rate of norepinephrine from storage granules of bovine adrenergic splenic nerves.

The precise location where the increased hydrogen ion concentration might exert its effect was not discussed. Barring the release by acidosis of a humoral factor capable of stimulating the medullary cells of the adrenals, it seems that an increase in hydrogen ion concentration might stimulate catecholamine release from the adrenal gland as it does from the sympathetic nerves of the isolated spleen(3). The mechanism involved in this release remains to be determined. In the present experiments the adrenal glands were not isolated from their spinal and splanchnic connections. It is not possible, therefore, to attribute the release of catecholamine from the adrenal gland to a direct effect of $[H^+]$. The stimulating effect of increased $[H^+]$ on catecholamine release contrasts with the lack of response caused by hypoxia.

Conclusion. In 8 spinal dogs adrenal blood flow and adrenal catecholamine output (ACO) were measured. Hypoxia (PaO_2 36 mm Hg) without acidosis did not change significantly ACO, while hypercapnic acidosis ($PaCO_2$ 127, pH 6.93) produced a significant increase in ACO. These findings confirm previous observations indicating that acidosis more than hypoxia stimulates catecholamine release.

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Erythropoietin Effects on Fasted Rats as a Function of Time of Injection.* (31155)

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It has been suggested that erythropoietin (EP)-containing extracts not only produce differentiation of stem cells into erythrocyte precursors(1) but also affect the precursors already present at time of injection(2,3,4,5), stimulating Hb synthesis and the release of reticulocytes(6). The purpose of the experiments described here was to evaluate the ef-

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fect of EP on iron uptake by erythrocyte precursors, when injected at different times after irradiation of fasting, *i.e.*, in recipients with varying rates of erythropoiesis.

Methods. Male A×C rats, from the Instituto de Biología Juan Noé, weighing between 160-180 g were used. In the experiments with fasted animals, fasting was started at zero time and continued throughout the experiments. Animals were injected with 10 U (St B) of erythropoietin-containing plasma at different intervals, and groups of 5 animals were injected with ^{59}Fe, 24 and 48 hours after EP injection, killed 3 hours after tracer injection, and the ^{59}Fe distribution(7) in plasma, erythrocytes (RBC) and femurs and plasma iron concentration measured(8). Iron uptakes by RBC and total erythroid tissue were estimated as follows:

RBC iron uptake ($\mu g/hr$):

$$\frac{^{59}\text{Fe RBC}}{100 - ^{59}\text{Fe Plasma}} \times \text{Kx Pv} \times \text{Fe}$$

Erythroid tissue iron uptake ($\mu g/hr$):

$$\frac{^{59}\text{Fe RBC} + 7 \times ^{59}\text{Fe Femur}}{100 - ^{59}\text{Fe Plasma}} \times \text{K} \times \text{Pv} \times \text{Fe}$$

Where:

$$\text{K} : 1/3\ 1\text{n}\ \frac{1}{^{59}\text{Fe Plasma}}$$

Pv : plasma volume (3.3 ml/100 g)
Fe : plasma iron concentration ($\mu g/ml$)

Spleen uptake is not taken into account since in this strain of rats it is very low and is not markedly affected by EP.

Plasma with a high titer of EP (8 U St B/ml) was obtained from irradiated phenylhydrazine-treated rats(9) and assayed as previously described(10).

The same pool of plasma was used throughout and was kept frozen in ampoules until injected.

Results. Fig. 1 shows iron uptake ($\mu g/hr$) by circulating RBC and by total erythroid tissue of fasted rats injected with EP at different times. Fasting rapidly reduces iron uptake which after 3 days reaches a plateau of 0.03 $\mu g/hr$ for RBC and 0.7 $\mu g/hr$ for total erythroid tissue. The drop in RBC (93%) uptake is proportionately greater than that of femurs (83%). The effect of EP measured

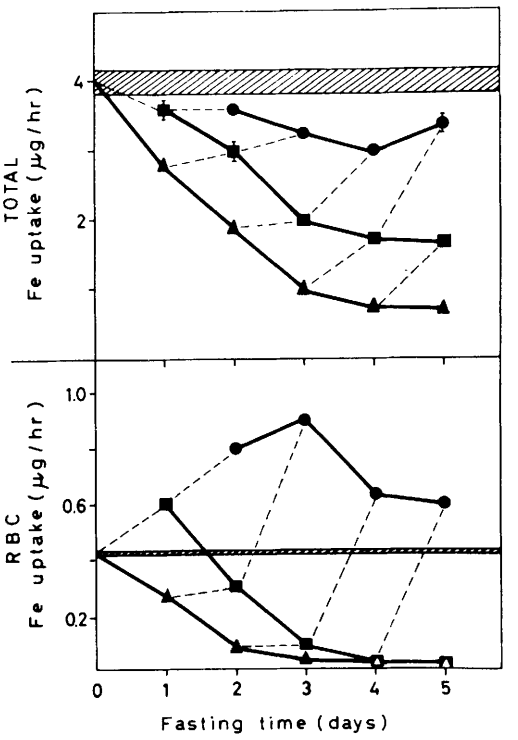

FIG. 1. Iron uptake in EP (10 U) injected rats. Top ordinate: Total iron uptake by erythroid tissue. Bottom ordinate: Iron uptake by circulating RBC. Abscissa: Fasting time in days. ▲——▲ Iron uptake in fasted, non EP injected, animals. ■——■ Iron uptake measured 24 hr after EP injection. ●——● Iron uptake measured 48 hr after EP injection. Dashed line (- - - - -) joins the iron uptake at moment of EP injection with corresponding uptakes measured 24 and 48 hr after. Standard errors of mean, I, are indicated only when they are larger than the symbols. Hatched area indicates uptake in non fasted rats.

24 hours after injection clearly depends on the time of injection. The longer the injection is delayed the smaller the effect. This is especially clear in the case of RBC iron uptake, which shows no increase at 24 hours, when EP is given after 3 days of fasting. The response to EP measured 48 hours after injection is much less affected by the time of injection (Fig. 1). It is to be noted that while the uptake by RBC of fasted rats, 48 hours after EP, is greater than that of non-fasted controls, total iron uptake by erythroid tissue is always smaller.

The effect produced by 10 U EP in RBC of non-fasted rats (Table I) at 48 hours, 1.4 $\mu g/hr$, is greater than that observed in fasted

TABLE I. ^{59}Fe Distribution in Erythrocytes (RBC) Femur and Plasma 3 Hr After Tracer Injection in: Normal Rats, Normal Rats 24 Hr and 48 Hr After Injection of 10 U of Erythropoietin (EP).

Group	Plasma iron (μg/ml)	K (%)	% ^{59}Fe			Total iron uptake (μg/hr)	RBC iron uptake (μg/hr)
			Plasma	RBC	Femur		
Normal	2.2	68.0 ± 2.0	13.0 ± .7	4.6 ± .3	5.4 ± .3	4.0 ± .17	.4 ± .01
Normal + 10 U EP (24 hr effect)	1.8	78.0 ± 7.0	10.0 ± 1.4	8.2 ± 1.6	6.0 ± .3	4.2 ± .3	.66 ± .12
Normal + 10 U EP (48 hr effect)	1.7	97.0 ± 6.0	5.5 ± .75	18.3 ± 1.5	6.0 ± .06	6.2 ± .8	1.8 ± .3

± Standard error.

PITR Plasma iron turnover rate: $1/3\ 1\ n \frac{1}{^{59}Fe_{pl_3}}$

$^{59}Fe_{pl_3}$ Fraction of tracer remaining in plasma after 3 hr.

rats. The increase in total erythroid uptake at 48 hours in normal EP-treated rats is similar to that observed in fasted rats injected with EP at the start of fasting.

Plasma iron concentration initially drops slightly on fasting, then rises (Table II). EP injection lowers it in fasted animals, but has only a slight effect in non-fasted rats.

Discussion. The effect of EP on iron uptake by erythropoietic tissue in fasted rats can be divided into 24-hour and 48-hour responses.

The response measured 24 hours after EP injection depends on the state of the animal at the time of injection. This is specially clear for the effect on iron uptake by circulating RBC which drops to zero by the third day of fasting. Iron uptake by circulating RBC depends on the reticulocytes present, but is more a function of their immaturity than of their number(11). The rise in iron uptake by circulating RBC can be interpreted as reflecting an increase of release of reticulocytes from the marrow(6) by EP containing plasma. This response would be expected to diminish as erythropoiesis of the recipient animal is decreased by fasting. Total iron uptake measured 24 hours after EP clearly diminishes with time of fasting, while that measured after 48 hours is much less affected. While RBC uptake measured 48 hours after EP is much greater than that of non-fasted controls, total iron uptake is smaller. This indicates that EP, in the doses used, changes the proportion in which circulating reticulocytes and marrow erythroid tissue contribute to total iron uptake (10 and 90% in the normal) (25 and 75% in EP-treated). This can be interpreted to reflect the delivery into circulation of reticulocytes of greater degree of immaturity. On the basis of these findings, it is conceivable that the response at 24 hours is mainly due to the stimulation of Hb synthesis by precursors already present at the time of injection, and that the uptake at 48 hours reflects uptake by precursors formed as a consequence of stem cell differentiation. In relation to this it is relevant to point out that, when EP is injected in polycythemic mice which have no RBC precursors in their erythropoietic tissue, no effect on ^{59}Fe uptake into Hem is observed until after 24 hours, and the maximum response is obtained at 48 hours(12).

Since EP is incapable of stimulating Hb synthesis in non-nucleated reticulocytes(4) it can be concluded that the stimulation of Hb by EP requires the presence of a functional nucleus capable of RNA synthesis.

TABLE II. Plasma Iron Concentration (μg/ml) as a Function of Duration of Fasting in Control Rats and in Rats Treated with Erythropoietin (10 U).

Group	Days of fasting					
	0	1	2	3	4	5
Control	2.2	1.8	2.1	1.9	2.3	2.6
24 hr after EP 10 U		1.0	1.1	1.0	1.0	1.0
48 hr after EP 10 U			1.1	.7	.6	.8

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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