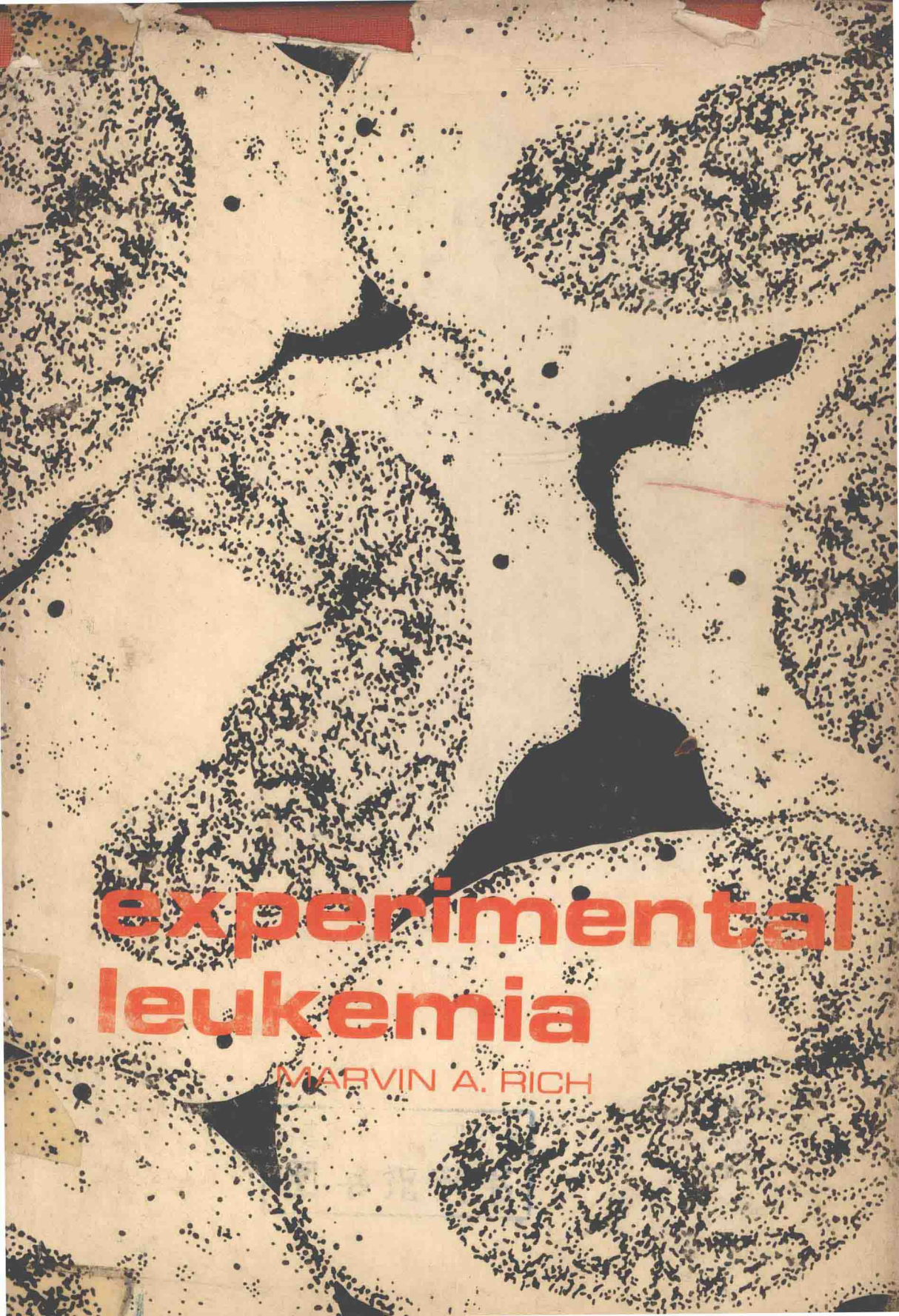
experimental
leukemia
MARVIN A. RICH

EXPERIMENTAL LEUKEMIA

editor

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PREFACE

Research in experimental leukemia has expanded rapidly both in abundance and in scope. The early leukemia workers, trained primarily in clinical pathology or hematology, acquired skills in animal genetics, virology, physiology, and bio-chemistry. The areas opened by these disciplines initiated still further diversification and specialization. At present the armamentarium of experimental leukemia research includes almost all of the disciplines of biology, chemistry and selected areas of physics. This widening of horizons in leukemia research suggested a need for the consolidation of information previously available only from diverse sources. To fulfill this need, EXPERIMENTAL LEUKEMIA was written.

In the opening chapter, Jacob Furth—one of the last of the early leukemia workers, provides a warm and interesting chronicle of the early days of leukemia research. In the chapters which follow, scientists active in the major areas of experimental leukemia research describe the current status of their own fields. EXPERIMENTAL LEUKEMIA is aimed at providing the specialist in experimental or clinical leukemia research with insights and perspectives in research areas removed from his own. It is hoped that this book will also provide a useful guide for scientists and students who may in the future bring their special interests and abilities to this exciting field.

In choosing the topics to be included in this book, it became obvious that the line which separates *experimental* from *clinical* leukemia research was neither distinct nor static. Emphasis has been placed, therefore, on those systems which are currently being used to study the nature and mechanisms of the leukemogenic process. It was not my intention for EXPERIMENTAL LEUKEMIA to duplicate the current literature reviews that are available. The contributors were invited to emphasize their *interpretation* of significant experimental findings as opposed to exhaustive documentation of the literature.

I am indebted to many of my colleagues, and especially to Dr. Richard Siegler for helpful discussions during the formative stages of this project. The assistance of Mrs. Barbara Flynn, Mrs. Phyllis Bleiweis, and the staff of Appleton-Century-Crofts is gratefully acknowledged.

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Marvin A. Rich

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AN HISTORICAL SKETCH OF EXPERIMENTAL LEUKEMIA*

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Introduction

This chapter is intended to place on record for historians the birth and growth of modern experimental leukemia as I have witnessed it. When, in 1928, I began the study of the etiology and pathogenesis of leukemia, it was an almost virgin

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field, ready for exploration (Opie, 1928). A virus causing leukemia in chickens had been reported by Ellermann and Bang (1908). Rous (1910) and, independently, Fujinami and Immamoto (1910) (cited by Fujinami, 1930) isolated avian tumor viruses. Soon the excellent contribution of Rous led the field, giving new directions to cancer research in general. His work overshadowed the pioneer contributions of Ellermann, whose viruses were lost following his untimely death. Fujinami, summarizing his contributions (1930) stressed biologic aspects of transplantable avian tumors and did not consider the evidence for a causative filterable agent to be conclusive.

In 1928, leukemia was known to exist in many species of animals, and to have diverse manifestations similar to those seen in man (Opie, 1928). This made it possible to depart from the then prevalent speculative communications on the nature and etiology of leukemias and to undertake experimental studies. Two possibilities were evident. Leukemia was either caused by a virus and, if so, it might be induced by cell-free material from leukemic animals, or it was an autonomous neoplasm induced by nonviral agents, such as radiation. In the latter case, leukemia would be transplantable by cell grafts either to genetically related animals or to animals whose resistance was lowered by pre-irradiation (Murphy, 1926). At that time, inbreeding mice for cancer research was well under way, begun by the mammalian geneticists of the Castle-Little School. Lowering host resistance to tumors by irradiation (and the relationship of immunologic defense to lymphocytes) had been well established by the now almost forgotten James Murphy (1926).

Initially, our own leukemia research was financed by an industrialist who generously supported medical research but who wished to remain anonymous. In 1932, the Lady Tata Memorial Trust, established to further leukemia research throughout the world, lent support to most newly established centers for leukemia research. One of these was led by Julius Engelbreth-Holm, whose comprehensive monograph, "Spontaneous and Experimental Leukaemia in Animals," (1942) lists the many workers aided by this fund.

Prior to this, Claude E. Forkner published a monograph "Leukemia and Allied Disorders" (1938), which is noteworthy for its comprehensiveness in recording the major clinical and experimental observations and views on leukemia and hemopoiesis.

In 1953, the Ciba Foundation held an International Symposium on Leukemia Research, initiated by Sir Henry Dale and other members of the European Scientific Advisory Committee of the Lady Tata Memorial Trust. This symposium signified a landmark in leukemia research. The 35 participating scientists discussed problems of leukemogenesis (chemical, radiation, and viral), chemotherapy (phenomena of resistance and dependence and drug design), the fundamental nature of the leukemias, and "Where do we go from here?"

In the course of the evolution of knowledge, the contributions of most toilers who prepared the ground for major discoveries pass into oblivion. Now that I seem to be the last of the early band of experimental workers on leukemia, I welcomed the assignment to write this chronicle in honor of those gone from us.

Leukemia research prior to 1910[1]

The history of clinical knowledge of human leukemia has been thoroughly reviewed by Forkner (1938). The description of the first cases by Craigie and Bennett (1845), Bennett (1845) and Virchow (1845; 1847) marked the beginning of a new era. As more human cases were studied, the complexity of leukemia became evident. This period is characterized by speculation, glorified with the label "theories" on the nature and pathogenesis of the disease, doctrinaire debates of classification, and lack of a successful experimental approach. Lymphoid leukemia was recognized first, and cases with were distinguished from cases without blood involvement. After the myelopoietic functions of bone marrow were recognized (Neuman, 1870), myeloid leukemia as a disease entity (earlier characterized by conspicuous splenomegaly) was established. The precise morphologic, biologic, and chemical characterization of leukocytes by Ehrlich and the discovery of the oxidase reaction of myeloid cells were important advances.

Numerous attempts to transmit human leukemia to animals and animal leukemia of one species to another species met with failure. Attempts to transfer leukemia from man to man were also unsuccessful. (Some transfers possibly occurred later, incidental to transfusions to combat anemia and leukopenia.)

Spontaneous leukemia in many species of animals was reported, notably in the dog, cattle, and mice, and the similarity between human and animal leukemia was pointed out in many reports. Even cases resembling Hodgkin's disease were described in mice. Isolated cases of leukemia were observed in the monkey, pig, horse, rabbit, cat, and guinea pig.[2] Lymphosarcoma was generally believed to be a neoplastic disease different from leukemia, the latter generally thought to be either some unusual infection or hyperplasia. This situation lasted until the late twenties, when transplantation studies indicated that leukemic cells behave like common cancer cells.

Viral neoplasia

Extensive research on viral leukemia began with the discovery by Ellermann (1908) of a filterable agent in chickens capable of producing "systemic" leukemia as well as solid tumors of lymphoid cells. Earlier, several investigators described various types of leukemia and their common occurrence in fowl (Butterfield, 1905; Warthin, 1907). Ellermann thought that they were produced by variants of one virus. Rous undertook a systematic search for a virus in all sorts of solid tumors and leukemias in chickens. A study of his original protocols suggested to me that he also transmitted leukemias, but his leukemic birds were wiped out by infections (probably *Salmonella pullorum*) which in those days was a common disease

[1] For references to this section see Forkner (1938).

[2] For references on leukemia prior to 1928 not given here, consult Opie (1928), Engelbreth-Holm (1942), and Donner Foundation Index to Literature of Experimental Cancer Research 1900 to 1935 (1948). The latter is a bibliographical work with a splendid subject and authors index, a painstaking, dedicated job of Mildred Schram for the Donner Foundation.

plaguing the poultry industry. The sarcoma viruses of Rous did not produce leukemia, nor did Ellermann (1922) report production of sarcoma by his leukemia virus. Rous passed on his virus to numerous investigators, while Ellermann's viruses were lost. The many interesting contributions of Rous and coworkers formed the basis for tumor virus research, which in the thirties became solidly linked to leukemia virus research.

Neither avian leukemia nor avian sarcoma were generally accepted to be diseases identical with human cancers, and it required individuals who believed in *l'art pour l'art,* or in the "usefulness of useless knowledge," and with courage to undertake the laboratory study of these diseases. Not until mammalian tumors, notably Shope's rabbit papilloma (which can turn into carcinoma) and the common mouse mammary carcinoma yielded causative viruses (Bittner, 1940) did the pendulum swing the other way.

Beard (see Ch. 9) reviewed fully Ellermann's work and views and the subsequent studies of the other investigators. Beard's own contributions are historic because they were solid in substance, based on experiments done in a period when avian leukosis research and the virus theory were unpopular and most reported viruses were lost. When the Regional Poultry Research Laboratory was established in East Lansing, Michigan, it became an avian tumor virus "bank" as well as an institute for the study of avian leukosis.

Numerous efforts (reports of which are mostly unpublished) to discover a virus capable of producing leukemia in a mammal remained unsuccessful. The closest to accomplish this was Engelbreth-Holm, who described one in the mouse, but since he could not confirm the results of his own experiments he modestly assumed that he may have made a mistake. He probably did not.

The groundwork for investigating mammalian leukemia was laid with the development of inbred strains of mice. Little's students at The Jackson Laboratory, Bar Harbor, Me., concentrated on genetic studies that included those of solid tumors, especially mammary cancer. During these experiments the "milk factor" (Bittner, 1940) was discovered and it turned out to be a virus. There is a great similarity in biologic behavior between Bittner's mammary tumor virus and Gross' leukemia virus. Virtually all investigators working on avian leukosis and sarcoma searched for virus in rodent leukemias without success. The discovery of the sensitivity of mice to mammary tumor virus during the neonatal period and their resistance to it during adult life opened the door to the discovery of murine viruses. Gross, who firmly believed in the viral etiology of leukemia, persisted in searching for the causative virus and became the first to prove the thesis. A similar situation exists today with respect to the still elusive presumed viruses in human leukemias.

Infectious "lymphosarcoma" of dogs[2]

Wehr (1888) reported a transmissible vaginal tumor of a dog, and Geissler (1895) a tumor of the prepuce, which could be transferred by implantation into other dogs. (It is possible that the transmissible tumor, primary in the nostril of a

dog, described by Nowinsky in 1876 was of similar type.) The first extensive studies of this type of tumor were made by Sticker (1904-1907). Sticker transferred this tumor to two foxes out of three inoculated, but failed in passing it on to five other species of mammals. This tumor was given many names by investigators who argued about its nature and whether or not it was a "true" neoplasm. (The same skepticism was voiced later against Rous' sarcoma, but given as an unknown, neither the "venereal" tumor of dog nor the viral lymphomas of chickens can be differentiated from accepted mammalian neoplasms.)

The literature until 1927 on this interesting tumor in dogs was well reviewed by Opie (1928). Among the many pioneer cancer investigators who studied it were Bashford, Murray and Cramer, Beebe and Ewing, and Loeb (cf. Opie, 1928).

Studies of Stubbs and Furth (1934) and those of De Monbreun and Goodpasture (1934) failed to clear up the basic problems of the character of this readily graftable tumor and its causative agent. The "infections" often seen are apparently grafts of neoplastic cells on mucosal surfaces, which can metastasize widely. Tumors grafted by natural contact or experimentally often regress as do other tumors grafted on heterozygous animals.

The research possibilities of that era being exhausted, interest in this tumor faded. Now, a reinvestigation of its etiology, notably a search for a causative virus, may be rewarding (see Ch. 7).

Transplantation research

In addition to the discovery of leukemia viruses, the development of inbred strains of mice enabled numerous basic findings in leukemia to be made. The most important antecedent was the demonstration that cancer cells were autonomous. When Hanau (1876) transplanted a rat cancer, he defined autonomy as the basic feature of cancer cells. He said that grafted normal cells survive to the extent of their need (later known as "Halstead's Law") but that cancer cells can grow autonomously, even on new hosts on which they are grafted.

The first indisputable transmission of leukemia was reported by Snijders (1926) and more fully described by his student Tio Tjwan Gie (1927). They reproduced malignant lymphoma with or without blood involvement by cell grafts in guinea pigs through 58 successive passages. They recognized that leukemia was due to proliferation of the introduced lymphoid cells in the new host and thus demonstrated the essential similarity between a leukemic and a cancer cell. They failed to transmit this leukemia with material free from living cells. Not until the fifties was research on transmissible leukemia of the guinea pig resumed (Congdon and Lorenz, 1954) and confirmation and extention of these findings were begun. However, a reinvestigation of the possible causation of leukemia in guinea pigs by virus is overdue.

Earlier, Miguez (1918) transmitted a lymphomatous tumor of the guinea pig that was extensively subpassaged by Fischer and Kantor (1919, 1921). Their publications appeared in Spanish and escaped the deserved attention.

Prior to development of inbred strains of mice, murine leukemia research made extensive use of the discovery of Murphy (1917a, 1917b) that radiation lowers the host's resistance to transplanted tumors. Application of this idea to leukemia (Furth et al., 1933) led to several interesting observations, such as the relation of lymphoid leukemia to lymphosarcoma and enhancement of the spread of tumor cells by radiation.

Inbreeding of mice led to the isolation of a high leukemia strain, of which MacDowell's C58 (1937) is best known in the United States. The lack of general availability of this strain led us to attempt to develop one (Ak) by persistent inbreeding from a pair of commercially obtained mice in whose ancestry we found leukemias (Cole and Furth, 1941).

We also attempted to breed a leukemia-free strain but had limited success. This strain (now known as the Rf strain) is sensitive to induction of myeloid leukemia, but its features have changed.

Genetic research

MacDowell (1937), Cole and Furth (1941), and others seemingly supported the thesis that the leukemias in the high leukemia strains were due to genetic factors, the number and character of which varied with different high- and low-leukemia strains as ascertained by hybridization tests. This concept was abandoned as newer findings came to the fore with newer techniques, such as visualization of virus by electron microscopy, use of germ-free strains, and assays of embryos for virus. The genetic factors became subordinated to those of virus in the thinking on the subject. The time of viral infection—whether embryonal, neonatal, or postnatal —is critical in the "expression" of leukemia.

Some other contributions of the early transplantation studies still stand today, even though gaps appeared in their comprehension. Transplantability indicated "autonomy" of the leukemic cells, but the basis for autonomy is still being debated; mutations, abnormal differentiations, or acquisition of viral genes are contending explanations.

Transplantability by single cells and failure to transfer leukemia with crushed cells (Furth and Kahn, 1937) was interpreted as the strongest evidence for the neoplastic nature of leukemia. They also suggested lack of immunologic deviation. In retrospect, it may be significant that the single-cell grafts were done only with viral Ak leukemias. Would nonviral leukemic cells behave the same way? Virus-induced Friend leukemic cells are certainly virus-dependent in the original generation. (Related problems of antigenic modification will be discussed in the closing section.)

Other lessons from transplantation studies include the recognition of the relation between lymphosarcoma (tumor formation) and leukemia, the disturbance in differentiation characteristic for the different strains of transplantable leukemia, other markers of their individuality, the usefulness of various transplanted strains in chemotherapeutic research (the different transplantable strains exhibiting differ-

ent individualities), the induction and analysis of drug resistance, differentiation between leukemoid reaction and leukemias.

Induction of leukemia by radiation and chemicals

Research on the induction of leukemia by ionizing radiation was motivated by the relatively frequent occurrence of leukemia among radiologists. Krebs and his associates (1930) were the first to verify the capacity of x-rays to produce lymphoid leukemia in mice. Our studies, begun independently, soon indicated that the incidence of myeloid leukemia and of numerous solid tumors was also increased by exposure to x-rays. It was also shown that among the lymphomas thymic tumors were most common and occurred the earliest (Furth et al., 1933; Furth and Furth, 1936). This led to entertainment of the possibility that thymectomy could prevent lymphoma development.

The many major observations which followed, reviewed by Law (1954), soon obscured these early studies.

Leukemogenesis by pluripotent carcinogens was first reported in mice by Morton and Mider (1938). The cocarcinogenic influence of chemicals was extensively studied by Kirschbaum et al. (1953). Among the most far-reaching findings concerned the indirect mechanisms of induction of radiation leukemias, the role of latent viruses in leukemogenesis (which may explain the high induction rate of murine leukemias by radiation), and the complexities of leukemogenesis. These advances are sketched in the presidential address of the American Association of Cancer Research by Kaplan (1967), and in other recent reviews (marked with an asterisk in the bibliography).

Induction of leukemia with benzol was reported by Lignac (1932), and of reticulum cell tumors with trypan blue by Gillman et al. (1949), and Simpson (1953). The proleukemogenic effects of x-rays were noted by Gardner and Rygaard (1954). A marked inhibition after development of spontaneous leukemia by underfeeding was demonstrated by Saxton et al. (1944). In rats, Shay et al. (1956) pioneered in the induction of myeloid leukemia with a polycyclic aromatic hydrocarbon. Recently, Huggins and Sugiyama (1966) expanded this line of research, believing that in the rat neither leukemia nor mammary tumors are caused by viruses.

The possible relation of chemical to viral carcinogenesis has been a target for research since the early era of Rous. The conclusion that the two can act synergistically is well founded, but it is unlikely that induction of all neoplasms require a virus.

The thymus and the leukemias

Prevention of leukemia by thymectomy created a fertile field of research, still not fully explored. The senior author of the first publication on this subject, McEndy (1944), did not live long enough to see its importance. Our paper summarizing the early contributions (Furth, 1946a) pointed out that removal of the thymus

in a high-leukemia strain, when the mice were 6 weeks of age, raised the mean length of life in females from 9.5 to 13.6 months and in males from 9.7 to 14.4 months. Leukemia incidence decreased from 83.5 percent to 10.8 percent. Most leukemias occurring in thymectomized mice were atypical, myeloid, or monocytic. The successive generations of Ak mice can be leukemia-free if thymectomized. The growth of grafted malignant lymphocytes or myelocytes is not influenced by the thymus.

In regard to thymectomy, certain facts were recognized but misinterpreted by us. It was assumed that spontaneous leukemia in Ak mice was brought about by two events: (1) the presence of cells in the thymus with high hereditary susceptibility to a leukemogen (intrinsic factor) and (2) the transformation of such cells to malignant cells. This, the extrinsic factor, was seen as related to the nursing influence but its viral nature was not suspected. This experience with thymectomy led us to the generalization that other neoplasms may also be prevented by removal of their organ of origin after this survived its usefulness.

Soon others, notably Law (1954) and Kaplan (1954) demonstrated the role of thymus in induction of leukemia by chemicals, radiation, and virus and the restoration of thymic deficiencies by thymus grafts. Analysis of the latter findings disclosed the existence of several thymic factors, one of which is related to immunologic competence (Miller, 1964; Good and Gabrielsen, 1964), another to a thymic lymphocytosis-stimulating factor (Gregoire and Duchateau, 1956; Metcalf, 1956). The importance of this area of research is indicated by several monographs written on this subject in recent years (Miller, 1961; Good and Gabrielsen, 1964; Metcalf, 1966; Ciba Symposium, 1966).

Lessons from the past—a glimpse into the future

Historical events are beacons which project light on events to come. With distance, the illumination gradually fades. Some discoveries become "inevitable" and are often reported simultaneously by two or more investigators, giving rise to painful priority debates.

Leukemia research began over a century ago with the recognition of the existence of various white blood cells and the different sites of their formation. Much in this area remains to be clarified, such as the interrelationship of hemopoietic cells, notably lymphocytes to monocytes, and the relationship of monocytes to "reticular" cells. Until recently, the small lymphocyte was thought to be short-lived and without a definite function. Presently, it is considered the central cell in immunology, linked to autoimmune disorders and, via the latter, to some leukemias.

Are there several lymphocyte types? Is the bone marrow the source of embryonal lymphocytogenesis and a reserve source of lymphocyte in postnatal life? What is its relation to other types of cells (stem cells, plasma cells, and "immunoblasts" of Dameshek, cf. Ciba Symposium, 1965)? What is the daily traffic of lymphocytes? What is the relation of short-lived to long-lived lymphocytes and of long-lived lymphocytes to chronic leukemia? Solution of some of these problems, including that of production of antibodies in vitro, were brought closer by recent technological