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AGRICULTURAL EXPERIMENT STATION

AVIAN LEUKOSIS AND LYMPHOMATOSIS

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*In cooperation with U. S. Department of Agriculture.
†On leave.
Avian Leukosis and Lymphomatosis

By
A. M. Lee, L. H. Scrivner,* and Mack O. North

INTRODUCTION

For many years a disease complex of poultry, confined chiefly to chickens, has been recognized by poultry owners and poultry pathologists. Because of a lack of knowledge concerning the causative agent, and because of a diversity of symptoms and lesions exhibited by affected birds, a great many different names for the disease complex have been introduced and used in recent years.

The disease has been variously designated as "fowl leukemia," "fowl leukosis," "range paralysis," "fowl paralysis," "iritis," "lymphomatosis," "lymphocytoma," "neuro-lymphomatosis," "leucotic tumors," "leukemoid disease," etc.

In order to attempt to standardize somewhat the nomenclature for the disease, a conference of investigators, all of whom were working in this particular field, at a meeting in East Lansing, Michigan, in July, 1940, recommended that the disease complex become known as the "avian leukosis complex," with a further sub-division of "lymphomatosis" and "leukosis" according to whether organs of the body, or the blood and blood forming tissues were affected. These terms could be further clarified by prefixing a descriptive adjective which was taken from the name of the tissue affected, such as: ocular lymphomatosis, neural lymphomatosis, visceral lymphomatosis, and osteopetrosis which indicate affection of the eyes, the nerves, the viscera, and the bones, respectively.

While this nomenclature may not comprise the best possible classification in light of future findings, and does not attempt a classification on the basis of the etiological agent, yet it is a commendable effort toward standardizing terminology which has, in the past, been decidedly confusing. So far as possible, the terminology used in this publication follows the recommendations of the aforementioned committee.

*Resigned April 30, 1943.
DEFINITION

The avian leukosis complex is a highly fatal, acute or chronic, apparently contagious disease of all breeds of domestic chickens, characterized by paralysis of the legs and wings, infiltration of various organs to the extent of causing tumor-like formations and marked changes in the cellular structure of the blood. It is quite generally agreed that the cause is a filtrable virus.

Whether the disease ever occurs naturally in birds other than the chicken in sufficient numbers to be economically significant appears to be doubtful, although there are limited reports of successful experimental transmission to turkeys, guinea fowl and pheasants by injecting them with infective material.

ECONOMIC IMPORTANCE

Although this disease complex was recognized in the United States as early as 1914, it was apparently of little economic importance at that time. However, comparatively soon after this the disease became of such importance that by 1925 it was causing increased losses and both poultrymen and poultry pathologists showed increased interest in it. Between 1925 and the present time the incidence has become increasingly greater and numerous investigational projects have been instituted in an attempt to discover the cause and nature of the disease, means of dissemination and methods of control.

In addition to numerous state experimental stations which are working with the disease complex, the Bureau of Animal Industry of the United States Department of Agriculture is making an intensive study of the disease in a recently established Regional Poultry Research Laboratory at East Lansing, Michigan.

At the veterinary diagnostic laboratory of the University of Wyoming Agricultural Experiment Station, records of diagnostic services prior to 1925 are not available. However, one diagnosis of "leg weakness" was made during that year and during the ten-year period from July 1, 1925 to July 1, 1935 a total of 9.7 per cent of all chickens, excluding baby chicks, submitted for diagnosis were found to have some form of the disease.
During the seven-year period extending from July 1, 1935 to July 1, 1942, 38.3 per cent of all chickens, exclusive of baby chicks, which were submitted for diagnosis were affected with the disease.

This indicated increase in incidence appears similar to the development of the disease in other sections of the country; although, it would seem that its appearance here as an important disease was somewhat delayed as compared with middle western states. In 1926, Doyle in Indiana (1), (2)* reported the prevalence of the disease and again in 1927 reported that “a number of poultry pathologists are now of the opinion that it is one of the most important diseases affecting chickens. Practical poultry-men have already recognized the seriousness of the disease and are asking for help in its control.”

CAUSE

As previously indicated, it is rather generally agreed that the cause is a filtrable virus or a substance so small that it will pass through filters of such fine density that they will hold back or filter out all of the bacteria which are large enough to be seen with ordinary means of magnification.

Whether there is more than one virus concerned in the production of the various manifestations of the disease remains a debatable question. Some investigators believe that there is more than one infective agent concerned, while others hold that the various forms of the disease are simply different manifestations of an infection with one distinct type of causative agent. Lee et al (3) reported that through injections of suspensions of infected liver into susceptible ten-day old chicks they were able to reproduce experimentally all of the various manifestations of the disease within 13 months. The same was true when affected nerves, ovarian tumor and tumorous kidney were used as the inoculating agents.

On the other hand there are some investigators who have indicated their belief that lymphomatosis, in its various manifestations, is caused by one or more viruses, while leukosis is caused

*The numbers in parentheses refer to citations in the list of references at the end of the bulletin.
by a virus or viruses which are separate and distinct from the lymphomatosis virus or viruses. Durant and McDougle (4) of the Missouri Agricultural Experiment Station, who have contributed greatly to the knowledge of the disease complex, seem to be definitely of the opinion that there are at least two different viruses concerned, one of which is the cause of leukosis and the other the cause of “fowl paralysis.”

These are things which must of necessity be taken into consideration by the poultry pathologist in attempting to formulate methods for bringing the disease under control. Which of the theories proves correct is a question which must greatly influence future work upon the disease, and must, for the present, remain unanswered.

SYMPTOMS

The symptoms exhibited by affected birds depend upon the part of the body affected. One of the most common symptoms is paralysis, partial or complete, which has been responsible for one of the more common names of the disease complex, i.e., “fowl paralysis,” and is due to involvement of various portions of the nervous system.

In this form of the disease, one or both legs may be affected. Either one or both wings may likewise be affected and the muscles of the neck may also be involved. The paralysis may be so slight as to cause only slight lameness, drooping of one wing or slightly wry neck, in the initial stages. However, the paralysis seems to be quite constantly progressive and within a few days after first symptoms of lameness are noticed, a curling of the toes of the foot on the affected side is in evidence, and the bird usually becomes unable to walk at about this time. If both legs are affected the bird goes down earlier than when only one leg is affected. Drooping of one or both of the wings and paralysis of the neck may appear during this time but these symptoms are not constant. They may, however, appear independently without there being any outward appearance of leg involvement.

The paralysis usually progresses until the bird becomes unable to get around to obtain feed and water. The legs often extend outward, forward or backward in peculiar positions and
although the appetite commonly remains good, the bird loses weight rapidly, becomes emaciated and dies. Shrinking of the muscles in the affected region is usual, and, in some cases, shrinking is extraordinarily severe, leaving the leg or wing decidedly withered and smaller than the normal. Not infrequently in affected birds, the crop becomes pendulous due to involvement of the nerves supplying the muscles of that organ. (4) It should be made clear, however, that this is not the only cause of pendulous crop. The vagus nerve which innervates numerous internal organs is quite commonly affected, and when this is the case, the symptoms exhibited are those of failure of the organ or organs affected. Under these conditions digestive disorders are frequent.

Another symptom of the disease which is quite frequently thought of as closely related to the paralysis complex, is iritis. This is commonly referred to as "white eye," "pearly eye," or "gray eye" by poultrymen. In this form of the disease, which may occur singly or in combination with other types, the iris of the eye becomes sufficiently infiltrated with internal body cells to make the eye assume a whitish or grayish appearance as compared with the characteristic color of the eyes of normal birds of the same breed. In addition the pupil very commonly becomes fixed and is unable to respond to light. The eye may bulge or twitch and vision is impaired, often to the extent of blindness. Many times the affection of the eye is the only visible symptom and many birds so affected live for long periods of time without apparent inconvenience, particularly if vision is not sufficiently impaired to prevent the bird from obtaining food and water.

In other forms of lymphomatosis in which the internal organs are affected, varying degrees of emaciation are encountered, with dullness, lack of appetite and sluggishness extending over a period of one or two days up to a week or two before death. The comb is commonly pale and varying degrees of anemia are usually present.

In the leukotic type of the disease the blood is affected as well as the blood-forming organs. This form of the disease may manifest itself in a general disturbance, weakness, and anemia.
Such cases can only be diagnosed by laboratory examination, as gross lesions are often absent, or are recognized only by the trained pathologist.

The form of the disease affecting the bones is called osteopetrosis. The shanks of the long bones of the legs and wings become thickened and hard. Deformity and impairment of gait may be noticed.

LESIONS

As previously indicated the lesions produced are extremely variable, and such variation has led to a great amount of confusion regarding proper nomenclature, etiological agent or agents and possible methods of control. In the “leukosis” type of the disease there is very little macroscopic change in the various tissues and organs of the affected birds. The blood may be thin and watery, but determining this is not always an easy task. When this form of the disease occurs in combination with other forms, it is often overlooked entirely. From the standpoint of recognition and diagnosis, this form of the disease can only be suspected until the diagnosis can be confirmed by microscopic examinations as carried out in the laboratory.

On the other hand, the “lymphomatosis” type of the disease usually produces sufficient changes in the organs affected so that it is not particularly difficult for the trained observer to recognize them, without the aid of magnifying instruments.

When the so-called “range paralysis” or neural lymphomatosis type of the disease is in evidence, it is usually possible to demonstrate some macroscopic alteration in the structure of the nerves which supply the affected part or parts. Such nerves are frequently enlarged as much as four to five times their usual diameter. They lose the natural greyish color and cross striations and assume a slight yellowish color. Microscopic examinations of such affected nerves shows them to be heavily infiltrated with internal body cells, usually lymphocytes. A great many different nerves have been found to be affected. Hence, any complete examination must be dependent upon a rather thorough knowledge of the nervous system. A knowledge of the symptoms and parts affected before death are also of considerable help.
In the visceral type of the disease the internal organs of the body are affected. Infiltration of cells into these organs causes them to assume undue proportions. Uneven distribution of these infiltrating cells causes the affected organs to appear mottled with greyish white areas intermingled with areas of more or less normally appearing tissue in the swollen organ. The liver, spleen, kidneys, and ovary are most commonly affected; although tumorous masses and infiltrations are common along the lymphatics of the intestines, the mesentery, the peritoneum, and the heart muscle and lungs. Occasionally the lymph glands along the neck are affected and show varying degrees of enlargement. Atrophy or shrinking of muscles in the affected region is common. Tumorous masses are frequently found in different parts of the muscular system or in connection with the skin. Although the significance of these tumorous growths has not definitely been determined, they have in some instances been regarded as a portion of this disease complex.

In the ocular type of the disease, the macroscopic changes are usually limited to the grayish appearance of the iris of the eye. Recognition of this condition necessitates familiarity with the normal pigmentation of eyes, and must not be confused with the entirely normal lack of pigmentation of the eyes of young birds. This form of the disease occurs frequently in combination with other forms, in which case the latter are usually the cause of death of the affected bird.

PROBLEMS

Since its inception, investigational work has been handicapped by numerous problems which are not inherent in investigations of other diseases. For example, no other known disease or disease complex of chickens appears to be capable of manifesting itself in so many different forms, and as previously indicated, this has led to confusion not only in classification of the disease but has led many investigators to believe that there is at least more than one causative agent. Added to this is the difficulty experienced by practically all workers, in successfully transmitting the disease to a high proportion of experimental birds. Likewise, experience shows that following inoculation, experi-
mental birds are extremely variable in the time required for visible symptoms and lesions to occur. This time may be from a relatively few weeks to many months. In the meantime, occurrence of spontaneous cases among control birds is confusing, and the interpretation of results becomes a relative matter.

Perhaps of more importance to the poultry raiser are questions concerning the mode of transmission and how the disease complex can most effectively be combatted. For example, how do birds become naturally infected, at what age are they most likely to become infected and from where does such infection come, are some of the questions which require answers before it is possible to outline any effective procedure in controlling the disease and avoiding infection. How long does the virus live inside and outside the body of the bird under natural conditions, and how and when it is eliminated from the body so that it is capable of infecting other birds, are important questions which remain unanswered.

Since, under natural conditions, the various symptoms of the disease ordinarily manifest themselves when the bird is from three to four or more months of age, and, since under experimental conditions it usually requires some months to reproduce the disease, many investigators feel that it is likely that the greatest amount of infection takes place at an early age. It is thought by some that artificially infected birds are much more likely to contract infection if they are inoculated as baby chicks.

It occurred to us that, if the disease is transmitted in significant amounts only when the birds are very young, then extreme sanitary precautions during that period should be a very potent factor in its control. It was with this idea in mind that we began an experimental project on the disease.

EXPERIMENTAL WORK

THE EFFECT OF WHEAT GERM OIL ON THE INCIDENCE OF LEUKOSIS FROM CONTACT EXPOSURE OF PULLETS

Forty-eight apparently normal pullets about six months old, from the poultry flock of this station in which this disease had not been encountered to any extent, were placed in equal numbers in four pens on November 5, 1938. A hemoglobin and a differ-
ential leucocyte count were made. The results did not indicate presence of the disease. Pen 1 was given a regular laying mash; pen 2, a regular laying mash and wheat germ oil; pen 3, a low vitamin E ration; and pen 4, a low vitamin E ration and wheat germ oil. Table I shows these rations.

**TABLE I RATIONS (1938-39)**

**Pen 1**

<table>
<thead>
<tr>
<th>Item</th>
<th>lbs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying mash:</td>
<td></td>
</tr>
<tr>
<td>Ground yellow corn</td>
<td>34.3</td>
</tr>
<tr>
<td>Barley, ground</td>
<td>10</td>
</tr>
<tr>
<td>Ground oats</td>
<td>10</td>
</tr>
<tr>
<td>Ground wheat</td>
<td>10</td>
</tr>
<tr>
<td>Wheat shorts</td>
<td>10</td>
</tr>
<tr>
<td>Mill run bran</td>
<td>10</td>
</tr>
<tr>
<td>Meat scrap</td>
<td>7</td>
</tr>
<tr>
<td>Dried buttermilk</td>
<td>3</td>
</tr>
<tr>
<td>Dehydrated alfalfa leaf meal</td>
<td>5</td>
</tr>
<tr>
<td>Salt (iodized)</td>
<td>.5</td>
</tr>
<tr>
<td>Cod liver oil (400 A. O. A. C.) D</td>
<td>.2</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

**Pen 2**

Laying mash (same as Pen 1) 50 lbs.

Wheat germ oil 7.1 grams

**Pen 3**

Low vitamin E laying mash lbs.

<table>
<thead>
<tr>
<th>Item</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground yellow corn</td>
<td>44.3</td>
</tr>
<tr>
<td>Ground oats</td>
<td>20</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20</td>
</tr>
<tr>
<td>Dried buttermilk</td>
<td>10</td>
</tr>
<tr>
<td>Dehydrated alfalfa leaf meal</td>
<td>5</td>
</tr>
<tr>
<td>Salt (iodized)</td>
<td>.5</td>
</tr>
<tr>
<td>Cod liver oil (400 A. O. A. C.) D</td>
<td>.2</td>
</tr>
</tbody>
</table>

**Pen 4**

Low vitamin E laying mash

(Same as Pen 3) 50 lbs.

Wheat germ oil 7.1 grams
Corn and wheat were fed to all pens so that the same amount of grain was consumed as of mash.

To these pens, 26 pullets were added as contact cases. These were from the vicinities of six different towns in this state. They came from flocks in which a diagnosis of leukemia had recently been made at this station. Many of these had symptoms of the disease varying from very slight to severe. They were divided as equally as possible among the four pens. Every Tuesday and Friday these supposedly infected birds were advanced from pen to pen to make as uniform an exposure as possible. The period of contact exposure was from November 5, 1938, to March 28, 1939. Four of the control birds developed symptoms and lesions of the disease. Two of these were in pens 2 and 4 receiving the wheat germ oil. (See Table II).

**TABLE II**
The Effect of Wheat Germ Oil on the Incidence of Leukosis
From Contact Exposure of Pullets (1938-39)

<table>
<thead>
<tr>
<th>PEN NO.</th>
<th>RATION</th>
<th>INCIDENCE OF DISEASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Basal plus wheat germ oil</td>
<td>1 developed symptoms and lesions</td>
</tr>
<tr>
<td>3</td>
<td>Low vitamin E</td>
<td>2 developed symptoms and lesions</td>
</tr>
<tr>
<td>4</td>
<td>Low vitamin E plus wheat germ oil</td>
<td>1 developed symptoms and lesions</td>
</tr>
</tbody>
</table>

From these results it can be seen that neither wheat germ oil nor vitamin E were factors in the development of the disease. The incidence of the disease was as great in the pens receiving them as in the control pens.

**THE EFFECT OF TISSUE INJECTIONS UPON TRANSMISSION OF LEUKOSIS AMONG ADULT PULLETS**

Seven pens, (Nos. 5-11), containing fifteen pullets to the pen, were placed on this experiment in October, 1939. Birds in each pen had no contact with other birds. Birds were fed and
March, 1944 Avian Leukosis and Lymphomatosis

watered without the attendants entering the pens. All were healthy and from a flock in which neither leukemia nor lymphomatosis had been observed. Blood smears were made from the pullets at the start and examinations did not indicate presence of the disease. Pen 5 was placed on erythroleukosis material, using a hen with a blood picture of erythroleukosis which showed no paralysis or eye lesion. A suspension was made of the blood, spleen, and liver. One cc. was injected intraperitoneally into each of eight birds, and one cc. was injected into the bone marrow of the tibia of each of three birds. Four birds were left un.injected. Pen 6 was exposed for 32 days to contact with a bird with an erythroleukosis blood picture. In Pen 7, material obtained by making a suspension of an affected eye was injected intraperitoneally into ten birds in one cc. doses and five birds were left as controls. Pen 8 contained all control birds which were uninjected and unexposed. Pen 9 was placed on the neural form of the disease. Suspensions from enlarged nerves were injected intraperitoneally in one cc. doses into ten birds and five were left as controls. Pen 10 had pen contact exposure to three birds with paralysis of the legs or the neural form of the disease. These three birds remained in the pen for nine, twelve, and 39 days, respectively. They were then autopsied and found to have enlarged nerves. In Pen 11, the enlarged liver or visceral lymphomatosis form of the disease was used. Five were injected intraperitoneally with one cc. of a suspension of such liver material. The other ten were left uninjected to determine possibility of transmission by contact and for controls.

The pullets were approximately five months of age at the time of the exposure and injections. The experiment was carried for seven months after injection. In Pen 5 two birds injected intraperitoneally developed erythroleukosis. One died in 47 days and one was killed in 129 days. In Pen 6, exposed by contact to erythroleukosis, one case of the neural complex developed in 21 days, and one case of visceral lymphomatosis developed later. In Pens 7, 8, and 9, no case of the disease complex developed, while one case of visceral lymphomatosis developed in an uninoculated bird in each of Pens 10 and 11. These latter two pens had contact exposure. These results are tabulated in Table III.
TABLE III
Transmission of Leukosis Among Adult Pullets (1939-1940)

<table>
<thead>
<tr>
<th>Pen. No.</th>
<th>Inoculations and contacts</th>
<th>No. inoculated and control</th>
<th>Incidence of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Blood, liver, spleen emulsion of erythroleukosis case intraperitoneally and bone marrow</td>
<td>8 intrap. 3 bone m. 4 controls</td>
<td>2 erythroleukosis none none</td>
</tr>
<tr>
<td>6</td>
<td>Contact with erythroleukosis case</td>
<td>15</td>
<td>1 neural, 1 visceral</td>
</tr>
<tr>
<td>7</td>
<td>Eye emulsions intraperitoneally</td>
<td>10 intrap. 5 controls</td>
<td>none</td>
</tr>
<tr>
<td>8</td>
<td>Controls</td>
<td>15</td>
<td>none</td>
</tr>
<tr>
<td>9</td>
<td>Enlarged nerve emulsion intraperitoneally</td>
<td>10 intrap. 5 controls</td>
<td>none</td>
</tr>
<tr>
<td>10</td>
<td>Contact with leg paralysis cases</td>
<td>15</td>
<td>1 visceral lymphomatosis</td>
</tr>
<tr>
<td>11</td>
<td>Enlarged liver emulsions intraperitoneally</td>
<td>5 intrap. 10 controls</td>
<td>none</td>
</tr>
</tbody>
</table>

THE EFFECT OF AGE ON SUSCEPTIBILITY TO LEUKOSIS

BY INJECTIONS AND CONTACT

Seven pens (Nos. 12-18) of non-sexed chicks hatched June 15, 1940, were placed on the experiment as day old chicks. Each pen was isolated so that there were no contacts with any birds outside the individual pen. In Pen 12, 22 birds were injected when 23 days of age, with blood from field cases of erythroleukosis. Eighteen were left as uninjected controls. In Pen 13, 23 birds were injected when 23 days of age with suspensions of affected organs from field cases of visceral lymphomatosis. Seventeen controls were in this pen. In Pen 14, 24 birds were injected when 26 days of age, with blood from field cases of visceral lymphomatosis. There were 16 controls. In Pen 15, 23 birds were injected when 47 days of age with blood from field cases of neural lymphomatosis. There were twelve controls. In Pen 16, 14 pullets were injected when 83 days of age, with blood
and nerve emulsion from field cases of neural lymphomatosis. There were 22 controls. In Pen 17, there were 40 unexposed birds serving as a control pen. In Pen 18, 26 birds were injected when 39 days of age, with dried avian blood of a visceral lymphomatosis case received from the Regional B. A. I. Poultry Research Laboratory at East Lansing, Michigan. There were twelve controls. Injections were made by the intravenous and intraperitoneal routes. See Tables IV to VIII, inclusive, and summaries for Pens 16 and 17.

There were six birds which developed the disease complex in Pen 12. Three were injected birds and three were contact birds. The three injected birds developed visceral lymphomatosis and one of them developed a leukosis blood picture of the monocytosis and eosinophilia type. One of these was injected intraperitoneally and the other two were injected intravenously. Two of the three uninjected birds that developed the disease, showed neural lymphomatosis, one of them also having erythroleukosis. The other uninjected bird developed visceral lymphomatosis with a leukosis of the monocytosis type.

TABLE IV

PEN 12—ERYTHROLEUKOSIS (1940-1941)
(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1869X</td>
<td>1 cc Ip</td>
<td>23 days</td>
<td>died</td>
<td>212 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1836X</td>
<td>½ cc Iv</td>
<td>23 days</td>
<td>died</td>
<td>217 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1854X</td>
<td>½ cc Iv</td>
<td>23 days</td>
<td>killed*</td>
<td>298 days</td>
<td>leukosis and visceral lymphomatosis</td>
</tr>
<tr>
<td>1874X</td>
<td>uninjected</td>
<td>23 days</td>
<td>died</td>
<td>276 days</td>
<td>tumor and neural lymphomatosis</td>
</tr>
<tr>
<td>No band</td>
<td>uninjected</td>
<td></td>
<td>killed</td>
<td>292 days</td>
<td>erythroleukosis and neural lymphomatosis</td>
</tr>
<tr>
<td>1873X</td>
<td>uninjected</td>
<td></td>
<td>killed*</td>
<td>297 days</td>
<td>leukosis and visceral lymphomatosis</td>
</tr>
</tbody>
</table>

*At termination of experiment.

Total of 40 birds hatched June 15, 1940.

Twenty-two were injected on July 8, 1940, with blood from typical cases of erythroleukosis by the intraperitoneal and intravenous routes. Eighteen were left as uninjected controls. About half of each group were cockerels. These were removed when 14 weeks of age, September 15th to 20th.
### TABLE V

**PEN 13—VISCERAL LYMPHOMATOSIS (1940-1941)**

(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1899X</td>
<td>1 cc Ip</td>
<td>23 days</td>
<td>died</td>
<td>94 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>¼ cc Pv</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1911X</td>
<td>½ cc Iv</td>
<td>23 days</td>
<td>died</td>
<td>110 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>1884X</td>
<td>1 cc Ip</td>
<td>23 days</td>
<td>died</td>
<td>171 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1893X</td>
<td>1 cc Ip</td>
<td>23 days</td>
<td>died</td>
<td>260 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>⅛ cc Iv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ocular lymphomatosis</td>
</tr>
<tr>
<td>1910X</td>
<td>1 cc Iv</td>
<td>23 days</td>
<td>killed*</td>
<td>290 days</td>
<td>leukemia and visceral lymphomatosis</td>
</tr>
<tr>
<td>1890X</td>
<td>1 cc Iv</td>
<td>23 days</td>
<td>killed*</td>
<td>297 days</td>
<td></td>
</tr>
</tbody>
</table>

*At termination of experiment.
Total of 40 birds hatched June 15, 1940.
Twenty-three were injected on July 8, 1940, with suspension of an infiltrated liver from a typical case of visceral lymphomatosis, by the intraperitoneal and intravenous routes.
Seventeen were left as uninjected controls.
About half of each group were cockerels. These were removed when 14 weeks of age. September 15th to 20th.

### TABLE VI

**PEN 14—VISCERAL LYMPHOMATOSIS (1940-1941)**

(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1924X</td>
<td>1 cc Iv</td>
<td>26 days</td>
<td>died</td>
<td>168 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>1939X</td>
<td>1 cc Pv</td>
<td>26 days</td>
<td>died</td>
<td>203 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1942X</td>
<td>1 cc Ip</td>
<td>26 days</td>
<td>died</td>
<td>203 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>1932X</td>
<td>1 cc Iv</td>
<td>26 days</td>
<td>died</td>
<td>209 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1964X</td>
<td>uninjected</td>
<td>26 days</td>
<td>died</td>
<td>228 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>1962X</td>
<td>1 cc Iv</td>
<td>26 days</td>
<td>died</td>
<td>259 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1963X</td>
<td>1 cc Ip</td>
<td>26 days</td>
<td>killed*</td>
<td>297 days</td>
<td>leukemia and visceral lymphomatosis</td>
</tr>
</tbody>
</table>

*At termination of experiment.
Total of 40 birds hatched June 15, 1940.
Twenty-four were injected on July 11, 1940, with blood from a typical case of visceral lymphomatosis, by the intraperitoneal and intravenous routes.
Sixteen were left as uninjected controls.
About half of each group were cockerels. These were removed when 14 weeks of age. September 15th to 20th.
TABLE VII
PEN 15—NEURAL LYMPHOMATOSIS (1940-1941)
(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993X</td>
<td>1 cc Iv</td>
<td>47 days</td>
<td>killed</td>
<td>279 days</td>
<td>erythroleukosis and neural lymphomatosis</td>
</tr>
<tr>
<td>1979X</td>
<td>1 cc Iv</td>
<td>47 days</td>
<td>killed</td>
<td>277 days</td>
<td>ocular and visceral lymphomatosis</td>
</tr>
<tr>
<td>1997X</td>
<td>1 cc Iv</td>
<td>47 days</td>
<td>killed*</td>
<td>297 days</td>
<td>ocular lymphomatosis and granuloblastic leukemia</td>
</tr>
<tr>
<td>1974X</td>
<td>1 cc Iv</td>
<td>47 days</td>
<td>killed*</td>
<td>297 days</td>
<td>erythroleukosis and visceral lymphomatosis</td>
</tr>
<tr>
<td>2002X</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>226 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>1981X</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>227 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2001X</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>230 days</td>
<td>visceral lymphomatosis</td>
</tr>
</tbody>
</table>

*At termination of experiment.

Total of 35 birds hatched June 15, 1940.
Twenty-three (of which 13 were pullets) were injected on August 1, 1940, with blood from typical cases of neural lymphomatosis by the intraperitoneal and intravenous routes.

Twelve (of which seven were pullets) were left as uninjected controls.
The cockerels were removed when 14 weeks of age. September 15th to 20th.

SUMMARY
PEN 15—NEURAL LYMPHOMATOSIS (1940-1941)

Total of 36 birds hatched June 15, 1940.
Fourteen pullets were injected on September 6, 1940, with blood and nerve suspension from typical cases of neural lymphomatosis by the intravenous route. Age at injection was 83 days.

Twenty-two cockerels were left as uninjected controls.
The cockerels were removed when 14 weeks of age. September 15th to 20th.
No cases of the disease complex developed and all 14 of the injected pullets remained alive and healthy to the end of the experiment. (298 days).

SUMMARY
PEN 17—CONTROL (1940-1941)

Total of 40 birds hatched June 15, 1940.
The cockerels were removed when 14 weeks of age. September 15th to 20th.
No birds developed symptoms or died of lymphomatosis.
There were eleven healthy hens remaining at the close of the experiment. (298 days).
Six injected birds developed the disease complex in Pen 13. None of the contact cases in this pen developed the disease. Four cases of visceral lymphomatosis, one of neural lymphomatosis, or greatly enlarged nerves to one leg, and one case of ocular lymphomatosis were found in this pen during the time the birds were on this experiment.

Seven birds developed the disease complex in Pen 14, six of which were injected birds, and one an uninjected contact case. Four of the injected birds had visceral lymphomatosis, one of them having a leukosis picture of the monocytosis type. Two of the injected birds and the uninjected one had neural lymphomatosis with greatly enlarged nerves to parts.

Seven birds in Pen 15 developed the disease. Four were injected and three were contact exposure birds. Two of the four had visceral lymphomatosis, one of these two also having erythroleukosis and the other also having the white eye type of the disease complex. One of the four had neural lymphomatosis with enlarged nerve and also erythroleukosis. One of the four had granuloblastic leukemia and grey eyes. The three uninjected contact birds had lymphocytic infiltration of the liver.

TABLE VIII
PEN 18—VISCERAL LYMPHOMATOSIS (1940-1941)
(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2076X</td>
<td>½ cc Iv</td>
<td>39 days</td>
<td>killed</td>
<td>117 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2070X</td>
<td>½ cc Iv</td>
<td>39 days</td>
<td>died</td>
<td>268 days</td>
<td>erythroleukosis and visceral lymphomatosis</td>
</tr>
<tr>
<td>2094X</td>
<td>¼ cc Iv</td>
<td>39 days</td>
<td>killed*</td>
<td>297 days</td>
<td>leukosis and visceral lymphomatosis</td>
</tr>
<tr>
<td>2095X</td>
<td>uninjected</td>
<td></td>
<td>killed*</td>
<td>297 days</td>
<td>leukosis and visceral lymphomatosis</td>
</tr>
</tbody>
</table>

*At termination of experiment.

Total of 38 birds hatched June 15, 1940.

Twenty-six (of which 13 were pullets) were injected on July 24, 1940, by the intravenous route, with dried blood received from the Regional Bureau of Animal Industry Poultry Research Laboratory of Lansing, Michigan.

Twelve (of which seven were pullets) were left as uninjected controls.

The cockerels were removed when 14 weeks of age. September 15th to 20th.
None of the injected or contact cases developed the disease in any form in Pen 16. All the injected birds remained alive throughout the experiment.

The control pen, Pen 17, was uninjected and no case of the disease was seen.

Four birds developed the disease in Pen 18. Three were injected ones and one was a contact case. Among the injected group one had neural lymphomatosis, one had visceral lymphomatosis and erythroblastic leukosis, and one had lymphocytic infiltration of the cervical lymph glands and leukosis of the monocytosis type. The uninjected bird had erythroblastic leukosis, lymphomatosis, and lymphocytic infiltration of lymph glands.

Four different types of the disease were reproduced experimentally in from 94 to 297 days in the series of birds for the year 1940-1941. There were 30 cases reproduced in five out of seven pens of 20 birds each at the start. These birds were from 23 to 47 days old when injected or exposed. The control pen had no cases of the disease. The remaining pen was injected at the age of 83 days and no cases appeared in it. These results for the year 1940-41 are shown in Tables IV to VIII, inclusive, and in the summaries for Pens 16 and 17.

FORMS OF THE DISEASE PRODUCED BY INJECTION AND ORAL EXPOSURE OF BABY CHICKS WITH TISSUES OF THE SEVERAL TYPES

Another group of non-sexed day-old baby chicks, 300 in number, were placed on this experiment in April, 1941. They were divided into seven pens numbered 19 to 25 inclusive. The chicks came from the poultry flock of this station, where fowl leukosis had not been a problem and where effort had been made to breed a flock resistant to the disease. The chicks were placed in the pens when taken from the incubator. The female birds remained in these same pens for the duration of the experiment, approximately one year. The cockerels were removed from the pens and from the experiment when 13 weeks old. The birds had no access to outside ground or runs. The pens had concrete floors and were separated by solid partitions about three feet
The birds were fed and watered without the attendants entering the pens. When it was necessary to enter a pen disinfected rubbers were worn.

In Pen 19, two-thirds were injected, when ten days old and again when thirteen days old, with blood and nerve suspension from neural cases. Injections were given to each bird both intravenously and intraperitoneally. The other third was uninjected and was to serve as contact cases for possible contraction of the disease from the injected birds. About 50 per cent of the injected pullets and about 44 per cent of the uninjected pullets developed the disease complex. None of the cockerels showed evidence of the disease when removed and killed at 13 weeks of age. The following forms of the disease complex developed: neural lymphomatosis, visceral lymphomatosis, ocular lymphomatosis, and erythroblastic leukosis. Table IX shows the amounts injected, ages, and lesions.

### TABLE IX

**PEN 19—NEURAL LYMPHOMATOSIS (1941-1942)**

(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2865U</td>
<td>0.1 cc Iv</td>
<td>10 days</td>
<td>killed</td>
<td>362 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>0.5 cc Ip</td>
<td>13 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2870U</td>
<td>0.1 cc Iv</td>
<td>10 days</td>
<td>died</td>
<td>105 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>0.5 cc Ip</td>
<td>13 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2879U</td>
<td>0.1 cc Iv</td>
<td>10 days</td>
<td>killed</td>
<td>336 days</td>
<td>ocular lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>0.5 cc Ip</td>
<td>13 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2883U</td>
<td>0.1 cc Iv</td>
<td>10 days</td>
<td>killed</td>
<td>128 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>0.5 cc Ip</td>
<td>13 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2890U</td>
<td>0.1 cc Iv</td>
<td>10 days</td>
<td>killed</td>
<td>362 days</td>
<td>ocular lymphomatosis</td>
</tr>
<tr>
<td></td>
<td>0.5 cc Ip</td>
<td>13 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2894U</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>114 days</td>
<td>neural lymphomatosis and erythroleukosis</td>
</tr>
<tr>
<td>2898U</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>232 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2931U</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>120 days</td>
<td>erythroleukosis</td>
</tr>
</tbody>
</table>

Total of 37 birds, numbers 2861-2900, hatched April 4, 1941, others April 12, 1941.

Twenty-two were injected on April 14-17 with blood and suspension of enlarged nerve from typical cases of neural lymphomatosis by the intravenous and intraperitoneal routes.

Fifteen were left as uninjected controls.

About half of each group were cockerels. These were removed when 13 weeks of age, July 1.
In Pen 20 one-third was injected, with blood and nerve suspension from neural lymphomatosis cases when six to 13 days old. The injections were made intravenously and intraperitoneally. The other two-thirds of the chicks in this pen were uninjected. The entire pen was exposed to oral infection by placing the same materials in the feed and water as were used for the injections. Three such exposures were made when birds were 6, 18 and 26 days old. About 50 per cent of the injected pullets and 42 per cent of the ones which were not injected developed the disease complex. None of the cockerels showed evidence of the disease when removed and killed at 13 weeks of age. Two forms of the disease complex developed. These were neural lymphomatosis and visceral lymphomatosis, commonly called range paralysis, and leukemia, respectively. Table X shows the summary for Pen 20.

**TABLE X**

**PEN 20—NEURAL LYMPHOMATOSIS (1941-1942)**

(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2703U</td>
<td>uninjected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2721U</td>
<td>0.5 cc Ip 0.1 cc Pv</td>
<td>13 days</td>
<td>died</td>
<td>353 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2726U</td>
<td>0.3 cc Iv 0.5 cc Ip</td>
<td>6 days</td>
<td>died</td>
<td>207 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2733U</td>
<td>uninjected</td>
<td>13 days</td>
<td>died</td>
<td>106 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2736U</td>
<td>0.1 cc Iv 0.5 cc Ip</td>
<td>13 days</td>
<td>died</td>
<td>76 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2737U</td>
<td>0.25 cc Iv</td>
<td>6 days</td>
<td>died</td>
<td>284 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2738U</td>
<td>0.1 cc Iv 0.5 cc Ip</td>
<td>6 days</td>
<td>killed</td>
<td>376 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2732U</td>
<td>uninjected</td>
<td></td>
<td>killed</td>
<td>106 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2771U</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>262 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2267W</td>
<td>uninjected</td>
<td></td>
<td>died</td>
<td>199 days</td>
<td>visceral lymphomatosis</td>
</tr>
</tbody>
</table>

Total of 45 birds, numbers 2763-2776, hatched April 4, 1941, others April 12, 1941.

Seventeen were injected on April 10 and 17 with blood and suspension of enlarged nerves from typical cases of neural lymphomatosis by the intravenous and intraperitoneal routes.

Twenty-eight were left uninjected.

Oral exposures of the entire pen to blood and a suspension of enlarged nerves from neural lymphomatosis cases were made when the chicks were six, 18, and 26 days old. About half of each group were cockerels. These were removed when 13 weeks of age, July 1.
In Pen 21 about two-thirds of the chicks were injected when seven to eleven days old. Blood from cases of erythroleukosis to which sodium citrate had been added was used for the injections. Both intravenous and intraperitoneal injections were given to each bird. The other third was uninjected and was to serve as contact cases for possible contraction of the disease from the injected birds. About 30 per cent of the injected pullets and about 33 per cent of the uninjected contact pullets developed the disease complex. None of the cockerels showed evidence of the disease when removed and killed at 13 weeks of age. The affected birds were found to have neural and visceral lymphomatosis. Table XI shows the amounts injected, ages, total numbers and lesions.

**TABLE XI**

**PEN 21—ERYTHROLEUKOSIS (1941-1942)**

(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2784U</td>
<td>uninjected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2790U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>11 days</td>
<td>died</td>
<td>190 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2791U</td>
<td>0.1 cc Iv 0.5 cc Ip</td>
<td>10 days 7 days</td>
<td>killed</td>
<td>338 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2793U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>11 days</td>
<td>killed</td>
<td>376 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2804U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>11 days</td>
<td>died</td>
<td>136 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2272W</td>
<td>uninjected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2278W</td>
<td>uninjected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total of 44 birds, numbers 2782-2799, hatched April 4, 1941, others April 12, 1941. Twenty-nine were injected on April 11-15 with blood from typical cases of erythroleukosis by the intravenous and intraperitoneal routes. Fifteen were left as uninjected controls. About half of each group were cockerels. These were removed when 13 weeks of age, July 1.

In Pen 22, twenty chicks out of 45 were injected with blood from cases of erythroleukosis the same as for Pen 21. The birds were three days old when injected both intravenously and intraperitoneally. The remaining 25 chicks in this pen were uninjected. The entire pen was exposed to oral infection by placing
the same materials in the feed and water as were used for the injections. Two such exposures were made when the chicks were three days old and ten days old. About 55 per cent of the injected pullets and 15 per cent of the ones which were not injected developed the disease complex. None of the cockerels showed evidence of the disease when removed and killed at 13 weeks of age. Neural and visceral lymphomatosis were the types of the disease that developed. Table XII gives the summary for Pen 22.

**TABLE XII**

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2828U</td>
<td>uninjected</td>
<td>-</td>
<td>killed</td>
<td>350 days</td>
<td>neural lymphomatosis, visceral lymphomatosis (kidney)</td>
</tr>
<tr>
<td>2837U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>3 days</td>
<td>died</td>
<td>179 days</td>
<td>tumor and neural lymphomatosis</td>
</tr>
<tr>
<td>2844U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>3 days</td>
<td>killed</td>
<td>354 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2852U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>3 days</td>
<td>killed</td>
<td>354 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2855U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>3 days</td>
<td>killed</td>
<td>368 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2856U</td>
<td>0.25 cc Ip 0.1 cc Iv</td>
<td>3 days</td>
<td>killed</td>
<td>354 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
</tbody>
</table>

Total of 45 birds hatched April 12, 1941. Twenty were injected on April 15 with blood from typical cases of erythroleukosis by the intraperitoneal and intravenous routes. Twenty-five were left as un.injected controls. Oral exposure of the entire pen to blood from erythroleukosis cases were made when the chicks were three days and ten days old. About half of each group were cockerels. These were removed when 13 weeks of age, July 1.

Pen number 23 was a control pen. It was located between Pens 22 and 24. It contained 43 chicks at the start of the experiment. About half of these were cockerels which were removed when 13 weeks of age. One case of the disease complex developed in this pen. This bird had both visceral and neural lymphomatosis. Table XIII shows the summary for Pen 23.
TABLE XIII
PEN 23—UNEXPOSED CONTROLS (1941-1942)
(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2073U</td>
<td>uninjected</td>
<td>---</td>
<td>killed</td>
<td>178 days</td>
<td>visceral and neural lymphomatosis</td>
</tr>
</tbody>
</table>

Total of 43 birds hatched April 12, 1941.
About half of these birds were cockerels which were removed when 13 weeks of age, July 1.

In Pen 24, twenty-eight chicks out of 43 were injected with blood and suspension of the affected organs of typical cases of visceral lymphomatosis. Both intravenous and intraperitoneal injections were given to each bird when they were seven to eleven days old. The other 15 chicks were uninjected and were to serve as contact cases for possible contraction of the disease from the injected birds. About 31 per cent of the injected pullets and 28 per cent of the uninjected ones developed the disease.

TABLE XIV
PEN 24—VISCERAL LYMPHOMATOSIS (1941-1942)
(Affected Birds)

<table>
<thead>
<tr>
<th>Number</th>
<th>Injections</th>
<th>Age at injection</th>
<th>Results</th>
<th>Age at autopsy</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001U</td>
<td>0.25 cc Ip 0.07 cc Iv</td>
<td>11 days</td>
<td>killed</td>
<td>360 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2004U</td>
<td>0.25 cc Ip 0.075 cc Iv</td>
<td>11 days</td>
<td>died</td>
<td>220 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2924U</td>
<td>0.25 cc Ip 0.05 cc Iv</td>
<td>11 days</td>
<td>killed</td>
<td>180 days</td>
<td>neural lymphomatosis</td>
</tr>
<tr>
<td>2925U</td>
<td>uninjected</td>
<td>11 days</td>
<td>died</td>
<td>333 days</td>
<td>visceral lymphomatosis</td>
</tr>
<tr>
<td>2926U</td>
<td>0.25 cc Ip 0.05 cc Iv</td>
<td>11 days</td>
<td>killed</td>
<td>362 days</td>
<td>slight visceral lymphomatosis</td>
</tr>
<tr>
<td>2927U</td>
<td>uninjected</td>
<td>7 days</td>
<td>killed</td>
<td>361 days</td>
<td>visceral lymphomatosis (spleen)</td>
</tr>
<tr>
<td>2931U</td>
<td>0.05 cc Iv 0.25 cc Ip</td>
<td>11 days</td>
<td>died</td>
<td>167 days</td>
<td>visceral lymphomatosis</td>
</tr>
</tbody>
</table>

Total of 43 birds hatched April 4, 1941.
Twenty-eight were injected on April 11-15 with blood and suspension of affected organs from typical cases of visceral lymphomatosis, by the intraperitoneal and intravenous routes.
Fifteen were left as uninjected controls.
About half of each group were cockerels. These were removed when 13 weeks of age, July 1.
complex. None of the cockerels, which constituted about one-half of each group, developed the disease complex when removed and killed at 13 weeks of age. The neural and visceral types of lymphomatosis were the forms of the disease that developed. Table XIV shows the summary for Pen 24.

In Pen 25, twenty-three chicks out of 48 were injected with blood and a suspension of the affected organs of typical cases of visceral lymphomatosis. Both intravenous and intraperitoneal injections were given to each chick when they were 11 to 14 days old. The remaining 25 chicks were uninjected. The entire pen was exposed to oral infection by placing the same materials in the feed and water as were used for the injections. Two such oral exposures were made when the chicks were 15 days and 18 days old. The neural and visceral forms developed as shown in Table XV.

**TABLE XV**

<table>
<thead>
<tr>
<th>PEN 25—VISCERAL LYMPHOMATOSIS (1941-1942) (Affected Birds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>2943U</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2980U</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Total of 48 birds hatched April 4, 1941.

Twenty-three were injected April 15-18 with blood and a suspension of the affected organs from typical cases of visceral lymphomatosis, by the intraperitoneal and intravenous routes.

Twenty-five were left as uninjected controls.

Oral exposures of the entire pen to blood and a suspension of the affected organs of typical cases of visceral lymphomatosis were made on April 19 and April 22.

About half of each group were cockerels. These were removed when 13 weeks of age, July 1.

**DISCUSSION**

The contact exposure of pullets in 1938-39 might be considered severe. The 48 pullets were kept constantly in contact in small pens with a large number of pullets with various forms of the disease complex from various sources. The duration of the exposure was about five months. Under these conditions only four of the exposed pullets developed the disease complex. This
strongly indicates that the disease is not transmitted from adult by pen contact to any great extent. Work done the following year showed that the disease complex with several different manifestations could be transmitted to adult pullets by injections of tissues. Even by injections, however, pullets were quite resistant to infection.

During the year 1940-41 it was shown that chicks three to seven weeks old were more susceptible to the disease than were pullets. They were apparently infected in this age range by contact exposure as well as by tissue injections. Since in the control Pen 17, and in Pen 16, inoculated at 83 days of age, no cases of the disease appeared, it is probable that most of the cases that developed in the injected and uninjected chicks in Pens 12, 13, 14, 15, and 18 developed the disease as a result of the injections and pen contacts with the injected birds. Pen 16 inoculated at 83 days probably did not receive as virulent a virus as the five-months-old birds of the previous year. One outstanding thing which is easily seen in the work for the year 1940-41 is the enormous variation in susceptibility. This is well stated by Brandly, Waters, and Hall (5) when they say that some individuals of families or entire families develop the disease after apparently very mild exposure, whereas, others do not become affected even when severely exposed. This same variation in susceptibility is observed in other infectious diseases. The percentage showing an increased resistance may not be as high in some other infectious diseases as is shown in the case of the fowl leukosis complex. The use of this group of resistant birds for breeding stock may be of value in prevention of the disease.

It can be seen from the 1941-42 series of birds that chicks exposed when three to thirteen days old to infection by contact, as well as by injections, were more susceptible than older chicks and adults. In the case of Pen 25 it is interesting to note that only two chicks developed the disease and these were killed at 376 and 425 days in order to diagnose it. This pen had a southern exposure and received a great amount of sunshine through glass. It was the only pen which received any sunshine, but adequate cod liver oil was fed. An absence of clinical cases in this pen
was very evident during the experiment. Whether the sunshine raised the resistance or a virus of lower virulence was the cause cannot be stated. Although one case of the disease developed in the control pen during the 1941-42 year in Pen 23, it is felt that, with no cases developing in Pens 16 and 17 and only one case in Pen 23, most of the affected birds were experimentally infected by injections, oral exposure or by contact with the injected chicks. It would thus appear that the disease may have an age susceptibility somewhat similar to pullorum disease and that it may be transmitted by contact as well as by injections. Also that this susceptibility decreases from the baby chick a few days old to the adult bird which may occasionally be infected by injections or contact, as has been found to be the case in pullorum disease. Many workers now feel also that the disease may be transmitted through the egg. If this is so, the avian leukosis complex would simulate pullorum disease in epidemiology even more than is here indicated.

From work reported here it appears that several forms of the disease complex were produced: (1) by blood from the erythroleukosis cases, (2) with the neural lymphomatosis cases, and (3) with the visceral lymphomatosis cases. This is in agreement with the findings of the Iowa station (3). Johnson (6), and others considered erythroleukosis to be due to the same causal agent of lymphomatosis. On the other hand, workers in the Missouri station (4), and others believe that the leukosis complex involving blood changes such as erythroleukosis are of a different etiological agent than the one causing visceral lymphomatosis. Due apology is offered here for not mentioning many other workers who have made great contributions to both sides of this question.

NOTHING SPECIFIC HAS BEEN FOUND FOR TREATMENT, CONTROL, AND PREVENTION

To date no vaccine, serum, or medicine has been found to be of any value either for the prevention or for treatment. Even various control measures suggested probably are often of no value. Frequent cleaning and disinfection of houses when a few cases appear does not seem to reduce the number of cases that
may continue to show up. This is discouraging to the poultry raiser and probably is due to the birds becoming infected weeks or months before, and no amount of sanitation seems to be of value.

Even after the disease appears, there is no blood test or other means of determining which birds have already been infected as chicks and are not yet showing symptoms. The number, therefore, which will be lost may be large or small. The owner has either to market the flock or risk the loss and hope it will be small. In doing the latter it is well to cull out the visibly affected as soon as found and maintain reasonable sanitary practices.

The use of older birds which have demonstrated livability rather than pullets for breeding purposes would probably greatly reduce mortality from this disease. In commercial baby chick production this is not often possible, and most Wyoming poultry raisers purchase their baby chicks rather than raise them. For them it is indeed hard to give advice other than to keep chicks in clean brooders and on ground where infection from other chickens is not so likely. This is especially true during the first few weeks of age. However, if the chicks are infected at the time they are purchased, this will not keep out the disease but it may reduce its spread.

Probably as good preventative measures as have been suggested are those of Jungherr (7), of the Connecticut station, Brandly (5), and others. These consist of (1) selective breeding, (2) laboratory check-ups on mortality, (3) sanitary and quarantine measures, (4) keeping external parasites in check, and (5) purchase of chicks from two-year-old breeding flocks. The available facts concerning the disease suggest these measures. More specific methods of prevention and control await further knowledge.
SUMMARY

The method of approach and the results are given for four years of experimental work upon the disease complex. A total of 753 birds were used and were divided into 25 pens.

When laying pullets were exposed to birds which had the avian leukosis complex, wheat germ oil supplied no protection for the non-infected birds when it was fed as a supplement to a normal laying diet or when it was fed as a supplement to a diet low in vitamin E.

By inoculating 5-months-old pullets intraperitoneally with suspension material from the blood, liver, spleen, eyes and nerves of infected birds, it was possible to transmit the avian leukosis complex.

Chicks were more susceptible to inoculations when a few days to a few weeks of age than when older, indicating that most of the infection may be transmitted when the chicks are very young.

Inoculated chicks were capable of transmitting the disease to other non-inoculated chicks which were housed in the same pen. Non-inoculated chicks kept in nearby separate pens were not affected.

Oral infection as a supplement to inoculation did not produce a higher incidence of the disease than inoculation alone.

When the inoculations of leukotic material were made at a young age the disease appeared only after several months, generally after laying commenced, but sometimes before.

Susceptibility to infection by contact and by injections varies greatly and appears to be greatest at a few days of age and to gradually decrease to adult age, at which time birds are only very slightly susceptible.

The source of material, either from neural, ocular, visceral, or erythroleukotic cases, was no indication as to the type of disease which was produced in the inoculated birds. Any one or all of the four types of the disease were produced from material of any single type.
REFERENCES


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20th to 52d, inclusive (1900-10 to 1941-42, inclusive, except 21st, 22d, 27th, 31st, 32d, 33d, 34th, and 35th.)

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