COMPARATIVE SUSCEPTIBILITY OF EAST AFRICAN NON-HUMAN PRIMATES-VERVETS (*Cercopithecus aethiops*) AND SYKES (*C. mitis*) MONKEYS TO EXPERIMENTAL INFECTION WITH *LEISHMANIA DONOVANI*.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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This thesis has been submitted for examination with my approval as the University Supervisor.

28/8/88

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THE HOLY KORAN TEACHES:

"DO NOT COVER THE TRUTH WITH FALSEHOOD
AND DO NOT CONCEAL THE TRUTH WHEN YOU
KNOW IT"

THIS THESIS IS DEDICATED TO MY FATHER, AMIR BINHAZIM AND MY MOTHER, KURSUM AMIR WHO IMPRESSED UPON ME THE NEED TO BE PATIENT AND THE URGENCY TO BE KNOWLEGEABLE AND EDUCATED.

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ABSTRACT

Four syke (*Cercopithecus mitis*) and 4 vervet (*C. aethiops*) monkeys were inoculated with stationary phase culture derived promastigotes of *Leishmania donovani* strain NLB065K via the femoral vein at a dose of $2x10^7$ promastigotes per Kg body weight.

A complete haematological analysis, serum biochemistry determinations (alanine and aspertate aminotransferases, glucose, alkaline phosphatase and albumin/globulin ratio), examination of histopathological sections of liver biopsies, organ aspirates from the liver, spleen and inguinal lymph nodes for parasite culture and aspirate smears for parasite quantitation were done at fortnight intervals post infection to monitor the progress of the disease in both species

The disease produced mortality of 3 vervet and 2 syke monkeys at various intervals post infection. Following intravenous inoculation of the parasite, all animals developed granulomatous nodules of the liver, consisting of histiocytes, lymphocytes and plasma cells. In addition, one syke (Syk 11 showed giant cell formation during late stages of infection. This mononuclear cell infiltration in the liver resulted in destruction of lobular pattern and a disarray of hepatic cord Animals that died during the course of infection had hepatosplenomegaly and histopathological sections of the spleen showed a mononuclear cell infiltration of the red pulp sinuses and erythrophagocytosis.

There was leucopaenia and anaemia of varying degrees and severity in both monkey species. Both species had increased levels of Alkaline phosphatase, hypoalbuminaemia

and hyperglobulinaemia of varying severity. All dead animals were parasite positive as determined by cultures and smears from the liver, spleen, inguinal lymph nodes and bone marrow. Also there was uniform visceralization and parasite dissemination. In addition, one vervet (Ver 99) had a high parasite grade of 4+ from the liver and spleen respectively at day 140 post infection. Similarly one syke (Syk 198) had parasite grades of 5+ and 4+ from the liver and spleen respectively at day 158 post infection.

Surving animals displayed a self-cure phenomenon and were parasitologically negative by day 154 post infection. The disease pattern in these 2 species of monkeys was similar and had a similarity to the human cryptic or sub-clinical infections.

From this study, it was concluded that both syke and vervet monkeys could be used as a primate model in the study of the various aspects of visceral leishmaniasis as well as being used in the screening of new anti-leishmanial drugs. In addition, these 2 monkey species could also be used in studying the mechanisms behind the self-cure phenomenon which has been observed to occur in human patients suffering from visceral leishmaniasis in Eastern Africa.

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1. INTRODUCTION.

Visceral leishmaniasis is an infectious disease naturally occurring in humans, the domestic dog, and some species of rodents. The disease is caused by the intracellular protozoan parasite, *Leishmania donovani* and is characterised by a chronic progressive course, intermittent fever, enlargement of the spleen and sometimes the liver, emaciation, anaemia, leucopenia and hypergammaglobulinaemia. The disease is transmitted to man and animals by blood sucking sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World.

Visceral leishmaniasis is distributed widely geographically, and major endemic areas include South America, Africa, Asia, and the Mediterranean basin. Rodents are extensively used as experimental models for the human disease. They have been used for immunological studies, (Clinton *et al.*, 1969); evaluation of chemotherapeutic agents, (Hanson, 1977; 1978); pathological studies and a variety of other studies involving *L. donovani* (Grun and Stauber, 1958; Stauber *et al.*, 1958; Bradley *et al.*, 1973).

Laboratory animals such as non-human primates have been used to a limited extent. A variety of studies have been conducted utilising these species of animals to determine susceptibility to experimental infection. In this respect, both Old and New World non-human primates have been used. The species in which studies have been carried out are:- *Macacus rhesus* (Shortt, 1923); *Macacus*

sinicus (Korke, 1927), Cercopithecus aethiops (Kirk, 1945; 1956; Manson-Bahr and Wilson, 1976); Galago senegalensis senegalensis (Sati, 1963); the owl monkey, Aotus trivirgatus (Chapman et al., 1981b). However, only one of these C. aethiops is indigenous to Eastern Africa. In this species, the experimental infection produced a muco-cutaneous disease, which is not produced by L. donovani under natural challenge (Kirk, 1945).

The use of non-human primates in exploiting the fields of immunology, chemotherapy and clinico-pathological studies have been done to a limited extent. However, both the squirrel Saimiri scuirea (Madindou, 1982) and the owl monkey (Chapman et al., 1983) have recently been utilised in antileishmanial chemotherapy studies. It is essential that a suitable experimental model be established for various aspects of studies related to leishmaniasis. Although the golden hamster (Mesocricetus auratus) is generally regarded to be the most suitable laboratory host, (Sati, 1963) due to their small size, it is not possible to sample repeatedly and serially from the same animal. Therefore, the need to identify other suitable hosts is obvious. The development of suitable non-human primate models of visceral leishmaniasis would be desirable because of the phylogenetic closeness to humans. Studies in chemotherapy and other related areas involving higher species of mammals such as non-human primates would have greater validity relative to the problems in humans. Non-human primates are man's

closest relatives in the animal kingdom and are therefore indispensable in the effort to understand and control problems of human health.

In Kenya, several species of non-human primates exist naturally, and can be utilised as experimental models for Kenyan visceral leishmaniasis. In this project, the sykes (Cercopithecus mitis) and vervet (C. aethiops) monkeys are the designated experimental animals.

1.1. OBJECTIVES OF THE STUDY.

(i) To study the clinicopathological response of sykes (Cercopithecus mitis) and vervet (C. aethiops) monkeys to experimental L. donovani infection.

(ii) To determine how suitable these two monkey species are to experimental infection with L. donovani.

(iii) To compare the course of the disease between the two species.

2. LITERATURE REVIEW.

2.1. THE PARASITE.

2.1.1. Morphology and Taxonomy.

The Leishmaniases are diseases caused by protozoan parasites belonging to the genus Leishmania; family Trypanosomatidae order Kinetoplastida. The family, Trypanosomatidae has eight recognized genera, of which two are known to include species pathogenic for man and animals; these are the genus Leishmania and Trypanosoma. Members of the genus Leishmania complete their life cycles in two different hosts, a vertebrate host and an insect vector (McGhee and Cosgrove, 1980).

The various species of Leishmania are considered morphologically indistinguishable (Heyneman, 1971; Manson-Bahr, 1971; Schnur et al., 1973). However, minor size differences have been described for some species of Leishmania (Shaw and Lainson, 1976). They noted that amastigotes of Leishmania mexicana amazonensis are larger than those of Leishmania braziliensis braziliensis. Apart from size differences, differences in the diameter of kinetoplast and position of the kinetoplast were also noted (Shaw and Lainson, 1976).

In man and other vertebrate hosts, Leishmania occur as obligate intracellular parasites known as amastigotes. These multiply in the macrophages of the skin and viscera, and other phagocytic cells of the reticulo-endothelial system. The protozoan is injected into a vertebrate

host by sandflies as promastigotes, and become amastigotes in the mononuclear cells of the vertebrate host. These amastigotes are round, oval bodies that measure about 1.5 - 3 um wide by 3.0 - 6.5 um long, varying from one species to another (Lainson and Shaw, 1978).

The amastigotes are highly distinctive. It is usually an ovoid body having one blunt and one more attenuated end, but the shape varies considerably. It has a distinct round nucleus. The kinetoplast is a rod-shaped body, often lying at right angles to the nucleus. They have no free flagellum, although a rudimentary one has been described in the flagellar pocket, a small invagination of the parasite's surface (Garnham, 1971; Campo-Aasen, et al., 1973; Cheng, 1973; Shaw and Lainson, 1976).

The morphology of the promastigote varies according to the stage of growth in the fly. The body elongates to about 20 um and this stage has a free flagellum, thus distinguishing it from the amastigote stage (Garnham, 1971). Within the sandflies, the resulting promastigotes undergo extensive longitudinal binary fission. They vary from 16.0 to 40.0 um long, by 1.5 - 3.0 um wide. The flagellum of Leishmania species is an organ of attachment as well as for locomotion (Adler, 1964). Adler and Adler (1955) considered that the development of the flagellum takes place in 3 stages, the protrusion which attains a

length of 1 - 2 um beyond the anterior end of the organism; growth of this protrusion till it attains it's maximum size; and the appearance of waves along the whole length of the flagellum.

The kinetoplast is considered unique to the protozoa of the order Kinetoplastida. From electron microscopic studies, the kinetoplast is described as a specialised part of the mitochondrion containing a mass or band of DNA, the kinetoplast nucleoid. It is an enlarged region of the parasite's single branching mitochondrion (Simpson, 1973; Wallace et al., 1972). Reproduction of the parasite is by binary fission (Lainson and Shaw, 1978).

The taxonomy of the named species of *Leishmania* that are known to infect man:

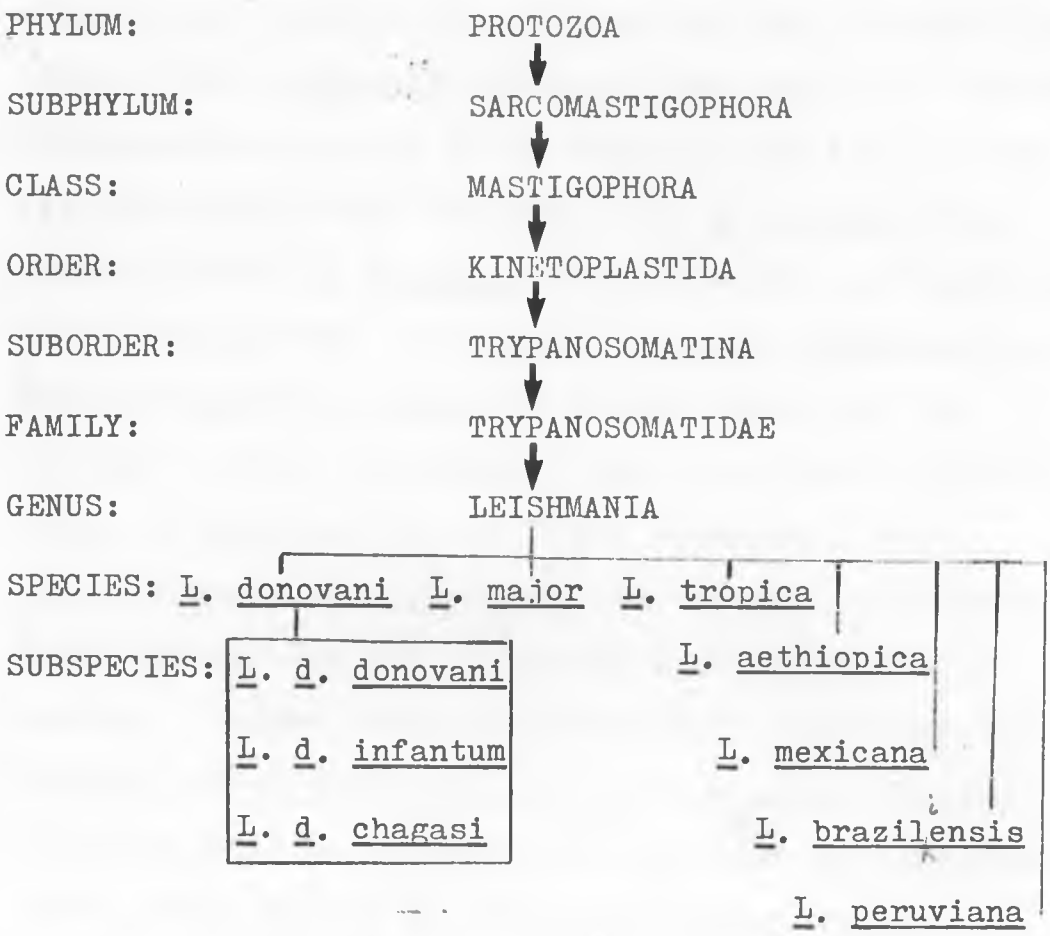


Fig. 1: A simplified diagram of the classification of *Leishmania donovani* and related *Leishmania* (modified after W.H.O. Technical report series 701, 1984).

2.1.2. Differentiation of strains and species.

Leishmania species have so far been recorded from mammals and lizards; none have as yet been recorded from birds. The Leishmania species of man were until recently differentiated mainly on the basis of the type of case from which they were isolated, e.g. L. tropica from oriental sore, L. donovani from Kala-azar, and partly on their geographical distribution, e.g. L. infantum from Mediterranean Kala-azar. It is now clear that the clinical picture is a useful, but by no means infallible guide to diagnosis (Adler, 1964; Zuckerman, 1975). Tissue tropisms of Leishmania in standard laboratory model systems may aid in distinguishing Leishmanial strains. Indeed, human leishmaniases are divided into disease groups (cutaneous, mucocutaneous and visceral leishmaniasis) on the basis of such tropisms (Zuckerman, 1975). What causes the different tissue tropisms is not clear.

Rates of growth, in vivo and in vitro, may differentiate Leishmanial strains from one another. Thus, a metastasizing strain, developing slowly in hamsters and scantily in vitro, was isolated in South America from nasopharyngeal lesions in man; while a strain developing rapidly in hamsters and floridly in vitro was isolated from cutaneous lesions (Lainson and Shaw, 1972). There are no morphological properties that clearly differentiate human Leishmanial strains

from one another by light microscopy. It had been hoped that electron microscopy might reveal such characters, but this has not occurred. With few exceptions, strains studied to date, have proved uniform in ultrastructure (Molyneux, 1973; Zuckerman, 1975). Recently however, differences have been established by electron microscopy and by techniques of biochemical taxonomy (Chance, 1981). This recent work demonstrates that L. tropica-like organisms can give rise to visceral disease (Chance et al., 1978; Schnur et al., 1981).

One approach to the differentiation of strains of Leishmania is serological typing. While using the Noguchi-Adler test, which is essentially an agglutination test, it was possible to show that a strain of Leishmania recovered from Phlebotomus martini in Kenya was indeed a Kenyan strain of L. donovani (Wertheim et al., 1970). Adler et al.(1966) showed that organisms recovered from a rodent viscera and human skin in the Sudan were all antigenically identical to L. tropica isolated from oriental sore in human beings. It has been reported that no antigenic variation occurred in strains kept for 4 months in culture or in hamsters by using the indirect haemagglutination test (Bray and Bryceson, 1969). While examining the antigenic constitution of 9 strains of L. tropica by the indirect haemagglutination test and cross-absorption of rabbit antisera by the strain antigens, Bray and Bryceson (1969) demonstrated that

strains of L. tropica from Ethiopia and Iran were distinct from strains from Iraq and lacked antigens possed by Iraq strains. Strains of Leishmania recovered from cutaneous leishmaniasis of human beings in Ethiopia were different from Ethiopian L. donovani (Bray and Bryceson, 1969; Bryceson, 1970).

Differentiation of species and subspecies has been based on, epigeographic features (Manson-Bahr, 1971; Barbosa et al., 1976), nuclear and kinetoplast dioxyribonucleic acid (kDNA) buoyant densities (Chance et al., 1974; 1978), serotypes and electrophoretic mobility of various enzymes (Zuckerman, 1975; Schnur et al., 1972; 1981).

At present, the methods used in the identification and classification of members of the genus Leishmania are based on the characters in Table 1.

Table 1: The biological, immunological, and biochemical characters used in the identification of Leishmania. (Modified after WHO technical report series 701 1984).

	Characters
Biological	Development in sandflies
	Virulence of clones in rodents
Immunological	Noguchi - Adler test
	Excreted factor serotyping
	Monoclonal antibodies
	In vivo cross-immunity test.

Biochemical	DNA sequence analysis DNA (nuclear) buoyant density DNA - RNA hydridization Restriction - endonuclease fragment analysis. Isoenzyme characterization Cell membrane structure Fatty acid analysis Radiorespirometry.

At the present time, by far the most widely used method for Leishmania characterization is the analysis of isoenzymes by electrophoresis. However, it is too early to say which of the methods available will prove the most useful. Newer and simpler techniques are being devised, and many characters are currently being examined for criteria of potential taxonomic value. The recognition that these identification procedures are of great value has created an urgent demand for typing of various isolates (W.H.O. technical report series, 701, 1984).

2.2. Hosts:

All known vertebrate hosts of Leishmania species are primarily confined to mammals, although some species of Leishmania are found in reptiles (Adler, 1964). No infections have yet been recorded in nature in any other vertebrate groups. There is usually a well balanced host-parasite relationship in the natural vertebrate

host, and the parasites are scattered in small numbers, in macrophages of the skin, viscera or blood, where they produce few or no pathological effects. However, there may be violent host-cell reaction to the parasite which results in skin lesions (cutaneous and mucocutaneous leishmaniasis) or severe pathological changes in the internal organs (Kala-azar and other forms of visceral leishmaniasis) (Lainson and Shaw, 1978).

2.3. Vectors:

All known vectors of the Leishmaniae are phlebotomine sandflies (Diptera: Psychodidae: Phlebotominae) which have a wide distribution in tropical and subtropical regions of both Eastern and Western hemispheres. Visceral leishmaniasis is transmitted in the Old World by the genus Phlebotomus and Lutzomyia in the New World.

When the female sandfly feeds on an infected host, amastigotes are taken up from the skin or peripheral blood. Within the first 72 hours, the amastigotes elongate, within the stomach contents, and the rudimentary flagellum grows out into a long whip-like structure. The resulting promastigotes undergo extensive longitudinal binary fission and vary in size from 16.0 to 40.0 um long, by 1.5 to 3.0 um wide. In the life cycle of most Leishmaniae, the parasites eventually migrate to the anterior parts of the sandfly gut, where they become attached in large numbers to the oesophageal wall, by the flagellae. The small free forms migrate further to

the pharynx and proboscis, with ultimate inoculation of some promastigotes into the skin of a further vertebrate host when the sandfly takes another blood meal. The time taken for the parasite to undergo its complete cycle in the insect vector depends on both the species of Leishmania involved, and the local environmental conditions (e.g. temperature). It may be transmitted in as little as 4 days after the infective blood meal (subspecies of L. mexicana), or only after 3 consecutive blood meals (L. infantum) (Lainson and Shaw, 1978).

In Kenya, Heisch et al. (1956) recorded 17 species of sandflies from the Kitui district and out of these, eight species are known to bite man. Although Phlebotomus garnhami was at first considered the most likely vector of Leishmaniasis in Kitui area, the infection rate was found to be rather low for a potential vector. The role of Phlebotomine sandflies as vectors of visceral leishmaniasis in Kenya has been investigated by several workers (Heisch et al., 1956; Wijers and Minter, 1962; Manson-Bahr et al., 1963; Mutinga and Ngoka, 1978) and they incriminated the vectors of visceral leishmaniasis in Kenya to be Phlebotomus martini, Phlebotomus vansomerinae and Phlebotomus celiae. It was evident that P. martini was the major vector, due to its presence in all endemic areas of Kenya (Wijers and Minter, 1962; Mutinga and Ngoka, 1978). In addition, there is probable evidence that Sergentomyia

bedfordi may also be involved in the transmission of Kala-azar in Machakos district of Kenya, although this fact remains to be confirmed by further experiments (Mutinga, 1981).

2.4. Reservoirs.

The ecological system in which the parasite population is maintained indefinitely may be regarded as a reservoir of Leishmania species. This system is normally composed of one or a small number of sandfly vector species and one or a small number of vertebrates the reservoir hosts.

Wild as well as domestic mammals have been proved to be reservoirs of Kala-azar and oriental sore in many parts of the world (Hoare, 1962; Lainson and Strangways, 1962; Ngoka and Mutinga, 1977).

In Kenya, work on animal reservoirs has been carried out extensively in various areas where visceral leishmaniasis is endemic (Heish, 1957; 1963; Mutinga et al., 1980). The dog is the traditionally zoonotic host in the Mediterranean, but recent investigations have shown canine infection to be more widely spread in Africa. In endemic areas, infection in dogs is more widespread than in man, and in some areas exists in the abscence of any human infection as in Senegal (Manson-Bahr and Apted, 1982). L. donovani has been isolated from dogs in Kenya as well as Senegal (Mutinga et al., 1980).

In addition, there has been occasional findings of L. donovani parasites in foxes, Vulpes vulpes from France, Italy, Iran and the Sudan (Kirk, 1956; Bettini et al., 1980; Manson-Bahr and Apted, 1982). The position of rodents as reservoirs of L. donovani has undergone considerable clarification as a result of the identification techniques described by Chance et al., 1978). Proven human strains of L. donovani have been isolated from black ra (Rattus rattus), spiny mice (Acomys albigenes), and unstriped grass rats (Arvicanthis nilotious) in the Sudan, which are identical with those isolated from P.orientalis, cases of Kala-azar and cutaneous lesions in American personnel (Hoogstraal et al.,I963; Hoogstraal and Heyneman, I969; Chance et al., I978).

In Kenya, there is also a widespread of Leishmanial parasites in rodents such as Mastomys rat (Mastomys), tatera gerbils (Tatera),and ground squirrels (Xerus) (Heisch, I963). However, this Leishmanial parasite is not the same as the one that causes Kalar-azar but is identica with the parasite that has been isolated from cutaneous lesions in Senegal, Sudan and Ethiopia (Chance et al.,I97

Nevertheless, all these animals have not been studied sufficiently to indicate that any of them constitutes the primary reservoir from which the secondary, synanthropic, canine reservoir could have developed (W.H.O. technical report series 701, 1984).

2.5. Host-parasite relationship.

There is limited information concerning the host-parasite relationship in both man and animals. Little

is known of the events that follow the inoculation of promastigotes, which may be critical to the eventual outcome of the infection. Presumably, many infections in man terminate subclinically, but nothing is known of the mechanisms that might be involved. Most of the work done are *in vitro* experiments (Akiyama and Taylor, 1970; Chang and Dwyer, 1976; Pearson *et al.*, 1981).

Infections in experimental animals and *in vitro* experiments however suggest the existence of several mechanisms that permit parasite survival and multiplication. Within the host macrophages, Leishmanial amastigotes are protected from antibodies and any other circulating substances that might be harmful to the parasite (Pearson *et al.*, 1981). *In vitro* experiments suggest that *L. donovani* are taken up by simple phagocytosis, and that the phagocytic activity of hamster macrophages is the primary agency leading to the ultimate intracellular entry of promastigotes (Chang and Dawyer, 1976; Chang, 1979). It has been demonstrated that phagosomes containing parasites, fuse with lysosomes, and the parasite not only survives but multiply within phagosome-lysosome vacuoles of hamster macrophages.

In vitro phagocytosis of *Leishmania* in macrophages induces increased oxygen uptake, and generates toxic oxygen metabolites such as H_2O_2. Promastigotes are susceptible to such components and, *in vitro*, they are presumably destroyed by these metabolites in macrophages.

Little is therefore known of the characteristics of the parasite that determine infectivity, virulence, dermotropism or viscerotropism, and the capacity to survive in man.

2.5.1. Immune response.

Leishmanial infection in man induces both humoral and cellular immune response but the balance of their expression varies with the type of disease. In the course of visceral leishmaniasis in man, the immunoglobulin titer rises very sharply, chiefly in the IgG compartment (Bray and Wilson, 1972; Zuckerman, 1975). Gamma globulins may reach 50 g/litre and may comprise 50% of the total serum proteins (Stauber, 1970). Levels of specific antibody and of polyclonal IgG and IgM are high, complement is activated, and immune-complexes involving immunoglobulin classes G, M, and A are formed in the serum. Cellular responses to tuberculin and other unrelated antigens are suppressed or absent (Zukerman, 1975). Patients who have recovered from infection are normally considered immune to reinfection with L. donovani, but relapse may be indistinguishable from reinfection (W.H.O. technical report series 701, 1984).

2.6. Geographical distribution.

It is not easy as such to plot accurately the present global distribution of visceral leishmaniasis and its prevalence in man. In addition, there are no

reliable data on prevalence and incidence. The disease occurs World wide and has so far been recorded in Africa, Asia, South America, the Mediterranean region and Europe; but so far not recorded in Australia, U.S.A. and some eastern European countries.

The main endemic areas of Kala-azar in East Africa are located in the Sudan, Kenya and Ethiopia. In Kenya, the main endemic foci are the Machakos, Kitui, Meru, West Pokot and the Baringo districts. These areas have suffered epidemics since the 2nd world war. In 1977-78 and 1980, more than 2,000 cases each were recorded in the Kitui and Machakos districts (W.H.O. technical report, series 701, 1984).

2.7. Clinical signs.

The incubation period ranges from 10 days to over one year. However, the Kenyan form has an estimated incubation period which lasts from 10 weeks to a maximum of 30 weeks. In an experimental infection, it varied from 4 to 8 months. However, considerable variation exists from one country to another (Manson-Bahr and Apted, 1982). In East Africa and India, the peak age is 5 - 9 years, but the disease occurs also among teenagers (W.H.O. technical report, series 701, 1984).

The common clinical signs are fever, marked splenomegaly, moderate hepatomegaly, lymphadenopathy, emaciation, anaemia, and pallor of mucus membranes. The onset of fever may be gradual or sudden, and is

usually intermittent. Hepatocellular jaundice can be observed in 10% of the cases. Signs of malnutrition such as oedema, skin and hair changes develop. Secondary infections such as pneumonia, dysentry and pulmonary tuberculosis are common.

Occasionally, post-Kala-azar dermal leishmaniasis occurs in East Africa. In addition, subclinical cases are thought to outnumber the clinical cases in countries like Kenya (Manson-Bahr, 1967; Leeuwenburg et al., 1983) and Italy (Pampiglione et al., 1974b).

2.8. Pathology.

In the vertebrate host, Leishmania occur as intracellular parasites known as amastigotes that multiply in macrophages and other phagocytic cells of the reticuloendothelial system (R.E.S.). At the site of inoculation with promastigotes, a small granuloma is formed consisting of histiocytes filled with amastigotes, and surrounding this are epithelioid and later giant cells (Manson-Bahr, 1959). Eventually, through the circulating lymph or peripheral blood, the parasites are transported to the various viscera of the body.

The parasites are then taken up by the reticuloendothelial cells in the spleen, liver, bone marrow and lymph nodes. In these organs, the parasite either multiplies or is killed depending upon the host response. If the response is minimal, the Kala-azar syndrome results; if it is maximal, inapparent

infections occur. Splenomegaly is observed in cases of visceral leishmaniasis and the enlargement is due to reticuloendothelial hypertrophy and a considerable amount of pulp is composed of amastigotes.

In addition, plasma cells are numerous. In some cases, granulomatous nodules of the Gandy Gamna type may be found with fewer parasites, and in the unusual cases, there may be extensive haemorrhagic necrosis (Pampiglione et al., 1974b; W.H.O. technical report series 701, 1984).

Involvement of the liver results to hepatomegaly and in inapparent infections, sarcoid-like granulomatous nodules are scattered throughout the liver. In the Kala-azar syndrome, the portal tracts contain Kupffer cells packed with amastigotes (Manson-Bahr, and Apted 1982). The liver function is normal and later prothrombin production decreases. Together with thrombocytopaenia, the prothrombin depletion may result in severe mucosal haemorrhage. Unusual cases reported from Italy described two types of lesions; extensive areas of haemorrhagic necrosis and the formation of granulomatous nodules (Pampiglione et al., 1974a, 1974b).

The lymphatic glands may be enlarged and contain numerous histiocytes filled with amastigotes. Oral, nasopharyngeal and retropharyngeal lesions have been described from the Sudan (Abdalla et al., 1975) and from India (Naik et al., 1978). Parasites are

usually more abundant in the oral form and amastigotes may be found in nasal and pharyngeal secretions.

Glomerular involvement in cases of Kala-azar has been described. The kidneys may show amyloidosis as described by Brito et al. (1975). The bone marrow is reddish and contain abundant amastigotes. The lungs show no parasites although, secondary infections are common as a result of the leucopenia (Manson-Bahr and Apted 1982).

2.9. Diagnosis and Treatment.

On the basis of clinical signs and symptoms, it is impossible to differentiate visceral leishmaniasis from other causes of febrile splenomegaly. Diagnosis should therefore be confirmed parasitologically.

Spleen puncture is the surest method of diagnosis, although the complications of splenic aspiration such as intraabdominal bleeding and splenic tear and rupture should not be overlooked.

After aspiration, the contents of the needle are blown on to a clean slide and a smear made. Alternatively, using sterile technique, the contents may be blown on to culture media for parasite culture. The media in use currently is Schneider's Drosophila medium (Githure et al., 1984). Liver puncture is nearly as good as spleen to demonstrate parasites.

When Leishmania are very scanty, they may be demonstrated by inoculating the material aspirated intraperitoneally into a hamster. After six months,

the hamster is sacrificed and impression smears from spleen and liver are examined for presence of amastigotes.

Serological tests have also been used for diagnostic purposes. Those that have been used in Kenya are formol gel test, compliment fixation test and the enzyme linked immunosorbent assay (ELISA) (Ho, et al., 1983). The formol gel test has the disadvantage of becoming positive late in the course of the disease and of being highly non-specific in areas where many other conditions are associated with splenomegaly and hypergammaglobulinaemia. Whereas the complement-fixation test is insensitive, it is inconvenient for handling mass samples, and has up to 20% uninterpretable results due to anti-complementary sera. However, the ELISA when evaluated as a diagnostic test for visceral leishmaniasis in the field was shown to have a sensitivity of 98.4% and a specificity of 100% when compared with parasite identification by splenic aspiration. It is therefore an accurate, safe and economical alternative to splenic aspiration for field diagnosis of Kala-azar.

2.9.1. Treatment.

Sodium stibogluconate (Pentostam(R) Burroughs, Wellcome) is the drug of choice in Kala-azar when a 30% solution of sodium stibogluconate containing 100 mg/ml is used. The dose used is 0.1 ml/kg daily by intramuscular injection, and therefore a maximum of 6 ml and a minimum of 2 ml for at least 30 days should be administered (Rees. et al., 1984).

Other drugs in use are Ethyl stibamine, Urea stibamine and Meglumine antimoniate. In cases whereby resistance to antimony develops, then diamidine drugs should be tried (Manson-Bahr. and Apted 1982). The drugs used for relapsed and unresponsive patients are pentamidine, amphotericin B, and allopurinol, but their use is strictly limited to patients who are unresponsive to antimonials (W.H.O. technical report, series 701, 1984).

2.10. Leishmaniasis of non-human Primates.

There has been continued efforts made to identify non rodent hosts with potential for use in experimental leishmaniasis. Due to the phylogenetic closeness of the non-human primates to humans, they appear attractive models for determining their potential susceptibility to experimental visceral leishmaniasis. To this effect, both New and Old World non-human primates have been utilized for various experiments.

Several New World primates have been identified as being highly susceptible to experimental L. donovani infections. Utilizing the owl monkey (Aotus trivirgatus and the squirrel monkey (Saimiri sciurea). Chapman and his co workers (1981a; 1981b) showed that fulminating visceral leishmaniasis developed following the intravenous injection of L. donovani amastigotes. These two species of New World primates appear to be potential candidates for use in further studies such

as immunization, immunopathology and chemotherapy trials in Leishmania infections. Both these species have since been utilized in antileishmanial chemotherapy studies (Madindou, 1982; Chapman et al., 1983).

No attempt has been made to identify Old World primates as potential models of the disease, and no recent studies have been conducted on the potential susceptibility of Old World primates to experimental infection with L. donovani. The studies that have been done in the past appear to have been limited to Macaques, Macaca rhesus (Shortt, 1923; Greig and Christophers, 1925) and M. irus (Meleney, 1925; Smith, 1933). In addition, the vervet monkey Cercopithecus aethiops has been described as susceptible (Archibald and Mansour, 1937; Kirk, 1942; 1945). Kirk (1945) reported an espundia-like lesion in a monkey (C. aethiops) inoculated intraperitoneally with material from a case of Sudanese visceral leishmaniasis. In addition, Kirk (1956), in an attempt to find a reservoir host of leishmaniasis in the Sudan, managed to identify a natural infection in one monkey (C. aethiops).

The only other Old World primate that has been used in susceptibility studies has been the bush baby (Galago senegalensis senegalensis). In search for new experimental animals as hosts for the study of Leishmaniasis, Sati (1963) described the bush baby to be more susceptible than the hamster to experimental L. donovani infection, and stressed its potential

usefulness as an experimental animal in the tropics.

However only one of these C. aethiops is indegeneous to Eastern Africa and Kenya. In this species, the experimental infection produced espundia, a condition that is not produced by Leishmania donovani (Kirk, 1945).

3. MATERIALS AND METHODS.

3.1. Experimental animals.

A total of 12 animals were utilized for this study; 6 sykes and 6 vervet monkeys. Two animals per species were allocated as controls. All the animals were wild caught and were aged as adults. The weights of the individual animals are shown in Table 1.

Prior to the study, these animals were subjected to routine screening procedures used at the Institute of Primate Research as recommended by the Committee on care and use of laboratory animals (1978). These animals were placed under; quarantine for a period of 3 months in separate cages, pre-experimental acclimatization, a complete clinical examination, a routine haematology, a check for blood parasites and a faecal parasite check. In addition, a Tuberculin test, and for this particular purpose, a Leishmanin skin test were also done (Githure *et al*. in press).

3.2. The parasite.

The organism used was originally isolated by a splenic aspirate from a human patient on 28th January, 1982. It had been identified as *Leishmania donovani* by isoenzyme analysis using malic enzyme and glucose phosphate isomerase as criterion (Kreutzer and Christensen, 1980; Kreutzer *et al*., 1983).

The strain used was designated Nairobi *Leishmania* Bank 065 (NLB 065) and has since been maintained by

passage through male golden hamsters (Mesocricetus auratus) using spleen homogenates. At the time when it was used to infect the primates used in this study, it had been passaged eleven times through golden hamsters and was therefore designated NLB 065 K.

Splenic aspirates from an infected hamster spleen were inoculated into 20 tissue culture flasks (25 cm^2) each containing 5 ml of Schneiders Drosophila Medium supplemented with 20% (v/v) Foetal calf serum. The parasite growth was monitored after every 24 hours by performing parasite counts using a standard haemocytometer (American Optical Co. N.Y. U.S.A.). Stationary phase promastigotes were harvested by centrifuging the contents of all the 20 flasks at 44 g (Beckman Inst. Inc. CA, U.S.A.). The supernatant was discarded and 9 ml of fresh media was then added to the centrifuge tube. The parasites were then adjusted to the required inoculum dose. The parasite dose for each animal is presented in Table 1. Each animal received 2 x 10^7 promastigotes/Kg body weight.

Table 2. Animal weight, parasite dose and route of infection used in vervet and syke monkeys experimentally infected with L. donovani

Animal Number	Body Weight (Kg)	Species	Total Parasite dose	Route of infection	Controls
198	7.5		1.4×10^8	Intravenously	All
116	3.9		0.7×10^8	via the	Controls
205	8.5	Sykes	1.6×10^8	femoral vein	received
214	6.7		1.4×10^8		1ml.of
86	8.6		Control		parasite
3	4.3		Control		free
					media
609	3.3		0.5×10^8	Intravenously	All
99	3.6		0.6×10^8	via the	Controls
137	3.6	Vervets	0.75×10^8	femoral vein	received
187	5.5		1.1×10^8		1 ml of
695	4.4		Control		parasite
673	4.7		Control		free
					media

3.3. Sampling.

All samplings from the animals were done after every 2 weeks as from the date of infection. However, base line data were obtained from the monkeys for 3 consequtive days prior to infection. A total of 17 samplings post infection (p.i.) were done.

At each interval p.i., sampling was done for haematology, serum biochemistry, organ aspirates for parasite culture, parasite density estimations and liver biopsies for histopathology.

Prior to each sampling, the animals were immobilised by a mixture of 700 mg ketamine hydrochloride (VETALAR(R) Parke-Davis, N.J. U.S.A.) and 60 mg Xylazine (Rompun (R) Bayer, Leverkusen, W. Germany).

Each animal was injected intramascularly with 1 ml of the mixture. The animals were then weighed and shaved at the sites from where samples were to be collected. One animal at a time was then placed on a table lying on it's back and all the four limbs secured to the table. The animal was then bled from the femoral vein and anticoagulated blood using dipotassium ethylenediamine tetraacetic acid (EDTA) was collected for haematology. The sites where organ aspirates were collected from were shaved and thoroughly disinfected using a 5% hibitane solution (Imperial Chemical Industries Ltd., Cheshire, England). The monkeys were fasted overnight prior to a next morning sampling.

3.4. Haematology.

All blood samples for haematological analysis were collected fortnightly. Using a 10 ml syringe and a 21 gauge 1½" needle, blood was collected from the femoral vein and 1 ml was immediately transfered into tubes containing dried dipotassium ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant at a concentration of 2.5 mg/ml of blood. About 9 ml of whole blood was collected from each animal for serum biochemical analyses. This blood was centrifuged at room temperature for 20 min. at 832g. (Beckman centrifuge, Beckman Inst. Geneva, Switzerland).

The serum was collected using a pasteur pipette and transferred into clean vials. It was used for the determination of glucose, alkaline phosphatase, serum aspartate aminotransferase (ASAT), serum alanine aminotransferase (ALAT) and Albumin-Globulin ratio (A/G).

3.4.1. Estimation of blood values.

A complete blood count was performed on every sample and comprised of Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell Counts (RBC), Total leucocyte counts, a differential leucocyte counts, total protein and fibrinogen. In addition the following values were obtained as per the following calculations (Schalm et al., 1975).

(i) Mean Corpuscular Volume (MCV)

$$MCV = \frac{PCV \times 10}{R.B.C.}$$

(ii) Mean Corpuscular haemoglobin (MCH)

$$MCH = \frac{Hb \times 10}{R.B.C.}$$

(iii) Mean Corpuscular haemoglobin concentration (MCHC)

$$MCHC = \frac{Hb \times 100}{P.C.V.}$$

3.4.2. Haemoglobin estimation.

This was determined using a Haemoglobinometer (Coulter Electronics Ltd. Herts, England). This method uses zap-o-globulin as a lysing agent and the results read in grammes percent.

3.4.3. Packed Cell Volume (P.C.V.).

Unheparinized microhaematocrit capillary tubes (VWR Scientific Inc. CA, U.S.A.) were used. The tubes were filled by capillary action upto $\frac{3}{4}$ full. The dry ends were then sealed using critoseal, a specially formulated vinyl plastic putty for sealing microhaematocrit tubes (Lancer, Brunswick Co. St, Louis, U.S.A.). The tubes were then centrifuged for 5 min. at 1872 g. in a microhaematocrit centrifuge (Heraeus-christ Gmbh, W. Germany). The percentage P.C.V. was determined from the scale of a microhaematocrit reader (Hawksley & Sons Ltd., England).

3.4.4. Red Blood Cell Count (R.B.C.).

The total RBC counts were obtained using a Coulter Counter Model DN (Coulter Electronics Ltd. Herts, England). EDTA blood was used and this was previously thoroughly mixed using a Coulter mixer (Coultronics France).

3.4.5. Total Leucocyte Count (W.B.C.).

The counts were determined using a Coulter Counter Model DN. This method uses zap-o-globulin as a lysing agent. Six drops of zap-o-globulin as a lysing sample immediately before counting, and the sample mixed gently. The counts are expressed in 10^3/ul of blood.

3.4.6. Total Protein (T.P.).

Serum obtained from PCV determinations was used in determination of T.P. The capillary tube was cut just above the buffy coat and a drop of serum was placed on the refractometer (Atago SPR-N, Japan). The values were then recorded in gm/100 ml of blood.

3.4.6. Fibrinogen.

Microhaematocrit tubes were used. After centrifugation of the blood for 5 min. at 1872 g., the capillary tubes were incubated in a waterbath at 56°C for 30 minutes. Then re-centrifuged for another 5 min. at 1872g. The protein was read on a refractometer and the values of fibrinogen were calculated using the following formula after Schalm et al. (1975).

$$\text{Fibrinogen (mg/dl)} = \text{T.P.} - \frac{\text{incubated protein gm/dl}}{\text{Fibrinogen gm/dl} \times 1000}$$

3.4.7. Differential Leucocyte Count.

Blood smears for differential leucocyte counts were prepared using standard procedures. The smears were stained with buffered Wrights stain for rapid staining of blood smears for 15 secs. using Camco quick stain (American Hospital Supply Corp. IL, U.S.A.).

Using the x40 objective, 200 cells were counted, and the figure obtained for each cell type was divided by 2 to give the relative percentage.

3.4.8. Neutrophils, Lymphocytes and Monocytes.

Absolute values of the above cells were calculated from total WBC Count and differential counts as described by Schalm et al. (1975). The formula is as follows: Absolute number/ul = Total W.B.C. x Cell differential.

3.5. Serum Biochemistry determinations.

3.5.1. Serum aspertate aminotransferase (ASAT) and Serum alanine aminotransferase (ALAT).

The above enzymes were determined following the method of Reitman and Frankel (1957) using the colorimetric method. The activities of ALAT and ASAT were estimated in a Spectrophotometer (Spectronic 20, Bausch & Lomb, U.S.A.) at a wavelength of Hg 546 nm. The reagents used were obtained from Mannheim Boehringer (Gmbh, W. Germany) and the values expressed in Units/Litre.

3.5.2. Alkaline phosphatase.

This was determined by the method of Bessy, Lowry and Brock (1946) using the colorimetric method. The activity of alkaline phosphatase was estimated in a spectrophotometer at a wavelength of Hg 405 nm. The reagents used were from Mannheim Boehringer (W. Germany) and the values expressed in Units/litre.

3.5.3. Total Protein, Albumin and Albumin-Globulin (A/G) ratio.

Total protein and albumin were determined with the biuret method as described by Coles (1967). The protein and albumin values for the optical densities recorded were read from the calibration graph.

Globulin was obtained by subtracting albumin from total protein. The A/G ratio was then calculated by dividing the total albumin by the value of total globulin. The values for total protein, albumin, and globulin were expressed in grammes per 100 ml of serum.

3.5.4. Blood glucose estimation.

Glucose was determined by the glucose-oxidase method (Kaneko and Cornelius, 1971), a specific method for blood glucose which employs the enzymes glucose oxidase and peroxidase, and a dye. For the above test, commercially available kits were used (Boehringer, Mannhein, W. Germany).

The activity of glucose was estimated in a spectrophotometer at a wavelength of Hg 578 nm and the values expressed in mg/100 ml of blood (Kaneko and Cornelius, 1971).

3.6. Organ aspirates for parasite culture and parasite density estimation.

Aspirates were collected from the spleen, liver, and the inguinal lymph nodes.

3.6.1. Splenic aspirates.

The spleen was first palpated around an area just behind the last rib of the left thoracic wall, and about 4 fingers breadth from the mid-ventral line. With a 21-gauge 1½" needle attached to a 5 ml syringe, the skin was penetrated and the syringe plunger was pulled back approximately 2 ml. to apply suction. Then with a quick in and out movement, the needle was pushed into the spleen to the full needle depth and then withdrawn completely, while maintaining suction throughout. This method yielded a tiny amount of material, but this was adequate for culture and smear.

The plunger was then pulled back to 4 ml, and using sterile technique the needle was inserted into the tissue culture flask containing ready to use medium, and briskly the plunger was pushed into the barrel of syringe to expel the contents of the needle into the flask. Additional material was then expelled gently onto a labelled glass slide and the material spread evenly with the needle using a linear motion.

The slides were allowed to dry and soon after the sampling, all slides were fixed with methanol for 1 min and stained with Giemsa stain.

3.6.2. Liver aspirate.

This was performed on the right thoracic wall. The area of aspiration was located on the 4th last intercostal space about 3 fingers breadth from the mid-ventral line. The aspiration technique was similar to that applied for the spleen.

3.6.3. Lymph node aspirate.

The inguinal lymph nodes were used for this purpose. Either the right or the left inguinal lymph node was first palpated, and then immobilized with the left hand against the pubic bone or the surrounding muscles. Using a 21-gauge 1" needle attached to a 5 ml syringe, the skin and the lymph node were penetrated. Once in the lymph node, suction was applied on the plunger, and maintained while the needle was pushed in different parts of the lymph node, until a whitish material was visible at the hub of the needle. Then the needle was completely withdrawn gently. The material in the needle was enough for inoculation into culture media and for the preparation of aspirate smears.

3.6.4. Examination of aspirate and impression smears.

All aspirate, and impression smears were routinely fixed in methanol for 1 min and stained with Giemsa stain. The smears were examined under oil immersion for parasites and then graded for parasite density estimations. The aspirate smears collected during sampling and at necropsy

were graded following the method of Chulay and Bryceson (1983) as outlined in Table 3.

Table 3. (Parasite grades).

1000 fields were examined, and the number of amastigotes counted per 1000 fields.

GRADE	AVERAGE PARASITE DENSITY
6 +	> 100 parasites/field
5 +	10-100 parasites/field
4 +	1 -10 parasites/field
3 +	1 -10 parasites/10 fields
2 +	1 - 10 parasites/100 fields
1 +	1 - 10 parasites/1000 fields
0	0 parasites/field

The impression smears collected at necropsy were examined for parasites and the parasite density estimations of the liver, spleen and inguinal lymph node was done following the method of Chulay and Bryceson (1983) as outlined in Table 3 and also following the method of Stauber (1958), as per the following formula.

$$\frac{\text{Parasites/organ cell nucleus} \times \text{wt. of organ in milligrams}}{} = \text{Parasite density of organ.}$$

3.6.5. Examination of culture flasks inoculated with organ aspirates.

All culture flasks that were inoculated with organ aspirates were incubated at 26°C and examined once daily for promastigote positivity for a period of 14 days.

Using an inverted microscope at x 32 objective, 40 fields were examined at three phases; the floating phase, the middle phase, and the floor phase. Therefore, a total of 120 fields were actually examined. If after examining 120 fields no promastigote was identified, the culture flask was regarded as negative for that particular day. Cultures were discarded by day 14 if still negative. Cultures were not examined any further once positive with promastigotes.

3.7. Pathology.

At each sampling, needle biopsies of the liver were obtained for histological examination. However, a complete necropsy was performed on all animals that died during the course of infection and at the termination of the experiment.

3.7.1. Liver biopsies.

The site from which the biopsy was collected was located at the same position as that for liver aspirates. After the skin was shaved and disinfected, a stab incision was made through the skin with a pointed scalpel to facilitate entrance of the biopsy needle. A 16 gauge 4" biopsy needle (Downs Surgical Ltd. Church path,

Surrey, England) attached to a 5 ml syringe containing 5 ml of physiological saline was used. The needle was inserted through the 4th intercostal space until the surface of the liver was felt opposing the needle. About 2 ml of the saline was pushed in to remove any tissue that could be found in the barrel of the needle. The plunger was pulled back to 1 ml and while maintaining suction, with a quick in and out movement, about 1 inch of the needle was pushed in. The biopsy material was then fixed in 10% formol-saline for histopathology sectioning, and stained with haematoxylin and eosin (H&E).

If after 3 attempts, no biopsy material could be obtained at any one time of sampling, this exercise was forfeited because of the excessive trauma that could result from many attempts. In this respect, biopsies of the liver during some of the sampling intervals were not obtained from some animals.

3.7.2. Necropsy.

Using standard procedures, necropsy was performed on all dead animals and at the termination of the experiment.

At necropsy, samples were collected for organ aspirate cultures, aspirate smears, and for organ impression smears. Furthermore, weights of the liver, spleen and inguinal lymph nodes were recorded. Sections of the liver, spleen, lymph nodes, and kidney were collected in 10% formol-saline for histopathology. After processing, they were stained with H&E and examined for histological changes. Other organs such as, intestines, lung, and bone marrow were also collected for histopathology and examined for histological changes.

3.7.3. Detection of non-specific acid esterase in macrophages.

Special staining techniques of macrophages of the liver to determine their population within the granulomas was performed using the esterase positive method as described by Mueller et al. (1975) with slight modifications. This method uses the demonstration of acid α-naphthyl acetate esterase (ANAE) activity in histological sections. After cytocentrifugation, cells were allowed to dry at room temperature before fixing in cold Baker's formocalcium. The activity of ANAE was ascertained by incubation in a medium consisting of phosphate buffer, hexazotized pararosaniline and α-naphthyl acetate in acetone. A macrophage was scored as esterase positive if it's cytoplasm contained a single of a few distinct spots of the reddish brown reaction product.

4. RESULTS.

4.1. Haematology picture.

The RBC counts, haemoglobin, PCV, WBC and differential white cell count values are summarized in the Tables of the Appendix.

4.1.1. Vervets.

There was a slight decrease in the RBC values of the infected animals as compared with the controls. The lowest values were observed at weeks 24 - 28 post-inoculation (p.i.). The mean RBC, % PCV and haemoglobin levels of 4 infected and 2 control vervets are shown in Figs 2, 3 and 4.

There was a significant decrease in the haemoglobin values of the infected animals as from weeks 4 - 20 p.i. The lowest levels were obseved at week 20 p.i. with a mean of 11.96 $\pm$ 4.4 gm/100 ml. One vervet (Ver 99) had the lowest value of 7 gm/100 ml at the time of death 20 weeks p.i.

There were no other significant reduction of the other erythron parameters, total protein and fibrinogen levels.

Changes in the WBC and differential leucocyte counts were marked in some infected animals. There was no significant change in total WBC values when the mean values were compared between the infected and control animals. However, 2 vervets (Ver 609 and 137) showed a decrease in values at various sampling intervals. Ver 609 had a significant drop in WBC values for a

period of 10 weeks with the lowest value of 1.7×10^3/ul being recorded at week 16 p.i.

There was no significant changes in the differential leucocyte counts.

4.1.2. Sykes.

There was a slight decrease in the RBC values of the infected animals as compared with the control animals. The lowest mean values were observed at week 2 and week 26 p.i. The lowest values were observed at the time of death of Syk 198, 22 weeks p.i. It's RBC value was 1.7×10^6/ul. The mean RBC and haemoglobin levels of the 4 infected and 2 control sykes are shown in Figs 2 and 3.

The changes in the haemoglobin values between the infected and the controls was variable in that there was a progressive decrease in values as from week 10 to week 22 p.i. The lowest values were recorded at week 22 p.i. with a mean of 9.37 ± 3.66 gm/100 ml.

In one of the infected animal, (Syk 198) Hb value was as low as 4 gm/100 ml. This was recorded just before death at week 22 p.i.

Other erythron parameters, total protein and fibrinogen values showed no significant changes between infected and controls.

There were marked changes in the mean total WBC values between the infected and control animals. The first significant drop was at week 6 p.i., and the lowest values were at week 14 p.i. with a mean value of

$2.42 \pm 1.72 \times 10^3$/ul. In addition, all infected sykes displayed leucopaenia of varying degrees, for periods ranging from week 4 to week 22. All the infected animals that died as a result of the infection had values below 2.6×10^3/ul WBC.

Syk 198 had a severe decrease in WBC values from the time of infection until death. It's lowest values were observed at week 14 and 20 p.i.

The changes in the differential leucocyte counts were not significant. The mean WBC values of 4 infected and 2 control syke monkeys are shown in Fig. 5.

4.3. Serum biochemistry.

The values of glucose, ALAT, ASAT and alkaline phosphatase are summarized in the tables of the Appendix.

4.3.1. Vervets.

There was no significant changes in the mean ALAT, ASAT, and glucose values of the infected and the controls. However, the mean values of alkaline phosphatase of the 4 infected and 2 control animals showed an increase to high levels of mean 126 ± 33.28 IU/litre at week 26 p.i. The highest level was observed from Ver. 187 at week 16 p.i., with a reading of 122 IU/litre. The mean values of the infected and control animals are shown in Fig.6.

4.3.2. Sykes.

Like the vervets, the only significant increase in the biochemistry determinations was in alkaline phosphatase values. The levels increased as from week

14 to 30 p.i. The highest mean values of 111.3 ± 17.0 IU/litre was observed at week 30 p.i. The highest value recorded was 224 IU/litre from Syk 205 at week 20 p.i. The mean values of alkaline phosphatase of the infected and control animals are shown in Fig. 6.

4.3.3. Serum total protein albumin, globulin and A/G ratio.

The total protein, albumin, globulin and the albumin/globulin ratio are summarized in tables of the Appendix.

4.3.4. Vervets.

All infected animals had an elevation of the total protein and a concomitant increase in the globulin levels. This occurred in the last 3 - 4 months p.i. In addition, albumin levels during the terminal stages of the infection were decreased. Thus, all infected vervets had a hyperglobulinaemia as a result of the L. donovani infection.

The mean values of infected and control animals are shown in Figs 7 and 8.

4.3.5. Sykes.

Like the vervets, all infected sykes displayed a hyperglobulinaemia with an elevated total protein and globulin values during the last 4 months of infection. During this time, albumin levels were slightly decreased.

The mean values of the infected and control animals are shown in Figs 7 and 8.

4.4. Parasitological findings.

The results of aspirate cultures and smears of vervet and syke monkeys infected with L. donovani promastigotes are shown in Tables 4 and 5.

4.4.1. Vervets.

Following intravenous needle inoculation with L. donovani promastigotes, all infected monkeys became positive within 22 - 35 days as demonstrated by positive smears and liver cultures.

The liver was the first organ to show positivity and throughout the course of infection, this organ remained with the highest parasite recovery followed by the spleen and inguinal lymph nodes. The positivity of lymph node cultures and aspirate smears were registered in all infected vervets. However, all inguinal lymph nodes registered positivity during the course of infection. One animal became positive in culture by days 56 p.i., and within days 84 - 98 p.i., 2 animals were positive in culture and smear while the third one was positive in smears only. In addition, the lymph nodes were positive by both culture and smear whenever the liver recorded a parasite grade of at least 3+ by a 6+ score system.

All vervets that died during the course of infection had positive cultures of the liver, spleen, inguinal lymph node and the femoral bone marrow. This demonstrated that there was a generalized dissemination

of the parasites throughout the reticuloendothelial tissues of the infected vervets. The total parasite densities of the liver and spleen of 2 vervet monkeys (Ver 99 and Ver 137) were 43 fold and 88 fold greater respectively than the total parasite dose given originally. The parasitological information regarding parasite grades and parasite densities are summarized in Tables 6 and 7.

Three infected vervets had peak parasite grades in the liver with a score of 3+ at days 84 p.i., while 1 vervet had a parasite grade of 3+ within days 112 p.i. In addition, one vervet had the highest parasite grade of 4+ at days 154 p.i., from the liver and 3+ from the spleen. However, other infected vervets had a parasite grade of at least 2+ and 1+ from the spleen and inguinal lymph node respectively during the course of infection.

The peak parasite grades reached was 4+ in the liver and spleen and 2+ in the inguinal lymph nodes from Ver 99 and Ver 137 at days 140 and 219 p.i. respectively (Table 7). In addition, impression smears of liver, spleen and inguinal lymph nodes at necropsy revealed parasite densities much higher than the size of the initial parasite inoculum from each of the animals that died. The total number of amastigotes in the spleen and liver of Syk 198 at days 158 p.i. were approximately 114 fold greater than the total parasite dose given originally (Table 7).

One vervet that survived the infection (Ver 187) was parasitologically negative by days 140 p.i. This particular animal displayed the self-cure phenomenon.

4.4.2. Sykes.

Following intravenous needle inoculation with L. donovani promastigotes, all infected monkeys became positive within 46 - 54 days as demonstrated by liver and splenic cultures. The results of aspirate cultures and smears are given in Table 5.

The inguinal lymph node cultures became positive at days 70 and 84 p.i., and all animals registered positivity by both cultures and smears during the course of infection. The inguinal lymph nodes of 3 sykes (Syk 214, 205 and 198) had positive cultures and smears within 84 days p.i. One syke (Syk 198) that died at days 158 p.i. had positive cultures of the liver, as well as the spleen, inguinal lymph nodes and bone marrow. This indicated that the disease in syke monkeys had a generalized dissemination in the reticuloendothelial tissues, a finding which was observed in the infected vervets. In addition, Syk 198 that died with positive organ cultures had a high parasite grade score of 5+ from the liver and 4+ and 3+ respectively from the spleen and inguinal lymph node.

The parasitological findings regarding parasite grades and parasite densities are shown in Table 6 and 7.

All the infected syke monkeys had peak parasite grade score of 3+ in the liver at days 84 p.i. In

at days 154 p.i. This was the highest parasite grade that was recorded during the course of infection and it is this same animal that registered a high parasite grade score of 5+ at the time of death as determined from impression smears, (Table 7). Similar to the vervets that died, this syke revealed parasite densities in either the liver, spleen or inguinal lymph nodes in excess of the size of the initial parasite inoculum as demonstratad by impression smears at necropsy.

Three syke monkeys that survived (Syk 214, 205 and 116) the infections were parasitologically negative by days 126 - 154 p.i. Two of these (Syk 205 and 116) survived until the termination of the experiment and were negative parasitologically. Syk 214 died after parasitological cure as a result of gastric ulcers. Therefore these 3 animals displayed the self-cure phenomenon. The parasite grades scored by each animal are summarized in the Tables of the Appendix.

The parasite grades scored during the course of infection of one vervet (Vervet 137) and one syke (Syk 198) are shown in Fig. 9.

Fig. 2. Mean Red Blood Cell (R.B.C.) values of infected and non infected control vervet and syke monkeys following intravenous inoculation with *L. donovani*.

Fig. 2.

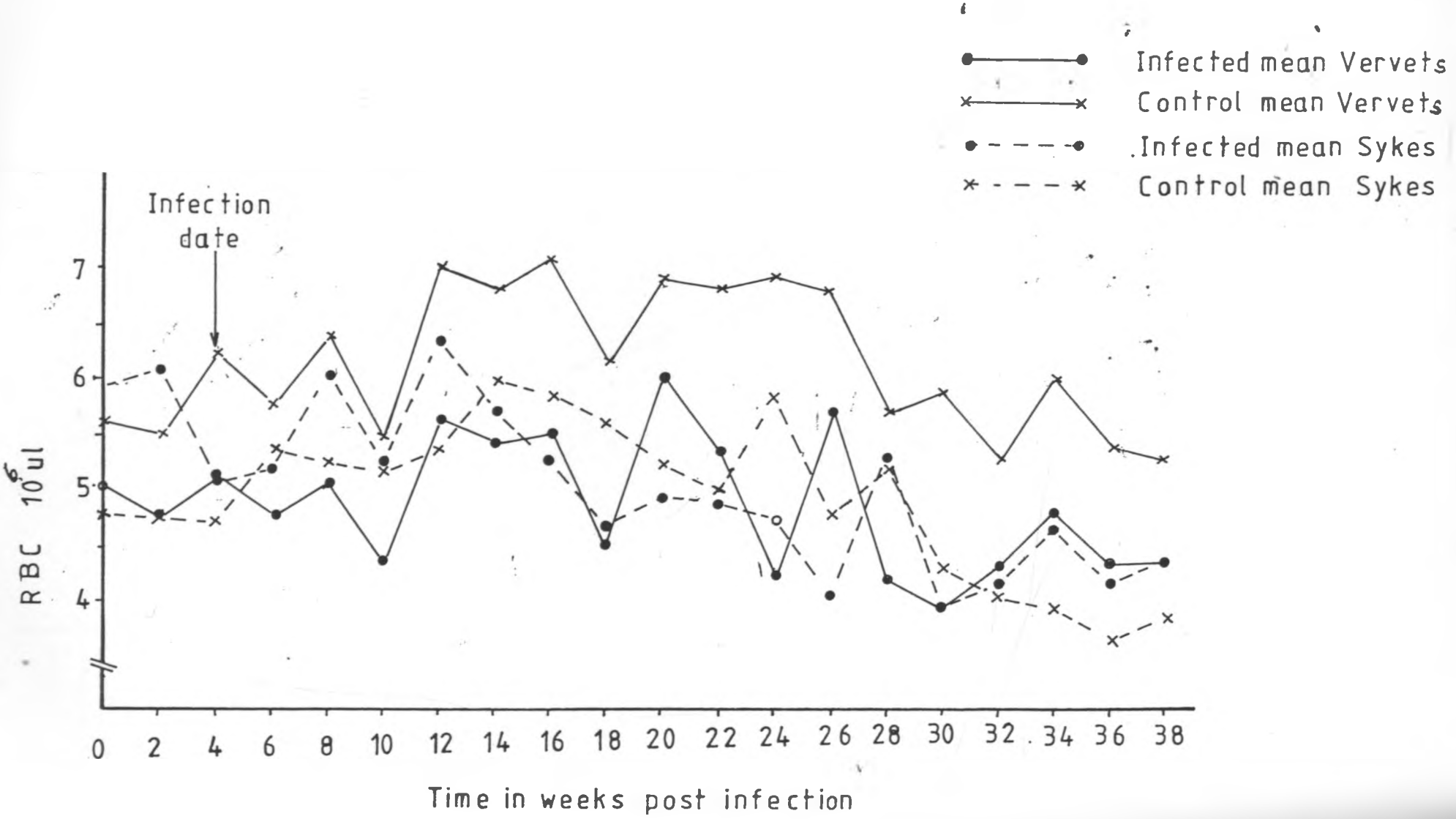

Infected mean Vervets
Control mean Vervets
Infected mean Sykes
Control mean Sykes
Infection date
RBC 10⁶ul
7
6
5
4
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Time in weeks post infection

Fig. 3. Mean haemaglobin values of infected and non infected control vervet and syke monkeys after infection with L. donovani.

Fig. 3.

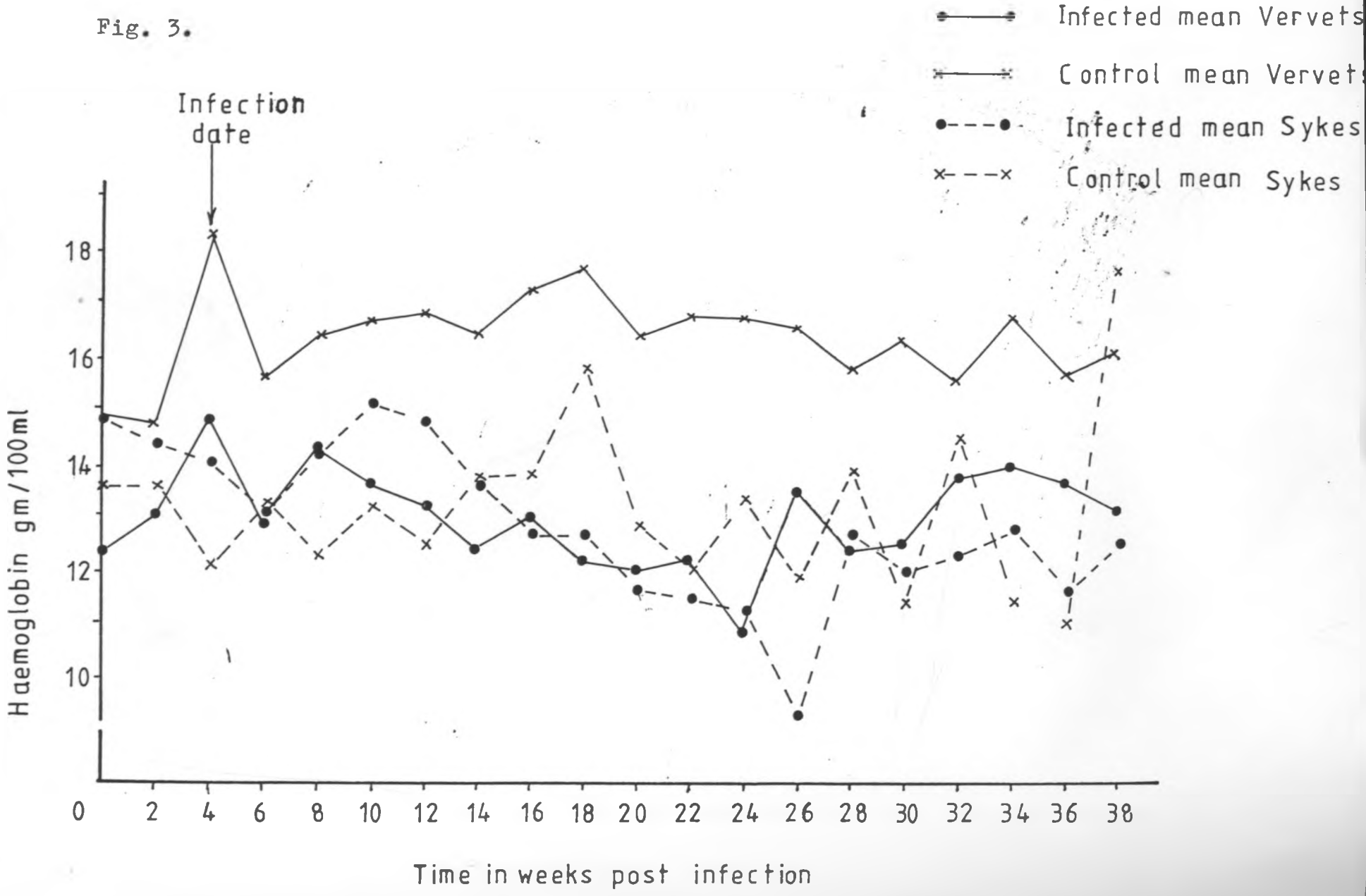

Infected mean Vervets
Control mean Vervets
Infected mean Sykes
Control mean Sykes
Infection date
Haemoglobin gm/100ml
18
16
14
12
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Time in weeks post infection

Fig. 4. Changes in the Packed Cell Volume (PCV) between infected and non infected control vervet and syke monkeys after infection with L. donovani.

Fig. 4.

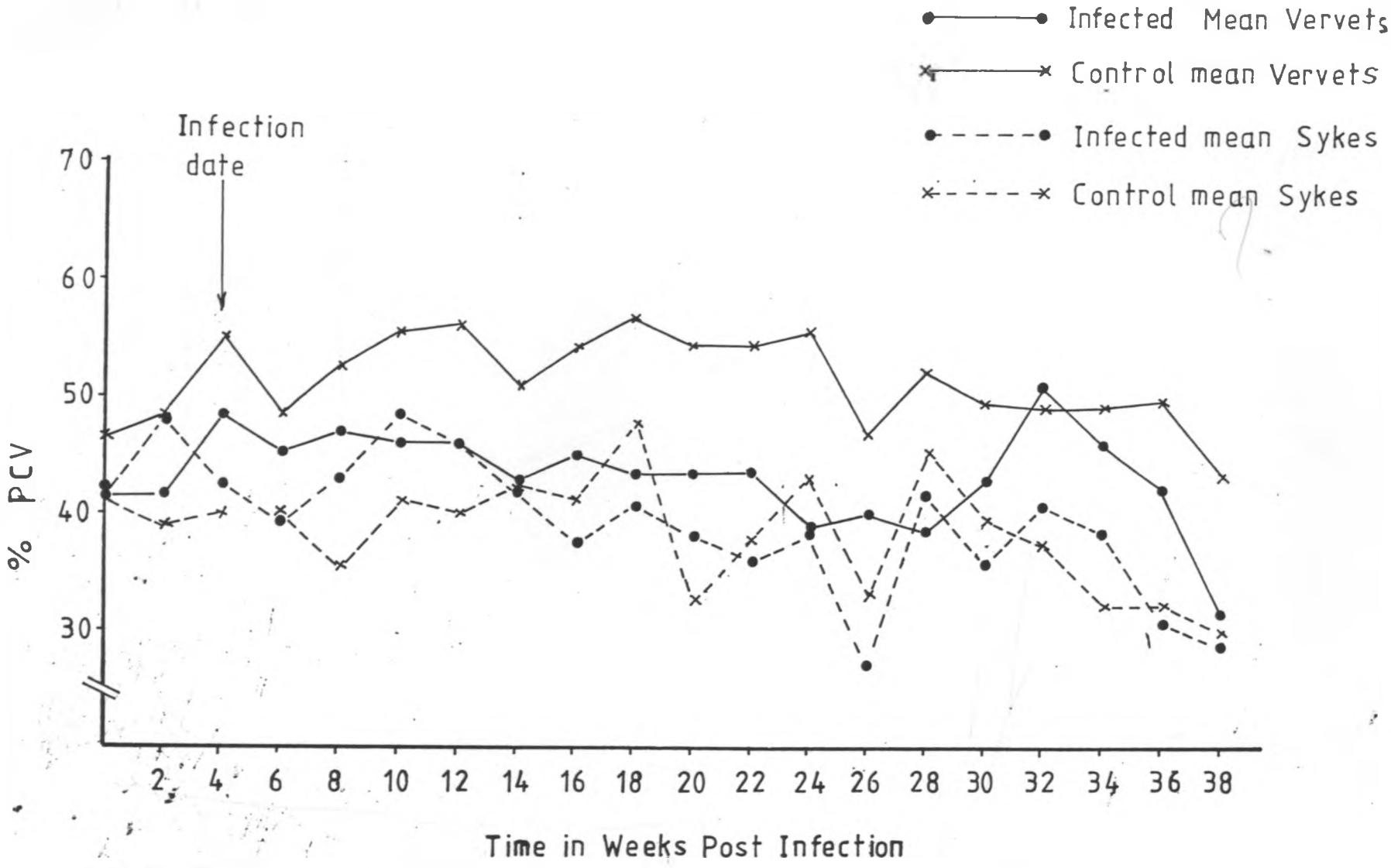

Infected Mean Vervets
Control mean Vervets
Infected mean Sykes
Control mean Sykes
Infection date
70
60
50
40
30
% PCV
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
Time in Weeks Post Infection

Fig 5. Typical changes in the mean WBC counts between infected and non infected control vervet and syke monkeys after infection with L. donovani.

Fig. 5.

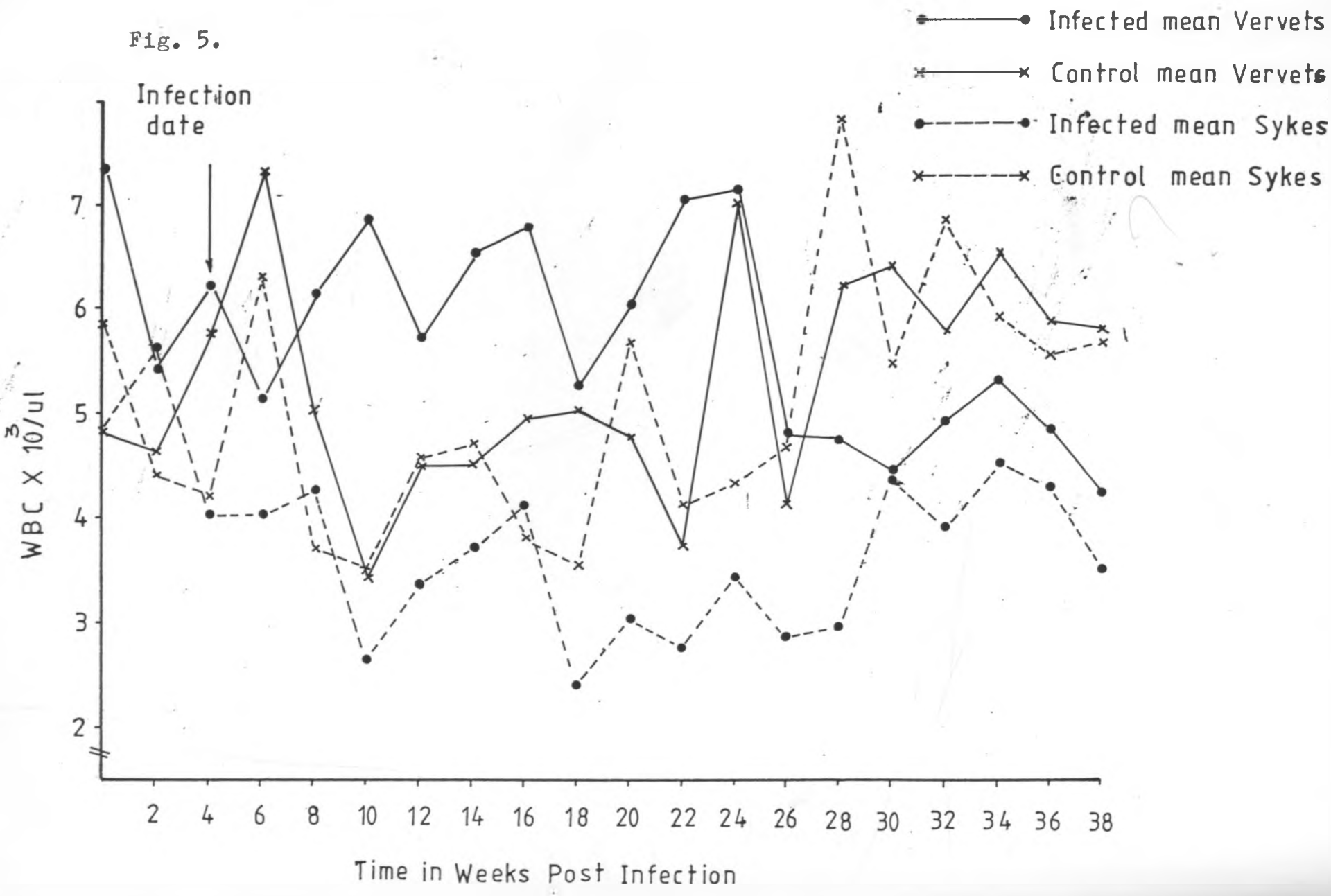

Infected mean Vervets
Control mean Vervets
Infected mean Sykes
Control mean Sykes
Infection date
WBC X 10/ul
3
7
6
5
4
3
2
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Time in Weeks Post Infection

Fig. 6. Mean alkaline phosphatase values of infected and non - infected control vervet and syke monkeys after infection with L. donovani.

Fig. 6.

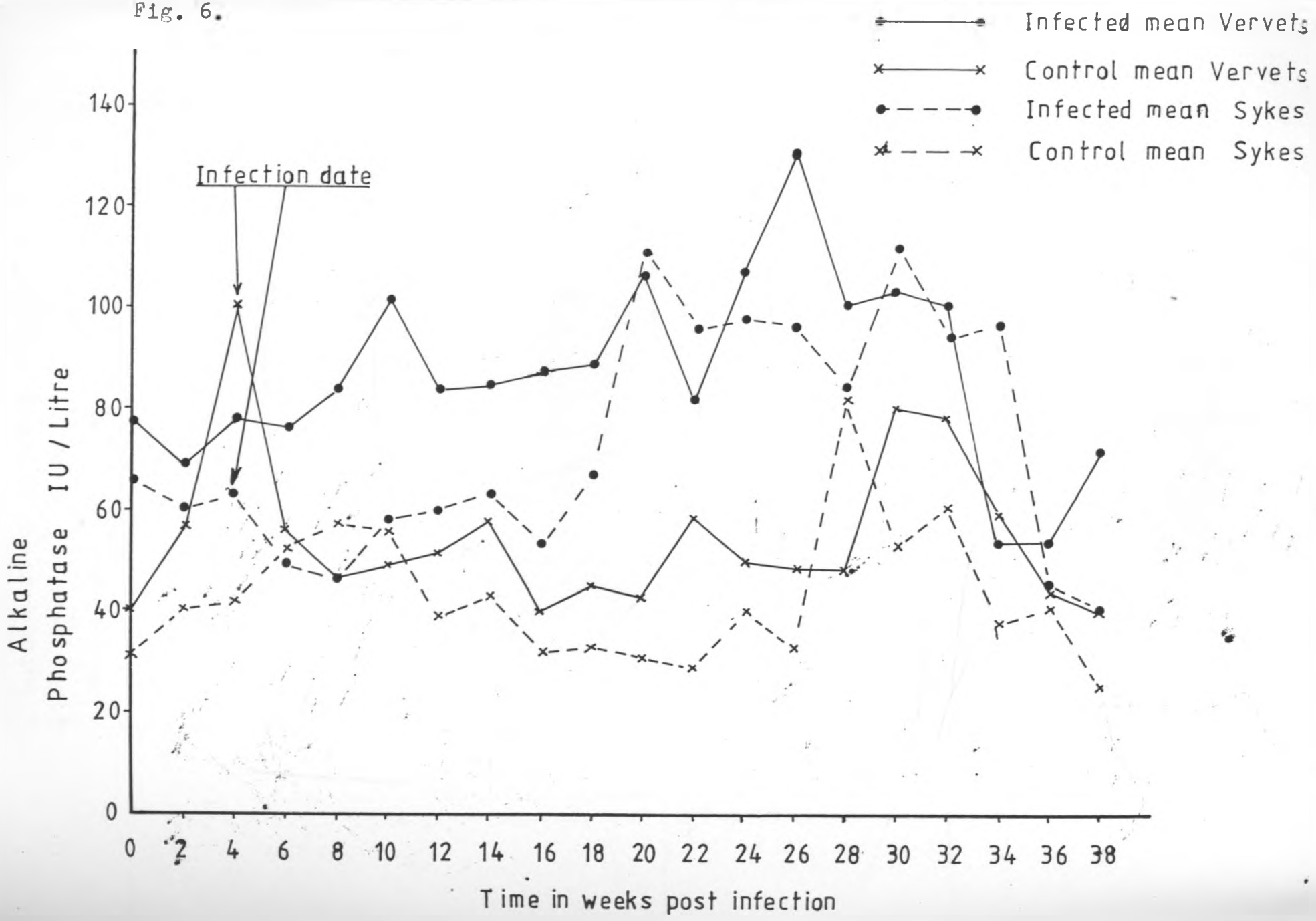
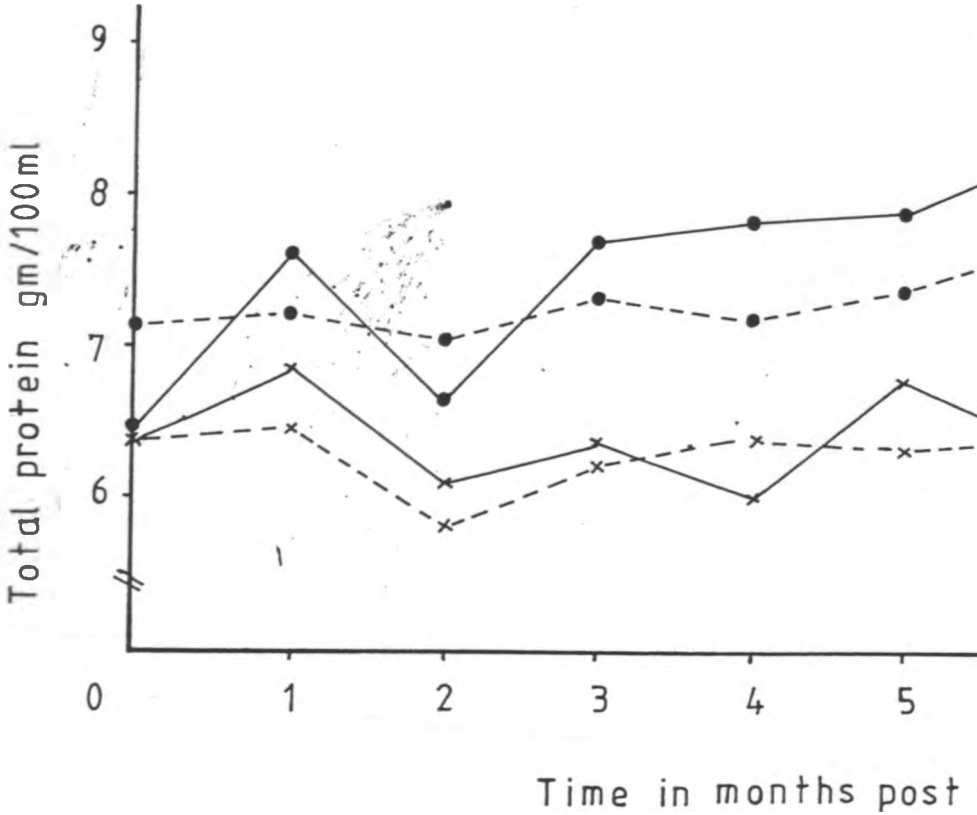
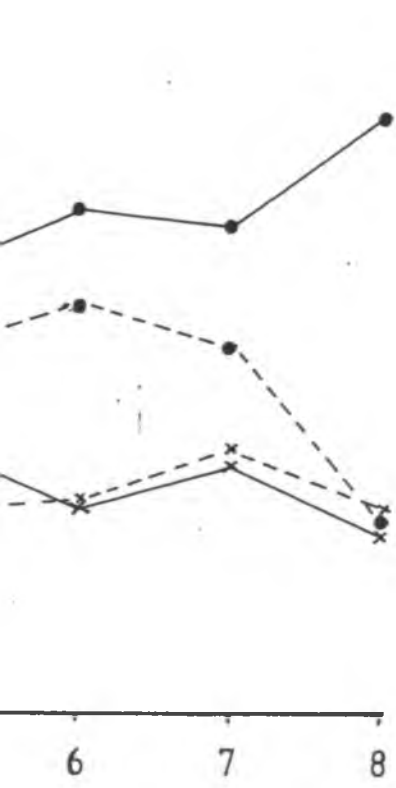

Infected mean Vervets
Control mean Vervets
Infected mean Sykes
Control mean Sykes
Infection date
Alkaline Phosphatase IU / Litre
0
20
40
60
80
100
120
140
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Time in weeks post infection

Fig. 7. Typical changes in the mean total serum protein of infected and non infected control vervets and syke monkeys after infection with *L. donovani*.

Fig. 7.





Infected mean Verve

Control mean Verve

Infected mean Syke

Control mean Syke

Fig. 8. Typical changes in the mean total serum globulin levels of infected and non infected control vervets and syke monkeys after infection with L. donovani.

Fig. 8.

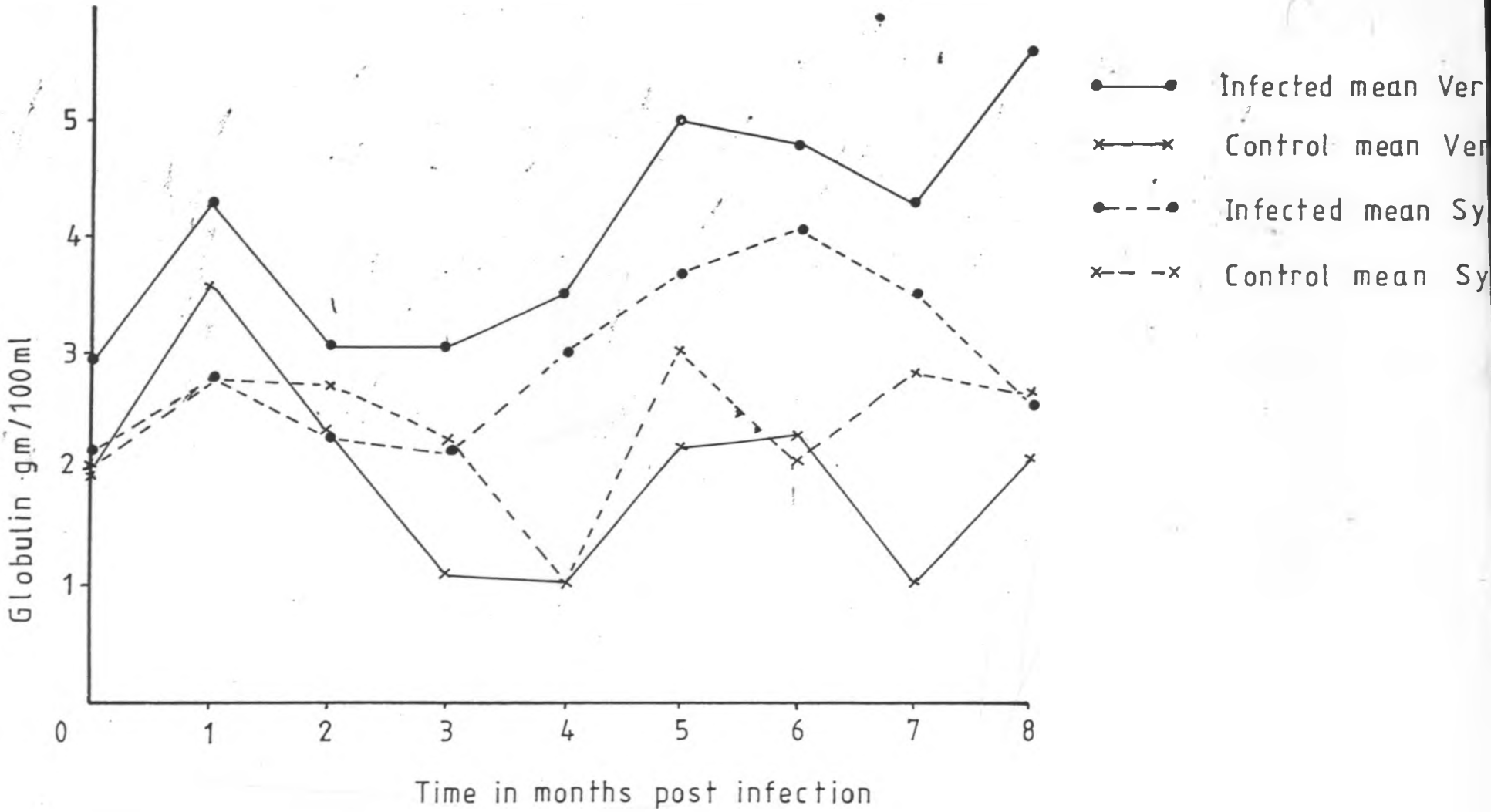

Globulin gm/100ml
0
1
2
3
4
5
6
7
8
Time in months post infection
Infected mean Ver
Control mean Ver
Infected mean Sy
Control mean Sy

Fig. 9. Changes in the parasite grades of the liver, spleen and inguinal lymph node in a vervet (Ver I37) and a syke (Syk I98) monkey following intravenous inoculation with L. donovani. Note that Syk I98 died at week 23 post infection

Fig. 9.

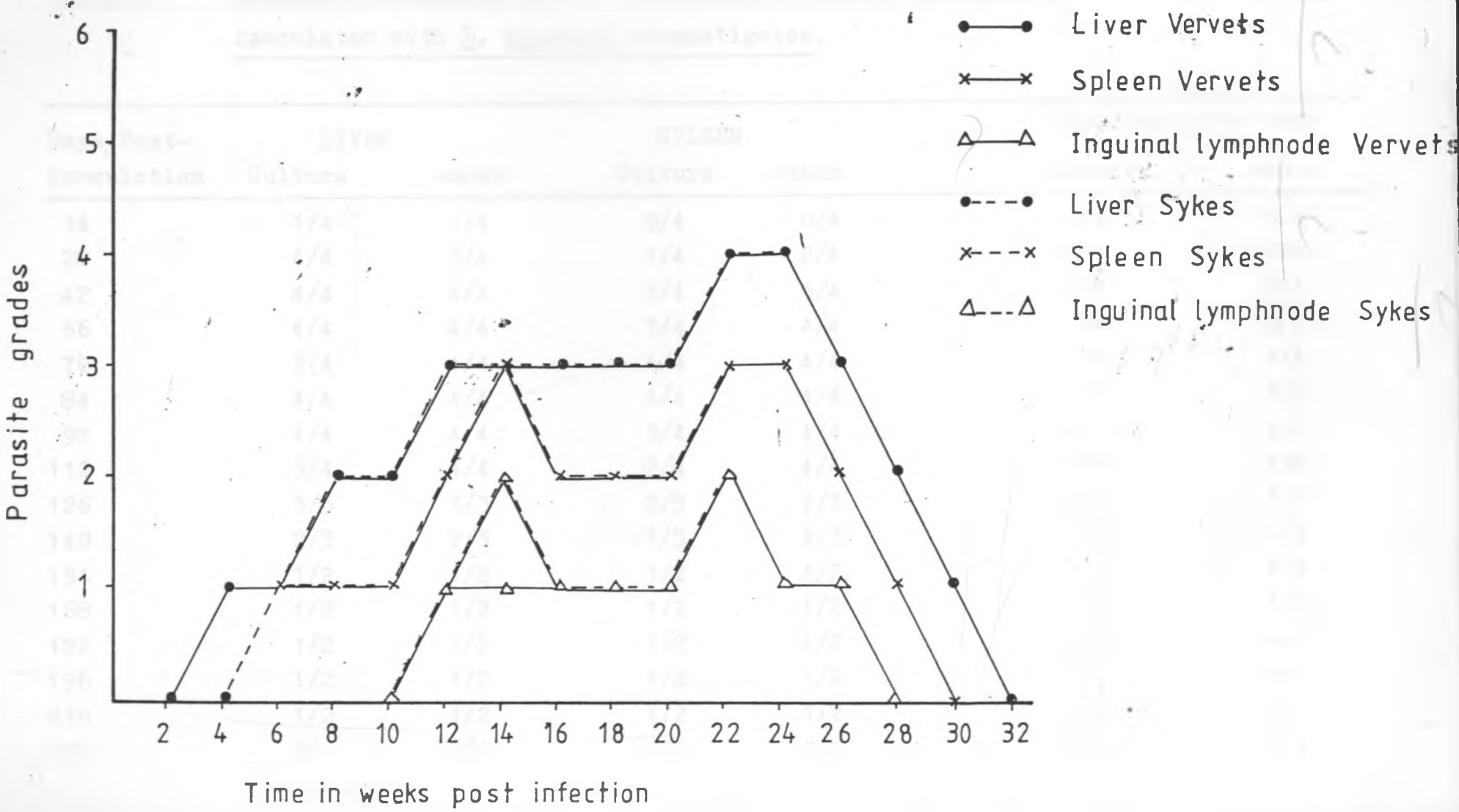

Liver Vervets
Spleen Vervets
Inguinal lymphnode Vervets
Liver Sykes
Spleen Sykes
Inguinal lymphnode Sykes
Parasite grades
1
2
3
4
5
6
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Time in weeks post infection

Table 4. Results of parasite culture and aspirate smears in Vervet monkeys intravenously inoculated with L. donovani promastigotes.

Days Post-inoculation	LIVER		SPLEEN		INGUINAL LYMPH NODE	
	Culture	smear	Culture	smear	Culture	smear
14	1/4	1/4	0/4	0/4	0/4	0/4
28	4/4	3/4	1/4	2/4	0/4	0/4
42	4/4	4/4	3/4	3/4	0/4	0/4
56	4/4	4/4	3/4	4/4	1/4	0/4
70	3/4	4/4	4/4	4/4	1/4	1/4
84	4/4	4/4	4/4	4/4	2/4	3/4
98	4/4	4/4	3/4	4/4	2/4	3/4
112	3/4	4/4	2/4	4/4	1/4	2/4
126	3/3	3/3	2/3	2/3	1/3	1/3
140	2/3	2/3	1/3	2/3	1/3	1/3
154	1/2	1/2	1/2	1/2	1/2	1/2
168	1/2	1/2	1/2	1/2	1/2	1/2
182	1/2	1/2	1/2	1/2	0/2	0/2
196	1/2	1/2	1/2	1/2	0/2	0/2
210	1/2	1/2	1/2	1/2	0/2	0/2
224	0/1	0/1	0/1	0/1	0/1	0/1

Table5 . Results of parasite culture and aspirate smears in Syke monkeys intravenously inoculated with L. donovani promastigotes.

Days Post-inoculation	LIVER		SPLEEN		INGUINAL LYMPH NODE	
	Culture	Smear	Culture	smear	Culture	smear
14	0/4	0/4	0/4	0/4	0/4	0/4
28	0/4	0/4	0/4	0/4	0/4	0/4
42	2/4	3/4	1/4	1/4	0/4	0/4
56	4/4	4/4	4/4	4/4	0/4	0/4
70	4/4	4/4	4/4	4/4	2/4	2/4
84	4/4	4/4	4/4	4/4	3/4	3/4
98	4/4	4/4	3/4	3/4	2/4	2/4
112	4/4	4/4	2/4	2/4	1/4	1/4
126	3/4	3/4	2/4	2/4	1/4	1/4
140	2/4	2/4	1/4	1/4	1/4	1/4
154	1/4	1/4	1/4	1/4	0/4	1/4
168	0/3	0/3	0/3	0/3	0/3	0/3
182	0/3	0/3	0/3	0/3	0/3	0/3
196	0/3	0/3	0/3	0/3	0/3	0/3
210	0/2	0/2	0/2	0/2	0/2	0/2
224	0/2	0/2	0/2	0/2	0/2	0/2

Table 6. Results of the incubation period, peak parasite grades scored by each animal, range of parasitic grades and time to parasitological cure in Vervet and Syke monkeys infected with L. donovani i.v.

Animal Number	Incubation period in days	Peak parasite grade	Range of parasite grade			Time to parasitological cure in days p.i.
			Liver	Spleen	Lymph node	
VER 187	22	3+	0-3+	0-2+	0-1+	140
VER 99	35	3+	0-3+	0-2+	0-1+	Died prior to cure
VER 609	34	3+	0-3+	0-2+	0-1+	Died prior to cure
VER 137	34	4+	0-4+	0-3+	0-2+	Died prior to cure
SYK 198	51	4+	0-4+	0-3+	0-2+	Died prior to cure
SYK 205	46	3+	0-3+	0-2+	0-1+	126
SYK 214	54	3+	0-3+	0-1+	0-1+	154
SYK 116	46	3+	0-3+	0-1+	0-1+	126

Table 7. Results of impression smears parasitic grades scored by all dead animals, organ parasite densities and time to death in vervet and syke monkeys infected with L. donovani i.v.

Animal Number	Parasite grade of impression smears at time of death			Parasite density at time of death			Time to death in days
	Liver	Spleen	Inguinal Lymph node	Liver	Spleen	Ing.Lymp node	
VER 187	0	0	0	0	0	0	
VER 99	4+	4+	2+	2.38×10^9	3.42×10^8	1.3×10^5	140 days
VER 609	3+	2+	1+	2.7×10^8	3.3×10^7	1.0×10^5	113 days
VER 137	4+	4+	2+	5.95×10^9	6.52×10^8	2.4×10^5	219 days
SYK 198	5+	4+	3+	1.21×10^{10}	3.9×10^9	9.36×10^6	158 days
SYK 214	0	0	0	0	0	0	177 days

Table 8. Organ weights in grammes at necropsy.

Animal Number		Liver	Spleen	Lt L/N	Rt L/N
**	SYK 86	183.38	7.68	0.22	0.18
**	" 3	133.47	5.66	0.36	0.38
	" 205	194.16	29.52	0.35	0.10
	" 116	90.00	6.52	0.52	0.29
*	" 198	253.00	60.3	0.52	0.28
*	" 214	193.71	18.71	0.30	0.20
**	VER 695	109.8	9.15	0.25	0.17
**	" 673	117.4	11.3	0.24	0.13
	" 187	157.56	24.78	0.3	0.23
*	" 609	46.2	8.42	0.1	0.2
*	" 99	152.61	28.55	0.11	0.15
*	" 137	186.00	22.04	0.10	0.10

* = Animals that died during the course of infection.

** = Uninfected controls.

Lt L/N = Left inguinal lymph nodes.

Rt L/N = Right inguinal lymph nodes.

4.5.3. Sykes.

The infected syke (Syk 198) that died during the course of infection with parasite grades of 5+ had a marked splenomegaly with rounded borders and the spleen was more than 8 times the normal weights when compared with that of the uninfected controls. The liver was also considerably enlarged, friable and had a prominent lobulation. At termination of experiment, when the animals were parasitologically negative, their gross lesions were unremarkable. In addition, there were no remarkable changes in other body organs.

4.5.4. Histopathology.

The histopathological picture of the liver biopsies and other organs at necropsy varied slightly among the infected animals.

4.5.5. Vervets.

4.5.6. Liver.

At week 2 - 4 p.i. the liver showed an early response to the infection. All infected vervets showed tiny foci of histiocytic cellular infiltration, some of these foci were periportal in their distribution (Fig. 10).

At week 6 to 10 p.i., there were prominent discrete granuloma foci scattered all over the section, some being associated with portal triads (Fig. 12). Hepatocytes were also incorporated into the reaction and had undergone pressure atrophy and necrosis. These granulomas were composed of histiocytes and few lymphocytes

at the periphery. The smaller foci had more of lymphocytes than macrophages.

At week 12 to 20 p.i., several granulomatous nodules were present. They were composed of histiocytes, lymphocytes and a few plasma cells. In addition, hepatocytes had been incorporated in the reaction where they were observed to be undergoing disappearance, loss of staining ability and with others showing cloudy swelling. Some of the reactions were periportal. Generally there was a severe granulomatous hepatitis (Fig. 11).

At week 22 to 26 p.i., a severe granulomatous hepatitis was still present with a histiocytic, lymphocytic cellular infiltration. These granulomas had resulted to disarray of liver columns and a disruption of lobular pattern as a result of the massive infiltration with mononuclear cells (Fig. 12) However, one vervet (Ver 137) was showing evidence of resolution of hepatic inflammation with tiny lymphocytic nodules, scattered here and there (Figs 13 and 14).

At week 28 - 32, the only surviving vervet (Ver 187) was still showing a mild granulomatous reaction, with the cellular composition being more of lymphocytes and a few plasma cells (Fig.13).

At the time of death, all the infected vervets that died at week 16 (Ver 609), week 20 (Ver 99) and week 27 (Ver 137) showed a classical granulomatous

reaction, some of these granulomas in one vervet (Ver 609) had coalesced to form large areas of mononuclear cellular infiltration.

4.5.7. Spleen.

All the vervets that died during the course of infection (Ver 609, Ver 99 and Ver 137) showed similarities in their histopathological findings. Generally, there was a depletion of periarteriolar sheath of lymphocytes. There was also a moderate infiltration with plasma cells and lymphocytic cells in the cords and sinuses of the red pulp. In addition, congestion of the red pulp was also evident. The vervets (Ver 99 and Ver 137) showed erythrophagocytosis by histiocytes. One vervet (Ver 99) showed, small and inconspicous splenic follicles due to the dilution by congestion and hyperplasia of histiocytic cells (Fig. 15).

4.5.8. Kidney.

Two vervets (Ver 99 and Ver 609) showed a mild lymphocytic infiltration adjacent to glomeruli and in between the tubules. Generally, there was a very mild interstitial nephritis (Fig.16).

The other organs and the control animals had indistinct histopathology.

4.5.9. Sykes.

The histopathological changes observed in the liver were very much similar to those of the vervet monkeys.

4.5.10. Liver.

At week 2 to 4 p.i., there were no evident changes. However, at week 6 to 10 p.i., the biopsies showed a few tiny foci of histiocytic cellular infiltration and few lymphocytes forming clusters scattered here and there. The bigger clusters had anything between 15 to 30 histiocytes, while other smaller aggregates had anything between 2 - 7 histiocytes. Some of these foci were periportal (Fig. 17).

At week 12 to 20 p.i., the reaction was much severe, there was a moderate to severe multifocal granulomatous hepatitis. The granulomas consisted of histiocytes, epithelioid cells, lymphocytes and a few plasma cells, (Figs 18 and 19). Some of these granulomas were adjacent to portal triads (Fig. 20). The centre of these granulomas had incorporated hepatocytes that were showing some degree of necrosis and their cytoplasma had lost their staining ability. As a result of the massive mononuclear cellular infiltration, there was a disarray of hepatic cords and a disruption of lobular pattern (Fig. 20).

In week 22 to 26 p.i., one syke (Syk 116) showed several foci of granulomas; one of these had inflammatory giant cells which were composed of nuclei resembling those of hepatocytes (Figs 21 and 22). In addition, the hepatocytes around the foci had undergone pressure atrophy and stretching as a result of the granulomatous reaction. Three sykes (Syk 205, Syk 198 and Syk 214)

showed tiny foci scattered here and there. These foci had mixtures of histiocytes, lymphocytes and some plasma cells; some of these foci were periportal.

At week 28 to 32 p.i., there was resolution of hepatic inflammation, there were few tiny foci scattered here and there, these foci were mainly lymphocytic. Generally, a mild focal hepatitis was evident. At sacrifice (week 32 p.i.) 2 sykes (Syk 205 and Syk 116) showed a mild non-suppurative lymphocytic hepatitis (Fig. 23).

4.5.11.Spleen.

Two syke monkeys that died during the course of infection (Syk 198 and Syk 214) showed a moderate plasma cells infiltration and congestion of the sinuses of the red pulp, the splenic corpuscles showed a reduction of lymphocytes and had no active germinal centres. In addition, some mononuclear phagocytic cells showed erythrophagocytosis (Fig. 24).

4.5.12.Kidney.

One syke (Syk 198) had a mild lymphocytic infiltration of the tubular interstitial tissue. In these areas, the tubules had been replaced by inflammatory cells (Fig. 25).

There was no remarkable histopathological changes in the control animals and other organs of the infected animals.

4.5.13. Detection of non-specific acid esterase in macrophages.

Due to the non-specific esterases the hepatocytes showed, it was not possible therefore to assess the quantitative response of the tissue macrophages within the liver. It was hoped that this particular staining technique would indicate a crude estimate of the involvement of macrophages in the pathological response of the liver and the subsequent occurence of self-cure as has recently been described by Githure *et al*. (in press).

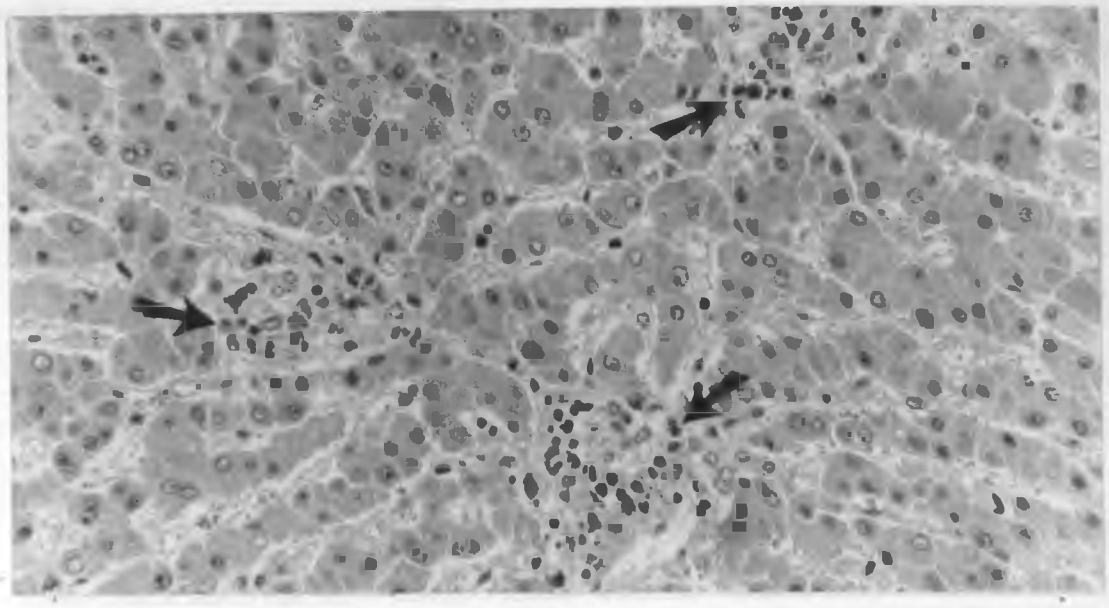


Fig. 10. Early forming histiocytic-lymphocytic nodules (arrows) in the liver of a vervet, (Ver 609) monkey infected with L.donovani

H&E x 250

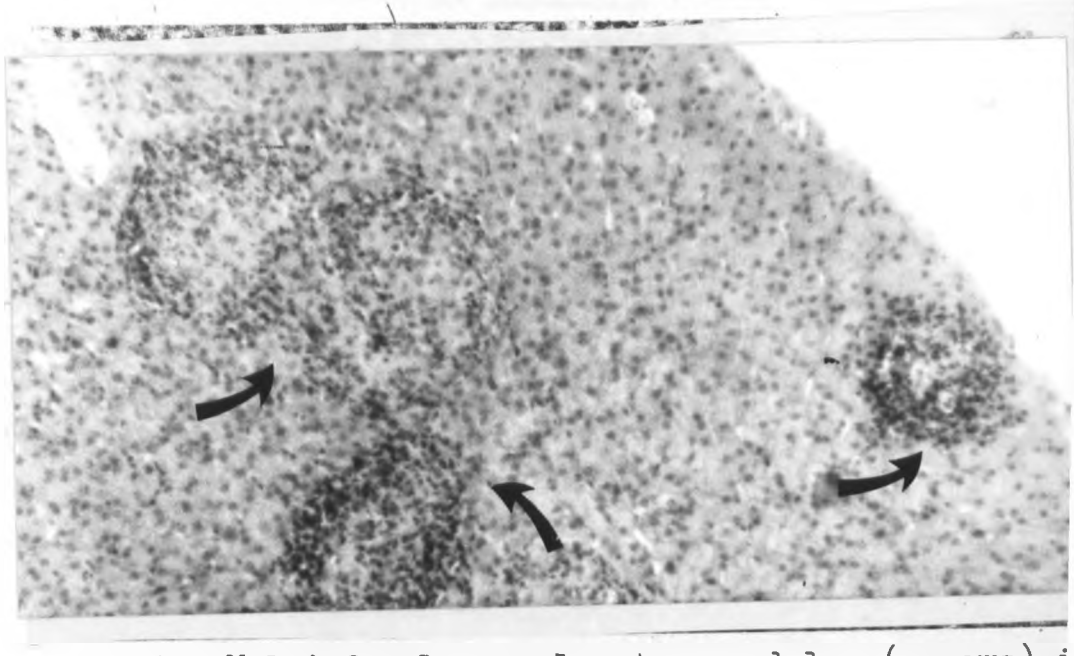


Fig. 11. Multi-focal granulomatous nodules (arrows) i the liver of a vervet (VerI87) monkey infected with L. donovani H&E X I00

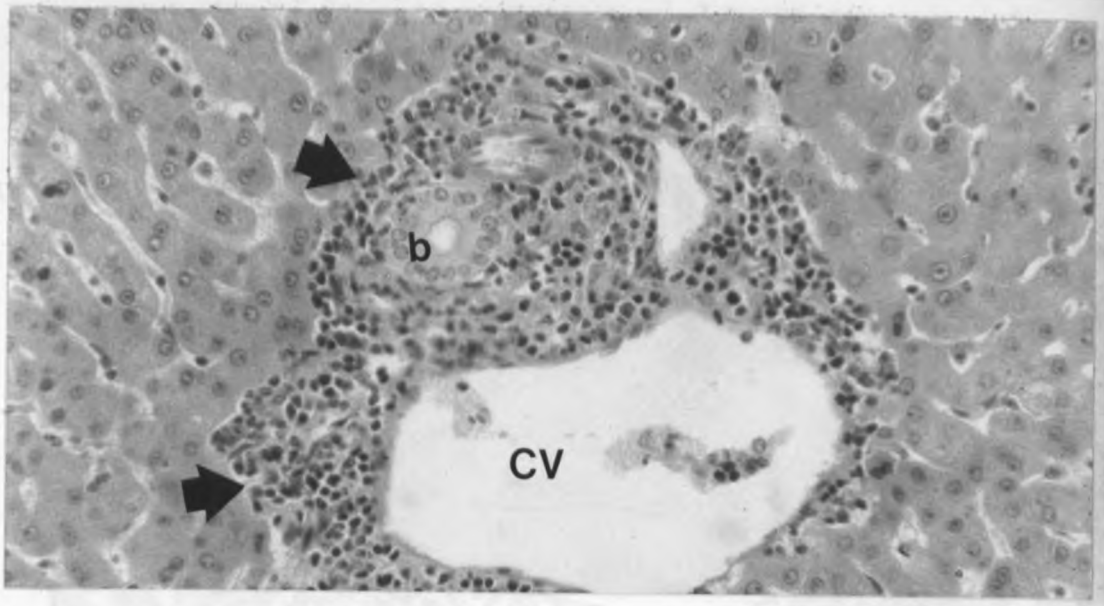


Fig. 12. A granulomatous reaction around a portal triad, showing a central vein (cv), a bile duct(b) and a mixture of inflammatory cells around the portal triad, (arrows) in the liver of a vervet (Ver I87) monkey infected with L. donovani . H&E × 250

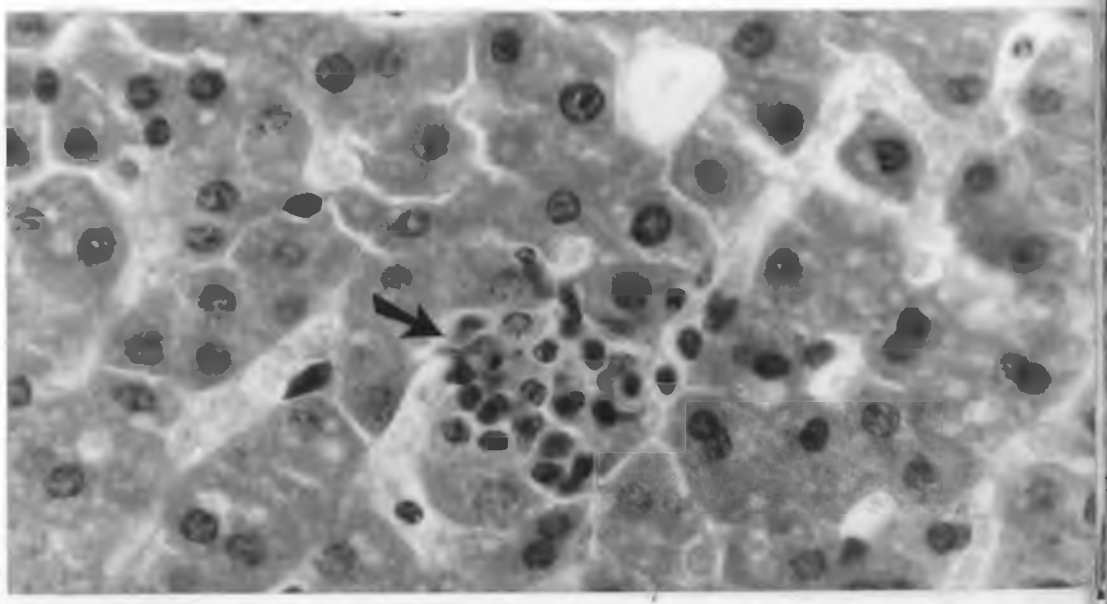


Fig. 13. A regressing granulomatous nodule (arrow) consisting of mainly lymphocytes (dark nuclei) in the liver sinusoids of a vervet (Ver I37) infected with L.donovani. H&E × 400

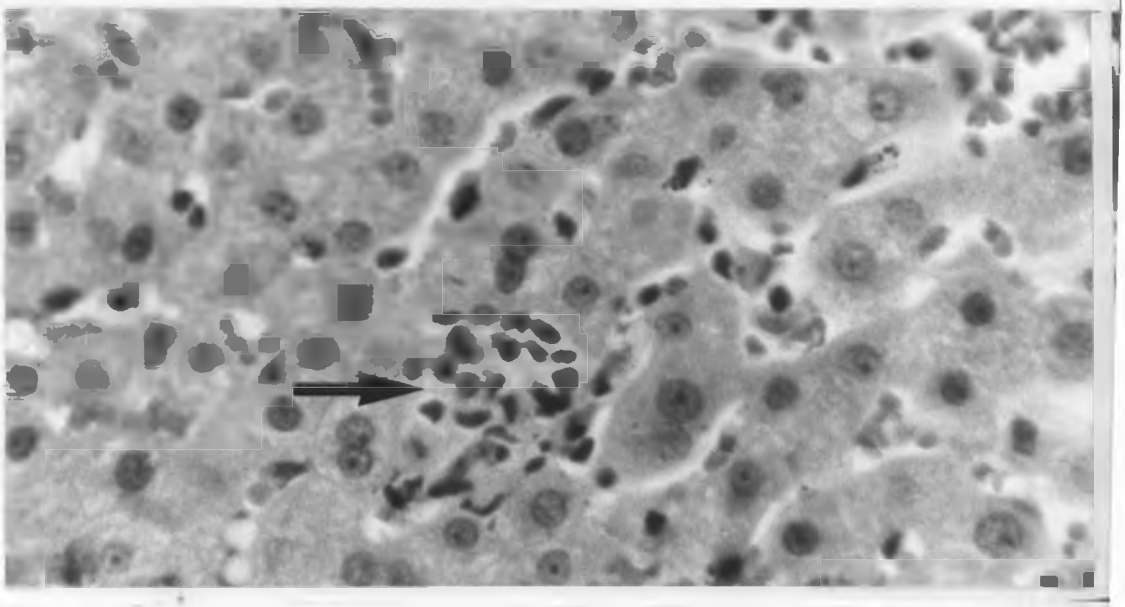


Fig. 14. A tiny lymphocytic nodule (arrow) during late stages inflammation in the liver of a vervet (Ver I37) monkey after L.donovani infection. H&E x 400

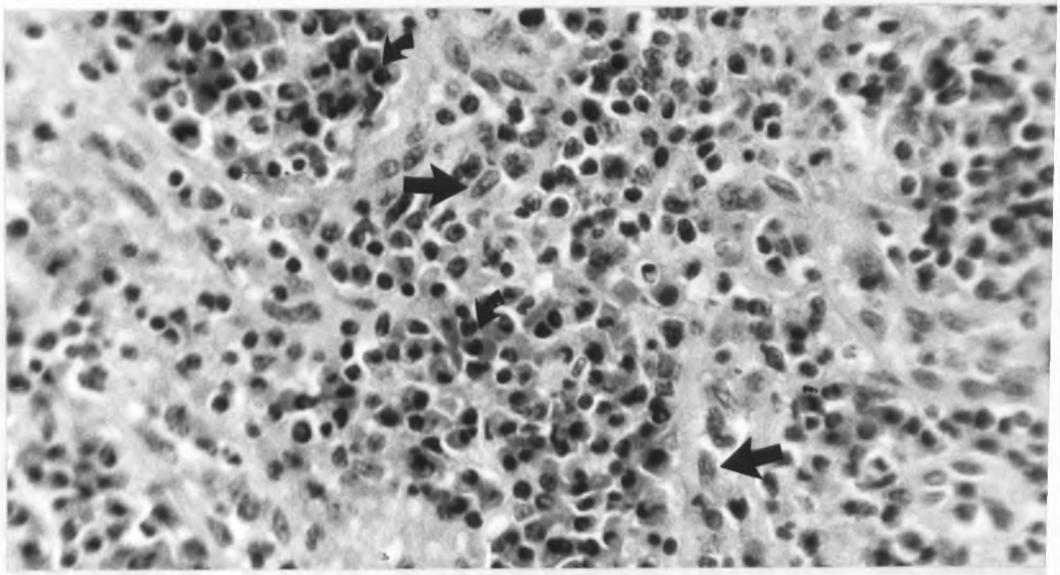


Fig. 15. Plasma cells (small arrows) and histiocyte (large arrows) infiltration in the sinusoi of the spleen red pulp of a vervet (Ver 99 monkey infected with L.donovani. H&E X400

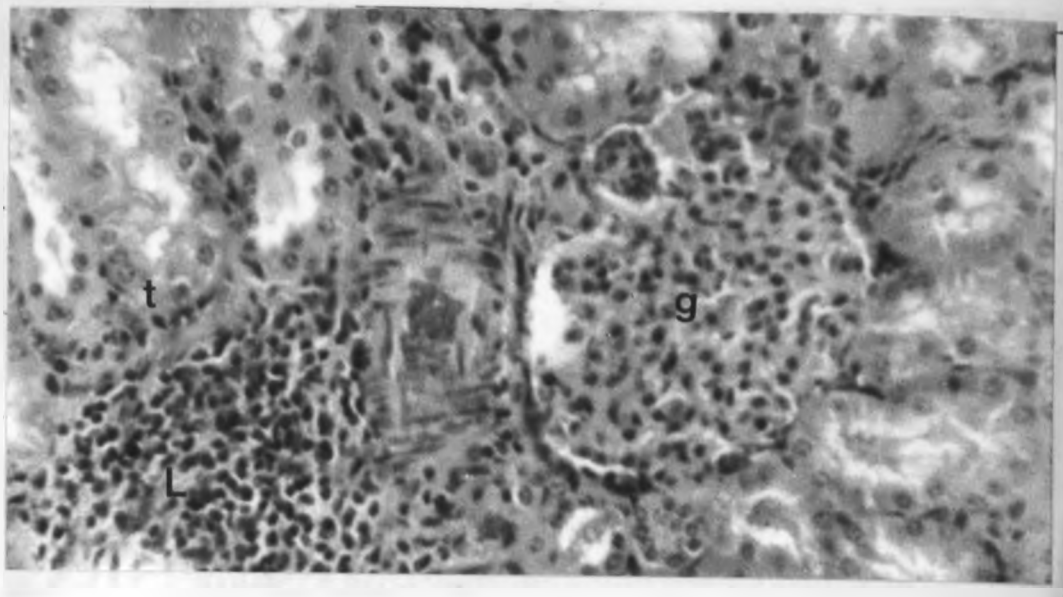


Fig. 16. A lymphocytic infiltration (L) in the interst tissue of the tubules (t) and adjacent to a glomeruli (g) in the kidney of a vervet (Ver monkey infected with L.donovani. H&E x 250

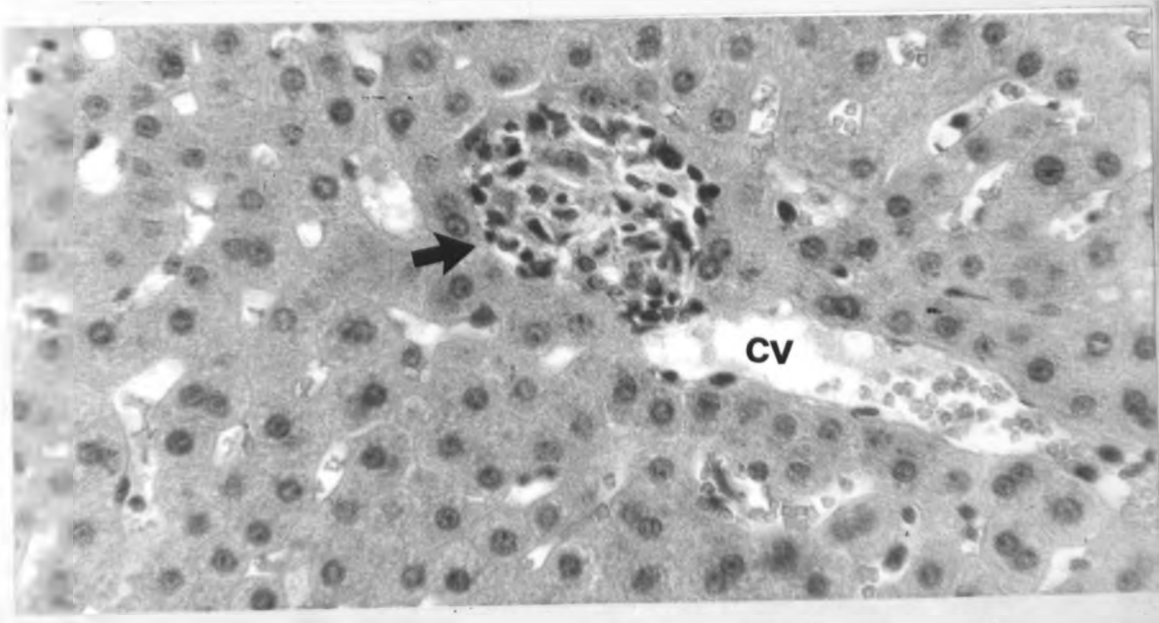


Fig. 17. An early forming histiocytic-lymphocytic granuloma (arrow) adjacent to a central vein (c.v) in the liver of a syke (Syk 205) monke infected with L.donovani. H&E X250

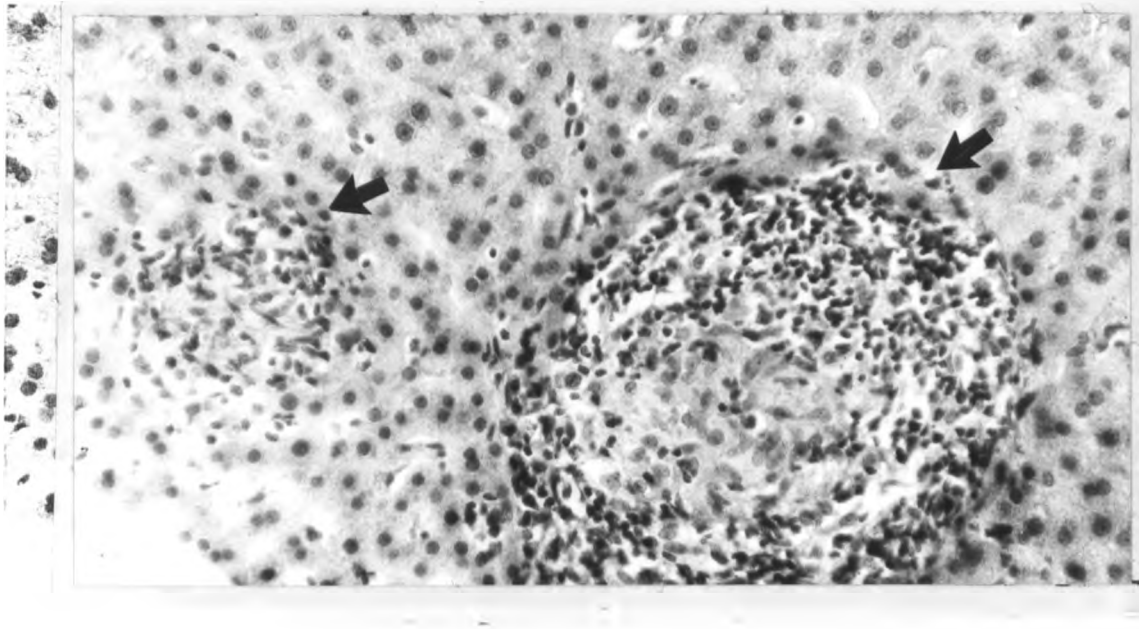


Fig. 18. Multifocal granulomas (arrows) consisting of mixed inflammatory cells in the liver of a syke (Syk I98) monkey. Note the interfearance of lobular pattern. H&E X 250

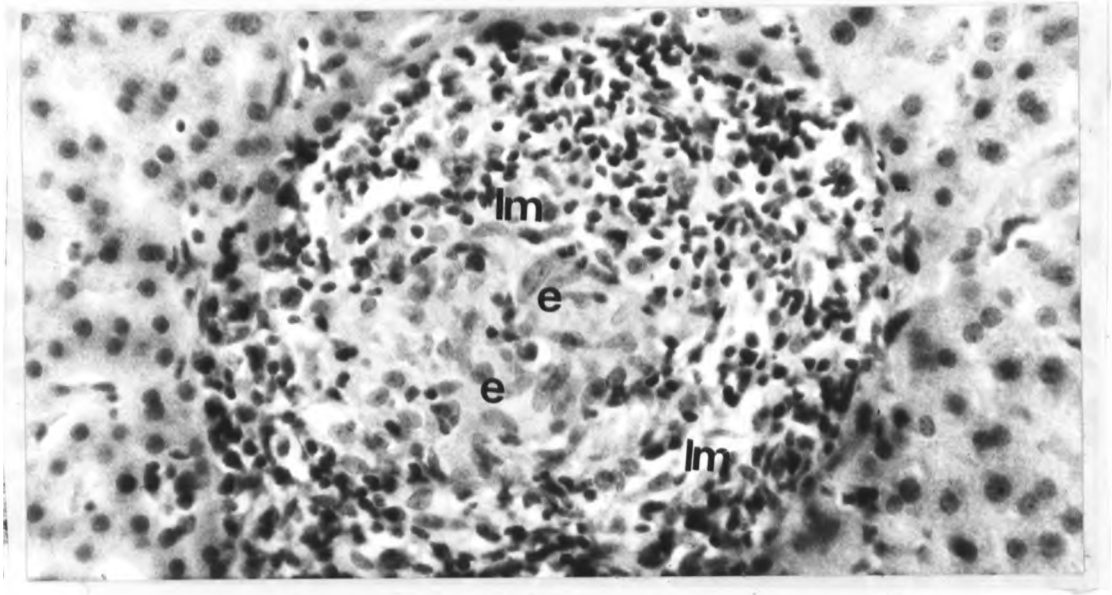


Fig. 19. A higher magnification of a granuloma consisting of epithelioid cells (e) at the centre and lymphocytic zone at the periphery (lm) in a liver of a syke (Syk I98) monkey. H&E X 400

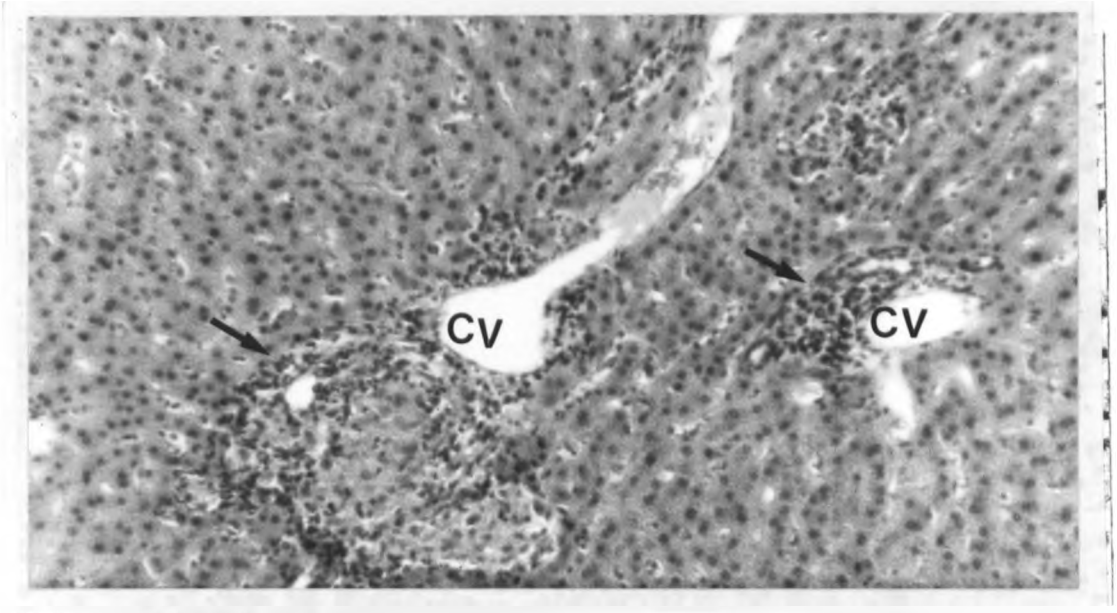


Fig. 20. A mononuclear cellular infiltration (arrows) adjacent to a portal triad and central vein (c.v.) in the liver of a syke (Syk 2I4) monke
H&E X IOO.

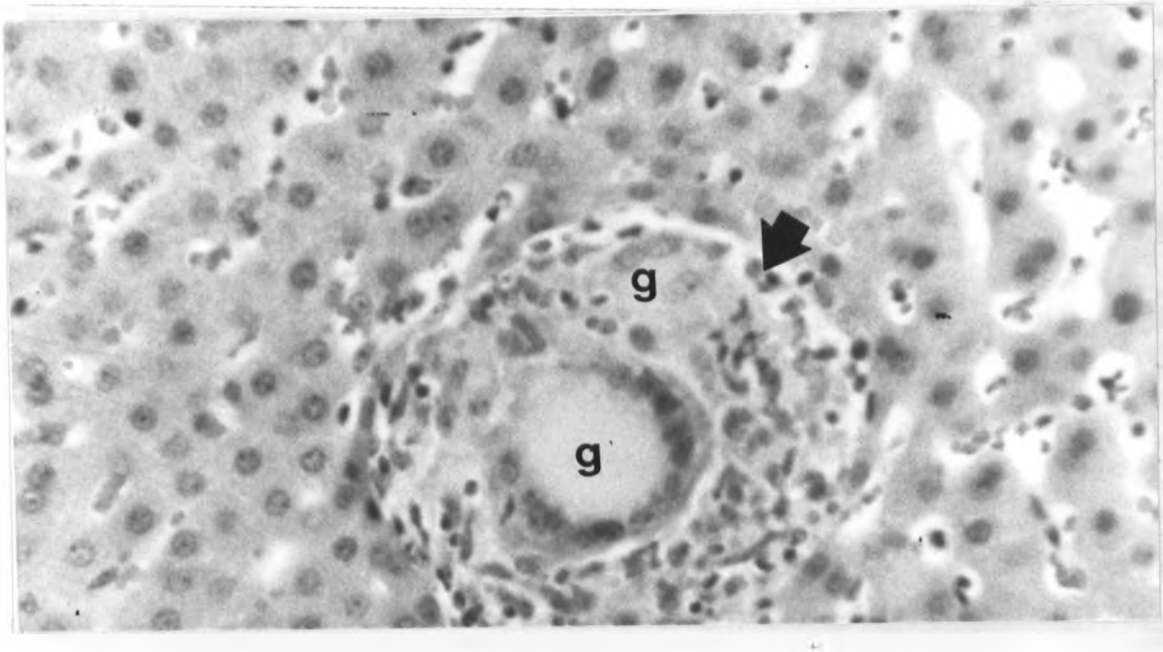


Fig. 21. A granulomatous nodule (arrow) consisting of mixed inflammatory cells at the periphery and giant cells (g) in the centre of the granulom in the liver of a syke (Syk II6) monkey. H&E X

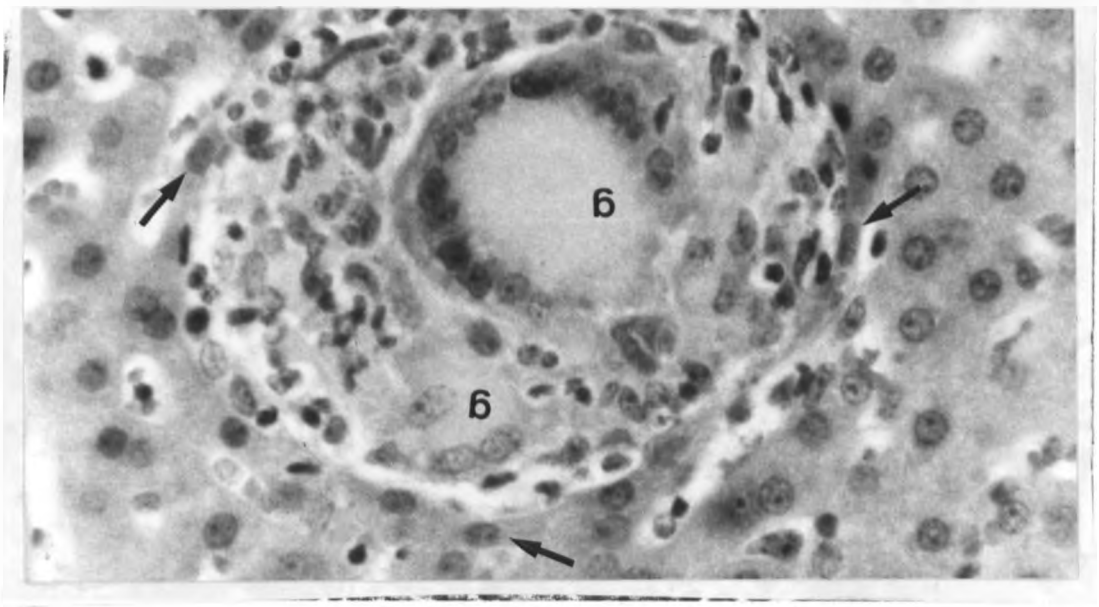


Fig. 22. A higher magnification of a granuloma (Fig. 21) consisting of giant cells (g) in the centre. The hepatocytes at the periphery of the reaction are undergoing stretching (arrow). H & E x 400.

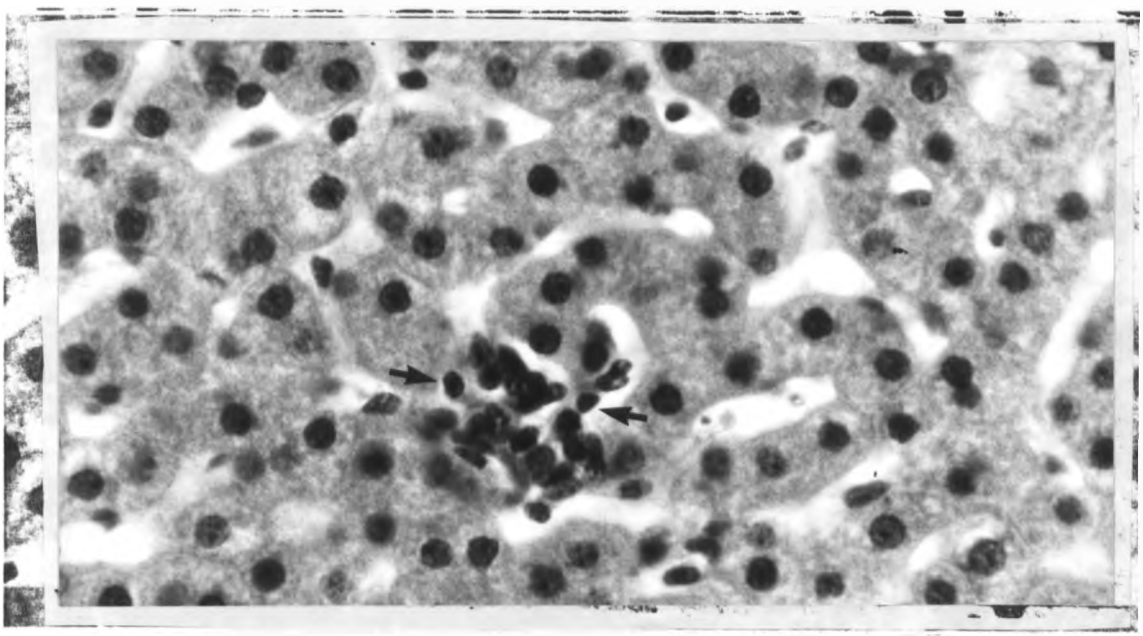
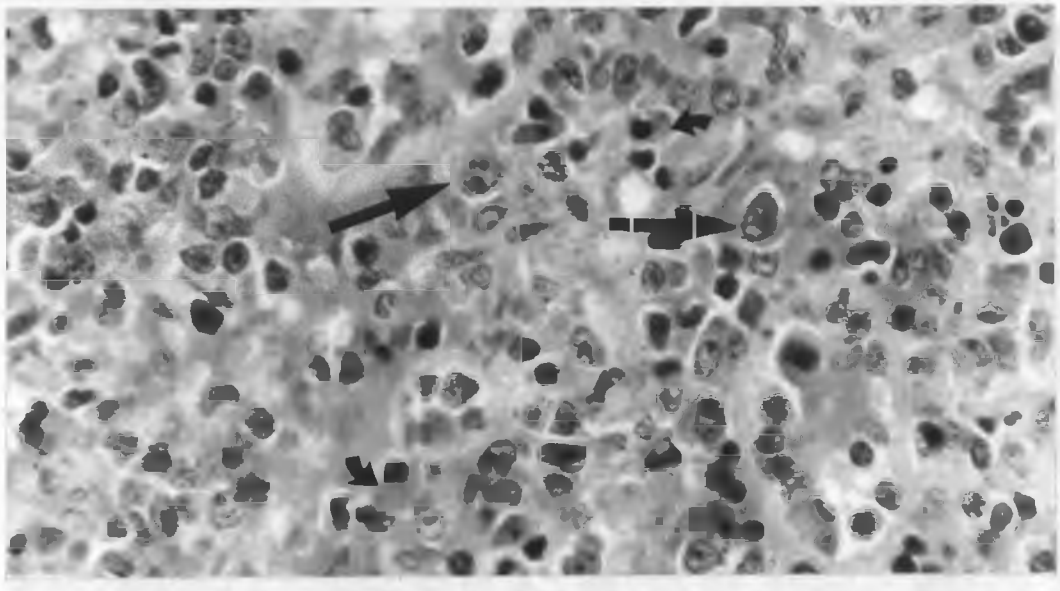


Fig. 23. A sinusoidal lymphocytic (arrows) cellular infiltration during late stages of infection in the liver of a syke (Syk 205) monkey. H&E X400



Figs 24 . A moderate plasma cells (small arrows) and histiocytes (large arrows) infiltration in the sinusoids of the spleen red pulp of a syke (Syk 205) monkey. H&E X400

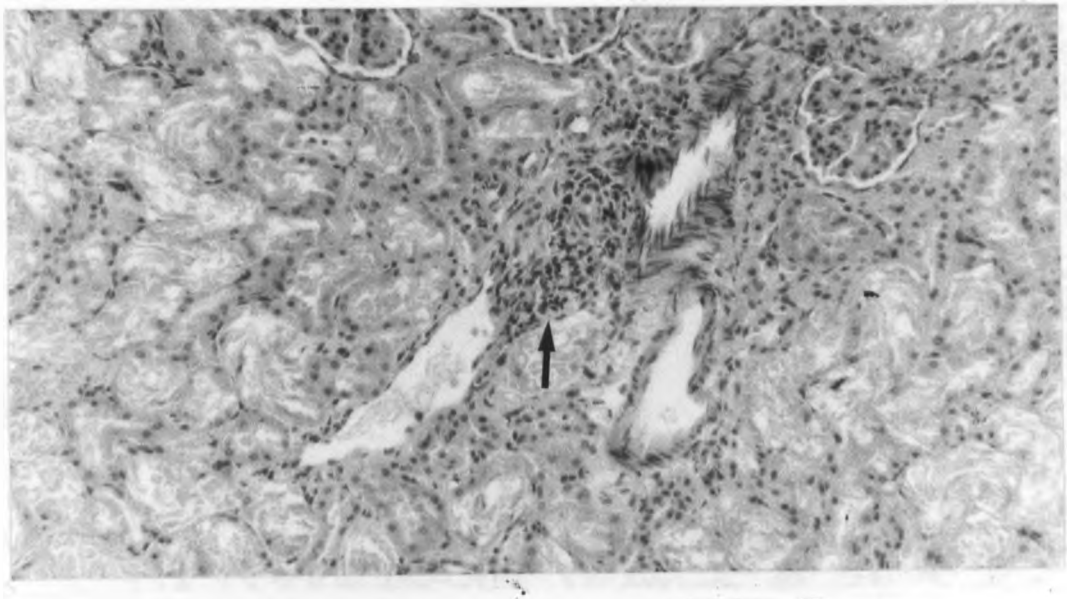


Fig. 25. A mild lymphocytic (arrow) infiltration in the interstitial tissue of the kidney of a syke (Syk I98) monkey. H&E X I00

5. Discussion and conclusion.

5.1. Discussion.

The presence of anaemia and agranulocytopaenia is almost invariable in established visceral leishmaniasis (Woodruff et al., 1972; 1973; Musumeci et al., 1976). Several theories and explanations have been suggested to explain the anaemia that is commonly observed in visceral leishmaniasis patients. The precise causes of these cytopaenias are less understood, little studied, and probably complex and many. The theory of bone marrow overcrowding due to reticuloendothelial proliferation and toxic depression as a result of parasite toxins does not offer satisfactory explanation (Cartwright et al., 1948; Knight et al., 1967). Thakur et al. (1981) demonstrated the relation of anaemia to the duration of the disease. However, Henderson (1937) in the Sudan had earlier reported that the most extreme cases of anaemia were seen in the most acutely sick patients, but not necessarily after a long illness.

Van Peenan (1962) and Mallede Maru (1979) reported a normocytic, normo - or hypochromic anaemia in visceral leishmaniasis cases from the Sudan and Ethiopia. In addition Kasili (1980) reported normal sized cells with polychromasia, microcytosis and poikilocytosis in Kenyan visceral leishmaniasis patients. Most investigators on the disease agree that the anaemia

is normocytic and hypochromic (Moskovsky and Southgate, 1971). Other workers (Rigg, 1957; Veress et al., 1977; El-Hassan, 1981) attributed the anaemia to the erythrophagocytosis seen in histological sections of the spleen and suggested an autoimmune aetiology. Chatterjea and Sen Gupta (1970) demonstrated anti-red cell antibodies and a positive Coomb's test and suggested an autoimmune mechanism. This was further supported by Woodruff (1972).

The reasonable well established factor contributing to anaemia in visceral leishmaniasis is haemolysis (Woodruff et al., 1972; 1973) as demonstrated by the markedly reduced T50. The haemolysis is possibly of immune origin with positive complement reacting direct Coomb's (Musumeci et al., 1976). However, Burchenal et al. (1947) and Turkman (1949) had described visceral leishmaniasis in patients who finally became cured after splenectomy and concluded that there was a haemolytic anaemia with pooling and increased destruction of red and white cells in the spleen; and that a correlation existed between rapid decrease in size of the spleen during treatment and the occurrence of leucocytosis. Hypersplenism or an important role of the spleen in pancytopaenia was further supported by results of splenectomies (Rigg, 1957; Manson-Bahr, 1959).

Kharazmi et al. (1982) investigated the presence of immune complexes in serum, and the existence of immunoglobulin and complement on the surface of red

blood cells of ten anaemic visceral leishmaniasis patients. They detected IgG, C3b and C3d on the surface of red blood cells of some of the patients, suggestive of an immune aetiology of anaemia. In addition, Abdalla *et al.* (1983) reported C3, C4 and IgG on the red cells of Kenyan patients and questioned the significance and clinical implication of these findings. O'Daly and Aso (1979) demonstrated the production of haemolysis by *Leishmania* which could be of relevance and possibly requires further exploration. However, Kager and Rees (1983) demonstrated the presence of various antibodies in Kenyan Kala-azar patients but argued against the absence of a direct correlation between haematological values and presence or absence of antibodies and/or their titres. They therefore, argue against a primary role of these antibodies in the causation of the pancytopaenia.

Keenan *et al.* (1984a) reported a normocytic and normochromic anaemia of increasing severity in German Shepherd dogs infected experimentally with visceral leishmaniasis using *L. donovani* and *L. chagasi*.

In the present study; the anaemia observed in the monkeys was normocytic similar to the findings reported by Keenan *et al.* (1984a), in dogs.

The demonstration of erythrophagocytosis in histological sections of the spleen lends additional morphological support to the findings of autoimmune haemolytic anaemia in visceral leishmaniasis. Similar observations were noted in visceral leishmaniasis patients in the Sudan (EL-Hassan et al., 1981). In order for complement to be bound, an antigen-antibody complex should be present on the red blood cell surface and once the complement sequence is activated, haemolysis occurs and the damaged cells are removed in the spleen (Decker-Jackson and Honigberg, 1978). In this regard, a similar situation could also have taken place in the infected monkeys under the present study. However, no haemolysis was observed to allow the above hypothesis to fully fit the two.

Leucopaenia due to granulocytopaenia, although reported by several workers is still poorly understood. Rogers (1907) in India considered the changes in WBC much more characteristic and important than those in the red blood cells. In Mediterranean countries and Sudan, leucopaenia and an increase of mononuclear cells was observed to be less constant and to be of limited value in the diagnosis of leishmaniases (Bousfield, 1911; Wenyon, 1912). The myelodepression theory suggested by Menon (1939) and Kuroya et al., (1939) is no longer acceptable (Adler, 1940; Cartwright et al. 1948). The leucopenia observed in Kala-azar patients

has been attributed to the degree of splenic enlargement or hypersplenism (Cartwright et al., 1948) which was supported in further publications from China (Chung, 1953). In addition, several other reports on splenectomized patients have suggested that the spleen plays an important role in the development of leucopaenia (Burchenal et al., 1947; Das and Sen Gupta, 1950; Manson-Bahr, 1959; Bada et al., 1979). Immune mechanisms have also been implicated as the cause of leucopaenia (Chatterjea and Sen Gupta, 1970). However, the obsevations that haematological improvement proceded the fall in the level of circulating immune complexes seemed to argue against a major role of immune mechanisms and in the development of leucopaenia (Kager and Rees, 1983). Keenan et al.(1984a) reported leucopaenia in German shepherd dogs experimentally infected with visceral leishmaniasis.

Leucopaenia was observed in the majority of the infected monkeys in this study and in some animals it was mostly severe with values going below 2.6 x 10^3/ul WBC. Generally, the haemotological changes in this study are close to what is observed in human visceral leishmaniasis patients i.e. normocytic, normo-or hypochromic anaemia and leucopaenia.

It was not possible however, to ascertain the exact causes of anaemia and leucopaenia in the infected monkeys. However, the erythrophagocytosis observed

suggest an autoimmune aetiology for the anaemia and leucopaenia observed in this study. Autoimmunity in visceral leishmaniasis has been described previously (Chatterjea and Sen Gupta, 1970; Kharazmi et al. 1982).

The haematological changes observed in this study resembled closely what is described in both human subjects and experimental animals. Therefore, the occurence of anaemia and leucopaenia in these two species of monkeys maybe utilized in establishing the exact causes behind the haematological changes observed in visceral leishmaniasis subjects.

It is well known that the liver is commonly enlarged in kala-azar, though this does not necessarily mean. there is any hepatic dysfunction. The pathological changes that occur in the liver, such as proliferation of Kuppfer cells, mononuclear cell infiltration together with alteration of lobular pattern has been reported in many pathological studies in both human subjects and experimental animals. The pathological changes that occur in the liver of Kala-azar cases indicate that the liver is markedly involved in the disease process. Chakravarty et al. (1949) in a study of hepatic function in Kala-azar reported 6 positive hepatic function tests in a high proportion of Kala-azar cases. He suggested that their being positive was an indication of hepatic damage and the possible explanation of certain clinical features observed in Kala-azar. In addition Al-Saffar

and Al-Mudhafer (1979) reported 40% of the 49 Iraqi Kala-azar patients to have elevated SGPT levels and 90% with raised SGOT levels. Low albumin and increased globulin concentrations are known features of visceral leishmaniasis (Manson-Bahr, 1982). Kager and Rees (1983) also reported biochemical values which were slightly above normal in 14 visceral leishmaniasis patients, with 3 of the patients having raised alkaline phosphatase values. Keenan et al. (1984a) in a clinical pathological study of visceral leishmaniasis in experimentally infected German shepherd dogs found high globulin levels, normal SGPT, SGOT levels and significantly low tryptophan levels.

Biochemical tests in the present study did not give remarkable results. However, there was an increase in alkaline phosphatase levels in both syke and vervet monkeys to 111.3 $\pm$ 17.0 and 126 $\pm$ 33.28 IU/litre respectively. These values were much higher than those of the uninfected controls whose values never exceeded 90 IU/litre. This increase in alkaline phosphatase values in both species of monkeys maybe indicative of liver damage as evidenced and supported by histopathological sections of liver biopsies and of other tissues collected at post mortem.

Hyperglobulinaemia and hypoalbuminaemia which is characteristic of the human and canine disease, developed in the majority of the infected monkeys. It was previously believed that this increase in

globulins (gamma globulins) was not productive or protective (Zuckerman, 1975; George et al., 1976). However, more recent work has suggested that complete cure and immunity depend on both antibody production and T-cell response (Poulter, 1980). Furthermore, the presence of high globulin levels observed in this study signifies in part, the response of the animals to antigenic stimulation and a humoral response to the infection.

Although the precise manner by which specific T. lymphocytes play a role in the elimination of Leishmania parasites is still unknown, one possibility is that activation by Leishmania antigens, specific T. cells might release lymphokines responsible for macrophage activation resulting in parasite destruction. Furthermore, the formation of certain anti-Leishmania antibodies has been demonstrated to be under the influence of T. cells, (Belehu et al., 1980).

Bray (1974) showed that anti-leishmanial antibody, or leishmanial antigen - antibody complexes, may assist in cytotoxicity for Leishmania infected macrophages in tissue culture. It could therefore be suggested that antibody might play an important role in conjunction with cell mediated immunity in the self-cure observed in some of the infected monkeys in this study. More sykes showed self-cure than did the vervets, but the significance of this cannot heavily be considered considering the few number of experimental animals that were used.

The pathological changes as a result of visceral leishmaniasis in human patients and experimental animals has been well documented (Shortt, 1923; Meleney, 1925; Smith, I933; Kirk, I945; I956; Manson-Bahr, I959; Manson-Bahr and Apted, I982). It is now universally accepted that hepatosplenomegaly is characteristic of the disease (Oomen, I970; Manson-Bahr and Apted, I982).

In addition, lymphadenopathy has also, been described by a number of workers (Angevine et al., 1945; Bell et al., 1958; Sen Gupta and Chatterji, 1961). Shortt (1923) described hepatosplenomegaly in monkeys (Macacus rhesus) experimentally infected with Indian Kala-azar, and Smith (1933) observed cancrum oris in a monkey infected subcutaneously with amastigotes of L. donovani. In a naturally infected vervet monkey in the Sudan where leishmania parasites were demonstrated, Kirk (1956) observed hepatomegaly. Manson-Bahr (1959) described a leishmanioma, a skin reaction that precedes the visceralization of the disease.

While searching for new experimental host-of L. donovani, Sati (1963) observed splenomegaly in bush-babies (Galago senegalensis senegalensis) experimentally infected with L. donovani. Other reports of hepatosplenomegaly in other experimental animals infected with L. donovani have been reported in the owl monkey, (Aotus trivirgatus) (Chapman et al., 1981b) and in the opossum (Didelphis marsupialis) (Hanson and Chapman, 1980). Furthermore, Keenan et al. (1984b) described hepatosplenomegaly and lymphadenopathy in German shepherd dogs

experimentally infected with visceral leishmaniasis. However, Marsden et al. (1981) in an attempt to evaluate the marmoset (Callithrix jacchus jacchus) as a model for studies of American visceral leishmaniasis, observed the absence of hepatosplenomegaly and the human Kala-azar syndrome despite the animals becoming infected. In the present study, using vervet and syke monkeys experimentally infected with L. donovani splenohepatomegaly was observed in those monkeys that died during the course of infection. The enlargement of these organs was as a result of reticuloendothelial hyperplasia as evidenced by the histopathological changes of the liver and spleen. It therefore appears that in these animals, the observed Kala-azar syndrome of hepatosplenomegaly was in agreement with what other workers have described in non-human primates and in the German Shepherd dog (Shortt, 1923; Sati,1963; Chapman et al., 1981a, Keenan et al., 1984b).

All the histopathological studies on visceral leishmaniasis, whether in experimental animals or in human cases have called attention to the marked parasitic invasion and hyperplasia of the reticuloendothelial organs, including the liver, spleen, lymph nodes and bone-marrow (Meleney, 1925; Goha, 1932; Manson-Bahr and Apted, 1982). Undoubtely, this is the most important and characteristic feature of the histopathology of this disease as confirmed in the studies reported here.

In his histopathological studies, Christophers (1904) demonstrated the affinity of the parasite for

endothelial tissues of the spleen and the liver where they underwent intracellular multiplication followed by rupture of the infected cells and invasion of the neighbouring cells. These observations were made by Meleney (1925), Menon (1939) and Manson-Bahr and Apted (1982); and indeed they were in agreement with observations made here. In addition, Hu (1933) reported an increase of plasma cells to L. donovani bodies and suggested their possible role in detoxification. The liver is greatly involved in resistant cases and in inapparent or asymptomatic infection, sarcoid like-granulomatous nodules are scattered throughout this organ. In an out-break of human visceral leishmaniasis in Italy, Pampiglione et al. (1974a) observed epithelioid granulomata from all liver biopsies. They considered the histological picture in the liver to be very characteristic with intralobular granulomatous nodules that varied in number, but always multiple. These rounded nodules had accumulations of histiocytes, lymphocytes and epithelioid cells.

In a vervet monkey experimentally infected with L. donovani, numerous scattered granulomas were observed in liver biopsy material examined histologically (Manson-Bahr and Wilson, 1976). Githure et al. (in press) reported similar histiocytic nodules in vervet and syke monkeys. In the Sudan, El-Hassan (1981) described liver granulomas consisting of epitheliod cells, lymphocytes and plasma cells in visceral leishmaniasis patients. In

the present study, hepatic granulomas consisting of histiocytes, lymphocyte, epithelioid cells and plasma cells were observed and this is in agreement with similar findings that have been made previously by other workers and reported in asymptomatic cases of visceral leishmaniasis in Italy, (Pampiglione et al., 1974a), and in liver biopsies of American visceral leishmaniasis subjects, (Rodrigues et al., 1961). In a recent study, Githure et al. (in press) reported histiocytic nodules in some East African primates.

The multiple hepatic granulomas seen in liver biopsies during the course of infection similar to those reported here, have been described in cryptic or asymptomatic forms of visceral leishmaniasis (Pampiglione et al. (1974a). In the syke and vervet monkeys used in the study reported here, there was reduction in size of the granulomas and resolution of hepatic inflammation with the appearance of lymphocytic infiltration during late stages of infection. These morphological changes lend support to a self-curing disease during experimental infection in these monkeys with L. donovani. In addition, the presence of plasma cells in hepatic granulomas and in splenic sinusoids signifies the response of the host to antigenic stimulation. Plasma cells also refered to as B-cells, are known to play an active role in antibody production in response to infections. In this context, it is suggested that these East African primates could be useful in immunopathological investigations, and

could serve as models for the self-cure phenomenon or cryptic infections of visceral leishmaniasis as previously described by several workers in some human subjects within Eastern Africa (Manson-Bahr, 1967; Hoogstraal and Heyneman, 1969; Cahill, 1970; Heyneman, 1971), and in Sicily (Pampiglione et al., 1974b). Consequently, the possible existence of this phenomenon in these two species of monkeys, under the present study may be of epidemiological significance since one naturally infected vervet monkey has previously been reported by Kirk, (1956) in the Sudan.

An immuno-histochemical study incooperating an in vivo (Leishmanin skin test) and an in vitro (lymphocyte transformation) correlates of cell-mediated immunity may help in elucidating the mechanisms behind the previously reported self-cure, which has been documented by several workers (Napier and Das-Gupta, 1930; Corkhill, 1948; Berbarian, 1959; Pampiglione et al., 1974a). In addition, the study of self-cure may offer possible explanations for the high incidence of leishmanin sensitivity found in the endemic areas of visceral leishmaniasis in Kenya and the Sudan (Manson-Bahr and Southgate, 1964; Heyneman, 1971).

The changes in the spleen with atrophy of the splenic follicles in Kala-azar has been described previously (Meleney, 1925; El-Hassan, 1980; 1981). The erythrophagocytosis may explain in part the anaemia observed in some of the infected animals during this

study. However, auto-immune haemolytic anaemia has previously been described in Kala-azar patients by some other workers (Chatterjea and Sen-Gupta, 1970; Woodruff, 1972).

The morphological changes in the liver and spleen of the infected animals used in this study are considered to be due to visceral leishmaniasis since control animals had no observable gross or even histopathological changes in the biopsy materials examined before, during and at termination of experiment.

There has been several previous attempts to experimentally infect non-human primates to find newer experimental hosts other than rodents. These studies have been carried out using both New and Old World primates. Shortt (1923) inoculated M. rhesus with splenic amastigotes derived from a human patient, and described an acute disease with demonstration of the parasites in smears from liver puncture within 38 days post-infection. Smith (1933) reported Cancrum oris and demonstrated the presence of the parasites within 4 months after infection of Macaca irus with L. donovani amastigotes derived from a human spleen. In another attempt, Macaca sinicus became infected with L. donovani with the parasite being demonstrated within 52 days p.i. after producing localized lesions on the forehead (Korke, 1927). In the Sudan, Archibald and Mansour (1937) inoculated monkeys via the intradermal route and by intranasal swabbing, they demonstrated the parasites by 60 and 80 days p.i., from the

enlarged spleens. In the same study, utilizing 4 clean and 4 infected monkeys kept together, these workers reported Kala-azar from 2 of the clean ones within 85 and 87 days p.i.

Kirk (1942) had variable results from monkeys inoculated with Leishmania. However, he demonstrated leishmaniasis in a vervet monkey experimentally infected with saline emulsion from a patient nasal secretion. In another experiment, Kirk (1945) reported Espundia (mucocutaneous leishmaniasis) in a vervet monkey experimentally infected with L. donovani. In an attempt to identify reservoir hosts of Sudanese-leishmaniasis, Kirk (1956) examined baboons, red hussar monkeys (Erythrocebus patas) and colobus monkeys (Colobus polykomos) for leishmania parasites with negative results. However, he found one vervet monkey originating from an endemic area of Kala-azar to be positive with Leishmania and suggested a natural infection.

Sati (1963) described bush babies (Galago senegalensis senegalensis) to be highly susceptible to experimental intraperitoneal infection and demonstrated parasites from spleen, liver, bone-marrow, and peripheral blood by 15 weeks p.i.; he suggested their possible use as models of visceral leishmaniasis. Hanson and Chapman (1980) reported the opossum (Didelphis marsupialis to be highly susceptible to L. donovani and demonstrated parasites from various internal organs 19 days p.i.

In other studies utilizing New World primates, Chapman et al. (1981a) described the squirrel monkey (Saimiri sciurea) to be susceptible to experimental infection and found parasite numbers of the liver and spleen to be in excess of the initial inoculum dose. In a similar study utilizing the owl monkey (Aotus trivirgatus), (Chapman et al., 1981b) described it's susceptibility with high parasite densities of the liver and spleen as early as 24 days p.i. These 2 New World primates have since been used in further chemotherapy studies (Chapman et al., 1983; Madindou, 1982). Other experiments describing the infectivity and the usefulness of the marmoset (Callithrix jacchus jacchus). have also been described (Marsden et al., 1981).

The present study has reported the susceptibility of the vervet and syke monkeys to infection with culture derived promastigotes of L. donovani, producing a disease ranging from moderate, (cryptic or self cure) to a fatal disease with hepatosplenomegaly, leucopaenia and anaemia. Monkeys infected with promastigotes intravenously allowed a uniform parasite visceralization from the site of infection as evidenced by positivity of the bone-marrow, peripheral lymph nodes and some internal organs. This suggested a haematogenous dissemination of the parasites resulting with visceral localization. In addition, both the vervet and syke monkeys were capable of supporting amastigote replication in the liver and the spleen during a duration of 3 - 4 months.

The incubation periods of 22 - 35 days in vervet monkeys and 46 - 54 days in syke monkeys observed in this study resemble closely what has been described in Macacus rhesus and Macacus sinicus with incubation period of 38 days and 52 days respectively (Shortt, 1923; Korke, 1927). Furthermore, the positivity of the liver, spleen, lymph nodes and the bone marrow suggestive of a generalized dissemination with visceral localization has been reported in the bush baby (Sati, 1963) and in the opossum, Didelphis marsupialis resulting to a fulminating visceral disease, (Hanson and Chapman, 1980).

The use of promastigotes capable of producing an infection, may be of value in infectivity studies, since previous experiments in non-human primates have widely used hamster spleen amastigotes (Shortt, 1923; Smith, 1933; Sati, 1963; Chapman et al., 1981a; 1981b; 1983).

The variation in the disease pattern in individual animals may have a genetic explanation (Bradley and Kirkley, 1977). On speculation, this could then explain why some experimental animals died while others self-cured.

The early demonstration of the parasite in the liver and later the spleen during very early stages of infection signifies the ability of the parasite to produce an infection with a short incubation period. Therefore, a weekly or bi-weekly collection of organ aspirates for the demonstration of parasites is necessary in giving information related to the

incubation period and levels of parasitaemia. In this context, early detection of parasites with high peak levels in the liver could be of diagnostic value (Napier, 1927). Rodrigues and Padla (I96I) recommended the use liver puncture for the diagnosis of Kala-azar in cases without splenomegally, or where splenic puncture was not advisable.

Although the opossum and the owl monkey (Hanson and Chapman, 1980; Chapman et al., 1981b) are the most satisfactory non-rodent experimental hosts currently available for studies on visceral leishmaniasis, the high degree of susceptibility of these animals to L. donovani suggest that they may not be ideal for all types of studies on leishmaniasis. Thus, continued efforts were made to identify non-rodent hosts with potential use in experimental visceral leishmaniasis.

A succesful infection in vervet and syke monkeys and the more protracted course of infection in these 2 species may make them laboratory models superior to the opossum in such studies as preclinical testing of new anti-leishmanial compounds. In addition, the occurrence of parasitological cure that has been reported in monkeys previously (Kirk, 1945; Githure et al., in press), is of significance. As described earlier, the presence of parasitological cure in human subjects has been documented from Eastern Africa and Italy. Subclinical cases are thought to outnumber clinical cases by about 5:1 (WHO technical report, series 701, 1984). Thus, a study

initiated and specifically designed in solving the self-cure phenomenon will throw light and possible explanation for the subclinical cases; and in addition to explaining the high rates of leishmanin positivity observed in endemic areas in subjects with no clinical disease (Leeuwenburg et al., 1983).

5.2. Conclusion.

Within the limits of the experimental procedures employed, and the results obtained; the conclusions that can be drawn from this study are as follows:

1. Both vervet and syke monkeys are susceptible to infection with promastigotes of *L. donovani* when administered intravenously.
2. The infection in both species produces uniform visceralization and dissemination of the parasites resulting to leucopenia and anaemia both of which could have an autoimmune aetiology.
3. Promastigotes administration of both species produces liver damage with hepatosplenomegaly, and Sarcoid-like granulomatous nodules in the liver.
4. The infection produces death in some monkeys and self-cure in surving monkeys, resembling closely human sub-clinical cases.
5. The disease has a similar pattern in syke and vervet monkeys, but vervets have a shorter incubation period and time to death.

In general, these non-human primate species are readily available in Kenya and could be utilized for studying various aspects of visceral leishmaniasis and the self-cure phenomenon.

APPENDICES

APPENDIX I (MEDIA)

Schneiders drosophila medium.

This media is prepared as outlined below:-

Solution A.

The contents of 1 packet (21 gm) of Schneiders drosophila medium (Gibco Labs, Grand Island, N.Y) was placed into a 1 litre beaker containing 500 ml distilled water. The beaker was placed on a magnetic stirrer and contents stirred at room temperature until completely dissolved.

Solution B.

0.1 gm of L.-Cystine anhydrous (Sigma Chemical Co. St. Louis, MO.) was placed into a 200 ml beaker containing 40 ml distilled water. The beaker was placed on a hot plate magnetic stirrer and contents stirred. While stirring, 3N HCl was placed into the beaker drop by drop until all the particles are completely dissolved.

Solution C.

0.5 gm of L - Tyrosine anhydrous (Sigma Chemical Co. St. Louis, MO.) was placed into a 200 ml beaker containing 40 ml of distilled water. The contents were stirred on a magnetic stirrer while adding drop by drop 3N KOH until all particles dissolved and solution was clear.

Solution D.

2.0 gm of Yeastolate powder (Difco Labs, Detroit, Michigan, U.S.A.) were placed into a 200 ml beaker containing 100 ml distilled water. The contents were stirred until completely dissolved.

Solution E.

0.4 gm of $NaHCO_3$ was placed into a 200 ml beaker containing 50 ml distilled water. The contents were stirred until completely dissolved.

1. Solution B, C, D and E are placed into beaker of solution A. The contents stirred until uniformly mixed.
2. The pH is then adjusted to pH 6.45.
3. The beaker of solution A was topped up to 1 litre with distilled water and the pH re-checked and adjusted if necessary.
4. The media is then filtered through sterile Nalgene membrane filters (Nalge Co., Sybron Corp. N.Y.) of 3 different pore sizes, 0.8 u, 0.45 u and 0.2 u.
5. After filtration, the sterile media is transfered into a sterile 1 litre bottle and stored at $4^{o}C$ until when ready to use.
6. Prior to use, the media is reconstituted by supplementing it with 20% (v/v) Foetal Calf Serum which has been inactivated at $56^{o}C$ for 30 minutes. After reconstitution, it is refiltered through 0.8 u, 0.45 u and 0.2 u sterile filters. It is then stored in 80 cm^2 sterile tissue culture flasks (Nunclon-Delta, Denmark).
7. 5 ml of reconstituted media is then dispensed into each sterile 25 cm^2 tissue culture flask

(Nunclon-Delta, Denmark). These cultures were modified by the addition of 500 I.U. of penicillin and 500 ug streptomycin* per millilitre of media.

* (Dawa pharmaceuticals Nairobi).

APPENDIX 2 (DYNAMICS OF INFECTION IN VERVET MONKEYS)

The parasitic grades scored by each animal during the course of *L. donovani* infection administered intravenously on a scale of 0 to 6+. Animal Number - Vervet 609.

Weeks Post infection	Parasite grades: Liver	Spleen	Inguinal lymph node
2	0	0	0
4	1+	1+	0
6	1+	1+	0
8	2+	1+	0
10	2+	1+	0
12	2+	1+	0
14	2+	2+	0
16	3+	2+	1+
18	died	died	died

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Vervet 187.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	1+	0	0
4	1+	1+	0
6	1+	1+	0
8	2+	1+	0
10	2+	1+	0
12	3+	2+	1+
14	3+	1+	1+
16	2+	1+	0
18	1+	0	0
20	0	0	0
22	0	0	0
24	0	0	0
26	0	0	0
28	0	0	0
30	0	0	0
32	0	0	0
34	0	0	0
36	0	0	0
38	0	0	0

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Vervet 137.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	1+	0	0
6	1+	1+	0
8	2+	1+	0
10	2+	1+	0
12	3+	2+	0+
14	3+	2+	0+
16	3+	2+	0+
18	3+	2+	1+
20	3+	2+	1+
22	4+	3+	2+
24	4+	3+	1+
26	3+	2+	0+
28	2+	1+	0+
30	2+	2+	0+
32	died		

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Vervet 99.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	0	0	0
6	1+	0	0
8	2+	1+	0
10	2+	2+	1+
12	3+	2+	1+
14	3+	1+	1+
16	2+	1+	0
18	2+	1+	0
20	1+	1+	0
22	died	died	died

APPENDIX 3 (DYNAMICS OF INFECTION IN SYKES MONKEYS)

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Sky 116.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	0	0	0
6	1+	0	0
8	2+	1+	0
10	2+	1+	1+
12	3+	1+	0
14	1+	0	0
16	1+	0	0
18	0	0	0
20	0	0	0
22	0	0	0
24	0	0	0
26	0	0	0
28	0	0	0
30	0	0	0
32	0	0	0
34	0	0	0
36	0	0	0
38	0	0	0

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Syk 214.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	0	0	0
6	0	0	0
8	1+	1+	0
10	2+	1+	1+
12	3+	2+	1+
14	2+	1+	1+
16	2+	1+	0
18	1+	1+	0
20	1+	0	0
22	0	0	0
24	0	0	0
26	0	0	0
28	0	0	0
30	0	0	0
32	0	0	0
34	0	0	0
36	0	0	0

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Syk 205.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	0	0	0
6	1+	0	0
8	2+	1+	0
10	2+	1+	0
12	3+	2+	1+
14	2+	1+	0
16	1+	0	0
18	1+	0	0
20	0	0	0
22	0	0	0
24	0	0	0
26	0	0	0
28	0	0	0
30	0	0	0
32	0	0	0
34	0	0	0
36	0	0	0

The parasitic grades scored by each animal during the course of L. donovani infection administered intravenously on a scale of 0 to 6+. Animal Number - Syk 198.

Weeks Post infection	Parasite grades Liver	Spleen	Inguinal lymph node
2	0	0	0
4	0	0	0
6	1+	1+	0
8	2+	1+	0
10	2+	1+	0
12	3+	2+	1+
14	3+	3+	2+
16	3+	2+	1+
18	3+	2+	1+
20	3+	2+	1+
22	4+	3+	2+
24	died	died	died

APPENDIX 4 (TOTAL PROTEIN AND ALBUMIN/GLOBULIN RATIO IN SYKES MONKEYS)

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN SYKE MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATI
0	116	7.6	5.3	2.3	2.3
	214	6.3	4.2	2.1	2.0
	198	7.4	5.4	2.0	2.7
	205	7.0	4.9	2.1	2.33
	Mean	7.07	4.95	2.125	2.33
	± Sd	0.57	0.54	0.12	0.28
	86	6.8	4.8	2.0	2.4
	3	6.0	4.0	2.0	2.0
	Mean	6.4	4.4	2.0	2.2
	± Sd	0.56	0.56	0.0	0.28
1	116	7.2	6.0	1.2	5.0
	214	7.4	5.4	2.0	2.7
	198	7.2	2.4	4.8	0.50
	205	6.8	3.8	3.0	1.26
	Mean	7.15	4.4	2.75	2.36
	± Sd	0.25	1.62	1.55	1.97
	86	7.0	3.5	3.5	1.0
	3	5.9	3.9	2.0	1.95
	Mean	6.45	3.7	2.75	1.475
	± Sd	0.77	0.28	1.06	0.67

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN SYKE MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
2	116	6.8	4.6	2.2	2.09
	214	8.4	5.1	3.3	1.54
	198	6.3	4.2	2.2	2.0
	205	6.5	4.9	1.6	3.09
	Mean	7.0	4.7	2.32	2.18
	± Sd	0.95	0.39	0.70	0.65
	86	6.5	4.2	4.3	0.97
	3	5.1	4.0	1.1	0.22
	Mean	5.8	4.1	2.7	0.595
	± Sd	0.98	0.14	2.26	0.53
3	116	7.6	6.8	0.8	8.5
	214	6.5	4.7	1.8	2.61
	198	7.4	5.1	2.3	2.21
	205	7.6	4.0	3.6	1.11
	Mean	7.27	5.15	2.12	3.60
	± Sd	0.52	1.19	1.16	3.32
	86	7.4	5.4	3.0	1.8
	3	5.0	3.5	1.5	2.3
	Mean	6.2	4.45	2.25	2.05
	± Sd	1.69	1.34	1.06	0.35

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN SYKE MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
4	116	7.4	6.5	0.9	7.22
	214	6.5	2.9	3.6	0.8
	198	6.8	3.9	2.9	1.34
	205	7.8	3.2	4.6	0.69
	Mean	7.12	4.12	3.0	2.51
	± Sd	0.58	1.63	1.56	3.15
	86	6.8	5.8	1.0	5.8
	3	6.0	5.0	1.0	5.0
	Mean	6.4	5.4	1.0	5.4
	± Sd	0.56	0.56	0	0.56
5	116	8.0	2.4	3.6	0.66
	214	6.3	2.5	3.8	0.65
	198	6.8	3.6	3.2	1.12
	205	8.2	4.0	4.2	0.95
	Mean	7.32	3.12	3.7	0.845
	± Sd	0.92	0.79	0.41	0.23
	86	6.7	2.1	4.6	0.45
	3	5.9	4.5	1.4	3.21
	Mean	6.3	3.3	3	1.83
	± Sd	0.56	1.69	2.26	1.95

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN SYKE MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
6	116	8.2	4.5	3.7	1.21
	214	7.0	3.2	3.8	0.84
	198				
	205	7.8	3.2	4.6	0.69
	Mean	7.66	3.63	4.03	0.91
	± Sd	0.61	0.75	0.49	0.26
	86	6.7	4.0	2.7	1.48
	3	6.0	4.5	1.5	3.0
	Mean	6.35	4.25	2.1	2.24
	± Sd	0.49	0.35	0.84	1.07
7	116	6.7	4.0	2.7	1.48
	214				
	198				
	205	8.0	3.7	4.3	0.86
	Mean	7.35	3.85	3.5	1.17
	± Sd	0.91	0.21	1.13	0.43
	86	6.7	3.7	3.0	1.23
	3	6.7	4.0	2.7	1.48
	Mean	6.7	3.85	2.85	1.355
	± Sd	0	0.21	0.21	0.176

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN SYKE MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
8	116	5.9	3.5	2.4	1.45
	214				
	198				
	205	6.5	3.7	2.8	1.32
	Mean	6.2	3.6	2.6	1.38
	± Sd	0.42	0.14	0.28	0.09
	86	6.1	3.7	2.4	1.54
	3	6.5	3.5	3.0	1.16
	Mean	6.3	3.6	2.7	1.335
	± Sd	0.28	0.14	0.42	0.24

APPENDIX 5 (TOTAL PROTEIN AND ALBUMIN/GLOBULIN RATIO IN VERVET MONKEYS)

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN VERVET MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RA
0	609	7.0	3.0	4.0	0.75
	187	5.5	4.6	0.9	4.11
	137	7.4	3.9	3.5	1.11
	99	5.99	2.5	3.4	0.73
	Mean	6.47	3.5	2.95	1.92
	± Sd	0.87	0.93	1.39	2.13
	695	7.8	4.4.	2.4	1.83
	637	5.0	3.5	1.5	2.33
	Mean	6.4	3.95	1.95	2.08
	± Sd	1.97	0.63	0.63	0.25
1	609	7.8	2.4	3.4	0.7
	187	7.0	3.8	3.2	1.18
	137	9.6	4.5	5.6	0.8
	99	6.1	1.1	5.0	0.22
	Mean	7.62	2.95	4.3	0.725
	± Sd	1.48	1.51	1.18	0.39
	695	7.6	3.5	4.1	0.85
	637	6.1	3.0	3.1	0.96
	Mean	6.85	3.25	3.6	0.905
	± Sd	1.06	0.35	0.7	0.07

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN VERVET MONKEYS INFECTED WITH L. DONOVANI INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
2	609	6.8	4.0	2.8	1.42
	187	7.4	4.0	3.4	1.17
	137	6.8	3.7	3.1	1.19
	99	5.5	2.1	3.4	0.61
	Mean	6.625	3.45	3.175	1.09
	± Sd	0.80	0.91	0.28	0.34
	695	6.8	4.2	2.6	1.61
	637	5.5	3.5	2.0	1.75
	Mean	6.15	3.85	2.3	1.68
	± Sd	0.91	0.49	0.42	0.09
3	609	7.2	4.4	2.8	1.57
	187	7.6	4.2	2.8	1.23
	137	7.2	4.4	2.8	1.57
	99	6.8	3.5	3.3	1.06
	Mean	7.2	4.125	3.07	1.35
	± Sd	0.32	0.42	0.32	0.25
	695	6.8	5.4	1.4	3.85
	637	5.9	5.0	0.9	5.55
	Mean	6.35	5.2	1.15	4.7
	± Sd	0.63	0.28	0.35	1.20

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN VERVET MONKEY INFECTED WITH L. DONOVAN INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
4	609	8.4	5.1	3.3	1.54
	187	8.7	5.1	3.6	1.41
	137	7.8	5.4	3.4	1.58
	99	6.3	2.6	3.7	0.7
	Mean	7.8	4.55	3.5	1.30
	± Sd	1.06	1.30	0.18	1.41
	695	6.7	5.4	1.3	4.5
	637	5.3	4.5	0.8	5.62
	Mean	6.0	4.95	1.05	4.865
	± Sd	0.98	0.63	0.35	1.03
5	609				
	187	7.8	2.7	5.1	0.52
	137	7.0	3.5	3.5	1.0
	99	8.7	2.2	6.5	0.33
	Mean	7.83	2.8	5.0	0.61
	± Sd	0.85	0.65	1.50	0.34
	695	7.2	4.7	2.5	1.88
	637	6.3	4.55	1.75	2.6
	Mean	6.75	4.625	2.12	2.24
	± Sd	0.63	0.10	0.53	0.50

MEAN ± STANDARD DEVIATION (Sd) OF TOTAL PROTEIN (T.P.) AND ALBUMIN/GLOBULIN RATIO IN VERVET MONKEY INFECTED WITH L. DONOVAN INTRAVENOUSLY.

MONTHS POST INFECTION	ANIMAL NUMBER	TOTAL PROTEIN gm/100 ml	ALBUMIN gm/100 ml	GLOBULIN gm/100 ml	A/G RATIO
6	609				
	187	8.4	4.1	4.3	0.95
	137	8.2	2.9	5.3	0.54
	99				
	Mean	8.3	3.5	4.8	0.745
	± Sd	0.14	0.84	0.70	0.28
	695	6.8	3.6	3.2	1.12
	637	5.9	4.5	1.4	3.21
	Mean	6.35	4.05	2.3	2.165
	± Sd	0.63	0.63	1.27	1.47
7	609				
	187	8.2	2.4	4.3	0.55
	137				
	99				
	Mean				
	± Sd				
	695	7.2	4.0	3.2	1.25
	637	6.0	5.0	1.0	5.00
	Mean	6.6	4.5	2.0	3.125
	± Sd	0.84	0.70	1.41	2.65

MONTHS p.i.	ANIMAL NUMBER	T.P.	ALBUMIN	GLOBULIN	A/G RATIO
8	609	8.9	3.3	5.6	0.58
	187				
	137				
	99				
	Mean				
	± Sd				
	695	6.8	3.2	3.6	0.88
	637	5.5	4.9	0.6	8.16
	Mean	6.15	4.05	2.1	4.52
	± Sd	0.91	1.20	2.12	5.14

APPENDIX 6 (SERUM BIOCHEMISTRY VALUES IN VERVET MONKEYS)

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatas
	187	86	19	20	100
	137	82	27	23	58
0	99	82	19	17	50
	609	82	52	17	100
	MEAN	83	29.25	19.25	77
	± Sd	1.99	15.62	2.87	26.75
	695	114	41	18	48
	673	100	47	18	32
	MEAN	107	44	18	40
	± Sd	9.89	4.24	0	11.31
	187	69	10	10	38
2	137	69	27	17	76
	99	82	13	20	66
	609	65	27	17	96
	MEAN	71.25	19.25	16	69
	± Sd	7.41	9.03	4.24	24.13
	695	82	23	17	58
	673	73	13	6	56
	MEAN	77.5	18	11.5	57
	± Sd	6.36	7.07	7.77	1.41

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
4	187	69	23	10	84
	137	46	36	5	54
	99	62	19	6	79
	609	62	36	5	98
	MEAN	59.75	28.5	6.5	78.75
	± Sd	9.74	8.81	2.38	18.35
	695	115	36	10	156
	673	90	47	11	70
	MEAN	1025	41.5	10.5	113
	± Sd	17.67	7.77	0.70	60.81
6	187	99	89	14	40
	137	110	89	32	90
	99	94	36	32	70
	609	84	80	20	105
	MEAN	96.75	73.5	24.5	76.25
	± Sd	10.81	25.35	8.99	28.09
	695	94	76	17	70
	673	73	89	22	42
	MEAN	83.5	82.5	19.5	56
	± Sd	14.84	9.19	3.53	19.79

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	70	67	13	48
	137	50	19	11	68
	99	55	23	13	62
	609	55	24	11	160
8	MEAN	57.5	33.25	12	84.5
	± Sd	8.66	22.60	1.15	51.02
	695	85	45	18	50
	673	70	25	20	45
	MEAN	77.5	35	19	47.5
	± Sd	10.60	14.14	1.41	3.53
	187	36	19	8	54
	137	45	41	17	110
	99	45	16	17	68
	609	40	41	28	176
	MEAN	41.5	29.25	17.5	102
10	± Sd	4.35	13.6	8.18	54.77
	695	68	47	20	68
	673	54	19	11	30
	MEAN	61	33	15.5	49
	± Sd	9.89	19.79	6.36	26.87

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	72	27	19	48
	137	63	23	23	82
	99	108	41	33	48
	609	58	41	27	160
12	MEAN	75.25	33	25.5	84.5
	± Sd	22.58	9.38	5.97	52.82
	695	104	27	33	62
	673	50	31	33	40
	MEAN	77	29	33	51
	± Sd	38.18	2.82	0	15.55
	187	100	23	15	42
	137	109	31	17	98
	99	73	23	21	64
	609	55	47	25	136
	MEAN	84.25	31	19.5	85
14	± Sd	24.78	11.31	4.43	41.06
	695	100	27	20	58
	673	100	27	20	58
	MEAN	100	27	20	58
	± Sd	0	0	0	0

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	38	64.	62	122
	137	42	23	15	94
	99	33	31	19	72
	609	29	52	19	64
16	MEAN	35.5	42.5	28.75	88
	± Sd	5.68	18.84	22.24	
	695	46	27	19	40
	673	46	27	19	40
	MEAN	46	27	19	40
	± Sd	0	0	0	0
	187	68	41	45	122
	137	100	32	27	90
	99	92	25	25	66
	609	68	28	25	80
	MEAN	82	31.5	30.5	89.5
18	± Sd	16.49	6.95	9.71	23.79
	695	75	20	45	50
	673	75	20	45	40
	MEAN	75	20	45	45
	±	0	0	0	0

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	76	89	42	240
	137	62	47	27	84
	99	53	31	23	72
	609	53	31	24	32
	MEAN	61	49.5	28.75	107
20	± Sd	10.86	27.39	8.34	91.41
	695	100	100	27	56
	673	76	40	18	30
	MEAN	88	70.5	22.5	43
	± Sd	16.97	41.71	6.36	18.38
	187	76	89	38	160
	137	68	47	25	118
	99	64	27	13	52
	609	68	52	28	28
	MEAN	69	53.75	26	82.5
22	± Sd	5.03	25.86	10.29	60.47
	695	88	89	40	76
	673	56	47	16	42
	MEAN	72	68	28	59
	± Sd	22.62	29.69	16.97	24.04

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	80	16	18	150
	137	90	13	22	120
	99	80	16	13	52
	609	-	-	-	-
24	MEAN	83.33	15	17.66	107.33
	± Sd	5.77	1.73	4.50	50.21
	695	120	16	8	60
	673	70	10	4	40
	MEAN	95	13	6	50
	± Sd	35.35	4.24	2.82	14.14
	187	66	47	19	88
	137	91	67	27	140
	99	116	89	17	150
	609	-	-	-	-
	MEAN	91	67.66	21	126
26	± Sd	24.99	21.00	5.29	33.28
	695	116	47	15	62
	673	100	52	34	36
	MEAN	108	49.5	24.5	49
	± Sd	11.31	3.53	13.43	18.38

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	79	20	27	120
	137	87	25	47	110
	99	-	-	-	-
	609	-	-	-	-
	MEAN	83	22.5	37	115
28	± Sd	5.65	3.53	14.14	7.07
	695	96	17	10	52
	673	58	11	7	46
	MEAN	77	14	8.5	49
	± Sd	26.87	4.24	2.12	4.24
	187	63	79	40	144
	137	75	90	45	152
	99	-	-	-	-
	609	-	-	-	-
	MEAN	69	84.5	42.5	148
30	± Sd	8.48	7.77	3.53	5.65
	695	108	67	20	90
	673	75	47	15	72
	MEAN	91.5	57	17.5	81
	± Sd	23.33	14.14	3.53	12.72

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	76	76	25	184
	137	100	100	60	44
	99	-	-	-	-
	609	-	-	-	-
	MEAN	88	88		114
32	± Sd	16.97	16.97	24.74	98.99
	695	75	59	15	90
	673	76	59	11	68
	MEAN	75.5	59	13	79
	± Sd	0.70	0	2.82	15.55
	187	97	67	25	44
	137	-	-	-	-
	99	-	-	-	-
	609	-	-	-	-
	MEAN				
34	±				
	695	116	52	19	76
	673	97	47	20	42
	MEAN	106.5	49.5	19.5	59
	± Sd	13.43	3.53	0.70	24.04

The Mean Serum biochemistry values ± standard deviation (Sd) of vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	187	93	76	27	54
	137	-	-	-	-
	99	-	-	-	-
	609	-	-	-	-
	MEAN				
36	± Sd				
	695	107	16	30	46
	673	73	47	20	42
	MEAN	90	31.5	25	44
	± Sd	24.04	21.92	7.07	2.82
	187	72	16	27	72
	137	-	-	-	-
	99	-	-	-	-
	609	-	-	-	-
	MEAN				
38	± Sd				
	695	90	31	30	48
	673	54	13	6	32
	MEAN	72	22	8	40
	± Sd	25.45	12.72	16.97	11.31

APPENDIX 7 (SERUM BIOCHEMISTRY VALUES IN SYKES MONKEYS)

The Mean Serum biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphat
0	205	68	27	27	72
	214	64	41	23	38
	116	85	23	38	96
	198	86	36	27	56
	MEAN	75.75	31.75	28.75	65.5
	± Sd	11.38	8.22	6.44	24.62
	86	42	89	34	20
	3	68	19	27	44
	MEAN	55	54	30.5	32
	± Sd	18.38	49.49	4.94	16.97
2	205	78	16	16	60
	214	78	16	23	30
	116	73	27	83	96
	198	69	13	17	54
	MEAN	74.5	18	34.75	60
	± Sd	4.35	6.16	32.31	27.27
	86	50	19	4	22
	3	79	23	50	58
	MEAN	64.5	21	27	40
	± Sd	20.50	2.82	32.52	25.45

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	54	27	15	102
	214	46	27	21	62
	116	63	52	83	28
	198	77	27	17	58
4	MEAN	60	33.25	34	62.5
	± Sd	13.29	12.5	32.76	30.39
	86	89	31	26	21
	3	78	52	85	62
	MEAN	83.5	41.5	55.5	41.5
	± Sd	7.77	14.84	41,71	28.99
	205	78	59	58	40
	214	52	47	43	44
	116	46	27	84	54
	198	68	27	50	58
6	MEAN	61	40	58.75	49
	± Sd	14.65	15.79	17.91	8.406
	86	73	19	23	35
	3	46	36	93	70
	MEAN	59.5	27.5	58	52.5
	± Sd	19.09	12.02	49.49	24.74

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	50	26	24	40
	214	45	19	29	36
	216	60	19	37	66
	198	70	14	26	42
8	MEAN	56.25	19.5	29	46
	± Sd	11.08	4.93	5.71	13.56
	86	68	16	23	34
	3	36	39	42	80
	MEAN	52	27.5	32.5	57
	± Sd	22.62	16.26	13.43	32.52
	205	50	31	20	44
	214	81	27	34	44
	116	59	10	34	50
	198	68	16	32	96
	MEAN	64.5	21	30	58.5
10	± Sd	13.22	6.69	6.73	25.5
	86	54	16	28	22
	3	76	31	62	90
	MEAN	65	23.5	45	56
	± Sd	15.55	10.60	2404	48.08

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	63	31	40	44
	214	92	31	47	74
	116	63	27	64	60
	198	58	27	58	62
	MEAN	69	29	52.25	60
12	± Sd	15.51	2.30	10.78	12.32
	86	90	19	23	22
	3	82	27	31	56
	MEAN	86	23	27	39
	± Sd	5.65	5.65	5.65	24.04
	205	100	27	25	52
	214	55	23	41	90
	116	82	31	50	56
	198	118	36	27	54
	MEAN	88.75	29.25	35.75	63
14	± Sd	26.87	5.56	11.87	18.07
	86	42	7	25	30
	3	33	27	30	56
	MEAN	37.5	17	27.5	43
	± Sd	6.36	14.14	3.53	18.38

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	42	41	32	50
	214	29	27	27	50
	116	38	27	25	44
	198	42	23	30	70
	MEAN	37.75	29.5	28.5	53.5
16	± Sd	6.13	7.89	3.10	11.35
	86	68	18	23	18
	3	75	15	80	46
	MEAN	71.5	16.5	51.5	82
	± Sd	4.94	2.12	40.30	19.79
	205	75	45	78	66
	214	108	23	32	44
	116	92	23	54	48
	198	75	23	34	112
	MEAN	87.5	28.5	49.5	67.5
18	± Sd	15.84	11.0	21.43	31.17
	86	76	19	28	14
	3	84	23	32	52
	MEAN	80	21	30	33
	± Sd	5.65	2.82	2.82	26.87

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	69	76	49	224
	214	69	41	34	66
	116	53	27	34	28
	198	46	52	39	124
	MEAN	59.25	49	39	110.5
20	± Sd	11.61	20.70	7.07	83.34
	86	72	19	25	20
	3	76	27	47	42
	MEAN	74	23	38	31
	± Sd	2.82	5.65	15.55	15.55
	205	64	89	52	136
	214	60	52	27	90
	116	72	67	38	46
	198	60	59	28	110
	MEAN	64	66.75	36.25	95.5
22	± Sd	5.65	16.04	11.61	37.99
	86	170	7	13	16
	3	200	7	32	42
	MEAN	185	7	22.5	29
	± Sd	21.21	0	13.43	18.38

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	200	23	22	110
	214	130	10	15	82
	116	130	13	32	50
	198	140	19	27	150
	MEAN	150	16.25	24	98
24	± Sd	33.66	5.85	7.25	42.45
	86	66	19	34	32
	3	58	23	23	48
	MEAN	62	21	28.5	40
	± Sd	5.65	2.82	7.77	11.31
	205	66	47	27	120
	214	75	31	20	110
	116	83	36	50	46
	198	66	47	36	110
	MEAN	72.5	40.25	96.5	
26	± Sd	9.18	8.05	12.94	33.99
	86	188	13	67	16
	3	158	10	59	50
	MEAN	173	11.5	63	33
	± Sd	21.21	2.12	5.65	24.04

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	116	17	94	90
	214	145	26	94	100
	116	183	10	94	46
	198	120	25	115	100
	MEAN	141	19.5	99.25	84
28	± Sd	3080	7.50	10.49	25.76
	86	67	31	23	60
	3	92	59	31	104
	MEAN	79.5	45	27	82
	± Sd	17.67	19.79	5.65	31.11
	205	63	76	90	112
	214	79	130	47	128
	116	113	160	90	94
	198	-	-	-	-
	MEAN	85	122	75.66	111.3
30	± Sd	25.53	42.56	24.82	17.0
	86	72	31	28	32
	3	75	47	60	74
	MEAN	73.5	39	44	53
	± Sd	2.12	11.31	22.62	26.69

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	60	76	27	116
	214	70	90	41	90
	116	80	76	80	78
	-	-	-	-	-
	MEAN	70	80.66	49.33	94.66
32	± Sd	9.99	8.08	27.46	19.42
	86	145	47	30	43
	3	145	190	30	78
	MEAN	145	118.5	30	60.5
	± Sd	0	101.11	0	24.74
	205	132	100	28	116
	-	-	-	-	-
	116	197	89	50	78
	-	-	-	-	-
	MEAN	164.5	94.5	39	97
34	± Sd	45.96	7.77	15.55	26.87
	86	67	31	26	36
	3	83	36	32	40
	MEAN	75	33.5	29	38
	± Sd	11.31	3.53	4.24	2.82

The Mean biochemistry values ± standard deviation (Sd) of sykes monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	Glucose mg/100 ml	ASAT IU/litre	ALAT IU/litre	Alkaline Phosphatase
	205	67	89	25	48
	214	-	-	-	-
	116	107	59	32	44
	198	-	-	-	-
	MEAN	87	74	28.5	46
36	± Sd	28.28	21.21	4.94	2.82
	86	86	23	47	40
	3	75	27	50	42
	MEAN	80.5	25	48.5	41
	± Sd	7.77	2.82	2.12	1.41
	205	162	16	28	42
	214	-	-	-	-
	116	100	7	45	40
	198	-	-	-	-
	MEAN	131	11.5	36.5	41
38	± Sd	43.84	6.36	12.02	1.41
	86	100	7	20	20
	3	178	10	20	30
	MEAN	139	8.5	20	25
	± Sd	55.15	2.12	0	7.07

APPENDIX 8 (HAEMATOLOGICAL VALUES IN SYKES MONKEYS)

Mean haematological values ± standard deviation (Sd) of Syke monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	RBC No.	Hb gm/100ml	PCV %	MCV U^3	MCHC %	MCH uug
0	198	7.02	13.1	50	71	26.2	18.7
	214	5	17.8	38	75	46.8	34.9
	205	6.46	15.4	42	65	36.7	23.8
	116	5.2	13	39	75	33.3	25
	MEAN	5.92	14.82	42.25	71.5	35.75	25.6
	± Sd	0.97	2.27	5.43	4.72	8.56	6.97
	86	5.27	16	47	89	34	30.4
	3	4.29	11.2	35	82	32	26.1
	MEAN	4.78	13.6	41	85.5	33	28.25
	± Sd	0.69	3.39	8.48	4.94	1.41	3.04
2	198	6.81	16.5	58	85.	28.4	24.2
	214	5.48	13.6	45	82	30.2	24.8
	205	6.98	15.4	51	78	30.1	23.7
	116	5.08	12.0	40	78	30	23.6
	MEAN	6.08	14.37	48.5	80.75	29.67	24.07
	± Sd	0.94	1.98	7.76	3.40	0.85	0.55
	86	5.3	14.8	43	81	34.4	27.9
	3	4.2	12.5	35	83	35.7	29.8
	Mean	4.75	13.65	39	82	35.05	28.85
	± Sd	0.77	1.62	5.65	1.41	0.91	1.34

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophis %		Lymphocytes %		Monocytes %		Total protein %	Fibrino-gen /ul
0	198	6.4	46	2944	54	3456	0	0	9.4	400
	214	3.4	57	1938	41	1394	2	68	7	200
	205	4.8	38	1824	62	2976	0	0	9.6	600
	116	4.8	38	1824	62	2976	0	0	8	400
	Mean	4.85	44.75	2132.5	5475	2700.5	0.5	17	8.5	400
	± Sd	1.22	8.99	543.6	9.91	899.91	1	34	1.22	163.29
	86	7	49	3430	51	3570	0	0	7.8	200
	3	4.8	34	1632	66	3168	0	0	8.4	200
	Mean	5.9	41.5	2531	58.5	3369	0	0	8.1	200
	±	1.55	10.6	1271.3	10.60	284.25	0	0	0.42	0
2	198	6.1	48	2928	52	3172	0	0	6	400
	214	6.9	49	3381	50	3950	1	69	7	200
	205	4.3	53	2279	47	2021	0	0	7.8	400
	116	5.2	71.5	3718	28.5	1982	0	0	6	200
	Mean	5.62	55.37	3076.5	44.37	2781.25	0.25	17.25	6.7	300
	± Sd	1.12	10.96	622.44	10.77	954.88	0.43	34.5	0.75	115.47
	86	4.6	51.5	2369	47.5	2185	1	46	8	200
	3	4.2	44	1848	56	2352	0	0	6.8	200
	Mean	4.4	47.75	2108.5	51.75	2268.5	0.5	23	7.4	200
	±	0.28	5.30	368.4	6.01	118.08	0.7	32.5	0.84	0

Interval in weeks	Animal Number	RBC $X10^6$/ul	gm/100ml	PCV %	MCV u^3	MCHC %	MCH u u g
	198	5.3	13.5	47	89	28.7	25.5
4	214	4.33	13.1	41	87	32	27.3
	205	6.04	16.8	50	83	33.6	27.8
	116	4.49	12.7	31	69	41	28.3
	MEAN	5.04	14.02	42.25	82	33.82	27.22
	± Sd	0.79	1.87	8.38	9.01	5.20	1.22
	86	4.9	11.5	40	82	28.8	23.5
	3	4.51	12.7	40	89	31.8	28.2
	MEAN	4.70	12.1	40	85.5	30.3	25.85
	± Sd	0.27	0.84	0	4.94	2.12	3.32
	198	4.3	13	40	93	32.5	30.2
6	214	4	10.9	35	88	31.1	27.3
	205	7.52	16.5	47	63	35.1	21.9
	116	4	11.8	34	85	34.7	29.5
	MEAN	4.95	13.05	39	82.25	33.35	27.22
	± Sd	1.71	2.45	5.94	13.25	1.88	3.75
	86	5.63	11.9	34	60	35	21.1
	3	5.07	14.7	46	91	32	29
	MEAN	5.35	13.3	40	75.5	33.5	25.05
	± Sd	0.39	1.97	8.48	21.92	2.12	5.58

N.B. Animals were infected at week 4.

Interval in Weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
4	198	42	66	2772	33	1386	1	42	8.2	200
	214	5	43	2150	57	2850	0	0	7	200
	205	3.2	44	1408	56	1792	0	0	8.4	400
	116	3.6	34	1224	63	2268	3	108	12	400
	MEAN	4.0	46.75	1888.5	52.25	2074	1	37.5	8.9	300
	± Sd	0.78	13.59	712.13	13.2	630.52	1.41	51.0	2.15	11.47
	86	4.8	56	2688	43.5	2088	0.5	24	6.2	200
	3	3.6	63	2268	36	1296	1	36	6.6	200
	MEAN	4.2	59.5	2478	39.75	1692	0.75	30	6.4	200
	± Sd	0.84	4.94	296.98	5.30	560.02	0.35	8.48	0.28	0
6	198	1.8	37	666	63	1134	0	0	7.2	600
	214	4.8	45	2166	55	2640	0	0	6.4	400
	205	4.5	49.5	2228	50.5	2270	0	0	9	200
	116	5	52	7600	48	2400	0	0	9	200
	MEAN	4.02	45.87	3165	54.12	2111.5	0	0	7.9	350
	± Sd	1.49	6.58	3043	6.58	669.28	0	0	1.31	191.48
	86	6	22.5	1350	77.5	4650	0.5	30	7.0	400
	3	6.6	75	4950	23	1518	2	132	6.4	200
	MEAN	6.3	48.75	3150	50.25	3084	1.25	81	6.7	300
	± Sd	0.42	37.83	2545	38.53	2214.65	1.06	72.1	0.92	141.42

Interval in weeks	Animal Number	RBC $X10^6$/ul	Hb gm/100ml	PCV %	MCV u^3	MCHC %	MCH u u g
8	198	5.88	14	44	75	31.8	23.8
	214	4.92	12.2	38	77	32.1	24.8
	205	7.88	17.3	54	69	32	22
	116	5.42	13.4	37	68	36.2	24.7
	MEAN	6.025	14.22	43.25	72.25	33.02	23.82
	± Sd	1.297	2.18	7.80	4.42	2.12	1.27
	86	5.67	12.7	37	65	34.3	22.5
	3	4.81	11.9	34	71	35	24.7
	MEAN	5.24	12.3	35.5	68	34.65	23.6
	±	0.60	0.56	2.12	4.24	0.49	1.55
10	198	5.4	16.2	50	93	32.4	30
	214	4.97	13.5	43	87	31.4	27.2
	205	6.08	17.2	58	95	29.7	28.3
	116	4.66	13.7	43	92	31.7	29.4
	MEAN	5.277	15.15	48.5	91.75	31.3	28.72
	± Sd	0.61	1.83	7.14	3.40	1.14	1.23
	86	6.22	13.9	44	71	31.6	22.3
	3	4.11	12.5	38	92	32.9	30.4
	MEAN	5.16	13.2	41.0	81.5	32.25	26.35
	±	1.49	0.98	4.24	14.84	0.91	5.72

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %		Lymphocytes %		Monocytes %		Total protein	Fibrinogen
8	198	2.9	41.5	1204	58.5	1699	0	0	7.2	600
	214	7.8	42	3276	56.5	4368	1.5	117	5.4	200
	205	3.4	50	1700	49	1666	1	34	6.2	200
	116	3	32	960	68	2040	0	0	7.2	200
	MEAN	4.27	41.37	1785	58	2443.25	0.62	37.75	6.5	300
	± Sd	2.35	7.36	1040.59	7.82	1294.25	0.75	55.2	0.87	200
	86	6.2	45	2790	55	3410	0	0	7.8	600
	3	1.2	34	408	64	768	2	24	7.4	200
	MEAN	3.7	39.5	1599	59.5	2089	1	12	7.6	400
	± Sd	3.53	7.77	1684.32	6.36	1868.17	1.41	16.9	0.20	282.84
10	198	1.9	29	551	71	1349	0	0	6.6	200
	214	4.4	28.5	1254	715	3146	0	0	6	200
	205	3.1	54	1134	46	966	0	0	6.4	200
	116	2.2	30	660	70	1540	0	0	6.6	200
	MEAN	2.65	35.37	899.75	64.62	1750.25	0	0	6.4	200
	±	1.17	12.43	346.15	12.43	960.62	0	0	0.28	0
	86	5	50	2500	50	2500	0	0	6.2	200
	3	2	38	760	62	1240	0	0	6.2	200
	MEAN	3.5	44	1630	56	1870	0	0	6.2	200
	±	2.12	8.48	1230.36	8.48	890.95	0	0	0	0

Interval in weeks	Animal Number	RBC $X10^6$/ul	Hb gm/100mm	PCV %	MCV u^3	MCHC %	MCH u u g
12	198	6.61	15	48	73	31.3	22.7
	214	4.89	12.4	40	82	31	25.4
	205	7.78	18.2	54	69	33.7	23.4
	116	6.22	13.6	45	72	30.5	21.9
	MEAN	6.37	14.8	46.75	74	31.62	23.35
	±	1.19	2.50	5.85	5.59	1.42	1.49
	86	5.82	13.8	44	76	31.4	23.7
	3	4.94	11.2	36	73	31.1	22.7
	MEAN	5.38	12.5	40	74.5	31.25	23.2
	±	0.62	1.83	5.65	2.12	0.21	0.70
14	198	6.28	15	42	67	357	23.9
	214	4.93	12.7	40	93	31.8	25.8
	205	6.3	14.5	49	78	29.6	22.9
	116	5.26	12.4	37	70	33.5	23.5
	MEAN	5.69	13.65	42	77	32.65	24.02
	±	0.70	1.29	5.09	11.63	2.58	1.25
	86	6.22	14.5	47	76	30.9	23.3
	3	5.7	13.2	38	67	34.7	23.2
	MEAN	5.96	13.85	425	71.5	32.8	23.25
	±	0.36	0.91	6.36	6.36	2.68	0.07

Interval in weeks	Animal Number	WBC $X10^3$/ul	Neutrophils %		Lymphocytes %		Monocytes %		Total protein	Fibrino-gen
12	198	2.1	21	441	79	1659	0	0	7.2	200
	214	3.8	49	1862	51	1938	0	0	6	400
	205	5.2	71.5	3718	28.5	1482	0	0	6.6	200
	116	2.4	33	792	67	1608	0	0	7	200
	MEAN	3.37	43.62	1703.25	56.37	1671.75	0	0	6.7	250
	± Sd	1.42	21.83	1472.87	21.83	192.45	0	0	0.52	99.99
	86	6.4	54.5	3488	455	2851	0	0	6.4	400
	3	2.7	36	972	64	1728	0	0	5.6	200
	MEAN	4.55	45.25	2230	54.75	2289.5	0	0	6	300
	± Sd	2.6	13.08	1779.08	13.08	794.08	0	0	0.56	141.42
14	198	3.7	52	1924	48	1776	0	0	9	200
	214	3.6	48.5	1746	51.5	1854	0	0	6.4	200
	205	4.2	50	2100	50	2100	0	0	8	200
	116	3.3	24.5	808	75.5	2491	0	0	6.2	200
	MEAN	3.7	43.75	1894.5	56.37	2055.25	0	0	7.4	200
	± Sd	0.37	12.91	805.94	12.83	321.64	0	0	1.32	0
	86	6.6	50.5	3333	49.5	3267	0	0	6.8	200
	3	2.8	31	868	69	1932	0	0	8.8	400
	MEAN	4.7	40.75	2100.5	59.25	2599.5	0	0	7.8	300
	± Sd	2.68	13.78	1743.01	13.78	943.98	0	0	1.41	141.42

Interval in weeks	Animal Number	RBC $X10^6$/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
16	198	5.37	12.5	36	67	34.7	23.3
	214	4.64	11.8	38	82	31	25.4
	205	5.48	13.8	38	69	36.3	25.2
	116	5.58	13	38	68	34.2	23.3
	MEAN	5.26	12.77	37.5	71.5	34.05	24.3
	± Sd	0.42	0.84	1.0	7.04	2.22	1.16
	86	6.13	14.4	42	69	34.3	23.5
	3	5.6	13.3	40	71	33.3	23.8
	MEAN	5.86	13.85	41	70	33.8	23.65
	± Sd	0.37	0.77	1.41	1.41	0.70	0.21
18	198	3.9	11.8	38	97	31.1	30.3
	214	5.3	13.1	42	79	31.2	24.7
	205	4.68	13.3	40	85	33.3	28.4
	116	4.9	12.9	42	89	30.7	27.4
	MEAN	4.69	12.77	40.5	87.5	31.57	27.7
	± Sd	0.58	0.67	1.91	7.54	1.17	2.33
	86	6.91	18.4	58	84	31.7	26.6
	3	4.29	11.6	37	86	31.6	27.0
	MEAN	5.6	15.0	47.5	85	31.65	26.8
	± Sd	1.85	4.8	14.8	1.41	0.07	0.28

Interval in weeks	Animal Number	WBC X10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
16	198	2.6	20	520	80	2080	0	0	7.8	400
	214	3.8	29.5	1121	70.5	2679	0	0	5.8	400
	205	5.6	19.5	1095	80.5	4508	0	0	8	200
	116	45	58	2610	41.5	1867	0.5	22	8.4	400
	MEAN	4.12	31.75	1336.5	68.12	2783.5	0.12	5.5	7.5	350
	± Sd	1.25	18.09	893.16	18.33	1199.95	0.25	11	1.16	99.99
	86	5	49.5	2475	50.5	2525	0	0	6.2	200
	3	2.6	36.5	949	636	1651	0	0	8	200
	MEAN	3.8	43	1712	57	2088	0	0	7.1	200
	±	1.69	9.19	1079.04	9.19	618.01	0	0	1.27	0
18	198	1.3	23	299	73	943	4	52	7.8	600
	214	1.7	24	408	76	1292	0	0	7.4	400
	205	5	19	950	79	3950	2	100	8	200
	116	1.7	20	340	79	1343	1	17	7	200
	MEAN	2.42	21.5	499.25	76.75	1882	1.75	42.25	7.55	350
	± Sd	1.72	2.38	303.84	2.87	1390.07	1.70	44.16	0.44	191.48
	86	5.4	555	2997	44.5	2403	0	0	7.8	400
	3	1.6	35	560	65	1040	0	0	6.2	200
	MEAN	3.5	45.25	1778.5	54.75	1721.5	0	0	7	300
	± Sd	2,68	14,49	1723.21	14.49	963.78	0	0	1.13	141.42

Interval in weeks	Animal Number	RBC X10^6/ul	Hb gm/100ul	PCV %	MCV u^3	MCHC %	MCH u u g
20	198	4.1	9	31	76	29	22
	214	4.74	11.9	41	86	29	25.1
	205	5.07	12.5	44	87	31.3	24.7
	116	5.9	13.7	36	61	38.1	23.2
	MEAN	4.95	11.77	38	77.5	31.85	23.75
	± Sd	0.74	1.99	5.71	12.06	4.30	1.42
	86	5.56	13.7	37	66	37	24.6
	3	4.88	12	28	57	42.9	24.6
	MEAN	5.22	12.85	32.5	61.5	39.95	24.6
	± Sd	0.48	1.20	6.36	6.36	4.17	0
22	198	4.32	10	31	72	32.3	23.1
	214	4.72	11.5	37	78	31.1	24.4
	205	5.18	12.2	38	73	32.1	23.6
	116	5.41	12.6	38	70	33.2	23.3
	MEAN	4.90	11.57	36	73.25	32.17	23.6
	± Sd	0.48	1.14	3.36	3.40	0.86	0.57
	86	5.41	13.3	42	78	31.7	24.6
	3	4.56	10.8	33	72	32.7	23.7
	MEAN	4.98	12.05	37.5	75	32.2	24.15
	± Sd	0.60	1.76	6.36	4.24	0.70	0.63

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fribrino-gen
20	198	1.9	29	551	71	1349	0	0	8.2	200
	214	3	26	780	71	2130	2	60	6.2	200
	205	4.7	19.5	916	79.5	3713	1	47	6	200
	116	2.6	29	754	69	1794	2	52	6.6	200
	MEAN	3.05	2087	750.25	72.62	2246.5	1.25	39.75	6.75	200
	± Sd	1.19	4.47	150.63	4.67	1028.66	0.95	27.03	0.99	0
	86	6	50.5	3030	49.5	2970	0	0	7	400
	3	513	19.5	1030	80.5	4266	0	0	6.4	400
	MEAN	5.65	35	2031.5	65	3618	0	0	6.7	400
	± Sd	0.49	21.92	1412.09	21.77	916.41	0	0	0.42	0
22	198	1.6	23	368	72	1152	5	80	6.4	200
	214	2.2	21	462	77	1694	2	44	5.6	200
	205	48	14	672	86	4128	0	0	6.8	200
	116	2.5	29	700	70	1750	2	50	6.6	200
	MEAN	2.77	21.75	550.5	76.25	2181	2.25	43.5	6.35	200
	± Sd	1.40	6.18	161.50	7.13	1325.71	2.06	33	0.52	0
	86	5.4	54.5	2943	44.5	2403	1	54	5.6	400
	3	2.8	41	1148	58	1624	1	25	5	200
	MEAN	4.1	47.75	2045	51.25	2013.5	1	41	5.3	300
	± Sd	1.83	9.54	1272.79	9.54	550.83	0	18.38	0.42	141.42

Interval in weeks	Animal Number	RBC X10^6/ul	Hb gm/100ml	PCV %	MCV u^3	MCHC %	MCH u u g
24	198	3.82	9.3	31	81	30	24.3
	214	3.33	8.4	38	114	22.1	25.2
	205	6.2	14.2	44	71	32.3	22.9
	116	5.77	13.1	42	73	31.2	22.7
	MEAN	4.78	11.25	38.75	84.75	28.9	23.77
	± Sd	1.41	2.83	5.73	19.97	4.62	1.18
	86	6.27	14.2	48	77	29.6	22.6
	3	5.43	12.6	38	70	33.2	23.2
	Mean	5.85	13.4	43	73.5	31.4	22.9
	± Sd	0.59	1.13	7.07	4.94	2.54	0.42
26	198	1.7	4	14	82	28.6	23.6
	214	3.93	10.1	28	70	36.1	25.8
	205	5	11.5	32	64	35.9	23
	116	5.63	11.9	34	60	35	21.1
	MEAN	4.07	9.37	27	69	33.9	23.37
	± Sd	1.72	3.66	9.01	9.59	3.56	1.93
	86	4.8	12.3	33	69	37.3	25.6
	3	4.74	11.3	33	70	34.2	23.8
	Mean	4.77	11.8	33	69.5	35.75	24.7
	± Sd	0.42	0.70	0	0.70	2.19	1.27

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
24	198	1.3	24	312*	71	*923	5	65	7	600
	214	2.3	53	1219	45	1035	2	46	6	200
	205	5.8	43	2494	57	3306	0	0	7.4	200
	116	4.3	20	860	79.5	3418	0.5	21	7	200
	MEAN	3.43	35	1221.2	63.12	2170.5	1.87	33	6.85	300
	±	2.01	15.64	926.84	15.23	1377.34	2.25	28.43	0.59	200
	86	6.2	66	4092	33.5	2077	0.5	31	6.6	400
	3	2.4	28	672	70	1680	2	48	6.4	200
	MEAN	4.3	47	2382	51.75	1878.5	1.25	39.5	6.5	300
	± Sd	2.68	2687	2418.3	25.80	280.72	1.06	12.02	0.14	141.42
26	198	2.6	8	208	92	2392	0	0	7	200
	214	1.4	38	532	62	868	0	0	7.2	200
	205	3.3	24	792	76	2500	0	0	8.8	200
	116	4.1	16	656	80.5	3300	3.5	143	8.4	200
	MEAN	2.85	21.5	547	77.62	2265	0.87	35.75	7.85	200
	±	1.14	12.79	249.7	12.40	1015.57	1.75	71.5	0.88	0
	86	6	22.5	1350	77.5	4650	0	0	9.4	400
	3	3.3	12	396	88	2904	0	0	7.4	400
	MEAN	4.65	17.25	873	82.75	3777	0	0	8.4	400
	± Sd	1.90	7.42	674.57	7.42	1234.65	0	0	1.41	0

Inteval in weeks	Animal Number	RBC $X10^6$/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
28	198	-	-	-	-	-	-
	214	5.93	12	35	59	34.3	20.2
	205	4.9	13.3	45	92	29.6	27.1
	116	5.3	12.9	45	85	28.7	24.3
	MEAN	5.37	12.73	41.66	78.66	30.86	23.86
	± Sd	0.51	0.66	5.77	17.38	3.00	3.47
	86	5.3	13.2	45	85	29.3	24.9
	3	5.07	14.7	46	91	32	29
	MEAN	5.18	13.95	45.5	88	30.65	26.95
	± Sd	0.16	1.06	0.70	4.24	1.90	2.89
30	198	-	-	-	-	-	-
	214	-	-	-	-	-	-
	205	4.46	12.8	38	85	33.7	28.7
	116	3.52	11.2	33	94	33.9	31.8
	MEAN	3.99	12	35.5	89.5	33.8	30.25
	± Sd	0.66	1.13	3.53	6.36	0.14	2.19
	86	4.59	12.6	43	94	29.3	27.5
	3	4	10.2	36	90	28.3	25.5
	MEAN	4.29	11.4	39.5	92	28.8	26.5
	± Sd	0.41	1.69	4.94	2.82	0.70	1.41

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
28	198	-	-	-	-	-	-	-	-	-
	214	1.3	40	520	56	728	4	52	6.6	200
	205	4.2	34.5	1449	64	2688	0	0	7.6	200
	116	3.3	28	924	72	2376	0	0	6.6	200
	MEAN	12.93	34.16	964.33	64	1930.66	1.33	17.33	6.93	200
	± Sd	1.48	6.00	465.81	8.00	1053.15	2.3	30.02	0.57	0
	86	9	61.5	5535	36.5	3285	2	180	9.8	800
	3	6.6	75	4950	23	1518	2	132	7.4	200
	MEAN	7.8	68.25	5242.5	29.75	2401.5	2	156	8.6	500
	± Sd	1.69	9.54	413.65	9.54	1249.45	0	33.94	1.69	424.26
30	198	-	-	-	-	-	-	-	-	-
	214	-	-	-	-	-	-	-	-	-
	205	3.3	23	759	75	2475	2	66	6.4	400
	116	5.4	17.5	945	81.5	4401	1	54	5.4	400
	MEAN	4.35	20.25	852	78.25	3438	1.5	60	5.9	400
	±	1.48	3.88	131.52	4.59	1361.88	0.7	8.48	0.70	0
	86	7.3	45.5	3321	54.5	3978	0	0	6.2	200
	3	2.6	15	390	85	2210	0	0	6.4	200
	MEAN	4.95	30.25	1855.5	69.75	3094	0	0	6.3	200
	± Sd	3.32	21.56	2072.5	21.56	1250.10	0	0	0.14	0

Interval in weeks	Animal Number	RBC X10^6/ul	Hb gm/100 ul	PCV %	MCV u^3	MCHC %	MCH u u g
32	198	-	-	-	-	-	-
	214	-	-	-	-	-	-
	205	4.4	13.1	42	95	31.2	29.8
	116	3.98	11.6	39	98	29.2	29.1
	MEAN	4.19	12.35	40.5	96.5	30.45	29.45
	± Sd	0.29	1.06	2.12	2.12	1.06	0.49
	86	4.2	12.8	37	88	34.6	30.5
	3	3.87	16.4	38	98	27.4	26.9
	MEAN	4.03	14.6	37.5	93	31	28.7
	± Sd	0.23	2.54	0.70	7.07	5.09	2.54
34	198	-	-	-	-	-	-
	214	-	-	-	-	-	-
	205	4.52	12.9	38	84	76	28.5
	116	4.8	12.8	38	79	33.7	26.7
	MEAN	4.66	12.85	38	81.5	54.85	27.6
	± Sd	0.19	0.07	0	3.53	29.91	1.27
	86	4.71	12.3	38	81	32.4	26.1
	3	3.09	10.6	26	84	40.8	34.3
	MEAN	3.9	11.45	32	82.5	36.6	30.2
	± Sd	1.14	1.20	8.48	2.12	5.93	5.79

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
32	198	-	-	-	-	-	-	-	-	-
	214	-	-	-	-	-	-	-	-	-
	205	3.4	30	1020	66	2244	4	136	6.8	200
	116	4.4	26.5	1166	72.5	3190	1	44	6	200
	MEAN	3.9	28.25	1093	69.25	2717	2.5	90	6.4	200
	± Sd	0.70	2.47	103.23	4.59	668.92	2.12	65.05	0.56	0
	86	10	60.5	6050	38.5	3850	1	100	6.8	400
	3	3.7	36	1332	63	2331	1	37	6.4	200
	MEAN	6.85	48.25	3691	50.75	3090.5	1	68.5	6.6	300
	± Sd	4.45	17.32	3336,12	17.32	1074.09	0	44.5	0.28	141.42
34	198	-	-	-	-	-	-	-	-	-
	214	-	-	-	-	-	-	-	-	-
	205	2.9	22.5	652	76.5	2218	1	29	8.6	200
	116	6.1	41	2501	56.5	3446	2.5	152	8.8	200
	MEAN	4.5	31.75	1576.5	66.5	2832	1.75	90.5	8.7	200
	± Sd	2.26	13.08	1307.44	14.14	868.32	1.06	86.97	0.14	0
	86	7.8	53.5	4173	44	3432	2.5	195	7.8	200
	3	4	25	1000	75	3000	0	0	10.6	600
	MEAN	5.9	39.25	2585	59.5	3216	1.25	97.5	9.2	400
	±	2.68	20.15	2241.52	21.92	305.47	1.76	137.8	1.97	282.84

Interval in weeks	Animal Number	RBC $X10^6$/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
36	198	-	-	-	-	-	-
	214	-	-	-	-	-	-
	205	4.42	12.9	31	70	41.6	28.5
	116	3.87	10.6	30	78	35.3	27.4
	MEAN	4.14	11.75	30.5	74	38.45	27.95
	± Sd	0.30	1.62	0.70	5.65	4.45	0.77
	86	3.87	11.6	35	90	33.1	30
	3	3.41	9.4	29	85	32.4	27.6
	MEAN	3.62	10.5	32	87.5	32.75	28.8
	± Sd	0.32	1.55	4.2	3.53	0.49	1.69
	198	-	-	-	-	-	-
38	214	-	-	-	-	-	-
	205	4.38	13.6	26	5.9	52.1	31
	116	4.41	11.6	31	70	3.7	26.3
	MEAN	4.39	12.6	28.5	64.5	44.55	28.65
	± Sd	0.02	1.41	35	7.77	10.67	3.32
	86	4.22	12.4	26	62	47.7	29.4
	3	3.56	11	33	93	33	30.9
	MEAN	3.89	11.7	29.5	77.5	40.35	30.15
	± Sd	0.46	0.98	4.94	21.92	10.39	1.06

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophis %	/ul	Lymphocytes %	/ul	Monocytes %	/ul	Total protein	Fibrino-gen
36	198	-	-	-	-	-	-	-	-	-
	214	-	-	-	-	-	-	-	-	-
	205	42	27.5	1155	72.5	3045	0	0	8.4	400
	116	4.4	15.5	682	83.5	3674	1	44	8	200
	MEAN	4.3	21.5	918.5	78	3359.5	0.5	22	8.2	300
	± Sd	0.4	8.48	334.46	7.77	444.77	0.7	31.11	0.29	141.42
	86	6.2	26	1612	72.5	4495	1.5	93	7.8	200
	3	4.8	33.5	1608	65.5	3144	1	48	6.8	200
	MEAN	5.5	29.75	16.10	69	3819.5	1.25	70.5	7.3	200
	± Sd	0.98	5.30	2.82	4.94	955.30	0.35	31.81	0.70	0
38	198	-	-	-	-	-	-	-	-	-
	214	-	-	-	-	-	-	-	-	-
	205	3.9	5.5	214	94.5	3685	0	0	8.6	200
	116	3.1	22.0	682	78	248	0	0	7	200
	MEAN	3.5	13.75	448	86.25	3051.5	0	0	7.8	200
	± Sd	0.56	11.66	330.8	11.66	895.90	0	0	1.13	0
	86	8.3	24.5	2033	75.5	6266	0	0	9.8	600
	3	3	21.0	630	79	2370	0	0	6.4	200
	MEAN	5.65	22.75	1331.5	77.25	4318	0	0	8.1	200
	± Sd	3.74	2.47	992.07	2.47	2754.88	0	0	2.40	0

APPENDIX 9 (HAEMATOLOGICAL VALUES IN VERVET MONKEYS).

Mean haematological values ± standard deviation (Sd) of Vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %
0	187	5.79	15.7	45	78	34.9
	137	5.66	12.2	40	71	30.5
	609	5.32	12.3	38	71	32.4
	99	5.38	13.2	42	78	31.4
	MEAN	5.53	13.35	41.25	74.5	32.3
	± Sd	0.22	1.62	2.98	4.04	1.89
	673	6.42	16.4	45	70	36.4
	695	5.8	15.4	48	82	32.1
	Mean	6.11	15.9	46.5	76	34.25
	± Sd	0.43	0.70	2.12	8.48	3.04
2	187	5.83	15.1	51	87	29.6
	137	5.5	15.1	41	74	36.8
	609	5.33	13.1	39	73	33.5
	99	4.52	12.7	36	79	35.2
	Mean	5.29	14	41.75	78.25	33.77
	± Sd	0.55	1.28	6.5	6.39	3.09
	673	6.53	16.2	50	76	32.4
	695	5.66	15.2	46	81	33.9
	Mean	6.09	15.7	48	78.5	35.15
	± Sd	0.61	0.70	2.82	3.53	1.76

Mean haematological values ± standard deviation (Sd) of Vervet monkeys infected with L. donovani intravenously.

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
0	187	5.2	42.5	2210	56.5	2938	1	52	8.4	400
	137	9.3	54	5022	44.5	4139	1.5	140	8.2	200
	609	5.0	20	1000	74.5	3725	4.5	225	7.8	200
	99	9.8	47	4606	52	5145	0.5	49	5.8	400
	Mean	7.32	40.87	2448.5	59.25	3532.5	1.87	116.5	7.55	300
	± Sd	2.57	14.69	1530.92	10.33	1218.73	1.79	83.74	1.19	115.47
	673	5.7	42	2394	51.5	2740	0.5	20	9	200
	695	4.0	31	1240	68.5	2935	1.5	86	7.2	200
	Mean	4.85	36.5	1817	60	2837.5	1	53	8.1	200
	± Sd	1.20	7.77	816.0	12.02	137.88	0.7	46.66	1.27	0
2	187	4.3	44	1892	56	2408	0	0	6	200
	137	4.3	46	1978	54	2322	0	0	7.4	400
	609	5.3	37	1961	61	3233	2	106	9.2	600
	99	7.8	59	4002	41	3198	0	0	5.8	400
	Mean	5.42	46.5	1458.25	53	2790.25	0.5	26.5	7.1	400
	± Sd	1.65	9.18	971.54	8.52	492.49	1	53.0	1.57	163.29
	673	4.6	41	1886	59	2714	0	0	7.8	200
	695	4.6	31	1426	69	3174	0	0	7.8	200
	Mean	4.6	36	1656	64	2944	0	0	7.8	200
	±	0	7.07	325.26	7.07	325.26	0	0	0	0

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u
4	187	6.63	18.1	52	78	34.8	27.5
	137	5.57	16.2	52	93	31.2	29.1
	609	5.49	16.4	50	91	32.8	29.9
	99	4.8	12.6	40	83	31.5	26.3
	Mean	5.62	15.12	48.5	86.25	32.57	28.2
	± Sd	0.75	2.31	5.74	6.99	1.63	1.61
	673	6.7	18.4	56	84	32.9	27.5
	695	6.87	20	54	79	37.0	29.1
	Mean	6.78	19.2	55	81.5	34.95	28.3
	± Sd	0.12	1.13	1.41	3.53	2.89	1.1
6	187	6.18	16.1	55	89	29.3	26.1
	137	4.9	13.5	41	84	32.9	27.6
	609	5.8	14.5	47	81	30.9	25
	99	4.3	11.3	38	88	29.7	26.3
	Mean	5.29	13.85	45.25	85.5	30.7	26.3
	± Sd	0.85	2.0	7.5	3.69	1.61	1.0
	673	6.63	16.9	46	69	37	25.5
	695	5.92	16.3	50	84	32.6	28
	Mean	6.27	16.6	48	76.5	34.8	26.7
	± Sd	0.5	0.42	2.82	10.6	3.11	1.7

N.B Animals were infected at week 4.

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u
4	187	6.63	18.1	52	78	34.8	27.5
	137	5.57	16.2	52	93	31.2	29.1
	609	5.49	16.4	50	91	32.8	29.9
	99	4.8	12.6	40	83	31.5	26.3
	Mean	5.62	15.12	48.5	86.25	32.57	28.2
	± Sd	0.75	2.31	5.74	6.99	1.63	1.61
	673	6.7	18.4	56	84	32.9	27.5
	695	6.87	20	54	79	37.0	29.1
	Mean	6.78	19.2	55	81.5	34.95	28.3
	± Sd	0.12	1.13	1.41	3.53	2.89	1.13
6	187	6.18	16.1	55	89	29.3	26.1
	137	4.9	13.5	41	84	32.9	27.6
	609	5.8	14.5	47	81	30.9	25
	99	4.3	11.3	38	88	29.7	26.3
	Mean	5.29	13.85	45.25	85.5	30.7	26.2
	± Sd	0.85	2.0	7.5	3.69	1.61	1.06
	673	6.63	16.9	46	69	37	25.5
	695	5.92	16.3	50	84	32.6	28
	Mean	6.27	16.6	48	76.5	34.8	26.7
	± Sd	0.5	0.42	2.82	10.6	3.11	1.76

N.B Animals were infected at week 4.

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
4	187	6.3	53.5	3370	46.5	2829	0	0	6	200
	137	4.8	33	1585	67	3216	0	0	8	200
	609	3.8	37	1406	65	2394	0	0	8.	200
	99	10	53.5	5350	46.5	4650	0	0	6.8	200
	Mean	6.22	44.25	2927.5	55.75	3297.25	0	0	7.2	350
	± Sd	2.71	10.8	1842.4	10.8	964.01	0	0	0.97	300
	673	6.6	39	2574	61	4026	0	0	9	400
	695	6.6	38	2574	61	4026	0	0	7.2	200
	Mean	5.75	42.25	240.5	57.75	3348	0	0	8.1	300
	± Sd	1.2	4.59	243.95	4.5	958.85	0	0	1.27	141.42
6	187	4	55.5	2220	44.5	17.80	0	0	6.8	200
	137	5.1	33.5	709	66.5	3391	0	0	7	400
	609	2.8	23	644	76	2128	1	28.	7	200
	Mean	5.12	39.75	1903.75	60	2964.2	0.25	7	6.55	300
	± Sd	2.49	14.37	1400.67	13.99	1268.0	0.5	14	0.77	115.47
	673	6	26.5	1590	73.5	4410	0	0	7.6	400
	695	8.6	51	4386	49	4214	0	0	7	200
	Mean	7.3	38.75	2988	61.25	4312	0	0	7.3	300
	± Sd	1.83	17.32	1977.07	17.32	138.59	0	0	0.40	141.41

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
	187	7.69	17.9	54	70	33.1	23.3
	137	5.53	16.4	48	87	34.2	29.7
8	609	4.4	13.6	41	93	33.2	30.9
	99	4.74	13.2	40	84	33	27.8
	Mean	5.59	15.27	47.75	83.5	33.37	27.92
	± Sd	1.47	2.25	6.55	9.74	0.55	3.33
	673	5.68	17.8	54	95	33	31.3
	695	8.06	17	51	63	33.3	21.1
	Mean	6.87	17.4	52.5	79	33.15	26.2
	± Sd	1.68	0.56	2.12	22.62	0.21	7.21
	187	5.82	17	54	93	31.5	29.2
	137	5.37	16.1	51	95	31.6	30
10	609	3.87	12.7	41	106	31	32.8
	99	4.4	12.6	40	91	31.5	28.6
	Mean	4.86	14.6	46.5	96.25	31.4	30.15
	± Sd	0.88	2.28	7.04	6.7	0.27	1.85
	673	6.1	18	58	95	31	29.5
	695	5.83	17.4	53	91	32.8	29.8
	Mean	5.96	17.7	55.5	93	31.9	29.65
	± Sd	0.19	0.42	3.53	2.82	1.27	0.21

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
	187	4.8	61	2928	38	1824	0	0	6.2	200
	137	5.8	32	1856	68	3944	0	0	8.4	600
	609	4.8	41	1968	59	2832	0	0	7.6	200
8	99	9.3	48	4464	52	4836	0	0	5.2	200
	Mean	6.17	45.5	2804	54.25	3359	0	0	6.85	300
	± Sd	2.13	12.23	1206.7	1265	1311.19	0	0	1.42	200
	673	5.2	42	2184	58	3016	0	0	6.2	200
	695	4.8	34.5	1656	65.5	3144	0	0	6	200
	Mean	5	38.25	1920	61.75	3080	0	0	6.1	200
	± Sd	0.28	5.3	373.35	5.3	90.50	0	0	0.14	0
	187	3.3	55	1815	45	1485	0	0	6.8	200
	137	6.2	51.5	3193	48.5	30007	0	0	8	200
	609	2.6	18	468	82	2132	0	0	7	600
	99	15.4	60	9240	39.5	6083	0.5	77	5.2	200
10	Mean	6.87	46.12	3679	53.75	3176.75	0.12	1925	6.75	300
	± Sd	5.89	19.07	3870.65	19.19	2035.4	0.25	38.5	1.15	200
	673	3.3	32	1056	68	2244	0	0	6.6	200
	695	3.6	31.5	1134	68.5	2466	0	0	7	400
	Mean	3.45	31.75	1095	68.25	2355	0	0	6.5	300
	± Sd	0.21	0.35	55.15	0.35	156.97	0	0	0.28	141.42

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
	187	6.79	16.4	54	80	304	24.2
	137	6.93	16.6	51	74	32.5	24
12	609	5.83	12.9	42	72	30.7	22.1
	99	4.98	11.2	38	76	29.5	22.6
	Mean	6.13	14.27	46.25	75.5	30.77	23.2
	± Sd	0.91	2.66	7.5	3.41	1.25	1.05
	673	7.22	17	55	76	30.9	23.5
	695	7.79	18.7	57	74	32.8	24
	Mean	7.5	17.85	56	75	31.85	23.75
	± Sd	0.4	1.20	1.41	1.41	1.34	0.35
	187	7.17	16.2	52	72.7	31.2	22.6
	137	5.84	13.4	43	74	31.2	22.9
	609	5.69	13	41	72	31.7	22.8
	99	5.01	11.1	37	74	30	22.2
14	Mean	5.92	13.42	43.25	73.17	31.00	22.62
	± Sd	0.9	2.10	6.3	0.99	0.72	0.30
	673	7.22	17	52	72	32.7	23.5
	695	7.4	18	50	68	36	24.3
	Mean	7.31	17.5	51	70	34.35	23.9
	± Sd	0.12	0.70	1.41	2.82	2.33	0.56

Interval in weeks	Animal Number	EG 10^3/ul	Neutrophils %	/ul	Lymphocytes %	/ul	Monocytes %	/ul	Total protein	Fibrinogen
	187	4.8	51.5	2472	48.5	2328	0	0	6.8	200
	137	6.8	56.5	3842	43.5	2958	0	0	6.8	200
12	609	3.4	33	1122	67	2278	0	0	6.6	200
	99	8.1	52	4212	46.5	3766	1.5	121	5	400
	Mean	5.77	48.25	2912	51.37	2832.5	9.37	30.25	6.3	250
	± Sd	2.08	10.41	1408.61	10.61	695.02	0.75	60.5	0.87	99.99
	673	4.5	45.5	2047	54.5	2452	0	0	6	600
	695	4.5	37	1665	63	2835	0	0	6.8	200
	Mean	4.5	41.25	1856	58.75	2643.5	0	0	6.4	400
	± Sd	0	6.01	270.11	6.01	270.82	0	0	0.56	282.84
	187	4.5	29	1755	61	2745	0	0	8	200
	137	7.6	49.5	3762	475	3610	3	228	7	400
	609	3.2	17	544	83	2456	0	0	7	200
	99	11	46	5060	53	5830	1	110	6	200
14	Mean	6.57	3787	2780.25	61.12	3710.25	1	84.5	7	250
	± Sd	3.47	1458	2017.67	15.60	1477.22	1.41	108.81	0.81	99.99
	673	4.2	28.5	1197	71.5	3003	0	0	8.6	400
	695	4.8	36.5	1752	63.5	3048	0	0	8.4	600
	Mean	4.5	32.5	1474.5	67.5	30255	0	0	8.5	500
	± Sd	0.42	5.65	392.44	5.65	31.81	0	0	0.14	141.42

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100ml	PCV %	MCV u^3	MCHC %	MCH u u g
	187	5.88	14.2	45	77	31.6	24.1
16	137	7.18	16.1	55	77	29.2	22.4
	609	5.37	12.7	38	71	33.4	23.6
	88	5.68	13	42	74	30	22.9
	Mean	6.02	14	45	74.75	31.05	23.25
	± Sd	0.79	1.54	7.25	2.87	1.85	0.75
	673	7.58	17.8	55	73	32.4	23.5
	695	7.57	18.6	53	70	35.2	24.6
	Mean	7.57	18.2	54.0	71.5	33.8	24.05
	± Sd	7.06	0.56	1.41	2.12	1.97	0.77
	187	4.64	13.6	43	93	31.6	29.3
18	137	6.41	15.8	52	81	30.4	25.9
	609	4.97	13,4	44	89	30.5	27
	99	4.31	10	35	81	28.6	23.2
	Mean	5.08	13.2	43.5	86	30.27	26.35
	± Sd	0.92	2.39	6.95	5.99	1.24	2.53
	673	6.91	18.4	58	84	31.7	26.6
	695	6.4	18.8	55	86	34.2	29.4
	Mean	6.65	18.6	56.5	85	32.95	28
	± Sd	0.36	0.28	2.12	1.41	1.76	1.97

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	/ul	Lymphocytes %	/ul	Monocytes %	/ul	Total protein	Fibrinogen
16	187	4	30	1200	70	2800	0	0	8	200
	137	67	51.5	3450	48.5	3249	0	0	7.8	200
	609	1.7	51.5	3450	48.5	3249	0	0	8	200
	99	14.6	47.5	6935	52.5	7592	0	0	7	200
	Mean	6.75	41.25	3049.25	58.75	3682.25	0	0	7.7	200
	± Sd	5.61	9.97	2864.70	9.97	2767.81	0	0	0.47	0
	673	5.9	40	2360	60	3540	0	0	7.8	400
	695	4	29.5	1180	70.5	2820	0	0	8.8	200
	Mean	4.95	34.75	1770	65.25	3180	0	0	8.3	200
	± Sd	1.34	7.42	834.38	7.42	509.11	0	0	0.70	141.42
18	187	4	31	1240	69	2760	0	0	8	200
	137	4.3	45	1935	55	2365	0	0	8.6	400
	609	2.5	14	350	86	2150	0	0	7.4	400
	99	10.2	58	5916	42	4284	0	0	6	200
	Mean	5.25	37	2360.15	63	2889.75	0	0	7.5	300
	± Sd	3.39	18.88	245765	18.88	963.21	0	0	1.11	115.47
	673	54	55.5	2997	44.5	2403	0	0	7.8	400
	695	4.6	42	1932	58	2668	0	0	7.6	200
	Mean	5.0	48.75	2464.5	51.25	2536.5	0	0	7.7	300
	± Sd	0.56	9.54	753.06	9.54	185.96	0	0	0.14	141.42

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100ml	PCV %	MCV u^3	MCHC %	MCH u u g
20	187	6.12	13.7	42	69	32.6	22.4
	137	8.5	16.7	50	59	33.4	19.3
	609	7.01	13.8	43	61	32.1	19.7
	99	4.52	10.4	39	75	30.6	23
	Mean	6.53	13.65	43.5	66	32.17	21.1
	± Sd	1.66	2.57	4.65	7.39	1.17	1.87
	673	7.79	18	56	72	32.1	22.8
	695	7.11	16.9	53	75	31.9	23.8
	Mean	7.45	17.45	54.5	73.5	32	23.3
	± Sd	0.45	0.77	2.12	2.12	0.14	0.70
22	187	5.83	13.9	45	77	30.9	23.8
	137	7.47	15.8	50	67	31.6	21.8
	609	-	-	-	-	-	-
	99	4.31	10	35	81	28.6	23.2
	Mean	5.87	13.23	43.33	75	30.36	22.73
	± Sd	1.56	2.95	7.63	7.21	1.56	1.36
	673	7.68	18	57	74	31.6	23.4
	695	6.97	17.6	52	75	33.8	25.3
	Mean	7.32	17.8	54.5	74.5	32.7	24.35
	± Sd	0.5	0.28	3.53	0.70	1.55	1.34

Interval in weeks	Animal Number	SBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
20	187	5.4	29.4	1593	69	3726	1.5	81	8	200
	137	7.3	57	4161	43	3139	0	0	7	200
	609	5.1	27.5	140.2	72.5	3697	0	0	7	200
	99	6.3	37.5	2393	60	3780	2	126	5.2	200
	Mean	6.02	37.85	2387.25	61.12	3584.75	0.87	51.75	6.8	200
	± Sd	0.99	13.46	1258.01	13.18	301.13	1.03	62.5	1.16	0
	673	5.4	50.5	2727	49.5	2673	0	0	6.2	200
	695	4.1	42	1722	56.5	2316	1.5	61	5.6	400
	Mean	4.75	46.25	22245	53	2494.5	0.75	30.5	5.9	300
	± Sd	0.91	6.01	710.64	4.94	252.43	1.06	43.13	0.42	141.42
22	187	6.8	47	3196	53	3604	0	0	7.8	200
	137	6.8	49.5	3366	50.5	3434	0	0	7.4	400
	609	-	-	-	-	-	-	-	-	-
	99	7.6	39.5	3002	60	4560	0.5	38	6.8	400
	Mean	7.06	45.33	3188	54.5	3866	0.16	12.66	7.33	333.33
	± Sd	0.46	5.20	182.13	4.92	607.00	0.28	21.93	0.50	115.47
	673	3.6	44	1584	56	2016	0	0	6.4	400
	695	3.8	46	1748	54	2052	0	0	6.2	200
	Mean	3.7	45	1666	55	2034	0	0	6.3	300
	± Sd	0.14	1.41	115.96	1.41	54799	0	0	0.14	141.42

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100m	PCV %	MCV u^3	MCHC %	MCH u u g
24	187	5.32	13.5	45	85	30	25.4
	137	6	15.4	49	82	31.4	25.7
	609	-	-	-	-	-	-
	99	2.87	7	23	80	30.4	24.4
	Mean	4.73	11.96	39	82.33	30.6	25.16
	± Sd	1.64	4.40 .	14	2.51	0,72	0.68
	673	7.63	17.8	56	73	31.8	23.3
	695	7.29	17.8	55	75	32.4	24.4
	Mean	7.46	17.8	55.5	74	32.1	23.85
	± Sd	0.24	0	0.70	1.41	0.42	0.77
26	187	5.9	15	40	68	37.5	25.4
	137	6.57	14	40	62	35	21.3
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	Mean	6.23	14.5	40	65	36.25	23.35
	± Sd	0.47	0.70	0	4.24	1.76	2.89
	673	7.3	17.8	45	62	39.6	24.4
	695	7.33	17.4	48	65	36.3	23.7
	Mean	7.31	17.6	46.5	63.5	37.95	24.05
	± Sd	0.02	0.20	2.12	2.12	2.33	0.49

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
24	187	4.8	10.5	504	88.5	4248	1	48	7	200
	137	4.0	34	1360	65.5	2620	0.5	20	7	400
	609	-	-	-	-	-	-	-	-	-
	99	12.7	50	6350	49.5	6286	0.5	63	8	200
	Mean	7.16	31.5	2738	67.83	4384.66	0.66	43.66	7.33	266.66
	± Sd	4.8	19.86	3157.22	19.60	1836.81	0.28	21.82	0.57	115.47
	673	8.9	17.5	1557	82.5	72.98	0	0	6.2	200
	695	5.1	305	1555	69.5	3544	0	0	6	200
	Mean	7	24	1556	62.75	2780	0	0	6.1	200
	± Sd	2.6	9.19	1.41	9.54	1080.45	0	0	6.14	0
26	187	6	21.5	1290	77.5	4650	1	60	7	400
	137	3.6	8	288	92	3312	0	0	10.2	200
	609	-	-	-	-	-	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	Mean	4.8	14.75	789	84.75	3981	0.5	30	8.6	300
	± Sd	1.69	9.54	708.52	10.25	946.10	0.7	0.42	2.26	141.42
	673	4	24	960	74	2960	2	80	10.8	600
	695	4.2	21.5	903	75.5	31.71	3	126	9.6	600
	Mean	4.1	22.75	931.5	74.75	3065.5	2.5	103	10.2	600
	± Sd	0.14	1.76	40.30	1.06	149.19	0.70	32.52	0.84	0

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100ml	PCV %	MCV %	MCHC %	MCH u u g
28	187	5.71	14.6	41	72	35.6	25.6
	137	3.71	12.2	36	97	33.9	32.9
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	MEAN	4.71	13.4	38.5	84.5	34.75	29.25
	± Sd	1.41	1.69	3.53	17.67	1.20	5.16
	673	6.42	17.1	49	76	34.9	26.6
	695	6.01	16.6	55	92	29.1	27.7
	Mean	6.21	16.85	52	84	32	27.15
	± Sd	0.28	0.35	4.24	11.31	4.10	0.77
30	187	5.27	15.1	50	95	30.2	28.7
	137	3.71	12	36	97	33.3	32.3
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	Mean	4.49	13.55	43	96	31.75	30.5
	± Sd	1.10	2.19	9.89	1.41	2.19	2.54
	673	6	16.4	41	68	40	27.3
	695	6.87	18.4	58	84	31.7	26.8
	Mean	6.43	17.4	49.5	76	35.85	27.05
	± Sd	0.61	1.41	12.02	11.31	58.6	0.35

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
28	187	8.2	27.5	2255	69.5	5699	3	246	8	200
	137	5.9	21	1239	78	4602	1	59	8	200
	609	-	-	-	-	-	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	Mean	7.05	24.24	1749	78.75	5150.5	2	152.5	8	200
	± Sd	1.62	4.59	718.42	6.01	775.69	1.41	132.2	0	0
	673	5.5	32	1760	68	3740	0	0	7.8	200
	695	5.2	29	1508	71	3692	0	0	6.8	200
	Mean	5.35	30.5	1634	695	3716	0	0	7.3	200
	± Sd	0.21	2.12	178.19	2.12	33.94	0	0	0.70	0
30	187	6.7	15.5	1038	79	5293	5.5	335	8	200
	137	2.9	7	203	87	2523	6	174	5.8	200
	609	-	-	-	-	-	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	Mean	48	11.25	6205	83	3908	5.75	254.5	6.9	20
	± Sd	2.68	6.01	590.43	5.65	1958.68	0.35	113.84	1.55	0
	673	5.6	22.5	1260	77.5	4340	0	0	7.8	400
	695	4.3	22.5	967	75.5	3246	2	85	6.4	400
	Mean	4.95	22.5	1113.5	76.5	3793	1	42.5	7.1	400
	± Sd	0.91	0	207.18	1.41	773.57	1.91	60.10	0.98	0

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
32	187	4.86	14.8	51	105	29	305
	137	-	-	-	-	-	-
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	673	5.59	16.4	42	75	39	29.3
	695	5.91	16.8	55	93	30.5	28.4
	Mean	5.75	16.6	48.5	84	34.75	28.85
	± Sd	0.22	0.28	9.19	12.72	6.01	0.63
34	187	5.3	15	46	87	32.6	28.3
	137	-	-	-	-	-	-
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	673	7.13	18.4	52	73	35.3	25.8
	695	6.03	17.4	45	75	38.7	28.9
	Mean	6.58	17.9	48.5	74	37	27.35
	± Sd	0.77	0.70	4.94	1.41	2.4	2.19

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul	Lymphocytes %	Lymphocytes /ul	Monocytes %	Monocytes /ul	Total protein	Fibrinogen
32	187	7.3	26	1898	74	5402	0	0	7.8	400
	137	-	-	-	-	-	-	-	-	-
	609	-	-	-	-	-	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	673	4.3	44.5	1913	55	2365	0.5	21	5.6	200
	695	7.4	26.5	1961	73.5	5439	0	0	6.4	400
	Mean	5.85	35.5	1937	64.25	3902	6.25	10.5	6	300
	± Sd	2.19	12.72	33.74	13.08	2173.64	0.35	14.84	0.56	141.46
34	187	9.2	21	1932	76	6992	3	276	9.6	200
	137	-	-	-	-	-	-	-	-	-
	609	-	-	-	-	-	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	673	6.8	47.5	3230	52	3536	0.5	34	9.6	200
	695	5	27.5	1375	72	3600	0.5	25	11.4	400
	Mean	5.9	37.5	2302.5	62	3568	0.5	29.5	10.5	300
	± Sd	1.27	14.14	1311.68	14.14	42.25	0	6.36	1.27	141.42

Interval in weeks	Animal Number	RBC 10^6/ul	Hb gm/100 ml	PCV %	MCV u^3	MCHC %	MCH u u g
36	187	4.82	14.7	42	87	35	30.4
	137	-	-	-	-	-	-
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	673	5.78	16.2	45	78	36.5	33.8
	695	5.83	17.4	53	91	32.8	29.8
	MEAN	5.8	16.8	49	84.5	34.65	31.8
	± Sd	0.03	0.84	5.65	9.19	2.61	2.82
38	187	4.22	14.2	31	73	45.8	33.6
	137	-	-	-	-	-	-
	609	-	-	-	-	-	-
	99	-	-	-	-	-	-
	673	5.84	17.4	44	75	39.5	29.8
	695	5.68	17	42	74	40.5	29.9
	MEAN	5.76	17.2	43	74.5	40	29.85
	± Sd	0.11	0.28	1.41	0.70	0.70	0.07

Interval in weeks	Animal Number	WBC 10^3/ul	Neutrophils %	Neutrophils /ul
36	187	7.7	19.5	1501
	137	-	-	-
	609	-	-	-
	99	-	-	-
	673	6	34	2040
	695	8.6	51	4386
	MEAN	7.3	42.5	32.13
	± Sd	1.83	12.02	1658.87
38	187	6.2	23.5	1457
	137	-	-	-
	609	-	-	-
	99	-	-	-
	673	5.5	18	990
	695	4.2	6	252
	MEAN	4.85	12	621
	± Sd	0.91	8.48	521.84

Lymphocytes		Monocytes		Total	Fibrino-
%	/ul	%	/ul	protein	gen
80.5	6198	0	0	10	200
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
65	3900	1	60	8.4	200
49	4214	0	0	7.2	200
57	4057	6.5	30	7.8	200
11.31	222.03	0.70	42.42	0.84	0
76.6	4743	0	0	9.8	400
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
82	4510	0	0	9.8	400
94	3948	0	0	9.4	400
88	4229	0	0	9.6	400
8.48	397.39	0	0	0.28	0

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