than did the smaller adrenals of the ACTH-treated rats. Our data do not explain why GH potentiates only the adrenotropic (not the steroidogenic) action of ACTH.

Growth hormone did not potentiate the effect of TSH or PMS on their respective target organs nor did it affect the iodide-trapping mechanism of the thyroid in association with TSH stimulation.

Summary. 1. The effect of administration to hypophysectomized rats of GH simultaneously with other pituitary hormones was investigated. 2. Growth hormone potentiates the effect of ACTH on adrenal weight but does not increase the steroidogenic response of the adrenal to ACTH. 3. GH did not enhance the effect of TSH on thyroidal weight or iodide uptake, nor did it augment the effect of PMS upon ovarian weight.

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A Comparison of Erythropoietin* Bioassays. (27418)

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Attempts to explain the mechanism by which the body reconstitutes its red blood cell mass after hemorrhage led investigators to the discovery of a humoral stimulant of erythropoiesis(1). This hormone, present in the plasma of anemic animals, has not yet been isolated, but characteristics of highly purified preparations suggest it is a glycoprotein(2). Jacobson and Goldwasser(3) have suggested that short periods of hypoxia initiate erythropoietin secretion which in turn stimulates erythropoiesis, and with frequent minute adjustments the equilibrium of the normal red cell mass is thus maintained within normal limits.

While Gurney *et al.* have demonstrated erythropoietic activity in 10-fold concentrated Borsook extract of normal human plasma by using the hypophysectomized rat assay(4), erythropoietin has not been found in raw plasma from humans. Critics of the theory that erythropoietin normally regulates red cell production argue that the hormone is absent from plasma of non-anemic animals and that it is elicited as a "panic mechanism" (5) to compensate for blood loss. Proponents of the theory contend that, because only slight steady stimulation is required to sus-

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tain homeostasis, a small concentration of the hormone is present normally and that the starved rat assay is not sufficiently sensitive to detect it at such a low level.

The enhanced sensitivity of the transfused plethoric mouse(6) to small quantities of erythropoietin is uniform in our experience; as a consequence, these mice have supplanted the starved rat as assay animals in our laboratory. Moreover, the increased sensitivity resulting from suppression of erythropoiesis occurs without apparent injury to the bone marrow(7). Purification of erythropoietin obtained from anemic sheep plasma(2) and the definition of an erythropoietin "unit" have enabled us to relate dose to erythropoietic response. Consequently, we will describe the accelerated erythropoiesis engendered by injecting incremental doses of erythropoietin into transfused plethoric mice and measuring the response as demonstrated by incorporation of radioiron (Fe^{59}) into circulating red cells. The results will be compared with those obtained using the more frequently employed starved rat method(8).

Materials and methods. Male Sprague-Dawley rats weighing 100-120 g were used in the starved rat assay. They were allowed only water for 32 hours before the first intravenous injection of erythropoietin and throughout the experiment. Erythropoietin was given once daily for 2 days and on the third day 1.0 μc of Fe^{59} (citrate) was administered intravenously. Sixteen hours later, 1.0 ml of blood obtained by cardiac aspiration was counted for radioactivity and reported as per cent incorporation of the injected dose (8).

Transfused plethoric CF No. 1 young adult female mice (2 injections of erythropoietin) were prepared by two 1.0 ml intraperitoneal infusions of an 80% saline suspension of washed packed red blood cells on consecutive days(6). Six days after the last infusion, hematocrits were at least 70%, and in animals untreated with erythropoietin, reticulocytes were absent from the peripheral blood and incorporation of Fe^{59} into red cells was less than 0.2% of the injected dose. Two subcutaneous injections of erythropoietin were made on the third and fourth days. Radioiron (Fe^{59}) was administered intravenously on the fifth day. Preliminary studies indicated that the blood volume of mice prepared for assay by this method was 7% of body weight, and radioactivity of the red cells, expressed as per cent of the tracer dose, could be determined on the eighth day using this information.

In a subsequent modification of the model, erythropoietin was given in a single subcutaneous injection (1 injection) 6 days after the transfusions. Fifty-six hours later, 0.5 μc of ferric (Fe^{59}) chloride was injected into the tail vein of each mouse. Three days thereafter, the animals were sacrificed, and blood from the thoracic aorta was counted in a well scintillation counter.

Erythropoietin was purified by repeatedly passing plasma from sheep, rendered anemic by phenylhydrazine injections, through DEAE (Diethylaminoethyl cellulose) and Amberlite IRC-50 resin‡(2). The preparation was dissolved and diluted to the desired strength with 0.9% saline and then injected subcutaneously in 0.2 ml volumes. One "unit of erythropoietin" causes the same degree of incorporation of Fe^{59} into the red blood cells of the starved rat as does 5 μM of cobaltous ion(2).

Hematocrits were determined with the micro-hematocrit method.

The starved rat assay distinguishes between injections of saline and 2 units of erythropoietin, but doses of less than 1 unit produce a response less than or equal to that provoked by saline (Fig. 1). The transfused plethoric mouse is sensitive to doses of 0.125 unit of erythropoietin by the 2-injection technic of assay, but the 1-injection technic detects a distinct effect with 0.05 unit of erythropoietin. When the volume of saline diluent is 5 times (1.0 ml instead of 0.2 ml) the usual amount, there is no appreciable change in response (Table I). Increasing the dose of erythropoietin above 6 to 8 units causes only a small increase in radioiron incorporation. Apparently, there is a sigmoidal relationship between Fe^{59} incorporation and the log-dose of erythropoietin.

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TABLE I. Relation of Incremental Doses of Erythropoietin and the Incorporation of Fe^{59} into Circulating Red Cells.

1. Starved rat (2 injections)			2. Transfused mouse (2 injections)			3. Transfused mouse (1 injection)		
Dose (units)	$\%Fe^{59}$ inc.	No. of animals	Dose (units)	$\%Fe^{59}$ inc.	No. of animals	Dose (units)	$\%Fe^{59}$ inc.	No. of animals
12	21.2 ± 4.2*	5	12	24.1 ± 4.2*	5	24	59.1 ± 7.0*	8
8	18.5 ± 6.8	5	8	20.5 ± 3.5	5	9	53.1 ± 16.0	8
4	12.9 ± 1.1	5	4	18.6 ± 3.4	5	6	47.1 ± 10.1	8
2	12.8 ± 2.2	5	2	17.8 ± 4.8	5	3	30.5 ± 4.7	8
1	8.6 ± 4.1	5	1	11.3 ± 2.9	5	1	14.7 ± 4.0	8
.5	4.7 ± 1.6	5	.5	7.2 ± 4.7	5	.5	7.6 ± 2.5	7
.25	6.6 ± 1.1	5	.25	3.7 ± 1.5	5	.1	1.8 ± 1.4	7
Saline	6.1 ± .8	5	.125	1.2 ± .5	5	.05	.9 ± .3	8
			.0625	.4 ± .3	5	Saline	.2 ± .0	8
			.03125	.3 ± .2	5	3 units/ml	26.8 ± 6.9†	7
			Saline	.2 ± .1	5	1 " "	15.3 ± 4.8†	7

* ± 1 standard deviation.
† Administered in 1.0 ml saline.

Discussion. It is evident that the transfused plethoric mouse is more sensitive to small increments of erythropoietin than the starved rat in which discrimination is possible with a minimum of about 1 unit in our hands. Although persistently variable results prevented us from distinguishing between doses of erythropoietin less than one unit and saline, Schleuter *et al.*(9) described a sigmoidal curve of Fe^{59} incorporation and log-dose of purified erythropoietin ranging from 0.6 unit and 25 units in the starved rat. In Schleuter's laboratory, standardization of erythropoietin is dependent upon slightly greater discrimination at lower doses than we can regularly obtain, although it is not clear from his data that less than one unit can be determined with precision.

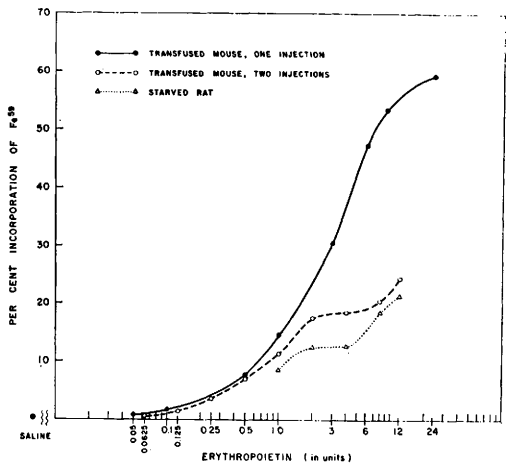

FIG. 1. Radioiron incorporation in the rat and mouse following administration of erythropoietin.

Previously, the erythropoietic stimuli were given in 2 daily injections, and the 16-hour erythrocyte incorporation of a tracer dose of Fe^{59} was determined 24 hours after the second dose of erythropoietin. This pattern was employed initially when the plethoric rat was explored as a bioassay animal(10,11). The results of recent physiological studies(6) suggested that using a single dose of erythropoietin and postponing the radioiron injection (into the mouse) to more than 24 hours later would yield greater differentiation of the erythropoietic effect. We therefore undertook a brief investigation to determine the optimal time of intravenous Fe^{59} administration after a single subcutaneous injection of erythropoietin. Maximum values occurred at 48 and 60 hours, followed by a rapid decrease in total incorporation of the radioiron.

We abandoned the transfused plethoric rat as an assay animal, because although reticulocytes and Fe^{59} incorporation diminished (10), the low base readily achieved in the plethoric mouse could never be obtained in the rat. Gastric bleeding associated with plethora in the rat is a further undesirable feature which may provide the stimulus for continuing erythropoiesis in longer experiments(12).

The relation between the log-dose of small amounts of erythropoietin and radioiron incorporation in the mouse has not been reported before. It is conceivable that the sig-

moidal dose-response curve would be considered linear if only 1- to 6-unit doses were employed. Perhaps the linear relationship observed by others using urine concentrates(13) and anemic plasma extracts(11,14) falls within that dose range.

Although a single dose of 6 units of erythropoietin yielded a maximum reticulocytosis in the plethoric mouse(6), our results suggest that increased erythropoiesis is produced by doses greater than 6 units. Larger groups of animals may be required to lend some degree of statistical significance to this assertion.

Proof of the concept that erythropoietin is the sole regulator of physiological erythropoiesis(3) (and not merely a "panic mechanism"(5)) requires a sensitive assay to demonstrate its presence in normal animal's plasma. The transfused plethoric mouse described here is to our knowledge the most sensitive assay animal available, and in our experience is 10 to 20 times more sensitive than the commonly employed starved rat when one is testing serum with slight erythropoietic potency. This enhanced discrimination doubtlessly will aid in demonstration of activity in human plasma in concentrations well below those detected using the starved rat. Currently, we are investigating the presence of erythropoietic factor in the plasma of normal and mildly anemic patients.

Summary. The transfused plethoric mouse is sensitive to quantities of erythropoietin as small as 0.05 unit as measured by radioiron administered 2½ days after challenge with the hormone. A sigmoidal curve describes the relationship between incremental log-doses of purified erythropoietin and incorporation of radioiron into circulating red blood cells.

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Cholesterol Metabolism in the Gerbil. (27419)

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Clarkson *et al.*(1) showed that the blood sterol level of the Mongolian gerbil increases after cholesterol feeding. It was subsequently shown that the increase in plasma sterol is roughly proportional to the amount of cholesterol fed(2). In spite of this, Gordon and Cekleniak were unable to produce atheromatous lesions in these animals(3). These results are in contrast to those in other animals, *i.e.,* the chicken and rabbit, in which cholesterol feeding leads to both an elevated blood sterol and atheromatous lesions(4,5). These data indicate that cholesterol metabolism in the gerbil may differ from that of other ani-