PATHOLOGICAL STUDIES ON LEUCOCYTOZOONOSIS IN CHICKENS

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(Received for Publication May 23, 1966)

In 1890, DANILEWSKY⁶⁾ first observed a type of protozoa in the blood of an owl and used the term "Leucocytozoon" to designate the parasite, believing that leucocytes were the host cells of the gametocyte. Later investigators believed that these host cells were immature erythrocytes. The hosts are primarily birds. The Leucocytozoon was reported by MATHIS and LEGER¹²⁾ to be in fowls at Tonkin in Indochina. Since then, similar reports have been made from Thailand, Formosa, London, America, and other places.

In Japan, outbreaks have been reported among wild birds since the beginning of the 20th century. AKIBA et al.⁸⁾ reported a new disease in fowls which had been caused by a type of protozoa observed in the blood vessel. AKIBA and his associates published serial reports as did ISHIGURO et al.¹⁰⁾ The life cycle of this protozoon, however, is not yet completely known. Very few papers have been published on pathological studies of the disease the report by ISHIGURO et al.¹¹⁾ being among them. In August, 1959, the authors investigated an epizootic of symptomatic cases among chickens which showed a very high fatality. The results of the histopathological investigation are described below.

MATERIALS AND METHODS

Two groups of New Hampshire chickens were investigated. Group A consisted of 16 chickens which died 17 days after hatching and 14 chickens which were killed because of emaciation, and group B of 4 chickens which died 20 days after hatching. Specimens were taken from each organ, fixed in 10% formalin, and embedded in paraffin. Sections were treated with hematoxylin-eosin stain, azan stain, WEIGERT's iron-hematoxylin stain, and PAS reaction.

CLINICAL COURSE

Group A: Green diarrhea, dullness, inappetence, and cramp and fall were observed in some chickens 15 days after hatching. Of 500 chickens, 150 died by 17 days after hatching and 200 by 27 days after hatching, while 70-days-old Rockhorns kept in the neighboring coop remained symptomless.

Group B: The same symptoms were observed among the fowls of this group. Of 350 chickens, 200 died by the 20th day after hatching.

FINDINGS IN THE INVESTIGATED CASES

Anatomical changes

Generally, the fowls were underdeveloped and malnourished. Their visible mucous

membranes were pale and anemic. Fat and muscle tissues were underdeveloped. In many cases, petechiae and irregularly-shaped ecchymosis were present in the subcutaneous tissues and muscles of the breast

Visceral cavity and organs

Hemorrhages were observed in the peritoneal cavity, under the capsules of the kidneys, and in the subdural space. Many miliary and beansized petechiae were present in the liver, kidneys, pancreas, thymus, and bursa fabricii. There were many grayish-white miliary and rice-grain-sized spots in the heart, liver, kidneys, pancreas, gizzard, and bursa fabricii. Most of the lungs showed varying degrees of congestion and several of them had coagulated blood adhered to their surfaces. There was an increase in the fluid in the pericardial cavity, but few or no clots in the right ventriculus of the heart.

Histopathology

Liver: Megaloshizonts were observed in veins, capillaries, and acini, and encapsulated with reticular fibers and endothelial cells of the blood vessels. In several cases, hemorrhages and infiltration of foreign-body giant cells and histiocytic cells were observed in the periphery of the megaloshizonts. Hepatic schizonts were found in the cytoplasm of hepatic cells and merozoites and gametocytes in sinusoids in the periphery of the schizonts. Endothelial and KUPFFER's cells were swollen and contained variable amounts of hemosiderin. Diffuse and focal infiltration of lymphocytes and heterophils were present in the interlobular connective tissue and sinusoids. In some cases, there was focal necrosis in hepatic cells.

Spleen: Schizogony took place to show various stages of schizonts from early ones which were parasitic in cells of the reticulo-endothelial system to megaloshizonts in which the merozoites had already been replaced by erythrocytes. Hemorrhages were seen on the periphery of ruptured megaloshizonts. Many of the megaloshizonts were found in red pulp and sheathed arteries. In most cases, the spleen showed varying degrees of congestion and proliferation of the cells of the reticulo-endothelial system. These cells were swollen and contained hemosiderin. In a few cases, hyalinization occurred in the walls of the sheathed arteries.

Kidneys: Remarkable hemorrhages were observed in the parenchyma. Megaloshizonts in various stages, like those in the spleen, and a large number of merozoites were seen around the capillaries. The periphery of ruptured megaloshizonts was infiltrated with foreign-body giant cells, histiocytes, and lymphocytes. Several cases exhibited cellular accumulation of lymphocytes, fibroblasts, and fibrocytes, hyaline degeneration of the glomeruli, and fatty degeneration of the epithelia of uriniferous tubules.

Pancreas: Megaloshizonts were observed in the acini and veins. The acini were pressed by groups of megaloshizonts. Hemorrhages and infiltration of lymphocytes and histiocytes were observed on the periphery of the megaloshizonts which merozoites were released.

Heart: Megaloshizonts with hemorrhagic peripheries were present in the myocardium and blood vessels. In several cases, megaloshizonts were enveloped by foreign-body giant cells, histiocytes, and lymphocytes. In those megaloshizonts which merozoites had been released, granulation tissues developed. Diffuse infiltration of lymphocytes and heterophils occurred in the connective tissues. In 3 cases, small, non-encapsulated, early schizonts were found in the capillary endothelium. In many cases, the heart muscle had undergone pressure atrophy and was torn.

Lungs: Large numbers of megaloshizonts were observed at several sites in the alveoli, veins, and capillaries. In some cases, there were groups of 2 or 3 megaloshizonts each exclusively in the outer coats of blood vessels. In all cases, degenerated and

necrotic megaloschizonts and capsules were enveloped by foreign-body giant cells, histiocytes, and lymphocytes. The alveoli and tertiary bronchi were remarkably congested, giving evidence of inhalation of blood. In 2 cases with *Aspergillus*, accumulations of giant cells, histiocytes, lymphocytes, and fibroblasts were shown inside the alveoli and in the walls of the parabronchi.

Proventriculus: Megaloschizonts were present in the tunica propria. The peripheries of the megaloschizonts were hemorrhagic. So-called non-capsulated schizonts were seen in the capillary endothelium of the tunica propria. The glandular epithelia were desquamated.

Gizzard: Megaloschizonts were found in the endothelia of small arteries and veins. The walls of these blood vessels in which large numbers of megaloschizonts had been parasitic were ruptured, and their surrounding tissues were hemorrhagic. Non-capsulated schizonts were observed in the capillary endothelia of the muscle layers. In several cases, granulomata had been formed.

Intestine: Megaloschizonts were present in the tunica propria and blood vessels in the tunica muscularis and tunica subserosa. Hemorrhages and cellular reaction occurred on the peripheries of these vessels. In the diverticulum caeci vitelli, hemorrhages and infiltration of heterophils, histiocytes, and lymphocytes were observed. Non-capsulated schizonts were found in the capillary endothelia in the tunica propria. There were parasitic coccidia in the glandular epithelia of the tunica mucosa in 2 cases.

Brain: Megaloschizonts were present in the subependymal portion of the ventriculus tertius, and in blood vessels of the pia mater, the cerebrum, cerebellum, and some other parenchyma of the brain. In several cases, non-capsulated megaloschizonts, 5 by 10 μ in size, were observed in the cerebrum and cerebellum. Merozoites had stagnated in the capillaries. There were a proliferation of glia cells and ependymal cells of the ventriculus and hemorrhage in the pia mater.

Other organs: Many groups of megaloschizonts were found in the thymus, ovary, testis, crops, bursa fabricii, cloaca, and breast muscles. Hemorrhage and cellular reaction occurred in the focal peripheries.

Findings in blood smears and stamp smears from organs: Blood smears were prepared from the heart and peripheral blood vessels (cervical and axillar veins) and stamp smears from some organs (liver, spleen, kidney, and lung). All the smears were stained with GIEMSA's solution. In some cases, parasitic merozoites were present in erythrocytes, and some erythroblasts appeared. The merozoites had already developed into micro- and macrogametocytes, both of which were isolated from the host cells.

DISCUSSION

O'ROKE,^{15,16} HUFF,⁸ and NEWBERNE¹⁴ stated that sporozoites which had been transmitted from gnats to ducks invaded the cells of the reticulo-endothelial system or the epithelial cells of various organs. The earliest schizonts appeared in the hepatic cells. Megaloschizonts grew in the heart, spleen, liver, intestine, and brain, causing enlargement of their host cells. The present authors found so-called "early schizonts", irregular in shape and finely granular, among cells of the reticulo-endothelial system in the heart, brain, proventriculus, gizzard, liver, kidneys, spleen, intestine, and thymus.

Hepatic schizonts, which HUFF⁸ and NEWBERNE^{13,14} observed in ducks and turkeys, were detected in the cytoplasm of hepatic cells in the present investigation. They resemble the "early schizonts" in morphology. The authors consider that the variation in hepatic schizonts results from the variation in host cells. Megaloschizonts were usually found either singly, as in the case of *Leucocytozoon smithi* and *L. simondi*, or in

groups in and adjacent to blood vessels in all the organs. Megaloszizonts at several stages of development were observed. They included early acidophilic megaloszizonts, mature basophilic megaloszizonts, and megaloszizonts which had already released merozoites. The megaloszizonts found in the brain were generally small and usually single.

NEWBERNE^{13,14)} stated that all megaloszizonts in the organs were enveloped by a capsular wall of reticular fibers. In the present investigation, megaloszizonts were enveloped not only by the capsular wall of reticular fibers but by endothelia of blood vessels. In addition, some of the grouped megaloszizonts were enveloped by a thicker capsule than that wall. In the brain, encapsulated and non-encapsulated megaloszizonts were discriminated. The former were larger in size and found beneath the pia mater and the ependyma of the ventriculus. The latter were smaller in size and irregular in shape and harbored in the deeper portions of the parenchyma. The cytome which NEWBERNE et al.^{13,14)} had observed in turkeys and ducks was found in only one case. In the present investigation, most megaloszizonts were filled with fine and granular merozoites.

HUFF⁸⁾ presented three hypotheses as to the relationship between the hepatic schizont and the megaloszizont: (1) The two kinds of schizonts may represent different stages in the same life cycle. (2) They may belong to essentially the same stage, except for location, as those entering the liver cells and may be limited in size by the type of host cells. (3) They may represent some stages in the life cycles of two different species of parasites. The authors consider that the hepatic schizonts which were found in the cytoplasm of the hepatic cells did not differ morphologically from the so-called "early schizonts" observed in cells of the reticulo-endothelial system and that, therefore, both types of schizonts are in the same stage. As to the relationship between the hepatic schizont and the megaloszizont, the authors support HUFF's hypothesis that both types of schizonts may represent different stages in the same life cycle. Hepatic schizonts and so-called "early schizonts" are in earlier stages than megaloszizonts. So megaloszizonts in the blood vessels may have developed from schizonts in the capillary endothelia. In two cases, many gametocytes were observed in cells of the reticulo-endothelial system.

NEWBERNE¹⁴⁾ observed, in ducks, infiltration of large mononuclear cells, forming one or more layers, around the schizont in the brain and infiltration of large mononuclear cells, fibroblasts, lymphoid cells, and plasma cells, forming layers, in the lungs, but not in any other organ. ISHIGURO¹¹⁾ found no host tissue reaction around growing megaloszizonts in fowls. In the present investigation, such tissue reaction as observed by NEWBERNE¹⁴⁾ in the lungs of ducks occurred in the gizzard and heart. Around megaloszizonts which had already released their merozoites, hemorrhages were seen in various organs, as reported by COWAN⁵⁾ and ISHIGURO¹¹⁾. They were infiltrated with foreign-body giant cells, histocytes, lymphocytes and heterophils. There were blood cells in the capsules. Moreover, capsules which had been destroyed rather early were replaced by fibroblasts and fibrocytes forming granulation tissue. Scar formations were observed in the heart. The grayish-white spots which were present in the various organs coincided with the parasitic sites of the megaloszizonts.

NEWBERNE^{13,14)} stated that morphological changes, if any, were very slight in the invaded hepatic cells inasmuch as there was no apparent malformation of either the host cells or their nuclei. In the present investigation, the nuclei of the invaded hepatic cells were pyknotic and depressed to one side. Hepatic cells which had been invaded by many merozoites were swollen, with their obscure nuclei. Sinusoids involved in petechiae and the peripheries of necrotic lesions were congested with merozoites which

had been released from hepatic schizonts. It is presumed that these findings may present some relationship between the necrosis and the hemorrhage. A proliferation of cells of the reticulo-endothelial system took place in the spleen and liver. COWAN⁵⁾ mentioned that the phagocyte in duck tissue was a major defence element in hosts infected with *Leucocytozoon*, because this cell increased in number with the advance in infection and returned to normal in the absence of continued stimulation by reinfection. As for the enlargement of the spleen, FALLIS⁷⁾ recognized in experimental ducklings that a maximum splenomegalia had been reached 10 to 14 days following exposure, possibly because of the presence of many megaloschizonts. The authors agree with NEWBERNE¹⁴⁾ and AKIBA¹⁾ who have asserted that the enlargement of the spleen results from the congestion and proliferation of reticuloendothelial cells, and that the activity of these cells is a reaction to the massive destruction of erythrocytes. Deposition of hemosiderin in the spleen and liver indicates that a large number of erythrocytes were destroyed.

Death which occurs in leucocytozoonosis may be attributed to severe anemia resulting from the massive destruction of erythrocytes and hemorrhages in liver, kidney, lung, spleen, pancreas, thymus, brain, muscle, and subcutaneous tissue. In addition, emboli produced by large numbers of gametocytes, megaloschizonts, and merozoites in blood vessels, lymphocytic infiltration and focal necrosis in the liver, and hyalinosis of the walls of the sheathed arteries in the spleen contribute to the fatalities. AKIBA et al.⁴⁾ observed hemorrhages and emboli produced by gametocytes and megaloschizonts in the brains of fowls infected with *Leucocytozoon caulleryi* which died with acute nervous symptoms.

According to AKIBA et al.⁴⁾, ISHIGURO et al.¹¹⁾, and SIMPSON et al.¹⁷⁾, most of the fowls infected with *Leucocytozoon* are also infected with lymphomatosis, blackhead, fowl cholera, pullorum disease, etc. In the present investigation, however, only two cases of mixed infection were detected, one case with aspergillosis and the other with coccidiosis.

SUMMARY

Pathological investigation was performed on 34 chickens naturally infected with leucocytozoonosis. The results are summarized as follows.

1) The characteristic macroscopic changes observed were hemorrhages and miliary grayish-white spots in various organs and enlargement of the spleen.

2) The following host-tissue reactions occurred: Hemorrhages; inclusion of megaloschizonts by foreign-body giant cells, histiocytes, lymphocytes, and fibroblasts; swelling and proliferation of cells of the reticulo-endothelial system in the liver and spleen; lymphocytic infiltration of the liver and kidneys; necrotic foci in the liver; deposition of hemosiderin in the liver and spleen; hyalinosis of the sheathed arteries in the spleen; and focal necrosis and histiocytic accumulation in the heart and ventriculus.

3) Megaloschizonts were found in the blood vessels of various organs and adjacent tissues. They were encapsulated by reticular fibers and endothelia of the blood vessels, and filled with fine granular merozoites. Only non-capsulated megaloschizonts were present in the brain.

4) Hepatic schizonts were seen in the cytoplasm of hepatic cells. So-called "early schizonts" were found in cells of the reticulo-endothelial system of the liver. Both hepatic and "early schizonts" were in the same stage and in an earlier stage than the megaloschizonts.

This report is dedicated to Dr. S. YAMAGIWA, the president of Obihiro Zootechnical

University (1962—), Professor Emeritus in Hokkaido University, Ex-professor in the Department of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University.

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鶏のロイコチトゾーン病に関する病理学的研究

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(昭和 41 年 5 月 23 日受付)

1959 年 7 月、多数羽飼育していた鳥取県内の養鶏場で、高い斃死率を示したロイコチトゾーン病の 34 例を、病理組織学的に検索した。

病鶏はニューハンプシャー種、20～27日令で、緑色下痢、元気沈衰、転倒および痙攣を主症状とし、短時日のうちに斃死した。

肉眼的には、皮下組織、筋肉および諸臓器に点状ないし不整形の出血、諸臓器の粟粒大の灰白色斑点の散在、および脾の腫脹が特徴的变化として認められた。

組織学的には、大小区々の出血巣、時期を異にする Megalosphizont に対する組織球、異物巨細胞、リンパ球、線維芽球などの細胞反応、肝および脾の

細網内皮系の活性化、ならびにヘモジデリン沈着、肝の巣状壊死、肝、心、腎、および筋肉の円形細胞の集積、放出された Merozoite の血管栓塞、脾の莢動脈の硝子化などが認められた。

多数の Megalosphizont が全身に認められ、多くは被膜または血管内皮に被包された微細な顆粒状の Merozoite を満たしていたが、脳実質には被膜に被包されない Megalosphizont が認められた。またアヒルや七面鳥には認められている Hepatic schizont、および細網内皮系細胞に、いわゆる Early schizont を認めた。これは生活史の上では、時期を同じくし、Megalosphizont よりも初期の段階のものと考えられた。

EXPLANATION OF PLATE

(All specimens were stained with hematoxylin and eosin.)

- Fig. 1. Various megaloschizonts in blood vessels of the lung. Groups of megaloschizonts are enveloped by the endothelium. $\times 180$.
- Fig. 2. Groups of mgaloschizonts beneath the ependyma of the ventriculus tertius. Proliferation of glia cells is seen around the megaloschizonts. $\times 160$.
- Fig. 3. Ruptured megaloschizonts surrounded by giant cells and lympho-histiocytic cells in the lung. $\times 340$.
- Fig. 4. Released merozoites around the renal tissue. Note hemorrhages and cellular infiltration. $\times 160$.
- Fig. 5. Severe infiltration of lympho-histiocytic cells and fibrocytic production in the heart. $\times 160$.
- Fig. 6. Hepatic schizont (arrow) in hepatic cells. $\times 580$.
- Fig. 7. So-called "early schizonts" in a glomerulus. $\times 580$.
- Fig. 8. So-called "early schizont" in cells of the reticulo-endothelial system of the spleen. $\times 1,200$.