Erythropoiesis and Hyperoxia (37637)

J. W. Fletcher, N. I. Gallagher, M. A. Warnecke, and R. M. Donati

St. Louis Veterans Administration Hospital, Nuclear Medicine and Internal Medicine Services, and the Department of Internal Medicine, St. Louis University School of Medicine, St. Louis, Missouri 63125

Diminished red blood cell production after prolonged exposure of animals or man to a high oxygen tension results from an alteration of the marrow regulatory system (1–3). Possible explanations include: a diminished production of the erythropoietic hormone, erythropoietin; active suppression of erythropoiesis as suggested by the demonstration of an erythropoietin antagonist in the plasma of plethoric animals by Krzymowski and Przala (4) and by Whitcomb and Moore (5), or a diminished marrow productive capacity due to a direct toxic effect of oxygen. In order to explore the mechanisms by which hyperoxia produces a decrease in erythropoiesis, the erythropoietic response and plasma erythropoietin titers of rats exposed to a sustained increase in environmental oxygen were studied. In addition, erythropoiesis was assessed in each partner of parabiotic pairs maintained in such a manner that one partner breathed a normal oxygen environment and the other a hyperoxic environment. The results indicated erythropoietic suppression following hyperoxic exposure and suggest the production of a humoral erythropoietic antagonist. The latter conclusion is supported by the suppression of erythropoiesis in the parabiotic partner maintained in a normal oxygen environment. These data support the hypothesis that erythropoiesis is regulated by the dynamic interaction of a humoral accelerator of erythropoiesis and an erythropoietic inhibitor.

Methods and Materials. Hyperoxic experiments. Female Sprague–Dawley rats weighing 180–200 g maintained on a diet of Purina Laboratory Chow and allowed tap water *ad libitum* were utilized in all experiments. Animals exposed to hyperoxia were maintained in a specially constructed Plexiglas chamber[1] with temperature and humidity control[2] and a source of oxygen regulated by an automatic oxygen analyzer[3] in order to maintain the oxygen concentration within the chamber at the specified level. The animals were in the chamber continuously except for 30–60 min each day at which time the chamber was serviced and food and water replenished.

Six groups of 10 animals each were exposed to a 95–100% oxygen environment for intervals of 24, 48, 72, and 120 hr. Eighteen hr prior to each specified time interval and the termination of each experiment, 1 μCi ^{59}Fe per 1 μg ^{56}Fe citrate was administered iv via tail vein. Eighteen hr thereafter, the animals were removed from the chamber, anesthetized with ether, and exsanguinated by cardiac puncture under direct visualization by means of a preheparinized plastic syringe. The 18-hr RBC radioiron incorporation was determined in a manner previously described (6). Appropriate control groups were maintained at atmospheric conditions under similar temperature and humidity.

In a similar fashion, 14 groups of 20 animals each were exposed to 50% oxygen for intervals of 0–20 days. Twenty-four-hr RBC radioiron incorporations were determined at daily intervals on day 0–3, 5–9, 12–16, and on day 20. Appropriate control rats were maintained at atmospheric conditions. In selected groups, the red blood cell mass was also determined (7). Plasma samples obtained at the time of sacrifice were pooled for each

[1] Specially constructed by E & M Consulting Service, St. Louis, Missouri.

[2] Temperature was maintained at 72°F and humidity at 60%.

[3] Beckman Automatic Ozygen Control System—G-2-984.

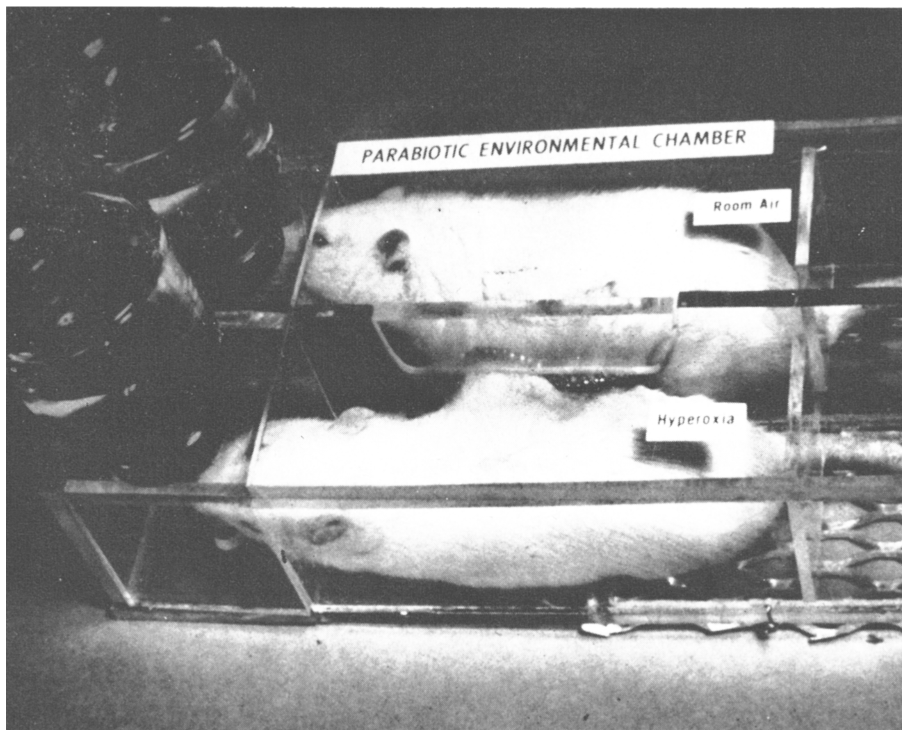

FIG. 1. Parabiotic environmental chamber: 20% oxygen was delivered by compressed air 1.5 liter/min to one side of the chamber and 80–90% oxygen delivered as 100% oxygen to the other side.

day and frozen at 4°. Erythropoietin levels in the plasma samples were determined in the exhypoxic plethoric mouse bioassay by a modification (8) of the method of Cotes and Bangham (9). Selected groups of 16 animals per group exposed to 50% O_2 hyperoxic environments for 7 days were challenged with known erythropoietic stimulants including: 6 hr of hypoxia (low pressure chamber at 0.5 atm), injection of exogenous erythropoietin laboratory standard, or 100 μg D-triiodothyronine or L-triiodothyronine. The erythropoietic response to stimulation was monitored by determining the 18-hr erythrocyte radioiron incorporation.

Parabiotic experiments. Animal pairs, matched by age and weight, were anastomosed by skin, muscle, and fascia from the inferior tip of the scapula to the root of the tail (10) under Nembutal anesthesia. Preliminary experiments utilizing ^{59}Fe-labeled donor red blood cells (7) indicated essentially no exchange of erythrocytes between parabionts up to 72 hr after the operation, although there was significant plasma transfer as determined by ^{131}I-labeled albumin. Therefore, 24 hr after anastomosis, the parabionts were placed in a divided Plexiglas chamber[4] (see Fig. 1) as follows: 13 pairs with both partners breathing 20% oxygen; 13 pairs with both partners breathing 90–95% oxygen; and 14 pairs with one parabiont exposed to 20% oxygen and the other parabiont exposed to 90–95% oxygen. Percent oxygen saturation was monitored by an oxygen analyzer[5] and remained within the ranges stated above during the period of chamber confinement. All animals remained in the chamber for a total of 68 hr. Eighteen hr prior to termination of the experiment, the animals were separated

[4] 20% oxygen was delivered as compressed air 1.5 liter/min to one side of the chamber and 85–90% oxygen was delivered as 100% oxygen 0.8 liter/min to the other. The differences in flow rates effectively separated the two oxygen concentrations although slight dilution of the 100% O_2 concentration did occur.

[5] Beckman D-2 Oxygen Analyzer.

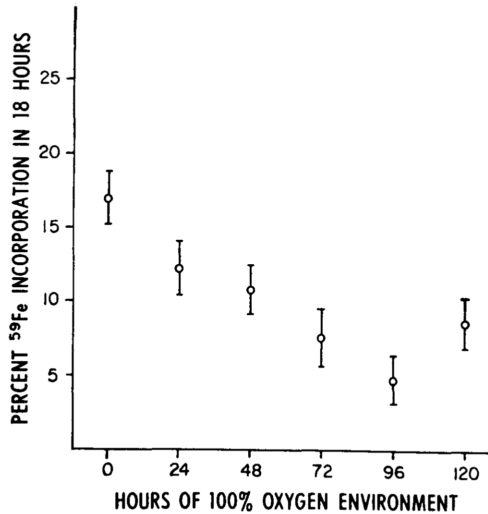

FIG. 2. RBC ^{59}Fe incorporation following exposure to a 100% oxygen environment: Each point represents the mean of values obtained in 10 rats with the exception of 96 and 120 hr (9 rats and 6 rats, respectively). The standard deviations are indicated by the vertical lines.

under Nembutal anesthesia, each animal was given 1 μCi ^{59}Fe per 1 μg ^{56}Fe citrate iv and returned to the chamber. The animals were sacrificed 18 hr thereafter and the 18-hr erythrocyte incorporation of radioiron determined. Arterial pO_2 (11) were determined on blood samples obtained from the abdominal aorta of selected parabionts.

Results. The effect of exposure to 100% oxygen environment for 0–120 hr on RBC ^{59}Fe incorporation is shown in Fig. 2. Radioiron incorporation decreased as early as 24 hr after exposure and continued to diminish to a low point at 96 hr. After 120 hr of exposure, iron incorporation was noted to increase toward normal, but continued exposure to 100% oxygen led to a high mortality rate, and further values were not obtained.

Figure 3 shows the effect on RBC ^{59}Fe incorporation as a result of exposure to 50% oxygen environment for periods ranging from 1–20 days. Diminution of radioiron incorporation (to values of 12%) was initially evident on the 3rd day. Continued exposure led to further decline and a low value of 3% incorporation was reached on day 8; thereafter, RBC ^{59}Fe incorporation increased and continued increasing to day 20 when it exceeded control.

The alterations in radioiron incorporation following 50% hyperoxic exposure were paralleled by changes in reticulocyte counts with low values of 0.9 ± 0.4% (8 animals) on day 8 and values of 5.0 ± 1.2% (8 animals) on day 20. Although there were no significant changes in the individual microhematocrit index, the red cell mass as assessed by ^{59}Fe-labeled donor erythrocytes after the manner of Huff (7) followed the pattern of diminution of RBC radioiron incorporation. The fall in red cell mass was slower, however, and a low point was not achieved until day 17 (2.23 ± 0.6 ml/100 g on day 0 to 1.78 ± 0.12 ml/100 g on day 17 in 16 rats).

The effect of 50% hyperoxia on plasma erythropoietin activity as assessed by the ex-

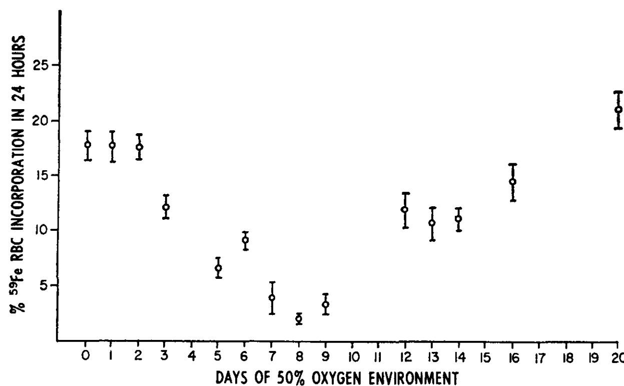

FIG. 3. RBC ^{59}Fe incorporation following exposureto a 50% oxygen environment: Each point represents the mean values obtained in 20 rats. Thestandard deviations are indicated by the vertical lines.

TABLE I. Bioassay of Plasma Erythropoietic Stimulating Activity in the Plethoric Mouse.

Substance injected	48-hr ^{59}Fe RBC incorporation (% ± SD)
Saline	2.1 ± 0.4
Plasma from normoxic rats	3.3 ± 0.3
Plasma from hyperoxic rats (days of 50% O_2)	
5 days	2.0 ± 0.4[a]
8 days	2.3 ± 0.4
9 days	2.7 ± 0.3
12 days	4.2 ± 0.6
16 days	5.5 ± 1.2[a]
18 days	4.3 ± 0.4

[a] $p < 0.05$ as determined by Student's t test.

hypoxic plethoric mouse bioassay is shown in Table I. Plasma recovered on days 5, 8, and 9 did not increase iron incorporation in the mice to the level seen with normal plasma, however, only the plasma on day 5 revealed a statistically significant variation from normal ($p < 0.05$). In marked contrast, plasma obtained from rats killed on days 12, 16, and 18 indicated radioiron incorporation greater than normal; again, only that plasma obtained on day 16 showed statistically significant increase ($p < 0.05$). The changes in erythropoiesis as directly measured in hyperoxic animals were accompanied and paralleled by changes in mouse erythrocyte radioiron incorporation (erythropoietin) induced by the hyperoxic plasma.

Rats with depressed erythrocyte radioiron incorporation following 7 days of continuous exposure to 50% oxygen demonstrated unequivocal response to known erythropoietic stimulating agents (Fig. 4). All of the agents that were administered accelerated and reversed the diminution in RBC production that followed hyperoxic exposure. The pattern of response to these erythropoietic stimulants was similar to the response which has been reported in animals with erythropoiesis suppressed by hypertransfusion, starvation, or hypoxia induced plethora.

Alterations in erythropoiesis in the parabiotic rats are shown in Fig. 5. The 18-hr erythrocyte radioiron incorporation of the control group with both partners exposed to 20% oxygen was 19.7%. Exposure of both partners to hyperoxia led to significantly depressed erythropoiesis ($p < 0.001$) with erythrocyte radioiron incorporation values of 8.9%. In the third group, exposure of one partner to hyperoxia (90–95% O_2) and the other partner to 20% oxygen resulted in significant depression of erythrocyte radioiron incorporation in both ($p < 0.001$). Arterial pO_2 determinations on partners exposed to 90–95% oxygen were elevated to 404 ± 53 mm Hg, whereas, partners exposed to 20% oxygen had a pO_2 of 96 ± 17 mm Hg.

Discussion. Concentrations of oxygen above atmospheric levels produce a marked alteration of erythropoiesis in man and experimental animals. Possible mechanisms advanced in explanation of this phenomenon include erythrocyte hemolysis due to oxidation, direct bone marrow suppression due to hyperoxia, or bone marrow suppression secondary to a decreased production or altered character of erythropoietin. The demonstration of hemolysis and morphologic alterations of the red blood cell similar to Heinz body anemia

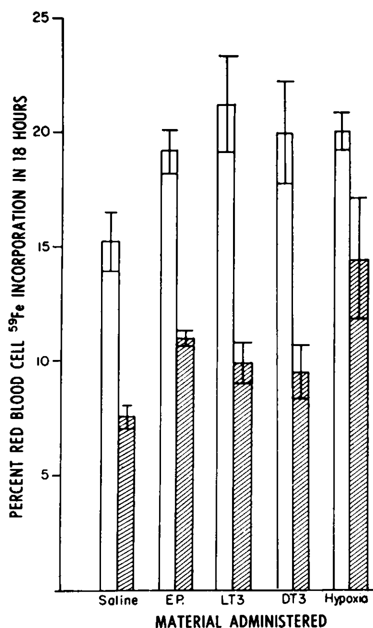

FIG. 4. Effect of erythropoietic stimulants: Open bars represent rats maintained in a 20% oxygen environment, crosshatched bars represent rats maintained in a 50% oxygen environment for 7 days. The vertical lines indicate the standard deviation of the 16 rats in each bar.

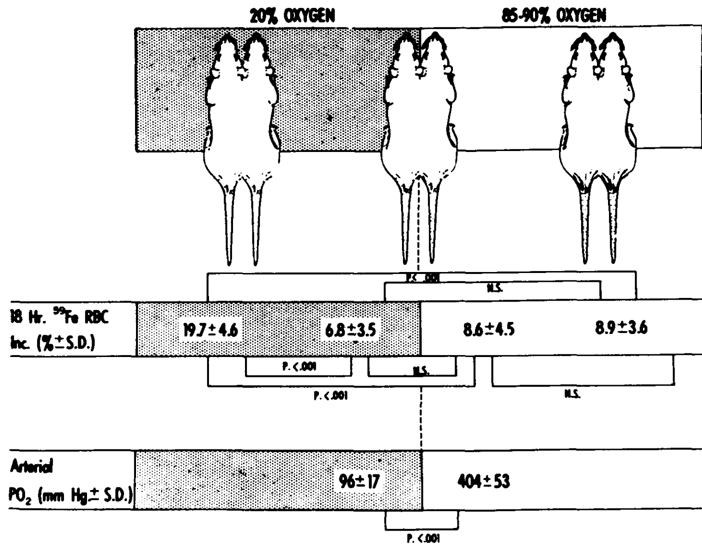

FIG. 5. Parabiotic experiments: Three groups of parabionts: 13 pair both breathing 20% oxygen; 13 pair both breathing 85–90% oxygen, and 14 pair with one partner breathing 20% oxygen and the other partner breathing 85–90% oxygen. Arterial oxygen values determined in each partner of 11 pair of the latter group. Standard deviations indicated following the value. Probability values obtained by Student's *t* test.

upon incubation of erythrocytes in hyperbaric hyperoxic environments supports the hypothesis of oxidative hemolysis (12). However, such oxidative changes have not been reported in normobaric hyperoxic conditions. The reticulocyte measurements and ferrokinetic data of the present study suggests that oxidative hemolysis does not play a significant role in the erythropoietic decompensation attendant to exposure to a hyperoxic environment, although a secondary role cannot be completely ruled out. Furthermore, the effect of a variety of erythropoietic stimulants on erythropoiesis in rats maintained in a hyperoxic environment suggest that the diminution in red blood cell production produced by hyperoxia can be reversed, and is not due to direct marrow oxygen toxicity.

Diminished oxygen tension produces an increase in plasma erythropoietin and a resultant erythrocythemia suggesting that the production of the erythropoietic hormone by the kidney is responsive to variations in blood oxygen content. The demonstration of a circulating antagonist to erythropoietin in the plasma of polycythemic animals (4, 5) suggests a dynamic interaction between the erythropoietic hormone and a circulating erythropoietic inhibitor in the regulation of erythropoiesis. The results of the present study support this hypothesis. In the parabiotic pairs, the exposure of one partner to hyperoxia and the other partner to 20% oxygen resulted in significant depression of erythropoiesis in both. Their individual iron incorporation of 8.6% and 6.8% were not statistically different. Arterial pO_2 determinations on partners exposed to hyperoxia were elevated to 400 mm Hg; whereas, partners exposed to 20% oxygen had a pO_2 of 90 mm Hg. These arterial oxygen results indicate that there was essentially no oxygen interchange between the parabiotic partners during the period of exposure. The depression of erythropoiesis in the normoxic partners of hyperoxic rats, in a situation where there is no significant oxygen interchange as established by the arterial pO_2 values, suggests that an erythropoietin inhibitor may have been induced in the hyperoxic animal and transferred to the room air partner resulting in depression of erythropoiesis in both partners. An alternate possibility is that there was a compensation of erythropoietin levels of both partners during the period of parabiosis with redistribution and dilution of nor-

mal erythropoietin levels in the 20% oxygen partner leading to depressed erythropoiesis. The degree of erythropoietic depression in the parabiont exposed to normal oxygen tensions while the partner was exposed to a hyperoxic environment as well as in the parabionts with both partners exposed to hyperoxia mitigates the latter explanation.

Summary. Exposure to a 50% oxygen environment produced a decrease in erythropoiesis which was paralleled by a decrease in plasma erythropoietic stimulating activity. Similar results were obtained following exposure to a 100% oxygen environment. Marrow depression produced by hyperoxia could be reversed by erythropoietic stimulants. The suppression of erythropoiesis in both partners of parabiots maintained so that one breathed 20% and the other 90–95% oxygen suggests that a humoral inhibitor is produced in the hyperoxic animal and supports the hypothesis that erythropoiesis is regulated by the dynamic interaction of erythropoietin and an erythropoietic inhibitor.

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