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Comparative Effects of Neutrons and X-rays on Three Tumors Irradiated *in vitro*.*

DOROTHY AXELROD, PAUL C. AEBERSOLD AND JOHN H. LAWRENCE.
From the Crocker Radiation Laboratory, University of California, Berkeley.

Introduction. Per unit of ionization, neutrons have been demonstrated to be more effective than X-rays in producing biological changes. This increased effectiveness is undoubtedly due to the great difference in distribution of the ionization produced by the rays, *i. e.*, the ionization caused by neutrons is much more intense and localized than that caused by X-rays. Table I summarizes the results obtained in previous work. It will be noted that the X-ray/neutron dosage ratios, *i. e.*, ratios of the X-ray/neutron doses required to produce 50% lethal effect, vary considerably for different organisms, as well as for different tissues of the same organism, and that the ratio is always in favor of neutrons. This suggested the possibility that the X-ray/neutron ratios of the tumors would vary and thus that neutrons may be selective in their action on different tumors. The work reported here is the study of the comparative effects of neutrons and X-rays on 3 tumors of the Strong-A strain mouse,[1] a mammary carcinoma,[2] a lymphoma,[3] and a lymphosarcoma.† All of these tumors are very suitable for experimental work because they are easily transplantable and grow 100% in the Strong-A strain mouse.

Methods. 1. *Preparation of Tumor Particles.* A mouse with subcutaneous, bilateral tumors was killed, the tumors removed aseptically and divided into small particles weighing approximately 10 mg. These particles were first wrapped in sterile filter paper moistened with sterile, buffered, physiological saline and then wrapped in cellophane. Twenty particles were placed in each package; for each experimental point, 2 packages were irradiated (40 implants).

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1 Strong, L. C., *J. Hered.*, 1936, **27**, 21.

2 Gardner, W. U., Smith, G. M., Allen, E., Strong, L. C., *Arch. Path.*, 1936, **21**, 265.

3 Lawrence, J. H., Gardner, W. U., *Am. J. Cancer*, 1938, **33**, 112.

† Induced by Dr. W. U. Gardner after prolonged folliculin injections.

TABLE I.
Summary of Previous Work on Comparative Effect of X-rays and Neutrons on Various Biological Objects.

Biological effect	X-ray neutron ratio
Inhibition of growth of wheat seedlings (6)	5
" " development of Drosophila eggs (6)	2.1
" " " " Fern spores (6)	2.5
Lethal effect on mammary carcinoma (7)	5.1
" " " normal mice (8), (9)	4
" " " sarcoma 180 (8)	4
Production of lymphopenia in normal rats (10)	10
Chromosomal abnormalities (11), (12)	6
Inhibition in development of Drosophila eggs (13)	2, 2.8, 3.2
Retardation of growth of wheat seedlings (root) (13)	6.2-11.8
" " " " " " (shoot) (13)	5.9-9
Inhibition of mitosis in rats (14,a)	6
Lethal effect on *B. mesentericus* spores (14,b)	5.3
" " " *B. coli* (14,c)	3.2
" " " bean roots (14,d)	11
Production of sterility in male mice (15)	5-6

Controls (10 implants) were prepared in the same manner and kept under as nearly the same conditions as the experimental material. After irradiation, the tumor particles were implanted subcutaneously, bilaterally into the axillary regions of mice by the trocar method (No. 13 hypodermic needles). Three hours was the limit set for the completion of the experiment, the controls being implanted last. The mice were observed weekly for 10 weeks and the number of tumor growths noted.

2. *Irradiation Technic. a. Neutron Irradiation.* Neutrons were produced by the bombardment of a beryllium target with deuterons accelerated to 16 million volts in the 60-inch cyclotron.[4] The neutron

[4] Lawrence, Alvarez, Brobeck, Cooksey, Corson, McMillan, Salisbury, Thornton, *Phys. Rev.*, 1939, **56**, 124.

[5] Aebersold, P. C., *Phys. Rev.*, 1939, **56**, 714.

[6] Zirkle, R. E., Aebersold, P. C., Dempster, E. R., *Am. J. Cancer*, 1937, **29**, 556.

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[8] Lawrence, J. H., Aebersold, P. C., Lawrence, E. O., *Proc. Nat. Acad. Sci.*, 1936, **22**, 543.

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[11] Marshak, A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 176.

[12] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.

[13] Zirkle, R. E., and Lampe, I., *Am. J. Roent. and Rad. Therapy*, 1938, **39**, 613.

[14] Unpublished data. (Reported in *Summary Reports from Research Centers for 1938* (London), Medical Research Council). (a-c) Spear, 1938; (d) Mottram, Gray, Read, 1939.

[15] Snell, G. D., Aebersold, P. C., *Proc. Nat. Acad. Sci.*, 1937, **23**, 374.

beam was collimated by an arrangement similar to that previously described.[5] The material to be irradiated was placed at the end of the channel through the collimator at a distance of 85 cm from the target. The packets of tumor particles were mounted on cellophane stretched over the opening of the irradiation port. A filter of 3 cm of lead was used to suppress gamma radiation. Deuteron currents of 100-150 microamperes were used. The intensity produced was about 10-15 n per minute, the n-unit being the quantity of neutrons

TABLE II.

Dose	No. of regressions	% regressions
1000 r	1	2.5
2000 r	2	5.0
320 n	10	25.0
300 n	1	2.5

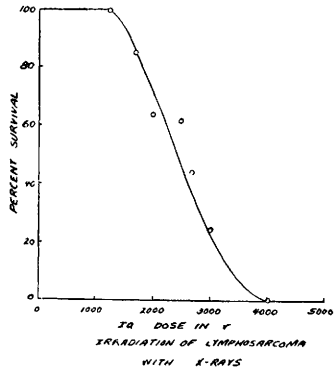
None of the regressed tumors were counted as "takes."

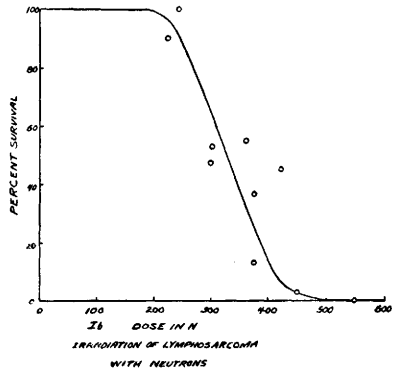
TABLE III.
Survival of Tumors After Irradiation.

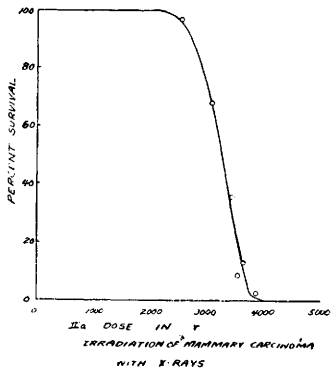
Neutrons				X-rays			
Dose in "n"	No. implants	No. takes	% takes	Dose in "r"	No. implants	No. takes	% takes
			Lymphosarcoma.				
225	40	36	90	1270	40	40	100
245	36	36	100	1700	40	34	85
300	30	16	53	2000	28	18	64
300	40	19	47	2500	40	25	62
360	38	21	55	2700	38	17	44
375	40	15	37	3000	40	10	25
375	40	5	12	4000	40	0	0
420	37	17	45				
450	40	1	2				
550	30	0	0				
			Lymphoma.				
150	40	39	97	1000	40	35	88
220	40	32	60	1200	40	32	80
300	40	18	45	1500	40	25	62
300	40	23	57	1600	40	32	80
340	40	15	37	1750	40	10	25
400	60	10	16	1800	40	6	15
475	40	0	0	2000	40	26	65
				2000	40	4	10
				2500	40	1	2
			Mammary Carcinoma.				
450	40	24	60	2500	40	39	97
495	40	34	85	3050	34	23	68
500	42	15	34	3500	40	3	7
550	40	11	27	3850	40	1	2
550	40	13	32	3400*	100	36	36
600	32	6	18	3600*	78	19	24
600	40	4	10	3600*	74	10	13
675	40	0	0	3600*	40	9	22
				4000*	38	12	31

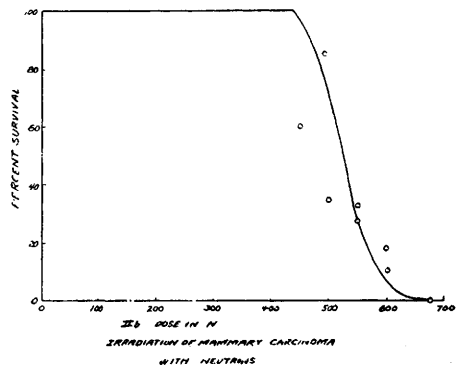
*Earlier unpublished data.

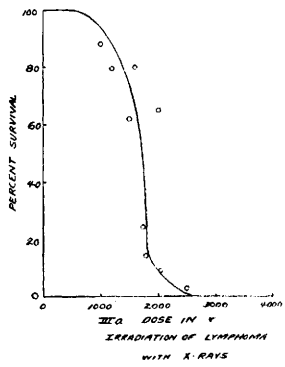
which gives the same reading on a Victoreen r-meter as does one roentgen of X-ray. The wet filter paper and cellophane wrapping around the tumor was sufficient to produce an equilibrium of the secondary ionizing particles. To avoid scattering from immediately surrounding material the tumor packages were suspended on the cellophane sheets.

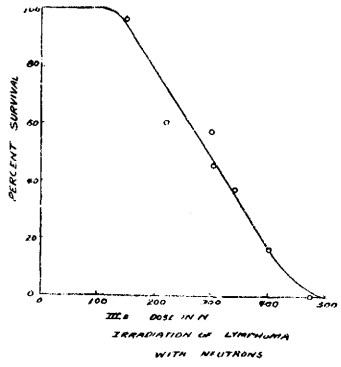












b. X-ray Irradiation. The source of X-rays was a General Electric 220 K.V. Maximar X-ray tube. With a target distance of 45 cm (10 cm from the end of treatment cone to packets) the output was 125 roentgens per minute. Occasionally, 0.5 mm copper filter was added to cut down the intensity; the output was then 61 r per minute. To minimize scattering from immediately surrounding material the packets were mounted on cellophane stretched between two hoops. The Victoreen r-meter was used for all dose measurements.

Results. Under control conditions, the 3 tumors grew 100%. The rate of their growth varied but slightly—lymphosarcoma appeared 2-3 weeks after inoculation, lymphoma within 2 weeks, and mammary carcinoma, 2-4 weeks. With respect to the surviving, irradiated tumors, 95% of the lymphosarcoma tumors appeared within 4 weeks after inoculation, the remainder by 5 weeks after inoculation. Of the lymphoma tumors, 99% appeared 2-4 weeks after inoculation, and 1% appeared at the sixth week. Eighty percent of the mammary carcinoma tumors which survived appeared 3-5 weeks after inoculation, the remaining 20% over a period of 6-10 weeks. Compared to the controls, the appearance of the irradiated tumors was delayed, the mammary carcinoma being more so than the other 2 tumors.

Due to the fact that none of the tumors used in these experiments spontaneously regress makes them especially valuable for experimental work. In 4 experiments regressions in the lymphoma tumor were noted in both X-rayed and neutroned material, Table II.

The results obtained for the survival of the tumors are summarized in Table III. Figures Ia and Ib show the survival values plotted for lymphosarcoma, IIa and IIb for mammary carcinoma, and IIIa and IIIb for lymphoma. When the neutron and X-ray dosages at the 50% survival point are compared, the ratio for lymphoma is 5.8, lymphosarcoma, 7.5, and mammary carcinoma, 6.1 (Table IV).

Summary and Conclusions. 1. The comparative effect of X-rays and neutrons on the survival of 3 mouse tumors was studied. 2. Both X-ray and neutron irradiation *in vitro* resulted in delaying the appearance of the tumors—mammary carcinoma being the most af-

TABLE IV.
X-ray-Neutron Ratios at 50% Survival.

Tumor	X-ray dose	Neutron dose	Ratio (r/n)
Lymphosarcoma	2450 r	325 n	7.5
Lymphoma	1700 r	290 n	5.8
Mammary Carcinoma	3250 r	525 n	6.1

fected. 3. Some cases of regressions in the growth of lymphoma were noted after neutron and X-ray irradiation. 4. When the doses at the 50% survival point were compared, the X-ray/neutron ratios of the 3 tumors, lymphoma, mammary carcinoma, and lymphosarcoma, were found to be 5.8, 6.1, and 7.5 respectively. These ratios do not seem to indicate a significant difference in the sensitivity of the tumors to the radiations, but do show that per unit of ionization neutrons are more biologically destructive than X-rays.

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Bone Fractures Due to Low Calcium Diets.

R. F. LIGHT AND C. N. FREY. (Introduced by C. A. Smith.)
From the Fleischmann Laboratories, Standard Brands Incorporated, New York City.

Several years ago[1] we reported the observation that a diet, adequate with respect to vitamin D and phosphorus but deficient in calcium, will produce a skeletal structure which is hard but light and fragile, in young growing animals. The thin-walled bones, showing no evidence of rickets, do not bend easily, have a nearly normal bone ash, and are not enlarged at the ends. Spontaneous fractures are common and are located at the point of greatest stress which varies with different species. In calves and dogs the fractures usually occur in the femur. In rabbits we find the vertebrae are the common site of fracture. Boelter and Greenberg[2] recently reported that rats on a low calcium diet frequently have spontaneous fractures of the long bones. According to our interpretation of their observations the vertebrae of rats are also susceptible to fracture.

During our study of this problem we carried out an experiment, using 5 litters of rabbits. Four of the litters were divided at weaning (3-5 weeks of age) each into 2 groups. Half of each litter was placed on a basal diet composed of whole oats 60%, alfalfa 18%, molasses 2%, whole wheat 10%, cracked yellow corn 8.5%, NaCl 0.5%, Na_2HPO_4 1%, plus irradiated yeast to supply 6.5 U.S.P. units of vitamin D per gram of ration. The other half of each litter received this basal diet plus 1% $CaCO_3$. The basal ration contained

[1] Presented at the American Chemical Society meeting, Dallas, Texas, April, 1938.

[2] Boelter, M. D. D., and Greenberg, D. M., *J. Nutrition*, 1941, **21**, 61.