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The History of Avian Medicine in the United States
V. Insights into Avian Tumor Virus Research

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INTRODUCTION

This historical review describes and discusses the discoveries that have been most important to the understanding of avian tumor viruses from the viewpoint of a biologist and a poultryman. It emphasizes the discoveries that were "firsts" in tumor virology, and, where appropriate, compares the state of the art in avian tumor virus research with that in tumor virus research of other animals. We will interject personal understandings, opinions, and philosophies, as well as a few experiences. This report is not a review of research on avian leukosis and related neoplasms. Such reviews are available elsewhere (9,16,22). Furthermore, to keep this article a reasonable length, we have omitted many reports and experiences.

AVIAN TUMORS IN BIOLOGY

Research concerning avian tumor virology has been the leader in cancer virus research. By this we mean that many discoveries about tumor viruses were first made with avian species, and the biology of tumor viruses in chickens has often served as a model for the study of tumor viruses of other animals, including man. The leadership of avian tumor virology in the viral etiology of cancer is in contrast to the areas of chemical- and radiation-induced cancer and surgical, chemical, and immunological therapy of cancer, where the avian system has been of limited use.

Chickens have played a leading role in tumor virology because viruses causing cancerous growths were discovered and isolated from chickens many decades before they were discovered in any other animal. Secondary favorable factors are that chickens have a relatively short life cycle, produce a large number of off-

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Table 1. Classification of avian leukosis complex as reported by Biggs (3).

Common names	After Cottral (1952)	After Chubb and Gordon (1957)	After Biggs (1962)
Gray eye or Pearly eye disease	Ocular lymphomatosis	Ocular — Lymphomatosis	Marek's disease — Ocular
Fowl paralysis or Range paralysis	Neural lymphomatosis	Neural (*Fowl paralysis*) — Lymphomatosis	Marek's disease — Neural (*Fowl paralysis*)
		Visceral — Lymphomatosis	Marek's disease — Visceral
Big liver disease	Visceral lymphomatosis	Diffuse — Lymphoid leukosis — Leukosis	Leukosis — Lymphoid leukosis — Diffuse
		Discrete — Lymphoid leukosis — Leukosis	Leukosis — Lymphoid leukosis — Nodular
Fowl leukemia	Myeloblastosis or granuloblastosis	Diffuse — Myeloid leukosis — Leukosis	Leukosis — Myeloid leukosis — Diffuse
White tumors	Myelocytomatosis	Discrete — Myeloid leukosis — Leukosis	Leukosis — Myeloid leukosis — Nodular
Fowl leukemia	Erythroblastosis	Erythroleukosis — Leukosis	Leukosis — Erythroleukosis
Marble bone or Big bone disease	Osteopetrotic lymphomatosis or Osteopetrosis	Osteopetrosis	Osteopetrosis

Conditions interrelated with leukoses:
- Sarcomas
- Endotheliomas
- Renal tumors

spring, and are easy to grow and maintain under laboratory conditions; and the embryonated chicken egg is one of the easiest and most commonly used hosts for study of viruses. In addition, poultry is an important agricultural commodity, making tumors an important economic factor in production.

While many of the advances were being made in avian tumor virology, great progress was being made in poultry husbandry. One result was the rearing of very large numbers of birds under crowded conditions, leading to much bird-to-bird contact and contaminated environment. Tumors became a major source of death loss; and when federal inspection of slaughtered poultry became mandatory, tumors were a major reason for condemnation. Under domestic rearing conditions, chickens have a higher incidence of tumors than any other animal; and losses have been reported of half the chickens in flocks numbering 1,000 or more. Also, tumors are detected at an earlier age in chickens than in any other domestic animal or in man, i.e., 7–8 weeks of age.

HISTORICAL ERAS

The history of research on avian transmissible neoplasms can be divided into three eras. The first began in the late 19th century, with work of pioneering biologists having broad medical and veterinary interests. They had a great curiosity about cancer and its causes and were permitted freedom to do basic research to satisfy that curiosity. During that era many clinical and pathological descriptions of tumors were recorded, and the transmissibility of some of them was first discovered.

The second era was during the second, third, and fourth decades of this century. During that period there were great advances in poultry husbandry and much expansion in the poultry industry. Because of the resulting population density, losses increased from infectious diseases of all types. Research was conducted primarily at land-grant universities in the United States, and studies were directed largely toward practical control of those diseases.

The third era, which still continues, is characterized by publicly supported goal-oriented agricultural and medical research. Because of the long incubation period of the neoplastic diseases, the expensive nature of the research, and the importance of the diseases, the governments of several countries took the lead. Notably, first, the United States established the Regional Poultry Research Laboratory (RPRL), in 1939, and then England established the

Leukosis Experimental Unit at the Houghton Poultry Research Station (HPRS), in 1959. Those national research laboratories were established for the express purpose of conducting research on avian leukosis and related neoplasms and with the goal of developing control measures. During that era, the war against human cancer was launched, and research funds became available for studies of avian neoplasms as experimental models for human cancer. The expansion of publicly supported research led to our present understanding of the nature, cause, and control of avian transmissible neoplasms.

The Regional Poultry Research Laboratory (RPRL)

Establishment of the RPRL had a profound effect on investigations of avian leukosis. It quickly acquired worldwide recognition for its advanced research on avian leukosis.

The impetus behind the concept of a central laboratory devoted to the study of leukosis was the lack of progress of many experiment stations in developing some sort of controls. Losses due to this disease complex were becoming more devastating year by year.

Initial plans for the Laboratory were laid at a conference called on April 5, 1937, by Prof. E. L. Dakan, of Ohio State University. The conference resulted in the appointment of a project committee by the experiment station directors of the Northeastern and North Central States. This important committee consisted of Prof. J. C. Graham, Massachusetts; Dr. F. B. Hutt, New York; Prof. H. C. Knandel, Pennsylvania; Dr. D. C. Warren, Kansas; Dr. B. H. Edington, Ohio; and Dr. L. E. Card, Illinois, Chairman.

The project plan called for the establishment of one cooperative regional project on poultry disease control. It was approved by the directors of the two regions, by Dr. James Jardine, Chief, Office of Experiment Stations, and by Dr. J. R. Mohler, Chief, Bureau of Animal Industry, USDA. On December 23, 1937, Henry A. Wallace, Secretary of Agriculture, approved establishment of the Laboratory, with funds provided by the Bankhead-Jones Act of 1935.

The year 1939 saw the dedication of the Laboratory, appointment of the Laboratory staff, and a meeting with collaborators representing the Northeastern and North Central state experiment stations to review current and proposed studies. Dr. J. Holmes Martin was the Laboratory director during the first difficult year,

followed in 1940 by Mr. Berley Winton. Dr. N. F. Waters was appointed to head studies in research in genetics, and Dr. C. F. Brandly was appointed to plan and direct research in virology and pathology. In the next year Dr. B. R. Burmester was appointed to work on the physiological aspects of the disease complex, and in 1944 Dr. A. M. Lucas was appointed to do basic work on the gross and microscopic anatomy of the chicken.

Because the Laboratory was established as a regional one, investigators at experiment stations of the two regions collaborated extensively with those at the Laboratory. Cooperative studies were initiated at 8 of the 25 state experiment stations. The regional programs were gradually reduced in importance, however, giving way to informal cooperative research with many laboratories throughout the United States as well as in a few foreign countries.

Through 1977 the scientists of the Laboratory published 339 original research papers, 81 research review manuscripts, 113 abstracts, and 62 miscellaneous articles related to avian leukosis.

CLASSIFICATION AND TERMINOLOGY

During the first era, the terms used in referring to neoplasms of the fowl varied widely with different investigators. Much confusion resulted. At the outset, pathologists used descriptive terms similar to those common in human medicine. Names such as lymphosarcomatosis, aleukemic lymphadenoma, visceral lymphomata, lymphatic leukosis, lymphocytoma, or simply leukemia were used to refer to what we now call lymphoid leukosis (LL) and laymen commonly call "big liver disease." Marek's disease (MD) was first referred to as polyneuritis, neuritis, neurolymphomatosis, or neural-lymphomatosis. Poultrymen referred to it as range paralysis or fowl paralysis.

For a long time, LL and MD could not be etiologically separated. Gross lesions in both diseases were similar. Transmission experiments with LL were contaminated with the infectious agent of MD. Transmission experiments with MD frequently failed because of the cell-associated nature of the causal virus, and LL frequently occurred in transmission experiments because the chicks used were infected congenitally. These confusing results led to the term "avian leukosis complex," which was adopted by a group of pathologists meeting at the RPRL in 1940 and reported by Dr. George Cottral in 1952 (see Table 1).

Many investigators had pointed to marked differences between fowl paralysis and leukosis, and in 1954 Dr. J. G. Campbell,

Edinburgh, Scotland, presented strong arguments that the two are distinct diseases. Largely because of that presentation, Drs. L. G. Chubb and R. F. Gordon (9), of HPRS, suggested the classification shown in the third column of Table 1. However, both Campbell and Dr. P. M. Biggs, also of HPRS, thought that retaining the term lymphomatosis would continue to contribute much confusion, so the terminology given in the fourth column of Table 1 was presented by them and adopted by the First Conference of the World Veterinary Poultry Association, in 1961 (3).

Many people, particularly at field diagnostic and poultry-meat inspection levels, did not, and still do not, use that terminology. A few scientists, notably Dr. Martin Sevoian, continued to use pathology nomenclature. They have even broadened the term lymphoid leukosis to include tumors caused by the reticuloendotheliosis group of viruses. Thus, they refer to lymphoid leukosis types I, II, and III. Those views persisted even after discovery of the causal agent of MD, in 1967 (10,31).

Another group of scientists, led for the most part by some working with murine RNA tumor viruses, held the view that Marek's disease virus (MDV) played only a secondary role in tumor development.

With the discovery of other herpesviruses causing mammalian neoplasms, e.g., herpesvirus saimiri of monkeys and the Epstein-Barr (EB) virus of Burkitt's lymphoma, and the revelation that a herpesvirus of turkeys (HVT) protected chickens against the herpesvirus-induced MD, the herpesvirus as a cause of tumors became accepted.

Three groups of avian tumor viruses are currently recognized: 1) the leukosis/sarcoma group of RNA tumor viruses, which cause LL (a tumor that originates in the bursa of Fabricius), erythroblastosis, myeloblastosis, myelocytomatosis, sarcomas, and related neoplasms; 2) MDV, a DNA-containing group-B herpesvirus causing lymphoproliferation in various tissues; and 3) the reticuloendotheliosis group of RNA tumor viruses, causing reticuloendotheliosis in turkeys, ducks, chickens and many other avian species.

The Leukosis/Sarcoma Group

The pathologic condition that was likely caused by this group of viruses was first recorded in Europe, by Dr. F. Roloff (26) in 1868, when he reported on a chicken that had "lymphosarcomatosis." Leukosis of the fowl was first described in 1896, by Dr. U. Caparini, in Italy. Although the tumors described by Roloff and

Caparini may have been of the type now known to be caused by leukosis/sarcoma viruses, Drs. E. E. Butterfield and Mohler, in 1905, described several cases from the District of Columbia and Michigan which were called "aleukemic lymphadenosis" and were the first clearly reported cases of LL. Those reports were soon followed by reports of Drs. Jutaka Kon, in Germany, and A. S. Warthin, in the United States. The latter used the term "lymphocytoma" and was the first to recognize the aleukemic and leukemic conditions as two forms of the same disease process and to consider both to be malignant neoplasms.

Other early contributors were Drs. E. E. Tyzzer and T. Ordway, of the Rockefeller Institute, New York, who described seven cases of lymphoma of the fowl in 1909, and Dr. M. Hobmaier, in Germany, who described lymphomatosis of the skin and viscera.

Filterable transmission. The transmissibility of tumors of this group was first demonstrated by Drs. H. Hirschfeld and M. Jacoby, in 1907, and was later confirmed in Germany by Drs. J. L. Buchard, in 1912, and H. Magnusson, in 1916, and in the United States by Dr. H. C. Schmeisser, in 1915. Drs. V. Ellermann and O. Bang (14), working in Copenhagen in 1908, reported the first successful transmission with a filterable agent.

Table 2 lists some of the various transmissible strains.

Although transmissibility was shown early, even with filtrates, many well known investigators over a period of 30 years considered LL to be a nontransmissible neoplasm. That hypothesis was based largely on negative results of transmission experiments with various materials from affected chickens. The view was expressed during the period 1928 to 1934 by Drs. C. W. Anderson and O. Bang, of Denmark; by F. P. Mathews, F. L. Walkey, W. H. Feldman, C. Olson, and R. Fenstermacher, of the United States; and by Drs. Ch. Oberling and M. Guerin, of France. Those and other scientists held that the intravascular forms of leukosis (erythroblastosis and myeloblastosis) were transmissible but that the extravascular form (LL) was a nontransmissible neoplasm.

At the Cornell Medical School, Dr. Jacob Furth (15) and coworkers, during the period 1931-1937, provided good experimental evidence that a transmissible filterable agent was the cause of LL, and extensive research at the RPRL between 1946 and 1947 (5) provided conclusive proof that a virus caused LL. The contagious nature of LL was suggested by Drs. C. W. Barber and Waters, in 1942 and 1949. Later, Burmester, between 1954 and 1957, provided

proof and delineated various factors influencing contact transmission; also, he was the first to show that the Rous sarcoma virus was readily transmitted by contact.

As early as 1910, Dr. Peyton Rous (29), working at the Rockefeller Institute, showed that the avian fibrosarcoma was transmissible with cell-free filtrates. That discovery and subsequent research with the Rous sarcoma virus proved to be so significant to progress in cancer research that, in 1968, Dr. Rous was awarded a Nobel Prize. This neoplasm and its causal agent were long considered completely separate from leukosis. The first suggestion of a close relation was in the 1930's, when a number of investigators, including Drs. Oberling, M. Guerin, K. Jarmai, A. Rothe Meyer, J. Engelbreth-Holm, J. Furth, E. L. Stubbs, Erwin Jungherr, and A. B. Wickware, consistently produced both leukosis and sarcomas with the same preparations. This response suggested either a polyvalent virus or a mixture of two or more mono-potential viruses. This close relation was confirmed by Drs. B. R. Burmester and T. N. Fredrickson; however, proof was provided by Dr. Harry Rubin's discovery in the 1960's of resistance-inducing factor (RIF) and Rous-associated virus (RAV) and their role as "helpers" to Rous sarcoma virus (see next section).

Egg transmission and virus-free flocks. Many investigators had suggested that virus was transmitted through the egg, but egg transmission was not established until the results of a series of experiments were reported by Cottral *et al.* (12) and Burmester and Waters (6), 1954 to 1956. Egg transmission was later confirmed many times, particularly when rapid *in vitro* tests for leukosis viruses became available and the cycle of infection of the virus was uncovered. Egg transmission is now recognized as the major means of persistence of the virus from one generation to the next.

By fortuitous selection and rearing in strict isolation, Dr. Cavett Prickett and Waters, working at the RPRL in 1944, were the first to develop a line of chickens free of LL virus. A more definitive method became available when Rubin developed the RIF test; in 1963 he and Dr. Walter Hughes, in work to develop virus-free flocks (17) at Kimber Farms, California, demonstrated its efficacy in the detection and elimination of hens that transmit virus in their eggs. Subsequently, many pharmaceutical companies and other organizations, using the RIF test, have developed flocks free of virus to supply eggs for vaccine production and for research.

Recently, Dr. J. L. Spencer, Agriculture Canada, Ottawa, Ontario, Canada, while on a sabbatical leave at the RPRL, found that infected hens that produce infected progeny have large amounts of virus or group-specific (gs) antigen in the egg albumen and in the vagina and cloaca. Moreover, the gs antigen can be detected directly by use of the complement-fixation test. Thus, simple, rapid methods now available might make possible the eradication of infection from commercial breeder flocks.

RIF, RAV, COFAL, and the like. In 1956, Drs. R. A. Manaker and Vincent Groupe (19) developed an *in vitro* method of assaying the Rous sarcoma virus. That discovery paved the way for Rubin, who in 1960 found that chick embryo fibroblasts infected with leukosis viruses were resistant to superinfection with the Rous sarcoma virus. That observation soon led to development of the resistance-inducing factor (RIF) test and to the discovery of Rous-associated virus (RAV), which was soon discovered to be an LL virus. That discovery led to recognition of the close relation between sarcoma and leukosis viruses, and the RIF test was used to confirm many of the observations, e.g., egg transmission, that had previously required a more laborious *in vivo* assay. The RIF test was shortly replaced by the complement-fixation test for avian leukosis viruses (COFAL test) developed by Dr. P. S. Sarma in 1964. The COFAL test, and the newer phenotypic mixing (PM) test developed by Dr. William Okazaki, are currently used to detect and assay for leukosis viruses. Discovery of those *in vitro* tests set the stage for a rapid increase in new knowledge on all aspects of the leukosis/sarcoma viruses and related neoplasms.

Subgroups and defectiveness of leukosis/sarcoma viruses. In 1962 and 1963, Drs. H. Hanafusa, P. K. Vogt, and H. Rubin discovered the defectiveness of Rous sarcoma virus. The studies of those investigators, together with studies of Drs. Ron Ishizaki and R. G. Duff, from 1964 through 1969, led to the recognition of four leukosis/sarcoma virus subgroups: A, B, C, and D. This subgrouping was based on differences in host range, interference with virus of the same subgroup, and viral envelope antigens detected by serum neutralization. Another important finding was the characteristic of defectiveness of some sarcoma viruses, i.e., they lack the genetic information necessary to reproduce themselves. They require a "helper virus" to co-infect the same cell to provide the missing components in the viral genome to render the sarcoma virus capable of reproduction. The characters of the sarcoma virus envelope are those contributed, hence the name "helper virus."

Genetic resistance. Waters *et al.* (33), in 1958, were the first to identify the mode of inheritance of genetic resistance to a leukosis virus. Strangely, in their work in both 1958 and 1963, they found that resistance to erythroblastosis appeared dominant. Dr. L. B. Crittenden was unable to confirm the dominance of resistance even with similar matings of the same lines of chickens. He has speculated often on whether the populations of chickens changed and the genes were lost, or whether errors in recording or interpretation of experimental results could have been responsible. Resistance to leukosis/sarcoma viruses was dominant in two other instances. The first was a single male observed by Drs. L. N. Payne and Biggs at HPRS, which sired all resistant progeny. Unfortunately, the male died, and the progeny had been disposed of before the significance of the observation was realized. The researchers were therefore never able to publish on it. The second instance was with subgroup E, the endogenous viruses.

Drs. Waters at the RPRL, A. M. Prince at Yale, and A. W. Nordskog at Iowa State University described resistance to Rous sarcoma virus. Their matings were sufficient to eliminate maternal effects and show that the segregation patterns fit a single-gene hypothesis. In 1961, Waters and Burmester found that resistance to Rous sarcoma virus was mediated by a single autosomal recessive gene. Shortly thereafter, Crittenden, Okazaki, and Payne showed that the host range, i.e., cellular resistance to infection with leukosis/sarcoma viruses, is controlled by the same genes in cell culture, in embryonated eggs, and in hatched chickens, and that there are different genes for the different subgroups of leukosis/sarcoma viruses. Thus, there was a basis for selection of breeders for resistance to infection from embryo or cell-culture inoculation.

In 1965, Crittenden and Okazaki (13) found that chickens resistant to Rous sarcoma virus were resistant also to the viruses of erythroblastosis or LL, i.e., the same gene was operating for resistance to each of these viruses. They also found that it operated at the level of preventing infection of the host by the virus. Resistant chickens also lacked antibody to the virus. Crittenden later postulated a second level of resistance. Chickens susceptible to virus infection could be susceptible or resistant to tumor development after virus infection. This may have been because the target cells of resistant chickens were unable to become infected with the virus, or the immunological system of the susceptible chicken was unable to reject the nascent tumor.

In recent years our understanding of the genetics of the avian tumor viruses and the genetics of virus-host interaction in the induction of disease has grown by leaps and bounds. Resistance to different subgroups of leukosis viruses, of which there are now seven (two are of pheasant origin), is controlled by different loci, some with several alleles. There are also dominant genes for gs antigen production, chick helper-factor production, and production of endogenous virus.

Role of bursa of Fabricius in lymphoid leukosis. In 1962 the RPRL was visited by a pediatrician, Dr. R. D. A. Peterson (see Peterson *et al.*) (25), who was working with an immunology group under the direction of Dr. R. A. Good at the Medical School of the University of Minnesota. He suggested that the bursa of Fabricius might be directly involved in the immune system of the chicken and alter the pathogenesis of LL. Experiments showed that bursectomy completely prevented LL, and this technique more than any other pointed to the central role of the bursa in LL and in humoral antibody production. Subsequently, Payne, while on sabbatical at the RPRL, together with Drs. M. D. Cooper, of the University of Minnesota, and H. G. Purchase, of the RPRL, in a detailed study of the pathogenesis of LL, found that the first signs of transformation were clearly visible in the bursa of Fabricius at 8 weeks of age. Later studies by Burmester and, subsequently, Dr. C. H. Romero with hormonal or chemical bursectomy confirmed that the bursa is the primary target organ. Dr. Douglas Gilmour, of New York City, and Purchase, now at Beltsville, Maryland, have recently shown that the bursa also plays a role in genetic resistance to LL.

Immunobiology. Attempts to produce a vaccine effective against some form of leukosis began with Ellermann, in the early 1920's. Since then, most investigators of avian leukosis have attempted to produce such a vaccine. In 1932, Furth found neutralizing activity in serum, and Jarmai induced passive immunity. In 1937, Dr. E. Uhl reported on attempts to actively immunize with aluminum hydroxide adsorbate, and in 1945, Dr. E. P. Johnson obtained "promising" results with desiccated material. More recently, Dr. J. W. Beard, at Duke University, immunized birds with inactive virus. All of the foregoing research was done with the intravascular, i.e., leukemic, forms of leukosis.

The first tangible results with LL were obtained by Burmester *et al.* (7), who reported in 1957 that repeated injection of

hens with virus of LL induced significant immunity in progeny chicks. The hens and chicks had high levels of neutralizing antibodies, and treatment of viral material with propiolactone or formalin reduced the immune responses. Later work showed that when treatment resulted in complete inactivation, there was no measurable immune response. An effective vaccine continues to elude the best efforts of investigators.

Oncogene and protovirus hypotheses and molecular biochemistry. As early as 1964, Okazaki and Crittenden noted that the avian leukosis virus gs antigen without infectious virus was present in certain line 15I embryos. Its occurrence changed with males, suggesting an inherited control. At that time, Payne of HPRS was spending a sabbatical leave at the RPRL. After he returned to England he worked on this curious situation, and in 1968 he reported that gs antigen was indeed inherited as a single gene. This classic publication, along with evidence provided by work of Dr. Robert Dougherty and others, gave strong impetus to Dr. Robert Huebner's "oncogene" and Dr. Howard Temin's "protovirus" hypotheses to explain the origin of cancer. Briefly, the hypotheses state that all of the information necessary for the formation of tumors (oncogenes) or tumor viruses (provirus) is present in every normal cell but is repressed. Various factors act to derepress this information, after which tumors result. Those concepts and additional research led to discovery of the endogenous viruses.

Integration of provirus in normal cells was essentially accepted with the discovery of reverse transcriptase by Temin and Dr. David Baltimore. That momentous discovery with avian tumor virus, which was the basis for a Nobel Prize jointly awarded those investigators, led to great strides forward in the molecular biology of tumor viruses in general. The development of hybridization techniques soon followed, with contributions by Drs. Paul Neiman, Michael Bishop, Harold Varmus, and others. Even more recently, the discovery and use of restriction endonucleases has allowed genome sequencing. A key to these discoveries was the development of many virus mutants by Vogt and co-workers. Mutants with specific deletions are required to determine the function of various segments of the genome.

Before long we will understand the molecular events of viral infection and replication; and much progress is being made toward the more important goal of understanding how viruses cause their characteristic tumors.

Lymphoid tumor transplants. One of the proponents of the nontransmissibility of LL was Dr. Carl Olson, but he found an outlet for experimentation by developing the now famous Olson tumor transplant. That experiment started with a Rhode Island Red by Plymouth Rock crossbreed hen in the backyard of a librarian at Massachusetts State College. According to Dr. K. L. Bullis, the hen, Case No. 452, had LL. Blood was obtained from the hen's heart, and Olson injected a few young chickens by the wing vein. One of the chickens developed a tumor at the site of injection.

The tumor was successfully transferred to other chickens by injection of tumor suspensions. This tumor started a long series of passages, first by Olson. Then, when he was called into the armed services, in 1942, he gave the tumor to Brandly of RPRL, and when Brandly was called into service to work with Jungherr on fowl plague, the work of maintaining Olson's tumor fell to Prickett and Burmester, who transferred the tumor once each week to a new batch of chickens. In other studies with this tumor strain, they found that all birds having a growing tumor that regresses are solidly immune to challenge with a second or third transplant, although, surprisingly, such birds may later develop LL. Perhaps more important was the finding that the Olson tumor transplant, also identified as RPL 12 at the RPRL, contained a virus that caused not only LL but also erythroblastosis and osteopetrosis.

These studies suggested an examination of other naturally occurring cases of LL. Using inbred line 15, developed by Waters as especially susceptible to LL, Burmester and Prickett, in 1946 and 1947, obtained good transmission with 13 of 17 cases and developed eight transplantable strains. Most of these strains contained a virus very similar to the one found in the Olson tumor.

In a laboratory in Italy, Dr. F. Pentimalli transplanted a chicken lymphosarcoma. He reported this transplant in 1941 in Volume 1 of *Cancer Research,* which contained Olson's (23) first report on his chicken tumor.

Transmissible fowl leukosis. Although many investigators long questioned the transmissibility of LL, they did not question the so-called "transmissible leukoses." Some of the strains isolated and studied were reported by Olson and are shown in Table 2.

Most of these strains produced more than one type of leukosis or other neoplasm, i.e., erythroblastosis, myeloblastosis, and LL, and some even produced fibrosarcoma, endothelioma, osteopetrosis, or myelocytoma. Classical isolation, transmission, and descriptive

Table 2. Synopsis of various transmissible strains of fowl leukosis agent as reported by Olson in 1940 (22).

Author	Year described	Designation of strain	Number of serial passages	Type of disease produced
Ellermann and Bang	1908	A	6	Leukosis
		B	1	Leukosis
		C	2	Leukosis
Schmeisser	1915	*	5	Leukosis
Magnusson	1915	*	1	Leukosis
Ellermann	1921	D	2	Leukosis
		E	6	Leukosis
		F	1	Leukosis
		G	1	Leukosis
Ellermann	1921	H	12	Leukosis
Stazzi	1927	*	**	Leukosis
Andersen and Bang	1928	*	**	Leukosis
Battaglia and Leinati	1929	*	2	Leukosis
Furth	1929	1	Numerous	Leukosis
Jarmai	1930	*	Numerous	Leukosis (erythroblastic) and sarcoma
Engelbreth-Holm	1931	R	Numerous	Leukosis (erythroblastic) and sarcoma
Engelbreth-Holm and Rothe Meyer	1932	T	Numerous	Leukosis (erythroblastic)
		T_1	16	Leukosis (granuloblastic)
Jarmai, Stenszky and Farkas	1932	*	5	Leukosis
Patterson, Wilcke, Murray and Henderson	1932	*	**	Leukosis, lymphomatosis and fowl paralysis
Olson	1932	I	3	Leukosis
		II	4	Leukosis
Furth	1933	2	Numerous	Lymphomatosis, myelocytomatosis, endothelioma
Rothe Meyer and Engelbreth-Holm	1933	E-S	Numerous	Leukosis and sarcoma

Table 2. continued

Author	Year described	Designation of strain	Number of serial passages	Type of disease produced
Oberling and Guerin	1933	*	Numerous	Leukosis and sarcoma
Johnson	1934	*	3	Leukosis, hemocytoblastosis, fowl paralysis
Nyfeldt	1934	*	Several	Leukosis (granuloblastic)
Engelbreth-Holm and Rothe Meyer	1935	ø	14	Leukosis (erythroblastic)
		AA	3	Leukosis (erythroblastic) and anemia
Stubbs and Furth	1935	13	Numerous	Leukosis and sarcoma
Furth	1936	12	Numerous	Lymphomatosis and osteochondrosarcoma
Schaaf	1936	*	**	Leukosis
Olson	1936	III	20	Leukosis
Lee, Wilcke, Murray and Henderson	1937	*	**	Leukosis, lymphomatosis, fowl paralysis
Pikowski and Doljanski	1938	*	7	Leukosis, sarcoma, reticuloendotheliosis
Fitch	1938	*	1	Leukosis
Hamilton and Sawyer	1939	*	12	Leukosis (erythroblastic)

*No specific designation given.

**Number of serial passages not indicated.

pathology studies were conducted by many investigators from 1908 to 1939.

Of outstanding significance was work of Beard (1) and his group at Durham, North Carolina, on the virology and pathology of myeloblastosis, erythroblastosis, and myelocytomatosis. The virus of myeloblastosis, on which Beard spent so much time, had a most interesting origin. In the late 1930's, Drs. W. J. Hall, C. W. Bean, and Morris Pollard, working in the Beltsville poultry laboratory of the USDA, Bureau of Animal Industry, obtained two chickens with fowl paralysis. One also had a large number of "lymphoid" cells in the blood. A suspension of enlarged nerves was injected into Rhode Island Red chickens. Subsequent passages were made with heparinized blood given intravenously. By the 17th passage, all birds died of what Hall called erythroblastosis, with an average latent period of 20 days. He named the causal virus BAI Strain A. When the RPRL started, in 1939, Brandly obtained from Hall chickens infected with this BAI Strain A. During the next 5 years it was passaged intravenously at the RPRL with heparinized blood of leukotic birds. During that period it caused a combination of erythroblastosis and myeloblastosis (the latter was also called granuloblastosis) in all birds. In 1944 Dr. E. P. Johnson requested the strain from the RPRL, and Burmester sent him a vial of heparinized blood. Johnson reproduced erythromyeloblastosis. In 1950 when he attended a lecture given by Beard on equine encephalitis, Johnson told Beard about his virus that caused different types of leukosis. The finding greatly interested Beard, so Johnson supplied him with a sick chicken. Blood from that chicken was the start of a long series of investigations by Beard. During the first 3 years the leukosis induced was called erythromyeloblastic leukosis. In 1952, Burmester visited Beard's laboratory and examined blood smears. He could find no evidence of erythroblasts. The immature elements were all of the myeloid series; in fact, they were uniformly myeloblasts. Hence, from then on the strain became known by Beard as myeloblastosis.

Beard investigated a second "pure" strain, Strain "R," causing a very acute erythroblastosis. Although both strains cause only one type of leukosis under usual experimental conditions, Burmester found that when birds were infected with very small doses of either virus and the experimental period was long, the incidence of LL was high, and birds inoculated with Strain A also developed kidney tumors and osteopetrosis.

Beard found two important advantages in working with Strain A, now called AMV (for avian myeloblastosis virus): 1) many chickens developed such high concentration of virus that the plasma was milky (1×10^{13} or more particles/ml); and 2) virus concentrations could easily be determined biochemically because the virus had on its surface an enzyme that acts on adenosine triphosphate. Strain A was the first tumor virus that could be produced in gram quantities for biochemical, immunological, and molecular studies. Beard and his group exploited the properties of this virus to the fullest, publishing over 150 papers. Beard also provided the means by which many other investigators made important discoveries regarding the properties and action of the RNA tumor viruses.

Cures for "leukosis." While reputable laboratories were attempting to understand the biology of LL, many unproved remedies for the disease were recommended. For example, in the 1920's and 1930's a good poultry veterinarian's pharmacy had an assortment of potions for the control of lymphomatosis. Iron and liver treatment was thought to reduce the incidence of leukosis.

In 1938, Drs. W. J. Butler and D. M. Warren of Kansas reported on the prophylactic and curative value of vitamin E. That finding was soon refuted by experiments of Drs. R. K. Cole, Jungherr, L. W. Taylor, and Johnson. Waters noted a reduction of lymphomatosis when the chicken diet was supplemented with a synthetic vitamin D rather than codliver oil. Olson, in 1962, reported that one batch of codliver oil increased the incidence of LL but had no effect on erythroblastosis or on neural lymphomatosis. Ten percent potassium iodide was at one time thought to be beneficial against fowl paralysis, and at one time the RPRL stored and processed tons of tomatoes and fed gallons of puree to chickens because of a claim that feeding tomatoes would prevent lymphomatosis. In 1950, Winton reported on those unsuccessful experiments, and in 1952 Dr. R. F. Gentry was unable to show that krebiozen, a substance isolated from horse serum, had any effect on LL. In the 1960's, Mr. Carlton Nash, of Nash Dinosaurland, in Massachusetts, promoted a secret formula which he claimed prevented the disease.

MAREK'S DISEASE

Marek's disease was first described in 1907, under the term polyneuritis, by Dr. Joseph Marek, a Hungarian (20). He attributed the characteristic lameness to the mononuclear infiltration found in peripheral nerves and spinal nerve roots. In 1921, Dr.

B. F. Kaupp found a similar disease in the United States and observed a frequent association with blindness. From 1926 through 1929, Drs. L. P. Doyle and A. M. Pappenheimer (24) coined the term neurolymphomatosis and studied the pathologic aspects of the disease. They found it to be frequently associated with tumors of the viscera, especially the ovary, and lesions in the brain and iris. They considered this disease to be unrelated to LL. Furth expressed a similar view in 1935. The type cell was a small lymphocyte.

Experimental transmission. The first reported successful transmission was in 1924, by Drs. N. Van der Walle and E. Winkler-Junius. In 1935, Furth found that transmission was successful only with viable cells whereas transmission of LL was successful with cell-free inoculums.

In the 1940's and 1950's results in studies on the etiology of MD were confusing, and progress was not significant. The lack of progress was due largely to frequent adventitious infections and to the low or erratic level of disease apparently induced. Not until work of Sevoian *et al.* (30), at Massachusetts in 1962, and of Biggs and Payne (4), at the HPRS, was the disease transmitted with regularity. Sevoian's results were at first not generally accepted, because, in trying to repeat them, others filtered their inocula and lost infectivity. The dogma prevailing at the time was that all viruses had to be filterable. When the cell-associated nature of MDV became known, the significance of Sevoian's findings was apparent. Sevoian's JM strain of MD became widely used as a standard pathogenic strain. More than 65 strains (or isolates) are now reported in the literature. The ability to transmit the disease soon led to the development of an *in vivo* assay system useful for quantitative studies and studies on the etiology, epizootiology, and pathogenesis of the disease.

New, acute form of disease appears in the United States. In 1949 and the early 1950's an acute form of MD appeared on the eastern seaboard of the United States, characterized by high mortality with tumors of the viscera as the dominant lesion, first described by Drs. W. J. Benton and M. S. Cover (2) of the Delaware Experiment Station. This disease, known as "acute leukosis," spread southward to Georgia and then westward to Arkansas, where it was first known as the "red leg" syndrome. The appearance of this acute form of MD and the beginning of compulsory inspection of poultry at slaughter, in 1961, resulted in enormous losses to the poultry industry, so that in the 1960's the major cause of economic loss to

the poultry industry was MD. The industry cried out for help, and researchers responded with increased emphasis on studies of the etiology of MD.

Virus etiology. One of the momentous breakthroughs in the study of MD came in 1967, when the virus was successfully grown in cell culture. This procedure did not come overnight, even in laboratories with experts in cell-culture work. The right combination came together at the HPRS when Drs. A. E. Churchill and P. M. Biggs (10) placed tumor cells of an MD bird on a chicken kidney cell culture. In the United States both Dr. B. W. Calnek, at Cornell University, and Dr. R. L. Witter, at the RPRL, tried infecting all kinds of chicken cell cultures, but without significant success. About that time Dr. Bart Rispens, of The Netherlands, visited the RPRL. He had been working with duck embryo cell cultures and suggested a trial. Immediately Dr. J. J. Solomon (31) set up duck embryo fibroblast cultures, seeded them with blood infected with the JM strain, and in a few days observed a cytopathic effect. Witter then inoculated chickens with those cultures and reproduced MD at a high rate. At the same time Dr. Keyvan Nazerian prepared cultures for examination under the electron microscope and was able to identify the discrete particles as herpes virions. He could relate them directly to MD because, several months earlier, he had identified similar particles in a gonad tumor of a bird with MD.

Biggs was on his way from England to Dallas, Texas, to attend the annual meeting of the American Veterinary Medical Association. Because he was a personal friend of Burmester and wished to renew acquaintances with scientists at East Lansing, he stopped at the Laboratory and showed workers his electron micrographs of the herpesvirus detected in kidney cells inoculated with blood from chickens with MD. Nazerian was able to show Biggs almost identical photographs of the same agent in duck embryo fibroblasts. Biggs and Nazerian were able to present their findings to those attending the annual meeting; however, the group from England got its work published in 1967, whereas the group in the United States chose a slower journal, and its results were not published until 1968.

Convincing evidence for etiologic agents of MD was found in considerable experimental data reported during 1968 and 1969 by investigators at the RPRL and the HPRS, but the final proof was provided by Calnek, when he reproduced MD with cell-free virus obtained from feather follicles.

Genetic resistance. Drs. F. B. Hutt and R. K. Cole (18) had for many years bred different lines of chickens at Cornell University for susceptibility and resistance to lymphoid neoplasms. Their selection, however, was based on a method of exposure and diagnosis that favored MD. Through continued selection, chickens genetically susceptible to MD were developed and were known as the Cornell S Line. Those chickens have proved very useful in MD research.

To confirm those observations and attempt a practical demonstration, Cole started a series of experiments on divergent selection of a random-bred population for resistance and susceptibility to MD. His efforts, reported in 1968, were surprisingly successful, and in three generations he developed stocks that differed greatly in susceptibility to MD. The Cornell N line was highly resistant to the disease, and the Cornell P line highly susceptible. Using those stocks, B. M. Longenecker, at Iowa, and Howard Stone, at the RPRL, working with Elwood Briles, of Northern Illinois University, were able to show an association between the B^{21} blood group antigen and resistance to MD. Although this pleiotropic association had great potential for industry application, it has not been used because of the great success of other methods of MD control.

Horizontal transmission. During the 1960's the epizootiology of MD was enigmatic. It appeared strictly cell-associated *in vitro* and could be transmitted only with intact cells. Any treatment that destroyed the viability of the tumor or blood cells would prevent transmission. Yet, the disease was highly contagious in nature. In 1968, Witter found that infection in droppings persisted much longer than one would expect from intact cells. This conundrum demanded an explanation, and Calnek *et al.* (8) provided the answer. They literally took the chicken apart and examined every organ for the presence of virus, using the fluorescent-antibody test. They found that the virus matured to its infectious form only in the feather follicles.

Purchase and Nazerian, at the RPRL, soon confirmed those findings. As feathers grow they shed dander, and that is the source of the virus contaminating the environment. Dr. J. N. Beasley and co-workers, at the University of Arkansas, and other investigators have shown that MD could be reproduced by poultry house dust and feather dander.

Attenuated Marek's disease vaccine. Churchill *et al.* (11), at HPRS, attenuated the virulent HPRS 16 MDV in 1969. That resulted in the first successful vaccine against MD and was the first

time that a cell-associated vaccine had been used. Churchill, recognizing the economic significance of the discovery, established a biologics company.

Herpesvirus of turkeys vaccine. While searching for the means of persistence of MDV in the environment, Witter examined turkeys as a possible intermediate host and reservoir for MDV in nature. From turkeys sent to the RPRL from Indiana by Dr. Joseph Ostendorf, a practicing veterinarian, Witter isolated a nonpathogenic virus. Shortly before that discovery, Drs. H. Kawamura and David Anderson, at the University of Wisconsin, also isolated a herpesvirus from turkeys. Further research was interrupted because Kawamura left for Japan and Anderson left for Georgia.

Soon after Witter *et al.* (34) isolated the HVT, he inoculated chicks with it, and they remained healthy; but serum agar-gel precipitin tests revealed cross-reactions with MD antigens. These cross-reactions prompted Witter to suggest to Okazaki that he run a protection test. Okazaki had been running protection tests with MDV virus he had attenuated by various protocols and was all set up for appropriate MD protection tests. The first tests resulted in 100% protection by HVT. In the meantime, Purchase, using fluorescent-antibody tests, confirmed the close antigenic relation between MDV and HVT; and Nazerian, using the electron microscope, characterized the ultrastructure of the virion and found it to be very similar to the MD virion.

This information and the finding that the HVT grew rapidly in duck as well as chicken embryo fibroblasts suggested the possibility that it might be a good vaccine against MD. Burmester, then the director of the RPRL, immediately recognized the economic importance of such a vaccine and requested that all effort on vaccine research be placed on HVT. He directed Purchase and Okazaki to organize and conduct a research program that would in a single year prove the efficacy of HVT as a vaccine and provide sufficient data to satisfy the requirements of the Biologics Division of the Animal and Plant Health Inspection Service of USDA. The assignment was fulfilled through the dedicated efforts of Purchase *et al.* (27), Okazaki *et al.* (21), and many other members of the Laboratory staff, and the vaccine was licensed by the State of Michigan in November 1970 and by the USDA in March 1971. At about the same time, Dr. C. S. Eidson, working at the Poultry Disease Research Center, University of Georgia, obtained some HVT from RPRL and vaccinated over 3 million chickens in a number of trials.

The high protection obtained provided the basis for the State of Georgia to issue a license in 1970. Similar herpesviruses were isolated from turkeys in England, in many other European countries, and in Australia. At least 18 strains or isolates of HVT are now reported in the literature.

The attenuated MD vaccine was superseded by the HVT vaccine because the latter was somewhat more effective and easier to grow than the former. Also, cell-free virus can be extracted from HVT-infected cells and a lyophilized vaccine prepared from it, whereas almost no cell-free virus can be extracted from the attenuated MDV. The cell-free lyophilized vaccine is now used most widely in Europe. Predominant in America is the cell-associated, wet, frozen preparation, because of its greater apparent efficacy.

The HVT vaccine has resulted in a remarkable economic benefit to human food production. The benefit-cost ratio has been estimated at 44.3, which means that the average dollar spent on research will return $44.30 in economic benefits. In terms of reduced cost of production it amounts to 0.56¢ per pound of broiler and 2.22¢ per dozen eggs.

Widely used in The Netherlands is a vaccine developed by Rispens that was naturally of low pathogenicity and then further attenuated.

Immunobiology. In 1973, Drs. B. T. Rouse, in Australia, and Payne, in England, independently showed that most of the cells in MD tumors were thymus (T) cells. A little later, Drs. Yoko Akiyama and Shiro Kato, in Japan, developed lymphoblastoid cell lines from MD lymphomas. These grow continuously in cell culture, and all have T cell and tumor-specific surface antigens as detected by Drs. P. L. Powell and Payne, of HPRS, Drs. Nazerian, Sharma, and Witter, of the RPRL, and Drs. O. H. Matsuda and Kato, of Japan. Witter *et al.* (35) discovered MATSA, an MD-tumor-associated antigen which appears to be related to conversion of cells to the neoplastic state. The foregoing and other cell lines developed at the RPRL, the HPRS, Cornell University, and Life Sciences, Inc., in Florida, have played a significant role in elucidating the complex picture of the immunobiology of MD. No less important has been the use of immunosuppressive drugs, irradiation, and surgical removal of immune active organs in studies on how the vaccine prevents the development of MD lymphomas.

This immunity is apparently quite complex; both the thymus and bursa of Fabricius play important parts in providing cellular

and humoral immunity, although the importance of each type varies with the situation.

Some enigmas of Marek's disease research. Research on MD, as with LL, has been fraught with many disappointments, discouragements, and blind alleys, where time, money, and other resources were spent without apparent value received. In most instances, however, something was gained, and eventually the remarkable HVT vaccine was discovered. Following is a view of some of the many enigmas of MD research.

1) "Chick disease" virus. On sabbatical leave from the Veterinary Laboratory, Weybridge, England, Dr. F. D. Asplin came to the RPRL in 1945 to investigate "chick disease." During the period 1939-1941, Drs. F. Blakemore and R. E. Glover had reported a high rate of transmission of fowl paralysis (MD) in young chickens. Morbidity and mortality were high, with focal necrotic lesions in the liver, spleen, and heart. The connection with MD was in the observation that some birds surviving 3 weeks developed typical MD lesions in peripheral nerves. Also, the original inoculum was a suspension of enlarged nerves. A number of strains that caused "chick disease" were isolated. Blakemore and Glover found that the infectious agent was filterable, and Asplin, in 1944, found that sulfonamides prevented macroscopic lesions and mortality.

The first question concerning the validity of Blakemore and Glover's earlier claims came to light when Asplin was unable to reproduce the "chick disease" with chickens of the RPRL having typical MD lesions. After Asplin returned to England he sought further for the "chick disease" agent. Some isolates caused the disease and some did not. One of them, strain A, caused MD lesions in 40% of birds inoculated, but no "chick disease." Also, sulfadiazine had no effect on the occurrence of MD. Other experiments were conducted, including neutralization tests. In 1947, after 8 years and much work, Asplin finally concluded that the "chick disease" was unrelated to MD.

Apparently, the Blakemore "chick disease virus" was probably a bacterial contaminant in the nerve-suspension inoculums. It was carried along with each passage, and the MD that occurred in survivors after 4 weeks was due to horizontal spread of infection from contaminated environment.

2) Isolation of lymphoid leukosis virus. With support of a grant from the American Cancer Society and in response to the

poultry industry's request for help to reduce losses from leukosis, Burmester and Fredrickson, in 1957 to 1960, collected tumors of chickens of 22 flocks located in all parts of the United States. Some were broiler-type chickens, and most were less than 5 months old; some had lesions of only ocular and of neural lymphomatosis, but most flocks and donor chickens had lymphoid tumors of the viscera and some nerve enlargement. Undoubtedly we would now give a diagnosis of MD to most or all of those problem flocks.

The material collected from selected donor birds was frozen slowly to preserve the infectious agents and stored at –70 C until prepared for inoculation into the laboratory line-15 chickens highly susceptible to leukosis (now known to be susceptible to both LL and MD). The collected material was processed to preserve a viral agent but to eliminate viable cells, so that the transmitted disease was induced by an agent that caused the disease in nature rather than a transplanted cell. The net effect of inoculating 84 different cell-free preparations was to induce the "big liver disease," i.e., LL, and some erythroblastosis and osteopetrosis. There were only a very few cases of MD.

Thus, LL was successfully transmitted and studied, but the important field-disease problem we now know as MD was not transmitted because viable cells were carefully eliminated. We now know that the herpesvirus of MD as it occurs in tumors is extremely cell-associated. Only in the feather follicle is it infectious outside the living cell. Thus, earlier studies of MD were thwarted because of what was considered a desirable procedure for preparing viral inoculum.

3) Egg transmission. At the American Veterinary Medical Association meeting in July 1968, Sevoian first reported that MDV was transmitted by the egg. His subsequent publication, in *Poultry Science,* did not contain enough detail to determine why and how he obtained so high a rate of transmission although many other investigators had failed. Sevoian's results were in contrast to much indirect evidence presented by Hutt and Cole in 1948 to 1951 and by others who observed a lack of MD in progeny reared in isolation from parents with a high incidence of disease. The question was laid to rest when Solomon, with others at RPRL, reported in 1970 on experiments designed to detect embryo transmission. They found no virus in tissues of 1,180 embryos of nine infected parental flocks, and all of the 3,387 progeny chickens were serologically negative. One can only conclude that if MD is transmitted

through the egg, such transmission is not important in the epizootiology of the disease.

4) Transmission to mammalian cells. During 1972 and 1973 many reports from Sevoian's laboratory and from that of Dr. Arthur Elliott claimed the growth of MDV in mammalian cells with either cell-associated or cell-free HVT cells. That finding was contrary to earlier reports by Calnek and could not be reproduced in 1974 by Drs. Hlozanek and Sovova or by Witter and Sharma. The last 2 investigators found that infection persisted only as long as heterokaryons with avian cell nuclei were present in the hamster kidney cultures. Also, the fact that Elliott's cultures were contaminated with herpes simplex virus could explain the cytopathic effects in mammalian cell cultures. Much other evidence, including a lack of specific antibodies in persons heavily exposed to contamination, refutes the contention that mammalian cells are susceptible to infection with MDV or HVT.

5) JMV and its vaccine? By rapid passage of the JM strain in chicks, Sevoian increased its pathogenicity so that it killed almost all chickens within 14 days. He referred to this variant as JMV. Of even greater interest was his claim of attenuation of this agent by rapid passage in embryos. When the attenuated virus was used as a vaccine against MD, Sevoian claimed that it was effective in the cell-free state, that it produced no persistent viremia, and that it prevented an MD viremia. Others, including investigators of several commercial companies, have failed to confirm those claims. Investigators in several laboratories in North America have now shown that most sources of JMV are a transplantable lymphoid tumor of MD origin. Ms. Ann Stephens, with others at the RPRL, was not able to recover MDV from JMV tumor cells, and the cells were devoid of MDV-specific antigens. Some sources of JMV may have been contaminated with or may have produced MDV, but most sources are now free of the virus.

6) "Darkling beetle" of Georgia. During the early 1960's the broiler industry of the South experienced increasing losses from condemnation for leukosis. Experiment station researchers were besieged with complaints and requests for relief. Drs. C. S. Eidson and S. C. Schmittle were taken to several new broiler houses in which many broilers had died of lymphoid tumors. When they examined carcasses soon after death, they found a large number of larvae and adult beetles among the feathers and in the subcutis. The litter of many broiler houses was found teeming with beetles.

These were identified as the "darkling beetle," *Alphitobius diaperinus*. In experiments reported in 1966, Eidson and Schmittle were able to produce a high incidence of MD by feeding or injecting suspensions of beetles collected from a broiler house. Whether the beetles played an active part or served only as a motile passive carrier of virus was not established. Other studies failed to show infectivity of those or other arthropods.

Reticuloendotheliosis

A viral agent was isolated from a moribund turkey submitted to the Kansas State diagnostic laboratory in October 1957 because of unexplained deaths in a Kansas commercial flock. Drs. F. R. Robinson and M. J. Tweihaus (28) transmitted the leukosislike lesions to young chickens and turkeys, and deaths occurred in 10 days. They tentatively called it visceral lymphomatosis (actually LL) because the tumorous liver and spleen of the original donor resembled the usual lesions in chickens with that disease. Because the work was supported in part by the USDA, Burmester visited Tweihaus. After examining gross lesions and microscopic sections and considering the very short latent period, he suggested caution in calling this a strain of lymphomatosis. Publication was delayed, and, unfortunately, other work was given higher priority. In 1964 Sevoian obtained the tumor from Tweihaus, called it acute lymphomatosis (T strain), obtained 100% mortality in various genetic lines of chickens, and was able to passage it in embryos. Studies by Dr. G. H. Theilen and others (32) revealed it to be an oncornavirus similar but not identical to the leukosis/sarcoma virus and to cause what they identified as a reticuloendotheliosis in chickens, turkeys, and Japanese quail. Similar lesions were produced in ducklings, goslings, turkeys, pheasants, and guinea keets. The virus was distinct from the Mill Hill 2 or Murray Begg strain of avian tumor virus, which belongs to the C subgroup of leukosis/sarcoma viruses and produces a similar reticuloendotheliosis.

Viruses have long been associated with bird passages of the malaria parasite, *Plasmodium lophurae*. Dr. W. Trager, in 1959, isolated a virus that produced a rapidly fatal disease characterized especially by enlargement and necrosis of the spleen. He referred to the virus as spleen necrosis virus. Dr. C. G. Ludford and others isolated from *P. lophurae* a virus that causes severe anemia in ducklings. Purchase found that all of those *P. lophurae* viruses and also a virus that Dr. M. K. Cook isolated from a stock of MD tumor

preparation are antigenically indistinguishable from the strain T virus.

Reticuloendotheliosis virus has been demonstrated in turkeys of Australia, Great Britain, Israel, and the United States. It has caused overt disease in turkeys of those same countries, and lesions due to reticuloendotheliosis virus contamination of HVT vaccines have been reported in Australia and Japan.

Conclusion

One of the greatest products of avian disease research is undoubtedly the discovery and development of the herpesvirus of turkeys vaccine, which has ended the great losses from Marek's disease. That vaccine has proved so effective that it is now used almost universally in all countries where poultry is produced commercially. Despite its great success, sporadic flocks experience "vaccine breaks" that result in unusually high rates of mortality. Also the vaccine virus and the virulent virus persist in vaccinated exposed birds. Research must continue in an effort to resolve the vaccine breaks and to develop a nonpersistent vaccine.

Research has not resulted in a useful vaccine against lymphoid leukosis. Nevertheless, investigators have developed new techniques and information which can reduce the impact of the virus infection by preventing it from developing in the bursa of Fabricius. In addition, eradication of the disease and its causal virus is possible. Eradication, in the end, would be much more satisfactory than continuous use of a tumor prophylactic or a vaccine. The poultry breeders in the United States will likely voluntarily eliminate avian leukosis viruses from their flocks in the near future.

Research on the avian tumor viruses has contributed greatly to an understanding of the basic mechanisms of cell-virus interactions and transformation to human cancer. It continues to contribute to the molecular biochemistry of cancer viruses. Genetic engineering with cancer viruses, being pioneered in the avian tumor virus field, is one of the most likely routes to better understanding and prevention of cancer in man.

Research on avian tumor viruses has already contributed much to both agriculture and human medicine. Advances in this field will likely continue to improve the wellbeing of man on this planet.

REFERENCES

1. Beard, J. W. Etiology of avian leukosis. Ann. N. Y. Acad. Sci. (Art. 2) 473. 1957.

2. Benton, W. J., and M. S. Cover. The increased incidence of visceral lymphomatosis in broiler and replacement birds. Avian Dis. 1:320. 1957.

3. Biggs, P. M. The avian leukosis complex. World's Poult. Sci. J. 20: 78-91. 1964.

4. Biggs, P. M., and L. N. Payne. Transmission experiments with Marek's disease (fowl paralysis). Vet. Rec. 75:177-179. 1963.

5. Burmester, B. R. Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell-free preparations. Cancer Res. 7:786-797. 1947.

6. Burmester, B. R., and N. F. Waters. The role of the infected egg in the transmission of visceral lymphomatosis. Poultry Sci. 34:1415-1429. 1955.

7. Burmester, B. R., W. G. Walter, and A. K. Fontes. The immunological response of chickens after treatment with several vaccines of visceral lymphomatosis. Poultry Sci. 36:79-87. 1957.

8. Calnek, B. W., H. K. Adldinger, and D. E. Kahn. Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Dis. 14:219-233. 1970.

9. Chubb, L. G., and R. F. Gordon. The avian leukosis complex. A review. Vet. Rev. Anat. 3(2):97-120. 1957.

10. Churchill, A. E., and P. M. Biggs. Agent of Marek's disease in tissue culture. Nature 215:528-530. 1967.

11. Churchill, A. E., L. N. Payne, and R. C. Chubb. Immunization against Marek's disease using a live attenuated virus. Nature 221:744-747. 1969.

12. Cottral, G. E., B. R. Burmester, and N. F. Waters. Egg transmission of avian lymphomatosis. Poultry Sci. 33:1174-1184. 1954.

13. Crittenden, L. B., and W. Okazaki. Genetic influence of the Rs locus on susceptibility to avian tumor viruses. I. Neoplasms induced by RPL12 and three strains of Rous sarcoma virus. J. Natl. Cancer Inst. 35:857-863. 1965.

14. Ellermann, V., and O. Bang. Experimentelle Leukamie bei Huhnern. Zentralbl. Bakteriol., parasitenkd., Infektionskr. Hyg., 46:595-609. 1908.

15. Furth, J. Lymphomatosis, myelomatosis, and endothelioma of chickens caused by a filterable agent. I. Transmission experiments. J. Exptl. Med. 58: 253-275. 1933.

16. Hofstad, M. S., B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder. Diseases of Poultry. 7th ed. Iowa State Univ Press, Iowa City. 1978.

17. Hughes, W. F., D. H. Watanabe, and H. Rubin. The development of a chicken flock apparently free of leukosis virus. Avian Dis. 7:154-165. 1963.

18. Hutt, F. B., and R. K. Cole. The development of strains genetically resistant to avian lymphomatosis. Official Rept. Eighth World's Poultry Cong., Copenhagen. 719-723. 1948.

19. Manaker, R. A., and V. Groupe. Discrete foci of altered chicken embryo cells associated with Rous sarcoma virus in tissue culture. Virology 2:839-840. 1956.

20. Marek, J. Multiple Nervenentzung (Polyneuritis) bei huhnern. Deut. Tierarztl. Wschnschr. 15:417-421. 1907.

21. Okazaki, W., H. G. Purchase, and B. R. Burmester. Protection against Marek's disease by vaccination with a herpesvirus of turkeys. Avian Dis. 14: 413-429. 1970.

22. Olson, C., Jr. Transmissible fowl leukosis. A review of the literature. Mass. Agr. Expt. Sta. Bull. 370. 1940.

23. Olson, C., Jr. A transmissible lymphoid tumor of the chicken. Cancer Res. 1:384-392. 1941.

24. Pappenheimer, A. M., L. C. Dunn, and S. M. Seidlin. Studies on fowl paralysis (neurolymphomatosis gallinarum). II. Transmission experiments. J. Exptl. Med. 49:87-102. 1929.

25. Peterson, R. D. A., H. G. Purchase, B. R. Burmester, M. D. Cooper, and R. A. Good. The relationships among visceral lymphomatosis, bursa of Fabricius and bursa-dependent lymphoid tissue of the chicken. J. Natl. Cancer Inst. 36:5855-5898. 1966.

26. Purchase, H. G., W. Okazaki, and B. R. Burmester. Long-term field trials with the herpesvirus of turkey vaccine against Marek's disease. Avian Dis. 16:57-71. 1972.

27. Roloff, F. Multiple Lymphosarkome beimhuhn. Magazin f. tierheilkde. 34:5190. 1868.

28. Robinson, F. R., and Twiehaus, M. J. Isolation of the avian reticuloendotheliosis virus (strain T). Avian Dis. 18:278-288. 1974.

29. Rous, P. A sarcoma of the fowl transmissible by an agent separable from tumor cells. J. Exptl. Med. 13:397-411. 1911.

30. Sevoian, M., D. M. Chamberlain, and F. T. Counter. Avian lymphomatosis. I. Experimental reproduction of the neural and visceral forms. Vet. Med. 57:500-501. 1962.

31. Solomon, J. J., R. L. Witter, K. Nazerian, and B. R. Burmester. Studies on the etiology of Marek's disease. I. Propagation of the agent in cell culture. Proc. Soc. Exptl. Biol. Med. 127:173-177. 1968.

32. Theilen, G. H., R. F. Zeigel, and M. J. Twiehaus. Biological studies with RE virus (strain T) that induces reticuloendotheliosis in turkeys, chickens and Japanese quail. J. Natl. Cancer Inst. 37:731-743. 1966.

33. Waters, N. F., B. R. Burmester, and W. G. Walter. Genetics of experimentally induced erythroblastosis in chickens. J. Natl. Cancer Inst. 20: 1245-1256. 1958.

34. Witter, R. L., K. Nazerian, H. G. Purchase, and G. H. Burgoyne. Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. Am. J. Vet. Res. 31:525-538. 1970.

35. Witter, R. L., E. A. Stephens, J. M. Sharma, and K. Nazerian. Demonstration of a tumor cell-associated surface antigen in Marek's disease. J. Immunol. 115:177-183. 1975.

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