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**Research Article**

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# On the In Vivo Action of Erythropoietin: A Quantitative Analysis

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ABSTRACT The composite response of the erythron to exogenous erythropoietin has been studied in normal, splenectomized, and polycythemic mice. After stimulation the normal animal doubled its marrow nucleated red cells by the 3rd day with little further change by the 5th. Nucleated red cells within the spleen began to increase sharply on the 2nd day and, by the 5th, exceeded those in the marrow. The total nucleated erythroid response represented a fourfold increase. Reticulocytes lagged behind the expansion of the nucleated red cell mass, but by the 5th day the original ratio was re-established. Hemoglobin synthesis was increased, but the ratio of hemoglobin synthesized in nucleated red cells and reticulocytes was basically unchanged. Early displacement of marrow reticulocytes into circulation and the production of a larger red cell also occurred. No evidence of a change in the number of erythroid mitoses was found; only a slight decrease in the average cell cycle time was demonstrated. Thus, whereas erythropoietin stimulation induced several changes in erythropoiesis, the increased number of cells entering into the maturing pool appeared to be of greatest quantitative significance.

Splenectomy reduced the proliferative response of the erythron over 5 days of stimulation to three-fourths that found in the normal animal. This difference, also reflected in a proportionately lower reticulocyte response and increment in circulating red cell mass, suggests that erythropoiesis within the mouse marrow is spatially or otherwise restricted and that the spleen provided a supplemental area of erythroid expansion.

## INTRODUCTION

Erythropoietin has been demonstrated in vivo to regulate the entry of precursor cells into the maturing erythroid pool (1, 2), to affect the mitotic interval (3, 4) and the rate of hemoglobin synthesis in the individual cell (5, 6), and to alter the release of the marrow reticulocyte into circulation (7–9). This study attempts to evaluate the quantitative importance of these different effects of erythropoietin and to provide an integrated picture of erythropoietic response to erythropoietin in an animal model.

## METHODS

The experimental animals were $C_3H$ virgin mice, 12–16 wk of age. Normal mice were studied before and after erythropoietin stimulation. The source of erythropoietin was a lyophilized urinary concentrate from a patient with aplastic anemia. The mice received intraperitoneal injections of 5 U[1] every 12 hr for a period of 3–5 days. The effect of erythropoietin was also evaluated in mice whose erythropoiesis was eliminated by hypertransfusion and in splenectomized ones. Splenectomy was performed at the 6th wk of age, and the animals were studied 6–8 wk after the operation. "Sham-operated" animals whose spleen was exteriorized during the operation and not removed served as controls. At the end of the study, the mice were exsanguinated under ether anesthesia and their spleen and the two femurs were removed.

The concentration of red cells was determined in a Coulter Counter. Hemoglobin determination was done by the cyanmethemoglobin method. Reticulocytes stained with brilliant cresyl blue were counted from among 4000 red cells on blood films. *Marrow* was flushed out of the femoral cavity with cold Hanks' solution. A homogeneous suspension was prepared by gently aspirating through a 1 ml syringe. A portion of the dispersed cells was diluted further in Isoton, treated with Zap-Isoton, and its cellularity was measured employing a Coulter Counter. The cell-counting settings were determined empirically on the basis of matching counts obtained by hemocytometer. The suspension was then concentrated by centrifugation and exposed to reticulocyte-staining fluid (brilliant cresyl blue in saline plus mouse serum). Smears made from the incubated concentrate were

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[1] Material was calibrated against Erythropoietin Standard B, kindly provided by the National Institute for Medical Research, London, England.

fixed in methanol and stained with Wright-Giemsa. To determine the proportion of erythroid, myeloid, and other elements, about 3000 nucleated cells, including at least 1000 red cell precursors, were counted. About 5–15% were too damaged for recognition but were included in the count. Nucleated erythroid cells display less cytoplasmic maturation than the corresponding cells in man; therefore they were classified in early, intermediate, and late normoblasts on the basis of their size and stage of nuclear maturation. The proportion of reticulocytes was related to the nucleated red cells and to the nonreticulated red cells present in marrow films. Assuming all nonreticulated erythrocytes in the marrow to be derived from circulating blood, it was possible to determine the number of blood reticulocytes contaminating the marrow reticulocyte pool. In five normal animals this amounted to 1.82±0.46%, and in five phenylhydrazine-injected animals with reticulocyte counts between 22 and 26% the contamination amounted to 13.82±2.02% of the pool estimate. Since the studies to be reported fell between these two extremes, no correction was made for the presence of circulating reticulocytes.

*Total marrow cellularity* was determined from the quantitative relationship, established through the use of $^{59}$Fe as a marrow label, between a counted femoral aliquot and the total marrow (10). 1 μCi of radioiron was bound to fresh plasma and injected intravenously. The animal was sacrificed 4 hr later. A femoral marrow aliquot was prepared and counted, and its radioactivity was compared to that of the intact femur. The ratio of radioactivity in the femora was compared to that of the total skeleton. This was accomplished by first autoclaving the carcass, removing all bones, and then directly measuring their activity as compared to that of the femora (10). The ratio was found to be 1 : 13.5±1.8 SD in four normal animals and 1 : 13.8±3.1 SD in four animals stimulated for 24 hr with erythropoietin.

*Splenic cellularity* was determined from a suspension of splenic cells. Splenic tissue was minced with scissors and placed in a tube containing Hanks' solution. A uniform cellular suspension was obtained by repeatedly and gently refluxing particles in a syringe. The stroma was allowed to settle out for 1 min after which the supernatant was removed and centrifuged. Smears were prepared from the concentrated suspension in a small amount of mouse serum. The proportion of nucleated red cells in a population of 3000 nucleated cells was determined as well as the number of reticulocytes among 1000 nucleated red cells. The total number of erythroid cells in the spleen was determined by relating the amount of radioactive iron in a counted cell aliquot to that in the total spleen.

*Body radioiron distribution* was determined before and after erythropoietin stimulation. 1 μCi of plasma-bound $^{59}$Fe was injected intravenously, and after 4 hr the uptake was measured in femur, spleen, and washed red cells. Red cell activity was translated to total blood volume previously measured with the $^{51}$Cr dilution technique in normal control animals and after 5 days of stimulation. In the hypertransfused animal the blood volume was assumed to be about 7% of body weight (11, 12). Femoral marrow was converted to total marrow activity as described above.

*Iron radioautography* of marrow smears was performed to determine the distribution of iron uptake among erythroid cells. 7 μCi of $^{59}$Fe was first bound to fresh plasma and then injected intravenously. Mice were sacrificed 1 and 4 hr later. Smears of femoral marrow were fixed in methanol and subsequently processed for radioautography. For each slide, the average grain count of at least 500 nucleated red cells (100 early, 200 intermediate, and 200 late) and 300 reticulocytes was determined. The radioactive uptake by nucleated cells at different stages of development and by noncirculating reticulocytes was calculated from the total amount of radioiron assimilated by the marrow and spleen, the number of these cells in marrow and spleen, and the relative uptake of each cell type on the basis of grain counting.

*DNA synthesis time* was determined with two different techniques. In the first, using a single label, 1.3 μCi/g body weight of thymidine-$^{3}$H (SA 3.7 mCi/mmole) was injected intraperitoneally. At hourly intervals thereafter mice were killed, and smears prepared from the femoral marrow were processed for radioautography. The per cent of labeled erythroid mitoses (metaphase-telophase) was determined in each preparation, and DNA synthesis time and median cell cycle time were estimated from the labeled mitoses, on the basis of previously employed rules for hand-drawn curves (13). In the double-labeling technique (14), DNA synthesis time (TS) was calculated from the ratio of the number of cells labeled with $^{14}$C, with and without $^{3}$H labeling (NC), and the number labeled with $^{3}$H alone (NH), according to the formula:

$$TS = \frac{NC}{NH} \times TA$$

where TA = 1 hr. A total of 2000 cells was counted for the above determination.

*Red cell mass* was determined by the injection of $^{51}$Cr-labeled red cells. In this procedure, red cells were incubated with radioactive chromium in the form of sodium chromate for 30 min at room temperature, were washed three times with saline, resuspended in saline to a hematocrit of about 37%, and injected intravenously in a volume of 0.2 ml per animal. Recipients were exsanguinated 8 min later and a measured quantity of their blood was counted in the scintillation counter against a standard. The red cell mass was calculated in the usual fashion from the degree of dilution of injected cells, correcting for the volume of cells injected.

## RESULTS

*Studies in normal animals.* The cellularity of the normal mouse erythron is characterized in Table IA. In unstimulated animals, tissue and circulating reticulocytes outnumbered the nucleated red cells by about 5 : 1. The spleen contained about one-tenth of the total nucleated red cells and one-fourth of tissue reticulocytes.

Erythron cellularity increased sharply after stimulation by erythropoietin (Table IA). The distribution of normoblasts according to their stage of maturation is shown in Table IIA. The increased proliferation in nucleated red cells was followed later by an increase in red cell output. The red cell mass measured 3 days after the completion of the 5 day stimulation period was increased by 15.47 ml/kg body weight. Changes in reticulocyte concentration and in red cell size and hemoglobin content occurring with stimulation are shown in Table III. A moderate increase in both mean cell size and cell hemoglobin content was observed.

Data on labeled marrow erythroid metaphases-anaphases with time after single-labeling technique are

TABLE I

*Erythron Composition (Cells×10⁹/kg of body weight)*

| Hours after erythropoietin stimulation | | Normoblasts | | | Reticulocytes | | | |
|---|---|---|---|---|---|---|---|---|
| | | Marrow | Spleen | Total | Marrow | Spleen | Circulating | Total |
| A. Normal animals | | | | | | | | |
| 0 | *22** | 3.1 ±0.6‡ | 0.35±0.09 | 3.45 | 6.0 ±1.7 | 1.82±0.6 | 9.80±2.3 | 17.62 |
| 24 | *17* | 4.2 ±0.9 | 0.6 ±0.18 | 4.80 | 3.6 ±0.8 | 1.25±0.3 | 13.43±3.1 | 18.28 |
| 48 | *23* | 6.15±1.2 | 3.0 ±0.6 | 9.15 | 4.16±0.9 | 3.32±0.7 | 17.04±3.5 | 24.52 |
| 72 | *16* | 6.3 ±1.0 | 4.7 ±0.7 | 11.0 | 4.04±0.9 | 3.10±0.5 | 29.6 ±5.2 | 36.74 |
| 120 | *8* | 6.33±1.46 | 7.53±1.37 | 13.86 | 8.9 ±2.06 | 12.44±2.65 | 56.30±6.42 | 77.64 |
| B. Polycythemic animals | | | | | | | | |
| 0 | *8* | | | | | | | |
| 24 | *9* | 0.9±0.4 | 0.1±0.04 | 1.0 | | Virtually absent | | |
| 48 | *12* | 3.2±1.0 | 1.3±0.14 | 4.5 | 2.9±0.6 | 1.0±0.1 | 1.9±0.5 | 5.8 |
| 72 | *11* | 5.5±1.0 | 4.7±0.5 | 10.2 | 5.4±1.2 | 4.5±0.5 | 14.6±2.0 | 24.5 |
| C. Splenectomized animals | | | | | | | | |
| 0 | *5* | 3.53±0.85 | | | 5.55±1.64 | | 10.26±1.32 | 15.81 |
| 24 | *8* | 3.9 ±0.87 | | | 2.82±0.45 | | 16.40±2.59 | 19.22 |
| 48 | *8* | 7.41±1.21 | | | 4.15±0.67 | | 19.82±2.99 | 23.97 |
| 72 | *7* | 7.33±0.93 | | | 5.06±0.9 | | 29.06±4.22 | 34.12 |
| 120 | *8* | 9.43±2.64 | | | 6.86±1.43 | | 40.83±6.98 | 47.69 |

* The numbers in italics indicate number of animals studied.

‡ ±1 SDM.

shown in Fig. 1. In the normal animal there are more scattered values and not well-defined second peaks. The early normoblasts had an S-phase of about 6 hr duration, and the estimate of the median cell cycle time was about 7.25 hr. In the late normoblasts a high percentage of labeled mitoses is maintained even after 7 hr, and the curve is spread out thereafter. In the stimulated animal the S-phase was estimated at about 5.25 hr for early normoblasts and 6.0 hr for intermediate and late forms. Median cycle times were estimated at 6.75 and 7.5 hr respectively. With a double-labeling technique, the S-phase duration was 8.1±0.52 SD hr for marrow normoblasts at all phases of development in four normal animals and 6.6±0.78 SD in four animals stimulated for 3 days.

The amount of hemoglobin synthesized in different locations of the erythron was monitored by the radioiron uptake of individual tissues. 4 hr after intravenous in-

TABLE II

*Normoblast Distribution (Per Cent of Total)*

| Hours after erythropoietin stimulation | | Marrow | | | | Spleen | | |
|---|---|---|---|---|---|---|---|---|
| | | Early | Intermediate | Late | | Early | Intermediate | Late |
| A. Normal animals | | | | | | | | |
| 0 | *44** | 3±1‡ | 29±4 | 68±4 | *13** | 1±0.5 | 31±4 | 68±4 |
| 24 | *15* | 8±3 | 37±2 | 55±5 | *7* | 4±1 | 37±4 | 61±4 |
| 48 | *23* | 6±3 | 32±5 | 60±6 | *13* | 10±2 | 48±4 | 42±6 |
| 72 | *19* | 5±1 | 37±4 | 58±5 | *8* | 4±1 | 42±5 | 54±7 |
| 120 | *10* | 3±1 | 39±3 | 58±4 | *3* | 3±1 | 45±3 | 52±2 |
| B. Polycythemic animals | | | | | | | | |
| 24 | *11* | 47±6 | 38±6 | 15±7 | *4* | 60±9 | 24±6 | 16±7 |
| 48 | *9* | 8±4 | 38±4 | 57±4 | *4* | 11±0.3 | 51±1 | 38±2 |
| 72 | *12* | 5±2 | 38±2 | 57±5 | *4* | 5±0.5 | 44±3 | 51±3 |

* The numbers in italics indicate number of animals studied.

‡ ±1 SDM.

TABLE III

*Red Cell Changes with Erythropoietin Stimulation*

| Days of stimulation | Reticulocytes | Red cell indices | | | Red cell mass |
|---|---|---|---|---|---|
| | | MCV | MCH | MCHC | |
| | % | | | | *ml/kg* |
| A. Normal animals* | | | | | |
| 0 | 2.18±0.5‡ | 53.47±2.46 | 19.08±1.10 | 35.69±0.70 | 25.15±4.03 |
| 1 | 2.89±0.67 | 52.39±0.82 | 18.96±0.26 | 36.20±0.46 | |
| 2 | 4.1 ±0.5 | 54.02±0.80 | 18.97±0.19 | 35.13±0.65 | |
| 3 | 5.8 ±2.3 | 56.11±1.39 | 19.28±0.24 | 34.38±0.68 | |
| 5 | 10.1 ±0.97 | 64.10±3.30 | 21.04±1.10 | 32.91±0.36 | 40.62±2.35 |
| B. Splenectomized animals | | | | | |
| 0 | 2.38±0.36 | 55.10±1.90 | 19.56±0.74 | 35.53±0.19 | 18.49±1.08 |
| 1 | 3.75±0.63 | 53.68±3.0 | 19.53±0.97 | 36.27±1.23 | |
| 2 | 4.83±1.35 | 56.24±0.61 | 20.09±0.30 | 35.73±0.51 | |
| 3 | 6.28±0.76 | 59.28±1.98 | 20.90±0.55 | 35.26±0.42 | |
| 5 | 8.47±1.3 | 62.22±2.4 | 21.48±0.68 | 34.54±0.41 | 28.92±1.97 |

* The data represent the mean of 10 normal and 8 splenectomized mice for each day of stimulation. Data referring to the red cell mass were derived from 12 normal and 4 splenectomized controls and 9 normal and 5 splenectomized animals stimulated for 5 days and sacrificed 3 days later.

‡ ±1 SDM.

jection, 57% of the radioiron injected was taken up by the erythron of the normal animal, 37% being distributed to the marrow, 11% to the spleen, and 9% to circulating red cells. With erythron stimulation, the uptake of the total erythron increased to more than 80% by 3 days. Within the erythron itself, the proportion localized in the marrow showed progressive decrease while the proportion taken up by the spleen and circulating blood increased (Fig. 2A).

The differential uptake of radioiron by marrow erythroid forms at various stages of maturity as determined

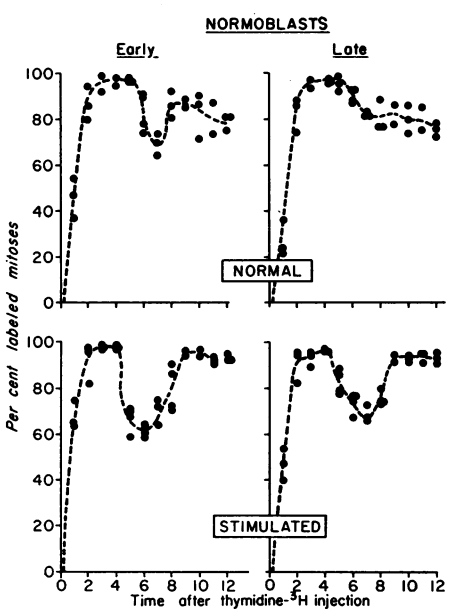


FIGURE 1 Fraction of labeled mitoses (metaphase-telophase) with time for early and late erythroid precursors in normal and ESF-stimulated mice for 3 days. Each point represents the determinations of labeled mitoses in an individual animal. Curves were hand-drawn through the experimental points.

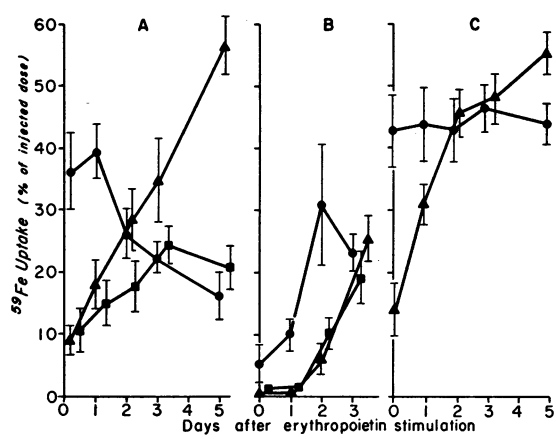


FIGURE 2 Erythron distribution of plasma-bound radioiron, 4 hr after intravenous injection in normal (A), polycythemic (B), and splenectomized (C) animals. Erythropoietin was injected at 0 time and at subsequent 12-hr intervals. Radioiron was injected at 24, 48, 72, and 120 hr poststimulation and the animals sacrificed 4 hr later. Each point represents the mean of 10–22 normal, 8–10 polycythemic, and 7–8 splenectomized animals. Vertical lines indicate 1±SDM. Bone marrow, ●; peripheral blood, ▲; spleen, ■.

TABLE IV

*Relative Radioiron Uptake* Per Cell in Marrow Erythroid Cells*

| | | Normoblasts | | | |
|---|---|---|---|---|---|
| | | Early | Intermediate | Late | Reticulocytes |
| 1 hr after radioiron injection | | | | | |
| Controls | *2*‡ | 25.3 | 37.4 | 14.5 | 22.7 |
| ESF Stimulated | *2* | 25.0 | 36.3 | 15.4 | 23.3 |
| 4 hr after radioiron injection | | | | | |
| Controls | *5* | 26.9±0.96§ | 33.9±2.56 | 17.7±1.52 | 21.5±2.26 |
| ESF Stimulated | *5* | 21.9±2.76 | 32.9±2.20 | 18.2±2.2 | 27.6±1.83 |

* Average grain count per cent for all cells, labeled or not. The grain count per cell was almost doubled between 1 and 4 hr after radioiron injection in the normal animal, whereas in the stimulated animal, lower levels of labeling were observed at both times. In the latter, however, conspicuous differences in the labeling indices of the early forms were found at 4 hr.

‡ The numbers in italics indicate number of animals studied.

§ ±1 SDM.

by radioautograph grain count at 1 and 4 hr is shown in Table IV. While the uptake was slightly more complete by the 4th hr, the longer time interval permitted some movement of radioiron from one compartment to another; thus both values are presented. Iron uptake by the individual cell was greatest in the intermediate normoblast. The total amount of radioiron assimilated at any stage of development depended also on the number of cells at that stage of development. Radioiron uptake among the major compartments of the fixed and circulating erythron is summarized in Table V. Most of the radioiron was taken up by the reticulocyte population, 73% before stimulation and 67% on the 3rd day of stimulation. This difference was attributed to the fact that the nucleated red cell had at that time increased proportionately more than had the reticulocytes. The greatest change was in the distribution of radioiron between the tissue and circulating reticulocytes. Whereas most of the reticulocyte activity was in tissues before stimulation, after stimulation most of the reticulocyte activity was in circulation.

*Studies in the polycythemic animal.* Animals with induced polycythemia showed no recognizable immature erythroid cells within either marrow or spleen. Within 24 hr after erythropoietin stimulation, nucleated red cells at all stages of development appeared in the marrow, but reticulocytes were virtually absent. The subsequent rate of increase in erythroid forms over 72 hr is shown in Table IB. Changes in normoblast differential (Table IIB) are similar to the normal stimulated animal except for the first 24 hr. By the 3rd day the erythroid marrow of the polycythemic animal was nearly as large as that of the normal mice stimulated for an equivalent period. The 4 hr uptake of intravenous radioiron by the erythron of the polycythemic animal at 0, 24, 48, and 72 hr after erythropoietin stimulation is shown in Fig. 2B.

TABLE V

*Radioiron Distribution in Fixed and Circulating Erythron 4 hr after Intravenous Injection of Transferrin-bound $^{59}Fe$)*

| | Normal controls | | | Normal stimulated (72 hr) | | |
|---|---|---|---|---|---|---|
| | Nucleated red cells | Tissue reticulocytes | Circulating reticulocytes | Nucleated red cells | Tissue reticulocytes | Circulating reticulocytes |
| Total cells ($\times 10^6$) | 71.0 | 180.0 | 212.0 | 226.0 | 145.0 | 615.0 |
| Total activity per cent* | 27.0 | 57.0 | 16.0 | 33.0 | 24.0 | 43.0 |
| Relative activity per cell‡ | 50.0 | 41.0 | 9.0 | 39.0 | 43.0 | 18.0 |

* Total activity per cent in nucleated red cell and tissue reticulocytes was calculated from the amount of radioiron localized in marrow and spleen, the total number of these cells in both tissues, and the activity per nucleated red cell and reticulocyte derived by grain count in marrow smears (see Table IV). It was expressed in per cent.

‡ Relative per cell activity was calculated by dividing the total cell activity by the total number of cells present and it was expressed in per cent.

*Studies in splenectomized animals.* Splenectomized animals had a residual anemia, which persisted for at least 8 wk postoperatively. Their immature erythroid population, higher than that of the unoperated controls on day 0, was increased further over a 5-day stimulation period (Table IC). The increase in the marrow erythroid precursors was greater than that observed in the nonsplenectomized animals and continued over a longer time period. After a 5-day stimulation period, the circulating red cell mass was increased by 10.42 ml/kg of body weight, in contrast to a 15.47 ml increase observed in normal and sham operated animals. Red cell indices and reticulocyte concentration before and after stimulation are presented in Table III. The normoblast differential after stimulation was similar to the nonsplenectomized animals. Data on sham-operated animals were similar in all respects to that obtained in the nonoperated animal group.

## DISCUSSION

Erythropoietin stimulation brings about a number of changes in the erythron, the most important being a rapid increase in the number of developing cells. 24 hr after stimulation of polycythemic animals, normoblasts were seen in all stages of development but virtually no reticulocytes had appeared. By the end of 5 days of stimulation, there was a fourfold expansion of the basal nucleated red cell population. Although the increase in reticulocytes lagged behind that of the nucleated red cell mass, a normal ratio of reticulocytes to nucleated red cells was re-established by the 5th day.

Evidence concerning the effect of erythropoietin on the number of mitotic divisions was obtained by comparing normal and polycythemic animals. In the polycythemic animal the first observable changes induced by erythropoietin must represent its effect on the undifferentiated erythroid precursors, since no maturing population exists. On the other hand, in normal animals erythropoietin has the opportunity to act on both undifferentiated erythroid precursors and cells maturing at the time of stimulation. If the number of mitotic divisions during the maturation sequence were increased (15), there should be an increase in the number of nucleated red cells beyond that derived from precursor input. The first 24 hr is critical because changes in terminal mitoses cannot be detected after a new maturation pattern is established. In the experiments reported here, the increase of nucleated erythroid cells in normal animals differed from that of polycythemic animals by only 10% at 24 hr, less than that which would be expected if an additional mitosis had occurred. Furthermore, the lack of change in total reticulocyte pool of normal stimulated animals over the first 24 hr suggests that the enucleation process is not altered. Finally, the re-establishment of the original ratio both in cell number and hemoglobin synthesis between nucleated red cells and reticulocytes is against any change which does not apply equally to nucleated and nonnucleated cells.

Although no perturbation in the number of divisions was demonstrated, a slight shortening of about 15% in the length of S-phase of normoblasts after stimulation was found by the two techniques employed. With the double-labeling technique, more difference in the duration of DNA synthesis phase was found, but the times concerned practically intermediate and late populations in the two groups, while the comparison in the labeled mitoses method concerned mainly early cells. With the latter technique the estimated median cell cycle times did not differ significantly after stimulation; however, it is clear that the erythroid population under stimulation is going through DNA synthesis and mitosis in a more synchronized fashion and with less inter-cell and/or inter-animal variation. Previous studies have demonstrated a decrease in mitotic interval of undifferentiated red cell precursors with erythropoietin stimulation. Nagai and Hara (3) demonstrated in bled rabbits a transient shortening of cell cycle after bleeding, while Hanna, Tarbutt, and Lamerton (4) showed in acute and protracted anemia in rats differences in cell cycle of about 20%. These various studies including those reported in this paper seem sufficient to indicate that the degree of shortening in mitotic interval would account for only a small fraction of the fourfold production increase.

An unusual feature of erythroid maturation in the mouse is the amount of hemoglobin synthesis, about 70%, which occurs in the reticulocyte phase of development in the normal and in 3-day stimulation animals. This is consistent with a much shorter time of normoblast development as compared with reticulocyte development, and is reflected in a 1:5 ratio between these cellular forms.

One of the earliest effects of erythropoietin was an accelerated release of reticulocytes in the circulation. At 24 hr, about one-half the marrow reticulocytes were so displaced. This erythropoietin effect has been previously described with in vitro perfusion (16–18) and in vivo studies (7–9). In addition to providing an exaggerated impression of red cell production in the peripheral blood, the reticulocyte shift is also associated with an increase in the amount of hemoglobin synthesis occurring in the peripheral blood (from 16% at day 0 to 43% after 3 days of stimulation).

The resting spleen has a small population of nucleated red cells and a proportionately larger reticulocyte pool, suggesting some degree of sequestration of circulating reticulocytes in that organ. This surplus, however, only amounts to 10% of the total reticulocyte pool and would seem to be of little quantitative importance. With initial

erythropoietin stimulation, the reticulocyte pool in the spleen decreased somewhat at a time when the circulating blood contained an increased number of immature reticulocytes (Table IA). Thus, instead of acting as an intermediary depot for reticulocytes prematurely discharged from the marrow, the spleen also appeared to respond to erythropoietin by shifting reticulocytes into the blood. Over a period of 5 days, the normoblast population of the spleen increased to 54% of the total population. This increase, however, lagged behind that of the erythroid marrow. This is in keeping with the observation of Rencricca, Rizzoli, Howard, Duffy, and Stohlman (19), suggesting a migration of stem cells to the spleen from the marrow.

In the mouse the erythroid marrow increases only twofold with stimulation, and further erythropoiesis takes place in the spleen. When the spleen of mice has been previously removed, the erythropoietin response of the nucleated red cell population, reticulocytes and circulating red cell mass at 5 days was 68, 62, and 66% of that of nonsplenctomized, stimulated animals. Similar quantitative data, concerning red cell mass increase after bleeding in splenectomized vs. nonsplenectomized mice were reported by Boggs, Geist, and Chervenick (20). This indicates that the spleen in the mouse is required for maximal erythropoietic response.

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