THE TRANSMISSION OF AVIAN VISCERAL LYMPHOMATOSIS

TO RATS

by

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A Dissertation Submitted to the Department of Biology of Carroll College in Partial Fulfillment of the Requirements for Honors

Carroll College
Helena, Montana
April, 1966
This Thesis for Honors Recognition Has Been Approved
for the Department of Biology by
Dr. James J. Manor
Date 3-31-66
PREFACE

As the table of contents indicates, this thesis has three main divisions. Some of the facts that led to the author's hypothesis are discussed in the introduction. Also included in this section are some findings by other investigators which give added support and meaning to this hypothesis.

The second chapter is simply a discussion of the incidence, symptoms, causes and treatment of the various kinds of lymphomatosis.

The third and final chapter is an account of the research the author performed and some of the conclusions that can be drawn.

The author would like to express his sincere gratitude for the suggestions and technical assistance rendered by the following people: James J. Manion, Ph. D., Associate Professor of Biological Sciences, Carroll College; Rev. Joseph D. Harrington, Ph.D., Assistant Professor of Biological Sciences, Carroll College; J. Allan Miller, M.D., Director of Laboratories, St. Peters Hospital, Helena, Montana; O.J. Anderson, M.D., Pathologist, St. Johns Hospital, Helena, Montana; B.R. Burmester, D.V.M., Director
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CHAPTER I

INTRODUCTION

Evidence has been steadily accumulating that cancers are perhaps more readily transmissible from one species to another than was previously thought. For example, in 1964 the National Cancer Institute published the notes from a symposium on malignant lymphoma. These notes disclosed the following findings:

1) Three monkeys who received an inoculum of human leukemia have developed leukemia. This is the first evidence of human to animal transmission of leukemia.

2) The Rouse cell sarcoma (RCS) virus has been successfully transmitted from chickens to both monkeys and hamsters.

3) Leukemias in the human may be related to the bites of dogs which could be harboring and shedding leukemia viruses in the saliva and perhaps in urine.

4) A cluster of human cases of leukemia recently occurred in Green Bay, Wisconsin and a high incidence of bovine leukemia was also found in the area. Leukemia also occurred frequently in dogs in the vicinity. A virus capable of inducing leukemia in normal cattle has been isolated from the milk of leukemic cows.

It was findings like these that initially led me to speculate about the possibility of transmitting visceral lymphomatosis in chickens to rats. As will
be explained in greater detail later on in this paper, visceral lymphomatosis in chickens is known to be caused by a virus. Further, a chicken infected with this disease passes the virus into the eggs she lays; consequently a good share of her brood are automatically doomed to a death from lymphomatosis. The virus has also been found in the feces and oral and nasal passages of chickens. It occurred to me then that perhaps other animals could contract cancer by direct contact with this virus.

My original hypothesis has led to far-reaching research plans. For simplicity's sake, these plans can be broken down into phases.

In the first phase of research, I plan to investigate two things: 1) whether or not the experimental line of rats used in this research are genetically susceptible to lymphomatosis and 2) whether or not lymphomatosis can be transferred from chickens to rats by injecting the rats intraperitoneally with an homogenate of the viscera of a chicken afflicted with visceral lymphomatosis. It is only this first phase of the research on which I am reporting in this paper.

Providing there is a successful intraperitoneal transmission of lymphomatosis in the first phase,
I secondarily plan to investigate whether rats can also contract the disease from infected chickens via drinking water, by ingesting infected hatching eggs, and through contact with an environment previously occupied by infected chickens.

In the event this latter phase meets with some success, I ultimately hope to imply that human beings can develop lymphomatosis or related malignant diseases by coming in contact with the virus of lymphomatosis in one of the ways mentioned in the second phase.

Since the incubation period for the virus causing visceral lymphomatosis in chickens has been found to be 100 to 250 days, time has limited my research to just the first phase, that is, investigating the genetic susceptibility of the laboratory rats to lymphomatosis. Ironically, however, the third phase of my research, implicating humans in the transmission of lymphomatosis, has received the greatest impetus. An article in the medicine section of the April 6, 1964 issue of Newsweek magazine implied that a Purdue University zoologist, [Letter from B.R. Burmester, Director, Regional Poultry Research Laboratory, East Lansing, Michigan, October 15, 1964.]
Dr. Olive S. Davis, had contracted cancer while experimenting with the lymphomatosis virus. Since this article so accurately reflects my own theory, I have chosen to reproduce the entire article in this thesis.

Dr. Olive Stull Davis's report bore the drily technical title, "Transmissibility of Avian Lymphomatosis to Mammals," but there was nothing abstract about what the Purdue University zoologist was saying. Dr. Davis was one of the mammals; she apparently "caught" cancer while experimenting with a virus—the first such case ever reported.

As the mild-mannered, 59-year-old researcher reconstructed her case, during World War II she was working with the so-called avian visceral lymphomatosis virus, an organism that causes 90 per cent of malignant tumors in chickens and is a major flock killer. At that time, no one suspected that contact with the virus could be dangerous for humans. "I had worked for years," she explained, "with my bare hands in contact with... infected chickens and chick embryos almost daily." The virus, she thinks, could have entered through a scratch.

The first sign of trouble came in 1953, when she noticed that the lymph glands in her neck, under her arms, and at her groin were swollen. A year later, a physician took a biopsy sample from the swollen glands. The verdict was lymphoblastoma, a type of growth which under the microscope appears virtually identical with the chicken tumors.

For the past decade, Dr. Davis has regularly traveled to the Ontario Cancer Foundation London Clinic for radioactive cobalt treatments which have controlled the lymph-node tumors. But during the past year, she reported matter-of-factly, tumors have appeared under the skin on both her legs. No one can predict the outcome in her case, but the zoologist is resolved to continue her work. To learn how easily the chicken cancer can be transmitted to animals of other species, she is inoculating the virus into
guinea pigs, mice, and hamsters. Her research is incomplete, but she notes that one other chicken virus—the Rous sarcoma virus—has already been shown to cause tumors in mammals, including newborn monkeys.

Does her case mean that many persons may "catch" cancer from animals outside the lab? Dr. Davis is extremely cautious about the question; her infection could have been a medical fluke. Nonetheless, the case supports the growing belief that cancer viruses can move from one species to another.2

It is unfortunate that a human life has to be endangered to provide evidence for a theory. However, in my research for this thesis, I may have stumbled across a finding which suggests that Dr. Davis may not be the only person to contract cancer from working with lymphomatosis. In October, 1965, I was in Bozeman, Montana and I happened to mention my research topic to Dr. William Sippel, a Bozeman general practitioner. He seemed interested and asked me to explain my theory. I soon found out the reason for his interest. He himself has treated four cancer patients from the diagnostic laboratory of the Livestock Sanitary Board in Bozeman. Three of these patients have succumbed to cancer; the fourth is considered a terminal patient. None of the cancers are the same, that is, none show identical manifestations. However, all four patients

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had one thing in common: each came in "frequent contact" with lymphomatosis while performing the duties of his job. Bear in mind that these four people all worked together in the diagnostic laboratory where chickens and other animals are received to be autopsied and diagnosed. In no other department of the Livestock Sanitary Board in Bozeman is the incidence of human cancer nearly as high as it is in the diagnostic laboratory. I have watched the employees of this laboratory perform their duties and I have never seen them take protective measures, such as wearing gloves, when working with lymphomatosis.

Five humans who worked with lymphomatosis have contracted cancer. This fact was suggestive enough for me to inaugurate my research into the interspecial transmission of avian lymphomatosis.
CHAPTER II

INCIDENCE, SYMPTOMS, CAUSES AND
CONTROL OF LYMPHOMATOSIS

Types of Lymphomatosis and Their Clinical Symptoms

Lymphomatosis is the most destructive disease of chickens in the United States. Millions of chickens die each year with the typical symptoms of lymphomatosis. However, many millions more die annually without showing the typical clinical and post mortem findings. These latter deaths are often attributed to latent infections of lymphomatosis. This conclusion is based upon the observations that deaths from undetermined causes often parallel deaths from lymphomatosis, and that a large proportion of the chickens in infected flocks, although they appear healthy, are actually infected. 3

The three common forms of lymphomatosis that are of great economic importance to the poultry industry are termed visceral lymphomatosis (big

liver disease), neural lymphomatosis (fowl or range paralysis), and ocular lymphomatosis (iritis). Oftentimes osteopetrosis (big bone disease) and fowl leukemia are classified as types of lymphomatosis. All the types are limited primarily to chickens although it has been reported in turkeys and pheasants.

The most common type of lymphomatosis is that affecting the viscera. The presence of neoplastic or cancerous tumors in the liver, spleen, gonads, kidneys, heart, lungs, mesenteries, glands or even skin is indicative of visceral lymphomatosis. Primary tumors may originate in any one of these organs and metastasize to other organs. Generally this disease is accompanied by immature lymphocytes in the blood, and subsequent anemia. The disease can be superficially diagnosed by observing loss of appetite, loss of flesh, diarrhea, or abnormal palpitations of the organs. At present there is no known method of arresting a lymphomatous growth once it has started; consequently death will inevitably ensue.

Visceral lymphomatosis may be either acute or chronic. Fast growing pullets may become droopy and succumb within a few days; laying hens may cease laying abruptly and die within a short time.
In contrast to this, other birds may be droopy and emaciated for a relatively long time before they succumb.

Neural lymphomatosis affects the nerves of chickens and often results in paralysis. Clinical manifestations are a drooping of one or both wings and a weakness and lack of coordination of the legs which usually leads to an inability to stand or walk. Oftentimes these fowl have difficulty in breathing because their vagus nerve has become infected with the disease.

Gross anatomical examinations of chickens with neural lymphomatosis will usually reveal localized swellings of peripheral nerves (the central nervous system is occasionally involved). Most commonly affected are the brachial and lumbar plexuses and the sciatic and femoral nerves. The nerves are generally infiltrated with lymphoid cells. There are little, if any, blood changes.

The ocular form is the least common of the three main types of lymphomatosis. This particular disease results in impairment of the vision or complete blindness in one or both eyes. In most breeds or varieties of chickens the color of the iris is orange or red. In ocular lymphomatosis,
however, the iris is usually invaded by lymphoid cells, rendering the iris gray. Often the pupil becomes irregular and loses its power of light accommodation. No deviation from the normal blood picture has ever been observed in a chicken infected with this disease. Interestingly, ocular lymphomatosis occurs primarily in flocks affected with the neural form.

Now a few words about the two diseases often associated with lymphomatosis-osteopetrosis and fowl leukemia.

An enlargement of the leg bones is usually the first clinical symptom observed in chickens with osteopetrosis. As the disease progresses, other bones of the body become involved. Recent research at the U.S. Regional Poultry Research Laboratory, East Lansing, Michigan, has disclosed that there may be a relationship between osteopetrosis and visceral lymphomatosis. According to this laboratory’s report, a variable amount of osteopetrosis has occurred in young chicks inoculated with a particular strain of inoculum from a chicken which apparently had only visceral lymphomatosis.\(^4\)

\(^4\)Ibid., p. 7.
As in humans, fowl leukemia is a neoplastic blood condition resulting from uncontrollable proliferation of either the immature red or white blood cells or both. The liver and spleen are usually enlarged and have a gray or bright red color, depending on the cell type involved. The disease can be readily reproduced in young chickens by inoculation with filtrates or blood from diseased birds. Most authorities of poultry diseases conclude that fowl leukemia is caused by a virus.

The Cause and Spread of Lymphomatosis

Lymphomatosis is an elusive, insidious disease. A couple examples will make this statement more meaningful. The incidence of lymphomatosis has greatly increased in the poultry population during the past thirty years while comparable diseases of the lymphoblastoma group in other domestic animals have not increased at such an alarming rate. The reason for the increase is not known. Further, there can be a high incidence of lymphomatosis in a flock one year and almost a complete absence of it the subsequent year, without any apparent change in hatching, brooding or maintenance procedures.

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Plate 2. Paralysis of the leg resulting from neural lymphomatosis
Plate 3. Two chickens of the same age. A, Cockerel with leg bones of natural size. B, Cockerel showing enlargement of leg bones, and posture indicative of osteopetrosis.
Plate 4. Enlarged brachial plexus nerves (A and B) leading to the wings, indicate paralysis or neural lymphomatosis. The condition is more pronounced in the right wing (A) than in the left wing of this chicken. Visceral lymphomatosis is also clearly shown by the ovarian tumor (C).
Plate 5. A, Enlarged tumorous liver and spleen taken from a bird with visceral lymphomatosis. B, Enlarged liver and spleen with small lesions. C, Enlarged liver and spleen; the minute individual lesions cannot be seen with the naked eye. D, A normal liver and spleen.

All five plates were taken from the U.S. Department of Agriculture circular Lymphomatosis in Chickens.
Incidences such as these have given rise to questions about the cause and transmission of the lymphomatosis complex. In the next few pages, evidence that sheds some light on the cause and spread of this complex will be given.

More is known about visceral lymphomatosis than the other manifestations. All evidence points to a virus as the causative agent of the visceral form. For example, experiments have shown that visceral lymphomatosis can be reproduced by inoculating young chicks with cellular material from diseased birds. Furthermore the inoculate may be passed through a filter capable of removing all bacteria and tissue particles and still the disease can be reproduced in young chicks. Other experiments have disclosed that such filtrates may be kept viable for several hundred days at low temperatures, or may be made inactive by temperatures of 125° to 135° Fahrenheit, by formaldehyde or by ultraviolet radiation.\(^6\)

Bits of evidence such as these presented here have led investigators to speak conclusively of a virus as the causative agent of visceral lymphomatosis. Evidently this virus is different from the agent or agents responsible for the neural and ocular forms of the disease.

\(^6\) *Lymphomatosis in Chickens*, p. 9.
The avenues of infection of lymphomatosis have demanded a great deal of research time. Some avenues have been definitely established; others are still being investigated. Infectious levels of the visceral lymphomatosis virus have been isolated in the saliva and feces of chickens.\(^7\) Hence the drinking water and feeding grounds are sources of infection. The nasal passages have also been found to contain the virus.\(^8\) This opens up the possibility of the disease being airborne.

Until recently the theory of egg transmission of lymphomatosis was based on rather circumstantial evidence. Briefly this theory stated that if a hen contracted visceral lymphomatosis and if her laying capacity was not arrested, the virus causing the lymphomatosis was passed into her eggs. Consequently a large percentage of her brood are doomed to die from the disease. This theory was rather widely accepted until a group of investigators whom we shall call the "isolationists," produced some evidence to the contrary. This group claimed that


\(^{8}\)Ibid., p. 499.
mortality in chicks hatched from "infected" eggs was practically nil when they were raised in isolation. They argued that if the virus causing visceral lymphomatosis were transmitted from hen to chick through the egg, or if any chicks were infected in the incubator, there should have been more serious losses than there actually were.\textsuperscript{9}

Investigators now agree that isolation is an important factor in reducing the incidence of visceral lymphomatosis in a flock. However, direct proof has now been obtained that the egg is an avenue of infection. Fertile eggs from infected hens whose progeny had died in large numbers from lymphomatosis were used to produce fifteen, eighteen and twenty-one day-old embryos from which inoculating material was obtained. These inoculums were injected into young chicks (averaging one day of age) from laboratory stock of relatively low incidence of lymphomatosis. The chicks were brooded under rigid control and isolation for 270 to 300 days.

Analysis of more than 2,500 chicks showed that the virus of visceral lymphomatosis was present in a significant number of chick embryos. In fact, in one lot of inoculated chicks the incidence of

\textsuperscript{9}Leukosis Not Transmitted Through Eggs\textsuperscript{9}, (American Veterinary Medical Association, October, 1951), p.292.
visceral lymphomatosis was as high as 88 percent. The average of 29 percent for all inoculated lots definitely established hatching eggs as a source of the infective virus.\textsuperscript{10}

Two other important observations were made in trying to prove the egg transmission theory. First it was noticed that hens may have an inapparent infection of visceral lymphomatosis and yet shed infectious levels of virus into their eggs, saliva, and feces. This finding warrants the classification of these hens as "carrier" hens.\textsuperscript{11} On the average the incidence of lymphomatosis in the progeny of carrier hens was not significantly greater than that in the progeny of non-carrier hens. However, the chicks of carrier hens are an important source of infection for chicks of noninfected stock. When an extract of dust, down, and other debris collected from an incubator containing chicks of an infected flock was injected into noninfected, susceptible chicks, it caused a high incidence of visceral lymphomatosis. Furthermore, when chicks of an infected flock were hatched and brooded with chicks

\textsuperscript{10} \textit{Lymphomatosis in Chickens}, p. 10.

\textsuperscript{11} Burmester, p. 497.
of a noninfected flock, the latter developed a high incidence of visceral lymphomatosis, whereas the former did not.

Upon further investigation it was found that hens with an inapparent infection may have circulating antibodies which are also transferred to their eggs. Hence the progeny of the carrier hens are afforded some degree of immunity.\(^\text{12}\) This is the second of the two observations arising from the attempt to prove the egg transmission theory.

The above discussion of transmission was primarily limited to visceral lymphomatosis. There is some evidence that neural lymphomatosis can be spread by contact during the early brooding period. Investigators have found no indication that neural lymphomatosis may be spread from parent to chick through the hatching egg.

The incidence of ocular lymphomatosis is so low that there has been little opportunity to study its transmission. However, Sevoian and Chamberlin claim they have reproduced the neural, visceral, and ocular forms of lymphomatosis in several strains and breeds of chickens with a single isolate. Their work has given impetus to the

\(^{12}\text{Burmester, p. 498.}\)
unitarian theory which claims that the three forms of lymphomatosis are caused by a single agent.\textsuperscript{13}

The fact that visceral lymphomatosis is spread by contact from diseased birds to healthy chickens after hatching and also from infected hens to chicks through the egg, establishes it as a contagious disease. Furthermore, it is the only known malignant disease that is spread by contact (although, as was stated in the introduction, evidence indicates that other malignancies may also be contagious). This feature more than any other makes visceral lymphomatosis a unique disease. In addition, transmission through the egg provides a chain of infection which cannot be broken so easily as that of other virus diseases which are not egg borne, or in which the virus is shed for only a short time.

Controlling Lymphomatosis

Results of experiments have shown that there are factors which affect the resistance of chickens to lymphomatosis, the three most important being genetic resistance, virus-stimulated resistance.

(resistance due to antibodies), and age of the chicken at the time of exposure. Resistance due to antibodies has already been discussed, so the following presentation will be limited to the remaining two resistance factors.

It has been firmly established that genetically resistant strains of chickens can be developed. In fact, one team of investigators were able to breed a strain which, when adequately exposed to lymphomatosis, had a mortality rate of only 0.8 to 1.5 percent. Conversely they were able to breed a susceptible strain in which the mortality rate was as high as 38.8 percent.¹⁴

The younger the chicks at the time of exposure to the virus, either under natural conditions or by inoculation, the greater the incidence of the disease. When day-old chicks isolated from infection were mixed with day-old infected chicks, 38 percent of the former lot developed lymphomatosis by the time they were five hundred days of age. When chicks from the same parental stock were held in isolation for thirty days and then mixed with infected chicks

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of the same age, the mortality rate was only 15 percent. 15

An interesting sideline might well be injected here. A relation does exist between the response of susceptible chickens and the dose of virus of visceral lymphomatosis injected intraperitoneally into the chickens. An intermediate increase in dosage of virus causes a corresponding increase in percentage of lymphomatosis. However, when the dosage was increased tenfold, the percentage of lymphomatosis did not increase in proportion. In one experiment where the highest dose of inoculum was 100,000 times the lowest dose, there was a difference of only 26.7 percent in the occurrence of visceral lymphomatosis. However the average age of death decreased rapidly with the increase in dose. 16

Since lymphomatosis is the most destructive disease in the poultry industry, the pressure has been on researchers to come up with some means of controlling the disease. Even though vaccination and other prophylactic measures designed to totally eradicate the disease have failed, there are practical

15 Lymphomatosis in Chickens, p. 12.
16 Ibid., p. 12.
methods of reducing the incidence of lymphomatosis.

As was previously mentioned, the poultry raiser can practice selective breeding to develop a resistant line of birds. If this selective breeding is accompanied by prompt removal from the flock of all chickens that have lymphoid tumors or display the typical clinical symptoms of lymphomatosis, infection of the premises will be reduced and exposure of the chicks to the causative agent will be limited.

A considerable measure of control can also be effected by brooding the chicks in isolation, as far as possible from older birds during the critical first few weeks after hatching. Good isolation techniques would involve a caretaker who does not come in contact with other poultry.

One team of investigators have found that the incidence of visceral lymphomatosis may even depend on the hatch date, there being a higher incidence in birds hatched in January, February and March than in April, May or June. If a poultry raiser were desperately trying to reduce the mortality rate from

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lymphomatosis in his flock, perhaps he could even practice a type of birth control.
CHAPTER III

DETERMINING THE GENETIC SUSCEPTIBILITY

This third and final chapter is an account of the research the author performed to determine whether or not the experimental line of rats used in the research are genetically susceptible to the visceral lymphomatosis virus. As was stated in the introduction, the time factor limited the research to just this first phase.

On December 31, 1964, the first group of experimental rats were born. From this litter, four male rats were culled and numbered respectively 2, 5, 7, and 8. Three days later, on January 3, 1965, another litter was born from which four more male rats were culled. These rats were numbered 1, 3, 4, and 6. In addition, two females and one male were culled from the two litters to preserve the genetic strain. The mothers of these two litters were from the same litter and both were bred to the same male. Hence the young males used in this experiment are all from the same genetic stock.
Rats 1, 2, 3, and 4 became the controls; rats 5, 6, 7, and 8, which will for the sake of simplicity be called the experimental rats, were chosen to receive an injection of chicken visceral homogenate presumably containing the visceral lymphomatosis virus.

These eight rats were isolated from each other in steel cages. The four control rats were placed in cages above the four experimental rats, so that if the virus were shed into the feces of the experimental rats, there could be no avenue of fecal contamination.

As there is some evidence that visceral lymphomatosis is transmissible (at least in chickens) through the water dispenser, the cages, water bottles, water bottle necks, and corks were each carefully numbered. Then whenever the rats were watered, the corresponding necks and bottles were put together and inserted into the corresponding cages. By following this method at each watering, the possibility of viral contamination was virtually eliminated.

The lymphomatous tissue itself was taken from the liver and spleen of a White Leghorn chicken which had been brought for diagnosis to the diagnostic
laboratory of the Montana State Livestock Sanitary Board in Bozeman, Montana. Dr. A.M. Jasmin diagnosed the chicken as having visceral lymphomatosis. The fowl's liver and spleen were greatly enlarged and infiltrated with lymphoid cells. The liver and spleen were removed from the chicken and frozen until the time of injection.

In addition, a normal-appearing chicken was culled from a flock in the Helena valley and eviscerated. Macroscopically the viscera appeared normal: the liver and spleen were of normal size and lacked any lymphomatous lesions. The liver and spleen of this chicken were also removed and kept frozen until the time of injection.

Histological sections were made of both the lymphomatous tissue and the normal-appearing tissue. Microscopic examination of the sections by a Helena pathologist verified that the tissues were indeed respectively lymphomatous and normal.

To prepare the homogenate, 15.93 grams of normal liver and spleen were placed in a sterilized Waring blender. To this, 15.93 grams of 0.8 percent saline solution, 200,000 units of penicillin (prepared beforehand from crystalline penicillin and sterile water) and 100 milligrams of streptomycin
were mixed in. The antibiotics were added to insure against bacterial infection. The above ingredients were thoroughly mixed and homogenized in the Waring blender.

The exact same procedure was followed in the preparation of the lymphomatous homogenate: 15.93 grams of lymphomatous tissue, 200,000 units of penicillin, 100 milligrams of streptomycin, and 15.93 grams of saline solution were all blended together.

Before the rats were injected with their respective homogenates, they were weighed, a peripheral blood sample was taken, and a leucocyte differentiation was made by the author. The blood was taken from the tip of each rat's tail and smeared on separate slides. The slides were stained with Wright's stain for one minute and buffered with distilled water for three minutes.

Approximately one gram (0.9 cc.) of normal liver-spleen homogenate was injected into the control rats. The injection was intraperitoneally on the right side, near the bladder. Likewise, 0.9 cc. of lymphomatous homogenate was injected intraperitoneally into the experimental rats.
After completing the injections, the rats were again isolated. The animals were watered and fed each day by Mr. Clifford Gillespie until the experiment was terminated. The author developed an allergy to the rat hair and Mr. Gillespie graciously consented to take over watering and feeding the rats.

Three leucocyte differentials were done at regular intervals on the peripheral blood of all eight rats. The rats were also weighed four times and at the completion of the experiment, a total white blood count was made. The results of these weighings, differentials and count are reproduced in the following tables. Because of a space shortage, the following abbreviations will be used in the leucocyte differential tables: prolymph. for prolymphocyte; lymph. for lymphocyte; neutro. for neutrophil; eosin. for eosinophil; and baso. for basophil.

**TABLE 1**

RAT WEIGHT AS TAKEN ON FEBRUARY 20, 1965

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Average Weight of Controls .... 137.2 grams
Average Weight of Experimental Rats .... 142.9 grams

The first litter, being three days older, was slightly heavier at the beginning of the experiment.

**TABLE 2**

**LEUCOCYTE DIFFERENTIAL AS TAKEN ON FEBRUARY 20, 1965**

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<td>4</td>
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<td>44</td>
<td>37</td>
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</tr>
<tr>
<td>5</td>
<td>34</td>
<td>25</td>
<td>38</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>42</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td>32</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>34</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The above two tables express the results of the weighings and differentials of the rats before they were injected with their respective homogenates. The figures expressed in the following tables were taken after the rats had been injected.
TABLE 3
RAT WEIGHT AS TAKEN ON MAY 31, 1965

Rat Number  Weight (in grams)
1  441.9
2  428.6
3  394.6
4  390.0
5  419.9
6  391.3
7  404.8
8  421.1

Average Weight of Controls .... 413.8 grams
Average Weight of Experimental Rats .... 409.2 grams

TABLE 4
LEUCOCYTE DIFFERENTIAL AS TAKEN ON MAY 31, 1965

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>prolymph</th>
<th>lymph</th>
<th>neutro</th>
<th>eosin</th>
<th>base</th>
<th>mono</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>18</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>17</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>16</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>4</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>20</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>6</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>7</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>84</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Note that there is a general increase in the number of prolymphocytes in table 4 over table 2.
All blood samples were drawn at approximately the same time of day; hence a time differential cannot
account for the difference in the number of prolymphocytes.

TABLE 5
RAT WEIGHT AS TAKEN ON SEPTEMBER 10, 1965

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Weight (in grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>515.6</td>
</tr>
<tr>
<td>2</td>
<td>513.3</td>
</tr>
<tr>
<td>3</td>
<td>478.7</td>
</tr>
<tr>
<td>4</td>
<td>438.7</td>
</tr>
<tr>
<td>5</td>
<td>503.9</td>
</tr>
<tr>
<td>6</td>
<td>505.2</td>
</tr>
<tr>
<td>7</td>
<td>467.9</td>
</tr>
<tr>
<td>8</td>
<td>479.0</td>
</tr>
</tbody>
</table>

Average Weight of Controls .... 486.6 grams
Average Weight of Experimental Rats .... 489.0 grams

TABLE 6
LEUCOCYTE DIFFERENTIAL AS TAKEN ON SEPTEMBER 10, 1965

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>prolymph</th>
<th>lymph</th>
<th>neutro</th>
<th>eosin</th>
<th>baso</th>
<th>mono</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>12</td>
<td>48</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>4</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>10</td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>7</td>
<td>24</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>19</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>81</td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>13</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Note that the over-all prolymphocyte count is still high as compared to the count in table 2.

It was after this third differential had been
completed that Mr. Gillespie remarked that the experimental rats had stopped eating and drinking as much as they normally do. In view of this observation and the fact that the prolymphocyte count was high, the author decided it was time to sacrifice and autopsy the rats.

Before killing the rats, approximately 3.0 cubic centimeters of blood was drawn from each one by means of a cardiac puncture. Each sample was drawn with separate needles and syringes and put into separate vacuum tubes containing sodium oxylate. The anticoagulate sodium oxylate was added to each vacuum tube to keep the blood from clotting until a total white count could be taken. The total white count was done on the autoanalyser of St. Peters Hospital Laboratory. The following counts were recorded.

**TABLE 7**

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Leucocyte Number (per cc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14,000 - 14,500</td>
</tr>
<tr>
<td>2</td>
<td>15,000 - 15,500</td>
</tr>
<tr>
<td>3</td>
<td>15,000 - 15,500</td>
</tr>
<tr>
<td>4</td>
<td>8,500 - 9,000</td>
</tr>
<tr>
<td>5</td>
<td>15,000 - 15,500</td>
</tr>
<tr>
<td>6</td>
<td>9,000 - 9,500</td>
</tr>
<tr>
<td>7</td>
<td>12,000 - 12,500</td>
</tr>
<tr>
<td>8</td>
<td>14,500 - 15,000</td>
</tr>
</tbody>
</table>
After drawing the blood samples, the rats were killed by putting them to sleep with ether; then they were autopsied. In the autopsy the author first examined all the viscera for neoplasms. The eyes, and brachial and lumbar plexuses were also scrutinized. If in gross examination, any tissue looked abnormal, it was removed and preserved in 10 percent formaldehyde. The liver and spleen were routinely removed, weighed and preserved for all eight rats. Later, the liver and spleen and any other preserved tissues were sectioned, stained with hematoxylin-eosin stain, and mounted on slides for histological examination.

Two of the rats had abnormally appearing livers. Rat number 2 had calcified nodules imbedded in the substance of its liver. Histological examination revealed these nodules were bacterial cysts which had calcified. Rat number 7 had a liver whose caudal lobe was superficially infiltrated with lymphocytes. At first glance the lobe resembled a lymphoma; however microscopic examination confirmed that the liver was indeed normal. Three different pathologists were unable to suggest any reason why the caudal lobe should have a superficial conglomeration of lymphocytes.

Drs. Sharman and Jasmin of the Montana
Livestock Sanitary Board observed that some centri-lobular veins in the liver of rat number 1 were lined internally with lymphocytes. Apparently, however, this finding is not particularly significant.

As for the blood smears dated May 31 and September 10, Drs. Sharman and Jasmin refused to commit their opinion because of too much stain precipitation on the slides. Dr. J. Allan Miller of St. Peters Hospital felt a leukemia was indicated in rats 6 and 7 because of the high incidence of prolymphocytes. Dr. O.J. Anderson of St. Johns Hospital could not concur. He did not feel the chromatin net of the prolymphocytes was such that it indicated a leukemia. All other blood smears and histological sections appeared normal to the latter two doctors.

Conclusions

Perhaps this particular subheading would be more appropriately entitled "Inconclusions," because decisive evidence to indicate a transmission or nontransmission of avian lymphomatosis seems nonexistent. If there was a transmission, it apparently manifested itself in the blood as a leukemia. On the one hand the high incidence of prolymphocytes
indicated a leukemia; on the other hand, the pro-
lymphocytes did not appear structurally to be the
same as those prolymphocytes usually associated with
a lymphocytic leukemia.

If one were to assume that the transmission
was unsuccessful, one could only conclude that
the transmission did not succeed with this particular
genetic stock. Genetic susceptibility seems to be
a critical factor in transmitting lymphomatosis in
both chickens and mammals. The author recently
learned in a letter from Dr. B.R. Burmester that
Dr. Ludwik Gross of the Veteran’s Memorial Hospital,
Bronx, New York, has succeeded in breeding a strain
of mice that is highly susceptible to lymphomatosis.
Dr. Burmester went on to say that Dr. Olive S. Davis
of Purdue University (the doctor who claims she may
have contracted lymphomatosis from working with
lymphomatous chickens) injected large amounts of
lymphomatous tumor specimens into various mammalian
species and has reportedly induced some atypical
tumors.

A more general conclusion to be reached is that
the transmission of avian visceral lymphomatosis to
mammalian species is meeting with some success.
Perhaps the research performed for this thesis would be more feasible if a known susceptible strain of rats could be obtained for use.
BIBLIOGRAPHY

Articles and Periodicals


Letters
