Role of egg "cytoplasm" in transmission of resistance to an avian leukosis tumor

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ROLE OF EGG "CYTOPLASM" IN TRANSMISSION OF RESISTANCE TO AN AVIAN LEUKOSIS TUMOR

by

Eugene Chen Seu

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major Subject: Genetics

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INTRODUCTION

The resistance of an animal to a particular disease may be dependent upon several types of factors. It may depend upon the presence of genes for resistance, inherited in the Mendelian fashion. Factors inherited through the cytoplasm of the egg may also affect resistance. In any disease an interaction exists between the pathogen and the host so that the degree of resistance manifested is also dependent upon the virulence of the pathogen. Similarly in cases where the pathogen can be transmitted through the egg it may affect the resistance of the infected progeny. A mild infection could stimulate antibody production in the young animal, or, on the other hand, it may render the animal more susceptible to further attacks by the pathogen. Considering these possibilities it is not difficult to imagine that the cytoplasm of the egg could have an important role in the transmission of disease resistance. The egg of the fowl, Gallus domesticus, with its large amount of yolk and albumen appears to be especially suitable for the transmission of extranuclear factors.

The avian leukemia complex of disease was classified in the following manner by Cottral (1952). The classification is based on clinical symptoms.
The Avian Leukosis Complex

<table>
<thead>
<tr>
<th>Scientific names</th>
<th>Common names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral lymphomatosis or Lymphocytoma</td>
<td>Big liver disease</td>
</tr>
<tr>
<td>Neural lymphomatosis or Marek's paralysis</td>
<td>Fowl paralysis or range paralysis</td>
</tr>
<tr>
<td>Ocular lymphomatosis or Iritis</td>
<td>Grey eye disease</td>
</tr>
<tr>
<td>Osteopetrosis</td>
<td>Big bone disease or marble bone</td>
</tr>
<tr>
<td>Erythroblastosis or Erythroleukosis</td>
<td>Fowl leukemia</td>
</tr>
<tr>
<td>Granuloblastosis or Myeloblastosis</td>
<td>Fowl leukemia</td>
</tr>
<tr>
<td>Myelocytomatosis or Leucochloroma</td>
<td>White tumours</td>
</tr>
</tbody>
</table>

Leukemia is generally not associated with the disease except in granuloblastosis and erythroblastosis, sometimes referred to together as transmissible fowl leukosis (Olson 1940).

The pathological feature of the aleukemic forms of avian leukosis appears to be an invasion of mononuclear cells into the involved tissues (Lee et al. 1937, Pappenheimer et al. 1926) and a proliferation of these cells in situ (Pappenheimer et al. 1926). In the leukemic forms the invasion and proliferation of the cells involves the peripheral blood. The particular form of the disease depends upon the type of invading cell. In the visceral form the undifferentiated lymphocyte appears to be the type cell (Feldman 1932, Lee...
et al. 1937). In the neural form, small cells, morphologically identical with lymphocytes, and larger cells with vesicular nuclei and frequent mitoses are found (Pappenheimer et al. 1926). Campbell (1956) considers these cells as mature lymphocytes, monocytes and plasma cells. In the case of granuloblastic and erythroblastic leukemia undifferentiated granular leukocytes and precursors of erythrocytes appear to be the type cells (Lee et al. 1937, Olson 1940). Pappenheimer et al. (1926) have shown that there is an unbroken series of transitions between the slightest lesions and those which approach true neoplasms in the intensity of cell proliferations for neural lymphomatosis. Discussions on the pathology of avian leukemia may be found in a number of books on animal pathology, Feldman (1932), McGowan (1928), Runnells (1954), and others. A chapter on the leukemia complex is included in Biester and Schwarte (1952).

The economic importance of the disease may be appreciated by reading the following statement of the director in the 13th Report of the Regional Poultry Research Laboratory (1955). "An estimated mortality from lymphomatosis of more than 53,000,000 adult chickens with a value of at least $73,000,000 occurred in the United States in 1953. This tremendous loss does not take into account the impairment of growth of infected young chickens, a reduction in egg production of laying stock caused by an onset of the disease, and inability
of poultry producers to keep their housing facilities filled to capacity." In the United Kingdom losses from all diseases of poultry in 1952 amounted to £20 million and leukosis was the most serious (Gordon 1953). A loss of £4 million per annum was estimated by Gordon (1954). Blaxland (1956) estimated the annual loss at £7 to £8 million.

This thesis deals primarily with a study of the mode of inheritance of resistance to visceral lymphomatosis. Special interest has been placed on the role of factors that might be inherited through the egg cytoplasm or egg albumen. Related studies on the histology of the tumor, the relationships of the visceral with the neural and ocular forms of the disease, and studies of the growth of the cells in cultures, were carried out to gain a broader understanding of the disease.
REVIEW OF LITERATURE

Historical

A paper by Roloff (1868), referred to by Chubb and Gordon (1957), appears to be one of the earliest papers dealing with the avian leukosis complex. Roloff described a case of "lymphosarcomatosis." Fowl leukemia was first described by Caparini in 1896. In 1905 the condition now known as visceral lymphomatosis was described by Butterfield. The earliest published account of fowl paralysis was by Marek (1907). In 1907 a case of lymphatic leukemia was described by Warthin.

Ellerman and Bang (1908) demonstrated that the etiological agent could be a virus. They showed that cases of typical leukemia can result from the inoculation of cell free filtrate from an organ emulsion.

Etiological relationships

Whether the complex of diseases can be caused by a single virus or several appears to be an unsettled question. Olson (1936) reported that transmissible leukemia, lymphocytoma, and neurolymphomatosis were not due to the same agent. Burmester and Gentry (1954a) were of the opinion that the agent of visceral lymphomatosis is different from that causing osteopetrosis and that causing neural lymphomatosis.
Waters (1954) was of the opinion that the visceral, neural, ocular and osteopetrotic forms of lymphomatosis may be caused by four distinct and separate agents.

Warthin (1907) regarded the leukemic and aleukemic conditions as being genetically related if not actually one and the same process in different stages.

Pappenheimer et al. (1926) noted that neurolymphomatosis was frequently associated with blindness and in two major outbreaks in large flocks the paralyzed and other fowl in the same flock showed gross tumors in an abnormally high proportion of cases. One case described which they felt was particularly significant, was that of an ovarian tumor which was found to have penetrated the spinal canal and invaded the cord. In the region where the growth had invaded the cord the appearance was that of a malignant neoplasm but as one passed away from this site and in the remainder of the cord, brain, and peripheral nerves the lesions were identical with those found in birds with neurolymphomatosis. Burmester (1952) reported that lymphoid tumor tissue can be propagated in the anterior chamber of the chicken eye. Ball (1944) reported that yellow carotinoid pigments in the ration and the vascular system of the iris can affect the color of the iris.

Pappenheimer et al. (1929) reported that neural lymph-
omatosis was transmissible. Lee et al. (1937) found that the various types of leukosis considered as expressions of the disease, neural and ocular lymphomatosis, erythroleukosis, myeloid leukemia, hemocytoblastosis and lymphocytoma resulted with injection of inoculum made from any one type. They concluded that the same etiological agent was responsible. Burmester et al. (1957) found a reduction in incidence of osteopetrosis as well as a reduction in visceral lymphomatosis when vaccines against visceral lymphomatosis were employed. Davis et al. (1947) reported that, among Barred and White Plymouth Rocks, Rhode Island Reds and White Leghorns, the Barred Plymouth Rocks had the highest incidence of visceral lymphomatosis but were most resistant to the neural and ocular forms. Greenwood (1951) observed that resistance to sarcomata was accompanied by an increase in death rate from neurolymphomatosis.

Studies of the disease agent

Mommaerts et al. (1954) and Sharp et al. (1954) found that certain particles in the plasma of chicks with erythromyeloblastic leukosis were associated with an enzyme dephosphorylating adenosine triphosphate. Eckert et al. (1954a) also observed a similar relationship and also a correlation between these particles and infectivity. They regarded the particles as the virus of avian erythromyeloblastic leukosis.
In chickens with visceral lymphomatosis, adenosine triphosphatase concentration is also high (13th Report, Regional Poultry Research Laboratory, E. Lansing, Michigan 1955).

Olson (1940) presents some additional properties of the leukosis agent. It is relatively resistant to drying, freezing, glycerin solution and to large doses of roentgen rays. It is thermolabile and susceptible to inactivation by oxidation. Poirier (1952) reported that the agent of visceral lymphomatosis may change in virulence.

Jarmai (1933) found that only healthy chicks were hatched from eggs which received injections of the agent of transmissible leukosis up to and including the eighth day of incubation, whereas chicks with leukosis were hatched from the eggs infected with the agent after the tenth day of incubation. The myeloid tissue of the bone marrow is developed on the tenth day of embryonic life. This was considered as evidence that the agent of transmissible leukosis was not capable of development independent from bone marrow tissue.

Furth and Breedis (1937) reported that their strain 1 fowl leukosis agent (pure erythroblastic and granuloblastic leukosis) would survive in a culture which contained mononuclear cells presumed to be myeloblasts, whereas the agent perished in a culture composed of sarcoma cells only. Ponten and Thorell (1957) showed histologically that the leukemia process started
as foci in some of the bone marrow sinusoids on the third day after viral inoculation. On the seventh day the bone marrow was diffusely involved and leukemic infiltration of the liver and spleen had started.

The Rous sarcoma (Rous 1910), another neoplasm of the fowl studied extensively, is also caused by a virus with a dependence on specific cells (Carrel 1926, Halberstaedter et al. 1941).

**Egg transmission**

The presence of the virus causing visceral lymphomatosis in the secretions of chickens with the disease was reported by Burmester and Gentry (1954b). The presence of the agent in the tissues of embryos and chicks from normal appearing dams was reported by Cottral et al. (1954). Burmester et al. (1955) reported that normal appearing hens may shed the virus of visceral lymphomatosis into their eggs. Shedding of virus was directly related to incidence of visceral lymphomatosis among sibs but no apparent relationship was found between shedders and the incidence of the disease among their progeny. The virus strain, RPL 12 was carried through five blind passages in chicken embryos by Gentry and Burmester (1955b). Chicks were obtained from eggs inoculated with transmissible leukosis by Jarmai (1933) and Van den Berghe and d'Ursel (1939). However some of these chicks developed the disease.
Ellerman (1921), Engelbreth-Holm (1931), Jarmai et al. (1932), Rothe Meyer et al. (1935), and Oberling and Guerin (1938) reported that no active immunity resulted after inoculation with leukotic material.

Furth (1932) reported that the plasma from spontaneously recovered chickens had a neutralizing effect against the cell-free agent of transmissible leukosis. Rothe Meyer and Engelbreth-Holm (1933) reported a similar effect on the cell free agent but none on leukemic whole blood. Rothe Meyer et al. (1935) reported that the plasma of a chicken with chronic erythroblastic anemia was also capable of neutralizing the cell free agent.

Burmester (1947) reported that antiserum from hyperimmunized birds had a cytotoxic effect against tumor strain RPL 12. Gentry and Burmester (1955a) reported that a series of 28 injections of the virus of visceral lymphomatosis into eight dams, selected for susceptibility, did not cause a higher incidence of the disease among chicks obtained after the injection series than among those hatched prior to inoculation. Burmester (1955a) reported that chicks from fourteen similar hens had a lower incidence of lymphomatosis after hyperimmunization. It was concluded that antibodies were transmitted to the chick via the egg. Similar results were obtained in another comparable experiment reported by
Burmester (1955b). Neutralizing antibodies were found in the serum after immunization. Several vaccine preparations were reported by Burmester et al. (1957) to be effective in stimulating an immunological response against visceral lymphomatosis in pullets 6 to 8 months of age. Evidence of passive immunity in chicks from such dams was found. Beard et al. (1957) reported that concentrates of the virus of avian erythroblastosis, inactivated with formaldehyde, had an immunizing effect. Uhl et al. (1956) found that the serum of ducks which had received multiple injections of chicken blood had a neutralizing action on the agent of leukosis.

Jarmai et al. (1932) found that two chicks killed at hatching and twelve that lived to a year or more of age, obtained from a hen with transmissible fowl leukosis, had no evidence of leukosis. They also found indications that the agent soon lost its potency in the chicken egg at room temperature. Lee et al. (1937) obtained eighteen chicks from hens with leukosis, four of which developed various manifestations of the disease over a period of eighteen months. They found that most fertile eggs from paralyzed birds died during the period of incubation. Cole (1949) reported that there was no difference in neoplastic mortality between daughters of dams that died of leukosis during the breeding season and daughters of pen mate controls. A similar situation was demonstrated for sires. Embryonic mortality was not adversely
affected.

Contact transmission

Lee et al. (1937) reported that the disease can be transmitted by contact with contaminated litter and surroundings. A similar conclusion was reached by Johnston and Wilson (1937). Reduction in mortality among pullets reared in isolation in 1936 was clearly evident. Mortality from fowl paralysis and total mortality are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Brooded in parent colony</th>
<th>Isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mortality</td>
<td>36.7%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Fowl paralysis</td>
<td>14.0%</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

Cole and Butt (1951) reported that mortality of pullets raised in isolation in 1948 was 9.4 percent in contrast to 54.0 percent for females raised under the usual procedures. Corresponding losses in 1949 were 5.8 percent and 63.8 percent, respectively. Burmester and Gentry (1954a) tested four strains of visceral lymphomatosis agents and found that only strain RPL 12 gave significant contact transmission and even with this strain it did not occur in all inoculations. High rates of contact transmission were reduced to insignificant levels by confining inoculated lots to separate cubicles having solid walls but an open top. Cubicle isolation to 90 days of age resulted in no apparent contact transmission.
Heisdorf et al. (1947) reported that two strains of White Leghorns, differing in susceptibility to spontaneously occurring lymphomatosis, also differed correspondingly in incidence of induced lymphomatosis following introduction of lymphomatous material into the crop, eye and left nostril of chicks 2 days of age. Difference between strains was not evident after subcutaneous inoculation.

**Genetic differences in resistance in the fowl**

Strains and breeds of chickens genetically different in resistance to leukosis have been frequently mentioned in the literature, Davis et al. (1947), Greenwood and Carr (1951), loc. cit. Pappenheimer et al. (1926) noted a high incidence of neural lymphomatosis in a particular strain of Silver Spangled Hamburgs. Lee et al. (1937) mentioned the use of resistant and susceptible strains of Leghorns in their experiments. Dudley et al. (1941) noted a difference in the incidence of the visceral and neural forms in Barred Rocks and in White Leghorns. Poirier (1952) reported that breeds differed in resistance. Eckert et al. (1954b) mentioned the discovery of a strain of White Leghorns which were high in susceptibility and homogeneity to the virus of erythromyeloblastic leukosis. Eckert et al. (1954c) reported chicks of a New Hampshire flock and White Leghorn chicks from two different commercial flocks were relatively resistant to the virus of avian ery-
thromyeloblastic leukemia. Among six inbred lines from the Regional Poultry Research Laboratory five were highly susceptible to the same virus and one was susceptible to neurolymphomatosis. Waters (1954) reported that these six inbred lines at the Regional laboratory differed in resistance to visceral and neural lymphomatosis.

The successful development of strains of chickens resistant to lymphomatosis have been reported by Hutt and Cole (1947, 1948, 1953), Poirier (1952), Moultrie et al. (1953) and Coles (1954). Poirier (1952) and Coles (1954) also reported the development of susceptible lines. King et al. (1952) reported that two resistant strains developed in New York and one from Alabama retained their resistance when exchanged and maintained at stations in those states.

Fenstermacher (1934) reported that birds not showing the disease may produce progeny that develop lymphocytoma. Two cases in particular were described. Bird 1943 had 17 chicks in 1931 that survived for six months or more. Eight of these developed the disease. Five progeny obtained in 1932 did not. The dam did not have the disease at four years of age. Bird 1897 produced 24 chicks in 1931 of which 10 developed the disease. The dam herself was free. Coles (1954) reported that although improvement in resistance to the disease seems established, families occur in which 70% of the progeny may
succumb to the disease by December of the year in which they were hatched.

A number of crosses of inbred lines of chickens selected for resistance and susceptibility to lymphomatosis were made at the Regional Poultry Research Laboratory, East Lansing, Michigan (13th Report, 1955). Mortality of progeny over a 500-day period from visceral and neural lymphomatosis were recorded. Crosses made extended over the period 1945 to 1952. Lines 6, 10, and 15 selected for resistance and lines 7, 9, and 15 selected for susceptibility were used. In general the data obtained did not follow any predictable course. In examining the mortality histograms it seemed that the cross 9 x 10, made in 1945 was intermediate between the parental lines. The cross 7 x 15 made in 1947 appeared to show a higher mortality than line 15. Both parental lines were susceptible. In 1951, reciprocal crosses were made between lines 6 and 15, 6 and 10, and 9 and 15. In the crosses between 6 and 15 the hybrids had a mortality near that of the susceptible parent, about 30%. In the crosses of lines 6 and 10 both resistant, and 9 and 15 both susceptible, dominance relationship in the hybrids was not clear. Mortality appeared to be lower with no cases of neural lymphomatosis observed in 1951. In 1952 reciprocal crosses, backcrosses, and four-way crosses were made. Mortality was also lower in comparison with observations for 1945 and 1947, though some cases of neural lymphomatosis were observed. The results were not
consistent with respect to dominance. In the backcrosses there appeared to be some tendency toward a higher mortality in backcrosses to susceptible line. However, the results were not clear-cut, as the authors have stated.

In surveying the literature one becomes aware of the numerous aspects of the leukosis problem that have been studied. Among these are the histological and pathological aspects, the nature of the causative agent, the mode of transmission of the disease, the etiological relationships between the various forms of the disease, i.e., whether a single virus can cause the different forms of the disease complex or different viruses are involved, and the genetic mode of resistance in the fowl. With regard to the histology and pathology of the disease, extensive studies have been made. However information is still wanting on the nature of the cells. The agent for the disease appears to be filterable and of a virus-like nature. The mode of transmission of the disease is a debated question with one school of thought favoring the idea of contact transmission and another school favoring transmission through the egg. The relationship of one form of lymphomatosis to another also appears to be an unsettled question. It is also evident that more information on the inheritance of resistance to the disease is desired.
MATERIALS AND METHODS

The genetic aspects of the problem, the mode of inheritance of resistance against the disease agent, is the topic of primary concern in this thesis. Other studies of a preliminary nature, on the histology, the effect of colchicine, cell cultures and on etiological relationships between the ocular neural and visceral forms of the disease were conducted. These studies have been included in the thesis because of their bearing on the leukosis problem as a whole.

Tumor material

Two substrains, A and C, of visceral lymphomatosis, RPL strain 12, obtained from the Regional Poultry Research Laboratory, East Lansing, Michigan and maintained at this laboratory through serial passages in chicks were used in these studies. In addition nervous tissue from birds with symptoms of ocular lymphomatosis was used for the studies on etiological relationships between the neural and visceral forms of the disease.

Chicks

Chicks employed for the testing of nervous tissue in the etiological studies were males of commercial stocks obtained from hatcheries that sell sexed chicks. In the genetic
All chicks tested were hatched at this laboratory.

**Inbred strains utilized in the genetic studies**

Lines 1, 2, 3 and 5 are strains of White Leghorn chickens inbred for a number of years at the genetics laboratory at Iowa State College.

Lines 9, 11, 15 and 16 are recently established lines of White Leghorns selected for leukosis tumor resistance or susceptibility at this laboratory. Lines 9 and 11 are tested birds selected from resistant and susceptible families, respectively. Lines 15 and 16 are untested birds related to those of lines 9 and 11.

**Inoculation procedures**

A 1 to 640 dilution of tumor cells in 0.85% saline was used in testing chicks for resistance. Each bird tested for resistance was inoculated in the pectoral muscle with 0.25 cc. of the inoculum. A tuberculin syringe was used for this purpose. In preparing the inoculum, tumorous breast muscle from serial passage chicks were rapidly dissected from the birds. The tumor was homogenized in a Waring blender at a concentration of one part tumor in five parts homogenate. Saline was then added to give the proper dilution.

**Histological procedures**

The technique found to be most satisfactory for staining of tumor smears and sections was a differential staining...
technique with a mixture of safranin and fast green (Bryan 1955). Acidic components of the cells, such as the nucleic acids of the nucleus and of the cytoplasm, were stained red by safranin while the basic components were stained green by the fast green. The Feulgen technique (Stowell 1945) was employed particularly for the staining of nuclei. Sudan black B was also used. Supravital staining with neutral red and Janus green B was tried.

Carnoy's fluid (3 parts absolute alcohol to 1 part glacial acetic acid) was used as a fixative.

Colchicine treatment

Tumor cells were treated with colchicine to obtain information on the rate of mitosis. Living tumor cells were suspended in chicken serum containing the following dilutions of colchicine: 1:2000; 1:4000; 1:8000; 1:16,000; and 1:32,000. The 1:2000 dilution was prepared by taking 1 cc. of a stock solution, containing one part colchicine to 500 parts distilled water, and diluting this with three cc. of serum. The subsequent dilutions were made by mixing equal volumes of the resulting dilution with serum.

Cell culturing procedures

Tumor cells and tissue were cultured in depression
slides and on the chorioallantoic membrane of chick embryos. For the culturing of cells in depression slides the best results were obtained by using a modified hanging drop technique. In preparing the cultures a drop of chicken serum was first placed upon the coverslip and tumor cells were added by touching the drop of serum with a piece of tumorous tissue. A piece of a coverslip, approximately 8 mm square, was placed over the drop of serum. The depression slide, with vaseline to hold the coverslip in place, was inverted over the coverslip. When the slide was righted the smaller coverslip was left hanging beneath the larger coverglass, held there by surface tension. The cells cultured between the two coverslips were thus provided with a physical means of support. The serum medium can be renewed simply by transferring the lower coverslip, with the cells adhering to it, to a new coverslip with a fresh drop of serum. Cells can be cultured for prolonged periods in this manner.

Nine to twelve-day old chick embryos were used in culturing cells on the chorioallantoic membrane. A window was first cut in the shell at the side of the egg without puncturing the white shell membrane beneath. The chorioallantoic membrane was then dropped away from the shell membrane by applying suction to a hole directly over the air sack at the end of the egg, using a rubber tube or bulb. The white membrane beneath the window was then perforated and a drop
of saline was introduced to moisten the chorioallantoic membrane before placing any tumor tissue on the membrane. Any excess moisture was removed with a pipette and the window was sealed with clean freshly drawn cellulose tape. Incubation was then continued.

**Etiological procedures**

In studies to determine whether ocular lymphomatosis is caused by the same agent as visceral leukosis tissue of the retina, optic nerves and brain of birds showing symptoms of ocular lymphomatosis were inoculated into the pectoral muscle of chicks to determine whether tumor cells of the visceral type were present. The development of a tumor at the site of inoculation and in the visceral organs was interpreted to mean that the same agent was responsible for both the visceral and ocular types of leukosis. The birds with ocular lymphomatosis from which nervous tissue was taken are from the various strains maintained at this laboratory.

**Genetic procedures**

Matings within resistant strains, susceptible strains and reciprocal crossing of these strains were made to investigate the role of nuclear genes and the role of the cytoplasm in this disease. Uninoculated birds and birds that have survived inoculations with tumor homogenate were used in the
respective matings. Progeny from these matings were inoculated with a 1/640 dilution of tumor homogenate at approximately a month of age. The homogenate consisted of a mixture of tumor strains A and C diluted with 0.85% saline. Chicks were inoculated in the pectoral muscle with 0.25 cc per bird. At the time when the birds were discarded or at death the sex and status of the breast muscles with regard to tumor growth were recorded. Birds that did not develop a tumor a month after inoculation were recorded as survivors. Those that had a tumor at one month were kept for further observation to determine whether they would survive or succumb to the tumor.

A series of three genetic experiments were conducted to investigate the role of the chromosomal and cytoplasmic inheritance in the transmission of factors affecting resistance to avian leukosis. The first experiment involved the four newly established lines. Lines 9 and 11, as previously described, consisted of tested birds selected for resistance and susceptibility, respectively. Lines 15 and 16 were made up of untested birds from the same commercial source and were related to lines 9 and 11 to some degree. Reciprocal crosses were made between lines 9 and 16, lines 11 and 15, and lines 15 and 16.

In the second experiment the same birds as in experiment
However the males were interchanged so that resistant females that were mated to resistant males were now mated to susceptible males and susceptible females that were mated to susceptible males were now mated to resistant males. Males with apparently the same degree of resistance were also interchanged. The purpose of interchanging was to determine whether resistance was transmitted by the male parent as well as by the female parent.

The third experiment was conducted to confirm the results of experiments 1 and 2. Birds from a subsequent generation of lines 9 and 11 were used. Line 3, a highly susceptible line, and line 5, a relatively resistant line, were included. Reciprocal crosses were made between lines 9 and 3, lines 5 and 11, as well as a cross of 3 x 11.

**Testcrosses**

Hybrids between resistant and susceptible strains were crossed to susceptible birds in the fourth experiment. On the basis of results obtained in the earlier experiments, it was hypothesized that susceptibility was recessive. These matings were designed as testcrosses to obtain information on the number of genes determining resistance. In these test crosses a hybrid male was mated to females from lines 1, 2 and 3. Hybrid females were mated to a male from line 3.
Study of progeny from inoculated dams

In the fifth experiment, highly resistant hens of line 9, hens less resistant of lines 5 and 16 and susceptible hens of lines 1, 2, 3, and 15 were mated to susceptible males of line 2. After a period of several weeks the hens were inoculated with 0.25 cc. of a 1:5 homogenate of a mixture of tumor substrains A and C. Resistance of the respective progeny in successive hatches, obtained before and after the dams were inoculated, were compared.

Experiment 6 was conducted to obtain further information on the resistance of chicks after their dams were inoculated. Hens of lines 1, 2, 3, and 9 were inoculated with 0.25 cc. of a 1:5 tumor homogenate and three hatches of chicks were obtained subsequently and tested for resistance. Hens of lines 1, 2 and 3 were mated to a male of line 3. The females of lines 5 and 9 were mated to males of those lines, respectively.
RESULTS

Histological

Several observations of interest resulted from the histological examinations of the tumor. The tumor cells resembled large lymphocytes in stained sections. Use of the safranin-fast green stain revealed that the cytoplasm of the tumor cells had a high concentration of acidic material that stained red. When the sections and smears were treated with perchloric acid before staining the acidic material was removed from the cytoplasm indicating that it is probably ribose nucleic acid.

Mitotic figures were also seen. Their frequency was fairly high in some preparations and low in others. The mitotic activity of the cells indicate that they apparently have not differentiated to such an extent that they have lost their ability to divide, as would be expected with mature leucocytes. In the pectoral muscle these cells are packed between the muscle fibers. The muscle fibers, observed in sections, appear to be degenerated in some cases and widely separated by the tumor cells.

Colchicine treatment

Arrest of mitosis at metaphase was apparent after colchicine treatment. Cells with elongated and bilobed nuclei resulted. At 1:8,000 concentration 5 to 10% of cells were
estimated as being affected 45 minutes after treatment. Very few if any cells were affected by the 1:32,000 concentration of colchicine following two hours exposure. However, examination twelve hours later showed that many of the cells were affected in both groups.

Cell cultures

Initial attempts to culture the tumor cells using the conventional hanging drop method met with limited success. Tumor cells could be kept alive for a week to ten days in chicken serum but the cultures had to be discarded when the medium deteriorated. The tumor was seen to consist of masses of free cells packed between muscle fibers in the case of tumors transplanted to the pectoral muscle. These were relatively large nucleated cells possessing a large refractile body besides the nucleus, and numerous smaller refractile granules in the cytoplasm. When free in the medium they assume a round shape. They appear to be larger than the cells of the thymus and are about the size of some of the largest cells in the bursa of Fabricius.

It was possible to culture the cells with greater success by using the modified hanging drop method described under the section on methods. In an experiment in which this method was employed eight cultures were prepared. Of these eight cultures four were discarded on the third day, three
because of poor condition of the cells and the fourth because no cells were alive. Cultures 4, 6, 7, and 8 were apparently in good condition and kept. A lower coverslip was employed for all except culture 8. Culture 4 was started with a drop of embryo juice added to the serum. Culture 6 employed a plasma coated coverslip but no embryo juice. Culture 7 employed no plasma or embryo juice. Culture 8 had embryo juice added to the medium. Little effect of embryo juice or plasma was detected. Culture 4 was carried on for four successive transfers from July 16, 1955 to September 12, 1955 at which time a patch of cells was present. The cells died shortly after. Culture 6 was also carried for the same length of time. The culture contained a patch of muscle fibers and the cells seemed to thrive best in its vicinity. The culture appeared to be in good condition at the fourth transfer on September 9th, but was lost on September 12, 1955 because of a crack in the coverglass. Culture 7 did not survive beyond the first transfer on July 23rd. Culture 8 was carried on by the modified hanging drop method after being started by the conventional method. Few cells were alive after the third transfer on September 1st. Following the initial establishment of the cultures only serum was used as a medium for all subsequent transfers. Increase in cell numbers or in the size of patches of cells were not easily detectable by gross observations.
The fact that the tumor cells have not differentiated to an extent that their ability to undergo mitosis had been reduced is also shown by successful transplants of the tumor to the chorio-allantoic membrane of 12-day-old chick embryos. Tumor fragments (about 1 mm cube) of strain A were transplanted on to the chorio-allantoic membrane of chick embryos on July 19, 1955. One of these died the following day. A second was opened on the second day after transplantation. The membrane appeared opaque and shiny with three areas of cell concentrations. The remaining five eggs were opened on the third day. One had a compact white colored growth the size of a pea located at the fork of two blood vessels, embedded in the membrane. The growth was penetrated by blood vessels. Three had opaque thickened areas where the tumor cells were growing. One embryo did not have any gross evidence of a tumor being established.

Tumor cells were cultured with India ink added to the medium to determine whether they are phagocytic. No evidence of phagocytization was observed after an hour and on the next day.

Studies of the ocular form of lymphomatosis

The initial study to determine the etiological relationship between ocular and visceral lymphomatosis did not yield
any significant results. Optic chiasmas obtained from four adult grey-eyed males, with a total weight of 0.25 gms, were separated into their cellular constituents by grinding in with a mortar. The material then was mixed with 10 cc. of 0.85% saline to make an inoculum. Twenty three fifteen-day-old male commercial chicks were inoculated in the pectoral muscle with the suspension. Not one developed a tumor in the pectoral muscle sixteen days after inoculation. At sixty days they were still tumor free. Three died from undetermined causes. The optic chiasma tissue was minced with difficulty and tumor cells, if present, may have been destroyed by the grinding procedure. For this reason the experiment is not considered of much significance by itself.

In the second experiment of this series the retina from a cock, with a pinkish, opaque, enlarged eye, and the brain were used in inoculating chicks. The retina of the enlarged eye was about 1 mm. thick and yellowish except for black pigment at the surface. The vitreous humor had a slight pinkish tinge. A homogenate was prepared of the retina and of the brain at a concentration of five cc. saline per gram of tissue. Twenty-four commercial male chicks, fifteen days of age, were inoculated in the pectoral muscle with retina tissue at a rate of 0.25 cc. per bird. Twenty of these developed breast tumors of which nineteen died. Fifteen of the nineteen that died showed evidence of metastasis of the tumor to the vis-
cereal organs. Metastasis to the liver resulted in enlarged livers as is often observed with visceral lymphomatosis. However in this case the areas near the edges often contained white cheesy lesions. In one, round reddish areas were seen on the surface. Three birds did not develop any tumor in the pectoral muscle. No record was obtained on one test bird.

Twelve male 15-day-old chicks were inoculated with the brain homogenate. One chick died four days after inoculation. Among the eleven other chicks nine were found to have a breast tumor sixteen days after inoculation or at the time of death. One of these nine survived. No tumors were observed in two of the chicks.

Tumors from two of the chicks inoculated with retinal material and one from chicks inoculated with brain material were maintained by serial transfer. Three New Hampshire chicks were used for each. All six chicks inoculated developed pectoral tumors. Four of these showed signs of fatty degeneration of the liver while one had an enlarged liver with lesions.

The retinal and brain tumors after two transfers, one in commercial chicks and the second in New Hampshire chicks, were tested again for virulence. Twenty commercial chicks, eight days of age, were inoculated with each tumor. Each chick received 0.25 cc. of a 1:5 tumor homogenate. All chicks
in both groups died with a pectoral tumor. Eighteen of the twenty chicks inoculated with the tumor from the retina also had enlarged livers of which nine showed lesions. Among the twenty chicks inoculated with the tumor from the brain eighteen also had enlarged livers of which fifteen showed necrotic lesions. Obviously the same tumor was present in both the retina and brain of the cock in which it was found. The same tumor was also able to grow in the pectoral muscle and metastasize to the visceral organs.

The brains from two other grey-eyed males and from a grey-eyed female were also tested for the presence of tumor cells capable of being transplanted to the pectoral muscle. An eye of one of the males was transparent, the opposite eye was grey. Both eyes of the female, A 28, were grey in appearance. Sixty-five fifteen-day-old commercial chicks were inoculated with 0.25 cc. each of a 1:5 homogenate prepared from the brains of both males. Twenty-seven fourteen-day-old commercial chicks were inoculated with a similar homogenate prepared from the brain optic lobe and optic chiasma of the female. None developed tumors in the pectoral muscle, the site of injection.

Pieces of the optic nerve and optic chiasma of another grey-eyed female and four other grey-eyed cocks were also tested for the presence of tumor cells capable of being
transplanted. The female, A124, had a grey right eye and a slightly grey left eye. The first male, Male A79, had both eyes of a light color with small pupils commonly seen in cases of ocular lymphomatosis. Male A80 had a yellowish right eye and a normal left eye. Male A1099 showed greying of both eyes. The fourth cock had one eye with a lifeless, grey, sunken iris, and a transparent pupil. Ten chicks were inoculated in the pectoral muscle with fragments of the optic nerve or optic chiasma from each of these birds. A number 22 needle and tuberculin syringe were used for this purpose. None of the chicks inoculated developed tumors.

Inheritance studies

Reciprocal crosses. The results of experiment 1 indicated that the resistance of a chick to lymphomatosis is strongly influenced by its genotype. Chicks of different lines showed different degrees of resistance. Line 9 was highly resistant and crosses involving this line appeared to be most interesting. Mortality was about equally distributed in both sexes. Mortality differences of inoculated chicks from lines 9 and 16 and their reciprocal crosses were highly significant statistically, \( X^2 = 70.7^{**} \), df = 3. The results are as follows:
Hybrids from both reciprocal crosses resembled the more resistant parent in resistance. Differences between these reciprocal crosses do not appear to be large enough to demonstrate that there were any strong maternal influence.

The following results were obtained in reciprocal crosses between lines 11 and 15.

<table>
<thead>
<tr>
<th>Mating</th>
<th>No. tested</th>
<th>No. died</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀ ♀</td>
<td>79</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>9 x 9</td>
<td>54</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>9 x 16</td>
<td>104</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>16 x 9</td>
<td>61</td>
<td>47</td>
<td>77</td>
</tr>
</tbody>
</table>

\[ x^2 = 38.6^{**}, \text{df} = 3. \]

The crosses between lines 15 and 16 gave the following results. (Data for lines 15 and 16 same as above)

<table>
<thead>
<tr>
<th>Mating</th>
<th>No. tested</th>
<th>No. died</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀ ♀</td>
<td>73</td>
<td>70</td>
<td>95.8</td>
</tr>
<tr>
<td>15 x 15</td>
<td>95</td>
<td>63</td>
<td>66.3</td>
</tr>
<tr>
<td>16 x 15</td>
<td>84</td>
<td>73</td>
<td>86.9</td>
</tr>
<tr>
<td>16 x 16</td>
<td>61</td>
<td>47</td>
<td>77.0</td>
</tr>
</tbody>
</table>
\[ x^2 = 26.1^{**}, \text{df} = 3. \]

It is evident that line 15 is relatively more susceptible than both lines 11 and 16. However, in reciprocal crosses between line 15 and 11 as well as between 15 and 16 no evidence of cytoplasmic influence was found. These crosses do not offer any support to the hypothesis that there might be a cytoplasmic effect and leads one to believe that the nucleus has a more important role in determining resistance. There is little clear evidence of dominance in these crosses. In the set of crosses involving lines 11 and 15, the cross 15 x 11 was more resistant than the line 11. The reciprocal cross however was intermediate. This was also true for the crosses between line 15 and 16.

Chi-square tests of independence of the reciprocal crosses only, in the three sets of crosses above, are as follows:

<table>
<thead>
<tr>
<th>Reciprocal crosses</th>
<th>Degrees of freedom</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 x 16 and 16 x 9</td>
<td>1</td>
<td>1.45 n.s.</td>
</tr>
<tr>
<td>11 x 15 and 15 x 11</td>
<td>1</td>
<td>10.67 **</td>
</tr>
<tr>
<td>15 x 16 and 16 x 15</td>
<td>1</td>
<td>9.25 **</td>
</tr>
</tbody>
</table>

Experiment 2. Males interchanged in matings. The results of experiment two provided additional information, substantiating the findings in experiment 1. The nuclear fac-
tors were of primary importance for resistance. A tendency was observed for resistance to be dominant over susceptibility. From the results of experiment 1 it was apparent that line 9 was relatively resistant and line 15 susceptible while lines 11 and 16 somewhat intermediate. When the matings in experiment 1 were changed so that the same females were mated to males that were relatively more susceptible, more resistant, or of about the same degree of resistance the results of Table 1 were obtained.

When two groups of resistant females from line 9, mated to males of lines 9 and 16, were mated to a new male from line 15 no significant change in rate of mortality occurred. However when line 16 females previously mated to a resistant male from line 9 were mated to the same male from line 15 a significant increase in mortality from the tumor resulted among the progeny. It is apparent that if one of the parents is resistant the progeny is resistant. Resistance is evidently dominant to some degree over susceptibility. When the more resistant parent is replaced by a relatively susceptible parent one finds a lowering of resistance.

When males of apparently intermediate resistance were interchanged the results were less predictable. When the male of line 16 was replaced by that of line 11 the progeny
Table 1.  Resistance of progeny of original matings and after males were replaced by those differing in resistance

<table>
<thead>
<tr>
<th>Matings within &amp; between strains</th>
<th>% mortality Orig. matings</th>
<th>Mortality after interchange of males</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chick tested</td>
<td>Observed dead</td>
</tr>
<tr>
<td>Original mating</td>
<td>Male strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>after replacement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 x 9 (susceptible)</td>
<td>15</td>
<td>15.2</td>
<td>18</td>
</tr>
<tr>
<td>9 x 16</td>
<td>&quot;</td>
<td>20.4</td>
<td>11</td>
</tr>
<tr>
<td>16 x 9</td>
<td>&quot;</td>
<td>30.8</td>
<td>23</td>
</tr>
<tr>
<td>16 x 11</td>
<td>11 (Intermediate)</td>
<td>77.0</td>
<td>62</td>
</tr>
<tr>
<td>11 x 16</td>
<td>&quot;</td>
<td>75.8</td>
<td>9</td>
</tr>
<tr>
<td>15 x 16</td>
<td>&quot;</td>
<td>44.7</td>
<td>5</td>
</tr>
<tr>
<td>15 x 16</td>
<td>11 (resistant)</td>
<td>66.3</td>
<td>44</td>
</tr>
<tr>
<td>11 x 15</td>
<td>9</td>
<td>80.4</td>
<td>23</td>
</tr>
<tr>
<td>15 x 15</td>
<td>&quot;</td>
<td>95.9</td>
<td>15</td>
</tr>
<tr>
<td>16 x 15</td>
<td>&quot;</td>
<td>86.9</td>
<td>5</td>
</tr>
</tbody>
</table>

Data before interchange is that of Genetic Experiment 1
"Expected" mortality is based on mortality of original matings

* = P < 0.05
** = P < 0.01
from the line 15 females showed a significant decrease in mortality and the progeny of line 16 females showed a highly significant decrease in mortality. The replacement of the line 11 male by that of line 16 did not result in any significant change in mortality among progeny from females of lines 11 and 15. Only a small number of progeny were tested from these two groups, however.

When the susceptible male from line 15 was replaced by the more resistant male from line 9, progeny from females of both line 11 and 15 showed a highly significant decrease in mortality. The mortality was less than expected from females of line 16 also but the change was not significant statistically. Only three chicks were tested from this latter group of hens.

The performance of some of these birds, especially those of line 9 and perhaps the male of line 15, were evidently quite consistent in both experiments though mated to different individuals.

**Experiment 3. Reciprocal crosses.** Young pullets and cocks destined as replacement for the generation of birds used in experiments 1 and 2 were used in this experiment. The results of experiment 3 generally confirmed the findings of the first two experiments. Reciprocal crosses between lines 9 and 3 once again resulted in hybrids that were approximately
as resistant as line 9. The number of chicks tested and the number dying for each of these crosses are:

<table>
<thead>
<tr>
<th>Mating</th>
<th>No. tested</th>
<th>No. dead of tumor</th>
<th>% dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 3</td>
<td>15</td>
<td>10</td>
<td>66</td>
</tr>
<tr>
<td>3 x 9</td>
<td>16</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>9 x 3</td>
<td>51</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>9 x 9</td>
<td>99</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

The differences in mortality between these four matings were highly significant statistically - Chi-square = 48.5. Difference between the two reciprocal crosses was not significant - Chi-square = 0.131. Although all four matings seemed to show higher resistance than usual, the relationships between these crosses appear to be the same as in experiment 1. Resistance was again largely dominant over susceptibility as shown in the 1st two experiments. Comparison of resistance of lines 5 and 11 and their reciprocal crosses also showed that their progeny were relatively more resistant than tests of the previous generation had indicated. Line 5 had a mortality after inoculation of 48% while line 11 had a mortality of 55% among chicks tested from the previous generation. In experiment one chicks tested from line 11 had a mortality rate of 75%. In experiment 3 the chick mortality after inoculation for these lines are:
Mating   No. tested   No. died   % dead of tumor
\[ \begin{array}{ccc}
\hline
5 \times 5 & 25 & 4 & 16 \\
5 \times 11 & 31 & 11 & 35 \\
11 \times 5 & 28 & 1 & 3 \\
11 \times 11 & 54 & 9 & 16 \\
\hline
\end{array} \]

Chi-square = 10.4*  df = 3  P 0.02

One of the crosses, 5 x 11, resulted in progeny less resistant than either parental lines while the reciprocal produced progeny more resistant. A similar situation was observed in experiment 1 in reciprocal crosses between lines 11 and 15 and between lines 15 and 16. The difference between reciprocal crosses 5 x 11 and 11 x 5 was highly significant—Chi-square = 6.2,  P 0.02. The hybrid chicks from the cross of line 3 females by line 11 males were intermediate in resistance between those of the parental lines.

Mating   No. tested   No. died   % dead of tumor
\[ \begin{array}{ccc}
\hline
3 \times 3 & 15 & 10 & 66 \\
3 \times 11 & 38 & 21 & 55 \\
11 \times 11 & 54 & 9 & 16 \\
\hline
\end{array} \]

Chi-square = 20.6**  df = 2

Experiment 4. Testcrosses. Hybrids between resistant and susceptible birds were backcrossed to birds of susceptible strains to test for segregation of the factors for resistance
to leukosis. The mortality of chicks tested are presented in Table 2. In earlier tests lines 9 and 3 were resistant and susceptible, respectively. In these testcrosses, 21 percent of the progeny from the F₁ male, resulting from a cross of lines 9 and 3, and 17 percent from the F₁ females involving these two lines were resistant. Nearly thirty percent of the chicks from the 15 x 11, F₁ females survived the tests. Line 15 was a susceptible line in the earlier tests. No survivors were observed among the limited number of progeny from the other F₁ females. If a single locus was responsible for the differences between resistant and susceptible lines one expects a 1:1 ratio of resistant heterozygotes and susceptible recessive types in these crosses to susceptible types. On this hypothesis mortality among the fifty percent that are heterozygous will determine the final percent that survive. Earlier data showed that mortality within the resistant lines varies widely. The numbers of chicks tested are also small. The testcross data does not support the hypothesis of a single locus for resistance but it is not possible to decide on the basis of these results that a single locus is not involved. The high mortality for the other testcrosses seem to indicate that two or more complementary loci or perhaps resistant alleles not fully dominant in the heterozygote are basic elements in the disease resistance.
Table 2. Mortality of testcross chicks

<table>
<thead>
<tr>
<th>From matings</th>
<th>Chicks tested</th>
<th>No. died</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁ male</td>
<td>Mates (susceptible)</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>F₁ females</td>
<td>Males from strain 3</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>9 x 3</td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3 x 9</td>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>11 x 9</td>
<td></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>11 x 5</td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5 x 11</td>
<td></td>
<td>17</td>
<td>12</td>
</tr>
</tbody>
</table>

Experiment 5. Tests of chicks obtained before and after their dams were inoculated. In all seven successive hatches were obtained and tested. Females of lines 5, 9, 15, and 16 were inoculated after the second hatch. Females of lines 2 and 3 were inoculated after the fourth hatch. The results are presented in Table 3. All lines were relatively resistant in the first hatch with line 9 being especially resistant. A general increase in susceptibility is evident in the second hatch. In the third hatch chicks from line 3, 9 and 15 showed a continued increase in susceptibility. The change was most marked in line 9. Lines 5 and 16 did not show any further
Table 3. Resistance of progeny from pullets before and after injection of the pullets with mixed tumor—percent mortality and number tested

<table>
<thead>
<tr>
<th>Hatch no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>7</td>
<td>60</td>
<td>36</td>
<td>--</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>No.</td>
<td>41</td>
<td>14</td>
<td>15</td>
<td>33</td>
<td>--</td>
<td>9</td>
<td>29</td>
</tr>
<tr>
<td>Pen 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>25</td>
<td>50</td>
<td>33</td>
<td>50</td>
<td>--</td>
<td>33</td>
<td>56</td>
</tr>
<tr>
<td>No.</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>--</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Pen 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>30</td>
<td>63</td>
<td>83</td>
<td>71</td>
<td>--</td>
<td>83</td>
<td>72</td>
</tr>
<tr>
<td>No.</td>
<td>30</td>
<td>27</td>
<td>12</td>
<td>35</td>
<td>--</td>
<td>12</td>
<td>36</td>
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<tr>
<td>Pen 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>22</td>
<td>40</td>
<td>42</td>
<td>44</td>
<td>--</td>
<td>67</td>
<td>54</td>
</tr>
<tr>
<td>No.</td>
<td>18</td>
<td>15</td>
<td>14</td>
<td>34</td>
<td>--</td>
<td>9</td>
<td>35</td>
</tr>
<tr>
<td>Pen 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>27</td>
<td>87.5</td>
<td>100</td>
<td>100</td>
<td>100.0</td>
<td>33.3</td>
<td>90</td>
</tr>
<tr>
<td>No.</td>
<td>15</td>
<td>8</td>
<td>10</td>
<td>14</td>
<td>1</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>

| Pen 2     |   |   |   |   |   |   |   |
| %         | 100.0| 100|   |   |   |   |   |
| No.       | 1   | 1  |   |   |   |   |   |

*Hatches 1 and 2 of lines 5, 9, 15 and 16 were obtained before inoculation. Hatches 1 to 4 and part of hatch 5 of line 3 and hatch 5 of line 2 were obtained before inoculation.
increase in susceptibility in the third hatch. The dams in all lines except 2 and 3 had been inoculated with the tumor after the second hatch. Following the third hatch there seemed to be no further change in resistance.

Differences in mortality before and after the dams were inoculated were highly significant for lines 9 and 15, \( \chi^2 = 29.2, \text{df} = 1 \) and \( \chi^2 = 11.9, \text{df} = 1 \), respectively, but not significant for lines 2, 3, 5, and 16. In view of the general trend toward an increase in susceptibility it is difficult to attribute the significant change in resistance for lines 9 and 15 to treatment of the dams. However it is interesting to note that line 9 showed a large increase in mortality which seemed to be maintained in the later hatches.

**Experiment 6.** Further tests of chicks obtained after inoculation of their dams. In order to obtain more information on the effect that inoculation of the dams will have on resistance of their progeny another experiment was conducted at the end of the regular hatching season in 1957. The results are summarized in Table 4. Chicks from the different hatches tested are also classified according to age tested. Rather marked differences between hatches was noted. Among the chicks of lines 5 and 9 a relatively large increase in susceptibility was observed in the third hatch. This does not appear to be entirely an age effect as the chicks in the second and third
Table 4. Mortality of chicks of different ages after inoculation with 0.25 cc. of a 1/640 homogenate of a mixture of tumor strains A and C; dams given 0.25 cc. of a 1:5 homogenate

<table>
<thead>
<tr>
<th>Line</th>
<th>Hatch</th>
<th>Age tested</th>
<th>No. tested</th>
<th>Died of tumors</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>33</td>
<td>11</td>
<td>8</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>33</td>
<td>7</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24</td>
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<td>3</td>
<td>100</td>
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<td></td>
<td>3</td>
<td>23</td>
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<td>100</td>
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</tr>
<tr>
<td></td>
<td>3</td>
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<td>4</td>
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hatches differed only by one day of age when tested. In experiment 5 no increase in susceptibility was observed for the corresponding period after the dams were inoculated (Table 3, hatches 4 and 6).
DISCUSSION

Study of the tumor by several approaches, standard histological techniques, supravital stains, treatment with colchicine, culturing of the cells in vitro and culturing the cells in the presence of India ink, yielded a picture of the tumor that agree with the previous descriptions of the disease. The tumor cell in the case of visceral lymphomatosis, with which we are dealing, resembles a large lymphocyte in fixed and stained sections and smears. The frequent mitotic figures observed also indicate that the cells are relatively undifferentiated. The presence of relatively large amounts of ribose nucleic acid in their cytoplasm seems to be a further indication that the cells are embryonal in character. No phagocytic behavior was observed.

Although some of the different types of leukosis appear to be caused by different strains or kinds of viruses, each associated with some specific type cell, the validity of classifying certain types of leukosis in separate categories appears questionable. Classifying some of the diseases on the basis of tissues and organs involved appears to have added to the complexity of classification. In studying this disease an attempt was made to determine whether the visceral, neural and ocular forms of lymphomatosis are definitely due to separate etiological agents. Osteopetrosis was rarely
encountered and no study of this form was made. Attempts to induce intramuscular tumor formation by inoculation of nervous tissue from a number of birds with the ocular form of the disease yielded no tumors except for tissue from one bird. In this case the eye of the bird was pink and cloudy with no normal pigmentation and was not a typical grey eye, although it could have been an advance stage of the ocular condition. However it did indicate that the agent of visceral lymphomatosis can be found in nervous tissue, the retina and brain. This particular case, in addition to that described by Pappenheimer et al. (1926) involving an invasion of the spinal cord by an ovarian tumor, adds further evidence that lesions in the nervous tissue may be caused by the same etiological agent as that responsible for visceral lymphomatosis. In the other cases where no tumors were found the experimental chicks were not kept long enough for observations to be made on the frequency of visceral tumors during their adult life to justify any definite conclusions.

Lack of tumor formation in experimental chicks for the other cases of ocular lymphomatosis does seem to support the view that the cells found in the nervous tissues of birds with neural lymphomatosis are not the same as those in visceral tumors. However, this does not necessarily mean that different etiological agents are involved. The ocular
symptoms generally occur in adult and older birds. It is quite possible that the cells have lost their ability to divide following prolonged periods in the environment of nervous tissue. On the other hand the lack of tumor formation could mean that the agent of ocular lymphomatosis affects only nervous tissue.

The initial question in mind was the role of the egg cytoplasm, particularly whether active immunity can be induced by virus transmitted through the egg. Studies of hyperimmunized hens by Burmester and co-workers have demonstrated that passive immunity may occur. In their studies they have demonstrated that chicks are protected at hatching. The literature surveyed provided no definite evidence of egg transmission of the virus. In cases where chicks were obtained from inoculated eggs a good number were infected. Progeny from dams with the disease did not appear to have an abnormally high incidence of the disease.

In my study no evidence of cytoplasmic influence was observed in reciprocal crosses. There is some question whether passive resistance is significant in normal birds that have not been hyperimmunized. It is also possible that protection due to passive immunity is effective only in young chicks. No evidence was found that active or passive resistance of chicks was influenced by a single inoculation of
their dams with a tumor homogenate.

Results obtained in the genetic experiments serve to emphasize the importance of nuclear factors influencing resistance to the disease. As far as the writer is aware, this is the first time that a predictable behavior with respect to dominance has been observed when inbred lines were crossed.

Resistance appears to be dominant over susceptibility. The observations by Fenstermacher and by Coles, loc. cit., support this view. These findings seem to show that genetic means for the control of visceral lymphomatosis, and other types as well, appear to be most practical. Control by prevention of contact transmission is effective but, as numerous workers have mentioned, impractical. The factor(s) for resistance do not appear to be sex-linked.

An understanding of resistance to a tumor in the chicken adds to our understanding of tumors in other species.

A review of the inheritance of immunity by Gowen (1948) has served to emphasize that difference between resistance and susceptibility to a particular disease, although often not understood, is in a number of cases due to one or a few
major genes. Furthermore, genes conferring resistance to one disease agent need not confer resistance to another. In man, a list of twenty-four benign, three precancerous conditions and ten malignant tumors in which chromosomal factors are important was included by Homburger (1957) in a monograph on cancer. Differences in reaction of certain inbred strains of mice to mammary cancer, leukemia, lung tumors, and other neoplasms were mentioned.

Testcrosses of F$_1$ birds to susceptible birds did not yield conclusive information on the number of genes differentiating the resistant and susceptible lines. Mortality among the small number of chicks tested was higher than expected for a one-gene hypothesis.

Large differences in mortality of chicks in different hatches were observed in these studies (Tables 3 and 4). Such changes were also reported by Poirier (1952).

The fact that the lines intermediate in resistance are unpredictable in crosses makes it appear that more than one locus is involved and that the loci are not fixed in these lines. However the apparently complete susceptibility in several lines make it seem improbable that the number of loci is large. Some degree of dominance in the F$_1$ also suggests that the character is determined chiefly by a small number of genes with major effects rather than by a large number of genes with additive effects.
In these studies a tumor homogenate, presumably containing both tumor cells and a virus-like agent, was used to test chicks for resistance. In terms of rapidity of tumor development following inoculation, the effect of a suspension of tumor cells is characteristically different from a cell free filtrate containing the virus-like agent. However, it is not certain that resistance to growth of tumor cells involves a different process from that determining resistance to the viral agent.
SUMMARY AND CONCLUSIONS

Several aspects of the leukosis problem were studied, the histology, the relationships between visceral, neural and ocular lymphomatosis, genetic factors, and the role of factors in the egg cytoplasm in influencing resistance. Visceral lymphomatosis tumor, Strain RFL 12, was used in these studies.

From a histological point of view the tumor cells were observed to be immature cells resembling large lymphocytes. Mitotic figures were frequent. Use of colchicine in serum cultures caused a large number of cells to have bilobed nuclei. This was interpreted to mean that mitosis was retarded at metaphase. The tumor cells were cultured in chicken serum; by a modified hanging drop method, for 33 days. They were also grown on chorioallantoic membranes of chick embryos. There appears to be a large amount of ribose nucleic acid in the cytoplasm of these cells. This seems to be further evidence of the embryonal nature of the cells.

Nervous tissue from birds showing signs of ocular lymphomatosis (grey eyes) were injected into the pectoral muscles of chicks to determine whether there was a relationship between the ocular, neural, and visceral forms of lymphomatosis. Only in one case did nervous tissue from a bird with disease symptoms cause tumor formation in the pectoral muscles of
chicks injected. This particular case supports the view that the neural and the visceral form of lymphomatosis, and possibly the ocular form as well, may be due to a single etiological agent.

In reciprocal crosses between resistant and susceptible lines no cytoplasmic influence was detected. Inoculation of dams also did not seem to influence the resistance of progeny obtained subsequently.

Testing procedures appear to be adequate for distinguishing the more resistant from the more susceptible groups of birds.

The importance of nuclear factors in determining resistance is strongly supported by the results of the genetic studies. The several inbred strains of chickens used in this study appear to fall into three categories; highly resistant, intermediate in resistance and highly susceptible. The highly resistant and highly susceptible strains behaved consistently in several experiments conducted in different years and involving more than one generation. These birds also behaved consistently when mated to different birds. The strains intermediate in resistance gave less predictable results.

Resistance in the highly resistant strain was evidently largely dominant over susceptibility. No evidence of sex-
linkage was observed.

These results suggest that the incidence of leukosis in flocks that include susceptible birds can be reduced by genetic methods.

The limited amount of data from the testcrosses do not justify any conclusion on the number of loci involved. It seems possible however that only a small number of loci are responsible. Inheritance of resistance to leukosis appears to be determined by a small number of genes with major effects rather than by a large number of genes with additive effects.

From a broader point of view these findings have a bearing on our understanding of the role of heredity in the development of cancer.
LITERATURE CITED


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