flushing followed injection into a patient with septal venous-arterial shunt of a dose of 5-HT unable to cause such effect in normal subjects. 4. It is postulated that injected 5-HT is quickly removed from the circulation, perhaps by platelets or by other cellular elements retained in capillary beds and released slowly at a later time.

1. Stefanini, M., and Magalini, S. I., *A.M.A. Arch. Int. Med.*, in press.

2. Magalini, S. I., and Stefanini, M., unpublished results, 1955.

3. Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F., and Vander Brook, M. J., *J. Pharmacol. and Exp. Therap.*, 1952, v105, 80.

4. Erspamer, V., *Pharmacol. Rev.*, 1954, v5, 425.

5. Humphrey, J. H., and Toh, C. C., *J. Physiol.*, 1952, v124, 300.

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Leukemia Induction in Mice by Fast Neutron Irradiation.* (22501)

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This investigation was undertaken for the purpose of studying development of lymphoid, myeloid, and other types of leukemia in mice exposed to fast neutrons, as compared to X rays. Most cases of radiation-induced leukemia in human beings have resulted from exposure to gamma or X rays(1); however, in Japanese atomic bomb survivors the possible importance of neutron irradiation in the production of leukemia has been cited(2). Because neutrons have been observed to be, in general, relatively more effective than X or gamma rays in causing delayed radiation injuries in mice(3,4) and in man(5), determination of the relative biological effectiveness (RBE) of neutrons for leukemia induction is of practical, as well as fundamental importance.

Materials and methods. Male and female mice of the RF strain 8-16 weeks of age were exposed to fast neutrons in the 86-inch cyclotron of the Oak Ridge National Laboratory or to X rays. The neutrons, produced by the bombardment of an internal beryllium target with 22-Mev protons, had an average effective energy of approximately 1 Mev. The factors of X radiation were as follows: 250 kvp, 30 ma, TSD 93.7 cm, filtration 3 mm of Al (plus beryllium window), hvl 0.4 mm of Cu. Neutrons and X rays were administered to the whole body at an intensity of 65-115 rad min in a single exposure or in two equal treatments separated by an interval of 2-8 days. Further details of the dosimetry and methods of exposure have been reported(6,7). After irradiation the mice were caged in groups of 8-10 in air-conditioned quarters, with Purina fox chow and drinking water available *ad libitum*. The animals were observed until natural death or were sacrificed *in extremis*. Postmortem examinations were performed on all mice.

Results. For a given dose of radiation the 30-day mortality was greater in neutron-exposed than in X-irradiated mice; in females the $LD_{50}/30$ days for a single exposure was approximately 335 rad of neutrons or 490 rad of X rays (RBE = 490/335 = 1.47)(7). With fractionation of the dose into two equal exposures, the $LD_{50}/30$ days for X rays and neutrons increased with the interval between treatments, being approximately 640, 710, 815, and 870 rad of X rays for intervals of 2, 4, 5, and 8 days, respectively(8). The acute injuries produced by neutron irradiation were indistinguishable from those caused by X rays except for acceleration of the 30-day mortality (the peak death rate occurred on the 5th day after single median-lethal neutron irradi-

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TABLE I. Incidence of Leukemia in RF Mice Exposed to Fast Neutrons and to X-Rays.

	No. of exposures	Time between expos., days	Neutrons							X-rays					
			Survival		Myeloid leukemia, %	Thymic lymphoma, %	Median survival, mo	Survival		Myeloid leukemia, %	Thymic lymphoma, %	Median survival, mo
			Dose, rad	30-day, %	No. of mice*				Dose, rad	30-day, %	No. of mice*			
Male	0	0	0	100	80	3	4	21	0	100	250†	4	6	21
	1	0							119	100	46†	19	9	18
	1	0	110–165	100	28	43	7	12						
	1	0	170–195	100	25	32	12	12						
	1	0	205–230	100	41	44	5	12	335	100	65‡	29	17	16
	1	0	245–320	19–100	68	38	10	13	475	95	89†	11	17	14
Female	0	0	0	100	99	3	17	21	0	100	197†	0	16	21
	1	0							119	100	106†	19	14	17
	1	0	200–230	100	34	29	21	12	335	100	60‡	18	33	13
	1	0	240–335	63–100	28	25	28	13	475	95	89†	4	35	15
	2	2							440–535	85–100	35	43	37	9
	2	2	345–370	50– 55	18	11	11	12	580–675	40– 70	22	17	37	9
	2	5	320–360	85– 95	32	19	25	11	490–580	85–100	31	17	64	9
	2	5	380–385	70– 95	19	16	32	11	625–720	75– 85	31	19	52	10
	2	7–8	355–420	65– 85	33	11	35	12	580–720	65–100	27	24	66	7

* No. surviving to 5 mo of age, when first case of leukemia was encountered. † Data from previous exp.(10). ‡ Data from previous exp.(9).

ation and on the 9th day after exposure to X rays). After the first 30 days postirradiation, the death rate dropped nearly to control levels; 4 or 5 months later the survivors began to die of leukemia. Two major types of leukemia were induced, myeloid leukemia and thymic lymphoma. Most deaths from thymic lymphoma occurred in mice 6-15 months of age (mean 10 months); the incidence and rapidity of development increased with the dose of radiation. Thymic lymphoma was consistently more common in females than in males (Table I). Myeloid leukemia developed slightly later in life, the peak mortality from this disease occurring in mice 10-14 months old (mean 12 months); as with thymic lymphoma, its incidence and rapidity of development increased with the dose of radiation. Myeloid leukemia was more common in males than in females and was induced by relatively low doses of radiation (110 rad of neutrons or 119 rad of X rays). Thymic lymphomas were significantly more numerous in X-irradiated than in neutron-exposed mice, but the two types of radiations appeared, in general, to be of approximately equivalent over-all leukemogenic potency when compared at dose levels comparable in 30-day lethality (Table I). On fractionation of either neutron or X irradiation, the incidence of thymic lymphoid tumors was noticably elevated when the interval between exposures was prolonged to 5-8 days; however, the induction of myeloid leukemia was not correspondingly enhanced by fractionation. The length of the life span of the mice in the various treatment groups appeared to be correlated more closely with leukemia incidence than with dose or type of radiation or 30 day lethality.

Discussion. The induction of lymphoid and myeloid leukemia by radiation has been reported in RF mice exposed to X rays(9) and to thermal neutrons(10). The enhanced thymic lymphoma formation noted on fractionation of the irradiation at intervals of 5-8 days confirms that observed in C57 BL mice (11). It is noteworthy that the induction of myeloid leukemia was not also increased by fractionation of the dose.

A comparison of neutron- and X-irradiated

mice showed a consistently lower incidence of thymic lymphoma, along with an equal or greater frequency of myeloid leukemia, in the neutron-irradiated mice. A lower induction rate of lymphomas in CF_1 mice exposed to reactor fast neutrons, in comparison to the rate in mice treated with comparable doses of γ rays (27-32% in the neutron-irradiated vs 60% or more in the γ-irradiated mice) has also been noted by Henshaw *et al.*(3). These observations suggest that the RBE of fast neutrons for leukemia induction may vary with the type of leukemia induced; this effect deserves further investigation, since it may shed light on differences in the mechanisms of myeloid and lymphoid leukemia induction. Further studies may disclose a correlation between the earlier mortality of the neutron-exposed mice dying within 30 days postirradiation, which has been attributed to relatively more severe intestinal injury and less severe marrow damage(7), and the lower rate of induction of lymphomas by neutrons, as compared to X rays.

The relative over-all leukemia-inducing effectiveness of fast neutrons was not greatly different from their RBE for 30-day lethality if allowance is made for slight variation with different hematologic types of leukemia. This is in agreement with the observations of Henshaw *et al.*(3), who noted that on acute irradiation the RBE of reactor neutrons for 30-day lethality approximated that for reduction of longevity. It is also consistent with similar observations on the constancy of the RBE of thermal neutrons for acute lethality, leukemogenesis, and most other delayed effects in mice(10,12). Since, however, the RBE of neutrons for some types of delayed injury increased significantly with prolongation of the period of exposure(3-5), the RBE for leukemia induction may under conditions of protracted irradiation greatly exceed the value noted in the present experiment.

Summary. 1. Leukemias induced in mice of the RF strain by acute fast neutron irradiation in the cyclotron resembled in frequency and hematologic type those induced by X irradiation. 2. Neutrons and X rays appeared to have an over-all leukemia-inducing effectiveness comparable to their 30-day lethality effectiveness, although thymic lymphomas were significantly less frequent in neutron-exposed than in X-irradiated mice. 3. The induction of thymic lymphomas appeared to be enhanced by fractionation of either neutron or X irradiation at intervals of 5-8 days.

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1. Furth, J., and Upton, A. C., *Acta radiol.* supplement, 1954, v116, 469.

2. Moloney, W. C., *New England J. Med.*, 1955, v253, 88.

3. Henshaw, P. S., Riley, E. F., and Stapleton, G. E., *Radiology*, 1947, v49, 349.

4. Evans, T. C., *ibid.*, 1948, v50, 811.

5. Stone, R. S., *Am. J. Roentgenol.*, 1948, v59, 771.

6. Hurst, G. S., Mills, W. A., Conte, F. P., and Upton, A. C., *Radiation Res.*, 1956, v4, 49.

7. Upton, A. C., Conte, F. P., Hurst, G. S., and Mills, W. A., *ibid.*, 1956, v4, 117.

8. Melville, G. S., Conte, F. P., Slater, M., and Upton, A. C., to be published.

9. Upton, A. C., and Furth, J., *Blood*, 1954, v9, 686.

10. Upton, A. C., Furth, J., and Christenberry, K. W., *Cancer Res.*, 1954, v14, 682.

11. Kaplan, H. S., and Brown, M. B., *J. Nat. Cancer Inst.*, 1952, v13, 185.

12. Storer, J. B., Furchner, J. E., and Krebs, A. T., *J. Aviation Med.*, 1954, v25, 368.

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