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Protection of Mice Against X-Irradiation by Spleen Homogenates Administered After Exposure.*† (19540)

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Protection of animals against the lethal or deleterious effects of ionizing radiations is now possible by means of several chemical substances administered prior to, or during X-irradiation: for example, cysteine(1), glutathione(2), or sodium nitrite(3). In addition, anoxic anoxia during irradiation is also effective(4). None of these measures, however, has been shown to be protective when carried out *after* irradiation. Recent studies by Jacobson *et al.*(5) suggest the presence of a spleen factor which appears to be effective *after* irradiation exposure. These workers transplanted 4 spleens from young non-irradiated mice (1-12 days old) into the peritoneal cavity of adult CF-1 mice immediately after exposure to 1025 r whole-body X-irradiation. Under these conditions, survival of the irradiated mice was increased, and regeneration of hematopoietic tissue was hastened.

Several initial experiments in this laboratory in which spleens from young mice were implanted into irradiated mice (650 r) confirmed the protective effect of this procedure in LAf_1 mice. The implants became vascularized, grew, and were still present and intact 6 weeks after implantation. As a first approach to isolating and ascertaining the nature of the splenic factor, studies were performed using spleen homogenates.

The preliminary data, described herein, indicate that mice receiving otherwise lethal doses of whole-body X-irradiation are protected by a single intraperitoneal injection of spleen homogenate administered either one hour or as long as 45 hours after radiation exposure.

Materials and methods. LAf_1 mice of both sexes, approximately 7 to 11 weeks old and weighing 20 to 26 g, were used in the irradiation studies. The animals were allowed free access to food (Purina Laboratory Chow) and to tap water. In all experiments the control and experimental animals were matched with respect to sex, age, and body weight; they were irradiated simultaneously and caged together (8 or 10 per cage). A Westinghouse Therapy Unit was used as the radiation source. The radiation factors were: 250 KVP; 15 ma; filter, 0.5 mm Cu plus 1 mm Al; HVL, 1.5 mm Cu; skin to target distance, 100 cm; dosage rate, 25 r per minute, as measured with a Victoreen r-meter placed in air at the position of the mice. Each radiation dose was delivered in a single exposure. During irradiation the mice were contained in individual, perforated lusteroid centrifuge tubes placed radially on a circular wooden turntable platform which rotated at 3.5 r.p.m. to obtain uniformity of radiation dosage.

In a typical experiment, 20 to 30 young LAf_1 mice (1 to 3 weeks old) were sacrificed by severing the cervical spine. The spleens were removed immediately and suspended in

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TABLE I. Protection of Mice by a Single Intraperitoneal Injection of Spleen Homogenate Administered after Whole Body X-irradiation.

Exp.	No. of mice*	Age (wk)	X-ray dosage (r)	Treatment†	No. of spleens	Survival at 30 days No.	Survival at 30 days % survival
I	9	7	650	Spleen homogenate	.9	9	100
	10	7		Phosphate buffer		0	0
II	14	8		Spleen homogenate	2	11‡	79
	19	8		Phosphate buffer		4	21
III	13	9		Spleen homogenate	1.7§	11	85
	13	9		Phosphate buffer		1	8
IV	10	10	700	Adult spleen homogenate	1.6	8	80
	10	10		Phosphate buffer		4	40
V	15	9		Spleen homogenate	1.3	14	93
	10	9		Phosphate buffer		1	10
VI	15‖	10–11	800	Spleen homogenate	.9	8	53
	14‖	10–11		" "	1.8	11	79
	11‖	10–11		Phosphate buffer		0	0

* Female mice were used in all experiments except as otherwise noted.
† Spleen homogenates were inj. within one hr after irradiation except as otherwise noted.
‡ Three animals died on 4th day post-irradiation.
§ Injected 45 hr post-irradiation.
‖ Male mice.

3 ml of cold M/15 phosphate buffer (pH 7.2). They were then homogenized in a motor-driven ground-glass Ten Broeck tissue homogenizer which was immersed in an ice-water bath. The homogenate was diluted with phosphate buffer to a volume suitable for injection, usually equivalent to 20 spleens per 10 ml of homogenate. Within 15 minutes after preparation the homogenates were injected into irradiated adult mice by the intraperitoneal route using a 26-gauge needle. Between 0.5 and 1.0 ml of homogenate was injected into each animal. Control irradiated mice were injected with equivalent volumes of phosphate buffer. The homogenate was injected within one hour after irradiation in all experiments except one, in which it was injected 45 hours after exposure. The mice were observed daily for mortality and gross signs, and were weighed at 24- or 48-hour intervals over the 30-day period following irradiation. Survival up to 30 days post-irradiation, and average body weight changes were used as criteria for evaluation of protection elicited by the spleen homogenates.

Microscopic examination of the homogenate revealed the presence of apparently intact cells, in addition to cell fragments and cell nuclei. Preliminary estimates indicate that 60 to 70% of the spleen cells were disrupted by the homogenization procedure.

Results. The survival data obtained in 6 separate experiments with spleen homogenates (a total of approximately 160 mice) are summarized in Table I. The radiation doses used in these experiments (650, 700 and 800 r whole-body X-irradiation) include the lethal and supra-lethal§ range for mice. The data reveal that a single parenteral spleen homogenate injection into adult X-irradiated mice within one hour after radiation exposure confers considerable protection in these mice. Each mouse received the equivalent of 1 to 2 spleens from young non-irradiated mice.

In Exp. I, 100% of the spleen-homogenate-treated animals survived a 650 r dosage of X-irradiation, whereas none of the control mice survived. In Exp. II, using a larger group of mice, only 4 out of 19 control animals (21%) survived a 650 r dose, and 11 out of 14 (79%) of the spleen-homogenate-treated

§ Defined here as that range of doses in excess of the minimum dose required to kill 100% of animals in 30 days.

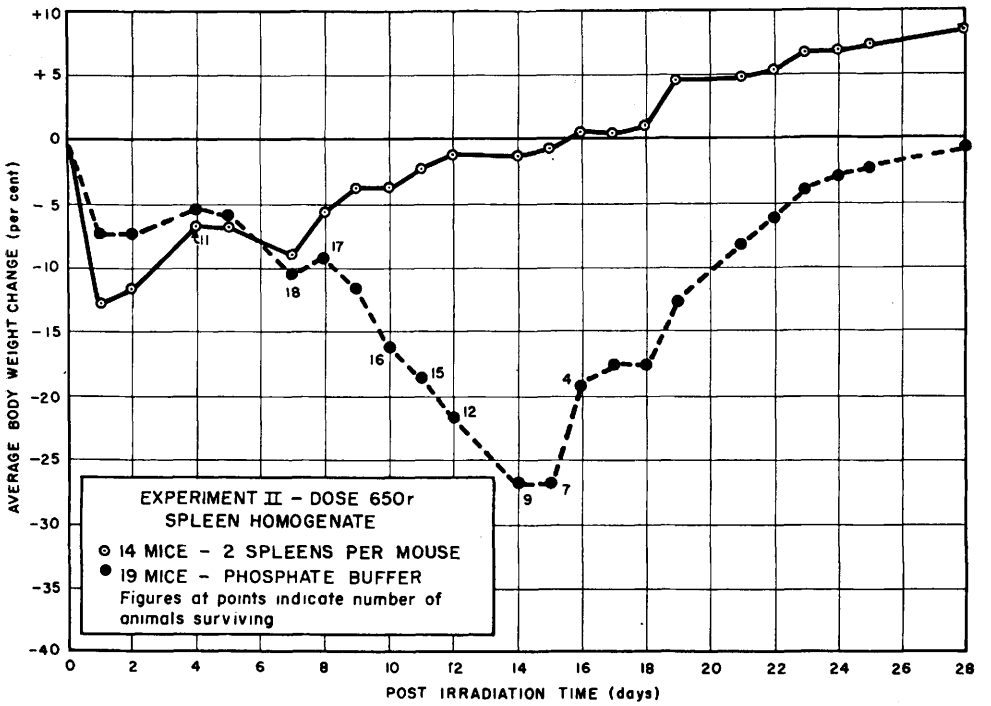

FIG. 1. Body weight changes in female mice injected with spleen homogenate one hour after 650 r x-irradiation.

mice survived.‖ Body weight changes, expressed as percent of initial body weight in Fig. 1, also illustrate the protective effect of the spleen homogenates.

In Exp. III, in which spleen homogenate was injected into irradiated mice 45 hours after 650 r X-irradiation, protection was also obtained. The survival data in Table I, as well as the body weight changes plotted in Fig. 2, indicate that the spleen homogenate is protective even when given approximately 2 days after irradiation. The efficacy of spleen homogenate protection was also demonstrated in mice subjected to 700 and 800 r whole-body exposure. Of the control group of 10 mice exposed to 700 r in Exp. V, one was alive at 30 days, and 14 out of 15 (93%) of the spleen-homogenate-injected group survived.

The possibility that the protective spleen factor is present, at least to some extent, in adult spleen tissue as well as in young spleens is suggested by the results of Exp. IV. The equivalent of 1.6 spleens from adult non-irradiated mice (5 weeks old) was injected into each of 10 mice within one hour after they had received 700 r. Twenty percent of this group was dead at the conclusion of the 30-day observation period, whereas 60% of the phosphate-buffer-injected group was dead at 30 days. The weight curves for these animals in Fig. 3 likewise illustrate this protection by homogenates of spleens from adult mice. Thus the weight loss of the control irradiated mice was 20% on the 12th day, compared with a weight loss of only 13% for the spleen-homogenate-injected group on the 12th day.

Finally, the effect of spleen homogenate from young mice on mice subjected to 800 r irradiation was investigated in Exp. VI. Of 14 mice receiving homogenate equivalent to 1.8 spleens, 11 were alive at 30 days (78%); whereas of 15 mice receiving homogenate equivalent to 0.9 spleens, 8 survived 30 days (53%). None of the control animals was

‖ Survival time of the 3 mice that died in this experiment was 4 days, as opposed to approximately 11 days as uniformly observed in this dose range at this Laboratory. It seems likely, therefore, that mechanisms other than radiation, *per se*, may have accounted for these deaths.

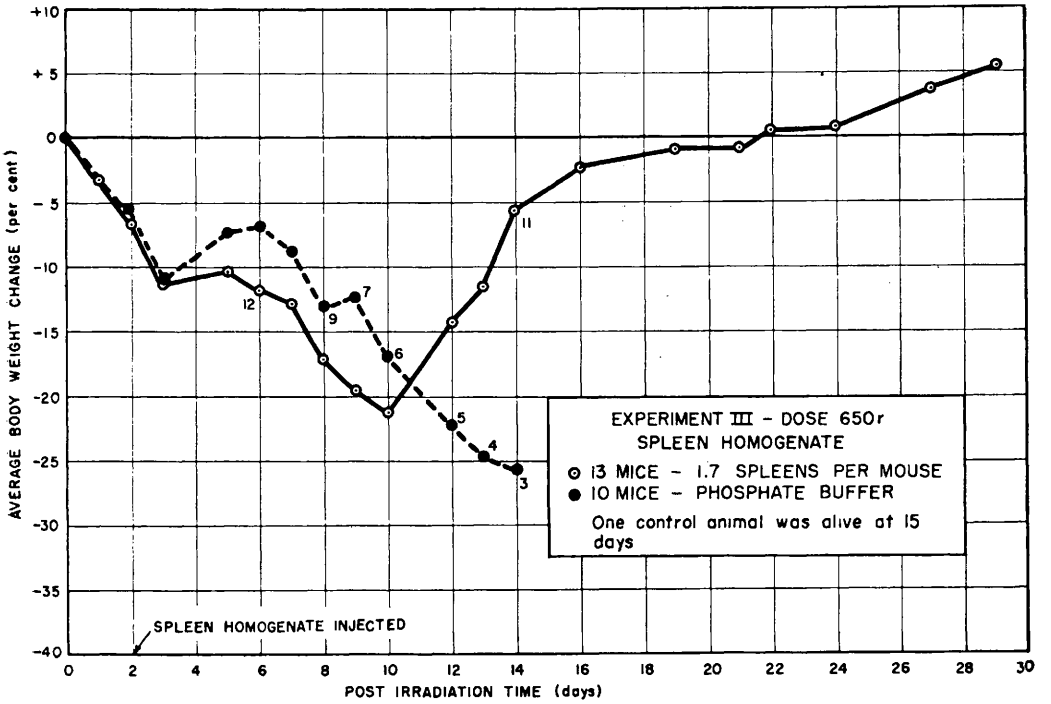

FIG. 2. Body weight changes in female mice injected with spleen homogenate 46 hours after 650 r x-irradiation.

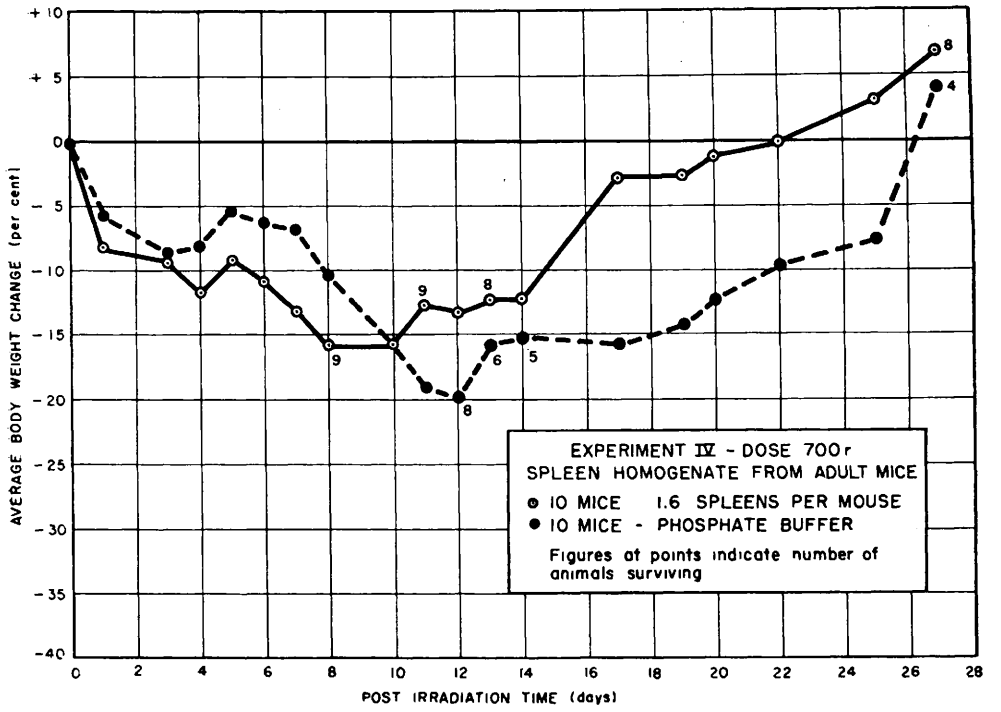

FIG. 3. Body weight changes in female mice injected with adult spleen homogenate one hour after 700 r x-irradiation.

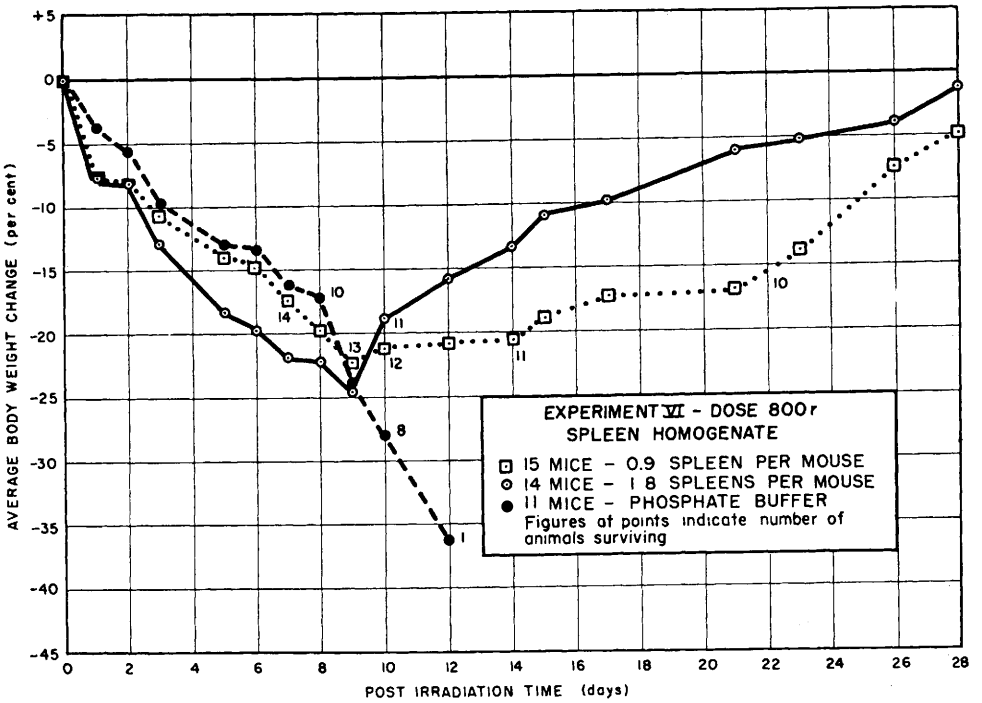

FIG. 4. Body weight changes in male mice injected with spleen homogenate one hour after 800 r x-irradiation.

alive at the conclusion of the 30-day observation period. Fig. 4 presents the post-irradiation body weight changes in these animals. The data suggest a possible quantitative relationship between the amount of spleen substance injected and the degree of protection against X-irradiation obtained; *i.e.*, 1.8 spleens elicited a greater degree of protection than did 0.9 spleens.

Discussion. The results appear to warrant a positive conclusion as to the presence of a factor in homogenates of spleens from young mice which greatly modifies the response of irradiated adult mice when injected parenterally after irradiation. This modification in response is reflected—as the present data demonstrate—in increased survival, minimal body weight losses and in earlier body weight recovery in spleen-homogenate-treated mice, as compared with control irradiated animals. The protection afforded by spleen homogenate injection is not attributable to a non-specific tissue effect, since spleen homogenates lose their protective activity after being subjected to ultra sound in a crystal Ultra-Sonorator.

The present positive finding of protective activity in *adult* spleen homogenate apparently is contrary to the negative findings of Lorenz *et al.*(6). These investigators reported the failure of post-irradiation adult mouse spleen brei injections (intravenous and intraperitoneal) to protect LAf_1 mice from 900 r whole-body X-irradiation. Each mouse received approximately 50 to 100 mg of spleen brei. In the present studies, it will be noted, the equivalent of 1.6 adult spleens (ca 150 mg) was required to protect mice which had received 700 r (Exp. IV). Thus in the Lorenz experiments both the higher radiation dosage and the smaller amounts of spleen substance administered may have been responsible for their negative findings.

Examination of the post-irradiation body weight data in the spleen-homogenate-treated mice reveals a turning point in their weight loss trend which occurred consistently at approximately 8 days after injection of spleen homogenate. It is at this time that a divergence in the average body weight losses between the spleen-homogenate-treated mice and

the corresponding control group of animals is noted. This finding is in agreement with that reported by Jacobson *et al.* for spleen-shielded mice exposed to 1025 r(7). By histological technics these workers have shown that bone marrow recovery appears to be complete in mice by the 8th day post-irradiation. Since, however, first evidence of a reversal of peripheral leucocyte and reticulocyte depression apparently occurs on the 6th day following irradiation(7), it would appear that body weight recovery of the spleen-homogenate-treated mice in the present investigation occurs only after the appearance of bone marrow recovery.

Multiple injections of spleen homogenates were not employed in the present investigation. It seems reasonable to suppose, however, that such treatment might confer greater protection upon irradiated mice than do single injections, especially at higher X-ray dose levels.

The present demonstration of the post-irradiation protection afforded by injectable spleen homogenates in irradiated mice provides a research tool of promise for investigation of the nature of the protective spleen factor. Studies along these lines are now in progress.

Summary and conclusions. 1. The mortality of adult LAf_1 mice, exposed to whole-body X-irradiation in doses of 650, 700 and 800 r, was either prevented or reduced markedly following a single intraperitoneal injection of spleen homogenate administered either one hour or as long as 45 hours after radiation exposure. The equivalent of 1 to 2 spleens was injected into each irradiated mouse. The protective factor was present in homogenates from spleens of young mice (1 to 3 weeks old), and to a smaller degree in homogenates of spleens from adult mice. 2. The post-irradiation protection afforded by spleen homogenate injection was reflected in minimized weight losses observed in the spleen-homogenate-treated mice as compared with the control animals. The data suggest a possible quantitative relationship between the amount of spleen substance injected and the degree of protection obtained.

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Human Assay of Three New Mercurial Diuretic Agents: A Promising Preparation for Oral Use.* (19541)

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A method for the bioassay of diuretic agents in ambulant patients with congestive failure

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