mouse hepatitis virus (MHV 1) were examined by conventional staining procedures and by electron and fluorescence microscopy using acridine orange fluorochrome and fluorescein-tagged antibody. Preparations stained with acridine orange showed abnormal accumulations of RNA-staining material in cytoplasm of hepatic cells. Inclusion bodies of similar size, shape, and location were stained selectively with fluorescein-tagged antibody. Observations by electron microscopy showed virus particles in cytoplasm which measured 90 ± 20 mμ in diameter.

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Influence of Age at Time of Irradiation on Induction of Leukemia and Ovarian Tumors in RF Mice. (25982)

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Among physiological variables influencing susceptibility to radiation-induced leukemia in mice is age at time of irradiation(1,2). In man also, according to epidemiological surveys, susceptibility to leukemia induction may be relatively high during embryonic and fetal development(3), and the hematologic type of leukemia induced may vary with age at the time of irradiation(4). The present investigation was carried out to extend earlier observations on the relation between age at irradiation and leukemogenesis in mice of the RF strain.

Methods. Male and female RF mice of several ages from 3 days before birth to 180 days after birth were exposed to whole-body X radiation in doses of 100, 200, and 300 r (Table I). Radiation was administered with a 250-kvp General Electric Maxitron machine at 30 ma, with 0.1 mm of inherent and 3 mm of added Al filtration (hvl, 0.44 mm of Cu), TSD 93.7 cm, and a rate of 75-100 r/min. Suckling young were exposed together in intact litters, whereas other mice were exposed individually by the technic described previously(5). The mice were necropsied on death and tissues fixed in Zenker-formol for sectioning.

Results. Incidence of granulocytic leukemia was not significantly increased by irradiation *in utero* or during the first 9 days after birth but was increased by irradiation later in life, susceptibility to induction of the disease rising gradually to a maximum at 70 days of

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TABLE I. Leukemia Incidence in Relation to Age at Irradiation.

Age at irradiation, days	X-ray dose, r	Mice		Mean after-survival time, mo	%		
		Sex	No.		Myeloid leukemia	Thymic lymphoma	Ovarian tumor
	0	♂	368	19	3	5	
		♀	161	20	3	6	1
1	100	♂	58	19	2	4	
		♀	55	18	2	18	44
	200	♂	67	17	8	9	
		♀	62	17	5	14	66
−1 to −3*	300	♂	21	18	0	0	
		♀	15	14	0	0	7
1–9	300	♂	124	12	5	15	
		♀	59	16	5	24	61
14	300	♂	101	14	26	13	
		♀	94	14	6	34	39
28	300	♂	80	12	42	14	
		♀	87	14	14	37	41
40	300	♂	104	10	43	19	
		♀	94	9	13	51	30
70	300	♂	65	12	54	8	
		♀	94	12	26	21	26
180	300	♂	111	10	32	9	

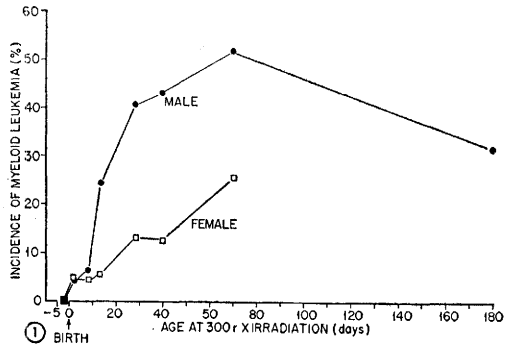
* Irradiated *in utero* 1-3 days before birth.

age (Table I, Fig. 1). Thymic lymphoma, likewise, was apparently not induced by irradiation *in utero* but was readily induced by irradiation immediately after birth (Fig. 2). Susceptibility to its induction decreased between 40 and 70 days of age coincidentally with spontaneous involution of the thymus (Fig. 3). The higher susceptibility of males to myeloid leukemia and of females to lymphoma agrees with earlier observations (2). Mortality during the first 30 days after irradiation was negligible in all age groups. Mean after-survival time was less consistently correlated with age at irradiation than with leukemia incidence but decreased with increasing radiation dose, as did mean induction time for leukemias. Ovarian tumors were significantly less common in the mice irradiated *in utero* than in those irradiated after birth (Table I).

Discussion. From earlier results(2), we expected susceptibility to induction of myeloid leukemia to be relatively low during the first few days of life, despite concomitantly high susceptibility to lymphoma induction. We did not expect, however, that susceptibility to both types of leukemia would be low in the fetus, especially in view of epidemiologic data suggesting a high susceptibility in the human fetus or infant(3). Although there was no evidence of radiation lethality in the exposed fetuses, as judged by number of viable offspring, it is conceivable that 300 r may have been too large a dose for maximum leukemogenesis at this age despite its leukemogenetic potency later in life. In irradiated adult RF mice, incidence of myeloid leukemia declines as dose is increased from 300 to 450 r(2). These experiments need to be extended, therefore, with smaller doses of radiation, exposures at other stages of gestation, and larger numbers of mice per group.

Since susceptibility to ovarian tumor induction was also relatively low in the mice irradiated *in utero* (Table I), radiosensitivity as such may vary appreciably with age of the host, for reasons yet to be elucidated. In this connection, it is noteworthy that sensitivity of the mouse ovary to sterilization by radiation is relatively low during the period shortly before birth(6, W. L. Russell, personal communication). In addition, of course, age-dependent variations in the $LD_{50/30}$ of radiation for mice are well documented(7).

Another factor of theoretical importance in susceptibility to leukemia development is the



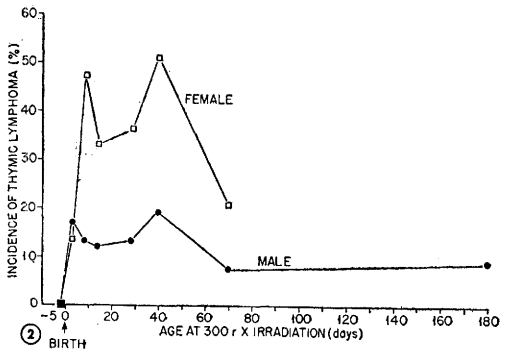


FIG. 1. Incidence of myeloid leukemia in relation to age at irradiation.

FIG. 2. Incidence of thymic lymphoma in relation to age at irradiation.

FIG. 3. Thymic wt in relation to age.

rate of turnover of cells in leukocyte-forming tissues, since selection and proliferation of leukemic variants are presumably favored under conditions of high mitotic activity. Consistent with this idea is the observation that susceptibility to induction of thymic lymphomas tends to decline with natural or hormone-induced thymic involution and to increase with thymic hyperplasia(8). Although rate of cell division in the fetal thymus is not known, irradiation *in utero* causes less depression of thymic weight than irradiation after birth (A.C. Upton and E. P. Sniffen, unpublished data). As regards myelopoietic activity, the number of myeloblasts in the body increases progressively with body weight throughout the first weeks of life (A. C. Upton and E. P. Sniffen, unpublished data). Thus, if the probability of myeloid leukemia induction varies with number of myeloblasts irradiated, susceptibility to radiation-induced leukemia should increase with age in the period of growth and development, as has been observed. That this correlation is not entirely consistent, however, is indicated by the rather sudden increase in susceptibility to myeloid leukemia induction between the first and second weeks of life (Table I), and by the disproportionate degree of protection against leukemia induction afforded by shielding a small amount of hemopoietic tissue(2).

An additional factor to be considered is the possible role of viruses in pathogenesis of radiation-induced leukemia(9-12). If leukemia is assumed to result from activation of a leukemogenic virus present at time of irradiation, age-dependent epidemiologic variations in exposure to the virus might account for the observed variations in susceptibility, as might passive immunization by maternal antibodies or the presence of other viruses that inhibit or enhance the leukemia agent.

Summary. RF mice exposed to 100-300 r of whole-body X-rays at various ages from before birth to 180 days after birth showed age-dependent variations in susceptibility to leukemia induction. Susceptibility to induction of granulocytic leukemia was minimal when irradiation was carried out during gestation or shortly after birth, rising later to a maximum at about 70 days of age. Susceptibility to induction of thymic lymphomas was, likewise, apparently minimal during gestation but maximal shortly after birth, declining later in life at the time of thymic involution. Susceptibility to induction of ovarian tumors was relatively low in mice irradiated *in utero*.

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Survival of Mice in Absence of Inert Gas.* (25983)

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It has been generally assumed, for lack of evidence, that the nitrogen gas of the earth's atmosphere plays no role as a respired gas in the metabolic function of higher animals. However, since N_2 at pressures above 2 atmospheres begins to interfere with the normal behavior of the nervous system(1), it appears justified to ask whether or not it may at .79 atmosphere exert some effect on animal systems. The answer to this question is to be sought by looking for any biological changes in animals maintained in absence of N_2 gas. As a means of freeing the environment of N_2 gas, substitution of another inert gas for N_2 is not satisfactory since the other gas may of itself modify the results. On the other hand an atmosphere of pure O_2 at normal pressure is unsuitable since this will prove lethal in a matter of a few days. To test the possible long-term effects of absence of any inert gas upon the function of animals it is therefore necessary to use an atmosphere of pure oxygen but at reduced pressure to avoid oxygen toxicity. Our experiments indicate that mice will survive for long periods of time in oxygen when its pressure is maintained at 197 mm Hg. This is the level which provides the same inspired O_2 tension (150 mm Hg at BTPS) as breathing air at 1 atmosphere. Furthermore, 197 mm Hg of pure O_2 is the atmosphere which provides for a satellite cabin in space the lowest pressure differential across the walls compatible with a normal inspired O_2 tension within.

Method. The major problem is a mechanical one, that of maintaining animals for long periods of time in an atmosphere of O_2 at a reduced pressure. To avoid introduction of N_2 gas from the air into the chamber, the arrangements must render access to the animals unnecessary during the course of an experiment lasting many weeks. Chamber pressure and oxygen ventilation must be automatically maintained within narrow limits by reliable means and temperature and humidity must be kept within the comfortable range. In addition food and water must be provided and excreta conveniently accommodated. Since it took many months of trial and error to arrive at a satisfactory solution, a brief description of our arrangement follows. With the exception of the watering device, the gas sampling system and the water vapor trap,† all other parts are commercially available. The apparatus is schematically represented in Fig. 1. A small cast-aluminum chamber‡ (10

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† An acrylic resin chamber sealed around the cooling coil of a small vapor absorption type refrigeration unit (Astral Refrigerette).