

SPONTANEOUS AND EXPERIMENTAL LEUKEMIA OF THE FOWL.

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During the past three years a transmissible leukemia of the fowl has come under observation, and, in the following paper, a summary of the literature, together with a brief presentation of the clinical and anatomical aspects of the disease will be given.¹

HISTORICAL.

Moore (1) reported infectious leukemia in fowls, but this has since been regarded as not true leukemia. Butterfield (2) and Mohler² recognized the condition from the postmortem findings, and the first careful study, including the clinical course and anatomical changes, was made by Warthin (3) in 1907. The predominating cell in the blood and tissues was the large lymphocyte,³ and the condition was diagnosed leukemic lymphocystostoma. Other cases described by both Butterfield and Warthin lacked the blood changes but showed the same tissue picture and were considered by these authors as examples of aleukemic leukemia.

Kon (4) and later Soshestrenski (5) each report a similar case of leukemia. They consider their case as true splenic leukemia.

Ellermann and Bang (6, 7), in 1908, were the first to transmit the disease successfully from a spontaneous case to other healthy fowls. They report two typical spontaneous cases in every respect similar to Warthin's first case. They transmitted the disease by inoculation of organic emulsion through three generations, producing blood picture and organic findings identical with the spontaneous cases. They also found by inoculation of organic extract from a pseudoleukemic fowl (used in the same sense as aleukemia of Warthin) that a picture of true leukemia resulted, and concluded that the two conditions are etiologically identical.

In several subsequent reports (8, 9) Ellermann and Bang call attention to the following points: (1) that in transmitted cases the disease may appear as

¹ A more detailed and fully illustrated study will appear in the *Rep. Johns Hopkins Hosp.*, 1915, xvii (in press).

² Cited by Butterfield (2). Mohler personally never reported his cases.

³ Obviously the large mononuclear of the classification presented in this paper.

typical leukemia, pseudoleukemia, or as an anemia with changes in the bone marrow; (2) that mitoses in the blood are pathological and always present in leukemia; (3) that the blood of leukemic fowls contains the virus; and (4) that the disease can be produced by a cell-free Berkefeld filtrate. They conclude (a) that leukemia must be an infectious disease, and (b) that it is to be placed among the diseases due to a filterable virus.

Schridde (10) questioned the infectious etiology of leukemia and claimed that chickens injected with extracts of entirely normal organs present the same changes as Ellermann and Bang reported for leukemia.

Hirschfeld and Jacoby (11, 12) in 1909 observed a typical case of leukemia, and the following year they reported (13) the transmission of the disease from a leukemic animal⁴ into the fifth generation.

Burckhardt's claim (14, 15), supported by Friedberger (16), that leukemia could be produced in the fowl by inoculation with pure culture of fowl tubercle bacilli is only of passing interest.

Ellermann (17, 18) in answer to the above objection of Schridde points out that in the latter's experiments only the blood picture was produced, and in the absence of the characteristic organic changes of which Schridde makes no mention, his experiments are of no importance. The injection of emulsion of normal organs Ellermann never found to cause any change in the blood.

Ellermann again reports the successful transmission of the disease with Berkefeld filtrates. Hirschfeld and Jacoby (19) and Burckhardt (15), on the other hand, report unsuccessful results with Berkefeld filtrate.

Ellermann claims to have shown that the leukemic virus can be separated from the virus of tuberculosis by filtration, and that, therefore, the two diseases are distinct. He further claims to be able to separate the spontaneous and transmitted leukemia into the types (a) myeloid and (b) lymphatic. He says that a myeloid type may occur in one generation and a lymphatic in the next, or both types in the same generation and that this is highly suggestive that both forms in man are due to one and the same infective agent.

The Normal Fowl.

The following data of the normal fowl are confined to those portions of the body which are involved in leukemia and have been compiled from a large number of young adult Plymouth Rock hens.

Blood.—The blood is readily obtained from the vein under the wing. From a small needle puncture the blood flows under pressure. It is thick, dark red, and clots quickly.

The number of red blood cells averages 3,000,000 to 4,000,000 per cmm. The number of white blood cells⁵ varies between 20,000 to 80,000 per cmm. Actually the proportion of white blood cells to red blood cells varies between 1 to 50 and 1 to 150. The hemoglobin (Sahli) averages 60 to 70 per cent.

⁴ This animal was given to them by Ellermann and Bang.

⁵ These were determined by the indirect method.

The blood cells stained by Wilson's method may be classified as follows:

Erythrocytes.—1. Normocyte: elliptical disk. Nucleus same shape as cell, deep blue, slightly pyknotic. Cytoplasm yellow and glassy. (Both cell and nucleus are uniform in size, shape, and staining.)

Blood Platelets.—Length of normocyte; width less than that of normocyte. Nucleus round, purple; chromatin diffuse; diameter equal to width of its cell. Cytoplasm pale gray with vacuoles about nucleus, frequently containing small circumscribed red structures. They may vary in size and shape.

Leucocytes.—1. Polymorphonuclear leucocyte with eosinophilic rods: round, diameter about length of normocyte. Nucleus, of two or more lobes, pale blue; chromatin diffuse. Cytoplasm colorless with bright red, spindle-shaped rods.

2. Polymorphonuclear leucocyte with eosinophilic granules: about the same in shape and size. Nucleus of two or more lobes, purple, slightly pyknotic. Cytoplasm faintly blue with dull red granules.

3. Lymphocyte: round, diameter about width of normocyte. Nucleus round, purple; chromatin diffuse. Cytoplasm small in amount, to one side of nucleus, pale blue. Same cell may be slightly larger. Thus a division into small and large lymphocyte may be made.

4. Large mononuclear cell: round or oval, diameter about length of normocyte (at times more or less). Nucleus round, oval, or slightly irregular, and larger; otherwise similar to nucleus of lymphocyte. Cytoplasm abundant, completely surrounds nucleus; pale blue. (A suggestion of fine granules.)

5. Mast cell: about same size and shape. Nucleus round or oval, very pale blue. Cytoplasm abundant, colorless, mostly to one side of nucleus with purple granules; some scattered over nucleus.

Differential count, 300 cells.	Per cent.
Polymorphonuclears with eosinophilic rods	29.6
" " " granules	4.3
Lymphocytes	42.3
Large mononuclear cells	19.4
Mast cells	2.2
Unclassified cells	2.2
	100.0

Gross Anatomy.—A fowl of average size and weight (1,760 grams) has abundant subcutaneous fat and large muscles. The inner surface of the skin is slightly yellow.

The cervical lymph glands⁶ are sometimes difficult to find. They are present as two chains of six to ten glands, one on each side of the neck, lying in the fat upon the internal jugular vein, and extend-

⁶ Kon (4) considers these structures the thymus. He (4) and Ellermann (17, 18) state that the fowl has no lymph glands. Soshestrenski (5) asserts their existence by stating that, in his case, they were not enlarged.

ing from the middle of the neck to the base of the heart. They are elliptical, flat, lobulated bodies, averaging $1 \times 0.5 \times 0.2$ cm. The lobules are pink and separated by narrow septa of fat. No other lymph glands were found in the entire body.

The peritoneal cavity contains an omentum rich in fat, averaging 1 cm. in thickness. It takes its origin from the anterior surface and lower margin of the gizzard, extending down the intestines, etc.

The liver is a bilobed organ, weighing 50 grams, or 2.8 per cent of the body weight. It extends down between the omentum and sternum, the margin of each lobe reaching as far as 3 cm. above the tip of the xiphoid. It is uniformly reddish brown, moderately soft, and friable. The lobulation is usually distinct, at times difficult to see. The cut surface shows small blood vessels in long and cross-section.

The spleen lies just behind the liver. It measures $2 \times 1.5 \times 1$ cm. and weighs 1 gram, or 0.05 per cent of the body weight. It is small, soft, and reddish brown. Beneath the capsule bluish white Malpighian bodies, slightly larger than a pin point, may be indistinctly seen. On section the capsule is very delicate, the trabeculae are few, but usually definite, containing small blood vessels. The Malpighian bodies at times are prominent. The pulp is not raised above the edge of the capsule. The kidneys weigh 12 grams, or 0.7 per cent of the body weight. They are uniformly reddish brown with a slightly nodular surface.

The bone marrow is mottled, bright red and yellow. It is very soft, at times semifluid. It is always rich in fat.

Histology.—The cervical lymph glands are divided into lobules of parenchyma, separated by fatty tissue. Each lobule is surrounded by a delicate fibrous capsule and has a very fine reticulum, in which the lymphocytes are diffusely scattered. These are small round cells with round, deeply staining, pyknotic nuclei and a narrow rim of pink cytoplasm, usually incompletely surrounding the latter.

Sometimes red blood cells are associated with the lymphocyte; but these are mostly confined to the capillaries. They appear as elongated yellowish pink cells with solid black nuclei, which are seen as rods or dots, according to whether they are in long or cross-

section. Scattered through the lobule are small, sharply outlined, hyaline structures, which Kon (4) considered as corresponding to Hassal's corpuscles of the thymus.

In the liver the lobule is difficult to limit. Periportal spaces are not easily made out. They contain the usual vessels, surrounded by very little acellular fibrous tissue, although lymphocytes may be present diffusely or in small follicles. The liver cells are arranged in trabeculæ, separated by capillaries. All blood vessels and capillaries are filled almost exclusively with red blood cells.

In the spleen the Malpighian bodies are numerous and indistinct. They are composed of the usual lymphocytes surrounding very small arteries. Red blood cells are limited to the pulp where they occur more or less in clusters, although sinuses or inclosures of any kind cannot be definitely demonstrated. The pulp is also diffusely infiltrated with lymphocytes.

The kidneys for all practical purposes have a structure similar to the human kidney.

The bone marrow under low power shows a framework of fatty tissue enclosing nests of marrow cells. Stained with hematoxylin and eosin these may be grouped as follows:

Erythrocytes.—1. Normocyte (a): elongated, varying in shape due to pressure. Nucleus a solid black rod or dot (long or cross-section). Cytoplasm yellowish pink and glassy.

2. Normoblast (b): round; diameter about width of normocyte. Nucleus uniformly black. Cytoplasm pink or faintly blue, and glassy. Frequently a narrow clear zone is seen about the nucleus.

3. Megaloblast (b): the same, larger, about the length of a normocyte. Nucleus slightly pyknotic.

Leucocytes.—1. Polymorphonuclear myelocyte with eosinophilic rods⁷ (c): round. Nucleus, two or more solid, black lobes. Cytoplasm colorless with bright red, spindle-shaped rods.

2. Polymorphonuclear myelocyte with eosinophilic granules⁷ (b): round; about same size. Nucleus two or more slightly vesicular lobes. Cytoplasm colorless with bright red granules.

3. Mononuclear myelocyte with eosinophilic granules (a): round or oval, varying in size, mostly larger than 1 and 2. Nucleus round, oval, or horseshoe-shaped, eccentric, slightly or very vesicular. Cytoplasm colorless with bright red granules.

⁷ These are obviously the polymorphonuclear with eosinophilic rods, the polymorphonuclear with eosinophilic granules, and the large mononuclear cell of the normal blood.

4. Large mononuclear myelocyte^r (d) : round, slightly larger. Nucleus round, very vesicular, one or more nucleoli. Cytoplasm basophilic, moderate in amount.

Reticular Cells.—Branched; nucleus elongated, vesicular; several nucleoli. Cytoplasm pink, giving off delicate fibers to form reticulum.

Lymphocytes, mast cells, platelets, mitoses of red and white cells were not seen. The letters in parenthesis indicate the order of predominance.

Spontaneous Leukemia.

On October 31, 1912, a typical case of leukemia of the fowl was brought to the pathological laboratory. The animal was a Plymouth Rock hen and had just been killed. Nothing is known of its clinical history.

Autopsy Findings.—Blood smears taken from the heart were stained by Wilson's method. A detailed description of the cells of leukemic blood will be reserved for the experimental leukemia where it was possible to study these more carefully. Suffice it here to comment briefly upon the most striking features.

There was an enormous increase in white blood cells, the proportion of white to red cells being 1 to 1.3. The predominating cells were the large mononuclear and the mononuclear myelocyte with eosinophilic granules. The latter is abnormal in the blood, normal in the bone marrow, and when found outside of the bone marrow is typical of leukemia. Lymphocytes and polymorphonuclears were strikingly decreased. Of the latter those with red granules and the mast cell were rare. Mitoses of the large mononuclears were common. The red blood cells appeared poor in hemoglobin, showed anisocytosis, poikilocytosis, and polychromatophilia. These cytoplasmatic changes were usually associated with an increase in the size of the nucleus. Premature red blood cells, normoblasts, and megaloblasts were present in large numbers.

Differential count, 300 cells.	<i>Per Cent.</i>
Polymorphonuclears with eosinophilic rods	8
" " " granules	0
Lymphocytes	2
Large mononuclear cells	30
Mast cells	0
Mononuclear myelocytes with eosinophilic granules	52
Unclassified cells	8
	100

Gross Examination of Tissues.—The comb, the featherless area about the eyes, the wattles, the buccal mucous membrane, and the conjunctivæ were very pale. The anterior chamber of the left eye was filled with an old blood clot. A moderately firm, slightly nodular tumor $3.7 \times 2 \times 1.5$ cm. occupied the triangular space on the left side of the head between the angle of the mouth, the ear, and the angle of the lower jaw, extending slightly below the ramus of the latter. Emaciation was extreme. Subcutaneous fat was practically absent. The muscles were greatly atrophied. A second, slightly smaller tumor $1.7 \times 1 \times 1$ cm. was found just inside of the ramus and below the orbit, apparently communicating over the ramus with the first growth and continuous below with a slightly larger third mass.⁸

The cervical lymph glands were somewhat enlarged and showed indistinct lobulation.

In the peritoneal cavity the omental fat was entirely absent and the lower margin of the right and left lobes of the liver extended almost to the pubis.

The liver was enormous. Its surface was extremely mottled. It was reddish brown, and specked with innumerable gray or slightly yellow spots from pin point to a few mm. in diameter, frequently closely packed to form irregular areas, the largest being 1 cm. in diameter. In addition there were scattered gray or slightly yellow nodules averaging about 2 mm. in diameter. The sectioned surface was similar in appearance. The blood vessels were surrounded by a gray zone.

The spleen was enormous. It was about the size of a small hen's egg and diffusely gray.

At the apex of the heart there were several gray spots, pin point to pin head in size.

The kidneys were greatly enlarged and both showed nodules similar to those in the liver.

The bone marrow was gray and poor in fat.

The remaining organs appeared normal.

Microscopical Examination of Tissues.—The normal structure of the cervical lymph glands was changed. The interlobular fat had

⁸ A more detailed description and study of these tumors will be reserved for a future communication.

entirely disappeared with approximation of the greatly swollen parenchymatous lobules. Here and there were intra- and interlobular foci of myeloid tissue. These frequently enclosed an artery or vein. There was a more diffuse infiltration of the parenchyma by myeloid cells, filling capillaries, veins, arteries, and in places breaking through the lobular capsule into the interlobular tissue. Of the infiltrating cells two predominated: (1) A large mononuclear, usually round, at times slightly polygonal, with a single round, oval or indented, vesicular, at times multiple nucleus with one or more nucleoli. Its cytoplasm was granular and slightly basophilic. (2) The mononuclear myelocyte with eosinophilic granules, described under normal bone marrow. Mitoses of both cells were common. Normoblasts and megaloblasts were also present.

In the liver the process was most extreme. Very little liver tissue remained. Everywhere were closely packed masses of myeloid cells, in which the liver trabeculae had completely disappeared. These masses of cells frequently surrounded blood vessels, both arteries and veins, infiltrating the walls of the latter and filling the lumina of both. Within the vessels, the white blood cells were present in about equal proportions with the normocytes. These cells also occurred everywhere between the liver columns, spreading them apart. It was usually difficult to demonstrate the capillary wall. The hepatic cells in these areas had not suffered so much. Some of the circumscribed infiltrations showed a coarse sclerosis. The infiltrating cells were the same as in the cervical lymph glands. A few polymorphonuclear cells of both types were present.

The pulp of the spleen was diffusely infiltrated with closely packed leukemic cells, crowding the reticulum and distending blood vessels, separating and compressing the Malpighian bodies. Mononuclear myelocytes with eosinophilic granules although present were somewhat scarce. The large mononuclear with mitoses was the common cell.

The bone marrow was greatly changed. It consisted of closely packed white marrow cells, with complete atrophy of the fat, and a great rarity of normocytes. One or two small areas of sclerosis were present. The marrow cells, both red and white, answered the same description as normal bone marrow, with the exception of the

large mononuclear myelocyte, which showed considerable variation in its nucleus. This was single or multiple, round, oval, or horse-shoe-shaped. The order of predominance had changed. The large mononuclear myelocyte was present in far greater numbers than any other cell, and showed extensive mitoses. Normoblasts and megaloblasts with mitoses followed next in frequency, then normocytes. Polymorphonuclear myelocytes, both with eosinophilic rods and granules, had disappeared entirely. The mononuclear myelocyte with eosinophilic granules could not be demonstrated with certainty. As in the normal marrow, lymphocytes, mast cells, and platelets were seen.

In the remaining organs, the blood vessels were filled with the characteristic blood. Cell infiltrations, both diffuse and focal, occurred in the heart, lungs, and kidneys.

Summary. Blood.—(a) Although a total count was not made, a great increase in the total number of white blood cells was evident from the appearance of the blood smears, and blood vessels in sections. The actual proportion of white to red cells (1 to 1.3) substantiated this fact. The differential count showed a marked increase of the large mononuclear cell at the expense of the other white blood cells of the normal blood. In addition, a true myelocyte appeared in the circulation. Mitoses of the large mononuclear in the circulation were common. (b) There was a corresponding decrease in the total number of red blood cells. These were poor in hemoglobin and showed variation in size and shape, basophilic staining of the cytoplasm, and swelling of their nuclei. Premature red cells also occurred.

Organs.—(a) Many organs contained diffuse or circumscribed infiltrations of myeloid cells. The large mononuclear and mononuclear myelocyte with eosinophilic granules predominated in these infiltrations although the other cells of the normal bone marrow were also present. This myelosis involved especially the liver, spleen, kidneys, and bone marrow, resulting in an extensive enlargement of the first three organs. Almost all the remaining organs show infiltration, but to a less degree. (b) The proportion of white to red cells was greatly increased in the blood vessels. The predominating cells were the same as in the infiltration.

Conclusion.—If we consider that the cells characteristic of both the leukemic infiltration and blood are the same, and that under normal conditions the mononuclear monocyte with eosinophilic granules occurs only in the bone marrow and the large mononuclear only in the marrow and blood, it is evident from the summarized facts that the above case must be considered a typical case of myeloid leukemia.

Experimental Leukemia.

From the above animal, the disease was transmitted into the 5th generation. A total of 105 animals was used in conducting many different kinds of experiments. Of this number, 22 in all developed leukemia. In 4 additional animals, a definite diagnosis could not be established, although they were highly suggestive.

This paper will be confined to a report of the simple transmission of the disease by injection of an organic emulsion.

Five series of experiments were conducted, each consisting of 5, 10, or 15 fowls, with 20 to 40 per cent of positive animals. Of the total number of 40 chickens injected, 13 developed leukemia; *i. e.*, 32.5 per cent. In addition, a definite diagnosis could not be made in 3, which were very suspicious.

For transmission pieces of liver, sometimes also spleen, were thoroughly macerated in 0.9 per cent salt solution and filtered through a single layer of fine linen or a small amount of raw cotton. This emulsion could be used for intraperitoneal injection. When used intravenously, however, instant death resulted, and it was necessary to filter it also through two layers of filter paper with the aid of a suction pump for successful intravenous inoculations. This filtrate still contained blood and parenchymatous cells. A 15 per cent emulsion gave the best results. 10 cc. of the emulsion were injected either intravenously or intraperitoneally. Both methods were sometimes combined when a total of 20 cc. was administered. The vein under the wing was selected for injection.

Animals used for transmission were young adult hens of the same breed as the spontaneous case and exemplified by the normal control.

Clinical History.—The incubation period is usually from 5 to 6 weeks, with a maximum of 16 weeks.

The onset is as a rule abrupt, beginning with a slight but progressive, at times a sudden and intense, pallor, affecting comb, featherless area about eyes, and wattles. This is almost invariably associated with jaundice, which is likewise progressive and usually reaches an extreme grade. A characteristic yellowish pink color results which gives the fowl a ghastly appearance. Comb, etc., may be surprisingly red or extremely pale without jaundice. The animal emaciates rapidly and to an extreme degree. The actual loss in weight during the disease is striking. No. 28 at the onset of the disease weighed 1,485 gm. The duration of the disease was 3 weeks and 4 days. Weight at death was 897 gm., a loss, therefore, of 588 gm. in 25 days, or at the rate of 24 gm. per day.

At first the fowl acts normally, or only slightly ill. In a few days, however, it is very sick, stops eating and drinking, and stands about with head retracted, eyes closed, and tail drooping. It prefers to assume a squatting position. If made to move, it does so very slowly and carefully. Weakness becomes more and more marked, until on the last day it lies on the floor, wings drooping, eyes closed, and is at times dyspneic. Fever has never been noticed.

After the animal is observed to be sick the illness lasts from 1 to 2 weeks. One case, exceptionally acute, lasted only 33 hours. The longest course was 4 weeks.

Only in one case, out of 23 leukemic animals, did a spontaneous cure result.

Blood.—With the onset of anemia, the vein under the wing collapses and the blood undergoes a change. It soon flows with the greatest ease, is pale yellow and watery, and shows no tendency to clot. The animal apparently would bleed to death from a pin point wound if hemorrhage were not artificially arrested.

In most cases there is a progressive and extreme decrease in the total number of red blood cells. At the onset the count may be but slightly below the normal, 2,224,000, while just before death it at times reaches 630,000 per cmm. In one case the count remained normal. The white blood cells are invariably high, 131,200 to 210,000 per cmm. The ratio of white blood cells to red blood cells varies between 1 to 3 and 1 to 9.

The hemoglobin usually falls steadily and reaches a very low point. From slightly below normal, 40 to 50 per cent at onset, it frequently drops to 10 to 15 per cent just before death. In one case there was no change at all.

The morphology of the leukemic blood is exceptionally interesting. In addition to the enormous increase in the number of white cells, all the cellular elements show marked changes and many new forms make their appearance.

The blood cells stained by Wilson's method may be classified as follows:

Erythrocytes.—1. Normocyte: (a) normal, except for variation in the amount of hemoglobin; (b) showing anisocytosis, poikilocytosis, and polychromatophilia, mostly associated with a swelling of the nucleus and a separating of its chromatin.

2. Normoblast: round; diameter less than length of normal normocyte. Nucleus of the same color, or slightly purple, with more scattered chromatin than nucleus of normal normocyte. Cytoplasm greenish blue and glassy, frequently with a clear zone about the nucleus.

3. Megaloblast: the same, except that the diameter is equal to or greater than the length of the normal normocyte.

4. Mitotic cells: all stages, from monaster to complete division of nucleus. (a) Round or elliptical; diameter about the length of the normal normocyte. Dense, deep blue chromosomes, massed in center of cell. Cytoplasm greenish blue and glassy. (b) The same, with two masses of chromosomes in opposite extremes of cell. (c) The same (at times with a slight constriction in the middle of the cell) with irregular dense, deep blue or slightly purple chromatin masses in place of individual chromosomes. (d) The same, with two nuclei, similar in appearance to those of the normoblast. (e) The same as the normal normocyte with two nuclei, similar in appearance to those of the normoblast, but only slightly larger than nucleus of normal normocyte. a, b, c, and d may show polychromatophilic cytoplasm.

Blood Platelets.—1. The same as normal, except that both cell and nucleus are larger.

2. Larger than normal with two nuclei.

Leucocytes.—1. Polymorphonuclear leucocyte with eosinophilic rods: the same as in normal blood, at times possibly a little smaller.

2. Polymorphonuclear leucocyte with eosinophilic granules: the same as in normal blood, at times possibly a little smaller.

3. Lymphocyte: the same as in normal blood.

4. Large mononuclear cell: (a) as described in normal blood with a little less cytoplasm. (b) The same size to one-half times larger than (a), with both nucleus and cytoplasm paler. (a) and (b) cannot be separated absolutely. Every gradation. (c) Mitoses: all stages from monaster to complete separation of nucleus: (a') elliptical; diameter about 1.5 times the length of the normal normocyte. Dense, purple chromosomes centrally massed. Cytoplasm pale bluish gray, granular. (b') The same, with two masses of chromosomes in opposite extremes of the cell. (c') The same, with two purple nuclei. (d') The same, with three purple nuclei.

5. Mast cell: as described in normal blood.

6. Mononuclear myelocyte with eosinophilic granules: round or slightly elliptical, diameter about length of normal normocyte or longer. Nucleus oval, pale blue with dense chromatin, eccentric. Cytoplasm colorless with small and large bright red granules; some scattered over nucleus.

Cytoplasmatic masses without nuclei: round or slightly oval; diameter varies, usually about width of normocyte. No nucleus. Cytoplasm grayish blue, sometimes with vacuoles.

The above is the blood picture common to all leukemic animals. The order of predominance of the white cells is typical of leukemia, differing from the normal, but agreeing with that of the spontaneous case. The large mononuclear is present in by far the greatest number. The other cells of the normal blood are decreased. Polymorphonuclears with eosinophilic granules and mast cells are very scarce. The mononuclear myelocyte with eosinophilic granules, although not as common as in the spontaneous case, can usually be demonstrated in every leukemic animal. The platelets are greatly increased in number.

Differential count, 300 cells.	<i>Per cent.</i>
Polymorphonuclears with eosinophilic rods	6
" " granules	0
Lymphocytes	4
Large mononuclear cells	86
Mast cells	1
Mononuclear myelocytes with eosinophilic granules	1
Unclassified cells	2
	<hr style="width: 100%; border: 0.5px solid black;"/> 100

Autopsy Findings. Gross and Microscopical.—The weight of the animal in every case was far below its weight before injection.⁹ The two lowest weights, at autopsy, were 675 and 897 grams.

The jaundice, at times, involved the skin of the entire body.¹⁰ The conjunctivæ and buccal mucous membrane were always very pale. Emaciation was extreme. There was a great scarcity or entire absence of subcutaneous fat. Muscular atrophy was usually marked; at times practically only the skeleton remained.

The cervical lymph glands, macroscopically, were, as a rule, uninvolved. In two cases, they were definitely enlarged, in the one very much so. They measured 1.5 × 8 × 4 cm. and 2 × 1 × 5 cm., respectively. They appeared uniformly gray with absence of lobulation due to parenchymatous swelling and atrophy of interlobular fat. In a third case they were of normal size and appearance, but showed several gray nodules 1 mm. in diameter. Microscopically, those glands which appeared normal in gross were usually free

⁹ Animals which remained negative invariably gained weight.

¹⁰ Probably this was more frequent but not recognized, except when very grave, because of the normal yellow tint of the skin.

of myeloid infiltration, although their blood vessels contained leukemic blood. The diffusely enlarged glands showed a marked swelling of their parenchyma with complete atrophy of the interlobular fat. Myeloid cells, both the large mononuclear and the mononuclear myelocyte with eosinophilic granules, were scattered through the lobules. Foci, entirely of the first, or exclusively of the second, were localized principally in the interlobular connective tissue. They were rich in mitotic figures. The gray nodules proved to be a mass of proliferating myeloid tissue.

In the peritoneal cavity, the omental fat was greatly decreased. At best, it was present only in moderate amount. As a rule, it was replaced by a thin yellow membrane. Ascites occurred in 6 out of 13 cases, at times in sufficient amount to distend the abdomen. It was always associated with a serofibrinous mass which covered the liver and most of the other abdominal viscera and the outer surface of the pericardium. With the exception of Nos. 31 and 70 every animal of the thirteen had an enlarged liver. The margins of the right and left lobes of the smallest of the enlarged livers were respectively 1 and 3 cm. above the xiphoid, while the margins of the largest liver extended 3.5 and 3 cm. respectively below the xiphoid. In fact, the latter organ *in situ* was so enormous that it filled the entire peritoneal cavity and was the only viscus visible on opening the abdomen.

The liver was frequently enormous. Its weight ranged from 80 to 265 grams; *i. e.*, over five times normal, or 6.2 to 10.3 per cent of body weight, an increase of 3.6 times normal.

The external appearance of the liver was usually very characteristic. It frequently appeared diffusely gray, due to very closely packed subcapsular spots, pin point to 2 mm. in diameter. These were usually more scattered and translucent, at times slightly yellow and opaque. They were often fused to form larger areas with a diameter of 0.5 to 1 cm., or they were arranged in a delicate gray network. In addition, numerous gray or slightly yellow nodules ranging from 0.2 to 0.5 cm. in diameter were often present. The whole gave the surface an extremely mottled appearance. Lobulation at times was visible, usually indistinct. The liver was either slightly firm or friable. The sectioned surface appeared very similar

to the external surface. Dots and lines often formed gray borders along the blood vessels. The latter also occupied the centers of some of the larger, circumscribed gray areas. The perivascular connective tissue in rare instances was bile-stained. Microscopically, there was usually a diffuse infiltration of myeloid cells. They were closely packed within and without the intralobular capillaries. The liver trabeculae showed fatty degeneration and atrophy. The large mononuclear, rich in mitotic figures, was by far the predominating cell. The mononuclear myelocyte with eosinophilic granules was very scarce. Scattered through the section were foci of large mononuclears, or of mononuclear myelocytes with eosinophilic granules. Some foci contained both types of cells. They abounded in mitotic figures. The liver cells in these foci had entirely disappeared. The small, gray nodules consisted of circumscribed masses of myeloid tissue, usually occupying the periportal spaces and composed of a central portion of large mononuclears surrounded by the mononuclear myelocyte with eosinophilic granules. In the blood vessels and capillaries the large mononuclear at times seemed to exceed greatly the normocyte in number.

The spleen with the exception of Nos. 31 and 70 was, in every case, enlarged, usually extremely. The smallest of the enlarged organs measured $3 \times 2 \times 1.7$ cm., the largest $4 \times 3 \times 2.3$ cm. The latter was just twice normal in every dimension. The lowest weight was 6 grams, the highest 18 grams; *i. e.*, 18 times normal, or 0.6 to 1.6 per cent of body weight; *i. e.*, 32 times normal.

The surface of the spleen was sometimes normal. More frequently, it was studded with scattered gray or slightly yellow spots and nodules. The first was from 1 to 3 mm., and the largest of the second from 0.5 to 1 in diameter. Again, the organ appeared diffusely gray. On section it was usually similar to the surface. When diffusely gray, the pulp was somewhat granular and in excess. Microscopically the entire pulp was often diffusely infiltrated with the large mononuclear cell separating and compressing the Malpighian bodies. In addition, circumscribed closely packed masses of pure large mononuclears or mononuclear myelocytes with eosinophilic granules were scattered through the section. Occasionally, the myelosis was only present in foci, with practically no disturbance

of normal splenic structure. Mitoses were abundant wherever the large mononuclear occurred.

The kidneys of 5 of the 13 cases were enlarged. They weighed from 12 to 26 grams; or 0.8 to 1.4 per cent of the body weight.

The surface and section of the kidney were entirely normal, but usually there were below its capsule scattered, gray, pin point dots, delicate lines, or even one or two nodules, the largest 0.5 cm. in diameter. Again, they appeared diffusely gray. Microscopically, the kidneys always showed more or less distention of their blood vessels and intertubular capillaries with the characteristic leukemic blood in which white blood cells, at times, even seem to exceed the normocytes, and in which the large mononuclears predominated. When capillary distention was moderate, the tubules appeared normal, but at times they were so enormously overfilled as to cause extreme atrophy and degeneration of the renal epithelium, resulting frequently in circumscribed areas devoid of any parenchyma. These areas were more pronounced about the larger blood vessels. The walls and the perivascular tissue of some of the vessels were infiltrated with actively generating myeloid cells, especially the large mononuclear.

The bone marrow was always involved, usually more or less characteristically. It was increased in amount, moderately soft, and red, with countless gray dots to slightly larger areas; or it was diffusely gray. Microscopically, the normal structure of the marrow was greatly changed. The fatty tissue had completely disappeared and the marrow cells formed a solid mass. The description of the red and white marrow cells agreed with that given under normal marrow. Possibly the large mononuclear was more commonly polygonal, due to pressure. Mitoses of both the large mononuclear and the erythrocytes were common. The order of predominance was changed from normal. The large mononuclear myelocyte was by far the most abundant; the normoblast, the megaloblast, and the mononuclear myelocyte with eosinophilic granules followed in about equal numbers; the normocyte was least numerous. Both types of polymorphonuclear as well as the lymphocyte, mast cells, and platelet were not seen at all. Mitoses of the mononuclear myelocyte with eosinophilic granules were not demonstrated.

In the remaining organs and tissues the blood vessels were filled with the characteristic blood; and in many, infiltrations of myeloid cells occurred in moderate degree.

Summary.—The essential points deduced from the simple transmission of leukemia by injection of an organic emulsion may be stated as follows:

Clinical History.—After an incubation period, usually from 5 to 6 weeks, the fowl suddenly becomes pale, jaundiced, emaciates rapidly, loses weight, and shows signs of extreme weakness, followed almost without exception by death in 1 to 2 weeks.

Blood.—(a) The total number of white blood cells is greatly increased, resulting in a proportion of one white to three red blood cells. The differential count showed a marked increase and predominance of the large mononuclear over the other white cells of the normal blood, which have decreased. The mononuclear myelocyte with eosinophilic granules is present in more or less numbers in practically every case. Besides the normal appearing large mononuclear, one sees many larger pale forms. In these cells, typical and atypical, mitoses in all stages are common. (b) The total number of red blood cells is correspondingly decreased, with a marked fall in hemoglobin. They present varieties in size, shape, and staining, associated with swelling of the nucleus. Normoblasts and megaloblasts, with mitoses in all stages, make their appearance. (c) There is an increase in the number of blood platelets, associated with an increase, both in size of the cell and its nucleus. The cells frequently contain more than one nucleus. (d) The clotting power of blood is greatly decreased.

Organs.—(a) A diffuse or focal infiltration of marrow cells occurs in many organs. The large mononuclear and mononuclear myelocyte with eosinophilic granules predominate and may occur separately or together in the same nodule. Extensive mitoses occur in both these cell types. This myelosis affects more particularly the liver, spleen, kidneys, and bone marrow, causing a great increase in the size of the first three organs. Rarely the cervical lymph glands are also very much enlarged. Most of the remaining organs and neighboring tissues may contain infiltrations, but not so extensive. (b) The proportion of white to red blood cells is greatly increased

in the blood vessels. The same cells predominate as in the infiltration. (c) The atrophic and degenerative changes of the parenchyma and of the adipose tissue are marked; ascites occurs.

Conclusion.—From the above experimental study the following conclusions may be drawn: The injection of an organic emulsion causes a picture of myeloid leukemia in every respect similar to spontaneous leukemia as it occurs in the fowl. The clinical picture and changes produced in blood and organs are analogous to those which occur in human leukemia.

CONCLUSIONS.

1. The spontaneous occurrence of myeloid leukemia of the fowl is confirmed.

2. Myeloid leukemia of the fowl is transmissible by intravenous or intraperitoneal injection of an organic emulsion.

The latter is in confirmation of the work of Ellermann and Bang (6, 7), who first successfully transmitted the disease. They were followed by Hirschfeld and Jacoby (13), whose successful transmissions, however, seem to be limited to a strain which had its origin in a fowl presented to them by Ellermann and Bang. Burckhardt (14, 15) likewise transmitted the disease, but here again the stock animal came from Hirschfeld and Jacoby and therefore indirectly from Ellermann and Bang.

The transmission reported above is of special interest, because it originated in an animal absolutely unrelated to that of the previous investigators.

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