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Humoral Regulation of Erythropoiesis XII. Effect of Erythropoietin and Iron on Cell Size in Iron Deficiency Anemia.* (28552)

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High doses of exogenous erythropoietin, when given to normal or hypertransfused rats, result in production of short-lived macrocytes (1-3). Similarly, after intense erythroid stimulation from bleeding, phenylhydrazine anemia or exposure to hypoxia, there is a macrocytosis, presumably mediated by high levels of endogenous erythropoietin(4). We have suggested that the macrocytic response is due to shortening of maturation time with a resultant skipped division(1-3). Iron deficient animals, of course, produce microcytes; the effect of erythropoietin on cell size in iron deficient animals has not been studied, although it has been shown that treatment with iron in the face of substantial anemia results in macrocytic response(5). This report concerns the effect of erythropoietin in the iron deficient animal and the relationship of iron to production of macrocytes.

Materials and methods. Weanling Sprague-Dawley females were placed on a milk powder diet to which essential vitamins and minerals with the exception of Fe were added. Control groups received a similar diet but with the addition of 200 mg of $FeCl_3$/kg. Animals were treated by adding 0.2-1.0 g of Fe/kg of diet or by parenteral injection with 5 mg of Fe as Imferon.† Erythropoietin was obtained by alcoholic extraction from the urine of a patient with a hypoplastic anemia; it was assayed in the starved rat and compared with the standard of Erythropoietin A(6). Standard techniques were used for measuring red cell values. The frequency distribution of red cell size was measured with a Coulter model B Counter and graphout. Differential centrifugation was used to separate out mixed cell populations(3) where the frequency of one cell population was sufficiently low that it could be detected from distribution curves on whole blood.

Results. After 3 months the animals receiving the iron deficient diet had a severe hypochromic microcytic anemia; the hemoglobin values were 4-5 g%; mean corpuscular volume (MCV), 35-42 μ^3; mean corpuscular hemoglobin concentration (MCHC), 21-24%; compared to values for Hgb. of 13-15 g%; MCV, 52-57; and MCHC of 32-36% in animals receiving Fe supplemented diets. There was a significant macrocytic response when 35 units of erythropoietin were given every 12 hours, for a total of 175 units, to animals on iron supplemented diets (Fig. 1). In contrast a similar amount of erythropoietin had no significant effect on cell size distribution in the iron deficient animal (Fig. 1).

When iron deficient animals were treated by supplemental dietary iron, in amounts equivalent to that in the control diet, there was a normocytic response. Blood was separated by differential centrifugation in an effort to detect any small proportion of macrocytic cells which might be present. None were found. The pre-treatment microcytes, however, were seen in the upper fraction of the centrifuged column of blood, while the post-treatment normocytes with a higher MCHC

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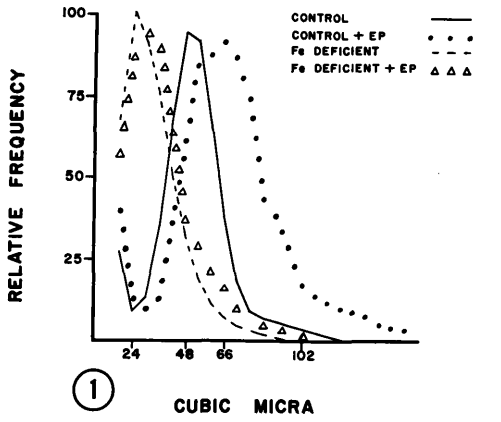
† Iron dextran complex kindly furnished by Lakeside Laboratories.

were present in the lower fractions. In animals given higher doses of iron, either by mouth or parenterally, there was a clearcut macrocytic response (Fig. 2); thus the presence or absence of a macrocytic response after treatment of animals with iron deficiency anemia was a dose (of iron) related phenomenon.

Discussion. Erythropoietin in high doses failed to affect the size distribution of emerging cells in iron deficient animals. After treatment with iron, the type of erythroid response in the anemic iron deficient animals was related to the dose of iron as well as the intensity of erythroid stimulation. Macrocytes were produced after high doses, and normocytes after lower doses. This points to the fact that iron is a limiting factor in the macrocytic response to erythropoietin.

The above studies permit speculation on the mechanism of action of erythropoietin and, particularly, the mechanism by which macrocytes or microcytes are produced. There is substantial evidence pointing to the fact that erythropoietin promotes the differentiation of stem cells into erythroid precursor(7-9). Other studies(10,11), however, suggest that erythropoietin exerts an effect on the differentiated erythroid cell. In part, the latter may be explained by changes in the rate of maturation of stem cells after differentiation; there is some evidence, however, for qualitative differences in the response of hypertransfused animals and normal animals to erythropoietin(12). Rather than to invoke a dual action for erythropoietin as has been suggested, it seems preferable to postulate a single mode of action. We would suggest that erythropoietin enters the cell, either the undifferentiated stem cell or differentiated erythroid precursor, and interacts with some unidentified component of the cell to form an enzyme capable of initiating hemoglobin synthesis. The amount of the latter would determine the rate of hemoglobin synthesis. Thus, increasing the available erythropoietin results in increased formation of the hemoglobin synthesizing enzyme in both differentiated and undifferentiated cells; in fact, in the latter the induction of this enzyme would lead to differentiation. From this it can be seen that the rate of hemoglobin synthesis



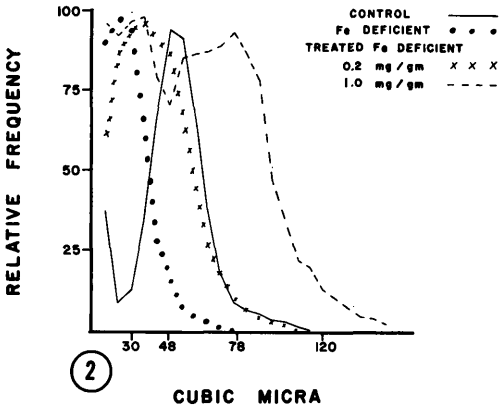


FIG. 1. Cell size distribution curves in control and iron deficient rats before and after treatment with erythropoietin. Measurements were made on the top fraction of centrifuged column of blood.

FIG. 2. Red cell size distribution curve on whole blood of control, iron deficient rats, and treated iron deficient rats. The latter were obtained 7 days after beginning of oral iron therapy at indicated dose levels. Note macrocytic response in animals receiving high doses of iron.

and, hence, maturation would be dependent in part on the availability of erythropoietin. Further, it might be suggested that when the MCHC, rather than the MCH as suggested by Lajtha(13) reaches a critical level, it triggers a feedback mechanism which "shuts off" division. Since the generation time of erythroid cells appears to be constant(14,15), the average number of divisions after differentiation will be dependent on the rate at which the critical "shut off" level of hemoglobin concentration is achieved. By increasing the available erythropoietin and thus the rate of hemoglobin synthesis, the time interval to achieve the critical MCHC would be reduced. This in

the face of fixed generation time would result in fewer divisions, i.e., skipping of the terminal division. Macrocytes would result. In the presence of iron deficiency, the rate of hemoglobin synthesis would be limited by the amount of iron available. Thus, even though large amounts of erythropoietin are available as in the experiments described, the relative unavailability of iron would prevent rapid hemoglobin synthesis. Under these conditions, the slower rate of hemoglobin synthesis would permit additional divisions before the critical shut-off level is achieved and microcytes would result. After treatment of the iron deficient subject, where iron is the limiting factor in hemoglobin synthesis, the type of cell produced would reflect the available iron; after high doses, macrocytes would be produced, and after lower doses, normocytes.

The above hypothesis appears to be in accordance with the available information on red cell production. Further, it would appear to explain the occurrence of microcytosis before hypochromia in the patient with developing iron deficiency anemia(5,16). The macrocytosis seen in certain diseases, such as pernicious anemia, after treatment with antimetabolities, etc., presumably results from interference with divisions *per se* rather than changes in the hemoglobin synthetic rate and the interval at which the critical MCHC is achieved. The macrocytes seen in acute hemolytic anemia and after exposure to altitude, however, would appear to be explained by the above hypothesis.

Summary. Erythropoietin did not affect the size distribution curve of iron deficient animals even when given in high doses. The type of erythroid response occurring after treatment with iron was related to the dose of iron administered, a macrocytic response being evoked after high doses and a normocytic response after low doses. The mechanism of action of erythropoietin and its relationship to available iron is discussed and a hypothesis presented to explain the above observations.

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