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**Delayed Radiation Injury of
Gut-Exposed and Gut-Shielded Mice.**

MASTER

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DELAYED RADIATION INJURY OF GUT-EXPOSED AND GUT-SHIELDED MICE.

II. THE DECUREMENT IN LIFE SPAN

by

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ABSTRACT

Two mouse strains (RF/J and C57Bl/6J) were exposed to x-ray doses totaling 400, 800, and 1200 rad. Total doses were given in 200-rad fractions at 7-day intervals to the whole body, gut only, or bone tissue with the gut shielded. Animals were anesthetized during exposure. Two control groups were used. A sham control group was anesthetized but not exposed to x rays, and another control group received neither anesthesia nor x-radiation. All mice were retained in a standard laboratory environment for observations on life span and histopathology at death. Life shortening was observed in all irradiated groups of strain RF/J mice and was attributed primarily to an increase in incidence and/or earlier onset of neoplasia. Life shortening was observed in the C57Bl/6J whole-body exposed mice, but the effect appeared to be noncarcinogenic. Shielding of the bone or gut tissue proved to have a 100% sparing effect in strain C57 mice and none in strain RF mice. In both mouse strains, the sham control groups (anesthetized but not irradiated) showed ~8% life shortening below the non-anesthetized control groups and increased incidences of neoplasia of ~40%, suggesting that sodium pentobarbital may be as carcinogenic as x-radiation.

I. INTRODUCTION

Although late effects studies have been performed on experimental animals with portions of their bodies shielded during exposure to ionizing radiation,¹⁻⁵ life shortening and cause of death have not been reported on mammals in which all osseous tissues were either exposed to or shielded from x rays.

Part I of this investigation⁶ to observe the possible site origin and nature of late radiation effects utilized mean after-survival time during a continuous 50-rad/day gamma-ray exposure as the effect end point. The cause of death was attributed to hemopoietic hypoplasia during the x-ray challenge exposures. However, gut-conditioned mice (all bone tissue shielded) showed late effects related to a hemopoietic decrement in resistance to the 50-rad/day gamma-ray challenge exposure. Thus, it appeared from this earlier study that late effects in the hemopoietic system may be provoked indirectly in nonosseous tissue and/or elements of

the circulating blood. In the investigation reported here, life shortening and incidence of neoplasia were used as a measure of late radiation effects.

II. METHODS AND MATERIALS

Strain C57Bl/6J (290) and strain RF/J (280) 3-month-old virgin female mice were used. Mice of each strain were divided randomly into 11 groups of approximately 24 mice each. Nine of the 11 groups of each strain were exposed to x-ray conditioning doses of 400, 800, or 1200 rad to the whole body, gut only, or whole body with gut shielded as shown in Table I. Radiation doses were given while the mice were anesthetized (~0.02 mg sodium pentobarbital/g body weight) and positioned in plastic jigs (Fig. 1). Two control groups of each strain, one sham control which received anesthesia and one which did not, were used. The total conditioning doses shown in Table I were given in 200-rad fractions (at approximately 200 rad/min) at 7-day

TABLE I
EXPOSURE DATA FOR TWO STRAINS OF MICE USED TO DETERMINE
THE GENERAL SITE ORIGIN AND MAGNITUDE OF DELAYED RADIATION INJURY

Region Receiving X-Ray Conditioning	Total Dose Accumulated ^a (rad)	Number of Mice Used		Acute Mortality ^b		Body Weight Exposed ^c (%)
		C57	RF	C57	RF	
Control (unanesthetized)	0	26	24	0	0	0
Control (anesthetized)	0	25	24	0	0	0
Whole-body	400	25	24	0	0	100
	800	32	28	9	0	100
	1200	34	32	65	0	100
Gut only	400	26	24	0	0	26
	800	24	24	4	0	26
	1200	26	28	4	0	26
Gut-shielded	400	24	24	8	0	74
	800	24	24	0	0	74
	1200	24	24	8	4	74

^aRad dose accumulated in 200-rad fractions at 7-day intervals.

^bPercent lethality from acute doses prior to challenge exposure.

^cBoth strains are represented by these figures (mean values of 20 animals).

intervals. The 7 days between exposures permitted sufficient repair⁷ to allow the accumulation of 1200-rad whole-body doses. A typical exposure schedule used in human radiation therapy might be 200 rad/day for 5 days/wk.

The exposures were scheduled so that each group received the last 200-rad exposure on the same day. One control group in each strain and all x-ray conditioned groups were anesthetized at each exposure time whether or not the group was scheduled for a 200-rad exposure.

The exposure factors used included: dose rate 208 rad/min; voltage 250 kVP; filament current 30 mA; filter Thoraeus II; half-value layer 2.6 mm Cu; and target-to-specimen distance 230 mm. The dose rates were measured with Victoreen high-energy 100-R chambers in the Lucite exposure jigs (Fig. 1). The Victoreen readings were corrected for barometric pressure and temperature and converted to tissue dose by the factor 1 R = 0.96 rad. Scatter radiation beneath the delineation of a 6.35-mm lead shield used for partial-body shielding was determined insignificant as measured by lithium fluoride thermoluminescent dosimeters.

Twenty-four mice were quick-frozen while under anesthesia in exposure jigs (Fig. 1) and dissected at the delineation of the exposed/shielded area to determine body weight and organs and/or tissue exposed during the 2 shielding conditions. Fractions of whole-body and of organs or tissue which were exposed are given in Table II.

Fig. 1. Plastic restraint and positioning jigs used to expose or shield mouse gut tissue during x-ray conditioning doses. The shaded area indicates the region shielded with 6.35 mm of lead.

TABLE II
WEIGHT OF BODY, ORGAN, OR TISSUE EXPOSED
TO X-RAY CONDITIONING DOSES^a

Whole				Kidneys				
<u>Body</u>	<u>Stomach</u>	<u>Gut</u>	<u>Bone</u>	<u>Right</u>	<u>Left</u>	<u>Liver</u>	<u>Spleen</u>	<u>Pelt</u>
<u>Gut-shielded</u>								
74	4	2	100	100	77	53	15	66
<u>Gut-exposed</u>								
26	96	98	0	0	23	47	85	34

^aIn percent.

Necropsies and gross examinations were performed on all animals that did not show post-mortem autolysis, and microscopic examination of the tissues was performed on ~30% of the animals as they succumbed.

III. RESULTS AND DISCUSSION

Acute lethality, which occurred during or within 60 days after x-ray conditioning exposures, is shown in Table I. Strain C57 mice were more sensitive to acute x-ray injury than were RF strain

mice. This observation is consistent with Part I of this project.⁶

Mean life span, reduction in life span, and neoplasia incidence at death for the various groups are shown in Table III. Life-shortening data for strain RF/J mice are shown in columns 2, 4, and 6. Life shortening was observed in all 9 x-ray-conditioned groups. Whole-body-exposed animals showed life-span reductions of 17 days (4%), 27 days (6%), and 39 days (8%) per 100-rad conditioning dose for the 1200-, 800-, and 400-rad conditioned groups, respectively. In whole-body-conditioned strain RF/J mice, the life-shortening effect was inversely proportional to dose (increasing dose was accompanied by decreasing effect per unit dose). The incidence of neoplasia in whole-body-conditioned RF/J mice was markedly greater than sham control mice (column 8, Table III).

Gut-conditioned (all bone tissue shielded) RF/J mice also showed life shortening at all 3 dose levels but, unlike whole-body-conditioned mice, the dose-effect relationship was more nearly linear with dose (increasing dose was accompanied with increasing effect). The incidence of neoplasia was again

TABLE III
MEAN LIFE SPAN OF X-RAY-CONDITIONED RF/J AND C57BL/6J STRAIN MICE

Conditioning Treatment	Mean Life Span (days)		Reduction in Life Span ^a				Neoplasia Incidence ^b at Death	
			Uncorrected Effect		Corrected to Whole-Body Exposure ^c			
	RF/J	C57Bl/6J	RF/J	C57Bl/6J	RF/J	C57Bl/6J	RF/J	C57Bl/6J
Control ^d	509	785					36	11
Sham Control	468	727	8 ^e	7 ^e			60	20
<u>Whole-body</u>								
1200 rad	264	425	4	3			76	28
800 rad	250	650	6	1			86	19
400 rad	312	637	8	3			67	14
<u>Gut only</u>								
1200 rad	306	754	3	0	11	1 ^f	83	25
800 rad	363	764	3	0	11	2 ^f	65	17
400 rad	409	732	3	0	12	1 ^f	65	17
<u>Gut-shielded</u>								
1200 rad	262	661	4	1	5	1 ^f	53	25
800 rad	306	759	4	0	6	1 ^f	53	10
400 rad	312	763	8	0	11	2 ^f	70	15

^aExpressed as %/100-rad conditioning dose.

^bMostly lymphoproliferative disease.

^cGut-exposed x 3.85; gut-shielded x 1.35.

^dNo anesthesia.

^eTotal reduction (in percent).

^fIncrease.

greater than with the sham controls (see column 8, Table III).

In gut-shielded mice (all bone tissue exposed), life shortening was similar in amount and type of response to whole-body-exposed mice. Thus, in the strain RF/J mice with a normal neoplasia incidence of 43%,⁸ late effects from radiation injury were observed when only gut tissue was irradiated and when all bone tissue was exposed with the gut shielded. Life shortening in all exposed groups was attributed primarily to an increase in incidence and/or earlier development of neoplasia (column 8, Table III). When the life-shortening effect is corrected to whole-body exposure or expressed as per volume dose effect (column 6, Table III), gut tissue appeared to be more radiation-sensitive than either whole-body-exposed or gut-shielded mice. Lymphoproliferative disease in RF/J mice appears to be provoked by x-ray exposure of the gut only. However, shielding of gut tissue did not contribute a significant sparing effect in terms of life shortening. It was also observed that sham control mice (anesthetized 1 day/wk for 6 wk) had shorter life spans and a higher incidence of neoplasia than did the nonanesthetized control groups.

Strain C57 mice responded quite differently to x-ray injury than did strain RF mice. The strain C57BL/6J mouse reportedly has a very low natural neoplasia incidence.⁸ We observed ~11% neoplasia in nonanesthetized mice. Whole-body-exposed C57 mice showed life-shortening effects from x-ray exposure which were apparently noncarcinogenic, as neoplasia incidence was not different from the sham control group (column 9, Table III). Gut-exposed and gut-shielded mice with all bone tissue exposed showed no life-shortening effects of x-ray exposure at any of the doses used. In fact, with the exception of the 1200-rad gut-shielded group, gut-exposed and gut-shielded groups had somewhat longer mean life spans than did sham control mice. In C57 mice, shielding the gut or the bone tissue had a 100% sparing effect on radiation-induced life shortening. It is interesting to note that the life span in sham control C57 mice (anesthetized mice) is shorter, by approximately the same percentage as RF mice, than nonanesthetized control mice, and again neoplasia

incidence is also higher by ~45%. This life-shortening effect of sodium pentobarbital in two strains of mice with grossly different natural neoplasia incidences would appear significant.

From the results of this two-part project, one may conclude that persistent injury incurred from exposure to ionizing radiation (a) may be provoked in both soft and osseous tissue; (b) may be carcinogenic and/or noncarcinogenic in nature; (c) is significantly greater in degree for carcinogenic response in animals with a genetic history of a naturally high neoplasia incidence than in animals with a naturally low neoplasia incidence and approximately the same for noncarcinogenic response; and (d) for all practical purposes, the noncarcinogenic effect on life span of mammals would be statistically insignificant.

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