

STUDIES ON THE LESIONS AND PATHOGENESIS OF EAST  
COAST FEVER (*THEILERIA PARVA* INFECTION) IN CATTLE,  
WITH SPECIAL REFERENCE TO THE LYMPHOID TISSUE.

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INTRODUCTION.

The study of the protozoal parasite, *Theileria parva*, the cause of East Coast fever, has engaged the attention of many investigators since it was first noticed by Koch in 1898. Studies on the life-cycle of the parasite in ticks have been carried out by Cowdry and Ham (1932), whilst Gonder (1909-1910, 1911), Meyer (1909-1910), Steck (1928), and Cowdry and Danks (1933) studied the development of the parasite in susceptible cattle, and the lesions it provoked.

Gonder described the agamogenous and gamogenous cycles of the parasite within the host, and these were well illustrated in several of his plates. The appearance of the schizont or "Koch Body" in the large mononuclear lymphocyte was referred to, and Gonder maintained that the segmentation of the parasite in the lymphocyte led in most cases to the destruction of the cell. Only towards the end of the disease, did the erythrocytic stage of the parasite cause a slight decrease in the number of red cells. He was of opinion that phases of development (so-called micro- and macro-gametocytes), occurred in the red cells and mentioned that the youngest stage of development of the schizont in the lymphocyte might easily be mistaken for cell granules, e.g., azure granules.

According to Meyer, the haemopoietic tissues were mostly affected as regards the distribution of the Koch bodies, or Koch granules. The secondary lesions were of the nature of a metastasis in the liver, kidney, lungs, etc., and often showed the same bodies. He maintained that the endothelial cells of the small capillaries became infected under the influence of the Koch granules, and the secondary endarteritis gave rise to haemorrhages, invasion of the cells and proliferation of the lymphoid elements with an increase of Koch granules.

Steck stressed that marked emaciation was a characteristic symptom in protracted cases of East Coast fever. The follicles of the lymph nodes were enlarged and a large proportion of lymphoblasts and lymphocytes was infected with Koch bodies. The foci of round cells in the kidney were situated either around a small artery or in the immediate neighbourhood of a glomerulus. These foci consisted of infiltrating lymphocytic cells (the majority containing Koch bodies) and sessile cells which formed a reticulum. The whole focus, according to Steck, was nothing else but a rapidly developed lymph follicle in the adventitia of a blood vessel. In the liver periportal interstitium there was a similar, more or less extensive, infiltration with lymphocytes and lymphoblasts, the majority infected with Koch bodies. The lymphopenia, which he observed, was due not only to a retention of lymphocytes in internal organs, but also to their destruction.

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As a result of the infection of the lymphocytes there was a compensatory hypertrophy of the lymphoid tissue. The wandering lymphocyte carried the parasites to a site favourable for their development. The focus was not inflammatory but rather resembled that of a lymphatic aleucaemia. He finally stated that, even when the majority of the red cells were infected, there was very little or no anaemia.

According to Wenyon (1926) Koch blue bodies or schizonts were seen either free, or within endothelial cells of the capillaries. Their extra-cellular position was probably due to the breaking of the cells in the process of film-making. In sections they were always intra-cellular. It was doubtful whether the many *Theileria* in the red cells represented division stages.

Cowdry and Danks described the developmental stages of *Theileria parva* in the bovine. The sporozoites passed in the lymph channels to the nearest lymph nodes, where they gave rise to agamogenous and gamogenous cycles in lymphoblasts and large lymphocytes. The nuclei of the gamonts were smaller, more regularly spherical and had less affinity for blue (Giemsa stain) than the agamonts. The duration of the free life-cycle of the agamont and gamont, how often the cycle was repeated and what facilitated or retarded the changing of agamonts into gamonts were not determined. The swelling of the lymph nodes was ascribed to oedema, and not to hyperplasia. They could not support the view that the parasite acted as a powerful stimulus to the multiplication of lymphocytes. Whether the lymphocytic infiltration in the liver were derived from an emigration of lymphocytes from the blood stream or a proliferation of lymphocytes previously present in the perivascular tissue could not be determined. These authors viewed East Coast fever as an acute infection in which the disturbance of the lymphocytes was probably the most important feature. They also noted the reduction of lymphocytes in the peripheral blood and stated that there was only slight anaemia despite gross infection of the red cells.

Neitz (1948) was of the opinion that the intra-muscular degeneration seen in several cases of East Coast fever was the cause of the muscular wasting, emaciation and general weakness. He also stressed the occurrence of oligocythaemia and icterus and maintained that, besides the hypertrophy and hyperplasia of the cells of the lymphocytic series in the lymph node smears, there was also a hyperplasia of the cells of the histiocytic and monocytic types. According to him the monocytes also contained Koch bodies.

Neitz (1943) stated that in *Theileria parva* infection active multiplication of the lymphocytes occurred, whereas in *Theileria mutans* infection such an active mitosis did not manifest itself.

De Kock *et al.* (1937) and De Kock (1937) were of opinion that the lesions provoked by *Theileria mutans*, under certain conditions, resembled those of East Coast fever. In both there were rapid emaciation and cachexia, the appearance of foci of lymphocytic cells in the kidneys and liver, depletion of lymphocytic cells in the lymph nodes and the presence of erosions in the abomasum. A homogeneous substance occupied the greater part of the lymph nodes in advanced cases. Developing Koch bodies were found only in cells of the lymphocytic series and small lymphocytes became progressively less numerous as the disease developed. Lymphoblasts seemed to predominate in the smears. An oligocythaemia was significant in all the *Theileria mutans* cases, the red cell counts varying from 2.38 to 4.5 million per cubic mm. The main difference between *Theileria parva* and *Theileria mutans* infection was that the red cells were parasitized to a lesser extent in the latter disease.

The relationship of "Turning Sickness", investigated by Mettam and Carmichael (1936), to *Theileria parva* infection has apparently not yet been clarified. In this disease of calves, lesions developed in the brain and often led to a partial or complete occlusion of the blood vessels of the pia, caused by masses of lymphocytic cells, often accompanied by perivascular cuffing. Apparently the Koch bodies in the brain did not lead to an infection of the red cells. There was also a scarcity of schizonts in the lymph nodes and spleen.

The following issues raised in the above brief review of the literature were subjected to further investigation and this work forms the basis of this paper:—

- (1) The first pre-erythrocytic infection of the lymphocyte is produced by the sporozoite. What is the nature and duration of the intra- and extra-cellular agamogenous cycles? Are histiocytes and monocytes also involved in the development of the parasite?
- (2) What changes occur in the "primary" lymphoid tissue, namely, in the lymph nodes, thymus, tonsil, spleen, or in solitary or aggregate nodules in the mucous membranes of the respiratory and digestive systems? Which stage of the lymphocytic series is more prone to infection, the mature or immature lymphocytic cells?
- (3) Is the reaction within the lymph node of the nature of a lymphatic aleucaemia or does the parasite provoke serious regressive changes, and a depletion of the lymphocyte cells, which markedly interfere with the function of the lymphoid tissue? Can this explain the characteristic symptoms of the disease, namely, the emaciation and cachexia and the subsequent death of the animal?
- (4) (a) Are the foci of lymphocytic cells in the so-called "secondary" situations, namely in the liver, kidneys, etc., of the nature of a compensatory hyperplasia following the loss of the lymphoid tissue in the lymph nodes, spleen, etc.? Do these secondary foci of lymphocytic cells also become infected with the merozoites present in the blood?  
or
- (b) Are the secondary foci in the kidneys, liver, etc., formed as a result of the proliferation of infected lymphocytic cells that have migrated from the blood?  
or
- (c) Do the schizonts, present in the blood, evoke a proliferation of the lymphocytic cells previously present in the perivascular tissues of the kidneys, liver, etc.?
- (5) At what time and at what rate does *Theileria parva* infect the red cells of the blood and what is the significance of the various shapes of the parasites in these cells? Does a multiplication of the parasite occur within the red cells? (The majority of cells contain several parasites at the peak of infection.)
- (6) A slight oligocythaemia is apparently present during the last stages of the disease. What is its relation to the icterus observed at autopsy? To what extent does lymphopenia occur?
- (7) Are the changes of waxy degeneration, etc., observed in the skeletal muscles, a lesion of East Coast fever or a complication?

MATERIALS AND METHODS.

The strain of *Theileria parva* used for the infection of the cattle, from which specimens and smears were collected for this study, had been maintained by several passages through cattle and *Rhipicephalus appendiculatus* ticks (Neitz, 1948). For the transmission experiments infected nymphae were placed in the ears of cattle, the ticks being controlled by the use of calico bags according to the method described by Neitz. The period of incubation varied from 8-15 days (average 10 days) and the duration of the disease from 10-23 days (average 15 days).

Blood and lymph node biopsy smears were collected from groups of cattle from the 6th day after infestation with ticks until the animal died of East Coast fever or was killed *in extremis*. During the period that the cattle were under observation, biopsy smears were made from the right and left subparotid, prescapular and precrucial lymph nodes. These lymph nodes were pierced with a stout syringe needle through the skin, the contents of the needle transferred to a glass slide and spread out in the usual manner. A drop of blood was also drawn for the preparation of ordinary smears and for differential counts, according to Nesor's method (1923). Impression smears were prepared from the different organs. In the case of the bone marrow, impression smears were made from the ribs. These smears proved of great value in the study of the various cell types, the nature of the pre-erythrocytic infection of the lymphocytic cells and the parasites in the red cells.

In the case of the lymph nodes, specimens and impression smears were collected from the subparotid, prescapular, precrucial, mediastinal and mesenteric lymph nodes in order to ascertain whether there were any differences in the lesions of the lymphoid tissue in various parts of the body. All the blood and organ smears were dried as quickly as possible, fixed with methyl alcohol and stained with Giemsa.

Sections of embedded material were stained with haematoxylin-eosin (H.E.), carbol-iron-haematoxylin (F.E.H.), phosphotungstic-acid-haematoxylin (M.P.A.H), Giemsa (G), Gomoris silver impregnation (G.R.I.), and berliner blue (B.B.).

PATHOLOGICAL ANATOMY.

The following are the chief autopsy findings in respect of the bovines infected with *Theileria parva* in the tick transmission experiments.

Various degrees of cachexia, anaemia and icterus were observed. In a number of cases serous effusion was recorded in the subcutaneous connective tissue, between certain muscle groups, as well as hydrothorax, ascites and hydropericardium. The lymph nodes were markedly swollen and their cut surface revealed a good deal of fluid. Most of them had lost the prominent nodular-like appearance in the cortex. In a few cases there was tumor splenis with hyperplasia of the malpighian bodies. Characteristic grayish white foci were present in the cortex of the kidneys but in the liver these were less apparent macroscopically. They were nodular-like and varied from 0.5 to 1.5 cm. in diameter. Some appeared as slightly raised foci on the surface of the organ under the capsule. They were usually circumscribed. Sometimes congestion, degeneration and small haemorrhages were also present in the kidneys and liver. In a number of cases there were congestion and oedema of the lungs and, in some areas of atelectasis, and/or emphysema. Significant changes were present on the

mucous membrane of the abomasum, and in a few cases the intestine was also affected. These lesions were of the nature of erosions, about 1-2 cm. in diameter, irregular in outline and sometimes covered with small haemorrhages. Various parts of the gastro-intestinal tract revealed hyperaemia, diffuse or in patches, and small haemorrhages. Slight acute catarrhal inflammation was sometimes present.

From the above it would appear that the following were the more constant and specific changes in acute East Coast fever: cachexia, icterus, oedema of the subcutis, skeletal muscles and serous cavities, swelling and regressive changes of the lymph nodes, foci in the kidneys and liver and erosions in the abomasum.

*Changes in the Blood and Lymph Node Smears Prior to Death.*

Many controversial matters relating to the origin, development, classification and function of the lymphocytic series of cells will not be dealt with. In this study it is accepted: (a) that the "free" reticulum cells, lymphocytes, and plasmacytes, are derived from the "fixed" reticulum cells of the reticular tissue; and (b) that the mature cells of the blood do not change into other cell types, neither do the small mature lymphocytic cells differentiate into plasmacytes, monocytes, etc.

The free reticulum cell is characterised by a large amount of a light grayish cytoplasm, with a less dense, small spherical leptochromatic nucleus with nucleoli. The large and medium immature lymphocytic cells are believed to differentiate from the reticulum cells. They show the presence of a larger round nucleus with denser chromatin and nucleoli, surrounded by a fair amount of intense blue cytoplasm, when stained with Giemsa. The small and medium mature lymphocytic cells resemble the lymphocytes of the blood and are characterised by the presence of a round trachychromatic nucleus, surrounded by a narrow seam of light blue cytoplasm in which azure granules are not infrequently present.

The distribution of the various cell types in a good impression lymph node smear from a clinically healthy bovine, 1-2 years old was found to be more or less uniform. The majority of the cells were small and medium mature lymphocytic cells with a few large immature lymphocytic cells were dispersed amongst them. Neutrophiles, monocytes and macrophages were present from time to time. The average number of lymphocytes, monocytes and granulocytes in the blood of a young clinically healthy bovine was found to be: lymphocytic cells, 55-65 per cent; monocytes, 5 per cent; neutrophiles, 25-40 per cent; eosinophiles, 1-5 per cent; basophiles, 0.5 per cent.

Unfortunately the majority of the lymph node impression smears, made from the same lymph node at various intervals during the course of the disease were unsatisfactory. The developmental stages of the different types of cells could not be followed in most of the cases, neither was it possible to ascertain how long the life-cycles of the gamonts and agamonts lasted. This was mainly due to the presence of a large amount of blood in a number of the films, which almost completely swamped the lymphocytes and the other cell types. In some of the smears there were fragments of cells, and it was not possible to indicate to what extent this was due to damage caused by the pre-erythrocytic stage of the parasite in the lymphocytic cell, or due to the technique applied in making the biopsy smears. It was, however, possible to observe certain changes in a number of smears of the lymph node and blood in this series.



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From the earliest or youngest stage of infection of the lymph node by the sporozoite of *Theileria parva*, the appearance of the lymphocytic cell types became more heterogeneous. This was mainly due to a gradual decrease in the number of small mature lymphocytic cells, and an increase in the number of the larger immature lymphocytic cells (see Plate I). In these cells the earliest or youngest schizont development could be traced. At this stage the infection was slight and Koch bodies could only be found after prolonged search.

The youngest stage of the agamont in the lymphocyte was represented by one or a few well-developed chromatin granules, surrounded by a small indefinite amount of a light grayish cytoplasm. They were usually situated in the perinuclear area of the cytoplasm of the immature lymphocytic cell. As the disease progressed, more lymphocytic cells became infected and the agamonts increased in size and numbers. Their cytoplasm assumed a deep blue colour, and they became well circumscribed in the cytoplasm of the lymphocytic cell. It was unfortunately impossible to state how long the agamogenous cycle lasted and how often it was repeated, because the first cycle of gamonts gradually overlapped that of the agamonts. During the last stages of the disease the gamonts dominated the picture. They could readily be differentiated from the agamonts by their smaller, more frequent and round chromatin granules, embedded in a cytoplasm that was lighter in colour when compared with that of the agamont. During the course of the development of these stages the medium-sized immature cell became more frequent, and was the cell type mainly infected with Koch bodies. In the lymph node smears, cell debris manifested itself as fragments of bluish cytoplasm and as irregular leptochromatic remains of the nuclei of disintegrated cells (see Plate II). Schizonts were sometimes found attached to these damaged nuclei (see Plate I). The presence of extracellular Koch bodies became more prevalent, and some of these were in the process of dissemination. It is doubtful whether the majority of these freed bodies were dislodged during the film making process, as similar extracellular agamonts and gamonts were also identified in the sections of the lymph nodes. Pre-erythrocytic stages were also seen in dividing cells of the lymphocytic series (see Plate IV).

Prior to and during the early development of the schizonts, some of the lymphocytic cells in the lymph node smears revealed irregular eosin-stained granules in their cytoplasm (see Plate I). These were irregular in size, and appeared in clusters of 6-12 granules. They were unlike the usual azure granules of the lymphocytic cells. The nuclei of the youngest typical agamont could be differentiated from these eosin-stained clusters by their larger and more regular size and their being surrounded by cytoplasm as described above. Similar eosin-stained clusters were also observed in some of the lymphocytic cells in the blood smears. The significance of these granules at certain stages of the disease is at present not understood.

At no stage of the disease could schizont development be identified in monocytes or histiocytes. Phagocytosed cell debris with schizonts, as well as red cells with *Theileria*, were, however, occasionally seen in macrophages.

Before and during the development of the pre-erythrocytic stages of the parasite in the lymph nodes, macrophages and neutrophils were observed in varying frequency. In this series of impression smears from some of the lymph nodes made at frequent intervals, the repeated insertion of the needle caused haemorrhage and injury of the tissue. This resulted in phagocytosis of disintegrated, injured cells and of the freed red cells. In some cases this was observed prior to any possible injury by the schizonts.

One of the earliest changes observed in the blood smears, made from the jugular vein during the course of the disease, was a reduction in the number of the mature lymphocytic cells. In some of the blood smears there was an apparent increase in the number of the monocytes and it was often difficult to differentiate some of these from the larger lymphocytic cells. In some cases an increase in the number of the eosinophiles was present during the early stages of the disease. During the later stages and prior to death, the majority of the lymphocytic cells showed the presence of Koch bodies (see Plate II). At this stage disintegrated free nuclei of lymphocytic cells were not infrequent in the blood smears. If the number, distribution and nature of these nuclei were taken into consideration, it would seem that some of them were not caused during the process of film-making. In the sections of internal organs, such as the liver and lungs, infected lymphocytic cells with schizonts and cellular debris were of frequent occurrence in the sinusoids and central veins of the liver and in the alveolar capillaries of the lungs. Presumably the presence of infected lymphocytic cells, and fragments of disintegrated nuclei in the blood, was due to a discharge of these cells from internal organs.

Theileria usually appeared in the red cells before Koch bodies were seen in the lymphocytic cells of the blood. At first the Theileria were rare but from then onwards a fairly rapid increase occurred, not only in the number of red cells infected, but also in the number of parasites in the individual red cells. In the majority of cases just prior to death, practically all the red cells were parasitized. The Theileria in the red cells during the peak of infection were of variable size and shape being ring-shaped, oval-shaped, pear-shaped or long and narrow, either straight or bent in form. In the same red cell several shapes were present at the same time, and it was not possible to state whether these were associated with developmental stages within the erythrocyte or not (see Plate II).

The red cells, even at the peak of infection, presented very slight morphological changes. From time to time a slight anisocytosis was present and in a few cases a small number of polychromatic erythrocytes was observed. From counts carried out, however, there was a reduction of from 50 per cent to 60 per cent in the number of red cells. There was never any evidence of intravascular haemolysis as seen in babesiosis in cattle.

#### *Changes in the Lymphoid Tissue.*

The changes observed in the lymphoid tissue and in the aggregates of proliferated lymphocytic cells in the different organs could best be considered under two headings:

- A. In those organs in which lymphoid tissue was permanently and primarily present, and functioned under normal conditions, namely: lymph nodes, spleen, thymus, tonsils, the solitary or aggregate nodules in the mucous membranes of the gastro-intestinal tract and bone marrow.
- B. In organs or tissues in which aggregates of lymphocytic cells formed secondarily, namely in the kidneys, liver, adrenals, lungs, and occasionally in the myocardium, skeletal muscles, pancreas, hypophysis, etc. As lymphoid tissue is not regularly encountered in the bovine lung, it was deemed advisable to include the lung in this group. Apart from the permanent centres of the reticular tissue (e.g. lymph nodes, spleen, etc.) there appeared to be a great potential reserve throughout the body, which under stimulation can proliferate and produce the cells found in the lymphoid tissue.

*Changes in the Lymph Nodes.*

There appeared to be some variation in the frequency, size and distribution of the nodules in the lymph nodes in various parts of the body. For that reason five sets of lymph nodes were selected for the collection of material in later cases, namely, subparotid, prescapular, precrucial, mediastinal and mesentric lymph nodes. In the bovine the distribution of the nodules is not only cortical, but also central. The nodules appeared as dense accumulations of lymphocytic cells embedded in the reticular tissue of the lymph node. These nodules passed through cyclic changes and varied from those with the so-called "germinal centres" of Flemming to those which consisted of a compact mass of lymphocytic cells. The central area, or germinal centre, was surrounded by a zone of closely packed, lymphocytic cells, mainly of the small mature type. The germinal centre appeared to be less compact, lighter in colour, and consisted mainly of larger and medium sized immature lymphocytic cells. In the bovine lymph nodes examined in this study the so-called "reaction centres" of Hellman did not seem to manifest themselves.

Lesions were identified in the lymph nodes of all the cases of East Coast fever studied. These changes varied from very slight, in those cases killed during the early stages of the infection, to very extensive. The earliest changes were of the nature of a depletion of the lymphocytic cells. The periphery of the nodules became less dense, probably mainly due to the loss of the small mature lymphocytic cells. The nodules gradually lost their identity, so that the lymph node as a whole assumed a more open reticulum, filled mainly with irregularly distributed large and medium sized lymphocytic cells. As the disease progressed, a homogeneous hyaline-like material, which stained with eosin, appeared, first as small foci in the cortical and central portions of the lymph node. Later larger areas were found, often in and around the original nodule, and along the periphery of the cortex (see Plate VI). The smaller foci consisted of clusters of necrosed lymphocytic cells. These stained a very faint pink with eosin, and in some of them faint outline of their nuclei or fragments of their nuclei could be recognised. In places only fragments of cytoplasm and nuclei were observed. The number of schizonts in large but more especially in medium-sized lymphocytic cells, increased. This was more or less comparable with the findings in the lymph node smears described above. Between these cells and cell debris, extra-cellular Koch bodies could be identified. These intra-cellular and extra-cellular bodies could best be demonstrated in the sections stained with G.R.I. stain.

Oedema and fibrin threads in variable amounts were present, and were usually associated with the damaged lymphocytic cells in and around the original nodules, the periphery of the cortex, and the region of the hilus. Some of the thickened threads were probably swollen reticulum fibres. With the G.R.I. stain, it appeared as if some of the reticulum cells were swollen and showed a number of small bluish granules.

The subcapsular and medullary sinuses of the lymph nodes, especially in the later stages of the disease, stood out as prominent "channels" in contrast to the much depleted cortex. The dilation of these sinuses was due to the presence of large numbers of cells, mainly of the lymphocytic series. These were apparently mainly discharged from the rest of the lymph node. In these sinuses lymphocytic cells with schizonts could be identified, as well as fragments of disintegrated cells, and extra-cellular Koch bodies.



Macrophages, with the remains of damaged cells, were sometimes not infrequent in these sinuses. In a few cases Koch bodies of the disintegrated cells, and even red cells with *Theileria*, could be identified in the cytoplasm of a few of these phagocytes. When the extent of the damage of the lymphocytic cells, caused by the schizonts, was compared with the number of macrophages present, it would appear that phagocytosis was not a striking feature and that it did not play a significant part in the removal of the damaged cells.

In the capsule of a number of the lymph nodes, and to a certain extent in the trabeculae, aggregates of mature and immature lymphocytic cells were observed around the walls of the small blood vessels. In some cases these cells were present in large numbers, with intra- and extra-cellular Koch bodies, and a certain amount of cell debris. In certain pathological conditions in bovines, Furuta (1949) referred to the presence of lymphocytic and reticular cells in the capsule of some of the lymph nodes, where they formed intracapsular islands. He was of opinion that these cells emigrated from the subcapsular sinus, through eroded portions of the capsule. It would, however, appear that these lymphocytic cell aggregates in the capsule and trabeculae of the East Coast fever lymph nodes were of the nature of a proliferation of perivascular stem cells.

#### *Changes in the Spleen.*

Snook (1950) regarded the lymph nodules of the Malpighian corpuscles (or white pulp) as rounded extensions of the lymphoid sheath that enveloped the central artery. From this artery, the capillaries of the nodules arose, and in the bovine the emerging penicilli pursued rather straight courses through the red pulp. According to him the lymphoid sheaths in the bovine were broad, and there were usually several nodules present. These were separated from the red pulp by a diffuse zone of reticular tissue, the so-called marginal zone which was distinct in the bovine.

The lesions observed in the spleen of cattle infected with *Theileria parva* were mainly associated with lymphoid tissue of the white pulp. They were similar to those described in the lymphoid tissue of the lymph nodes. The changes varied from slight in the early stages of the disease, to very extensive lesions in the white pulp during the later stages, and prior to death. The earliest change was of the nature of a gradual depletion of the lymphocytic cells in the white pulp, and a gradual change in the appearance of the lymphoid nodules, probably mainly due to the disappearance of the mature lymphocytic cells. The periphery of the white pulp assumed an irregular and frayed-out appearance, and eventually it was not possible to demarcate the limits of the white and red pulps, i.e. the marginal zone could no longer be defined. There was an irregular discharge of lymphocytic cells from the white into the red pulp, where they were irregularly dispersed amongst the red cells (see Plate VII).

In some cases there was a good deal of blood in the red pulp and small haemorrhages were seen. Some nodules only revealed the lymphoid sheath with a small number of lymphocytic cells in its meshes. In the lymphocytic cells Koch bodies could be identified, and they were of frequent occurrence in the later stages of the disease.

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Small aggregates of necrosed lymphocytic cells with a variable amount of oedema and fibrin were also present. This presented itself as a hyaline-like, eosin-stained material in and around the former nodule. This material sometimes extended into the red pulp. Debris of cellular disintegration and extra-cellular schizonts were also present. The frequency of *Theileria* in the red cells varied from rare, to cases in which the majority of red cells were infected with several parasites of different sizes and shapes in the same red cell. The extent of infection of the red cells was well illustrated in the spleen smears, which also showed the frequency of intra-cellular and extra-cellular schizonts. These were mainly of the gamont type, and immature medium-sized lymphocytic cells were mainly infected. Many of the gamonts were in the process of dissemination into the so-called merozoites.

Macrophages were infrequently present in the sections and smears, and showed the presence of debris of disintegrated cells. The macrophages of the red pulp did not reveal any excessive erythrophagocytosis. Here again it would appear that the majority of damaged lymphocytic cells disintegrated and shed their cytoplasm, instead of being disposed of by the process of phagocytosis (see Plate V).

### *Changes in the Thymus and Tonsils.*

In the limited number of cases in which specimens and smears were collected from these organs, lesions were seen. These lesions to a certain extent, resembled the less advanced changes, they varied from a slight to a fairly well-marked depletion of the lymphocytic cells, and in places the lymph spaces were dilated with discharged cells. In the lymphocytic cells schizonts could be identified, and in some cases a large number of cells was infected. This was well illustrated in the impression smears, in which the majority of lymphocytic cells showed the presence of Koch bodies. Fragments of disintegrated cells, as well as extra-cellular schizonts, were also present. In the sections from some of the cases, clusters of necrosed lymphocytic cells with oedema and fibrin were observed. In the case of one thymus, small haemorrhages were present, and in one of the tonsils, there was a complication of an acute tonsillitis.

### *Changes in the Bone Marrow.*

Unfortunately the majority of the sections of the bone marrow specimens were not satisfactory, and an opinion had to be based mainly on the results of the examination of the impression smears. In the available sections it was not possible to identify any nodular-like proliferation of lymphocytic cells as seen in some of the other organs. In the sections of some of the cases there appeared to be a depletion of the immature cells of the erythrocytes and the granulocytes, and an apparent increase in the frequency of the cells of the lymphocytic series. The impression smears of the bone marrow from the rib showed a good deal of variation. Lymphocytic cells with schizonts were present in all of them. In some cases the majority of the lymphocytic cells, mainly immature cells, were infected. There were also numbers of extra-cellular schizonts present, and debris of disintegrated cells. The lymphocytic cells usually predominated; on the other hand there was a paucity of cells of the erythrocytic and granulocytic series.

### *Changes in the Abomasum.*

Practically every bovine that died of East Coast fever, or was killed *in extremis*, showed multiple erosions (not ulcers) on the mucous membrane of the abomasum, that did not penetrate deeper than the muscularis mucosae. Their

average size was about 1-1½ cm. in diameter; they were very irregular in outline and sometimes showed slight superficial haemorrhage. Erosions were rare in the intestines.

The erosion was of the nature of a depression on the mucus membrane, as a result of a localised superficial necrosis. Between the base of this necrotic area and the muscularis mucosae, which was usually intact, there was an extensive proliferation of lymphocytic cells in the propria. This caused a certain amount of pressure atrophy of the glandular tissue. The necrosis of the mucous membrane was probably due to circulatory disturbances, caused by the pressure exerted by the clusters of lymphocytic cells on the vessel walls. In the majority of these lymphocytic cells, mostly of medium size, schizonts were seen as well as cells in the process of disintegration. In some cases, this proliferation of lymphocytic cells was also present in the submucosa, where it was associated with the walls of small blood vessels. Proliferation of lymphocytic cells were also observed in sections of the abomasum and intestine, without any evidence of any erosion or superficial necrosis.

#### *Changes in the Kidney.*

Except in one case, the proliferations of lymphocytic cells only occurred in the cortex of the kidney, and extended from the region of the blood vessels between medulla and cortex to the capsule. In the one case referred to above, there was an aggregate of lymphocytic cells in the medulla close to the cortex. These aggregates in the cortex varied in size and shape. (See Plate III.) In some cases they were nodular-like, whilst in others they were distributed in streaks from the medulla to the capsule. In some instances the lymphocytic cells were irregularly dispersed between the tubuli in the cortex. These proliferations were usually found around the walls of the interstitial blood vessels, and distributed around a number of the glomeruli (see Plate VIII). When large, these aggregates caused a certain amount of atrophy of the tubuli and degeneration of the tubular epithelium. Sometimes the degenerative changes were fairly extensive. In a number of cases there was congestion present and occasionally small haemorrhages were seen.

The majority of the lymphocytic cells appeared to be of medium size and immature. They were embedded in a reticulum, which had apparently been formed from the stem cells of the reticular tissue. The lymphocytic cell types were probably derived from this tissue as in the primary lymphoid tissue depots. Mitosis was not infrequent and intra- and extra-cellular schizonts could be identified. The type of lymphocytic cell most frequently involved in this infection with Koch bodies was the immature, medium-sized cell as verified in the impression smears. In some cases practically all the lymphocytic cells present in the smears showed the presence of schizonts. Freed, vacuolated epithelial cells, as well as their freed nuclei, neutrophiles and macrophages were not infrequently seen in the impression smears of the kidney.

#### *Changes in the Liver.*

In the liver, proliferations of lymphocytic cells were regularly found in the perilobular or periportal zone, where they were associated with the walls of the blood vessels and bile-ducts. In some cases these cell aggregates extended into the capsule of the liver. They varied in frequency, size and shape. These nodular-like aggregates never assumed the structure of a typical nodule of a lymph node.

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As in the kidney these newly-formed lymphocytic cells were embedded in a reticulum. They were not associated with any proliferation of the connective tissue or of the bile-ducts. In a few cases a slight reticulosis together with aggregates of lymphocytic cells were observed. These cell accumulations encroached on to the liver parenchyma, where they caused a certain degree of atrophy and degeneration of the adjacent liver cells. In certain cases there was schizonts, and extra-cellular Koch bodies and remnants of disintegrated cells were without nuclei. In the uncomplicated cases there was no evidence of any undue biliary stasis in the liver. Congestion was also observed and, infrequently, small haemorrhages. The majority of the lymphocytic cells showed the presence of schizonts, and extra-cellular Koch bodies and remnants of disintegrated cells were also observed. In the impression smears, it was seen that a certain percentage of these cells was immature, medium-sized lymphocytic cells. In the congested sinusoids and central veins, large numbers of lymphocytic cells, and less frequently macrophages, were present in a number of cases.

### *Changes in the Adrenals.*

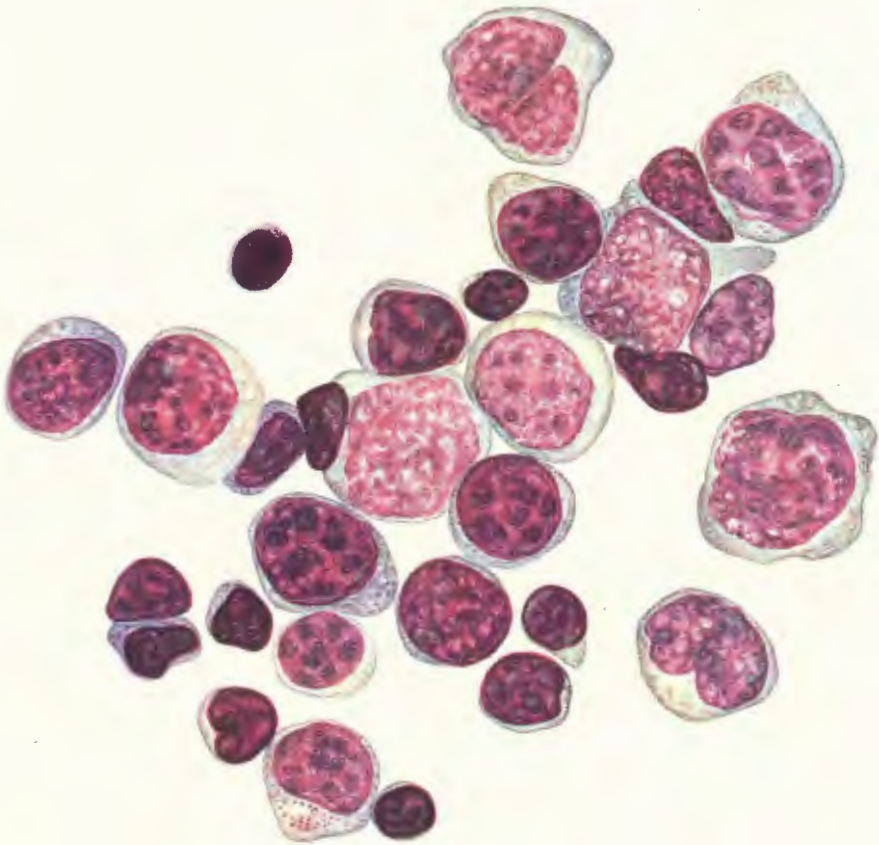
The adrenals were regularly affected with proliferations of lymphocytic cells which varied in distribution, frequency, size and shape. Sometimes they were nodular-like in appearance but more often irregularly distributed in streaks between the columns of the parenchymal cells. In some cases the medulla and all the zones of the cortex, as well as the capsule, were affected. In practically every instance the zona reticularis was implicated. In one case practically the whole of the gland was involved, except for small irregular portions of the zona fasciculata. The distribution of these cell accumulations should be studied in serial sections in the different organs to obtain a more accurate picture of their frequency and distribution. In the impression smears lymphocytic cells were present almost exclusively, besides a few freed nuclei of the gland cells. The majority of these lymphocytic cells were immature, medium-sized, and showed the presence of schizonts. Large aggregates of these cells caused a certain amount of atrophy and degeneration of the adjacent adrenal parenchyma.

### *Changes in the Lungs.*

The lungs were sometimes involved in the formation of aggregates of lymphocytic cells, which were associated with the walls of bronchi, bronchioles, or the walls of the related blood vessels. They varied in frequency, size and shape and were sometimes focus-like in distribution. Cells of the lymphocytic series were predominant and as in the kidney, embedded in a reticulum. In a few cases small clusters of necrosed lymphocytic cells were seen in these aggregates. Macrophages and neutrophils, if present, were rare. Koch bodies could be identified, as well as extra-cellular schizonts and debris of disintegrated cells.

The extent of infection of the lymphocytic cells, as well as the presence of the other cell types were well depicted in the impression smears referred to above. There was no evidence of any connective tissue proliferation, but sometimes the newly-formed lymphocytic cells infiltrated between the smooth muscle fibres of the bronchi and bronchioles, and caused a certain amount of atrophy. In the congested alveolar capillaries, numbers of lymphocytic cells, sometimes infected with Koch bodies, were often present. In a number of cases there was a good deal of oedema and hyperaemia of the lungs. In some there were areas of emphysema, and/or areas of atelectasis.

PLATE No. I.

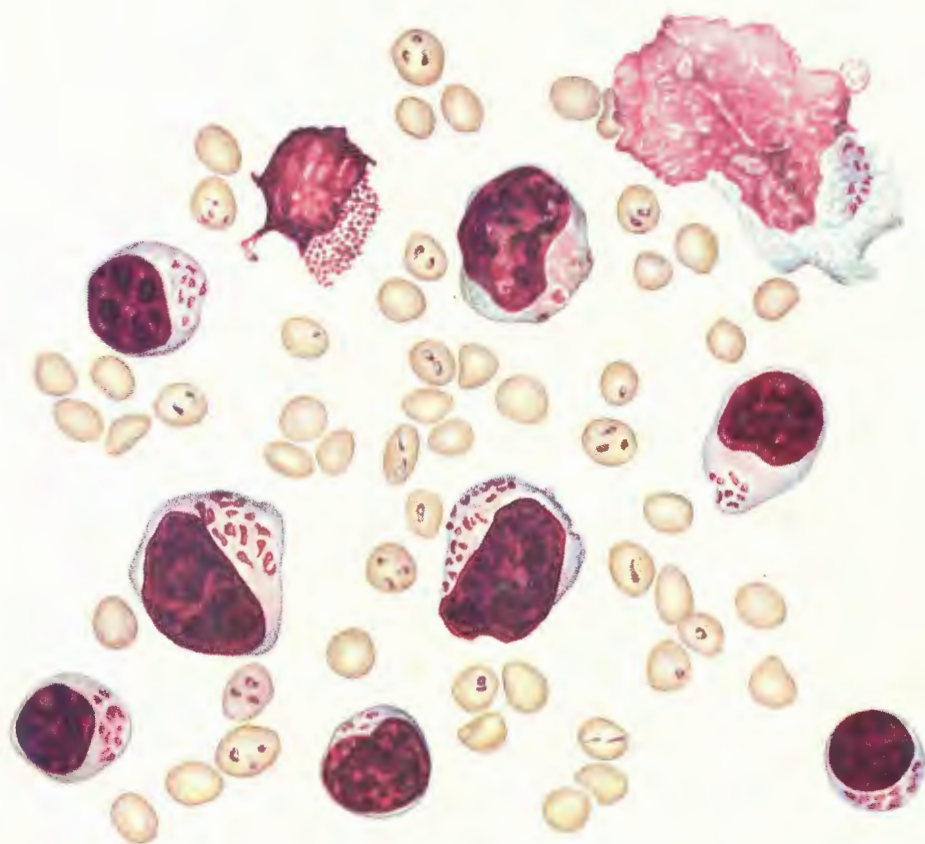


Bovine No. 7964 infected with ticks 27.3.41. Biopsy *lymph node* smear made 1.4.41, and stained with Giemsa. Note the diversity in the sizes and nature of the cells. The small lymphocytic cells have to a fair extent been replaced by the larger immature lymphocytic cells.

(X. 1400.)

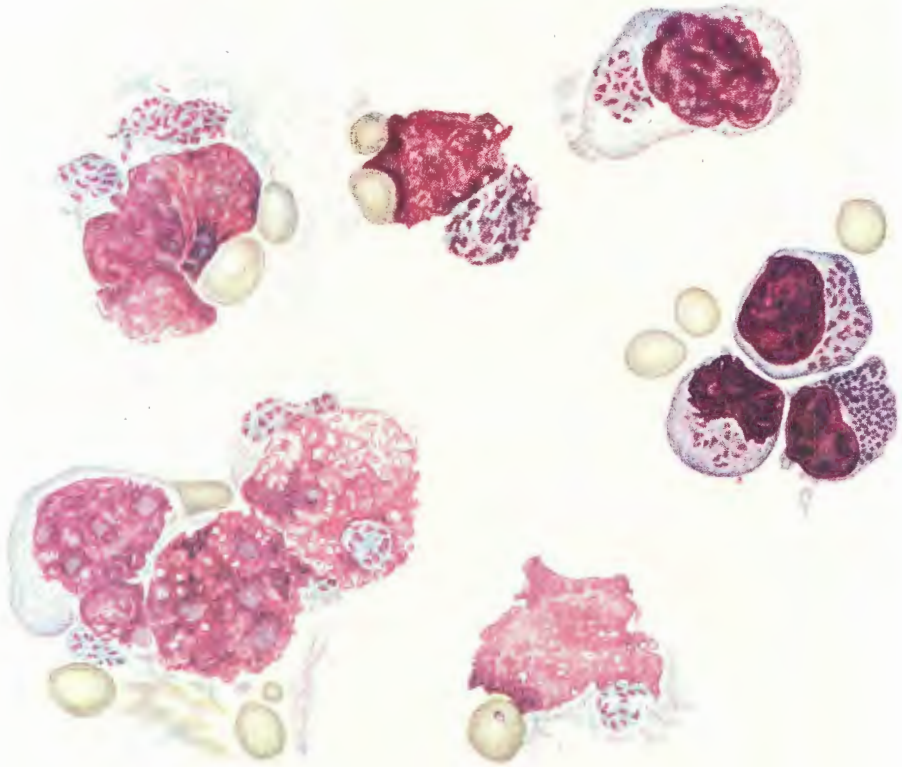


PLATE NO. II.



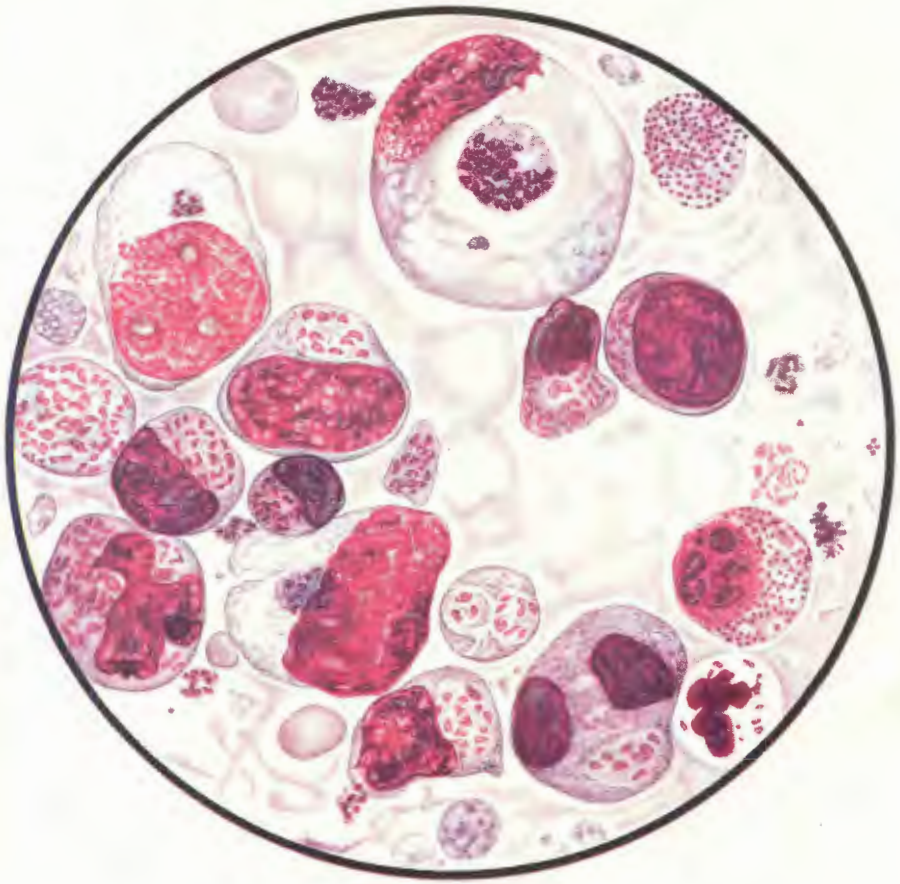
Bovine No. 7964 infected with ticks 27.3.41. *Blood* smear made 7.4.41, and stained with Giemsa, shows about 40 per cent erythrocytes infected with one or more *Theileria*, of various shapes. All the lymphocytic cells show agamonts, except one with a gamont. Note one disintegrating lymphocytic cell with an agamont. (X. 1400).

PLATE NO. III.



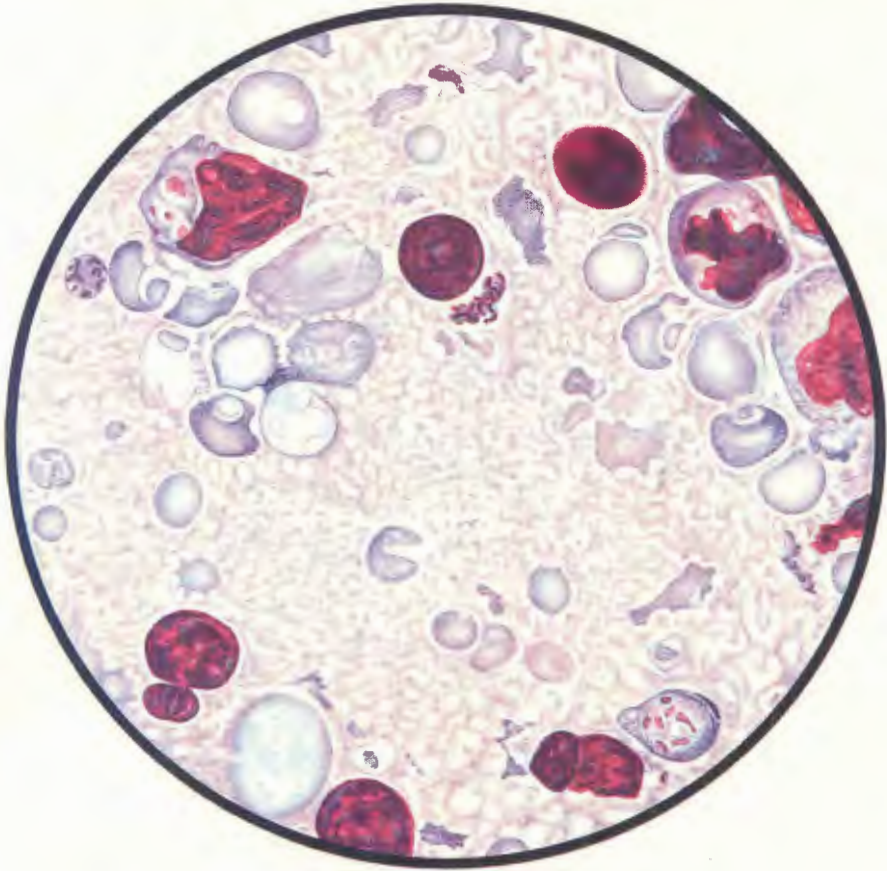
Bovine No. 7964 infected with ticks 27.3.41. Biopsy *lymph node* smear made 8.4.41, and stained with Giemsa, shows a number of disintegrating lymphocytic cells, some immature, with schizonts. Note the dark blue colour of the cytoplasm of the intact lymphocytic cells. (X. 1400).

PLATE NO. IV.



Specimen No. 39698. Bovine No. 5044 infected with ticks 8.9.50, and killed *in extremis* 1.10.50. Impression smear from *prescapular lymph node*, and stained with Giemsa, shows a diversity of sizes of immature lymphocytic cells. Practically all infected with schizonts. Several extracellular schizonts present. (X. 1400).

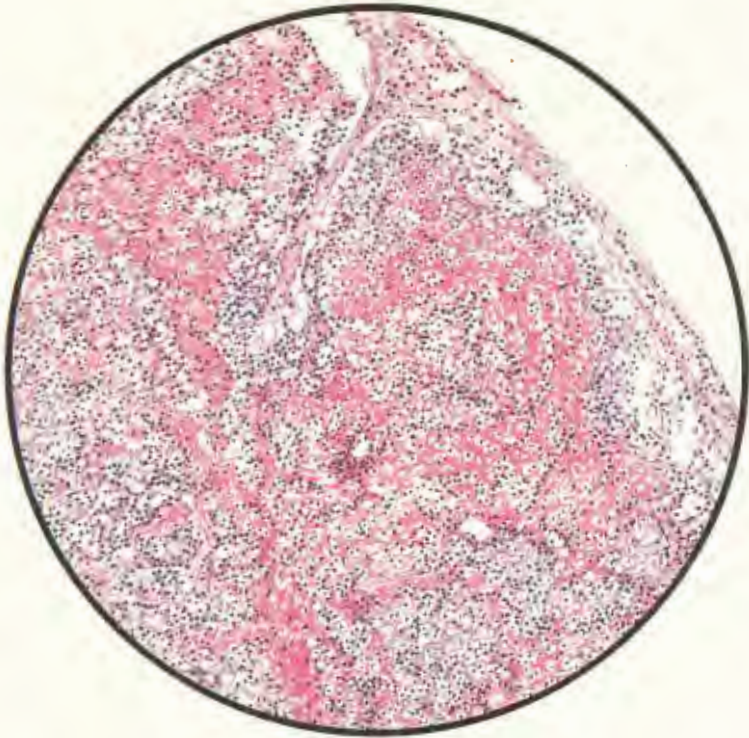
PLATE NO. V.



Specimen No. 39697. Bovine 5038 infected with ticks on 8.9.50, and killed *in extremis* 4.10.50. Impression smear from *mesenteric lymph node*, and stained with Giemsa, shows the presence of disintegrated cytoplasm of lymphocytic cells, and a few free nuclei. (X. 1400).



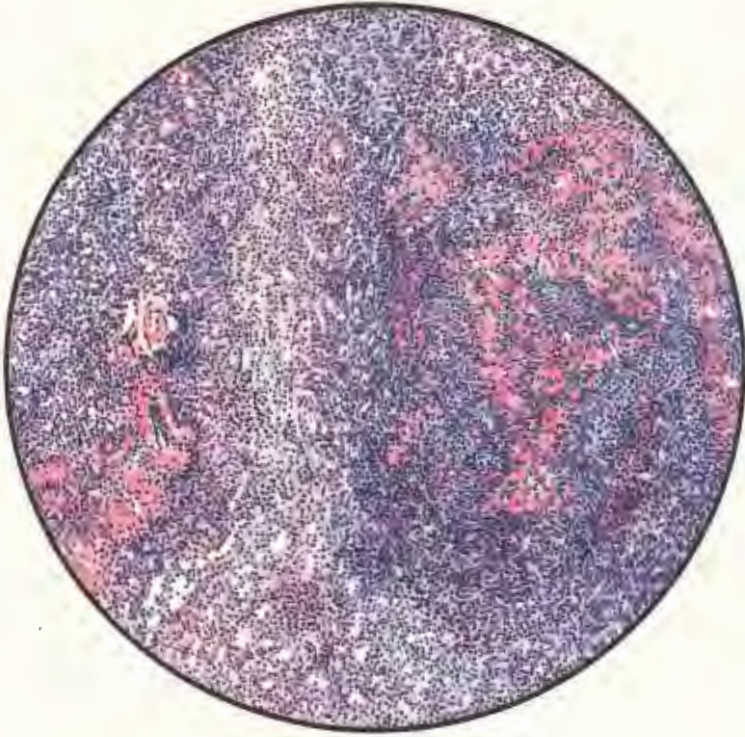
PLATE NO. VI.



Specimen No. 19759. Bovine 7396 infected with ticks 27.5.37, and killed 5.6.37. Section of a *lymph node*, and stained with H.E., shows the loss of lymphoid tissue, and nodular structure. Note the presence of the eosinophilic material. (X. 85).



PLATE No. VII.



Specimen No. 20425. Bovine No. 5620 infected with ticks 6.10.37. Died 1.11.37. Section of the *spleen*, and stained with H.E. As a result of the disintegration of the lymphoid tissue, it is not possible to define the nodular structure, and to demarcate the red and white pulps. Note the presence of the eosinophilic material, and the extension of mononuclears into the red pulp.

(X. 85).

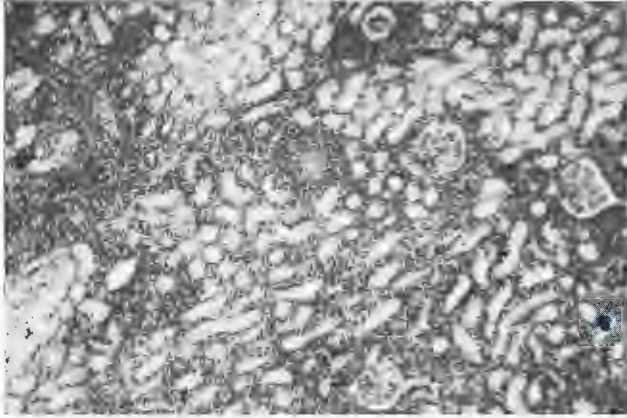


PLATE No. VIII.

Specimen No. 34730. Bovine 2892, infected with ticks 4.6.47, and killed 1.7.47. Section of the *kidney* shows the presence of mononuclears, mainly lymphocytic cells, in the cortex. (X. 80).



PLATE No. IX.

Specimen No. 21234. Bovine 6794 infected with ticks 31.3.38. Died 27.4.38. Section of the *liver* shows the presence of clusters of mononuclears, mainly lymphocytic cells, in the perilobular zone. (X. 80).

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In the remaining organs studied, namely myocardium, skeletal muscle, pancreas, hypophysis and thyroid, proliferation of lymphocytic cells was not seen regularly. In the skeletal muscle, thyroid and pancreas it was rarely observed. Only in one case did the pancreas show extensive proliferation of lymphocytic cells around the blood vessels in the interstitial tissue of the lobules which caused a certain degree of atrophy of the adjacent epithelial cells. Here and there these cells were associated with the presence of fibrin. In the majority of the lymphocytic cells schizonts could be identified.

In two of the eighteen cases examined, the skeletal muscle showed characteristic lesions. These comprised congestion with a few haemorrhages, waxy degeneration and fibrosis. In one case there was also calcification. These lesions were considered to be of the nature of a complication (see later).

In 50 per cent of the cases examined the myocardium showed proliferations of lymphocytic cells. These proliferations were never nodular-like in distribution, nor very extensive, and were associated with the walls of the blood vessels of the interstitium. Koch bodies could be identified in a certain percentage of these lymphocytic cells. It is quite possible, however, that these proliferations might have proved to be more frequent and extensive, if a greater number of specimens of the organs and tissue had been examined in serial sections.

Unfortunately specimens of the hypophysis were collected from only four cases. In two of these, aggregates of lymphocytic cells were present in the stalk of the pars nervosa. They were usually in clusters around small blood vessels, and around the so-called acini (cysts), which were lined with a single layer of cuboidal cells. In the lymphocytic cells Koch bodies could be identified. In the one case the proliferations were fairly extensive, and amongst them disintegrated lymphocytic cells were seen. This was confirmed in the impression smears, which also showed extra-cellular schizonts.

## DISCUSSION.

Steck was of opinion that proliferation of lymphoid tissue was the most characteristic change in East Coast fever. From the present study it would appear, however, that regressive changes in the lymphoid tissue are the striking pathological feature of this disease. The large immature lymphocytic cell appeared to be primarily involved in the earliest development of the schizont, causing a change in the appearance and distribution of the cell types in the lymph node. The number of the larger immature types as seen in the impression smears appeared to be increased at the expense of the small mature forms. Apparently a certain percentage of the damaged immature lymphocytic cells did not reach maturity. Steck referred to the infection of a large proportion of lymphoblasts and lymphocytes, whilst Gonder mentioned the development of Koch bodies in the large mononuclear lymphocyte, which in most cases led to the destruction of the cell. He was of opinion that the youngest stage of the schizont could be mistaken for cell granules, i.e. azure granules. Reference has been made to the occurrence of eosinophilic granules in some of the lymphocytic cells during certain stages of the disease. By their size, staining characteristics, shape and number they could, however, be differentiated from the nuclei of the earliest agamonts, and they did not resemble the azure granules of the lymphocytic cells. They were, however, not unlike the granules observed by Jackson and de Boom (1951) in medium lymphocytes, and designated by them as fine and coarse azure granules (see their Figs. 5 and 6, Plate I).

As stated above the lesions of East Coast fever in the lymphoid tissue were of a regressive nature. These varied from slight changes, such as a depletion of the lymphocytic cells, to those in which the lymphoid tissue, especially in the lymph nodes, was extensively damaged. The development and multiplication of the schizonts within the lymphocytic cells brought about their disintegration with shedding of the cytoplasm and fragmentation of the nuclei. The nodules of the lymphoid tissue in the lymph nodes and spleen lost their identity and the reticular tissue became more prominent. A homogeneous eosinophilic material formed in the meshes of the reticulum, especially in and around the disintegrated nodules. This material was variously composed of oedema, fibrin, necrosed cells, and the debris of disintegrated cells. Some of the reticulum fibres were apparently also damaged. The subcapsular and medullary sinuses of the lymph nodes were dilated and filled with infected lymphocytic cells, remnants of these cells, extra-cellular schizonts, and macrophages, which were, however, never prominent or frequent. In the impression smears of the lymph nodes made prior to death or a post mortem, the majority of the immature and mature lymphocytic cells were infected with schizonts. At this stage the gamogenous cycle predominated.

These changes observed in the lymph nodes of East Coast fever cases and referred to above, resembled those described by Dougherty and White (1945) in rabbits and mice, after the injection of adrenotropic hormone, or adrenal cortical extract. The whole lymph node in their cases was enlarged and distended with fluid. Lymphocytes were in all stages of disintegration. The migration and disintegration of lymphocytes resulted in so marked a depletion of the cortex, that reticular cells were observed more easily, probably unaffected by the hormones. In a further paper Dougherty (1952) indicated that the destructive changes were grouped under the term dissolution of lymphocytic cells, with cytoplasmic budding, which eventually resulted in a lymphocytolysis. The oedematous fluid of hormone treated animals, was loaded with cytoplasmic fragments.

Many schizonts in East Coast fever apparently became freed and led to a further infection of the intact lymphocytes in the same node, or they were carried away in the circulation to infect lymphoid tissue, or newly-formed aggregates of lymphocytic cells in other parts of the body. In the later stages of the disease gamonts apparently became disseminated into merozoites, responsible for the infection of the erythrocytes. During the last stages of the disease, the latter were frequently present in large numbers in the impression smears of the spleen, and apparently many erythrocytes were infected in the red pulp of the spleen. The infection of the red cells progressed rapidly during the last stages of the disease. The view of Wenyon, namely, that the extra-cellular position of the schizonts was due to a breaking up process of the film making, could not be accepted. In these studies extra-cellular schizonts could easily be demonstrated in a large number of sections of the lymph nodes.

Partly due to the overlapping of the agamogenous and gamogenous cycles of the schizonts, it was not possible to indicate how long these lasted, or how often they were repeated. Cowdry and Danks were also not able to determine the extent of the free life of these stages nor how frequently they occurred.

The presence of aggregates of lymphocytic cells in other organs in East Coast fever, has also been described by Meyer (1909-1910), Steck, Cowdry and Danks and others. Meyer was of opinion that the secondary lesions were of the nature of a metastasis in the liver, kidney, etc. The endothelial cells of small capillaries, according to him, became affected under the influence of the Koch bodies, and the

secondary arteritis gave rise to an invasion of the cells and proliferation of lymphoid elements. Steck could not observe any inflammatory changes, but was of opinion that the foci of round cells in the kidney were composed of infiltrating lymphocytic cells (the majority with Koch bodies), and sessile cells, which formed a reticulum. He was inclined to regard East Coast fever to be of the nature of a lymphatic leucaemia.

According to Engelbreth-Holm (1942) the lymphogenous form of leucaemia in cattle prevalent in Germany, was characterised by a considerable hyperplasia of some of the lymph node groups, with only slight changes in the liver, spleen and bone marrow. Typical infiltrations of lymphocytic cells were, however, observed in the heart, lungs, digestive tract and less frequently in the liver and spleen. According to Feldman (1932) lymphoblastoma, common in the bovine species, was confined to the lymph nodes and other depots of lymphoid tissue. Extensive metastasis and secondary involvement occurred in the lungs, adrenals, heart, liver, kidney and even in the brain.

In the text reference has been made to the regular and frequently extensive occurrence of aggregates of lymphocytic cells in the so-called secondary groups of organs such as the kidney, liver, adrenal, lung, etc., in East Coast fever. It was furthermore stated that in the myocardium, skeletal muscle, pancreas, hypophysis, and thyroid, such lesions were infrequent and less extensive. There was no evidence of the brain lesions described by Mettam and Carmichael in theileriosis (1936), and in none of the cases investigated could a proliferation of lymphocytic cells be demonstrated in the central nervous system.

In the kidneys, liver, adrenals, etc., these lesions manifested themselves in definite situations, such as the adventitia of the blood vessels, walls of the bile-ducts and walls of bronchi and bronchioles. They were even identified in the capsule of a number of the lymph nodes. These lymphocytic cells, immature and mature, appeared in the meshes of a reticulum and in no instance did they form typical nodules, with secondary nodules, or "germ centres". They bore no resemblance to the multiple lymphoid nodules with secondary nodules described by de Kock (1929) in the livers of a certain number of splenectomised bovines. In these cases, typical nodules were associated with a system of sinuses, and resembled a haemolymph node.

In the East Coast fever aggregates of lymphocytic cells, mitosis was not infrequent and the majority of the lymphocytic cells, of which many were immature, showed the presence of schizonts. Extra-cellular schizonts, as well as the presence of disintegrated cells and cell debris, could be identified as in the lymph nodes. Again phagocytosis did not play a significant rôle. The breaking up of the damaged cells was of the nature of a dissolution. These changes were well illustrated in the impression smears. Moreover, these aggregates were never associated with a fibrosis. In a few cases a slight reticulosis was identified in the liver. Many infected and disintegrated lymphocytic cells were from time to time seen in the sinusoids and central veins of the liver, and in the alveolar capillaries of the lung. Although the capsule, cortex, and medulla of the adrenal in cases of East Coast fever from time to time showed the presence of aggregates of lymphocytic cells, it was found that the zona reticularis was most frequently involved. Bloom and Meyer (1945) in describing the lymphoma of dogs, stated that besides nodules in the capsule of the adrenal, cellular collections occurred particularly in the zona reticularis, and less frequently in the other zones of the adrenal.



Furthermore, they stated that, although portal lymphomatous infiltrations in these dogs might involve 10-70 per cent of the liver tissue, the liver cells were well preserved but occasionally showed mild fatty changes, and sometimes atrophy in the region of heavy infiltrations.

Apart from congestion, and/or small haemorrhages met with from time to time in some of the organs in cases of East Coast fever, it was rather remarkable that these extensive aggregates of lymphocytic cells, e.g. in the liver, kidneys, lungs, etc., did not cause more advanced regressive changes in these organs. In respect of the erosions on the mucous membrane of the abomasum, it was not clear how the superficial necrosis was caused.

Difficulty was experienced in interpreting the changes found in the bone marrow of East Coast fever cases, especially in the impression smears. Serial sections would have been a decided advantage. In none of the sections of the bone marrow could nodular-like aggregates of lymphocytic cells be identified. In all the impression smears, intra-cellular, as well as extra-cellular schizonts could be identified, and the immature lymphocytic cells appeared to be more frequent. There was an increase of lymphocytic cells in these smears, and an apparent reduction of the granulocytic and erythrocytic cell types. It could not, however, be determined whether there was a suppression of the production of the usual cell types in the bone marrow, and whether the stem cells were involved in a proliferation of lymphocytic cells, or whether this apparent increase of lymphocytic cells was due to a greater influx of lymphocytic cells from the circulation. In this respect the findings of Drinker and Yoffey (1941) and Yoffey (1954) were of interest. According to them lymphoid nodules in mammalian bone marrow were found chiefly under pathological conditions. Scattered lymphocytes could, however, accumulate in the bone marrow in considerable numbers, without any evidence of multiplication under normal conditions. Kracke (1947) was of opinion that under normal conditions, lymphocytes originate from reticulum cells of lymph nodules, but under abnormal conditions they might probably arise from the primitive cells of the bone marrow. The appearance of scattered nodules and lymphoma cells in the bone marrow of some of the dogs affected with lymphoma, was referred to by Bloom and Meyer (1945). They were of opinion that sectioned material gave a more accurate picture of lymphomatous infiltrations in the bone marrow than biopsy preparations.

In the examination of the skeletal muscle of a large number of East Coast fever cases, only two revealed lesions of a muscular dystrophia. It seems doubtful, therefore, whether these lesions could explain the muscular wasting, emaciation, and general weakness, as believed by Neitz (1948). The possibility of a deficiency such as the tocopherol deficiency producing muscular dystrophia in calves reported by Safford *et al.* (1954) merits attention.

In view of the presence of the aggregates of lymphocytic cells found in the pars nervosa of a few pituitaries of East Coast fever cases, the remarks of Shanklin (1951) were of particular interest. He described areas of lymphocytes and lymphoid tissue in 43 out of 100 pituitaries of humans examined. Those for instance found in the pars nervosa were free in the spaces, and he suggested that these areas were a normal finding.

Some of the lymphocytic cells, as believed by Steck, could carry parasites to sites favourable for their development. It seemed doubtful, however, whether such damaged lymphocytic cells present in the circulation were able to emigrate, and

proliferate and form aggregates of these cells in the secondary localities. It seemed more likely that the "stem cell" in the perivascular tissue was stimulated *in situ* to form reticulum and aggregates of lymphocytic cells. In these situations cells in all stages of development could become infected, in the same way as in the lymph nodes.

Aschoff (1926) believed that under certain pathological conditions "lymphoid tissue", as described by him, could be found anywhere in the connective tissue, and as a rule, out of these lymphocytic reactions no real "lymphatic tissue" formed. Maximow and Bloom (1941) stated that new foci of lymphoid tissue could develop in any part of the loose connective tissue in the adult organism. In such cases, lymphocytic cell developed from the mesenchymal elements of adult connective tissue. Thus, apart from the permanent centres of reticular tissue, there appeared to be a great potential reserve throughout the body, which under stimulation could proliferate to produce cells found in the lymphoid tissue. The aggregates of lymphocytic cells in the kidneys, liver, adrenals, etc., in cases of East Coast fever were, therefore, apparently not of the nature of an infiltration of lymphocytic cells from the circulation, but a proliferation of the stem cells *in situ*. Proliferations of lymphocytic cells in the so-called lymphoma or/and lymphatic leucaemia or aleucaemia of the bovine, appeared in the same situations and organs as seen in East Coast fever.

The disintegration and the much reduced function of the primary lymphoid depots probably led to a compensatory formation of lymphocytic cells in various parts of the body. The view of Cowdry and Danks were supported, namely, that the parasite of East Coast fever did not seem to act as a powerful stimulant for the proliferation of the lymphocytic cells. They considered East Coast fever to be an acute developing disease, and the wasting, so characteristic of the disease, might have been associated with the progressive failure of numerous lymphocytic cells to perform their normal function.

The study of the function of the lymphocyte and lymphoid tissue has in recent years received a great deal of attention. Drinker and Yoffey (1941) stated that, if the lymphocytes and the lymphoid tissue suffered extensive destruction, death ensued. Complete blockage of the lymph into the circulation of dogs led to the disappearance of the lymphocytes from the blood. The animals lost weight rapidly, and were killed *in extremis*. Bloom (1948) in his summary of the histological changes in rabbits, guinea-pigs, rats, etc., variously irradiated by both external and internal sources, showed that the organs with lymphoid tissue (lymph nodes, spleen, etc.) were found to be highly susceptible to irradiation.

Dougherty and White (1945) referred to the increased dissolution of lymphocytes in the lymph nodes, following cortical activation. They suggested that this dissolution was a normal process, and that its rate was under the control of the pituitary and adrenal cortex. They mentioned the important rôle of lymphoid tissue in protein metabolism and as a source of blood globulin. In a further paper White (1948) stressed the rôle of the lymphatic tissue in the synthesis and release of certain serum proteins, and its rôle in the body economy. It seemed, therefore, possible that the extensive destruction and elimination of lymphatic cells in East Coast fever interfered with a vital function in the animal body, resulting in the rapid wasting and emaciation, so characteristic of this disease, and the subsequent death of the animal.

Large numbers of lymphocytes formed in the lymphoid tissue reach the circulation, where they apparently have a very short sojourn. The fate of the lymphocytic cells in the blood stream has been raised frequently in the literature without any unanimity having been reached. According to Drinker and Yoffey (1941), and Ehrlich (1946), lymphocytes in the circulation of the dog were replaced twice a day. Ehrlich referred to the various theories that have been advanced as regards the fate of these lymphocytic cells, e.g.: (a) transformation into other cell types, such as monocytes, macrophages, and in the bone marrow into erythrocytes; (b) rapid disintegration within the blood stream; (c) disappearance from the blood stream through the gastro-intestinal tract; (d) they left the blood stream, and returned to the lymph nodes, where they were according to the Heiberg theory, destroyed in the so-called "reaction centres" or "germ centres" of Flemming. According to the latter theory, macrophages engaged in the phagocytosis of the fragmented lymphocytes collected in these "reaction centres" of the lymph nodes. In the present study phagocytosis apparently played an insignificant rôle in the removal of damaged lymphocytes and cell debris. As a result of a so-called "dissolution" of the lymphocytic cells, fewer mature lymphocytes reached the circulation, and this would seem to explain the lymphopenia observed during certain stages of the disease. The views expressed by Neitz, that there was also a hyperplasia of the cells of the histiocytic and monocytic types, and that monocytes showed Koch bodies, could not be substantiated in these studies. The transformation of lymphocytic cells into macrophages or other cell types could not be identified in the primary lymphoid depots, nor in the secondary aggregates of lymphocytic cells, formed in several organs.

It was not possible to establish the nature of the anaemia and icterus described at post mortem in cases of East Coast fever. Only towards the end of the disease did the erythrocytic stage of the parasite, according to Gonder, cause a slight decrease in the number of red cells. Cowdry and Danks indicated that almost all the red cells might be parasitized, and this only resulted in a slight anaemia. Although the majority of the erythrocytes were usually parasitized with several *Theileria* (in the same cell) at the peak of infection, yet only slight morphological changes, such as a slight anisocytosis, and the presence of a few polychromatic erythrocytes, were sometimes observed in these studies. In some animals with evidence of anaemia and killed *in extremis*, only a very moderate *Theileria* infection was present in the blood. These latter cases resembled the acute cases of *Theileria mutans* infection described by de Kock *et al.* (1937) namely extensive destruction of the lymphoid tissue and only a moderate infection of the red cells. It was not possible to state how the infected and presumably damaged red cells were disposed of in the body. According to Wintrobe (1944), it would appear that the manner of the destruction of the red cells was not fully understood, and that a phagocytosis did not seem to suffice as a general explanation of normal blood destruction. In cases of uncomplicated East Coast fever, it was not possible to identify any abnormal pigmentation in the liver microscopically. Neither did the spleen reveal an abnormal erythrophagocytosis and haemosiderosis.

In these studies the significance of the various shapes and sizes of *Theileria parva* could not be assessed. In view of the irregular occurrence of their pleomorphism in the red cells, it was not possible to postulate a multiplication of the parasite in the red cells, neither was it possible to identify sexual stages as described by Gonder.

SUMMARY AND CONCLUSIONS.

1. Regressive changes in the lymphoid tissue appeared to be the striking pathological feature in cattle that died of East Coast fever.

2. The earliest stages of *Theileria parva* seemed to occur mainly in the immature lymphocytic cells. The developing stages of the schizonts brought about a disintegration of most of the lymphocytic cells and the nodules lost their identity. The meshes of the reticulum, and the dilated subcapsular and medullary sinuses showed the presence of infected and disintegrated lymphocytic cells and dislodged schizonts, as well as a certain amount of fibrin, oedema, debris of cells, and infrequently, macrophages.

3. During the last stages of the disease, the impression smears of the lymphoid tissue showed practically all the lymphocytic cells infected with schizonts, chiefly gamonts. Due to the overlapping of the agamogenous and gamogenous cycles, it was not possible to say how long each cycle lasted, or how frequently it was repeated.

4. The damaged lymphocytic cells were apparently eliminated by a process of dissolution, whereas phagocytosis did not seem to play a significant part.

5. Schizonts, carried away in the circulation, probably infected lymphoid tissue, or newly-formed aggregates of lymphocytic cells in other parts of the body. The merozoites, during the last stages of the disease, were frequently present in large numbers in the impression smears of the spleen. It was, therefore, possible that many erythrocytes might have become infected in the red pulp of the spleen.

6. Regular and often extensive aggregates of lymphocytic cells appeared as a secondary involvement in the kidneys, liver, adrenals, etc., and less frequently and less extensively in such organs as the lungs, myocardium, skeletal muscle, pancreas, hypophysis and thyroid. The central nervous system was not involved in these cases.

7. These secondary aggregates of lymphocytic cells were found associated with the walls of the blood vessels, of the bile-ducts or of the bronchi, or bronchioles. They were lodged in a reticulum, and mitosis was not infrequent. These newly formed lymphocytic cells became infected with schizonts and disintegrated, in the same way as described in the lymph nodes.

8. It seemed doubtful whether these infected and damaged lymphocytic cells present in the circulation, were able to emigrate and proliferate to form aggregates in the so-called secondary localities.

9. There appeared to be a "potential stem cell" widely distributed throughout the body, which under stimulation could proliferate to produce cells found on the lymphoid tissue. It would, therefore, seem to be more likely that "stem cells" in various parts of the body were stimulated to form aggregates of lymphocytic cells *in situ*.

10. The elimination of the lymphocytic cells, in the primary lymphoid depots, probably led to a compensatory formation of these cells in other parts of the body.

11. From the brief discussion of a number of papers dealing with the function of the lymphoid tissue, it was possible that in the extensive dissolution of lymphocytic cells in East Coast fever, a vital function in the animal body was interfered with. This apparently was an important contributory factor to the rapid wasting, the emaciation, and the subsequent death of the animal.

12. At present it is not understood how the many infected erythrocytes at the peak of infection were eliminated in the body.

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