Effect of Leukocyte-Promoting Factor (LPF) on Survival of X-Irradiated Mice.* (19933)

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The beneficial effect of spleen shielding on survival of X-irradiated mice has led to the hypothesis that a "humoral" substance is produced by the protected spleen, and that this substance stimulates rapid recovery of the hematopoietic tissues(1). Implantation of normal spleens in irradiated mice following radiation exposure is also reported to increase survival. The therapeutic effect of the implantation is considered additional evidence of a "humoral" mechanism of protection(1). As yet the only cell-free factor that has been isolated from biological material which might produce this protective effect following radiation is the factor described by Menkin which induces release of leukocytes into the peripheral blood and produces hyperplasia of granulocytes and megakaryocytes in the bone marrow(2,3).

The present study was undertaken to determine whether or not Menkin's leukocytosis-promoting factor (LPF) when given therapeutically would increase survival in irradiated mice.

Methods. A total of 200 female white Swiss mice 8 to 10 weeks of age were used in the present study. Prior to treatment the mice were randomly distributed into treatment groups by a random number table. Prior to and following treatment the mice were kept 10 to a cage and given water and Purina laboratory chow *ad libitum*. The leukocytosis-promoting factor used was freshly prepared by V. Menkin from a sterile pleural exudate induced in a dog. The material obtained was a fluffy white powder. It was stored in a desiccator *in vacuo* over phosphoric anhydride. Fresh solutions were made up for each injection by dissolving a suitable amount of the LPF in sterile saline. With the exception of some of the injections in the preliminary portion of the study which were done intravenously into the tail vein, all injections were made intraperitoneally. Injection volumes varied from 0.1 to 0.5 cc. Blood counts were performed in some of the mice by cutting of the tip of the tail to obtain freely flowing blood which was then aspirated into standard white cell pipettes. Counts were made in standard hemocytometer chambers. *X-rays* were delivered from a Picker industrial model machine operated at 250 KVP and 15 ma. The other factors were: added filtration of ¼ mm Cu and 1 mm Al, 55 cm tube-specimen distance, dose rate—70 r/min. The mice were exposed 20 at a time in shallow lucite cages curved on a radius of 55 cm to minimize differences in radiation intensity between the center and periphery of the cage.

Results. In preliminary studies a dose of LPF was established which would produce maximal leukocytosis. Eight groups of 5 mice each were injected either intravenously or intraperitoneally with various doses of LPF. Four hours later white cell counts were performed and the degree of leukocytosis determined. These data are given in Table I, and they show that a dose of 50 mg/kg produces maximal leukocytosis at 4 hours and that intraperitoneal injections produce as great a rise as intravenous injections. For these reasons 50 mg/kg given intraperitoneally were used in the study on therapy.

Four groups of 40 mice each were irradiated with 450, 500, 600, and 700 roentgens of X-rays, respectively. After irradiation, 10 mice from each dosage group were set aside for treatment with LPF, and the remaining 30 in each group were used as controls. Beginning the day of radiation and continuing for the following 5 days the mice were injected with 50 mg LPF/kg intraperitoneally. The control mice were injected similarly with saline. Survival was then followed for 30 days.

Table II shows the 30-day survival of the various groups. It can be seen that treatment with LPF did not significantly increase sur-

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TABLE I. Degree of Leukocytosis Produced in Mice at 4 Hours by Various Doses and Routes of Administration of LFP. 5 mice in each exp.

Route of administration	Dose LPF (mg/kg)	Avg change in white cell count at 4 hr (% of pre-inj. count)
Intraperitoneal	5	110
	10	119
	50	153
	100	119
	*	96
Intravenous	10	105
	50	156
	*	107

* Saline control.

TABLE II. 30-Day Survival of Irradiated Mice Treated with LPF or Saline Intraperitoneally.

No. mice	Dose of X-rays (roentgens)	Treatment	30-day survival	% survival
10	450	50 mg LPF/kg for 6 days	9	90
	500		4	40
	600		2	20
	700		0	0
30	450	Saline inj. controls	22	73
	500		13	43
	600		2	7
	700		0	0

vival. Analysis of the data by the probit analysis method described by Finney(4) gives an LD_{50}^{30} value of 489 r for the control mice and 518 r for the mice injected with LPF. These differences are not considered significant.

Discussion. Treatment of irradiated mice with leukocytosis-promoting factor did not significantly increase their 30-day survival. This fact strongly suggests that if survival of irradiated mice into which normal spleens have been implanted is due to a "humoral" factor released by the spleen, such a factor is unrelated to the specific leukocyte-promoting factor obtained from sterile exudates.

Summary. The present study does not contribute to the discussion as to whether or not a "humoral" substance is secreted by the spleen. It does show, however, that LPF, a logical therapeutic agent, is ineffective in reducing X-ray mortality in mice.

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Mechanism of Renal Glycosuria in ACTH-Treated Premature Infants.* (19934)

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In a group of 7 premature infants given ACTH in the treatment of early retrolental fibroplasia, 5 developed glycosuria, and in none of these was there an associated hyperglycemia. These data implied a glomerulotubular functional imbalance in the handling of glucose. Dustan *et al.*(1) concluded that glycosuria during cortisone and ACTH administration results from a depression of the glucose Tm. However, Earle *et al.*(2) concluded from their studies that there was no consistent decrease in the maximum ability (Tm) of the tubules to reabsorb glucose under the influence of ACTH and believed that the glycosuria results from a rise in glomerular filtration rate producing an increased glucose

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