Failure of Cell-Free Spleen Extracts to Protect Irradiated Animals.* (24968)

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It is a current hypothesis that effectiveness of postradiation treatment with hematopoietic containing tissues is a function of "cellular repopulation" and/or "transitory activity" of injected cells pending recovery of recipient marrow rather than any elusive "humoral" factor which stimulates recovery. Evidence presented by Ford *et al.*(1), Odell *et al.*(2), Makinodan(3), Smith *et al.*(4), and Nowell *et al.*(5), has demonstrated the replacement of circulating erythrocytes, platelets and leukocytes in x-irradiated recipients by parenterally introduced cellular components from non-irradiated donors. Jacobson(6) has reported that at least 50,000 spleen cells are required to enhance survival of irradiated mice. On the other hand, Ellinger has reported protection of mice(7) and guinea pigs(8) against death following radiation by injection of a cell-free mouse spleen extract. In this report the results are given of experiments conducted to determine the value of cell-free mouse spleen extracts employed as a protective agent for mice and guinea pigs exposed to whole body x-irradiation.

Materials and methods. (a) Spleens from 350 normal albino mice (*Mus musculus*) of mixed gender, age and size were removed following ether anesthesia. The spleens were placed in a precooled mechanical blender containing 350 ml of 0.85% saline and homogenized in 2 periods of 1 minute each with cooling in between. The homogenate was alternately frozen and thawed 3 times for maximum cellular disruption. The mixture was then placed in a refrigerator for 24 hours after which it was centrifuged in a refrigerated centrifuge at high speed. The supernate was passed through several layers of Whatman No. 1 filter paper in a Buchner funnel under light negative pressure. Microscopic examination of numerous fields of the resulting filtrate failed to reveal any intact cells, therefore the extract was assumed to be cell-free. The extract was then lyophilized and stored at −20°C. The entire procedure was conducted under aseptic conditions at low temperatures. Forty-five mice similar to those used for preparation of the splenic extract were exposed to approximately 600 r total body x-irradiation by means of a Westinghouse Quadrocondex x-ray machine. The radiation factors were: 250 KVP; 15 ma., 1.0 mm Al and 0.5 mm Cu filters in addition to an inherent filtration of 2.5 mm Al and 0.25 mm Cu; size of field, whole-body; target distance, 16 inches. The mice were irradiated for 6.7 minutes at approximately 90 r/min in groups of 9 in a cylindrical cardboard container 17 cm in diameter and 3 cm high. The dosage corresponded to a $LD_{100}/10$ days. The mice were randomly divided into 2 groups of 20 and 25; the larger group served as controls. The lyophilized spleen extract was resuspended in 0.85% sterile saline to give a total volume of 1 ml per 4 spleens. This extract was injected intraperitoneally, 0.1 ml immediately following exposure the day of radiation and 0.6 ml administered at a dose of 0.2 ml per day on the first, second and third post-irradiation days for a sum total of 0.7 ml. (b) Thirty-three guinea pigs of mixed gender, each weighing about 600 g were irradiated for 4.4 minutes at approximately 90 r/min in groups of 4 under the same conditions as the mice, each receiving a total body dose of about 450 r. The radiation intensity in this case, as with the mice, was checked with a Victoreen r meter. This dosage corresponded to a $LD_{85}/10$ days under these conditions. These animals were randomly divided into 2 groups of 20 and 13 each, the smaller group serving as controls. The treated animals were injected intramuscularly with 0.5 ml of the spleen extract immediately after irradiation and 2.0 ml was administered at a dose of 0.5 ml per day given on the first,

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second, third, and fourth post-irradiation days for a sum total of 2.5 ml.

Results. The results of the experiments are shown in Figs. 1 and 2. It can be seen that post-irradiation treatment of whole body x-irradiated mice and guinea pigs with cell-free spleen extracts under the conditions noted, did not result in protection against postradiation mortality. In fact, treated animals died at an accelerated rate when compared to the death rate of controls.

The cell-free spleen extract preparation used was similar to but different from that of Ellinger, and the induced x-irradiation mortality was higher and more rapid with both guinea pigs and mice than in Ellinger's experiments. Irradiated animals died within 10 days whereas in Ellinger's experiments significant differences in mortality rates of spleen-treated animals and their saline controls were not evident prior to the fifteenth day after irradiation. Other significant deviations from Ellinger's technic were reconstitution of lyophilized suspensions to yield injectable material equivalent to 4 spleens rather than 5, the injection of this material for 3 or 4 days after irradiation rather than for 5 days, the use of repeated freezing and thawing of spleen cell suspensions to obtain cell-free extracts and the use of ether anesthesia to sacrifice the animals from which spleen extracts were prepared. Finally, the intraperitoneal rather than intramuscular route was employed for injection of mice although not guinea pigs. It is possible that the differences in preparation and administration of the cell-free extract as well as the method and time of storage may have been destructive to the "humoral spleen factor(s)" and/or that the higher dosage of irradiation was sufficient to mask the effects of the factor(s) if present. Under the experimental conditions employed protective humoral factors could not be shown to exist in cell-free mouse spleen extracts. These results suggest that strict adherence to Ellinger's technic methods is critical.

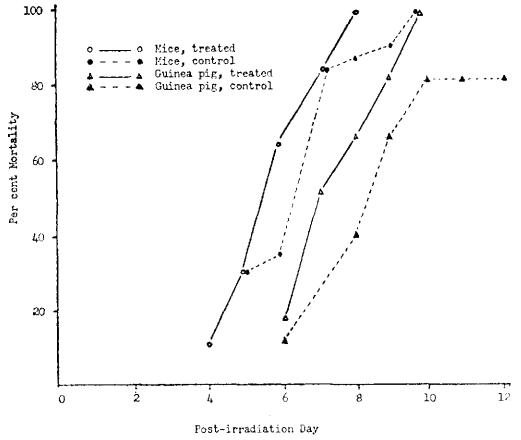

FIG. 1. Mortality of adult albino mice treated intraper. and guinea pigs treated intramusc. with cell-free spleen extract following 600 r and 450 r total body x-irradiation, respectively.

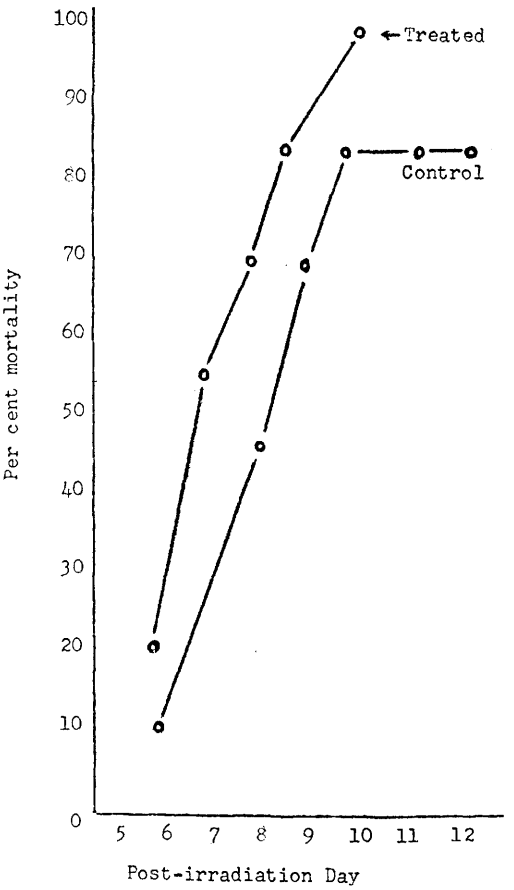

FIG. 2. Mortality of guinea pigs treated intramusc. with cell-free spleen extract following 450 r total body x-irradiation.

Summary. Postradiation administration of cell-free mouse splenic extract to whole body x-irradiated mice (intraperitoneally) and guinea pigs (intramuscularly) did not result in protection against postradiation death.

The treated animals died at an accelerated rate when compared to the controls.

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Some Relationships Between Peripheral Monoamine Oxidase Inhibition and Brain 5-Hydroxytryptamine Levels in Rats.* (24969)

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Recently Sjoerdsma *et al.*(1) suggested *in vivo* assay in man of monoamine oxidase (MAO) inhibitors based on ability to suppress excretion of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-hydroxytryptamine (5-HT). Since rats excrete 5-HIAA(2), it seemed probable that the Sjoerdsma procedure could be modified into routine method of evaluation of *in vivo* peripheral MAO inhibition in the rat. In addition, by measuring the effect of same MAO inhibitors on brain 5-HT levels a comparison of peripheral with central effects would be obtained.

Methods and materials. 5-HIAA excretion: Male albino rats (175-225 g) were obtained from Carworth Farms. Compounds were administered orally to minimum of 6 rats. At various times after administration of inhibitor, 10 mg/kg of 5-HT (free base) was administered orally. Rats were then placed in metabolism cages and urine collected over subsequent 6 hours. Urines were stored in refrigerator overnight and analyzed next day. The nitroso-naphthal method(3) was employed to measure 5-HIAA levels with following modification: One ml of urine was placed in 50 ml glass-stoppered bottle containing 5 ml of water, to which was added 6 ml of 2,4-dinitrophenyl-hydrazine reagent. Optical measurements were performed with Beckman DU spectrophotometer and Pyrocell microcuvettes. *Brain 5-HT concentrations*: The procedure of Bogdanski *et al.*(4) was employed. 5-HT was determined with Farrand spectrofluorometer. The following test compounds were employed: Phenylisopropylhydrazine: PIH or JB-516 (Dr. J. Biel, Lakeside Labs); 2-isopropyl-1-isonicotinyl hydrazine: IPH or iproniazid (Dr. L. O. Randall, Hoffmann-LaRoche); harmine, harmaline, and tetrahydroharmine (Dr. F. H. Clarke, Schering Corp.); the authors are indebted to these investigators for these compounds.

Results. 5-HIAA excretion: Excretion of 5-HIAA by normal rats is 8.9 γ/100 g BW (95% confidence limits: 7-10.8 based on 18 determinations). Rats given 10 mg/kg of 5-HT orally will excrete 65.8 γ/100 g BW (95% confidence limits: 58.3-73.3 based on 83 determinations) or 5.6% of dose in urine as measurable 5-HIAA over 6 hours. Erspamer(2) reported that the rat excretes 5.5% of orally administered 5-HT (6 mg/kg) as 5-HIAA. Preliminary experiments indicated that inhibitor studies were more reliable when employing the exogenous 5-HT test rather than measuring only the endogenous 5-HIAA.

Fig. 1 indicates efficacy of various substances in diminishing excretion of 5-HIAA. The most potent compound was PIH. Harmine, harmaline, and tetrahydroharmine were somewhat less potent. Lysergic acid diethyl-

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