states in the radiation-induced oxidation of solid peptides which on the basis of the present work appears to have interesting implications both in radiation chemistry and in radiation biology (*14*).

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14. High values for oxygen uptake under γ-radiolysis have also been observed with other highly dispersed macromolecular structures. Of particular interest is the finding that solid DNA obtained on freeze-drying a 1 percent solution gives $G(-O_2)$ about 40. Oxygen uptake by DNA is remarkably sensitive to the degree of dispersion of the solids; material prepared by freeze-drying a several-percent solution showed $G(-O_2) < 10$.
15. Work performed under the auspices of the U.S. Atomic Energy Commission.

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Chromosome Aberrations: Their Role in the Etiology of Murine Leukemia

Abstract. *If the association between chromosome aberrations and leukemia is a causal one, the aberrations should be present before the appearance of tumor. In a virus-induced murine leukemia in which the pre- and early leukemic stages were defined, aneuploidy was observed only during the later stages of the disease. This suggests that the chromosome alteration results from, rather than initiates, the neoplastic transformation.*

Since the speculations of Boveri in 1914 (*1*) there has been abundant demonstration of the association between chromosome alteration and neoplasia. Chromosome aberrations in solid and ascites tumors in vitro and in vivo, in a variety of species including man, have been extensively reviewed (*2*). The suitability of the leukemias as a model system stimulated numerous studies on chromosome abnormalities or their lack in human leukemia as well as those induced in animals by viral, chemical, and physical agents. Despite these efforts, reports to date do not conclusively establish a causal relationship between chromosome alterations and neoplasia. Proof for a causal hypothesis in opposition to chromosome alterations which occur as a result of neoplastic transformation would require the presence of these alterations during the earliest pre-neoplastic stage, before the histologic appearance of tumor (*3*).

The virus-induced murine leukemias offer a convenient model system for a test of the "causal" hypothesis. The system provides a transition from the normal to the neoplastic state under controlled conditions with a reproducible interval between inoculation and the appearance of histologically recognizable tumor. Recently we described a potent murine leukemia virus which induces lymphoid leukemia in 80 to 100 percent of the inoculated animals within 50 to 70 days (*4*). This thymic lymphoma is a rapidly proliferating, undifferentiated tumor histologically similar to the other murine lymphomas. The development of this thymic lymphoma is not preceded by an early erythroblastic splenic response as in Friend (*5*) and Rauscher leukemia (*6*). The increased incidence of secondary chromosome constrictions which we observed (*7*) during the early splenic phase of Friend and Rauscher leukemia was not observed in the disease initiated by our agent.

The appearance of thymic, and subsequently generalized lymphoma in the system reported here, was accompanied by aneuploidy with a predominantly hyperdiploid mode (*8*) similar to that observed during the later, lymphomatous phase of Rauscher leukemia. No characteristic "marker" chromosomes were observed in the leukemia induced by our agent.

During a detailed study of the pathogenesis of this leukemia (*9*), tumor evolution occurred in only one of the two paired thymus glands. The appearance of histologically detectable, proliferating tumor was consistently preceded by unilateral depletion of thymic lymphocytes and by an intermediate preleukemic histological alteration which we termed "lymphoma *in situ*." It was thus possible in this system to study the incidence of chromosomal aberrations at various stages of neoplastic transformation in tissue whose ultimate neoplastic fate had been predetermined.

Newborn (less than 24 hours old) Swiss HaICR mice were inoculated with 0.05 ml of the leukemia virus stock (*9*). At regular intervals thereafter beginning at day 22 the animals were injected with colchicine (*10*). The mice were autopsied and examined thoroughly according to the procedure previously described (*9*, *11*). The right and left thymus were removed for studies of the chromosomes (*12*). In this way, chromosome characteristics could be directly correlated with the stage of leukemogenesis in specific animals. The chromosomes were prepared directly, without being cultured (*7*).

In accord with observations in previous studies of the pathogenesis of this and other virus-induced leukemias (see *9*, *11*), the mice were divided into four groups depending on the stage of the leukemogenic process (Fig. 1).

1) No gross or microscopic pathology: This group included inoculated animals which showed no pathologic changes at the time they were killed.

2) Unilateral thymus depletion: Mice in which one thymus (right or left) showed more than a 30 percent loss of weight relative to the other thymus. The weight loss was reflected by the histologic evidence of unilateral lymphocyte depletion. No evidence of lymphoma or abnormal cell proliferation was observed in this group. Those depleted thymuses which histologically suggested lymphoma but did not fulfill the other criteria for malignancy were also included in this group.

3) Unilateral lymphoma: Animals in which overt lymphoma could be detected in one thymus. In addition to increased cell size, nuclear staining,

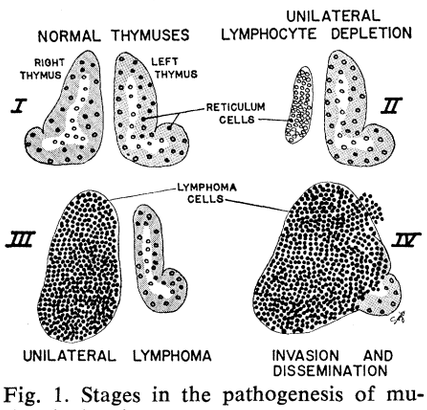

Fig. 1. Stages in the pathogenesis of murine leukemia.

and mitoses, architectural disorganization and cellular proliferation resulted in significant enlargement of the affected organ.

4) Generalized lymphoma: Animals in which the proliferating lymphoma had invaded the opposite thymus and disseminated to spleen, liver, and peripheral nodes.

In chromosome counts, a significant alteration was considered to occur when at least 10 percent of the cells of that organ exhibited a chromosome number different from the normal diploid mode of 40. Chromosome counts were carried out independently and without prior knowledge of the pathologic diagnosis.

The nonproliferative "preleukemic" changes described for group 2 invariably preceded the development of overt lymphoma. This was observed consistently in the murine leukemias induced by a variety of viruses (*9, 11*). If the change in chromosome number is part of the sequence leading to or causing the neoplastic transformation, it should be observed prior to the histologic appearance of tumor. Table 1 shows that this is not the case. Significant numbers of aneuploid cells were not observed in the thymuses of animals with nonproliferative preleukemic thymic changes (group 2) and only rarely even in mice with early thymic lymphoma (group 3). This is in contrast to animals exhibiting late proliferating lymphoma (group 4), in which the thymus cells of six out of ten animals showed alterations in chromosome number.

Table 1. Incidence of aneuploidy during various stages of leukemogenesis. Group 1, no pathologic changes; group 2, unilateral thymus depletion (pre-lymphoma); group 3, unilateral lymphoma; group 4, generalized lymphoma.

Incidence of aneuploidy	Group			
	1	2	3	4
	Right thymus			
In animals*	0/15	0/6	0/7	4/10
In cells†	2/181	0/73	2/87	64/283
	Left thymus			
In animals*	0/15	0/6	1/7	3/10
In cells†	2/159	3/65	52/149‡	54/209

* Number of animals whose thymus cells showed 10 percent or more aneuploidy/number of animals examined. † Number of aneuploid cells/number of cells examined. ‡ Fifty-one aneuploid cells were observed in one animal with late, though unilateral, lymphoma (51/53).

The sparsity of aneuploidy in pre- and early leukemia compared with the high incidence in late disseminated lymphoma suggests that the chromosome changes which are associated with the "thymic" group of murine leukemias are not causal but rather represent one of the consequences of the neoplastic transformation.

At present, the noncausal role of observed chromosome aberrations may be properly applied only to the murine viral leukemias. The clinical similarity of murine leukemia to that in man may offer insight into the role of chromosome aberrations in human leukemia.

The results of studies with chemically induced neoplasia are equivocal (*13*). In a study of aneuploidy in murine leukemia induced by irradiation, Nadler (*14*) suggested that these leukemias were not suitable for evaluation of the significance of aneuploidy.

In chromosome studies of the Shope virus papilloma-carcinoma system during the transition from benign to malignant tumors, no consistent abnormalities were observed (*15*). Hellström *et al.* (*16*), studying primary polyoma tumors, suggested that the observed aneuploidy was secondary to the neoplastic transformation.

The data presented here do not bear upon the possible role of undiscernible chromosome aberrations as discussed by Nichols (*17*), or the role of chromosome aberrations in enhancing the progression of tumor (as opposed to its initiation). They do, however, add experimental proof to the hypothesis that chromosome aberrations do not play a primary role in the etiology of the virus-induced leukemias.

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Serotonin Rhythm in the Pineal Organ: Control by the Sympathetic Nervous System

Abstract. *The serotonin content of the pineal organ of the rat varies diurnally in relation to the photoperiod. When the sympathetic nerves to the pineal are interrupted by the removal of the superior cervical ganglia, no such fluctuation in the serotonin content of the pineal occurs.*

On the basis of careful anatomical studies, Kappers (*1*) has proposed that the pineal organ is innervated chiefly, if not completely, by fibers of the autonomic nervous system. Of these, the nerves leaving the superior cervical ganglia, passing in the tentorium over the dorsal surface of the cerebellum as the nervi conarii, and penetrating the pineal organ, constitute the primary supply.

There is some biochemical evidence supporting the view that such a neural