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Received September 4, 1957. P.S.E.B.M., 1957, v96.

Modification of Antibody Response in X-irradiated Rats by Injection of Spleen Homogenates. (23572)

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Sublethal doses of x-radiation are capable of inhibiting antibody response in experimental animals if antigen is given before or after irradiation(1,2). This inhibition can be reversed by lead shielding of the spleen or appendix during irradiation even if the spleen is removed soon afterward(3,4). Injections of homogenates of spleen and bone marrow have been shown to accelerate recovery from the hematopoietic damage caused by total-body x-irradiation(5-7). It was demonstrated recently that this protection by cell transfer is characterized by a "recolonization" process in which the injected cells proliferate and substitute for some of the animal's own tissues that were destroyed by x-rays (8,9). There has been relatively little study of the effects of heterologous and homologous post irradiation cell transfer upon the immunological process. The present work was undertaken to study antibody response of rats receiving homologous spleen cell injections up to 24 hours after 500 r total-body x-irradiation and stimulated from 3 to 48 hours later with an intravenous dose of sheep erythrocytes. Other investigators have attempted to restore or protect the immune mechanism of experimental animals by injection of various materials following irradiation. Of these, Jaroslow and Taliaferro have been able to produce considerable protection in rabbits by means of injection of whole spleen cells and of HeLa and yeast extracts when the material is given *mixed with the antigen* 24 hours after irradiation(10). The present experiments, on the other hand, have always involved separate administration of spleen cells and antigen from a *few hours to 24 hours* apart at different intervals after irradiation. A previous study, with a somewhat similar pattern, is that of Smith and his associates(11) in which spleen or bone marrow cells were given immediately after x-irradiation, immunization was carried out from 1 to 7 weeks later, and the circulating antibody titer was studied on the day of peak response. Under these conditions no difference in the rate of recovery of the antibody-forming capacity was noted between "protected" mice and mice receiving x-irradiation alone. *Spleen cells* were transferred into the irradiated animals in this study because of the demonstrated importance of the spleen in antibody formation in the rat. Under these circumstances, splenectomized animals form only a very small amount of circulating antibody(12). Rats injected intravenously with a particulate antigen show characteristic cellular changes in the spleen which appear to be closely correlated with the appearance of circulating antibody(13). These changes can be inhibited by a variety of treatments known to depress antibody formation(2,14,15).

Materials and methods. Young adult Sprague-Dawley albino rats were used throughout. Irradiation was administered by means of a General Electric Maxitron thera-

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TABLE I. Types of Treatment Administered to Rats Receiving 500 r of Total-Body X-irradiation. Several different combinations of the indicated variables were used in 9 experiments.

Time of inj. of spleen cells or erythrocytes (days after total body x-irradiation)	Sheep erythrocytes (1.0 ml of a 0.25% suspension given intrav.)	Spleen cells (2×10^8)												
			Harvested after antigenic stimulation (days)						Homogenization method*					
		Normal	1	2	3	4	5	6	1	2	3	4	Washed	Unwashed
0		x		x	x	x	x	x	x	x	x	x	x	x
1	x	x	x		x	x		x	x					x
2	x								x					x
3	x								x					x

* Homogenization methods: 1—Crushing in a porcelain mortar with pestle in small amt of Locke's solution. 2—Homogenization by hand with Potter type homogenizer. 3—Homogenization by motor driven Potter type homogenizer followed by straining through a U.S. Standard Sieve No. 100, 0.149 mm, Dual Mfg. Co., Chicago, Ill. 4—Cells forced out of the spleen by inj. Locke's solution into the organ after removing it. All cell preparations were filtered through a double layer of gauze and then aspirated through a gauge 28 needle to eliminate connective tissue strands and large cellular aggregates which may cause embolization of pulmonary vessels.

peutic machine. Irradiation factors were: 250 KVP, 30 ma., with 0.5 mm Cu and 1 mm Al added filtration. Target distance was 95 cm. The animals were irradiated in groups of 12 in a circular aluminum box with perforated top, each animal being housed separately in a small section. The box was rotated slowly during exposure. Each rat received a total of 500 r at the average dose rate of 29.4 r per minute. Irradiated rats received one injection of spleen homogenate containing 2 x 10^8 cells and one intravenous injection of 1 ml of 0.25% suspension of sheep erythrocytes at varying times up to 3 days after irradiation. Spleen homogenates were prepared from "normal" animals and from rats that had received an earlier intravenous injection of 1 ml of *S. typhi* vaccine. Several methods of preparing spleen homogenates were tried. It became apparent, however, that the most effective method of preparation was crushing the spleen in a mortar. Therefore, only this technic was used in the later experiments. Cells were counted in a hemocytometer after dilution in Hayem's solution. Smears of the original preparations were stained with methyl green-pyronine and percentage of different cell types was determined. Homogenates of "normal" spleens were examined as stained smears and contained a mixture of small lymphocytes, macrophages and primitive reticular cells. Homogenates of spleens from stimulated animals contained all of these cell types and a large proportion of the immature pyroninophilic cells ("antibody forming cells") implicated in antibody formation(13). The percentage of "antibody forming cells" increased from 10% of the total on the 2nd day to 50% on the 4th day after antigen injection and returned to 5-10% on the 6th day. In one experiment, a group of rats was also given the spleen cells in 3 divided doses which totaled 2 x 10^8 cells. In another experiment, spleen cells were given intraperitoneally. Table I summarizes the various types of treatments that were used in a number of different combinations. Rats were bled for antibody determination at 4, 6, 12, and 18 days. Hemolysin titrations were carried out as described by Cannon and coworkers(16). The double dilution method was employed starting with a serum dilution of 1:15. The last tube showing complete hemolysis was read as the end point rather than employing the more critical 50% hemolysis end point because only large differences in antibody-forming capacity were being considered (*i.e.*, some or none).

Results. The best protective effect on the mechanism of antibody formation was always observed in animals receiving spleen homogenates prepared by crushing the spleen with mortar and pestle. Homogenization by hand or by a motor driven homogenizer with subsequent straining through a #100 screen, or collection of cells by forcing Locke's solution in the spleen with a needle and syringe yielded preparations which, although effective, protected to a lesser degree than cells from

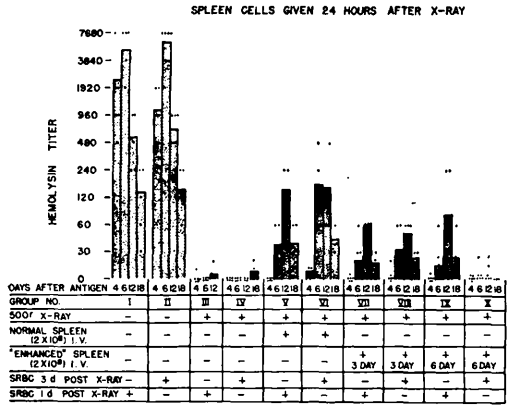

FIG. 1. Hemolysin titer in serum of rats receiving 500 r total-body x-irradiation and given spleen homogenates 24 hr after x-ray.

crushed spleens. A certain toxicity was observed in the homogenates. Washing with Locke's solution abolished toxicity almost entirely but also decreased the protective effect. Fractionating the cell infusion into 3 equal portions or changing the injection route from intravenous to intraperitoneal resulted in less protection.

The protective effect of cells from normal spleens as well as from spleens stimulated by antigen injection 3 or 6 days earlier is shown in Fig. 1. Normal rats stimulated with an intravenous injection of a particulate antigen will reach a peak in the titer of circulating antibody about the sixth day after injection. A delay in reaching the peak is apparent in the irradiated, spleen-injected rats as compared with normal control animals in the present series of experiments. Usually this peak is not reached until the 12th day after injection.

Fig. 2 indicates that essentially the same results are obtained by using normal spleen cells and cells taken from spleens stimulated with another antigen 2 to 6 days earlier. A substantial protection of the antibody-forming mechanism appears to be afforded by injection of either type of spleen cells, although the peak of circulating antibody is always considerably lower than in normal rats. It is apparent also that spleen cells collected during an active antibody response do not protect the antibody-forming mechanism of rats given 500 r of total-body x-irradiation to an extent greater than normal spleen cells. This is true regardless of the time of cell collection relative to antigen administration to the cell donors.

Discussion. Injections of homogenates prepared from "normal" spleens or from spleens of rats stimulated antigenically 6 days earlier may be somewhat more effective in restoring the antibody-forming mechanism of the irradiated rat than cells from the spleen of rats stimulated 2 to 5 days earlier. It appears definite that cells engaged in antibody synthesis are not more effective than those from "normal" spleens. The fact that the antibody response of x-irradiated, spleen-cell injected rats is not as great as that of normal animals may be the result of insufficient time for the transferred donor cells to recolonize completely, insufficient number of cells transferred, or other factors. In most instances, the appearance of the peak titer has been delayed beyond the usual time of 6 days after antigen injection. This delay can be interpreted as the time necessary for the injected cells to exercise their effect on the antibody-forming mechanism.

It should be noted that while the injection of spleen homogenates restores at least partially, the antibody-forming mechanism in the x-irradiated rat, a similar type of injection has been reported to be ineffective in the mouse (11). Comparative studies between the rat and the mouse during active antibody response and after x-irradiation may help to explain these differences.

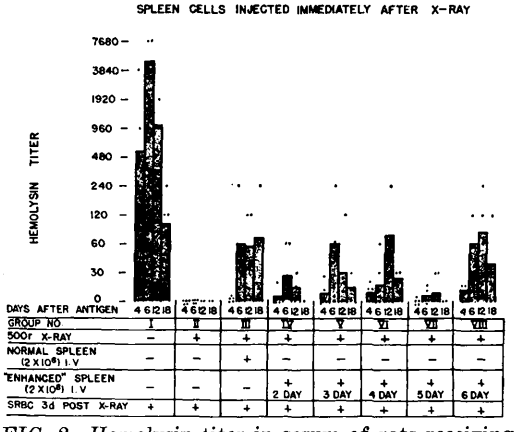

FIG. 2. Hemolysin titer in serum of rats receiving 500 r total-body x-irradiation and given spleen homogenates immediately after x-ray.

Summary. Antibody formation, which is inhibited by 500 r of total body x-irradiation into the rat, can be restored partially by spleen homogenates injected soon after irradiation. No striking difference in the degree of recovery is observed when spleen cells from rats stimulated earlier with a different antigen are used in place of "normal" spleen cells.

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Received September 5, 1957. P.S.E.B.M., 1957, v96.

Comparative Lipotropic and Lipide Phosphorylating Effects of Choline, Betaine, and Inositol.* (23573)

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It has been concluded that lipotropic action is more specific than stimulation of lipide phosphorylation since evidence showed that those substances which exert a marked lipotropic action also stimulate the formation of phospholipides, but that other substances stimulating phosphatide turnover may not necessarily cause a lipotropic effect(1). The lipotropic effect of choline, betaine, and inositol under specific dietary regimes is well known(2). However, the present study is, to our knowledge, the first to compare the lipotropic and phospholipide turnover actions of these compounds under identical experimental conditions. These conditions include the feeding of low protein-low fat and low protein-high fat diets to the experimental animals.

Methods. Male albino rats of Sprague-Dawley strain weighing 141 ± 43 g were divided into 2 groups. *Group I:* Ninety-nine rats, 26 of which served as controls, were maintained on a 5% casein-5% fat diet(3) or a 5% casein-32% fat diet(4) for a duration of 3 weeks. The animals were fed *ad libitum*. At the end of the dietary regime, the rats were stomach-tubed with a single dose of lipotropic agent (0.4 mM in 1 ml H_2O). All animals were then injected intraperitoneally with 1 ml of physiological saline containing 4 μc of P^{32} as NaH_2PO_4. Six hours later the animals were killed by decapitation. The liver was removed, rapidly weighed, and

* Supported by grants from Lipotropic Research Fn. and Amer. Cyanamid Co. Preliminary report presented at 126th meeting of American Chemical Society.