HISTOPATHOLOGICAL STUDIES ON CHICK MALARIA INFECTED WITH *P. GALLINACEUM*

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The occurrence of malarial parasites in the various species of monkeys, birds and rats have facilitated the understanding of pathogenesis of malarial infection in these animals. This gives some insight into the probable mechanism in human malaria, where similar lesions occur. Taliaferro (1932) has already drawn attention to the macrophage activity, the general lymphoid hyperplasia in the spleen and tissue localisations met with monkey malaria. Row, Dalal and Gollerkeri (1933) have observed enormous clusters of parasites and pigment in the spleen and other organs. Ray (1953) has reported fatty cystic deposits in liver in monkey malaria when the animals were on low protein diets. It is only in recent years attention has been drawn towards basic disturbances of the host's physiological make-up that develop during the disease (Maegraith, 1951). Any attempt to interpret the various pathological processes that are observed in the host must take into account the parasite, especially from the point of view of its possible competition with the host for nutriments, essential metabolites and oxygen. Again the condition under which parasite exists will inevitably change the physiological environment of the host's tissues. This applies not only to the humoral and cellular aspects, but to the effects of such things as prevailing anæmia and anoxemia and the carriage of and distribution of oxygen by hæmoglobin.

The observations recorded in this paper are based on the histopathology of chicks infected with *p. gallinaceum* and partly on alterations of blood picture observed during the acute phase of malarial infection.

Materials and Methods

Chicks, 8–12 weeks old, were used for the purpose of this study. The strain of the malarial parasite *p. gallinaceum*, was originally obtained from the Southern Branch of the Malaria Research Institute, Coonoor. The method of maintaining the strain in chicks was the same as was reported previously (Ramaswamy *et al.*, 1950).

Histological.—The animals were sacrificed at varying intervals after the appearance of parasites in the peripheral blood and the various tissues like liver, lungs, spleen, kidney and suprarenal were dissected out and fixed in Bouin's fluid as a routine and some were fixed in Zenker-Formol for comparison. Paraffin sections were stained as a routine with Delafield's Hæmatoxylin and Eosin. Iron hæmatoxylin and Van Gieson's stain were used to display connective tissue.

Hæmatology.—Blood and bone marrow smears at varying intervals were made and stained with Leishmann stain as a routine and some of the bone marrow smears were stained with peroxidase stain of Lepehne to identify primordial cells.

Experimental

The material was obtained from 15 cases of acute malaria in chicks in which the dose of parasite was slightly higher than the normal dose (16×10^6 parasites per 0·1 ml. blood) so that the animal usually succumbed before the 10th day of infection. The birds were sacrificed at different periods from the 5th day when usually the parasites appeared in peripheral blood until the last so that events in the pathological process could be followed. The histopathological picture obtained has been illustrated in the microphotographs (Plates I, II and III).

Microscopic Appearances

The Spleen.—The enlargement varied from 2 to 3 times the normal size. The capsule was tense and stretched. Microscopic examination showed no clear-cut capsulitis with cell infiltration, but some times the collagen bundles were separated by œdema. There was no increased trabacular branching, but in a few cases the trabacular bundles were swollen by œdema and the muscular and collagen bundles split up. Cases of frank necrosis were rare.

The malpighian bodies were generally large, numerous and discrete. The definition of the marginal zones was variable. Peri-malpighian hæmorrhage was not observed but a zone of congestion was present around the follicles in many cases. An important feature was the central necrosis in the follicles, but more often it was seen at the periphery of the follicles. The marginal necrosis seemed to affect the cytoplasm of the surrounding cells as a diffuse hyaline transformation with swelling of the cytoplasm and karyolysis of the nuclei. The large eccentric artery of the tuft showed no intimal hyaline transformation, but some swelling of the endothelium was present in the smaller vessels. The cells of the follicle were predominantly lymphocytes, but in active follicles numerous large lymphocytes were also present. Apart from occasional plasma cells, other cells were not met with. Pencillar sheaths described by Bilroth (1861, 1862) and Schweigger-Seidel (1862, 1863) could occasionally be demonstrated. The arterioles as in Plate VI, Fig. 1 were very much dilated and appeared in places distended by masses of parasites and erythrocytes within the lumen so that an actual blockage of the vessel was regarded as possible. There was no thrombosis. A large proportion of red cells showed small clusters of pigment, showing varying stages of development of the parasite. Many of the cells were adhering to the reticular mesh, while some were phagocytosed by the reticular syncitium. The red cells were irregular in shape partly due to fixation and possibly from hæmolysis.

The venous sinuses of the pulp were clearly defined in some cases, the lining endothelium was often flat or ovoid with plump nuclei and sometimes they were seen projecting into the lumen. Many of these littoral cells showed phagocytosis

of parasites and pigment. The free cells were often rounded, sometimes irregular in shape, with faintly staining nucleus. They were markedly phagocytic, loaded with granules and irregular clumps of pigment and with parasites in some cases. In some cases the cytoplasm was vacuolated and nuclear staining was obscured by collections of pigment. The ingested pigment appeared as fine granules, rodlets and bars, while from repeated accumulation as dense irregular clusters and blocks looking like irregular lumps of coal. Erythrophagocytosis was well marked and many of these cells were parasitised, while some of them were disintegrating. Other cells in the sinuses were mostly of the lymphoid series. Eosinophils were sometimes met with while the lymphoblast series and plasma cells were very rarely seen.

The Liver.—Degenerative changes varying from cloudy swelling to necrosis of individual cells are seen in the liver where groups of cells are found necrosed, it is mainly confined to the inner two-thirds of the hepatic lobules being most prominent in the cells around the central vein and sinusoids. The sinusoids are distended and full with red cells of which many are parasitised when anæmia is severe. There was no evidence of thrombosis or stasis. Parasites were found in numbers, but many appeared phagocytosed by the Kupffer cells. Some of these were loaded with pigment granules. Hæmosiderin was found in large quantities in the form of small granules, or little clumps around the central vein of the lobule gradually lessening towards the periphery. Malarial pigment was chiefly confined to the phagocytic cells of the liver sinusoids, especially in the Kupffer cells. In these cells, the nature and amount of pigment depends upon the history of the case. Thus the engulfed pigment in Kupffer cells were in thick coarse clumps of varying sizes. Hyperplastic changes affecting the reticulo-endothelial system were slight.

The Adrenals.—The histopathological changes in the adrenals consisted in the presence of parasitic collections in the capillaries both of the cortex and of the medulla. The cortical and medullary cells showed signs of degeneration, but frank areas of necrosis were not met with. In the medulla, there appeared diffuse areas where the cells showed no nuclear staining. Phagocytic activity was not very marked as in the case of liver and spleen.

Kidneys.—The degenerative changes in the kidney was most marked in the convoluted tubules, particularly in the proximal ones. Such changes are not unique for malaria, but have been observed in other acute infections and are generally considered to be associated with the presence of toxins. The capillaries in between the tubules showed numerous parasites. Proliferative glomerulonephritis was present, with adhesion to the Bowman's capsule in some cases. Some of the glomerullii exhibited avascular thinned out tufts.

The Lungs.—Histopathological changes in the lungs showed numerous parasites in the alveolar capillaries. Swelling of the capillary endothelium was the rule. The pigmentation of the lungs was much less than in other organs like the liver or brain and there was no evidence of appreciable phagocytosis.

Bone Marrow.—The bone marrow was studied in few cases. It showed typical erythroblastic reaction. Microscopically normoblasts and myeloblasts were seen. The reticulo-endothelial tissue showed here and there large round cells which were phagocytic. Large clumps of pigment were not met with. Numerous eosinophilic cells were present.

Peripheral blood.—In striking contrast to the numerous phagocytic cells which occur in such organs as the spleen and liver in malaria, comparatively few phagocytic cells are found in the peripheral blood. Occasionally, however, in cases of extremely actue malaria two types of eosinophils are observed, the one having coarsely granular eosinophil leucocyte with acicular granules and the other with lozenge-shaped granules. These types of cells have been reported by Knowles *et al.* (1929) to be normally observed in the blood films of hawk and vulture. The significance of these eosinophilic cells are not known (Plate VIII, Figs. 7 and 8).

Discussion

Every addition to our knowledge of the mechanism of pathogenesis and immunity in malaria is important because the proper understanding of this subject may prove of great value, not only in devising measures to combat malaria in severely infected localities, but also in seeking advancement in the rational treatment of the disease.

Considerable advances have been made in our knowledge of the immunology of malaria by Taliaferro (1932) in America using the quartan parasite *P. brasilianum* for his experimental work. In India, a species discovered in a macacus monkey by Napier and Campbell (1932) and named *P. knowelsi* (Sinton and Mulligan, 1932) has been used with many advantages for experimental work, since it is easily available and produces a severe infection with most of the clinical features of hyperacute malaria, such as enlargement of the spleen, remittent fever, progressive anæmia and great prostration and in some cases hæmoglobinuria. Infection is almost invariably fatal in the common rhesus monkey.

Detailed studies of the organs and tissues at all stages of different malarial infections in birds and monkeys [Taliaferro and Mulligan (1937); Bhaskara Menon (1939)] have revealed a very striking correlation between the acquisition of immunity in malaria and the cellular changes that occur in certain organs. The disappearances of parasites at all stages of infection can be directly correlated with the activity of the differentiated macrophages of the spleen and liver and to a less extent within the bone marrow. The acute enlargement of the spleen with the distension of the capsule and separation of trabaculæ may result either from mechanical distension from vascular engorgement or from cell infiltration, from degenerative swelling of the splenic pulp from a paralysis of innervation of the capsule-trabacular system or from a direct toxic effect on the muscle and collagen bundles which would result from vascular trauma. The presence of œdema of the trabaculæ, occasional trabacular softening with loss of staining of the nuclei of the muscle and collagen bundles suggest a direct toxic effect, in addition to

marked vascular engorgement in the present series of cases. The meshes of the pulp are so packed with red blood cells that the venous sinuses are often compressed and collapsed so that an active hyperæmia might be held to occur. There are also evidences of the distension of venous sinus of the spleen pulp. Local circulatory variations developing in malaria depend upto a certain extent on the anatomical structure of the tissue concerned. Organs in which there are sinusoidal elements are severely affected while others with more simple circulatory paths relatively escape from damage. Again the trauma to the endothelial walls—either mechanical, humoral or toxic causes—and accumulations of parasitised cells or their aggregates and macrophages and cell debris from the damaged endothelial wall or lysed cells of erythrocytes and leucocytes may cause physical obstruction to the blood flow through the small vessels [Maegraith (1948), Mægraith (1951), Maegraith *et al.* (1951)].

Maegraith *et al.* (1947) has suggested from an extensive study of circulation of blood flow in the kidney and liver in normal and pathological states that the various pathological changes develop as a consequence of stagnant anoxia arising from reduction of flow in the intralobular vessels following active constriction of the tributaries due to local or central vascular reflexes. The role of tissue anoxia in the development of the changes has recently been emphasized by Delorme (1951) who studied the effects of anoxia in liver perfusion experiments.

Knisely and his associates (1947) made the observation that under pathological conditions and in experimental animals the red cells often appear to stick together giving a granular or clumpy appearance when the blood streams into the capillaries. They also observed that anæmia appears to enhance clumping. Bloch and Powell (1952) demonstrated the coating material on the surface of human red cells by electron-micrographs. They used the term 'Sludging' for the pathological change in the blood flow. The intravascular erythrocytic aggregation which is found is caused by the fibrin or antibody coating on the red cells which renders them susceptible to phagocytosis and in consequence cause red cell destruction. The evidence that Knisely's 'sludging factor' actually results in red cell agglutination, phagocytosis and hæmolysis is rather scanty (Rigdon, 1950). However, he is of the opinion that the disproportion between the amount of plasma and number of red blood cells—hæmoconcentration—to be the factor involved and escape of tissue electrolytes being due to anoxia. In the cases now studied parasitic localisation and vascular enlargement were marked in the spleen and liver and kidney than in the adrenal glands, bone marrow and the lungs. It is not possible to decide whether the vascular reaction is due to central or local reflexes without further detailed study. Adhesion of free parasites and infected red cells to the reticular mesh is so well marked in the spleen and the liver, as to suggest a general fixing effect on the parasite laden red cells.

The occurrence of marginal annular necrosis and occasionally central necrosis is of some significance. Feitis (1921) and Meuret (1925) associated the changes to arteriosclerosis while Geipe and Mathias (1924) and Wilton (1925) have

described cases associated with acute infection. The presence of annular necrosis within the marginal zone is of some importance since according to Thoma (1924) the small pencillar branches of the central artery end in ampullary terminations at the marginal zones beyond which sinuses commence. Degenerative changes in this region suggest a toxic lesion on the capillaries. Bhaskara Menon (1939) in his study observed that in the case of monkey malaria, though the occlusion of capillaries by parasitic plugs together with cell debris cannot be overruled, it is quite unlikely to be the sole mechanism. In the sinuses, this is still more improbable although hyperplastic changes affect the littoral cells.

Napier, Krishnan and Lal (1932) in human malaria, Krishnan and Ghosh (1935), Taliaferro and Mulligan (1937) in monkey malaria have reported the phagocytic mechanism comes into action rather early in infectious stage and appears to be present throughout the period of infection. Free parasites, damaged parasites and parasitic and cell debris and damaged erythrocytes all appear to be equally ingested. There is no crisis with a sudden drop in the number of parasites in the blood, but the infection increases in intensity until the animal succumbs. Toxic lesions affecting the lymphoid cells and the reticulum of the spleen appears, while the fixed reticular cells and littoral cells show marked differentiation into free amœboid cells exhibiting marked phagocytic activity.

Active ingestion of parasites and pigment granules was present in all the specimens studied and this was not only by the free cells, but by the cytoplasmic reticulum and by the living cells of the sinuses. The phagocytic reaction was equally well marked in the sinuses and showed itself in littoral differentiation into large mononuclear cells which ingested parasites and pigment granules. Bilroth cords showed marked engorgement due to the accumulation of blood within the meshes of the pulp. The red cells were irregular in shape possibly due to hæmolysis and partly from the effect of fixation. Many of the parasitised red cells appeared adhering to the reticular mesh, while some were actually phagocytosed by the primitive reticular syncitium. The histological picture on the whole was not that of a lack of reticulo-endothelial response, but that of an overwhelming infection that developed in-spite of reticulo-endothelial reaction.

The tissue lesions in the various organs arising from circulatory interference indicated by dilatation, congestion and frequently stasis of the smaller vessels, though seem to be largely developing as a consequence of tissue anoxia is not adequate enough to explain all facts. Oxygen lack though mild could result in inefficient action of various enzymes so that incomplete reconversion with failure of energy production results. A similar state could be caused by some lack of essential substance, *e.g.*, glucose or calcium or a catalyst. Preliminary observations have shown that adenosine compounds are released in parasitised blood than in the case of normal blood and the possibility of purine compounds and their degradation products having ætiological significance cannot be ruled out in the light of known action of these adenylic and uridylic compounds in the body

[Drury and Szent-Gyorgi (1929), Fiske (1924), Billings and Maegraith (1937), Stoner and Green (1944). Zahl and Albaum (1951) and Hoffmann *et al.* (1951)].

SUMMARY

1. A histopathological study of 15 cases of fatal malaria in chicks infected with *P. gallinaceum* has shown (1) the presence of vascular mechanism that localises the parasites in large numbers in the spleen, (2) the activation of lymphoid reaction in the spleen and the role of reticulo-endothelial system in active phagocytosis.

2. The degenerative swelling of the capillary endothelium with necrosis of cytoplasmic reticulum around follicles suggests a toxic factor.

3. The severity of the infection appears to be due, not to a failure of the reticulo-endothelial response but to some other factor. The present work does not establish a role for adenyl and uridyl compounds in the genesis of the lesions observed. It is probable it may along with other unpublished observations prove to be one link in the chain of evidence to prove the proposition.

4. The lesions met with other organs besides the spleen suggest that such localisations may be responsible for the varied changes observed in different organs.

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Explanation of Plates

PLATE VI

Comparative phagocytic activity of the spleen, liver, lungs and bone marrow, after infection with *P. gallinaceum* in chicks.

Fig. 1. A splenic nodule showing ring of necrosis in the marginal zone. The red pulp showing large number of macrophages engorged with malarial pigment. (A) Follicular artery. H. & E., × 240.

Fig. 2. The liver showing heavily pigmented Kupffer cells, which however contain appreciably less pigment per cell than the macrophages of the spleen. Note the radial distribution of pigment around the central vein. H. & E., × 120.

Fig. 3. The lung showing macrophages (dust cell) containing small amount of malarial pigment. There is no ingestion by the flat alveolar epithelium. The dense collections of pigment seen in this figure is contained within circulating phagocytic leucocytes, H. & E., × 240.

Fig. 4. The bone marrow showing a few pigmented macrophages. It shows typical erythroblastic reaction. (B) Parasite in red blood cell. C and D. Erythroblasts. H. & E., ×540.

PLATE VII

Pathological changes following infection with *P. gallinaceum* in chicks.

Fig. 1. Spleen showing the concentration of parasites in a Bilroth cord (B) and the phagocytic reaction in the sinus (S). H. & E., × 1100.

Fig. 2. Spleen, a portion of a venous sinus (S) showing phagocytosis (P) of malarial parasites and the vacuolation of the macrophages. H. & E., × 1100.

PLATE VIII

Fig. 3. Liver showing fatty change degeneration and necrosis around the central vein. Some of the liver cells show vacuolation. The dark dots within the sinusoids represent Kupffer cells engorged with pigment and unphagocytosed parasites. H. & E., × 120.

FIG. 4. Adrenal gland showing foamy degeneration of the cortex. The capillaries are loaded with parasites. Phagocytic activity is not marked. Note in the avian suprarenal gland the cortex and medulla are scattered and diffuse. (C) Adrenal Cortex. M = Medulla. H. & E., × 250.

PLATE IX

FIG. 5. The kidney shows parasites in the glomerular capillaries. Degenenerative cloudy swelling of tubular epithelium is seen. The glomerulii are also affected. H. & E., × 250.

FIGS. 6, 7 & 8. Blood smears showing parasitised erythrocytes (A, B), Round erythroblast (C), Eosinophil leucocytes (D), Coarsely granular leucocytes with acicular granules and (E) the same with lozenge-shaped granules. Leishmann Stain, × 1100.

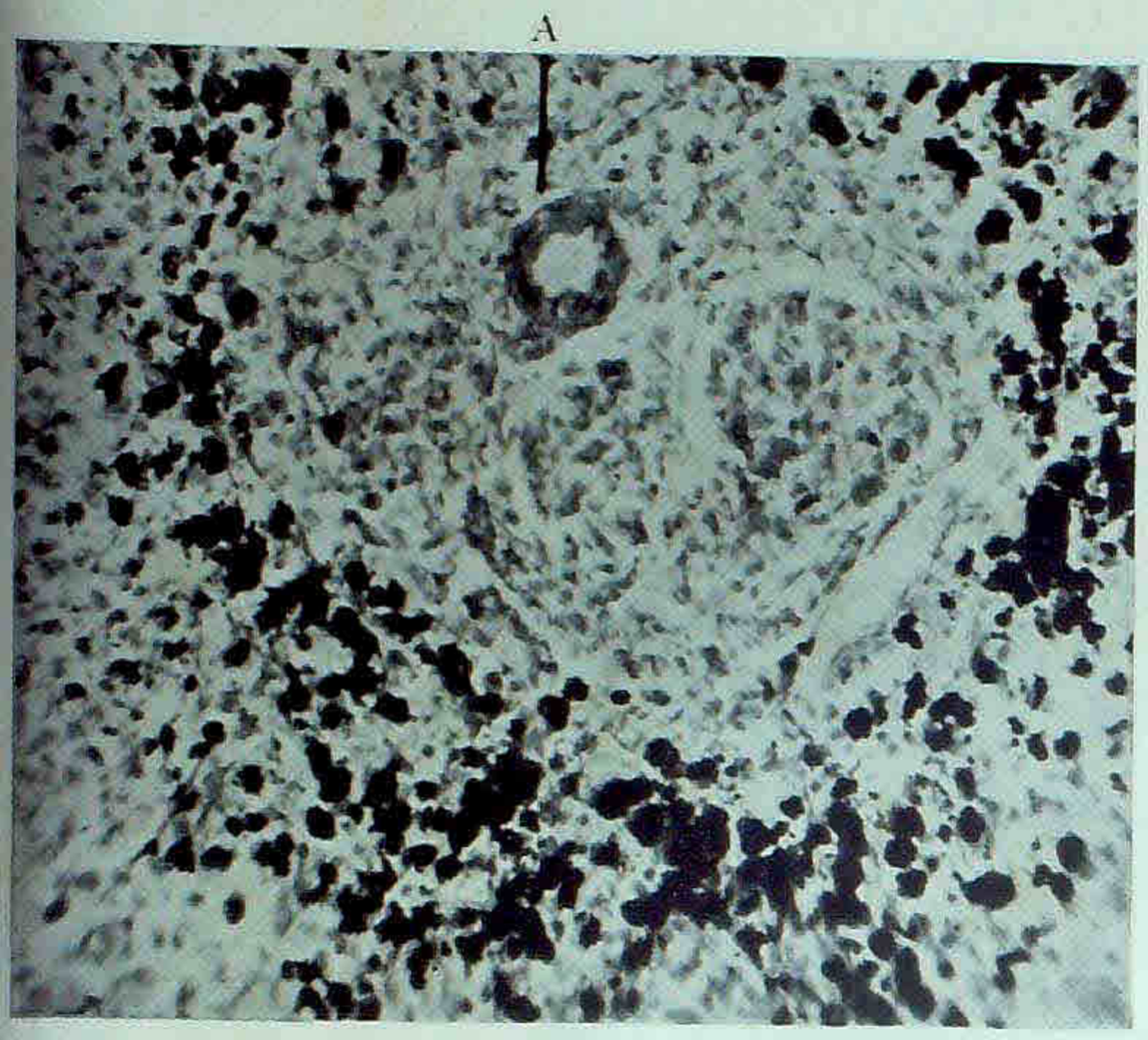

FIG. 1

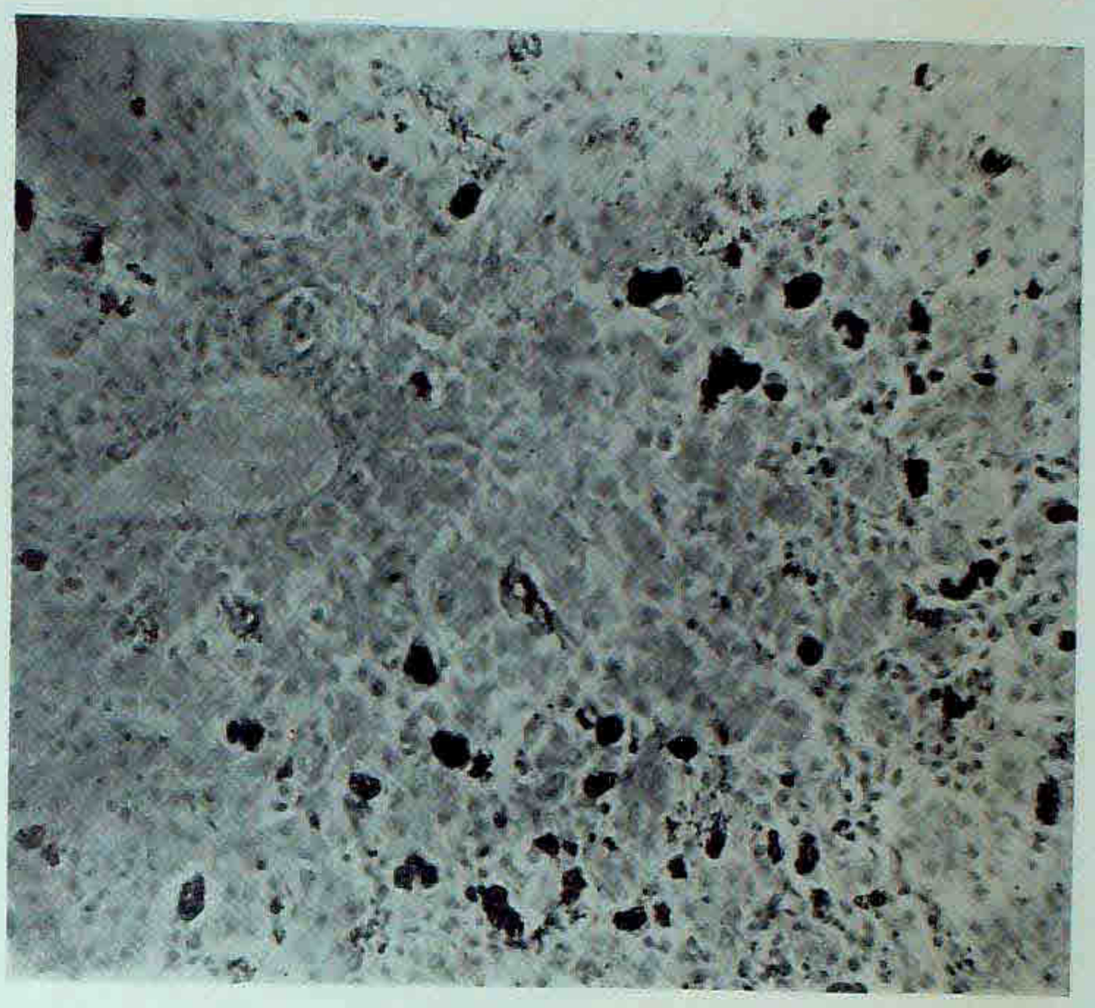

FIG. 2

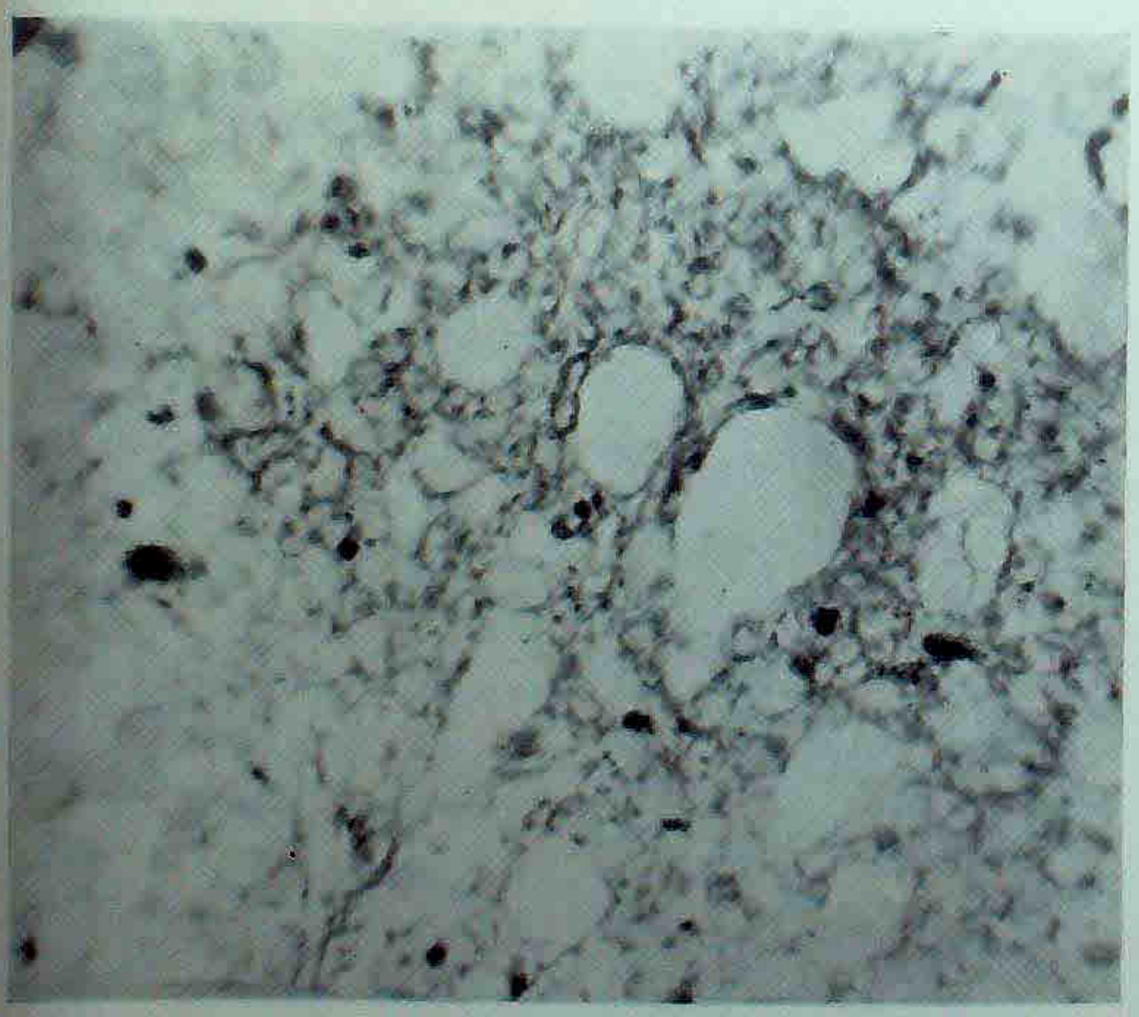

FIG. 3

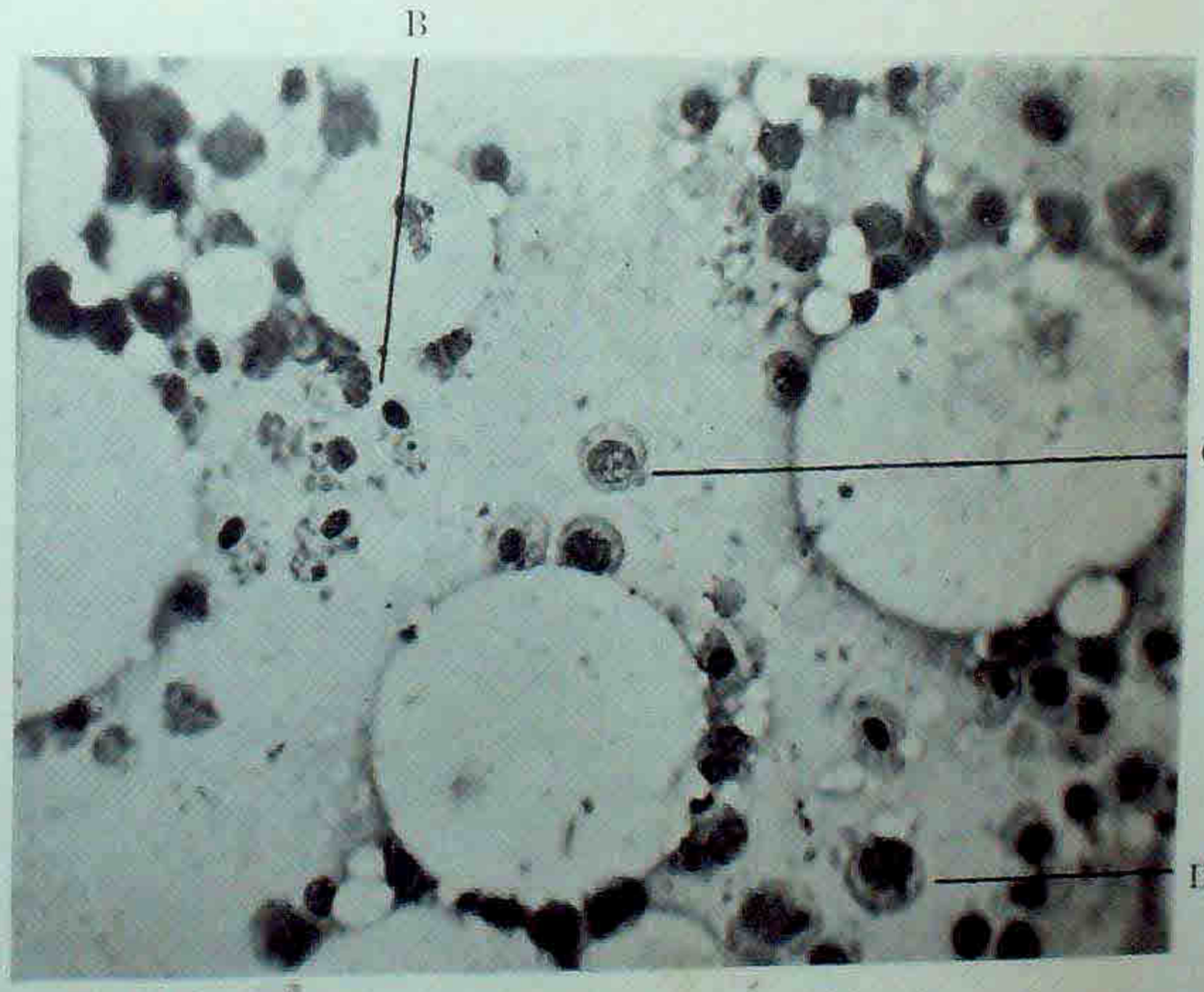

FIG. 4

Comparative phagocytic activity of the tissues in chicks after severe infection with *P. gallinaceum*.

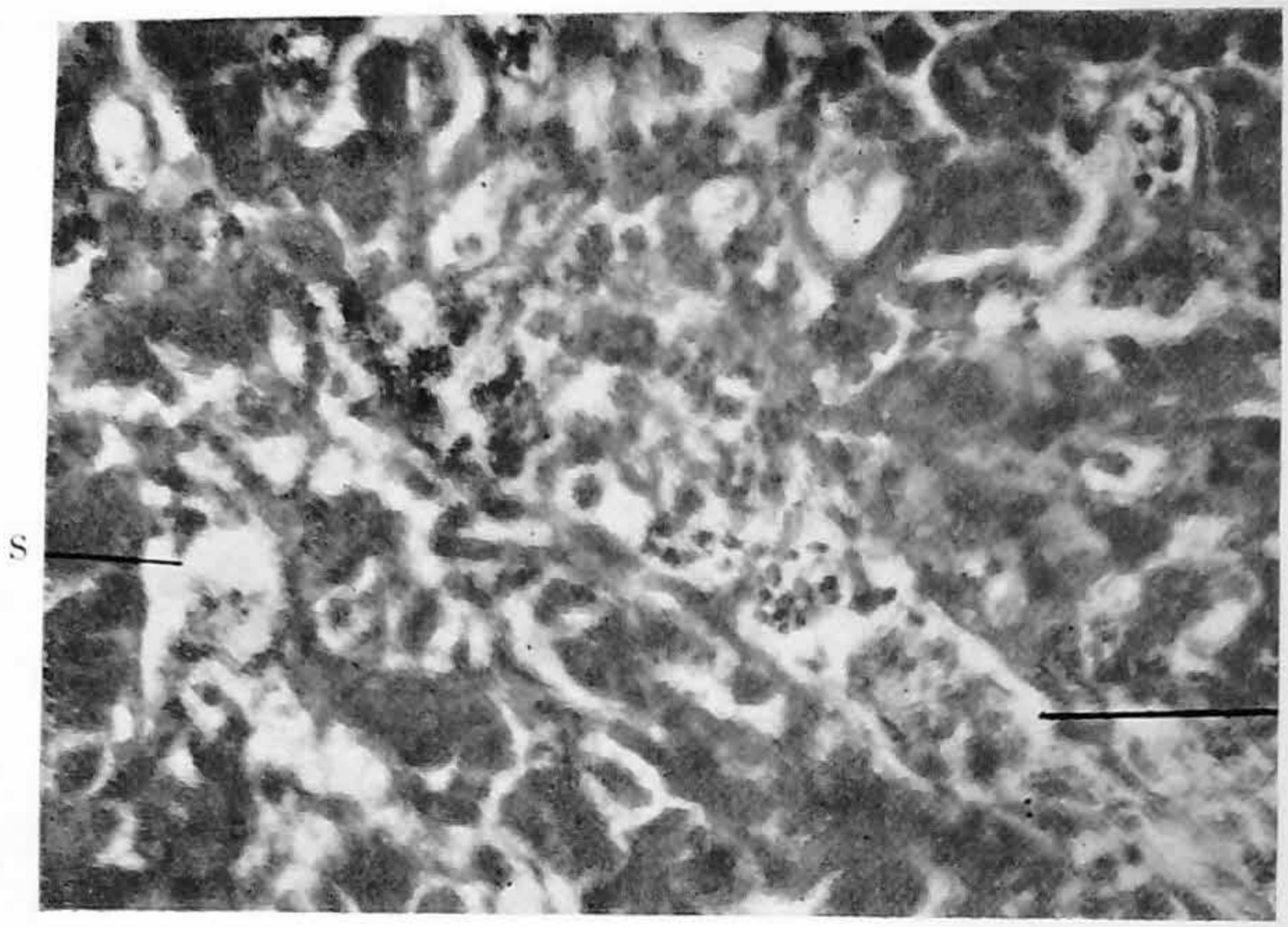

FIG. 1

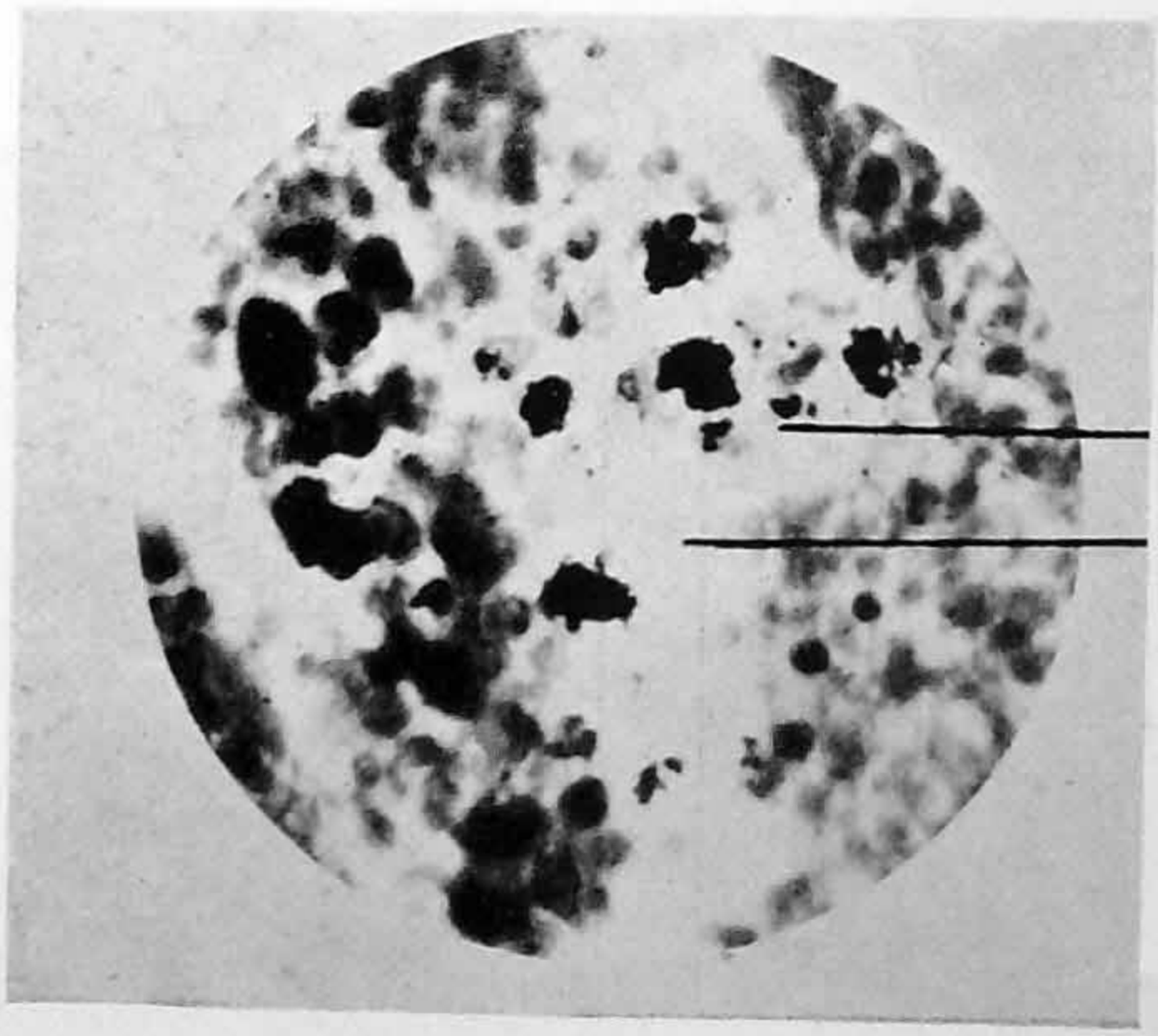

FIG. 2

Pathological changes following infection with *P. gallinaceum* in chicks

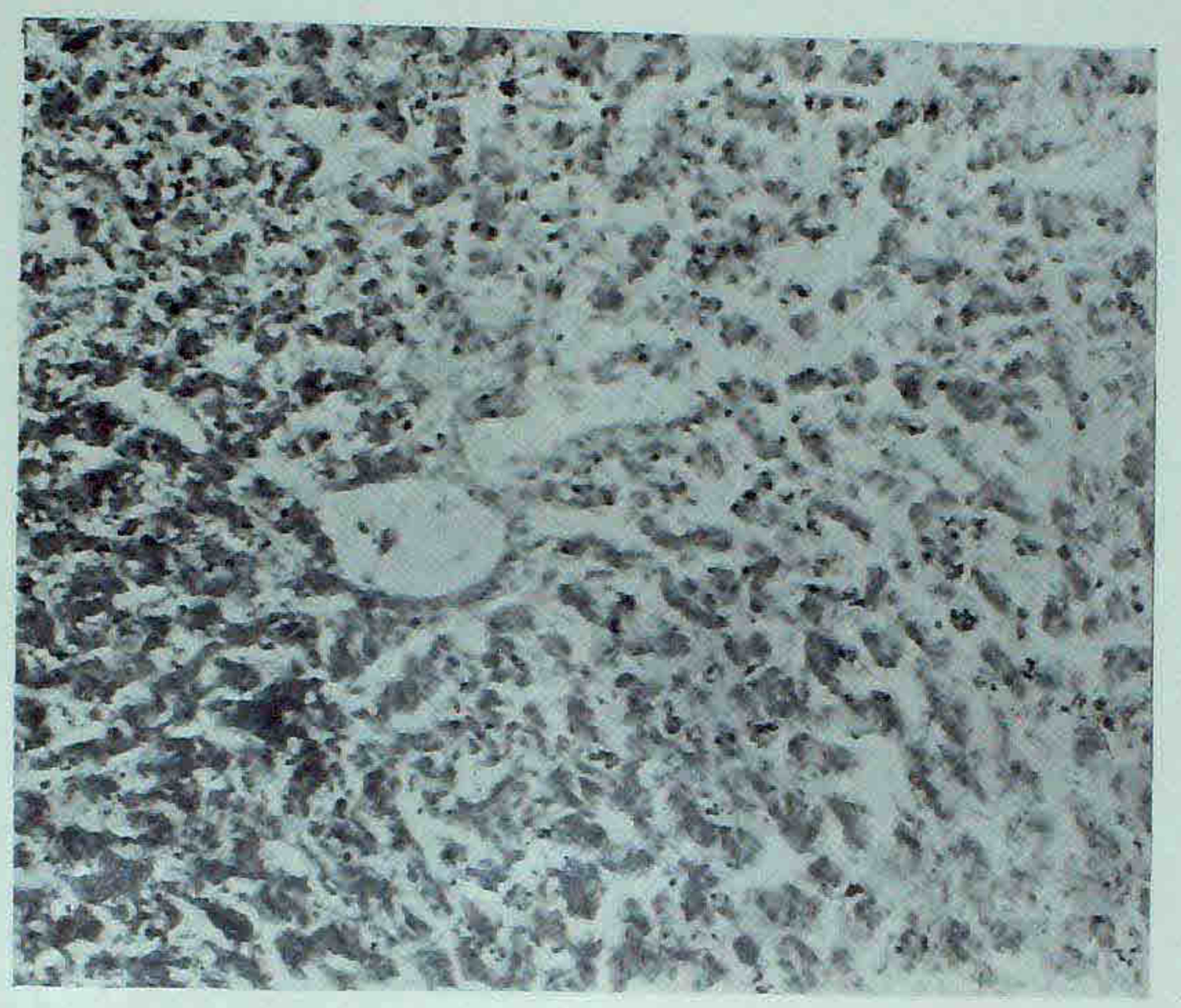

FIG. 3

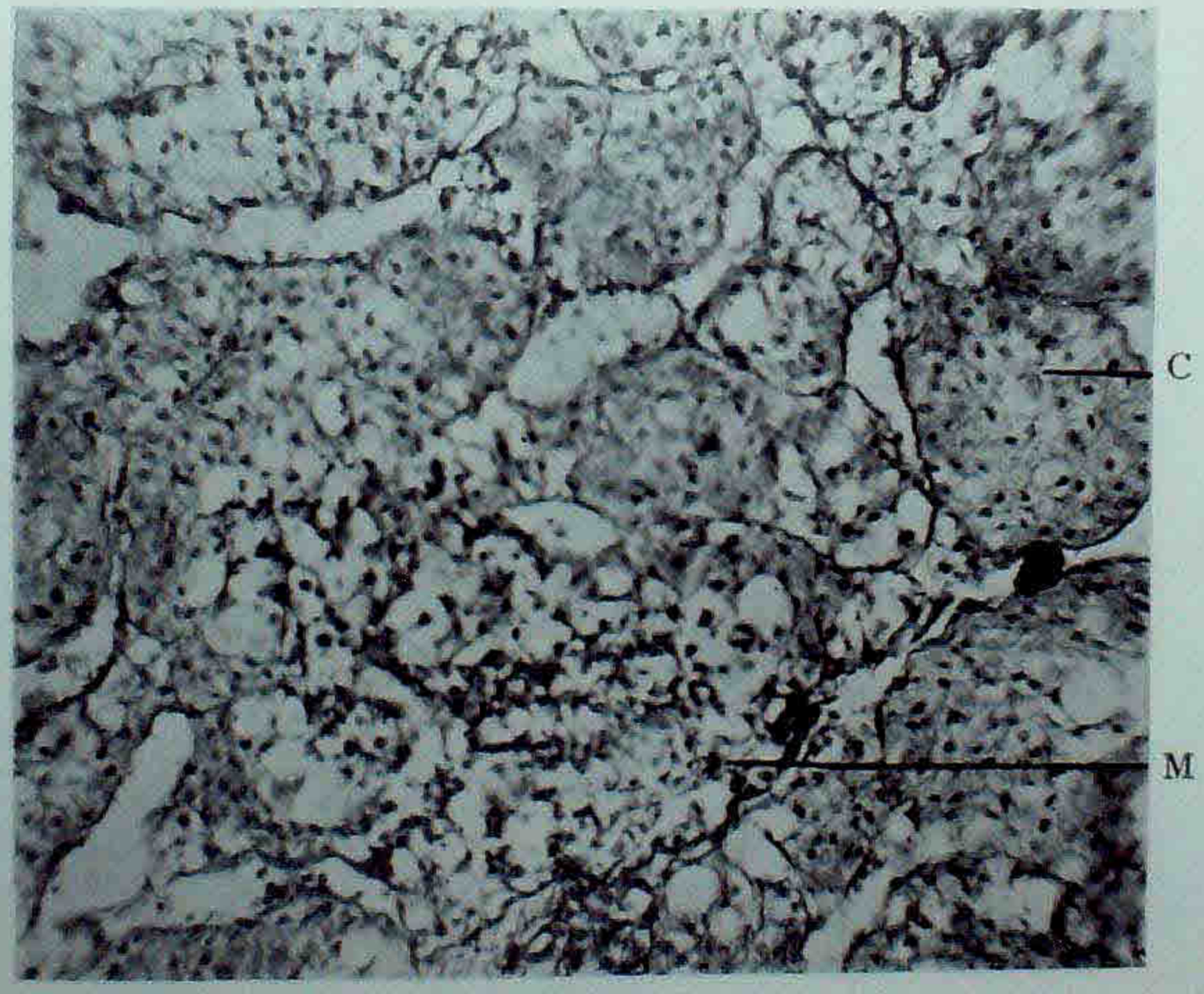

FIG. 4

Pathological changes following infection with *P. gallinaceum* in chicks

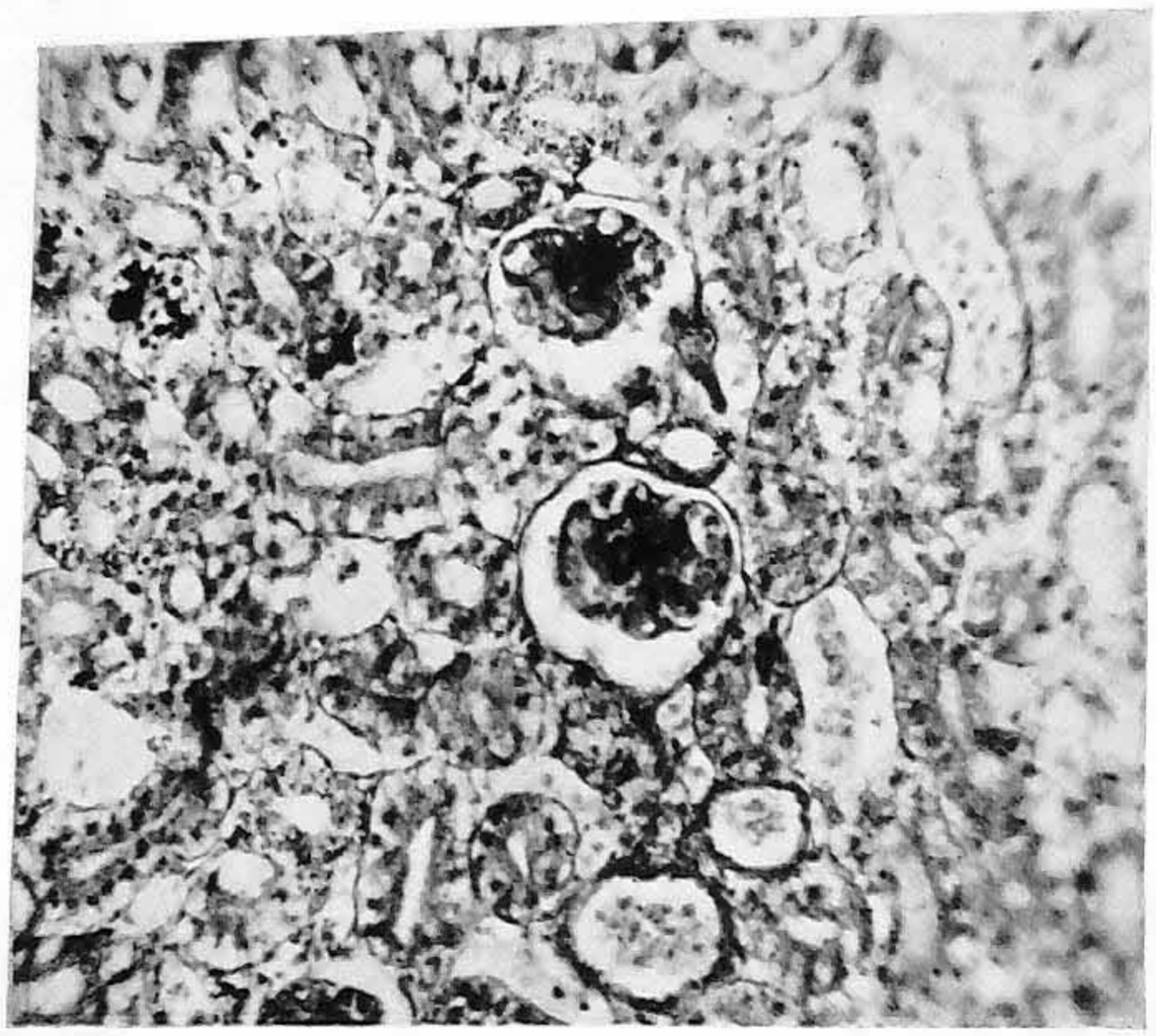

FIG. 5

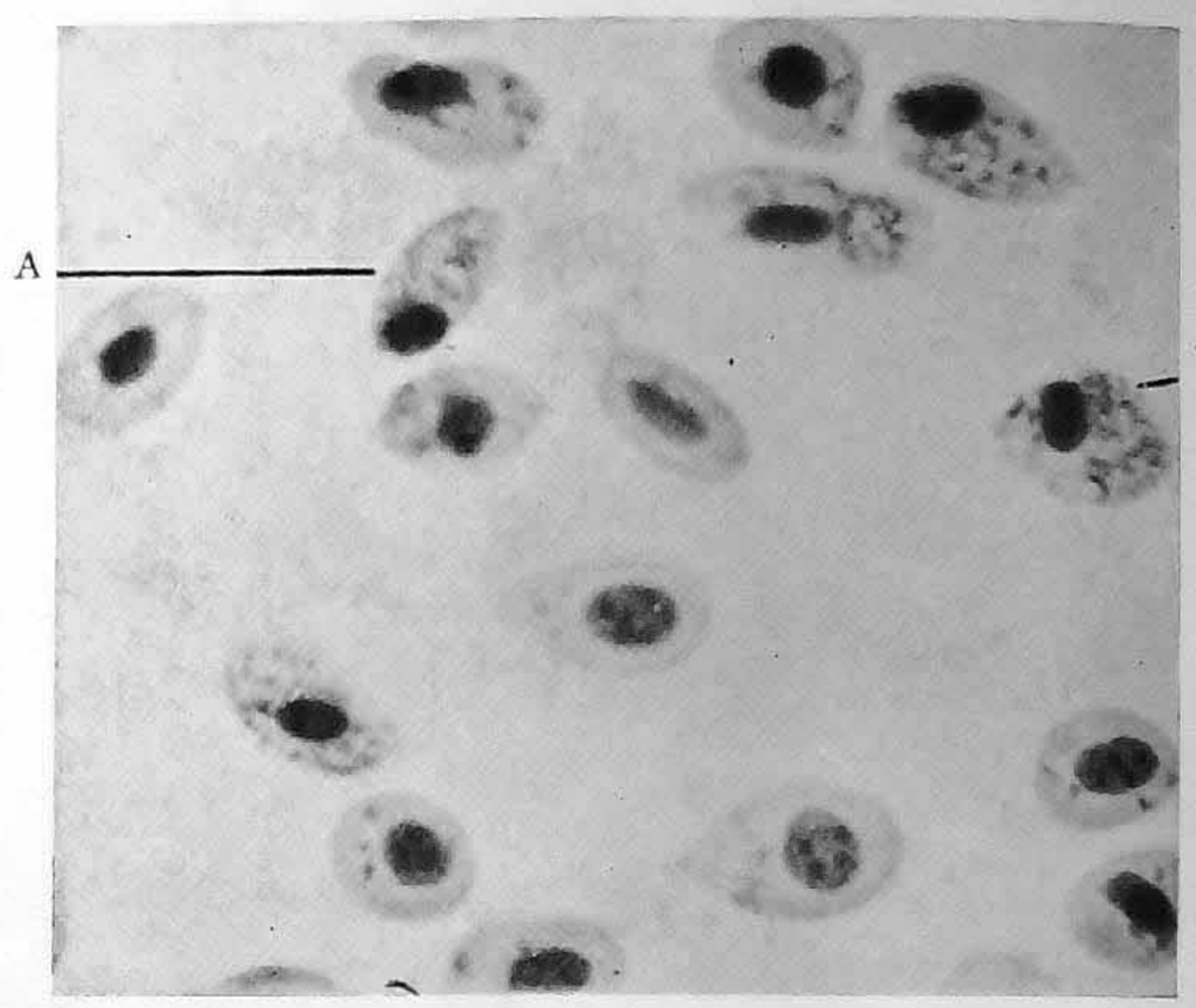

FIG. 6

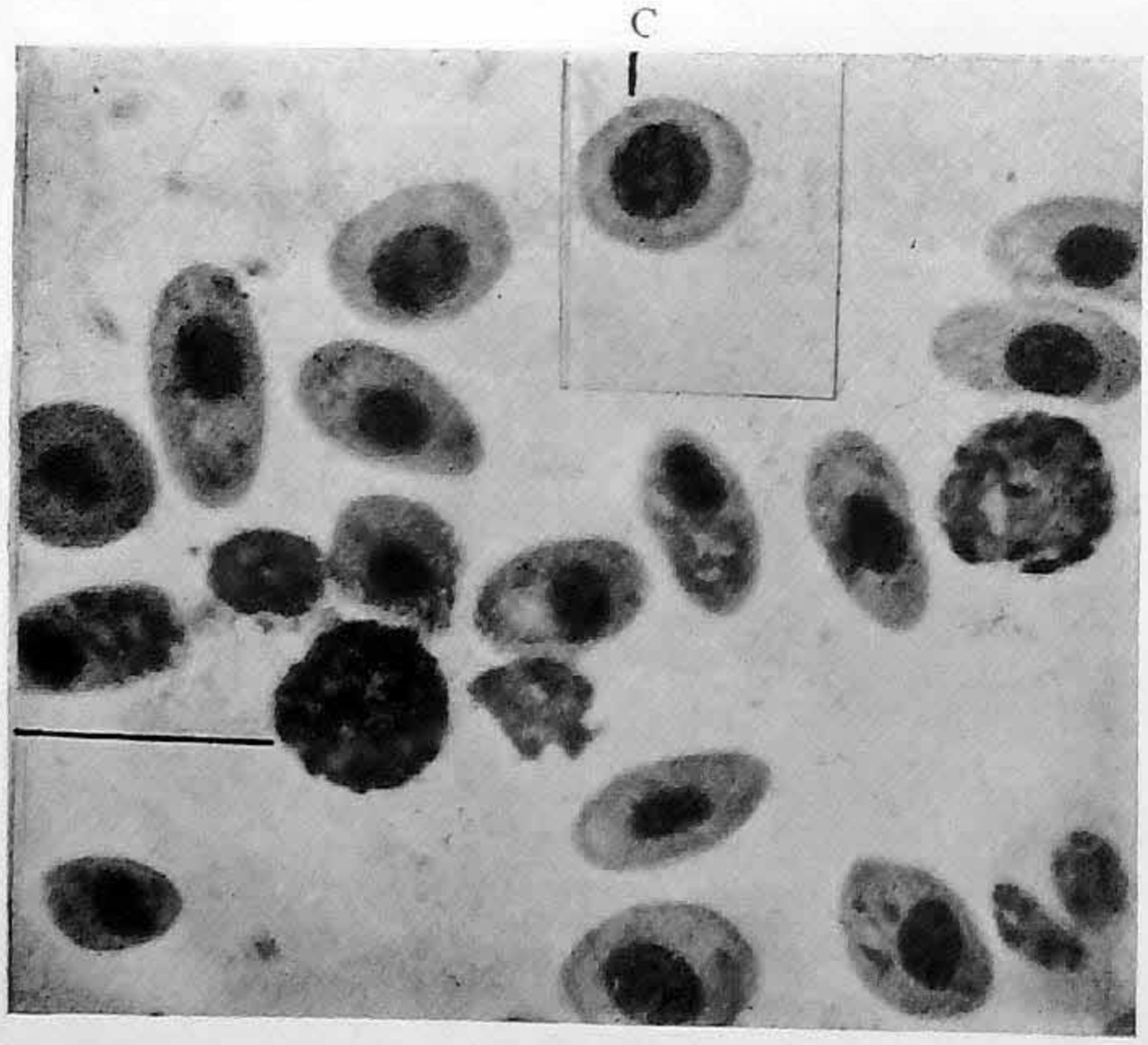

FIG. 7

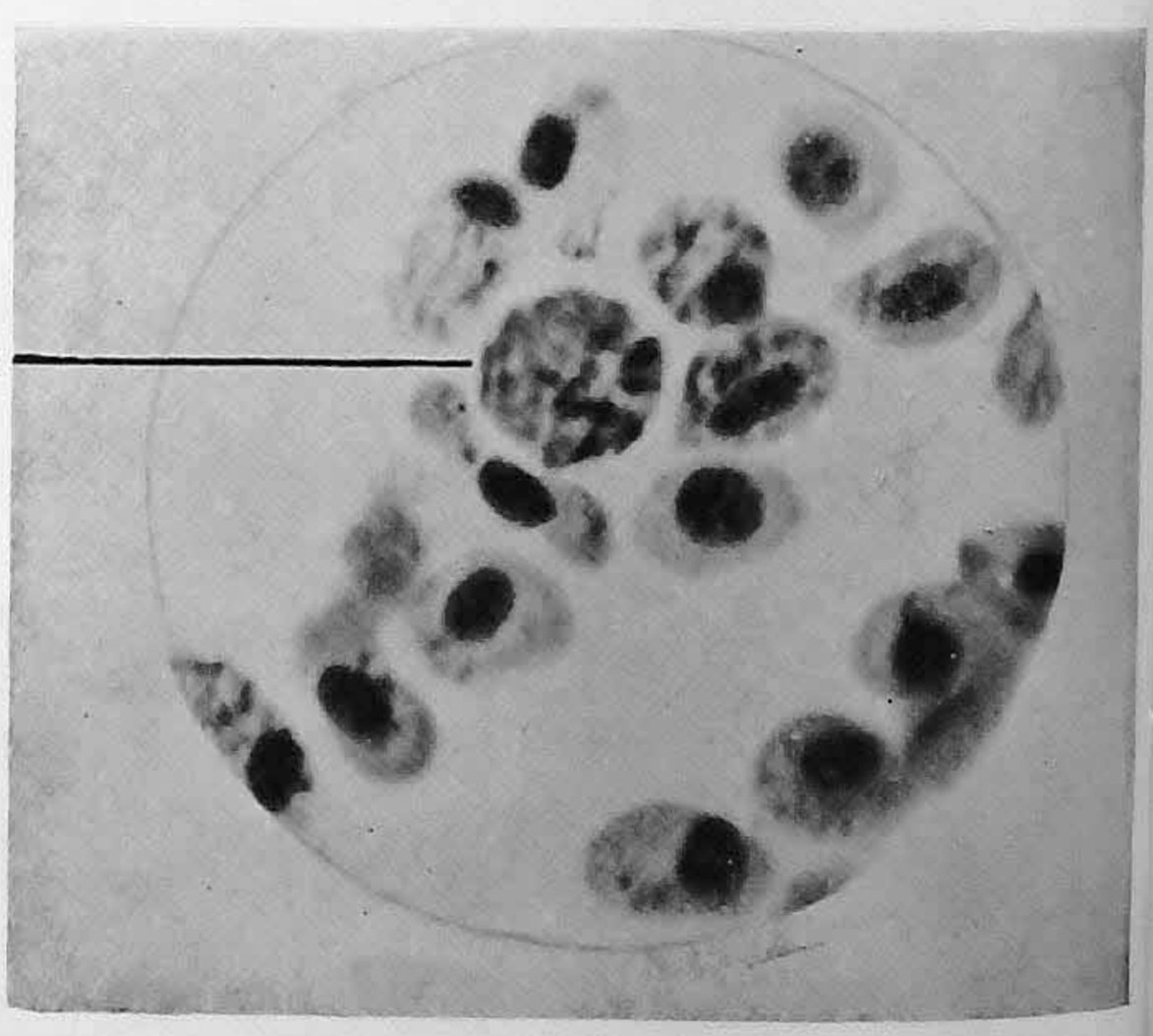

FIG. 8

Pathological changes following infection with *P. gallinaceum* in chicks