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Response of Starved Rats and Polycythemic Rats to Graded Doses of Erythropoietin.* (26259)

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Present studies of the humoral regulation of erythropoiesis are based on diverse *in vivo* bio-assay technics, described by Gordon(1). Gordon also noted that methodology influenced results. Few specific attempts have been made to compare results obtained by different assay procedures(2,3).

This report details results of assays carried out using starved rats or rats rendered polycythemic by hypertransfusion. Reticulocyte counts and red cell Fe^{59} incorporation were measured; erythropoietic response of both types of assay animal to human and rabbit erythropoietin was assessed. Serially graded doses of erythropoietin were assayed and the relationship between response in red cell iron incorporation or reticulocyte production and dose of erythropoietin was determined.

Methods and materials. Erythropoietin was prepared from rabbit plasma and human urine: a) Hemolytic anemia was produced in albino rabbits by daily subcutaneous injections of 1 ml of 2.5% phenylhydrazine solution. When the hematocrits were 15% or less the rabbits were exsanguinated into heparinized flasks. The separated plasma was pooled, and extracted by the method of Borsook(4). This extract was concentrated 10-fold, dialyzed for 12 hours against distilled water at 4°C and stored frozen. The erythropoietic stimulating activity of such extracts had been previously demonstrated(4,5). b) Urine was obtained from a patient with severe anemia due to erythroid failure. His hematocrit ranged between 17 and 20%; there was persistent, absolute reticulocytopenia, and marrow was devoid of red cell precursors. Urine was dialyzed against running tap water for 18 hours, concentrated by flash evaporation to one-twentieth of the original volume and stored at −18°C.

Two types of bio-assay were used. On the first day of a 4-day assay period, female Sprague-Dawley rats weighing 180 to 200 g were started on starvation(6) or rendered polycythemic by a single transfusion of homologous packed red cells equivalent to 50% of the animals' original predicted red cell mass(7). Starvation was continued for the entire 96 hour assay period; the polycythemic animals were fed a regular rat chow diet. The remainder of the assay technic was standard. Plasma extract or urine concentrate was injected intravenously 30 and 54 hours after transfusion or institution of starvation. Although the dose of plasma extract or urine concentrate varied, volume of injection was kept constant by dilution with saline.

Eighteen hours prior to completion of the assay, each rat was given an intravenous injection of approximately 1 μc of Fe^{59} citrate in saline. An appropriate standard was also

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TABLE I. Hematocrit, Red Cell Fe^{59} Incorporation, Reticulocyte Count and Total Red Cell Mass of Normal Rats and Assay Rats 96 Hours after Transfusion or Initiation of Starvation.

No. of rats	RBC Fe^{59} incorporation, %	Reticulocyte count, %	Hematocrit, %	Red cell mass,† cc/100 g
28 Normal	27.72 ± 6.19*	2.83 ± 1.15	44.28 ± 1.73	2.21
21 Starved	6.05 ± 1.77	.32 ± .08	52.85 ± 1.52	2.64
27 Polycythemic	5.00 ± 1.64	.38 ± .08	54.53 ± 2.59	2.83

* Stand. dev.
† Calculated with blood volumes described in reference 7 (normal rats) or text.

prepared. The assay was completed by determining red cell radio-iron incorporation, reticulocyte count and hematocrit of blood obtained from each rat by cardiac puncture at 96 hours.

Red cell radio-iron incorporation was determined by the following formula:

$$\% \text{ incorporation} = \frac{\text{Counts/ml blood} \times \text{total blood volume}}{\text{Total counts inj.}}$$

One ml of blood was counted in a well scintillation counter. Blood volume determinations by Fe^{59} tagged homologous cells(8) on representative groups of assay rats revealed that the starved rats had a blood volume of 4.87 ± 0.19% (SD) of final body weight and the polycythemic animals 5.23 ± 0.26% (SD) of body weight. Hematocrits were determined by the micro technic. Reticulocyte counts were determined by the methylene blue technic(9). Number of reticulocytes per 1000 red blood cells were enumerated by 2 observers.

In all assays each dose of extract or concentrate was tested in 5 animals to obviate differences due to individual variation. Control values for iron incorporation, reticulocyte count and hematocrit were obtained for assay animals injected with saline. Results are recorded by plotting response in Fe^{59} incorporation or reticulocyte level against the log of the dose of urine concentrate or plasma extract in milliliters. Regression lines(10) have been drawn for all sets of data and correlation coefficients(10) for the relation between dose and response have been determined.

Results. Table I records the hematocrit, reticulocyte count and red cell iron incorporation for both normal rats and saline injected control rats 96 hours after transfusion or commencement of starvation. Both procedures produced elevation of hematocrit and a concomitant partial arrest of erythropoiesis, manifested by a fall in circulating reticulocyte count and iron incorporation.

The erythropoietic response of transfused assay rats to graded doses of erythropoietin obtained from both human urine and rabbit plasma is depicted in Fig. 1 and 2 respectively. In each instance iron incorporation increased as dose was raised; the log dose response relationships were linear and both showed high degrees of correlation. In contrast the changes in reticulocytes were more irregular, especially at lower dose levels; correlation was less than that seen for dose and response measured by Fe^{59} incorporation.

The response of starved rats to rabbit plasma erythropoietin is shown in Fig. 3. The extract injected was prepared from a different pool of anemic rabbit plasma. The relationship between dose of rabbit erythropoietin and both iron incorporation and reticulocyte response was linear and high degrees of correlation were noted.

Finally, comparable doses of urine concentrate were assayed in starved rats or transfused rats to test the relative sensitivity of these 2 types of assay animals. Red cell radio-iron incorporation alone was measured and results are recorded in Table II. Regres-

TABLE II. Comparison of Erythropoietic Response of Polycythemic and Starved Rats Injected with Human Erythropoietin.

Dose in ml	Red cell Fe^{59} incorporation, %	
	Starved	Polycythemic
.050	9.3 ± 2.85*	10.2 ± 1.65
.100	10.9 ± 3.51	12.6 ± 2.31
.200	15.9 ± 4.61	14.7 ± 1.43
.500	23.9 ± 4.18	24.0 ± 2.58
1.000	22.9 ± .96	28.1 ± 1.56
2.000	31.1 ± 2.87	31.2 ± 3.48

* Stand. dev.

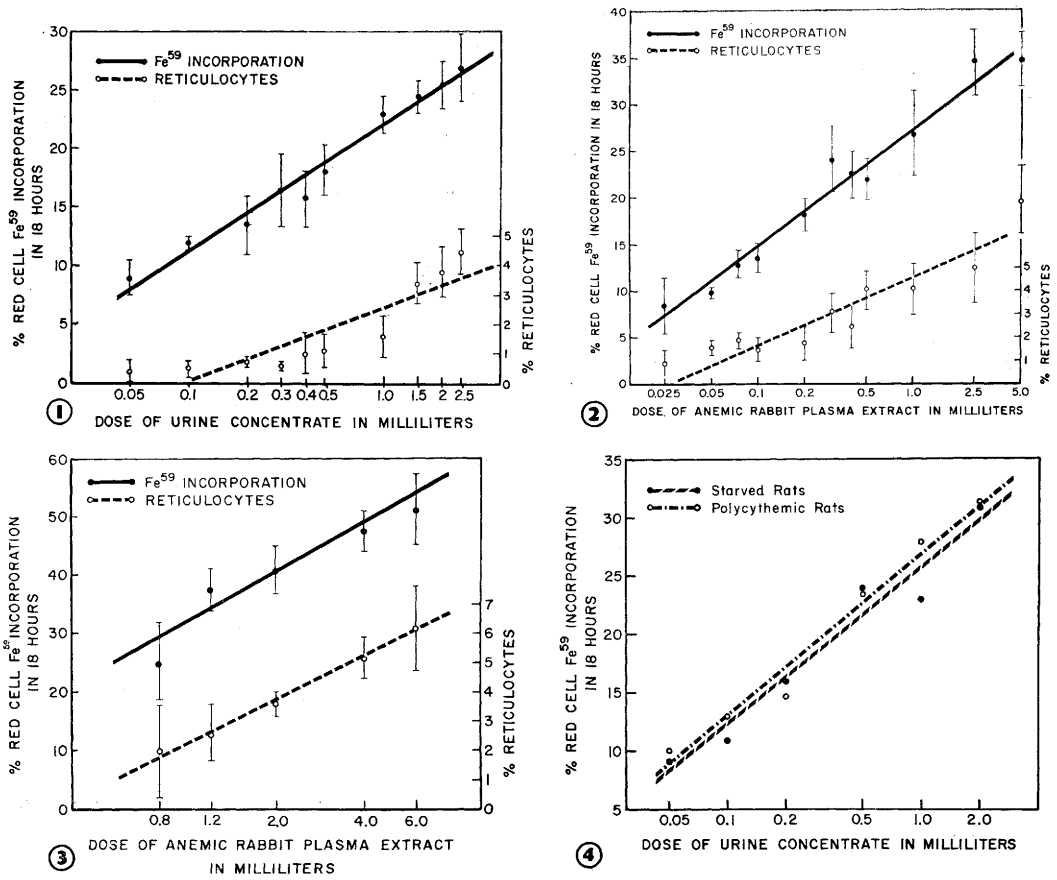

FIG. 1. Response of transfused rats to human erythropoietin. Each point represents mean (± one S.D.) response of 5 assay animals. Correlation coefficients: dose-iron incorporation, .986; dose-reticulocytes, .884.

FIG. 2. Response of transfused rats to rabbit erythropoietin. Each point represents mean (± one S.D.) response of 5 assay animals. Correlation coefficients: dose-iron incorporation, .983; dose-reticulocytes, .942.

FIG. 3. Response of starved rats to rabbit erythropoietin. Each point represents mean (± one S.D.) response of 5 assay animals. Correlation coefficients: dose-iron incorporation, .964; dose-reticulocytes, .994.

FIG. 4. Response of starved rats and transfused rats to human erythropoietin. Each point represents mean response (S.D. recorded in Table II) of 5 assay animals. Correlation coefficients: starved rats, .974; transfused rats, .985.

sion lines for both dose-response relationships were constructed (Fig. 4). Results were similar for both types of assay animal.

Discussion. Few attempts have been made to compare the response of the starved rat to an erythropoietic stimulus with the response of rats in which erythropoiesis has been arrested by other means. Gurney and Pan(2) found that starved rats were more sensitive than hypophysectomized rats, whereas Garcia and Van Dyke(3) found hypophysectomized rats and starved rats of equal sensitivity. In the present study measurement of red cell radio-iron incorporation indicated that erythropoietic responsiveness of acutely starved rats was essentially the same as that of animals with erythropoiesis arrested by transfusion induced polycythemia (Fig. 4, Table II). The latter observation supports the thesis(11) that diminished erythropoiesis in the acutely starved rat is due to the relative plethora associated with starvation and hemoconcentration and not to the effect of food deprivation on the rat's ability to synthesize erythropoietin.

Comparison of dose-response relationship

for reticulocyte levels and red cell Fe^{59} incorporation showed that both increased as the dose was augmented, but reticulocyte response gave more variable results especially at lower dose levels in the transfused rats. This variability, thought to result from the method of reticulocyte counting, restricts use of this parameter to assay of relatively high doses of erythropoietin. Although measurement of red cell iron incorporation provided a simple and reliable means of measuring both high and low doses of erythropoietin, the amount of blood required for determination of Fe^{59} incorporation at the dose of radioiron employed precluded the use of a single animal for serial determinations.

The dose-response analyses in these experiments were similar to those of Garcia and Van Dyke(3) and Hodgson *et al.*(5) in that the relationship between the log of the dose of urine concentrate or plasma extract in milliliters and the erythropoietic response was linear. Schleuter *et al.*(12) found a sigmoid relationship between Fe^{59} incorporation in erythrocytes of starved rats and the log dose of purified erythropoietin obtained from anemic sheep plasma. Within the range tested in the present study a sigmoid dose-response relationship was not noted.

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Automatic Screening of Normal and Abnormal Electrocardiograms by Means of a Digital Electronic Computer.* (26260)

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In a digital computer program for automatic analysis of electrocardiograms (ECG) a screening procedure for separation of normal and abnormal records appears an essential preliminary step. A more detailed analysis of pathological tracings may then follow. Since the ECG consists of several distinct wave forms (P, QRS and T waves) with different electrophysiological significance a single screening procedure should preferably encompass analysis of all electrical processes which accompany each heart beat. The spatial ventricular gradient ($SV\hat{G}$) appeared as the most comprehensive single parameter known for this purpose.

Wilson *et al.*(1) developed the concept of $V\hat{G}$. It has been defined as the net electrical effect of the differences in time course of ventricular depolarization and repolarization. As predicted by simple theory, the time integral ($\hat{A}$) of depolarization potentials of a muscle should be equal to those of repolarization but with opposite sign. For the ECG, the sum of the time integrals of QRS and T should be

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