LD-50 and Mitotic Rate of Animals at Time of Exposure to X-Radiation.* (26067)

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It has long been known that rapidly growing tissues are the most susceptible to lethal effects of X-radiation. In studies of urethane carcinogenesis, a precise correlation was found between rate of growth of the pulmonary tissue in animals of varying age at time of injection of urethane and number of tumors initiated(1). It was further demonstrated that the variation in responsiveness between animals matched as to age, weight, sex, and strain and was directly related to the individual animal's growth rate at time of urethane injection(2). Growth rate of individual mice was estimated by number of mitoses occurring in unit lengths of ear epithelium biopsied immediately prior to injection, it having been previously demonstrated by Bullough in studies of diurnal variation (3) that though the absolute mitotic rate of various tissues was highly variable from tissue to tissue at a given time, mitotic rates in the various tissues rose and fell in concert. Therefore, it seemed worthwhile to find whether growth rate of individual mice at time of exposure to X-radiation would be related to whether they lived or died, that is, is such individual variation in growth rate the overriding factor determining the existence of an LD-50 under general laboratory conditions?

Methods and materials. "A" strain mice raised in this laboratory, but procured originally from Jackson Memorial Lab., Bar Harbor, Me., were matched as to age, weight, and sex. Immediately after biopsy of the ear of each mouse the animals were irradiated in groups of 8 in cages rotating at 11 rpm. The dose is indicated for each experiment in Table I. Multiple sections were made of each ear biopsy and number of mitoses occurring in a total of 4 cm of ear epithelium determined for each individual mouse. All recognizable states of mitoses were counted. Time of death for each of the animals was recorded for 30 days.

TABLE I. Mitotic Rate and Lethal Effect of X-Irradiation.*

Group	Avg mitotic rate†		No. of mice		
	Dead mice	Live mice	Dead	Alive	Total
A	10.54	4.6	19	11	30
B	11.5	5.1	27	13	30
25 ± 1 g mice, 697 r, approx. LD 65/30 da.					
C	9.7	5.1	11	29	40
17 ± 1 g mice, 600 r, LD 28/30 da.					

* Whole body irradiation 71 r/min.; 18 ma at 200 KV with ½ mm Cu and 1 mm Al filtration, T.S.D. 50 cm.

† Total No. mitoses in 4 cm ear epithelium.

Results. It is evident in Table I that the mice that died had considerably higher mitotic rates at time of irradiation than those that survived. No relation was found between mitotic rate of the individual mice and time interval to death. Variation in growth rate of groups of animals from cage to cage occurred which was uniformly associated with number of animals surviving in the individual cage.

These results make plain that relative mitotic rate of individual mice is an important factor in individual variation in susceptibility to lethal effects of X-radiation among mice matched as to age, weight, sex, and strain.

When put in terms of numbers of surviving or dying mice for various mitotic rates (Table II), it becomes evident that there are other factors more critical than growth rate. For example, 4 mice survived which had a growth rate higher than the mean of these that died and 10 mice died whose mitotic rate was lower than the mean of survivors.

These influences should be taken into account by those studying the mechanism of agents or environmental conditions relating to radiation protection. In addition, advan-

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TABLE II. Distribution of Mice and Mitotic Rates of Mice that Died or Survived.

															Total
No. of mice dying	5	5	2	5	5	3	7	1	2	6	1	2	0	1	46
Mitotic count	0-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	
No. of mice surviving	10	5	4	1	0	2	1	1	0	0	0	0	0	0	24

tage might well be taken of the influence of diurnal variation in therapeutic utilization of X-radiation, for it is generally known that neoplasms do not undergo the degree of diurnal variation in mitotic activity that normal tissues do, remaining instead at a relatively high level.

Summary. A correlation is demonstrated between mitotic rate of individual mice at time of exposure and lethal effect of X-irradiation. The significance of this finding is discussed.

1. Rogers, S., *J. Exp. Med.*, 1951, v93, 427.
2. ———, *Fed. Proc.*, 1957, v16, 370.
3. Bullough, W. S., *Proc. Roy. Soc. London*, Series B, 1948, v135, 212.

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Deposition of C^{14}-Labelled Cholesterol* in the Atheromatous Aorta.† (26068)

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This laboratory has been concerned with the relationship between cholesterol metabolism(1,2,3,4) and development of atheromatous conditions in patients. During investigation of the "biological halflife" of C^{14}-cholesterol in various patients, it was felt that the aorta might be permeable to circulating tagged cholesterol. For this reason 5 terminal patients received intravenous C^{14} tagged cholesterol and the aortas were assayed post-mortem to determine amount of C^{14}-cholesterol in various parts of the aorta, especially near the atheromatous plaques.

Methods. Radioactive labelled cholesterol-4-C^{14} (1-3 mg) obtained from a commercial source was dissolved in a minimal quantity of ethanol (0.3 ml). This cholesterol was introduced by use of a micro-syringe into a sterile ampule containing 2-3 ml of human serum albumin solution and a sterile glass-covered magnetic stirrer. While the stirrer was rotated by means of an external magnet, the alcoholic-cholesterol solution was slowly added. The sterile suspension of cholesterol in human serum albumin was used immediately after preparation(5). (The preparation has a tendency to settle after long standing.) This preparation containing the cholesterol (with 15 to 20 μc C^{14} radioactivity) was injected slowly (10 to 15 minutes) into the antecubital vein of selected patients. Terminal patients with life expectancy of from 2 to 10 days were selected for this study. These patients were aged and had clinical evidence of arteriosclerosis. Postmortem examination revealed that all patients had well defined atheromatous plaques. Some of these plaques, in addition to being calcified, had adjacent ulcerations. Samples of the aorta of each individual were carefully obtained by use of a metal punch. Disks were obtained at intervals throughout the length of each aorta, representing an even

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