; mitochondria, golgi complex, endoplasmic reticulum and free ribosomes, which probably perform the same functions as those found in other cells (Florey 1966; Cotran 1967b). In addition, multivesicular bodies, dense granules and Weibel-Palade bodies are found in endothelial cytoplasm (Florey 1966; Rhodin 1968; Santolaya and Bertini 1970). A characteristic of venules, is the abundance of lysosomes, which are rare in other small blood vessels (Movat and Fernando 1964).

One of the most prominent features of vascular endothelium though found in other epithelial cells, is the presence of a large number of spherical or ovoid structures called pinocytotic (micropinocytotic; plasmalemmal) vesicles. These structures were first described by Palade (1953), who suggested that they might be involved in the transport of materials from the blood vessel to the extravascular tissue, by a process Moore and Ruska (1957) termed cytopempsis (transmission by cell). The vesicles are about 600-700Å in diameter, bounded by the typical unit membranes and distributed both on the blood (luminal) and tissue (abluminal) surfaces of the endothelial cells (Moore and Ruska, 1957; Cotran 1967b; Rhodin 1967, 1968; Bruns and Palade 1968). In venules, the vesicles can be as large as 1700Å in diameter (Movat and Fernando, 1964). Several investigators have reported the presence of intracytoplasmic microfilaments (60 - 90Å in diameter) in vascular endothelium (Fernando and Movat 1964a,b; Majno 1965; Giacomelli *et al* 1970). It has been suggested that they may be involved in cellular contractility and movement (Gabbiani *et al* 1972).

Intercellular junctions

Earlier morphologists, using light microscopic techniques suggested

that adjacent endothelial cells were joined to each other by an inter-cellular cement substance (see Chambers and Zweifach 1947). However, electron microscopy has shown that there are no cement substances between endothelial cells; rather apposing endothelial membranes are connected to each other by junctional complexes (Fawcett 1963). The relationship of endothelial cells to one another at their junctions, varies from one microvessel to another (Simionescu. M. *et al*, 1975). The margins of adjacent endothelial cells either abut sharply, overlap or interdigitate (Bruns and Palade 1968). Occasionally in 'small' capillaries, an endothelial cell rolls upon itself to form a single endothelial junction (Fernando and Movat 1964b).

The ultrastructure of endothelial cell junctions have been described by several workers (Farquhar and Palade 1963; Majno 1965; Cotran 1967b; Bruns and Palade 1968; Simionescu. M. *et al* 1975). The apposing endothelial cell membranes are either fused (tight junctions or zonula occludens) or are separated by 20$\overset{\text{o}}{\text{A}}$ intercellular gap (gap junctions) or by a 200$\overset{\text{o}}{\text{A}}$ gap filled with glycoprotein (zonula adherens). Along the intercellular junctions in some cases, there are focal areas of increased electron density called attachment belts. Although typical desmosomes are rare, they are found in the vascular endothelium of lower vertebrates (Fawcett 1963).

The pathways by which materials are transported across the walls of small blood vessels have occupied the attention of physiologists and morphologists for several years (reviewed by Renkin, 1977).

On the basis of physiological experiments, it was postulated that, the blood vessel wall had two sizes of pores; small pores (40-50$\overset{\text{o}}{\text{A}}$ radius) which allowed the passage of water and small lipid-insoluble molecules and large pores (120-350$\overset{\text{o}}{\text{A}}$) which account for the passage of molecules of varying molecular weights, including large proteins (Pappenheimer 1953; Mayerson *et al* 1960). Several ultrastructural studies, using tracer

particles or molecules of graded sizes (e.g. ferritin, saccharated iron oxide, horse radish peroxidase, myoglobin and dextran), have been carried out, in an attempt to identify structural equivalents of these pores (Jennings *et al* 1962; Marchesi 1965; Karnvosky 1967; Florey and Sheppard 1970; Simionescu. N. *et al* 1973). These authors, suggested that in small blood vessels with continuous endothelium, the small pore system is represented by intercellular junctions and the large pore system by the pinocytotic vesicles in the endothelial cytoplasm.

These suggestions were based on the fact that, the injected probe molecules were detected in these vascular structures. However, this morphological classification is not absolute. Some workers have shown that the intercellular junction cannot be regarded as the only site of the small pore system (Williams and Wissig 1975), while others consider the vesicles as representing both the small and large pore systems (Simionescu N. *et al* 1973, 1975).

(b) **Basement membrane (Basal lamina)**

The endothelial cells of all blood vessels are lined on their abluminal surfaces by an acellular basement membrane. This membrane, which also supports pericytes and vascular smooth muscle cells, is either continuous or discontinuous, depending on the type vessel (Bennett *et al* 1959).

The ultrastructure of the basement membrane has been described by several workers (e.g. Majno 1965; Bruns and Palade 1968; Latta 1970). It varies in thickness from 200 to 600$\overset{o}{A}$ and has a filamentous or fibrillar appearance. Most of the filaments are approximately 50µm in diameter and are similar to the fine fibrils found in the connective tissue.

Earlier workers thought that the basement membrane was made up essentially of polysaccharides (see Florey 1961; Majno 1965). However, because it was almost completely hydrolysed by collagenase, it has been

suggested that its major component is probably collagen or collagen-like material (Majno 1970). Baumgartner *et al* (1971) found that the pattern of interaction between the basement membrane and platelets was different from that of collagen and platelets and suggested that it was made up mostly of myofibrils and not collagen alone. Apart from its obvious function of providing mechanical support for the vascular endothelium, the basement membrane is regarded as a filtration barrier for large molecules (Majno and Palade 1961; Cotran *et al* 1967).

(c) Pericytes

These cells are found in the walls of capillaries and venules, where they are separated from the endothelium by basement membrane. They are also surrounded by basement membrane, which in most cases is continuous with that of the endothelium itself. There are more pericytes in the walls of venules than in capillaries (Movat and Fernando 1964; Rhodin 1968). The fine structure of pericytes closely resembles those of endothelial cells and fibroblasts; except that they are highly branched and have abundant rough endoplasmic reticulum (see Rhodin 1968). The functions suggested for pericytes include, mechanical support for the vascular endothelium; a smooth muscle-like function (Rhodin 1968); and transformation into other mesenchymal elements or cells connected with defence mechanisms (Movat and Fernando 1964).

(d) Smooth muscle cells.

Arterioles and muscular venules have smooth muscle cells in their media (Fernando and Movat 1964a; Movat and Fernando 1964; Rhodin 1967, 1968; Simionescu M. *et al* 1975). The ultrastructure of vascular smooth muscle cells has been described in detail by Rhodin (1967, 1968).

(e) Adventitia

The adventitia of small blood vessels include macrophages, plasma cells, fibroblasts, a few mast cells, collagen fibres and other fibrils

(Fernando and Movat 1964b; Bruns and Palade 1968).

1.3.2. MICROVASCULAR INJURY

Inflammation

Inflammation may be defined as the response of living tissue to injury. The inflammatory response may be acute or chronic, depending on the severity and persistence of the stimulus eliciting the injury and on the defence capacity of the host.

The inflammatory process can be divided into the following stages: dilatation of blood vessels (hyperaemia), increased vascular permeability and mobilization of white blood cells (leucocytes) into the exudate (Taussig 1979). These events are interrelated and occur in a regular sequence, irrespective of the stimulus initiating the reaction or the organ involved.

(I) Increased vascular permeability

One of the most important events in the inflammatory reaction, is injury to small blood vessels, manifested by increase in permeability; which results in substantial losses of fluid and solutes, including plasma proteins into the extravascular compartment.
This phase of inflammation has been extensively studied using various stimuli to induce microvascular injury; e.g. thermal (Cotran, 1965; Hurley *et al* 1967), known mediators of inflammation i.e. histamine, bradykinin and serotonin (Majno *et al* 1967; Northover and Northover 1969) and mechanical trauma (Marchesi, 1962).

The vascular leakage induced by thermal injury is biphasic, an early/immediate leakage, which is followed by a delayed and prolonged response. It is well documented that the early/immediate phase is induced by histamine-type mediators of inflammation while the mediators for the delayed and prolonged phase remain obscure (reviewed by Shea *et al* 1973).

Several workers, using the carbon labelling technique to indicate

the portion of the vascular tree damaged, (see Cotran *et al* 1967), have

shown that, in vascular leakage elicited by histamine-type mediators, venules

are predominantly affected (Majno *et al* 1961; Majno *et al* 1967; Buckley

and Ryan 1969), while both venules and capillaries are involved in the

delayed and prolonged leakage (Cotran and Majno 1964a; Spector *et al* 1965;

Cotran 1967a; Wells 1971).

The ultrastructural changes underlying the inflammatory phenomenon

of increased vascular permeability have been investigated by several

workers, using different models of inflammation. There is good agree-

ment that the formation of gaps, at or near endothelial intercellular

junctions, is the morphologic basis of the increase in vascular perm-

eability in almost all models used to elicite vascular injury (see Movat

1966; Cotran 1967b, 1969).

These endothelial gaps occur principally in venules in injuries induced

by histamine-type mediators (Majno and Palade 1961; Majno *et al* 1969;

Hultström and Svensjö 1977; Northover 1978), antigen/antibody interactions

(Movat and Fernando 1963; Uriuhara and Movat 1964) and mechanical trauma

(Marchesi 1962). However, in vascular leakage resulting from direct

injury to vascular endothelium, these gaps are not restricted to venules,

but also occur in other small blood vessels (Cotran and Majno 1964b;

Cotran and Remensnyder 1968). The gaps allow the passage of cellular and

dissolved blood elements, as well as injected tracer particles (e.g.

colloidal carbon and ferritin) into the extravascular tissue. It has

been shown that in immunological inflammation, due to antigen and anti-

body reactions, immune complexes pass through gaps in venules and locate

within the blood vessel wall (Movat and Fernando 1963; Movat 1966).

Apart from the intercellular gaps, other endothelial alterations

are observed in inflammatory foci; they include vacuolation, development

of cytoplasmic processes (Williamson and Grisham 1961; Hammersen 1972),

dilatation of cytoplasmic organelles, swelling, necrosis and sloughing

of endothelium (Cotran 1965; Cotran and Remensnyder 1968). It has

also been shown that in injured small blood vessels, the endothelial cells

and pericytes become phagocytic, picking up tracer particles injected

into the blood vessels (Cotran 1967b).

In injured small blood vessels, the basement membrane in most

cases remain unaltered. However, injected tracer particles have been

shown to traverse the basement membrane through small gaps or tears,

mostly in those areas where the membrane splits to surround the pericyte

(Cotran *et al* 1965; Cotran 1967b).

The mechanism of gap formation in leaking blood vessels following

injury remain controversial. Several attempts have been made to elu-

cidate the mechanisms for the gaps induced by histamine-type mediators

of inflammation (see Majno 1965; Cotran 1969). Rowley (1964) postulated

that, the gaps were formed by an increased hydrostatic pressure in

venules consequent to constriction of the larger veins. However, *in-vivo*

studies by several workers, using the vascular labelling technique

(Majno *et al* 1967; Buckley and Ryan 1969; Northover and Northover 1969)

have shown that vascular leakage occurs independently of venous spasm

and consequent increased hydrostatic pressure.

Some workers have suggested that the injurious agents caused the endo-

thelial gaps by their direct action on endothelial membranes at the

intercellular junctions (Majno and Palade 1961; Majno 1965; Hammersen

1972). Majno and Co-workers (Majno and Leventhal 1967; Majno *et al* 1969;

Majno 1970) advanced the hypothesis that, the gaps were formed as a

result of endothelial contraction. These authors found that the nuclei

in endothelial cells adjacent·to the intercellular gaps showed numerous

infoldings and pinches reminiscent of nuclei in contracted smooth muscle

cells. This hypothesis is supported by the observation that, the vascular

endothelium has cytoplasmic microfilaments (Cotran 1967b; Giacomelli *et al*

1970) which have been suggested to be involved in cellular contractility.

However, there is no conclusive evidence that the endothelial cells can

contract when damaged. Moreover, gaps have been found in blood vessels

without signs of endothelial contraction (Cotran 1969).

(II) Cellular response

In inflamed small blood vessels, particularly venules and capillaries,

formed elements of blood (leucocytes, platelets and red blood cells)

interact with the vascular endothelium.

Leucocytes emigrate from the blood vessels into the extravascular tissue,

where they accumulate. However, this emigration is preceded by their

margination (pavementing) on to the vascular endothelium (Clark and

Clark 1935; Cliff 1966). Since this process is only observed in in-

flammation, it is assumed that a change in the endothelium renders it

'sticky' for leucocytes at the time. Calcium is required, but details of

the mechanism of leucocyte adherence are unknown. The interaction

between leucocytes and the vascular endothelium in inflammatory foci

have been studied most extensively with the electron microscope (Marchesi

1961, 1964; Williamson and Grisham 1961; Movat and Fernando 1963; Wiener

et al 1969). Marchesi (1961) using serial-sections showed that the

leucocytes usually flatten against the vascular endothelium and then

penetrate the intercellular junctions by means of pseudopodia. Although

Williamson and Grisham (1961) observed that the inflamed vascular endo-

thelium developed cytoplasmic processes which enmesh the leucocytes and

push them towards the extravascular space, they did not exclude the

intercellular junction as the route of emigration.

The mechanisms suggested for the induction of leucocyte emigration in inflammatory foci, include increased vascular permeability to plasma, chemotaxis, specific changes in the leucocytes themselves and alterations of the blood vessels (see Spector and Willoughby 1963; Movat 1966).

Several workers have shown that leucocytes, particularly the neutrophils contain substances in their granules e.g. cathepsins, cationic proteins, collagenase, elastase and Kallikrein, which might be involved in causing vascular injury (reviewed by Mustard *et al* 1970).

Platelets interact with injured vascular endothelium by initiating a series of responses which lead to the formation of a haemostatic plug or intravascular thrombosis. The initial reaction is the adhesion of the negatively charged platelets to subendothelial components of the vessel wall, followed by their aggregation and the release of substances e.g. 5HT, lysosomal enzymes, histamine and prostaglandins which escalate the vascular injury (see Gingrich and Hoak, 1979).

Several workers have described thrombosis of small blood vessels, following vascular injury elicited by various stimuli (Uriuhara and Movat 1964; Cotran 1965; Cotran and Remensnyder 1968). Various stimuli are implicated in the behaviour of platelets (i.e. adhesion, aggregation and release reaction) following vascular injury; they include collagen, fibrinogen/fibrin degradation products (FDP), thrombin, Adenosine diphosphate (ADP) and immune complexes (reviewed by Mustard and Packham 1970).

1.3.3. VASCULAR CHANGES IN TRYPANOSOMIASIS

In experimental *T. brucei* infections, one of the most important pathological changes known to occur, particularly in rabbits, is increased vascular permeability. (Boreham and Goodwin 1967; Goodwin 1967; Goodwin and Hook 1968; Goodwin 1970; Boreham 1974).

Goodwin and Hook (1968) used angiographic and histological techniques

to study the changes in the blood vessels of the ear, viscera and

cremaster muscles of rabbits infected with *T. brucei*. It was found

that the venous channels in the muscles and viscera were congested

and the tissues oedematous. The blood vessel walls were fragile and

surrounded by granulomatous tissue, a feature of chronic inflammation.

The endothelium of venules and capillaries were lined by phagocytes,

suggesting long-standing damage. Their angiographic experiments showed

constrictions of the central arteries in the ears and evidence was pro-

duced to show that this was mediated through the autonomic nervous

system.

These authors suggested that the arterial spasm could result in

areas of anoxia, with subsequent tissue necrosis.

Goodwin (1971) extended these observations to the small blood vessels

in ear-chambers of rabbits infected with *T. brucei*, by using a com-

bination of *in-vivo* microscopy, histological and electron microscopic

techniques.

Changes were observed in the ear-chambers about 14 days after infection,

which confirmed that the vascular lesion in trypanosomiasis was a chronic

angiitis. The extravascular tissue was heavily infiltrated by mono-

nuclear cells and phagocytes marginating on the endothelial lining of

venules caused obstructions to blood flow, which led to vascular stasis

and eventually disintegration of the small blood vessels.

Electron microscopic studies showed damage to venular endothelium;

increase in the space between the periendothelial cells and basement

membrane and deformation of endothelial nuclei. There was extensive

degeneration of extravascular connective tissue, which was filled with

trypanosomes and their remnants; an observation which confirmed that

T. brucei subgroup organisms were essentially tissue parasites, Goodwin

(1971) also provided ultrastructural evidence to show that the try-

panosomes in the extravascular tissue were phagocytosed by macrophages.

Various factors in trypanosome infections enhance the phagocytosis of

the parasites. For example, Cook (1977) has shown by *in vitro* studies

that serum opsonin and macrophage cytophilic antibody activities in-

creased in rabbits infected with *T. brucei;* with maximum activities

2-3 weeks after infection.

The vascular lesions described by Goodwin and his colleagues

(Goodwin and Hook, 1968; Goodwin 1971) are not soley restricted to the

tissues they examined, but occur in other parts, e.g. in the heart, liver,

brain and kidney (Murray *et al* 1974; Murray. M.*et al* 1975; Poltera *et al*

1977; Morrison *et al* 1979).

In infections of *T. congolense* and *T. vivax* in cattle, in addition

to leucocyte infiltration and inflammatory oedema, there is widespread

dilatation of small blood vessels, thrombosis and damage to the walls

of blood vessels in many organs e.g. the liver, lungs, kidneys and the

brain (Fiennes 1970; Losos *et al* 1973; Kaliner 1974; Van den Ingh *et al*

1976; Valli and Forsberg, 1979; Mwambu and Losos 1979). In cattle infected

with *T. vivax*, there is histological evidence of DIC in vessels lining

renal tubules and also fibrinoid deposition in vessel walls which is in-

dicative of immune complex lesions (Facer, personal communication). Many

blood vessels in infected animals are clogged by trypanosomes. Recently,

Banks (1978) showed by *in-vivo* microscopic observations of the mesenteric

microcirculation in rats and rabbits infected with *T. congolense* that the

parasites attached themselves to the endothelial lining of small blood vessels.

It is conceivable that the parasites could cause direct damage to the

vascular endothelium.

The similarity between the Arthus reaction and chronic trypanosomiasis

was suggested by Goodwin and Hook (1968); who also postulated that

pharmacologically active substances, released as a result of allergic

reactions were probably involved in the vascular changes. Since

trypanosomiasis is basically an inflammatory reaction and increased

vascular permeability is a feature of inflammation (see Section 1.3.2),

it is likely that mediators of inflammation are involved in the path-

ogenesis (see Goodwin 1974, 1976).

It is known that there is an increase in plasma kinins in mice,

rabbits, cattle and humans infected with *T. brucei* subgroup organisms

(Goodwin and Richards 1960; Boreham 1968a; Boreham 1970). It has also

been suggested that kinins are released in all pathogenic infections

caused by African trypanosomes (Boreham, 1977). Although kinins are

released in goats infected with *T. vivax*, there is decrease in levels

of serotonin (Veenendaal *et al* 1976).

Levels of the proteolytic enzyme kallikrein which releases kinins

from inactive precursors (kininogens) are also raised in rabbits

infected with *T. brucei* (Boreham and Parry 1979).

These substances are known to be involved in the inflammatory response

by causing increased vascular permeability, hypotension, chemotaxis of

leucocytes and release of other vasoactive substances (see Boreham 1979b).

The mechanisms of kinin release during trypanosomiasis was elucidated

by Boreham and co-workers (Boreham 1968b; Boreham and Goodwin 1970;

Boreham and Wright 1976). Since kinin release appeared to be associated

with peaks of parasitaemia, it was suggested that antigen/antibody re-

actions might be involved. These authors suggested that immune complexes

of trypanosomal antigen and antibody absorb and activate Hageman factor

(factor XII) which causes the conversion of prekallikrein to kallikrein,

with subsequent production of kinins. This is supported by the *in vitro*

observation, which showed that heating the kininogen substrate to $65^{\circ}C$

(not $56^{\circ}C$) prevented the release of kinins by immune complexes; since

Hageman factor is destroyed at $65^{\circ}C$ (see Boreham 1977, 1979b).

Recently, circulating soluble immune complexes have been found in

man and animals infected with *T. brucei* subgroup organisms (Fruit *et al*,

1977; Lambert and Castro 1977; Boreham and Parry 1979). Immune complex

deposits have also been found in the kidney during trypanosomiasis

(Lambert and Houba 1974). It is likely that the immune complexes con-

tribute to the proliferative glomerulonephritis (Nagle *et al* 1974;

Facer *et al* 1978) which is secondary to other pathological lesions.

Other pharmacologically active substances implicated in the path-

ogenesis of trypanosomiasis include histamine and serotonin (5 - Hydro-

xytryptamine) (reviewed by Goodwin, 1976). Although Richards (1965)

found increases in histamine in mice infected with *T. brucei*, Yates (1970)

could not detect any significant increases in either histamine or the

precursor enzyme (histidine decarboxylase) in mice or rats. Slots *et al*

(1977) have shown that *T. vivax* antigen and specific antibody (i.e. immune

complexes) caused the release of serotonin from goat platelets.

Other substances known to cause increased vascular permeability,

such as fibrinogen/fibrin degradation products have been reported in

clinical and experimental trypanosomiasis (see Section 1.2.2.).

Seed (1969) obtained a protein fraction from homogenates of trypanosomes,

which caused increased vascular permeability and other inflammatory re-

actions in rabbits and guinea-pigs. Goodwin (1974) suggested that this

permeability factor probably acts by releasing kinins.

Recently, many investigators have shown that trypanosomes release

biologically active substances, which might contribute to the overall

pathogenesis of trypanosomiasis (reviewed by Tizard *et al* 1978) Autolysates

of *T. congolense* and *T. brucei*, among other trypanosomes studied were

able to generate active phospholipase A which acts on endogenous phos-
phatidylcholine to produce free fatty acids (FFAs). Several biological
effects suggested for the FFAs and phospholipase A include the causing
of microvascular damage, with thrombotic consequences; immunosuppression
and myocarditis.

These assumptions are attractive, since during trypanosomiasis, the host
is constantly exposed to products of dead and disrupted trypanosomes.
However, there is as yet, no definite evidence to support this hypothesis.

1.4. OBJECT OF STUDY

To investigate the changes that occur in the small blood vessels of
T. brucei infected rabbits; with emphasis on ultrastructure. This is
intended as a further contribution towards an understanding of the pathology
and pathogenesis of the disease.

PART 2. MATERIALS AND METHODS

2.1. ANIMALS

2.1.1. Mice

Male CD-1 mice weighing 18-25 g were obtained from Charles River (UK) Ltd.

2.1.2. Rats

Albino CD male rats weighing 225-250 g were purchased from Charles River (UK) Ltd.

2.1.3. Rabbits

New Zealand white male rabbits weighing 2.5-3.0 kg were obtained from Morton Commercial Rabbits, Parsonage Farm, Essex.

All animals were kept in the animal house and fed standard laboratory diet and given water *ad libitum*.

2.2. TRYPANOSOME

2.2.1. History and Passage

The trypanosome strain used throughout was *Trypanosoma (Trypanozoon) brucei*, 427, obtained from the Lister Institute of Preventive Medicine, Elstree, Herts.

The strain was originally isolated from a sheep in South East Uganda in 1960 and subsequently passaged in mice.

In this laboratory, the strain was maintained by syringe passage of infected blood through mice every two to three days.

2.2.2. Separation of Trypanosomes and Infection of Animals

Trypanosomes were separated from heavily infected rat blood obtained by cardiac puncture using the DEAE - Cellulose anion - exchange column of Lanham (1968). Phosphate buffered glucose (PSG) at pH 8.0 was used as eluant.

The rats erythrocytes were separated from the trypansomes by centrifugation (1,600 g for 10 min) before applying the trypanosome suspension onto the Cellulose in a glass Buchner funnel of porosity 1 (Anderman and Co. Ltd., London).

The separated trypanosomes were then washed five times in PSG, to remove all plasma components. Separated and washed trypanosomes were counted in an improved Neubauer haemocytometer, as used for counting erythrocytes.

Male rabbits were infected by subcutaneous inoculation of 10^8 trypanosomes previously separated from infected rat blood as described above.

2.3. LIGHT MICROSCOPY

2.3.1. Paraffin Wax Technique

a. Fixation

Autopsy materials obtained from control and infected rabbits were cut into small pieces (2-5 mm thick) and fixed in 10% formol-saline for 24 hr. The specimens were dehydrated in graded series of ethanol (50%, 70%, 90% and absolute alcohol) and cleared in two changes of Xylene. Tissues remaining opaque after treatment with Xylene were returned to absolute alcohol for further dehydration.

b. Embedding and Examination of Sections

The cleared materials were transferred to beakers containing molten paraffin wax (m.pt 56-58°C) in a heated vacuum embedding bath (Gurr Ltd) to effect impregnation. They were subjected to three changes of wax for 1 hr each.

After impregnation, the tissues were embedded in watch glasses with fresh molten paraffin wax and left to solidify in cold water.

Sections (5-10 μm thick) were cut on a Reichert rotary microtome, collected on clean microscope slides and dried on a hot plate at 45-50°C.

After removing the wax from the sections with Xylene, the slides were stained with haematoxylin and eosin, dehydrated through graded ethanol, cleared in Xylol and then mounted with Canada balsam. The permanent slides prepared were examined under a Zeiss RA light microscope with X10 and X40 objectives and photographs taken with an attached 35 mm camera.

2.3.2. Cremaster Muscle Preparation

The cremaster muscle is a laminar extension of the internal oblique of the adbominal wall. Each cremaster forms a pouch containing one testis and is loosely attached to the skin of the scrotum on the ventral and lateral sides and to the peritoneal region on the dorsal aspect.

Although the two cremaster muscles are partially joined by loose connective tissues, they are anatomically separate.

The technique for transparent whole mount preparation of rat cremaster muscle described by Majno, Palade and Schoefl (1961) and adapted for rabbits by Goodwin and Hook (1968) was used.

Rabbits under anesthesia (See 2.4.1. a.) were given 1 ml/kg body wt of Pelikan ink (Gunther Wagner, Hannover, W. Germany) or Shellac-free colloidal carbon (Taab Lab., Reading) diluted 1 in 4 with normal saline intravenously through the marginal ear vein.

One hour after injecting the carbon suspension, the scrotal skin was removed and the exposed cremaster muscle was quickly excised with the testis it surrounds and immersed in 10% formol-saline. The cremaster muscle was separated from the testis in fixative, pinned out on dental wax and the remaining connective tissue gently removed.

The prepared cremaster muscle was further fixed in formol-saline for 24-48 hr, dehydrated in graded ethanol and cleared in methyl benzoate for 24-48 hr. The transparent preparation was trimmed, mounted on a clean microscope slide with DePex (Gurr Ltd). The slides were examined with a Zeiss RA microscope and Wild M8 stereo-dissector. Photographs were taken with a 35 mm camera.

2.4. ELECTRON MICROSCOPY

2.4.1. Mesentery

a. Anaesthesia

Rabbits held in a restrainer were either given 1.2 g/kg of urethane (ethyl carbamate) or 26.4 mg/kg of pentobarbitone sodium (Sagatal) intravenously through the marginal ear vein.

The effect of the anaesthetic was monitored by observing the animal's eye reflexes and slightly pinching between the paws.

b. In-vivo Microscopy

The set up for in-vivo microscopic observation of the mesenteric terminal vascular bed is shown in Fig. 2a. The rabbit under anaesthesia was laid on it's side on a dissecting board supported by an adjustable laboratory jack.

An incision about 5 cm long was made in the lower abdominal region and a small portion of the small intestine exteriorized and draped over a perspex ring (mesentery chamber) (Fig. 2b). Care was taken not to touch the surface of the mesentery, as the small blood vessels could be affected by mechanical trauma.

A suitable area of the mesentery was selected and bathed with physiological saline at $37^{\circ}C$. The other parts of the exposed intestine not required were wrapped with cotton wool soaked in warm saline. The mesentery was allowed to settle for 30 min before observations of the microvessels were made under a wild M20 light microscope equipped with a polaroid 3000 camera.

The following were noted:

(a) Direction of blood flow in the vessels; to determine whether the blood vessels were venular or arterial segments of the terminal vascular bed.

(b) Diameters (from wall to wall) of the blood vessels, using an occular micrometer previously calibrated with a stage micrometer.

(c) Behaviour of formed elements of blood, particularly leucocytes.

The blood vessels observed were photographed with the polaroid camera attached to the microscope.

c. Fixation

The observed microcirculatory bed was fixed *in situ* by dripping warm 2.5% Glutaraldelyde in 0.1 M sodium cacodylate buffer, pH 7.2-7.4 (Sabatini *et al.*, 1963) for 30 min. After this initial fixation, the mesentery was excised from the animal, pinned on flat embedding mould (Emscope Laboratories Ltd., London) and fixed further for 90 min in Glutaraldelyde at 4°C. Following 1 hr post fixation in buffered 1% Osmium tetroxide at 4°C, the specimen was dehydrated in ascending concentrations of ethanol (50%, 70%, 90% and absolute alcohol).

d. Infiltration, Embedding and Polymerization

The specimen was further dehydrated in two changes of propylene oxide, infiltrated with a mixture of equal parts (V/V) of propylene oxide and Epon 812 for 1 hr and passed through pure Epon 812 for 15 hrs on a rotator at room temperature (Luft, 1961).

The mesentery was finally embedded in flat embedding mould in fresh Epon 812 and polymerized at 60°C for 24-48 hr.

The blocks containing the specimens were allowed to 'cure' at

Fig. 2A : Set up for *in vivo* microscopic observation of mesenteric terminal

vascular bed.

Fig. 2B : Mesentery chamber: a perspex ring (arrow) over which the

exteriorized mesentery is placed for *in vivo* microscopic

observation.
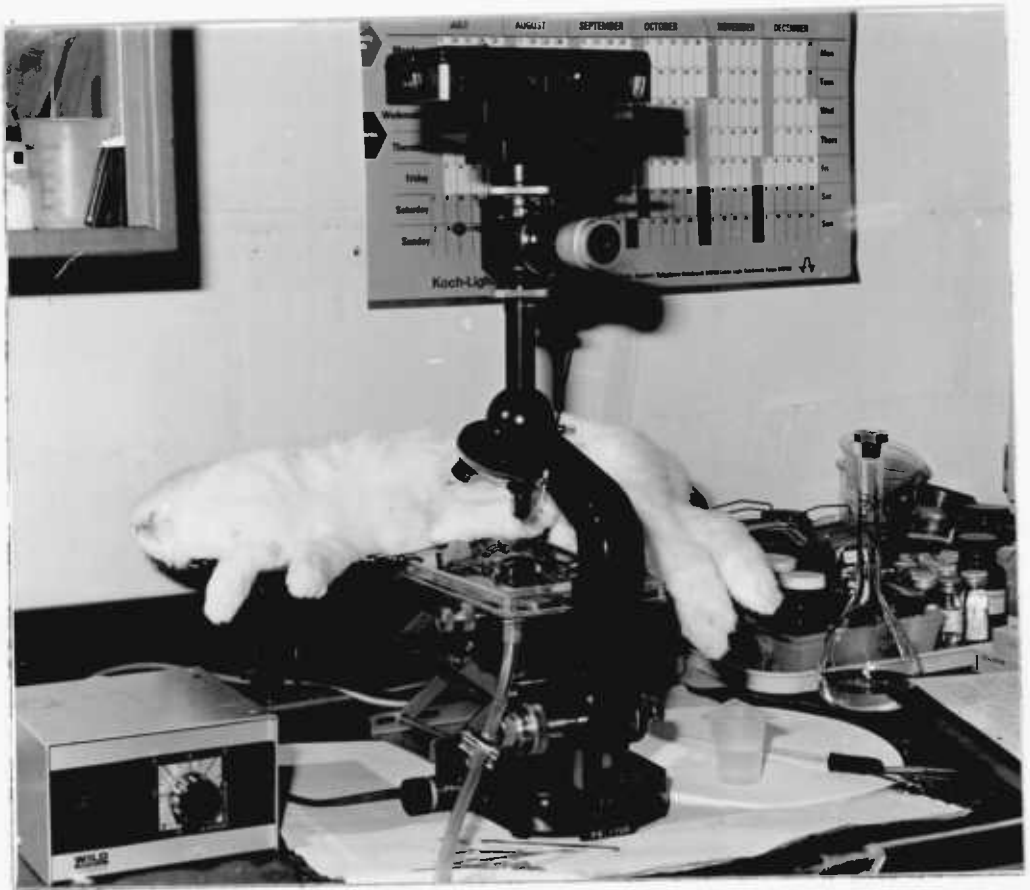
room temperature for 2-4 days before microtomy.

2.4.2. Cremaster Muscle

a. Dissection and Fixation

Cremaster muscles from uninfected control and infected rabbits were used. The animals were subjected to anaesthesia as described in Section 2.4.1. a.

The dissection procedure adapted was that described by Baez (1973) for rat cremaster muscle. The fur around the scrotal areas was gently removed by shaving. A longitudinal incision of the scrotal skin was made in the mid line over the ventral aspect of the scrotum, by holding these structures away from the cremaster muscle with fine forceps (Fig. 3a).

The scrotal skin and the connective tissue joining the cremaster to it were retracted from the exposed cremaster muscle which was quickly flooded with chilled 2.5% Glutaraldelyde in 0.1 M sodium cacodylate pH 7.2-7.4 (Majno & Palade, 1961).

A suitable portion of the cremaster muscle was selected avoiding anastomosing blood vessels and another incision made towards the distal end (Figs. 3b & c). The testis and epididymis were separated from the opened cremaster muscle which was excised after clamping the base and immersed in cold fixative.

The tissue was cut into small segments (1-2 mm^2) in a pool of fixative on dental wax, under a dissecting microscope. The pieces of tissue were further fixed in glutaraldelyde for 4 hr at $4^{\circ}C$, washed in three changes of 0.1 M sodium cacodylate buffer and post-fixed in buffered 1% Osmium tetroxide at $4^{\circ}C$ for 1 hr.

Fig. 3 : Dissection of cremaster muscle

3A : Shows the initial stage in the dissection of rabbit cremaster muscle.
An incision is made at the tip of the scrotal skin at X and extended
towards the base (Y).

3B : Shows the exposed cremaster muscle (CM) covered with connective tissue,
after retracting the scrotal skin (SS).

3C : Shows the completed dissection of the cremaster muscle (CM) with the
testis and epididymis (TE) retracted.
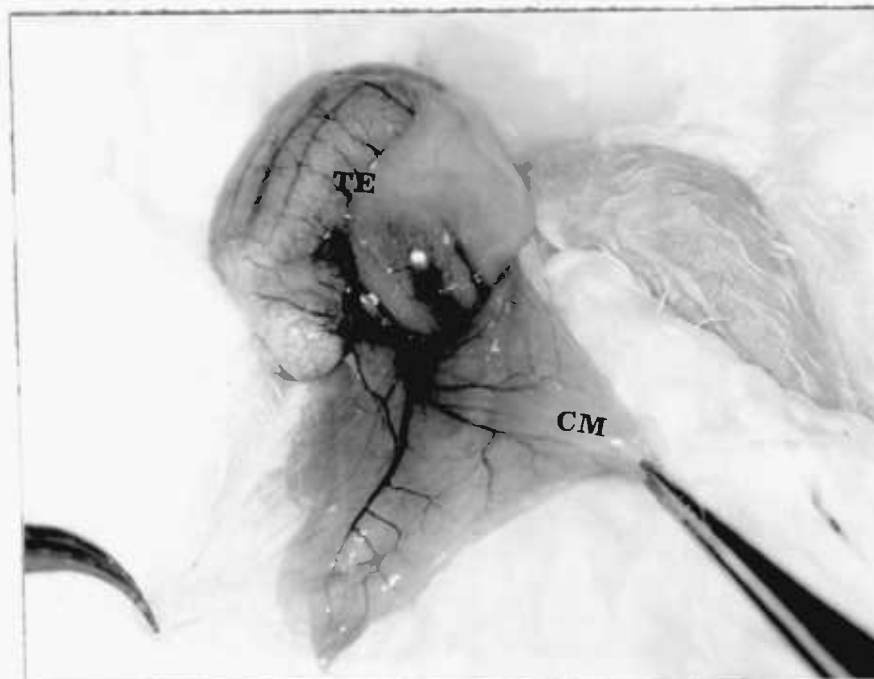
Dehydration and infiltration of the cremaster muscle segments were carried out as described in Section 2.4.1. d. The specimens were embedded in Effa flat preshaped embedding moulds (Ernest Fulham Inc., Schenectady, N.Y.) and polymerized as described for the mesentery.

2.4.3. Preparation of Specimen Blocks for Examination

a. Microtomy

The specimen blocks prepared as described in Section 2.4.1. were cut into smaller ones with a jeweller's saw and mounted on 'dummy' blocks with rapid Araldite (Ciba Geigy). The cremaster muscle blocks were sectioned as embedded, since preshaped embedding moulds were used (Section 2.4.2.).

An LKB Ultrotome III equipped with glassknives made with an LKB knifemaker was used for sectioning the specimen blocks.

After trimming the blocks, 0.5-1 µm thick sections were cut, collected with a platinum loop onto a clean microscope slide, stained with 1% toluidine blue in 1% borax and examined with a light microscope, for survey purposes. Some of the slides were photographed with a 35 mm camera and used as adjunct to the ultrastructural observations.

Ultrathin sections (60-150 Å) with gold to silver colour inter- ference (Peachey, 1958) were cut and collected on formvar-coated (0.3% formvar in ethylene dichloride or chloroform) Cu/Rh 150-200 meshed specimen grids (Taab. Labs., Reading).

The grids were always sonicated in chloroform and dried before coating with formvar.

b. Staining and Examination of Specimen Grids

The double staining technique described by Knight (1977) was

adapted for staining the specimen grids obtained.

All stain solutions were freshly prepared and centrifuged before use.

A clean piece of dental wax was placed in the bottom of a petri dish with a lid and with a pasteur pipette drops of 8% uranyl acetate in 70% ethanol corresponding to the number of grids to be stained were squeezed on it. The grids were placed section side down individually onto a drop of stain and left for 10-15 min. After staining, the grids were washed in 70% ethanol and carbon dioxide-free distilled water, dried and transferred to another petri-dish containing drops of lead citrate (Reynolds, 1963) on dental wax. Sodium hydroxide (NaOH) pellets were placed on the dental wax, to remove atmospheric carbon dioxide. The grids were left for 10 min to stain and later washed under a jet of carbon dioxide-free distilled water, dried on filter paper and stored in grid holders.

The stained grids were examined with a Philips EM 300 transmission electron microscope at 60 KV. Records were made using 35 mm and plate cameras in the microscope.

PART 3. EXPERIMENTAL

3.1. GROSS PATHOLOGY OF RABBITS

Rabbits infected with *Trypanosoma brucei* were examined for external (morphological) changes and compared with uninfected control animals.

Figs 4a and b show an uninfected rabbit. There are no necrotic lesions on any part of the animal and the ears are held upright. All control animals remained in good health throughout the period of observations, which was 4-6 weeks.

About 21 days after infection, rabbits became emaciated, debilitated and showed gross oedema which increased with time (Fig. 5). The ears become heavy and swollen especially at the base and finally droop as the animal is unable to hold them upright (Fig. 6a). There is swelling of the face and tissues around the eyes and necrotic crusts appear on the eyelids, skin of the ears, external nares (Fig. 6a) and the scrotum (Fig. 6c). In a few rabbits infected for more than 28 days the testicles were swollen and in some cases ruptured, probably due to oedema or secondary infections. At the late stage of the infection, the eyes may become completely closed (Fig. 6b). Purulent exudates from the eyes and nostrils were also observed.

After 28 days of infection, the blood vessels (e.g. the marginal ear veins) in many rabbits became very fragile, such that it was difficult to inject substances into them.

The animals die usually 6-8 weeks after infection. However, a few rabbits were observed to be asymptomatic several weeks after infection. Although bacteriological examinations were not carried out in these rabbits, it is likely that secondary bacterial infections contributed to

the lesions observed, as trypanosomiasis is known to have immuno-
suppressive effects on infected rabbits (Goodwin *et al.*, 1972) cattle
(Holmes *et al.*, 1974) and humans (Greenwood *et al.*, 1973).

These changes are in agreement with the descriptions given for rabbits
(Goodwin & Hook, 1968) and sheep (Ikede & Losos, 1975) infected with *T.
brucei* and indicate an inflammatory response.

Figs. 4-6 : Gross pathological observations of rabbits.

Figs. 4A & 4B : Show two views of an uninfected rabbit. The ears are held

upright and there are no necrotic lesions on any part of

the animal's body.

Fig. 5 : Rabbit infected for 21 days. The ears are heavy, with one (left)

almost drooping. The face and eyelids are swollen.

Fig. 6(A-C) : Rabbit infected for 35 days.

6A : There are necrotic crusts on the skin of the ears, eyes and external

nares. The face is swollen, especially above the eyes and both ears

droop, as the animal is unable to hold them upright.
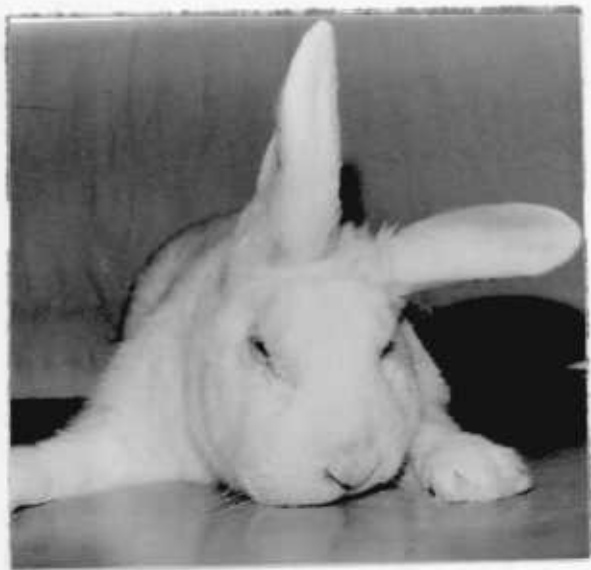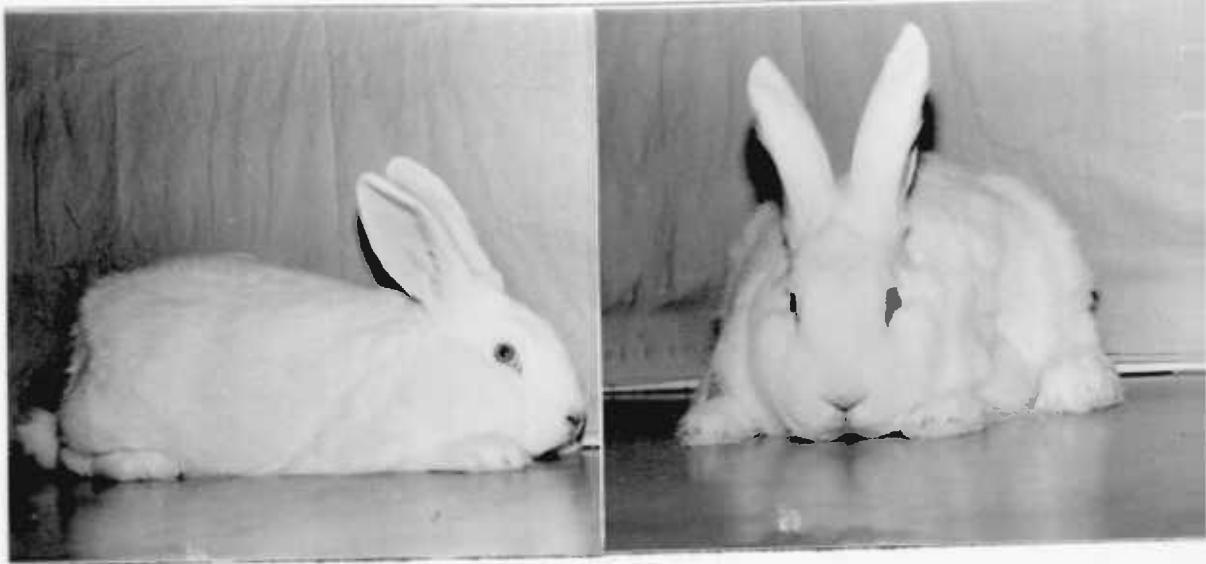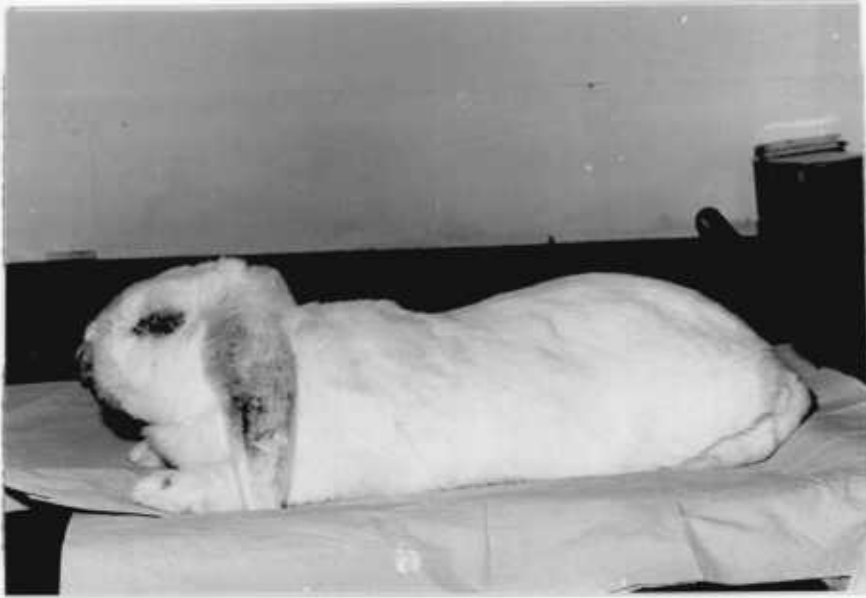
Fig. 6B : Same rabbit as in Fig. 6A.   The eye is completely closed.
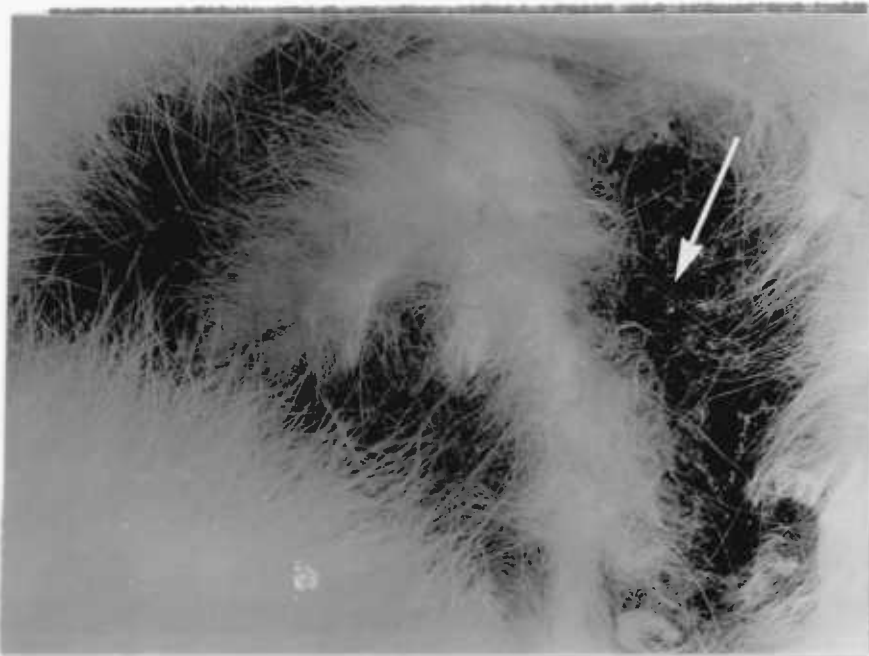
Fig. 6C : Same rabbit as in Fig. 6B, showing necrotic lesions (arrow) on
the scrotal skin.

3.2. HISTOPATHOLOGY OF RABBIT TISSUES

Thirty rabbits (8 controls and 22 infected) were used for histo-pathological studies. The animals were killed by intravenous injection of pentobarbitone sodium 48 mg/kg into the marginal ear vein. The infected animals were sacrificed at 10 (2), 14 (4), 21 (4), 28 (6), 35 (4) and 42 (2) days after infection. The figures in parenthesis show the number of animals used for each infection period. The uninfected control rabbits were sacrificed at different days throughout the experimental period.

At necropsy, pieces of tissues were quickly removed from different parts of the ear, liver and kidney. The tissues were fixed in 10% formol-saline, dehydrated in graded alcohols and embedded in paraffin wax after infitration as described in Section 2.3.1.

Sections (5-10 μm thick) of the tissues in paraffin blocks were cut on a Reichert rotary microtome, stained with haematoxylin and eosin (H and E) and examined with a Zeiss RA light microscope. Photographs were taken with a 35 mm camera attached to the microscope.

RESULTS

(a) Ear

Figs 7a and b show sections through the ear of an uninfected rabbit. The ear consists of an outer epidermal layer made up of squamous epithelium and an inner dermal layer. In the central part is the cartilage which is covered on both sides by connective tissue (Perichondrium) and it's matrix shows chondrocytes with collagenous fibres between them. The chondrocytes are mostly elliptical in shape.

Fig. 7b also shows both the epidermal and dermal layers of the ear. In the dermal layer are small blood vessels (small veins, venules,

arterioles and capillaries), glandular ducts, hair follicles and cartilage.

In control rabbits there was no infiltration of the tissues by leucocytes. Apart from occasional fixation artefacts, the connective tissue fibres were not separated.

About 14 days after infection, the ear was oedematous and infiltrated by leucocytes (Fig. 8).

Leucocyte margination and emigration (diapedesis) occurred in venules and small blood vessels were surrounded by an accumulation of these emigrated leucocytes and cellular debris (Fig. 9). The lumina of many venules were clogged by leucocytes which were mostly mononuclear cells.

By day 35 massive cellular infiltration and severe oedema manifested by extensive separation of the connective tissue fibres were observed (Fig. 10).

There were no alterations in the cartilage matrix of all the animals examined.

(b) Liver

Sections through the liver of control rabbits are shown in Figs 11 and 12.

Fig. 11 shows the centrilobular zone with the central vein and hepatocytes which are radially arranged in plates. Between these rows of hepatocytes are sinusoids. Fig. 12 is an enlarged area showing liver parenchyma consisting of hepatocytes and sinusoids.

In control rabbits, the sinusoids were not dilated and there were no cellular infiltration of the tissue. The nuclei of the hepatocytes were not vesiculated.
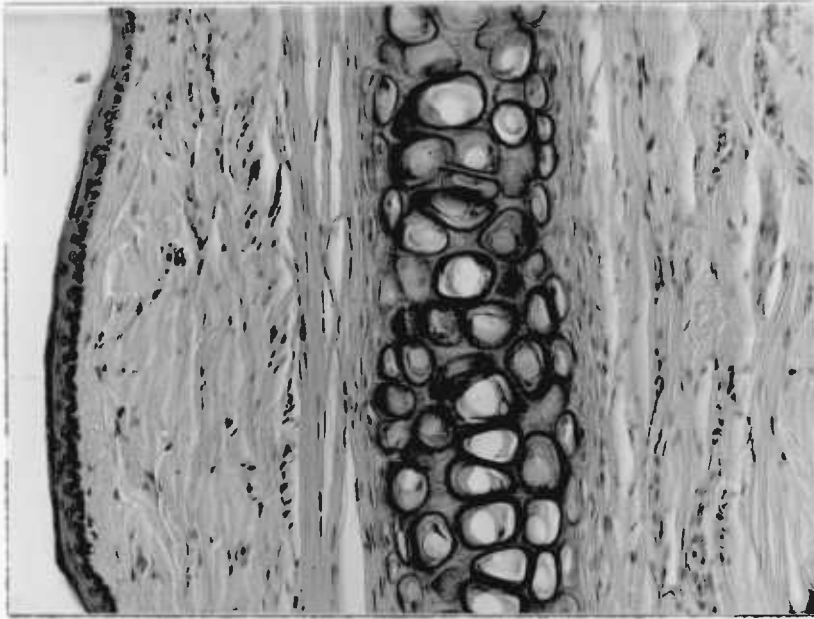
Fig. 7A : Section through the ear of an uninfected rabbit. X 215.
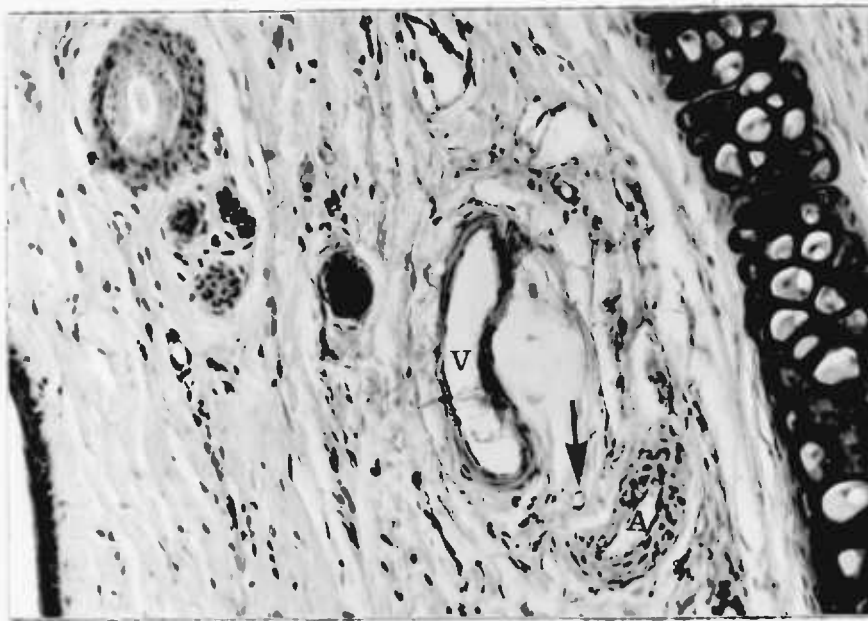
Fig. 7B : Section through the ear of an uninfected rabbit showing small
blood vessels (venule - V, capillary (arrow) and Arteriole - A)
in the dermal layer. X 215.

Fig. 8 : Section through the ear of a rabbit infected for 14 days. The

tissue is oedematous and shows slight cellular infiltration

(arrow). X 215.

Fig. 9 : Section through the ear of a rabbit infected for 28 days showing

marginating and emigrating leucocytes in a venule (V). The

tissue is oedematous and the small blood vessels are surrounded

by leucocytes and cellular debris (arrow). X 860.

Fig. 10 : Section through the ear of a rabbit infected for 35 days. The

tissue is oedematous and heavily infiltrated by leucocytes.

Leucocytes also marginate on the venule (V) and block the lumen

of another venule (arrow). X 215.
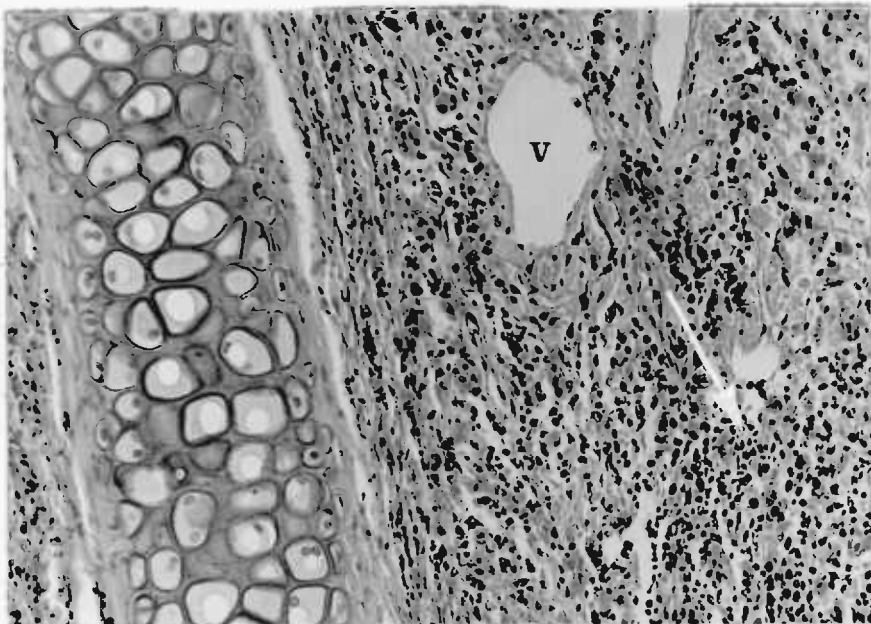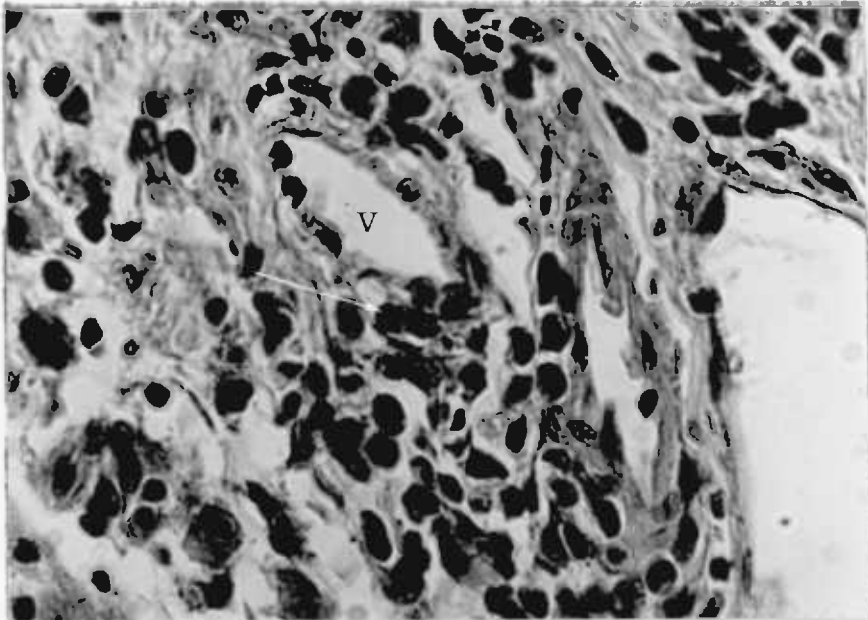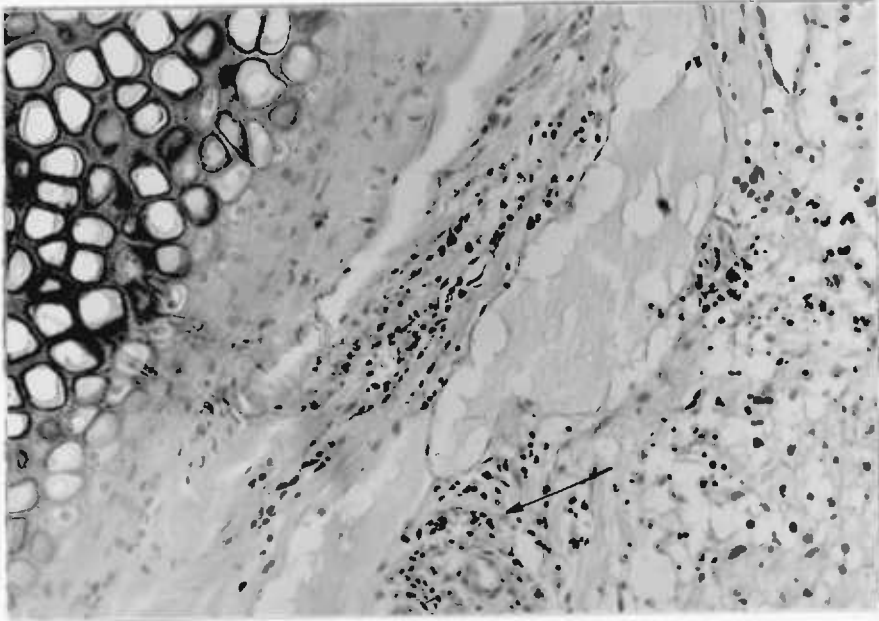
The liver showed degenerative changes about 14 days after infection. The hepatic sinusoids and venous channels were dilated and congested. The centrilobular zone showed degeneration of hepatocytes and slight infiltration by leucocytes (Figs.13 & 14).

After 21 days of infection, venous congestion, extensive hepatocellular necrosis, dilatation of hepatic sinusoids and cellular infiltration were marked (Figs. 15 and 16). The hepatocytes were infiltrated by fat globules and many showed vesiculated nuclei (Fig. 17).

Fig. 18 is a section through the liver of a rabbit infected for 35 days showing extensive hepatocellular degeneration (the structures are barely discernible) and fatty infiltration. The fat globules coalesced in many areas.

(c) Kidney

Figs. 19 and 22 show sections of kidney from an uninfected rabbit. In Fig. 19, there are glomeruli, proximal and distal convoluted tubules. The glomeruli are made up of capillary tufts surrounded by Bowmans' capsules. The convoluted tubules are lined by cuboidal epithelium, with uniform nuclei. In the section are renal blood vessels.

Fig. 22 is a section through the medulla showing collecting tubules. They are mostly lined with cuboid epithelium.

Changes were observed in the kidneys of the rabbits 14 days after infection. The glomeruli and the convoluted tubules (especially the proximal ones) were dilated and the capsular space increased in many instances (Fig. 20). The capillary loops were lobulated and in some cases were almost obliterated (Fig. 21).
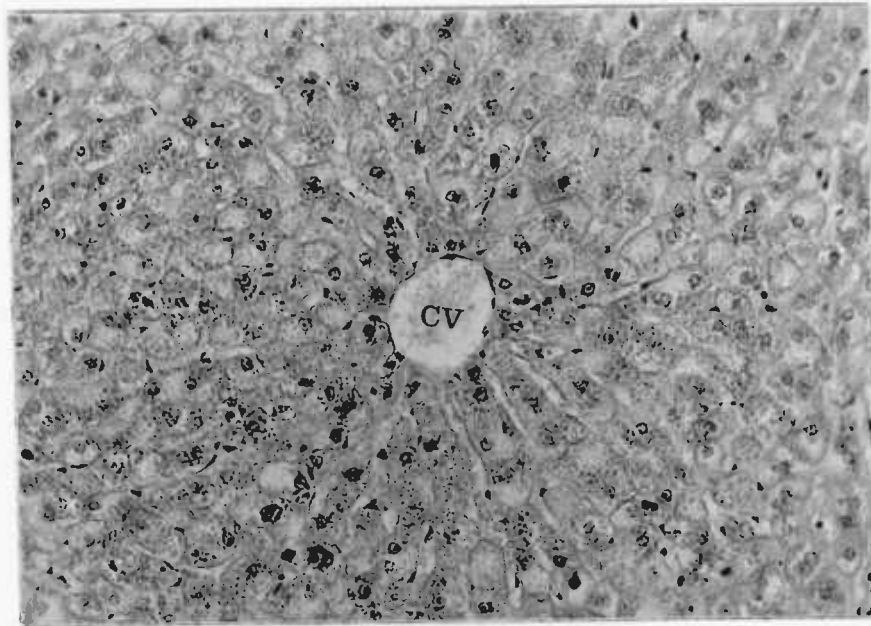
Fig. 11 : Light micrograph of a section through liver of an uninfected
rabbit, showing the central vein (CV) surrounded by hepatocytes.
X 215.
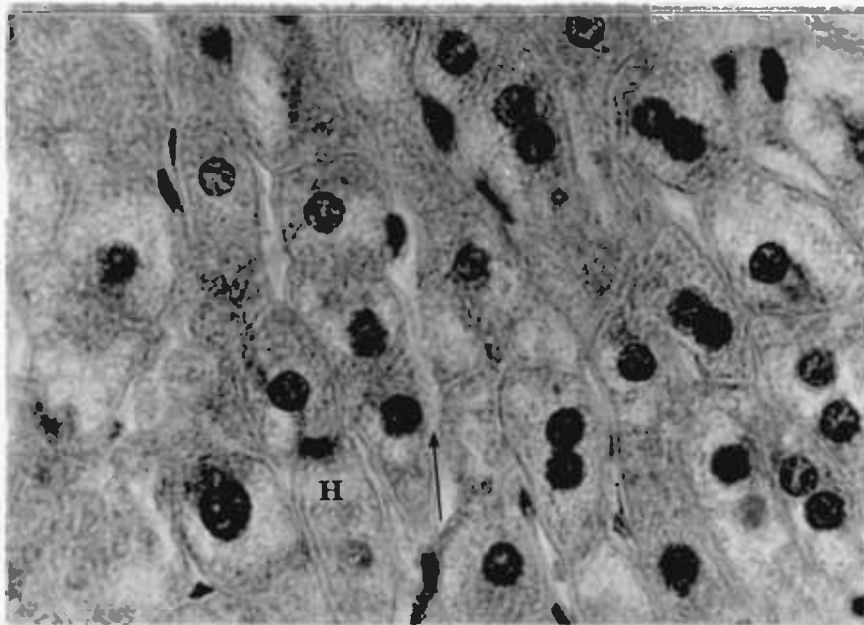
Fig. 12 : High magnification light micrograph of liver parenchyma from an
uninfected rabbit showing hepatocytes (H) separated by sinusoids
(arrow). X 860.

Fig. 13 : Section through the liver of a rabbit infected for 14 days

showing congestion of the central vein (CV) and focal degener-

ation of hepatocytes and slight cellular infiltration (arrow).

The sinusoids are dilated. X 215.

Fig. 14 : Section through the liver of a rabbit infected for 14 days

showing dilatation of hepatic sinusoids (S). The nuclei of the

hepatocytes (H) are vesiculated. X 860.

Fig. 15 : Section through the liver of a rabbit infected for 21 days

showing extensive cellular infiltration (arrow) and dilatation

of sinusoids. X 215.
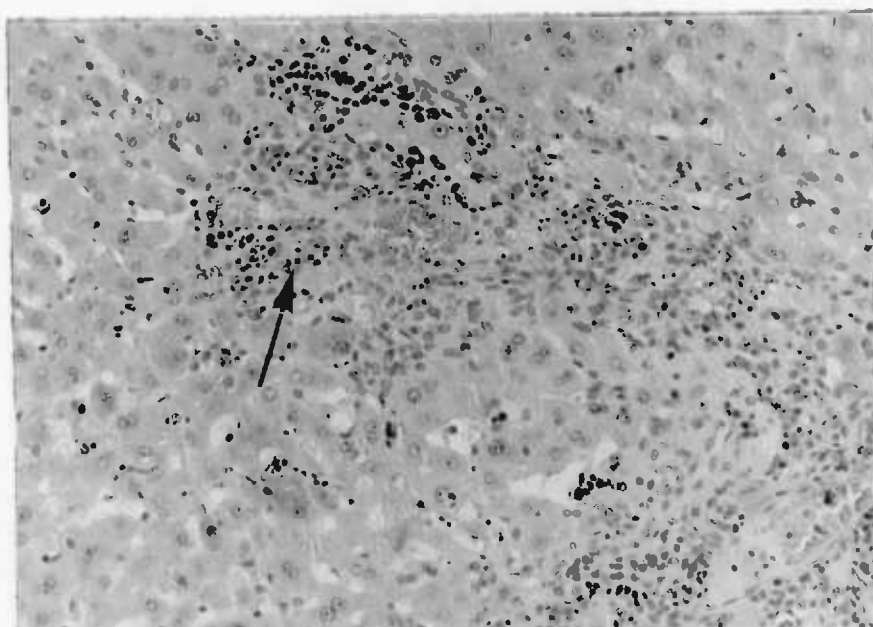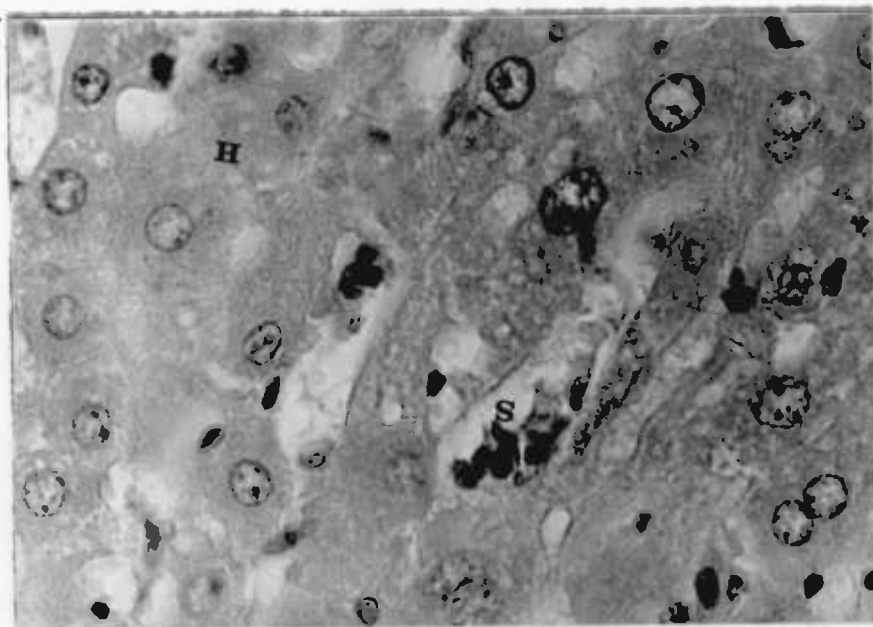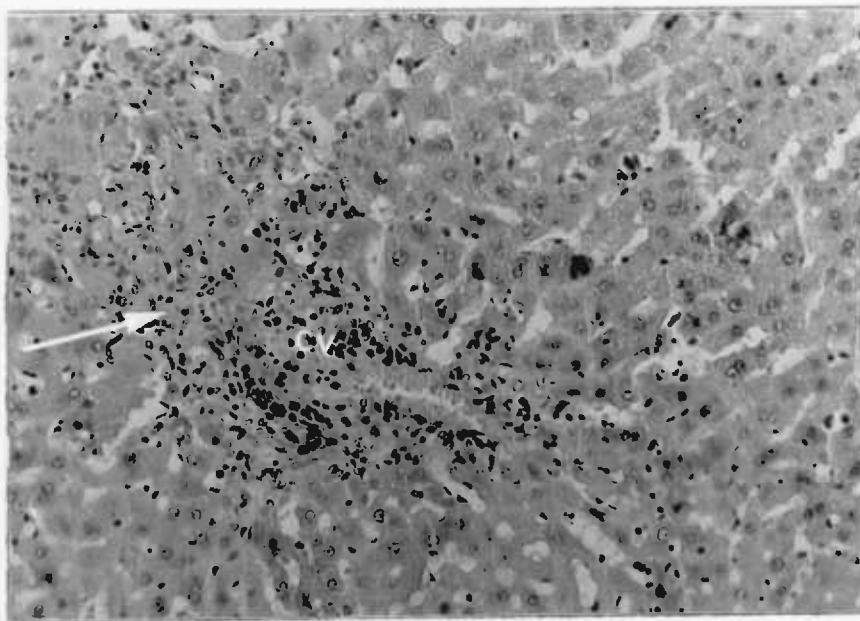
Fig. 16 : Section through the liver of a rabbit infected for 21 days

showing massive cellular infiltration of hepatic parenchyma

(arrow) and degeneration of hepatocytes (H). X 860.

Fig. 17 : Section through the liver of a rabbit infected for 28 days.

The hepatocytes (H) are degenerate and infiltrated by fat

globules (arrow). X 860.

Fig. 18 : Section through the liver of a rabbit infected for 35 days

showing extensive degeneration of hepatocytes. The hepatic

parenchyma shows fatty infiltration (arrow). X 215.
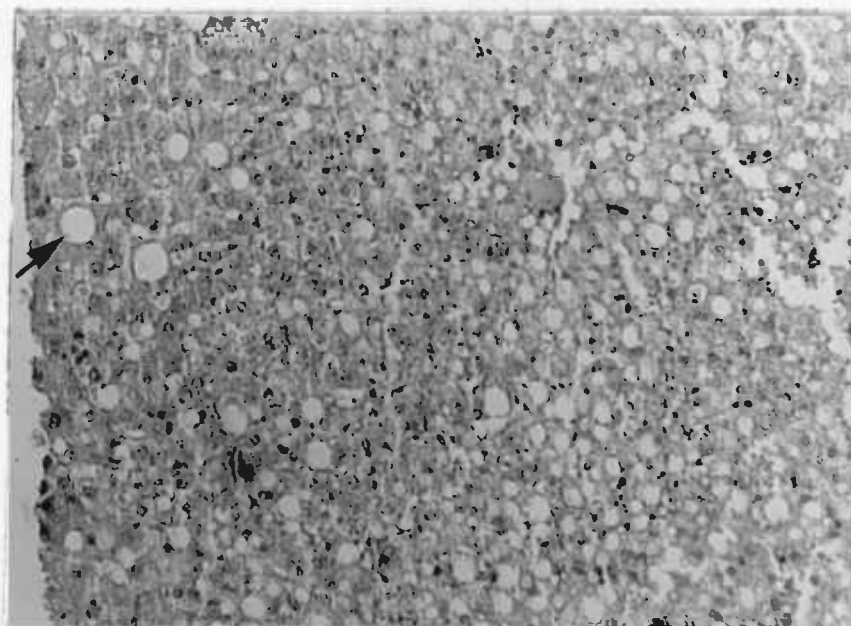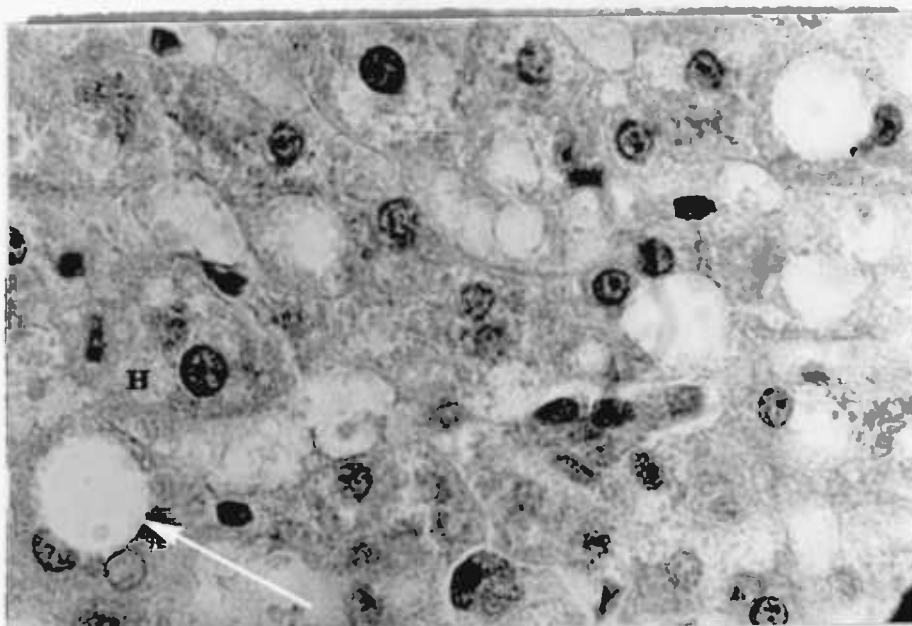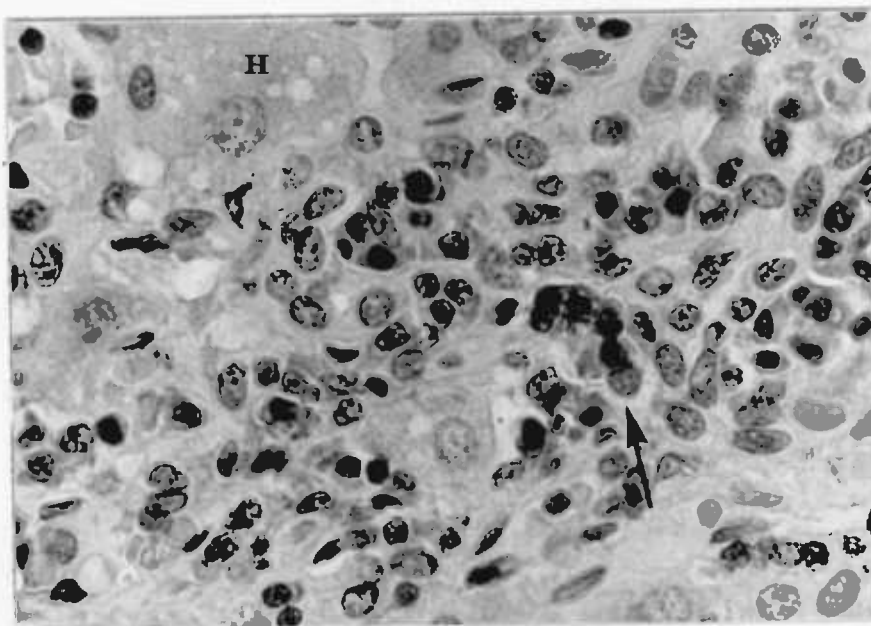
Figs. 19-24 :   light micrographs of sections through kidneys of

uninfected and infected rabbits.

Fig. 19 : Section through the cortical region of kidney from an uninfected

rabbit, showing glomeruli (G), tubules (T) and renal vessels

(arrow). X 860.

Fig. 20 : Section through the cortical region of kidney from a rabbit

infected for 21 days. The glomeruli (G) and tubules (T) are

dilated. The glomerulus (G) is lobulated. X 860.

Fig. 21 : Section through the cortical region of kidney from a rabbit

infected for 28 days. The glomeruli (G) and tubules (T) are

dilated. The glomerular loop (G) is almost obliterated. X 860.
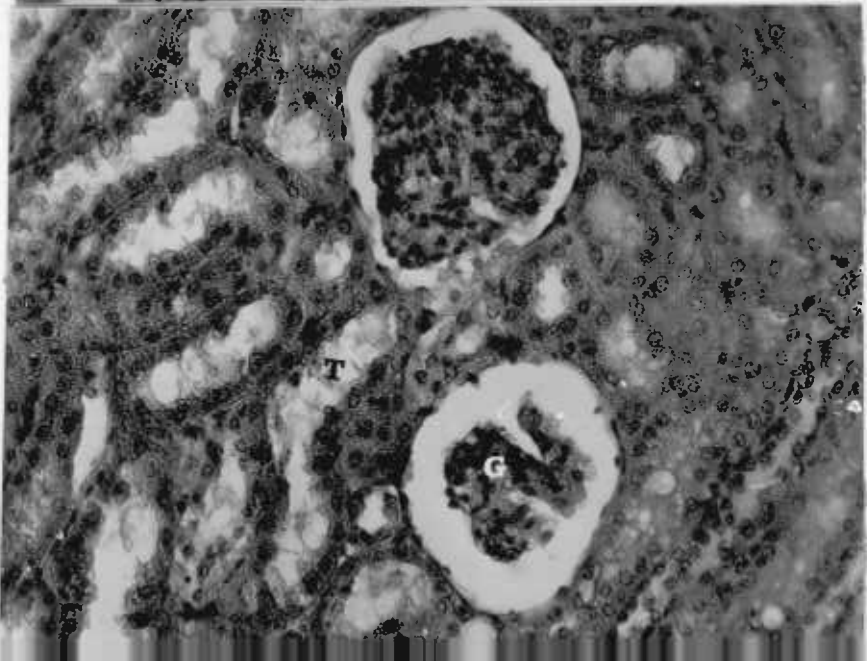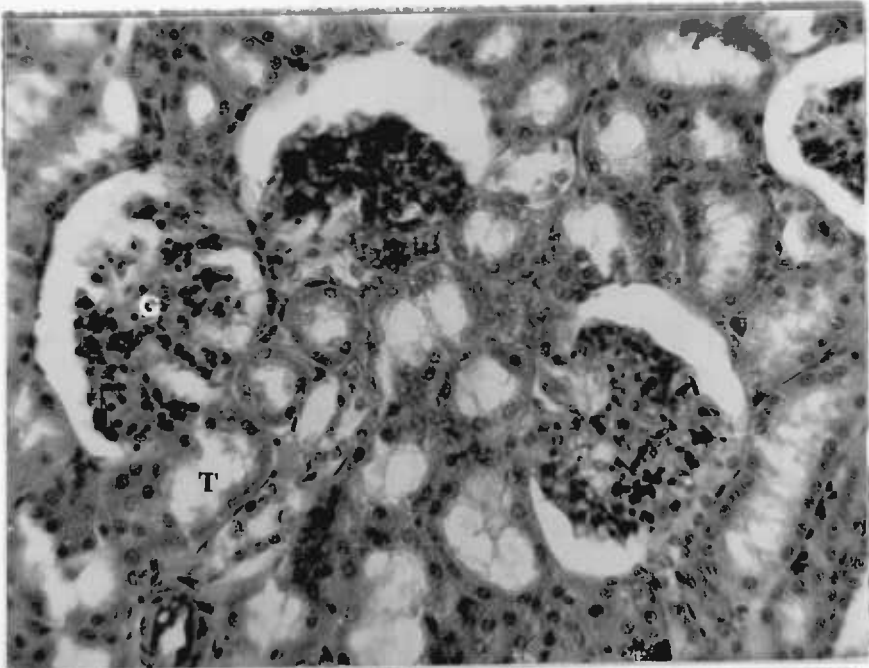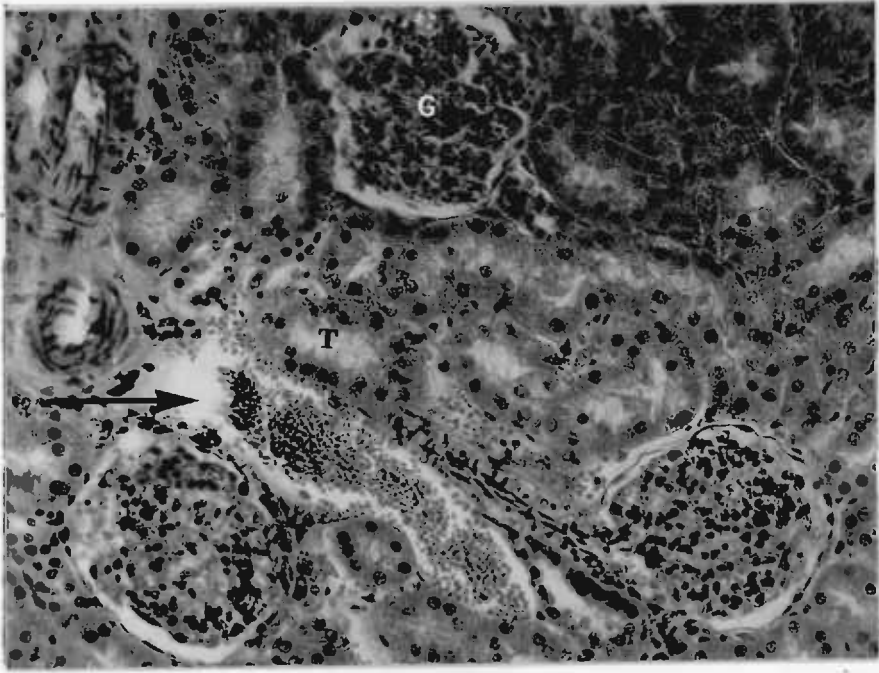
Fig. 22 : Section through the medulla of kidney from an uninfected rabbit,

showing tubules (T). X 860.

Fig. 23 : Section through the medulla of kidney from a rabbit infected for

28 days, showing degeneration of the tubules (arrow). The nuclei

of the cells lining the tubules are pyknotic. X 860.

Fig. 24 : Section through the medulla of kidney from a rabbit infected for

42 days, showing extensive degeneration of tubules (T). Most

tubules are devoid of nuclei and contain materials in their

lumina (?) which are probably casts. X 860.
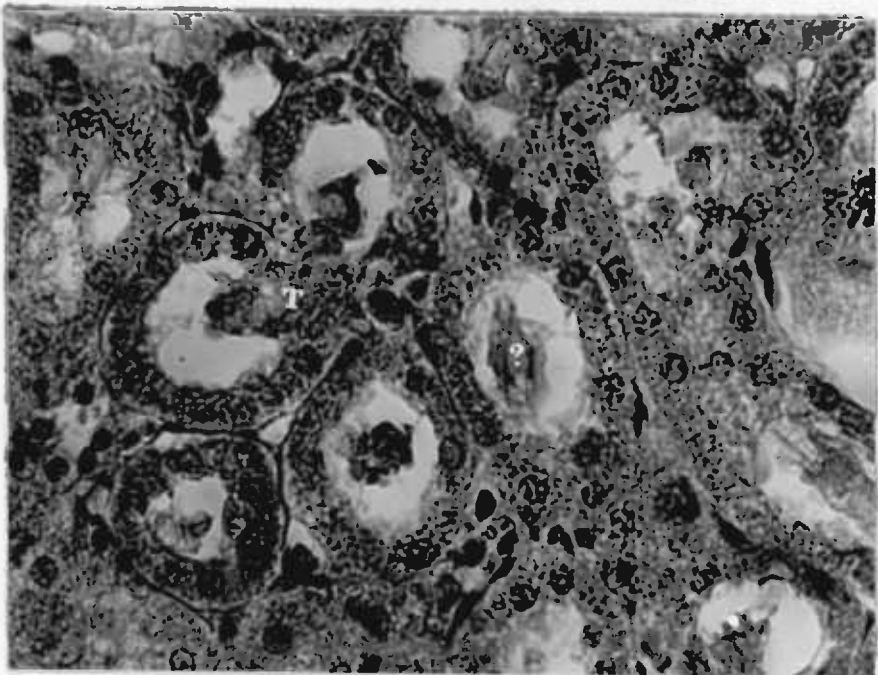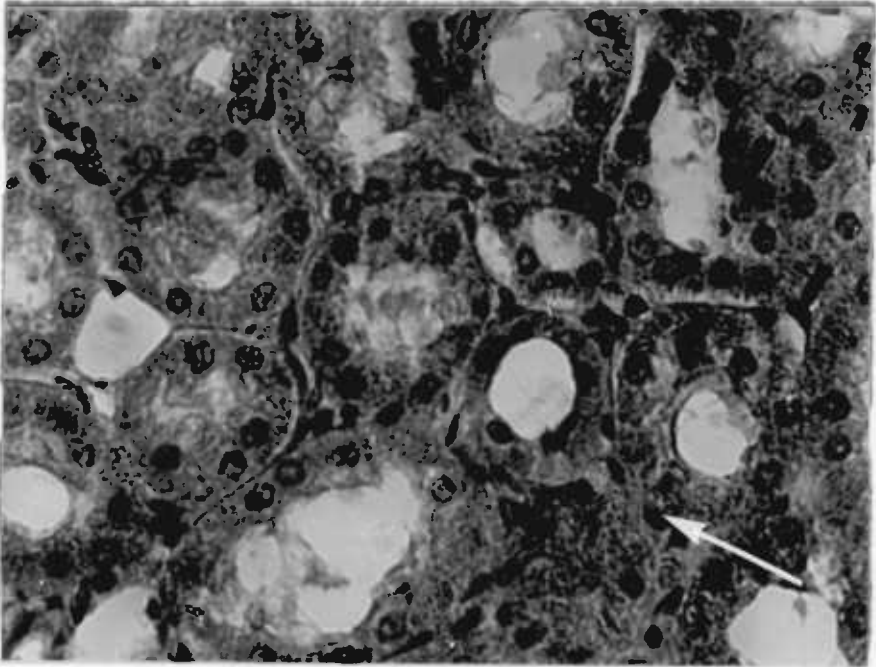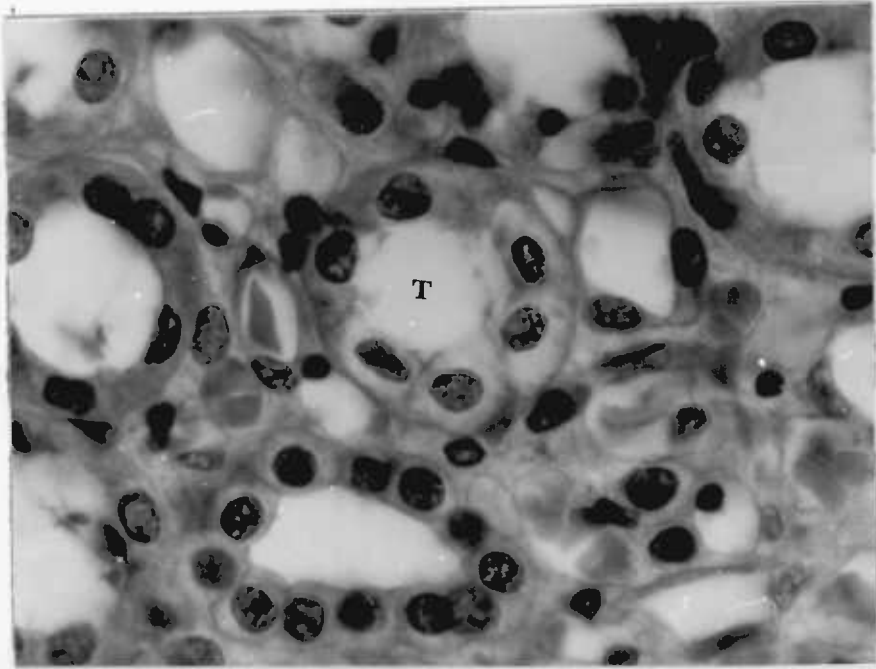
The collecting tubules were also dilated and degenerate in places and most had pyknotic nuclei (Fig. 23). These changes were marked in late infected rabbits. In Fig. 24, for instance most of the tubules in the section are necrotic and devoid of nuclei.

DISCUSSION

The results obtained above suggest that rabbits infected with trypanosomes show an inflammatory response, manifested by leucocyte mobilization, microvascular damage, inflammatory oedema and subsequent tissue necrosis.

The lesions observed in the ears are consistent with the light microscopic descriptions given by Goodwin (1971) for the ear chamber tissues of rabbits infected with *T. brucei*.

The changes in the kidney and liver are also similar to those described for cattle infected with *T. congolense* (Kaliner, 1974; Losos *et al.*, 1973; Valli & Forsberg, 1979) and *T. brucei* infection in rabbits (Facer *et al.*, 1978; Van den Ingh, 1976) and mice (Murray *et al.*, 1975).

The leucocytes observed in the venous channels suggest injury to these blood vessels, as alteration in the morphology of the vascular endothelium is a pre-requisite for leucocyte margination and subsequent emigration. As the number of leucocytes marginating on the endothelial lining increased, the lumen of such blood vessels would be occluded and therefore blood flow through them impeded. This could lead to haemodynamic changes that would be detrimental to the host's tissues.

The venous and sinusoidal congestion observed in the liver of infected rabbits also indicate defects in the flow properties of blood and consequent damage to the blood vessels. The fatty infiltration of hepatocytes suggest hepatic dysfunction especially in the metabolism of

free fatty. acids, probably due to hypoxia.

The inflammatory oedema in the tissues point to an increase in vascular permeability or decreased albumin and therefore injury to the blood vessels, since changes in the structure of the blood vessel walls are necessary to bring about the increase in vascular permeability and loss of plasma proteins. The cartilage matrices in the ears of infected rabbits showed no abnormalities, suggesting that the 'floppy' ears is due to the inflammatory oedema.

The lesions and cellular necrosis observed in the tissues were probably due to inadequate blood supply as a result of damage to the blood vessels and haemodynamic changes in the blood.

3.3. STUDIES ON THE TERMINAL VASCULAR BED

3.3.1. Mesentery

Twelve rabbits (4 controls and 8 infected) were used to study the terminal vascular bed of the mesentery. The studies were undertaken, in order to elucidate the morphological changes in the small blood vessels.

The infected rabbits were sacrificed as follows: two each at 14, 21, 28 and 35 days post infection.

The mesentery was prepared for light and electron microscopic observations as detailed in Section 2.4.1.

a. In-vivo Microscopy

The small blood vessels (arterioles, venules and capillaries) in the area of the mesentery selected from the exteriorized small intestine were observed under the light microscope. The behaviour of the formed elements of blood, particularly leucocytes were noted.

b. Light Microscopy

The area of the mesentery observed *in-vivo* was identified in the polymerized specimen blocks, cut out with a jeweller's saw and mounted on "dummy" blocks with rapid araldite (Ciba Geigy) and sectioned as described in Section 2.4.3.

Thick sections (0.5-1μm) cut from the blocks were stained with toluidine blue and used either as survey sections i.e. to delineate the areas of the specimen suitable for ultrastructural studies or examined and photographed with a Zeiss RA microscope.

c. Electron Microscopy

Attempts to cut ultrathin sections suitable for examination under

the transmission electron microscope were unsuccessful.

<u>RESULTS</u>

(1) <u>In-vivo Microscopy</u>

In small blood vessels of uninfected rabbits the formed elements

of blood flowed intermingled in the central part of the venular blood

vessels. At no time during the observations were leucocytes seen to leave

the axial stream to occupy the plasmatic zone.

Stasis or reduced blood flow was not observed in blood vessels

in the control animals. In 'true' capillaries (about 3-8 μm in diameter)

red blood cells were seen to flow in a single file.

Blood flow was reduced in blood vessels of the venular segments

14 days after infection. Leucocytes were observed to leave the central

part of the venules to adhere to the endothelium. These marginating

leucocytes and other formed elements of blood obstructed the vascular

lumen, resulting in disturbed flow. Stasis occurred in many of these

venules as the number of marginating leucocytes increased. Occasionally,

flow was also disturbed in capillaries by leucocytes clogging the lumen.

These events were marked in rabbits infected for more than 28 days.

(2) <u>Light Microscopy</u>

Fig. 25 shows a venule from an uninfected control rabbit. There

are no leucocytes on the endothelial lining of the blood vessel and in the

extravascular tissue.

Leucocyte margination and emigration occurred in the venules 14

days after infection (Fig. 26).

The emigrated leucocytes were located between the endothelium

Figs. 25-28 :   Photomicrographs of thick sections of rabbit mesentery,

stained with 1% toluidine blue in 1% borax.

Fig. 25 : Longitudinal section (LS) through venule of an uninfected rabbit.

There are no leucocytes on the vascular wall or in the extra-

vascular tissue. X 1200.

Fig. 26 : L.S. through venule of a rabbit infected for 21 days showing

leucocytes marginating on the vascular wall (a) and at various

stages of emigration (b-d). X 1066.

Fig. 27 : L.S. through an arteriole. Rabbit infected for 21 days. There

are no leucocytes on the vascular wall. The vessel wall has

smooth muscle lining (arrow). X 960.

Fig. 28 : Shows section through a venule (V) and a capillary (Cap) of a

rabbit infected for 28 days. Erythrocytes (arrow) removed from

the blood vessel are deposited in the subendothelial space. The

tissue is infiltrated by leucocytes (Le). X 1380.
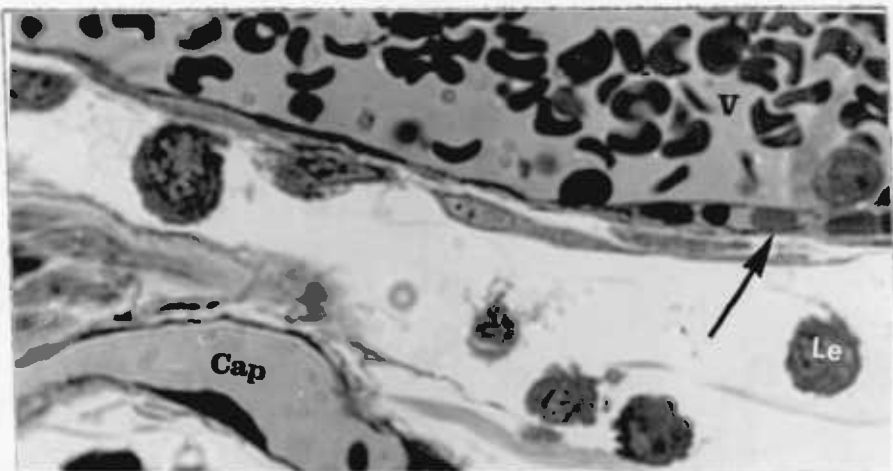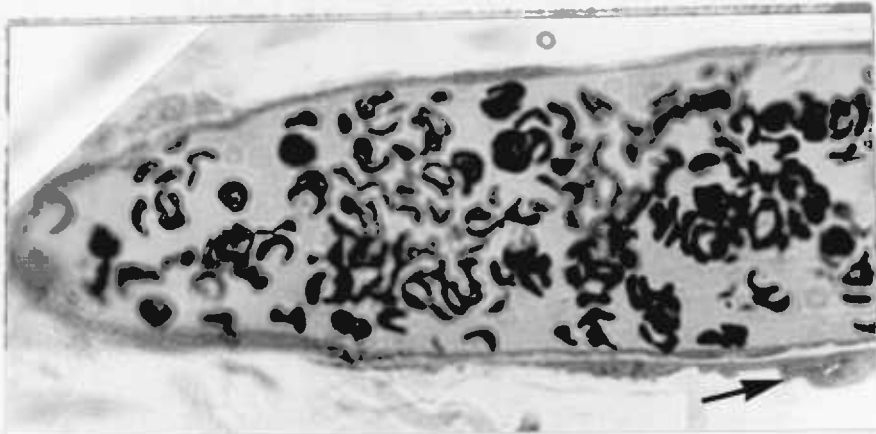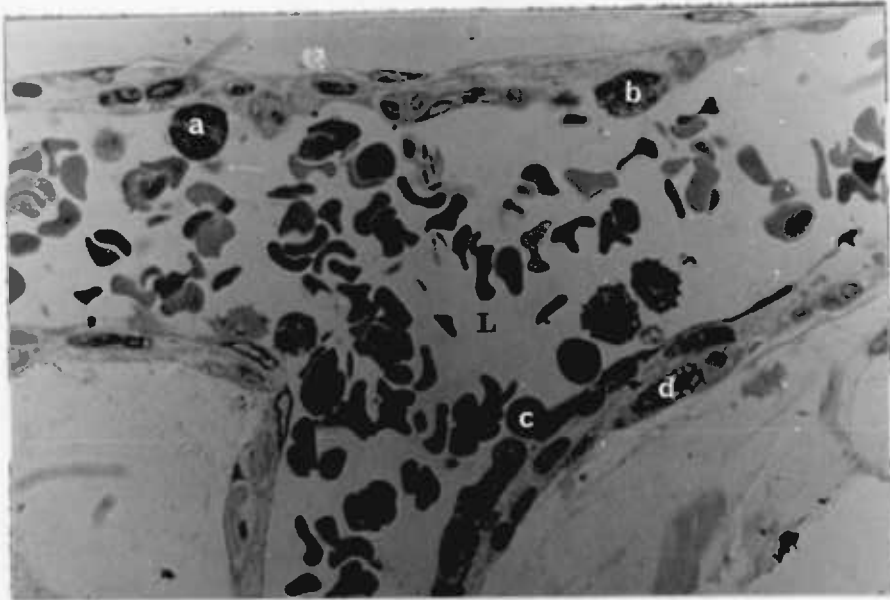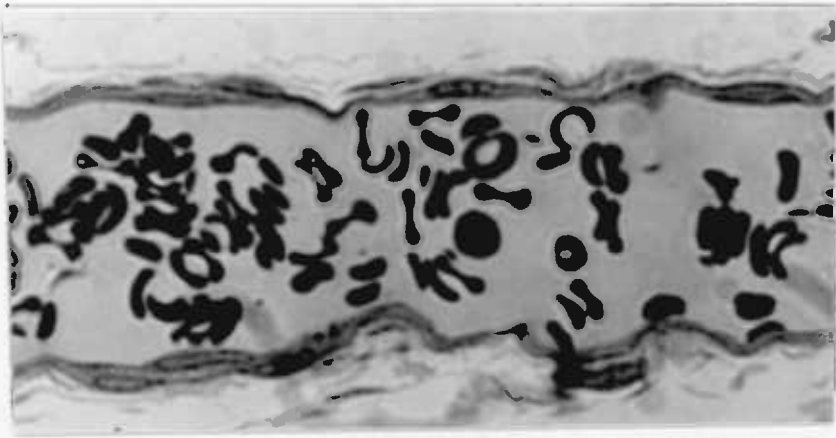
and perivascular elements (subendothelial space) until they infiltrated

the extravascular tissue (Figs. 26, 28).

Cellular infiltration of the tissue was marked in rabbits

infected for more than 14 days. The leucocytes were mostly mononuclear

cells, with a few polymorphs. The mononuclear cells were of various

shapes and sizes and most had small projections on their surfaces.

These stages of leucocyte mobilization were not observed in

blood vessels of the arterial segments (Fig. 27). Red blood cells which

probably escaped from damaged blood vessels were found in the subendo-

thelial spaces (Fig. 28).

(3) Electron Microscopy

No results were obtained from the ultrastructural studies. (See

Discussion)

DISCUSSION

Disturbance in the venous flow observed by *in-vivo* microscopy

indicate defects in the rheological properties of blood and possible

venular damage which were not discernible because of the low resolving

power of the light microscope.

The *in-vivo* observations were similar to the descriptions given by

Goodwin (1971) for regenerating blood vessels in the ear chambers of

rabbits infected with *T. brucei*. However, disintegration of the blood

vessels were not seen, contrary to Goodwin's observations.

Leucocyte mobilization which is a cardinal feature of the inflammatory

response, suggests likely derangement of the venular walls and are

consistent with the histopathological studies (see Section 3.2.). The

results are also in agreement with those of Goodwin and Hook (1968).

The extravasation of red blood cells through the venules show clearly that these blood vessels were probably damaged and therefore leaky.

Results were not obtained from the ultrastructural studies, since no suitable ultrathin sections were available for examination with the transmission electron microscope (TEM). The specimen was so thin that it was difficult to cut ultrathin longitudinal sections. Attempts to cut transverse sections were also unsuccessful, as excess embedding resin around the specimen hampered effective ultramicrotomy.

In order to eliminate improper embedding as a source of error, different formulations of the embedding resin (Epon 812) obtained from the manufacturers and other workers (e.g. Simpson, personal communication) were used (See Appendix). The recommended duration and temperature for polymerization of the specimen blocks were strictly followed.

Since increasing the ratio of the hardners (i.e. Methyl Nadic Anhydride (MNA) to Dodecenyl Succinic Anhydride (DDSA)) increases the hardness of the final block, several formulations were used to obtain "medium hard" blocks and thus eliminate the texture of the block as the factor affecting the sectioning procedure, yet no good sections were obtained.

The thickness of the sections was increased i.e. from gold to purple and blue colour interferences (150-240 Å) but resolution of the structures under the TEM was very poor as the sections were too dark.

Factors that could affect ultramicrotomy such as clearance angle, knife angle and cutting speeds were carefully checked and altered accordingly during each sectioning session. Methodical sectioning also did not

not yield good sections. All glass knives were prepared fresh and thoroughly examined under a stereo-dissecting microscope before use, as blunt knives are known to affect the quality of sections for ultra-structural observations (Reid, 1975).

After all attempts made to section the mesentery blocks failed, an alternative tissue was chosen for the ultrastructural studies (See Section 3.3.2.).

The terminal vascular bed of the mesentery would have been ideal for electron microscopic studies, since one could identify the same blood vessels observed *in-vivo* after embedding the specimens and examining the sections under the TEM. This would have made identification (i.e. whether a venule or an arteriole), measurements and quantitative analysis of changes in the blood vessels easy to carry out by the observer.

3.3.2. Cremaster Muscle

The cremaster muscle was chosen as an alternative to the mesentery as it is rich in small blood vessels, fairly thick and easy to prepare without traumatic effects on the blood vessels.

a. Whole Mount Preparation of Cremaster Muscle for Topographic Study

Experiments were carried out to ascertain the parts of the terminal vascular bed in the cremaster muscle likely to be involved in the inflammatory response in rabbits infected with *T. brucei*.

Ten rabbits (2 controls and 8 infected) were used in these experiments. Two infected animals each were sacrificed at 14, 21, 28 and 35 days post infection.

The animals were given 1 ml/kg of Pelikan ink (Günther Wagner,

Hannover, W. Germany) or colloidal carbon (Taab Laboratories, Reading)

diluted 1 in 4 with normal saline intravenously into the marginal ear

vein, under pentobarbitone anaesthesia. The carbon suspension was slowly

injected into the animals' ears, since it's been shown that rapid injection

results in unusual labelling of blood vessels, even under normal conditions

(Majno *et al.*, 1967).

Whole mounts of the cremaster muscle were prepared 1 hr after

injecting the carbon suspension, as described in Section 2.3.2. After

this period, the carbon particles should have been cleared from the

circulation and therefore blood vessels (apart from those in the reti-

culoendothelial system) showing carbon in them on light microscopic

examination were considered labelled and abnormal (Cotran, 1967a).

When the carbon suspension was injected, the animals turned

greyish especially on the skin of the face. This greyish-tinge persisted

in animals sacrificed after 21 days of infection. In some instances, the

carbon leaked out of the marginal ear veins. This leakage was marked in

rabbits infected for 28 and 35 days. The cremaster muscles in some of

these animals were so flaccid that it was difficult to obtain good trans-

parent preparations.

Fig. 29 shows a whole mount preparation of cremaster muscle from

an uninfected control rabbit. The muscle shows the usual vascular pattern,

consisting of small arteries, arterioles, small veins, venules and

capillaries, most of which run parallel to the muscle fibres.

In control animals, carbon particles were not found in the blood

vessels. However, a very few carbon particles occurred in the extra-

vascular tissue.

In infected rabbits, small veins, venules and a few capillaries showed patches of carbon particles in their lumen and walls and in the extravascular tissue (Figs. 30, 32).

Fig. 31 is a cremaster muscle preparation of an infected rabbit showing scanty labelling of capillaries arranged parallel to the muscle fibres.

The arterial segments were almost free of carbon particles and leucocytes (Fig. 32). On closer microscopic examination of the labelled blood vessels, the carbon particles were found to be mostly enclosed in leucocytes lining these blood vessels and in the extravascular tissue (Fig. 33).

The observations above suggest that venules and capillaries were damaged during trypanosome infection of rabbits. The results also showed that towards the end of infection, blood vessels on the venous aspect of the circulation became leaky; a feature reflecting the increase in vascular permeability in *T. brucei* infection of rabbits (Boreham & Goodwin, 1967; Goodwin & Hook, 1968; Goodwin, 1970, 1971; Boreham, 1974).

The fact that the carbon particles were mostly enclosed in leucocytes lining the blood vessels strongly suggests an inflammatory response and likely damage to the vascular endothelium.

These results conformed to the topographic observations in rabbits infected with *T. brucei* (Goodwin & Hook, 1968) and are consistent with vascular injury due to histamine-type mediators, in which predominantly venules and a few capillaries are labelled with carbon (Majno *et al.*, 1961; Majno *et al.*, 1967; Cotran, 1967a).
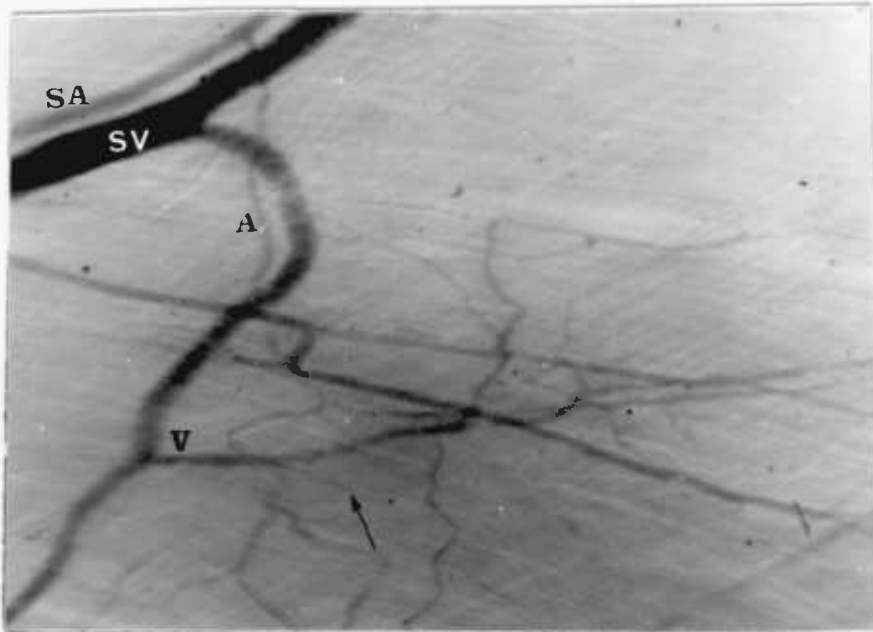
Fig. 29 : Whole mount preparation of cremaster muscle from an uninfected
rabbit showing small blood vessels - Arterioles (A), Venules (V)
and Capillaries (arrow); i.e. branches of the small vein (SV)
and artery (SA). The blood vessels are not labelled with
carbon. X 150.
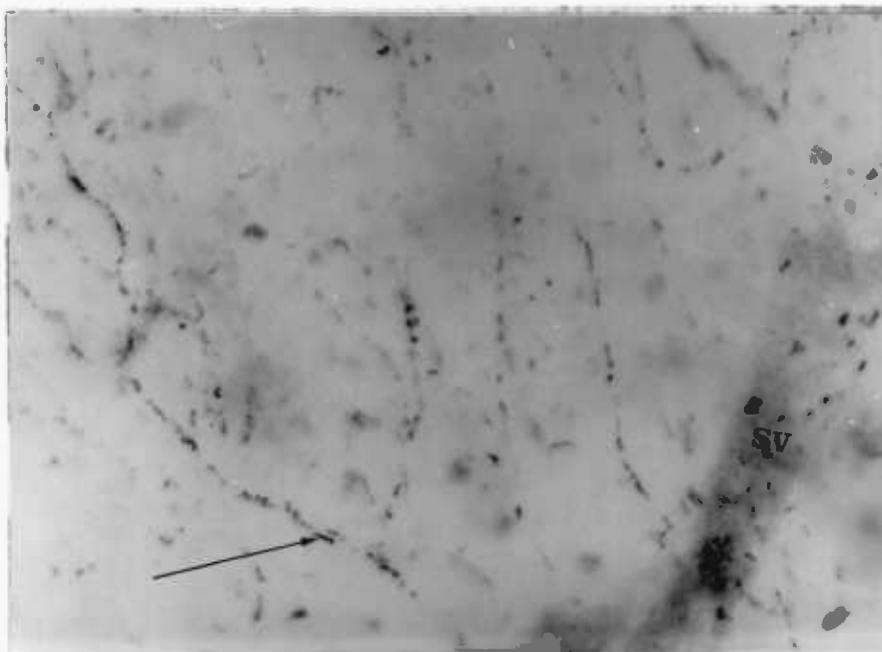
Fig. 30 : Whole mount preparation of cremaster muscle from a rabbit
infected for 14 days showing carbon labelling of small blood
vessels - small vein (SV) and venules (arrow). X 150.

Fig. 31 : Whole mount preparation of cremaster muscle of a rabbit infected

for 14 days, showing scanty labelling of capillaries (arrow)

running parallel to the muscle fibres. X 215.

Fig. 32 : Whole mount preparation of cremaster muscle of a rabbit infected

for 21 days, showing carbon labelling of small vein (SV) and its

branches. The small artery (SA) is not labelled. X 350.

Fig. 33 : Whole mount preparation of cremaster muscle of a rabbit infected

for 28 days showing venules (V) labelled with carbon particles

(arrow) most of the carbon particles are in leucocytes lining

the blood vessels and in the extravascular tissue (arrowhead).

X 480.
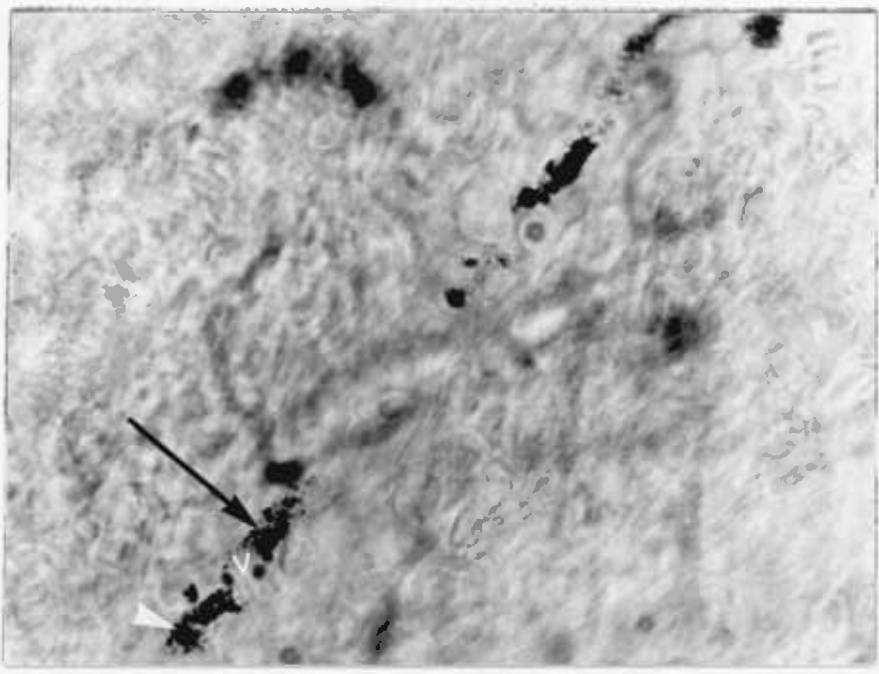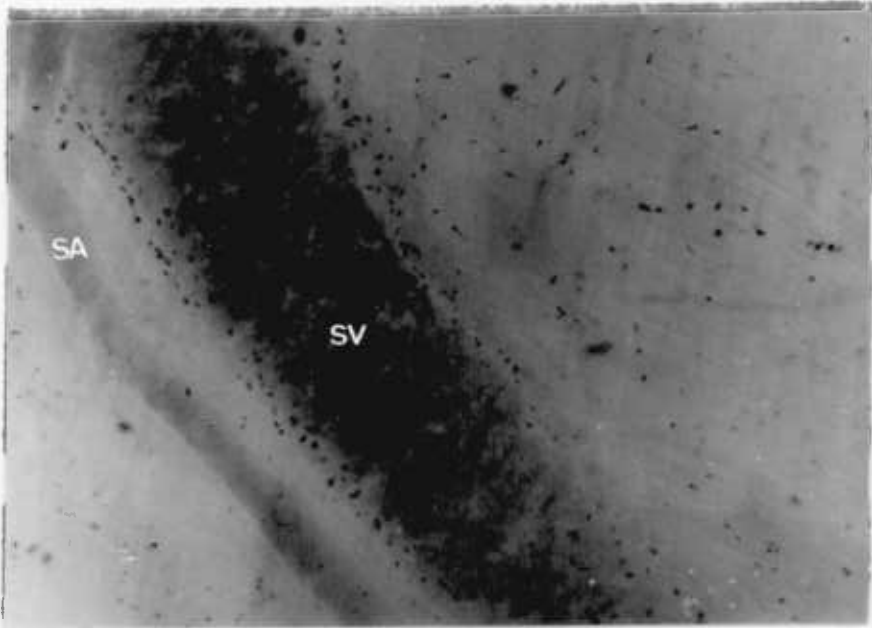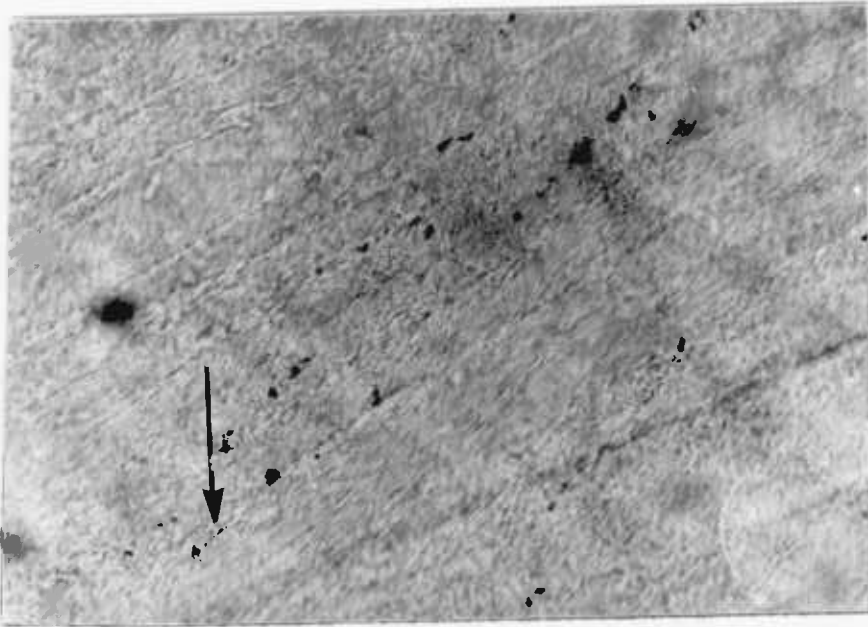
b. <u>Light and Electron Microscopic Studies on Cremaster Muscle Small</u>

   <u>Blood Vessels</u>

Thirty five rabbits (10 controls and 25 infected) were used in these studies. The infected rabbits were sacrificed as follows: two at day 10, five at day 14, five at day 21, six at day 28, four at day 35 and three at day 42. Five control rabbits were sacrificed before the infected animals while others were used at different times during the experimental period.

Under pentobarbitone sodium (26.4 mg/kg) or urethane (1.2 g/kg) anaesthesia, the cremaster muscles from the rabbits were dissected, fixed with 2.5% glutaraldelyde in 0.1 M sodium cacodylate buffer pH 7.2-7.4 and processed for microscopic examination as detailed in Section 2.4.2.

Twenty to thirty samples were taken randomly from the cremaster muscle of each animal.

(1) <u>Light Microscopy</u>

After trimming the specimen blocks, 0.5-1 μm thick sections cut on an LKB ultrotome III equipped with glass knives were collected on microscope slides, stained with 1% toluidine blue in 1% borax, mounted with glycerol and examined under a light microscope. Some of the slides were used for survey purposes, i.e. to ascertain suitable areas of the block from which ultrathin sections could be cut for examination under the transmission electron microscope. Others were examined and photographed with a Zeiss RA microscope and used as adjunct to the ultrastructural studies.

Fig. 34 shows a section through the cremaster muscle of an uninfected control rabbit. The cremaster muscles from the control

animals showed the usual microvascular pattern consisting of small veins, venules, capillaries, arterioles and small arteries with accompanying nerve fibres. The muscle fibres show cross striations and are separated by connective tissue.

In infected rabbits, changes were observed in the cremaster muscle about 14 days after infection. There was slight infiltration of the tissue by leucocytes and the venules were also lined by these cells (Fig. 35). Later in the infection, there was massive cellular infiltration of mononuclear cells and marked oedema shown by extensive separation of the tissue components (Fig. 36a).

Leucocyte margination and emigration occurred in the venules (Fig. 37). The small blood vessels were surrounded by emigrated leucocytes and cellular debris (Fig. 36b). The leucocytes observed at all stages of infection were predominantly mononuclear cells and showed various shapes. At the late stage of infection, many of these mononuclear cells were vacuolated. These changes were similar to those observed in the ears of infected rabbits (Section 3.2.).

Red blood cells which probably escaped from damaged blood vessels were observed in the extravascular connective tissue in rabbits sacrificed after 14 days of infection (Figs. 38a & b). In many cases no blood vessels were seen near these extravasated red blood cells.

There was extensive degeneration of the cremaster muscle fibres shown by aggregation and separation of myofibrils and the loss of other internal structures about 21 days after infection. In many sections only the outline of the sarcoplasmic membrane and a few internal structures such as myofibrils were visible (Fig. 38a). As in other tissues, there
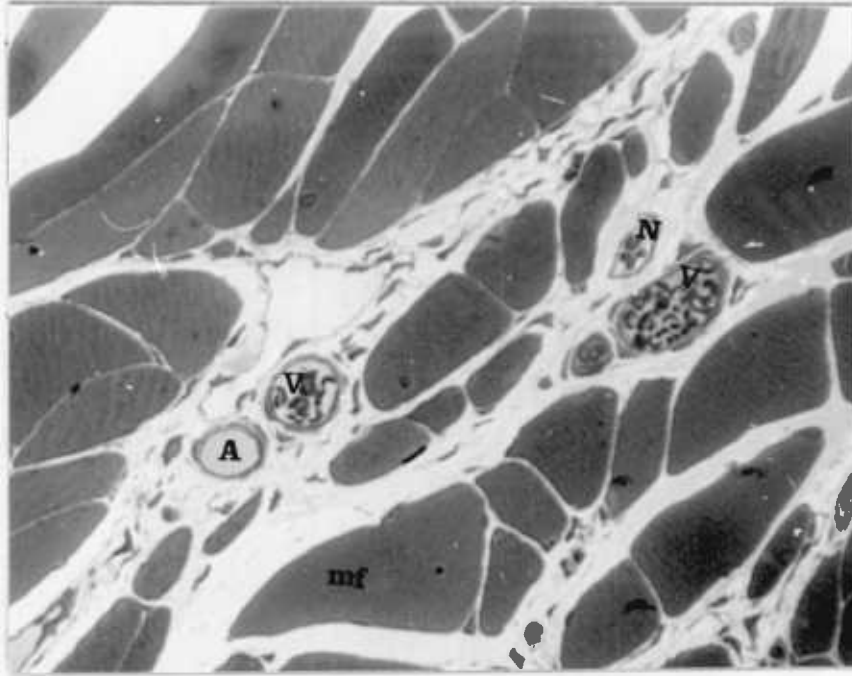
Fig. 34 : Section through cremaster muscle of an uninfected rabbit showing
venules (V), an arteriole (A), nerve (N) and muscle fibres (mf)
separated by connective tissue. X 480.
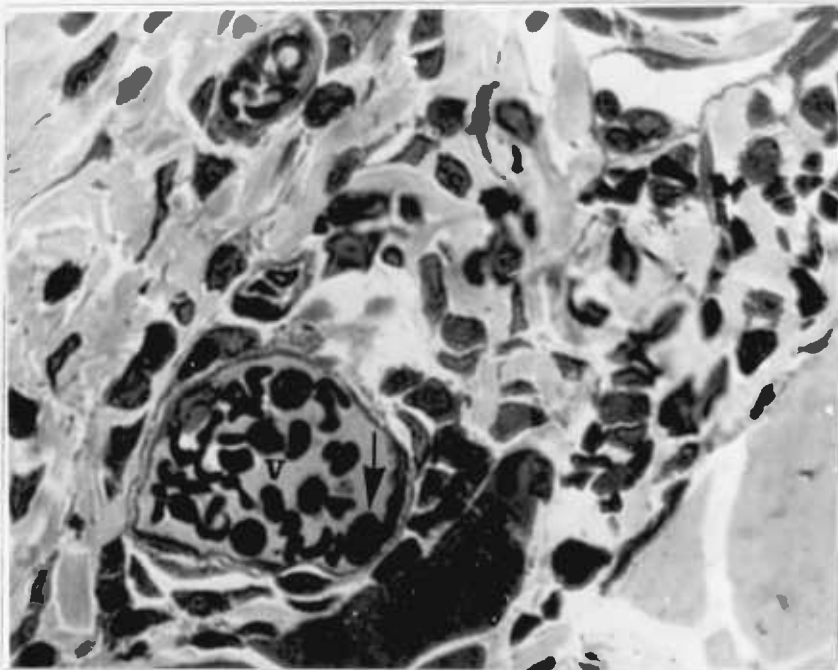
Fig. 35 : Section through cremaster muscle of a rabbit infected for 14
days. The tissue infiltrated by leucocytes is slightly oedematous.
Leucocytes (arrow) marginate on a venule (V). X 860.

Fig. 36A : Section through cremaster muscle of rabbit infected for 28 days.

The tissue is oedematous and heavily infiltrated by leucocytes.

The muscle fibres (mf) are degenerate. X 300.

Fig. 36B : Englarged area (enclosure) in Fig. 36A, showing a venule (V)

surrounded by leucocytes and cellular debris, some of which

are probably trypanosome materials (arrow). X 900.

Fig. 37 : Section through cremaster muscle of rabbit infected for 35 days.

There is massive infiltration of the tissue by leucocytes and

cellular debris, some (arrow) are probably trypanosomes. The

venule (V) is lined by leucocytes.

mf - degenerate muscle fibres.

X 860.
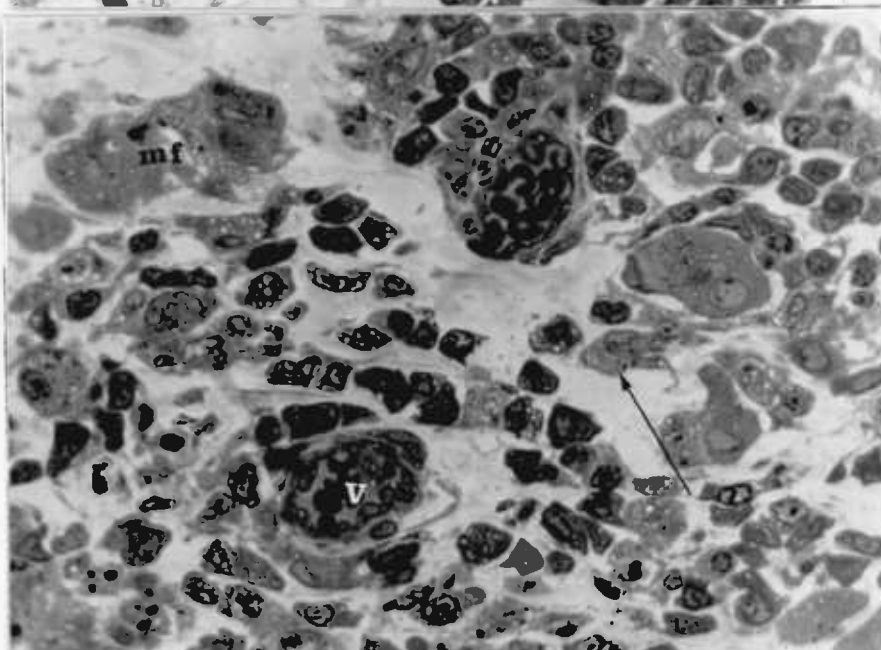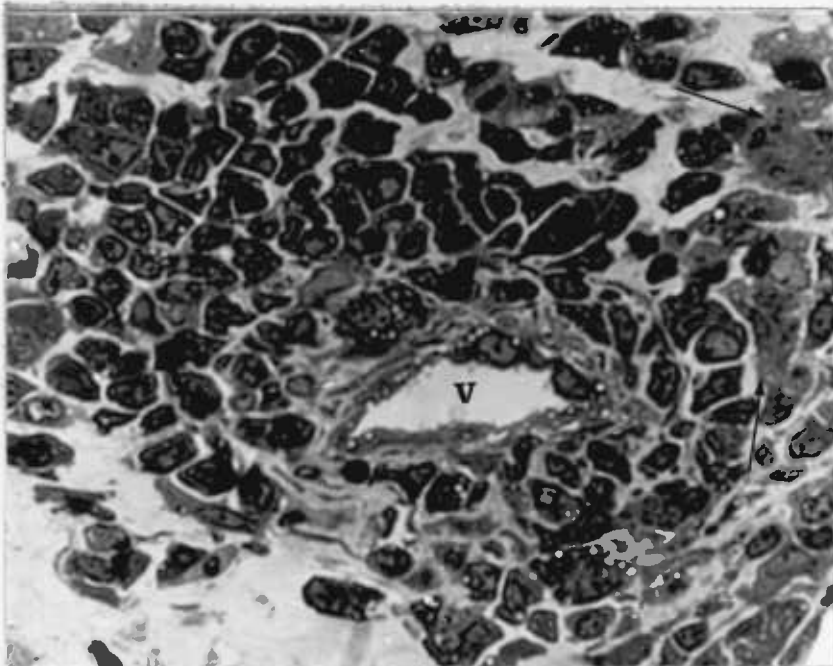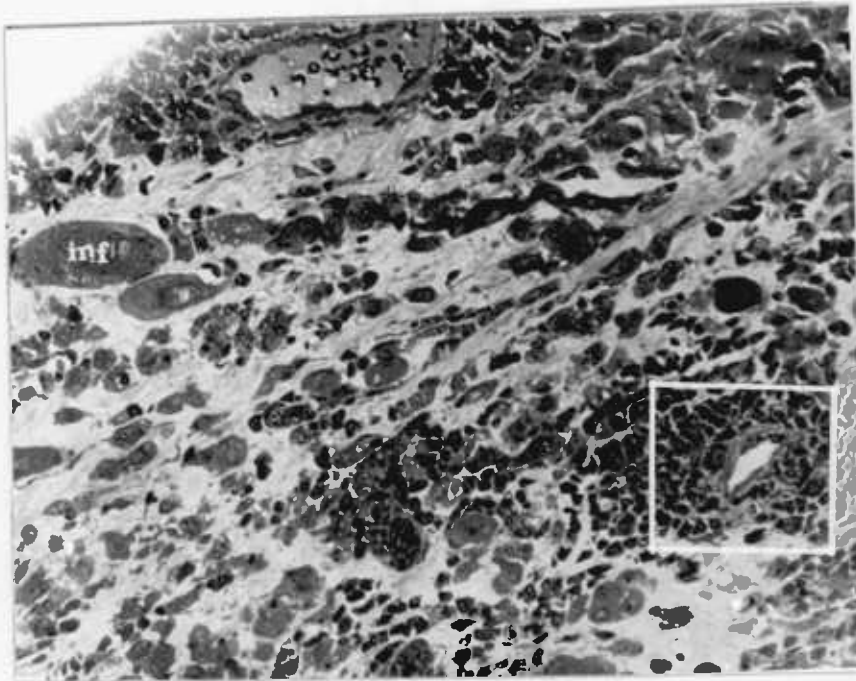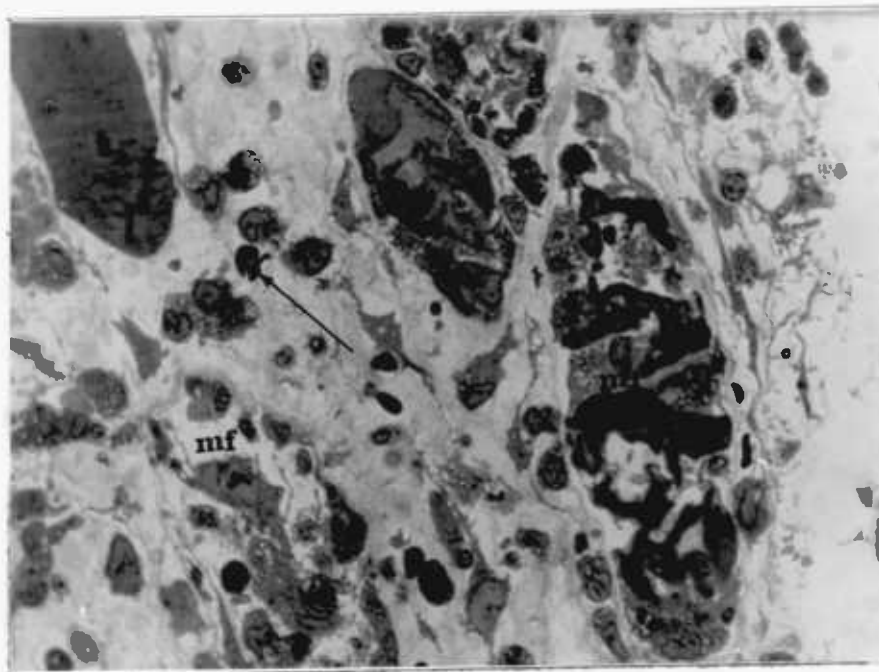
Fig. 38A : Shows section through cremaster muscle of a rabbit infected for

35 days. The muscle fibres (mf) are degenerate and the tissue

is infiltrated by leucocytes. Erythrocytes (arrow) which

escaped from damaged blood vessels are in the interstitium. X 860.
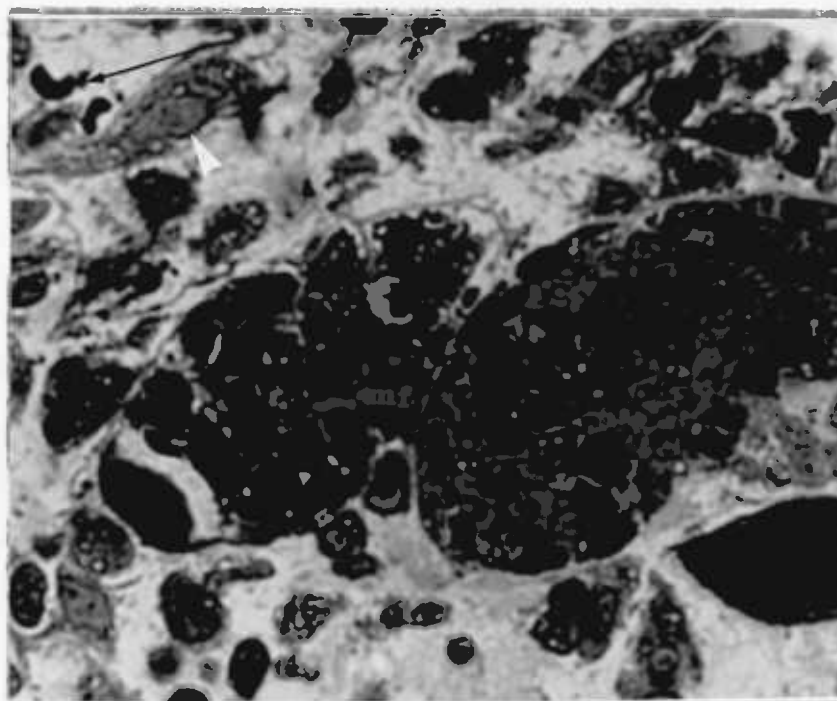
Fig. 38B : Section through cremaster muscle of a rabbit infected for 35

days, showing degenerate muscle fibres (mf) invaded by phago-

cytes. Erythrocytes (arrow) and probably trypanosome materials

(arrowhead) are found in the extravascular tissue. X 1168.

was a gradation, as the number of degenerate muscle fibres increased towards the end of infection. For example all the rabbits infected for 42 days had many degenerate muscle fibres in every section taken from their cremaster muscles.

These degenerate muscle fibres were invaded by phagocytes which probably remove cellular debris (Fig. 38b).

Other changes observed include vascular stasis, which was recognized by closely packed red blood cells with amorphous material in the vascular lumen, degenerate and thrombosed blood vessels (see Ultrastructural Studies).

(2) Ultrastructural Studies

Ultrastructural studies with the transmission electron microscope were undertaken in order to obtain details of the vascular changes observed with the light microscope.

Ultrathin sections (60-150 Å) from areas of the specimen blocks delineated by examination of the initial survey sections were cut as described in Section 2.4.3. a. Many blocks were remounted (the other side up) on 'dummy' blocks with rapid Araldite (Ciba Geigy) and sectioned again. This was to ensure that both sides of the specimen were examined, thus compensating for the inherent sampling problem in electron microscopy.

Blood vessels were cut either as transverse or longitudinal sections, depending on their positions in the vascular tree and the specimen blocks. At least ten specimen grids filled with sections were collected from each block. The grids were stained with uranyl acetate and lead citrate and examined with the transmission electron microscope (see

Section 2.4.3. b.). Occasionally specimen grids were stained only with
lead citrate for 15 min. and washed with 0.2 M sodium hydroxide and carbon-
dioxide free distilled water.

In some cases grid-bars covered parts of a blood vessel
appearing in a section and as such only the exposed areas were examined.
Attempts were made to eliminate this by using single-slot grids (Taab Labs,
Reading) but these were difficult to coat with formvar and the specimens
were easily burnt under the electron beam, even at the lowest illumination.
However, many sections were collected on each meshed grid and examined
thoroughly.

Since previous observations and the light microscopic
results indicate that the venular segments of the terminal vascular bed
were most likely to be damaged; emphasis was placed on venules and
capillaries. All the blood vessels appearing in each section were care-
fully studied under the transmission electron microscope at low and high
magnifications. Photographs were taken with 35 mm and plate cameras in the
microscope.

CONTROL RABBITS

The morphology of venules and capillaries from the
cremaster muscles of uninfected rabbits conformed to the ultrastructural
descriptions given for microvessels in skeletal muscles and related tissues
(see Section 1). These are shown in Figs. 39-43. All the blood vessels
examined under the microscope throughout the duration of these studies had
the continuous-type of endothelial lining.

INFECTED RABBITS

For convenience, the changes observed in the components
of the blood vessels will be described individually:
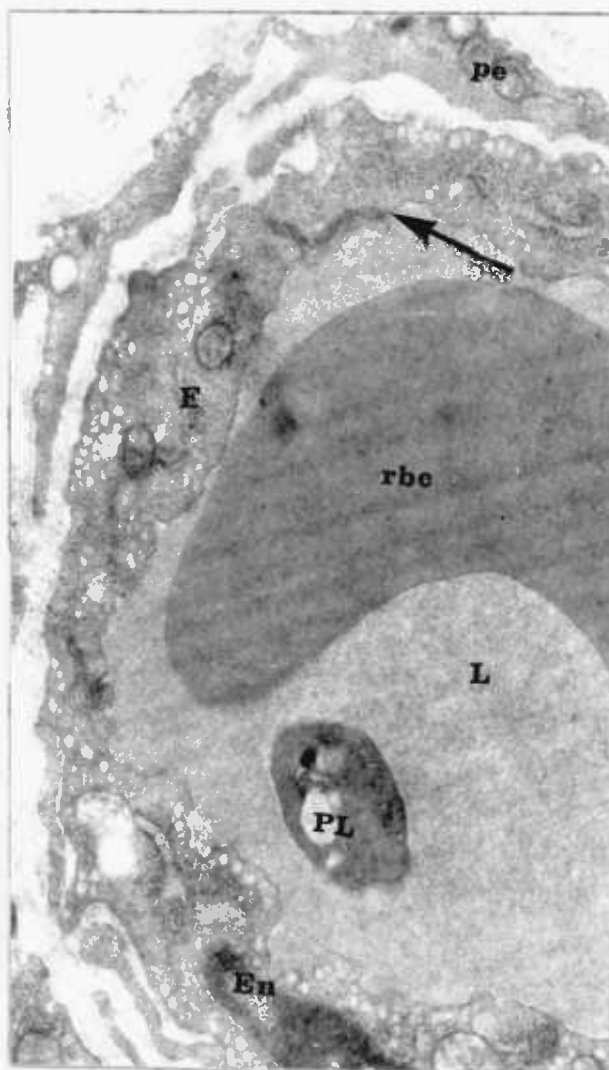
Fig. 39 : Electronmicrograph showing a venule from an uninfected rabbit.

The endothelial cells (E) are separated by an intercellular

junction (arrow).

PL - Platelet, En - Endothelial nucleus, L - Vascular lumen,

rbc - Erythrocyte, pe - Pericyte.

X 8,000.

Fig. 40 : Electronmicrograph of a section through a capillary from an

uninfected rabbit.

E - vascular endothelium, En - endothelial nucleus, rbc -

erythrocyte.

X 14690.

Fig. 41 : Electronmicrograph of a section through a venule from an

uninfected rabbit showing the vascular endothelium (E) surrounded

by pericytes (pe). The lumen (L) contains erythrocytes (rbc)

and platelets (PL). X 5470.

Fig. 42 : A low magnification electronmicrograph of a section through a

large venule from an uninfected rabbit. X 5470.
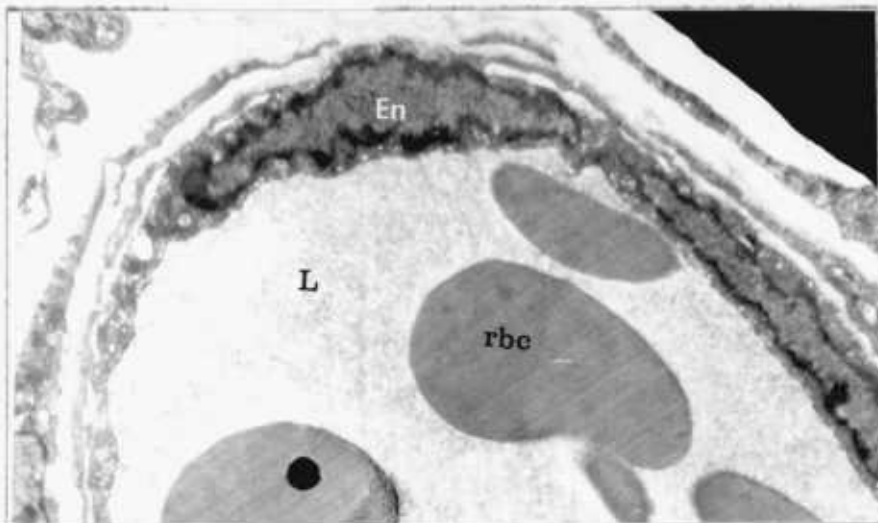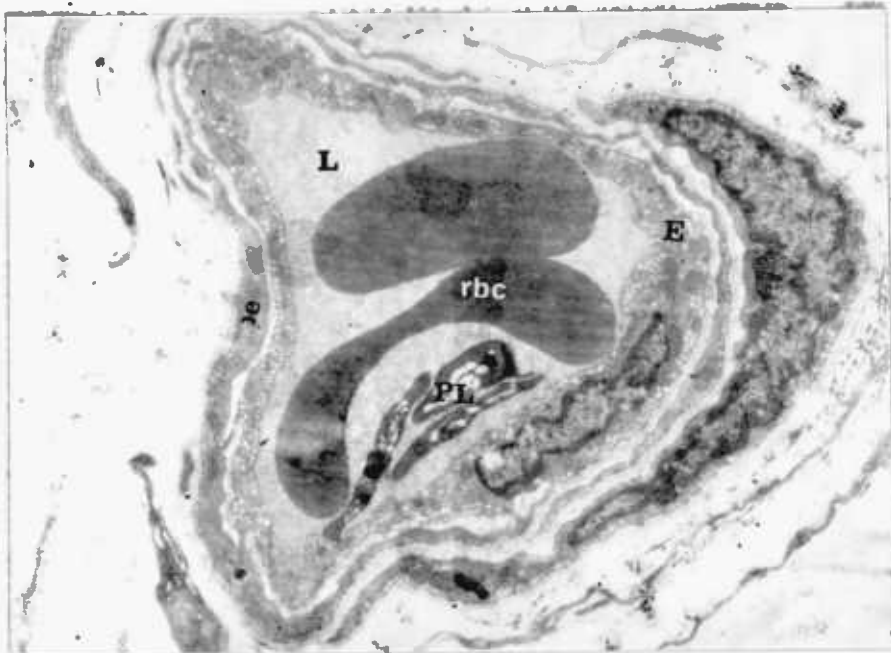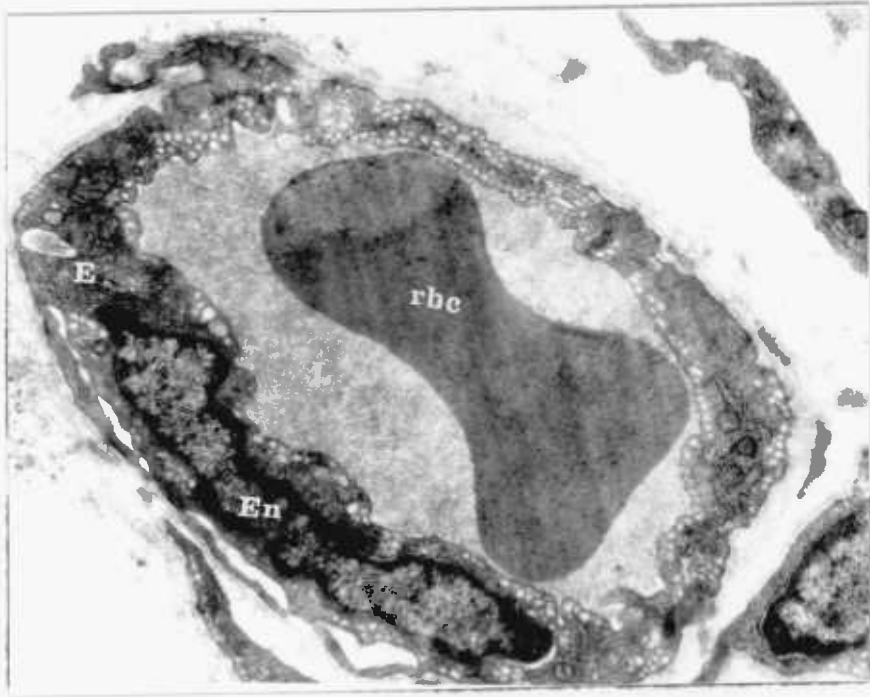
Fig. 43 : High magnification electronmicrographs showing junctions between

endothelial cells in blood vessels of an uninfected rabbit.

43A : Wall of a capillary showing an intercellular junction (arrow) between

endothelial cells (E_1 and E_2). The vascular endothelium is

surrounded by a basement membrane (bm). X 19400.

43B : Wall of a venule showing an intercellular junction (arrow) between

endothelial cells (E_1 and E_2). There is a pericyte (pe) outside the

endothelium. X 20134.

Fig. 44 : Electronmicrograph of a section through a venule of an infected

rabbit. The endothelium (E) is swollen and occludes the vascular

lumen (L). The endothelial nucleus (En) shows clumping and

margination of chromatin. The pericyte (pe) is vacuolated.

X 10108.
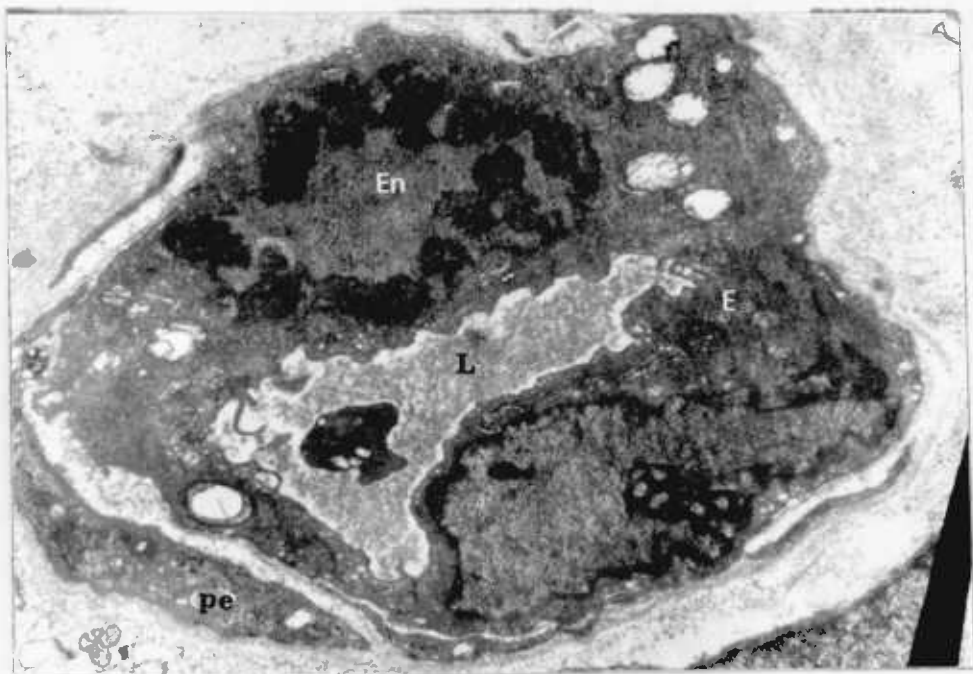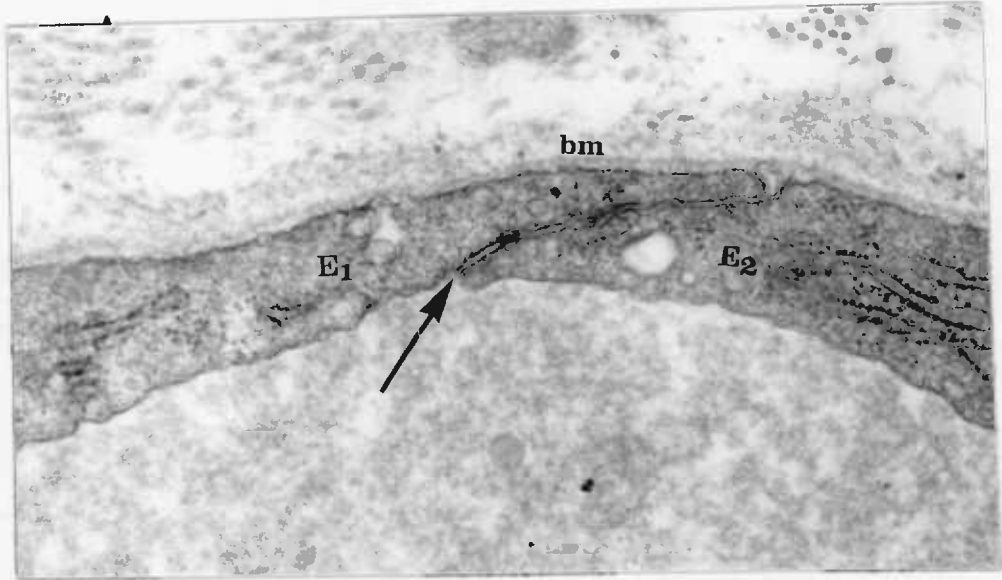
(a) Endothelial changes

In rabbits infected for at least 14 days, venules and capillaries showed focal or diffuse swelling of endothelial cells which almost occluded the vascular lumen (Figs. 44, 46, 47). This feature was prominent in blood vessels cut in transverse section.

Changes observed in the endothelial cytoplasm include vacuolation, rarefaction i.e. loss of cytoplasmic inclusions and dilatation of organelles such as endoplasmic reticulum and mitochondria in places (Figs. 48, 49, 75). These cytoplasmic changes which were predominant in venules sometimes occurred only in one endothelial cell with others appearing normal. Cytoplasmic processes were found on the luminal and abluminal surfaces of the endothelial cells in venules and capillaries (Figs. 47, 59, 60, 65). The cytoplasmic processes on the luminal aspect of the endothelial cells aided the margination of leucocytes on the vascular endothelium. In some venules, these luminal processes were found without leucocytes marginating on the endothelial lining.

After 14 days of infection, occasional fragmentation of endothelial cytoplasm was observed in the venules (Figs. 50, 66).

Apart from occasional clumping and margination of chromatin (Figs. 44 and 45) and focal contortion in a few venules and capillaries (Figs. 46, 51), the endothelial nuclei showed no other marked structural alterations.

Gaps occurred between contiguous endothelial cells at or near the intercellular junctions in the venules 14 days after infection (Figs. 51-55). These gaps were not found in blood vessels considered to be capillaries and in arterioles examined. The number of venules with gaps

between their endothelial cells increased in late infected rabbits.
Although the gaps were qualitatively similar in all infected animals,
quantiative differences occurred in animals of the same infection period.
For example, out of the five rabbits infected for 14 days only three
animals showed gaps between the endothelial cells of their venules.

Occasionally more than one gap was found in the
section of a blood vessel (Fig. 83b).

The gaps measured on electron micrographs or negatives
were on average 0.4-1 μmwide, although a few larger ones were found.

Mention must be made of the fact that in most cases
where these gaps were observed, the endothelial cells were not swollen.

These endothelial gaps allowed the escape of amorphous
material (probably plasma proteins) and other endogenous elements such as
erythrocytes, platelets and leucocytes into the subendothelial space.

Fig. 56 is a typical leaky venule from a rabbit
infected for 28 days showing large deposit of amorphous material in the
subendothelial space. The endothelial lining and pericyte are vacuolated.
The basement membrane is not visible because it probably has the same
electron density as the deposit.

Complete disintegration of venules and capillaries were
observed 21 days after infection. Most of these degenerate blood vessels
had only the basement membrane, a few cellular fragments (probably endo-
thelial cells) and formed elements of blood (Fig. 57).

The number of such blood vessels, like other features
of vascular damage observed were marked towards the later stages of

infection. In rabbits infected for 42 days for instance, at least two out of five blood vessels appearing in a section were degenerate. These degenerate blood vessels were almost always invaded by phagocytes which probably remove cellular debris and trypanosome material (Fig. 58). There was no ultrastructural evidence to suggest regeneration of the degenerate blood vessels in any of the infected animals examined.

(b) Pericytes

In venules and capillaries (with pericytes) the pericytes were vacuolated and showed dilatation of cytoplasmic inclusions similar to the endothelial cells (Figs. 56, 59, 61, 64). Those in venules were almost always separated from the endothelium (vessel wall proper) by emigrating or extravasated formed elements of blood, leading to an increase in the subendothelial space (Figs. 64, 65). Occasionally, the pericytes were fragmented in both venules and capillaries.

(c) Basement membrane (Basal lamina)

The basement membranes were thrown into folds by cytoplasmic processes on the abluminal surfaces of the endothelial cells of the venules and capillaries (Figs. 60 and 65). They were disrupted in areas where gaps occurred between endothelial cells by leucocytes in diapedesis (Figs. 64, 65, 66a) and extravasated red blood cells and their fragments (Figs. 73, 75, 76).

Apart from these alterations, the basement membrane was intact in many blood vessels even in degenerate ones (Figs. 57 and 58).

FORMED ELEMENTS OF BLOOD

The changes and behaviour of the components making up the formed elements of blood in infected animals will also be described individually:

Fig. 45 : Electronmicrograph of a section through the wall of a capillary,

showing clumping and margination of chromatin (arrow) in the

endothelial nucleus. X 8489.

Fig. 46 : Electronmicrograph of a section through a venule. The endo-

thelial cells (E) are swollen and occlude the vascular lumen (L).

The endothelial nuclei (En) are slightly distorted. X 10,200.

Fig. 47 : Electronmicrograph showing a section through a capillary. The

vascular endothelium (E) is swollen and occludes the lumen (L).

The pericyte (pe) is slightly separated from the vessel wall.

X 14690.
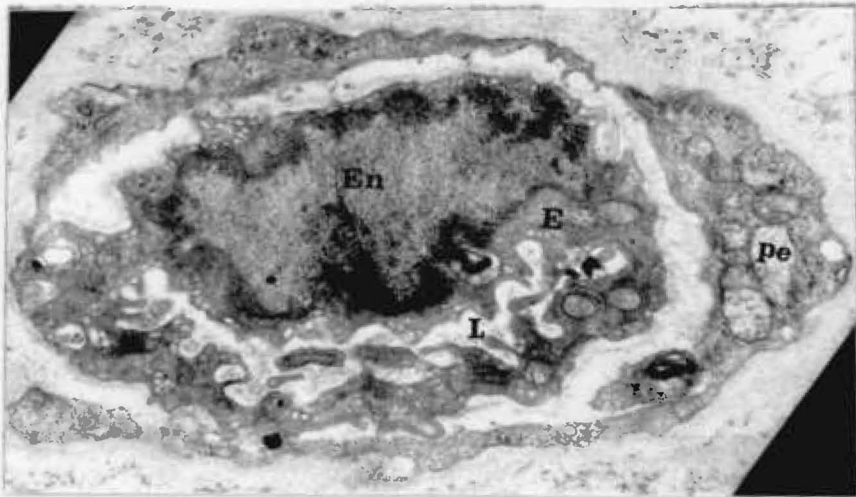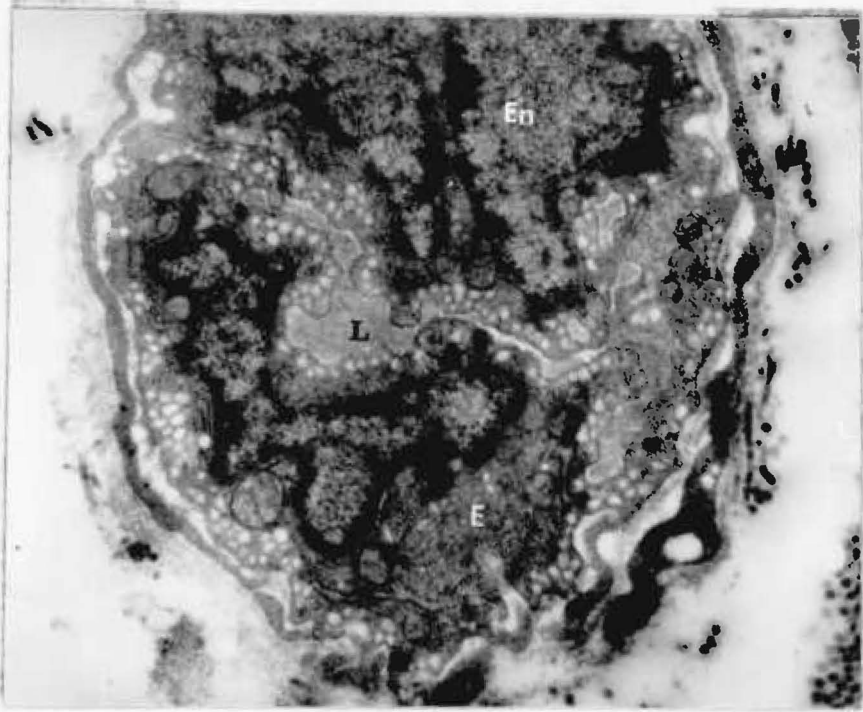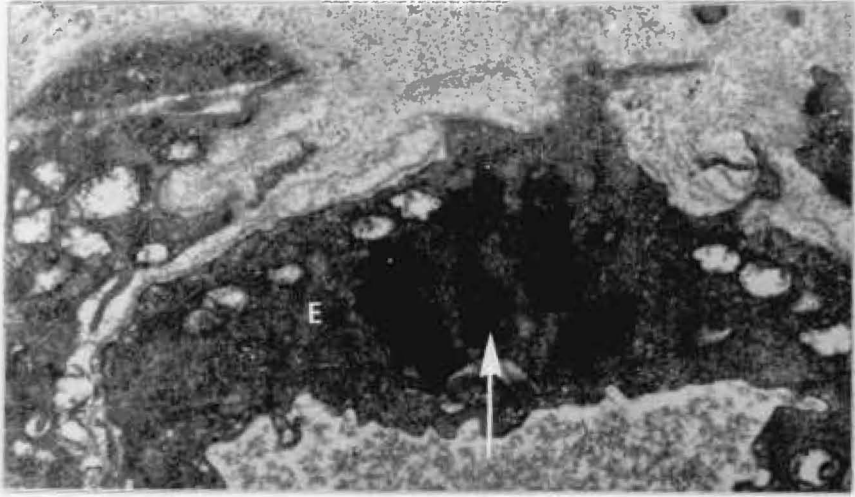
Fig. 48 : Electronmicrograph of a section through a capillary. There is
rarefaction of the endothelial cell (E_1) i.e. loss of cyto-
plasmic inclusions while E_2 is normal. X 10108.

Fig. 49 : Electronmicrograph of a section through a venule showing cyto-
plasmic processes (arrow) on the vascular endothelium. The
cytoplasmic inclusions in the endothelial cell (E) are dilated.
X 10108.

Fig. 50 : High magnification electronmicrograph showing a section through
the wall of a venule. The endothelial cytoplasm (arrow) is
fragmented. X 12950.
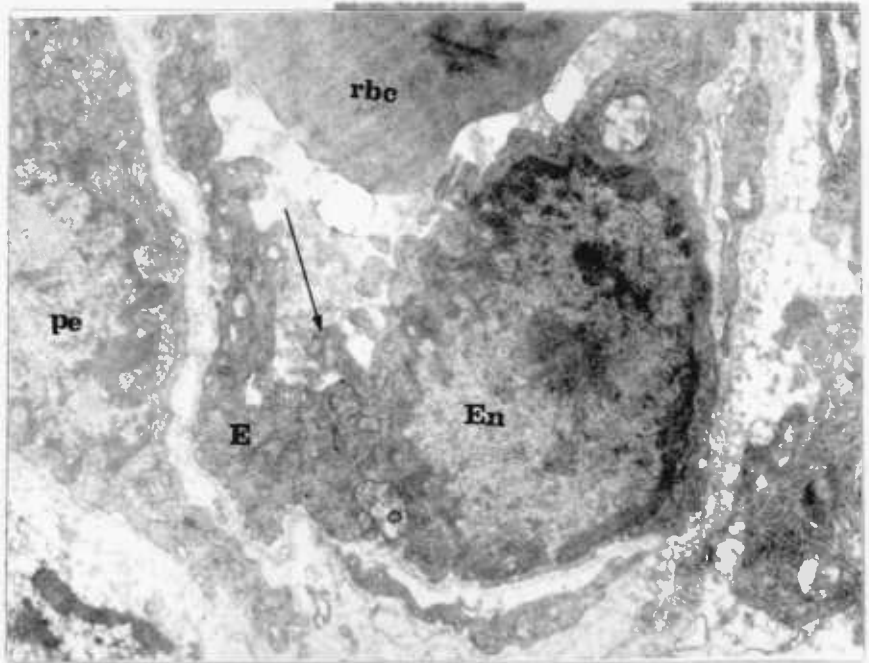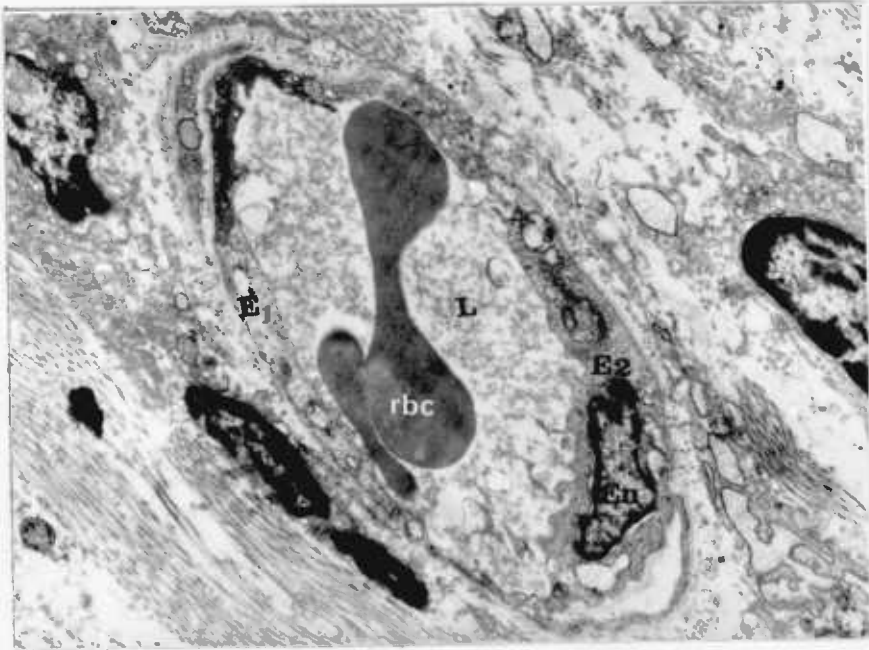
Fig. 51 : Electronmicrograph of a section through a venule showing a gap

(arrow) between the endothelial cells. The endothelial nucleus

(En) is contorted. X 9562.

Fig. 52 : Electronmicrograph of a section through a venule showing a gap

(arrow) between the endothelial cells (E_1 and E_2). X 10600.

Fig. 53 : Electronmicrograph of a section through a venule showing a gap

(arrow) between the endothelial cells (E). The gap allowed the

passage of erythrocyte fragments (arrowheads) and amorphous
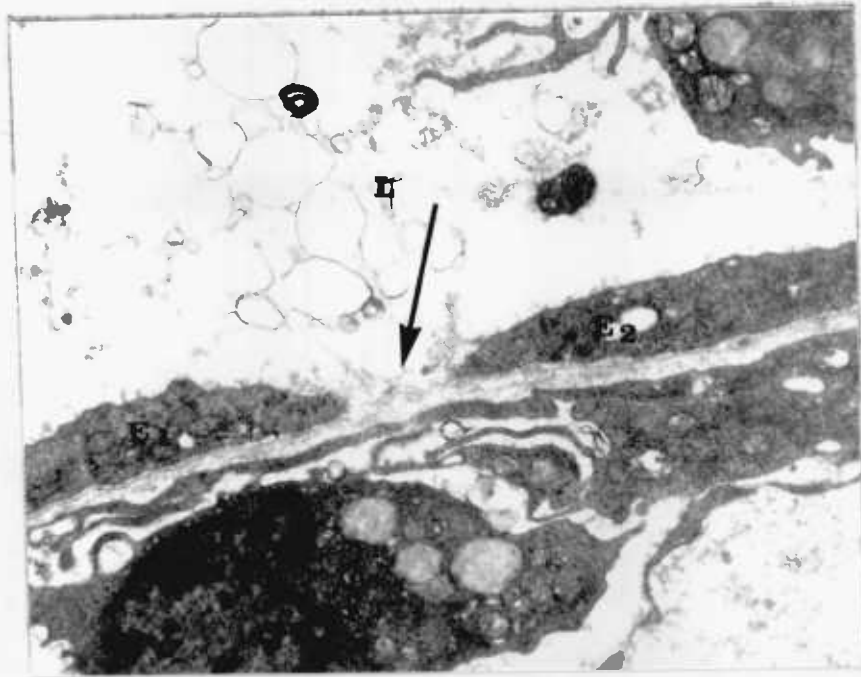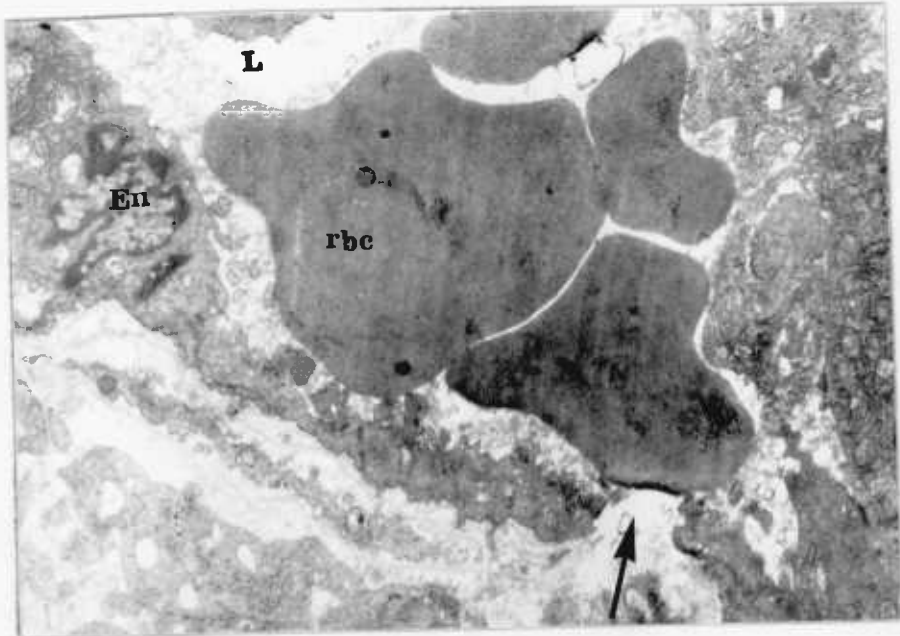
deposit (d) into the subendothelial space. X 16,780.
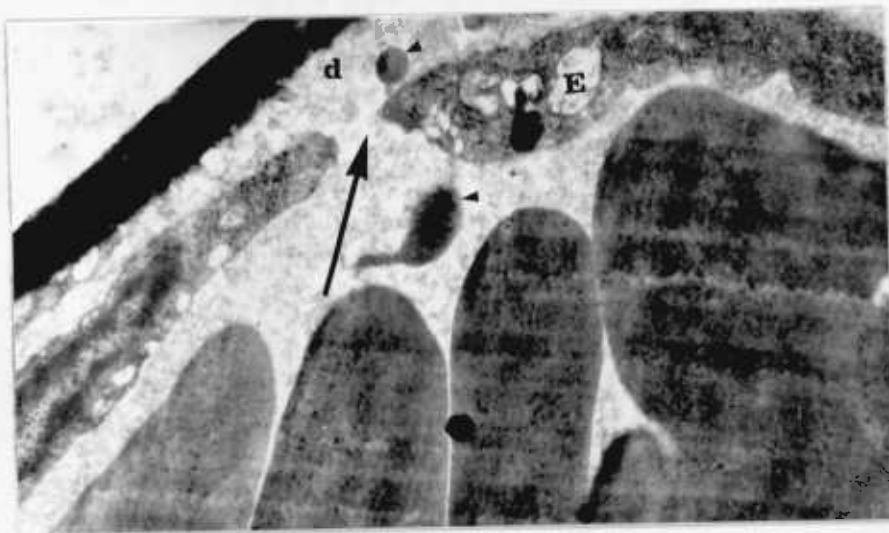
Fig. 54 : High magnification electronmicrograph of a venular wall, showing

a gap (arrow) between endothelial cells (E_1 and E_2). The gap

allowed the passage of amorphous material (d) and a leucocyte

(Le) into the subendothelial space. The endothelial cell E_1 is

vacuolated. X 25,418.

Fig. 55 : Electronmicrograph of a section through a venule showing a gap

(arrow) in the vessel wall. The gap leads to an intramural

deposit (d).

ef - probably endothelial fragment.

X 14732.

Fig. 56 : A low magnification electronmicrograph of a section through a

leaky venule from a rabbit infected for 28 days. There is a

large deposit (d) in the subendothelial space. The pericyte

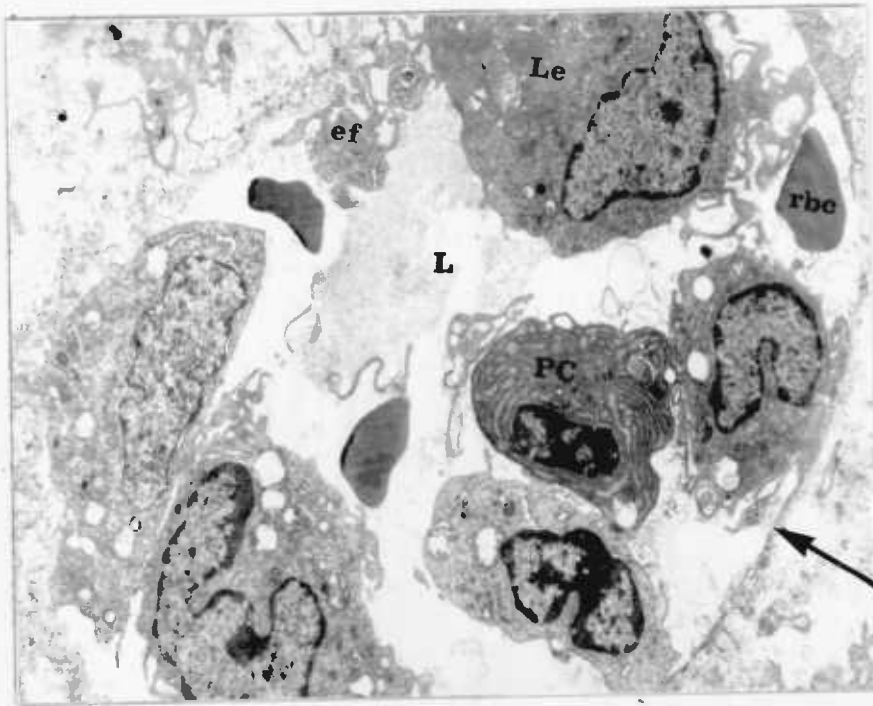(pe) separated from the vessel wall is vacuolated. X 5470.
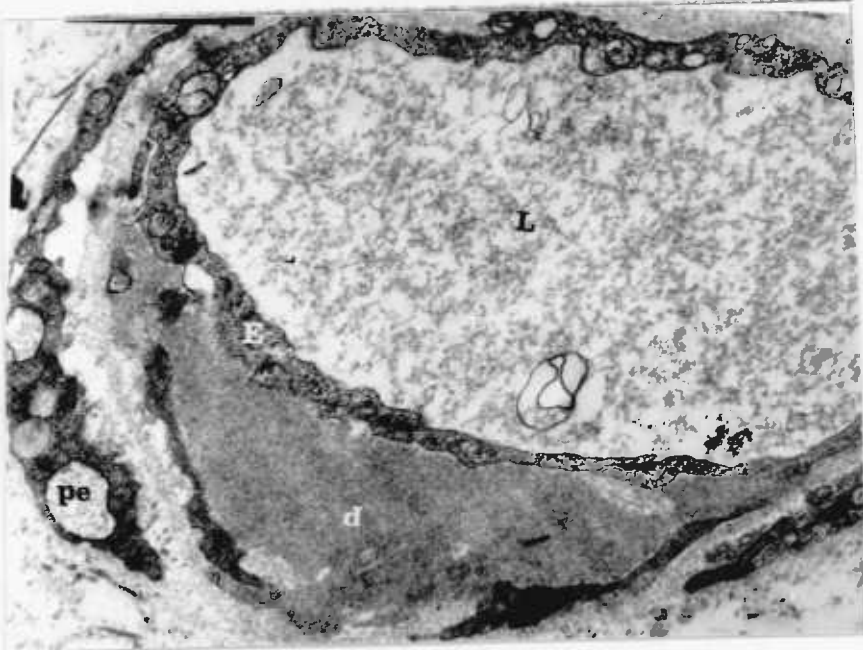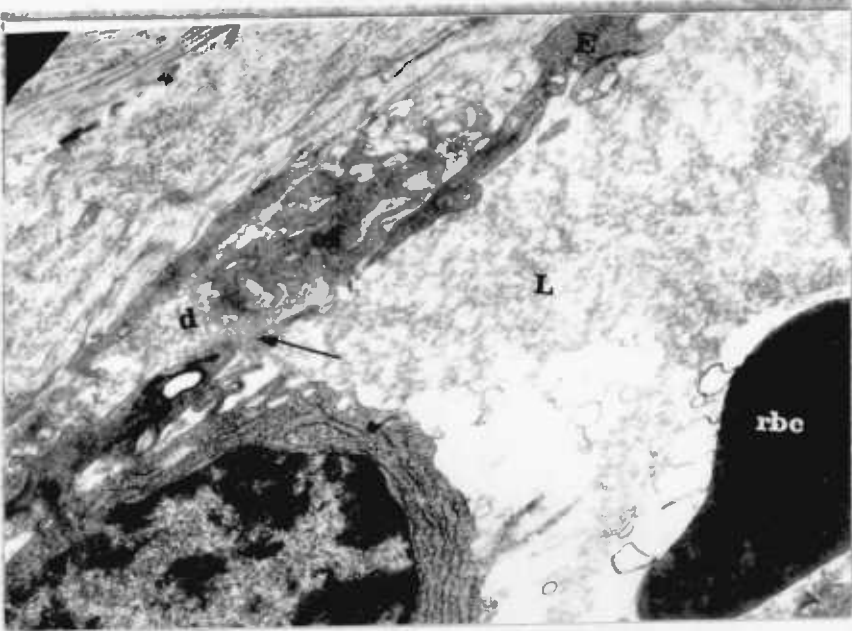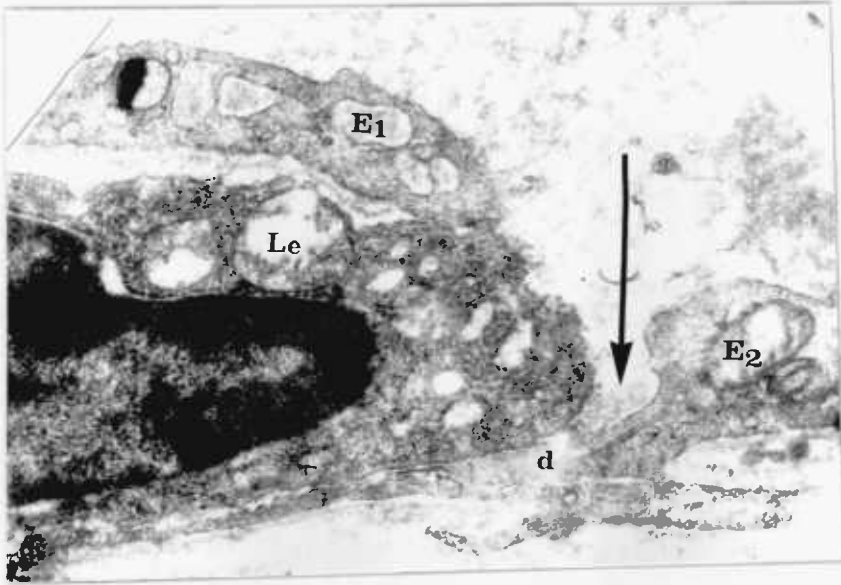
Fig. 57 : Electronmicrograph showing a section through a degenerate blood
vessel, probably a venule. The basement membrane (arrow) is
intact. The vascular lumen (L) contains leucocytes (Le), plasma
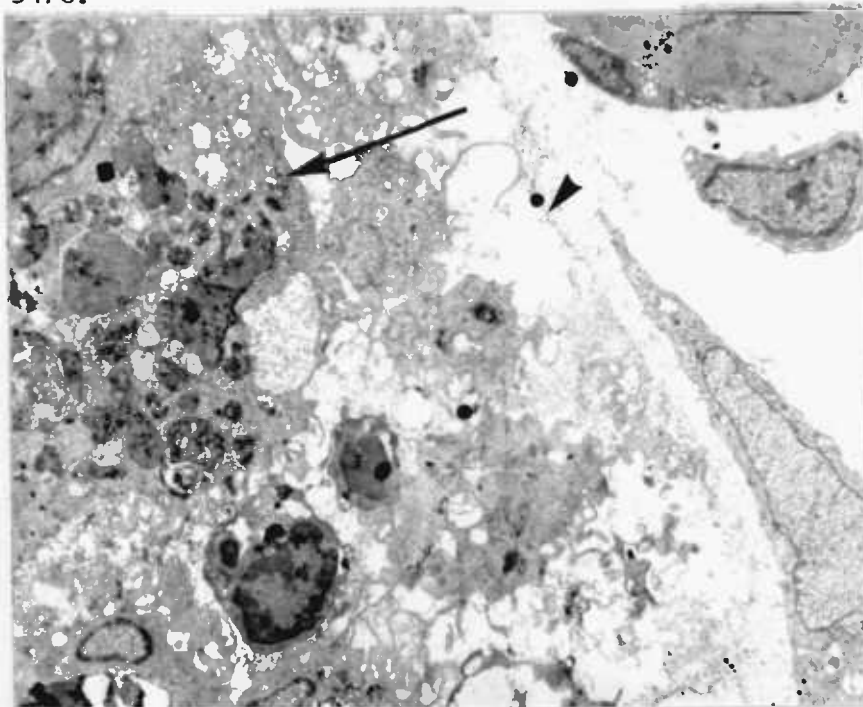cells (PC), endothelial fragments (ef) and erythrocytes (rbc).
X 5470.

Fig. 58 : Electronmicrograph of a section through a degenerate blood
vessel invaded by phagocytes (arrow) probably to remove cellular
debris. The basement membrane (arrowhead) is intact. X 5470.

(a) Leucocytes

Leucocytes were observed marginating on the endothelial cells of venules and a few capillaries 14 days after infection. These cells adhered to the vascular endothelium by means of cytoplasmic processes which interlocked or touched those on the luminal surfaces of the enothelial cells (Figs. 59-61).

Leucocyte margination by cell to cell contact, i.e. without structural modifications were seen occasionally (Fig. 62). In a few cases leucocytes adhered to the endothelium by producing a large pseudopodium (Fig. 63).

In many instances, leucocytes were seen to fill the vascular lumen, almost occluding such blood vessels. These leucocytes also adhered to each other by means of cytoplasmic processes (Figs. 67 and 68). Red blood cells in blood vessels clogged with leucocytes were squashed and fragmented.

Leucocyte emigration occurred through the gaps between endothelial cells in venules (Figs. 64 and 65). This event was marked in rabbits infected for more than 21 days. Degenerate leucocytes recognised by the loss of their limiting cell membranes and cytoplasmic contents were also observed in the endothelial gaps, especially in rabbits infected for 28 days (Figs. 66a and b). There was no evidence to suggest that the leucocytes formed the endothelial gaps as a result of their diapedesis.

The emigrating leucocytes were initially located in the subendothelial space i.e. between the endothelial lining and perivascular elements (Figs. 68 and 69). They eventually left the sub-

Fig. 59 : Electronmicrograph showing leucocytes (Le) sticking to the endo-
thelium (E) in a venule; by means of cytoplasmic processes. The
leucocytes also stick to each other. X 5470.

Fig. 60 : Electronmicrograph of a section through a venule showing a
leucocyte (Le) sticking to the vascular endothelium (E) with
cytoplasmic processes (arrow). X 7816.

Fig. 61 : High magnification electronmicrograph of a section through a
capillary. A leucocyte (Le) marginates on the vascular endo-
thelial cells (E_1 and E_2) with cytoplasmic processes (arrow).
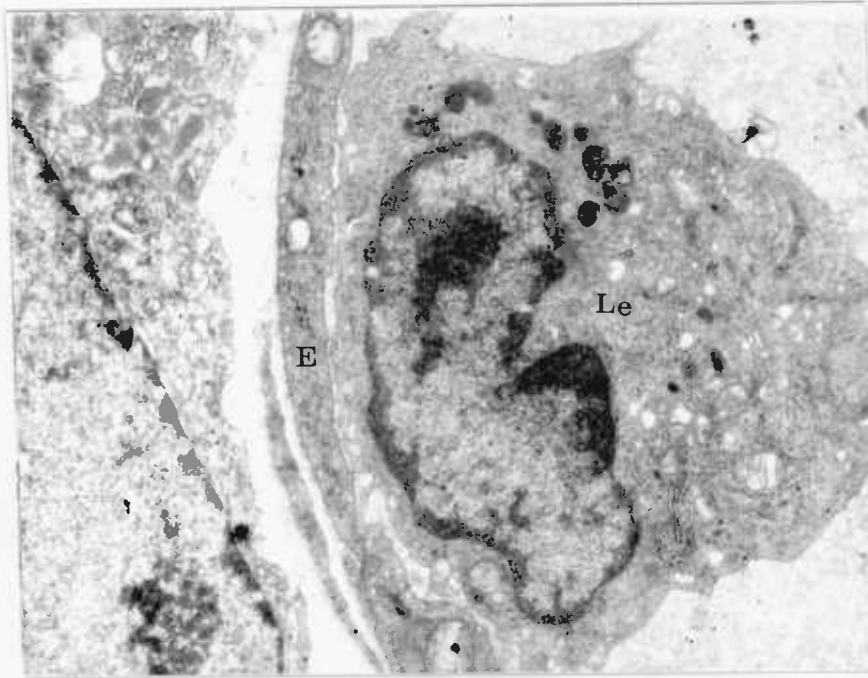The lumen (L) is blocked by the marginating leucocyte. X 14,440.
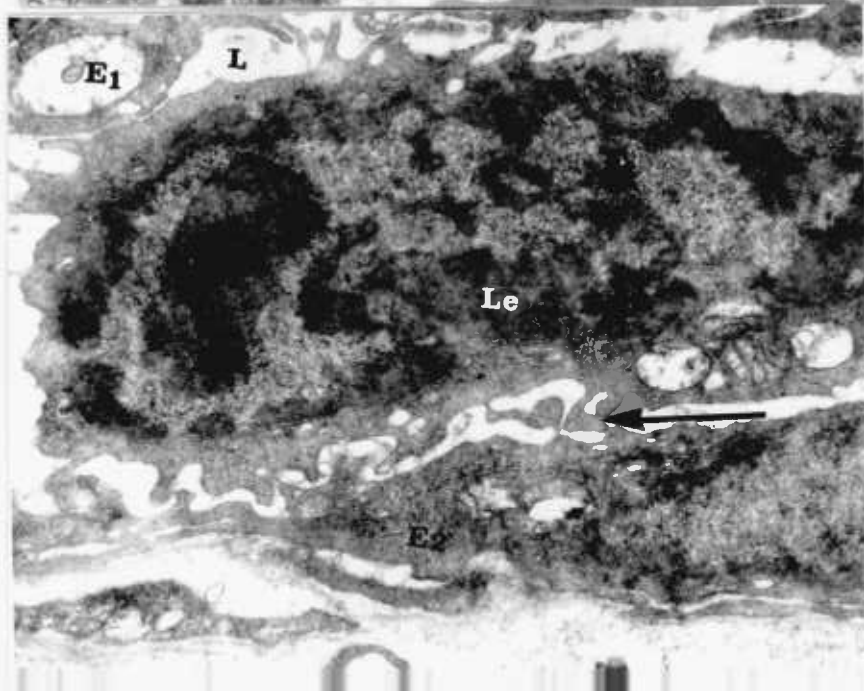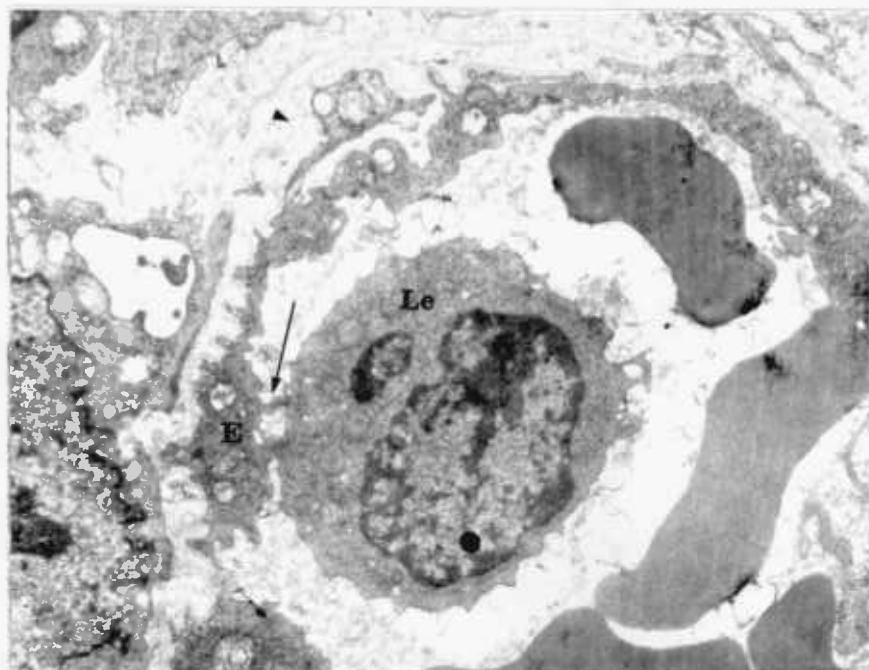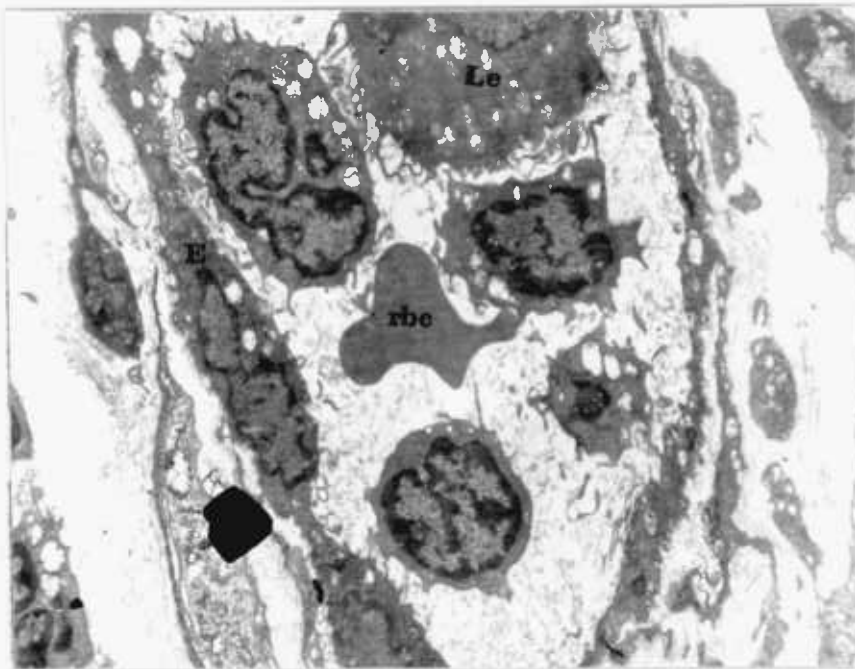
Fig. 62 : Electronmicrograph showing a leucocyte (Le) marginating on
vascular endothelium (E) in a venule. There are no structural
adaptations on either the leucocyte or the endothelium. X 14698.
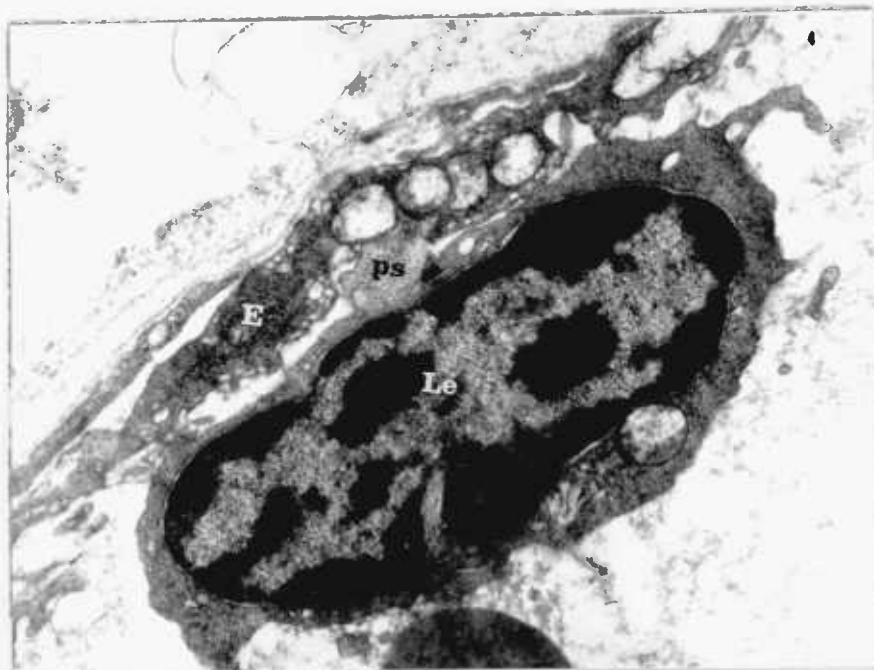
Fig. 63 : Electronmicrograph showing a leucocyte (Le) marginating on
vascular endothelium (E) in a venule, with a pseudopodium (ps).
X 12897.

Fig. 64 : Electronmicrograph showing section through a venule. A leucocyte

(Le) is emigrating through a gap (arrowheads) in the endothelium

(E). The pericyte (pe) is vacuolated and separated from the

vessel wall. X 10108.

Fig. 65 : Electronmicrograph showing section through a venule. A leucocyte

(Le) is emigrating via a gap between endothelial cells (E_1 and

E_2). The pericyte (pe) is separated from the vessel wall,

leading to an increase in the subendothelial space (arrow).

The basement membrane (arrowheads) is thrown into folds and

disrupted by the emigrating leucocyte. X 10108.
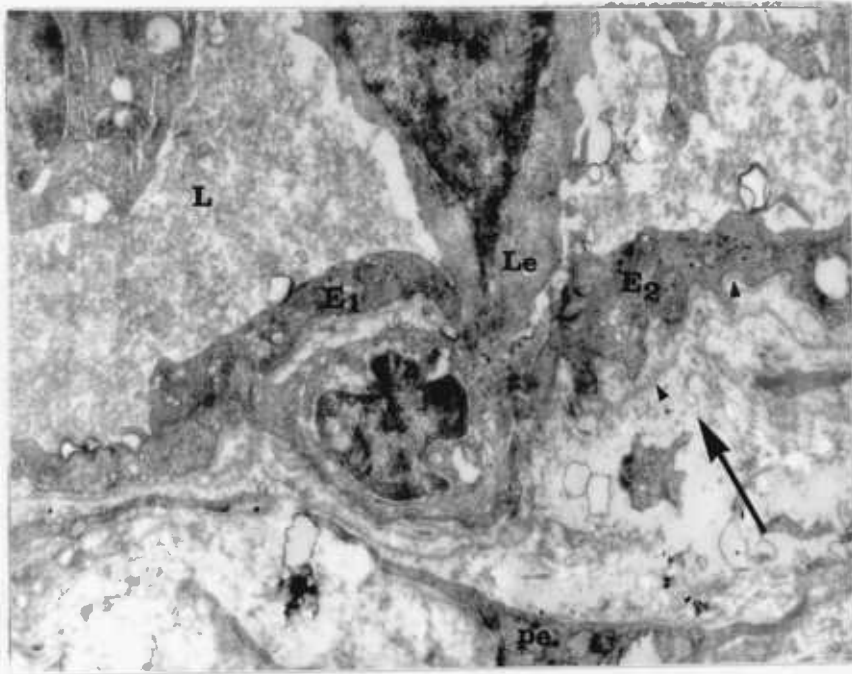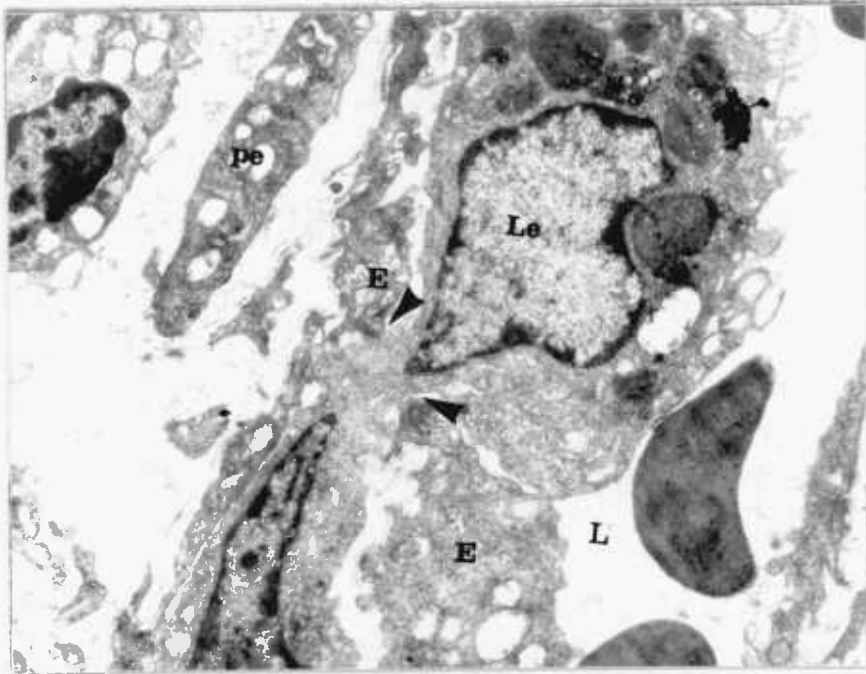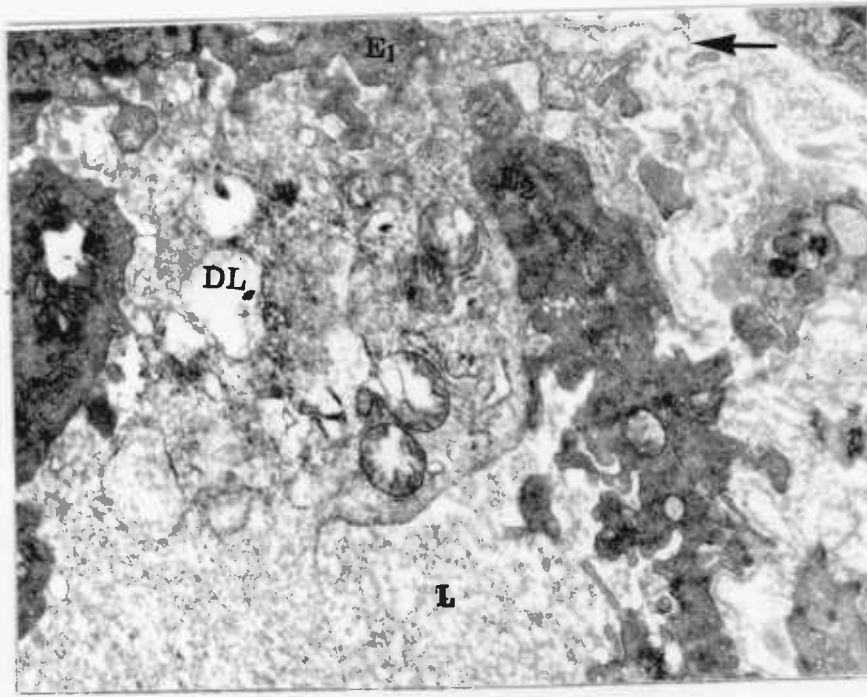
Fig. 66A : Electronmicrograph showing a degenerate leucocyte (DL) blocking

a gap between endothelial cells (E_1 and E_2) in a venule. The

basement membrane (arrow) is displaced. X 13766.
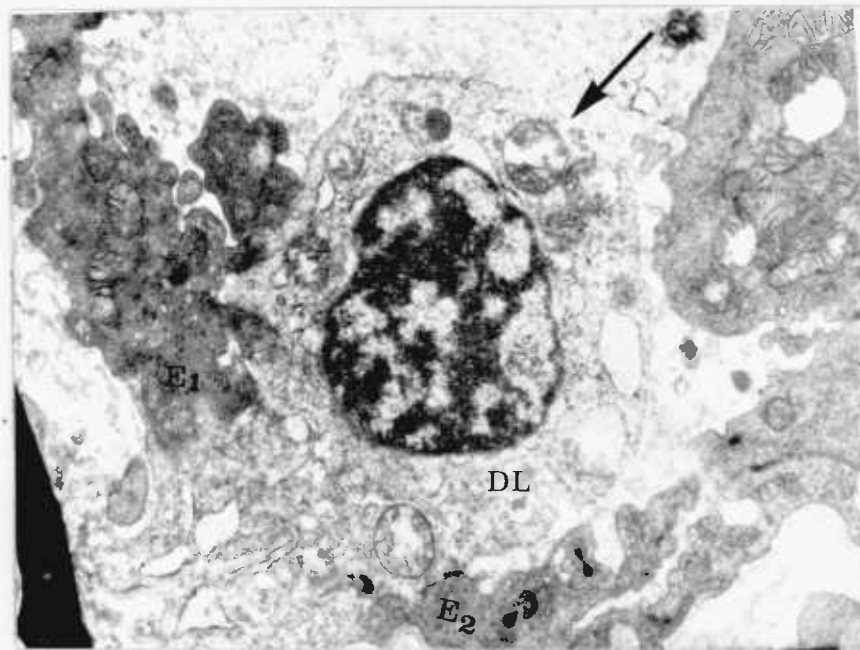
Fig. 66B : Electronmicrograph showing the same leucocyte (DL) as in Fig.

66A, blocking a gap. The limiting cells membrane of the leucocyte

is lost (arrow). X 14698.

Fig. 67 : Electronmicrograph showing a section through a venule. The

vascular lumen (L) is blocked by leucocytes (Le) marginating on

the vascular endothelium. An erythrocyte fragment (fr) is

trapped between the leucocytes. X 5470.

Fig. 68 : Electronmicrograph showing leucocytes (arrows) clogging the

lumen (L) of a venule. Some leucocytes (Le) have emigrated into

the subendothelial space. X 5470.

Fig. 69 : Electronmicrograph showing section through a venule. Leucocytes

(Le) marginate on the vascular endothelium (E) and infiltrate

the extravascular tissue. Some leucocytes (arrow) have emigrated

into the subendothelial space. X 5470.
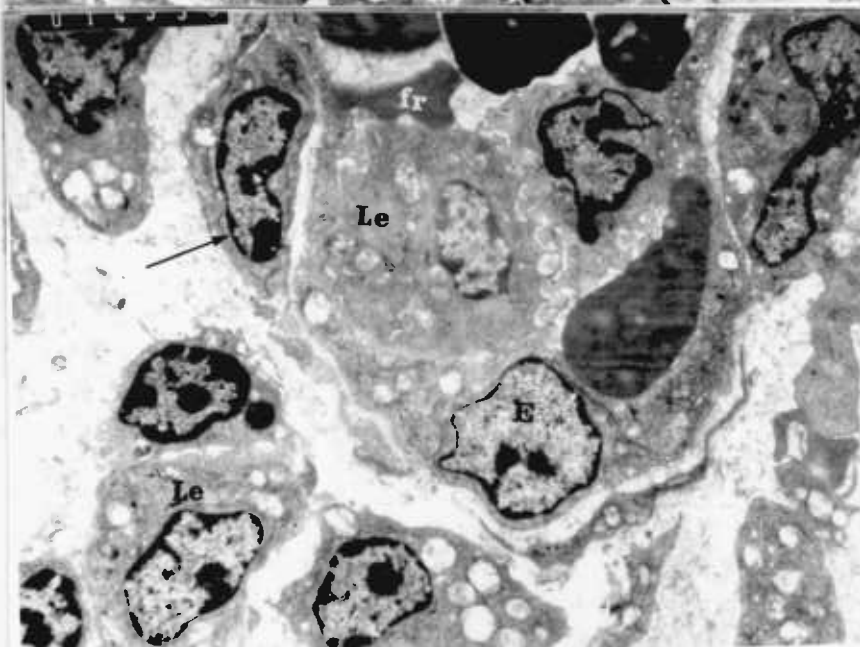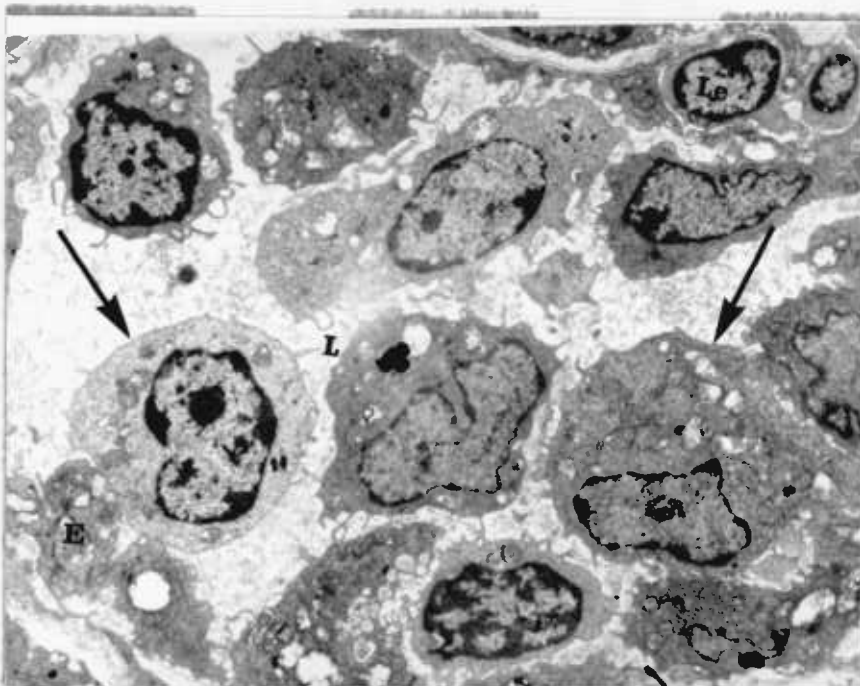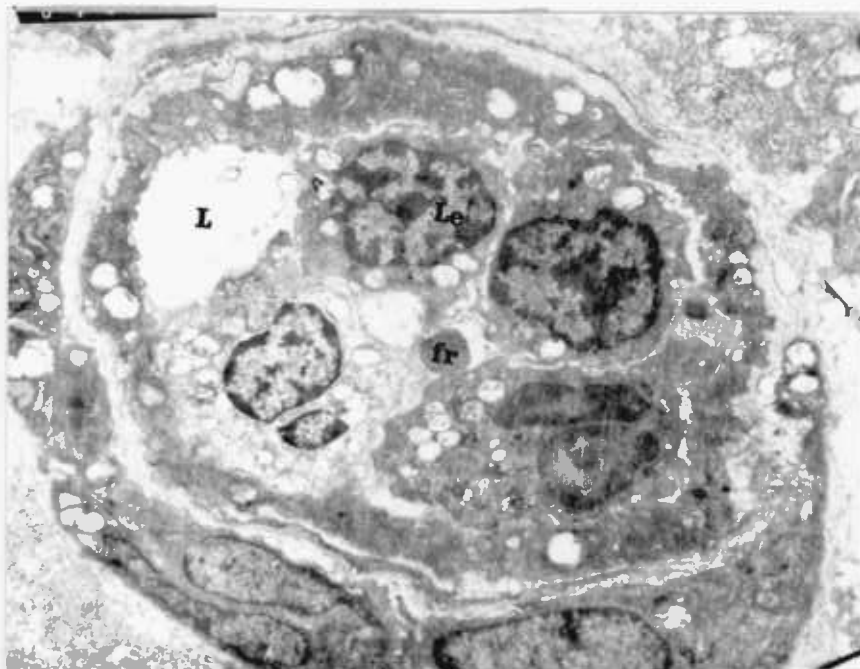
endothelial space probably by passing through the perivascular elements to

infiltrate the extravascular tissue (Figs. 69). Infiltration of the

tissue by these cells was marked towards the end of the infection (see

Light Microscopic Results).

The leucocytes which were mostly mononuclear cells

were vacuolated and had numerous cytoplasmic processes. Occasionally,

polymorphonuclear leucocytes were found.

(b) Red blood cells (Erythrocytes)

After 14 days of infection many venules and a few

capillaries were filled by closely packed erythrocytes with amorphous

material such that the vascular lumen was barely patent; suggesting reduced

blood flow or stasis in these blood vessels (Figs. 70-72). However,

extravasation of erythrocytes and their fragments occurred through endo-

thelial gaps predominantly in the venules (Figs. 73 and 74). The extra-

vasated erythrocytes and their fragments were initially deposited in the

subendothelial space (Figs. 75 and 76) and then in the extravascular

tissue (Fig. 77) where they are eventually phagocytosed by macrophages

(Fig. 78 and 79). The macrophages phagocytosed seemingly normal erythrocytes

and in most cases multiple phagocytosis of erythrocytes and their fragments

were observed (Fig. 79).

Erythrocytes were numerous in the extravascular

(interstitium) tissue with no blood vessels near by in rabbits infected

for 28 days and more.

(c) Platelets (Thrombocytes)

Platelets were observed aggregating in the lumen of

venules 14 days after infection. Many of the platelets were at various

Fig. 70 : Electronmicrograph showing a section through a venule. The

lumen (L) is filled with closely packed erythrocytes (RBC) and

fragments (fr).

E - vascular endothelium; Pe - pericyte.

X 5470.

Fig. 71 : Section through a venule showing the vascular lumen (L) filled

with erythrocytes (rbc) held together by an amorphous material.

X 5470.

Fig. 72 : Section through a capillary. The lumen is filled with closely

packed erythrocytes (rbc). X 12045.
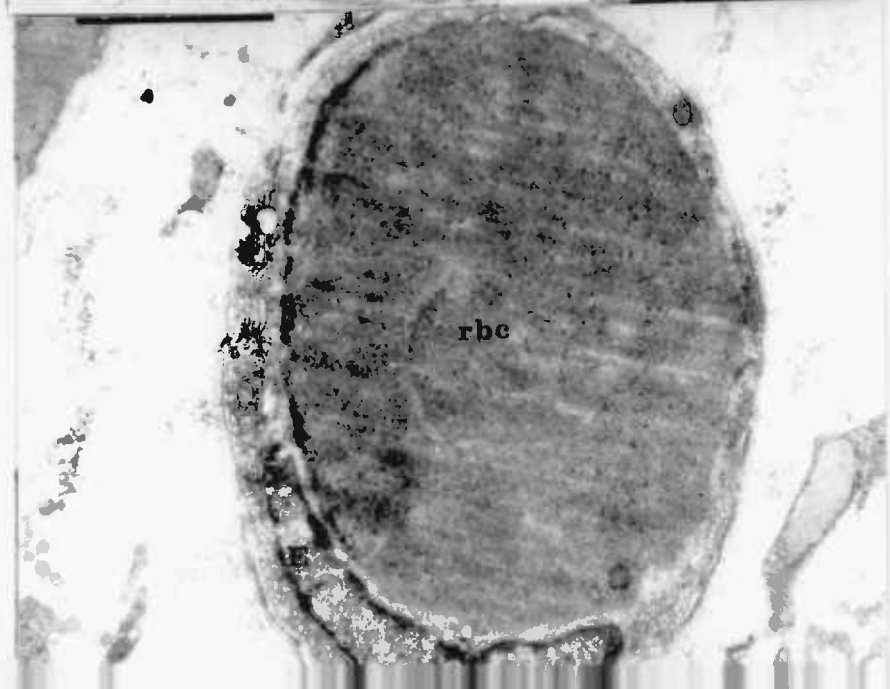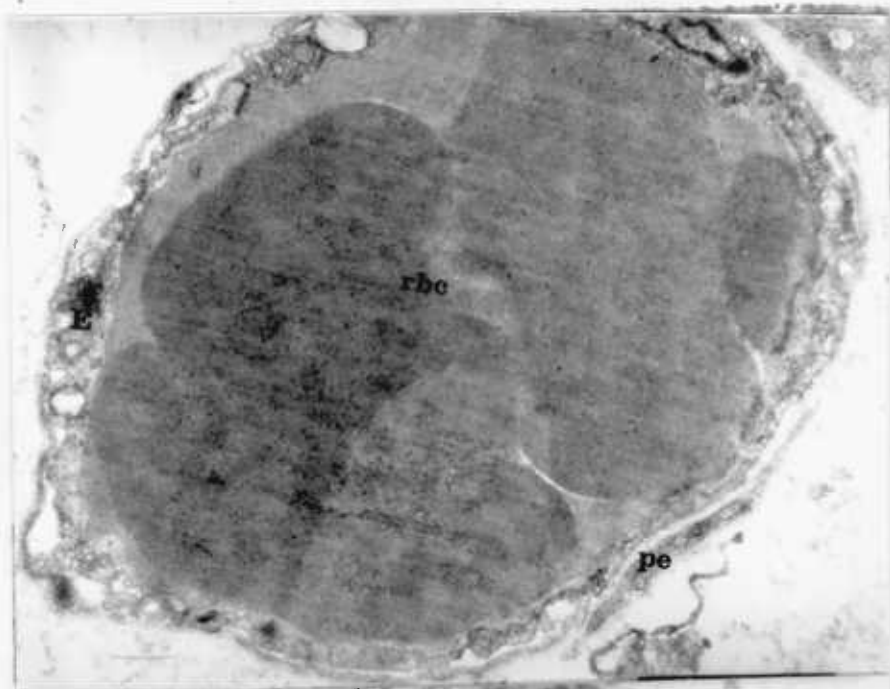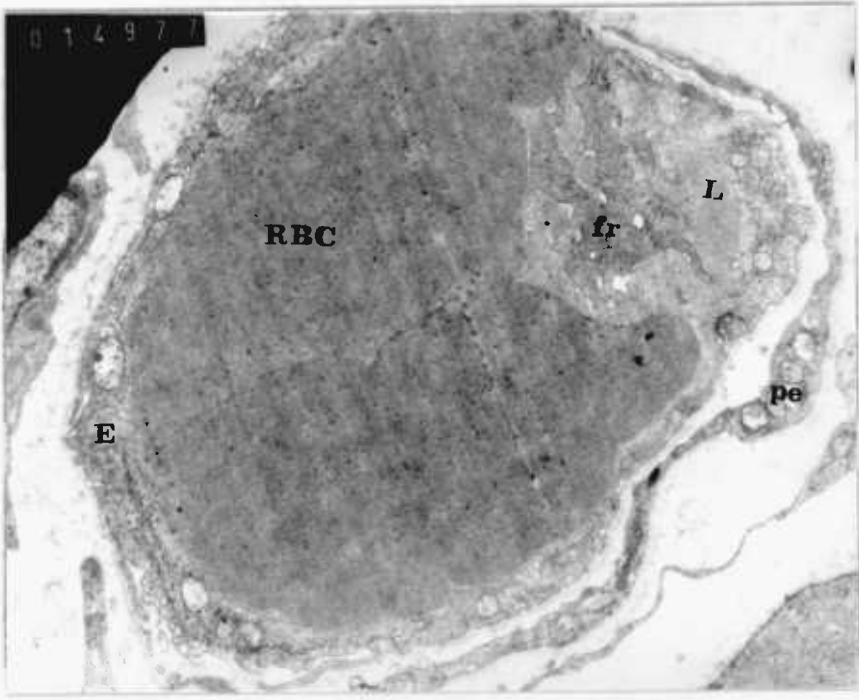
Fig. 73 : Section through the wall of a venule, showing extravasation of

erythrocyte (RBC) through a gap (arrow). The basement membrane

(arrow head) is displaced by the erythrocyte. X 14698.

Fig. 74 : Section through the wall of a venule, showing extravasation of

erythrocyte fragments (arrow) through a gap between endothelial

cells (E_1 and E_2). There is an intramural deposit (d) in the

subendothelial space.i.e. between the endothelium (E) and

pericyte (pe). X 22260.

Fig. 75 : Electronmicrograph of section through wall of a venule, showing

extravasated erythrocyte (RBC) in the subendothelial space.

There is rarefaction (i.e. loss of cytoplasmic inclusions) of

the endothelial cell (E_1) while E_2 is normal. X 14698.
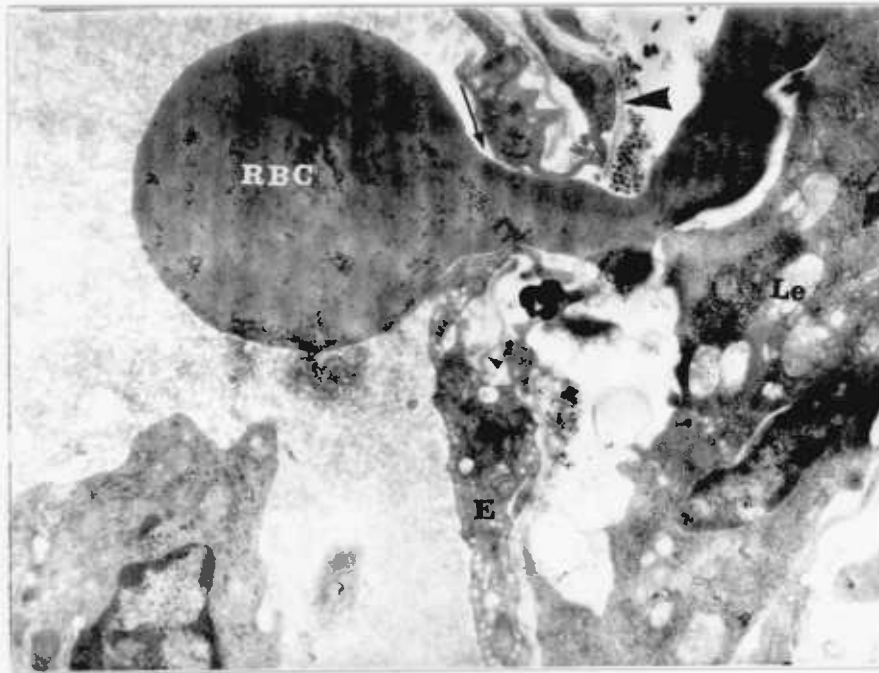
Fig. 76 : High magnification electronmicrograph of a section through the

wall of a venule, showing extravasated erythrocyte fragment (FR)

in the subendothelial space (arrow). The pericyte (pe) is

separated from the endothelium (E). X 23104.

Fig. 77 : Electronmicrograph showing extravasated erythrocytes (rbc) in

the extravascular tissue. The connective tissue is disrupted

(arrow).

M - Macrophage.

X 5470.

Fig. 78 : Electronmicrograph showing a macrophage (M) about to phagocytose

an erythrocyte (rbc) in the extravascular tissue. X 10108.

Fig. 79 : Electronmicrograph showing multiple erythrophagocytosis by a

macrophage (M). X 14698.
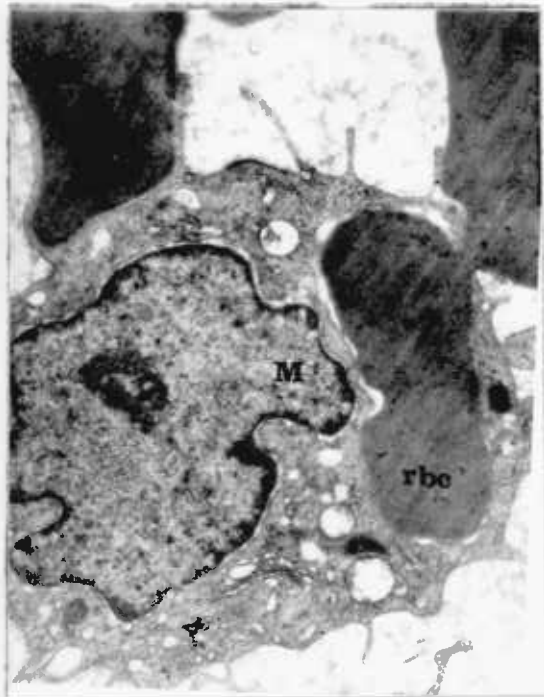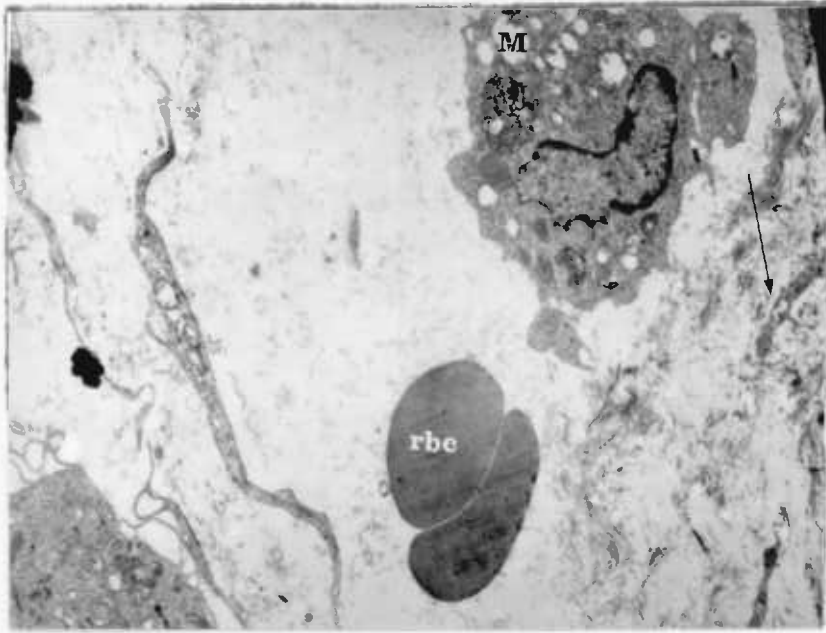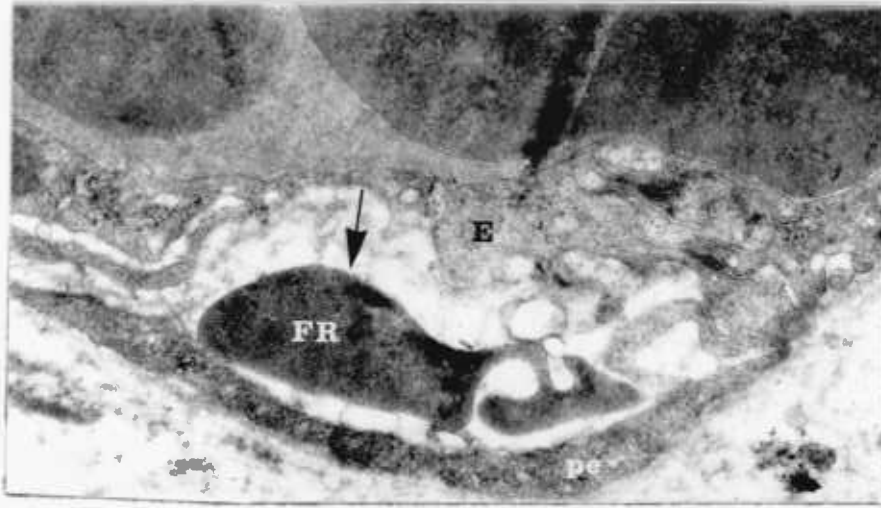
stages of degranulation (Fig. 80). In a few instances, platelets in some blood vessels were not aggregated. Fibrin strands were deposited in the vascular lumen in addition to the platelets, in many venules (Fig. 81).

Platelets like other endogenous elements were observed to pass through the gaps between endothelial cells in blood vessels of the venular segments and become deposited in the subendothelial space (Fig. 82). When these platelets plugged a gap, they come in contact with the subendothelial components (e.g. basement membrane) of the blood vessel and as more platelets are incorporated, a thrombus is formed. In most cases the platelets undergo degranulation as they aggregate.

Thrombosis of the venules was evident 21 days after infection. The thrombi were either mural or occlusive and consisted of platelets, fibrin and red blood cells in varying proportions and different patterns.

Fig. 83a and b show a mural thrombus of degranulated platelets held together by fibrin strands, in a venule. In Fig. 84a and b the occlusive thrombus is made up of platelets (many of which are degranulated) and red blood cells at the periphery of the venule.

Many occlusive thrombi were made up essentially of degranulated platelets and large deposits of fibrin strands (Figs. 85 and 86). Occasionally, occlusive thrombi consisting of platelets only were observed (Fig. 87). The occlusive type of microthrombi were predominant, and the number of thrombosed blood vessels were more in animals sacrificed after 28 days of infection.

Fig. 80 : Electronmicrograph of a section through a venule. The vascular

lumen (L) contains platelets (PL) some of which are degranulated.

X 5470.

Fig. 81 : Electronmicrograph of a section through a venule, showing fibrin

(F) deposits and platelets (PL) in the vascular lumen (L). A

leucocyte (Le) marginates on the vascular endothelium (E).

X 5470.

Fig. 82 : Section through the wall of a venule showing platelets (PL)

lodged in the subendothelial space, i.e. between the endothelium

(E) and the pericyte (pe). X 12045.
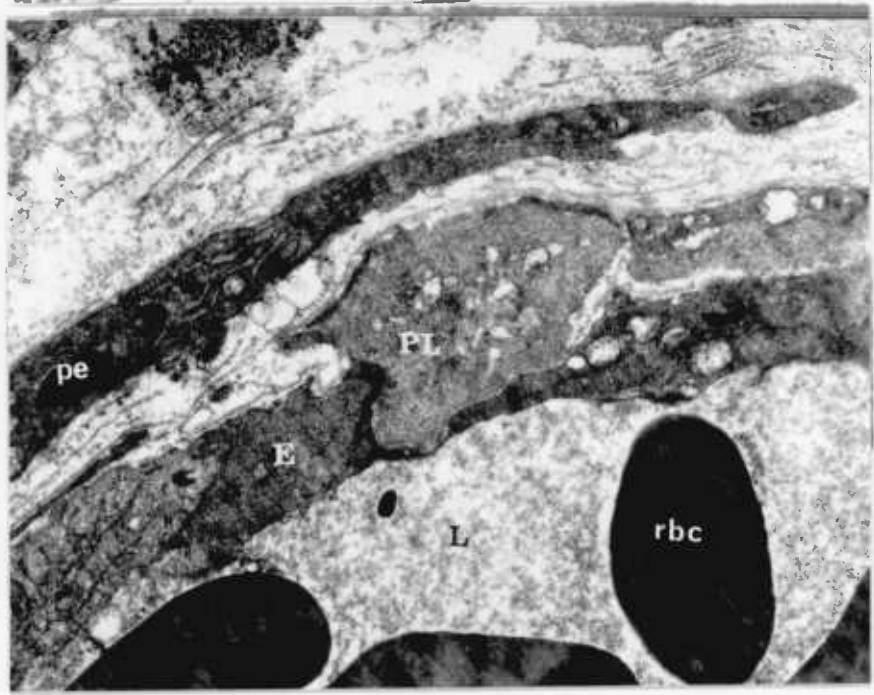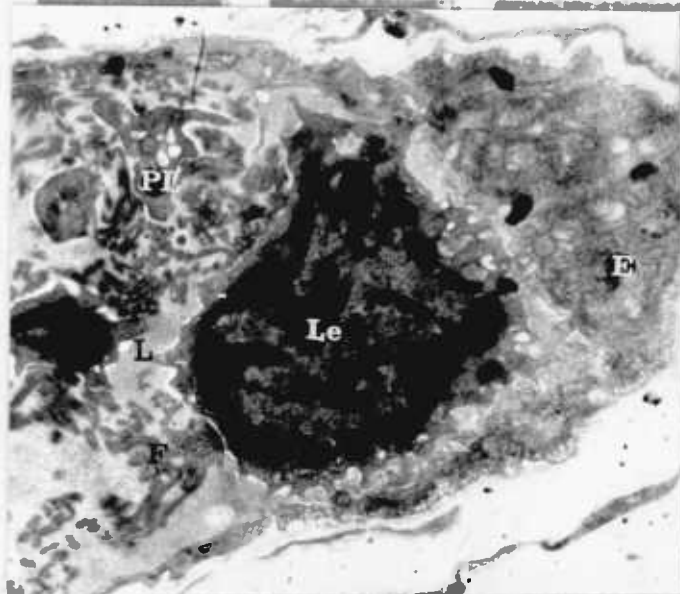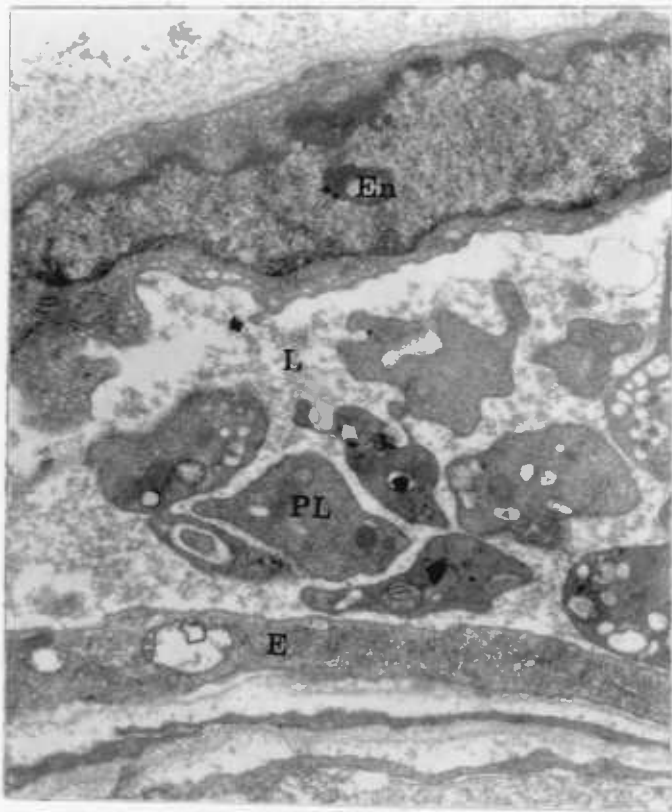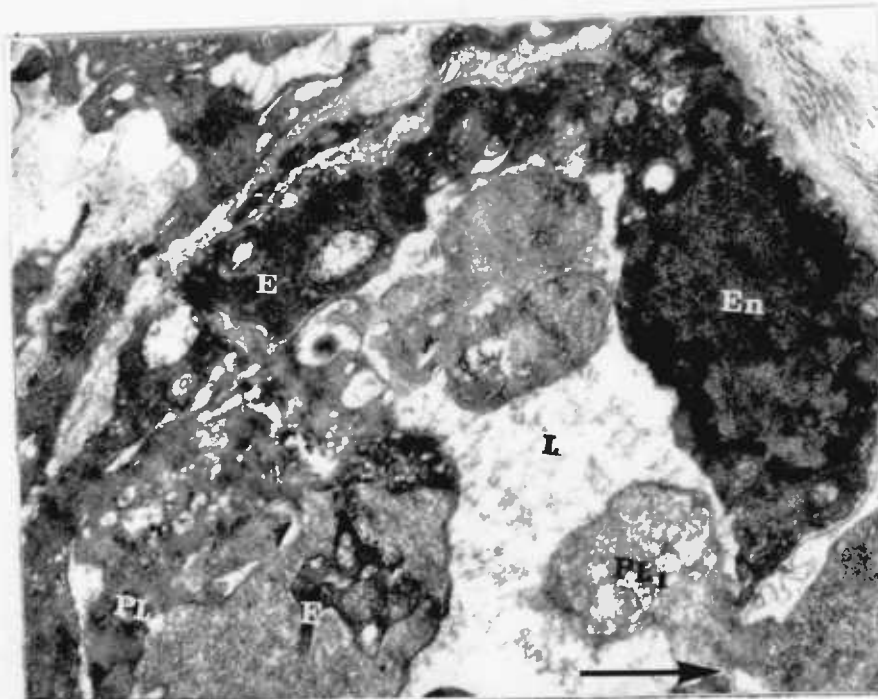
Fig. 83A : Low magnification electronmicrograph of section through a
thrombosed venule. The thrombus consists of platelet mass (PL)
held together with fibrin strands (F). The platelet mass (PL$_1$)
plugs a gap (arrow) in the vessel wall. X 5470.
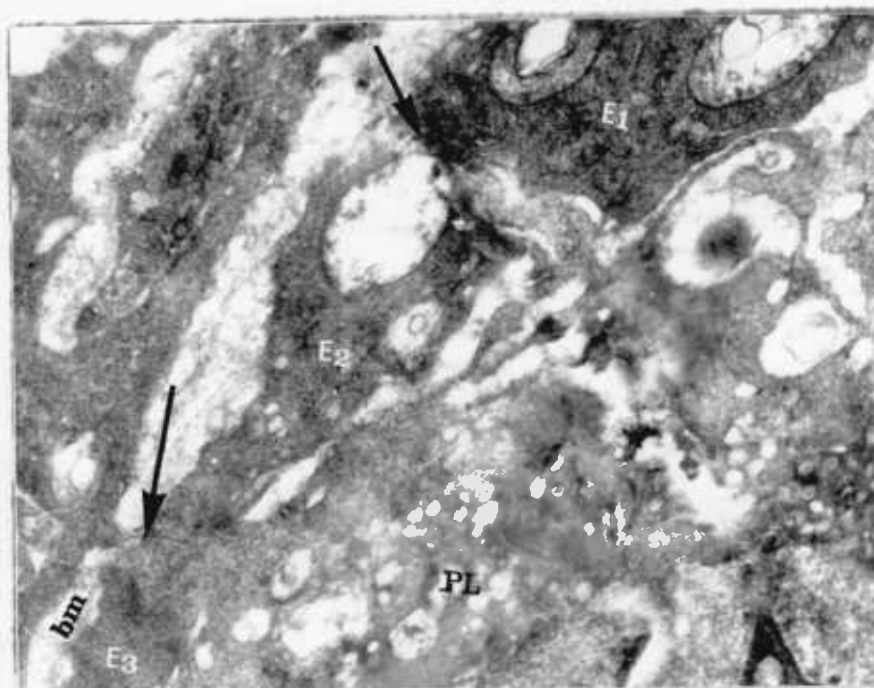
Fig. 83B : Higher magnification electronmicrograph of an area in Fig. 83A.
There are gaps (arrows) between the endothelial cells (E$_1$ and
E$_2$ and E$_3$) plugged by the platelet mass (PL). The basement
membrane (bm) is displaced where the gaps occur. X 10924.
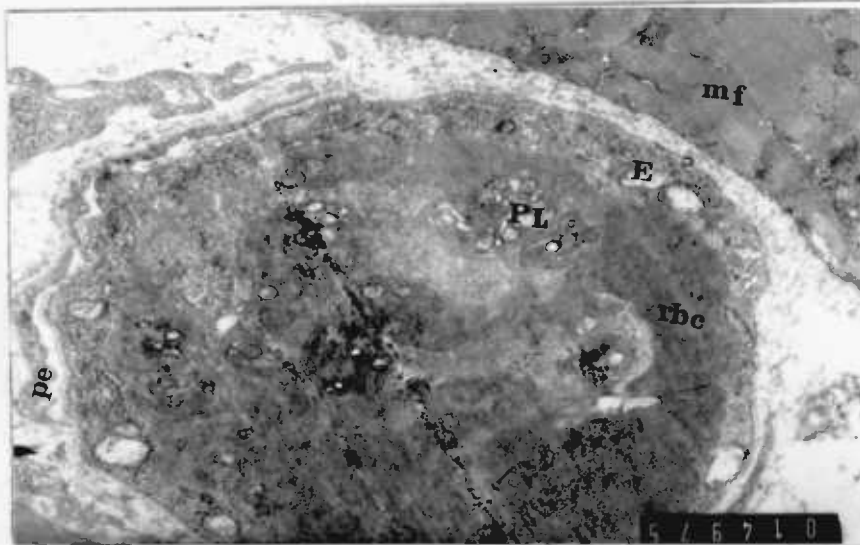
Fig. 84A : Low magnification electronmicrograph of a section through a
thrombosed venule. The thrombus consists of platelets (PL) and
erythrocytes (rbc) at the periphery of the blood vessel.
X 5470.
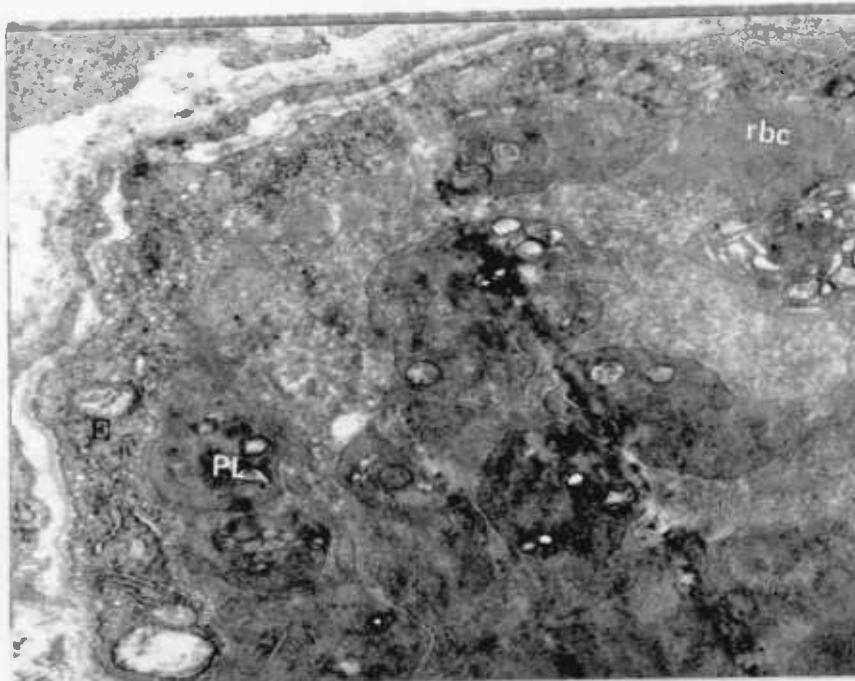
Fig. 84B : Higher magnification electronmicrograph of an area in Fig. 84A
showing details of the thrombus (platelets - PL; erythrocytes -
rbc). Cytoplasmic inclusions in the vascular endothelium (E) are
dilated. X 10940.

Fig. 85 : Low magnification electronmicrograph of a section through a

thrombosed venule. The thrombus consists of platelets (PL)

stabilized with fibrin strands (F). The basement membrane

(arrow) is the only visible part of the blood vessel. X 5470.

Fig. 86 : High magnification electronmicrograph of part of a thrombosed

venule showing platelet mass stabilized with fibrin strands (F).

An erythrocyte (RBC) is surrounded by fibrin strands. X 14440.

Fig. 87 : Electronmicrograph of section through a thrombosed venule. The

thrombus consists of platelets (PL) only. E - Endothelium.

X 7860.
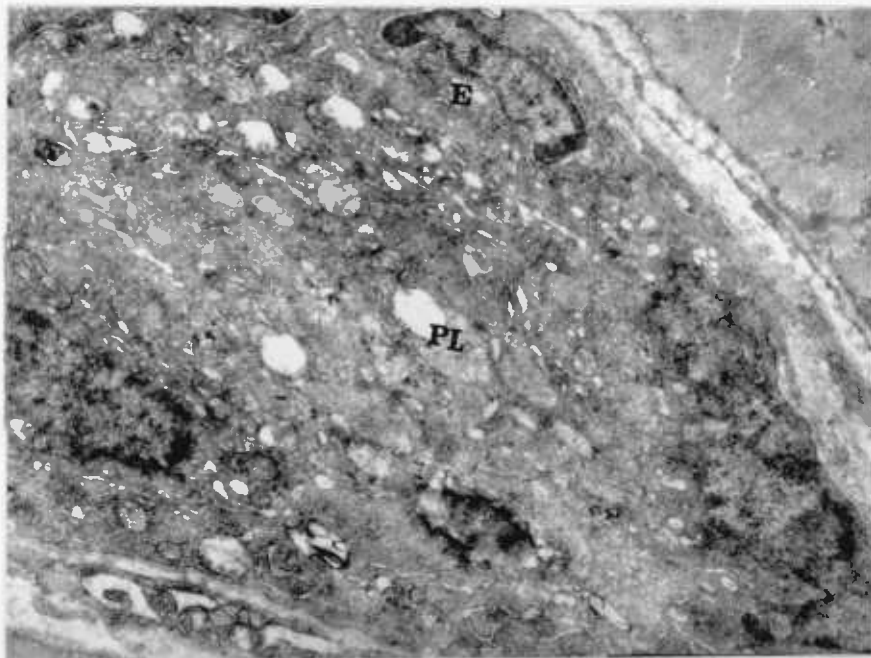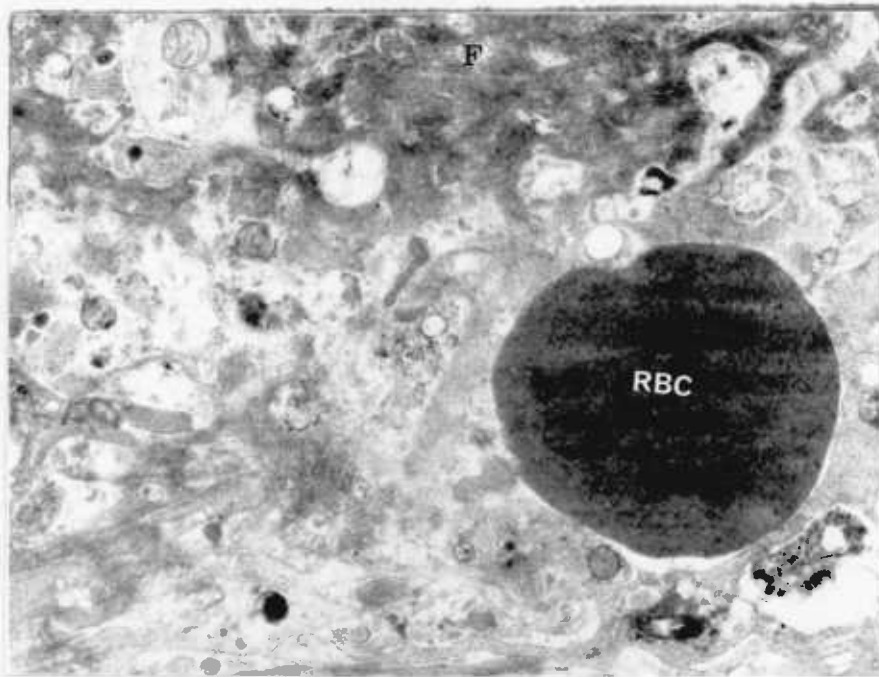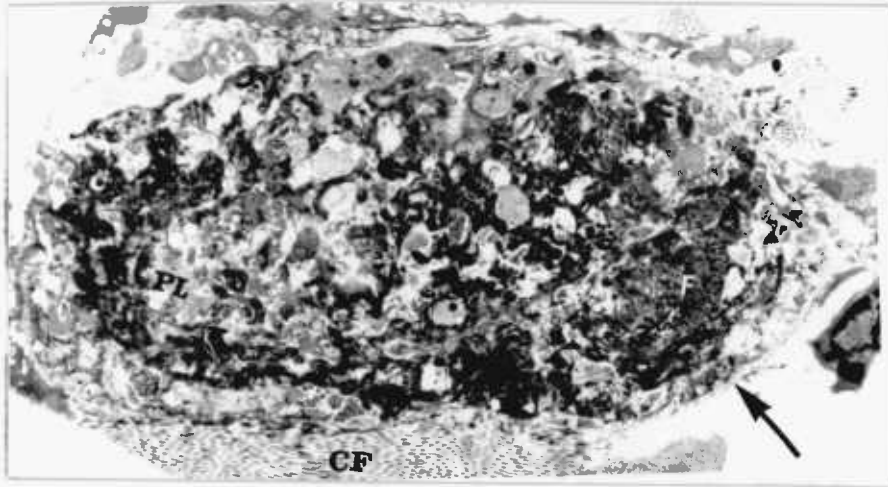
EXTRAVASCULAR TISSUE

(a) Muscle

Fig. 88 shows section of cremaster muscle from an uninfected control rabbit.

In infected rabbits degenerative changes in the muscle occurred 21 days after infection. The muscle fibres were swollen and there was extensive separation and clumping of the myofibrils, loss of internal structures (e.g. mitochondria) and vacuolation (Fig. 89). In some cases only the sarcoplasmmic membranes and a few internal structures were present in sections (See light microscopic observations). Phagocytes were observed to invade these necrotic muscle fibres, probably to clear the cellular debris (Fig. 90).

The number of degenerate muscle fibres increased towards the end of the infection, although individual variations occurred. In rabbits infected for 35 and 42 days respectively most muscle fibres appearing in the sections observed were at one stage of degeneration or another.

(b) Trypanosomes

At no stage of the infection were trypanosomes or their fragments seen in the lumen or walls of the blood vessels examined. However, trypanosomes and their fragments (mostly flagella), phagocytes, plasma cells and cellular debris were observed in the extravascular connective tissue, 14 days after infection (Figs. 91 & 92b).

There was extensive separation and degeneration of connective tissue components, particularly collagen fibres and fibroblasts towards the end of the infection.

Fig. 88 : Electronmicrograph showing section through cremaster muscle of

an uninfected rabbit. The muscle fibre (mf) is normal. X 14698.

Fig. 89 : Electronmicrograph showing section through cremaster muscle of

an infected rabbit. The muscle is degenerate and shows clumping

(mf) and vacuolation (arrow) of myofibrils. X 10108.

Fig. 90 : Low magnification electronmicrograph of a section through

cremaster muscle of an infected rabbit. The degenerate muscle

is swollen and vacuolated (arrow). A phagocyte (Le) invades the

muscle fibre, probably to clear cellular debris. X 5470.
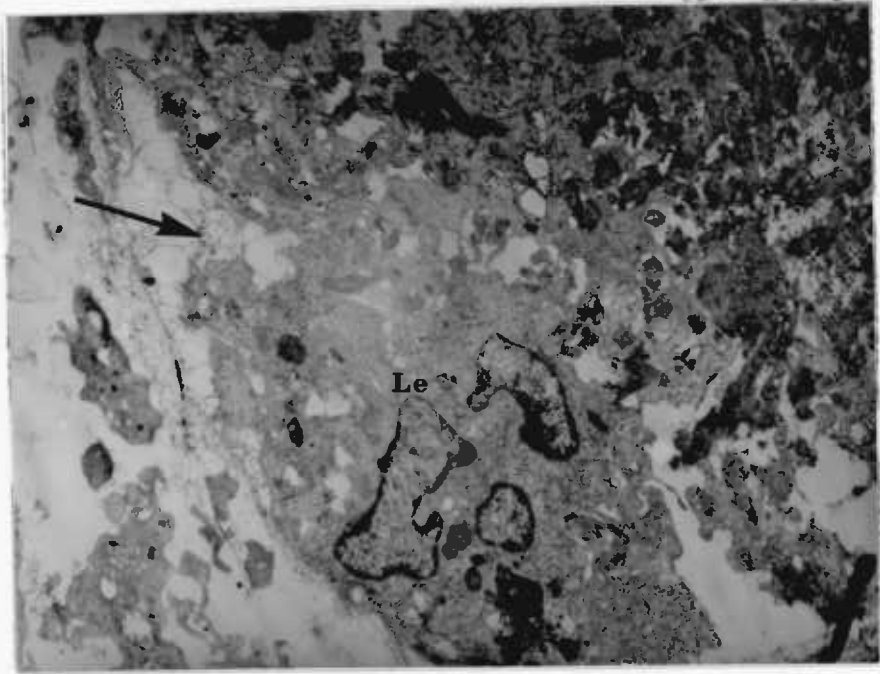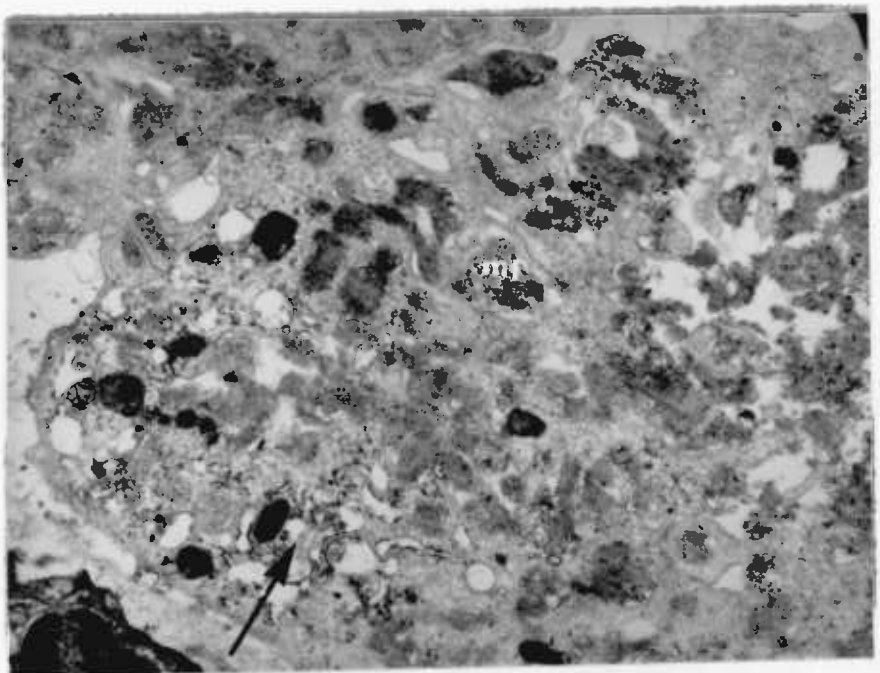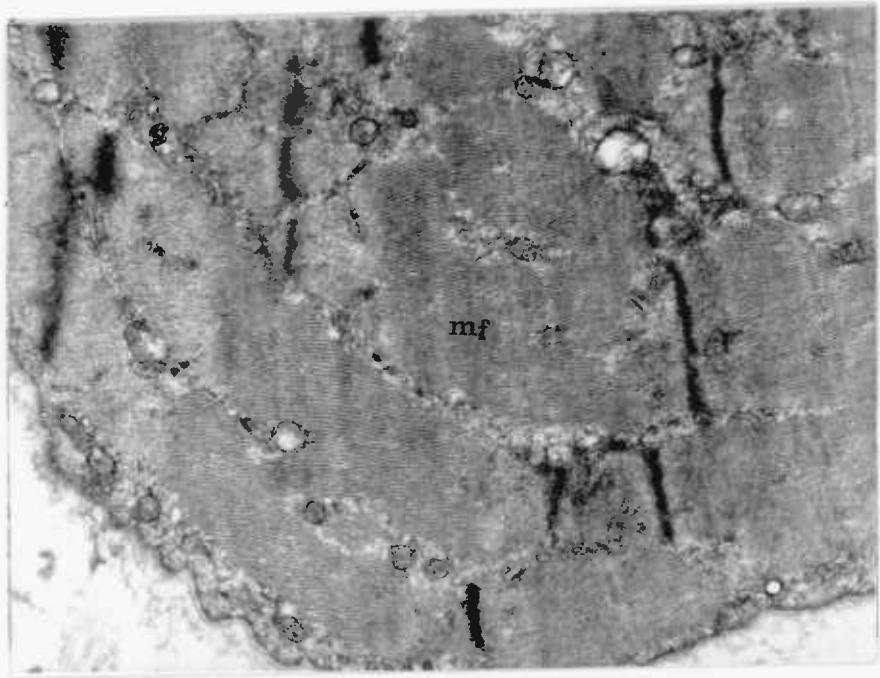
Fig. 91 : Electronmicrograph showing trypanosomes (arrow) and a macrophage

in the extravascular tissue.   The tissue is oedematous.   X 10108.

Fig. 92A : Low magnification electronmicrograph showing trypanosomes

(arrow), macrophages (M) and cellular debris in the extra-

vascular tissue.   Most trypanosomes are in close contact with

macrophages.   X 5470.

Fig. 92B : Higher magnification electronmicrograph of an area in Fig. 92A

showing trypanosomes (arrows) and cellular debris in the extra-

vascular tissue.   Most trypanosomes aggregate and show vacu-

olation.   X 14698.
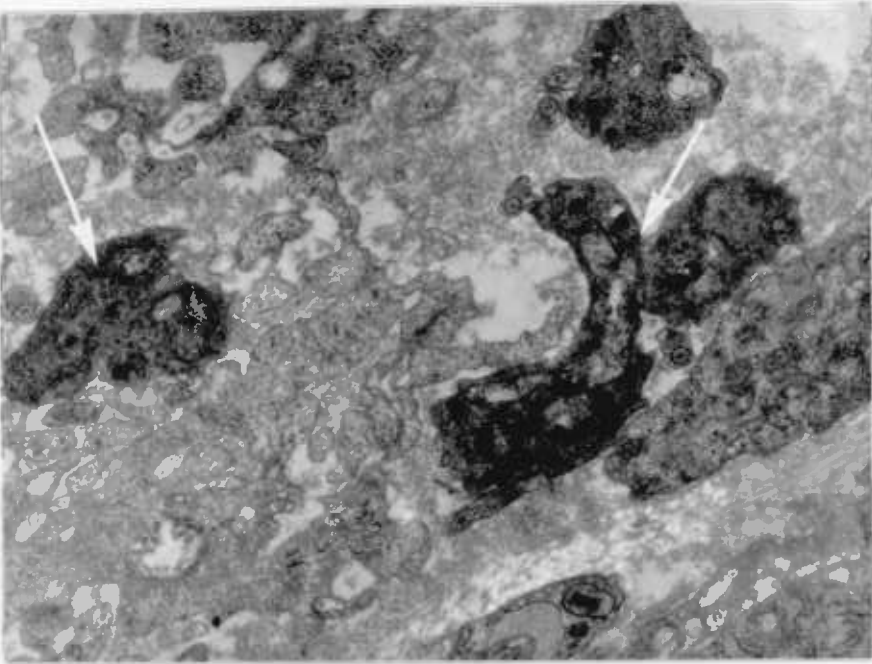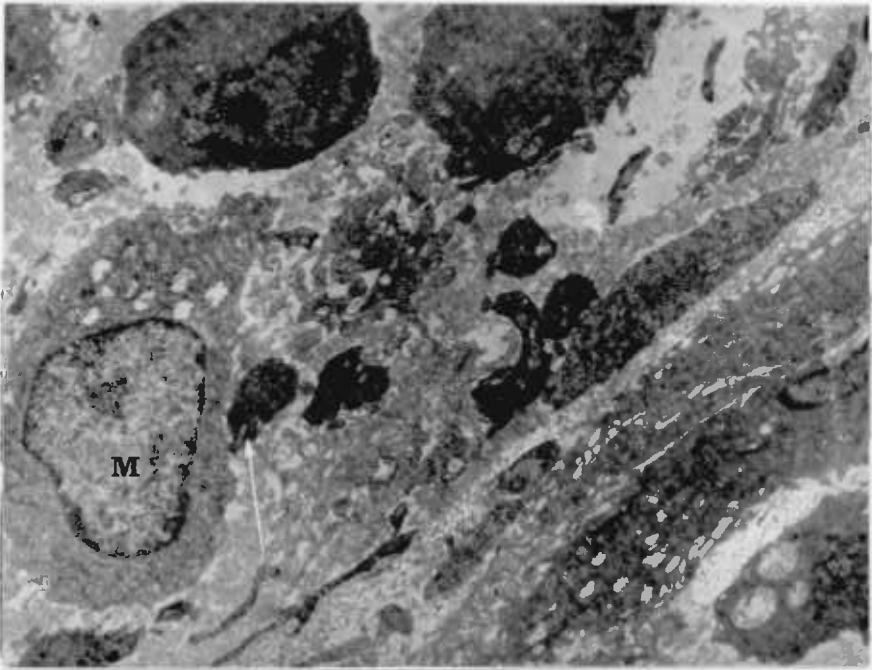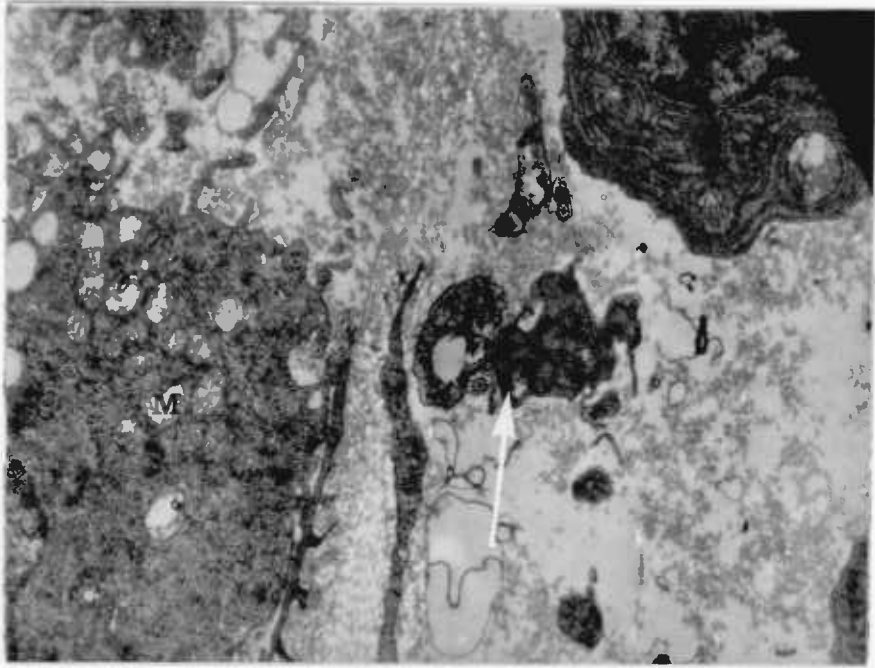
The trypanosomes found in the connective tissue were almost always vacuolated and aggregated. Many of them were in close contact with phagocytes (Fig. 92a).

The trypanosomes and their fragments were phagocytosed by macrophages. This event was observed at all stages of infection after 14 days.

Figs. 93 and 94 show macrophages with vacuoles containing materials which are probably phagocytosed trypanosomes and their fragments. The macrophages in the extravascular tissue were mostly irregular in shape and had extensive cytoplasmic processes (pseudopodia).

DISCUSSION

The light and electron microscopic observations of the cremaster muscle show that venules and to some extent capillaries, were damaged during *T. brucei* infection of rabbits. The light microscopic results are similar to the descriptions for regenerating blood vessels in ear-chambers (Goodwin 1971) and the cremaster muscles (Goodwin and Hook, 1968) of rabbits infected with *T. brucei*. The results also show that the lesions produced as a result of *T. brucei* infection in rabbits occurred in many parts of the animals and were essentially similar.

The endothelial changes are consistent with those given for endothelial reactions to various inflamatory stimuli : immunogenic (Uriuhara and Movat, 1964), thermal (Cotran 1965; Cotran and Remensnyder 1968), traumatic (Marchesi 1961) and chemical (Ham and Hurley 1965; Hurley *et al* 1967). The damaged endothelial lining of the blood vessels could provide a suitable surface for adhesion of formed elements of blood, in particular leucocytes and platelets. Since the vascular endothelium is known to have
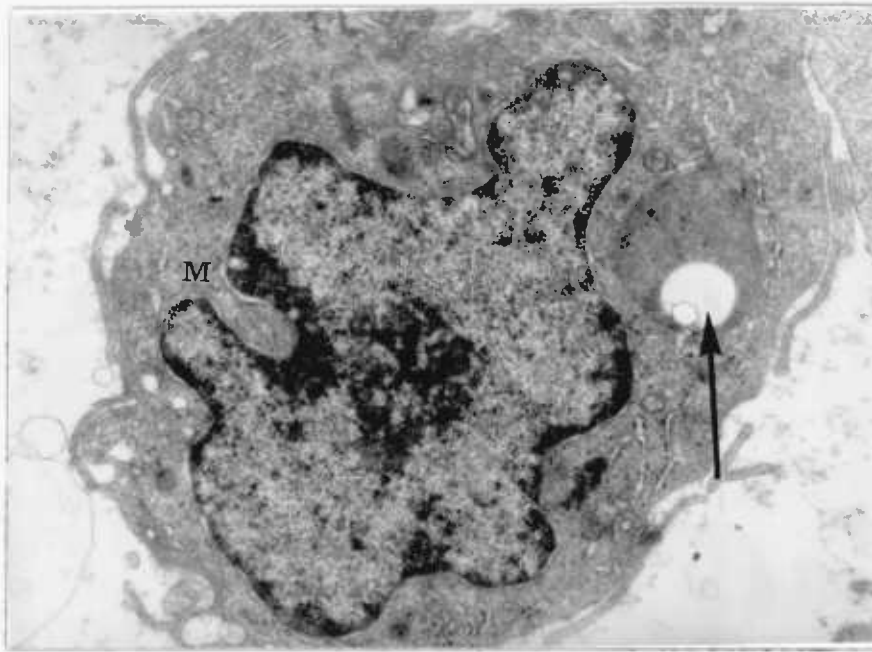
Fig. 93 : Electronmicrograph showing a macrophage (M) with materials
(arrow) which are probably phagocytosed trypanosomes. X 14698.
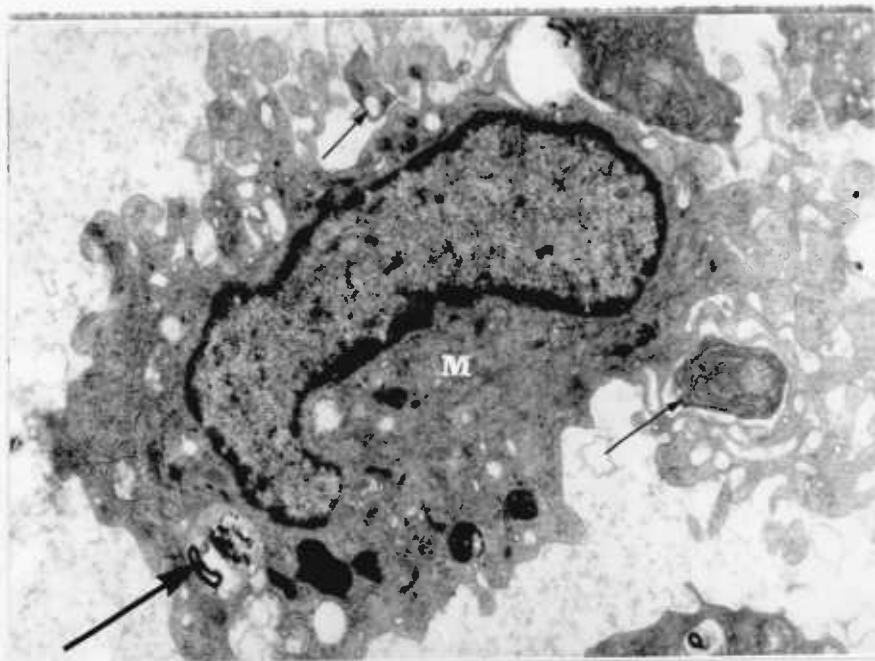
Fig. 94 : Electronmicrograph showing a macrophage (M) containing materials
which are probably phagocytosed trypanosomes and fragments in
it's vacuoles and between it's extensive cytoplasmic processes
(arrows). X 10108.

fibrinolytic activity, which helps to maintain the fluidity of blood
(Todd 1972; Warren and Khan 1974), its damage would certainly lead to
thrombotic consequences. The observed disintegration of venules and
capillaries towards the end of infection, suggests severe damage to
these blood vessels and provides ultrastructural support for the *in vivo*
observations of small blood vessels in rabbits infected with *T. brucei*
(Goodwin 1971).

The gaps formed at or near endothelial intercellular junctions in
the venules could account for the increase in the permeability of these
blood vessels; a feature of *T. brucei* infections in rabbits (Boreham and
Goodwin 1967; Goodwin and Hook 1968; Goodwin 1970, 1971). These gaps
are reminiscent of those found in venules, during injuries elicited by
histamine-type mediators (Majno *et al* 1961, 1969; Hultström and Svensjö
1977; Northover 1978), antigen/antibody interactions (Movat and Fernando
1963; Movat, 1966) and in thermal injury of skeletal muscle microvessels
(Cotran 1967a).

Since it has been shown that kinins and other inflammatory mediators,
such as histamine and 5HT are released during trypanosomiasis (Goodwin
1976; Boreham 1979b) it is likely that these substances contribute to the
vascular changes observed. Other substances known to occur in trypano-
somiasis infections which might also contribute to the vascular damage
include FDP (Boreham and Facer 1974a), a permeability increasing factor
released by the trypanosomas (Seed 1969) and products of disrupted try-
panosomes e.g. phospholipase A and free fatty acids (see Tizard *et al*
1978).

The present observation of the escape of amorphous materials (pro-
bably plasma proteins) from the endothelial gaps into the extravascular
space, strongly suggests that the blood vessels were leaking. The loss

of these proteins, particularly albumin into the extravascular space would certainly reduce the colloidal osmotic pressure (COP) of the blood and lead to fluid accummulation in the tissue (i.e. inflammatory oedema). Although the lymphatic vessels were not studied, the oedema suggests malfunction of these channels.

The behaviour of the leucocytes (i.e. margination and emigration) are consistent with those described in inflammatory foci (Marchesi 1961, 1964; Williamson and Grisham, 1961) and provides ultrastructural support for the observations made by Goodwin and Hook (1968) and Goodwin (1971) in experimental _T. brucei_ infection of rabbits. The lining of the venules and capillaries by these cells indicates damage to the vascular endothelium (Cliff 1966) as leucocytes rarely adhere to normal endothelium (Mayrovitz _et al_,1977). The predominance of mononuclear cells in the infiltrate indicates a chronic inflammatory response (see Goodwin 1970, 1971). Leucocytes clogging the lumina of the blood vessels could disturb blood flow and together with the increase in blood viscosity known to occur in infected rabbits (Facer 1976), contribute to blood stasis. This then would have ischaemic and anoxic consequences on the animals' tissues and organs.

The removal of red blood cells and their fragments from the gaps in the venules provides further evidence for vascular leakage and therefore damage to the endothelium, since alterations in the morphology of the vessel wall is a prerequisite for removal of red cells into the extravascular tissue.
The clumping of the red blood cells in the venules and some capillaries suggests stasis or decreased blood flow in these vessels. The increase in macroglobulins and plasma fibrinogen known to occur in rabbits infected with _T. brucei_, probably led to the clumping of the red cells

in the vessels. The stasis observed could result in tissue damage as a result of anoxia; predispose the blood vessels to microthrombosis and escalate the increase in blood viscosity.

Red blood cells were probably fragmented, either when trapped in blood vessels clogged by leucocytes or as they passed through fibrin strands in thrombosed venules. Since the typical blood picture in MHA is characterized by small red cell fragments or schistocytes, helmet cells and Burr cells, it is likely that the anaemia in rabbits infected with T. *brucei* is partly microangiopathic.
The observed phagocytosis of red blood cells provides ultrastructural evidence for extravascular haemolysis. It is likely that the red cells were either sensitized by trypanosome antigen/antibody or immune complexes, which made them susceptible to phagocytosis by the expanded and active mononuclear phagocytic system.

Microthrombi observed in venules provide morphological confirmation of the changes known to occur in the fibrinolytic system of rabbits infected with T. *brucei* (Boreham and Facer 1974a; Facer 1974). The microthrombi in these blood vessels could lead to further reduction in blood flow and damage to the vascular endothelium. Since platelets were involved in the formation of the microthrombi, it is likely to contribute to the thrombocytopenia known to occur in trypanosomiasis. The thrombosed venules are consistent with those described in inflammatory reactions (Movat 1966).

The degenerative changes observed in the muscle fibres of the infected rabbits, like other lesions are indicative of inadequate blood supply. This observation is consistent with muscular wasting, which is a feature of all forms of chronic trypanosomiasis (Goodwin 1970). The changes also parallel those described by Ham and Hurley (1965) in

turpentine-induced inflammation of the pleura and in thermal injury of
skeletal muscle (Cotran, 1967a).

The connective tissue changes, the location and phagocytosis
of the trypanosomes are in agreement with the ultrastructural observations
in the ear-chamber tissues of rabbits infected with *T. brucei* (Goodwin
1971). The observation that the trypanosomes were localised in the extra-
vascular tissue supports the contention that parasites of *T. brucei*
subgroup are essentially tissue inhabitants (see Losos and Ikede 1972).
The injured tissues probably contribute to the vascular injury, through
the release of tissue thromboplastin that can initiate activation
of the coagulation system. The dead trypanosomes plus the phagocytes
and resulting cellular debris would contribute to the necrotic lesions
in the tissues by the release of lysosomal enzymes. In fig. 95, an
attempt is made to correlate the vascular events observed with path-
ophysiological changes known to occur during trypanosome infections,
particularly in the rabbit. The representation is a simplication of
events, since many factors/processes are involved in bringing about the
changes and their consequences (see Part 4).

EXTRAVASATION OF
RED BLOOD CELLS

LOCAL HAEMORRHAGE
ERYTHROPHAGOCYTOSIS → Anaemia

Increased
vascular
permeability

Loss of plasma
Constituents

OEDEMA

VASCULAR
ENDOTHELIAL
DAMAGE

MOBILIZATION
OF
LEUCOCYTES

TISSUE
NECROSIS

Tissue anoxia

TRYPANOSOMES

Kallikrein-Kinin
System

Hypotension

MICROTHROMBI
FORMATION

Vascular
Stasis

Trypanosomes

Thrombocytopenia

Via
Activated
Hageman Factor.

Phagocytosis
of trypanosomes

Trypanosome
antigen/antibody
complex

Increase in
plasma proteins

Hyperviscosity
of whole blood

CLUMPING OF
RED BLOOD CELLS

Activated
Hageman
Factor

Via Plasminogen
Plasmin

Fibrinogen
FDP

Hyperviscosity
of plasma

Fig. 95 Schematic representation of events following vascular damage in rabbits infected with *T. brucei.*

PART 4.   GENERAL DISCUSSION

Changes in small blood vessels of rabbits experimentally infected with *T. brucei* have been investigated, using light and electron microscopic techniques. Although both venules and capillaries were damaged, changes were more marked in venules. Vascular injury was manifested by endothelial alterations which included gaps in venules, swelling, fragmentation and loss of cytoplasmic components; mobilization of leucocytes; microthrombi formation and tissue necrosis.

The events that could lead to vascular damage in trypanosomiasis are numerous and complex. In Fig. 96, an attempt is made to show some of these events, their interelationships and how they are probably initiated. Rabbits infected with *T. brucei* produce antibodies towards trypanosomal antigens. The immune complexes formed have been shown by *in vitro* experiments to activate Hageman factor (factor XII of the coagulation system), which in turn activates the Kallikrein-Kinin system to release Kinins (See Boreham 1979b). The release of Kinins probably occurs in all infections caused by pathogenic trypanosomes (Boreham 1977). Since one biological effect of the Kallikrein-Kinin system is to cause increased vascular permeability, Kinins are most likely to be involved in the vascular damage observed in the infected rabbits. In the present investigation, the initiation of vascular damage correlates with the peak levels of plasma Kallikrein (Boreham and Parry 1979) and Kinins (see Boreham 1968a) which are formed by the action of this protease on Kininogen. Moreover, salient features of the vascular damage e.g. gaps in venules, were consistent with those in injuries induced with products of the Kallikrein-Kinin system e.g. bradykinin (Majno *et al* 1969; Hultström and Svensjö 1977). Although Kinins have a short half life (Ferreira and Vane 1967), their release would be expected to occur in a recurring manner, since immune
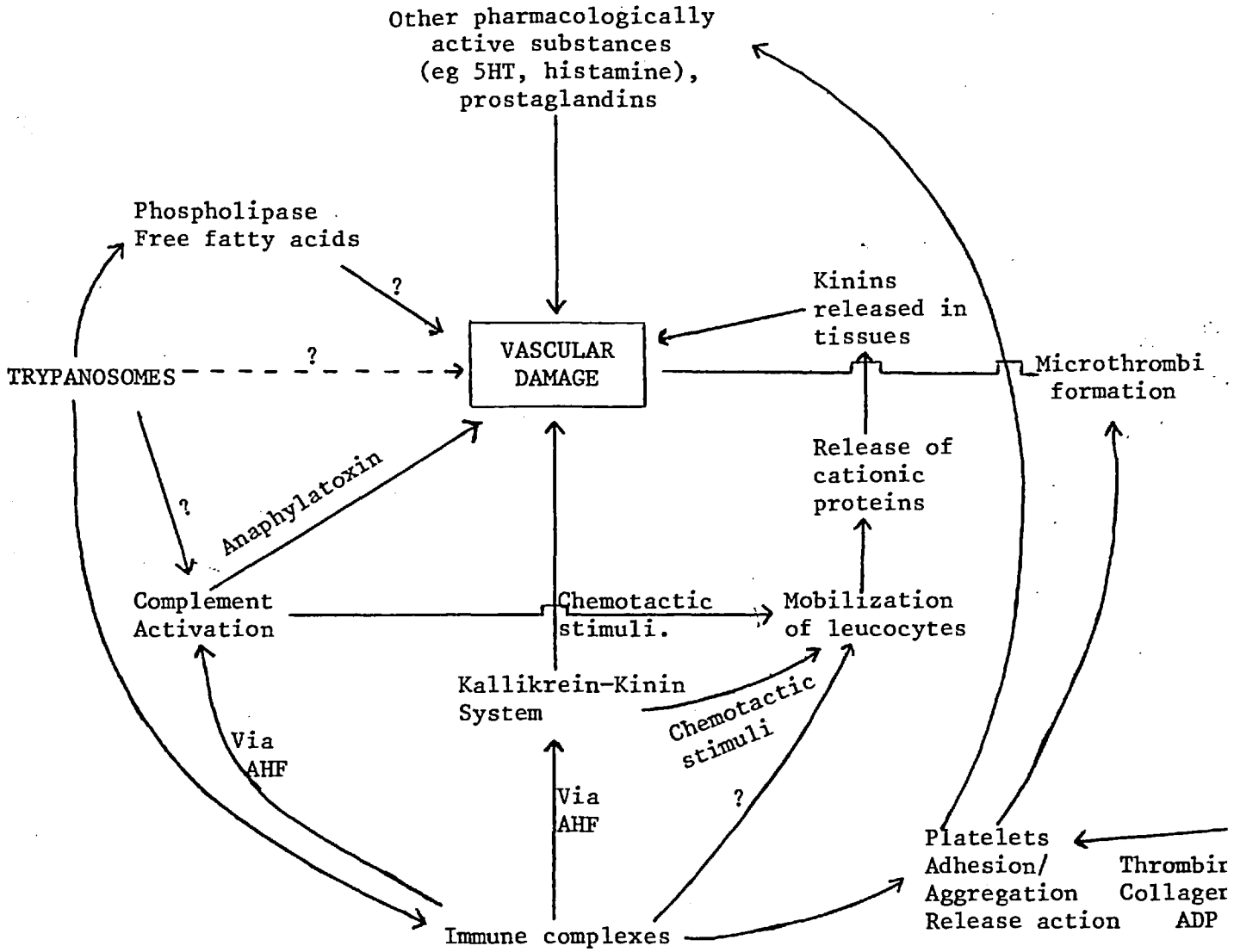
Figure 96. Schematic representation of possible events leading to vascular damage in rabbits infected with *T. brucei*.

complexes are formed each time the infected host produces antibody to each new trypanosome antigenic variant (Goodwin 1976). Since the complement system is activated during trypanosomiasis (see Greenwood and Whittle 1976b; Nielsen and Sheppard 1977), it is likely that histamine is released, following mast cell degranulation by fragments of C3 and C5. Although rabbits are insensitive to histamine (Boreham 1979b), the possibility that this substance contributes to vascular injury should not be ruled out.

Direct damage to the vascular endothelium by the trypanosomes is not likely, as in the present study, parasites were not seen in the blood vessels. However, in infections caused by *T. congolense* and *T. vivax*, where these parasites are known to aggregate and block the lumina of microvessels, direct vascular injury is feasible. For example, Banks (1978) has shown that trypanosomes attached themselves to vascular endothelium of small blood vessels in animals infected with *T. congolense*. It would not be difficult to demonstrate the effect of these parasites on the vascular endothelium with the electron microscope.

The role played by the products of dead trypanosomes is difficult to assess in the present work. However, the hypothesis advanced by Tizard and co-workers (reviewed by Tizard *et al* 1978), that trypanosomes might contribute to vascular injury via the biologically active materials (e.g. phospholipases, free fatty acids and proteases) they release when destroyed by the host's immune response or during autolysis should be considered.

The events following vascular damage in rabbit trypanosomiasis are shown in Fig. 95. An interesting ultrastructural observation in the present study, is the presence of gaps at or near endothelial intercellular junctions in venules. These gaps, which allowed the passage of amorphous materials (probably plasma proteins) and formed elements of blood, provide morphological evidence for the increased vascular permeability described by Cotran (1969).

Increase in vascular permeability is a consistent feature of *T. brucei*

infections in rabbits (Goodwin 1970, 1971). In allergic inflammation,

immune complexes have been shown to escape via gaps in venules, into the

extravascular compartment (Movat 1966). It is likely that a similar

process occurs in rabbits infected with *T. brucei*. The inflammatory

oedema, manifested by swelling of the ears and face and extensive dis-

ruption of connective tissue in rabbits infected with *T. brucei* is a

consequence of increased vascular permeability, due to the loss of plasma

proteins and fluid through the endothelial gaps. The mechanism for the

formation of these gaps was not apparent in the blood vessels studied.

The suggestion by Majno and co-workers (Majno and Leventhal 1967; Majno

et al 1969), that endothelial contraction could account for the formation

of gaps in injured small blood vessels is not likely in the present study,

since signs of contraction reflected by endothelial nuclear deformations

such as 'pinches' and 'infoldings' were not found in the blood vessels

with gaps. Although Goodwin (1971) described 'crinkling' of endothelial

nuclei in small blood vessels of rabbits infected with *T. brucei*, gaps were

not demonstrated. The present observation is probably the first description

of gaps in small blood vessels of rabbits infected with *T. brucei*.

It is likely that the gaps in the venules observed were formed,

as a result of the direct action of the injurious agent(s) on the vascular

endothelial cell membrane at the intercellular junctions as suggested

by Majno (1965) and Hammersen (1972).

Leucocytes are mobilized during trypanosomiasis, as in other in-

flammatory conditions. This is shown by the lining of small blood vessels

and infiltration of the extravascular tissue by these cells.

In the present study, no 'gluing substance' was found on the vascular

endothelium (either with the light or electron microscope) which would make

it sticky for the leucocytes. Most leucocytes marginated on the endo-
thelium by means of cytoplasmic processes. The observation is contrary
to the work of Williamson and Grisham (1961) who showed that the inflamed
endothelial cells develop cytoplasmic processes which enmesh the leuco-
cytes and push them across to the subendothelial space.

Since the essence of leucocyte mobilization, is to transfer the
cells into the extravascular tissue, the mode of adherence might not be
very important. However, they cause obstruction to blood flow by their
margination on endothelium and clogging of vascular lumina. It is difficult
to ascertain whether the leucocytes caused any damage to the blood vessels
directly, by sticking to the vascular endothelium. ·

There was no evidence in the present work to suggest that the
leucocytes themselves induced the endothelial gaps during diapedesis. How-
ever, the possibility that the cells formed the gaps, by thrusting their
pseudopodia between endothelial cells, as described by Marchesi (1961,
1964) cannot be excluded, since serial sections were not obtained from the
tissue (Cremaster muscle) used for ultrastructural studies.
Although several workers have described leucocyte mobilization in try-
panosome infections (e.g. Goodwin and Hook 1968; Goodwin 1971; Murray
et al 1974), the present work provides a detailed ultrastructural des-
cription of the events involved. The factors which induce leucocyte
emigration were not determined in the present study. However, it is
known that the complement fragments C3a and C5a and the complex C5b67
produced when complement is activated are chemotactic for leucocytes.
It has been shown that the conversion of pre-kallikrein to Kallikrein
results in the formation of cytotaxins (chemoattractants) for neutrophils
and human blood monocytes (Gallin and Kaplan 1974). Boyden (1962) showed
that antigen/antibody complexes exert a strong chemotactic effect on

rabbit polymorphonuclear leucocytes. *In vitro* studies by Cook (1977) have shown that immune complexes of trypanosome and antibody had a higher chemotactic index for leucocytes than trypanosome or immune serum alone. Leucocytes probably contribute to tissue necrosis and subsequent vascular injury through the substances released upon degranulation, e.g. Cathepsins, Collagenase, elastase, phosphatases, lysozyme, plasminogen and Kallikrein.

The factors involved in platelet behaviour (i.e. adhesion, aggregation and release reaction) during trypanosomiasis are not clearly delineated. The suggestion that *T. rhodesiense* trypanosomes alone were capable of aggregating platelets *in vitro* (Davis *et al* 1974) have been refuted by Greenwood and Whittle (1976a). However, immune complexes of trypanosome and specific antibody have been implicated in the aggregation and release-reaction of platelets both *in vitro* and *in vivo* (Slots *et al* 1977).

The elevated viscosity of blood, resulting from increases in globulins and plasma fibrinogen in rabbits infected with *T. brucei* (Facer 1976) leads to decreased blood flow and subsequent stasis in the microvascular bed; thus setting the stage for thrombus formation: as platelets and other formed elements of blood are brought closer to the vessel wall. In the present work, microthrombi were seen in vessels where platelets plugged gaps or adhered to damaged vascular endothelium. This observation is consistent with the fact that the loss of vascular endothelial lining with subsequent exposure of subendothelial structures promotes thrombus formation (Ashford and Freiman 1967).

It is known that activated Hageman factor is involved in the acti-vation of the Kallikrein-Kinin, coagulation, complement and fibrinolytic systems and also that these systems are interelated via the activities of Kallikrein, C1 esterase and plasmin (reviewed by Murano 1978). It is therefore likely that activation of Hageman factor by immune complexes

could account for most of the pathological changes induced by these systems (Boreham 1979b).

Fibrin deposits seen in thrombosed blood vessels in the present work indicate activation of the coagulation system. It is likely that this activation was initiated via thromboplastin released following tissue injury or by activated Hageman factor. There is also activation of the fibrinolytic system via plasminogen activation in rabbits infected with T. $brucei$ (Boreham and Facer 1974a; Facer 1974); resulting in the production of FDP. The biological properties of FDP include causing increased vascular permeability and interfering, with haemostasis by inhibiting the polymerization of fibrinogen by thrombin, platelet aggregation and thromboplastin activity (Triantaphyllopoulos and Triantaphyllopoulos 1970).

Platelets when damaged or stimulated are known to release substances which increase vascular permeability, such as 5HT and histamine. It is also known that platelets play a significant role in the biosynthesis of prostaglandins from arachidonic acid through activation of the phospholipase A_2 in their membranes by agents such as thrombin, collagen and ADP (reviewed by Gingrich and Hoak, 1979). Since some prostaglandins e.g. PGE_1 and PGE_2 cause vasodilation and increased vascular permeability, it would be worthwhile to investigate the role of prostaglandins in the pathogenesis of trypanosomiasis.

The vascular damage described in the present work could lead to tissue necrosis observed in this infection in various tissues (e.g. Cremaster muscle) and subsequent organ dysfunction, as a result of inadequate blood supply. Since most features of T. $brucei$ infections in animals are similar to those found in human trypanosomiasis, it is likely that the vascular changes observed in the present investigation occur in humans. For example, human trypanosomiasis is characterized by inflammatory

lesions in vital organs such as the brain and heart (Ormerod 1970; Hutt
and Wilks 1971). However, care should be taken in extrapolating findings
in laboratory animals to the human situation. It would be necessary to
look at the changes in small blood vessels of humans infected with try-
panosomes at the ultrastructural level; probably along the lines described
in the present work. Skin-snips and renal biopsies could be used for
such studies, as it is difficult to obtain necropsy materials.

The use of anti-inflammatory drugs such as Aspirin and indomethacin
should be considered as adjunct to specific trypanocidal therapy. For
example, Aspirin or Aspirin-like drugs have been shown to inhibit cyclo-
oxygenase, the first enzyme system involved in prostaglandin synthesis
in the platelet. It is also known that Aspirin inhibits the platelet-
release reaction induced by ADP and Collagen (Moncada and Vane 1979).

The changes in the blood during trypanosomiasis are not specific
for the disease, but occur in other inflammatory conditions and parasitic
infections such as malaria. For example D.I.C., increased vascular
permeability, constriction of renal arterial blood vessels and Kallikrein-
Kinin system activation have been reported in malaria infections (reviewed
by Maegraith and Fletcher, 1972).
Moreover, some features of chronic trypanosomiasis are similar to the
Arthus reaction (Goodwin and Hook 1968) and the Schwartzman phenomenon
(Boulton et al 1974).

The cause of death in rabbits infected with T. brucei is not clear.
Goodwin and Guy (1973) suggested renal failure as a contributing cause of
death from trypanosomiasis. There are histological and ultrastructural
evidences for renal damage in rabbits infected with T. brucei (Facer et al
1978). In the present work, light microscopic observation of kidneys
from infected rabbits showed damage to the glomeruli and tubules.

It is known that trypanosomiasis exerts an immunosuppressive effect on the host's response to unrelated antigens; thus exposing the animals to secondary bacterial, viral or helminthic infections, from which the animal rarely recovers (Goodwin 1974). Goodwin *et al* (1972) have shown that rabbits infected with *T. brucei* failed to elicit a proper immune response to an injection of sheep erythrocytes; indicating impairment of the humoral response. The cell mediated response is also affected, as Allt *et al* (1971) found that *T. brucei* infected rabbits failed to develop experimentally induced allergic neuritis; although more recently MacKenzie *et al* (1979) have presented results from trypanosome-infected rats which indicated that there was no suppression of allergic encephalitis as a result of infection.

The structural changes in small blood vessels described in the present study and those of Goodwin and his colleague (Goodwin and Hook 1968; Goodwin 1971); combined with other haemodynamic changes in rabbits infected with *T. brucei* are consistent with cardiovascular shock. It is therefore obvious that circulatory embarrassment, would contribute to events leading to death in rabbit trypanosomiasis.

PART 5.   CONCLUSIONS

1. Rabbits experimentally infected with *T. brucei* show a chronic
   inflammatory response. Small blood vessels viz venules and cap-
   illaries are damaged. The main features of damage include alter-
   ations in vascular endothelium and gap formation.
   The gaps allowed the passage of plasma proteins and formed elements
   of blood into the extravascular compartment. This provides ultra-
   structural evidence for increased vascular permeability, which
   accounts for the inflammatory oedema.

2. Several events are implicated in the pathogenesis of trypanosomiasis.
   The present study supports the hypothesis that the activation of the
   Kallikrein-Kinin system via Hageman factor absorbed by immune com-
   plexes is a major event leading to vascular injury. Other events
   such as complement activation and trypanolysis probably contribute
   to vascular damage.
   The formed elements of blood, particularly leucocytes and platelets
   escalate the vascular damage by clogging the vascular lumina and
   via the substances they release when damaged.

3. Mobilization of leucocytes confirms the inflammatory nature of
   T. brucei infections in rabbits. The predominance of mononuclear
   leucocytes is indicative of a chronic or long-standing inflammation.
   Leucocyte margination on vascular endothelium was mostly by means
   of cytoplasmic processes. It is probable that activation of the
   complement and Kallikrein-Kinin systems provided the chemotactic
   stimuli for leucocyte emigration.

4. Thrombosis of venules occur in rabbits infected with *T. brucei*.
   Several factors, such as immune complexes, vascular stasis and
   exposure of subendothelial components following vascular injury
   initiate the microthrombi formation.

The presence of fibrin strands in the thrombi demonstrates activation
of the coagulation system. Tissue injury may initiate activation of
the coagulation system via the release of thromboplastin.

The occurrence of microthrombi provides one mechanism for the dev-
elopment of thrombocytopenia in rabbit trypanosomiasis.

5. The observed erythrophagocytosis outside the blood vessels shows
    that the anaemia in rabbits infected with *T. brucei* is at least
    partly due to extravascular haemolysis. The presence of erythrocyte
    fragments suggests that the anaemia may be partly microangiopathic
    in origin.

    Clumping of erythrocytes in small blood vessels contributes to the
    increase in whole blood viscosity and together with the increase
    in globulins and fibrinogen known to occur in infected rabbits
    lead to reduced blood flow or stasis. Vascular stasis predisposes
    the blood vessels to thrombosis (4) by bringing the formed elements
    of blood closer to the vessel wall.

6. Vascular damage leads to tissue necrosis as a result of inadequate
    blood supply. This is reflected by the degenerative changes in
    the organs and tissues of rabbits infected with *T. brucei*. This
    could result in malfunction of vital organs, such as the brain and
    the heart.

7. Cardiovascular collapse as a result of vascular damage and haemo-
    dynamic alterations is an important event contributing to death in
    rabbits infected with *T. brucei*.

APPENDIX

RESIN FORMULATIONS USED FOR ELECTRON MICROSCOPY.

A.   TAAB 812 RESIN (Recommended by Taab. Lab. Reading)

Taab 812 Resin   -   48g

DDSA   -   19g

MNA   -   33g

DMP - 30   -   2g

B.   EPON 812 (Recommended by EMScope Labs. Ltd. London)

Epon 812   -   225ml

DDSA   -   100ml

MNA   -   175ml

BDMA (equivalent to DMP-30)   -   75ml

C.   Formulation recommended by Dr. J.G. Simpson (personal comm.)

Epon 812   -   20ml

DDSA   -   15ml

MNA   -   10ml

DMP - 30   -   20 drops.

D.   Recipe calculated for Epon 812 supplied by Taab Laboratories, Reading.

Epon 812   -   25.31g

DDSA   -   20.19g

MNA   -   4.50g

DMP - 30   -   .625g.

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