

A COMPARATIVE STUDY OF SOME
CHANGES IN THE BLOOD AND CEREBRAL
CAPILLARIES IN MICE DURING
INFECTION WITH BABESIA RODHAINI
AND BABESIA MICROTI



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A comparative study of some changes in the blood and
cerebral capillaries in mice during infection with
Babesia rodhaini and Babesia microti

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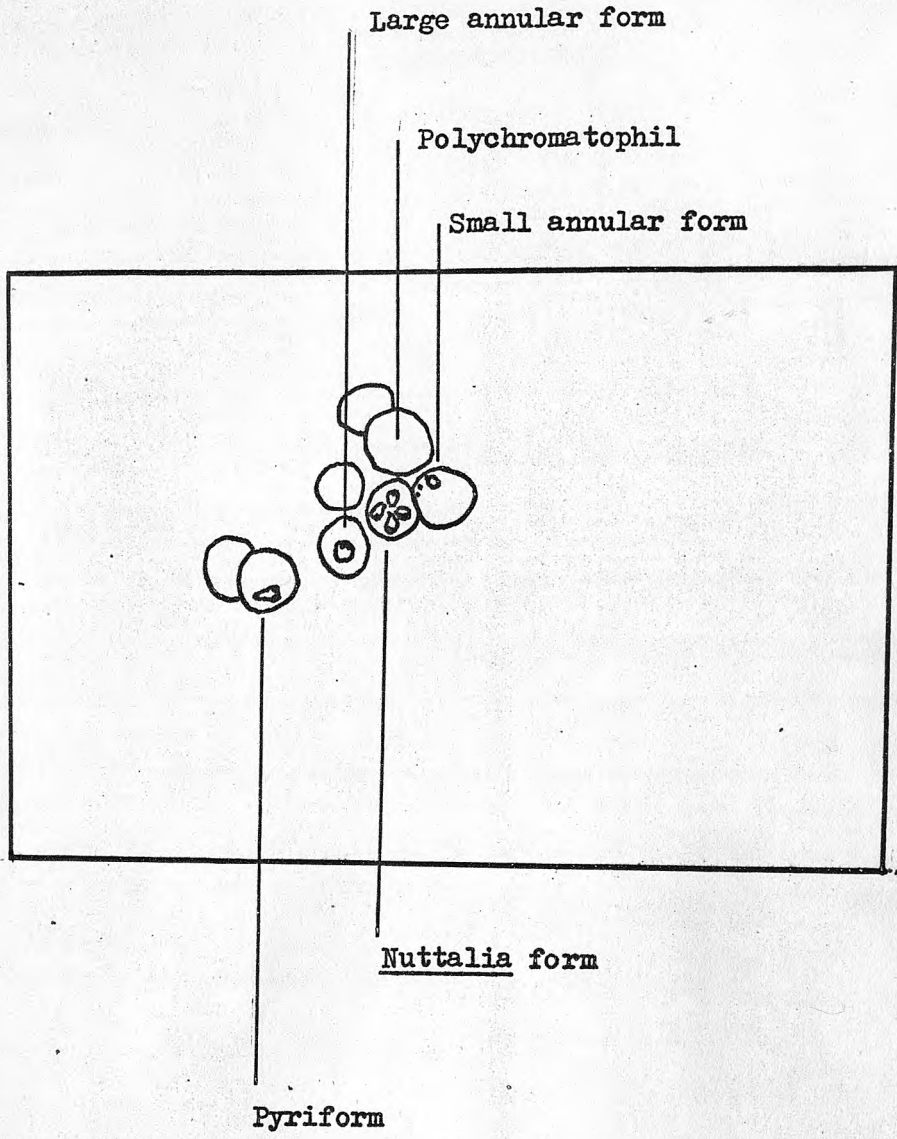
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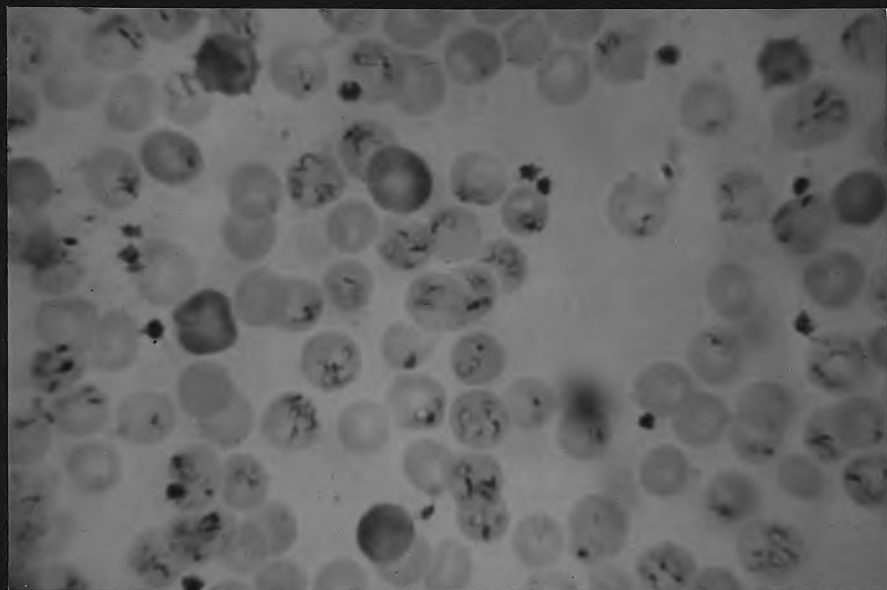
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Giemsa Stained blood smear

Magnification X 1750



Babesia rodhaini

SUMMARY

Methods of sampling mice for blood examination and brain crush preparation, are described.

Erythrocyte, leucocyte and brain capillary parameters were studied in the normal mouse, and these were measured throughout infections with Babesia rodhaini and B. microti in order to compare any differences in response to these parasites.

A number of differences between the infections were established. Babesia rodhaini produced a disease of high mortality with a normocytic normochromic anaemia. The white cell response was restricted to the neutrophil series. Babesia microti produced a non-fatal disease with a macrocytic, normochromic anaemia. The white cell response was massive, involving the neutrophils, lymphocytes and monocytes.

In neither disease was any evidence, to suggest that cerebral babesiosis played an important part in the pathology of the disease, found.

INTRODUCTION

Babesia rodhaini and B. microti are parasites of wild and laboratory rodents. Nowell, F. (1968) considered them to be two separate species, but Levine, N.D. (1971) reclassified them as the same organism.

Both these parasites are used in the laboratory as models for research work, leading to the increase in knowledge of the more economically important babesias of domestic animals. Nowell (1968) set out in his thesis to compare these parasites with reference to their use as laboratory models. His project covered a wide field of experimental hosts and experimental parameters.

The object of the work reported here was to study the changes in the formed elements of the blood throughout infection with these two parasites. Observations were confined to one experimental host, the mouse. This has not been attempted previously and is of value in understanding the reaction of the host to the parasites and also any differences in host response to each of the two parasites.

Cerebral babesiosis has not been found in these infections, and it was decided to examine brain crush preparations during the whole course of infection, to establish whether any abnormal brain capillary changes occur, and to relate them to the stage of infection.

The observations on infected mice were preceded by studies in the normal mouse. These were expected to add to the information already available but, more important, would support the pre-infection normal values using methods later to be employed in infected animals.

REVIEW OF THE LITERATURE

Babesia rodhaini:

Babesia rodhaini was discovered by Van den Berghe, Vincke, Chardome & Van den Bulke (1950) in Thomnomys surdaster surdaster. It was infective to white mice and white rats, (Beveridge, 1953; Matson, 1964); Cricolis auratus, (Rodhain & Demylder, 1951); Sigmodex hispidus, (Rodhain, 1950); Steotomys opinus, (Rodhain & Demylder, 1951) and Tatera myassae, (Van den Berghe et al, 1950).

No invertebrate host has been described, although it is probably a tick, Nowell (1968).

Mode of infection:

In the laboratory, infection was produced by blood inoculation, (Beveridge, 1953). A higher infection rate was achieved by the intraperitoneal, rather than subcutaneous route of inoculation, (Beveridge, 1953). Aeschliman & Suter (1965) discovered that the prepatent period decreased with higher dosage rates, yet the intensity of infection was not affected. Foetal infections in utero did not occur, (Colas-Belcour & Vervent, 1953).

Morphology:

The morphology of the parasite was described by Flewett & Fulton (1959); Van den Berghe et al (1950); Aeschliman & Suter (1965) and Nowell (1968), the parasites described as annular or pyriform. The annular ones were

1.8 to 3.6 microns in diameter, and the pyriform 1.8 to 2.7 microns by 2.7 to 4.5 microns. Parasites, in the mouse, were vacuolated and, with Giemsa stain, were blue with red nuclei. Babesia rodhaini did not produce pigment, (Goodwin & Richards, 1960). Reproduction, as described by Van den Berghe et al (1950) and Radzinsha & Trager, (1962), could be by budding, binary fission, or multiple fission, the last by way of Maltese cross forms. The parasite apparently ingested the cytoplasm of the invaded erythrocytes.

Course of infection :

The prepatent period of the infection is between three and 10 days (Van den Berghe et al, 1950). This period is inversely proportional to the inoculated dose (Aeschliman & Suter, 1965). Rodhain (1950) reported peak parasitaemia in mice between six and 13 days after infection, with parasitaemia up to a maximum of 84 per cent. He also stated that the parasites preferentially infected mature erythrocytes. Beveridge (1953) reported an 80 per cent mortality rate, with death following the peak of parasitaemia. This was, however, variable (Rodhain, 1950; Colas-Belcour & Vervent, 1953) but apparently depended on the size of the inoculum (Canache-Mata, 1959; Aeschliman & Suter, 1965).

Canache-Mata (1959) described pallor of the mucous membranes, anaemia, weakness, collapse, fever and hypothermia before death. He also recorded a loss of twenty to twenty five per cent in body weight and an increase in

heart and respiratory rates. Colas - Belcour & Vervent (1953) described an enlarged darkened spleen. Paget, Alcock & Ryley (1962) reported an increase of phagocytes, reticulum cells, giant cells and excess polymorphonuclear leucocytes in the splenic pulp. Canache-Mata (1959) recorded an increase in the haemopoietic centres of the spleen. The liver was enlarged and mottled with white spots. After vacuolation and reduction of glycogen cells, the liver showed focal and zonal necrosis. The presence of hyaline degeneration of the liver was suggested by Paget, Alcock & Ryley (1962) and Rodhain & Demylder (1951) suggested that fatty change occurred. The kidney showed enlargement and pallor, with cells of the proximal convoluted tubules being cloudy and swollen, and containing haemoglobin (Rodhain & Demylder, 1951; Canache-Mata, 1959; Paget, Alcock & Ryley, 1962). Nowell (1968) found no increase in kidney weight, and that the spleen started increasing in weight three days before the peak of parasitaemia. Haemoglobinuria occurred, with a maximum level of 2 gm Hb/100 ml urine, just prior to the parasitaemic peak. Albumin, erythrocytes and cylinders of kidney epithelial cells have also been found in the urine (Rodhain & Demylder, 1951).

Anaemia:

The anaemia of Babesia rodhaini infection has been described by Beveridge (1953); Matson (1964); Schroeder,

Cox & Ristic (1966) and Phillips (1966). The anaemia was normochromic, transmacrocytic and haemolytic. Nowell (1968) described a fall in erythrocytes to between 1.6 and 4.9 million per cubic millimeter in rats. Schroeder et al, (1966), working with rats, found that the degree of anaemia produced was not in proportion to the parasitaemia obtained. They suggested that the anaemia might be partially due to auto-immunisation, with serum auto-haemagglutinins acting as opsonins in erythrophagocytosis. Rodgers (1974) detected serum opsonins which seemed to be specific for infected erythrocytes. There was no phagocytosis of normal erythrocytes during the experiments. Sibinovic, Milar, Ristic & Cox (1969) found a β -globulin associated antigen in serum in acute parasitaemia. This antigen was bound to the erythrocytes within a few minutes. These erythrocytes were then removed, mostly from the peripheral circulation. Antiserum selectively agglutinated and disintegrated erythrocytes containing the more mature parasites, yet the parasites released were still intact. Dolan (personal communication) reported no phagocytosis in the peripheral blood, even with free Babesia in the circulation. Erythrophagocytosis by mononuclear cells was found to occur in the spleen and bone marrow. George, Stokes, Wicker & Conrad (1966) studying Plasmodium berghei in rats, could not demonstrate any anti-erythrocyte antibody. McHardy (1973) using rabbit anti mouse erythro-

cytic serum, which caused destruction of mature erythrocytes within the circulation during Babesia rodhaini infection, produced recovery in the mice. This suggested a preference of the parasite for mature cells. Nowell (1968) gave a preference for immature cells. R.A.M.E.S. caused shrinkage of erythrocytes and this change is also seen just before death in animals having a normal infection. McHardy (1973), from this, postulated that autoantibody against erythrocytes might also inhibit Babesia rodhaini. Maegraith (1966) working on malaria, revived the theory of "toxin production" producing cell damage which could play a part in the anaemia. This "toxin" was probably break down products of damaged erythrocytes, causing non-specific damage to other erythrocytes. There was no toxic depression of the bone marrow.

On recovery, parasites disappeared from blood smears (Colas et al, 1953). Blood, however, might still be infective to clean mice. Relapses to disease were not fatal and parasitaemic peaks were lower (Rodhain, 1950). Some animals had chronic parasitaemias for months and some were free from parasites and were not infective (Colas et al, 1953). Splenectomy did not alter the parasitaemia (Rodhain, 1950), but Roberts, Kerr & Tracey-Patte (1972) found that, although the spleen did not affect the multiplication of parasites in normal hosts, the ultimate fate of the host largely depended on an intact spleen producing

antibody. They also concluded that if phagocytosis was important then unstimulated non-splenic phagocytes were probably adequate.

Resistance:

Rodhain (1950) reported that some mice were refractory to infection. Goble (1966) found that females were more resistant than males, whereas Nowell (1968) found no age or sex differences.

Cod liver oil suppressed infection (Godfrey, 1957) and tetra ethyl thiuram disulphide, vitamins C and E reversed the suppression (Rodhain, 1950).

Babesia microti:

Babesia microti was discovered by Franca (1910) in blood from Microtus cincercus in Portugal.

Similar parasites have been discovered in small mammals of North West Europe and the United States of America. They have been found in Apodernus sylvaticus (Coles, 1914; Jacobs, 1953; Shortt, 1961): Arvicola amyhibius (Coles, 1914): Clethrionomys glareolus (Jacobs, 1953; Shortt, 1961; Baker, Chitty & Phipps, 1963): Microtus agrestis (Baker et al, 1963; Shortt & Blackie, 1965): Sorex araneus (Jacobs, 1953; Shortt & Blackie, 1965). Shortt & Blackie (1965) decided all were indistinguishable on grounds of morphology and immunology.

Invertebrate hosts:

The only recorded vector of Babesia microti is Ixodes

trianguliceps (Young, 1970).

Morphology:

The morphology of the parasite was described by Shortt & Blackie (1965) with annular forms 2.0 to 3.0 microns in diameter, pyriforms 6.5 microns x 2.0 microns, elongated ellipsoid and Maltese cross forms. They described ten possible stages based on size. The parasite was morphologically very similar to Babesia rodhaini.

Course of infection:

On inoculation of 5×10^6 parasites, disease was patent between two and four days and attained a peak at 12 days. It was subpatent by 16 to 18 days (Young, 1970), with parasitaemias reaching 50 to 80 per cent. Irvin & Brocklesby (1969) described mice with persistent parasitaemias up to 247 days. Anaemia has been described by Shortt & Blackie (1965) and Young (1970), with erythrocyte counts falling to 3.0×10^6 per cubic millimetre of blood.

Pathology:

The pathological changes in Babesia microti infection were described by Shortt & Blackie (1965). Liver cells showed cloudy swelling and fatty degeneration. There were concentrations of erythrocytes in the lungs. The spleen was large, dark and friable, with parasites present in the red pulp. There was an increase in foreign body cells with the malpighian corpuscles less obvious than normal. Granular swelling and degeneration of the kidney tubule cells

with debris in the lumina of the tubules were found. The total white blood cell count was increased. The enlarged spleen, with erythrophagocytosis in blood and spleen, was reported by Young (1970). He recorded that, before the peak of infection, phagocytosis was mainly of infected cells but in the recovery stage it was usually of non-infected cells. Mortality was low. Nowell (1968) found the lymphoid macrophage system to be enhanced.

Immunity:

Cox & Young (1969) stated that recovered animals were immune and that immunity was mainly sterile and cross immunising with Babesia rodhaini.

Normal mouse blood values:

Schalm (1967) reviewed the work done by Scarborough, Gardner, MacNamee, Shermer, Dougherty, De Kock & Russel. Erythrocyte counts averaged between 9.0 and 10.0 million per cubic millimetre of blood, but the range was from 5.5 to 13.9 million per cubic millimetre.

Haemoglobin estimations averaged between 15 and 20 grams per cent, but varied from 12 to 25 grams per cent.

Packed cell volumes averaged between 39 and 50 per cent.

Total leucocyte counts were between 7,000 and 32,000 per cubic millimetre of blood, of which neutrophils could be between five and 40 per cent of cells. Lymphocytes varied from 35 to 90 per cent, but usually averaged between 60 and 70 per cent. Monocytes fell within the range of nought to eight per cent and eosinophils averaged between two to three per cent, varying from nought to 15 per cent. Basophils were rarely seen, never going above one per cent.

Russel, Neufeld & Higgins (1951), using various strains of inbred mice, found mean cell volumes to be within the range of 41 to 52 cubic microns.

De Kock (1931) found leucocyte numbers in tail blood to be 1.5 to 5.0 times greater than in blood from large vessels.

Brown & Dougherty (1956) demonstrated marked diurnal

variations in tail blood leucocytes, the highest counts occurring during periods of relative inactivity.

Halberg, Hamerston & Bittner (1957) found similar diurnal variations in eosinophils and females had lower eosinophil counts than males. Russel et al (1951) found differences between 18 inbred lines in their leucocyte counts and that the granulocyte percentage was greater in males than females.

Leucocyte morphology:

The white cells of the mouse were described by Schalm (1967).

Lymphocytes were of small and large types, the small lymphocyte having darker blue cytoplasm than the large. Azurophillic granules would be seen in some large lymphocytes.

The monocyte nucleus is amoeboid with pale chromatin strands. The cytoplasm was basophilic and could contain a few small vacuoles.

The nucleus of the eosinophil was elongated and coiled to form U and ring forms. The cytoplasm was basophilic and the acidophilic granules were small and scanty and in clumps or patches.

The neutrophil had a diffusely pink cytoplasm, with delicate, dust-like granules. It had a polymorphous nucleus.

The basophil was rarely seen and no description has been found.

Leucocyte response to babesial infections:

In dogs with Babesia canis infection Saunders (1937) and Macgrath (1957) found a leucocytosis. Brody & Prier (1962) gave figures of between 19,500 and 85,000 per cubic millimetre of blood. Seibold & Bailey (1957) found that there was a 50 per cent neutrophilia and a 50 per cent eosinophilia, and Hindaway (1951) found that the neutrophils were increased more in chronic cases than in acute ones.

In cattle, Suteu & Guirgea-Jacob (1971) reported a lymphocytosis, monocytosis, basophilia, eosinophilia and a neutropenia which was persistent.

In rats infected with Babesia rodhaini there was a lymphocytosis and neutrophilia, which rapidly returned to normal, Dolan (1974) (personal communication). Paget et al (1962) reported an increase in phagocytes in rats infected with Babesia rodhaini.

Shortt & Blackie (1965) working with rats infected with Babesia microti recorded a leucocytosis.

Cerebral babesiosis:

Cerebral babesiosis has been reported in dogs and cattle.

In dogs, Purchase (1947) described free Babesia spp. present in the brain capillaries. Cerebral babesiosis has also been reported in dogs with Babesia canis by Malckerke & Parkin (1943).

In cattle, Dunmuth (1960) working in Venezuela reported sludging of the brain capillaries with Babesia argentina infection. Hartley (1968) found sludging in cattle in Turkey. Abeysen (1970) found the same in Ceylon and Callow & McGavin (1963) reported the condition in Australia. Folkes (1967), also working with Babesia argentina, described the condition in Nigeria and Tchermomoretz (1943) found the condition with Babesia berbera infection. Zlotnick (1953) describes cerebral babesiosis in Babesia bigemina infection in Nyasaland.

Nowell (1968) could find no capillary bed stages in animals infected with Babesia rodhaini.

MATERIALS AND METHODS

The experimental hosts:

For the establishment of normal haemograms and brain crush examinations, and for infection with Babesia rodhaini, mice obtained from the laboratory animal breeding section at Bush Estate, Roslin, Midlothian, were used. At the time of the Babesia microti experiment "Bush strain" mice were not available and Theilers original strain from A. Tuck & Sons Ltd., Laboratory Animal Breeding Station, Rayleigh, Essex, were used.

Only female mice were used throughout. All sampling of animals was carried out in the morning. The mice used all weighed between 20 and 30 grams.

All animals were sampled by the same method of blood withdrawal from the heart and the same method of brain crush preparation.

The animals were all fed, ad lib, on the same balanced ration. The same sized cages were used throughout, with seven animals in each cage.

The parasites:

Babesia rodhaini:

The parasite, designated TREU 812, used, originally came from Mrs. K. Adam, Zoology Department, University of Edinburgh. This parasite was obtained from a rat and passaged into six mice. When parasitaemia was at a peak, pooled blood from two infected mice was used to infect

experimental animals.

Babesia microti:

The parasite used was King strain, TREU 1122.

This parasite was obtained from a mouse and passaged once in mice and, at peak parasitaemia, as with the Babesia rodhaini, pooled blood from two infected mice was used to infect experimental animals.

Withdrawal of blood from mice:

Each mouse was put into a bottle with cotton wool and ether anaesthetic. The mouse was observed until it was adequately anaesthetised, depth of anaesthesia being judged by respiration rate. It was then removed and pinned out on a cork board, ventral side uppermost and a small piece of cotton wool, soaked in ether, placed over the animal's head to maintain depth of anaesthesia. Using small scissors and rat tooth forceps, the rib cage was reflected anteriorly, exposing the thoracic cavity and the heart.

Blood was then withdrawn from the right ventricle using a one ml. plastic syringe and a 26 g. $\frac{3}{8}$ " needle, previously rinsed in trisodium citrate solution to prevent coagulation. Approximately one ml. of blood could be obtained by this method. The blood, after removal of the needle, was transferred into a labelled, plastic 5 ml. E.D.T.A. tube.

Brain crush preparation:

After taking the blood, the mouse was turned over and

pinned out dorsal side uppermost. The skin was then reflected away from the cranium. A longitudinal cut, using scissors, was made through the mid-line of the skull. Forceps were introduced under the bone and by lifting in a lateral direction, one side of the cranium was removed, exposing the cerebrum. A small portion of cerebrum was removed with the forceps and placed on a glass slide, which had previously been soaked in 70 per cent Alcohol and dried with a clean cloth. Another slide was taken and placed over the portion of brain and compressed. As the brain was compressed the two slides were drawn apart longitudinally giving two brain crush preparations, which were left to dry in air after being labelled.

Preparation of thin blood films:

Blood samples were thoroughly mixed on a rotary mixer. From these samples a drop was transferred to the ends of two clean glass slides and two thin blood smears made, using a clean dry spreader. These films were rapidly air dried and labelled.

Staining of thin blood films and brain crushes:

Blood films and brain crushes were stained with Giemsa. Dried smears were ^{fixed} in methanol for two minutes. They were then transferred to a Coplin jar and stained for 45 minutes with 10 per cent Giemsa stain. The stain was made up in buffered distilled water at a pH of 7.0. After staining, slides were removed and washed in buffered distilled water

and blotted dry.

Processing of blood samples:

Blood samples were mixed using a rotary mixer and dilutions made, using a Coulter dual diluter model R. Dilutions were made up in Isoton (Coulter Electronics, Hight Street, South Dunstable, Bedfordshire), in 20 ml. disposable plastic containers. A 1/500 dilution was prepared. From this, a further dilution of 1/100 was made to give a 1/50,000 dilution. The 1/500 dilution was used for leucocyte and haemoglobin estimations and the 1/50,000 dilution for estimations of erythrocytes, mean cell volumes and packed cell volumes. These estimations were made with a Coulter counter model F.N., with a mean cell volume and haematocrit computer, using the 1/50,000 dilution. An aperture of eight was used, with an erythrocyte threshold of five and an attenuation of one. Using a coincidence correction chart erythrocyte counts were obtained. The cells were sized and the packed cell volume computed by the machine.

During the Babesia microti experiment, packed cell volumes were estimated using a Hawkesly microhaematocrit centrifuge, due to a fault in the Coulter computer. Microhaematocrit tubes were two thirds filled with blood and spun for five minutes, after heat sealing one end. The packed cell volumes were read using a Hawkesly reader.

Leucocyte estimations were carried out using the 1/500

dilution. The red cells were first haemolysed with Zapaglobin (Coulter Electronics), using six drops in 20 ml. and then counted at an aperture of eight, with a leucocyte threshold of 15 and an attenuation of 0.707.

The same sample was used for haemoglobin concentration estimation, in a Coulter haemoglobinometer.

Differential white cell counts:

Stained blood smears were examined using the x100 oil immersion lens. The slides were scanned using the battlement method and all cells seen were identified. Those cells which were badly damaged and unrecognisable were discarded and not counted. Two hundred cells from each sample were examined and the cell types recorded as percentages. These were related to the total leucocyte count and expressed in absolute figures.

Parasite counts:

Blood slides were examined using the x100 oil immersion objective. Fields, as near the centre of the smear as possible, were examined. Three fields were examined for each sample and infected and non-infected cells counted. The infected cells were recorded as a percentage parasitaemia.

Examination of brain crush preparations:

Stained slides were scanned using the low power objective in order to locate a good bundle of capillaries for examination. This bundle was examined for any abnormalities within the capillary bed, using the x100 oil immer-

sion objective. Each slide examined was scanned for a period of ten minutes. If no abnormalities were seen during this period the results were considered negative.

Inoculation of experimental animals:

Blood, for inoculation, was collected in the manner previously described. Total erythrocyte counts and parasitaemia estimations (where applicable) were done. In order to get the correct number of erythrocytes in an 0.2 ml. dose of inoculum the blood was diluted in phosphate buffered balanced salt solution A.B.P. 8.0, as described by Lumsden, Cunningham, Webber, K. Van Hoeve, Knight & Simmons (1965).

This inoculum was used as quickly as possible after preparation, 0.2 millilitres being injected intraperitoneally, using a one ml. plastic syringe and a 26 g. $\frac{3}{8}$ " disposable needle. Mice, after inoculation, were identified with picric acid.

Experiments:

The examination of normal mice:

"Bush strain" mice, from stock of the Centre for Tropical Veterinary Medicine, Edinburgh, were used to obtain normal haematological values and for observations on brain capillary morphology. In all, 41 mice were examined. Two were discarded as pre-experimental trials of technique. One other sample was unsuitable for leucocyte parameters, leaving 39 animals for red and 38 for white cell observations.

Factors which affect the haematological picture of normal mice are:-

Strain of mice used. (Russel et al, 1951).

Sex. (Russel et al, 1951).

Time of day. (Brown & Dougherty, 1956). (Halberg et al, 1957).

Age of mice. (Schalm, 1967).

Site and method of sample withdrawal. (De Kock, 1931).

Feeding and management. (Schalm, 1967).

In order to minimise these effects the previously described methods were kept as constant as possible.

Initially, tail blood was used but this method was not continued due to the abnormally high leucocyte counts obtained. It was decided that heart blood would be used throughout the experiments, as this gave more consistent results and a larger volume of blood would be obtained.

For each animal examined the following observations were made -

- (1) Erythrocyte count.
- (2) Packed cell volume.
- (3) Haemoglobin concentration.
- (4) Mean corpuscular volume.
- (5) Leucocyte count.
- (6) Differential leucocyte count.
- (7) Brain capillary morphology.

Babesia rodhaini experiment:

Six mice were infected intraperitoneally with blood from a rat with a 20 per cent parasitaemia. Each mouse was given 1×10^7 parasitised erythrocytes. Six days later all had high parasitaemias and a pooled sample from two mice gave an erythrocyte count of $6 \times 10^6 / \text{mm}^3$ of blood with a parasitaemia of 65 per cent. This blood was diluted in A.B.P. 8.0 buffered salt solution (0.25 ml. in 20 ml. final volume), to provide 100 doses of 0.2 ml. each containing 1×10^7 parasitised erythrocytes. This was used to infect experimental animals.

Two normal mouse blood samples were pooled, giving an erythrocyte count of $1 \times 10^7 / \text{mm}^3$. An 0.13 ml. volume of this blood was made up to 20 ml. with A.B.P. 8.0, to provide 100 0.2 ml. doses for the control animals, enabling each to receive the same number of erythrocytes as the test animals.

Fifty six mice were grouped in eight identical cages, seven mice per cage. The cages were numbered one to eight representing the days after infection on which sampling would be carried out.

Two mice in each cage were given normal blood intraperitoneally and marked with picric acid. The other five animals in each cage were given infected blood intraperitoneally.

From each sample the following observations were made:-

- (1) Parasitaemia.
- (2) Erythrocyte count.

- (3) Packed cell volume.
- (4) Haemoglobin concentration.
- (5) Mean corpuscular volume.
- (6) Leucocyte count.
- (7) Differential leucocyte count.
- (8) Brain capillary morphology.

The infected animals were sampled up to day seven, by which time the disease had caused death in all animals scheduled for sampling later. Sampling of control animals continued to day eight.

Babesia microti experiment:

From two infected mice, with a parasitaemia of 70 per cent, 1×10^7 infected erythrocytes were injected intraperitoneally into four further mice. Seven days later, two of these mice were sampled and blood pooled. This blood had an erythrocyte count of 3.64 million per cubic millimetre, with a parasitaemia of 50 per cent. The blood was then diluted in A.B.P. 8.0 to give 0.2 ml. doses each of 1×10^7 infected erythrocytes. This was used to infect experimental animals.

As in the Babesia rodhaini experiment, a pooled blood sample from two normal mice was also diluted in A.B.P. 8.0 to produce 0.2 ml. doses of 2×10^7 erythrocytes.

Seventy seven mice were grouped in 11 identical cages, seven mice per cage. The cages were numbered from one to 11, each cage representing one sampling day. Two mice from

each cage were given normal blood intraperitoneally and identified with picric acid. The other five animals in each cage were given infected blood intraperitoneally.

The animals were sampled on days two, four, six, eight, 10, 12, 14, 16, 20, 25 and 32 after infection and observations made as for the Babesia rodhaini experiment.

The experiment continued for the full 32 days, no animal dying from the disease.

Statistical analysis:

From each day's sampling means and standard deviations were calculated. For differences between control and test groups t tests (Snedecor, 1957) were applied. The standard deviation and t tests were calculated on a Monroe 1265 computer.

RESULTS

The normal mouse:

Haemograms:

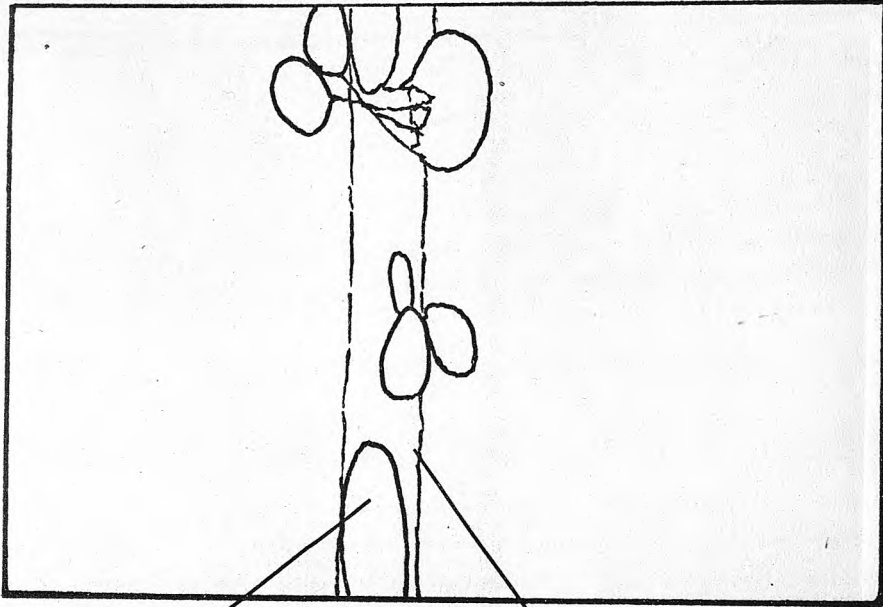
The following figures represent normal haemograms for "Bush strain" mice. The numbers of animals examined, the means and standard deviations are given.

	No. of animals examined	Mean values and standard deviations		
Red Blood Cell Count $\times 10^6$ per mm^3	39	8.46	\pm	0.79
Haemoglobin Concentration gm %	39	17.02	\pm	1.66
Packed Cell Volume %	39	44.85	\pm	3.23
Mean Cell Volume microns ³	39	54.9	\pm	4.2
White Blood Cell Count $\times 10^3$ per mm^3	38	5.64	\pm	2.48
Neutrophil Count per mm^3	38	1128	\pm	826
Eosinophil Count per mm^3	38	22	\pm	39
Lymphocyte Count per mm^3	38	4368	\pm	1790
Monocyte Count per mm^3	38	118	\pm	90
Basophil Count per mm^3	38	NONE		SEEN
Myelocyte Count per mm^3	38	NONE		SEEN

Normal brain capillary morphology:

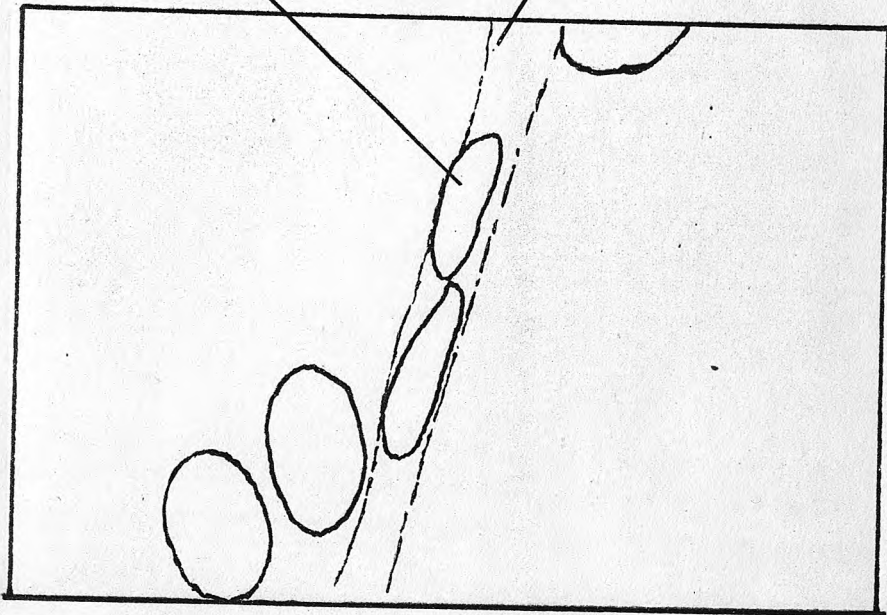
Figs. I & II show typical brain capillary morphology from normal mice.

The capillaries are not packed with erythrocytes. The nucleus of the endothelial cell is elongated and the cytoplasm of the cell contains no inclusions.



Endothelial
Nucleus

Capillary wall



Giemsa stained brain crush preparations

Magnification X 1750

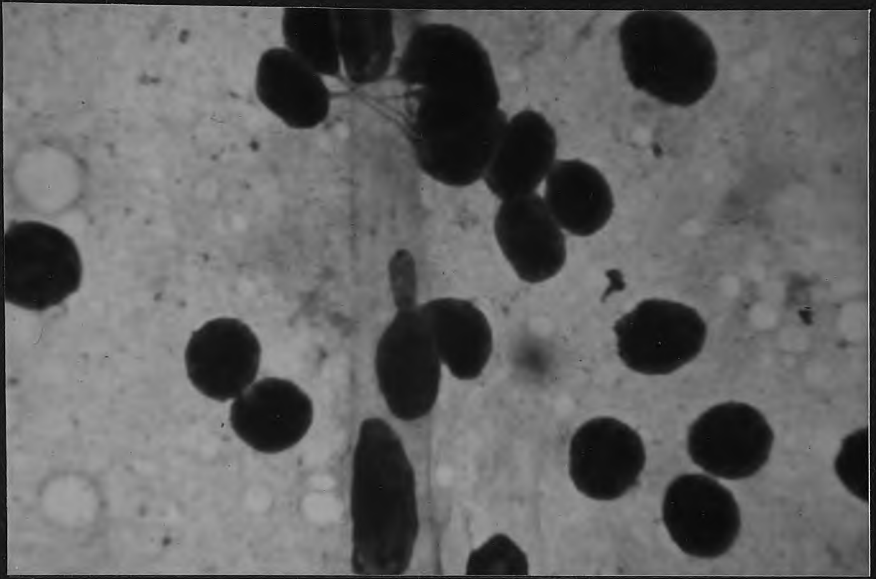


FIG. I.

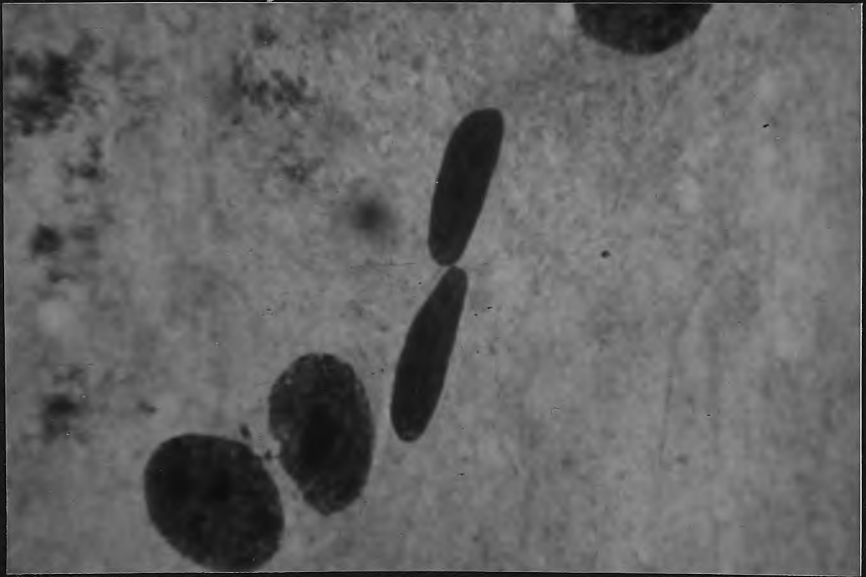


FIG. II.

The parasitaemia of Babesia rodhaini and Babesia microti infection:

Each graph, showing the various parameters measured, has the parasitaemia curve superimposed on it. Standard deviations for the Babesia rodhaini parasitaemia curve are shown in Table I, column 10, and these for Babesia microti in Table II, column 10.

t test results:

B. rodhaini. t tests between parasitaemias of consecutive days:

between days	2 - 3	t = 3.60 ^{**}	for 8 d.f.
"	"	3 - 4	t = 3.74 ^{**} for 8 d.f.
"	"	4 - 5	t = 3.43 ^{**} for 8 d.f.
"	"	5 - 6	t = 4.78 ^{**} for 8 d.f.

All these figures are significant to the 0.01 level of probability, giving a significant daily rise in parasitaemia from patency onwards.

B. microti. t tests between parasitaemia of consecutive sampling days:

between days	2 - 4	t = 3.17 ^{**}	for 8 d.f.	P = < .01
"	"	4 - 6	t = 1.93	for 8 d.f.
"	"	6 - 8	t = 3.40 ^{**}	for 8 d.f. P = < .01
"	"	8 - 10	t = 1.44	for 8 d.f.
"	"	10 - 12	t = 1.45	for 8 d.f.
"	"	12 - 14	t = 1.14	for 7 d.f.
"	"	14 - 16	t = 1.55	for 7 d.f.

between days 16 - 20 $t = 2.45^*$ for 8 d.f. $P = < .05$

" " 20 - 25 $t = 1.97$ for 7 d.f.

" " 25 - 32 $t = 2.10$ for 6 d.f.

As an extra t test, the values between days 12 and 16 were tested when $t = 2.64^*$ $P = < 0.05$ for 8 d.f.

0.05 Probability level for $t = 2.306$ for 8 d.f.

$t = 2.365$ for 7 d.f.

$t = 2.447$ for 6 d.f.

Although there are no significant differences for values between consecutive days there is a significant peak at day eight and the peak at day 16 is significantly greater than the trough on day 12.

Babesia rodhaini Red blood cell counts (Fig. III).

The changes in mean red blood cell count with relation to time and parasitaemia percentage are represented by Figure III. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, column 1 and t tests between infected and control groups are shown in Table III, column 2.

Babesia rodhaini Packed cell volume (Fig. IV).

The changes in mean packed cell volume with relation to time and parasitaemia are represented by Figure IV. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, column 2 and t tests between infected and control groups are shown in Table III, column 3.

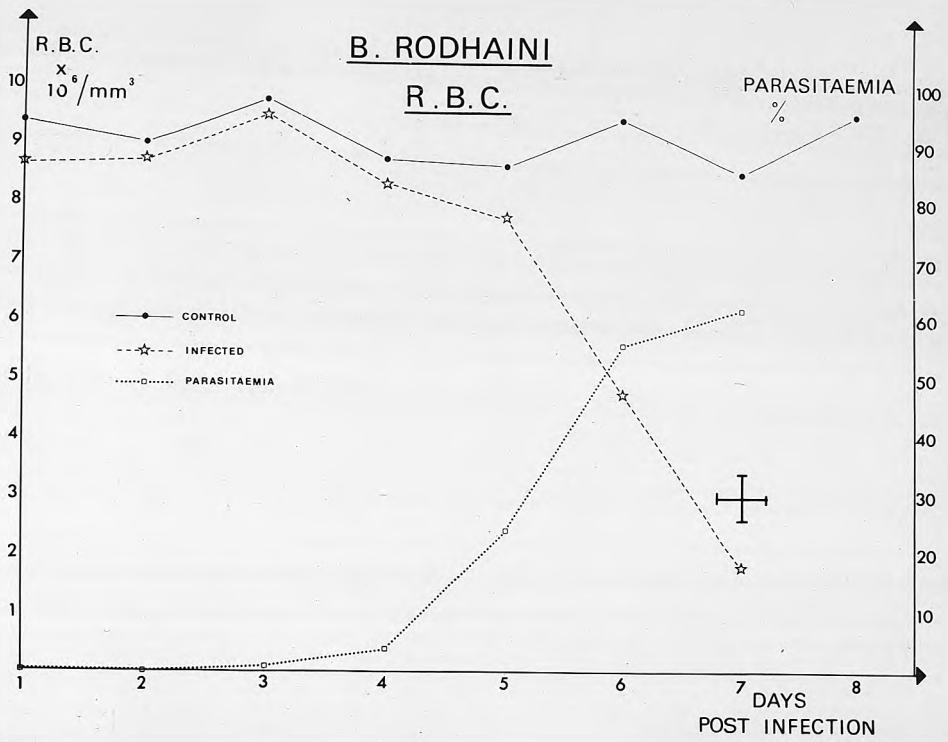


FIG. III.

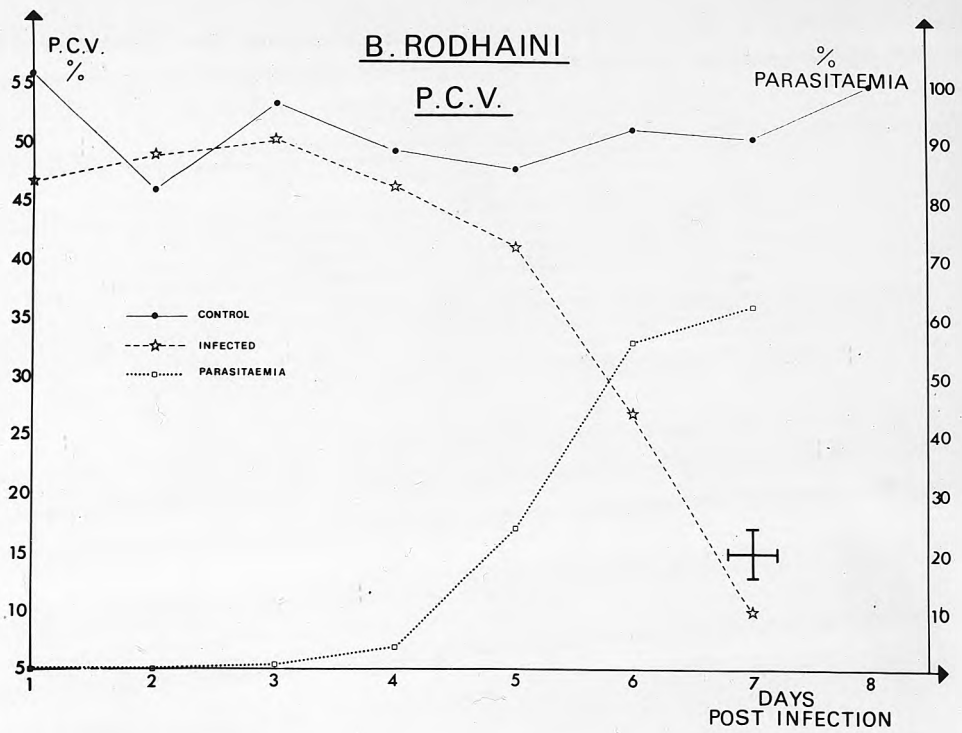


FIG. IV.

Babesia rodhaini Haemoglobin concentration (Fig. V).

The changes in mean haemoglobin concentration with relation to time and parasitaemia are represented by Figure V. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, column 4 and t tests between infected and control groups are shown in Table III, column 5.

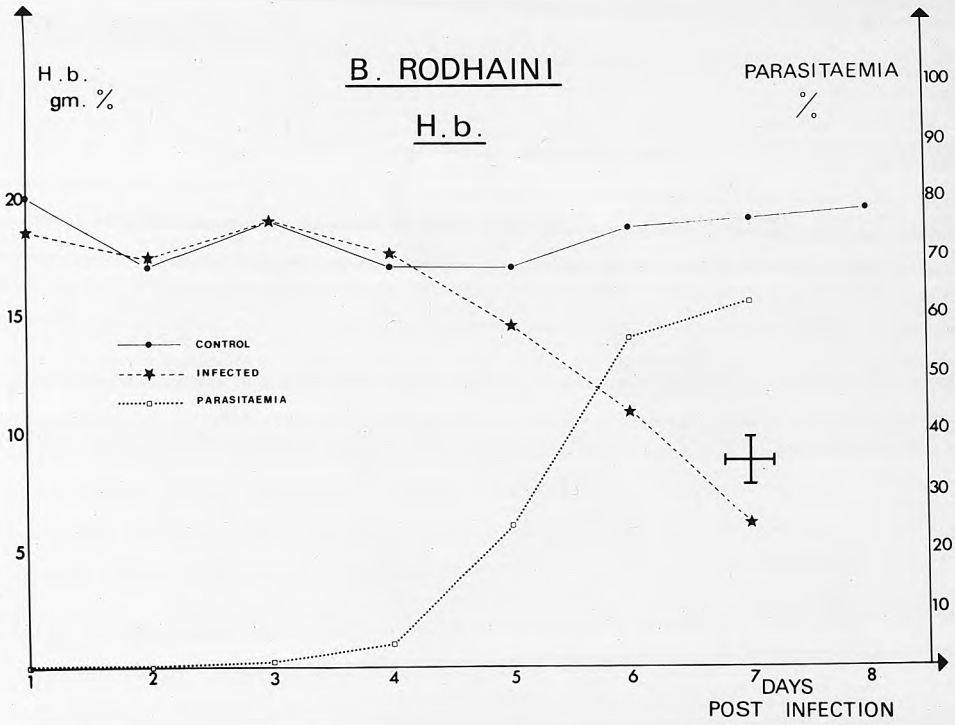


FIG. V.

Babesia rodhaini White blood cell counts (Fig. VI).

The changes in mean white blood cell count with relation to time and parasitaemia are represented by Figure VI. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, column 5 and t tests between infected and control groups are shown in Table III, column 6.

Babesia rodhaini Neutrophils and Myelocytes (Fig. VII).

The changes in mean neutrophil and myelocyte counts with relation to time and parasitaemia are represented by Figure VII. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, columns 7 and 9, and t tests between infected and control groups are shown in Table III, columns 7 and 10.

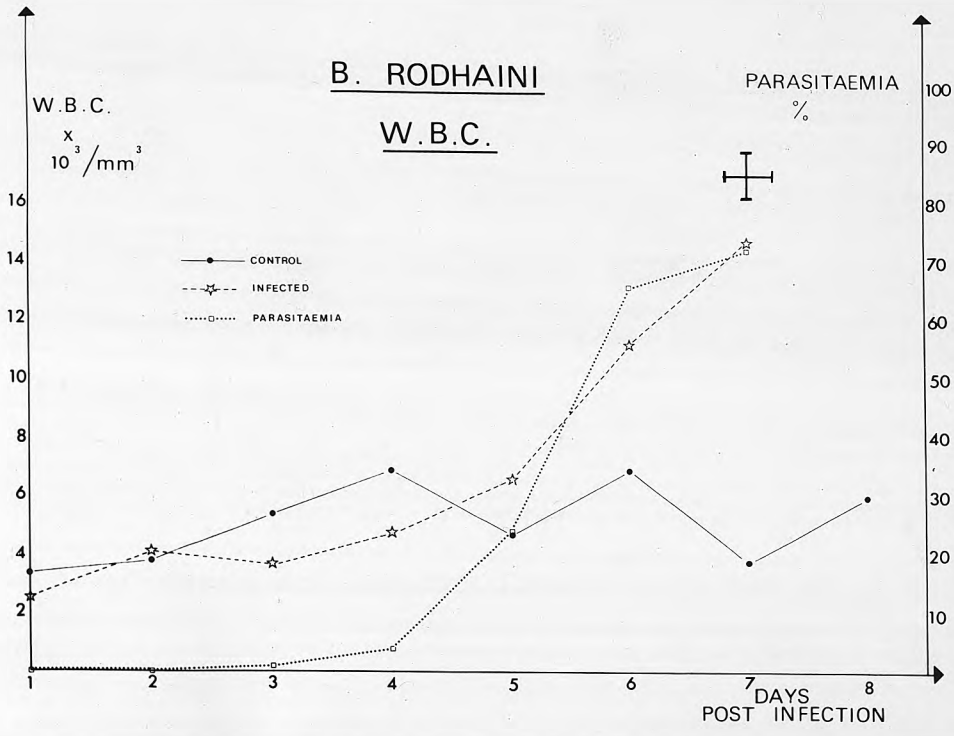


FIG. VI.

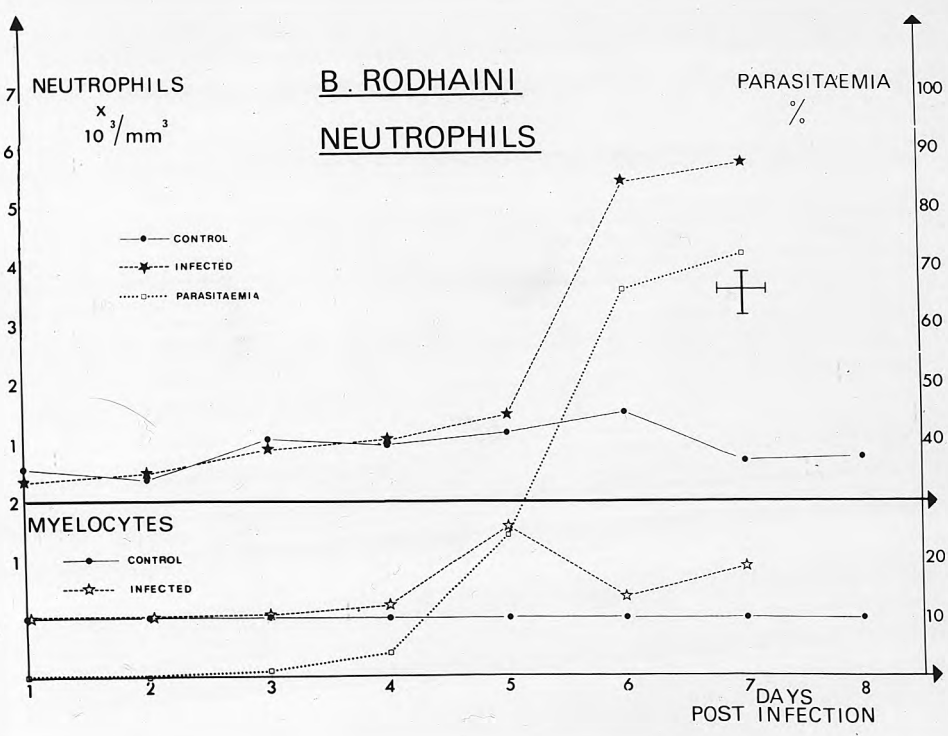


FIG. VII.

Babesia rodhaini Lymphocytes, monocytes, mean
cell volumes (Fig. VIII).

The changes in mean lymphocyte and monocyte counts and mean corpuscular volume with relation to time are shown in Figure VIII. From days one to six there were five animals in each test group. Day seven is represented by the one surviving test animal. Control figures are the means of two animals per day. Standard deviations are shown in Table I, columns 6, 8 and 3, and t tests between infected and control groups in Table III, columns 8, 9 and 4 respectively.

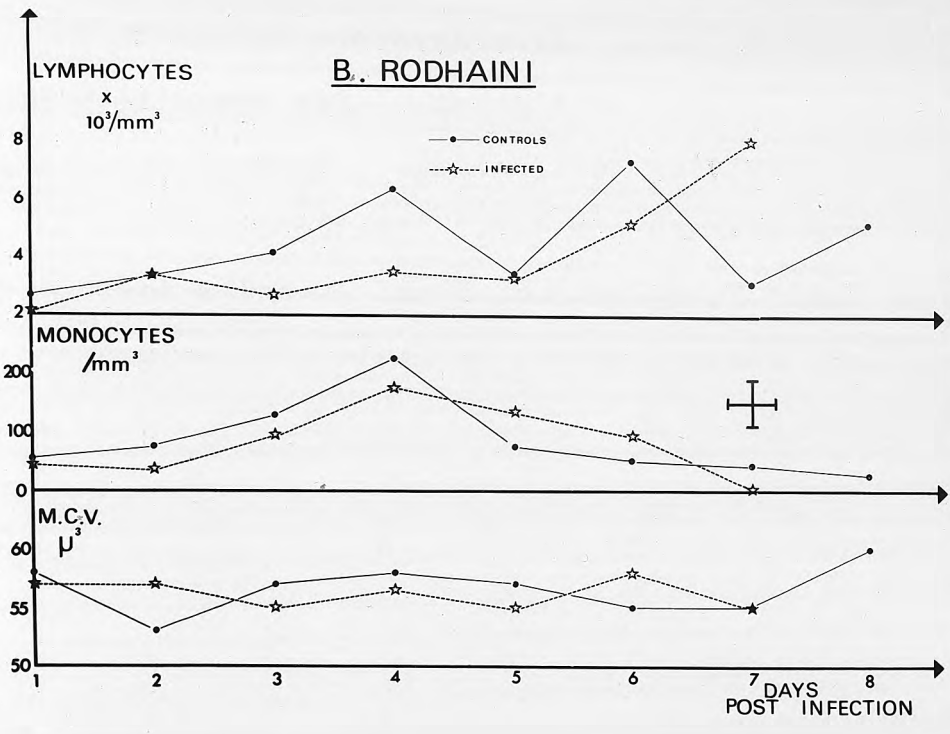


FIG. VIII.

Babesia microti Red blood cell counts (Fig. IX).

The changes in mean red blood cell counts with relation to time and parasitaemia are represented by Figure IX. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 1, and t tests between infected and control animals in Table IV, column 2.

Babesia microti Packed cell volume (Fig. X).

The changes in mean packed cell volume with relation to time and parasitaemia are represented by Figure X. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 2, and t tests between infected and control animals in Table IV, column 3.

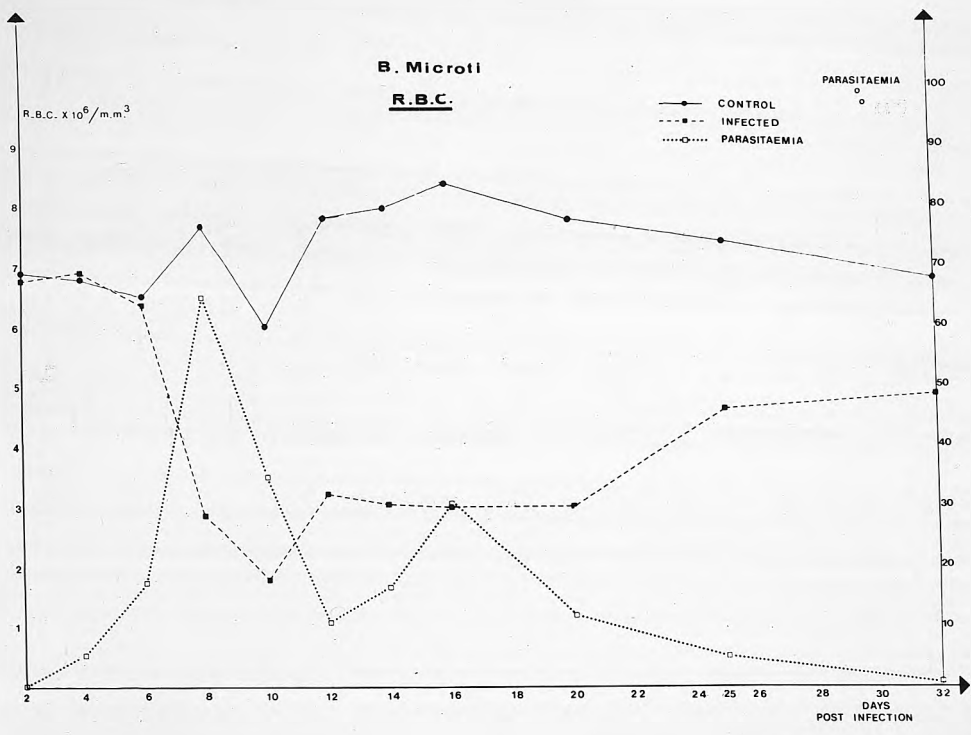


FIG. IX.

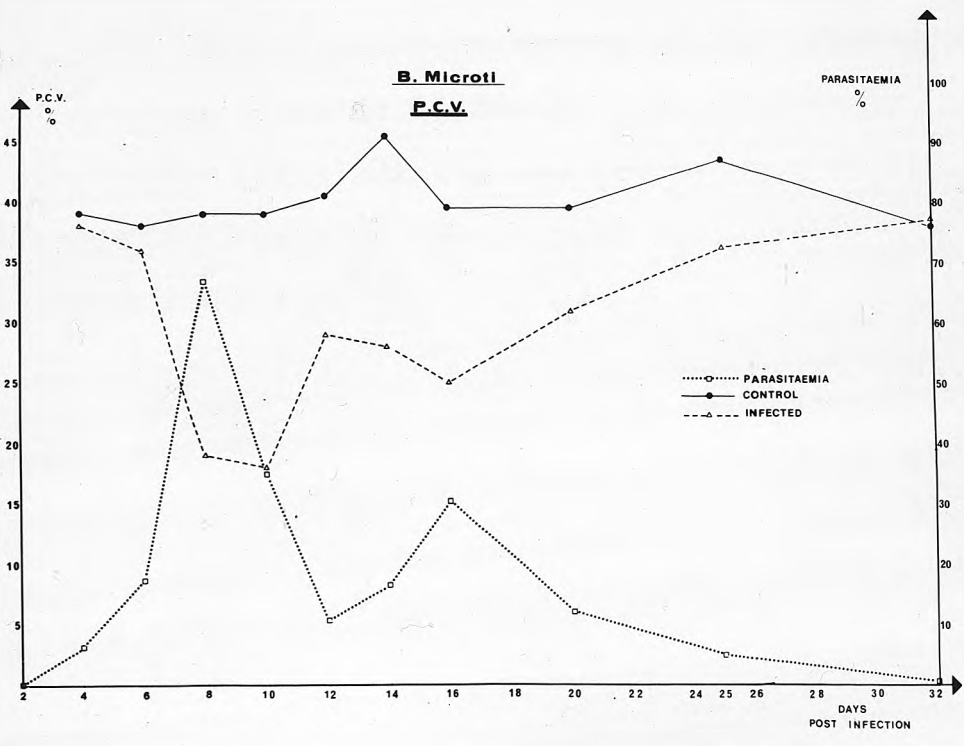


FIG. X.

Babesia microti Mean cell volume (Fig. XI).

The changes in the mean cell volume with relation to time and parasitaemia are represented by Figure XI. Test animals are in groups of five except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 3, and t tests between infected and control animals in Table IV, column 4.

Babesia microti Haemoglobin concentration (Fig. XII).

The changes in mean haemoglobin concentration with relation to time and parasitaemia are represented by Figure XII. Test animals are in groups of five except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 4, and t tests between infected and control animals in Table IV, column 5.

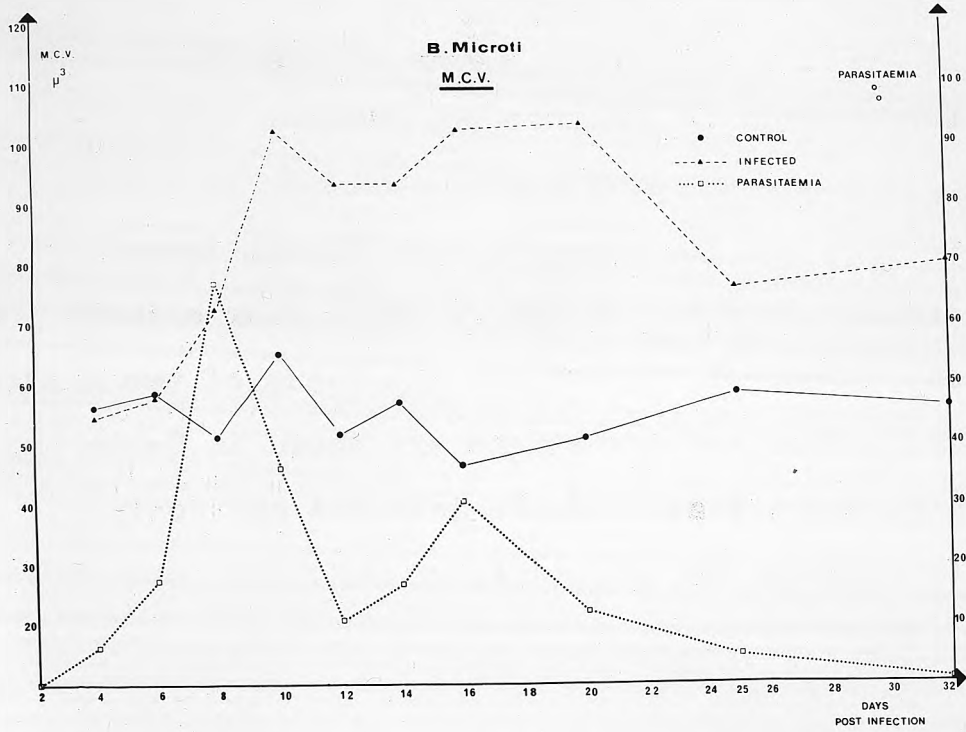


FIG. XI.

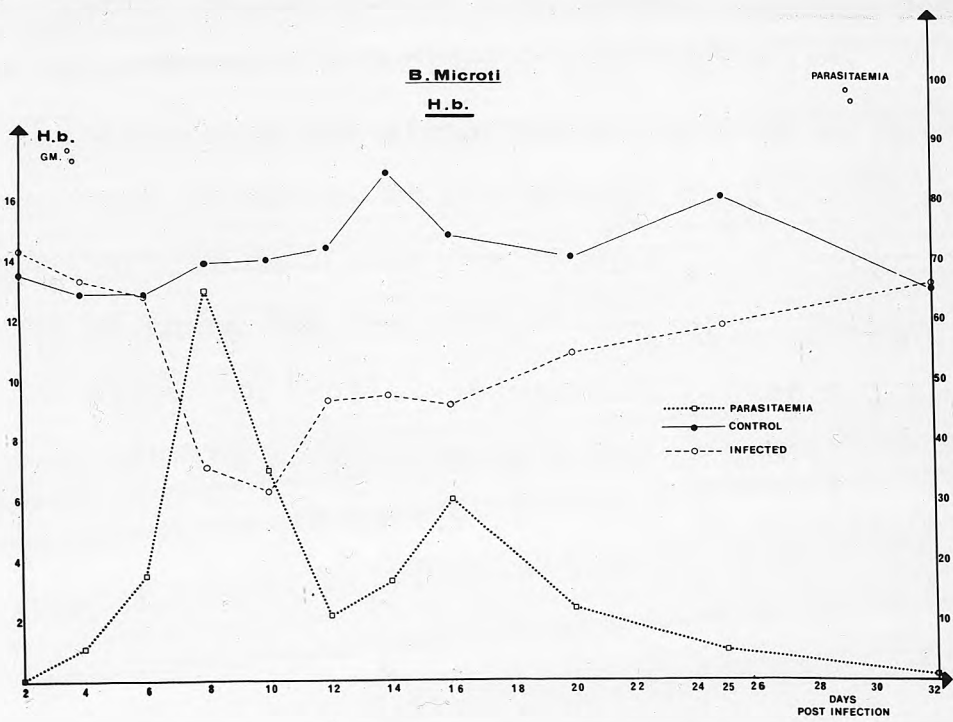


FIG. XII.

Babesia microti White blood cell count (Fig. XIII).

The changes in mean white blood cell counts with relation to time and parasitaemia are represented by Figure XIII. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 5, and t tests between infected and control animals in Table IV, column 6.

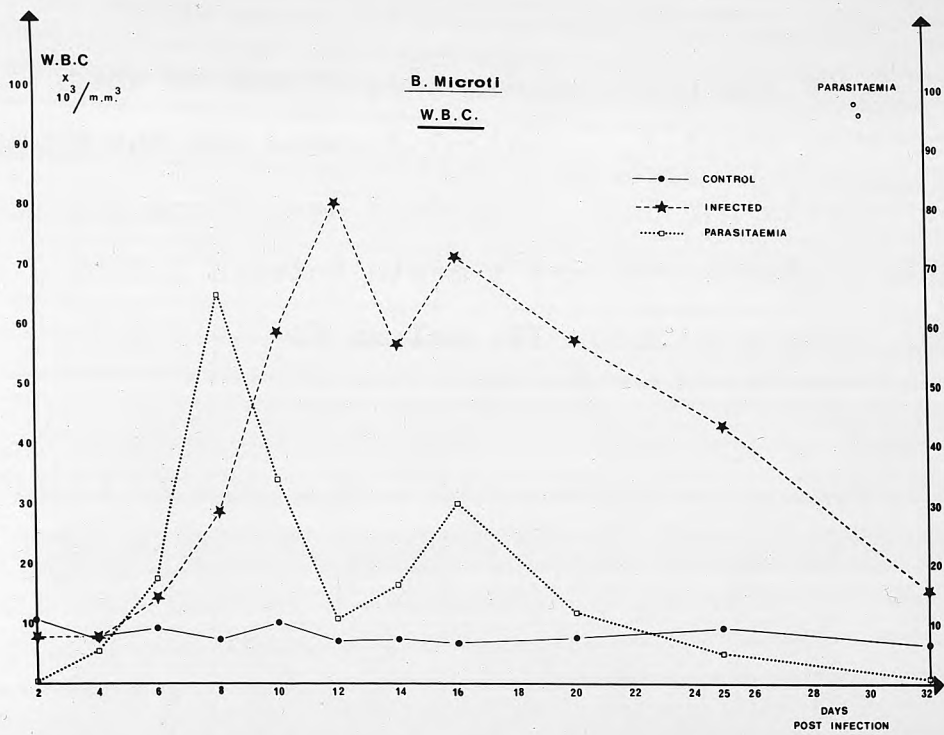


FIG. XIII.

Babesia microti Neutrophils (Fig. XIV).

The changes in mean neutrophil counts with relation to time and parasitaemia are represented by Figure XIV. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 7, and t tests between infected and control animals in Table IV, column 7.

Babesia microti Myelocytes (Fig. XV).

The changes in mean myelocyte counts with relation to time and parasitaemia are represented by Figure XV. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 9, and t tests between infected and control animals in Table IV, column 10.

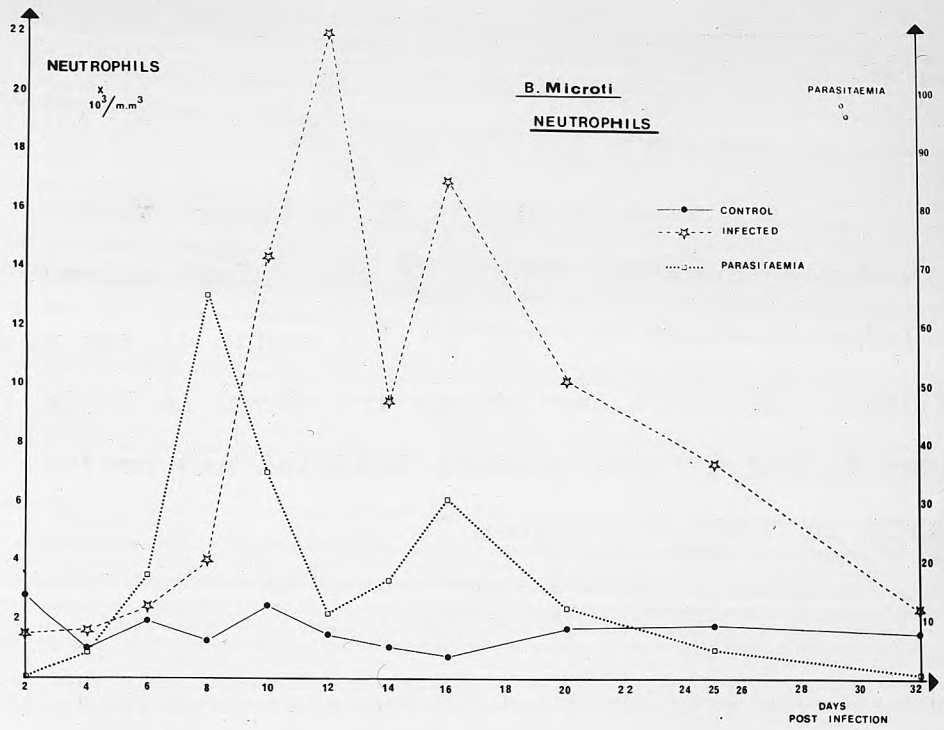


FIG. XIV.

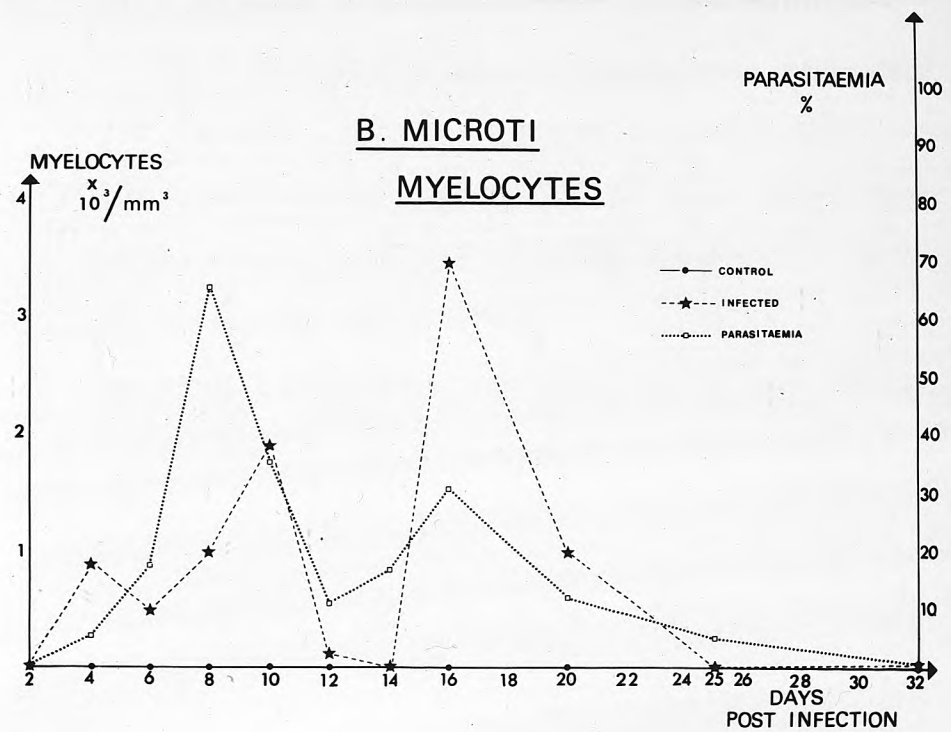


FIG. XV.

Babesia microti Lymphocytes (Fig. XVI).

The changes in mean lymphocyte counts with relation to time and parasitaemia are represented by Figure XVI. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 6, and t tests between infected and control animals in Table IV, column 8.

Babesia microti Monocytes (Fig. XVII).

The changes in mean monocyte counts with relation to time and parasitaemia are represented by Figure XVII. Test animals are in groups of five, except those representing days 14, 25 and 32 where four animals were sampled. Control figures are the means of two animals per day. Standard deviations are shown in Table II, column 8, and t tests between infected and control animals in Table IV, column 9.

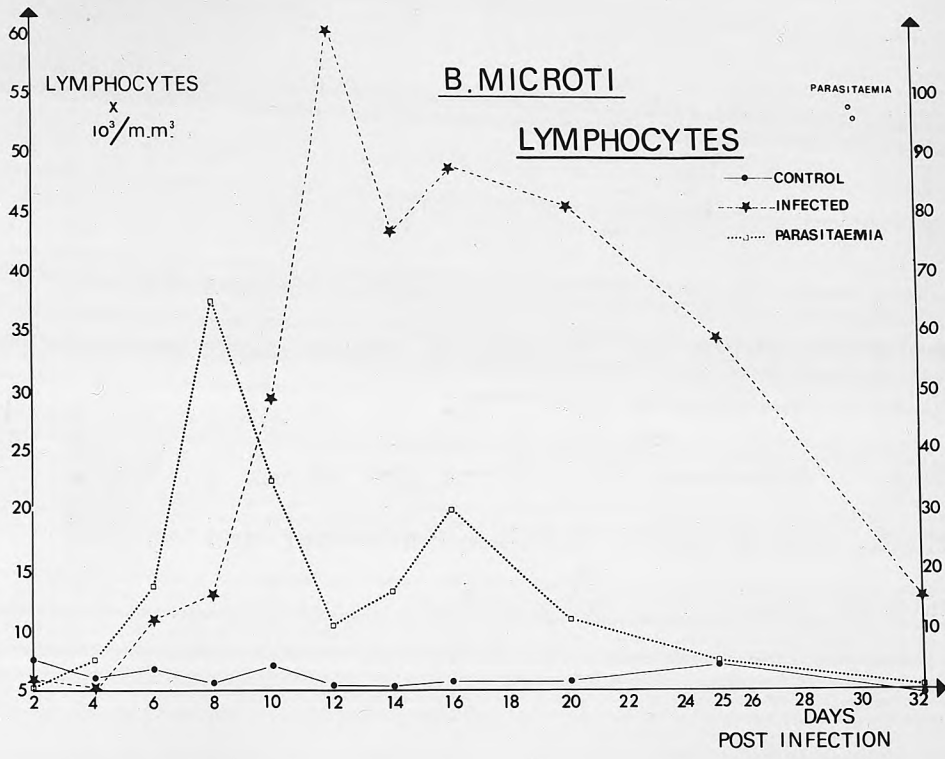


FIG. XVI.

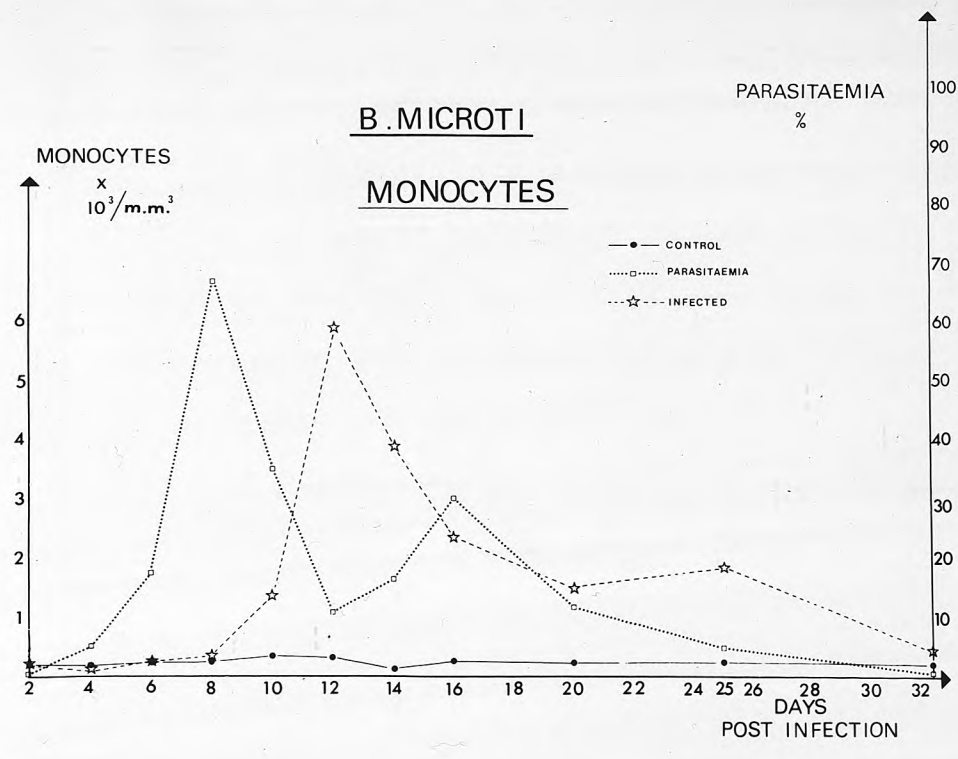


FIG. XVII.

Brain capillary bed examinations:

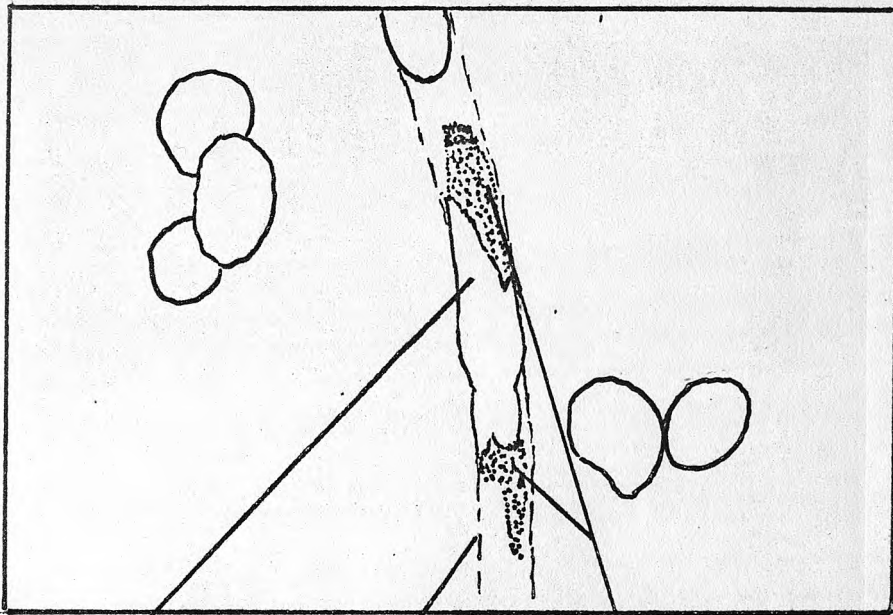
Babesia rodhaini:

Results are based on brain crush examination of all test and control animals. Animals were sampled on their respective days, i.e. five infected and two control animals per day, except on day seven when all test animals were dead and 10 infected animals were sampled at that time.

There was no evidence of sludging of red blood cells within the cerebral capillaries in any of the animals tested.

There was also no evidence of obvious high concentrations of parasites within the brain capillaries, compared with heart blood parasitaemias.

The only findings of noteworthy importance are shown in the two photographs overleaf. Fig. XVIII shows a blue amorphous mass in the vicinity of the endothelial nucleus, apparently within the capillary. This finding, although present in test animals, could also be seen in control slides and was not regarded as significant or relevant to the infections being studied. Fig. XIX shows focal eosinophilic bodies around the endothelial nucleus. These were seen in five test animals, two on day five and three on day six after infection. No control animals showed this.

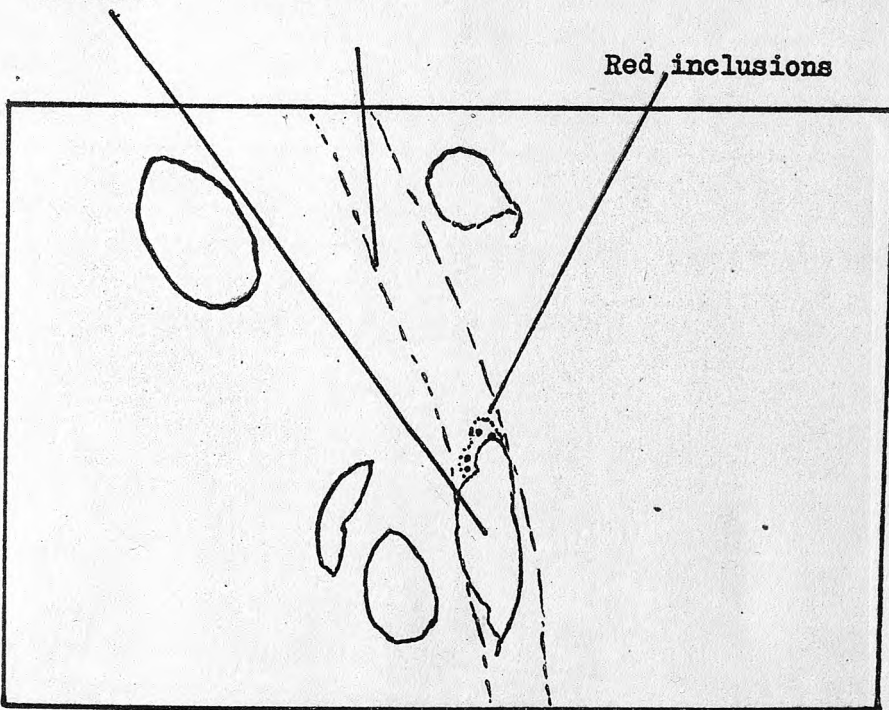


Endothelial

Nucleus

Capillary wall

Blue mass



Red inclusions

Giemsa stained brain crush preparations

Magnification X 1750

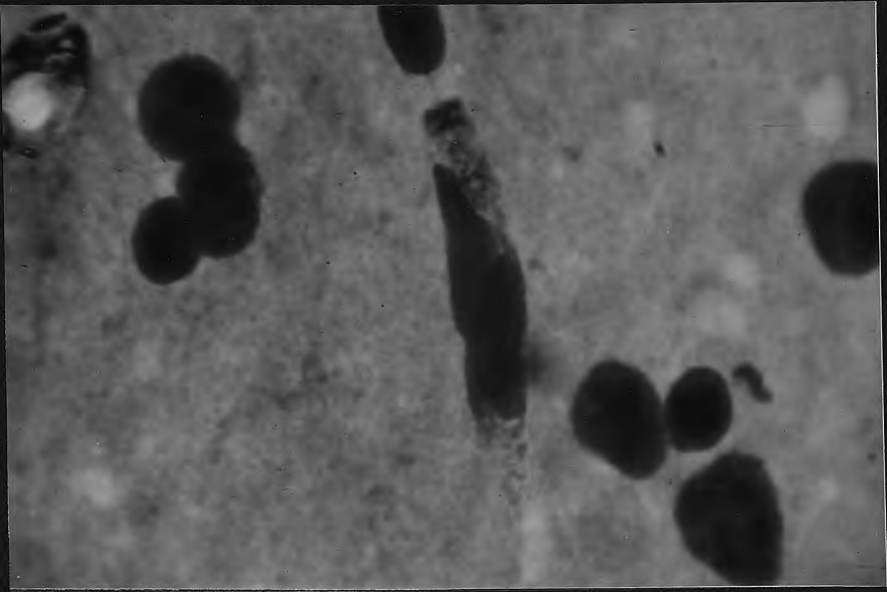


FIG. XVIII.

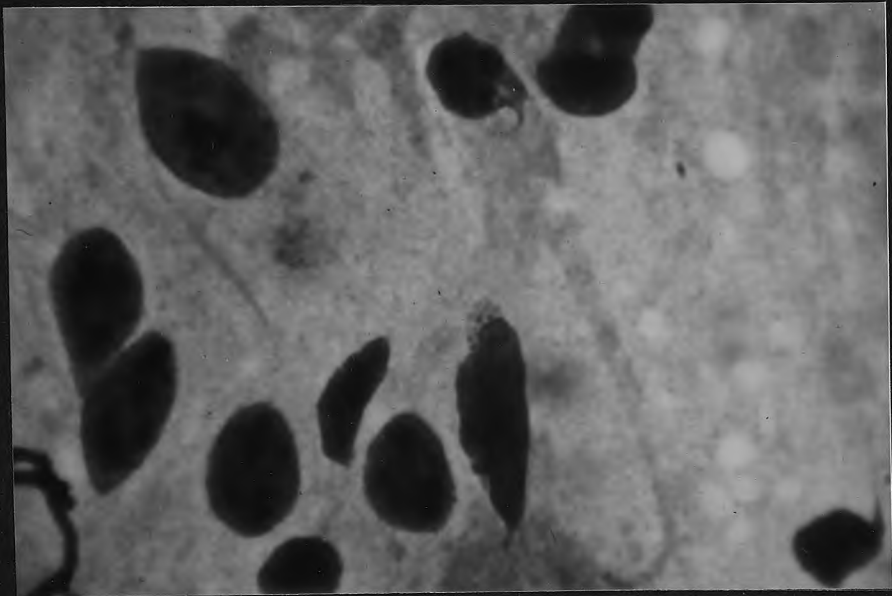


FIG. XIX.

Babesia microti:

Results are based on brain crush examinations on five test animals and two control animals, for each sampling day, except days 14, 25 and 32, where four test animals were sampled.

There was no evidence of sludging of red blood cells within the cerebral capillaries in any animal tested and no evidence of obvious high concentrations of parasites within the brain capillaries compared with heart blood levels.

No other noteworthy findings were recorded in infected or control animals.

TABLE I.

Standard deviations

Babesia rodhaini

	1	2	3	4	5	6	7	8	9	10	
Day	R.B.C. x10 ⁶ /mm ³	P.C.V. %	M.C.V. μ ³	Hb. gm/100ml	W.B.C. x10 ³ /mm ³	L/mm ³	N/mm ³	M/mm ³	My/mm ³	% Parasite	
1	T	8.68 ± 1.18	46.64 ± 3.59	56.2 ± 4.38	18.5 ± 1.09	2.54 ± 0.66	2189 ± 560	325 ± 128	42 ± 12.7	0	0 ± 0
	C	9.36 ± .33	55.8 ± 5.37	58.5 ± 0.71	20.0 ± 1.56	3.35 ± 0.77	2647 ± 223	550 ± 382	53 ± 35.4	0	0
2	T	8.73 ± 0.48	48.98 ± 2.23	57.6 ± 1.52	17.34 ± 0.81	3.9 ± 1.36	3276 ± 1290	410 ± 277	34.8 ± 44	0	0 ± 0
	C	9.0 ± 0.57	46 ± 7	53 ± 4.24	16.95 ± 1.77	3.75 ± 2.90	3340 ± 1772	347 ± 82	75 ± 58	0	0
3	T	9.5 ± 1.15	50.32 ± 7.39	54.8 ± 3.7	18.92 ± 0.92	3.7 ± 0.92	2686 ± 875	889 ± 571	95 ± 29	21 ± 29	0.72 ± .41
	C	9.76 ± 0.5	53.35 ± 2.33	57 ± 5.66	18.85 ± 1.34	5.4 ± 1.84	4110 ± 764	1075 ± 940	78.5 ± 78.5	0	0
4	T	8.32 ± 1.2	46.2 ± 6.35	57.4 ± 1.82	17.6 ± 0.75	4.8 ± 0.35	3432 ± 347	1012 ± 20929	136 ± 121	229 ± 179	3.6 ± 1.67
	C	8.75 ± .92	49.3 ± 5.23	58 ± 0	17.0 ± 0.99	6.95 ± 2.19	5740 ± 1852	957 ± 209	224 ± 164	0	0
5	T	7.76 ± 0.71	41.1 ± 4.69	55 ± 3.16	14.48 ± 1.79	6.64 ± 3.2	3352 ± 2208	1458 ± 5605	1334 ± 2036	1537 ± 712	21.8 ± 11.76
	C	8.64 ± 0.44	47.15 ± 1.34	56.5 ± 0.71	16.95 ± .49	4.65 ± 1.2	3415 ± 1393	1132 ± 124	75 ± 28	0	0
6	T	4.75 ± 1.61	26.86 ± 11.8	57.6 ± 9.45	10.84 ± 2.74	11.2 ± 2.48	5304 ± 795	5446 ± 2369	81.6 ± 124	276.5 ± 229	55.8 ± 10.69
	C	9.42 ± 1.18	51 ± 5.66	55 ± 0	18.7 ± 1.27	6.9 ± 4.95	5240 ± 4045	1515 ± 940	51 ± 72.12	0	0
7	T	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	62 ± 0
	C	8.52 ± 0.69	50.4 ± 3.25	60 ± 0	19.05 ± 1.2	3.75 ± 0.63	3010 ± 608	698 ± 38	42 ± 59.4	0	0
8	T	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	C	9.54 ± .84	55.2 ± 5.37	59.5 ± 0.71	19.5 ± 0.42	5.95 ± 0.77	5133 ± 923	790 ± 106	N/A	0	0

N/A - Not available.

T - Test animals.

C - Control animals.

R.B.C. - Red blood cell count.

P.C.V. - Packed cell volume.

M.C.V. - Mean cell volume.

Hb. - Haemoglobin concentration.

W.B.C. - White blood cell count.

L - Leucocyte count.

N - Neutrophil count.

M - Monocyte count.

My - Myelocyte count.

TABLE II.
Standard deviations
Babesia microti

Day	1	2	3	4	5	6	7	8	9	10
	R.B.C. $\times 10^6/\text{mm}^3$	P.C.V. %	M.C.V. μ^3	Hb. gm/100ml	W.B.C. $\times 10^3/\text{mm}^3$	L/ mm^3	N/ mm^3	M/ mm^3	My/ mm^3	% Parasite
2	T 6.8 ± 0.715	N/A	N/A	14.3 ± 1.03	7.64 ± 6.9	5972 ± 6702	1500 ± 870	214 ± 0	7 ± 16	.2 \pm .22
	C 6.9 ± 0.28	N/A	N/A	13.5 ± 1.2	10.5 ± 1.77	7565 ± 1011	2778 ± 735	199 ± 114	0	0
4	T 6.9 ± 0.41	38 ± 1.52	54.3 ± 1.92	13.3 ± 0.826	7.8 ± 3.0	5266 ± 2044	1621 ± 831	115 ± 80	821 ± 308	5.4 \pm 3.65
	C 6.8 ± 0	39 ± 0	56 ± 0	12.85 ± 0.212	7.5 ± 4.7	6250 ± 4144	1060 ± 481	192 ± 34	0	0
6	T 6.35 ± 0.839	36 ± 2.07	57.7 ± 4.35	12.8 ± 1.028	14.3 ± 4.256	10850 ± 3882	2433 ± 673	246 ± 136	479 ± 552	17.4 \pm 13.94
	C 6.51 ± 0.105	38 ± 0	58.5 ± 1.415	12.85 ± 0.071	9.1 ± 0.32	6825 ± 1025	1948 ± 205	222 ± 179	0	0
8	T 2.87 ± 1.834	19 ± 9.7	72.3 ± 18.9	7.1 ± 3.6	18.8 ± 4	13040 ± 2550	4062 ± 1271	370 ± 96	962 ± 597	64.8 \pm 28.2
	C 7.68 ± 1.018	39 ± 1.414	51 ± 8.1	13.9 ± 0.28	7.3 ± 0	5650 ± 71	1280 ± 156	255 ± 50	0	0
10	T 1.8 ± 0.31	18 ± 1.9	102 ± 10.8	6.3 ± 0.62	58.7 ± 11.9	29390 ± 10076	14397 ± 2911	1381 ± 1314	1884 ± 1239	35 \pm 36.8
	C 6.00 ± 0	39 ± 0	65 ± 0	14.0 ± 0.57	10.1 ± 3.4	7100 ± 1980	2470 ± 1103	365 ± 191	0	0
12	T 3.2 ± 0.95	29 ± 4.1	93 ± 18	9.35 ± 1.4	80.2 ± 37.2	60040 ± 29168	21013 ± 7989	5880 ± 5708	115 ± 258	10.8 \pm 6.2
	C 7.8 ± 0.65	40.5 ± 2.1	57.5 ± 2.1	14.4 ± 0.71	7.25 ± 0.07	5440 ± 566	1493 ± 470	326 ± 54	0	0
14	T 3.03 ± 0.95	28 ± 7.5	93 ± 8.46	9.5 ± 2.06	56.9 ± 4.95	43300 ± 6730	9427 ± 4944	3870 ± 1854	0	16.7 \pm 9.4
	C 7.96 ± 0.1	45.5 ± 0.71	57 ± 0	16.9 ± 0.28	7.5 ± 5.37	5305 ± 4448	1068 ± 824	130 ± 107	0	0
16	T 3.0 ± 0.6	25 ± 4.1	102 ± 14.9	9.2 ± 1.03	71.4 ± 25.8	48680 ± 21032	16910 ± 5671	2345 ± 1914	3448 ± 2058	30.4 \pm 15.4
	C 3.37 ± 0.47	39.5 ± 0.71	46.5 ± 0.71	14.8 ± 0.57	6.8 ± 1.34	5775 ± 1450	782 ± 82	281 ± 55	0	0
20	T 3.01 ± 0.3	30.9 ± 2.8	103 ± 13.3	10.9 ± 1.14	57.6 ± 14.6	45320 ± 12379	10106 ± 3483	1500 ± 591	983 ± 806	12.2 \pm 6.3
	C 7.76 ± 0.87	39.5 ± 0.71	51 ± 4.6	14.1 ± 0.14	7.7 ± 1.48	5740 ± 1584	1715 ± 92	252 ± 110	0	0
25	T 4.62 ± 0.36	36.4 ± 1.82	76 ± 4.6	11.8 ± 0.98	43.4 ± 15.9	34350 ± 12061	7332 ± 4010	1840 ± 514	0	5 \pm 4
	C 7.40 ± 0.28	43.5 ± 0.71	58.5 ± 3.5	16.1 ± 0.99	9.2 ± 6.2	7140 ± 4327	1808 ± 1672	264 ± 203	0	0
32	T 4.86 ± 0.84	38.75 ± 2.36	80 ± 7.81	13.2 ± 1.56	15.6 ± 3.7	12820 ± 4096	2355 ± 835	443 ± 359	57.75 ± 99.5	.75 \pm .55
	C 6.79 ± 2.14	38 ± 6.36	56 ± 8.1	13.0 ± 3.0	6.5 ± 1.41	4750 ± 1131	1533 ± 378	213 ± 88	0	0

N/A - Not available.

T - Test animals.

C - Control animals.

R.B.C.- Red blood cell count.

P.C.V.- Packed cell volume.

M.C.V.- Mean cell volume.

Hb. - Haemoglobin concentration.

W.B.C. - White blood cell count.

L - Lymphocyte count.

N - Neutrophil count.

M - Monocyte count.

My - Myelocyte count.

TABLE III.

t. tests between infected and control groupsBabesia rodhaini

	1	2	3	4	5	6	7	8	9	10
Days after infection	Degrees of freedom	R.B.C.	P.C.V.	M.C.V.	Hb.	W.B.C.	N	L	M	My
I	5	0.76	2.73*	0.70	1.50	1.42	1.31	0.05	0.66	0
II	5	0.64	0.93	2.36	0.43	0.10	0.30	0.05	1.02	0
III	5	0.38	0.54	0.63	0.08	1.75	0.34	1.99	0.44	0.95
IV	5	0.45	0.60	0.44	0.90	2.50	0.31	3.12**	0.80	1.71
V	5	1.57	1.70	0.42	1.83	0.82	0.77	0.04	0.38	2.88*
VI	5	3.65**	2.66*	0.37	3.74**	1.64	2.17	0.04	0.32	1.61
VII	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
VIII	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A - not applicable (infected animals dead).

* - significant at .05 level. $t > 2.571$.** - Significant at .01 level. $t > 3.365$.

R.B.C. - Red blood cell count.

P.C.V. - Packed cell volume.

M.C.V. - Mean cell volume.

Hb. - Haemoglobin concentration.

W.B.C. - White blood cell count.

N - Neutrophil count.

L - Lymphocyte count.

M - Monocyte count.

My - Myelocyte count.

TABLE IV.

t. testsBabesia microti

	1	2	3	4	5	6	7	8	9	10
Days after infection	Degrees of freedom	R.B.C.	P.C.V.	M.C.V.	Hb.	W.B.C.	N	L	M	My
II	5	0.19	N/A	N/A	.91	0.55	1.72	0.32	0.35	0
IV	5	0.31	0.88	1.18	0.73	0.10	0.87	0.45	0.86	3.56**
VI	5	0.26	1.29	0.24	0.07	1.64	0.95	1.37	0.2	1.16
VIII	5	3.39**	2.75*	1.05	2.52	3.85**	2.90*	3.87**	1.55	2.15
X	5	17.50**	14.79**	4.58**	15.1**	5.40**	5.38*	2.94*	1.03	2.03
XIII	5	6.13**	3.63**	3.08*	4.68**	2.62*	3.25**	2.50	1.45	0.60
XIV	4	6.94**	3.11*	5.67**	4.77**	11.28**	2.24	7.03**	2.69	0
XVI	5	11.19**	4.71**	4.98**	7.00**	3.35*	3.80**	2.72*	1.44	2.24
XX	5	12.18**	4.08**	5.15**	3.76*	4.56*	3.22*	4.26*	2.81*	1.63
XXV	4	9.27**	5.07**	4.64**	5.06**	2.80*	1.80	2.97*	4.02**	0
XXXII	4	1.89	0.23	3.51*	.11	3.2*	1.27	2.59	.85	0

N/A - not available

* - significant at .05 level $t > 2.571$ for 5 d.f. $t > 2.776$ for 4 d.f.** - significant at .01 level $t > 3.365$ for 5 d.f. $t > 3.747$ for 4 d.f.

R.B.C. - Red blood cell count.

P.C.V. - Packed cell volume.

M.C.V. - Mean cell volume.

Hb. - Haemoglobin concentration.

W.B.C. - White blood cell count.

N - Neutrophil count.

L - Lymphocyte count.

M - Monocyte count.

My - Myelocyte count.

DISCUSSION

The normal mouse:

Haemograms:

Figures obtained for normal mouse haemograms, using "Bush strain" mice, agreed with previously published data, apart from the mean cell volume and total leucocyte counts.

Russel et al (1951) quoted mean cell volumes between 41.5 and 51.5 cubic microns, with various strains of inbred mice. No figures were available for the strain used in these examinations but it would appear that, for Bush strain mice, using the Coulter counter, the figures obtained were higher than those quoted by Russel, with a mean of 54.9 ± 4.2 cubic microns.

White blood cell counts in these examinations were low compared with published data. A discrepancy in this parameter was not surprising due to the methods used in sampling animals. This conclusion is supported by evidence from other workers. Heart blood was low in leucocytes compared with tail blood (De Kock, 1931). Sampling at times of activity during the daytime gave lower counts (Brown & Dougherty, 1956). Females had lower eosinophil counts than males (Halberg et al, 1957) and the granulocyte percentage was less in females than males (Russel et al, 1951). It was also possible that strain difference was involved but no other figures were available for "Bush mice" as a comparison.

Brain capillaries:

In the normal mouse there was no evidence of high concentrations of erythrocytes within the capillaries. The morphology of the capillaries was similar to that in other animal species, the endothelial cells having the typically elongated nucleus.

Parasitaemias during Babesia rodhaini
and Babesia microti infection:

The parasitaemia curve of Babesia rodhaini infection showed the classical picture of an exponential rise, after a prepatent period of three days. Parasitaemia continued to rise until death supervened approximately four days later. Death occurred when the parasitaemia rose above the 60 per cent level. The highest parasitaemia obtained, when the animal was still alive, was 71 per cent.

Animals infected with Babesia microti, at the same dosage rate as animals infected with Babesia rodhaini, became patently infected by the second day, and followed a similar pattern of exponential rise. This pattern differed from that of Babesia rodhaini infected animals, in that parasitaemia was slower in rising, not passing the 60 per cent level until day eight after infection. Maximum mean parasitaemias obtained with both infections were not appreciably different. Those of Babesia microti infection reached 64.8 per cent and of Babesia rodhaini infection, 62.0 per cent.

Seven days after infection with Babesia rodhaini, of the ten animals scheduled for sampling, nine were dead. At no time during infection with Babesia microti did animals die from the disease. This illustrated the differing pathogenicity of the two organisms. The results from these experiments could indicate that pathogenicity was not related to the parasitaemia reached but to the rate of parasitic multiplication within the host. This gave, to Babesia microti infected animals, more time to respond to parasitic stimulus, than to Babesia rodhaini infected animals.

In Babesia microti infection the levels of parasitaemia fell rapidly, after the peak at day eight after infection, and appeared to rise again over the next four days. On applying a t test between the parasitaemias on day 12 and day 16 a significant difference was established. This suggested that the parasite produced a diphasic parasitaemia in the mice. However, the parasitaemias recorded on day 12 were from animals different from those recorded on day 16 and the significant difference between the two groups could have been caused by a prolonged prepatent period, or time to peak parasitaemia in animals sampled on day 16. Fluctuating parasitaemias in Babesia microti infection have been described by other workers and therefore it is quite possible that this was a true reflection of the course of parasitaemia.

After the second peak, the parasitaemia declined steadily until, by day 32, the parasites were present only in small numbers.

The anaemia during Babesia rodhaini

and Babesia microti infection:

Erythrocyte counts:

Babesia rodhaini infection produced very little difference in erythrocyte counts between control and infected animals up to five days after infection, when parasitaemia was at 20 per cent. After this there was a rapid fall in erythrocytes with death two days later. Animals infected with Babesia microti remained normal up to six days after infection, when parasitaemia was also approximately 20 per cent. Again there followed a rapid fall in erythrocytes to levels similar to those produced by Babesia rodhaini infection. Whereas Babesia rodhaini infected animals took only two days to fall from normal erythrocyte levels to minimum levels, those infected with Babesia microti took four days. After the minimum levels had been reached Babesia microti infected animals appeared to show a rise in erythrocyte count over a period of two days. This was probably due to the release of stored cells, but could also have been due to dehydration giving a falsely elevated count. Erythrocyte levels, over the next eight days, remained fairly constant, even during the second parasitaemic peak. From day 20 onwards there was a gradual

return towards normal levels.

Packed cell volumes:

In the Babesia rodhaini infected animals the packed cell volume reflected the erythrocyte picture, with a rapid depression after day five.

In Babesia microti infected animals levels fell to a minimum at day 10 but not to the low levels expected from erythrocyte counts. This can be explained by a macrocyte response by the host with release of immature cells. After the minimum levels had been reached, there was a rapid rise in packed cell volume over the next two days, due probably to release of stored cells. This was followed by a small depression corresponding to the second parasitaemic peak. After this, there was a rapid return to normal levels, which were attained by day 32.

Mean cell volumes:

Throughout infection with Babesia rodhaini no significant changes occurred in mean cell volume. This indicated a lack of response to the loss in functional erythrocytes.

Macrocytosis was marked during Babesia microti infection, from day eight onwards. There was a trend towards normality after day 20, but the cells were still significantly macrocytic at day 32.

Haemoglobin concentrations:

Again, the same pattern was shown in Babesia rodhaini infected animals with a rapid decline after day five and death within two days, at very low haemoglobin levels.

Haemoglobin concentrations showed the same pattern, as did the packed cell volumes, in Babesia microti infection. There was a fall after day six to a minimum at day 10, a rapid rise then a flattening of the curve corresponding to the second parasitaemic peak. This was followed by a steady return to normal by day 32.

When the anaemia was examined as a whole, the differing picture between the two infections became apparent. Babesia rodhaini infection in mice produced a rapid progressive anaemia, with no obvious compensatory host mechanism. This was possibly due to the acute nature of the infection which precluded the immune response of the host from operating. This phenomenon need not necessarily be a function of time but that the parasite itself could have contributed to the depression of the host's immune response.

Compensatory mechanisms in Babesia microti infection were marked but were not evident until day eight, after infection, by which time animals, with corresponding Babesia rodhaini infection, would have died. The recovery picture was typical of a haemolytic anaemia. The mouse has a normal erythrocyte life span of only 30 days and,

because of this, the return to normal following a haemolytic crisis is rapid. By day 32 a functional normality had been attained, although evidence of the anaemia was manifested by a significant macrocytosis. The larger cells had the same haemoglobin concentration as normal cells, giving low erythrocyte counts with normal haemoglobin concentrations and packed cell volumes.

The anaemia due to Babesia rodhaini infection in mice can be classified as normocytic and normochromic, whereas that of Babesia microti infection was macrocytic and normochromic.

The leucocyte response during Babesia rodhaini and Babesia microti infection:

Total leucocyte counts:

The leucocyte response in Babesia rodhaini infection was not marked. From inspection of the graph, a trend upwards could be seen towards the end of infection. This was, however, not significantly different from the picture in the control animals. When the response in Babesia microti infected animals over the first week was considered, it was almost identical with that of Babesia rodhaini infected animals. These animals, however, did not die and, four days following the parasitaemic peak, the total leucocyte counts showed a 10 fold increase over those of the controls. These fell rapidly but rose to a new peak coinciding with the second peak of parasitaemia.

After this, there was a rapid return towards normal levels, which were almost attained by day 32.

Granulocytic response:

Neutrophil levels in Babesia rodhaini infection appeared to rise towards the end of infection. This rise was only significant to the 0.05 probability level on a one tail 't' test. This result was somewhat doubtful but the presence of myelocytes, preceding this rise, which were not normally found in mouse blood, could be regarded as a significant finding. It would appear that the reason for the poor neutrophil response was that death had supervened and that had the animal lived, a more significant response might have been seen.

Over the first seven days, response to Babesia microti infection showed a similar pattern to that in Babesia rodhaini infection. There was a fairly insignificant initial rise in neutrophils, but there was a preceding myelocytosis. After this time, the response was considerable and followed the same pattern as the total leucocyte counts.

Eosinophil, basophil response:

In neither infection were raised levels found at any time, so that it appeared that these cells take no major part in the host's response.

Mononuclear cell response:

At no time during the Babesia rodhaini infection were

any significant changes seen in lymphocytes or monocytes.

In Babesia microti infection the response of both was marked. Over the first week of infection differences were slight but thereafter the lymphocyte numbers rose very rapidly to a peak at day 12. There was a second smaller peak corresponding with the second parasitaemic peak and this was followed by a rapid return towards normal levels, which were almost attained by day 32.

The changes in monocytes were delayed slightly, but came to a peak at day 12 and normality was attained by day 32.

It is possible that the different response observed was due to differences in the times taken by the two parasites to develop in the host.

It would appear that the times taken by the two parasites to develop in the host had an important significance in both white and red cell responses. It was impossible in these experiments to confirm this theory as no animals infected with Babesia rodhaini survived long enough for such a response to be studied.

Brain capillary bed examinations:

The absence of any sludging of erythrocytes within the capillaries of the brain during either infection led to the conclusion that if this does occur it is a rarity not found during these experiments. This confirms the findings of Nowell (1968). The finding of blue amorphous material

near the endothelial nucleus can be regarded as not significant or relevant to the infections being studied.

The presence of red dots around the nuclei of the endothelial cells could be significant. These were found in animals infected with Babesia rodhaini, but not in the corresponding control animals. It is possible that these particles are of babesial origin but could be host derived. In order to establish their exact identity further studies would be necessary, using different examination methods.

Anomalies in statistical analysis:

In Table III there are two significant t test results in places where they would not normally be expected. On day I, column 3 both test and control animal figures are within the normal range. The variance shown within each group, however, is so small that the difference indicated between the groups is not significant. The same has occurred in Table III, day IV, column 8.

In Table IV, day XII, column 8 and day XIV, column 7 the differences appear to be insignificant. On consulting the relevant graphs it can be seen that the differences between control and infected animals are considerable. The distorted value is caused by abnormally large variations within the infected groups.

CONCLUSIONS

Babesia rodhaini caused a rapidly progressive fatal disease of mice with a severe anaemia which was normocytic and normochromic. There was no evidence of host mechanisms compensating for the anaemia. There was only a very limited leucocyte response, restricted to the neutrophil series. There was no evidence to suggest that cerebral babesiosis was of any importance in the infection although further work is necessary to establish the identity of the red inclusions.

Babesia microti caused a severe anaemia which was macrocytic and normochromic. The disease was not fatal. Response to the anaemia was rapid and functional normality was attained by day 32 after infection. The leucocyte response was marked with a neutrophilia and associated myelocytosis, lymphocytosis and monocytosis. The response was short lived and normality was nearly attained by day 32 after infection.

No evidence of cerebral babesiosis could be found in either infection.

No evidence of any differences which could be classified as species differences was found.

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