Evidence has been obtained recently that indicates that EP stimulates marrow RNA synthesis *in vitro*(13,14) and *in vivo*(14) within 2 hours, and that the RNA formed may be of messenger type(15).

Summary. The iron uptake by circulating RBC and erythroid tissue of fasting rats measured 24 hours after erythropoietin injection, is clearly dependent on the state of the recipient at the moment of injection and drops off sharply with time of fasting. The uptake measured 48 hours after EP injection is not affected by the state of the recipient at the time of injection.

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Effect of Gamma Irradiation on Response to Erythropoietin of Non-Fasted Rats.* (31156)

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It has been shown(1) that the effects of irradiation on the response of fasted rats to erythropoietin (EP) depend on time of irradiation in relation to EP injection. Maximum depression of the response was obtained when irradiation was carried out 3 hours before EP injection and minimum effects when it was given 5 hours after EP. The fractional depression obtained by varying doses of gamma rays was independent of EP dose, when irradiation was given prior to EP, but varied clearly with EP dose, when irradiation was given after EP. In these experiments, however, the state of fasting at which irradiation was given may have affected the results. For this reason it was decided to carry out experiments in non-fasted rats to see if similar responses were obtained. The results are reported here.

Materials and methods. Male A×C rats from the Instituto de Biología Juan Noé, weighing between 160-180 g were used. ^{137}Cs gamma irradiation conditions and the assay of erythropoiesis were carried out as described previously(1).

Results. Fig. 1 shows the response to erythropoietin (20 U) in irradiated (200 r) rats as a function of the interval separating irradiation and EP injection. ^{59}Fe was always injected 48 hours after EP injection. The response is measured as the difference in iron uptake by erythroid tissue, between the EP-treated and the non-EP-treated irradiated rats. As in fasted animals, maximum depression of the response is observed when gamma irradiation is given 3 hours before EP injection and at about 24 hours after, while mini-

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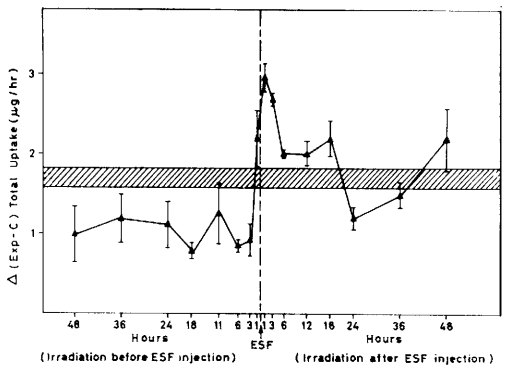

FIG. 1. Response of non-fasted irradiated rats to erythropoietin (EP). Ordinate: difference in iron uptake by erythroid tissue of EP-treated and non-treated rats. Abscissa: Time of irradiation (200 r) in relation to EP injection. Hatched area corresponds to response to EP of non-irradiated animals.

mum depression is obtained when EP is given one to 3 hours after irradiation. It is to be noted that when EP is given 1 to 3 hours post irradiation the response is greater than that of non-irradiated rats. This latter phenomenon had previously been observed by Stohlman(2).

Fig. 2 shows the ratio of iron uptake by erythroid tissue of irradiated rats to that of the respective non-irradiated controls, in animals injected with EP before (a) or after (b) irradiation. When EP is given before irradiation, depression of iron uptake by erythroid tissue is significantly smaller in the EP-treated than in the non-EP-treated animals. The curve for the former has a significant shoulder. However, when irradiation is given prior to EP, there is no difference between the groups. This phenomenon is analogous to that observed in fasted rats given 2 different doses of EP (5 and 20 U)(1).

Discussion. As in the case of fasted rats, the effects of irradiation on the response to EP of normal rats depend on the time relation of irradiation and EP injection. Target cells to EP action are more sensitive to irradiation given before injection than after injection of EP. The finding that the fractional depression of erythropoiesis by irradiation depends on EP dose, when EP is given before irradiation, also has been observed in the transfused mouse given EP immediately after irradiation(3); at this time the response to a given dose of EP is greater than when EP is given 3 hours after irradiation(1).

The observation of higher EP dose increasing resistance to irradiation has been interpreted as a consequence of the action of larger doses of EP sending more cells into the "skipped division pathway"(3) and thus reducing the probability of cell death at mitosis. If this is the mechanism, it would suggest that EP given hours after irradiation can only act on "non-damaged cells" on which shunting through the "skipped division pathway" would have no favorable effect. Furthermore, since EP also shows a dose effect when given immediately after irradiation but not 3 hours after, it is conceivable that the damage of irradiation on the cells capable of responding to erythropoietin develops with time.

Summary. The effect of gamma irradiation on the erythropoietic response to erythropoietin (EP) in non-fasted rats depends on

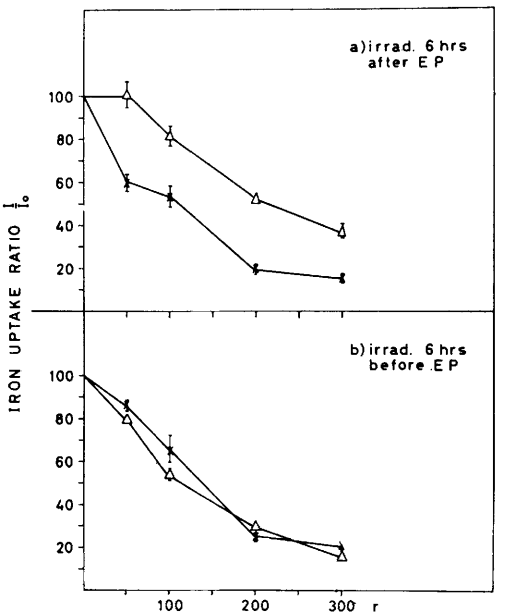

FIG. 2. Fractional iron uptake by erythroid tissue as a function of radiation dose. Ordinate: Iron uptake ratio = iron uptake of erythroid tissue of irradiated rats divided by uptake of the respective non-irradiated controls. Abscissa: Gamma dose in r. a) irradiation given 6 hr after EP. b) irradiation given 6 hr before EP. Δ——Δ EP (20 U) treated rats. x——x non-EP-treated rats. I ± 1 Standard error. When not indicated, it is smaller than the size of the symbol used.

the time of irradiation in relation to EP injection. There is maximum sensitivity to irradiation when it is given 3 hours before or 24 hours after EP. Radiosensitivity is at a minimum when irradiation is given one to 3 hours after EP injection. Fractional depression of erythropoiesis by varying doses of gamma rays is less in animals injected with EP, when it is given before irradiation. There is no effect of EP on fractional depression produced by irradiation given 6 hours before EP.

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Neutralization of Alpha-Staphylotoxin Cytotoxic Effect with Human And Monkey Immune Globulins. (31157)

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The data presented by Elek(1), Bernheimer(2), and Rogers and Melley(3) attest that the staphylococcal α toxin (SAT) is lethal, hemolytic, and leukocidal. Arenstein *et al*(4), Kienitz and Schmelter(5) and Cooper *et al*(6) reported that SAT is cytotoxic for monkey kidney tissue and human amniotic cell cultures. Thal and Egner(8) and Thal *et al*(9) observed very rapid cell damage in cultures of human esophageal epithelial cells and of rabbit fibroblasts. This activity reveals itself in alteration of the permeability of the cell membrane, swelling and vacuolization of the protoplasm, and nuclear changes, followed by cytolysis. Results with other cell lines were summarized by Korbecki and Jeljaszewicz(7). The outcome of the experiments varied. Hyperimmune sera employed in the neutralization tests often contained preservatives which interfered with the results (Kienitz and Schmelter(5)). It was postulated that a modified experimental technique may offer a better insight into the mechanism of toxin-antitoxin relationship.

Antibodies against SAT are being measured principally by the inhibition of the SAT hemolysin. The tissue culture cell damaging factor of SAT, however, is not identical with the α hemolytic principle (Bernheimer(2)). Experiments were set up to investigate further the possible relationship of these two factors by measuring the cytotoxic activity neutralizing property (CANP) of human sera and comparing the results with those of anti-SAT hemolysin determinations.

It had been noted that not only persons afflicted with clinically manifest staphylococcal disease but also the majority of adults without a history of staphylococcal infection have measurable antistaphylococcal antibodies in their sera (Elek(1)). To establish whether man is the only primate endowed with such circulating antibodies, parallel experiments were carried out with selected human and non-human primate sera.

It was also assumed that an inquiry into the role of the various immune globulins in the CANP of the sera (if any) might yield revelant results.

Materials and media. All materials were kept at $-20°C$ without a preservative.

Staphylococcal alpha toxin (SAT) was prepared by one of us (O.F.) at the Walter Reed Army Institute of Research in 1964 according to the method of Lominski and Arbuthnott(10) by methanol and Sephadex G75 fractioning from *S. pyogenes* strain S6. It contained 1.3% carbohydrate and 0.02% P. It was inactivated by trypsin. The molecular weight was 41,000. Immunoelectrophoresis at pH 6.5 revealed a strong 3 S and a weak 16 S component. The MHD for rabbit RBCs was 80,000/mg material; the LD_{50} 3 μg/20