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Stimulation of Erythropoiesis in Sublethally Irradiated Rats by a Plasma Factor.* (22152)

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It is well established that red cell production is severely depressed by low doses of whole body irradiation. Erythropoiesis can be significantly increased, however, when the animal is bled immediately before(1,2) or after(2) sublethal irradiation. A similar but less pronounced effect results from the post-irradiation administration of para-amino-propiophenone (PAPP), which produces a severe, transient methemoglobinemia. Recently evidence has been presented to indicate that in the normal animal hypoxia elicits the release of a humoral substance (hemopoietine) capable of stimulating erythropoiesis(3-6). Since both bleeding and PAPP produce hypoxia, it might be suggested that the subsequent release of hemopoietine is responsible for the accelerated regeneration of erythropoietic tissue in the sublethally irradiated animal. If such is the case, plasma collected from donors with post-hemorrhagic anemia might be capable of stimulating erythropoiesis in the sublethally irradiated animal.

Material and methods. Female Sprague-Dawley rats weighing 135-170 g were used. In each experiment 24-30 animals were randomly assigned to 3 or 4 groups. Animals were exposed to whole body irradiation, 200 r from a Co^{60} source in 4 π geometry(7) or 160-190 r from a 2.5 MEV Van de Graaff generator at an average dose rate of 50 r per minute measured with a Bendix ionization chamber known to be air equivalent at that energy. "Anemic" plasma was prepared by bleeding normal donor rats 2% of the body

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TABLE I. Effect of Plasma from Anemic Donors on Fe^{59} Incorporation in Sublethally Irradiated Rat.

Exp. #	Irradiated controls	Normal plasma	Anemic plasma* 24 hr	Anemic plasma* 48 hr	p†	Radiation Source	Radiation Dose, r
1	23 ± 2.6 (7)‡	36 ± 7.8 (7)		68 ± 2.5 (5)	<.01	Co^{60}	200
2	35 ± 1.7 (8)	32 ± 4.4 (7)		42 ± 2.5 (7)	<.05	2.5 MEV	160
3	31 ± 6.2 (5)	33 ± 6 (6)	51 ± 1.3 (5)	38 ± 2.9 (6)	<.01	2.5 "	160
4		39 ± 6.6 (6)	44 ± 2.7 (8)		n.s.	2.5 "	180
5		38 ± 5 (7)	53 ± 2.1 (8)		<.01	2.5 "	180
6	26 ± 4 (5)	37 ± 2.6 (6)	53 ± 4 (7)		<.01	2.5 "	190

* Plasma collected 24 or 48 hr after production of post-hemorrhagic anemia.

† p refers to probability that observed differences between groups receiving anemic and normal plasma occurred by chance. In the third experiment, 24 hr anemic plasma was compared with normal plasma. The p was determined using the one sided t test.

‡ Fe^{59} values given represent % of total injected dose present in circulating red cells 48 hr after injection ± a single stand. error computed as $\frac{\text{Range}}{K_n\sqrt{n}}(10)$. Number of animals in each group is included in parentheses.

weight (approximately 30% of the blood volume) by cardiac puncture. 24 or 48 hours thereafter, blood was collected in heparin or 5% Na_2 ethylene diamine tetraacetate and the plasma separated. Control plasma was obtained from normal donors not previously bled. The plasma preparation to be tested was injected into the tail vein within 90 minutes following irradiation. An amount equivalent to 30% of the recipient's blood volume, calculated as 7% of the body weight, was injected. For studies of heat stability, anemic plasma was adjusted to pH 5.4 with 1 N HCl and heated at 100°C for 10 minutes. The precipitated protein was separated by centrifugation at 2000 G for 2 hours. The pH of the supernatant was adjusted to 7.1 with 1 N NaOH and injected as before. Fresh anemic plasma was also dialyzed at 4° for 24 hrs. 25 cc of plasma was dialyzed against 1000 cc of saline with 3 changes of the dialyzing fluid during the 24 hr period.

Rates of erythropoiesis were originally estimated by reticulocyte levels, the histologic appearance of erythropoietic tissue on the 4th post-irradiation day and iron incorporation. Since there was an excellent correlation between these measures(2), subsequent estimates of red cell production were based on Fe incorporation alone. For this determination, Fe^{59} tagged normal plasma was injected on the 2nd post-irradiation day and 48 hours thereafter blood was collected by cardiac puncture for determination of Fe^{59} activity. The details of tagging of the plasma, determination of radioactivity, and the calculation of the % Fe incorporation have been given previously(2). At the completion of each experiment, the tails of the injected rats were checked for radioactivity. The rare animals in which more than 5% of the injected Fe^{59} was present in the tail, indicating substantial extravascular injection, were excluded from the series. Rats with pneumonia, which occurs in 2 to 5% of animals in our colony, were also excluded after verification of the diagnosis by autopsy.

Results. The average Fe^{59} incorporation in 48 hours by normal non-irradiated animals was 65%, with individual animals varying from 50-80%. The average Fe^{59} incorporation for animals receiving 160-200 r of whole body irradiation was 25% (Co^{60} source) and 30% (2.5 MEV source) with a range of 10-45%. The injection of normal plasma following irradiation produced a variable response. In 3 of 4 experiments, where animals receiving normal plasma were directly compared with irradiated controls (Table I), the Fe^{59} incorporation of those receiving normal plasma was greater than in the irradiated controls. In only one of these, however, was the difference significant (Exp. 6, Table I, $p < .02$). The variability within each group receiving normal plasma was greater than that observed with control animals or those receiving anemic plasma (Table I).

The injection of plasma from anemic don-

TABLE II. Studies on Stability of Erythropoietic Factor in Anemic Plasma.

Normal plasma	Anemic plasma*				
	Fresh	Stored 4°	Stored −20°	Dialyzed	Heated
38 ± 5 (7)†	53 ± 2.7 (8)	44 ± 5 (7)	51 ± 6 (5)		
39 ± 6.6 (7)	44 ± 2.7 (8)			44 ± 2.5 (5)	34 ± 5.7 (4)

* Plasma collected 24 hr after production of post-hemorrhagic anemia.

† Fe^{59} values given represent % of total injected dose present in circulating red cells 48 hr after injection ± a single stand. error computed as $\frac{\text{Range}}{K_n\sqrt{n}}(10)$. Number of animals in each group is included in parentheses.

ors into irradiated animals produced a significant increase in the rate of erythropoiesis as measured by Fe^{59} incorporation (Table I). In all experiments to date the average Fe^{59} incorporation values were greater in the animals injected with anemic plasma than in those receiving normal plasma or in untreated irradiated controls. The difference in the means of groups receiving anemic and normal plasma varied from 5-32% (Table I). In 4 of 6 experiments the differences in mean Fe^{59} incorporation between animals receiving anemic and normal plasma were significant at a 0.01 level, and in one at a 0.05 level (Table I). In the remaining experiment, the mean for anemic plasma was greater than for normal plasma, but the difference was not statistically significant ($p > 0.05$). There was marked variation in the response between experiments but the variability within a given experimental group was relatively small (S.E. 1.3-4).

In one experiment anemic plasma collected 24 hours after the donor animals were bled produced a greater response than that collected 48 hours after bleeding (Table I). The factor present in anemic plasma retained its activity during storage for 3 weeks at −20°C (Table II). The supernatant obtained after heating at 100°C at pH 5.4 for 10 minutes was inactive (Table II). There was no reduction in the activity of anemic plasma following saline dialysis at 4°C for 24 hours (Table II).

Discussion. It has been suggested that the modification by acute blood loss of the usual irradiation injury to erythropoiesis is due to the elaboration of hemopoietine which accelerates the regeneration of erythropoietic tissue. This interpretation has been supported by the observation that plasma collected from anemic donors, when injected into rats immediately following sublethal irradiation, stimulated erythropoiesis as evidenced by an increase in mean Fe^{59} incorporation of 5-30% above that of animals receiving normal plasma. As might be expected, the magnitude of response in animals receiving anemic plasma was not as great as had been noted previously in animals with acute blood loss following irradiation. Although there was a considerable variation in the degree of response between experiments, all groups receiving anemic plasma had a mean Fe^{59} incorporation greater than that observed following the administration of normal plasma. In 4 experiments the differences were significant at the 0.01 level, in 1 at the 0.05 level and in 1 there was no significant difference ($p > .05$). The response of the sublethally irradiated animals to anemic plasma, therefore, appears to be highly significant. The stability of the factor present in anemic plasma was similar to that reported by Erslev (8).

The variability of response to anemic plasma between experiments may be accounted for in part by differences in the plasma content of hemopoietine. The marked difference between the experiment in which a Co^{60} source was used and those with the 2.5 MEV source, suggests that irradiation factors may account for some of the variability.

Normal plasma produced an increase in Fe^{59} incorporation in 3 of 4 experiments where there was a direct comparison with irradiated controls but in only one was the difference statistically significant. This effect may have resulted from the presence of small amounts of the erythropoietic substance in

normal plasma or from a transient dilution anemia. The marked variability within groups receiving pooled normal plasma (S.E. of the mean 2.6-7.8) is thought to result from variations in responsiveness of individual animals to borderline amounts of hemopoietine.

It is of interest that only a single injection of anemic plasma is required to demonstrate erythropoietic activity in the sublethally irradiated animals, whereas multiple injections are required in normal animals(4,6). It has previously been observed that a single injection of PAPP, which fails to produce a significant alteration of erythropoiesis in the normal rat(9), will increase red cell production when administered to rats following a sublethal dose of irradiation (120-200 r from a 3 MEV source, 200 r from a Co^{60} source)(2). These observations suggest that immediately following irradiation an animal is more sensitive to erythropoietic stimuli than normally, provided that the dose of irradiation is not sufficient to destroy the majority of the erythropoietic tissue. It is tempting to suggest that the increased sensitivity of the irradiated animal results from the destruction of an inhibitor thus altering the normal balance between inhibitors and stimulators of erythropoiesis. The sublethally irradiated rat may serve as a useful animal for the further investigation of the regulation of erythropoiesis, since the method employed requires but a single treatment, allows a satisfactory estimate of red cell production by the Fe^{59} incorporation technic and requires only 5 days for an experiment. At present, the method requires the use of 5-7 animals per group and the inclusion of controls. The variability of response may be in part a reflection of the plasma content of hemopoietine rather than the lack of sensitivity of the test.

Summary. Injection of plasma from anemic donors into rats immediately following exposure to sublethal doses of X-rays produced a statistically significant increase in erythropoiesis as measured by Fe^{59} incorporation. The factor present in anemic plasma was nondialyzable and was not present in the supernatant obtained after precipitation of protein by heating. Normal plasma produced a variable response. Use of sublethally irradiated rat as a test animal in the study of regulation of erythropoiesis is suggested.

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Absorption and Elimination of Carbon^{14} Labelled Nicarbazin by Chickens. (22153)

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Nicarbazin, an equimolecular complex of 4, 4'-dinitrocarbanilide (DNC) and 2-hydroxy-4, 6-dimethylpyrimidine (HDP), is effective for prevention of coccidiosis in chickens(1). This complex is split into its components in the animal, and the HDP portion rapidly eliminated. By use of spectrophotometric methods, both moieties of nicarbazin have been detected in tissues of chickens receiving the drug(2). However, 2 days after