EXPERIMENTAL STUDIES OF BOVINE LEUKEMIA

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INTRODUCTION

Leukemia is essentially a blood disease in which there is more or less enlargement of the blood and lymph forming organs. There is usually a very marked increase in the number of white blood corpuscles with a possible decrease in the number of red cells.

Leisering is credited with being the first to demonstrate the presence of leukemia in animals in 1858, but from an investigational standpoint the disease received little attention until a much later date. Nocard, Mosler, Cadiot, Roger, Gilbert, Marek, Stockman, and a number of other European investigators have from time to time made studies of leukemia in animals, but it appears that comparatively little investigational work has been done on the disease in the United States.

The various studies of leukemia in mammals have failed to reveal the etiological factor involved, and all efforts made to transmit the disease from animal to animal, through experimental inoculations or otherwise, have thus far given no definite results.

On the other hand, certain European investigators have been able to transmit chicken leukemia through experimental inoculations, thus establishing the infectious nature of the disease in fowls. The present writers, however, have been unable to reproduce the disease in chickens by experimental inoculations of tissue emulsions from what appeared to be typical cases of avian leukemia.

In dealing with the subject of leukemia it becomes necessary to differentiate between the true leukemia, in which there are very marked numerical changes in the cellular elements of the blood, and the so-called pseudoleukemia, in which the blood and lymph forming organs are similarly affected but without apparent changes in the cell content of the blood.

It appears that leukemia is more rarely encountered, in some species of animals, at least, than pseudoleukemia. According to Ostertag, for example, pseudoleukemia is of more frequent occurrence in cattle and hogs than the true form of the disease.

ORIGIN AND HISTORY OF CASE STUDIED

The affected cow, which was the subject of these studies, originated in the State of Maryland, not very far from the District of Columbia. The attention of the writers was first called to her by the owner, who had observed an abnormal condition in the cow and offered to contribute her to this department for experimental purposes.
examination she was found to be typically leukemic in appearance. Subsequently she was purchased from the owner and brought to the experiment station at Bethesda, Md., for observation and study.

It is of some interest, and possible significance, to refer in this connection to the statement of the owner that another of his cows had been similarly affected about two years previously. The visible enlargement of the superficial lymph glands which was also noted in that case was thought by the attending veterinarian to be due to tuberculosis, but the cow failed to give a positive reaction to the tuberculin test, and, according to his statement, showed no lesions of tuberculosis upon subsequent slaughter.

CLINICAL EXAMINATION OF LEUKEMIC COW

The outstanding deviation from the normal noted on clinical examination of the cow was the very marked enlargement of the prescapular, precrural, and supramammary lymph glands. (Figs. 1 and 2.) The animal also exhibited some evidence of difficult breathing. The latter symptom was found later, on autopsy, to be due to chronic pneumonia. A preliminary blood count confirmed the belief that the case was one of true leukemia.

EXPERIMENTAL OBJECTIVES

Since it was definitely determined that the cow was affected with the true form of leukemia, the opportunity appeared favorable to study any possible etiological factor involved, by cultural methods and otherwise, and to endeavor to reproduce the disease through experimental animal inoculations.

BLOOD COUNTS

A limited number of blood counts were made, at short intervals, with a view to observing any possible change in the cytological ratio during the course of the disease. This study was terminated rather unexpectedly by the sudden crisis in the course of the disease which necessitated the slaughter of the animal. The number of counts made showed comparatively slight variations in the cell ratio with the exception of the last, in which it was found that the ratio of red to white cells had made a precipitous drop.
Table 1 shows the blood counts made on the respective dates. The chamber used in making these determinations, as well as other parts of the counting apparatus, had been approved by the United States Bureau of Standards. On two occasions when blood counts were being made, determinations were also made of the hemoglobin content of the blood, using Ernst Fleischl's hemoglobinometer.

**Table 1.** Blood cell counts, with ratio, and hemoglobin content of blood of leukemic cow

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of white cells</th>
<th>Number of red cells</th>
<th>Ratio</th>
<th>Hemoglobinometer reading</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 5, 1926</td>
<td>102,400</td>
<td>5,246,800</td>
<td>1 to 51</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Apr. 9, 1926</td>
<td>117,000</td>
<td>6,640,000</td>
<td>1 to 56</td>
<td>-</td>
<td>35</td>
</tr>
<tr>
<td>Apr. 14, 1926</td>
<td>101,875</td>
<td>5,840,000</td>
<td>1 to 57</td>
<td>-</td>
<td>35 Animal showed great weakness.</td>
</tr>
<tr>
<td>Apr. 19, 1926</td>
<td>205,250</td>
<td>5,520,000</td>
<td>1 to 27</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*The hemoglobinometer reading of a normal cow's blood, using the same apparatus and technic, was 60.

About three weeks after the cow arrived at the experiment station she was reported unable to stand even after being assisted to her feet. Besides the extreme weakness she also showed difficult respiration. Under the circumstances it was thought best to slaughter her immediately.

**AUTOPSY FINDINGS**

On bleeding the animal it was noted that the blood was pale in color and did not coagulate very readily. The mucous and serous membranes were very pale. There was evidence of emaciation.

The heart was paler than normal and showed numerous small whitish areas scattered through the musculature.

The lungs exhibited a well-defined chronic pneumonia, which accounted for the difficult breathing noted for some time prior to slaughter. The bronchial and mediastinal glands were enlarged to several times their normal size.
The liver was considerably enlarged, with thickened borders, and was rather light in color.

The spleen exhibited the usual leukemic appearance. The surface was uneven or slightly nodulated. The capsule was thickened and the cut surface showed thickly distributed whitish nodules (enlarged follicles) throughout the splenic pulp, which was rather dark or brownish red in color. On account of the very marked enlargement of the spleen and certain of the lymphatic glands these were measured and weighed. The spleen was found to be 10 inches wide at the middle and 26½ inches in length. The weight of this organ was 11 pounds and 5 ounces. Sisson\(^4\) gives the normal dimensions of this organ in cattle as 5 to 6 inches wide and 16 to 20 inches long. He also states that the average weight of the ox spleen is about 30 ounces.

The kidneys were lighter in color than normal, but did not appear to be very much enlarged.

The associated lymphatic glands of the organs described were very noticeably enlarged.

Little change was noted in the digestive tract other than the pronounced increase in the size of the mesenteric lymph glands.

A fetus weighing 8 pounds and 4 ounces was removed from the uterus. Autopsy showed no visible abnormalities either in the principal organs or lymphatic glands of the fetus.


Figure 3.—Section of heart of leukemic cow showing lymphocytic infiltration of the musculature. The section also shows evidence of degenerative changes in muscle fibers. Magnified 250 times.
The prescapular glands of the cow were the most markedly enlarged of the lymphatic glands. One of these glands measured 10 inches in length and 6½ inches in width, and weighed 2 pounds and 10 ounces. Sisson 3 gives the normal dimensions of these glands as 1 or more inches wide and 4 or 5 inches long.

The supramammary lymphatic glands were also greatly enlarged. Paired, these glands measured 10 inches in length and 6½ inches in width. The weight of the two glands was 4 pounds and 10 ounces.

The weight of the precrural glands singly was 14½ ounces.

**TISSUES CULTURED**

The mediastinal, prescapular, precrural, and supramammary lymphatic glands, and also the spleen and liver, were cultured on various media including serum-agar slants, 0.1 per cent agar, and Smith tubes of liver dextrose; the last mentioned for anaerobic microorganisms in particular.

Cultural results were entirely negative.

**HISTOPATHOLOGY**

**HEART.**—Sections from the heart muscle showed a limited lymphocytic infiltration, chiefly between the larger bundles of fibers. The musculature stained rather poorly and the fibers showed loss of striations and other evidences of degeneration. (Fig. 3.)

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Lungs.—There was evidence of a rather extensive lymphocytic invasion of the lungs, in addition to the well-marked catarrhal pneumonia present.

Liver.—The liver sections showed some capillary engorgement with small hemorrhages in places. There was quite an extensive white-cell invasion of the organ, the cells penetrating not only between the lobules but also between the columns of liver cells. There was also a cellular infiltration of the interstitial tissue. (Fig. 4.) The exces-

![Figure 5](image)

*Figure 5.—Showing the excessive number of white cells intermixed with the erythrocytes in an artery of the liver. Magnified 250 times*

sive number of white cells present in the blood could readily be seen in the larger vessels of the liver sections. (Fig. 5.)

Spleen.—The changes in the histologic appearance of the spleen were quite marked. There was a red-cell engorgement in addition to the large masses of white cells invading the splenic pulp. Most of the Malpighian corpuscles were completely obliterated by the lymphocytic infiltration. The trabeculae had largely disappeared, only portions being visible here and there. The capsule of the organ showed some cellular infiltration and was somewhat thickened. (Fig. 6.)
Lymph glands.—All the noticeably affected lymph glands presented practically the same histological picture. The accumulation of lymphocytes in the glands resulted in complete distortion of the normal structure. The lymph cords and sinuses were crowded with masses of the lymphocytes, and the germinal centers could no longer be recognized.

The capsules and trabeculae were thoroughly infiltrated and in some sections only traces of the latter could be seen. Some of the glands showed capillary engorgement which in places resulted in small hemorrhages. (Figs. 7 and 8.)

ANIMAL INOCULATIONS

A number of preliminary inoculations of small animals were made shortly after the cow was received at the experiment station. Four guinea pigs were inoculated intraperitoneally and two rabbits intravenously with varying amounts of blood obtained from the jugular vein of the leukemic cow.

The guinea pigs either died or were slaughtered at periods ranging from one month to more than a year. None of these showed any
lesions indicative of leukemic disease. The two rabbits, likewise, showed no evidence of leukemia on autopsy approximately one year after inoculation.

Just prior to the time of slaughter of the leukemic cow, blood was drawn from the jugular vein and another cow was inoculated immediately with 10 cubic centimeters of the warm blood, intravenously. A weanling calf and one sheep were also inoculated intravenously with 5 cubic centimeters each of the cow’s blood.

The autopsy findings on the experimental sheep, 10 months after inoculation, were negative for leukemia. The experimental cow and calf were slaughtered more than a year after inoculation and neither exhibited any lesions suggestive of leukemia.

Four guinea pigs were inoculated intraperitoneally and two rabbits intravenously with composite samples of emulsion of the enlarged lymph glands of the cow. The guinea pigs and rabbits either died or were slaughtered at periods varying from approximately one month to more than one year. None of these animals showed any evidence of leukemia on autopsy.
During the year that the experimental cow was held under observation a number of blood counts were made in order to note any possible numerical deviation from the normal of the cell content during this period which might give evidence of a leukemic condition. That no such changes occurred in the blood is indicated in Table 2.

![Figure 8](image)

**Figure 8.**—Section of lymph gland, showing excessive accumulation of lymphocytes and also cellular infiltration of a trabecula. The histological picture is typical of the various glands affected. Magnified 250 times.

**Table 2.**—Blood cell counts, with ratio, of blood from experimental cow intravenously inoculated with warm blood from the leukemic animal

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of white cells</th>
<th>Number of red cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 24, 1926 *</td>
<td>15,750</td>
<td>7,190,000</td>
<td>1 to 454</td>
</tr>
<tr>
<td>July 21, 1926</td>
<td>11,625</td>
<td>7,100,000</td>
<td>1 to 610</td>
</tr>
<tr>
<td>Oct. 5, 1926</td>
<td>13,750</td>
<td>6,840,000</td>
<td>1 to 497</td>
</tr>
<tr>
<td>Dec. 22, 1926</td>
<td>14,320</td>
<td>6,480,000</td>
<td>1 to 452</td>
</tr>
<tr>
<td>Mar. 9, 1927</td>
<td>11,250</td>
<td>5,900,000</td>
<td>1 to 524</td>
</tr>
</tbody>
</table>

* Preliminary count made on day cow was inoculated.
SUMMARY AND CONCLUSIONS

The very marked and definite change in the numerical ratio of the blood cells, as demonstrated by a number of cell counts, together with the typically leukemic changes noted histologically, are considered sufficient evidence that the disease of the cow studied by the authors was true leukemia.

Bacteriological studies made of the affected tissues of the leukemic cow gave negative results.

All efforts to transmit the disease to other animals of the same and different species, by inoculations of blood and glandular material from the affected animal, likewise gave negative results.

It is concluded, therefore, that no evidence was obtained through the experimental studies made of this case to indicate that bovine leukemia is of infectious origin.