Rodent Leukemia: Recent Biological Studies. A Review

ARTHUR KIRSCHBAUM†

(Department of Anatomy, University of Minnesota Medical School, Minneapolis 14, Minn., and of the College of Medicine, University of Illinois, Chicago 12, Ill.)

Understanding of the factors influencing the spontaneous development and the transplantation of experimental neoplasms has been sought in studies of rat and mouse leukemia. Since leukemia is induced readily in various strains of mice by either carcinogenic hydrocarbons, ionizing radiations, estrogens, or combinations of these agents (81, 66), the disease has been the subject of recent studies on experimental carcinogenesis. Because of their sensitivity to so-called "therapeutic" chemical agents, the leukemias of mice are being used extensively in screening programs (16) and for the study of cellular refractoriness to agents such as the folic acid antagonists (19, 7@).

Morphological types.—The usual type of systemic leukemia is lymphoid. Myeloid leukemia, often chlorotic, appears relatively infrequently (67). Spontaneous mediastinal (thymic) lymphosarcoma occurs primarily in young mice (49, 67). Unusual distribution of leukemic infiltrations may characterize the disease in hybrids, the patterns of disease being more uniform in the pure strain (49). In "high-leukemia" strains the incidence may be as high as 90 per cent, the majority of cases appearing by 1 year of age. Leukemia occurs spontaneously in old animals, beyond 500 days of age, of strains such as the CBA and Bagg albino which are considered "low-leukemia." If careful autopsies were done routinely on populations given good care, it would probably be found that in old mice of even the "leukemia-resistant" strains the disease develops in as many as 5 per cent. It can be very difficult to decide in old mice upon gross examination whether the large Malpighian corpuscles of the spleen are hyperplastic or actually representative of leukemic neoplasia.

The interactions of genetic and extrinsic factors determine the time of appearance and the type of lymphomatous and myelomatous disease. In the Ak stock and in certain hybrid crosses with a low-leukemia strain (F, and backcross to the Ak), neoplastic disease of the hemopoietic system appears early, and mediastinal lymphosarcoma is common. In backcrosses to the low leukemia stock, leukemia develops late, and localized mediastinal disease occurs rarely (49). In strains which develop spontaneous leukemia or lymphosarcoma only occasionally (as well as in certain ones with a high incidence), ionizing radiations, carcinogenic hydrocarbons, or estrogenic hormones may induce lymphomatous disease (65). Susceptibility to each of these agents is determined by genetic factors which are not identical with those determining susceptibility to spontaneous disease.

Rare types of rodent neoplasms of hemopoietic tissue are the hepatic histiocytoma (48), which on transplantation may express itself as a leukemia or sarcoma of the stromal reticulo-endothelial tissue, plasma-cell leukemia (100), and mast-cell tumors (24). Reticular cells may predominate in some rat and mouse lymphomas, the term "reticulum-cell sarcoma" having been applied. Al-

† Present address: College of Medicine, University of Illinois, Chicago 12, Ill.

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though atypical giant cells and fibrosis may appear in the infiltrations, the “Hodgkins” adjective is probably not appropriate.

**Cytology.**—The “immature” leukemic cells, as seen in blood and marrow smears, and imprints of the infiltrated organs, closely resemble the undifferentiated human leukemic cells (67). Distinctive cytological characteristics such as granulation may distinguish transfer lines.

An increase in the number of mitochondria occurred during potentiation of malignancy within one transfer line, whereas a second transfer line exhibited no similar cytologic modification (86).

Chromosomes of mouse leukemic lymphocytes are larger than those of homologous normal cells of the adult. Their size is greater in the more malignant cells of transplanted leukemia as compared to lymphocytes of spontaneous leukemia. Lymphocytes of the embryo also possess large chromosomes. The difference in the size of the chromosomes of normal and leukemic cells is due to amounts of pepsin-digestible protein (11). Sex hormones influence the size of the chromosomes in mouse leukemic cells (12).

The whole “nuclear fraction” of leukemic spleens is extremely rich in RNA, as compared to the similar fraction from normal spleens. This may be accounted for by the fact that the nuclei of leukemic spleens contain nucleoli which are not present in normal mature lymphocytes (95).

No cytologic alteration could be correlated with the development of refractoriness in a transfer line of leukemia to the inhibiting action of folic acid antagonists (19, 72).

Although folic acid antagonists inhibit the development of certain transplanted leukemias, there seems to be no “radiomimetic” effect, as seen in the action of nitrogen mustards (7), colchicine (77), and alpha- and beta-peltatin (44), where pyknosis and nuclear fragmentation occur. Massive doses (several times the LD₅₀) of nitrogen mustards may kill leukemic cells, so that transplantation is not possible from treated animals. On the other hand, treatment of leukemic mice with extremely large doses of the folic acid antagonists does not alter transplantability (14).

An increased percentage of mature cells appeared in the blood, and the total white blood cell count dropped to normal levels following the administration of urethan to mice with a transplanted myeloid leukemia. Similarly, there was a “shift to the right” in the bone marrow. Correlated with the accelerated maturation of leukemic cells there was a decreased number of mitotic figures (64).

Cellular morphology may indicate fundamental differences in leukemic cells. Myeloid leukemic cells of the F strain are usually sensitive to the action of urethan and potassium arsenite, although resistant to folic acid antagonists. The reverse is true for lymphoid leukemic cells (83). Within leukemias of one cell type, tremendous variations in response may occur.

**Leukemogenic Factors**

**Genetic.**—The most carefully controlled work reveals that the gene may definitely influence the development of mouse leukemia (81). Females of the low-leukemia StoLi strain were mated with a single C58 (high-leukemia) male, with 7 F₁ sons and with 50 backcross (to StoLi) grandsons. All mice were nursed by Bagg albino females. The incidence of leukemia in the offspring of StoLi females mated with backcross fathers varied from 0 to 42.8 per cent, indicating the genetic diversity of the backcross males and suggesting a segregation of the genes influencing leukemia.

The length of life of the offspring was a complicating factor in evaluating results. Length of life was affected by the genetic pattern of the backcross fathers, by a nursing influence from the females, and by sex itself, females living longer than males. Increasing the parturition age of the mother delayed the appearance of leukemia without influencing the other causes of death, so that potential leukemics died of other causes.

When high-leukemia C58 mice were originally reciprocally crossed with low-leukemia StoLi mice, a maternal influence analogous to that observed for mammary cancer was suggested (82), since the incidence of leukemia in genetically similar F₁ hybrids was higher if the female parent was of the high-leukemia C58 strain. It now appears that the maternal influence responsible for this difference consists of a definite resistance to leukemia contributed by the low-leukemia strain mothers (83). This may be supplied before birth or by nursing alone. It becomes an increasingly potent influence with advancing age. When this resistance is absent, the leukemic heredity of the C58 strain, whether introduced by the male or female, shows dominance. It appears that this “resistance factor” contributed by StoLi mothers extends to other causes of death.

Nongenetic factors influence the development of leukemia and complicate the situation sufficiently so that it is probably premature to conclude from one series of crosses that a single dominant gene controls the development of leukemia, and that leukemic cells develop as a result of somatic mutation in cells with this labile gene (49).
Leukemia has been generally observed to appear later in life in F1 hybrids and in backcrosses to the low-leukemia strain than in the pure strain or its backcrosses (49, 62). The time of appearance of leukemia in the heterogeneous F2 population is variable. Early development of the disease tends to be manifested as thymic lymphosarcoma.

The genes determining susceptibility to the development of "spontaneous" leukemia are not identical with those involved in the reactions to leukemogenic agents such as ionizing radiations, carcinogenic hydrocarbons, and estrogenic hormones. Strains with a relatively low incidence of spontaneous leukemia (e.g., CBA) may respond readily to a specific leukemogenic agent (e.g., estrogen), but not to another such as methylcholanthrene (65).

Viral.—Although in F1 hybrid reciprocal crosses between certain high- and low-leukemia strains the incidence of leukemia is higher and the age of onset earlier if the female parent is of the high-leukemia strain, foster nursing experiments do not support the suggestion that a "leukemia agent" is present in the milk, as in the case of mouse mammary cancer (65). Although a virus may be associated with certain transfer lines of leukemic cells (110), it is considered to be a contaminant in so far as the etiology of leukemia is concerned.

When very young mice of the low-leukemia C3H strain were inoculated with a noncellular extract of either leukemic tissue, or of embryonic cells from the high-leukemia Ak strain, leukemia appeared "spontaneously" (46). Susceptibility to the agent was expressed only by mice inoculated at least 12 hours of age. It is suggested that mouse leukemia may be transmitted by an agent analogous to that of chicken leukemia. Confirmation of these findings is required to establish mouse leukemia as a viral disease. Several investigators have suggested the possibility that mouse and rat leukemia may be transmitted by cell-free materials (119). Freezing followed by lyophilization at low temperatures inactivates the capacity of leukemic tissue to transmit the disease to animals susceptible to cellular transmission (34).

Hormonal.—Estrogenic hormones, when given in appropriate doses, are leukemogenic for certain strains of mice (58) and their hybrids (96, 87). The resulting type of disease is usually thymic lymphosarcoma. The leukemogenic effects of x-rays or methylcholanthrene may be potentiated by exogenous estrogen (66). In certain high-leukemia strains (88), but not in others (81), the incidence of spontaneous leukemia is higher in females than males.

Androgenic hormones are anti-leukemogenic in mice under certain conditions. The leukemogenic action of estrogens is nullified by the simultaneous administration of appropriate amounts of androgen (58). The incidence of radiation-induced lymphomas is likewise reduced by administering testosterone propionate (58, 58). Injections of androgenic hormone reduced the incidence of leukemia in females to that found in males of the RIL strain (88). Although orchidectomy increased the incidence of leukemia in RIL males, the incidence of the radiation-induced disease of C57 black mice was not significantly altered by gonadectomy (58).

Adrenalectomy increased the incidence of spontaneous leukemia in C58 mice (73) and of radiation-induced thymoma in C57 blacks (59). Administration of cortisone decreased the percentage of induced lymphomas in the latter strain (59). Adrenalectomy is said to increase susceptibility to transplantation of rat lymphosarcoma (115), whereas cortical hormone exerts inhibitory effects (91).2

Lymphosarcomas originating in the lung appeared in six of fifteen rats treated with growth hormone, whereas none appeared in controls. In all the injected animals the peribronchial lymphoid tissue was hyperplastic, and it was from this site that the lymphosarcomas developed (87).

Carcinogenic hydrocarbons.—The susceptibility of certain strains of mice to the induction of leukemia may depend primarily upon the response of the thymus. When small amounts of carcinogenic hydrocarbons were implanted directly into the various organs, local lymphoid tumor development appeared in the thymus but not in the other lymphoid organs (98). The most potent of the carcinogens upon subcutaneous injection was 9,10-dimethyl-1,2-benzanthracene. Since 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, and 20-methylcholanthrene are effective when injected directly into the thymus, it appears that rapidity of absorption may determine leukemogenic potency. The site of injection may be decisive in determining leukemogenic effectiveness (97, 99).

Removal of the thymus reduced the incidence of carcinogen-induced leukemia from 69.7 to 29.0 per cent (75). Removal of the spleen or transplantation of one or two thymuses into intact DBA mice did not alter the incidence of carcinogen-induced leukemia. If, however, thymectomy was followed by autotransplantation, the incidence of induced leukemia was 69.1 per cent, indicating that the presence of thymic tissue is necessary for the development of leukemia in a high percentage of

1J. Stasney, K. E. Paschkis, and A. Cantarow, personal communication, 1951.
mice of this strain. Although, in contrast to Ak mice (85), the thymus is apparently not ordinarily the primary locus for the development of spontaneous leukemia in C58 mice, its removal does reduce the incidence (76). Grafted thymuses did not appear to serve as the focus for lymphoma development. Absence of the thymus did not change the incidence of other types of induced tumors.

Although p-dimethylaminoazobenzene is usually considered carcinogenic for the liver alone, 5 of 28 rats receiving splenic implants developed lymphoblastic lymphosarcomas (78). No similar tumors appeared in controls, nor were neoplasms of this type induced by oral administration.

Following gastric instillation of methylcholanthrene into 59 Wistar rats, 6 developed lymphatic and 2 myelogenous leukemia (104). Leukemia appeared in young rats suckled by mothers which received methylcholanthrene by stomach tube. Labeled 20-methylcholanthrene was recovered from the milk of the stomachs of the offspring, indicating that carcinogenic agents may be transferred to the offspring by way of the breast milk (105).

Radiation.—Lymphosarcoma and/or leukemia may be induced by the whole-body radiation of mice. The thymus of C57 blacks seems to be primarily susceptible to this action of x-rays, resulting from secondary infiltration of other organs (53). Radiation of the thymus alone, or of the whole body except the thymus, did not result in the induction of thymic tumors (55, 56). That a humoral factor operates is indicated, since shielding of an extremity decidedly reduced the incidence of induced thymomas (56). Normal nonirradiated tissue may inactivate a humoral factor produced by radiation.

If a strain of mice is susceptible to the induction of leukemia by x-radiation, the younger the animal is at the time of exposure to x-rays (54), the greater its susceptibility. Estrogen enhances (35, 66) and androgen decreases susceptibility (35, 58). Certain strains are more susceptible to the induction of leukemia than others; in one the thymus may be the target organ (58), whereas in others the locus seems frequently to be extra-thymic. Treatment with estrogenic hormone may render lymphoid tissue susceptible to the induction of neoplastic change by local irradiation. In support of the idea that a humoral factor resulting from radiation is the actual leukemogen is the observation that in estrogen-treated mice thymic lymphosarcoma may be induced by radiation of the entire body except the thymus.

The incidence of lymphoma was proportional to the total dose of radiation in C57 black mice, whether fractionated or in one exposure (57). With fractionation treatment, if the intervals between radiation were 4–8 days, the incidence was greater than if treatments were given daily. If the intervals were 16 days the incidence was no greater than for daily treatments.

Nutritional.—Nutritional factors may influence the time of onset of either spontaneous or induced leukemia, or alter the growth of transplanted lymphoid tumors. Caloric restriction resulted in a later appearance and reduction of the total incidence of leukemia in mice of the Ak strain (101). The incidence of carcinogen-induced leukemia was remarkably reduced in DBA mice when the animals were fed a diet low in cystine (119). That the effect was not one resulting from nonspecific inhibition of growth is suggested by the observation that the restriction of other amino acids (lysine or tryptophan) decreased the body weight but not the incidence of leukemia.

Both pyridoxine and riboflavin deficiencies were associated with the inhibition of growth of certain transplanted lymphosarcomas (113, 114). Administration of a guanine analogue, 8-azaguanine (guanazolo), has been reported to inhibit the growth of transplanted leukemia (60, 71). It has been postulated that the effect is caused by interference with the utilization of guanine, which is essential for the growth of neoplastic cells (61). The inhibitory effect of folic acid analogues on transplanted leukemia has been attributed to interference with folic acid metabolism resulting in a diminished nucleic acid synthesis (107).

Transplanted Leukemia

Genetic factors control the transplantation of leukemic cells. Cells of specific lines proliferate only in mice of certain genetic constitution. Leukemic cells are usually 100 per cent transplantable into the mouse strain of origin and into F₁ hybrids between this and the foreign strain.

Transplantation patterns (susceptibility of hybrids of varied genetic constitution) of normal and malignant cells are different, an observation which has been interpreted to indicate that the genic composition of malignant cells differs from that of normal cells (32). The percentage of F₁ hybrid mice supporting the growth of leukemic cells indicates the number of genes involved in transplantation.

Malignant lymphocytes grow not only when transplanted into a suitable living animal medium, but also in tissue culture if a mesenchymal stromal tissue is present (23). The stromal tissue itself cannot be retransplanted into animals and grow as a tumor, which observation indicates that the lymph-
phoblastic round cell is the essential malignant cell. In peritoneal ascitic fluid malignant lymphocytes proliferate, apparently increasing in number in the absence of peritoneal implants (41).

The rate of growth of transplanted mouse lymphosarcoma cells was not more rapid than that of normal cells of 12-day-old hosts (98); when the same lymphosarcoma was inoculated into 42-day-old mice, the animals with tumors gained more weight than littermates without tumors—the tumors accounting for weight gain (92). When inoculated into a foreign strain, mouse leukemic cells may form a temporary growth which subsequently regresses. The hosts resist a second transplantation of the same line of leukemic cells (10). This experiment has been interpreted as demonstrating that humoral substances passing from the nonradiated to the radiated rat nullified the radiation-induced susceptibility.

Heterologous and cross-strain transplantation of mouse leukemia have been accomplished by x-irradiation of the foreign species or mouse strain prior to inoculation of leukemic cells (51). Attempts to transplant lymphomas into foreign species by inoculation into the anterior chamber of the eye have been unsuccessful (79). This lack of growth has been explained on bases other than nonmalignancy and/or absence of autonomy. Mouse lymphomas are malignant by all criteria other than growth in the anterior chamber of a foreign species. Failure to grow may be attributed to the lack of resistance of lymphoma cells to various types of trauma, the short life span of the cells, and the rapid development of immunity in the inoculated host. Mouse lymphomas are transplantable into the eyes of only homologous mouse strains.

When inoculated into a foreign strain, mouse leukemic cells may form a temporary growth which subsequently regresses. The hosts resist a second transplantation of the same line of leukemic cells, no “temporary” growth appearing. This capacity of certain leukemic cells to grow in foreign strains is said to be evidence of their high resistance to the action of antibodies. Inability of regressing leukemic cells to grow upon transplantation into an ordinarily susceptible host is attributed to adsorbed antibody. The serum of “immune” animals may inactivate leukemic cells in vitro, so that their ability to transmit the disease is lost (45). Similar in vitro inactivation of leukemic cells occurs when they are placed in contact with antiserum prepared by inoculating leukemic cells into a foreign species (118). Antigen is present in both normal and malignant cells, but to a greater extent in the latter (20). Cytoplasmic material is more highly antigenic than nuclear (4, 25). Nonspecific inactivating material of rabbit serum can be destroyed by heating at 56° C. (118). Antiserum against lymphosarcoma cells (produced in rabbits) inhibited the growth of a transplanted rat lymphosarcoma (94).

The temporary growth of leukemic cells in foreign strains of mice may be inhibited or stimulated by multiple prior injections of frozen, lyophilized tissue (109, 110). Stimulation or inhibition depends on both the source of tissue and the neoplasms transplanted. Animals may actually succumb as a result of invasion of cells which ordinarily grow only temporarily. Foster-nursing may render animals of otherwise resistant strains susceptible to the transmission of leukemia (69).

Immunity to mouse leukemic cells has been induced in mice by inoculation of normal tissue of a foreign strain or a sublethal dose of leukemic cells prior to injection of a lethal dose (84). The immunity produced in the latter manner was passively transferred by inoculation of spleen or liver of immune animals; that induced by the administration of normal tissue was not transferable (96). Although it was possible to immunize against transplanted leukemia, such immunity could not be induced against spontaneous leukemia nor the cells from a spontaneous case (80).

When a rat reticulum-cell sarcoma was transplanted after exposure to 3,000 r of x-rays, some of the transplants failed to grow (40). The hosts were immune to reinoculation of untreated grafts of the same neoplasm. Following regression of a lymphosarcoma grafted into riboflavin-deficient mice, subsequent tumor grafts did not grow (114). Regression of the same lymphosarcoma in pyridoxine-deficient mice did not induce immunity (113).

The first experiments on parabiosis in relation to susceptibility to transplanted leukemia revealed that if mice of a susceptible and of a resistant strain were united, inoculation of either parabiont with leukemic cells resulted in the development of leukemia by only the susceptible animal (30). Recent experiments utilizing the technic of parabiosis support the idea that humoral factors are involved in transplantation. Rats were made susceptible, by radiation with x-rays, to the heterologous transplantation of mouse leukemia. When an irradiated rat was united to a genetically similar untreated animal, the irradiated rat lost its susceptibility to the transplanted mouse leukemic cells (10). This experiment has been interpreted as demonstrating that humoral substances passing from the nonradiated to the radiated rat nullified the radiation-induced susceptibility.

Rats or mice which ordinarily do not develop leukemia when inoculated with leukemic cells from the same species may exhibit susceptibility if inoculated when relatively young with large doses of cells (45, 103). If genetic factors control the immunologic physiology determining transplantation, it might be concluded that transplants may be successful in otherwise resistant animals if
are inoculated before the ability to form antibodies is well developed. Genetically determined resistance is relative, modified by the age of the recipient and the dose of leukemic cells.

A virus which is not to be considered a causative agent of mouse leukemia has been associated with several transfer lines and has been studied especially in relation to line I of the C58 strain (116). In mice inoculated with extracts of leukemic cells a lymphocytosis appeared, and the animals became sick 8–10 days after inoculation. All animals recovered and were immune to reinoculations of the virus. Leukemic cells can be freed from the virus by inoculation of cells into immune animals. The virus shortens the latent period of the transplanted disease, and influences the development of immunity against leukemic cells of line I.

Leukemic cells may contain a viral agent of perhaps another type (22). By culturing a lymphosarcoma, the mesenchymal were separated from the round cells. A lymphopenia resulted from the inoculation of either the leukemic round cells or extracts, but not from stromal mesenchymal cells. Animals were immune to subsequent inoculations.

In experiments demonstrating the transmission of leukemia by single intact cells it was found that leukemia did not result when the fragments of leukemic cells ruptured by microdissection were inoculated (38). Successful transmission of rat leukemia with cellular constituents alone has been described (112). Although the presence of intact cells was considered unlikely with the procedure used, many tumors appeared at the subcutaneous site of inoculation. Local appearance of tumors in the subcutaneous tissue makes cellular transmission a distinct probability.

Chemical agents may influence the development of transplanted leukemia. This will be discussed below under "Therapy."

**Therapy**

There are three fundamental types of rat or mouse leukemia which can be used in assaying the anti-leukemic effect of therapeutic agents—spontaneous, induced, transplanted. The spontaneous disease is more the counterpart of human leukemia than are the other two types. For various reasons, however, it has not been used in routine testing. First, an extremely large colony of animals is necessary to obtain a sufficient number of test animals; second, there is considerable variation in survival from case to case within even inbred strains; and third, the date of origin of the disease is uncertain, since the external manifestations of leukemia might not bear an exact time relation to the onset of malignant transformation. However, patients are treated only after the clinical onset of leukemia, and, if comparable material were to be used for experimental study, spontaneous mouse leukemia should be tested.

In the case of induced leukemia, agents might be assayed for effectiveness in two ways: first, to test their effect on inhibiting leukemogenesis; second, to test their ability to increase survival time once the disease has appeared. Induced leukemia has been used only infrequently to test agents (estrogens, androgens, x-rays) for either their anti-leukemogenic action (35, 38, 66) or their effect on the established leukemic cell.

Transplanted leukemia is generally used in screening programs (39). It must be recognized that the transplanted leukemia is quite different from either the induced or spontaneous disease, in that the cells of the leukemic animal did not undergo their malignant transformation in the host, but are the progeny of cells which were malignant when introduced. Upon considering that it has been possible to immunize against transplanted, but not spontaneous, leukemia (80, 84), it should be realized that the leukemic cells of the two types of disease may be quite different. Transplantation may certainly affect the isogenic character of the leukemic cell.

After inoculation of leukemic cells a latent period exists before the animal may be considered leukemic—that is, a period before the inoculated leukemic cells have set up generalized foci of proliferation. Or, if a local tumor of lymphocytes is used for testing, as in the case of any other tumor, a latent period exists prior to the establishment of the tumor-host relation of the successful graft.

Although delaying or inhibiting the development of transplanted leukemia by instituting treatment within 1–4 days after transplantation may have significance, and although drugs demonstrating this effect have usually had the greatest effect upon human lesions, it must be recognized that inhibiting transplanted mouse leukemia is a far cry from successfully treating spontaneous mouse leukemia, not to mention human leukemia.

If an agent shows no inhibitory action against only certain transplanted mouse leukemias, this does not necessarily constitute evidence of its general ineffectiveness. Tremendous biological differences exist between the different transfer lines. Myeloid leukemia responds to certain drugs, lymphoid to others. It is encouraging that urethan and potassium arsenite, which are considered to influence human chronic myeloid leukemia, affect...
primarily the myeloid leukemias of mice, and similarly, the folic acid antagonists influence the acute lymphoid mouse leukemias (68). The essential findings of experimental therapy using transplanted leukemia are given below.

Radiomimetic drugs (nitrogen mustards, colchicine, urethan, alpha- and beta-peltatin, podophyllotoxin).—Administration of this group of chemicals results in profound cytopathological alterations in both normal hemopoietic tissue and malignant cells arising from this source. The question has been raised concerning the extent to which the action of these agents is direct, or mediated by way of the pituitary-adrenocortical mechanism (5), since these agents are so much more effective on lymphoid than other tumors. A nonspecific noxious stimulus may induce regression of a transplanted lymphosarcoma (8). Retardation or regression of growth of lymphosarcoma 6CSHEDP was induced in either intact or adrenalectomized animals by the administration of one of the nitrogen mustards or urethan (6). Cortisone does, however, inhibit the growth of certain transplanted lymphoid tumors. Inhibition of growth may be obtained only with relatively large doses which are not compatible with extended survival (18). Fasting induced hypoplastic alteration in normal lymphoid organs only in the presence of the adrenals, whereas transplanted malignant lymphoid tissue was affected in adrenalectomized animals as well, indicating its independence of adrenal cortical control (1).

Increase in the survival time of mice with transplanted mouse leukemia was obtained with only certain of the nitrogen mustards, others being completely ineffective, indicating the chemical specificity of these agents (17).

Complete regression of a transplanted lymphosarcoma resulted from the administration of colchicine (9). In earlier experiments in which a colchicine-treated lymphosarcoma regressed, recurrence appeared and refractoriness to the drug developed (77). Not all mouse lymphosarcomas are sensitive to the action of this drug.1

Although urethan retards the development of certain transplanted myeloid, but not lymphoid, neoplastic growths of F strain mice (63), the effect in other strains of mice and rats is not confined to myeloid transfer lines (70, 90). Fully developed transplanted leukemia with widespread infiltration was not favorably influenced from the standpoint of survival. The high white blood cell count dropped remarkably, the leukemic myeloid cells of the tissues showed a "shift to the right," and fewer mitotic figures were present (64). In affecting the normal hemopoietic tissues of the mouse and rat the lymphoid are more sensitive than the myeloid (47).

Temporary alkalosis follows urethan therapy. Administration of sodium bicarbonate and ammonium chloride to affect the acid-base balance temporarily did not alter the anti-leukemic effect (108). The effect of urethan on transplanted mouse leukemia is highly specific. Any change in the molecule either destroys or lessens the anti-leukemic action (108).

When carbonyl-labeled urethan was administered to both normal mice and animals with either leukemia or mammary cancer, the latter group retained more radioactivity in all tissues (18). There was a specific fixation of carbon for two groups in the urethan molecule to sperm, which has a high DNA content, suggesting that this compound combines with nuclear material (15).

Folic acid antagonists.—Following the report of effectiveness of folic acid antagonists in inducing remissions in human childhood leukemias, the inhibiting effects of these drugs were tested on transplanted mouse leukemias. If treatment is begun within 4 days after transplantation, the development of certain lines is delayed, whereas others are not affected (15, 63, 74).

Perhaps of more significance than the limited clinical benefits of therapy is the evidence that there develops a refractoriness of the leukemic cell per se to treatment. Sublines of transmitted leukemia were made resistant by passage for several generations through treated mice (19, 72). This resistance remained unchanged in thirty passages through treated animals (72). Morphologically, the sublines remained the same. Resistance to one 4-amino analog implies resistance to all, although the cells remain sensitive to the action of competitive antagonists of other chemical structure and to anti-leukemic agents such as alphapeltatin.

Concentrations of folic acid antagonists which inhibit the growth of a susceptible subline are essential for the growth of variant sublines, indicating "dependence." Morphology, antigenicity, and transplantability of the transformed (resistant) and untreated sublines of resistant cells were similar (72).

The resistance of the leukemic cells to folic acid antagonists appears to be a stable and irreversible change. Either genetic mutation is induced with resultant resistant forms developing, or the susceptible cells of a varied population of leukemic cells are eliminated by treatment, the resistant forms being left behind to propagate the constituents of the resistant subline (72).

Refractoriness to potassium arsenite therapy.
of a transplanted mouse myeloid leukemia could be induced by treatment of the host with the drug prior to transplantation of leukemic cells. The host rather than the leukemic cell was responsible in this case for resistance to drug treatment. The possibility that the leukemic cells participate in this "fastness" has not been eliminated, although cells which have been passed through seven generations of treated hosts continue to respond to arsenic therapy.

**Hormones.**—The growth of one line of mouse lymphosarcoma was retarded by the administration of cortisone (48). In another transfer line no increase in survival time was observed, but with 19.5 μg, given 8 times daily no leukemic infiltrations occurred (18). The white blood cell counts of leukemic mice dropped in conjunction with cortisone treatment; ACTH was less effective. The Murphy lymphosarcoma grew to a larger size in adrenalectomized than in intact rats; cortisone inhibited its growth if treatment was begun soon after transfer. Compounds F, A, and corticosterone, as well as cortisone, each reduced the rate of growth of a transplanted mouse lymphoid tumor (190). Androgenic hormone inhibits the leukemogenic action of estrogenic hormone (38) and x-rays (35, 58).

**Radiation.**—Secondary effects of x-radiation were apparently involved in the inhibition of growth of a transplanted mouse lymphoid neoplasm (50). The effect obtained was maximum if the whole body as well as the tumor was radiated; radiation of the body exclusive of the tumor did not induce tumor regression. Administration of radioactive colloidal gold intraperitoneally results in the prolongation of life in certain transfer lines, but the distribution of gold is such (greatest amount in liver and spleen, relatively little in lymph nodes and bone marrow) that optimum radiation of leukemic infiltrations is not obtained. The uptake of radioactive phosphorus by lymphomatous tissue is greater than by other tissues (102). Radioactive sodium, on the other hand, is not concentrated selectively in tumorous lymphoid tissue and is probably of no potential therapeutic value (28). Retardation or inhibition of growth of transplanted leukemia and/or lymphosarcoma has been achieved by miscellaneous methods: injection of heterologous antibodies (94), ingestion of benzene (29), intravenous administration of aqueous suspensions of carcinogenic hydrocarbons (111), subjecting host to low body temperature (27), intravenous vaccinia (117), injection of testicular extract (8), pyridoxine and riboflavin deficiency (113, 114), injection of 8-azaguanine (60, 61, 71).

The significance of these experiments is at present difficult to assess.

**SUMMARY**

1. Morphologic types of lymphoblastoma simulating those seen in man appear in rodents.
2. Interaction of genetic and extrinsic factors determine the time of appearance and type of lymphoblastoma. A nongenetic factor contributing towards resistance is transmitted from mother to offspring and may be supplied before birth or by nursing alone. Susceptibility to the development of "spontaneous" leukemia is not determined by genes identical with those involved in reactions to leukemogenic agents such as ionizing radiations, carcinogenic hydrocarbons, and estrogenic hormones. Specific genes may control susceptibility to each leukemogen. Leukemogenic agents may act synergistically in strains of mice susceptible to the independent action of each agent.
3. Leukemic cells of certain transfer lines may harbor a virus which is not an etiologic agent for leukemia, but which causes an illness hastening death from leukemia and alters the antigenicity of cells or the immune reaction of the host. Preliminary experiments have suggested that noncellular extracts of either leukemic tissue or of embryonic cells from a high leukemia strain may cause leukemia to appear "spontaneously" if injected into mice of a low-leukemia strain. Freezing and lyophilization of mouse leukemic cells render them inactive for transmitting leukemia.
4. Estrogen favors, androgen inhibits leukemogenesis in mice in certain situations. The leukemogenic effects of both x-rays and carcinogenic hydrocarbons may be potentiated by estrogenic hormone.
5. Susceptibility to the development of mouse leukemia may represent a property of the thymus, which serves not only as a primary locus for the development of the disease, but may influence its genesis in some unknown manner.
6. A humoral factor may be involved in the development of x-ray–induced leukemia. Radiation of the thymus alone, or of the whole body except the thymus, did not result in the induction of thymic tumors in animals which develop such neoplasms following whole body radiation. Since shielding of an extremity reduced the incidence of x-ray–induced thymoma, it appears that nonirradiated tissue may inactivate a humoral factor produced by radiation.
7. In a strain of mice susceptible to a particular leukemogenic agent, the younger the animal is at the time of exposure, the more the susceptibility is enhanced.
8. Nutritional factors such as caloric restriction or cystine deficiency may delay the development and reduce the incidence of spontaneous or carcinogen-induced leukemia. Pyridoxine or riboflavin deficiency are associated with the inhibition of growth of certain transplanted lymphosarcomas. It has been postulated that guanine is essential for the growth of neoplastic cells and that the growth of certain lymphosarcomas may be inhibited by the administration of 8-azaguanine, a guanine analog. The inhibitory effect of folic acid analogs on transplanted leukemia is attributed to interference with folic acid metabolism resulting in diminished nucleic acid synthesis.

9. Genetic factors control the transplantation of leukemic cells. The malignant transplantable elements are only the lymphoblastic round cells and not mesenchymal stromal elements. Antigen-antibody reactions may be involved in transplantability, since the gene presumably controls immunologic physiology. Homologous and heterologous immune sera may inactivate leukemic cells in vitro. Cytoplasmic constituents of leukemic cells are more highly antigenic than nuclei. Temporary growth of leukemic cells in foreign strains may be accelerated or inhibited by multiple prior injections of frozen, lyophilized tissue. Heterologous transplantation of leukemic cells can be accomplished by previous radiation of the foreign host, but not by inoculation into the anterior chamber of the eye. Humoral factors passing from normal to irradiated rats joined by parabiosis may nullify radiation-induced susceptibility. Immunity towards transplanted leukemia can be passively transferred by inoculation of spleen or liver of immune animals. Resistance to homologous transplantation of leukemic cells may be overcome by inoculation of large doses of cells into very young animals.

10. Various agents may inhibit or delay the growth of certain lines of transplanted leukemic cells. Among these are “radiomimetic drugs,” folic acid antagonists, cortical hormones, ionizing radiations, trivalent arsenic, benzene. Although the most active agents are those which have given the most encouraging clinical results, transplanted leukemia as generally used can at most be considered a helpful but not a critical testing medium. Wide variation exists in the response of different lines of leukemic cells to the same agent. Of greatest significance are the studies on refractoriness of leukemic cells and host to drug therapy.

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