

Studies on Recovery from Radiation Injury.

Part II. Observations on the Effects of Implantation of the Shielded Spleen.

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Lethally irradiated adult CF#1 mice received intraperitoneal implants of two sliced spleens taken from adult CF#1 mice at various intervals after lethal irradiation with the spleen-shielding procedure. The following results were obtained: the survival of mice implanted with shielded spleens removed three hours, one, ten and fourteen days after irradiation was enhanced significantly, whereas, the survival of mice implanted with spleens removed four and seven days after irradiation failed to increase survival rate.

In 1949 JACOBSON et al.¹⁾ reported that lead shielding of the spleen during whole body lethal irradiation of mice markedly enhanced their survival. JACOBSON's experiment initiated rapid advances in the field of blood forming tissue transplantation and the relationship to radiation injury. At present, it is generally accepted that the most effective way to promote recovery from radiation injury is by transplantation of blood forming tissue and the main mechanism of this effect lies in repopulation of the host animal's blood forming tissue by donor cells. Recent studies have established that only tissues containing active blood forming cells are effective, for example, red bone marrow,²⁾ spleen that contains extramedullary hematopoiesis³⁾, fetal liver,⁴⁾ and leukemoid blood.⁵⁾ Furthermore, COLE et al.⁶⁾ and Vos⁷⁾ have shown that the precursors of all the hematopoietic cell series are required for successful transplantation and that optimal proportions of the various stem cells are provided by bone marrow from normal animals. However, the mechanism of promoting recovery by the spleen-shielding procedure is not yet fully clarified.

The present study was designed to investigate this problem. Lead shielded spleens removed from mice at various intervals after the irradiation-spleen-shielding procedure, were implanted intraperitoneally into lethally irradiated mice. The effects on survival of the recipient animals were compared.

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MATERIALS AND METHODS

CF#1 mice of both sexes, 10 to 12 weeks of age, were used in this study. The radiation factors were: 180 KVP; 25 ma; filter, 0.5 mm Cu plus 0.5mm Al; HVL, 1.26mm Cu; skin to target distance, 60 cm; dose rate, 53 r per minute. The exposures were measured with a Radocon placed in air at the position of the mice.

The mice were exposed to a single dose of 930 r whole body irradiation with lead-shielding of their surgically exteriorized spleens. The technique used was essentially that of JACOBSON.¹⁾ The mice were sacrificed at various intervals after the irradiation-spleen-shielding procedure and their spleens were sliced prior to implantation.

The recipient mice, which were used for the evaluation of the effectiveness of the spleen implants, were exposed to 930 r whole body irradiation without shielding of their spleens. The radiation dose used in this experiment is in the lethal range for mice. At the completion of radiation, the slices of two spleens removed from donor mice were placed in the peritoneal cavity of each recipient mouse.

The animals were divided into nine groups as indicated in Table I. In Groups 1/8-ST, 1-ST, 2-ST, 4-ST, 7-ST, 10-ST and 14-ST, the spleens used for implantation were taken from donor animals three hours, one, two, four, seven, ten and fourteen days, respectively, after the irradiation-spleen-shielding procedure. Groups O-ST and C represent controls. Mice in Group O-ST received implants of two spleens from non-irradiated mice. Mice in Group C were irradiated but were not given implants.

RESULTS

Studies were made on survival up to twenty-eight days. The survival data are summarized in Table I. Of ninety-seven mice in the control Group C (930 r whole body irradiation without spleen implantation) two, or 2.1 per cent, survived. In Group O-ST four mice, or 9.5 per cent, of forty-two mice survived. This indicated that implantation of spleens from adult mice was slightly effective. In Group 1/8-ST, 1-ST, and 2-ST, five of thirty mice (16.7 per cent), six of twenty-one mice (28.6 per cent), and two of eighteen mice (11.1 per cent), respectively, survived, whereas none of sixteen mice in Group 4-ST and only one of twenty-two mice (4.5 per cent) in Group 7-ST survived. However, in Group 10-ST and 14-ST, three of seven mice (42.9 per cent) and eight of twenty-six mice (30.8 per cent), respectively, survived. These results indicated that the protective effect of the shielded spleens removed three hours, one, ten and fourteen days after the spleen-shielding procedure were even more effective than spleens from non-irradiated mice in enhancing survival. On the other hand, the shielded spleens removed

after the fourth and seventh days were less effective than spleens from non-irradiated mice.

Table I.

A Comparison of the Effect of Intraperitoneal Implantation of Donor Spleen, Shielded during X-irradiation, on the Survival of Recipient Mice Exposed to 930 r

Group	Time after irradiation of donor mouse when spleen implanted	Number of recipient mice	Survival at 28 days	
			No.	%
C	No spleen implantation	97	2	2.1
O-ST	Spleen from non-irradiated mouse	42	4	9.5
1/8-ST	3 hrs	30	5	16.7
1-ST	1 day	21	6	28.6
2-ST	2 days	18	2	11.1
4-ST	4 days	16	0	0
7-ST	7 days	22	1	4.5
10-ST	10 days	7	3	42.9
14-ST	14 days	26	8	30.8

DISCUSSION

In 1949 JACOBSON and his colleagues¹⁾ discovered that shielding of the spleen during lethal irradiation caused a considerable reduction of mortality in mice. Histologic studies on the shielded spleens of mice after 600 r whole body irradiation showed a remarkable increase in erythropoiesis, megakaryocytopoiesis and granulocytopoiesis apparent by eighteen hours after exposure.⁸⁾ This increase in extramedurally blood formation continued thereafter even though the cell population of the previously irradiated bone marrow had returned to normal. JACOBSON et al.³⁾ also reported that significant protection was retained even when the shielded spleen was removed one hour after the irradiation. Transplantation of spleens from young mice into the peritoneal cavity of mice immediately after a lethal dose of whole body x-irradiation significantly increased the survival of the irradiated mice, but transplantation of spleens from adult mice was less effective in enhancing survival.

The present study was designed to investigate the protective activity of shielded-spleen removed at various intervals after the irradiation-spleen-shielding procedure. We expected that the longer the interval from irradiation with the spleen-shielding procedure to removal of the shielded spleen used for implantation, the better would be the

protective effect, because the ectopic blood formation in the shielded spleen would increase with time as JACOBSON^{8), 9)} had reported. However, the present study has shown that the intraperitoneal implantation of shielded spleens from mice three hours, and one, ten and fourteen days after irradiation was effective, whereas shielded spleens taken from mice four and seven days after this treatment was considerably less effective. The increased protective activity of shielded spleens taken after the tenth and fourteenth days may depend on an intense increase in blood cell formation in these spleens. However, the increased protective activity of shielded spleens taken after three and twenty-four hours does not seem to be readily explained by colonization of hematopoietic cells alone, because increased cellular proliferation is not yet apparent within that short time interval. An alternative possibility is that non-cellular elements of the shielded spleen may play an important role in recovery from radiation injury during an early period following irradiation. Such an interpretation is consistent with results reported in a previous paper¹⁰⁾ that the O_2 -uptake of lead shielded spleen tissue increases markedly at two, three and twenty-four hours after irradiation. The reduction of the protective effect of the shielded spleen removed four and seven days after irradiation may be due to toxic substances in the spleen.

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