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Effect of Irradiation on Ferrokinetics of Cross-Circulated Dogs

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The notion of the transmission of radiation effects from the original site of irradiation to a distant, nonirradiated location is potentially important to many areas of radiation biology. Assuming that such a mechanism exists, it would seem logical that the medium of transmission is the vascular system.

One of the most dramatic effects of ionizing radiation is that of depressing erythropoiesis; in fact, remarkably small doses (25 r) have been shown to produce demonstrable changes (1, 2). Since the bone marrow is highly important in the recovery processes after irradiation, while at the same time very radiosensitive (3), the possibility of demonstrating that at least part of this effect is due to abscopal mechanisms is the purpose of this study.

An approach to the problem is to use the technique of cross-circulating a pair of experimental animals. By irradiating one of the partners while shielding the other, and subsequently studying the pair for radiation effects, the demonstration of "radiation changes" in the unirradiated partner would be highly suggestive of vascular transmission of the injury.

An ideal system for investigating erythropoiesis is blood radioiron ferrokinetics. Small doses of radiation will produce significant lengthening of the plasma radioiron disappearance half-time and depression of the normal erythrocyte radioiron reappearance (1, 2). The degree of perturbation of these measurements is linear with dose over a fairly wide range (1). Also, since the uptake of radioiron is a function of the bone marrow and not the circulating elements of the blood, considerable theoretical and practical difficulties in interpretation of the data are avoided.

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METHODS

Twelve adult female Beagle dogs (18 - 26 lbs) which had been paired on the basis of weight and blood type and cross-match compatibility were used. There was less than 2 lbs differential in weight between any of the pairs. Sodium pentobarbital anesthesia was used at the calculated dose of 30 mg/kg and maintenance doses were added as needed. Heparin anticoagulent was used at a dose of 2 mg/kg in each dog. The cross-circulation was established by a surgical carotid-to-jugular vein (one pair of dogs) or femoral artery to femoral vein anastomosis (the other 5 pairs) between the partners by means of polyethylene catheters (No. 280). These catheters provided a complete external vascular loop; *i.e.*, the artery of dog A was connected to the vein of dog B, while the artery of dog B joined the vein of dog A. The rate of cross-circulation was controlled by a rotary pressure pump external to the catheters; it propelled the blood by a milking action on the flexible catheter tubing. The pump was so constructed as to simultaneously advance equal amounts of blood into two partners; the direction of advance was physiological, *i.e.*, away from the artery into the vein. A delivery rate of 140 ml of blood per animal per minute was provided by the system. A detailed description of the cross-circulation method will be published as a separate report. Previous experiments in our laboratory using radiochromium tagged erythrocytes have shown that vascular equilibrium is established in less than 15 minutes (4).

Following the establishment of the cross-circulation, the animals were placed at opposite ends of a specially constructed nine foot rotary platform, the pump being located between the animals. One animal was positioned so as to be irradiated while the other was shielded from both primary radiation and scatter by concrete blocks and lead. A 2 mev Van de Graaff accelerator was used for the irradiation. The beam factors were: 150 cm f.s.d., 7.5 mm Pb HVL and a dose rate of 11.5 rads/min.

The exposure dose rate was established by a Victoreen R meter placed at the level of the axis of rotation. The absorbed dose rate was estimated by adjusting the exposure dose rate by 0.975 rads. A dose of 430 rads was delivered to the nonshielded member of the pair. Continuous monitoring of the shielded animal with a Victoreen R meter failed to detect any dose. The cross-circulation was started 5 minutes before irradiation and continued while the dose was delivered. During the irradiation the platform was rotated at 1 revolution per minute to improve depth dose distribution. After the dose had been delivered, rotation was stopped but the pump was allowed to maintain the cross-circulation for an additional 20 minutes. The catheters were then removed, and the wound surgically closed.

Twenty-four hours after irradiation all animals received intravenous doses of 6 μc of radioiron ($^{59}FeCl_3$, 10.7 mc/mgm). This dose was prepared by diluting the stock solution to the concentration of 6 μc/ml with sterile water. For each injection, a 1 ml volume of the solution was quantitatively drawn up into a 1 ml tuberculin syringe and injected through a 26-gauge needle into a superficial vein. Whole blood samples were taken by femoral venipuncture during the first four postinjection hours. From each sample a small specimen was taken

for hematocrit. This was placed in a previously calibrated centrifuge tube (Wintrobe), and then centrifiuged for 30 min; the measured hematocrits were corrected for trapped plasma (5). The remainder of the sample was immediately centrifuged, and 1 ml aliquots of plasma were quantitatively pipetted into precounted 10 ml glass counting tubes. Samples of blood were also obtained at 7 days postinjection; these were counted as whole blood; *i.e.*, the sameples were not centrifuged. Hematocrits on these samples were also obtained as described above.

A Nuclear-Chicago scintillation counter having an efficiency of 5 per cent for a 1 ml sample was used for all assays.

The plasma disappearance half-times and the 7-day erythrocyte radioiron reappearance percentages were established by standard means (5).

RESULTS AND DISCUSSION

Table I summarizes the experimental findings. The ranges of the plasma radioiron disappearance half-times and erythrocyte reappearance percentages of the unirradiated dogs are within normal ranges for our laboratory (plasma half-time 50 - 100 minutes, and 7-day erythrocyte reappearance 70 - 100 per cent of injected tracer dose).

The prolongation of the disappearance half-time and the marked depression of the erythrocyte reappearance percentage are expected for doses of the magnitude used in the study. It is apparent that depression of erythropoiesis did not occur in the unirradiated partners of the cross-circulated pairs. The cross-circulation studies of Lawrence, Valentine and Dowdy (6), which were based upon leucocyte counts as an index of depression of hematopoiesis, did not demonstrate any evidence of abscopal mechanisms. Partial body irradiation experiments (7, 8) have also demonstrated that the depression of the radioiron ferrokinetic parameters (plasma disappearance half-time and percent erythrocyte reappearance) are not influenced to any great degree by abscopal effects.

Because of the marked sensitivity of the bone marrow to irradiation and because of the size of the dose delivered to the irradiated animals, if a significant amount of circulating "toxic material" was formed as a result of the radiation, it should be delivered in sufficient quantities to the unirradiated partner to produce a demonstrable effect.

While it is theoretically possible that the "toxic material" is so short lived that it is inactivated by the time and distance required to cross from one animal to another, such an occurrence would be very unlikely because of the experience of those workers who have performed partial body irradiation experiments.

It should be mentioned, however, that minimal abscopal changes might be missed. For example, if 10 per cent of the primary irradiation effect was on the basis of an abscopal mechanism, this would amount to a 43 rad "dose" to the cross-circulated, shielded partner, which could go undetected without an extensive statistical investigation. It should also be stated that although a delayed appearance of "toxic material" cannot be excluded on the basis of data presented in this report since the cross-circulation was terminated 20 minutes after irradiation, the partial-body irradiation studies suggest that delayed depression of hematopoiesis did not occur. Therefore, the consensus of the available data is that, if

abscopal mechanisms are active, they are of minimal significance with respect to hematopoiesis, and that the action of radiation on hematopoiesis is direct.

SUMMARY

Six pairs of adult beagle dogs were cross-circulated by surgical arteriovenous anastomoses. Four of the pairs were treated as follows: following the establishment of the cross-circulation, one of the partners of each pair was irradiated with 430 rads of 2 mev x-rays while the other partner was shielded. The other two pair of dogs were cross-circulated but not irradiated. Twenty-four hours

TABLE I. EXPERIMENTAL RESULTS

Group	*Cross-Circulated*	*Rotated*	*Irradiated*	*Plasma Disappearance Half-Time*	*^{59}Fe Reappearance at 7 Days (% of Injected Tracer Dose)*
Group 1					
Dog 1 A	Yes	Yes	No	81 min.	100%
Dog 1 B	Yes	Yes	No	64 min.	100%
Group 2					
Dog 2 A	Yes	No	No	38 min.	100%
Dog 2 B	Yes	No	No	83 min.	83%
Group 3					
Dog 3 A	No	Yes	430 Rads	265 min.	11.3%
Dog 3 B	No	Yes	No (Shielded)	56 min.	85%
Group 4					
Dog 4 A	Yes	Yes	430 Rads	180 min.	7.9%
Dog 4 B	Yes	Yes	No (Shielded)	95 min.	89%
Group 5					
Dog 5 A	Yes	Yes	430 Rads	218 min.	1.2%
Dog 5 B	Yes	Yes	No (Shielded)	50 min.	82%
Group 6					
Dog 6 A	Yes	Yes	430 Rads	252 min.	2%
Dog 6 B	Yes	Yes	No (Shielded)	72 min.	75%

following irradiation (or cross-circulation in the case of the nonirradiated animals) radioiron ferrokinetics were performed. The expected depression of erythropoiesis occurred in the irradiated animals but not in the nonirradiated ones. Therefore, no evidence of an abscopal mechanism being responsible for radiation depression of erythropoiesis was demonstrated.

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REFERENCES

1. HENNESSY, T. G. AND HUFF, R. L.: *Proc. Soc. Exp. Biol. Med.* 73:436, 1950.
2. LOEFFLER, R. K.: *Am. J. Roentgen.* 77:836, 1957.
3. MIRAND, E. A., HOFFMAN, J. G., PRENTICE, T. C. AND REINHARD, M. C.: *Exp. Med. Surg.* 16:257, 1958.
4. BRODISH, A. AND LONG, C. N. H. *Yale J. Biol. Med.* 28:644, 1956.
5. LAJTHA, L. G.: The Use of Isotopes in Hematology, Charles C Thomas, Springfield, Ill. (USA), p. 83, 1961.
6. LAWRENCE, J. S., VALENTINE, W. N. AND DOWDY, A. H. *Blood* 3:593, 1948.
7. ROSEGOTTI, L.: *Radiat. Res.* 9:240, 1958.
8. CARSTEN, A. L. AND NOONAN, T. R.: *Radiat. Res.* 22:136, 1964.