TRANSMISSION STUDIES ON AVIAN LYMPHOMATOSIS

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W. J. Stapleton

Frank T. Hoff Jr.

Major professor

Date 5-24-50

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TRANSMISSION STUDIES ON AVIAN LYMPHOMATOSIS

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By

George Edward Cottral

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TABLE OF CONTENTS

INTRODUCTION

Avian lymphomatosis is a fatal infectious neoplastic disease of chickens caused by one or more virus-like agents. It is characterized by the infiltration and multiplication of lymphoid cells in the various tissues of the bird's body. The invading cells ultimately become neoplastic and result in the formation of tumors of the visceral organs and other tissues, or may cause paralysis, blindness, or skeletal changes.

Avian lymphomatosis has been divided into four types, according to the tissues involved: visceral, neural, ocular, and osteopetrotic lymphomatosis. All of these disease entities are included under the classification, the avian leukosis complex, which also embraces three additional neoplastic diseases, namely, erythroblastosis, granuloblastosis, and myelocytomatosis. The scheme of nomenclature, as worked out by a committee of poultry pathologists, is given in table 1 along with some of the common names that have been applied to these diseases (Jungherr, 1941).

TABLE 1.--Outline of nomenclature of the avian leukosis complex

Recommended nomenclature	Common names
The avian leukosis complex	
Lymphomatosis:	
Neural	Fowl paralysis, Marek's paralysis
Ocular	Grey eye, iritis
Visceral	Lymphocytoma, big liver disease
Osteopetrotic	Marble bone, big bone disease
Erythroblastosis	Fowl leukemia, blood disease
Granuloblastosis	" " " "
Myelocytomatosis	Leukochloroma, white tumors

Visceral and neural lymphomatosis are the two most important diseases in this group; the others occur much less frequently. These diseases are widespread throughout the world, and it has been estimated that they cause an annual loss to the poultry industry of about 60 million dollars in the United States alone (Winton, 1949).

The plan of this thesis is to present the history of the various etiological concepts of the avian leukosis complex, to review the literature on the transmission of avian lymphomatosis, and to present the results of recent studies on the problem of egg transmission of avian lymphomatosis.

The problem has been approached from the viewpoint that the various diseases embraced by the avian leukosis complex are separable and that at least some of them are probably caused by separate, filtrable oncogenic agents. The pathological changes and diagnostic aspects of these diseases have been omitted purposely because they have been adequately covered in other reports (Jungherr, 1948; Feldman and Olson, 1948; Runnells, 1948). For clarity, a standard nomenclature has been used throughout.

HISTORY OF THE ETIOLOGICAL CONCEPTS

In 1896 Caparini in Italy described fowl leukemia, in 1905 Butterfield reported on "Aleukemic lymphoid tumors of the hen," and in 1907 Marek in Hungary described "Multiple polyneuritis of fowl." From these beginnings two independent groups of workers evolved--one was the fowl leukosis group, and the other was the fowl paralysis group. The fowl leukosis group was composed of veterinary and comparative pathologists and medical men who were interested in tumors and in human leukemia. They found in fowl leukosis a means of studying, experimentally, a leukemic disease. The fowl paralysis group was composed of veterinary pathologists, poultrymen and other investigators who were interested in fowl paralysis primarily because of its economic importance to the poultry industry.

The Original Etiological Concepts (1896 to 1929)

The fowl leukosis group.--The disease entities originally included under the term fowl leukosis were: erythroblastosis, granuloblastosis, myelocytomatosis, and visceral lymphomatosis. In Ellermann's (1921, 1923) concept of fowl leukosis all of these diseases were caused by the same etiological agent. The studies by this group of workers were highlighted by the discovery of Ellermann and Bang, in 1908, that fowl leukosis could be transmitted with a filtrable agent. All of the workers agreed that there were several types of fowl leukosis, but they disagreed on the nomenclature and the transmissibility of the various types. Some investigators reported an inability to transmit "lymphatic" or "extravascular leukosis" and others were inclined to classify visceral lymphomatosis as a separate disease (Andersen and Bang, 1928; Furth, 1929). Mathews (1929)

believed that leukochloroma (myelocytomatosis) was also a separate disease. Attempts to discover the natural mode of spread of fowl leukosis failed. This was the status of fowl leukosis in 1929. Ellermann's concept of the disease was beginning to be seriously questioned.

The fowl paralysis group.--Originally fowl paralysis meant only neural lymphomatosis; later, ocular and visceral lymphomatosis were also included because of their frequent association with neural lymphomatosis. During the period 1907 to 1929 fowl paralysis spread throughout the major poultry producing areas of the world. It spread like an infectious disease, and the etiological agent was assumed to be a filtrable virus (Kaupp, 1921; Van der Walle and Winkler-Junius, 1924). Doyle (1929) suggested that fowl paralysis may be transmitted from dam to offspring through the egg. Pappenheimer and his associates (1926) demonstrated that this disease could be experimentally transmitted. The knowledge of fowl paralysis in 1929 was summed up quite well by Pappenheimer and his associates (1929a, 1929b): "Although the evidence, epidemiological and experimental, points to an infective agent, we are totally ignorant as to its nature, and as to the manner in which the disease is conveyed under natural conditions..... Our studies at least indicate that the agent which is responsible for the pathological changes in the nervous system is not exclusively neurotropic; it may in certain cases stimulate the proliferation of lymphoid cells in the viscera, and to a degree which makes them take on the morphological character of a neoplasm."

During the period 1921 to 1929 there were numerous comments in the literature that attempted to show a relation between fowl paralysis and intestinal parasites (coccidia, tapeworms, etc.) Eventually this

erroneous supposition was dropped as more facts concerning the disease became known.

The status of visceral lymphomatosis (lymphocytoma).--Visceral lymphomatosis was independently claimed by both the fowl leukosis and fowl paralysis groups. This dual relationship of visceral lymphomatosis was the result of its confusing association with the diseases studied by each group. Ultimately, visceral lymphomatosis became the common denominator that resulted in drawing the two groups closer together. Pappenheimer and his associates (1929a, 1929b) were the first to notice this overlapping. They saw that what Ellermann (1923) and his associates were calling "lymphatic leukosis," was similar to their "visceral lymphomata." However, they were emphatic in their denials of an association of visceral lymphomatosis with leukemia.

In the fowl leukosis group the later tendency was to exclude visceral lymphomatosis because it was thought to be a non-transmissible lymphoid tumor that was not accompanied by a true leukemic state. Feldman (1927) preferred to classify this disease as lymphocytoma, defined as a malignant lymphoblastic neoplasm of chickens (Furth, 1929).

Later Etiological Concepts (1929-1950)

The fowl leukosis and fowl paralysis groups maintained their separate identities up to 1932. At that time the two groups were united; however, many workers refused to join the union. The influence of the original groups has persisted up to the present time.

Unitarian view.--In 1932 Johnson came to the conclusion that fowl paralysis and fowl leucosis were all part of the same disease complex, after observing that all types of involvement resulted in his transmission

trials. In the same year, Patterson, Wilcke, Murray and Henderson (1932) presented the results of their study of "so-called range paralysis of the chicken," and they also concluded that these diseases were all part of the same disease complex. Johnson (1932) summed up this viewpoint as follows: "From this work it appears that the common cause of leucosis and lymphomatosis of fowls is a filtrable agent, and that the predominating cell in the lesions is the hemocytoblast which is a trivalent cell capable of differentiation into erythrocytes, granulocytes, and lymphocytes." Many workers adopted this viewpoint. Jungherr (1948) has termed this concept "the unitarian view."

Multitarian view.--Opposed to the unitarian view is the multitarian view, which as presented by Furth (1933) tended to demonstrate that each of the various diseases had its own etiological agent that performed quite true to type when maintained as a transmissible strain. Furth (1936) was inclined to view the few discrepancies that occurred as contaminants that were picked up in the course of serial passage. Feldman and Olson (1933, 1934) had a somewhat similar viewpoint based primarily on the pathological changes. They believed that lymphocytoma (visceral lymphomatosis) was non-transmissible, and that lymphocytoma, neural lymphomatosis, myelocytoma, and leukosis (myeloid and erythroleukosis) were all distinct, unrelated diseases.

Division of visceral lymphomatosis.--Furth (1934b) and Stubbs (1937) recognized "hepatolymphomatosis" (big-liver disease) as a separate disease, but classified it as lymphoid leukosis along with myeloid and erythroleukosis. Stubbs (1939) advocated placing the other visceral lymphoid tumors (i.e., other than hepatolymphomatosis) in the fowl paralysis category. Thus, a concept developed that there are two kinds

of visceral lymphomatosis: the visceral lymphomatosis associated with neural and ocular lymphomatosis, and a visceral lymphomatosis or lymphoid leukosis associated with erythroblastosis and granuloblastosis. This was essentially a return to the concepts of the two original groups.

Leukosis-sarcoma virus.--Oberling and Guerin (1933a, 1933b) presented evidence which suggested that fowl leukosis and sarcoma were caused by the same agent. Their viewpoint was as follows: "The virus of leukemia of fowls can give rise to sarcoma by affixing itself to connective or histiocytic cells, and this histiotropism is most plainly exhibited if, instead of making the transfers by intravenous injection of leukemic blood, one makes intramuscular or subcutaneous grafts of leukemic material, which hinders the virus from exteriorizing its always predominant hemotropism." Other workers also reported the apparent association of sarcoma and fowl leukosis or lymphomatosis (Troisier and Sifferlen, 1935; Rothemeyer and Engelbreth-Holm (1933). However, some workers felt that the association was simply a coincidence (Furth, 1934a), and Stubbs and Furth (1935) could not duplicate Oberling's claim that storage in glycerine modified the fowl leukosis agent, so that it would produce sarcoma. Nevertheless, the virus tumors described by Rous and his associates (1911-1914) had to be considered in subsequent studies on leukosis and lymphomatosis.

Mixed and complex strains.--Furth (1934b) found that endothelioma and myelomatosis were sometimes associated with lymphomatosis. This brought the transmissible endothelioma described by Begg (1927) into the already confused picture. Furth (1934b, 1936) postulated mixed and complex strains. A mixed strain had several agents each one of which

produced a different type of neoplasia. A complex strain had a single agent with multiple potentialities for producing different types of neoplasia.

Osteopetrosis.--Jungherr and Landauer (1938) observed that osteopetrosis was frequently associated with lymphomatosis. In their concept the agent of osteopetrosis was "non-dissociable from the agent of lymphomatosis."

Hemocytoblastosis.--Emmel (1934, 1937) thought that the basic process in this group of diseases was a hemocytoblastosis, which resulted from numerous non-specific factors, such as vitamin and mineral deficiencies and bacterial toxins, particularly of the salmonella group.

Chick disease virus.--In 1939 Blakemore reported on the discovery of a filtrable agent in the tissues of birds with lymphomatosis. This agent when inoculated into young birds produced an inflammatory response. Blakemore advanced the theory that this acute disease in chicks was caused by the same agent that was responsible for lymphomatosis in older birds, and that the lymphomatous involvements represented the end result or chronic stage of an acute infection. Glover (1940) and later Asplin (1947a, 1947b, 1947c) were also able to demonstrate this agent, which Asplin has since termed "'chick disease' virus." In his latest report Asplin (1947c) made the following comment, "Experiments were carried out in an effort to determine the validity of the theory that 'chick disease' is an acute reaction caused by the agent responsible for lymphomatosis. The results, whilst not conclusive, present evidence that this is not so."

The avian leukosis complex.--In 1940 a committee of pathologists met at the U. S. Regional Poultry Research Laboratory and suggested a tentative classification in which this whole group of diseases was

collectively called "the avian leukosis complex." (Jungherr, 1941) The classification was not intended to imply an etiological relationship of the various diseases, but was suggested as a means of arriving at a uniform terminology in the literature. In spite of the admonition, many people construed the classification to mean acceptance of the unitarian view.

Recent trends.--In the more recent literature the trend has been away from the unitarian viewpoint, especially in regard to separating lymphomatosis from erythroblastosis and granuloblastosis. Myelocytomatosis and sarcomatosis likewise have been set apart from the other diseases. Leukosis has been thought of as meaning only erythroblastosis and granuloblastosis, and lymphomatosis has been accepted generally as a term that includes neural, visceral, ocular and osteopetrotic forms. Recently there has been an attempt to separate the various types of lymphomatosis on an etiological basis, and the new evidence indicates that this eventually may be possible (Davis and Doyle, 1947a, 1947b, 1947c, 1949; Burmester, 1947c).

The difficulty in the separation of these agents in the past may have been due to the use of heterogeneous populations of birds, in which many agents may have been present in a latent form. The use of pedigreed inbred birds, whose strain and familial histories have been followed, and the use of isolated quarters may enable investigators to solve this problem of separation. Fortunately we have passed the day when an experimenter will rely on the results obtained with a crate of nondescript chickens from the local market.

REVIEW OF THE TRANSMISSION STUDIES ON AVIAN LYMPHOMATOSIS

Transmission of neural lymphomatosis.--Neural lymphomatosis has been transmitted by inoculating birds with suspensions of nerve, ganglion, spinal cord, and brain tissues taken from birds with lesions of neural lymphomatosis. The inoculations were made via the intraperitoneal, intracranial, intraneural, subcutaneous, intramuscular, subdural, and intravenous routes (Pappenheimer, et al., 1926, 1929b; Baumann, 1936; Jungherr, 1937; Beach, 1938; DeOme, 1943). Intravenous inoculation of fresh or heparinized whole blood from cases of neural or ocular lymphomatosis appears to be the method of choice in transmitting neural lymphomatosis (Durant and McDougle, 1937, 1939, 1945, 1947). When the transmission of neural lymphomatosis is attempted, the resulting incidence is usually not very high, and other forms of the avian leukosis complex are likely to be transmitted also, especially visceral lymphomatosis. Transmissible strains of neural lymphomatosis have been reported (Furth, 1934c, 1935). Freezing at -30° C and drying from the frozen state inactivates inocula from such transmissible strains.

Transmission of neural lymphomatosis with a filtrable agent has been claimed (Van der Walle and Winkler-Junius, 1924). The evidence to support this assertion is quite insufficient, yet most workers agree that it is very likely that neural lymphomatosis is caused by a filtrable agent. The hypothetical agent is thought to be present in the circulating blood and in the involved nervous tissue.

The lesions found in peripheral nerves of paralyzed birds that show typical symptoms of neural lymphomatosis may be either microscopic foci of small lymphoid cells that have an inflammatory appearance, or

large areas of definite neoplastic cells. Birds that have been destroyed within a few days after they first developed paralytic symptoms usually had the inflammatory type of involvement; whereas, paralytic birds that were allowed to live several weeks after the appearance of symptoms usually had the neoplastic type of involvement. Microscopic lesions suggestive of neural lymphomatosis have been seen frequently in the peripheral nerves, adrenal ganglia and other sympathetic ganglia in birds that apparently were normal and showed no symptoms of nerve disorder during life.

Transmission of ocular lymphomatosis.--Ocular lymphomatosis has been observed in the chickens of the inoculated groups by many workers (Johnson, 1932; Durant and McDougle, 1937, 1939, 1945). However, the incidence of this disease was usually so low that a significant difference between the inoculated and control groups could not be demonstrated. This has been a most disconcerting disease entity because of its rarity and sporadic occurrence. However, a rather high incidence of the disease has occasionally been reported in certain farm flocks.

Transmission of other types of lymphomatosis has resulted and tumor strains have been developed from donors that had ocular lymphomatosis (Brandly, et al., 1942). A series of transmission studies using whole blood from donors with ocular or neural lymphomatosis demonstrated that lymphomatosis could be transmitted in this manner. When a large amount of blood was transfused from such donors into chicks, a high incidence of neural lymphomatosis resulted (Durant and McDougle, 1945). Successful transmission of lymphomatosis, especially neural lymphomatosis, was accomplished using whole blood from normal chicks as the inoculum. The chicks were the offspring of birds with ocular lymphomatosis (Durant and McDougle, 1937, 1939).

Recently it has been demonstrated that a transplantable visceral lymphomatous tumor strain will survive and grow when inoculated into the anterior chamber of chicken eyes. In such cases the iris was often involved and presented a gross and histological picture similar to ocular lymphomatosis (Little, et al., 1949; Burmester, 1949).

Transmission of visceral lymphomatosis.--Visceral lymphomatosis has been transmitted by inoculating chicks with whole blood from birds with visceral, neural, and ocular lymphomatosis (Johnson, 1932; Furth and Breedis, 1933; Brandly et al., 1942). The use of cell-containing inocula prepared from blood or tumor tissue from birds with naturally occurring visceral lymphomatosis led to the discovery that transplantable lymphoid tumor strains could be developed and carried in serial passage. One of these strains produced visceral lymphomatosis, myelomatosis and endothelioma (Furth and Breedis, 1933). A filtrable agent was demonstrated in this tumor strain. This evidence was not readily accepted because in the other tumor strains that produced visceral lymphoid tumors (lymphocytoma), filtrable agents could not be detected (Olson, 1941, 1944). This led to the idea that lymphocytoma could be set apart from the other diseases of the avian leukosis complex as a non-transmissible but transplantable neoplasm. Meanwhile, others contended that visceral lymphomatosis was a part of the avian leukosis complex and that it could betransmitted by inoculating whole blood into chicks (Brandly et al. 1942). Thus, transmissible strains of lymphomatosis were developed and carried in serial passage, using blood as an inoculum; later, other lymphoid tumor strains were developed by inoculating into chicks tumor cell suspensions from naturally occurring cases of visceral lymphomatosis (Burmester and Prickett, 1945).

The Olson lymphoid tumor, RPL 12 (Olson, 1941) was carried in serial passage and studied at the U. S. Regional Poultry Research Laboratory (Burmester and Cottral, 1947d). A filtrable agent that produced lymphoid tumors and osteopetrosis was found in this tumor strain. Subsequently, this tumor strain was carried through six serial passages using cell-free inocula. Because the filtrable agent was found in a transplantable tumor strain and filtrable agents had not been demonstrated in naturally occurring visceral tumors, it was not accepted as the etiological agent of visceral lymphomatosis. It was believed that there was a difference between the naturally occurring visceral lymphomatosis and the filtrate induced lymphoid tumors. Recent studies have shown that this objection is no longer valid (Burmester and Denington, 1947e; Burmester, 1947f).

In a recent study it was found that visceral lymphomatosis (and in one group osteopetrosis) could be transmitted with cell-free inocula prepared from lymphoid tumors of donors with naturally occurring lymphomatosis (Burmester and Denington, 1947e). Four out of ten tumor bearing donors tested gave positive results using cell-free inocula. The types of lymphomatosis in the four donors were as follows: two had gross visceral and neural lymphomatosis; one had visceral lymphomatosis, only; and the other had gross visceral and ocular lymphomatosis and microscopic evidence of neural lymphomatosis. It was also found that lymphoid tumor strains could be developed and carried in serial passage using cell suspensions prepared from the tumors of some of these donors. Furthermore, transmission of visceral lymphomatosis and osteopetrosis using cell-free inocula could be demonstrated at different stages during the course

of serial passage of some of these and other tumor strains. Some of the tumor strains were carried through several serial passages using cell-free inocula. One tumor strain was carried through seven serial passages using cell-free inocula, but it did not produce typical visceral lymphomatosis, and hemangiomatosis was associated with it (Burmester, 1947f).

Many workers have reported on the transmission of lymphomatosis using whole blood or tumor cell suspensions taken from cases of the naturally occurring disease. These cell-containing inocula have dual potentialities and it is exceedingly difficult, if not impossible, to demarcate strictly between these lymphoid tumors resulting from the multiplication of transplanted tumor cells and lymphomatosis caused by the etiological agent. Undoubtedly, many of the transmission studies reported in the literature, where cell suspensions were used, should be regarded as true transmissions and not transplantations. The difference in the incubation periods may be one method of separation. In transmission studies, using cell-free inocula and inbred susceptible birds, the incubation period for visceral lymphomatosis varied from about 60 to 250 days, with an average of about 150 days. After serial passage with cell-containing inocula the transplantable lymphoid tumor strains had a much shorter incubation period, varying between 6 and 30 days. Thus, when a cell-containing inoculum is used, those birds that die of lymphomatosis after a long incubation period, are more likely to develop lymphomatosis from the etiologic agent than from the multiplication of the transplanted tumor cells but a strict differentiation cannot be made.

Recently, the relationship of the other forms of the avian leukosis complex to visceral lymphomatosis was reviewed and subjected to critical

study. The conclusion was reached that visceral lymphomatosis could be readily transmitted, and that neural and ocular lymphomatosis were not transmitted by the method employed by these workers. The inoculum consisted of cell suspensions prepared from the tumorous organs of birds with visceral lymphomatosis (Davis and Doyle, 1947a, 1947b, 1947c, 1949). Other studies have borne out this conclusion. Further work suggested that there was no marked concentration of the causative agent or absence of it in any of the organs tested in the transmission studies. The organs tested were the liver, heart, spleen, kidney, ovary, and blood from a bird with naturally occurring visceral lymphomatosis (Davis and Doyle, 1947a, 1949).

In other experiments, visceral lymphomatosis, and possibly neural lymphomatosis, were transmitted to young chicks by inoculating them intratracheally and intranasally with tracheal and nasal washings from donors with neural and ocular lymphomatosis (Cottral and Burmester, 1948; Winton, 1948, 1949). Filtrates of the respiratory washings were not effective in producing lymphomatosis. In the same study, respiratory washings rendered bacteriologically sterile with penicillin and streptomycin were found to be effective in producing lymphomatosis when inoculated intravenously. However, the same inoculum was ineffective when inoculated via the respiratory route. Peroral administration of tumor cell suspensions appeared to be effective in transmitting visceral lymphomatosis but not all workers have been successful in the transmission of lymphomatosis when the oral and respiratory routes were used (Brewer and Brownstein, 1946; Barber, 1948). Successful transmission has been reported with fresh and desiccated feces (Jungherr, 1937).

Studies with tumor strains induced with cell-containing inocula.--
The transplantable tumor strains have been carried in serial passage via the intraperitoneal, intravenous, intramuscular, or intraocular routes by adaptation. Serial passage reduced the incubation period of the tumor strain so that some of the strains produced tumors and caused the death of the recipients in 6 to 30 days (Olson, 1944; Burmester, 1945, 1947f). Dilute inocula containing as few as eight tumor cells still resulted in successful transplantation. The thermal death point of the tumor cells has been found to be between 45° and 50°C (Cottral and Burmester, 1948; Winton, 1947, 1948). Chicks 30 days or less of age were the most susceptible to the transplanted tumors. However, the resistance of many of the older birds was overcome by increasing the dosage of the inoculum. Birds that developed transplanted tumors and survived were immune to further implants, but their immunity appeared to be only against the cells, as many of them developed typical visceral lymphomatosis and died at a later date (Burmester, et al., 1946, 1947b, 1947c). In some tumor strains a "hemorrhagic disease" has been seen in some of the inoculated birds. The lesions appeared to be similar to those that have been described in studies with the Rous agent (Duran-Reynals, 1940).

Several lymphoid tumor strains have been successfully transplanted on the chorio-allantoic membrane of embryonated eggs, and the resulting tumors have been carried in serial passage. When embryonated eggs were inoculated with cell-free filtrates of visceral tumors no measurable reaction took place in the embryos. Thus, the available embryonic time prior to hatching is sufficient for transplanted tumor cells to form a tumor, but appears to be insufficient for the induction of tumors with

the filtrable agent. Another interpretation would be that other factors necessary for tumor production with the cell-free agent may be lacking.

Transmission of osteopetrosis.--Osteopetrosis has been a relatively rare disease. The incidence was reported to be less than 0.05 percent. In an endemic outbreak studied, the incidence was less than 2.0 percent (Jungherr and Landauer, 1938). Osteopetrosis has usually occurred in association with visceral lymphomatosis. When birds with spontaneous osteopetrosis were used as donors the disease was transmitted to recipient birds by using cell suspension prepared from blood, bone marrow, and visceral lymphomata. Four serial passages were made with this strain. The agent survived desiccation up to 105 days.

A "lymphomatosis-osteopetrosis" strain was developed from donors that had neural and ocular lymphomatosis, but showed no evidence of osteopetrosis (Brandly, Nelson, and Cottral, 1942). Osteopetrosis occurred in six successive serial passages of this strain. Heparinized whole blood was used as the inoculum.

Recently it was found that osteopetrosis could be transmitted with a filtrable agent (Burmester, 1947a; Burmester and Cottral, 1947d). The filtrable agent was first demonstrated with cell-free inocula prepared from the Olson visceral tumor strain (RPL 12). Filtrable agents producing osteopetrosis and visceral lymphomatosis were carried through six serial passages using cell-free inocula prepared from visceral tumors and blood derived originally from birds inoculated with strain RPL 12.

Osteopetrosis has been transmitted with a filtrable agent derived from an ovarian tumor of a bird with naturally occurring visceral and neural lymphomatosis (Burmester and Denington, 1947e). Cell suspensions prepared from this tumor also produced osteopetrosis. In other studies

osteopetrosis was produced with cell-free and cell-containing inocula prepared from visceral tumors and blood derived from birds inoculated with other lymphoid tumor strains. In many instances osteopetrosis has been latent or "masked" in donor birds and has been found in subsequent passages. The incubation period for osteopetrosis was quite variable in transmission studies. It ranged from about 60 to 300 days. Centrifugation studies indicated that it might be possible to effect a partial separation of the agents of osteopetrosis and visceral lymphomatosis (Burmester, 1947a). It has been suggested that these two diseases may be caused by separate agents.

A condition resembling osteopetrosis was found in chicks that received inoculations of bone marrow, both embryo and x-rayed adult marrow (Gohs, 1934). Osteopetrosis was occasionally seen in some of the complex strains of leukosis and lymphomatosis (Furth, 1936). Osteopetrosis was found in groups of chickens that had been inoculated with the Rous sarcoma (Pikovski and Doljanski, 1946). Recently a disease resembling osteopetrosis was observed in groups of chickens that had been inoculated when embryos with the blood of mice having mouse leukemia (Thiersch, 1944). In the same series of studies, osteopetrosis also occurred in groups that received chronic myeloid leukemic blood from man. These birds were also inoculated when they were embryos. In this particular study none of the other forms of the avian leukosis complex occurred.

Mechanical transmission.--Insect and rodent vectors for the agents of the avian leukosis complex have been postulated. It has also been suggested that accidental transmission may take place during surgical, blood testing, and vaccinating operations, and by the use of certain

vaccines prepared from embryonated eggs. However, none of these methods of transmission have been established.

Contact transmission.--The evidence indicates that naturally acquired lymphomatosis can be transmitted by contact of infected chicks with non-infected chicks in the incubator and during the first few weeks of life (Waters and Bywaters, 1949; Waters 1947a, 1947b). Contact transmission after the first month of life appears doubtful. Chicks artifically infected by inoculation via the intraperitoneal route usually do not transmit the disease to contact controls that are raised with them. On the other hand, chicks inoculated via the intravenous, oral, and respiratory routes in some instances appear to transmit the disease to chicks in the contact control groups. This suggests that the route of infection may play an important part in determining whether or not the disease is spread by contact. However, an important question not answered by the available data is: do these exposed birds pick up the etiological agent and become carriers not destined to die of the disease themselves, but capable of passing the agent on to the next generation? The transmission studies using the oral and respiratory routes of inoculation indicated that lymphomatosis can be transmitted via these routes and that the agents appear to be present in the respiratory tract of certain birds.

All of the evidence seems to favor the conclusion that erythroblastosis, granuloblastosis, and the Rous sarcoma are not transmitted by contact.

THE PROBLEM OF EGG TRANSMISSION

The natural mode of transmission of avian lymphomatosis has not been clarified. One of the main questions that needs to be answered is whether or not avian lymphomatosis can be transmitted from the dam or sire to the offspring through the egg. The second phase of this problem is to determine whether apparently normal birds, i.e., those without clinical or gross evidence of the disease, can be carriers of the agent or agents of lymphomatosis. With these points established, control measures for the disease may be more easily sought. An answer to this whole problem is of vital importance to the poultry industry.

Doyle (1929) was the first to suggest that avian lymphomatosis may be transmitted through the egg. Since then, many other workers have presented observations and experimental evidence both for and against this contention. The evidence favoring egg transmission of lymphomatosis may be classified as follows: epiornithic observations, flock isolation studies, family isolation studies, incubator exposure studies, histological studies, and transmission studies involving inoculation.

Epiornithological observations on the spread of lymphomatosis indicated to some workers that the disease may have been introduced into flocks by the purchase of hatching eggs from infected flocks (Doyle, 1929; Marginson and McGaughey, 1931; Warrack and Dalling, 1932).

Flock isolation studies, such as the establishment of the U. S. Regional Poultry Research Laboratory flock, also suggested egg transmission. In this flock only eggs were introduced into previously unused quarters and the first cases of lymphomatosis were found during the second and subsequent months after the chicks hatched (Waters, 1945).

Family isolation studies were carried out in which only the eggs of individual hens were placed in isolated incubators (Waters and Prickett, 1944). Thus, each family group of chicks was hatched and reared in an isolated pen. Gross manifestations of lymphomatosis were later evident in the birds of certain family groups, but not in others. This was further evidence of egg transmission.

The incubator exposure studies also indicated egg transmission (Waters and Bywaters, 1949). These experiments were carried out with the offspring of isolated dams. Control groups were hatched and reared in isolated pens and their sibs, comprising the test groups, were hatched and reared in incubators and pens with the offspring of non-isolated highly "contaminated" parent stock. Other test groups of sibs were hatched in isolation and then exposed to the non-isolated or "contaminated" stock at different ages following hatching. At the termination of the experiments a significant difference in the incidence of gross lymphomatosis was found between the groups exposed continually from hatching on to termination and the groups that were hatched in isolation and then exposed from one day of age on to termination. The incubator exposed groups had the highest incidence of lymphomatosis. These results were interpreted to indicate that the offspring of the non-isolated or "contaminated" stock were being infected with lymphomatosis through the egg and that they were capable of infecting the test group in the incubator at hatching time to a greater degree than at any time thereafter. Thus, those birds hatched in isolation and exposed at one day of age had a lower incidence of lymphomatosis because they missed the incubator exposure.

Histological studies have given information relative to egg transmission, especially dealing with a possible mechanism of transmission and the carrier-bird problem (Lucas, 1946; Lucas, et al., 1949a, 1949b, 1949c, 1950; Oakberg and Lucas, 1949). If the agent of lymphomatosis causes the formation of lymphoid areas in the viscera and nerves of birds, then there is histological evidence for the presence of the agent in embryos, since such areas have been found in some embryos.

Transmission studies that tended to indicate egg transmission were conducted by Durant and McDougal (1937, 1939). They inoculated the blood from chicks that were the offspring of diseased parent stock (ocular lymphomatosis) into other susceptible chicks. A higher incidence of neural lymphomatosis was found in the inoculated chicks than in their uninoculated control sibs.

The evidence against egg transmission is very weak. Some workers have noted a lower incidence of lymphomatosis in heavily infected stock when it was raised in isolation as compared to similar populations left in the exposed environment (Johnson and Wilson, 1937; May, Tittsler and Goodner, 1925). Other investigators have been impressed by the low incidence of lymphomatosis in the offspring of certain dams with ocular lymphomatosis, and they have shown that the offspring of such dams do not have any higher incidence of lymphomatosis than the offspring of other apparently normal dams (Madsen, 1937; Ball and Cole, 1946). Other workers have reported the opposite effect when infected dams were used (McClary and Upp, 1939).

Hutt and Cole (1947, using the results obtained with diallel and reciprocal crosses between lines that had been bred for resistance or susceptibility to lymphomatosis, concluded that the influence of egg

transmission, if it does occur, is not nearly as great as are genetic and environmental influences.

Recently, Cole (1949) made a study of several populations of birds, dividing them into two main groups. These two groups were as follows: (1) Chickens that were the offspring of birds that died of lymphomatosis during the breeding season or shortly thereafter, and (2) chickens that were the offspring of birds that survived throughout the breeding season. There was no significant difference between the two groups of chickens in respect to the incidence of lymphomatosis. This evidence was interpreted to indicate that if egg transmission of lymphomatosis does occur it is not of great importance in determining the viability of the progeny. These experiments were based on the premise that carrier birds are destined to die of lymphomatosis within a relatively short period of time--a conclusion that does not seem to be justified.

MATERIALS AND METHODS

In the present study conducted at the U. S. Regional Poultry Research Laboratory only single-comb White Leghorn chickens of both sexes were used. The experimental chickens were all from inbred line 15 of the laboratory flock. The history of this line, which has been raised in isolation, has been described by Waters, et al. (1944, 1949). This line of chickens has had a relatively low incidence of lymphomatosis when raised in isolated quarters, yet the birds were quite susceptible to lymphomatosis, as proved by their performance when exposed in a "contaminated" environment.

Fourteen hatches of inbred line 15 chickens were used in this study. All of the line 15 chickens were hatched in isolated incubators away from all other chickens. The chickens were brooded and raised in isolated pens; thus, they had no direct contact with any other chickens. In all of the experiments, except experiment No. 1, and the isolated controls for the other experiments, the following procedure for housing was used: When the birds were about 60 days old the males from the various experimental lots were removed and placed in a separate pen adjoining the pen of females. This was done to lessen the danger of cannibalism. Later, when the birds were about 100 days old, they were moved from their brooding pens and placed in laying house pens, still maintaining the separation of the sexes by placing them in adjoining pens. The diet fed to the birds was the standard laboratory poultry ration (Winton, Lucas, and Cottral, 1950). The experimental work was started in April, 1947, and was terminated in May, 1950. The experimental period for each group of birds was set at 310 days, except in the case of two groups which were terminated at 250 days. All birds that died during the course of the

experimental period were subjected to a necropsy examination. At the termination of the experimental period all surviving birds were killed and examined. The diagnosis of lymphomatosis was based on the gross necropsy findings.

Source and preparation of inoculum.--In order to provide inocula for these studies certain dams were selected as donors. The dams used were either picked at random from the exposed population or were selected on the basis of the incidence of lymphomatosis in their sibs and progeny. Eggs were saved from these selected dams and were then incubated. At the appropriate time these embryos or hatched chicks were killed and their tissues were aseptically removed. For the most part liver tissue was used in these studies.

Embryo and chick tissue.--In general the inocula were prepared as follows: The liver tissue was weighed, then ground in a sterile test tube grinder. Sterile 0.85 percent saline was added at the rate of 1 part tissue to 9 parts of saline. The cell suspension was then filtered through sterile gauze to remove the large clumps of tissue. The inoculum was then placed in rubber stoppered serum vials and held under refrigeration until used. The inoculum was never held in storage longer than two hours. Sterility tests were made at the time the inoculum was placed in the vials.

When filtered inocula were prepared the same procedure was followed except that the cell suspension was passed through a Seitz filter, using a No. 8 pad, and N_2 pressure. The filters were tested following filtration of the inoculum for their ability to hold back cultures of bacteria in broth medium. Usually a culture of micrococci or Serratia marcescens was used for this test. Thus, one could assume that no cells were able

to pass through to the filtrate and that the filter was in good working condition.

Material from donor hens.--In one experiment the blood of adult birds was used as an inoculum. The blood was drawn from the wing vein and was heparinized to prevent coagulation by using a solution of heparin at the rate of 1 mg. per 7.5 ml. of blood.

The respiratory washings were collected from the donor hens by holding the bird with its head down and washing sterile saline through its nasal passages. A syringe with a rubber tip was used for this purpose and the fluid was injected into the choanae and caught as it came out the external nares. Approximately 10 ml. of fluid was used to wash through the nasal passages of each donor. Penicillin (100,000 units) and streptomycin (33.0 mg.) were added to the respiratory washings to inactivate the bacteria. The material was held in the refrigerator for two hours before it was used.

All inoculations were made intraperitoneally, unless otherwise specified, and the amount used for this route was 0.25 ml. The birds were inoculated at one day of age.

Experimental groups.--There were eight separate experiments, each of which was composed of various lots or groups of chickens that received different treatments. Each hatch used was divided so that each lot received as nearly an equal family representation as possible.

Experiment No. 1

Donors.--In this preliminary experiment 9 donor chicks were taken from the incubator tray on the morning they hatched. These chicks were selected at random, taking one chick from each of nine different inbred

lines (Table 2). The chicks were sacrificed and inocula were made as follows:

Inoculum(A) was composed of the pooled liver tissue of all of the chicks.

Inoculum (B) was the pooled yolk sacs of all of the chicks.

Inoculum (C) was the pooled intestines and trachea of the chicks.

Recipients.--The inocula were administered to the recipient line 15 chicks when they were one day old as follows: Lot No. 1.--14 chicks, inoculum (A) given intraperitoneally, 0.25 ml.; inoculum (C) given intratracheally, 0.12 ml.

Lot No. 2.--14 chicks, inoculum (B) given intraperitoneally, 0.25 ml. inoculum (C) given intratracheally, 0.12 ml. Lots 1 and 2 were from a single hatch and were raised together in an isolated pen.

Lot No. 3.--34 non-inoculated control chicks from a single hatch, hatched and raised in an isolated pen adjacent to the pen in which lots 1 and 2 were located.

Lot No. 4.--28 non-inoculated control chicks from a separate hatch, also raised as an isolated group.

This experiment was started on June 30, 1947, and terminated about 300 days later.

Experiment No. 2

Donors.--The donors for this experiment were 15 day old embryos, 18 day embryos and newly-hatched chicks--all were the offspring of 5 selected dams. These dams were selected because there was a fairly high incidence of lymphomatosis among their offspring or their sibs. (See table 3). All of the dams and sires of the donor embryos lived for 600 days, at which time they were killed. None of them showed any clinical

or gross evidence of lymphomatosis. No abnormalities were noted in the donor embryos and chicks. In order to carry out this experiment, it was necessary to set eggs from these selected dams at specified intervals so that on a predetermined date there would be available, embryos 15 and 18 days old and chicks just hatching. In each age group two embryos (or chicks) were used from each of the five dams. Thus, each inoculum was composed of the liver tissue of 10 embryos (or chicks).

Recipients.--One hatch of line 15 chicks was divided into 5 experimental lots, i.e., lots 5 to 9 inclusive. All inoculations were made intraperitoneally.

Lot No. 5.*--17 chicks were inoculated with liver tissue from 10 newly-hatched donor chicks.

Lot No. 6.--14 chicks were inoculated with a cell-free filtrate of the liver tissue of the 10 newly-hatched donor chicks.

Lot No. 7.--17 chicks were inoculated with liver tissue from 10 embryos, 15 days old.

Lot No. 8.--17 chicks were inoculated with liver tissue from 10 embryos, 18 days old.

Lot No. 9.--17 chicks were used as uninoculated contact controls. All of the above groups of chickens, i.e., lots 5 to 9, inclusive, were hatched and reared together in an isolated environment. When the birds were about 90 days old all of the males were removed and placed in an adjacent pen.

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*Note: Lot numbers were assigned consecutively through all of the experiments. Thus, experiment No. 1 contains lots 1 to 4, experiment No. 2 contains lots 5 to 11, etc.

Lot No. 10.--20 chicks used as non-inoculated controls. This represented a single hatch and the birds were raised in complete isolation.

Lot No. 11.--25 chicks, likewise used as non-inoculated isolated controls.

This experiment was started on April 5, 1948, and terminated about 300 days later.

Experiment No. 3

Donors.--The information on the donors for this experiment is given in table 4. Liver tissue taken from embryos 15 days old was used as the inoculum. The embryos supplying inoculum for each experimental lot were from only one dam. At the time the eggs were laid and the embryos were used the dams and sires of the respective embryos were all clinically normal and apparently in good health. These dams and sires lived in an exposed environment where they had ample opportunity to become infected with lymphomatosis.

Recipients.--One hatch of line 15 chicks was divided into 10 lots with as nearly as possible an equal family representation in each lot. All inoculations were made intraperitoneally when the chicks were one day old. The protocol for this experiment was as follows:

	No. of birds	Liver tissue inoculum (dam No.)
Lot No. 12	19	J7010
" " 13	16	J430H
" " 14	17	J404N
" " 15	17	J716R
" " 16	17	J313N
" " 17	17	J310R
" " 18	18	J256C
		Filtrate of liver tissue
" " 19	18	J310R
" " 20	16	Amnionic fluid J313N
" " 21	18	Uninoculated contact controls

This experiment was started on Jan. 17, 1949 and terminated 220 days later.

Experiment No. 4

Donors.--This experiment was similar to experiment No. 3 except that all of the dams and sires but two, lived in an isolated environment where they had less chance of becoming infected with lymphomatosis. In addition these isolated dams and sires were closely related to the recipient chicks, because they came from the same inbred line. The information on these dams is given in tables 4 and 5. All were clinically healthy at the time their eggs were used.

Recipients.--One hatch of line 15 chicks was divided into 12 experimental lots, as in the previous experiment. All inoculations were made intraperitoneally when the chicks were one day old. The protocol for this experiment was as follows:

	No. of birds	Liver tissue inoculum (dam No.)
Lot No. 22	18	J726Q
" " 23	18	J709W
" " 24	18	I1414H2
" " 25	18	I1406U2
" " 26	19	J1318K2
" " 27	18	I1413B2
" " 28	18	J1318S2
" " 29	19	J1301W2
" " 30	19	J1301M2
" " 31	19	J1314W
" " 32	17	J1318B2
" " 33	19	Non-inoculated contact control

Lot No. 34.--Was composed of 34 chicks from a single hatch reared in complete isolation. All of the other birds in lots 22 to 33 lived together as a population, except that the two sexes were separated as in the previous experiment. This experiment was started on Jan. 31, 1949, and terminated 220 days later.

Experiment No. 5

Donors.--In this experiment 5 families were selected as donors (i.e., 5 dams and the males to which they were mated, table 6). Only high producing hens were used, and that was the main basis for the selection of the families. Eggs were set from these hens, so that on a predetermined day there would be available, embryos and chicks from these families as follows: embryos 1 day old, 8 days old, 15 days old, and chicks one day old, 8 days old, 15 days old, 29 days old and 43 days old. Two embryos or chicks from each respective age group and from each of the five families were sacrificed to prepare the inocula. Thus, a pooled inoculum was prepared from the livers of the birds in each age group. In addition, a filtered inoculum was prepared from the livers of the one day old chicks. Part of this filtrate was placed in a glass tube, sealed, and heated to 45°C for 30 minutes in a water bath. This treatment furnished another inoculum.

Recipients.--One hatch of line 15 chicks was divided into 11 experimental lots. All inoculations were made intraperitoneally when the chicks were one day old. The protocol for the experiment was as follows:

	No. of birds	Pooled liver tissue inoculum from:
Lot No. 35	22	Embryos 1 day old
" " 36	23	" 8 days old
" " 37	25	" 15 " "
" " 38	21	Chicks 1 day "
" " 39	26	" 8 days "
" " 40	24	" 15 " "
" " 41	25	" 29 " "
" " 42	22	" 43 " "
" " 43	20	Filtrate of livers - 1 day old chicks
" " 44	24	Heated " " " 1 " " "
" " 45	23	Uninoculated contact controls

All birds lived together in a common isolated environment with the two sexes in separate adjoining pens. The experiment was started March 21, 1949, and was terminated approximately 310 days later.

Experiment No. 6

Donors.--In this experiment a recheck was made on some of the donor dams previously used in experiments No. 3 and 4. In addition, other dams were tested. Eggs were set from the selected dams and when the embryos were 18 days old they were sacrificed to furnish liver tissue for the preparation of inocula. A pool of liver tissue from two sibling embryos was used for each inoculum. All of the donor hens and sires lived in the exposed population and they were clinically normal at the time the eggs were used. The information on the donors is given in tables 4 and 5.

Recipients.--A single hatch of line 15 chicks was divided into 11 experimental lots as in the previous experiments. The inoculations were all made intraperitoneally when the chicks were 4 days old. This experiment was initiated on May 2, 1949, and was terminated 310 days later.

The protocol for this experiment was as follows:

	No. of birds		Embryo inoculum (dam No.)
Lot No. 46	19	(Filtrate of liver)	J709W
" " 47	18		J709W
" " 48	18		J404A
" " 49	18		J716R
" " 50	18	(Amnionic fluid)	J716R*
" " 51	18	(Filtrate of liver)	J716R
" " 52	20		J306P
" " 53	20		J430H
" " 54	18		J709D2
" " 55	19		J310R
" " 56	18	Uninoculated contact controls	

All of the birds in this experiment were housed in a common isolated environment and the management practices used were similar to those in the previous experiments.

*In lot No. 50 the undiluted amnionic fluid from the J716R embryos was used as the inoculum.

Experiment No. 7

Donors.--In this experiment the donor hens used were some of the same ones that had been used in experiment No. 4. They were all housed in the isolated environment. They were closely related to the recipient chicks. From 2 to 5 embryos 18 days old were used to prepare each liver tissue inoculum.

Recipients.--A single hatch of line 15 chicks was divided into 6 experimental lots. The birds were inoculated when they were four days old. The birds were housed together in a common isolated environment as in the other experiments. This experiment was started on May 16, 1949, and was terminated 310 days later. The protocol for the experiment was as follows:

	No. of birds	Liver tissue inoculum (dam No.)
Lot No. 57	20	I1413B2
" " 58	22	J1318B2
" " 59	29	J1301W2
" " 60	24	J1314W
" " 61	15	J1301M2
" " 62	23	Uninoculated contact controls

Experiment No. 8

Donors.--In experiment No. 8 an attempt was made to determine what effect the inoculation of blood and respiratory washings from the dams, themselves, would have on the recipient chicks. Seven of the hens that had been previously tested by using their offspring were used in this study. The collection and preparation of the blood and respiratory washings have already been described. Two of the donor hens were from the isolated environment and the others were from the exposed environment. All of the hens were clinically normal when they were used as donors.

Recipients.--A single hatch of line 15 chicks was divided into 10 experimental lots. The housing of the birds was similar to that of previous experiments. The experiment was started on May 30, 1949, and was terminated 310 days later. The protocol for the experiment was as follows:

	No. of birds	Inoculum and donor No.	
Lot No. 63	19	Blood	J716R
" " 64	18	"	J404N
" " 65	19	Resp. washings	J7010
" " 66	20	"	J709W
" " 67	17	Blood	J7010
" " 68	18	"	J709W
" " 69	16	"	I1413B2
" " 70	18	"	J430H
" " 71	13	"	I1414H2
" " 72	19	Noninoculated contact controls	

Lot No. 73 was composed of 31 birds from a separate hatch that were used as a non-contact control group. This group of birds was hatched in isolation, but they subsequently had some degree of indirect exposure to lymphomatosis in the brooder house in which they were housed. During

their early life, these birds had more potential exposure to naturally occurring lymphomatosis than did any of the other groups of birds.

RESULTS AND DISCUSSION

The donor dams used in these experiments were all clinically normal at the time they were used as a source of eggs, blood, or respiratory washings. Later, some of them died of lymphomatosis, but most of them showed no clinical or gross evidence of the disease at the time of autopsy. Likewise, most of the sires were clinically normal. The donor embryos and chicks did not show any gross abnormalities when they were sacrificed.

The results of the eight experiments are given in table 7. The incidence of lymphomatosis in the various experimental lots is given in cumulative percentages, broken down into intervals of 30 days. The column marked "terminal" includes those birds that had lymphomatosis at the time they were killed. Experiments 3 and 4 were terminated at 220 days. Thus, the birds that survived to the end of the experiment, and showed lesions of lymphomatosis upon necropsy examination, were placed in the 221-250 age group. This was justified because observations have shown that it takes about 30 days for the tumors to develop from the microscopic stage (grossly invisible) to a large tumor mass (Davis and Doyle, 1947b; Lucas, 1950). Thus, the birds that had grossly evident tumors at 220 days would probably have died before 250 days had elapsed.

Both visceral and neural lymphomatosis occurred in the various experimental and control lots. The incidence of neural lymphomatosis varied considerably, but there was no significant difference in the incidence of neural lymphomatosis between the treated groups and the control groups. Thus, the difference in the incidence between the various lots was mainly due to visceral lymphomatosis. No cases of ocular lymphomatosis were found. In experiment No. 3, three cases of osteopetrosis occurred in combination

with visceral lymphomatosis during the 191 to 220 days age period (two in lot 13 and one in lot 15). Two uncomplicated cases of osteopetrosis were found in lot 23 of experiment No. 4 at the time of termination. One case of osteopetrosis was observed in the 281-310 days age period of lot 37 in experiment No. 5. No cases of osteopetrosis were observed in any of the control groups for these experiments or in any other line 15 control groups for other experiments. However, this line of inbred birds has been found to be quite susceptible to osteopetrosis when inoculated with tumor strain RPL 12 (Burmester and Cottral, 1947d).

Birds of both sexes were used and the sex ratio in the various lots was balanced for the most part. Due to the small number of birds used in each lot it was not possible to make an accurate test of the effect of the two sexes. An analysis of the entire group of birds led to the observation that more males than females died of lymphomatosis during the early age periods. However, more females than males died later in the experimental period. Thus, at the termination of the experiments the females had a slightly higher incidence of lymphomatosis than the males. This difference was not statistically significant.

The first procedure followed in making an analysis of the eight experiments was to determine whether or not the birds in the various lots of each experiment belonged to the same population in respect to the incidence of lymphomatosis. This was accomplished by using the chi-square test as in the two examples that follow:

Experiment No. 1

No. in group	No. dying of lymphomatosis	Probability of dying of lymphomatosis	Products PX
14	9	0.6428	5.7852
14	5	0.3571	1.7855
34	6	0.1765	1.0590
62	SX= 20	SPX=	8.6297

$$X^2 = \frac{SPX - \bar{P}SX}{\bar{P}\bar{Q}} = \frac{8.6297 - 6.4520}{(.3226)(.6774)} = 9.966^{**}$$

(with 2 D.F. at 1% level $X^2 = 9.210$)

Experiment No. 2

No. in group	No. dying of lymphomatosis	Probability of dying of lymphomatosis	Products PX
17	13	0.7647	9.9411
14	12	0.8571	10.2852
17	14	0.8235	11.5290
17	17	1.0000	17.0000
17	3	0.1765	0.5295
82	SX = 59	SPX =	49.2848

$$X^2 = \frac{49.2848 - 42.4505}{(.7195)(.2805)} = 33.866^{**}$$

(with 4 D.F. at 1% level $X^2 = 13.277$)

**Significant at the 1.0 percent level.

The results of the chi-square test for the various experiments were as follows:

Experiment No.	Inclusive lot Nos.	Degrees of freedom	Value of X^2
1	1 - 3	2	9.966 **
2	5 - 9	4	33.866 **
3	12 -21	9	42.801 **
4	22 -33	11	26.051 **
5	35 -45	10	13.409 N.S.
6	46 -56	10	35.560 **
7	57 -62	5	4.915 N.S.
8	63 -72	9	21.578 *

** Significant at the 1.0 percent level
* " " " 5.0 " "
N.S. Not significant

From these tests it can be seen that the various lots of birds within each of these experiments do not represent homogeneous populations with respect to the incidence of lymphomatosis. This suggests that some of the treatments given to the birds within each experiment were responsible for the rise in the incidence of lymphomatosis over what the controls showed. Experiments 5 and 7 were the only ones that failed to show a significant difference between the various experimental lots.

Since the population of birds within each experiment (i.e., except 5 and 7) was not a homogeneous one, the next logical test is to compare each lot within an experiment to its respective control group. The contact control was used for this purpose in all cases except in experiment No. 1, which did not have a contact control. In this experiment the isolated control with the highest incidence of lymphomatosis was used. Therefore, in all of the experiments except No. 1, the experimental lots were tested against the most comparable control group. Thus, the control group that was used was made up of birds from the same hatch, the same families, and

was subjected to the same environment as the inoculated groups. This comparison was made using the chi-square test as in the following example:

Experiment No. 1

(Test of lot 1 with lot 3)

Treatment	Take	Fail	Total
Lot 1 inoculated	9	5	14
" 3 control	6	28	34
	15	33	48

$$X^2 = \frac{((9)(28) - (6)(5) - 24)^2 (48)}{(15)(33)(14)(34)} = 7.986 **$$

** = significant at the 1.0 percent level.

The results of the chi-square test for each experimentare given in table 8. The analysis was made at the 250 day point and at the termination point (310 days).

In experiment No. 1 there was no significant difference between the two control groups or between the control lot 3 and the inoculated lot 2. However, there was a significant difference between inoculated lot 1 and control lot 3. Both lots 1 and 2 received inoculum C, the pooled intestines and trachea of the donor chicks. However, lot 1 received the liver inoculum and lot 2 received the yolk sac inoculum. Thus, the higher incidence of lymphomatosis in lot 1 was attributed to the liver inoculum. For this reason the liver of the donor chicks and embryos was chosen as the tissue to use as a source of inoculum for the experiments that followed.

In experiment No. 2 there was no significant difference between the three control groups. All of the inoculated groups had a high incidence of lymphomatosis, varying from 76.5 to 100.0 percent. Thus, lymphomatosis

was transmitted by using liver tissue from embryos 15 and 18 days old, chicks one day old, and by using a cell-free filtrate of the chick liver tissue.

The controls in experiment No. 3 had a rather high incidence of lymphomatosis, 22.2 percent. This was the highest incidence for all of the control groups at the 250 day point. Only two inoculated groups, lots 12 and 13, had an incidence of lymphomatosis that was significantly high.

The controls in experiment No. 4 had a low incidence of lymphomatosis and the incidence in the inoculated groups was also correspondingly lower than in the third experiment. Three inoculated groups (lots 23, 24, and 25) gave significant differences when compared to the controls.

Experiment No. 5 was designed to determine at what ages the embryos and chicks would have the agent of lymphomatosis in their liver tissue. However, when the experiment was terminated, none of the inoculated lots differed significantly from the controls.

In experiment No. 6, some of the previously used donor hens were retested. A filtrate prepared from the livers of embryos of J709M produced a significant increase in lymphomatosis in the inoculated birds, but the unfiltered preparation failed to give significant results. In the previous test of this hen's embryos the results were also positive (experiment No. 4).

The embryos of hen No. J716R gave positive results this time for both the liver preparation and the amnionic fluid, but the filtrate group did not show a significant increase in the incidence of lymphomatosis. In the previous test (experiment No. 3) the results with the offspring of this bird were not significant. Embryo J404A liver tissue gave significant

results. This hen was not previously tested. J430H, which gave positive results before, was negative in this test. J310R embryos were negative in both tests (experiments Nos. 3 and 6).

In experiment No. 7 none of the inoculated lots had as high an incidence as the control lot. The embryos of these hens had been previously tested and they were negative in both tests. Unfortunately, the hens, whose embryos had given significant results in experiment No. 4, had not produced any fertile eggs by the time this experiment was scheduled, so they were not represented in experiment No. 7.

The purpose of experiment No. 8 was to test the blood and respiratory washings of some of the donor hens that had been previously used in the other experiments. Hen No. J716R, which had given questionable results in experiment 3, and significant results in experiment 6, gave significant results this time. Hen J404N which also gave questionable results in experiment 3, was positive in this test. Hen No. J701O, whose embryos had shown significant results in experiment No. 3, gave only questionable results this time. The respiratory washings of this bird and those of hen No. J709W likewise produced questionable results. The results obtained with the other donor hens tested in this experiment were not significantly different from those of the control group.

A significantly high incidence of lymphomatosis was transmitted to experimental birds by inoculating them with the liver tissue of newly-hatched chicks and embryos 15 and 18 days old. The logical conclusion was that the donor chicks and embryos were infected with the agent of lymphomatosis. Thus, if the donor embryos and chicks were infected, they must have acquired the infecting agent from their parents. Lymphomatosis was transmitted by using the blood of some of these donor hens. The respiratory washings of two of these hens gave only questionable results.

At the time they were used, the donor hens and sires were all clinically normal and most of them remained clinically normal or free of gross evidence of lymphomatosis. (Some of them are still living.) There did not appear to be any definite correlation between the infectivity of the embryo or chick tissue or the dam's blood and the subsequent development of gross lymphomatosis by the dam or sire. Thus, clinically normal dams and sires may be carriers of the agent of lymphomatosis.

Some donor hens were checked several times, either by two tests with their embryos or by using the dam's blood for the re-test. The tests did not agree in all cases, however, one could not expect biological material to give constant results. Furthermore, the infected hens may be transmitting the disease to their offspring only at certain intervals or cycles. Thus, every egg laid by a carrier hen would not be infected. The work with pullorum disease brought out the fact that Salmonella pullorum could not be isolated at every attempt from carrier birds or their offspring (Rettger and Stoneburn, 1909).

In this study the work was greatly handicapped by the presence of naturally occurring lymphomatosis in the birds that were used. Thus, lymphomatosis was transmitted to some of the birds, but in others we apparently were only adding to the infection that they already had; therefore, the interpretation of the results depended upon the inoculated lots developing an incidence of lymphomatosis that was significantly higher than that observed in the control groups.

The concept of egg transmission of disease producing agents was established many years ago, in fact, the work of Pasteur in 1870 proved that pébrine, a protozoan disease of silkworms, caused by Nosema bombycis, was egg-borne. Since then it has been shown that nearly all types of

disease producing agents--protozoa, fungi, bacteria, rickettsia, and viruses--have been proved to be transmitted through eggs or seeds. This type of transmission takes place in certain helminths, arthropods, birds, and plants (Cottral, 1949).

There are a great many difficulties in unequivocally proving that a disease producing agent is transmitted through the egg. The ideal situation would be one in which the etiological agent could be demonstrated in the dam, isolated from the interior of the dam's eggs at different stages of incubation, isolated from the hatched offspring, and, finally, in the ideal situation the offspring would remain carriers of the agent or subsequently develop the disease. Not all of these conditions have been satisfied in the study of lymphomatosis. However, three of the most important steps have been accomplished; namely, demonstration that the agent is present in certain 15 and 18 day old embryos, and in newly-hatched chicks, and in the blood of certain dams.

SUMMARY AND CONCLUSIONS

The history of the etiological concepts of the avian leukosis complex is presented, and the literature dealing with the transmission of avian lymphomatosis is reviewed. The problem of egg transmission of avian lymphomatosis is discussed and new evidence is presented.

The problem of egg transmission of avian lymphomatosis was studied by attempting to transmit the disease to experimental birds by making use of inocula prepared from the tissues of newly-hatched chicks and embryos 15 and 18 days old, and from the blood and respiratory washings of certain selected donor hens. Eight experiments were conducted and the results indicate that certain clinically normal dams and sires may produce offspring infected with the agent of lymphomatosis. Furthermore, in two experimental groups lymphomatosis was produced with a filtrate prepared from embryo and chick liver tissue. In another test, the amnionic fluid from eggs of a certain dam was infective.

The blood from two donor hens produced lymphomatosis when inoculated into susceptible birds. The results obtained with respiratory washings from donor hens were not statistically significant.

The interpretation of the results is rendered difficult due to the presence of the agent of naturally occurring lymphomatosis in the experimental birds that were used. Thus, the disease produced by inoculation was superimposed on the infection already present in the birds. If stock free of the agent of lymphomatosis could be found, studies of this kind would be much easier to carry out and interpret.

Many experiments have been conducted which suggest that lymphomatosis may be egg-borne, such as: epiornithic observations, flock isolation

studies, incubator exposure studies, histological studies and transmission studies. The results of these studies in conjunction with the result of the present study justify the conclusion that avian lymphomatosis is transmitted from carrier parents to the offspring through the egg.

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TABLE 2.--History of parents of donor chicks for Experiment No. 1

Dams and Sires	Age at use	Days lived after use	Age killed or died	Diagnosis	Lymphomatosis in sibs of dams: Number of sibs	Lymphomatosis in sibs of dams: Percent lymphomatosis
Dam: Q1	489	115	604	Normal	15	13.33
Sire: J1	475	107	582	Visceral lymphomatosis		
Dam: M2	483	121	604	Normal	23	8.69
Sire: V2	868	113	981	"		
Dam: N3	483	120	603	"	19	5.26
Sire: W3	469	92	561	"		
Dam: N4	483	149	632	"	22	36.36
Sire: N4	476	149	625	"		
Dam: H5	462	149	611	"	16	18.75
Sire: W5	441	113	554	Testicular neoplasm		
Dam: R6	371	232	603	Normal	23	21.74
Sire: I6	399	58	457	"		
Dam: M7	483	121	604	"	13	15.38
Sire: C7	483	58	541	"		
Dam: N8	476	133	609	"	9	33.33
Sire: C8	434	58	492	"		
Dam: I9	441	141	582	Cannibalism	19	21.05
Sire: 09	469	92	561	Normal		

TABLE 3. History of parents of donor chicks and embryos for Experiment No. 2

Dams and Sires	Age at use	Days lived after use	Age killed or died	Diagnosis	Lymphomatosis in sibs of dams		Lymphomatosis in offspring	
					Number of sibs	Percent lymphomatosis	Number of offspring	Percent lymphomatosis
Dam: H1	448	156	604	Normal	10	00.00	14	28.57
Sire: A1	476	204	680	"				
Dam: 02	434	204	638	"	10	00.00	10	60.00
Sire: A2	476	204	680	"				
Dam: B3	490	156	646	"	12	66.67	19	21.05
Sire: S3	476	204	680	"				
Dam: M4	476	156	632	"	12	50.00	11	36.36
Sire: S4	476	204	680	"				
Dam: B5	378	249	627	"	16	6.25	Discarded	
Sire: J5	448	232	680	"				

TABLE 4.--History of parents of donor embryos
(Experiments 3, 6, and 8)

Dams and Sires	Age at first use	Days lived after use	Age killed or died	Diagnosis	Lymphomatosis in sibs of dams: Number of sibs	Lymphomatosis in sibs of dams: Percent lymphomatosis
Dam: J7010	301	259	560	Cannibalism	16	56.25
Sires: K	315	177	492	Normal		
T	301	177	478	"		
V	301	53	354	Visceral lymph.		
Dam: J430H	309	365+	alive	Normal	17	41.18
Sire: Q	350	152	502	Neural lymph.		
Dam: J404N	350	168	518	No gross lesions	15	46.67
Sires: P	350	177	527	Normal		
V	336	129	465	No gross lesions		
Dam: J716R	282	365+	alive	Normal	11	72.73
Sire: E	329	163	492	"		
Dam: J313N	309	365+	alive	"	10	60.00
Sire: G	321	178	499	"		
Dam: J310R	301	365+	alive	"	14	28.57
Sires: D	346	"	"	"		
N	320	"	"	"		
O	320	"	"	"		
Dam: J256C	350	365+	alive	"	12	25.00
Sire: D	350	177	527	Peritonitis		
Dam: J726Q	294	17	311	Reproductive disorder	11	36.36
Sire: E	329	163	492	Normal		
Dam: J709W	316	365+	alive	"	14	50.00
Sire: E	329	163	492	"		
Dam: J404A	496	365+	alive	"	15	46.67
Sires: J403P	455	72	527	No gross lesions		
V	440	25	465	Normal		
A2	440	73	513	"		
Dam: J306P	420	365+	alive	"	26	15.38
Sires: J316C	440	73	513	"		
J320G	426	73	499	"		
J321L	440	73	513	"		
Dam: J709D2	392	365+	alive	"	14	50.00
Sires: J707H	413	72	485	"		
J715I	413	72	485	"		

TABLE 5.--History of parents of donor embryos
(Experiments 4, 7, and 8)

Dams and Sires	Age at first use	Days lived after use	Age killed or died	Diagnosis
Dam: I1414H$_2$	714	365+	alive	Normal
Sire: R$_2$	696	31	727	Bacterial infection
Dam: I1406U$_2$	714	38	752	Visceral lymphomatosis
Sire: *				
Dam: I1413B$_2$	714	365+	alive	Normal
Sire: *				
Dam: J1318K$_2$	230	144	374	Visceral and neural lymphomatosis
Sires: T	230	144	374	Normal
T$_2$	230	21	251	Neural lymphomatosis
L	230	144	374	Visceral and neural lymphomatosis
V	230	9	239	Neural lymphomatosis
Dam: J1318S$_2$	203	136	339	Visceral lymphomatosis
Sires: **				
Dam: J1318B$_2$	259	365+	alive	Normal
Sires: **				
Dam: J1301W$_2$	231	365+	alive	Normal
Sires: **				
Dam: J1301M$_2$	259	365+	alive	Normal
Sires: **				
Dam: J1314W	231	365+	alive	Normal
Sires: Q$_2$	244	284	528	"
L$_2$	244	389	633	"
D$_2$	216	175	391	No gross lesions
V$_2$	230	215	445	Neural lymphomatosis
Y$_2$	244	320	564	Visceral lymphomatosis

* The sire used was I1414R$_2$.

** The four sires used were J1318T, T$_2$, L, and V.

TABLE 6.--History of parents of donor chicks and embryos for Experiment No. 5

Dams and Sires	Age at use	Days lived after use	Age killed or died	Diagnosis	Lymphomatosis in sibs of dams: Number of sibs	Lymphomatosis in sibs of dams: Percent lymphomatosis
Dam: J310R	384	365+	alive	Normal	14	28.57
Sire: J322G	321	178	499	"		
Dam: J403B	427	365+	alive	"	19	26.31
Sires: P	455	72	527	"		
V	440	25	465	No gross lesions		
A_2	440	73	513	Normal		
Dam: J403R	384	365+	alive	"	19	26.31
Sires: *						
Dam: J405Y	370	337	707	No gross lesions	19	26.31
Sire: J404Q	350	152	502	Neural lymphomatosis		

*The three sires used were J403P, V, and A_2.

TABLE 7.--Cumulative percentage of lymphomatosis at intervals of 30 days

	Treatment	Number of birds	Intervals of 30 days									
			41-70	71-100	101-130	131-160	161-190	191-220	221-250	251-280	281-310	Terminal
Exp. No. 1												
Lot 1	Inoc. A & C	14	14.3	35.7	35.7	35.7	42.9	42.9	50.0	64.3	64.3	64.3
2	" B & C	14		7.1	14.3	14.3	14.3	21.4	21.4	28.6	28.6	35.7
3	Isol. controls	34				2.9	5.9	5.9	8.8	8.8	14.7	17.6
4	" "	28							3.6	3.6	3.6	3.6
Exp. No. 2												
Lot 5	chick liv.	17				29.4	58.8	64.7	70.6	70.6	70.6	76.5
6	Filt. " "	14			14.3	28.6	42.9	50.0	78.6	78.6	78.6	85.7
7	15-day emb. "	17		17.6	29.4	47.1	64.7	70.6	70.6	70.6	76.5	82.4
8	18-day " "	17				17.6	58.8	76.5	82.4	82.4	88.2	100.0
9	Contact Controls	17				5.9	11.8	11.8	11.8	11.8	17.6	17.6
10	Isol. "	20				5.0	5.0	5.0	5.0	10.0	10.0	15.0
11	" "	25		4.0	4.0	4.0	8.0	8.0	12.0	12.0	16.0	16.0
Exp. No. 3												
Lot 12	Emb.liv. J7010	19	10.5	36.8	42.1	63.2	78.9	78.9	84.2			
13	" " J430H	16		18.7	25.0	31.2	43.7	75.0	81.3			
14	" " J404N	17				17.6	41.2	52.9	58.8			
15	" " J716R	17		11.8	11.8	11.8	17.6	41.2	47.0			
16	" " J313N	17			5.9	29.4	35.3	41.2	41.2			
17	" " J310R	17		5.9	11.8	11.8	17.6	35.3	35.3			
18	" " J256C	18				5.6	11.1	16.7	22.2			
19	Filt." " J310R	18			5.6	5.6	11.1	11.1	11.1			
20	Amn.fluid J313N	16				6.3	12.5	18.7	18.7			
21	Contact controls	18				5.6	5.6	16.7	22.2			

(continued)

TABLE 7. (continued)--Cumulative percentage of lymphomatosis at intervals of 30 days

	Treatment	Number of birds	Intervals of 30 days									
			41-70	71-100	101-130	131-160	161-190	191-220	221-250	251-280	281-310	Terminal
Exp. No. 4												
Lot 22	Emb.liv. J726Q	18					11.1	22.2	33.3			
23	" " J709W	18	5.6	16.7	22.2	27.8	33.3	38.9	50.0			
24	" " $I1414H_2$	18		11.1	27.2	27.2	33.3	44.4	55.5			
25	" " $I1406U_2$	18				5.6	5.6	16.7	38.9			
26	" " $J1318K_2$	19		5.3	15.8	15.8	21.1	31.6	31.6			
27	" " $I1413B_2$	18			5.6	5.6	5.6	16.7	22.2			
28	" " $J1318S_2$	18			5.6	11.1	16.7	16.7	16.7			
29	" " $J1301W_2$	19			5.3	5.3	10.5	15.8	15.8			
30	" " $J1301M_2$	19						5.3	15.8			
31	" " J1314W	19			5.3	5.3	5.3	10.5	15.8			
32	" " $J1318B_2$	17						5.9	11.8			
33	Contact controls	19							5.3			
34	Isol. "	34	2.9	2.9	2.9	2.9	5.9	5.9	5.9			
Exp. No. 5												
Lot 35	Emb.liv.(1 day)	22							9.1	13.6	27.3	27.3
36	" " (8 days)	23				4.3	4.3	4.3	8.7	8.7	13.0	17.4
37	" "(15 days)	25						4.0	4.0	4.0	12.0	20.0
38	Chick " (1 day)	21							0.0	4.8	14.3	14.3
39	" " (8 days)	26					3.8	3.8	3.8	3.8	7.7	15.4
40	" "(15 days)	24			4.2	4.2	8.3	12.5	12.5	12.5	16.7	16.7
41	" "(29 days)	25					8.0	16.0	16.0	20.0	24.0	28.0
42	" "(43 days)	22				4.5	13.6	22.7	27.3	36.4	40.9	40.9
43	" " filt.	20						5.0	5.0	5.0	5.0	5.0
44	" " " heated	24							0.0	0.0	12.5	12.5
45	Contact controls	23				4.3	4.3	4.3	8.7	8.7	13.0	13.0

(continued)

TABLE 7. (continued)--Cumulative percentage of lymphomatosis at intervals of 30 days

	Treatment	Number of birds	Intervals of 30 days									
			41-70	71-100	101-130	131-160	161-190	191-220	221-250	251-280	281-310	Terminal
Exp. No. 6												
Lot 46	Filt.emb.liv.J709W	19		5.3	15.8	26.3	31.6	47.4	52.6	63.2	63.2	63.2
47	Emb.liv.J709W	18					5.6	22.2	33.3	38.9	44.4	55.5
48	" " J404A	18				11.1	27.8	44.4	44.4	61.1	61.1	72.2
49	" " J716R	18			5.6	11.1	22.2	27.8	38.9	50.0	61.1	66.7
50	Amn. fluid J716R	18				11.1	11.1	27.8	33.3	44.4	44.4	61.1
51	Filt.emb.liv.J716R	18				5.6	11.1	16.7	22.2	27.8	44.4	44.4
52	Emb.liv.J306P	20				5.0	5.0	5.0	10.0	15.0	20.0	30.0
53	" " J430H	20						5.0	15.0	15.0	20.0	25.0
54	" " J709D$_2$	18					5.6	5.6	5.6	11.1	11.1	11.1
55	" " J310R	19							0.0	5.3	5.3	21.1
56	Contact controls	18						5.6	5.6	11.1	16.7	22.2
Exp. No. 7												
Lot 57	Emb.liv. I1413B$_2$	20							0.0	0.0	0.0	20.0
58	" " J1318B$_2$	22						4.5	9.1	9.1	9.1	18.2
59	" " J1301W$_2$	29							0.0	6.9	10.3	10.3
60	" " J1314W	24						4.2	4.2	8.3	8.3	8.3
61	" " J1301M$_2$	15							0.0	0.0	0.0	6.7
62	Contact controls	23			4.3	4.3	8.7	8.7	13.0	13.0	21.7	26.1
Exp. No. 8												
Lot 63	Blood J716R	19			10.5	21.1	26.3	42.1	47.4	68.4	68.4	68.4
64	" J404N	18			5.6	11.1	11.1	33.3	44.4	55.5	61.1	61.1
65	Resp.wash. J7010	19			5.3	5.3	5.3	15.8	26.3	36.8	36.8	52.6
66	" " J709W	20					10.0	20.0	25.0	30.0	45.0	50.0
67	Blood J7010	17					5.9	11.8	23.5	35.3	41.2	41.2
68	" J709W	18				11.1	22.2	22.2	27.8	33.3	33.3	33.3
69	" I1413B$_2$	16			6.3	6.3	12.5	12.5	12.5	18.7	18.7	31.2
70	" J430H	18	5.6	5.6	5.6	5.6	5.6	11.1	16.7	16.7	27.8	27.8
71	" I1414H$_2$	13						7.7	7.7	7.7	7.7	7.7
72	Contact controls	19						10.5	10.5	10.5	21.1	21.1
Lot 73	Exposed controls	31	3.2	3.2	6.5	6.5	6.5	6.5	16.1	19.4	19.4	19.4

TABLE 8.--Analysis of egg transmission experiments at 250 days and at termination 310 days
(Chi-square test for the significance of the incidence of lymphomatosis in the various experimental lots)

	Treatment	Number of birds	Analysis at 250 days for lymphomatosis: Take	Fail	Percent lymph.	X^2 value	Final analysis at termination 310 days for lymphomatosis: Take	Fail	Percent lymph.	X^2 value
Exp. No. 1										
Lot 1	Inoc. A & C	14	7	7	50.0	7.850**	9	5	64.3	7.986**
2	" B & C	14	3	11	21.4	N.S.	5	9	35.7	N.S.
3	Isol. controls	34	3	31	8.8	-	6	28	17.6	-
4	" "	28	1	27	3.6	-	1	27	3.6	-
Exp. No. 2										
Lot 5	chick liv.	17	12	5	70.6	9.836**	13	4	76.5	9.562**
6	Filt. " "	14	11	3	78.6	11.622**	12	2	85.7	11.647**
7	15-day emb. "	17	12	5	70.6	9.836**	14	3	82.4	11.764**
8	18-day " "	17	14	3	82.4	14.284**	17	0	100.0	20.521**
9	Contact controls	17	2	15	11.8	-	3	14	17.6	-
10	Isol. "	20	1	19	5.0	-	3	17	15.0	-
11	" "	25	3	22	12.0	-	4	21	16.0	-
Exp. No. 3										
Lot 12	emb. liv. J7010	19	16	3	84.2	12.037**				
13	" " J430H	16	13	3	81.3	9.562**				
14	" " J404N	17	10	7	58.8	N.S.				
15	" " J716R	17	8	9	47.0	N.S.				
16	" " J313N	17	7	10	41.2	N.S.				
17	" " J310R	17	6	11	35.3	N.S.				
18	" " J256C	18	4	14	22.2	N.S.				
19	Filt. " " J310R	18	2	16	11.1	N.S.				
20	Amn. fluid J313N	16	3	13	18.7	N.S.				
21	Contact controls	18	4	14	22.2	-				

(continued)

TABLE 8. (continued)--Analysis of egg transmission experiments at 250 days and at termination 310 days (Chi-square test for the significance of the incidence of lymphomatosis in the various experimental lots)

	Treatment	Number of birds	Analysis at 250 days for lymphomatosis				Final analysis at termination 310 days for lymphomatosis			
			Take	Fail	Percent lymph.	X^2 value	Take	Fail	Percent lymph.	X^2 value
Exp. No. 4										
Lot 22	emb. liv. J726Q	18	6	12	33.3	N.S.				
23	" " J709W	18	9	9	50.0	7.248**				
24	" " $I1414H_2$	18	10	8	55.5	8.913**				
25	" " $I1406U_2$	18	7	11	38.9	4.342*				
26	" " $J1318K_2$	19	6	13	31.6	N.S.				
27	" " $I1413B_2$	18	4	14	22.2	N.S.				
28	" " $J1318S_2$	18	3	15	16.7	N.S.				
29	" " $J1301W_2$	19	3	16	15.8	N.S.				
30	" " $J1301M_2$	19	3	16	15.8	N.S.				
31	" " J1314W	19	3	16	15.8	N.S.				
32	" " $J1318B_2$	17	2	15	11.8	N.S.				
33	Contact controls	19	1	18	5.3	-				
34	Isol. "	34	2	32	5.9	-				
Exp. No. 5										
Lot 35	emb. liv. (1 day)	22	2	20	9.1	N.S.	6	16	27.3	N.S.
36	" " (8 days)	23	2	21	8.7	N.S.	4	19	17.4	N.S.
37	" " (15 days)	25	1	24	4.0	N.S.	5	20	20.0	N.S.
38	chick " (1 day)	21	0	21	0.0	N.S.	3	18	14.3	N.S.
39	" " (8 days)	26	1	25	3.8	N.S.	4	22	15.4	N.S.
40	" " (15 days)	24	3	21	12.5	N.S.	4	20	16.7	N.S.
41	" " (29 days)	25	4	21	16.0	N.S.	7	18	28.0	N.S.
42	" " (43 days)	22	6	16	27.3	N.S.	9	13	40.9	N.S.
43	" " Filt.	20	1	19	5.0	N.S.	1	19	5.0	N.S.
44	" " " heated	24	0	24	0.0	N.S.	3	21	12.5	N.S.
45	Contact controls	23	2	21	8.7	-	3	20	13.0	-

(continued)

TABLE 8. (continued)--Analysis of egg transmission experiments at 250 days and at termination 310 days (Chi-square test for the significance of the incidence of lymphomatosis in the various experimental lots)

	Treatment	Number of birds	Analysis at 250 days for lymphomatosis				Final analysis at termination 310 days for lymphomatosis			
			Take	Fail	Percent lymph.	X^2 value	Take	Fail	Percent lymph.	X^2 value
Exp. No. 6										
Lot 46	Filt.emb.liv. J709W	19	10	9	52.6	7.681**	12	7	63.2	4.753*
47	Emb.liv. J709W	18	6	12	33.3	N.S.	10	8	55.5	N.S.
48	" " J404A	18	8	10	44.4	5.333*	13	5	72.2	7.133**
49	" " J716R	18	7	11	38.9	4.018*	12	6	66.7	5.512*
50	amn. fluid J716R	18	6	12	33.3	N.S.	11	7	61.1	4.114*
51	Filt.emb.liv. J716R	18	4	14	22.2	N.S.	8	10	44.4	N.S.
52	" " J306P	20	2	18	10.0	N.S.	6	14	30.0	N.S.
53	" " J430H	20	3	17	15.0	N.S.	5	15	25.0	N.S.
54	" " J709D2	18	1	17	5.6	N.S.	2	16	11.1	N.S.
55	" " J310R	19	0	19	0.0	N.S.	4	15	21.1	N.S.
56	Contact controls	18	1	17	5.6	-	4	14	22.2	-
Exp. No. 7										
Lot 57	emb.liv.I1413B2	20	0	20	0.0	N.S.	4	16	20.0	N.S.
58	" " J1318B2	22	2	20	9.1	N.S.	4	18	18.2	N.S.
59	" " J1301W2	29	0	29	0.0	N.S.	3	26	10.3	N.S.
60	" " J1314W	24	1	23	4.2	N.S.	2	22	8.3	N.S.
61	" " J1301M2	15	0	15	0.0	N.S.	1	14	6.7	N.S.
62	Contact controls	23	3	20	13.0	-	6	17	26.1	-
Exp. No. 8										
Lot 63	Blood J716R	19	9	10	47.4	4.606*	13	6	68.4	6.812**
64	" J404N	18	8	10	44.4	3.809*	11	7	61.1	4.604*
65	Resp.wash. J7010	19	5	14	26.3	N.S.	10	9	52.6	N.S.
66	" " J709W	20	5	15	25.0	N.S.	10	10	50.0	N.S.

(continued)

TABLE 8. (continued)--Analysis of egg transmission experiments at 250 days and at termination 310 days (Chi-square test for the significance of the incidence of lymphomatosis in the various experimental lots)

	Treatment	Number of birds	Analysis at 250 days for lymphomatosis				Final analysis at termination 310 days for lymphomatosis			
			Take	Fail	Percent lymph.	X^2 value	Take	Fail	Percent lymph.	X^2 value
Exp. No. 8										
Lot 67	Blood J7010	17	4	13	23.5	N.S.	7	10	41.2	N.S.
68	" J709W	18	5	13	27.8	N.S.	6	12	33.3	N.S.
69	" I1413B_2	16	2	14	12.5	N.S.	5	11	31.2	N.S.
70	" J430H	18	3	15	16.7	N.S.	5	13	27.8	N.S.
71	" I1414H_2	13	1	12	7.7	N.S.	1	12	7.7	N.S.
72	Contact controls	19	2	17	10.5	-	4	15	21.1	-
Lot 73	Exposed controls	31	5	26	16.1	-	6	25	19.4	-

** Significant at 1.0 percent level

* Significant at 5.0 percent level

N.S. Not significant