Suppression of Erythropoiesis in the Plethoric Rat by Antierythropoietin (34602)

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Within a week after the production of plethora by transfusion of isologous red blood cells, the hematopoietic tissues of the mouse are devoid of recognizable nucleated erythroid cells, and the 72-hr incorporation of radioactive iron into peripheral red blood cells is decreased from the normal value of about 25% to a value of about 0.2% or less. However, the production of a similar plethoric state in the rat suppresses the radioiron uptake into red blood cells to a lesser extent of about 3–5% (1, 2). The mechanism by which plethora suppresses erythropoiesis is poorly understood. Kilbridge *et al.* (3) have indicated that factors other than the ratio of oxygen supply to oxygen requirement play important roles in the regulation of erythropoiesis and presumably erythropoietin production. They emphasize that factors related to increased hematocrit rather than an expanded blood volume or red cell mass have an important role in the suppression of erythropoiesis in the dehydrated mouse. The present study demonstrates that appreciable erythropoietin still remains in the severely plethoric rat with an elevated hematocrit.

Materials and Methods. Female Buffalo/N rats were transfused by two consecutive intraperitoneal injections of washed isologous red blood cells. The hematocrit of the transfused blood was about 75, and each rat received an amount of blood equivalent to 3.0% of the body weight. The transfused rats were divided into three groups. One group received no injections. One group received daily subcutaneous injections of 2.5 ml of normal rabbit serum for 3 days, and the last group received similar injections of rabbit antihuman urinary erythropoietin immune serum. The preparation and properties of this immune serum have already been described (4). One ml of the immune serum neutralized 25 IRP units of human urinary erythropoietin. Such immune serum can neutralize the biological activity of rat erythropoietin *in vitro* (5).

Seven days and 8 hr after the last transfusion the rats received about 0.5 μCi of ^{59}Fe as the citrate (specific activity approximately 10 μCi/μg), and 72 hr later the rats were bled and the radioactivity in 0.5 ml of blood was measured. Hematocrits were measured at this time and, in some cases, smears of the bone marrow were prepared. The results are expressed as the percent of the injected dose of ^{59}Fe in the calculated blood volume; blood volume was assumed to be 7% of the body weight. The time of injection of ^{59}Fe and sampling was identical to that used in this laboratory when assaying for erythropoietin utilizing the plethoric mouse.

The erythropoietic activity of 1 ml of normal rabbit serum was determined in transfusion-induced plethoric mice by the schedule previously described (4, 5).

Results. The results are shown in Table I. It is evident that erythropoiesis is not completely suppressed by the production of plethora in the rat, but the unsuppressed erythropoietic activity can be virtually abolished by the injection of antierythropoietin immune serum. The ^{59}Fe uptake of plethoric rats receiving normal rabbit serum injections is significantly higher than uninjected rats ($p<0.001$). The 72-hr ^{59}Fe uptake observed after antierythropoietin injection in the rat is not significantly different from the value of 0.18 ± 0.03 found in the plethoric mouse when the same temporal patterns of transfusion, ^{59}Fe injection and blood sampling, are

TABLE I. Erythropoietic Activity of Plethoric Rats Receiving Antierythropoietin.

	No. rats	Body wt	Hematocrit	72-hr ^{59}Fe uptake
Uninjected	12	208 ± 3[a]	60 ± 1[a]	6.0 ± 1.0[a]
Normal rabbit serum	8	197 ±10	60 ± 2	20.0 ± 5.0
Rabbit antierythropoietin serum	10	199 ± 4	60 ± 1	0.35 ± 0.06

[a] Standard error of the mean.

used. Nucleated erythroid cells were markedly reduced in the uninjected plethoric rat compared to the normal rat. However, virtually no nucleated erythroid cells were observed in the bone marrow of plethoric rats receiving antierythropoietin.

Discussion. The results indicate that erythropoiesis, although markedly reduced by the production of plethora in the rat, is not abolished. The finding that virtually complete suppression of erythropoietic activity, as measured by ^{59}Fe uptake, occurred following antierythropoietin administration indicates that even though the hematocrit was elevated to about 60% some erythropoietin still was present.

The observation that the injection of normal rabbit serum significantly increased the ^{59}Fe uptake in the rat, although erythropoietin could not be demonstrated in this serum using the transfusion-induced plethoric mouse assay, remains unexplained. The finding, however, emphasizes the difficulties involved in using a plethoric animal for determining the presence of erythropoietin when endogenous erythropoietin is still present in the assay animal.

Summary. Production of plethora in rats by transfusion does not completely abolish erythropoiesis as measured by the uptake of radioiron into red blood cells, although a marked suppression of erythropoiesis occurs. This remaining erythropoietic activity can be abolished if rabbit antierythropoietin immune serum is injected.

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