The Experimental Transmission of Avian Leukosis: A Review

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INTRODUCTION

The first important studies were made by Ellerman1 (93), who showed that some forms of leukosis were transmissible. He also suggested a classification of the avian leukoses into the following main types: leukemic and aleukemic myeloid, intra-vascular lymphoid (erythroblastosis), and extra-vascular lymphatic leukosis. Later observers, on the basis of an apparently related etiology, added a variety of other conditions, including those involving bone and nerve tissue which do not remotely resemble leukemias. Recently in Britain there has been a return to a modification of Ellerman's classification (67).

Since there have been many recent reviews of the literature on fowl leukosis (6, 29, 56, 61, 68, 69, 78, 126, 189) and on other fowl virus tumors (115, 185), this paper will be limited to a discussion of more recent transmission experiments with the various leukosis viruses in chicks, chick embryos, and tissue culture.

It will be noted that the writer has found it convenient to follow Campbell (49, 50, 67) in using the term, lymphoid leukosis, rather than "lymphomatisis." Unfortunately, the latter term has become so firmly entrenched that its usage in some reports may cause some confusion. Furthermore, in some papers this current practice had led to the grouping of neural lymphomatosis with lymphoid leukosis, which appears to have a different etiological basis (35).

Leukosis Viruses

Since Ellerman's publications appeared, laboratory strains of leukosis viruses have been isolated in all parts of the world; only a few of these survive today. Many were obtained during the years 1930–1940 when interest in these conditions reached a peak. Engelbreth-Holm in Denmark, Jarmai in Hungary, Furth in the United States, and Oberling in France published numerous papers1 dealing with strains they had isolated. Except for one of Furth's strains, which appears to produce lymphoid leukosis as well (104), these were strains of either erythroblastosis or myeloblastosis virus. On first isolation the strains of myeloblastosis virus would produce cases of erythroblastosis and vice versa. However, the first passages of the B.A.I. strain of myeloblastosis virus induced predominantly lymphomatosis and hemocytoblastosis (87). With the many passages since their isolation, the laboratory strains of the leukosis viruses presently available induce a more uniform pathologic process than was obtained in earlier passages, provided the route of inoculation and the strain of chicks used are kept constant. However, when the route of inoculation is changed some of the strains of leukosis viruses are found capable of inducing a wider variety of tumor-like conditions than might have been anticipated. Sarcomas and endotheliomas may develop following subcutaneous inoculation of some strains of erythroblastosis virus (94), while intrarenal inoculation results in adenocarcinoma of the kidney (58).

Furth's claim to have transmitted lymphoid leukosis and Ellerman's earlier and similar claim were disputed, and for many years lymphoid leukosis was regarded as nontransmissible (94, 158). Many transplantable lymphoid strains have been obtained more recently. Tumor strain RPL 12, isolated by Olson in 1940 (159), and RPL 16, one of several similar tumors developed at the Regional Poultry Laboratory, East Lansing, Michigan (42, 82), have been studied in many laboratories. Filtrates of these tumors produce erythroblastosis, hemangiomas, and a non-neoplastic bone malformation (osteopetrosis) as well as lymphoid leukosis (39, 43, 107). Cellular suspensions inoculated intramuscularly or subcutaneously produce a local "lymphoid" tumor which appears within a few days to 3 weeks, depending on the dose and individual resistance. Metastasis to the visceral organs 2–4 days after the appearance of the local tumor, with the liver as a site of predilection, is the usual occurrence (65). The incidence of lymphoid leukosis may be raised (72, 123, 125, 134, 135) without the development of transplantable tumors. This can be accomplished by the inoculation of lym-

1 See Engelbreth-Holm (94).

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experiences are common in studies on these diseases obtained by others (39, 107). Such conflicting evidence has been claimed by some (75, 85, 165), it has not been resolved. Although the successful transmission has been provided osteopetrosis, suggesting that some birds have a high natural resistance.

Finally, one other group of transmissible agents has been described, although unfortunately they have not survived as laboratory strains. These are the “Chick Disease Viruses” of Blakemore (15) and Asplin (8) which were obtained by rapid passage of material from birds with the different forms of leukemia and which produce focal necrosis of the liver and myocardium. An etiological relationship with the fowl leukoses was not established (4). Interest in these strains today lies in the somewhat analogous finding of a mouse hepatitis virus which interferes with the successful transmission of a strain of mouse leukemia described by Furth (108).

Holmes (117) has confirmed the evidence that osteopetrosis can be successfully transmitted from field cases (127) to either chicks or embryos left to hatch, without noticeably increasing the incidence of other forms of leukemia. He used whole blood, bone marrow, and unfiltered plasma for inoculation. Only a percentage of inoculated birds developed osteopetrosis, suggesting that some birds might have a high natural resistance.

Two strains of leukemia virus are being intensively studied in Bead’s laboratory at Duke University: the B.A.I. strain of myeloblastosis virus and the Engelbreth-Holm ES strain of erythroblastosis virus obtained from a spontaneous case of erythromyeloblastosis (96, 97). In our laboratory we hold a sarcoma-inducing variant of an erythroblastosis virus strain which also originated from Engelbreth-Holm.

In Japan a strain of erythroblastosis and a strain of a transmissible anemia have been recently described (120, 121). It was thought that the latter might be the anemic form of erythroblastosis described by Furth (108).

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To explain the distribution of “Chick Disease Virus” in the poultry population, Asplin suggested that this agent might grow better in tumor tissue than normal tissue. Studies by Cox and associates on the growth of neurotropic viruses in the RPL lymphoid tumor recall this observation (141, 182). They found that the viruses grow better in the tumor and may lead to its regression without apparent effects on the host.

The B.A.I. strain of myeloblastosis virus was obtained from two cases of neurolymphomatosis, but no transmissible strain of neurolymphomatosis exists. Although the successful transmission has been claimed by some (75, 85, 165), it has not been obtained by others (39, 107). Such conflicting experiences are common in studies on these diseases and make difficult an assessment of the true picture. That neurolymphomatosis is connected in some tenuous way with the other fowl tumor viruses might be suspected, however, from an observed inverse relationship between the incidence of neurolymphomatosis and susceptibility to the Rous sarcoma (106). It appears, however, that a picture indistinguishable from neurolymphomatosis may be produced by methods designed to produce an allergic encephalitis (51).

While there have been many successful isolations of erythroleukosis and myeloid leukemia viruses, it should not be assumed that the isolation from spontaneous cases is easily accomplished. Campbell (48) has frequently observed spontaneous cases of erythroleukosis which were not transmissible even when massive doses of whole blood were given.

Progress in the field of avian leukemia has been delayed by the relative paucity of inbred lines and by the apparent failure of many investigations to realize that these are indispensable for primary isolations of the less virulent tumor viruses. Initial transfers of the Rous sarcoma were only successful in related stock (111), and the isolation of a large number of strains of erythroblastosis and lymphoid tumors by the East Lansing workers probably results from the availability of inbred lines. Their line 15 is especially useful in that it has proved very susceptible to the B.A.I. myeloblastosis virus for which many other strains of chicks show high resistance (10).

Natural Transmission

This has been the subject of several reviews (29, 33-35, 61) and will be discussed here only briefly. A paradox stressed by Burmester (35) is that, in spite of the apparent epizootic nature of the lymphoid diseases, experimentally they are not easily transmissible. Conversely, many avian tumor viruses are highly infective in the laboratory, yet their natural level of occurrence is much lower than that of the lymphoid tumors.

The causal agent of lymphoid leukoses has been shown to be carried by the egg (46, 62) and is effectively transmitted from carriers to otherwise healthy chicks by contact infection in the first few weeks of life. The importance of egg transmission in the natural spread of leukemia has been disputed, however, by Cole and Hutt (38).

It has not been possible to demonstrate contact transmission with other avian leukoses, but birds reared in contact with RPL 12 lymphoid tumor virus can develop leukemia. The different tissue responses to this and other leukemia viruses raises the question of whether one or more than one virus is...
present in the inoculum and also whether the erythroblastosis, myeloblastosis, and lymphoid leukemia viruses are variants of the same virus. If the leukemia viruses are forms of a single virus, then erythroblastosis and myeloblastosis must be considered as potentially contagious, although it has not been usual to consider them as such. Burgmester has suggested that lymphoid leukemia virus might be considered to be a "wild type," from which all other known avian neoplastic viruses arise by "mutations" (35).

Birds carrying the "wild type" virus would be expected to have a certain degree of resistance to further infection. Accordingly, when birds are encountered which are resistant to lymphoid leukemia—if this proved to be a conditioned neoplasm—then it may be difficult to decide whether the resistance is due to a lower level of the conditioning factor, to hereditary factors, or to a carried virus. This point has a bearing on the dilemma of the hatchery in the modern poultry industry. It can either supply chicks which are relatively free of the disease but, being susceptible, may develop a high incidence on a customer's infected premises, or it can supply "carrier" birds resistant to an infective environment which may introduce the disease to a farm where leukemia has not previously existed. Some of these practical problems are discussed by Coles (60).

**Variables Influencing the Outcome of Transmission Experiments in Chicks**

**Age.**—Age was recognized early as an important variable. Engelbreth-Holm found that older birds developed fewer cases of erythroblastosis than did chicks and that they showed a longer incubation period. For myeloblastosis to develop with the T1 strain, young chicks were necessary (97). In myeloblastosis Beard found the natural resistance of chicks increased about 40 times from the age of 3 to 40 days; in contrast, resistance to erythroblastosis decreased with age up to 77 days (10).

Age plays an important role in transmission studies with lymphoid leukemia. Following intravenous injection with RPL 12 filtrates, the incidence of both erythroblastosis and lymphoid leukemia declined with increase in age at inoculation. After intraperitoneal inoculation, the decline was noted for erythroblastosis but was not as obvious for lymphoid leukemia (39). Age appears to be very important in contact transmission in that natural resistance to infection by this route increases rapidly after the first few weeks of life (39). In the case of the transplantable lymphoid tumors, the age of the chick may determine the time of appearance, percentage of takes, and the extent of metastasis (64).

**Strain of birds used.**—This considerably influences the reaction to the different leukemia viruses. Of a number of strains of birds examined for their response to myeloblastosis virus, the East Lansing Line 15 White Leghorns were found the most susceptible and most homogeneous in their response (10). Different strains of chicks may also vary in their response to erythroblastosis. A crossbred strain inoculated subcutaneously with an erythroblastosis virus developed a high incidence of sarcomas, later dying of erythroblastosis. In a strain of White Leghorns similarly inoculated, only occasional cases of sarcoma were observed but many birds died of erythroblastosis. Inbred lines of White Leghorns and their crosses tested for their susceptibility to RPL 12 virus differed markedly in their behavior (39). The erythroblastosis and lymphoid leukemia ratio varied greatly in the different lines. Line 7 chickens were resistant to both, the 15 × 16 cross showed a high level of lymphoid leukemia, and Line 15 a high mortality from erythroblastosis. This would suggest that hereditary factors are important in determining the type of disease induced and that more than one such factor may be involved.

The incidence and pathological types of leukemia observed in different flocks show considerable variation. For example, outbreaks of specific types of leukemia such as myelocytoma may occur. Such outbreaks may indicate the emergence of a leukemia virus of exalted virulence and altered tropism rather than a hyper-susceptibility of the flock or strain of fowls to leukemia viruses in general.

**Route of inoculation.**—That route of inoculation had an important effect on the disease obtained was recognized by the earlier workers (97). Quantitative studies on erythroblastosis and myeloblastosis have been recently made by Beard and associates in which they have compared the relative sensitivity to inoculation by different routes. Taking the resistance to intravenous inoculation as unity, birds inoculated by the intramedullary, intramuscular, intraperitoneal, and subcutaneous routes were estimated to be 2, 15, 18, and 67 times as resistant to myeloblastosis (89), while for erythroblastosis the average relative resistance was found to be 1, 22, 393, and 1400, respectively (12).

With the RPL 12 virus, the pattern may be complicated by the different resultant pathologies. When routes such as intravenous, intramedullary, and intraperitoneal are used, there is such a high incidence of erythroblastosis that few birds survive long enough to develop lymphoid leukemia. Inter-
mediate levels of erythroblastosis and some cases of lymphoid leukosis follow intracranial, intramuscular, subcutaneous, and tracheal routes. Aerogenic infection and nasal administration produce few cases of erythroblastosis and a moderate percentage of lymphoid leukosis. These differences are believed by Burmester et al. (39) to be due for the most part to variation in the effective dose.

Pikovski and Doljanski (168, 169) suggested that, for sarcomas to develop following the intramuscular injection of the T1 strain of erythroblastosis, it was necessary to inoculate cellular material. With our strain of erythroblastosis virus, filtered plasma readily produced sarcomas in one strain of birds but not in another.2

**Endocrine factors.**—Endocrines have been found to play a significant role in the development of spontaneous and induced neoplasms. In the fowl spontaneous lymphoid leukosis occurs more frequently in females than in males (26), while castration increases the incidence (40). This is in contrast to the picture in man (70) and mice (154), where leukemia can occur more frequently in males. The influence of sex hormones on spontaneous and induced lymphoid leukosis has been investigated. In general it appears that both diethylstilbestrol and testosterone propionate may reduce the incidence (40, 72). Sex differences in the prevalence of lymphoid leukosis was found to disappear when the birds were given inoculations intravenously of the blood of birds with lymphoid leukemia (26).

Seasonal effects may be exerted through the endocrine system. Some of the earliest work in this direction is recorded by Engelbreth-Holm and Rothe-Meyer (97), who found that in older birds, but not in chicks, the number of takes dropped significantly in October and November.

The injection of anterior pituitary extracts increased the susceptibility to the Rous sarcoma virus (189), but studies of the effect of pituitary-derived hormones on the reaction to the leukosis viruses have been limited. Having observed that the adrenal cortex of birds with spontaneous lymphoid leukosis was significantly enlarged (132), Lannek conducted a series of experiments in which he found that ACTH and hydrocortisone exerted a marked but temporary inhibition on the growth of experimental lymphoid tumors, whereas cortisone had only a slight effect (139).

**Diet.**—Diet is a variable in experimental studies on the leukoses that might be expected to influence results. Vitamin E deficiency was earlier suspected to be a contributing factor in the development of spontaneous leukosis—but this could not be confirmed.4 Cater (55) in studies on the effect of vita-

min E deficiency on the growth of the Rous sarcoma noticed that the vitamin E-deficient birds “ate badly.” This fact alone might be expected to affect results, since reduction of caloric intake may depress the growth of the Rous sarcoma (136). A record of the food consumed should be kept, therefore, in studies of this kind.

The effectiveness of folic acid antagonists in the treatment of the human leukemias prompted a study of dietary folic acid levels on the growth of the Rous sarcoma (35), erythroblastosis, and a lymphoid tumor (184). The growth of these three tumor conditions was retarded on folic acid-deficient diets. Since the body may have reserves that mask the effects of a vitamin deficiency, experiments with antagonists of the vitamin concerned may give clearer results, provided nonspecific toxic effects can be controlled and there is effective absorption of the antagonist from the intestine. These qualifications are important. Conflicting results were obtained in studies with folic acid antagonists by Darcel (66) and Chubb and Laursen (57) on the growth of fowl lymphoid tumors. No tumor growth-retarding effect and no toxicity were obtained by the former, who administered the drugs directly into the crop, but both effects were obtained by Chubb and Laursen, who incorporated the drugs into the diet and probably obtained better absorption.

**Other factors.**—Dosage and individual variations in resistance are important factors which may affect the results of a transmission experiment, but there must be many other factors involved. For instance, the virus activity of the initial preparation may vary, depending on its rate of growth and the dose of virus with which the tumor was induced (8). Dosage may even determine the histological character of the response to a tumor filtrate. In one experiment with RPL 12 filtrates, lasting 270 days, one dosage level induced erythroblastosis in most inoculated birds; lymphoid leukosis attained a level of 51 per cent in contact controls, while only 3.6 per cent of the isolated controls developed the disease. Dose was considered to be the prime factor governing the emergence of either erythroblastosis or lymphoid leukemia, but the evidence for this was not considered conclusive (39).

Natural antibodies to tumor viruses present in the tumor tissues may also be expected to influence the results of transmission experiments, but this possibility has received little attention in the case of the leukosis viruses.

**Transmission in Chick Embryos**

There were many earlier attempts to use chick embryos for transmission experiments with leuko-
sis viruses, but Hall and associates (112, 113) were the first to make extensive investigations. They used the B.A.I. strain of myeloblastosis in a large number of chick embryos, utilizing the chorioallantoic, intravenous, and yolk sac routes of inoculation. Takes were obtained only by the intravenous route and even then with a low incidence. Although several passages were made with apparent enhancement of virus activity for chicks and the virus was transmissible to embryos of heterologous species, these passages were difficult to accomplish (170). The onset of leukemia in the embryos was indicated by the appearance of an increasing number of hemocytoblasts in the peripheral circulation. Chick embryos were inoculated at 11 days of age, and 6-8 days afterwards they were moribund or dying. At necropsy, the livers and spleens appeared enlarged and showed pin-point grey foci; microscopic examination showed that cellular infiltration of these organs had occurred. Atanasiu (5) obtained a higher proportion of infected embryos with intravenous inoculations of erythroblastosis virus, but infection of the chorioallantoic membrane was not successful except with cellular inoculum. Vigier and Guérin (190), on the other hand, were able to transmit the E/S strain of Engelbreth-Holm to embryos, using the chorioallantoic route. Typical leukemia developed by the 20th day in one-third of the embryos. In the affected embryos, small pin-point hemorrhagic nodules developed on the chorioallantoic membrane, which appeared to be erythroblastomas. Intramuscular inoculation of these into chicks produced sarcomas. A few further passages were made with both the leukemic form and the erythroblastomas, but the results indicated an attenuation of the virus with passage.

Cellular material (blood, etc.) from erythroblastosis and RPL 12-infected chicks can infect embryos when inoculated by the yolk sac route, but some chicks may not develop the disease until after hatching.2

With the RPL 12 virus, intravenous inoculation regularly produced infection of 12-day-old embryos; death occurred 3-8 days afterwards. In the first 24 hours, hepatic cells developed swollen nuclei containing conspicuous inclusion bodies. After 36-48 hours, the number of hepatic cells containing inclusions decreased, and perivascular infiltrations of round cells with a vesicular nucleus and intense basophilic cytoplasm appeared in the visceral organs. The spleen became considerably enlarged, and blood and bone marrow showed marked changes. At 5-5 days postinoculation mortality reached a high level, and massive, often hemorrhagic, areas of necrosis were observed (74, 183).

The successful experimental transmission of leukosis viruses in the egg embryo has an obvious bearing on the natural transmission of the disease. Since the embryonated egg will support virus growth, this favors egg transmission as a possible factor in the natural spread and maintenance of these diseases.

**Transmission in Tissue Culture**

Papers by Doljanski and Pikovski (79, 80, 168) provide access to earlier work on the growth of leukosis viruses in tissue culture. They noted that in plasma clot cultures of leukotic blood and bone marrow from birds inoculated with the T1 strain of erythroblastosis, the "stem cells" (probably proerythroblasts) multiply rapidly and liquefy the clot; many then die, some become polyblasts, while others retain their original characteristics for long periods. The infectivity of such cultures was found to last during the entire period, while the morphology appeared to have little influence on activity. Pure fibroblast cultures were as infective as cultures which were rich in "stem-cells." The virus persisted in cultures of normal chicken fibroblasts derived from adult fowl myeloblastoma and was maintained for 178 days. Apart from these workers, the tissue culture study of erythroblastosis appears to have received little attention in recent years.

The Rous virus has been found to produce cytopathogenic changes in tissue culture (138, 150) which can be used for virus assay (186). This has prompted attempts to demonstrate similar changes with the leukosis viruses. Beaudreau and associates (11) observed no obvious cytopathogenic effect with myeloblastosis virus. The cells continued to multiply despite virus liberation at a rate of almost 2,000 particles per cell being liberated in the initial 48-hour period. Intracellular virus could be demonstrated only infrequently by electron microscopy of the cells (166).

The lymphoid leukosis-derived RPL 12 virus has recently been grown successfully in tissue cultures of chick embryo liver cells and other embryonic chick cells. The agent also grew in cultures of duck embryo cells but not in mammalian cells (183). Extensive cytological investigations have since been made in which the virus was found to induce intranuclear inclusions similar to those seen with adenoviruses or the polyhedral virus of the silkworm (74). The inclusions follow enlargement of the nucleus, with alterations of the chromatin and an increase in DNA. The changes in tissue culture can be prevented by neutralizing the virus with specific antiserum (101, 189). Several passages of this virus have now been made in tissue.
culture, and spray counts with the electron microscope suggest that actual multiplication occurs (71).

**Titration of Virus Activity**

This is usually carried out by infectivity determinations in chicks and embryonated eggs, but other techniques have been employed. Tissue culture may eventually be used for this purpose, as in the case of the Rous virus (186), as well as in recent work on a leukemia virus (71). In myeloblastosis, the correlation of infectivity with adenosinetriphosphatase (ATPase) activity is so close that determination of the level of the enzyme can be used for approximations of virus activity (152, 153). There is also a possibility that, with the leukemia viruses, direct counts of virus particles with combinations of ultracentrifugation and electron microscopy could be made to serve such a purpose (90, 115, 179). The spray technique (119) for counting virus particles by electron microscopy has recently been employed for tissue culture studies of the growth of lymphoid leukemia virus (71).

There have been many recent reviews on the subject of infectivity titrations in chicks (21, 25, 119) to which the reader is referred for full details of the techniques employed. The infectivity of a preparation is usually assessed by the frequency with which disease is induced by different dosage levels of the virus or the time required for manifestation of disease. The frequency is used in determining 50 per cent infectivity end-points, usually by the Reed-Muench method (99) or by probit analysis. The time relationships are analyzed by linear regression of the log-dose against functions of the response time such as median in log days, mean time of appearance, or mean reciprocal time of appearance. The rankit transformation has also been suggested for analysis of this type of data (22). It is usual in these techniques to compare the activity of the unknown preparation against a standard. The development of standard preparations has received some attention in the case of the Rous virus (25). Virus-containing plasma from birds infected with erythroblastosis retains its activity for long periods in the dry ice chest, and material of predetermined activity can be used as a reference.

Chick embryos have been used for titrations of the Rous virus by virtue of the lesions produced on the chorioallantois. There are many variables involved, and in spite of refinements recently introduced (108, 173) the writer has had little success with it in his laboratory. Lesions of a similar type do not appear to follow chorioallantoic inoculation with the leukemia viruses. A titration method with the use of the hemorrhagic lesions produced in the viscera of embryonated eggs has been recently developed (19, 137) for the Rous virus. Hemorrhagic foci may occur in the livers of chick embryos inoculated by the yolk sac method with erythroblastosis and the RPL tumors, but this feature is not sufficiently regular as a basis for titration.2 Atanasiu (5) has observed that erythroblastosis virus, inoculated intravenously, successfully infects embryos, and this might be used in determinations of the infectivity of a virus preparation.

It does not yet seem possible to correlate the incidence of lymphoid leukemia with dose. If erythroblastosis is caused by the same virus, it is possible that the incidence of the disease may reflect the amount of virus present in an inoculum. Since, with the RPL 12 virus, most cases of erythroblastosis have developed by 100 days, this would seem a convenient observation period. However, 9 weeks has been recommended as a suitable post-inoculation period for the assessment of results (32).

The period of observation required for bioassay will vary according to the susceptibility of the chicks and virulence of the virus; in experiments with myeloblastosis, for instance, the average life after inoculation for New Hampshire Red chicks was 40.6 days, while the corresponding figure for the more susceptible East Lansing Line 15 White Leghorn chicks was 26 days (88).

In spite of difficulties of various kinds, the precision of tumor virus titrations nevertheless can approach that obtained with other animal viruses (2).

**Relation of Other Virus Infections to the Transmission of Avian Leukosis**

The presence of other viruses might be expected to influence the development of leukemia by: (a) interference, (b) cytopathogenic effect on tumor cells or (c) immunization due to antigenic relationships. The first and last of these possibilities do not appear to have been explored to any extent. The second has received much recent attention.

Sharpless and associates (182) examined the effect of a very large number of viruses on the growth of the RPL 12 tumor implants. Only Russian Spring Summer, West Nile, St. Louis, Japanese B. encephalitis, and louping-ill viruses had any visible influence on tumor growth. These viruses increased the chances of survival of tumor-bearing chicks, St. Louis encephalitis and louping-ill viruses being the most active in this regard. The effect was most pronounced when the viruses were injected into the tumor site 2–4 days after inoculation.

Love (142) in a study of cytopathological changes of
RPL 12 tumors infected with St. Louis encephalitis noted an increase in the number and size of lipochondria. These combined with the mitochondria to form artefacts similar to inclusion bodies during the processing of the tissue. Partial arrest of mitosis in metaphase was observed, together with increased numbers of mitotic abnormalities and binucleate cells. Regression following infection with this virus would appear to follow enhancement of phagocytosis of tumor cells and stimulation of the defense mechanism (141).

Unfortunately, most of the viruses which have so far been found effective in controlling tumor growth are highly dangerous in their unattenuated form. Sharpless and associates (182) recommended a search for agents without such pronounced pathogenic characteristics. Cole and Sharpless (59) have reported an unsuccessful field trial with such a virus in which, unfortunately, leukemia and neurolymphomatosis developed to a high level in virus-treated pullets.

THE EFFECT OF SOME CHEMICAL SUBSTANCES ON TRANSMISSION OF LEUKOSIS

There have been a limited number of studies dealing with the effect of antibiotics on the initiation of infection with the Rous sarcoma virus (52, 109). In some instances, when the virus was exposed to the agent before inoculation, inhibition of tumor growth occurred. If the chickens were treated with the agent, there was usually no detectable effect. No such studies seem to have been made with the leukemia viruses.

Structural analogs of amino acids have been examined for possible effects in the growth of other virus (131). This approach might prove highly successful with tumor and leukemia viruses. Since birds show a high natural resistance to these, it is conceivable that minor interference with the growth of the virus might “tip the scale” in the direction of complete failure of response.

There have been, however, a few trials of the effect of nitrogen mustard (124), triethylene melamine (68), and folic acid antagonists (57, 66) on the transplantable lymphoid tumors. It is unfortunate that these substances, if administered at effective levels, have toxic side effects.

Nitrofurazone, widely used in poultry feeds, was reported to have tumor-inhibitory effects on mouse sarcomas, apparently associated with an enlargement of the adrenal glands. This drug was administered for 75 days in an experiment in which chicks were inoculated with cell-free RPL 19 lymphoid tumor extract and maintained for 240 days. The incidence of disease developing suggested that the drug had not prevented leukemia.

More cases of leukemia occurred, in fact, in treated uninoculated controls than in the flocks from which the chicks originated. No inhibitory effect was noted on the growth of tumor RPL 12, and there was no enlargement of the adrenal glands; there was, however, a significant increase in body weight of drug-treated uninfected birds over control birds (63).

INFLUENCE OF IMMUNITY ON THE TRANSMISSION OF LEUKOSIS

Natural resistance.—

1. To the Viruses: That sera from adult fowls and newly hatched chicks may carry Rous virus neutralizing antibodies was established some time ago (1). There was an indication that the chicks acquired these antibodies through the egg, the amount depending on the antibody content in the blood of their dam (a point disputed by Bang and Haley [7]). The antibodies persisted in chick sera for only a few weeks, but chicks which had these antibodies in early life showed a tendency to develop potent Rous antibodies later. It was suggested that these naturally transmitted antibodies might be responsible for some difficulties in transmission of the virus in young chicks and in tissue culture (1).

The importance of the Rous-neutralizing antibodies to investigators in the leukemia field lies in the cross-immunity observed between sarcomas and the leukemia viruses (110); consequently, the presence of antibodies to one tumor virus may only indicate previous contact with another distinct but related one.

Antibodies for avian tumor viruses develop with aging in many normal chickens. Kenzy and Neuzil (129) forged another link between the fields of leukemia and Rous virus research by demonstrating a direct relationship between the incidence of lymphomatosis and the number of birds in which Rous virus-neutralizing antibodies could be demonstrated. Rearing birds in strict isolation reduced the prevalence of Rous-neutralizing antibodies in sera from 45 per cent to 0 per cent (12). Similar observations were made by Duran-Reynals and associates (84).

In both infected and normal chicks, the development of a globulin component, capable of flocculating saline or alcoholic extracts of various tissues from many species of animals has been observed (88). The writer has confirmed the flocculating capacity of normal fowl sera for such extracts. It is probable that these lipide precipitins are not true antibodies but they may interfere in other antigen-antibody reactions. Removal of these lipide substances from the tumor extracts...
might conceivably increase the specificity of serological reactions between these viruses and their corresponding antibodies.

Leuko-agglutinins have been described (130), but it is doubtful whether these were true agglutinins (64).

Strains of birds vary in their resistance to these viruses, and it seems possible that this resistance may follow the presence of natural antibodies in the circulating blood. Studies on the incidence in the sera of apparently normal fowls of natural antibodies to the viruses of erythroblastosis and myeloblastosis seems either to have been neglected or not to have been reported.

2. To the Tumor Cells: Natural antibodies to tumor cells, if they occur, would presumably take the form of isoantibodies or heteroagglutinins. The former can only rarely be demonstrated (81). The relation of the latter to the transplantability of fowl tumors does not seem to have received any attention, but heteroagglutinins do show variation in titer in the sera of rats following tumor inoculation (16).

Several workers have compared the susceptibility of different lines of birds to lymphoid tumor transplants with varying results: sometimes birds of a different genetic background behave similarly to a given tumor, sometimes with marked differences (54, 65, 92, 98, 193). In most instances the prime purpose has been to use the susceptibility to the transplant as a criterion of the susceptibility of the strain to spontaneous lymphoid leukemia. It is not surprising that such a relationship has yet to be convincingly demonstrated. Factors other than genetic influences may be operative in experiments of this kind. Acquired resistance may develop during the growth of the implant. Wood and Garren (193) found in such transplantation experiments that dosage levels were important, fine differences in resistance tending to be obscured by too heavy a dosage.

Eye and brain have been used as sites for the implantation of tumors in other animals, where the natural resistance to the transplant is high. This prompted Burmester (28) to attempt the passage of tumor strains RPL 12 and RPL 16 in the anterior chamber of the chicken eye. Tumors developed in the iris, ciliary body, and adnexa. In most cases there was a metastasis to the viscera, followed by death.

**Acquired resistance.**

1. To the Viruses: Engelbreth-Holm and associates noted on occasion a high percentage of recoveries following inoculation with their strain of erythroblastosis. Recovered birds were resistant to reinoculation with this and another strain (175). Recovery was accompanied by the development of neutralizing antibodies for the free agent. These antibodies were stable to heating at 55° C. for 30 minutes. Acquired immunity was not always permanent, since one bird refractory to nine inoculations died after the tenth. Ducks were found very suitable for the production of neutralizing antisera.

It has since been shown that rabbit antinormal chicken sera neutralizes both the erythroblastosis and myeloblastosis viruses (9). The serology of the leukemia viruses is complex, and this is discussed in detail for myeloblastosis by Eckert (86, 91). Burmester has demonstrated neutralizing antibodies in hens immunized with RPL 12 virus (31).

Regarding active immunization against leukosis, the findings of Engelbreth-Holm and associates have been repeatedly confirmed (110, 177, 188). Immunization with RPL 12 agent gave some promise that lymphoid leukemia may be controlled in the field by vaccination. Chicks less than 1 year of age, when given several injections of the fully virulent virus of visceral lymphomatosis or two injections when mixed with adjuvant, gave rise to progeny with a considerable resistance to lymphoid leukemia and osteopetrosis. Vaccines containing killed virus were less effective (30, 45, 105). Natural infection is acquired in the first few weeks of life so that, although immunity to the leukemia viruses is short-lived, there may be protection of the newly hatched chicks by passively transferred antibody. A disadvantage lies in the need for live virus and the consequent danger of spread to non-immunized flocks. An encouraging feature is the cross-immunity often obtained with the various leukosis and sarcoma viruses which suggests a close immunological interrelationship (98, 110). Unfortunately, vaccines directed against other viruses may be contaminated with lymphoid leukosis and be a factor in their distribution (37, 167).

2. To the Tumor Cells: Earlier workers noted that while the sera from birds which had recovered from erythroblastosis could neutralize the free leukemia agent, they were ineffective against agents within the leukemic cells (175). Cell-free plasmas or filtrates are commonly used in studies with the leukemia viruses, but with the transplantable lymphoid tumors, for reasons of the shorter incubation period, cellular transfers have been more common. An immune phenomenon, namely, the tendency of the implant to regress following small inocula or through the use of older birds, becomes quickly familiar to the student of these tumors.

Burmester and Prickett (41) noted the high incidence of regression with tumor RPL 12 and that birds, when this occurs, become resistant to large or repeated doses of the same agent over a long
period. In exploring the possibility of using the RPL tumors as vaccines against leukemia in the field, Olson confirmed these observations (160) and treated cellular inocula in various ways (161, 164) with a view to producing immunity in the absence of takes. The presence of cellular material but not necessarily tumor-producing activity was necessary for the development of immunity. Cross-immunity was obtained with other transplantable lymphoid tumors (36, 65), but normal tissues and infected material from spontaneous cases failed to immunize (36, 65). Material from field cases of lymphoid leukemia had only slight or no immunizing capacity against the RPL 12 implants. Unfortunately, lymphoid leukemia and lymphomatosis occurred at a higher level in birds immunized against tumor RPL 12 than in the nonimmunized controls (44).

Heat-stable (up to 66° C. for 30 minutes) cytotoxic antibodies, active in vitro and in vivo, developed in recovered birds receiving RPL 12 tumor material. This was taken to indicate that lymphoid tumor cells contain an antigen not normal to the host (27). Complement is required for the activity of cytotoxic antisera against some tumors (174, 192). Although the antibodies were heat-stable, the recipient’s complement would be present in the in vivo experiments with the RPL 12 tumor, and complement might be carried over from the donor of tumor cells in the in vitro experiments.

Two phases in the life cycle of implanted tumor cells were recognized by Love (140). For 10–17 days, growth was progressive. This was followed by a regression phase lasting for 3–4 days which was characterized by phagocytosis of intact cells. During the progressive phase, Love observed a reaction of both local tissue and distant organs to the implant, with associated changes in the lymphoid and plasma-cell series. Eventually, the regressing tumor disappeared to leave only a few lymphoid foci at the site of inoculation, with the spleen returning to normal. Concomitant immunity developed during the growth of the tumor.

**Heterologous Transplantation**

Numerous attempts have been made to transmit leukemia to other species of birds, such as ducks, geese, pigeons, pheasant, quail, canaries, water fowl, parrots, sparrows, peacocks, doves, guinea fowl, and turkeys, and also to guinea pigs, mice, and rabbits. In most cases disease has not followed inoculation, but guinea fowl, pheasants, and turkeys have shown transient anemia and sometimes a leukemia (171). The B.A.I. strain of leukemia has produced disease in a high percentage of embryonated eggs from five heterologous species—duck, turkey, guinea fowl, pheasant, and quail (171).

Turkeys were found to be tolerant to the Rous virus if the turkey embryos or 1-day-old poults were given inoculations of normal whole blood from the strain of fowls in which the tumor was propagated (116). No experiments of this type had been reported with the leukemia viruses, but in view of their resemblance in many respects to the Rous virus, a similar success might be expected. It is difficult to see, however, what practical purpose this would serve since the chicken is such an excellent experimental animal.

**Location of the Virus**

An early attempt to determine the location of the leukemia virus in infected birds was made by Furth (103), who by infectivity titrations measured the relative concentrations of erythroblastosis virus in the cells and plasma. The titration limits were extended for plasma, but the percentage of takes was greater for red cells. The infectivity of cellular and cell-free inocula have a different basis, and consequently there is a need to treat immunity to tumor cells and to tumor viruses separately (176). This is a limitation to the use of chick titration for the demonstration of the location of viruses. Contemporary virus workers are now using electron microscopy (191) or fluorescent antibody technics (156). The former but not the latter has been applied to the study of the leukemia viruses. If difficulties in preparing specific antisera could be surmounted, fluorescent antibody technics would have many advantages.

Studies with electron microscopy in erythroblastosis, myeloblastosis, and lymphoid leukemia have been made on ultrathin sections of pellets (14) and particles (6, 114, 178, 180) obtained by ultracentrifugation of infected plasmas, and also on sections of organs and tissues of infected birds and of tissue cultures (13, 76, 78, 166). The viruses are represented by spherical particles of 80–140 m in size, with central dense cores of 20–40 m. Some of the statements regarding the location of these particles have been rather contradictory (78, 166). The proportion of infected cells in which these particles can be found may be as low as 2 or 3 per cent (77, 78). Unfortunately for the interpretation of these findings, similar particles may be found in the plasma and tissues of normal birds (13, 114). Experiments with tissue culture of the RPL 12 virus, in which titrations have been made, lend weight to the suggestion that the particles observed by the electron-microscope are the actual virus particles. While considerable numbers of electron photomicrographs of the leukemia viruses
have appeared in various publications, the reviewer (who has no electron microscope) cannot resist quoting the cautionary remarks made by Williams (191): "With tissue cultured cells in general there will have to be a great deal of work done before notions gained from electron microscopy can be quantitatively sound. When sections are cut in fragments of tissue taken from portions of intact animals or plants, I believe the establishment of quantitative correlations is essentially impossible because of the heterogeneity of the samples observed and bioassayed."

There has been a tendency to excuse a lack of correlation between the found and the expected, as, for example, to explain the absense of demonstrable virus in myeloblasts in myeloblastosis; it has been suggested that this might be due to rapid formation and secretion or to the virus being present as in nondemonstrable precursor state (106).

Swedish workers have been interested in the distribution of virus within infected erythroblasts of birds infected with erythroblastosis. They have attempted the fractionation of the structural constituents by differential centrifugation in sucrose media (14) and fractionation of cytoplasmic extracts on silica columns (100, 172, 187).

DISEASE PROCESS

The pathology of these diseases has been described in detail elsewhere, and a further description would serve no useful purpose. In general, leukosis consists of an over-growth of primitive blood cells. The over-growth is usually looked upon as malignant and serving no useful purpose. This may be taking too much for granted. Erythroblastosis, for instance, has some features suggestive of a hemolytic anemia, i.e., depression of red cell count, circulating pro-erythroblasts, enlarged spleen and hemoglobin present in the plasma (18) in the terminal stages.

Leukemic sera or plasma from cases of erythroblastosis behaves electrophoretically like sera that have been altered through incubation at 37° or 57° C. The addition of hemoglobin accelerates this change, which appears as a "shift" in the globulins. It is unlikely that this alteration occurs in vivo, but if it does it would be expected that a variety of biological systems might be affected.

The close correlation of ATPase activity (181) with the infectivity of preparations of myeloblastosis virus has already been noted. Present evidence suggests that the enzyme is closely associated with, if not part of, the virus particle. It would seem important that the biological effect of large amounts of ATPase in the circulation should be determined. Alkaline and acid ATPases are found in leukocytes (17), and it is possible that the presence of ATPase in the plasma is due to destruction of leukocytes and is analogous to hemoglobin liberation during lysis of red cells.

Another important question is the relation of the lymphoid foci encountered in sections of organs and tissues of the fowl to the later development of lymphoid leukemia. The purpose of these foci is not known. Some regard them as abnormal. Lucas and associates have studied these lymphoid foci in detail in several species of birds (143–145, 157). The foci appear shortly after hatching, are more extensive in flocks with a high incidence of leukemia than in flocks in which leukemia is less important (148), and increase following injection of the RPL 12 virus (146, 147).

One of many possible explanations of the correlation would be that the lesions provide a suitable soil for the later development of lymphoid leukemia, since, by providing a larger number of cells to become diseased, it may increase the chances of this happening (122).

CONCLUSIONS

It seems certain that in the future the trend in leukosis research will be from in vivo to in vitro studies. Bioassay methods in chicks, intensively studied in recent years, have shown many limitations. Tissue culture seems likely to supplant chicks for this purpose, especially if better indicator systems become available.

There is the virtual certainty that infective nucleic acid will eventually be obtained from the leukemia viruses as it has been from other viruses (2, 118). This will lead to extensive physicochemical investigations on the isolated nucleic acid.

A better understanding of the pathogenesis of the leukotic process may emerge from the observations of increased ATPase and the presence of hemoglobin in the plasma of birds infected with myeloblastosis and erythroblastosis. This information may help to clarify the relationship between the different disease entities within the leukosis group and may show whether one or more viruses are involved. The latter problem has interested research workers in this field since Ellerman's time and is still a key problem. Possibly the answer may come from serological investigations, but satisfactory serological methods for the study of leukemia viruses have yet to be developed. Currently serology is limited to the demonstration of neutralizing antibodies. Zilber and associates (151) and Makari (149) were able to differentiate antigens in neoplastic cells from the antigens of normal
cells by means of in vivo and in vitro technics utilizing the anaphylactic response of guinea pigs. The potentialities of these technics should also be investigated in relation to the leukemia viruses, since it would be possible for the sensitized guinea pig or its uterine to be desensitized to antigens present in normal tissue, prior to the test with infected tissue.

There are innumerable other approaches that can be visualized and that will presumably be followed in the studies of fowl leukoses. A number of important questions remain unsolved, as for instance:

What is the relationship of the leukoses to each other, to lymphomatosis and to osteopetrosis?

What is their relationship to the other tumor viruses, to other spontaneous fowl tumors, and to carcinogen-induced fowl tumors?

What is their relationship to the animal and human leukemias?

What is the true nature of the condition?

At the present time the leukoses are generally accepted as neoplastic. If this is correct, it is doubtful whether the answer to the first three questions will be realized until the nature of neoplasia is known.

Engelbreth-Holm (96) considered the agents of fowl leukosis and chicken sarcoma as "endogenous," but if lymphoid leukoses and erythroblastosis are related, present evidence would suggest that the agents are of a contagious nature. This constitutes the most important known difference between the avian and animal leukoses. There are certain differences in the pathological process between the leukoses of the fowl on the one hand and man on the other, but it is usual to explain these on the histological and anatomical differences in the two species. It remains to be seen whether the analogies between leukosis in the fowl and in other species will be weakened or strengthened by further experiments.

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REFERENCES


42. The Development of Highly Malignant Tumor Strains from Naturally Occurring Lymphomatosis. Ibid., 5:652-60, 1945.
44. The Occurrence of Neural and Visceral Lymphomatosis in Chickens Proven Immune to Transplants of Lymphoid Tumor Strains. Poult. Sc., 25:616-21, 1946.
71. Davies, M. C., and Sharpless, G. R. Electron Micros-


74. Defendi, V.; and Sharpless, G. R. The RPL 12 Lymphosarcoma Virus in Tissue Cultures. Ibid., 100.


80. ———. Agent of Fowl Leukosis in Tissue Cultures. Ibid., 2:626-31, 1942.


97. ———. Variation in the Percentage of Takes in 3 Strains of Chicken Leukosis. Ibid., pp. 306-77.


110. Guérin, M., and Guérin, P. Étude sur l'immunisation...


132. LANKEE, N. The Effect of Adrenocortico-trophic Hormone (ACTH), Cortisone and Hydro-Cortisone on the Growth of Experimental Lymphoid Tumors in Chicks. Ibid., pp. 869-76.


162. ———. Immunization against a Lymphoid Tumor of the Chicken. II. Use of Centrifuged material. Ibid., pp. 309-18.


188. Vigne, P. Action favorisante de l'extrait antit hypophysaire total sur la réceptivité de la poule au virus du...


REFERENCES (ADDENDUM)


The Experimental Transmission of Avian Leukosis: A Review
C. le Q. Darcel

Cancer Res 1960;20:2-17.

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