PATHOGENESIS AND PATHOLOGY OF BOVINE PETECHIAL FEVER (ONDIRI DISEASE) IN CATTLE

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A thesis submitted in part fulfilment for the degree of Master of Science in the University of Nairobi.

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SEPTEMBER, 1974.

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ACKNOWLEDGEMENTS :

I wish to express my sincere gratitude to my two supervisors Prof. G.M. Mugera of the Department of Veterinary Pathology and Microbiology, and Dr. W.Z. Lutta of the Department of Veterinary Clinical Studies for their help, encouragement and technical advice throughout my study, and for their careful reading and constructive criticism of the Manuscript.

I should also wish to express my sincere appreciation for the great assistance offered by Messrs. James Mawaru and Kahara D. Njau both of the Department of Veterinary Pathology and Microbiology for their help in the processing of the material used in this study and for their assistance with the photography. The assistance of Mr. John Mungai of the same Department, in the handling of the experimental animals is gratefully acknowledged.

The co-operation and assistance offered by the technical staff of the Department of Clinical Studies, in particular Mrs. A. Missan and Mrs. M. Muitta in Haematology and Messrs. Philip Thuo and John Chege in Biochemistry are much appreciated.

My sincere thanks to Mrs. R.W. Mionge for typing both the draft and the final copies of the thesis.

This study was financially supported by a research grant from the University of Nairobi for which I am most grateful.

TABLE OF CONTENTS :

TABLE OF CONTENTS (CONT) :

APPENDIX 1 - 9

Appendix 1 : Daily Temperature Reaction (^{o}F) of six calves infected with Bovine Petechial Fever.

Appendix 2 : Daily temperature recordings (^{o}F) of calves used as controls in Bovine Petechial Fever.

Appendix 3 :

(a) Daily Haemoglobin Concentration (gm/100ml) of calves infected with Bovine Petechial Fever (Recovering calves)

(b) Daily Haemoglobin concentration (gm/100ml) of calves infected with Bovine Petechial Fever (Dying calves).

(c) Daily Haemoglobin concentration (gm/100ml) of six calves used as controls.

APPENDIX 4 :

(a) Daily Percentage Packed Cell Volume (%) of calves infected with Bovine Petechial Fever.

(b) Daily Percentage Packed Cell Volume of calves infected and died with Bovine Petechial Fever

(c) Daily Percentage Packed Cell Volume of calves used as controls.

APPENDIX 5 :

(a) Daily Measurement of Total Protein (gm/100ml) of calves (recovering calves)

(b) Daily Measurement of Total Protein (gm/100ml) of calves infected and dying with Bovine Petechial Fever.

(c) Daily Measurement of Total Protein (gm/100ml) of calves used as control.

APPENDIX 6 :

(a) Daily Red Blood Cell counts ($10^6/mm^3$) of calves infected with and recovering from B.P.F.

(b) Daily Red Blood Cell counts (10^6/cu.mm) of calves infected and dying with B.P.F.

(c) Daily Red Blood Cell counts ($10^6/mm^3$) of calves used as controls.

APPENDIX 7 :

(a) Daily White Blood Cell counts ($10^3/mm^3$) of calves infected and recovering.

(b) Daily White Blood Cell counts ($10^3/mm^3$) of calves infected and dying with B.P.F.

(c) Absolute Neutrophil Counts ($10^3/mm^3$) of calves infected and recovering from B.P.F.

(d) Absolute Neutrophil counts ($10^3/mm^3$) of calves infected and dying from B.P.F.

(e) Absolute Lymphocyte counts ($10^3/mm^3$) of calves infected and recovering from B.P.F.

(f) Absolute Lymphocyte counts ($10^3/mm^3$) of calves infected and dying from B.P.F.

APPENDIX 8 :

(a) Daily Measurement of the Mean Corpuscular Volume (μ^3) of six calves infected and recovering

(b) Daily Measurement of the Mean Corpuscular volume (μ^3) of six calves infected and dying with B.P.F.

(c) Daily Measurement of the Mean Corpuscular Haemoglobin concentration (%) of the recovering calves.

APPENDIX 8 :

(d) Daily Measurement of the Mean Corpuscular Haemoglobin concentration (%) of six calves infected and dying with B.P.F.

APPENDIX 9 :

Daily Measurement of Serum Alkaline Phosphatas of three calves infected with B.P.F., and two calves used as control.

LIST OF TABLES :

TABLE :

LIST OF FIGURES

FIGURE :

LIST OF FIGURES (CONT) :

FIGURE :

LIST OF FIGURES (CONT)

FIGURE :

LIST OF FIGURES (CONT) :

FIGURE :

30 Section of liver showing sinusoidal cell loaded with more than a dozen vacuoles (V) containing more than 40 parasites (ib) in all. Nucleus (N) is under grea pressure while some of the parasites are degeneratin X 12,600.

31 Section of Liver showing vacuoles in sinusoidal cell filling the sinus and forcing erythrocytes (E) to squeeze through narrowed. Several stages (1-5) of binary fission of the organism within the vacuole, X 12,600.

32 Section of Liver showing complete occlusion of the sinus lumen (L) arresting Erythrocytes (E). Numbere parasites are completing binary fission X 12,600.

33 Section of Liver showing sinusoidal cell with severa vacuoles. The organism in the center shows a double division (1,2,3) while the single organism below the nucleus (N) is degenerating. X 12,600.

34 A higher magnification of the same vacuole as fig. 3 showing the double membranes and the development of lines of division (→) X 31,000

35 Tangentially cut liver sinusoidal cells containing many vacuoles. The parasites in the vacuoles are fast multiplying by binary fissions X 12,600.

LIST OF FIGURES (CONT) :

FIGURE :

LIST OF FIGURES (CONT) :

FIGURE :

43 A still higher magnification of same vacuole (V) as fig. 42 with double membrane (dm) of each parasit clearly demarcated. Multiplication division is near complete on the organisms in the right side while th large one on the left is just showing the division lines and the whole mass still enclosed in one outer membrane X 31,000.

44 Heart section showing a granular mass (V) on capilla wall of a blood vessel. There are many erythrocytes (E) outside the vessel lumen (L) while the adjacent myocardial cells are showing stages of degeneration (d-Ht). X 4,000.

45 Heart section higher magnification of fig. 44 showin necrosis of the endothelial cells (End) and the distension of the capillary endothelial cell by the vacuole of the parasites. Other adjacent endothelia cells show the presence of cytoplasmic vacuoles whic indicate degenerative changes : X 12,600

46 Heart section higher magnification of fig. 45 showin the double membranes (dm) of the parasites. The parasites are showing lines of division(→) while other parasites seem to be attached to the vacuole membrane: X 31,000.

LIST OF FIGURES(CONT) :

FIGURE :

SUMMARY :

Research into the pathogenesis and pathology of Bovine Petechial Fever has been stimulated by the fact that the disease is an important limiting factor in keeping cattle in many areas of the Kenya Highlands. The clinical and gross pathological syndrome of the disease was studied both in field natural cases of the disease and in experimental bull calv The calves used were "high grade" animals (6-12 months old) and t were infected by intravenous inoculation using 80-250ml of infec whole blood from clinical cases. Some of the calves were infect with blood from the heart of a heifer that had died three hours previously from the natural disease in the field.

After a varying incubation period (from 5-10 days) there was a sudden elevation of body temperature (to 103°F or above) accompanied by serous discharges from the eyes and the nose with grinding of the teeth. One to two days after this elevation of temperature, some haemorrhages varying from pin-point petechiae to ecchymoses appeared on the visible mucous membranes. About 20% of the experimental animals developed a varying degree of diarrhoea with blood in the faeces. Some developed conjunctival oedema, pulmonary oedema and submandibular oedema. The pulmonar oedema was manifested by a very harsh cough, and sometimes by frothing from the mouth and the nose. Respirations became marke fast with the animal tending to be dyspnoeic. The haemorrhagic syndrome caused a tremendous reduction of both the leucocytes an erythrocytes of the blood with a persistent profound anaemia being observed in the recovering animals.

The animals also showed a leucocytosis in the period of the disease incubation and the recovering ones during convalescence. The duration of the disease course has been between four and two days.

In the early pyrexic stage of the disease blood smears stained by Giemsa Method showed intracytoplasmic Rickettsia in the cells of the leucocytic series. Smaller organisms were more common while giant bodies and morulae became predominant during the lat course of the disease. After the appearance of the extensive haemorrhages, the organisms became difficult to detect in the bl smear.

42.8% of the reacting experimental animals died while another 25% were sacrificed at various stages of the disease course to obtain fresh organ tissues for electron microscopic examination. At autopsy the most striking pathologic change was extensive haemorrhage into connective and muscular tissues. About 30% of those examined at post mortem showed a varying degree of oedema in the connective tissues of the sub-cutis. All the animals examined at post-mortem showed grossly haemorrhagic myocarditis and a varying degree of haemorrhagic involvement in other organs. Over 85% of them had mucosal and submucosal haemorrhages of the gall bladder.

Histological examination of organ sections showed the extensive haemorrhages as areas of capillary vasculitis with cell degenerat and an accompanying perivascular hystiocytic cell infiltration.

In the lungs and brain, these degenerative changes were accompanied by oedema of the tissues and congestion of the vessels. The liver cells showed degenerative changes ranging from vacuolation and fatty degeneration to extensive coagulative necrosis of the liver cord cells. The heart showed marked haemorrhagic myocarditis with extensive myocardial cell degeneration.

Biochemical investigation showed a marked increase in the serum glutamate oxalacetate transaminase (S.G.O.T.) which supports the observation of the tissue degeneration. Liver excretion test with Bromsulphalein revealed a very prolonged excretion time in sickness which indicated impaired hepatic function associated with the degenerative changes.

Fresh tissues obtained from sacrificed animals were processed for electron microscopic studies. Ultra-thin sections from the liver, spleen, heart muscle, kidney and third eyelid were examined together with buffy coat preparations. Different shapes and sizes of the Rickettsia representing different stages of development of the parasite were observed in the cytoplasm of different types of cells. Their presence in the organ cells was associated with degenerative changes of the host cells. They were observed either singly or enclosed in vacuoles within the cytoplasm of the host cell.

The Ultra-structure of the organism was found to consist of a double membrane enclosing a dense nucleoid mass with no noticable cytoplasm.

The inner layer of the membrane was firmly apposed to the nucleoid mass which was rich in ribosomes. The replication process of the organism observed showed a binary fission and multiple fission for the smaller and larger organisms respectively. Phagocytic action of the host cells was observed in some cases to cause changes in the parasite involving loss or degeneration of the double membranes. All the replicating parasites had their double membranes intact and no observation was made of parasites multiplying without the membrane.

The organisms found in the buffy coat cells were similar to those that were found in the cells of the other tissues.

INTRODUCTION.

I N T R O D U C T I O N :

Bovine Petechial Fever, commonly known in Kenya as "Ondiri Disease" is a disease of ruminants that occure in many areas of the Kenya Highlands, with altitudes between 5,000 ft. and 9,000 ft. (1,500-2,800 m). The natural occurrence of the disease is seen only in cattle, but other ruminants (Sheep and Goats) can be infected experimentally. The disease syndrome is characterised clinically by fever, appearance of petechial haemorrhages on the visible mucous membranes. Occasionally the disease also causes sudden collapse and death. In milking cows, the first noticeable sign is sudden drop in milk production and fever with anorexia. The duration of the disease course is very variable from two to ten days (Piercy 1953).

It is possible that Bovine Petechial Fever (B.P.F.) has been present in Kenya from time immemorial even though the disease is not known in any of the languages of the cattle-keeping tribes of the Kenya Highlands. Evidence of the existence of this disease were first noticed in 1929 when imported cattle on a quarantine station at Nairobi developed a haemorrhagic syndrome that was difficult to associate with any other known cattle disease (Nettam, 1929). Studies undertaken then revealed no causative agent and experimental transmission was not successful. The disease condition was tentatively called "Nairobi Quarantine Disease".

Four years later the disease assumed new significance when deaths occured among milking cows on Ondiri Farm at Kikuyu (Dept. of Agric. Ann. Rept. 1933). Studies were then carried out at the Veterinary Research Laboratories at Kabete which established this as a new disease entity different from all other known ailments affecting cattle (Danks 1933). The disease was then variously named "Ondiri disease", "Bovine Infectious Petechial Fever", Specific Transmissible Petechial Fever", "Haemorrhagic diathesis" and also "Ondiri-itis".

Piercy (1953) indicated that the disease is widely distributed in the Kenya Highlands, where cattle grazed in or near indigenous forests. He reported that the incidence of the disease was higher during the rainy season and further that "needle" passage increased the adaptation of the virus to artificial transmission and its virulence (The East African Agric. Journ, 1953). He attempted to transmit the disease naturally using ticks, stable flies and mosquitoes, but this gave negative results. He concluded that arthropod transmission must be responsible for the sporadic nature of the disease. Studies on this disease were then undertaken by several workers both at Muguga and Kabete. Plowright (1958) observed morula-like granular bodies in tissues of dying animals examined with the light microscope. Haig and Danskin working at Veterinary Research Laboratories at Kabete followed these observations and demonstrated the constant presence of these pleomorphic Rickettsia - Like Bodies (R.L.B), in the polymorphs and monocytes of circulating blood obtained from clinically reacting animals (Haig and Danskin 1962).

This demonstration then opened a new chapter in the study of this "Ondiri - disease" and stimulated further research into the study of the characteristics of this pleomorphic causative agent, with a view of classifying it and eventually finding methods for control of the disease.

The disease occurs sporadically with varying degrees of the clinical syndrome. Nettam (1929) had reported that the duration of the disease varied between four to eleven days. Piercy(1953) on the other hand reported acute cases where the animal collapses and dies after two to three days.

Danks and co-workers (Dept. Agric.Ann.Rept. 1935) had put the mortality of the disease as low as 5 or 6%. Workers at Kabete (Vet.Dept.Ann.Rept. 1956) carried out experimental transmissions and their findings showed a mortality higher than 55%. In one case during our investigation at a Naivasha farm, we examined a herd of 33 yearling heifers and found six of them with typical petechiation, listlessnessness and high temperature 105 - 107°F., three showed high temperature and no other sign, while two had few petechiae and pale mucous membranes without pyrexia. This confirmed earlier reports by Danskin and Burdin (1963) that in the field only the most severe cases are noticed and there is undoubtedly a high rate of inapparent infections.

In my experimental transmission, the incubation period has been found to vary from 4 to 10 days (average 6.2 days). One infected calf (No. 53) showed only temperature elevation to 104°F for two days and haematologic changes without other obvious manifestations of the disease.

In many areas of the Kenya Highlands, Bovine Petechial Fever continues to hinder cattle farming by causing actual deaths, and by retarding production. However, until more knowledge is obtained about the behaviour of the causative parasite, the control of the disease will still present difficulties.

This investigation was undertaken for two purposes. The first was to study the clinical syndrome of Bovine Petechial Fever (Ondiri - Disease) and its correlation with pathological changes in blood and other tissues provoked by the causative agent in infected animal. The second purpose was to study the morphology and the ultra-structure of the agent in the different tissues of the affected animal as a means of determining the most suitable material for diagnosis of the disease when it is suspected.

REVIEW OF LITERATURE

REVIEW OF THE LITERATURE :

Incidence and Clinical Signs :

Danskin and Burdin (1963) thought it was extraordinary that the Bovine Petechial Fever did not occur even in adjacent countries that have similar topography to the Kenya Highlands. The disease is enzootic in Kenya Highlands where it occurs sporadically. The incidence of the disease vary from one farm to the other and from year to year. Occurrence seems more frequent when short periods of rains follow bright sunny weather, (Plowright 1962). On the farms in the enzootic areas, the infection persists in the absence of cattle for many years and often only a particular small area of a farm or paddock appears to harbour the infection. The incidence of the disease is consistently associated with parts of farms covered with forest and thick scrub. In times of drought farmers are tempted to move animals into these thick scrub areas of their farms, and then the disease occurs.

Outbreaks of this "Ondiri-disease" have shown considerable variation in the clinical signs and the course of the disease (Danskin and Berdin, 1963). The severity of the disease in cattle varies from inapparent infection to a highly fatal syndrome (Haig 1966). The disease normally has an insidious onset in the herd which may go undetected when animals are not frequently handled (Nettam 1929). The most severe cases are noticed while a high proportion of inapparent infections go unnoticed (Danskin and Burdin 1963).

Danks (1933) reporting on its outbreak in dairy herd on Ondiri farm at Kikuyu said that the principal symptom was a sudden rise in temperature and a marked drop in milk yield. Later on (1936) he reported cases of sudden death where animals would look well one day and be found dead the next morning. The peracute forms of the disease with sudden death ar not often seen in the experimental disease. In experimental transmissions the course of the disease has often been acute or sub-acute; with duration of the disease syndrome varying from 4 to 11 days followed either by death or slow recovery. Incubation period in the artificial transmission have been between 5 and 14 days (Vet.Dept.Rept.1956). There are no figures for the incubation period of the natural disease because its mode of natural transmission to date is not known.

The first sign that is seen is a systemic disturbance manifested by a dry coat, anorexia and grinding of teeth (Nettam 1929). In a milking herd these signs accompany an immediate and striking drop in milk yield of the affected cows (Danks 1936). Mildly affected animals usually eat and behave normally during most of the period. In non-lactating animals high temperature 105-107°F. accompany the systemic disturbance, and it precedes all other signs by 1-2 days. Profuse serous nasal and lacrimal discharge are passed. One or two days following elevation of temperature, the visible mucous membranes (gums, lips, underside of tongue, vulva and conjunctivae and nares),

an even light areas of the skin, show the development of haemorrhagic areas varying from fine petechiae to extensive flecking, in a congested background (Plowright 1962). With the onset of these signs, respiration and pulse become fast. Some of the animals develop pulmonary oedema which is shown by a short harsh cough that later progresses to severe and prolonged coughing. Some animals develop slight diarrhoea with fluid faeces which may be tinged with blood. Others develop severe diarrhoea that progresses to frank dysentery. In a few of these animals oedema develops in limbs, trunk and neck. Animals may collapse and die due to heavy loss of blood and fluids, or some may progress in 2-3 days to prostration and weakness, that may lead to death or to prolonged recovery. In the recovering animals the course of the disease lasts 5 to 10 days with development of anaemia and disappearance of the petechiae. This is then followed by a protracted convalescence during which time animals are likely to go down with secondary complications.

Gelatinous oedema of the conjunctivae develops in about 5% of the animals affected with Bovine Petechial Fever (Piercy 1953). This syndrome when it occurs is seen after the stage of the development of petechiae. This gelatinous oedema of the conjunctivae causes the eversion of the eyelids and may be accompanied by haemorrhage into the aqueous humor. This appearance of the yellow gelatinous eye with haemorrhage is referred to as a "poached egg" eye.

H A E M A T O L O G Y :

Mettam (1929) in his investigation of Nairobi quarantine disease had noticed that there were circulatory disturbances in the capillaries of the skin. He observed a delayed coagulation time of the blood, and drew the conclusion that the disease set up specific changes in the peripheral vascular system. These changes led to hyperemia or stasis in the solid viscera, and to extensive haemorrhagic syndrome. Danskin and Burdin (1963) supported this observation and added that there was an accompanying basophilic stippling of the erythrocytes and a thrombocytopenia with a resultant increase in clotting time. Studies undertaken by Danke (1936) had confirmed an earlier observation made by Mettam that there was also an accompanying gross deficiency of fibrinogen.

Investigation of the behaviours of the various blood components was commenced after 1953. Piercy (1953) had reported that Bovine Petechial Fever had been causing problems in several areas of the country. Plowright (1962) briefly studied the haematologic picture in nine natural cases of the disease and found that there was a drop in both the erythrocyte counts and the packed cell volume. This view was later supported by Danskin and Burdin (1963) who recorded a drop in the erythrocyte count from about 7×10^6 to 4.5×10^6 cells/cu.mm. Plowright carried out most of his haematologic observations when the animals were already visibly sick and recorded white cell counts of 1,950 to 5,050 cells per cu.mm.

At this stage of the disease he observed that the leucopenia appeared to affect the monocuclear cells predominantly. In about half of the animals he examined he observed a complete disappearance of the eosinophils in the terminal stages of the disease.

Damskin and Burdin (1963) carried out their haemotologic examinations on fifteen experimental animals and numerous natural cases. They noticed that there was a decrease in the leucocyte count concomittant with pyrexia and before clinical signs appeared, and that this changed rapidly to a marked leucocyte increase, sometimes to as high as 19×10^3 to 20×10^3 cells/cu.mm. They recorded that the initial drop was due to reduction in the lymphocyte-monocyte groups. During their studies the granular series remained constant, till the appearance of clinical signs when the lymphocyte - monocyte groups increased with the appearance of many immature cells. There was also a proportional reduction in Haemoglobin concentration of the blood. Krauss et al (1972) in their experiments to determine the morphology and characteristics of the causative agent in the blood of sheep, supported these observations. They found that there was a drop in circulating lymphocytes from about 4×10^3 to below 2×10^3 cells per cu.mm. when temperature reaction started. Unlike Damskin and Burdin (1963) they observed that there was a reduction in the number of neutrophils during the course of the disease. This decrease was very marked on the 7th day post - infection and that there was a return to normal counts with the disappearance of the agent from circulating blood cells.

The Causative Agent :

When Danks (Vet.Dept.Ann.Rept. 1935) succeeded in transmitting the disease from natural cases of visibly reacting cattle, he noticed that the causative agent was present in the blood of reacting animals. He further observed that this agent could be preserved for up to 12 days "at cold - room temperature in the form of virulent blood". It was reported (Kenya Vet. Dept. Ann. Rept. 1953) that Rickettsia - like bodies had been seen in sections of heart muscle from a severe case of the disease but this incidental observation was not immediately pursued. Burdin and Danskin, working at Kabete established the fact that the infective agent was present only in the leucocyte portion of the blood (Kenya Vet. Dept. Ann. Rept. 1957). Brocklesby (1958) observed the Rickettsia - like bodies in the leucocytes of blood from animals of Muguga estate, but he believed these were the agent of tick-borne fever and he did not associate them with "Ondiri- Disease." Subsequently, Burdin and Danskin (Kenya Vet. Dept. Ann. Rep. 1957, 1958, 1959), Haig and Danskin (1962) and Plowright (1962) investigated the disease and observed the presence of the pleomorphic causative agent in the blood cells using the light microscope.

Krause et. al. (1972) employing both the light and the electron microscopes went further to study the morphology and ultra-structure of this pleomorphic agent. Their studies were carried out on the agent present in granular leucocytes of the circulating blood. In the light microscope they could readily recognise the blue-stained rickettsia - like bodies in the cytoplasm of the neutrophils, monocytes and eosinophils.

The organism appeared either as single pleomorphic forms or as morulae in the later stages of the disease.

With the electron microscope, they studied the morphology of the single pleomorphic forms and the small organisms forming a morula. They took measurements of these organisms and observed that multiplication was by binary fission which appeared to take place at any stage of the development of the small organisms to giant bodies. From their observation, they concluded that the morula was a group of small organisms (initial bodies) released by a breakdown of a giant body. They also observed that there were phagocytic cellular activity against the parasite with degenerative changes induced in the organism. In their studies, they suggested that this agent be placed tentatively in the genus Cytoecetes (Tyzzer 1938) within the Tribe Ehrlichieae.

Plowright (1958) observed morula-like granular bodies in Von-Kupfer cells and vascular endothelium in other organs. Plowright (1962) later observed vascular proliferation with endothelial swelling and mononuclear and hysticcytic infiltration. These observations supported Mettam's suggestion (Mettam, 1929) that the organism set up changes in the peripheral vascular system.

PATHOLOGIC CHANGES :

Gross Lesions :

The lesions of Bovine Petechial Fever are very characteristic, the most striking changes being profuse localised subcutaneous, submucous and subserous haemorrhages (Piercy, 1953). These haemorrhages vary in extent and size from fine petechiae on a background of congested or pale mucous membranes to extensive extravasations into muscular and connective tissue.

Plowright (1962) reported on the post-mortem appearance of 25 cases he examined, most of them being steers. He observed that in the acute form of the disease there were extensive flecking in a congested background on the underside of the tongue, lips, gums and conjunctivae. He further observed that the haemorrhagic condition involved many other organs of the body including lymph node cortices and spleen capsule, besides the haemorrhages he observed gelatinous oedema and swelling which caused these organs to bulge on incision. The peritoneal, pleural and pericardial cavities showed excessive blood-tinged yellowish fluid. The rumen had large splash haemorrhages on its serosal surface and the abomasum showed extensive gelatinous oedema of the wall and folds. All animals that died showed extensive haemorrhages of the heart involving epicardium, myocardium and endocardium. About half of the animals he examined showed congestion and oedema of the lungs, with frequent observation of froth in the trachea and bronchi.

Haig (1966) states that lung oedema is seen in most peracute cases and it appears to be the immediate cause of death. The central nervous system presents congestion of the Meninges with occasional haemorrhages in the dura mater (Plowright, 1962). Plowright (1962) also observed that the liver showed a marked congestion with a distinct greyish mottling. Histologically this revealed an acute congestion and parenchymal degeneration or necrosis of the liver cell cords. The gall bladder showed a characteristic swelling of the wall with submucosal and mucosal haemorrhages. This haemorrhage of the gall bladder is a very characteristic pathologic change of this disease.

He further examined the kidneys and reported cases of cortical haemorrhages with flecking around the hilus. Petechiae and larger haemorrhages were constantly found in the mucosa and submucosa of the urinary bladder with very little exsanguination into the urine. He then said that these haemorrhages of the urinary bladder must be regarded as one of the most constant lesions of the disease.

Danskin and Burdin (1963) examined animals dying from acute and subacute syndrome of the disease and found that animals dying during the anaemic stage show few of the haemorrhagic lesions.

Microscopic Lesions :

Nettam (1929) in the study of Nairobi quarantine disease had found no naked eye lesions on the blood vessels. He thought that the haemorrhagic syndrome was due to circulatory disturbances in the capillaries. In conclusion he stated that the causative agent must have specific injuries in the endothelial lining of the peripheral vascular system which were so slight as to escape detection even by the microscope, but which led to stasis or hyperemia and extensive haemorrhages in the tissues. This observation is supported by Plowright (1962) and Haig and Danskin (1962) who observed morula-like granular bodies in the von-Kupfer cells of the liver and vascular endothelium of other organs.

Plowright further stated that the presence of these granular bodies on the endothelium caused vascular proliferation with endothelial swelling and some mononuclear and hystiocytic infiltration.

A part from these preliminary histological examinations and reports by Plowright (1962), very little else has been reported on the histological appearance of other organs and tissues.

EXPERIMENTAL WORK

MATERIALS AND METHODS :

The Causative Agent :

The causative agent was initially obtained in blood from clinically sick natural cases of "Ondiri-Disease" at Naivasha Government Experimental Farm. One instance was infected with blood obtained from the heart of a calf that had died of the disease at Limuru. Subsequent transmissions in the animal compound were done using blood from clinically sick experimental calves.

Routine clinical examinations (taking temperature, checking mucous membranes, lymph nodes, checking pulse and respiration) were carried out on the natural cases. The animals that were at their early reaction phase with high temperature (over 105°F) and few petechiae were chosen as the donor patients. They were then bled into bottles containing disodium Ethylenediamine-tetra-acetic acid (E.D.T.A.) as the anticoagulant in the basis of 2 mg. E.D.T.A. per c.c. of blood. The volumes collected varied between 80 ml. and 250 ml. of whole blood. After gently mixing the blood and the anticoagulant, the bottle containing the blood was quickly put in an ice-packed flask for transportation to Kabete. At the same time fresh lymph node and blood smears were made from the donor animal, then air dried and brought to Kabete. Where they were first fixed with methanol for 3 minutes, then stained with fresh Giemsa Stain for 30 min. and examined microscopically for the presence of the Rickettsia - like bodies, and also for the presence of athroped-borne diseases (Anaplasmosis, Babesiosis, Trypanosomiasis and East Coast Fever).

When these slides were found negative for other disease and positive for Rickettsia, then the sample blood was administered intravenously into a "susceptible" experimental calf. In the subsequent transmission of the disease using blood from clinically sick experimental calves, these preliminary screening stages were always used before blood was administered into a "susceptible" calf. In this respect, no anticoagulant was used but transfer of blood was done with calves standing side by side using 50 c.c. syringes.

2. THE EXPERIMENTAL ANIMALS :

These experiments were carried out using 27 cattle. The cattle were "high grade" bull calves of the various exotic breeds (Guernsey, Friesian, Ayrshire and Jerseys); aged 6 to 12 months. These cattle were bought from farmers in the Uthiru area near Kabete from where Bovine Petechial Fever has not been reported over the last ten (10) years. The calves were routinely examined both clinically and haematologically for tick-borne diseases. They were housed in stalls in the animal compound in groups of 4 bulls per stall. Faecal samples were examined in the laboratory for helminth eggs and coccidial oocysts. Those that were positive for these parasites were routinely treated with tetramisole (Nilverm - I.C.I) for helminths and sulfadimidine tablets (vesadin-May & Baker); the dosage given depending on the wright of the calf. Faecal examination was repeated weekly after to check the parasites, and any that were still positive were treated with a higher dosage of those drugs.

During this period, the calves were bled for haematological determination of the baseline. Once weekly the calves are sprayed with toxaphene spray to prevent tick infestation.

3. METHODS OF INFECTION :

A measured amount of whole blood between 80 ml. and 250 ml, from the donor patient was administered into the jugular vein of the experimental calf using a flutter valve. In the stalls, the transmission was done using 50 ml. syringe and the needle with calves standing side by side. The day of infection is recorded as day Zero (0) for the purposes of subsequent examination and clinical reaction of the calf. Infection was routinely done after the collection of the days' haematologic sample was obtained. The control calves were not given any blood, but clinical examination was always done.

4. CLINICAL EXAMINATION :

Daily clinical examination was carried out. The appearance of the coat of the calf and its demeanor were observed. Rectal temperatures were taken and recorded in Farenheit degrees. Then the respirations and pulse rates were counted per minute. When these clinical examinations were done, the mucous membranes of the gums, underside of tongue and conjunctivae were examined for moistness, paleur and the presence of petechiae.

Then the digestive system was examined with special emphasis on the consistency of the faeces, frequency and strength of ruminal movements. These clinical examinations were always done in the mid-morning. Lymph nodes were examined for changes in size and consistency.

5. COLLECTION OF BLOOD SAMPLES :

All samples for haematological analysis were collected after the clinical examination in the morning. Blood samples for haematology were collected from the jugular vein using a 1½"x18" gauge disposable needle and a plastic (5cc.) syringe. About 4 c.c. of whole blood was transferred into a bijou bottle containing approximately 4-5 mg. of dried E.D.T.A. as anticoagulant and shaken gently to allow the blood and the anticoagulant to mix. For serum determination, about 20 ml. of whole blood was collected at the same time into universal bottles with no anticoagulant, and then allowed to stand in an incubator at 37°C for about 30 minutes to clot and the serum immediately processed.

For electron microscopic studies, about 20 ml. of whole blood was collected into a universal bottle containing about 30 - 40 mg. E.D.T.A. and taken to the laboratory for the preparation of the buffy coat layer.

6. PREPARATION OF THE BUFFY COAT LAYER FOR ELECTRON MICROSCOPY :

The 20 ml. of whole blood was transferred into centrifuge tubes and centrifuged at 2000 g for 15 min. in a Koto uni centrifuge. The plasma was then decanted and the buffy coat on top of the erythrocytes layer was overlaid with "Yellow-Fix (ITO-Karnovsky, 1968).

This was then placed at+4°C for 15 min. to solidify for the preparation of blocks.

The fixing of blood and tissue specimens was according to the method of Ito-Karnovsky (1968). In this method, the fixative referred to as "yellow-fix" was prepared as follows:-

4 gm. of paraformaldehyde were added to 100 ml. of distilled water which was then heated to near boiling. The solution was then cleared by adding IN NaOH dropwise and shaking well. The clear formaldehyde solution was diluted with an equal amount of 0.2 M phosphate buffer of pH 7.2. Then a few milligrams of 0.02% trinitrophenol was added and the solution stored in the refrigerator . To make the final fixative 2% glutaraldehyde was added to the stock solution just before use.

The specimens (blood) were fixed in this "Yellow-fix" for 2 hours at +4°C. This was then followed by 3 changes of 15 minutes each in 0.2 M cold (+4°C) phosphate buffer. They were then post-fixed in cold 1% osmium tetroxide (OsO_4) in 0.2M phosphate buffer for 4 hours. The specimens were then rinsed in physiological saline with three changes of fifteen minutes each and dehydrated in 30% acetone concentration at 4°C for 15 minutes. The specimens (buffy coat) was then dehydrated further through stages of increasing acetone concentrations of 50%, 70%, 90% and dry acetone (100%) each stage taking 30 minutes.

For embedding the specimens, DURCUPAN ACM (Fluka AG, Buchs SG, Switzerland) was used in various concentration in acetone, through three stages at room temperature :

(a) 3 parts dry acetone to 1 part - Durcupan No. IACM. for I hr.

(b) 2 parts dry acetone to 2 parts Durcupan No. 1 ACM for 1 hr.

(c) I part dry acetone to 3 parts Durcupan No. 1 ACM for 1 hr.

The specimens were then passed through undiluted Durcupan No. 1 for 4 hours at 50°C (122°F) with a change of the Durcupan at 2 hours; and then through Durcupan No. 2 for another 2 hours at 50°C.

The pieces of tissue (buffy coat) were then removed from the last stage and placed in dry gelatine capsules which are then filled with No. 2 Durcupan mixture and the capsules closed. They are here after hardened in the drying cupboard at 50-80°C (122-176°F) for 48 hours. Sections from the hardened blocks were cut with glass knives on the REICHERT OM U2 ultramicrotome. Phase sections of about 1 u were cut and stained with 3% toluidine blue in 1% borax solution and examined with the light microscope for orientation of the respective tissue blocks. Ultra-thin sections (60-90nm) were then cut with glass knives from the selected area of the block and these ultra-thin sections were picked up on formvar-coated copper grids, stained with 2% aqueous uranyl acetate for 8 minutes and 0.4% lead citrate for 3 min.

The specimens were then ready to be examined and photographs taken in a Carl Zeiss EM 9A electron microscope. Two samples of blood from two reacting animals were subjected to partitioning by centrifugation then plasm was decanted. The corpuscular elements of the buffy coat were haemolysed by being washed and centrifuged in distilled water with two changes. The precipitate from these washed buffy coat were prepared for examination under the electron microscope. The fixation, embedding and section preparation were as described above.

7. ESTIMATION OF THE BLOOD VALUES :

Routine haematology was done to determine the red blood cells counts (Rbc), white blood cell count (both total and differential), Haemoglobin concentration, (Hb), total protein (TP) and the packed cell volume (PCV).

(i) THE PACKED CELL VOLUME (PCV)

This was done using the Microhaematocrit Method. Commercially available unheparinized microhaematocrit capillary tubes (Arthur H. Thomas Co. Philadelphia 5, U.S.A.) with lengths 75 mm and internal diameter of 1.3 - 1.5 mm were used. These capillary tubes were filled with the uncoagulated (E.D.T.A) blood by capillary action until about $\frac{3}{4}$ full, then the dry sealed over a Bunsen flame.

The sealed tubes were then placed in a microhaematocrit centrifuge (Measuring and Scientific Equipment Ltd., Sussex, England) with their sealed ends towards the periphery. The MSE microhaematocrit operates at a fixed speed of approximately 12,400 r.p.m (15,000 g). The tubes were then spun for 15 minutes and the centrifuge stops automatically. The tubes were then removed and placed on an MSE microhaematocrit reader and the packed cell volume read.

(ii) THE DETERMINATION OF THE HAEMOGLOBIN CONCENTRATION (Hb):

The haemoglobin concentration of blood was determined by the cyanmethaemoglobin method using a Coulter Hemoglobine-meter (Coulter Electronics Inc. Hialeah, Florida, U.S.A). Forty Lambda of whole blood was diluted with ISOTON*, then six drops of Zap-Oglobin * (Containing 300 mg. $K_3Fe(CN)_6$, per 100 ml.)was added and gently shaken to mix. The mixture was left to stand for about 10 minutes and then the solution was placed in the cuvette of the Coulter Hemoglobinometer. The values of haemoglobin were then observed on the numerical Readout of the Hemoglobinometer. The readings were directly recorded as grams per 100 ml. of blood (with ± 0.1 gm. error).

*(ISOTON & ZAP-OGLOBIN are available from Coulter Electronics Inc. Hialeah, Florida, U.S.A.).

-23-

(iii) THE RED BLOOD CELL (Rbc) COUNTS :

The red blood cell count was done using the Coulter Electronic Counter(Coulter Electronics Inc. Hialeah, Florida, U.S.A.), Model Z_B with a mercury manometer. For the red blood cell count, a blood dilution of 1 : 50,000 is required. This dilution is prepared in two steps. The first step involves making a 1 : 500 dilution by mixing 40 Lambdas (λ) whole blood to 20ml of ISOTON. The blood is measured by the sucking action of the mercury manometer. Then 0.2 ml of this dilution is further mixed with 20ml of Isoton to give the desired dilution of 1:50,000. The diluted blood in an auxiliary beaker is placed on the beaker platform and the electrodes are inserted into the fluid. The Aperture Current Switch is then adjusted to diameter between 0.177 and $\frac{1}{2}$ to control the Aperture Current and eliminate counting small particles that pass through the aperture. The Threshold is also adjusted to 6-8 setting position (for bovine) to ensure that only particles of the right diameter (Mean Particle Volume) are counted. The readings on the Readout of the Coulter Counter multiplied by 100 is equal to the number of red cells per cu.mm. of the sample counted and the figure computed thus was expressed as the number of red blood cells (in millions, 10^6) per cu.mm.

Erythrocyte indices were calculated to determine the size and hemoglobin content of the erythrocytes using the method described by Benjamin (1961).

..24..

(a) THE MEAN CORPUSCULAR VOLUME (MCV)

Was calculated to find the average volume of the individual erythrocyte in cubic microns (U^3) from the formula :

$$MCV = \frac{PCV \times 10}{Rbc \text{ count (Millions/cu.mm)}}$$

From the figure obtained one determines the type of anaemia in Bovine Petechial Fever as normocytic (normal MCV), Macrocytic (increased MCV), or Microcytic (decreased MCV).

(b) THE MEAN CORPUSCULAR HAEMOGLOBIN CONTENT (MCHC)

The mean corpuscular haemoglobin concentration was calculated to determine the concentration of the haemoglobin in the average erythrocyte. Since this is a saturation degree its value is expressed as percentage. The figure is determined by dividing the amount of haemoglobin (gm/100 ml. blood) by the Packed cell volume and multiplying the result by hundred.

$$MCHC = \frac{\text{Haemoglobin} \times 100}{PCV}$$

From the figure obtained, the cell can either be normochromic (normal concentration) or hypochromic (reduced concentration).

(iv) THE WHITE BLOOD CELL (Wbc) COUNT :

Both the total and differential white blood cell counts were done. The total white cell count was also done using the Coulter Electronic counter (Coulter Electronics Inc. Hialeah, Florida, U.S.A.).

For the white blood cell count, a blood dilution of 1:500 is required. This dilution is prepared as the first step of the red blood cell count, by mixing 40 Lambdas (λ) whole blood to 20 ml. ISOTON. The blood is measured by the sucking action of the Mercury Manometer. The diluted blood is put in an auxiliary beaker on the beaker platform and the electrodes are inserted into the mixture. The aperture current switch is then adjusted to between 0.177 and ½ and the Threshold is raised to 16 for white cell counts. The reading on the Readout of the coulter counter is equal to the number of white cells per ml. of the sample counted, and were expressed as an absolute count of the white blood cells ($10^3/mm^3$).

For the differential white cell count, blood smears were made, air dried and stained with Wright's Stain. Then the differential white cell count was done under the microscope using the Battlement Method as described by Schalm (1965). Two hundred white blood cells were counted, recording lymphocytes, neutrophils, eosinophils and monocytes with a Marbel Blood - Cell Percentage Calculator (MARBLE BLOOD CALCULATOR CO. ILL,. U.S.A.). The total count of each blood cell type was then expressed as a percentage of the total number of the cells counted. The number of immature leucocytes with various maturation stages of development were also counted and expressed as seperate percentage of the total leucocyte count.

(v) THE DETERMINATION OF TOTAL PROTEIN (TP) CONCENTRATION

Determination of total protein content was done using a refractometer (ATAGO, JAPAN).

A single drop of plasma from the Wintrobe Microhaematocrit tube was placed on the prism of the refractometer and viewed through the eyepiece by electrical illumination. The reading was made at the point where the dividing line between the bright and dark fields crosses the scales. The total protein value was obtained directly from the scales and recorded as grams per 100 ml. of blood.

8. DETERMINATIONS ON SERUM AND LIVER FUNCTION TEST

Preliminary studies were done on the activity of serum glutamate oxalacetate transaminase (S.G.O.T.) and serum Alkaline Phosphatase (A-P), and some studies were done on the Bromsulphalein dye excretion by the liver. Samples of 20ml of whole blood was collected from the jugular vein into universal bottles without any anticoagulant. The samples were then incubated at 37°C for 30 minutes to clot and the serum pipetted off and used for serum enzyme determinations.

(i) SERUM GLUTAMATE OXALACETATE TRANSAMINASE (S.G.O.T).

This was done following the modified method of Reitman-Frankel (1957). 0.2 ml. of serum was mixed with 1.0 ml. of substrate (aspartic & α- Ketoglutaric acid) and incubated at 37°C for 1 hour. The activity of the S.G.O.T. was estimated by measuring the optical density in an "EEL spectronic 20" (BauschALomb, Inc. Rochester 2, N.Y., U.S.A). The readings were taken at a wavelength of 505 mm and the results were expressed in Sigma-Frankel (S-F) units.

...27.

(ii) SERUM ALKALINE PHOSPHATASE (A-P) :

This was done following the method of Bessey, Lowry and Brock (1946). 0.1ml of serum was added to 1.0 ml. of buffered substrate (4 - nitrophenyl phosphate) that has been incubated at 37° for about 15 min. The mixture was further incubated for 30 minutes, and the final pH 10.5 was established by adding 10.0 ml. of 0.02N, NaOH. The optical density of the yellow 4-nitrophenol produced is read at 400 mμ against a water blank, using the "ESL spectronic 20". The readings obtained were expressed in Sigma-Frankel (S-F) units.

(iii) BROMSULPHALEIN (BSP) DYE EXCRETION TEST :

This was done following the method described by Benjamin (1961). The clearance test was performed at three separate stages of the experimental disease: (a) the preinfection period, (b) the period of the height of the fever (c) the period when the animal was prostrate due to advanced sickness. The animals were weighed and a 5.0 ml. pre-injection blood sample was taken followed by intravenous injection of Bromthalein at the rate of 2 mg. per Kg. body weight. Four 5 ml. blood samples were then taken at 3 min., 5min, 10min., and 30min., after injection. The blood samples were then incubated at 37°C till clotting occurred and serum was seperated. 0.5 ml. of the serum was then taken and an equal volume of 0.2 N NaOH was added to the test serum. In the alkaline solution the dye gives a purple color.

0.5 ml. of 0. 2 N HCI was added to the blank to retain acidity. The amount of dye in the test serum is measured by reading its optical density at 546 nm using an Eppendorf Photometer (Eppendorf, Geratebau Nether + Hins GmbH, Hamburg, Germany). The results obtained were plotted on a semilog paper and the half-time ($T\frac{1}{2}$) was calculated. This is the time required for the dye concentration in the serum to be halved and is directly related to the rate of the dye excretion.

9. NECTOPSY PROCEDURE :

All the animal that died from both the experimental and natural infection of the disease were examined by standard necropsy procedures. Gross changes in the organs were noted and tissue sections were obtained and prepared for histopathologic examination. Routinely these tissues (2x2x2 cm) were collected from the liver, heart, spleen, kidney, lungs, lymph nodes, intestines and brain. Tissues were fixed in 10% formalin for 48 hours, then sections prepared were routinely stained with Haematoxylin and Eosin (H&E) and Giemsa. The histologic preparations and staining procedures were according to the Manual of Histologic and Staining Methods (3rd. Edition, 1968) of the Armed Forces Institute of Pathology.

A few of the animals were sacrificed either at the height of the fever or when in extremis. Fresh tissues were obtained from these animals and immediately fixed with "yellow Fix" (ITO-Karnovsky, 1968) for 2 hours at 4°C.

Pieces of tissues about (2x2x2 mm) for this procedure were routinely obtained from the liver, spleen, kidney, heart muscle and the third eyelid. The steps followed in the preparation of these tissues after fixation with the "yellow fix" were similar to those described for buffy coat preparation in section 6. Examination of the ultra-thin sections similarly was the same as described in section 6.

RESULTS

R E S U L T S

Clinical Observations :

Twenty bull calves of ages 6 - 12 months were infected. The volumes of blood administered varied from 80 ml. to 250 ml. depending on the weight of the recepient animal. Severn other bull calves were kept under similar conditions as the controls, but these were not given blood.

Of the twenty infected bull calves, fourteen (70%) showed the typical reaction, two showed elevated temperature (above 103°F) for two days only and the remaining four did not show any clinical reaction to the disease. Six of the fourteen animals (42.8%) that reacted clinically died of the experimental disease. Another four of those visibly sick were sacrificed at the height of the sickness to obtain tissues for ultra-microscopic studies with the electron microscope.

The incubation period varied between 5 and 9 days (average 6.2 days). The first clinical signs were high temperature (above 103°F) with appearance of staring coat, grinding of teeth and a profuse serous nasal and lacrimal discharge. The animal showed no other abnormality at this period and will eat and behave like the non-infected animals. The following day this temperature elevation is even more marked and most animals showed a temperature higher than 106°F.

Late the same day or on the third day of reaction, the mucous membranes showed pinpoint haemorrhages which were soon replaced by extensive extravasations brought about by confluence of the petechiae or by occurence of more extensive haemorrhages. With the appearance of these petechiae on the mucous membranes the respirations and the pulse rates become very fast (over 40 and 100 respectively). The grinding of teeth becomes more frequent and the animal grunted - apparently due to some abdominal pain. Rumination ceased and ruminal and intestinal movements became weak and irregular giving the animal a constipated appearance. The animals then showed varying degree of anorexia. The respirations became very harsh and were accompanied by a short cough which were later prolonged. The anorexic animal prefered standing at corners of the stall, away from light and disturbance. After the appearance of the haemorrhages the faeces became loose and at times blood-tinged; which condition later progressed to frank diarrhoea with tarry black faeces.

Three experimentally sick animals developed oedema of the conjunctivae of both eyes, but the degree of the oedema varied in each case. One of them had bilaterally advanced oedema with protrusion and eversion of the eyelids and bleeding into the anterior chamber; giving a typical "poached egg eye" appearance. This gave the incidence of the "poached egg eye" appearance at about 7% of the visibly sick animals.

In the observation of the natural cases in the field, only two out of 34 animals examined showed a mild degree of oedema of the conjunctivae; but without eversion of the eyelids.

The duration of the sickness also varied between 4 and 11 days (average 7.2 days), with the animal either progressing into death or to slow recovery. The animals that were advanced in sickness to the advanced stage of the disease appeared tired and depressed. This was the time when petechiation and ecchymoses were very advanced on the mucous membranes. The six animals that died of the experimental disease died at the height of the petechiations. They become recumbent prior to death but the recumbency did not progress to coma. Examination of the recumbent animal revealed a very fast weak pulse rate, and fast respirations that were accompanied by very loud grunting and groaning. Recovering animals showed an initial fading of the petechiae leaving pale mucous membranes followed by a drop in temperature. The pale mucous membranes persisted for about 5 to 6 days after the disappearance of petechiae. Some animals showed the development of submandibular and brisket oedema during this anemic stage of the disease. A summary of the clinical observations made are shown in Table I. Lymph nodes showed slight enlargement which was rather pronounced in the cases that showed oedema in other organs.

Haematologic Examinations :

Results of the haematologic studies are summarised in Table II. When the animals were incubating the disease, there was a slight elevation of both the Packed Cell Volume and the Haemoglobin levels.

Once visible clinical reaction had occurred, these parameters showed a drop which was very marked in the animals that died of the experimental disease (Appendix 3(b) & 4(b)). Recovering animals showed a slight drop during the clinical reaction and slow return to normal values. The control calves showed little variation in these parameters during the same period. Total protein levels dropped markedly in the dying animals while it showed a slight drop in the recovering animals followed by a slow return to normal values.

A striking change in the haematological studies is the marked drop in the erythrocyte count after the appearance of the petechiae. Values dropped from an average cell count of $7x10^6$ per cu.mm. to an average cell count below $4x10^6$ per cu.mm. on the day prior to death or disappearance of the petechiae in recovering animals. Five of the six animals that succumbed to the disease died at this critical period of anaemia. At this same time there was marked leucopenia which was both a neutropenia and a lymphopenia.

MICROSCOPIC EXAMINATION OF BLOOD SMEARS :

Blood smears were examined for the presence and the behaviour of the Rickettsia organisms. Changes in the blood cells were also noted.

The blood cells themselves showed various changes during the course of the disease.

...34..

At the time of the thermal reaction there was an increased number of immature neutrophils in circulation, with the nuclei showing several stages of segmentation. There was also an increase in the number of circulating monocytes and eosinophils. Following the haemorrhages the erythrocytes showed some immature stab cells with several showing basophilic stippling of the cytoplasm when stained with Wright's stain. Examination of Giemsa-stained blood smears at the time of the thermal reaction showed the initial bodies (small organisms) of the causative organism in the cytoplasm of the neutrophils and at times monocytes and eosinophils. These organisms appeared as pleomorphic purple-staining intra-cytoplasmic bodies (about 0.2 u). different from the reddish staining cellular granules. The initial bodies were more predominant during the early stage of the disease, but later other stages of development were seen. They appeared as large bodies (giant bodies, about 3 u) of homogenous purplish intracytoplasmic inclusions different from the deeply staining nuclear segments of the host cell. They could also be seen as a purple staining granular mass with a mulberry appearance, enclosed in a halo within the cytoplasm. Some of these morular-like bodies had a peripheral position in the cytoplasm with the end towards the cytoplasmic periphery often pointed which indicated an area of weakness that would soon break to release the initial bodies that form the morula.

The occurrence of these organisms was more frequent in the neutrophils and the various shapes and sizes of the initial bodies were seen.

The morula is a giant body whose inner mass has segregated into the tiny initial bodies but these are still contained within one vacuole. It was common to see two or three giant bodies within the cytoplasm of the same neutrophil, and at times an initial body and a giant body could be seen at different positions in the cytoplasm of the same neutrophil. Intermediate stages of the organism between an initial body and a giant body were occasionally observed.

As the disease advanced into the time of the appearance of petechiae, the intracytoplasmic inclusions of the parasite become rare on slide examination. The internal characteristics of the various stages of this parasite are revealed more by the electron microscope.

Gross Lesions :

The animals that died of the disease and those that were sacrificed were all subjected to routine post-mortem examination. Six animals died of the experimental disease and four others died of the natural disease in the field. These animals and the four that were sacrificed were subjected to systematic study of the gross and microscopic pathological changes. The pathologic appearance showed variation from animal to animal. The animals that died after a prolonged course of illness had a general emaciation of the body. The other animals which died of the acute disease had a carcasse of good body condition.

The mucous membranes had many areas of haemorrhages on a pale background. Three animals in the experimental group had developed varying degrees of gelatinous oedema of the conjunctivae. When the skin was removed the subcutaneous and the intermuscular tissues were involved in extensive extravasation and haemorrhagic zones intermingled with areas of gelatinous oedema. The areas of the peripheral lymph nodes showed gelatinous oedema involving the lymph nodes and the connective tissue. In five animals (Nos. 42, 52, 58, 81, 97) the cutaneous trunci muscles had extensive sheets of haemorrhages running longitudinally along the muscle.

In ten of the fourteen carcasses examined the intercostal muscles were involved in extensive extravasations (Fig. 6). These haemorrhages were more pronounced in the area of the muscles of costochondral junction. The thoracic cavity presented varying sizes of haemorrhages on the parietal pleurae and the thoracic organs with blood - tinged transudate. The pericardial sac contained excessive blood-tinged pericardial fluid which measured about ½ litre in two cases. The mediastinum and the mediastinal lymph nodes were involved in gelatinous oedema of the lymph nodes and the connective tissue. The heart presented a striking appearance of extensive epicardial haemorrhages. All the animals that were examined at the post-mortem showed haemorrhages of the heart ranging from numerous petechiae to extensive ecchymoses. On incision of the heart these haemorrhages were found to involve the epicardium, myocardium and endocarduim.

On the endocardium they appeared more extensive in the pillars supporting the chordae tendinae.

The lungs exhibited varying degree of oedema with froth in the bronchioles, bronchi and trachea and occasionally covered by few petechiae. On opening the trachea, this organ was found to contain petechiae on its mucosal surface especially at the areas of the muscular dorsal aspect. The diaphragm showed areas of longitudinal haemorrhagic striations in the hiatus muscles.

The abdominal organs were covered by petechiae, ecchymoses and extensive blotches. The rumen showed large areas of haemorrhage on the dorsal sac in the region close to the paralumbar fossa (Fig. 8); this being the area of maximum rumen movements. There were no mucosal or submucosal haemorrhages noticed on opening the rumen, reticulum and omasum. The rest of the alimentary canal from abomasum to large intestines showed both subserosal and submucosal haemorrhages with oedema that is marked in abomasal folds. The serosal surfaces of the intestines appeared as if sprayed with blood and these haemorrhages had a constant pattern running longitudinally down the organ. Mesenteric lymph nodes showed varying degree of oedema.

The liver grossly appeared congested and very dark brown, and in four animals showed many subcapsular haemorrhages on the parietal surface. It was always difficult to judge the changes in the size of the livers. The gall bladder was distended with bile. Five animals showed subserosal haemorrhages that varied in extent from petechiae to extensive ecchymoses.

Incision of the gall bladder showed oedema and thickening of the wall. Eleven cases showed extensive submucosal petechiations and ecchymoses. In one case the haemorrhage had been so severe that the contents of the gall bladder were a clotted tarry-red mass of blood and bile (Fig. 10).

The spleen showed both capsular and subcapsular haemorrhages on both the parietal and visceral surfaces. The degree of haemorrhage varied between small petechiae to extensive sheet extravasations covering most of the organ surface. The spleen generally did not show appreciable change in its size.

The kidneys showed congestion and a few subcapsular petechiae. The capsule always peeled off well and showed few cortical haemorrhages. One animal had extensive haemorrhage and oedema of the capsule. The adrenal glands showed capsular haemorrhages and oedema. The urinary bladder on the other hand showed extensive submucosal petechiation without any evidence of discoloration of the urine. The petechiae tended to be confluent towards the neck of the organ.

When the joints of the carcass were opened they showed many petechiae on the articular surface but the joint fluid did not show alteration in its volume or color. The meninges showed oedema with a few petechiae often seen in the area of the foramen magnum.

In the animals that were sacrificed the meningeal changes were marred by the bleeding due to the captive bolt. In those that died, these changes were often noticed with an accompanying congestion of the organ vessels. A summary of the gross pathologic observations made is shown on Table III.

Microscopic Examination of the Tissues :

Histologic sections from organs of the dying and the sacrificed animals were examined for pathologic alterations.

In the tissue sections, the main pathologic change observed were the extensive exsanguination into muscular tissues, connective tissues and occasionally into epithelial tissues. The muscular tissues showed extensive areas of tissue necrosis accompanying the extensive haemorrhages. Muscle cells showed degeneration with lysis of the cell nuclei and the cell cytoplasm losing its continuity and staining properties. This tissue necrosis was very marked in the haemorrhagic areas of the heart muscle. In spite of the extensive haemorrhage the capillary endothelium showed no detectable break in the continuity of the capillary endothelium.

The lungs showed areas of lymphoid cell infiltration especially around the walls of the pulmonary blood vessels. In these areas the perivascular tissue and the infiltrating lymphocytic cells showed degenerative changes ranging from pyknosis to cell necrosis. There was an increased interalveolar septal wall because of the lymphocytic cell infiltration.

This interstitial pneumonia was accompanied by the presence of froth in the alveoli. The bronchi and bronchioles showed a tendency to lymphoid cell infiltration but this was not as marked as it was with the pulmonary blood vessels.

The alimentary canal showed areas of submucosal haemorrhages with tissue necrosis in the submucosal areas of the tongue, abomasum and the intestinal villi. The submucosal haemorrhages were seen to occur in the vicinity of the muscularis mucosae. It was difficult to detect any haemorrhagic areas in the liver, but the sinusoidal spaces showed a degree of congestion. Liver cells showed many degenerative changes especially around the portal veins. The parenchymal cells showed swelling, fatty degeneration, piknosis and liquefactive necrosis. The sinusoidal cells were proliferative and some showed necrosis.

The spleen showed areas of hyperplasia of the lymphocytic cells. Many cells in the white pulp showed degenerative changes ranging from pyknosis to cell degeneration. Haemorrhagic zones separated areas of lymphocytic cell proliferation. Some of the lymphocytes in the haemorrhagic areas and in the red pulp were pyknotic.

The kidneys showed areas of interstitial lymphoid cell infiltration and sometimes there were haemorrhages. The most affected area in these lymphocytic cell infiltration were the area of renal cortical capillaries and especially in the periglomerular tissue.

Kidney tubules showed areas of extensive hyaline degeneration and haemorrhagic zones. The renal pelvis showed areas of haemorrhage and interstitial lymphoid cell infiltration. Generally the organ vessels showed marked congestion. The urinary bladder showed submucosal haemorrhages in a number of cases with perivascular hystiocytic cell infiltration.

The brain showed extensive congestion and an accompanying perivascular tissue necrosis. Oedema was very marked with the glial cells undergoing degenerative changes with marked pikmosis, vacuolation and necrosis. There were also many areas of lymphocytic cell infiltration around the meningeal vessels.

The adrenal glands showed haemorrhagic areas involving the zona fasciculata of the cortex with an accompanying cortical cell degeneration.

Giemsa stained sections of the liver and spleen revealed the presence of the bluish - pink intracytoplasmic organism. The liver showed granular inclusions in the von Kupfer cells and the spleen showed these granular bodies in the macrophages. Their position was always intracytoplasmic as were revealed more by the electron microscope.

STUDIES WITH THE ELECTRON MICROSCOPE I

Introduction :

Although the morphological appearance of the stages of the Rickettsial organism in the blood of cattle and experimentally infected sheep and goats have been described by many workers using the light microscope, no work has been done to study the ultra structure and behaviour of this causative organism in the organs of the affected animals. The original work to use the electron microscope to observe the Rickettsia - Like Bodies (R.L.B) was that of P rof. W.O. Weits (Haig and Danskin, 1962), followed more recently by that of Krauss et. al. (1972). These studies were done using blood obtained from clinically reacting cattle and sheep.

In this study, the morphological appearance of the Rickettsial organism was studied under two sections; the first dealing with the study of the organism in the Bovine Blood, and the other section dealing with the organism within other tissues of the animals when these tissues were obtained at the height of the sickness and at the advanced stages of the disease course.

The Blood :

In the studies on blood, most of the organism that were seen were elongated, ovoid, round or comma shaped. They were seen either singly, in groups or at various stages of division.

In the buffy coat where the corpuscles were haemolysed these organisms were demonstrated either singly or in clumps. These forms and shapes are demonstrated in Fig. 26 to 29.

The single organisms are small initial bodies. Those found in groups would appear in the light microscope either as morulae or as giant bodies. The initial body has a double membrane which encloses the inner denser mass of uniformly dense protein. This inner mass is not differentiated into nuclear and cytoplasmic components. The shape and size of this inner mass varies greatly as shown in Fig. 27 where 4 organisms can be seen inside one vacuole within the cytoplasm of a neutrophil. Some of the initial bodies in this vacuole have been cut tangentially and these appear as empty membranes. The large organism within the vacuole would appear to be a stage of development between the initial body and the giant (large) organisms. The multiplication of these organisms appears to be by binary fission which appears to occur at any stage of development of the initial body. The two medium sized organisms in the same vacuole appear to have just completed this process of binary fission resulting in the two initial bodies. The double membranes of these initial bodies are not very clearly defined. The host cell appears to have a membrane lining the inner side of the vacuole. The cytoplasmic membrane towards the periphery shows an area of weakness that is depressed inwards. These observations would indicate that the organisms have been phagocytosed and the host cell is in the process of enclosing them at the periphery.

Fig. 28 to 29 are micrographs of the organisms obtained from washed buffy coat of a sick bovine. The single organism has its outer membrane slightly detached. There are some double membranes released from other parasites that have broken down. The other figure shows clumps of the organisms precipitated with cellular membranes. It appears that their outer membranes have endured the lysing action of water even though their denser inner masses seem to have been affected by the action of the distilled water used for washing. The organisms in Fig. 29 seem to have come from one granular mass as they are still enclosed to some extent by a membrane.

The Liver :

The electron microscopic studies of liver sections of infected cattle revealed the presence of the Rickettsial organism in the sinusoidal (von Kupfer) cells, in the endothelial lining cells of the hepatic capillaries, and in the liver acinar cells. The stages of the organism found in these cells were the single initial bodies, giant bodies and the intermediate forms. Many of the organisms, however, were found in vacuoles enclosing several (at times as many as 20) organisms. The organisms were found in the cytoplasm of the affected cells. The number of vacuoles in each liver cell also varied from one to as many as a dozen. In this respect one liver cell could at times be seen to contain up to fifty initial bodies and intermediate stages, enclosed in about eight to 10 vacuoles.

At times the presence of these vacuoles could distend the cytoplasmic membrane so much that the sinusoidal spaces or capillary lumen were occluded the distension. The nucleus of the affected cell would appear very squeezed. With the distended endothelial or sinusoidal cell filling the capillary or sinusoidal space, the erythrocytes are either forced to squeeze through or escape into the perivascular spaces. Fig. 31 shows these erythrocytes squeezing their way through the narrowed capillary. At times many endothelial cells could be loaded in series with vacuoles containing the organisms. Fig. 35 shows a capillary cut tangentially with about six vacuoles in three cells that are adjacent.

Fig. 37 shows a giant body enclosed in the cytoplasm of an acinar cell of the liver. The giant body has internally divided into about five initial bodies which have not separated. The lines of division can be seen as thin intersecting dark lines. The host cell has laid about three layers of cytoplasmic membranes to enclose this giant body, and there are four little empty spaces around the organism.

Fig. 36 show a vacuole in another acinar cell which contains about eighteen initial bodies. Some of these organisms are at various stages of binary fission. Some of the small organisms have been cut tangentially and the empty membranes are seen. The presence of these organisms in the parenchymatous cells of the liver was not as common as their presence in the endothelial and sinusoidal cells.

This finding of the organism in the non-phagocytic acinar cells would indicate that the rickettsia invaded the cell, unlike their presence in the von Kupfer cells and circulating neutrophils where it could be due to cellular phagocytosis of the parasite.

The presence of these organisms in the von Kupfer cells and endothelial cells was a common finding in the liver sections that were examined. In Fig. 31 there are about five stages of division by the organisms. The middle larger body is just starting the process of binary fission seen by the kinking of the organisms both sides of the middle. The second, third and fourth stages are just intermediate between this stage and stage five where the two resultant bodies are separating. Fig. 33 and 34 show another vacuole with about 14 organisms. The organism in the middle is dividing by binary fission, but another stage of division has already commenced before the first one is completed. This would indicate that multiplication of this organism is a very rapid process.

The Spleen :

In Fig. 38 to 40 there are three vacuoles containing organisms, one giant body and two initial bodies within a macrophage cell of the spleen. One of the small organisms is about to be extruded out of the cell while another appears to have undergone degeneration. The giant body contained in an adjacent parenchyma cell contains about 4 initial bodies one of which is about to divide by binary fission. The higher magnification brings out the detail of the lines of division of the components of the giant body.

In Fig. 40 the higher magnification shows the uncompleted stage of division of one of the organisms into two initial bodies of about equal sizes and similar shapes. The same spleen cell contains an initial body that has enlarged to about a giant body stage within the vacuole. Its inner mass has not divided into more than one organism. Some of the organisms have been cut at a tangent and the empty membrane is seen inside the vacuole. Higher magnification shows clearly the two membranes enclosing each initial body of the organisms. The host cell has secreted some dark granules into the cytoplasm around the vacuole. Some of the parasites in the vacuoles seem to have degenerated as seen by the absence of an enclosing membrane and loss of density in the inner mass. This condition is more pronounced in the organism that is about to be extruded by the host cell.

The Kidney:

The examination of the kidney sections revealed the presence of this organism in the interstitial kidney cells and the endothelial cells of the renal capillaries. Fif. 41 shows a kidney interstitial cell with a vacuole that contains about 10 organisms of various shapes and sizes. Fig. 42 is a higher magnification of the same vacuole that shows the presence of three giant bodies that are starting to divide into many initial bodies. One is just starting to form the membranes that divide the organism while another has already formed four complete small organism of various sizes and shapes. Another giant organism is just dividing into two organism.

....48..

The enclosing membranes around these organisms are more clearly defined. The outer membrane is loosely attached around the organism and is seen as a wavy continuous line. The inner membrane is more closely apposed to the inner denser mass of the organism. Some of the organisms have been cut tangentially and shows their empty outer membrane in the picture. The initial bodies that can be seen to result from the division are round, ovoid and comma shaped. The denser inner mass of the initial body has denser and lighter areas. The dark areas that give the organism a granular appearance are the ribosomes and nucleic acids of the denser inner mass.

In the division process the organism seems to develop a distinct line of cytoplasm to separate the two portions of the granular mass. Then the inner membrane is developed along this cytoplasmic line, while the outer loosely attached membrane still enclose the whole organism. The final stage of division is the seperation of the outer membrane to release each separate initial body.

The Heart :

The heart muscle sections examined under the electron microscope revealed the presence of the rickettsial organism in the endothelial cells of the cardiac capillaries and in the interstitial cells between the muscle cells. These forms found in these situations were initial bodies individually and small organisms enclosed in morulae-like vacuoles on the endothelial cells. The endothelial cells in the vicinity of the morulae and elsewhere where the organism were not detected showed cellular degenerations which include vacuolation of the cytoplasm and loss of cell continuity.

In the myocardial cells that were not affected there were notable changes that had been observed in the light microscope, namely cellular degeneration.

Figures 44 to 48 are micrographs of the organism when examined in the myocardial tissues. In the lower magnification, many erythrocytic cells are found outside the capillary and spread into the intermuscular connective tissue. The endothelium in many areas has lost most of its continuity with areas of distinct cellular necrosis. The myocardial cells in the vicinity of the haemorrhage show some degree of degeneration. Their cytoplasmic myofibrils show degenerative changes with loss of continuity.

Figures 44 and 45 show these degenerative changes of the myocardial cells and the intermuscular connective tissue cells. Figure 45 further show areas where there is a breach in the integrity of the endothelium and gives an indication of the escape route through which the extra - vascular erythrocytes must have escaped. Figure 45 further shows areas of the endothelial cells where there are no parasites but the endothelial cells still show degenerative changes including vacuolation and increased intercellular spaces within the capillary wall. Some of the parasites enclosed in the vacuole seem to be attached to the membrane of the vacuole wall as there is no separation visible between their outer membranes and the inner wall of the vacuole formed in the endothelial cell.

When the vacuole and its organisms are magnified in Fig. 46, there are several stages of development showing division in these organisms.

The organisms present variation in both their size and their shapes as have been seen in other tissues. The double membrane of each individual initial body of the parasite is visible. The wavy outer membrane that is loosely attached to the organism is still continous for those organisms that have not completely separated. The nucleus of the affected endothelial cell has been pushed to one side and shows some degenerations. The bulging colony of the morula has in effect occluded about one quarter of the volume of the capillary lumen. In addition to causing cell degeneration, the distension of the cell cytoplasm has brought about degenerative changes in the nucleus due to pressure.

Figure 47 shows an initial body in a vacuole in one of the myocardial cells. In addition to the vacuole that contains the parasite the cell cytoplasm shows the presence of other vacuoles that would indicate the degenerative changes brought about in the affected cell. Some of the parasites in the vacuole have been cut tangentially and their empty membranes are visible. Some of the organisms also are undergoing degeneration within vacuoles in the same cell cytoplasm and in another adjacent cell. The affected cells are undergoing vacuolisation while the parasite within the big vacuole seems to still retain both of its double membranes. Some parasites have also been cut tangentially and their empty membranes are visible within the vacuole. The presence of the parasite both in the endothelial lining of the heart capillaries and the interstitial cells, and the visible necrotic changes observed on the endothelial lining demonstrate the cause of the presence of erythrocytes outside the capillaries.

The rickettsial parasite seems to attack both the cells lining the endothelial wall and other organ cells. The parasite also does not seem to undergo degeneration when the host cell degenerates. Figure 48 is an advanced stage of cell degeneration in a heart cell as a result of the parasite invasion. Many initial bodies are seen within vacuoles in a host cell that has undergone necrosis. Many empty vacuoles have also been cut with only the vacuole membranes but without parasites. The host cell shows degeneration of the cell cytoplasm, nucleus and their membranes. The parasites within the collapsed vacuoles and those that occur singly in the cytoplasm of the cell seem to have lost their double membranes. This appearance indicates that these organisms had been overcome by the defence mechanisms of the host cell. The cell membrane shows a breached area that is continuous with an intracytoplasmic vacuole. The other side of the cell shows areas where the cytoplasmic membrane has disentegrated completely exposing the cytoplasmic contents to the outside.

Serum Glutamate Oxalacetate Transaminase (S.G.O.T.) And Serum Alkaline Phosphatase (A-P)

Serum Glutamate Oxalacetate Transaminase levels increased in the sick animals during the course of the sickness. The increases were recognised after the animals developed the haemorrhagic syndrome. Increased levels rose from an average levels of 80 Sigma-Frankel units at the beggining of the sickness to levels between 140 and 190 Sigma-Frankel units at the height of the sickness. During the recovery period of the disease these levels showed a gradual return to the original levels.

The results of the preliminary investigation are summarised in Table IV.

Serum Alkaline Phosphatase levels did not change in the sickness. The recorded levels remained generally the same during the course of the disease, although there were day to day variations. The daily variations that were observed with the sick animals were similar to the variations that were seen in the cases of the control animals (Appendix 9).

BROMSULPHALEIN EXCRETION BY THE LIVER :

This test was undertaken after the observation that there was an extensive involvement of the sinudoidal cells of the liver by the organisms. The investigation was carried out on three experimental animals. The pre-infection excretion rates of the dye were determined. Then the animals were again tested for the excretion rates at the height of the fever and when they had showed extensive extravasations. There was a marked increase in the clearance time when the animal showed advanced sickness. The clearance time was about double that of the pre-infection test and the results are summarized in Fig. 4 (for No. 40).

DISCUSSION

D I S C U S S I O N

The disease syndrome of Bovine Petechial Fever in the experimental transmission showed remarkable similarity to the cases of the disease reported from the field and experimental transmission by earlier workers. There is agreement throughout the literature that the disease in cattle produces a haemorrhagic syndrome with the depression of both the erythrocytes and leucocytes. In this study a mortality rate of 42.8% was recorded which compares favourably with the findings of Burdin and Danskin (1956) who reported a mortality rate of 55.6% in 27 experimental animals. Danks (1936) had reported that the mortality in the natural outbreaks of the disease varied considerably and put the figure at about 20% of the affected animals. He thought the mortality rate in experimental infection was about 5 to 6% Fieroy (1953) thought that the disease was very virulent but gave no figures of what he considered the rate of virulence. This variability in the clinical response of the disease and its mortality has been observed by many of the workers on this disease.

After the experimental administration of blood into experimental animals, the disease has shown a variable clinical manifestation. Some animals have shown temperature reaction for 2 days then recovered while several have shown an acute and fatal haemorrhagic syndrome characteristic of the Bovine Petechial Fever. The perio between infection and the elevation of body temperature has also been very variable, between 4 and 11 days. The temperature rise has preceeded the appearance of the haemorrhagic syndrome by one or two days.

Several animals have shown grinding of the teeth, grunting, profuse serous discharge from the nose and the eyes. This eye and nose discharges indicate an irritation of the mucous membranes before the development of the haemorrhagic signs. The petechiations start as small pinpoint haemorrhagic areas that are caused by the injury of the fine capillary walls of the mucous membranes. They also start on the organs that are very actively contracting and expanding (tongue, eyelid, lips) as observed by Burdin and Danskin (Kenya Vet. Dept. Ann. Report 1956). The extensive haemorrhages and the high temperature have been the very outstanding and constant clinical observations. A varying degree of anorexia and the development of oedema both on the conjunctivae and later on in the submandibular and brisket areas have been observed. Piercy (1953) has reported the incidence of "poached egg" eye as being between 5 and 10%. In this experiment the incidence has been found to be about 7%, but unlike the observations of Danskin and Burdin (1963) the oedema observed has been bilateral, even though the degree of involvement varied in each eye.

The oedema seen clinically and at post mortem both grossly and histologically may be a resultant from the damage of the blood vessels by the parasite leading to the leakage of plasma. It may also be due to tissue cell distruction and anaemic changes which disturb the interstitial fluid balance. The injured blood vessels are permeable to blood fluid which then accumulate in the interstitial spaces.

The degeneration of the endothelial and interstitial cells aggrevates an already serious situation where haemorrhage had caused many blood cells to escape into the interstitial spaces. The toxic substances released by cellular breakdown accumulate and cause further tissue damage.

The oedema of the brain with congestion of the organ vessels would seem to indicate the origin of the dull demeanor that is seen in the protracted cases of the disease. This situation is aggrevated by the muscle weakness due to cellular degeneration and tissue hypoxia.

Haemorrhagic areas on the skin have been observed constantly on the underside of the tail, the inside of the ears and other light coloured areas of the skin. In these areas and other situations, the skin haemorrhages, on recovery of the animal, have shown a regression without showing any other pathologic abnormality, unlike the loss of hair and skin necrosis reported by Nettam (1929). Danskin and Burdin (1963) indicated that death invariably followed in cases where pulmonary oedema developed or where there was a heavy loss of blood in the faeces. Several of the experimental animals and some observed in the field have shown marked coughing and frothing from the mouth, which would indicate pulmonary oedema, but some of these cases have later recovered. There have also been cases that died without diarrhoea and showed no signs of pulmonary oedema at post mortem.

In the animals confined to the stalls, there are no cases that have shown sudden death as described by Danks (1936). The severity of the clinical signs however have shown no guide to prognosis, as several of the surviving animals had advanced to recumbency while a few died after the extensive haemorrhages without first going down.

In the natural and experimental disease, weakness and a profound anaemia have been found to follow on the haemorrhagic stage of the disease. The profound anemia had been observed by Burdin and Danskin. The weakness that is observed in the disease could due to the extensive muscle degeneration that is seen histologically. It could also be due to the hypoxia of muscles resulting from the endothelial damage and anaemia, or it might come about as the result of a combination of both muscle tissue degeneration and hypoxia.

The post mortem examinations have revealed the extensive haemorrhages to be more pronounced in those organs or areas of organs that are actively contracting and expanding. Plowright (1962) had found that the haemorrhages of the heart were a constant feature. In my experiment, all the animals examined have shown a varying degree of heart haemorrhages from many fine petechiae to extensive ecchymoses and suffusions. This results from the injury to their capillary walls which then become weak and cannot withstand the normal changes of blood pressure brought about by muscular contractions. This conclusion is supported by the observation that the rumen has the haemorrhages more pronounced on the area of the paralumbar fossa and the heart endocardium has haemorrhages more pronounced on the pillars supporting the chordae tendinae.

The injury of the endothelial cells leads to tissue reaction in the perivascular areas. This reaction is then observed as infiltration of the area by cells of the hysticocytic mononuclear series. The involvement of the causative parasite in the interstitial cells causes the infiltration of the organ by histiocytic cells.

..57..

Plowright (1962) had suspected that the hyperplasia of the larger cells of the lymphocytic series was due to a direct effect of the causal agent. He further reported cases of mononuclear and hysticocytic infiltration around the blood vessels in the haemorrhagic areas. The hysticocytic cell infiltration around blood vessels have been confirmed in the lungs, kidneys and the urinary bladder. The lymphoid organs including the spleen have shown a proliferation of the lymphoid cells.

Studies undertaken with the electron microscope have demonstrated the direct injury to the tissues of the affected bovine host. In the light microscope the tissue damage are seen as extensive cell degenerations accompanying the extensive haemorrhages but the endothelial lining appear to retain their internal continuity. Mettam (1929) had suggested that the organism had a specific damage to the endothelial lining but that the damage was so slight as to escape detection by the microscope. These studies with the electron microscope have revealed the presence of the causative agent in the cells lining the capillary wall. In these situations the organism has been observed to multiply and cause degeneration of the affected cells. Several endothelial cells in one area have been observed to undergo necrosis because of the invasion by these parasites. The physical growth of the parasitic agent in the cytoplasmic environment causes a spatial dislocation of the cellular organelles and ultimately induces cell damage. Nuclei are forced to a peripheral position and caused to shrink.

Other cellular organelles become affected by the dislocation and the rupture of the cellular membranes are the final stages of the disintegration of the affected cell.

In the examination of the blood smears stained with Giemsa, the parasitised circulating cells show the distensions of the cytoplasmic membranes. The studies carried out using the electron microscope indicate that the phagocytic cells phagocytose the elementary bodies and the initial bodies of the parasite. Once inside the cytoplasmic vacuoles, the parasites start to grow and multiply by several processes including binary fission and multiple fission. By multiplication the parasites fill the vacuole and distend the cell cytoplasmic membrane in addition to displacing the cell organelles. The process of multiplication by binary fission seems to occur at a very fast rate. Krauss et. al. (1972) had observed the multiplication of this organism by binary fission and found that it occurred at any stage during the development of the small organism to the giant body. They further observed that the giant body could break down its protein to release several elementary bodies. The present study has revealed the multiplication of this organism in the liver spleen kidneys and heart of the sick bovine, in the endothelial cells and in other interstitial and acinar cells.

The studies revealed that the phagocytic action of the macrophage cells does not have much effect on the multiplication of the parasite as long as the parasite retains its double membrane. The organism has been observed to divide when the outer and inner membranes are still intact.

In some cases the organism has been observed to have lost the outer wavy membrane and the inner denser mass has shown degenerative changes. There is thus a very close relationship between the multiplication of the organism and the degree of damage the host cell can inflict on the invading parasite. In the washed buffy coat the double membrane is separated from the denser inner mass by the lysing action of water. It is difficult to determine the possible alterations in the antigenic properties of these separated membranes and the use to which this can be put in preparation of a vaccine.

Organ sections have been examined with electron microscope and the organisms have been found to cause a direct damage on the organ cells and the capillary endothelium. Giant bodies in the cells of the heart endothelium, heart muscle, spleen macrophages and kidney interstitial cells and liver acinar cells have been found in the same situations as the elementary and initial bodies. The division of the various sizes of the organism, observed by the electron microscope, into elementary, initial and giant bodies becomes difficult because of the different sizes and shape observed. Within one vacuole in any of the organs, the organism could be observed together as initial bodies, elementary bodies and large ones which could be termed giant bodies. Single initial bodies could also be seen singly in vacuoles within the cytoplasm.

In the light microscope, the giant body appears as uniformly dense mass, whereas in the electron microscope the giant body is seen to be many small organisms, that have resulted from a multiple division, but the various components are still held together within one outer membrane. Another giant body would appear in the electron microscope as a group of small initial bodies which have little space in the vacuole to show a distinct separation.

Multiplication by binary fission in these organisms seems to start by the invagination of the denser inner mass and the inner membrane on both sides. Then a thin plate of a cytoplasmic disc is developed joining the belt of invagination across the inner denser mass. This line is further reinforced to be continuous with the inner membrane of the parasite. The denser inner masses so separated then move apart while the two daughter organisms are still enclosed together by one outer and loosely attached membrane. In the multiplication of the initial bodies this separation of these small organisms produces a bipolar appearance when examined with the low magnification of the electron microscope. The final stage of multiplication is the separation of each organism by the severing of the outer membrane. In the multiplication of the giant body into many elementary and initial bodies, the la down of the thin layer of cytoplasm occurs in several directions and planes. The small organism produced by this multiplication are morphologically similar to those that result from the binary fission of the initial bodies and the elementary bodies.

..61..

The modes of multiplication observed in the blood cells heart, liver, spleen and kidney sections were similar.

The cellular damage inflicted by the parasite impairs the normal function of the affected organ. In this respect, myocardial cell degeneration with the hypoxia resultant from the haemorrhages and endothelial damage would be sufficient to cause heart failure. On the other hand, the loading of the Reticulo-endothelial cells especially the liver sinusoidal cells and macrophages in other situations would essentially be sufficient to hasten death. The delayed bromsulphalein excretion by the liver confirms further the impairment of the reticuloendothelial system of the liver. The decrease in the blood protein content and blood haemoglobin concentration indicate further an impaired synthesis of these compounds. Biochemical investigation have shown an increase in the levels of S.G.O.T. which would be due to increased cellular permeability from degeneration leading to the escape of these enzymes into circulation.

Having therefore established the cause of the haemorrhages in Bovine Petechia Fever as being due to endothelial damage by the parasite and escape of the blood into the perivascular areas, the cause of death is still difficult to determine. Cellular degenerations in various organs complicated by tissue hypoxia due to anaemia would lead to organ failure in the heart, liver and kidneys which would seem to be responsible for the final of death of the animal.

CONCLUSION

CONCLUSION:

Bovine Petechial Fever is essentially a haemorrhagic cattle disease in which there is extensive extravasation of blood into epithelial, muscular and connective tissue followed by a disturbed tissue metabolism. The haemorrhagic syndrome is caused essentially by the invasion of the capillary endothelial cells by the Rickettsial parasites leading to marked capillary permeability. The organism also attacks interstitial cells and acinar cells in glandular organs. The resultant cell degenerations leads to metabolic impairment and organ function. The initial inflammatory process of the disease causes a leucocytosis with the presence of an increased number of immature neutrophils in circulation. The haemorrhagic syndrome precipitates a marked haemorrhagic anaemia with a marked hypoxia and tissue degeneration.

Complex phenomena including heart failure due to myocardial degeneration, hepatic impairment and a probable renal impairment are suggested to be contributory factors in causing death. Tissue hypoxia and excess fluid loss from haemorrhage and oedema are further incriminated as contributory factors to organ failure in infection with Bovine Petechial Fever.

At post morten the carcasses of the animals have demonstrated extensive haemorrhages in many organs of the body, and about 30% have shown an accompanying tissue oedema. Histologically there is extensive cell degeneration accompanied by a varying degree of hysticocytic cell infiltration in the perivascular areas due to peripheral vasculitis.

The parasitic rickettsia appear in various stages of development within the cytoplasm of circulating leucotytes and other tissue cells.

Electron microscopic studies reveal the presence of these Rickettsia in many organ tissues. The individual organism is found to possess a double membrane, the inner layer more firmly apposed to the inner denser mass while the outer one is a loosely attached wavy membrane. There is no distinct separation of the inner denser mass into cytoplasm and nucleus, but it is observed to be rich in ribosomes. Various stages of development and the intracytoplasmic presence of the parasite are concluded as the cause of cell degeneration and cell death which precipitate impairment of the organ function.

TABLES

TABLE I : SUMMARY OF CLINICAL OBSERVATION IN CASES INFECTED WITH BOVINE PETECHIAL FEVER

Animal No.	Days to Temp. Reaction (after infec).	Days to Petechiation.	Duration of Visible Sickness (Days)	Maximum Temp. Recorded (°F)	Results
40	5	6	6	107.4	D
41	9	11	6	104.9	R
42	5	6	5	107.0	(K)
49	6	8	8	103.6	R
50	7	9	10	105.1	R
52	6	7	7	107.2	D
54	7	8	8	106.4	R
55	6	8	4	107.4	D
58	5	7	10	106.0	D
59	7	8	8	106.1	R
72	6	7	7	107.3	(K)
74	5	7	11	106.4	R
81	8	10	6	106.8	(K)
97	5	7	4	105.4	(K)
MEAN	6.22	7.78	7.22	106.2	

D = Died

R = Recovered

(K) = Sacrificed (Killed)

TABLE II : MEAN HAEMATOLOGIC VALUES OF 12 CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	PCV.	T.P.	Hb.	Rbc.	Wbc.	TN.	LYMP.
0	29.04	6.73	10.01	7.32	10.32	2.62	7.71
1	28.22	6.81	10.46	7.58	10.67	2.26	8.70
2	32.33	6.82	10.77	7.91	10.91	3.02	7.55
3	31.00	6.52	10.74	7.44	10.07	2.25	7.41
4	29.50	6.57	10.07	6.83	9.11	2.32	5.76
5	28.83	6.33	9.56	6.89	8.02	2.06	5.40
6	28.58	6.23	9.52	6.59	7.45	2.10	4.77
7	27.74	5.32	7.82	6.44	6.59	1.29	5.10
8	24.16	5.19	8.13	5.44	7.20	1.87	4.88
9	21.41	4.59	6.53	4.68	6.98	1.16	5.71
10	21.41	4.29	6.42	4.24	7.35	0.79	7.29
11	15.20	5.62	5.55	3.82	8.94	0.84	8.75
12	16.41	5.92	5.37	3.57	10.38	1.69	9.07
13	23.17	5.90	7.55	5.06	10.47	1.51	10.31
14	22.67	5.95	7.52	5.01	11.08	1.73	9.91
15	24.17	6.27	8.02	5.20	12.1	1.67	10.01

1. Day '0' = average of 5 days preceeding infection.
2. Pcv = Packed cell volume (%)
3. TP. = Total protein (gm/100ml)
4. Hb. = Haemoglobin concentration (gm/100 ml.).
5. Rbc) = Red blood cell counts ($10^6/mm^3$)
   Wbc.) = White blood cell count($10^3/mm^3$)
6. TN&LYMP. = Absolute counts of neutrophil (TN) and Lymphocytes(LYMP). ($10^3/mm^3$).

TABLE III : POST-MORTEM CHANGES OBSERVED IN CASES OF BOVINE PETECHIAL FEVER

	ANIMAL NUMBERS						
ORGAN CHANGES	40	42	52	55	58	60	72
Conjunctival Oedema	+	+	-	-	-	-	++
Costochondral Extravasation	-	+	++	+	++	-	+
Cardiac haemorrhage	+	++	++	+	++	+	++
Pulmonary Oedema	+	++	+	++	+	-	-
Subserosal Rumen Haemorrhage	++	++	++	+	+	-	+
Splenic haemorrhage	+	+	++	+	++	-	-
Gall bladder Haemorrhages	++	++	++	+	++	-	+
Urinary Bladder Haemorrhages	+	++	++	+	+	+	++
Meningeal Haemorrhage	-	+	+	-	+	-	-

+= positively present

++= extensive involvement

-= not present

TABLE III : POST-MORTEM CHANGES IN ANIMALS DYING OF BOVINE PETECHIAL FEVER

ORGAN CHANGES	ANIMAL NUMBERS 81	97	L20	N529	N34	373
Conjunctivae Oedema	-	-	-	-	+	-
Costochondral Extravasation	+	++	+	++	++	-
Cardiac Haemorrhages	++	++	++	++	++	+
Pulmonary Oedema	+	+	-	+	+	-
Subserosal Rumen haemorrhage	+	+	+	++	++	+
Splenic Haemorrhages	+	+	+	-	++	-
Gall Bladder Haemorrhages	++	++	++	-	+	+
Urinary bladder Haemorrhage	++	++	+	+	+	+
Meningeal Haemorrhages:	+	++	-	-	+	-

+ = positively present

++ = extensive involvement

- = not present.

TABLE IV : MEASUREMENT OF DAILY SERUM GLUTAMATE OXALACETATE TRANSAMINASE (S.F. UNITS) OF THREE CALVES INFECTED WITH BOVINE PETECHIAL FEVER AND TWO CALVES USED AS THE CONTROLS :

	ANIMAL NUMBERS				
	INFECTED			CONTROLS.	
DAYS OF SICKNESS.	41	42	74	43	44
1	97	84	86	96	58
2	110	77	80	50	50
3	116	87	77	68	52
4	104	86	86	60	50
5	147	Died.	103	68	58
6	190		116	80	42
7	147		127	84	52
8	127		142	68	40
9	137		143	99	50
10	93		147	88	42
11	80		147	72	52
12	96		96	84	55
13	92		104	68	47
14	105		35	62	53
15	90		116	78	48
	Recoverd		Recovered		

No. 42 - Died after 5 days sickness.

Fig. 7

Typical Temperature [illegible]

F I G U R E S

Fig. 8. [illegible]
[illegible]

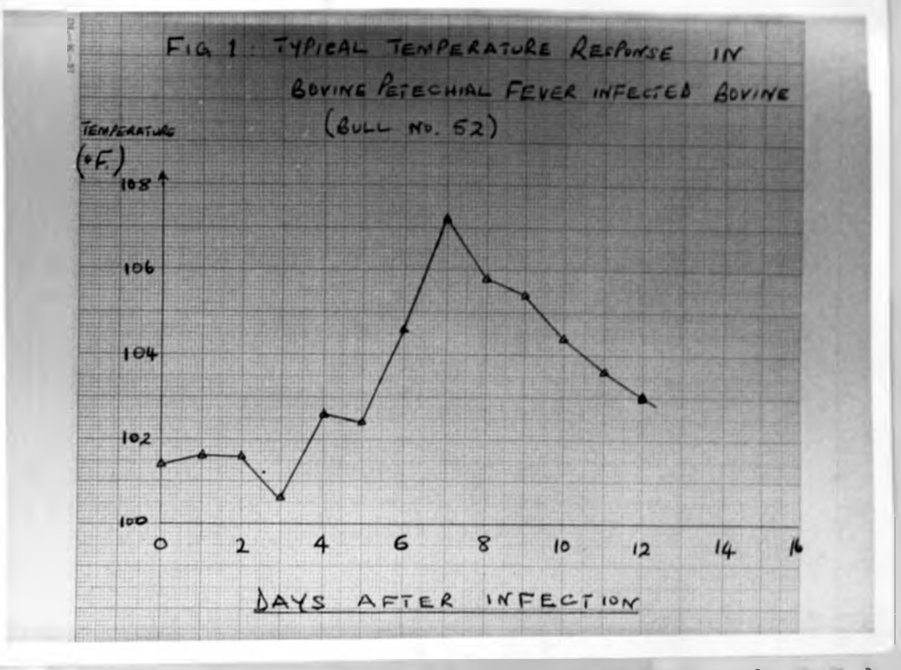

Fig. 1

Typical Temperature Response in Bovine Petechial Fever,(B.P.F.)

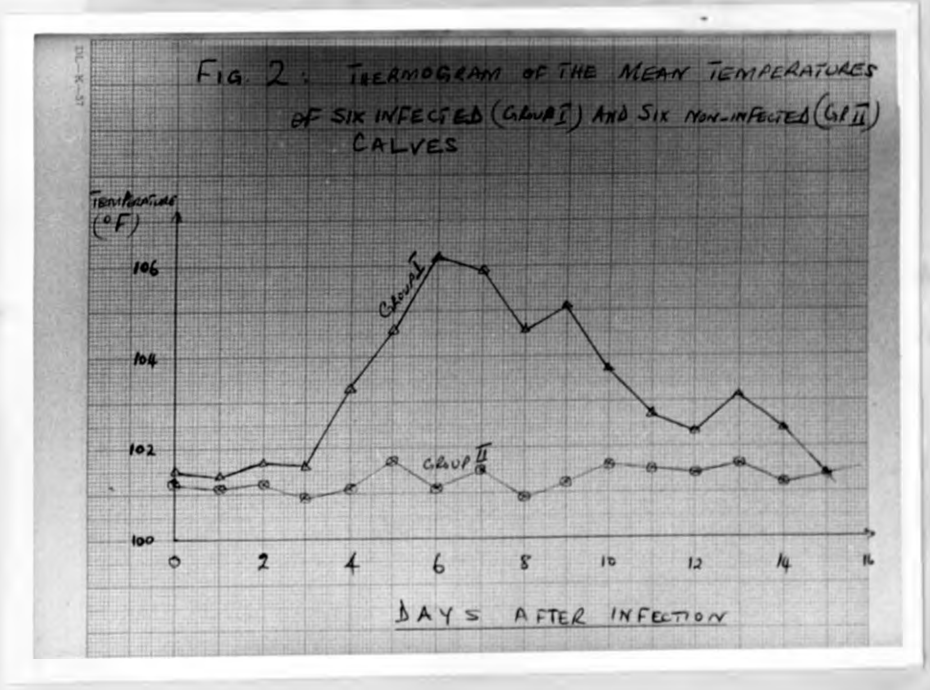

Fig. 2. Thermogram of the Mean Temperatures of Six B.P.F. infected and six non-infected bull calves.

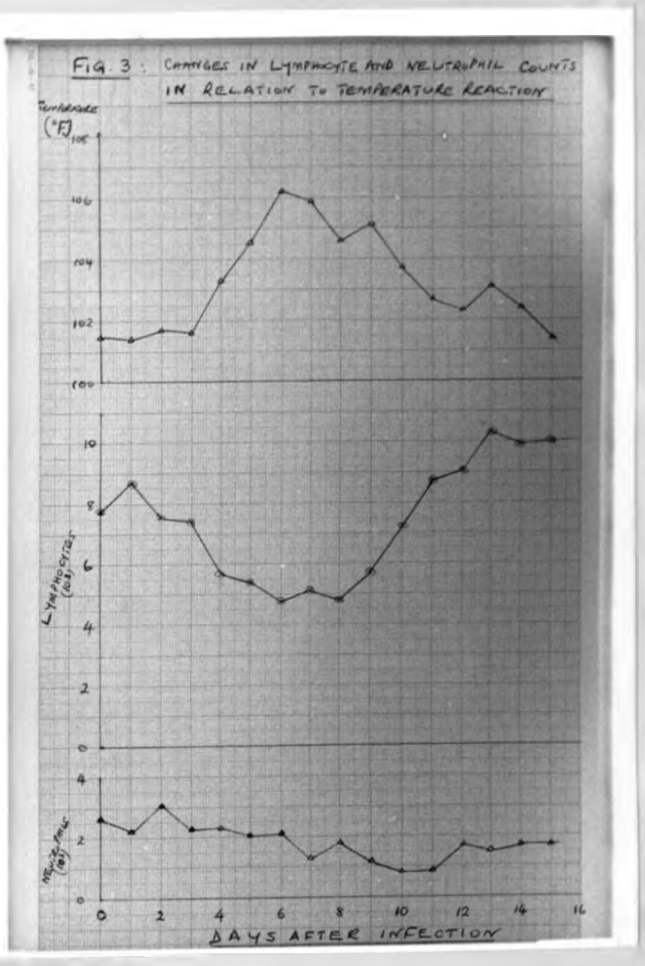

Fig. 3. Changes in Lymphocyte and Neutrophil counts in relation to the temperature reaction in cases of B.P.F.

Fig. 5. Extensive haemorrhages on the thoracic wall of a c ase of B.P.F.

Fig. 6. Extensive petechiation and marked pneumonia in a case of B.P.F.

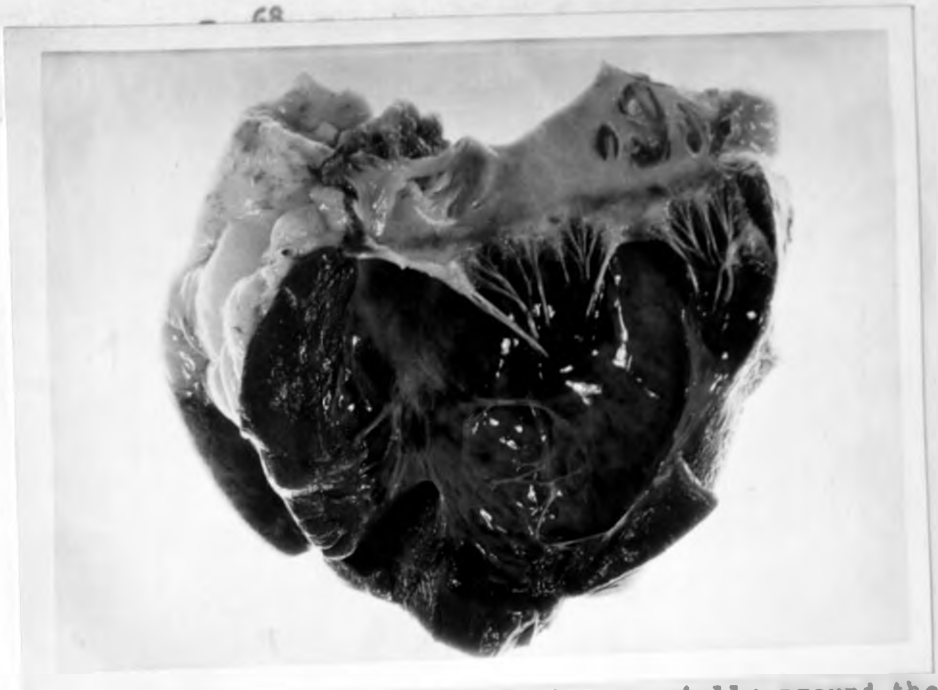

Fig. 7. Marked haemorrhagic endocarditis especially around the pillars of heart.

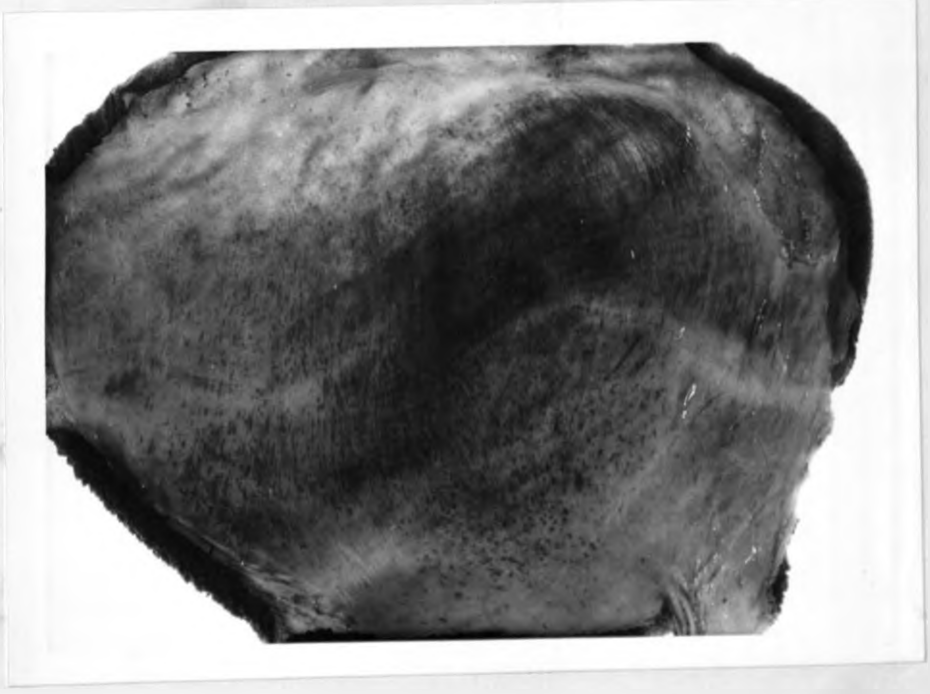

Fig. 8. Typical sub serosal haemorrhages on the rumen in B.Q.F.

Fig. 9. Extensive subcapsular splenic haemorrhages in a case of B.P.F.

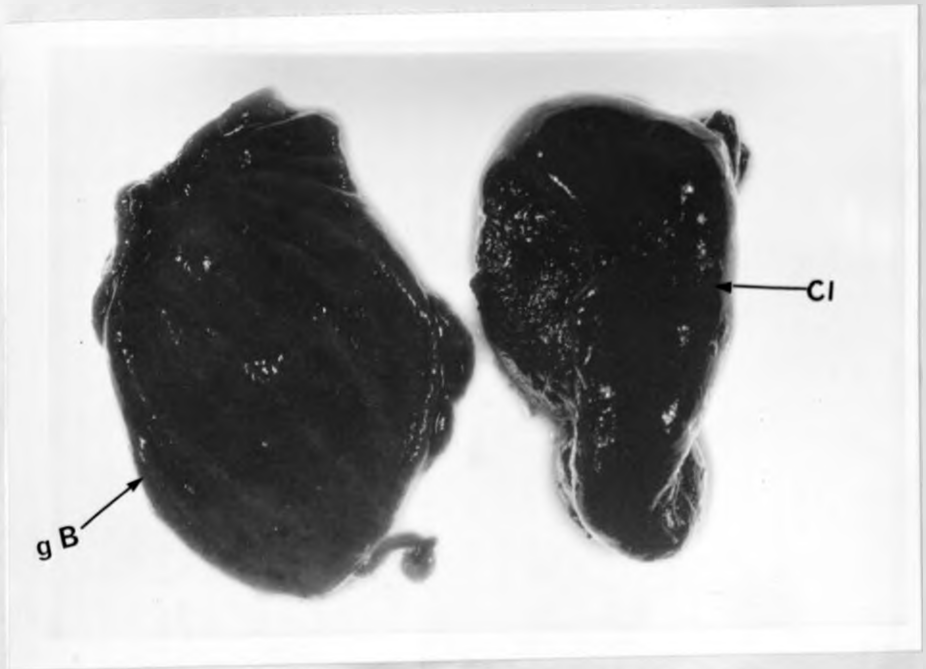

Fig. 10. Extensive mucosal haemorrhages of the gall bladder (gB) with clotting of the contents (Cl).

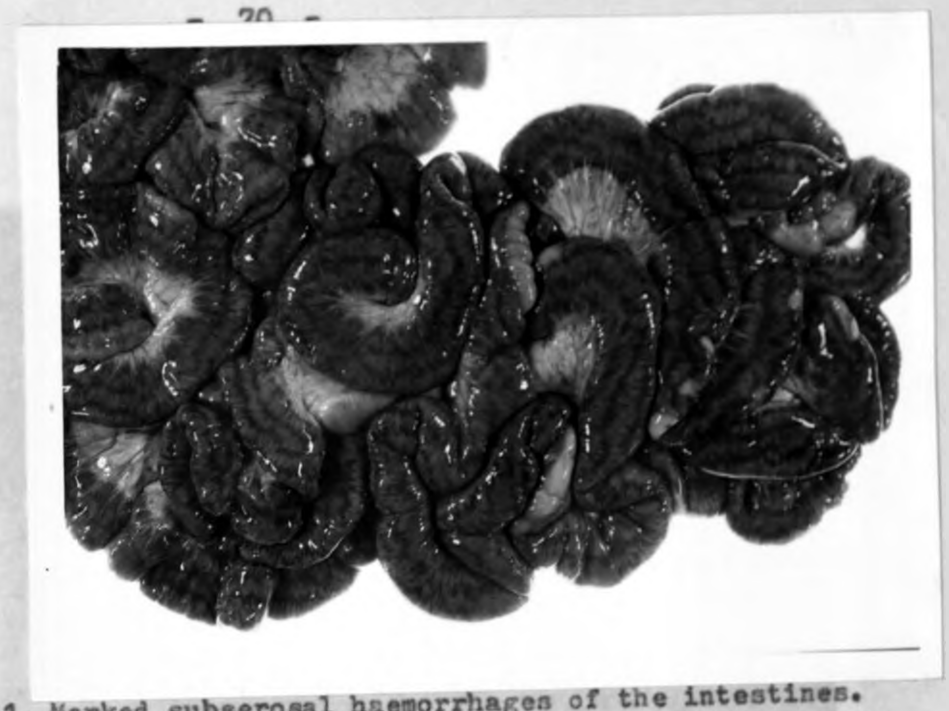

Fig. 11. Marked subserosal haemorrhages of the intestines.

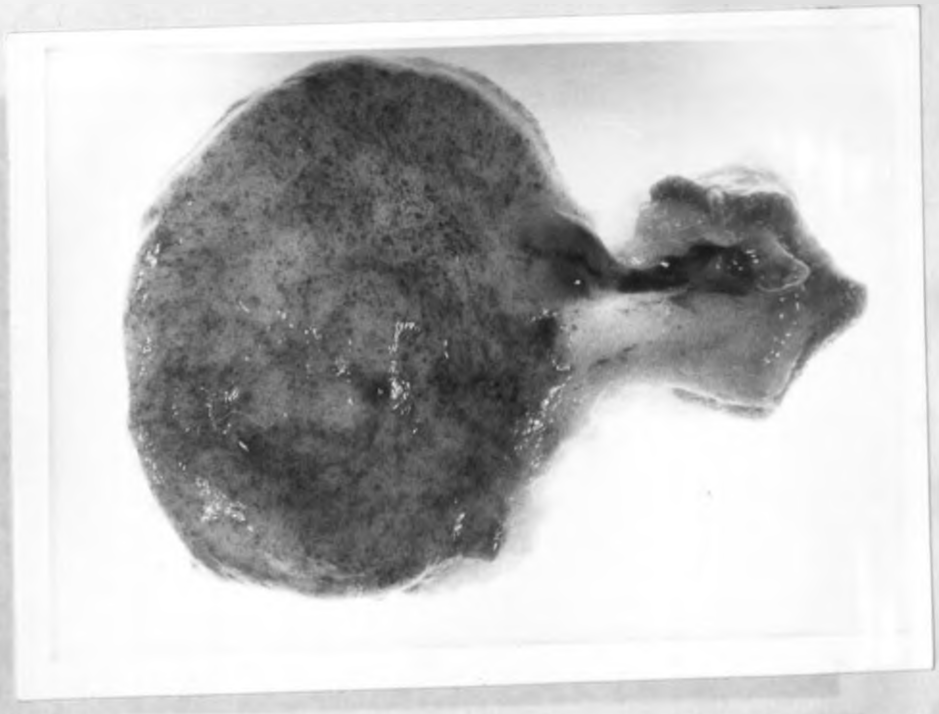

Fig. 12. Typical petechiae and ecchymoses in the urinary bladder.

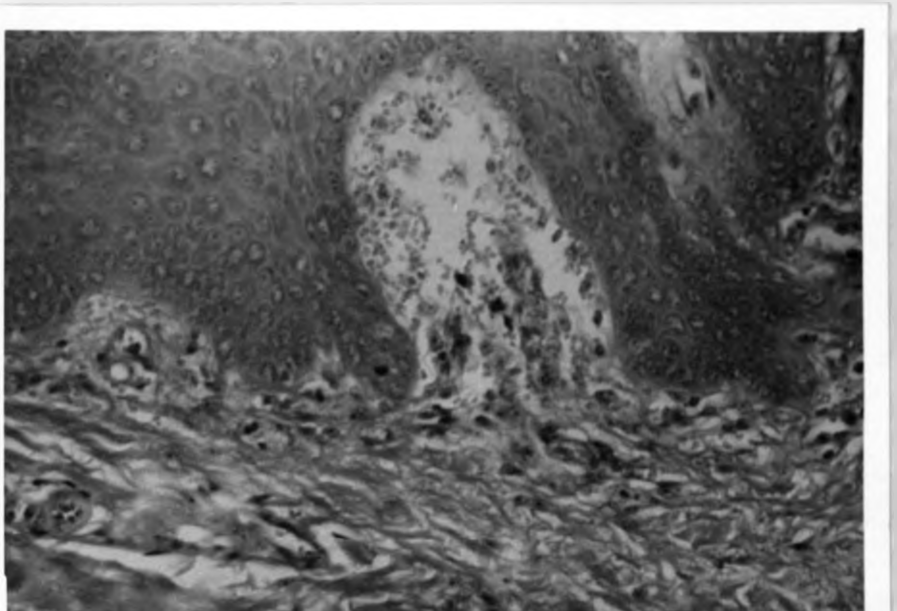

Fig. 13. Submucosal haemorrhages and cell necrosis in the tongue of a E.P.F. case. Giemsa X 390.

Fig. 14. Extensive haemorrhagic myocarditis with marked myocardial cell degenerations in a case of E.P.F. X 63

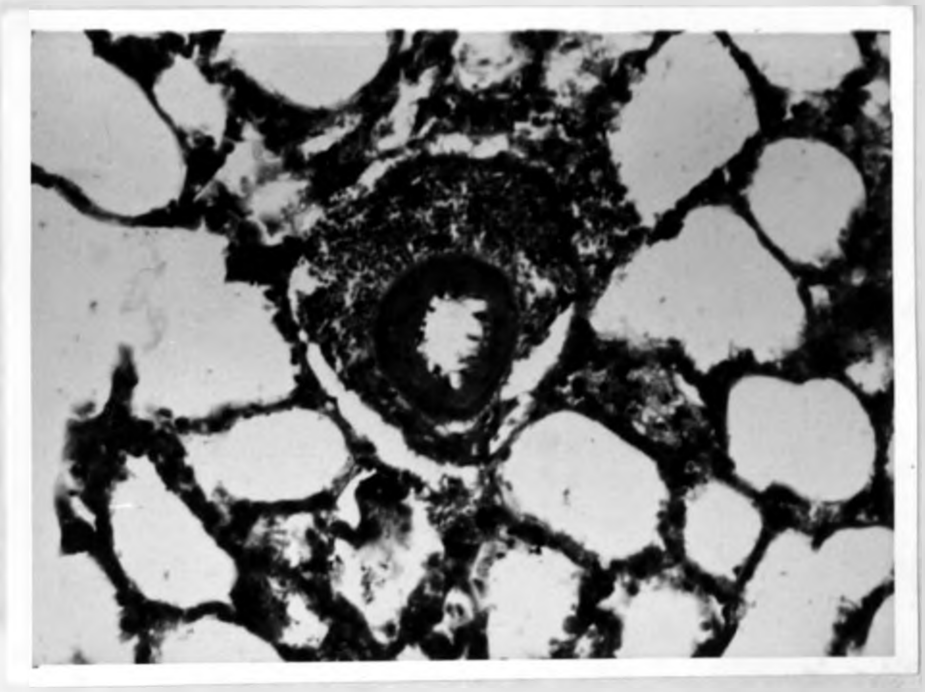

Fig. 15. Marked perivascular hystiocytic cell infiltration with cell degeneration in the lung of a B.P.F. case Giemsa Stain X 390.

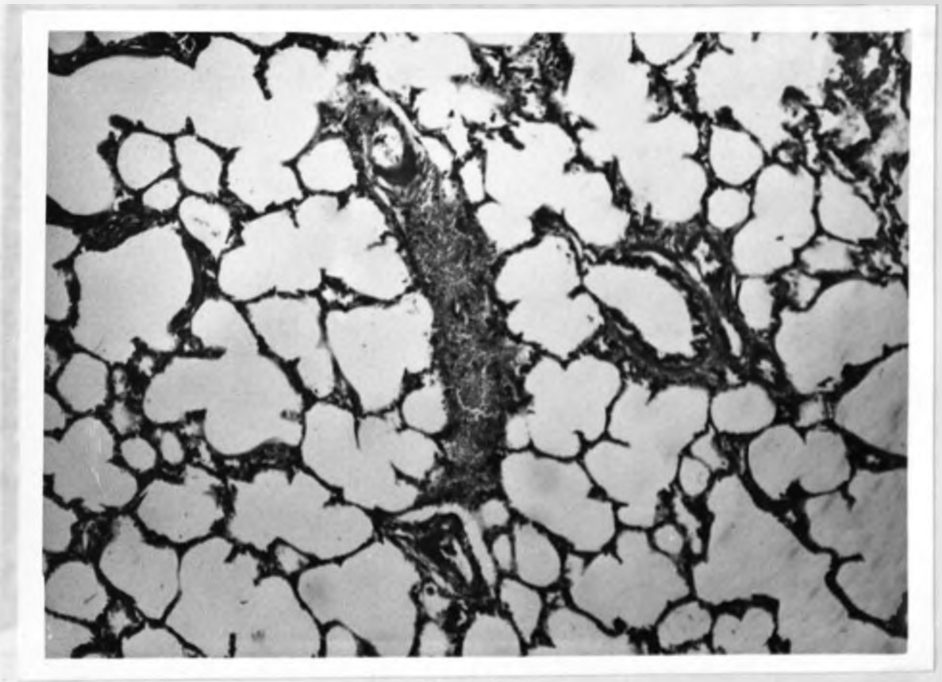

Fig. 16. An oblique section showing marked infiltration of hystiocytic cells in the perivascular and the interalveolar septal walls of the lung. Giemsa Stain X 390.

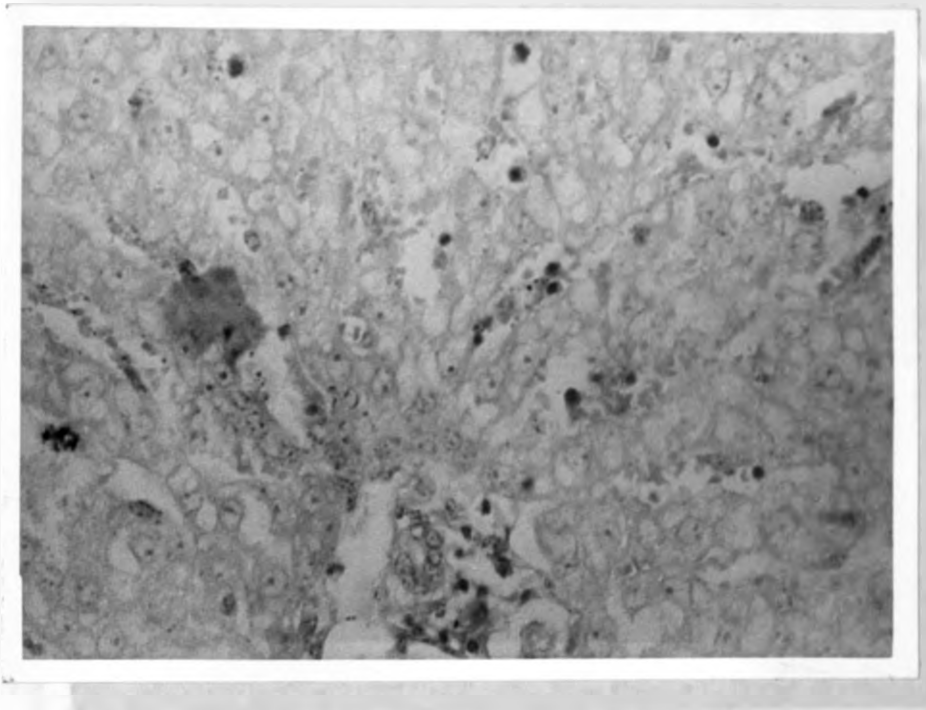

Fig. 17. Section of liver showing area of coagulative necrosis in the liver cell cords. H. & E. X 390.

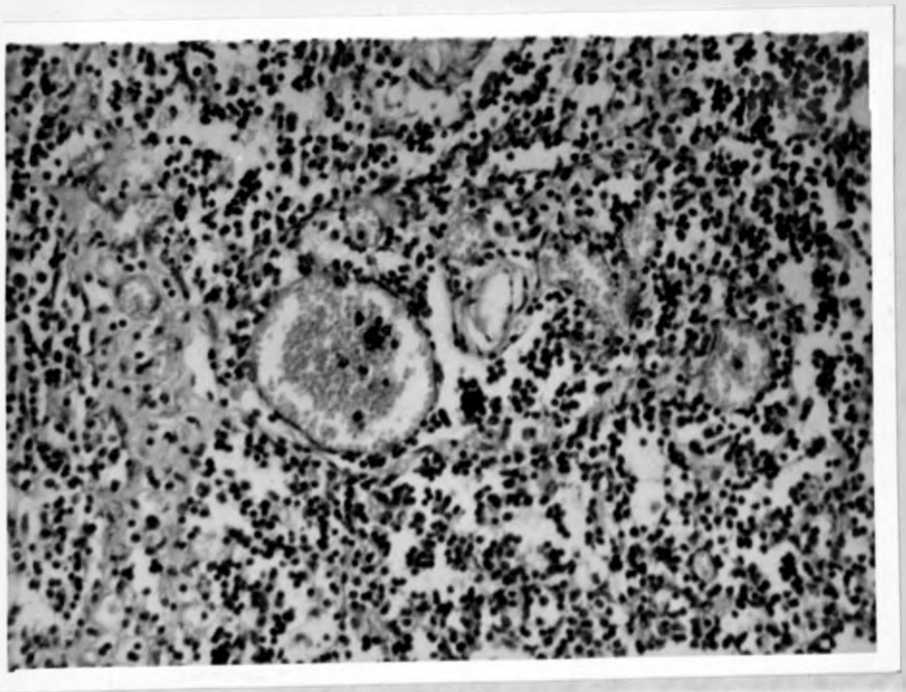

Fig. 18. Spleen section showing congestion, pyknosis and cell degeneration. H&E. X 390.

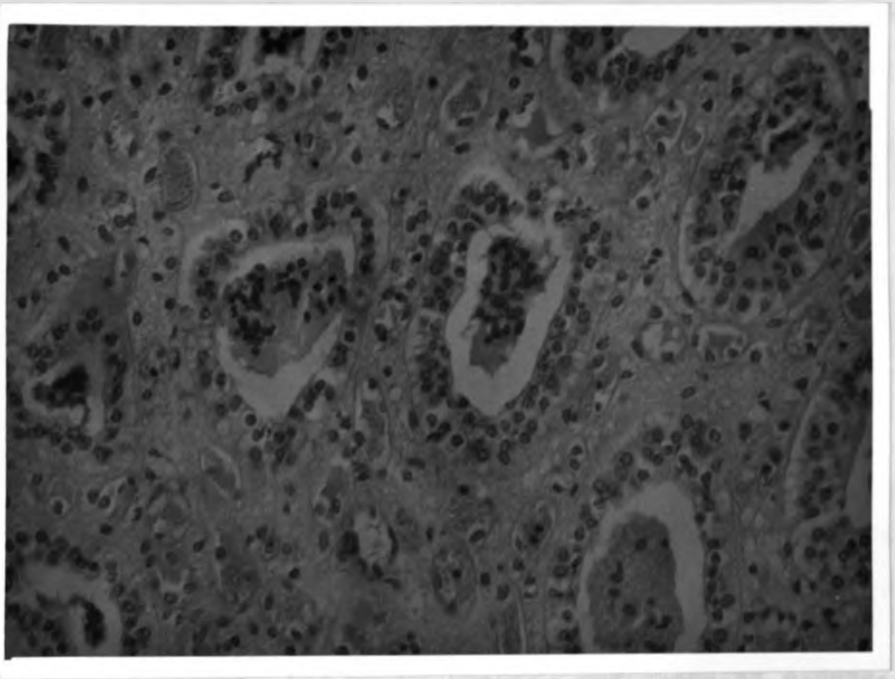

Fig. 19. Section of kidney showing cell degeneration with hyaline and cell casts in the tubules : H&E. X 625.

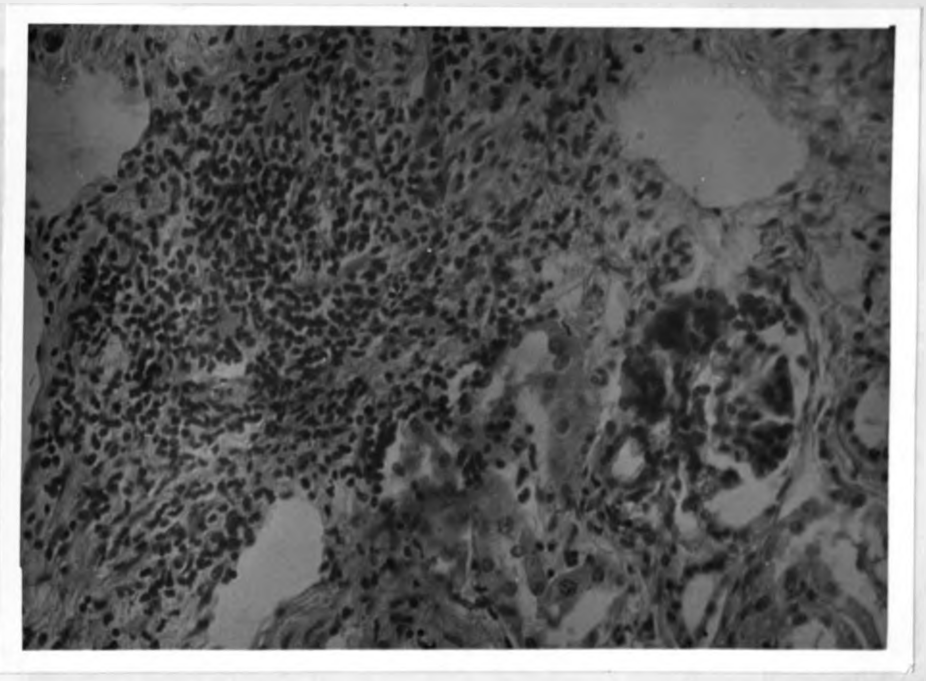

Fig. 20. Section of Kidney showing cell degeneration with hytiocytic cell infiltration and congestion of the vessels. H&E. X 625.

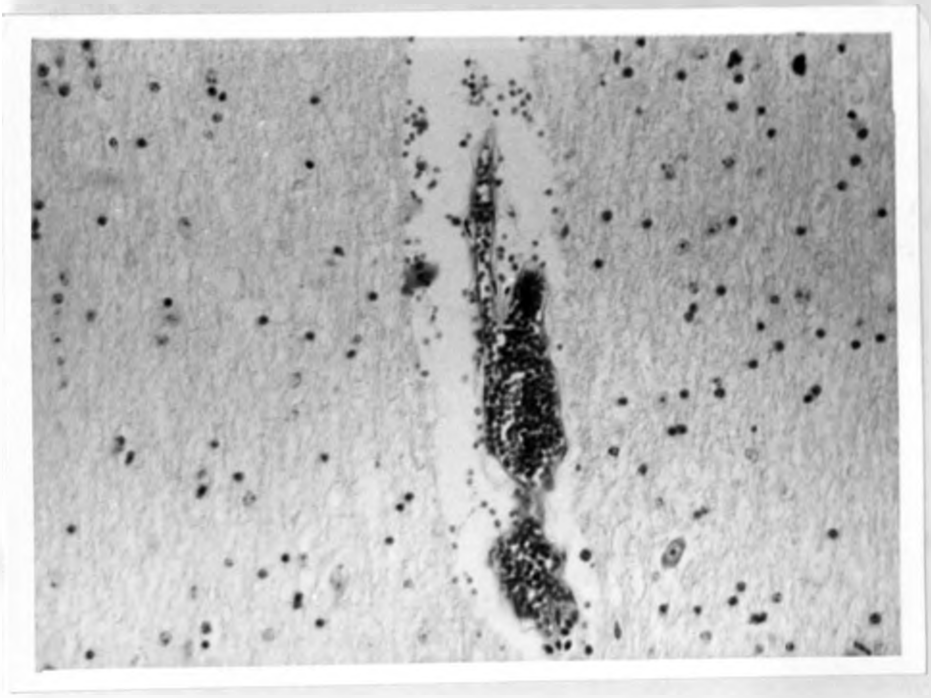

Fig. 21. Section of the mid brain showing perivascular oedema, pyknosis of glial cells and congestion of the vessels H & E. Stain X 166.

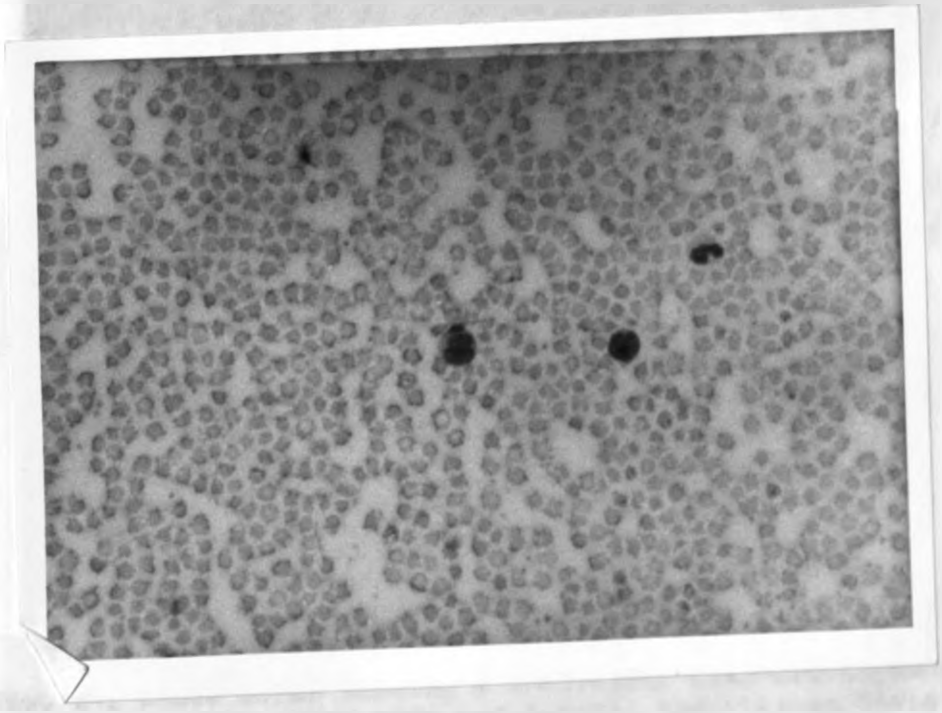

Fig. 22. Blood smear showing an immature neutrophil with an intracytoplasmic giant body : Giemsa Stain X 625.

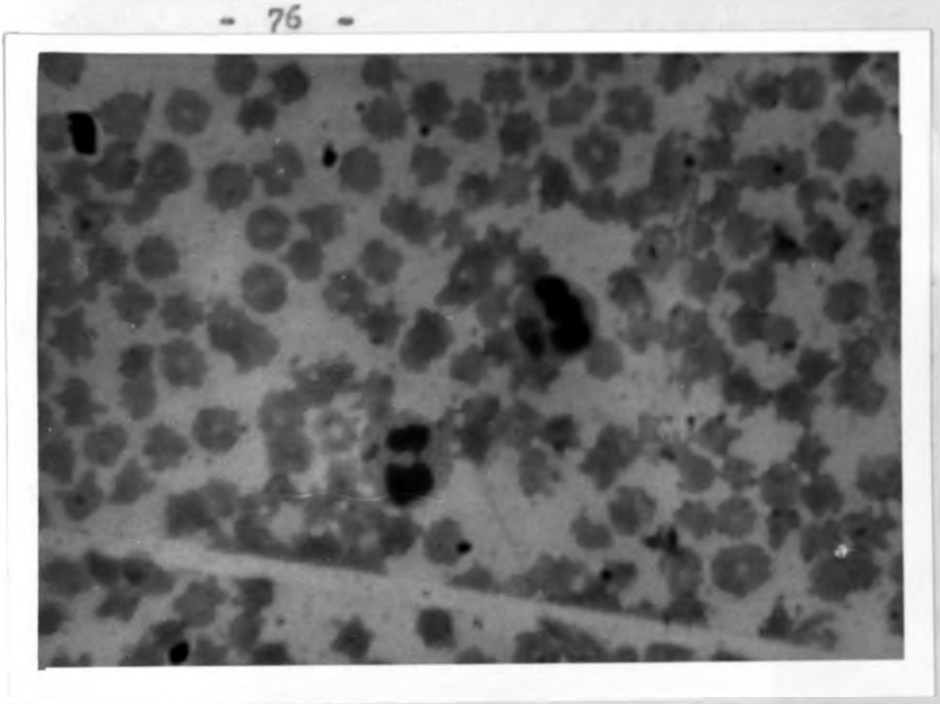

Fig. 23. Blood smear showing two neutrophils, one with a giant body and one with a comma shaped initial body : Giemsa Stain X 1563

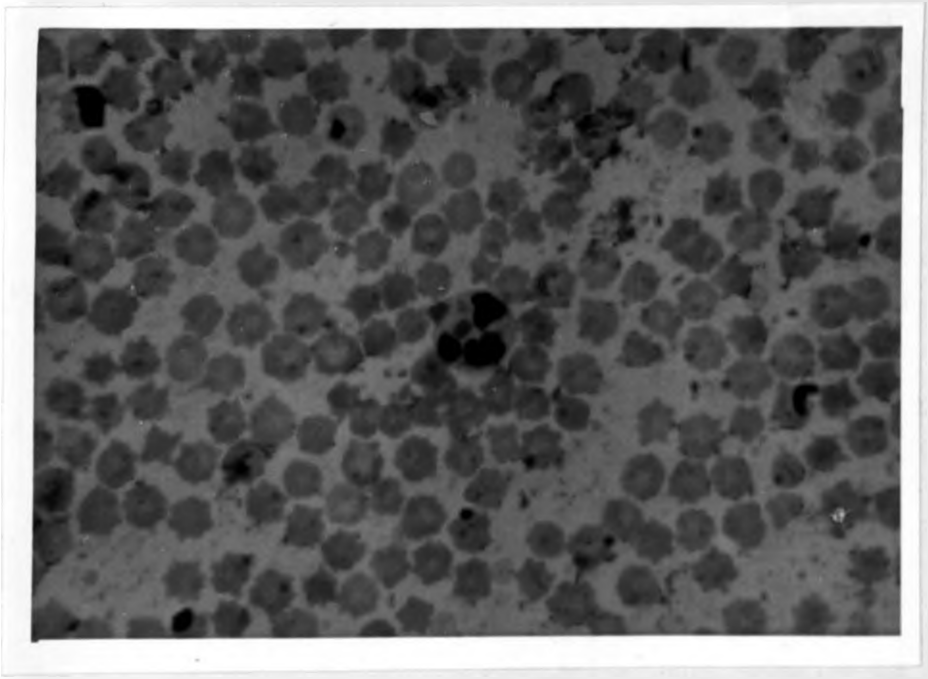

Fig. 24. Blood smear showing a neutrophil with intracytoplasmic morula, giant body and initial body: Giemsa Stain X 1563.

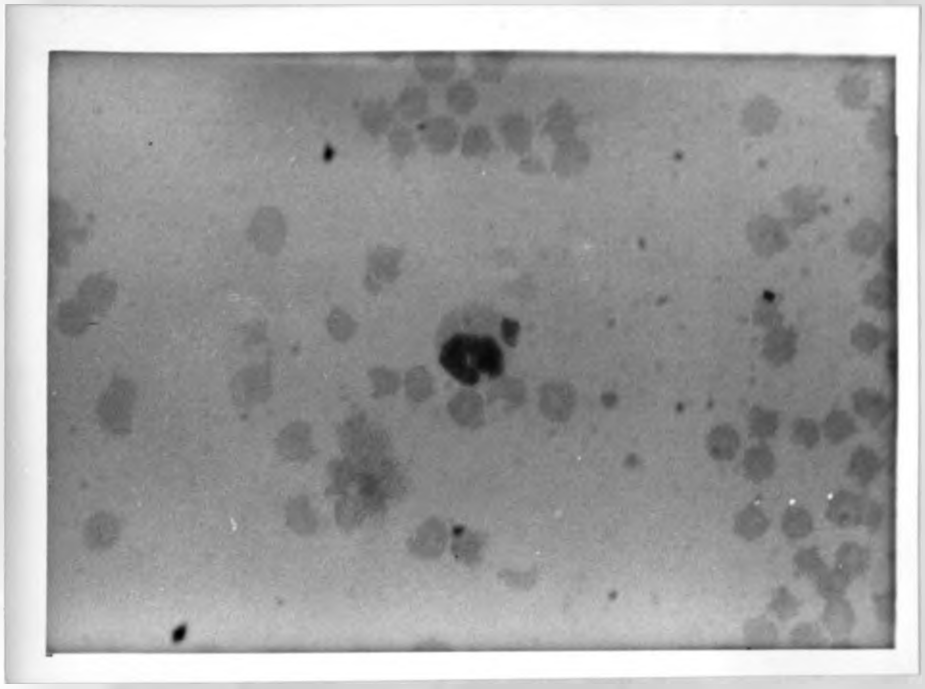

Fig. 25. Buffy coat smear showing a neutrophil with a peripheral intracytoplasmic morula ready to rupture the cytoplasmic membrane. Giemsa Stain X 1563.

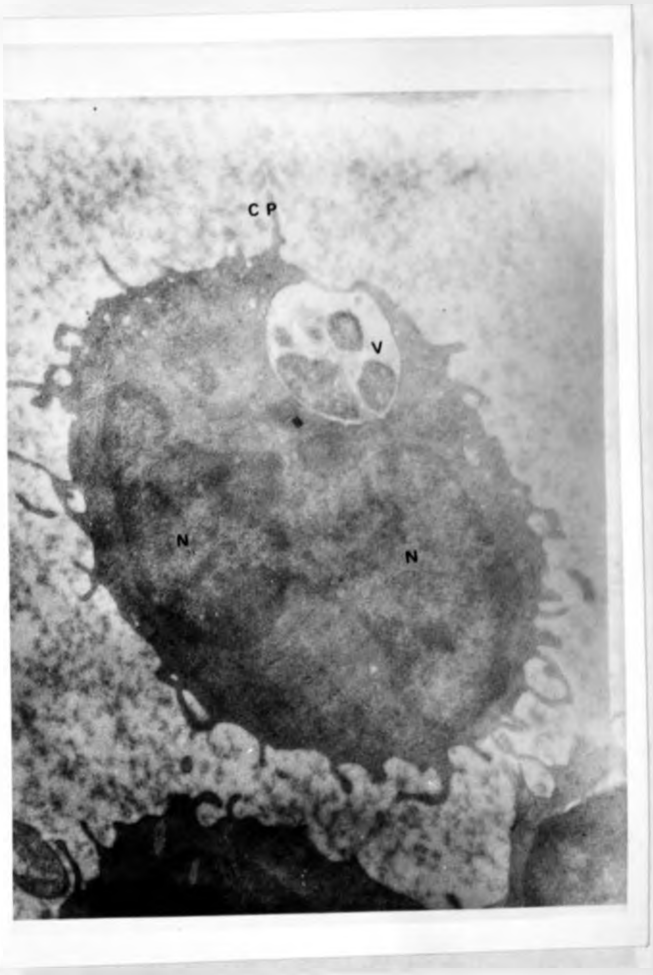

Fig. 26. Neutrophil of an experimental bovine, five parasites of different sizes and shapes are visible within a large cytoplasmic vacuole (V). Cytoplasmic processes (CP) and cytoplasmic vesicles indicate phagocytic activity. (N=Cell nuclear segments) X 12,600.

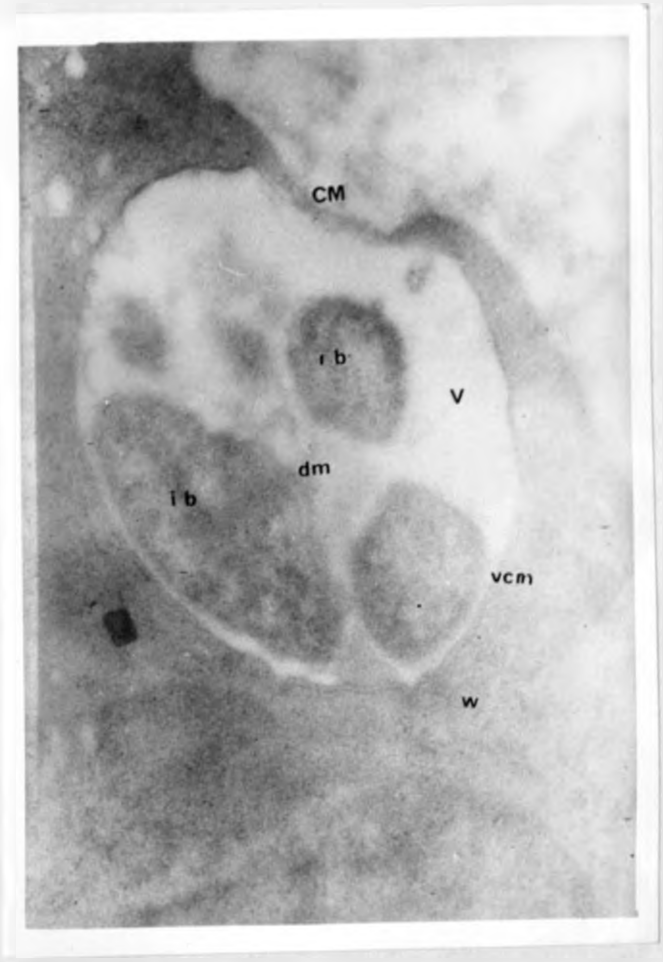

Fig. 27. Higher magnification of the same vacuole (V) as Fig. 26 showing initial bodies (ib) with double membranes (dm). Cytoplasmic membrane (CM) is being closed where it was invaginated. Note also the vacuole membrane (vcm) is similar to and continuous with cytoplasmic membrane: X 31,000.

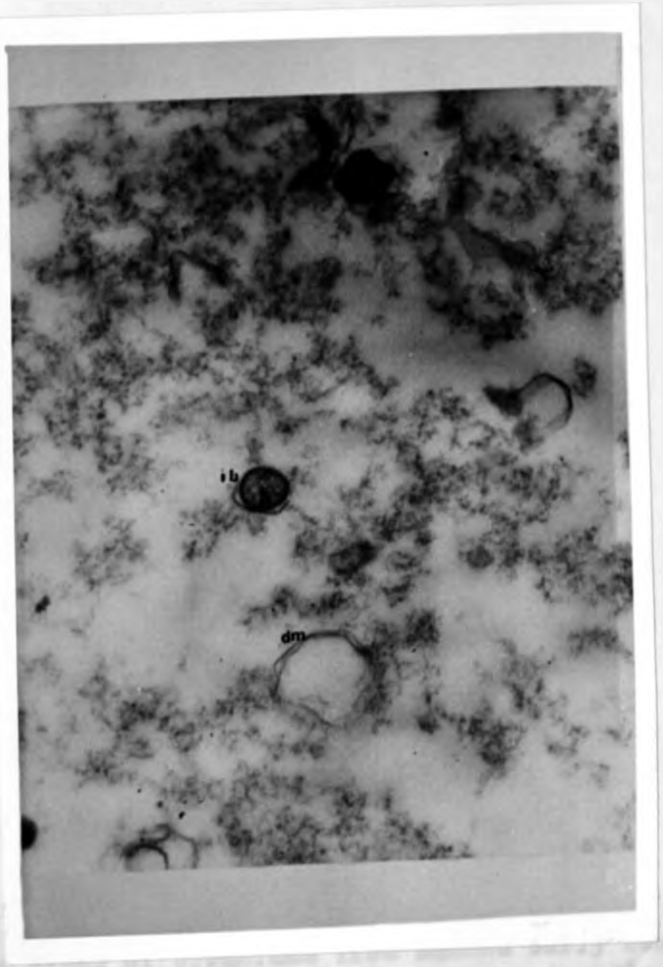

Fig. 28.

A single elementary body (ib) from the washed buffy coat. Note the presence of separated double membranes (dm) from lysed organisms: X 31,000.

...81...

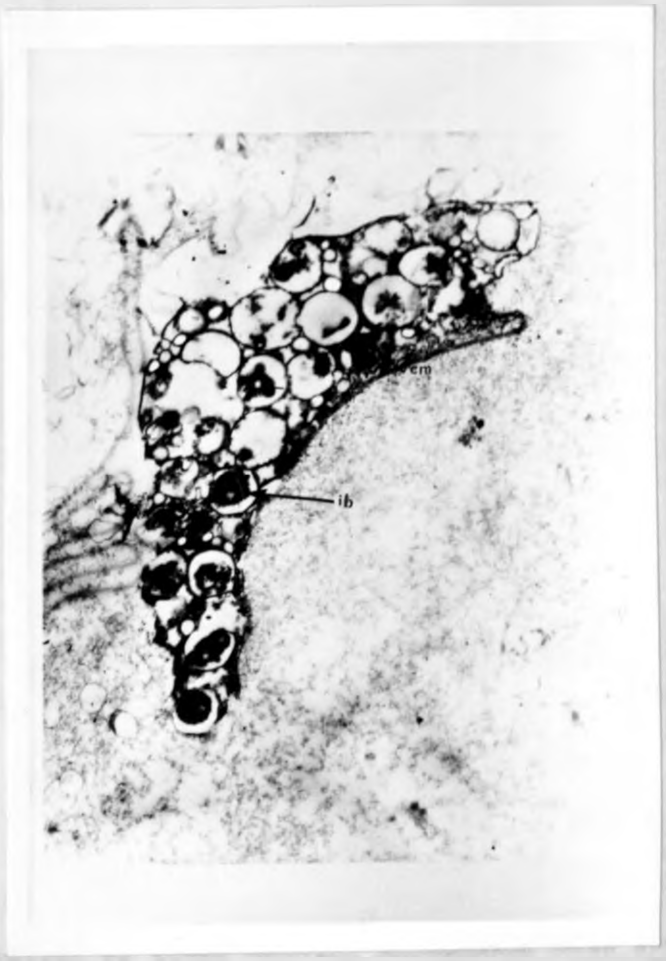

Fig. 29. A clump of organisms from washed buffy coat. The initial bodies (ib) have been disintegrated while the double membranes are still intact. They appear to have come from a single morula by the presence of vacuole membrane (vom) X 12,600.

...82..

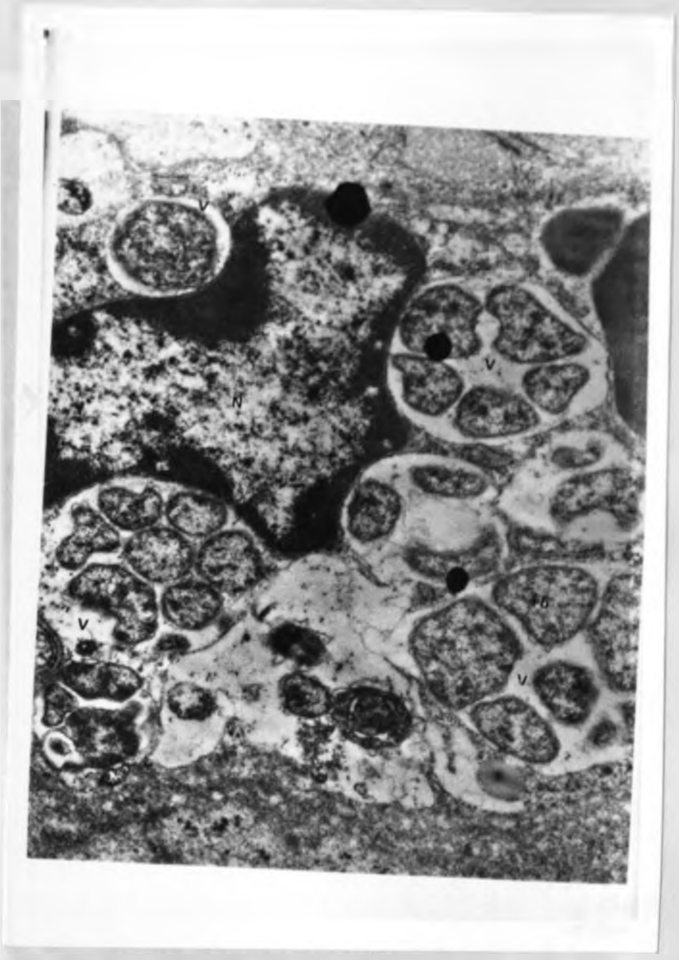

Fig. 30. Section of liver showing sinusoidal cell loaded with more than a dozen vacuoles (V) containing more than 40 parasites (ib) in all. Nucleus (N) is under great pressure while some of the parasites are degenerating : X 12,600.

Fig. 31. Section of Liver showing vacuoles in sinusoidal cell filling the sinus and forcing erythrocytes (E) to squeeze through narrowed Sinus. Several stages (1-5) of binary fission of the organism within the vacuole X12,600.

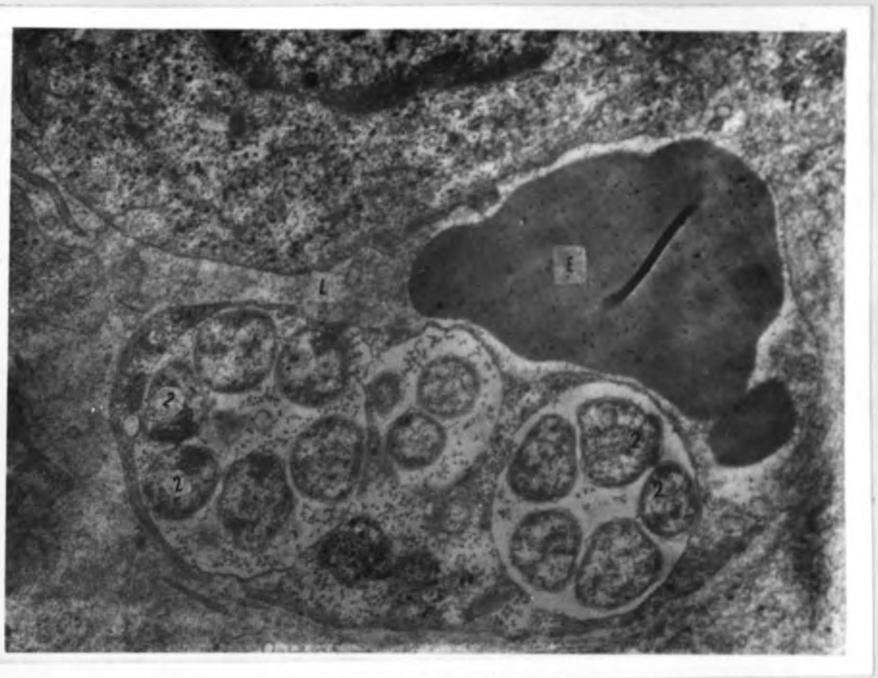

Fig. 32. Section of Liver showing complete occlusion of the sinus lumen (L) arresting Erythrocytes (E). Numbered parasites are completing binary fission : X 12,600.

Fig. 33. Section of Liver showing sinusoidal cell with several vacuoles. The organism in the center shows a double division (1,2,3) while the single organism below the nucleus (N) is degenerating X12,600.

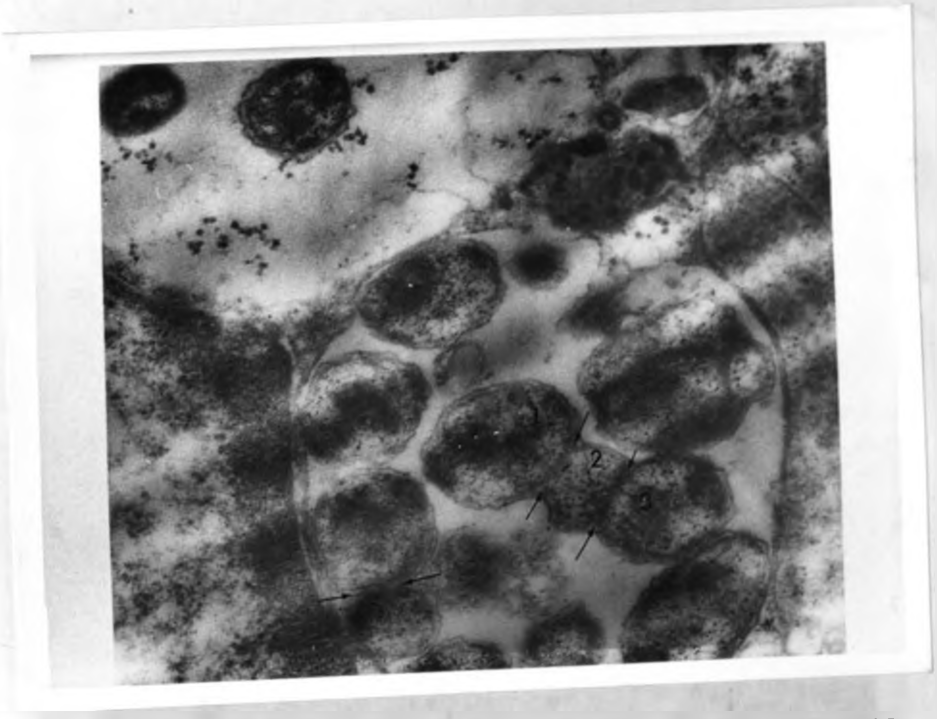

Fig. 34. A higher magnification of the same vacuole as Fig.33 showing the double membranes and the development of Lines of division (→) : X 31,000.

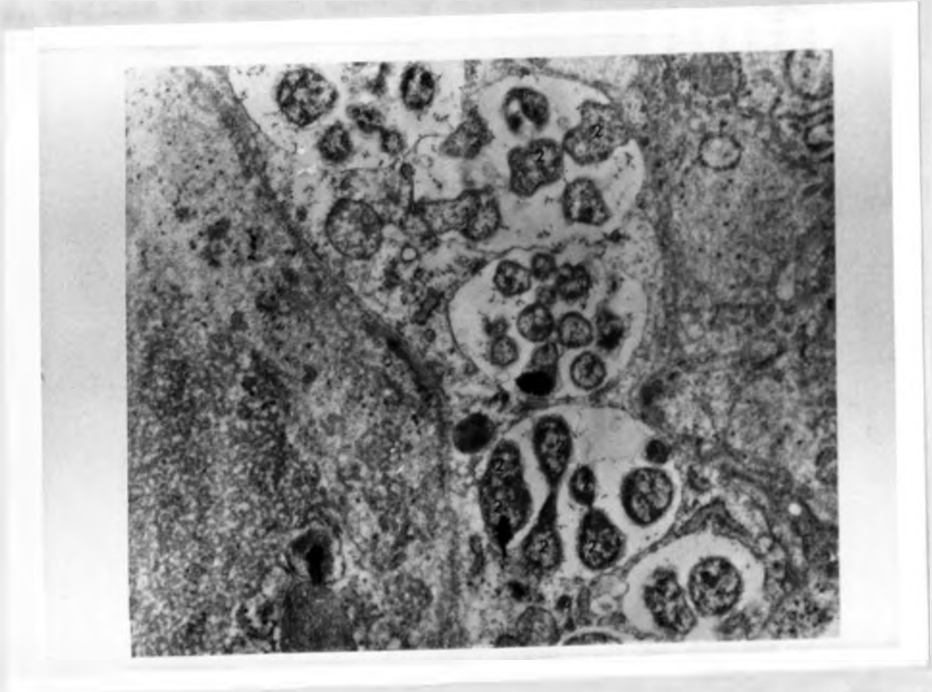

Fig. 35. Tangentially cut liver sinusoidal cells containing many vacuoles. The parasites in the vacuoles are fast multiplying by binary fission : X 12,600.

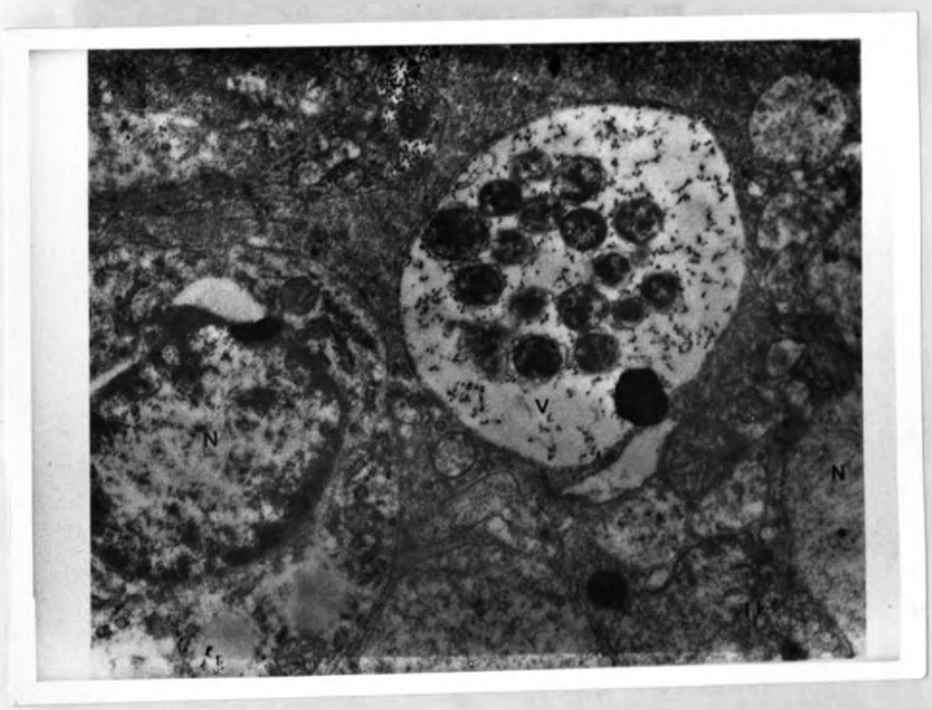

Fig. 36. Section of Liver showing a large vacuole (V) within an acinar cell of the liver (Ac). The outer membrane of each elementary body is clearly demarcated. X 12,600.

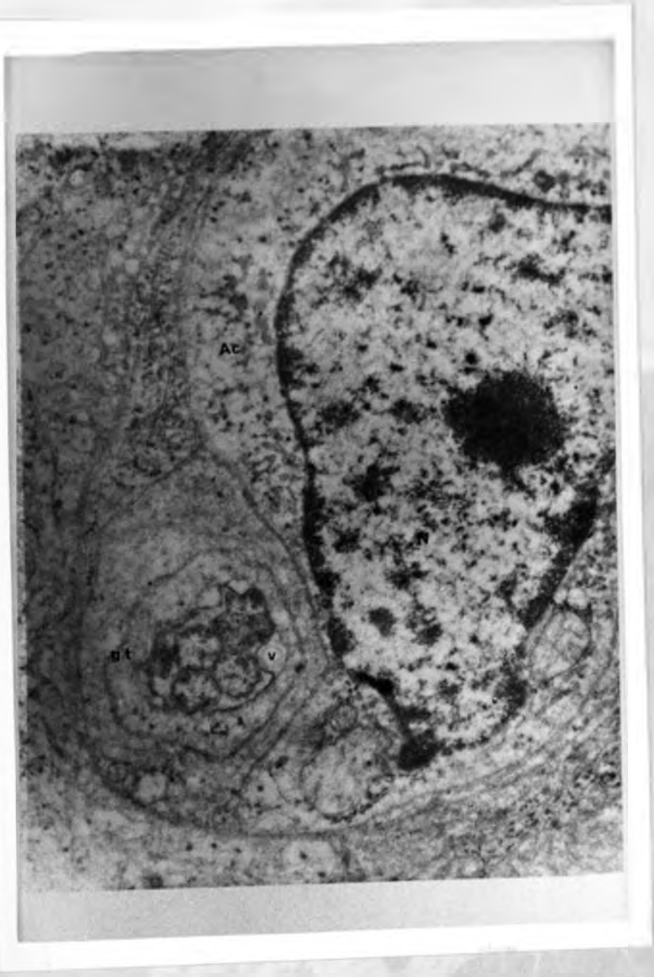

Fig. 37. Section of Liver showing a Giant body (gt) in an acinar cell (Ac). The host cell has laid down about 4 layers of membranes to enclose the vacuole (V) and the parasite. Note the lines of multiple fission in the parasite X31,000

Fig. 38. Spleen section showing a macrophage cell loaded with parasites in vacuoles (V). A giant body (gt) is seen within an acinar cell X 4,000.

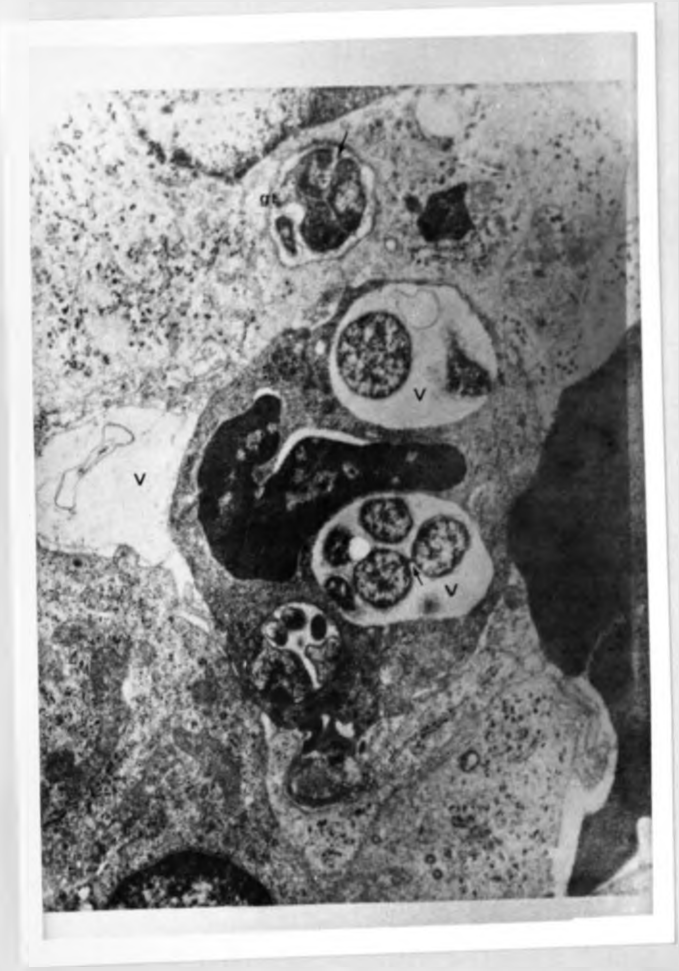

Fig. 39. Higher magnification of same as Fig. 38 showing division lines (⟶) of the parasite. Note also the empty membranes of parasites cut tangentially within the vacules: X 12,600.

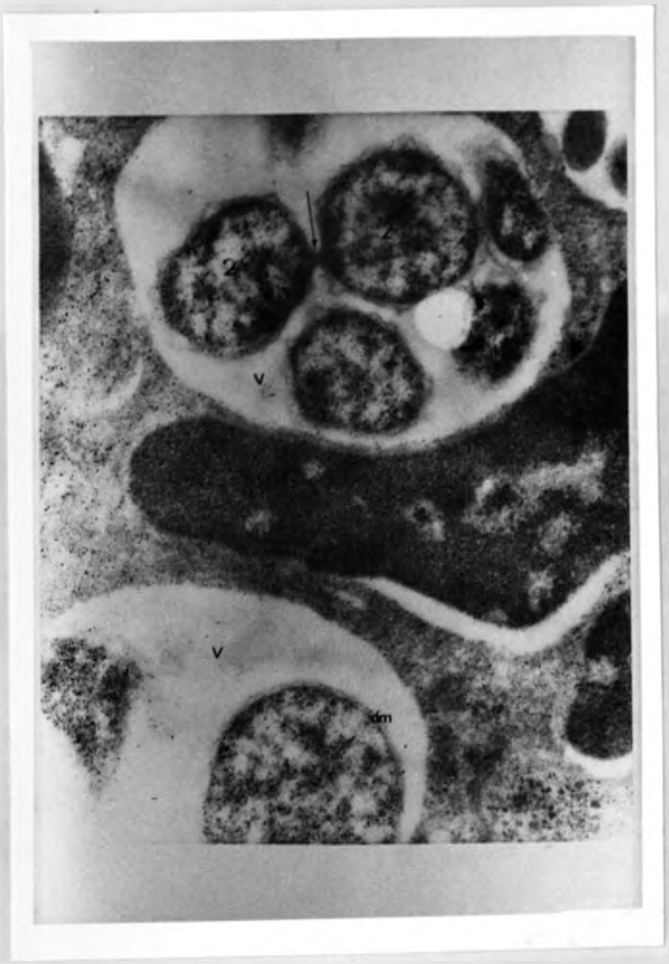

Fig. 40. Higher magnification of same as Fig. 39- showing the double membrane (dm) of each parasite in the vacuoles, X 31,000

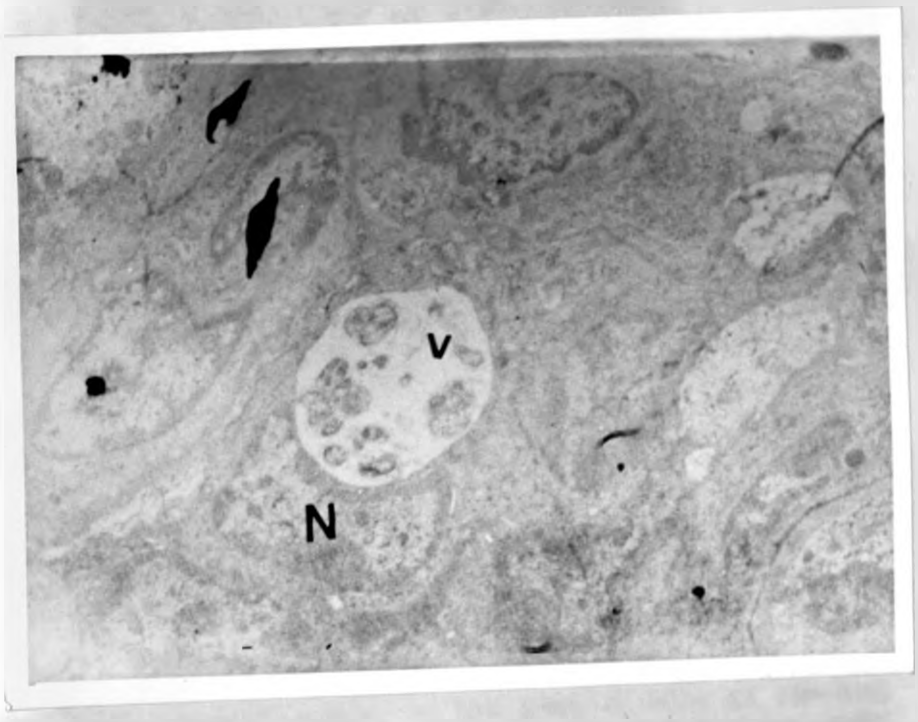

Fig. 41. Kidney section showing a vacuole (V) in Kidney interstitial cell. The cell nucleus (N) is compressed by the presence of the vacuole X 9,600.

Fig. 42 Higher magnification of the same as Fig. 41 showing the loosely attached outer membranes enclosing many organsims that are formed by multiple fission and binary fission X 12,600.

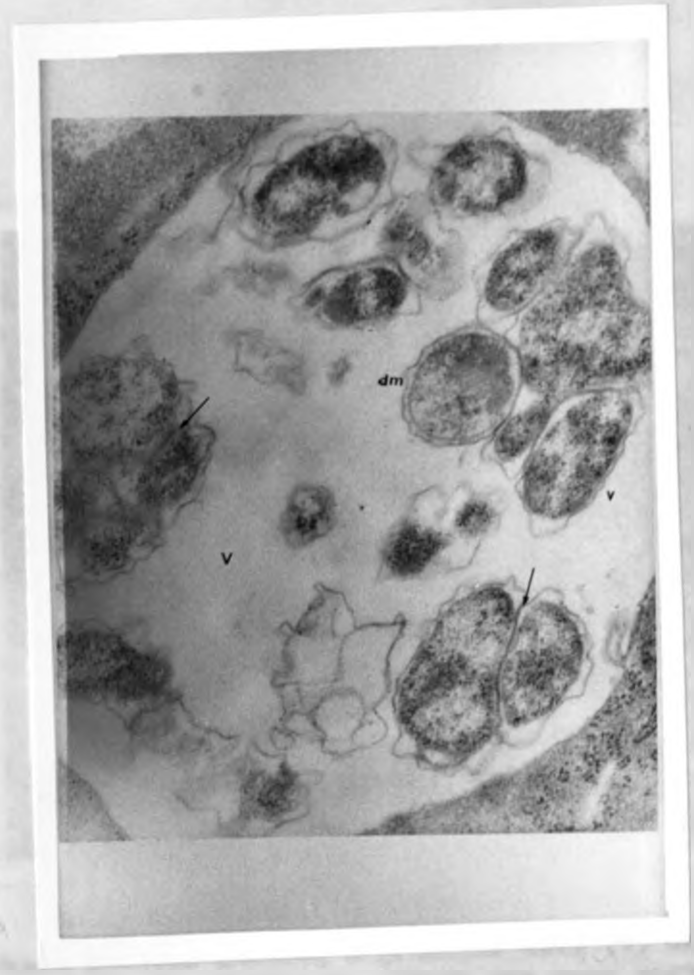

Fig. 43. A still higher magnification of same vacuole (V) as Fig. 42 with double membrane (dm) of each parasite clearly demarcated. Multiplication division is nearly complete on the organisms in the right side while the large one on the left is just showing the division lines and the whole mass still enclosed in one outer membrane : X 31,000.

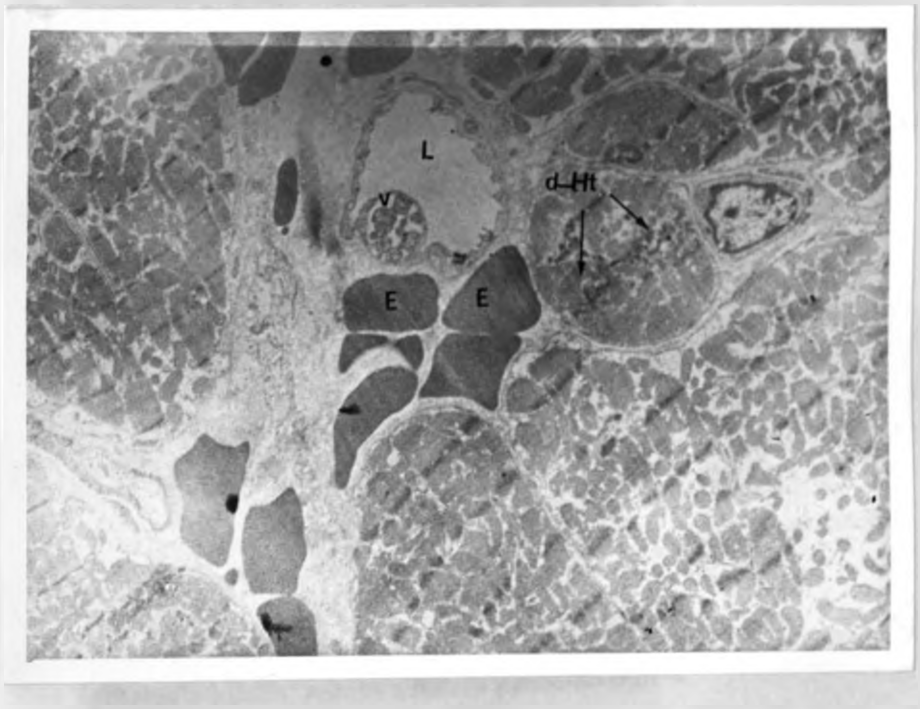

Fig. 44. Heart section showing a granular mass (V) on capillary wall of a blood vessel. There are many erythrocytes(E) outside the vessel lumen (L) while the adjacent myocadial cells are showing stages of degeneration (d-Ht). X 4,000.

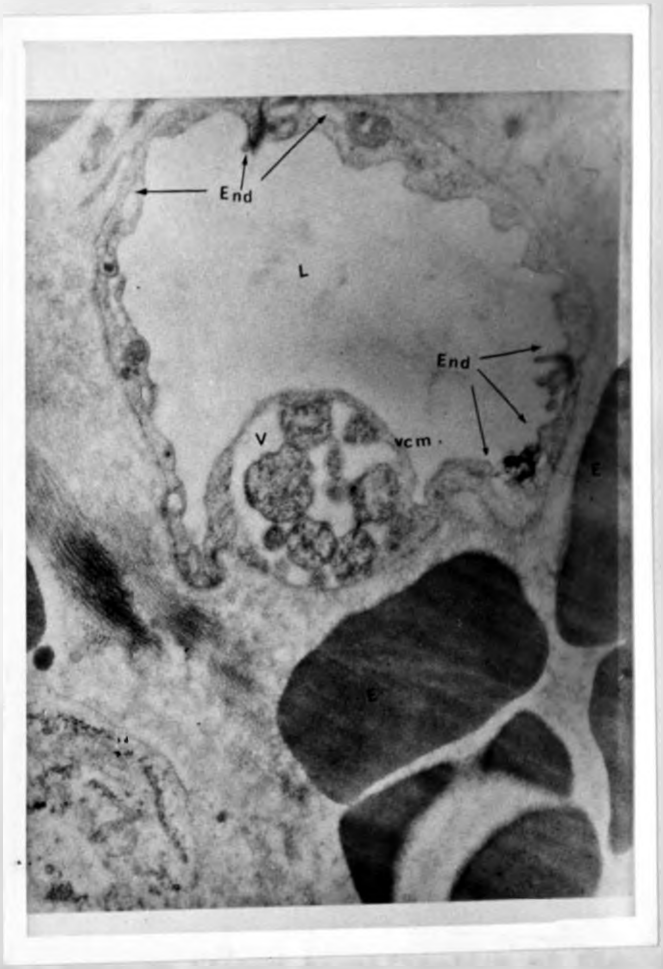

Fig. 45. Heart section higher magnification of Fig. 44 showing necrosis of the endothelial cells (End) and the distension of the capillary endothelial cell by the vacuole of the parasites. Other adjacent endothelial cells show the presence of cytoplasmic vacuoles which indicate degenerati changes. X 12,600

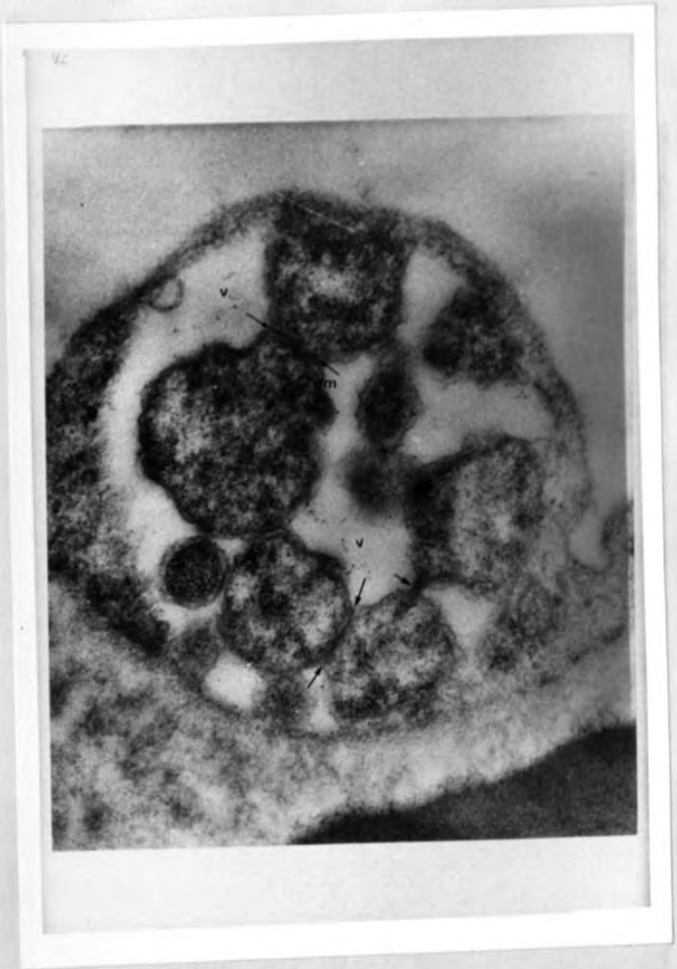

Fig. 46. Heart section higher magnification of Fig. 45 showing the double membranes (dm) of the parasites. The parasites are showing lines of division(→) while other parasites seem to be attached to the vacuole membrane: X 31,000.

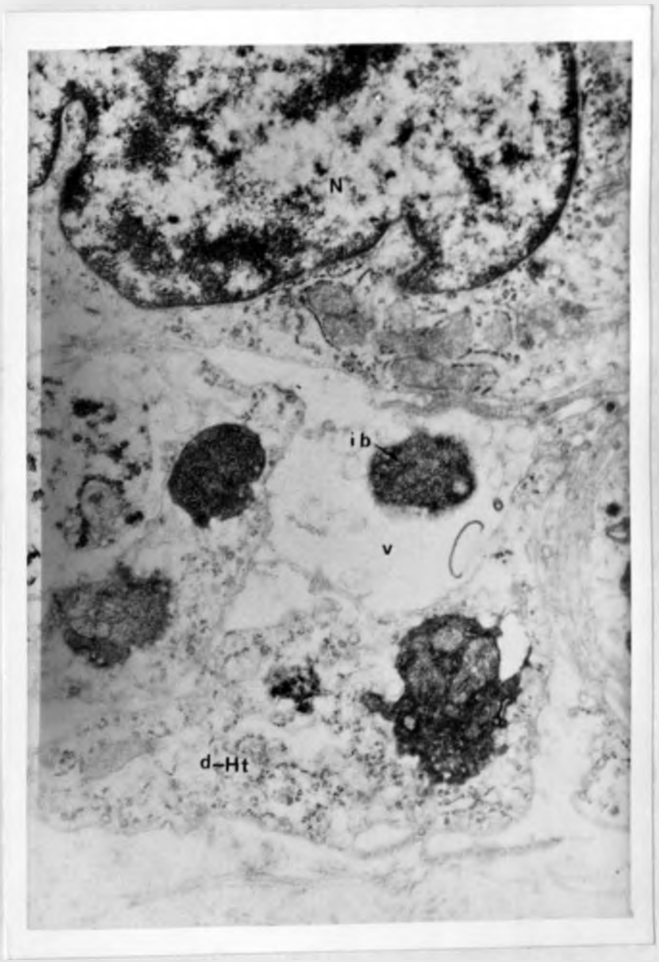

Fig. 47. Heart section showing advanced degeneration of the heart cells (d-Ht) and the presence of degenerating parasite(ib) within an vacuole (V). The parasite has lost its outer membrane : X 12,600.

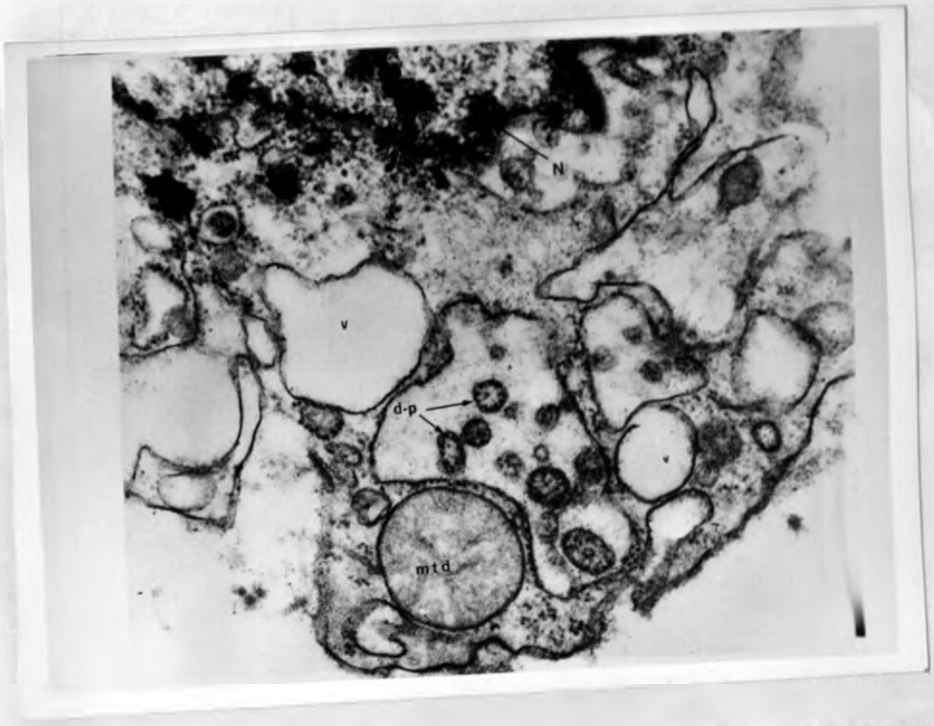

Fig. 48. Heart section showing many vacuoles (V) within a degenerating heart cell. The cell nucleus (N) has also degenerated. Many degenerating initial bodies of parasite (d-p) are seen in some of the vacuoles. The parasites have lost their outer membranes. (Mtd-Mitochondrion) X 31,000.

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APPENDIX

APPENDIX 1 : DAILY TEMPERATURE REACTION (e_n) OF SIX CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS						MEAN.
	40	42	52	54	59	74	
0	101.2	101.2	101.4	101.6	101.8	101.8	101.5
1	101.6	101.4	101.6	101.0	101.8	101.2	101.4
2	102.1	101.7	101.6	101.4	101.6	101.7	101.7
3	101.8	101.3	100.6	101.0	102.5	102.7	101.6
4	103.5	103.1	102.6	102.2	103.3	104.9	103.3
5	106.3	107.0	102.4	103.4	102.4	106.4	104.6
6	107.4	106.8	104.6	106.4	105.7	105.0	106.2
7	105.1	105.2	107.2	105.7	106.1	105.8	105.9
8	104.9	99.0	105.8	102.0	105.0	105.3	104.6
9	98	-	105.4	104.0	105.6	105.4	105.1
10	-		104.4	103.2	105.0	102.2	103.7
11			103.6	102.3	103.3	101.8	102.7
12			103.0	102.0	102.8	101.6	102.3
13			-	101.9	103.0	104.4	103.1
14				101.8	102.8	102.8	102.4
15				101.2	101.4	101.8	101.4

APPENDIX 2 : DAILY TEMPERATURE RECORDINGS (°F) OF CALVES USED AS CONTROLS IN BOVINE PETECHIAL FEVER

	ANIMAL NUMBERS.						
DAYS.	23	43	44	61	62	64	MEAN
1	101.2	101.3	101.6	101.2	101.0	101.4	101.28
2	101.8	101.8	101.0	101.4	100.8	101.0	101.16
3	101.6	101.4	100.8	101.8	101.2	100.6	101.23
4	100.8	101.2	101.0	101.2	100.4	100.8	100.9
5	101.4	101.8	100.8	101.0	100.6	101.0	101.10
6	101.8	101.8	101.6	101.6	102.0	101.0	101.63
7	100.6	101.6	102.0	100.6	101.4	100.6	101.13
8	102.0	102.0	102.0	101.4	100.6	100.6	101.43
9	101.6	101.2	101.3	100.4	100.0	101.4	100.98
10	101.4	101.3	101.6	100.6	101.4	101.4	101.28
11	101.8	101.8	101.8	101.4	101.6	101.6	101.66
12	101.9	101.5	101.8	101.6	100.6	101.8	101.5
13	101.6	101.7	101.1	101.4	101.6	101.2	101.43
14	102.1	100.8	101.9	101.3	101.4	101.2	101.6
15	101.1	101.6	101.3	100.8	101.2	100.8	101.30
16	101.5	102.0	101.7	101.0	100.4	101.8	101.40

APPENDIX 3 (a): DAILY HAEMOGLOBIN CONCENTRATION(gm/100ml) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER (RECOVERIES).

DAYS AFTER INFECTION.	ANIMAL NUMBERS						
	49	50	53	54	59	74	MEAN.
0	7.8	8.8	10.9	7.0	9.5	11.5	9.25
1	9.4	9.0	12.9	7.3	10.4	12.5	10.25
2	9.4	8.6	12.5	9.8	9.8	11.2	10.21
3	10.4	9.2	10.7	8.8	12.6	12.2	10.65
4	9.8	11.0	9.7	9.2	10.2	11.5	10.23
5	9.9	8.4	10.6	8.1	8.8	11.7	9.58
6	9.2	10.5	10.9	7.8	8.6	-	9.40
7	9.9	11.1	11.4	6.3	8.0	10.8	9.58
8	9.5	10.7	9.6	6.4	5.8	8.2	8.37
9	9.2	10.4	9.7	5.1	4.5	7.0	7.65
10	8.9	10.2	10.4	6.3	4.9	6.3	7.84
11	8.8	10.3	9.1	4.9	4.9	6.4	7.40
12	9.2	10.3	9.8	5.7	5.1	6.3	7.74
13	7.9	9.8	10.1	6.0	4.8	6.7	7.55
14	7.7	10.1	9.8	5.9	4.8	6.8	7.52
15	7.9	11.7	10.6	5.5	5.4	7.0	8.02

APPENDIX 3(b) : DAILY HAEMOGLOBIN CONCENTRATION(gm/100ml) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER (DEATHS).

DAYS AFTER INFECTION.	ANIMAL NUMBER.						
	40	42	52	55	58	72	MEAN
0	12.8	9.6	11.1	9.1	9.6	12.4	10.77
1	13.9	9.4	12.3	8.6	10.0	9.8	10.67
2	15.7	9.9	12.8	8.6	10.6	10.4	11.33
3	13.5	12.0	12.3	8.6	7.8	10.8	10.83
4	12.8	10.0	12.0	8.3	5.9	10.5	9.92
5	12.2	10.1	11.7	7.0	5.8	10.5	9.55
6	11.7	10.9	11.6	7.0	5.5	11.2	9.65
7	9.9	9.9	11.7	8.3	5.3	9.5	6.07
8	6.7	7.4	9.9	10.2	4.9	8.3	7.90
9	5.9	4.3	7.3	-	4.2	-	5.42
10	-	-	5.0		-		5.0
11			3.7				3.7
12			3.2				3.2
13			-				
14							
15							

APPENDIX 3 (c) : DAILY HAEMOGLOBIN CONCENTRATION(gm/100ml) OF SIX CALVES USED AS CONTROLS IN BOVINE PETECHIAL FEVER

	ANIMAL NUMBERS.						
DAYS.	23	43	44	61	62	64	MEAN
1	9.1	12.4	10.2	10.3	9.2	10.0	10.2
2	9.6	12.2	10.4	8.0	10.3	10.1	10.1
3	8.7	13.5	10.8	11.0	9.1	9.7	10.4
4	9.0	12.0	10.3	11.1	9.6	9.6	10.2
5	8.8	12.0	9.8	9.9	11.1	9.1	10.1
6	9.2	12.2	9.4	11.7	9.3	9.8	10.2
7	8.9	10.6	9.9	10.8	9.6	10.3	10.0
8	9.4	12.1	10.2	10.0	9.2	10.2	10.1
9	8.9	11.2	10.0	10.1	9.6	8.7	9.7
10	10.2	11.3	9.8	10.5	9.2	8.6	9.9
11	10.2	11.9	9.5	10.1	8.9	8.5	9.9
12	10.1	9.40	9.1	10.3	9.9	9.9	9.7
13	9.7	9.54	10.3	8.0	10.1	9.4	9.5
14	9.2	12.4	9.8	9.3	9.4	8.0	9.6
15	9.8	11.2	9.9	9.8	9.8	8.3	9.6
16	10.1	10.6	9.4	9.6	9.2	9.4	9.7

APPENDIX 4(a) : DAILY PERCENTAGE PACKED CELL VOLUME OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	49	50	53	54	59	74	MEAN
0	23	31.5	31	23	33	34	29.25
1	26	31.5	37	24	33	34	25.78
2	27	37.9	36	22	30	36	31.50
3	25	35	31	23	35	35	30.67
4	27	31	30	20	32	34	29.00
5	27	24	33	20	28	35	27.83
6	-	30	33	21	26	-	27.50
7	25	31	34	19	25	33	27.83
8	27	30	30	18	18	27	25.00
9	23	30	28	18	14	21	22.33
10	26	30	32	20	14	21	23.83
11	26	29	28	18	-	19	20.40
12	25	28	30	19	16	19	22.83
13	26	27	32	18	16	20	23.17
14	23	28	29	19	15	22	22.67
15	23	31	31	22	16	22	24.17

APPENDIX 4 (b) : DAILY PERCENTAGE PACKED CELL VOLUME OF CALVES INFECTED & DIED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	35	28	31	25	24	30	28.83
1	38	30	32	25	28	30	30.66
2	43	35	36	26	28	31	33.16
3	39	30	34	26	28	31	31.33
4	36	31	33	25	24	31	30.00
5	36	31	35	25	20	32	29.83
6	34	31	33	24	24	32	29.66
7	29	22	33	25	24	33	27.66
8	20	14	28	20	28	30	23.33
9	17	-	22	-	16	27	20.50
10	-	-	16	-	16	25	19.00
11			10		-	-	10.00
12			10				10.00
13							
14							
15							

APPENDIX 4 (c) : DAILY PERCENTAGE PACKED CELL VOLUME OF CALVES USED AS CONTROLS IN BOVINE PETECHIAL FEVER

	ANIMAL NUMBERS						
DAYS.	23	43	44	61	62	64	MEAN
1	26	39	27	30	27	27	29.3
2	28	37	29	27	29	28	29.6
3	26	41	31	32	25	30	30.8
4	27	40	27	33	31	30	31.3
5	29	36	28	31	30	26	30.0
6	26	37	31	35	27	26	30.3
7	27	37	30	32	29	26	30.1
8	28	36	31	30	27	30	30.3
9	28	35	32	31	28	28	30.3
10	27	34	27	29	27	29	28.8
11	29	35	29	30	29	25	29.5
12	26	36	28	30	27	28	29.1
13	28	37	29	26	28	26	29.0
14	30	37	30	27	30	28	30.3
15	28	34	28	29	28	27	29.00
16	29	33	27	32	29	28	29.6

APPENDIX 5 (a) : DAILY MEASUREMENTS OF TOTAL PROTEIN (gm/100ml) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 49	50	53	54	59	74	MEAN
0	5.3	7.4	6.2	6.0	7.4	7.4	6.62
1	6.6	6.6	7.0	6.0	7.8	6.3	6.72
2	5.6	6.1	6.8	6.0	7.2	6.8	6.42
3	5.2	5.9	6.2	6.2	7.0	6.2	6.12
4	6.2	6.4	6.2	5.9	6.6	6.8	6.35
5	6.2	5.6	6.2	5.9	6.4	5.4	5.95
6	5.9	6.7	6.4	5.8	6.2	4.5	5.92
7	5.2	6.4	6.7	5.8	5.8	4.3	5.07
8	4.9	6.4	6.2	5.4	5.4	4.6	5.48
9	4.8	5.6	6.4	5.4	5.4	5.0	4.68
10	4.8	6.0	6.6	5.6	5.8	5.0	4.88
11	4.4	5.6	5.8	5.4	5.9	5.6	5.45
12	5.4	5.8	6.4	5.4	6.1	6.4	5.92
13	5.0	5.8	6.4	5.4	6.4	6.4	5.90
14	5.4	6.0	6.0	5.4	6.6	6.3	5.95
15	5.4	6.6	6.4	5.6	6.8	6.8	6.27

APPENDIX 5 (b) : DAILY MEASUREMENTS OF TOTAL PROTEIN(gm/100ml) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS 40	42	52	55	58	72	MEAN
0	6.6	7.0	7.2	6.0	6.8	7.4	6.84
1	6.8	7.4	7.6	6.0	6.6	7.0	6.90
2	7.3	8.2	8.0	6.4	7.2	6.3	7.23
3	6.8	7.2	7.4	6.6	6.8	6.7	6.92
4	6.6	7.1	7.4	6.6	6.4	6.6	6.80
5	6.4	7.0	7.7	6.4	6.2	6.6	6.72
6	6.4	6.2	7.6	6.4	6.2	6.4	6.54
7	5.3	4.8	6.4	5.3	6.2	5.4	5.57
8	4.4	4.8	5.7	3.6	6.0	-	4.90
9	4.2	-	4.6	-	5.2	4.0	4.50
10	-		4.0			3.4	3.70
11			5.8				5.80
12			5.8				
13			-				
14							
15							

APPENDIX 5(e) : DAILY MEASUREMENT OF TOTAL PROTEIN(gm/100ml) OF CALVES USED AS CONTROLS IN BOVINE PETECHIAL FEVER.

DAYS.	23	43	44	61	62	64	M E A N
1	6.2	6.9	7.0	6.4	6.6	6.9	6.6
2	6.0	7.1	7.4	6.0	6.5	6.6	6.6
3	5.8	6.8	7.2	6.8	7.0	7.0	6.7
4	6.2	7.2	6.9	7.4	7.0	7.0	6.9
5	6.6	6.0	7.4	7.0	7.2	6.4	6.7
6	7.2	6.4	7.0	8.0	7.0	6.0	6.9
7	6.8	7.0	7.2	7.6	6.8	6.0	6.9
8	6.8	6.7	7.4	7.4	6.8	7.0	7.0
9	7.3	6.9	7.4	7.0	7.6	6.6	7.1
10	6.9	7.2	7.0	7.8	6.8	6.2	6.9
11	6.6	6.8	6.9	7.6	6.9	6.4	6.8
12	6.7	7.0	7.1	8.4	6.8	6.7	7.1
13	7.0	6.8	7.2	7.6	6.6	6.4	6.9
14	6.5	6.8	7.6	6.4	6.8	7.2	6.8
15	6.8	7.2	7.6	6.8	7.0	6.6	7.0
16	6.6	6.8	7.4	7.0	6.9	6.3	6.8

APPENDIX 6 (a) : DAILY RED BLOOD CELL COUNTS(10^6/mm3) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	ANIMAL NUMBERS						
	49	50	53	54	59	74	MEAN
0	4.36	7.8	7.53	4.8	7.59	8.92	6.83
1	5.24	7.31	8.66	4.76	7.79	8.24	7.00
2	4.66	8.33	8.00	5.64	7.85	8.62	7.18
3	6.12	8.0	7.00	5.54	7.05	8.36	7.01
4	5.40	7.10	6.72	5.50	-	8.17	6.58
5	5.54	4.66	7.44	4.82	7.27	8.82	6.42
6	5.15	6.86	7.38	4.65	5.55	8.07	6.28
7	5.65	7.07	7.9	4.09	5.52	8.11	6.39
8	5.55	6.59	6.71	4.07	4.21	6.31	5.57
9	5.66	6.12	6.23	3.62	3.01	4.54	4.86
10	5.15	6.94	6.59	3.7	3.42	5.05	5.14
11	4.93	6.8	6.55	3.27	3.60	4.74	4.98
12	5.30	6.9	6.77	3.61	3.53	5.01	5.02
13	4.48	7.02	6.67	3.92	3.27	5.03	5.06
14	4.06	7.0	6.80	3.54	3.40	5.30	5.01
15	4.78	6.90	7.23	3.66	3.26	5.37	5.20

APPENDIX 6 (b) : DAILY RED BLOOD CELL COUNTS ($10^6/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS						
	40	42	52	55	58	72	MEAN
0	8.81	6.85	7.4	6.48	9.6	7.79	7.82
1	9.40	7.67	8.0	5.82	10.0	8.11	8.17
2	10.76	7.42	9.8	6.74	10.6	6.51	8.64
3	9.23	8.49	8.85	6.13	7.8	6.79	7.88
4	8.78	6.92	8.36	5.69	5.9	6.91	7.09
5	8.24	7.87	8.0	-	5.8	6.89	7.36
6	8.10	7.79	7.9	5.43	5.5	6.66	6.90
7	6.74	5.38	8.12	6.08	5.3	7.42	6.50
8	4.78	3.14	6.76	6.18	4.9	6.18	5.32
9	4.20	-	4.69	-	4.2	4.91	4.50
10	-		3.35		-	-	3.35
11			2.66				2.66
12			2.12				2.12
13							
14							
15							

APPENDIX 6 (c) : DAILY RED BLOOD CELL COUNTS ($10^6/mm^3$) OF SIX CALVES USED AS CONTROLS IN BOVINE PETECHIAL FEVER

	ANIMAL NUMBERS						
DAYS.	23	43	44	61	62	64	MEAN
1	7.25	8.55	6.88	6.51	6.13	7.10	7.01
2	7.12	9.40	7.56	5.04	6.06	6.48	6.94
3	6.67	9.54	7.67	6.35	6.83	6.35	7.23
4	6.88	8.11	7.52	7.26	6.17	6.27	7.03
5	7.67	8.71	8.11	6.19	6.35	6.00	7.00
6	6.51	8.12	7.87	7.49	7.18	6.45	7.27
7	6.91	7.84	7.26	6.71	6.52	6.85	7.01
8	7.26	8.25	7.56	6.53	6.43	6.71	7.12
9	6.89	8.40	8.03	7.23	6.25	6.88	7.28
10	6.32	8.59	6.26	6.55	6.31	6.94	6.82
11	7.42	8.38	7.18	6.32	6.60	6.75	7.10
12	6.74	8.42	6.83	6.60	6.00	6.40	6.83
13	7.23	8.36	7.25	6.55	6.17	6.35	6.98
14	6.88	8.30	7.53	6.95	6.83	6.64	7.18
15	8.03	8.11	6.64	6.55	7.22	6.23	7.13
16	7.85	7.05	6.05	7.19	6.98	6.48	6.93

APPENDIX 7 (a) : DAILY WHITE BLOOD CELL COUNTS ($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	49	50	53	54	59	74	MEAN
0	5.8	12.3	13.4	10.0	14.4	11.2	11.18
1	6.3	14.5	12.1	9.2	14.2	12.3	11.43
2	8.0	11.0	12.0	8.8	13.7	10.7	10.70
3	7.9	11.8	12.0	7.8	13.2	8.3	10.17
4	8.5	14.6	11.0	7.2	11.4	7.2	9.98
5	6.7	7.2	14.0	7.7	9.6	4.0	8.20
6	6.5	11.8	17.0	6.2	7.2	4.0	8.78
7	8.4	10.0	15.8	5.4	5.4	4.1	8.18
8	6.4	7.9	16.6	6.0	6.9	3.65	7.90
9	6.0	6.3	10.4	8.0	9.1	5.0	7.46
10	9.1	6.7	11.3	10.7	10.6	6.5	9.15
11	11.9	8.9	12.0	10.6	12.1	9.8	10.88
12	15.3	10.6	10.6	8.6	14.6	9.7	11.57
13	10.3	10.3	10.8	8.4	15.6	7.4	10.47
14	7.1	12.8	11.9	9.5	14.4	10.8	11.08
15	8.0	14.2	12.4	11.6	13.8	12.6	12.1

APPENDIX 7(b) : DAILY WHITE BLOOD CELL COUNTS ($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	10.5	7.2	9.9	10.9	6.7	11.6	9.46
1	11.7	7.4	10.4	11.6	6.1	12.3	9.91
2	9.0	10.3	11.6	15.6	8.8	11.5	11.13
3	9.5	8.8	11.5	10.6	7.8	11.7	9.98
4	7.8	7.2	11.0	5.7	7.3	10.5	8.25
5	6.2	6.6	11.1	8.9	8.1	6.2	7.85
6	3.6	4.9	6.25	8.9	6.2	6.9	6.12
7	3.1	3.1	4.4	8.8	5.9	4.8	5.01
8	2.5	8.9	4.3	12.0	5.6	5.7	6.50
9	2.4	-	5.0	-	5.7	9.1	5.55
10	-		7.0		-		7.0
11			9.2				9.2
12			10.8				10.8
13							
14							
15							

APPENDIX 7 (c) : ABSOLUTE NEUTROPHIL COUNTS($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 49	50	53	54	59	74	MEAN
0	1.16	2.46	2.52	1.80	5.18	5.66	3.13
1	1.63	3.19	2.72	1.66	3.83	4.60	2.93
2	1.60	3.08	6.48	1.76	3.97	3.24	3.35
3	1.03	3.07	3.32	1.49	3.96	2.45	2.55
4	1.28	7.15	1.25	1.96	-	1.04	2.53
5	1.07	1.51	2.03	1.74	5.09	-	2.28
6	-	7.32	2.16	0.89	-	0.61	2.74
7	1.11	2.70	1.38	0.60	0.86	0.40	1.17
8	3.69	2.28	1.62	1.62	0.83	0.05	1.68
9	1.85	0.88	2.14	0.88	1.36	0.78	1.31
10	1.02	0.82	1.24	0.43	1.38	0.49	0.89
11	1.82	1.34	0.75	0.21	2.07	1.16	1.22
12	1.79	1.42	1.14	0.69	2.03	1.63	1.45
13	1.14	1.06	0.86	1.12	1.66	3.24	1.51
14	1.75	0.62	2.10	1.93	1.88	2.14	1.73
15	1.28	1.28	1.48	1.90	1.70	2.43	1.67

APPENDIX 7 (d) : ABSOLUTE NEUTROPHIL COUNTS ($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	2.20	1.11	3.96	1.53	1.27	2.64	2.11
1	2.22	1.34	2.28	2.20	0.73	0.82	1.59
2	1.71	2.64	2.20	4.52	2.46	2.62	2.69
3	1.87	1.44	2.30	2.76	-	1.43	1.96
4	2.05	1.65	4.07	1.31	2.12	1.52	2.12
5	1.15	0.88	4.77	-	1.94	0.48	1.84
6	0.34	1.24	2.19	2.76	1.70	0.54	1.46
7	0.42	1.96	0.53	3.43	1.53	0.63	1.141
8	0.29	1.02	0.56	6.36	1.96	2.18	2.06
9	-	-	0.25	-	1.43	1.40	1.02
10			0.70		-		0.70
11			0.46				0.46
12			1.94				1.94
13			-				
14							
15							

APPENDIX 7 (e) : ABSOLUTE LYMPHOCYTE COUNTS ($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 49	50	53	54	59	74	MEAN
0	4.58	9.84	11.48	7.80	9.07	6.64	8.23
1	4.47	11.31	14.28	7.27	9.51	5.99	8.80
2	6.24	7.81	9.01	6.86	9.59	4.90	7.40
3	6.87	8.73	13.28	7.91	9.24	4.68	8.45
4	6.97	7.01	9.15	-	-	2.96	6.52
5	5.43	5.62	9.27	-	4.52	-	6.21
6	-	4.37	9.84	-	4.53	3.32	5.51
7	5.20	7.30	9.22	4.81	4.37	3.14	5.67
8	4.70	8.01	9.18	4.38	6.07	0.45	5.46
9	4.54	6.95	9.76	7.12	7.73	5.72	6.97
10	4.86	5.29	11.04	10.27	9.22	9.02	8.28
11	7.28	5.36	8.65	10.39	12.73	8.34	8.77
12	10.11	7.48	11.43	7.40	13.57	5.77	9.29
13	13.16	9.54	13.44	6.38	12.14	7.24	10.31
14	8.65	9.48	12.90	7.50	10.62	10.33	9.91
15	5.75	11.39	13.17	8.12	11.40	10.24	10.01

APPENDIX 7 (f) : ABSOLUTE LYMPHOCYTE COUNTS ($10^3/mm^3$) OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER.

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	8.19	6.29	5.94	8.94	5.43	8.39	7.19
1	9.36	8.96	8.32	8.81	5.37	10.88	8.61
2	7.41	5.98	9.40	9.52	6.34	7.66	7.71
3	5.77	5.76	8.85	6.78	-	4.71	6.37
4	4.15	4.75	6.93	3.99	5.11	5.17	5.01
5	2.45	4.02	6.33	-	6.07	4.08	4.59
6	2.76	1.86	3.88	6.05	5.60	-	4.03
7	2.08	6.94	3.78	5.01	4.37	5.07	4.54
8	2.11	-	3.48	5.40	3.64	6.91	4.30
9	-		4.65	-	4.27	-	4.46
10			6.30		-		6.30
11			8.74				8.74
12			8.86				8.86
13			-				
14							
15							

APPENDIX 8 (a) : DAILY MEASUREMENT OF MEAN CORPUSCULAR VOLUME OF SIX CALVES INFECTED WITH BOVINE PETECHIAL FEVER (B5)

(RECOVERING CALVES).

DAYS AFTER INFECTION.	ANIMAL NUMBERS.						
	49	50	53	54	59	74	MEAN
0	44.8	32.5	45.0	43.0	42.0	41.3	42.82
1	45.0	34.5	43.7	48.2	42.4	45.5	44.95
2	46.3	35.4	44.7	42.6	38.4	42.0	41.92
3	43.6	33.2	46.2	44.8	40.0	41.7	42.63
4	41.5	30.7	44.7	43.6	38.6	40.0	41.10
5	48.2	40.3	43.0	45.6	45.2	41.0	43.06
6	47.8	43.9	44.8	44.2	42.4	42.8	43.33
7	49.0	44.0	45.0	46.5	46.7	46.2	46.68
8	48.0	45.5	54.0	44.3	41.0	41.5	45.76
9	41.5	49.0		51.0	45	41.6	44.77
10	46.0	43.2	43.0	42.5	52.0	39.0	44.50
11	51.0	43	44.5	55.0	45.2	40.0	47.14
12	51.0	39.9	48.0		46.2	40.0	46.30
13	49.0	39	43.0	53.5	47.0	41.5	46.80
14	51.0	40.5	42.7	46.0	49.0	41.5	46.04
15	48.0	41	47.3	51.0		42.8	47.27

APPENDIX 8 (b) : DAILY MEASUREMENT OF MEAN CORPUSCULAR VOLUME OF SIX CALVES INFECTED WITH BOVINE PETECHIAL FEVER (DYING CALVES). (D^3)

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	40.5	45.0	39.2	33.0	38.4	43.4	40.05
1	40.0	41.5	38.2	43.0	39.0	45.6	41.82
2	42.2	43.4	36.7	40.0	38.2	44.7	40.86
3			38.0	42.5		46.5	42.33
4	43.7	39.4	39.5	44.0	37.8	47.0	41.90
5	42.0	39.8	43.8	42.4	40.7	44.5	42.16
6	43.0	41.0	42.0	44.2	43.1	42.4	42.55
7	42.0	44.0	41.0	42.0	43.3	43.7	42.66
8	40.5	43.2	41.5	43.7	39.4	51.0	43.22
9			47.0		42.2		44.60
10			47.8				47.8
11			37.6				37.6
12			47.0				47.0
13							
14							
15							

APPENDIX 8 (c) : DAILY MEASUREMENT OF THE MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION OF CALVES INFECTED WITH BOVINE PETECHIAL FEVER. (%)

DAYS AFTER INFECTION.	ANIMAL NUMBERS.						
	49	50	53	54	59	74	MEAN
0	33.9	30.5	35	33.2	35.0	33.1	33.45
1	36.0	33.2	34.5	32	28.8	34	33.10
2	34.8	34.5	32.3	40	31.5	32.8	34.30
3	41.6	32.5	32.1	33.1	32.7	33.2	34.2
4	39.2	33.5	33	35.6	36	33.4	35.1
5	37	35.6	33.5	28.4	31.4	32.7	33.1
6	37	35	32.0	31.4	32	30.5	33.0
7	37	36	35	27.1	32.2	33.3	35.1
8	41.5	35.5	32.5	30	32.1	30.0	31.9
9	35.4	34.6	33.0	33.6	35.0	33.7	34.2
10	34	34.7	32.5	32.8	30.6	33.1	32.9
11	35	35	31.6	29	31.9	33.4	32.6
12	35	36.8	34	30.8	32	33.4	33.6
13	34	35	34.2	27.7	30	30.8	33.6
14	33.5	37	39.6	30	31.2	31.7	32.0
15	34.3	39.0	33.5	31.3	27	31.8	32.6

APPENDIX 8 (d) : DAILY MEASUREMENT OF THE MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION OF SIX CALVES INFECTED WITH BOVINE PETECHIAL FEVER. (%)

DAYS AFTER INFECTION.	ANIMAL NUMBERS. 40	42	52	55	58	72	MEAN
0	33.6	32.9	36	36.5	35.5	33.8	34.7
1	36.5	34.1	36.4	34.4	34.4	33.5	34.9
2	34.6	33.3	35.6	33.0	36.5	35	34.6
3	33.8	35	38	33.0	31.0	33.0	33.9
4	34.4	35.1	35.5	33.2	36.2	32.8	34.5
5	34.1	33.6	33.4	29.2	31.3	34.0	32.6
6	33.2	30.7	35	33.3	32.6	35.1	33.3
7	34.8	29.8	35	38.0	32.3	33.0	33.8
8	32.6	32.7	35	35.8	33.8	32.6	33.7
9			33				33.0
10			31.2				31.2
11			37				37.0
12			32				32.0
13							
14							
15							

APPENDIX 9 : DAILY MEASUREMENT OF SERUM ALKALINE PHOSPHATASE (S-F.Units) OF THREE CALVES INFECTED WITH BOVINE PETECHIAL FEVER AND TWO CALVES USED AS CONTROLS

DAYS OF SICKNESS.	ANIMAL NUMBERS. INFECTED 41	42	74	CONTROLS 43	44
1	1.00	0.90	1.38	0.70	1.45
2	1.10	0.98	1.44	2.50	1.23
3	1.10	1.00	1.60	1.36	2.24
4	1.38	1.35	1.90	1.37	2.08
5	1.20	Died.	0.90	0.75	1.80
6	1.07		0.80	1.50	0.80
7	1.07		0.80	0.60	1.20
8	0.90		0.70	0.60	0.75
9	1.20		0.90	1.10	1.25
10	1.10		1.62	1.20	1.15
11	1.08		1.55	0.96	1.37
12	1.12		1.70	1.13	0.98
13	1.00		1.60	0.75	1.15
14	0.98		1.30	1.30	1.25
15	1.05		1.45	1.20	1.08
	Recovered.		Recovered.		