A HISTOPATHOLOGICAL STUDY OF FOWL LEUCOSIS

by

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INTRODUCTION

The term leucosis is used to designate a widespread disease of fowls, characterized by paralysis of legs and wings, the formation of tumorlike masses in various parts of the body and by changes in the cellular structure of the blood. Fowl leucosis is the cause of a large part of the mortality of adult birds on many commercial poultry farms and in the past few years it has become increasingly more important as the cause of death in many farm flocks. Leucosis (or leukemia) is a general term including such names as fowl paralysis, range paralysis, neuritis, pearly eye and big liver disease. The similarity of leucosis to fatal leukemia of man has attracted many investigators interested in its oncologic aspect, as well as many veterinary research men. In the early work, investigators were interested in the paralysis and eye manifestations but the frequent concurrence of tumors in the various visceral organs which were of similar structure as those of the nerves, showed the conditions to be closely associated.

The selection of fowls for heavy production has served to place a heavy load on the metabolism of the fowl. Eggs are high in proteins, fats, vitamins and minerals so

hens must consume and digest large quantities of food. The usual poultry rations are of limited variety, doubtful quality and often of questionable vitamin content. The constant housing places them in an artificial environment and increases the probability of contact infection. Avitaminoses and other deficiency diseases are quite common and were involved in about twelve percent of the cases presented for diagnosis at this college in 1938.

THE BLOOD OF CHICKENS

The cells of the blood are divided into two main groups, the red cells or the erythrocytes, and the white cells or the leucocytes. The blood contains in addition the blood platelets. The erythrocytes contain hemoglobin and their principal function is to carry oxygen to the body cells. Anemia is a deficiency of blood. There may be a decrease in any of the blood elements but usually it is a diminished number or a poor quality of the red blood cells. This may be caused by excessive hematolysis, by defective hematogenesis or by hemorrhage. The erythrocytes of fowls are nucleated and oval in shape. The red blood cells in adult life are mostly formed in the bone marrow

at the ends of the long bones, principally the femur. The leucocytes are divided into two classes, the uninuclear and the polynuclear. The uninuclear leucocytes are the large and small lymphocytes and the large mononuclears. With ordinary stains the cytoplasm of the uninuclears is non-granular. The large and small lymphocytes are thought to neutralize toxins. The uninuclears are formed in the spleen, lymph glands and reticulo-endothelial system.

The polynuclear leucocytes stain with granules in their cytoplasm and are named, by many, the granular leucocytes. By the staining of their granules they are separated into three groups; the basophiles, the neutrophiles and the eosinophiles. Basophile granules are round and take the blue stain. Eosinophile granules take the red stain, are round in shape but not as large as the basophile granules. Neutrophiles are also called pseudo-eosinophiles, as their granules take the red stain, however, the neutrophilic granules are rod shaped. The polynuclear leucocytes are formed in the bone marrow at the ends of the long bones. The large monocytes of the uninuclear elucocytes and the polynuclear leucocytes are phagocytes and a part of the body's defense against infection. Blood platelets of fowls are nucleated, their cyto-

plasm stains very faintly and at the periphery they contain a few red granules. They are about the size of the erythrocytes and they assist in the clotting of the blood.

The nucleation of all the blood cells makes the counting of the white cells quite difficult, and that probably accounts for the great differences in the normal counts as given by various investigators. The following counts given by Professor L. D. Bushnell at Kansas State College are about midway between the extremes: basophiles 1.8 to 3.5 percent, eosinophiles 3.1 to 6.0 percent, neutrophiles 13.2 to 24.7 percent, monocytes 7.6 to 11.4 percent, lymphocytes 60.6 to 71.1 percent. The total white cells per cu. mm. varies from 27,320 to 40,330.

HEMATOLOGY OF THE DISEASE

Leucosis in chickens is a disease in which there is an increase in number of leucocytes in the blood, anemia and patholgoical changes in the nerves, spleen, liver, bone marrow and occasionally in the other internal organs. Blood changes may be shown by increases of the uninuclears or the polynuclears or by anemia and the presence of undifferentiated blood cells in the circulating blood. The

The age of the blood cells is considered of more importance than the relative numerical increase, according to Forkner (1).

The liver shows lesions so frequently that leucosis is commonly known as big liver disease. Various terms are used by the investigators to describe the predominating lesions and cells as neuroleucosis, visceral leucosis, lympholeucosis, myeloleucosis and, if the immature blood cells are not differentiated, erythroleucosis.

OCCURRENCE

The disease has been reported from practically all countries and is thought to be the cause of about ten percent of the mortality in chickens in the United States. Young adult birds, from four to eighteen months of age, are most commonly affected. Losses are frequently greatest shortly after pullets get into egg production. Leucosis is seldom of epidemic form, and usually one or two birds sicken at a time in the average flock while the seasonal mortality may vary from five to fifty percent. The sick birds may die in a few days to week, while some may linger a month and a few may apparently recover.

ETIOLOGY

Various opinions are held as to etiology of leucosis in chickens; some investigators believe it to be a virus; others that it occurs as a result of infection with bacteria and parasites; some contend that it is nutritional disturbance; while others consider it a true neoplastic formation. Many have reported data to show its transmissibility, while others have been unable to produce the disease in much greater numbers than occurred in the controls. In general, it may be said that leucosis has not been produced by many of the investigators with a uniformly high degree of regularity.

Transmission

In 1908, Ellerman and Bang (2) produced leucosis of fowls by experimental inoculation. They showed that transmitted cases were of two types; the blood and the tumor forms. They were able to transmit the disease with cell free Berkefeld filtrate, through three generations with blood changes and pathological lesions similar to those of

spontaneous cases. In 1930, Bayon (3) reported that blood and tissue of leucotic chickens, when inoculated into healthy fowl, produced anemic symptoms in some birds similar to those of the donor while in others an increase in number of red cells with chronic hyperemia of the liver and spleen was observed. He stated that when the proliferative process originates in the marrow, it leads to definite blood changes, but when proliferation arises in other organs, tumors are formed and the blood changes are fitful and limited.

Stubbs (4) reported studies on a blood type of leucosis that had remained constant for a period of six years. It reproduced itself irrespective of age or breed of fowl. This strain did not produce paralysis or tumors. Two of four chickens, however, developed tumors at the wing vein at the point of inoculation. Intravenous infections of blood of these two birds produced leucosis, some with and some without tumor formation. Intramuscular injection of tumor material more frequently produced tumors. Stubbs feels that the chickens had the tumor producing agent without any evidence of tumor. It may have in this way gotten into the blood strain and then been subsequently transferred with it.

The work of Johnson (5) showed that erythroleucosis, neurolymphomatosis, and myeloleucosis are different manifestations in response to a common filterable agent. He mentions hyperplasia of the bone marrow of the radius, ulna and femur as almost constant lesions in the early stages. He considers the stem cell or hemacytoblast as arising from the reticulum of the bone marrow. These cells which are potentially red or white blood cells predominate in the first stages of leucosis. They may later differentiate to myeloid or lymphoid cells. He found the oxidase test was of no aid in distinguishing between the myeloid and the lymphoid series when the stem cells were undifferentiated. Johnson believes that the visceral masses are heterotopic blood cell formations and that the neuro-tumors metastasize from bone marrow by way of blood stream.

Lee and Wilcke (6) found that the etiological agent of leucosis resembled colloids in its electrophoretic action. At a pH value of 6.01 to 7.01 it was electrostatic, at an acid pH of 4.01 to 6.01 electronegative, and at a basic pH 7.01 to 9.01 electropositive. They produced the three forms of leucosis by cell free filtrate.

Stubbs and Furth (7) reported that young birds were more susceptible than adult chickens and that guinea fowl and pigeons were refractory to their strain. In 1932,

Patterson, Wilcke, Murray, and Henderson (8) reported production of all types of leucosis by injections of suspensions of lesions and by pen contact. Biely, Palmer, Lerner, and Asmundson (9) believed that there was an inherited resistance to paralysis and that success in transmission would require the causative agent and a genetically susceptible host. Gildow, Williams, and Lampman (10) produced the disease by pen contact. They found that the incidence of disease was greater in the progeny of clean stock and that some individual hens had progeny which were resistant to leucosis. That different strains of chickens regardless of breed or variety show marked difference in regard to their resistance to leucosis was reported by Patterson (11). The incidence of iritis was reported by Madsen (12) to be less in strictly iritic progeny than in the general flock progeny.

Dalling and Warrack (13) found that incubation and raising played no part in causing leucosis. Pullets which produced the most cases of leucosis have remained healthy and birds in all stages of leucosis have failed to infect others in the same pen. Olson (14) failed to produce paralysis by transplantation of affected nerve tissue or by cross circulation of the blood between paralytic and normal

chickens.

Professor L. D. Bushnell of this college inoculated fowls parenterally and by feeding and has not been able to get a significantly greater number of infections than in the controls. Little greater incidence in inoculated birds than in the controls was also reported by Beach (15).

Bacterial Causes

Winternitz and Schmeisser (16) observed a typical case of leucosis in a chicken with fowl typhoid. They found that fowls which did not die as a result of the first injection of fowl typhoid frequently developed leucotic changes in the blood with subsequent infiltration of the visceral organs with myeloid cells. Cash and Doan (17) observed spontaneous development in undernourished pigeons of a fatal disease showing marked myeloid hyperplasia of the bone marrow, increase of myelogenous cells in the blood and infiltration of these cells into the kidneys and liver. They cultivated Salmonella aertycke from the pigeons and were able to produce disease by oral administration of cultures or by inoculation of liver emulsion of infected birds. Emmel (18) produced leucosis by re-

peated injections of bacteria of the Salmonella group, heat killed bacteria, benzene, phenol, xylol, by normal homologous tissues and by suboxidation. He advanced the hypothesis that these substances stimulate tissue proliferation and autolysis, which when fully established, is a self perpetuating process and is transmissible within the species.

Fowls seem to develop leucosis very easily and many have reported its production from injection of various foreign bodies.

Nutritional Causes

Tumors are most common in old chickens when the body has lost some of its power to utilize vitamin A. A avitaminosis may cause a proliferation of the cells of the skin and of the mucous membrane. Ackert, McIlvaine and Crawford (19) have shown that chicks on vitamin A deficient rations were less resistant to worm infestations. Ackert and Nolf (20) showed that chicks on vitamin B deficient rations were less resistant to worm infestations. Ackert and Spindler (21) showed that vitamin D deficient rations did not lower resistance to worm infestations.

Bayon (3) found small quantities of raw chopped liver beneficial in treatment of valuable birds. Johnson (5) and Durand (22) also mention the improvement shown by addition of liver to the ration. There is little doubt that rations containing proper vitamins will contribute to the health of the flock. One of the latest articles on leucosis and nutrition is by Butler, Warren and Hammersland (23). They report results of experimental work under field conditions in Montana. They maintain that natural resistance is a practical means of reducing flock mortality. They are of the opinion that the egg yolks of hens on vitamin sufficient rations will be dark golden in color, and that pale yolks indicate vitamin deficient rations. They found that many rations contained rancid fish or marine oils which was the cause of chronic enteritis. By removing the rancid oils and adding fresh 0.5 percent cod liver oil, 0.2 percent wheat germ oil and allowing free access to fresh cut alfalfa, they reduced the mortality about 75 percent. They also reported treatment of 25 clinical cases with 18 recoveries by use of wheat germ oil, in one mil doses, intramuscularly every other day for three doses, in addition to rations containing one percent cod liver oil, one percent germ oil and fresh alfalfa. Jungherr (24)

found that wheat germ oil was not a specific antileukotic. Many researchers have observed the presence of parasites in leucosis cases. So many cases, however, have been shown to be free of parasites and though they may contribute to the infection, they are not considered to be an etiological factor.

SYMPTOMS OF LEUCOSIS

Where the poultryman has had previous cases of leucosis in the flock, it is rather easy to pick out most of the leucotic birds by their clinical symptoms. Dropped wing, extended leg, white eye, slightly greenish diarrhea, icteric and anemic comb and wattles, slow clotting of blood, absence of fever, subcutaneous tumors, going light and out of production, any, all or combinations of these symptoms mark the leucotic fowl. The birds retain their appetite to a great degree and their condition may improve if they are separated and well fed. However, leucotic birds usually die in a few days.

At post mortem the nerves of the paralyzed leg or wing may show swelling, a greyish color and sometimes nodules. The liver, spleen and kidneys may be greatly enlarged and have a nodular appearance, or they may be dark in color,

soft swollen, and filled with blood, even when the bird was well bled out. At times, internal hemorrhages due to rupture of these organs may cause death.

MATERIALS AND METHODS

Histopathological studies were made of gross lesions of chickens diagnosed as leucosis at the Kansas State Poultry Diagnostic Laboratory. Parts of liver, spleen, kidney and bone marrow were fixed in Zenker's fluid, blocked in paraffin, sectioned and stained with (a) hemotoxlyn and alcoholic eosin, (b) Unna's alkaline methylene blue and alcoholic eosin. Blood smears were made from cases showing clinical symptoms, these were stained by Wright's method and with Unna's alkaline methylene blue and eosin. White and red cell counts were made on two clinical cases. Serum from these chickens was injected into pregnant rabbits with no ill effects to the rabbits. In the tissue slides, the alkaline methylene blue and eosin stain brought out the granules in the granulocytes better than the hemotoxlyn eosin method.

HISTOPATHOLOGY

The tissue slides are divided into three groups: (a) lymphoid nos. 1-2-3-4-5-15, these slides show few or no granulocytes in the lesions; (b) mixed nos. 6-7-8-10-11-16-18-20, these slides show a number of granulocytes; (c) myeloid nos. 12-13-14-17, in these slides, granulocytes compose half or more of the cells in the lesions. Slides nos. 9 and 19 show degeneration of the liver cells, the blood vessels are engorged with blood, while the bone marrow of number 19 shows large areas which are producing red blood cells. On practically all slides, the functional cells of the liver and kidney show degrees of degeneration varying from cloudy swelling to fatty degeneration and rupture of the cell. No increase in supporting connective tissue was seen in any of the slides. Pigment in any quantity was not seen in any of the slides.

Lymphoid cells are present in all slides but nos. 9 and 19. These cells are quite similar to the cells of the spleen at the periphery of the germinal center. Lymphoid cells are found in the blood and blood vessel walls inside many of the severe lesions. The nuclei of the lymphoid cells are vesicular or bladder shaped and vary in size from

the nucleus of a small lymphocyte to that of a large lymphocyte.

The nuclei contain considerable chromatin and usually have three to five nucleoli, which are supported by a network of fibrils. The cytoplasm of lymphoid cells is about the same in quantity as that of large lymphocyte. It stains a very faint blue and the cell outline is not distinct.

The myeloid cells are similar to the mature polynuclear neutrophiles of the blood. Some have unsegmented pale staining nuclei, these are called myelocytes. The myeloid cells contain faintly eosinophilic rod granules and are called by some pseudo-eosinophiles.

Lymphoid Leucosis

The livers of mild lymphoid cases show the lymphoid cells surrounding the portal vein in the interlobular connective tissue. There may be five to fifteen layers of cells about the vein forming a lymphoid cuff where the lymphoid cells are deeply layered; they also surround the hepatic artery and the bile ducts. In three of the lymphoid cases the bile ducts are thickened, and in areas

appeared to be convoluted, resembling some of the tubules of the kidney. The few granulocytes seen are polynuclear neutrophiles.

Where lymphoid areas are large the lymphoid cells invaded the lobules and the functional cells seemed to disappear as though they were dissolved by the lymphoid cells. There are a few blood vessels in the lymphoid areas but the bile ducts are practically gone. Lymphocytes usually the small ones are seen in areas adjacent to the blood vessels. In number 1 slide, a few lymphoid areas show necrosis of the central cells.

The lymphoid cells of the kidney vary from a few groups of cells between the tubules and in the glomeruli to areas where half of the tissue is made up of lymphoid cells. The germinal areas of the spleen are not distinct, the pulp is increased and contains many blood cells. The supporting trabeculae are decreased. In the bone marrow the normal fat and germinal areas for red blood cells and granulocytes are almost entirely replaced by the lymphoid cells although a few germinal areas remain in the center of the marrow.

Mixed Leucosis

The lesions of the mixed group vary from those of the lymphoid group in that there are fewer large areas of lymphoid cells. In the vicinity of blood vessels are groups of neutrophiles, many of which are myelocytes. The bone marrow shows increased germinal areas for granulocytes and a few lymphoid areas. In number 8 of the mixed group, a lesion of the heart muscle is found. Here the lymphoid cells accumulate between the muscle fibers and in some places the muscle fibers are about gone, yet there were no signs of heart weakness.

Myeloid Leucosis

In the myeloid group, the granulocytes make up about half of the cells of the lesions. The sinusoids of the liver and spleen are filled with blood containing many neutrophiles. The liver lobules are quite distinct. About half of the neutrophiles are myelocytes having a pale staining unsegmented nucleus. The bone marrow is almost completely made up of germinal areas of granulocytes.

Blood

The blood slides may be divided into three groups: (a) lymphoid, where more than sixty percent of the cells are lymphocytes; (b) myeloid, where more than twenty-five percent of the cells are neutrophiles; and (c) erythroid, where there are stem cells and immature red blood cells in the circulating blood stream. Forkner (1) states that in acute leukemia, eighty to ninety percent of the white blood cells are of a uniform and immature type. Using this as a standard, all the smears were from chronic leucotic cases. The small number of blood platelets accounts in part for the slow blood clotting in the severe anemic cases. Forkner (1) reported that the low platelet count is almost constant in cases of leukemia. The red blood cells are decreased in number and vary in size and shape. They may contain vacuoles and stain poorly due to the decreased amount of hemoglobin.

In the lymphoid group, the small lymphocytes are usually the predominating type of cell. These small lymphocytes have a small amount of cytoplasm. With the alkaline methylene blue stain, the nuclei of about half the

small lymphocytes take a pink stain. There are varied degrees of staining from pink to dark purple on the same slide. These pink nuclear lymphocytes are thought to be pathological cells. In the myeloid group there are increased numbers of neutrophiles, many of which have pale staining nuclei. Few myelocytes are seen in the blood slides. Where the increase in neutrophiles is not marked, there are frequently numbers of pink nuclear lymphocytes showing that there are probably mixed types.

The erythroid group shows the most marked blood changes. The red blood cells are of all sizes and shapes, (poikilocytes). About half of them are round and contain no hemoglobin. The cytoplasm of the round red blood cells takes a light blue stain (polychromophilia). Other red blood cells are long, narrow and contain vacuoles which take a mottled stain. The nuclei of many of the immature cells are large, round and take a somewhat paler stain. There is an increase in the neutrophiles and monocytes but the small lymphocytes are only about half their normal number.

Table 1. Differential leucocyte counts of chicken blood.

	Smear number																			
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Basophiles		1				4				1								1		1
Eosinophiles		5															3	5		
Neutrophiles	66	17	24	64	47	24	48	44	55	68	50	33	25	38	51	25	12	19	29	19
Myelocytes								1	1						1			1		
Monocytes	2	6	1		1	24	14	5	7	2	1	1	4	20	3	3	5	12	4	2
Lymphocytes large	10	12	6	7	14	17	5	14	22	21	10	24	20	20	25	22	11	20	10	18
Lymphocytes small	22	59	69	29	38	31	33	36	15	8	39	42	51	22	20	50	69	42	57	60
Classification*	M	L	L	M	M	E	M	M	M	M	E	M	L	M	M	L	L	L	L	L

* M = myeloid
L = lymphoid
E = erythroid

Table 2. Total cell count of chicken blood.

Number of chicken	Red blood cells	White blood cells
56	1,544,000	160,000
61	2,356,000	84,000

SUMMARY

1. Fowl leucosis is the cause of a large part of the mortality of adult birds on many commercial poultry farms and in the past few years, it has become increasingly important as the cause of death in farm flocks.

2. The lesions of the visceral organs were divided into three groups: the lymphoid, the mixed, and the myeloid. They were differentiated according to the approximate number of lymphoid cells found.

3. The bone marrow of the femurs showed hyperplasia correlated with the predominating type of cell found in the lesions.

4. The blood changes were also divided into three groups: the erythroid, the lymphoid, and the myeloid. In the erythroid group, there were varying degrees of immature red blood cells. The lymphoid and the myeloid groups were differentiated by the predominating type of cell found in the blood smears.

5. As none of the smears showed great numbers of uniform, immature cells, they were considered to have been from chronic cases of leucosis.

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