Physiologic Response to Increased Oxygen Partial Pressure III. Hematopoiesis

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ABSTRACT

The influence of increased partial pressure of oxygen on hematopoiesis was studied in 8 normal subjects: 4 subjects were exposed to a total pressure of 700 mm. Hg, 33 per cent O_2 and 62.3 per cent N_2; and 4 subjects to a total pressure of 258 mm. Hg, 98.5 per cent O_2 and 0.2 per cent N_2. Two control subjects remained outside the chamber during each 30-day study. Measurements were designed to determine whether the increased arterial P_{O_2} (177.7 mm. Hg in the 700 mm. Hg group, and 169.7 mm. Hg in the 258 mm Hg group) affected circulating red blood cells and/or red blood cell production. Except for mild changes in red cell values with hematocrit reduction of 6.7 per cent and 9.1 per cent in the 700 mm. Hg group and 258 mm. Hg group, respectively, most of the hematopoietic studies were normal. It appears that 30-day exposure to the increased oxygen partial pressures used in this study does not significantly alter hematopoiesis.

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HYPOXIA ACTS AS a potent stimulator of red blood cell production in the mammal. Most evidence indicates that this stimulus is mediated through a humoral factor, erythropoietin.[17] On the other hand, there are relatively few studies concerning the influence of increased oxygen concentration on erythropoiesis. Conflicting data exists suggesting that hypoxia may either suppress [7, 8, 16, 18] or have little influence [6] on red blood cell production. In an occasional report, an increase in red cell values has been noted.[19] Because of pulmonary pathology resulting from excessively high oxygen concentrations, the latter have been attributed to relative hypoxemia.

We had the opportunity to study erythropoiesis in a group of healthy young subjects who were being maintained in an environment of increased oxygen concentration for one month. From practical considerations, it became important to determine whether the proposed gaseous environment would have any adverse influence on hematopoiesis during prolonged space flight. The studies were designed to estimate whether hyperoxia of this degree would have a deleterious effect on circulating red blood cells, resulting in a hemolytic anemia, or, perhaps more importantly, whether suppression of erythropoiesis occurs resulting in the gradual development of anemia through the normal attrition of senescent red cells.

METHODS

The subject groups and environmental conditions used in this study are given in another report.[10]

Hematologic and Blood Chemistry Technics:—Determination of hematocrit, hemoglobin, red and white blood cell and differential counts were performed by routine methods. Reticulocytes were enumerated by the "wet method" and expressed as per cent of mature red cells. Red blood cell osmotic fragility curves and Heinz body counts[3] were obtained weekly. Sternal marrow aspirates were performed on all chamber subjects just prior to oxygen exposure and immediately following return to sea-level conditions. Fecal urobilinogen and urine urobilinogen excretion[20] were determined on 3-day and 24-hour collections, respectively. Serum bilirubin values were obtained about twice a week. Red cell glutathione stability and glucose-6-phosphate dehydrogenase (G-6-PD) levels were measured on heparinized blood which was kept in an ice bath until analyzed. Time from venepuncture to analysis rarely exceeded two hours. Both determinations were performed three times a week on all subjects. The glucose-6-phosphate dehydrogenase assays were performed according to the method of Zinkham *et al.*[22] using a Beckman DU Spectrophotometer. During the enzyme reaction, the temperature in the cuvette was kept constant at 30°C, using a water jacket in the spectrophotometer. Reduced glutathione (GSH) and glutathione stability were measured by the method of Beutler *et al.*[5] Lactic dehydrogenase (LDH) was determined by the method of Wroblewski *et al.*[21] on the same red cell hemolysate used for the G-6-PD assay.

Isotope Technics:—Seven days prior to increased oxygen exposure, 30 ml of the subject's blood was collected in a Unitag® bag (Abbott Laboratories) containing 5 ml A-C-D solution and 100 μc of Cr^{51} (as $Na_2\ Cr^{51}\ 0_4$). After 45 minutes incubation at room temperature, the cells were centrifuged and the supernatant plasma with excess Cr^{51} discarded. This was repeated three times following washing of the cells with normal saline. The cells were then reconstituted to their original hematocrit with normal saline and after taking a 5 ml aliquot as a standard, 25 ml were reinjected into the subject for determination of red cell survival time. Samples were obtained at 15 minutes for determination of the red cell mass, 5 hours, then every other day for 2 weeks, and every third day for the final 2 weeks. The apparent red cell half life (T 1/2 Cr^{51}) was estimated by plotting the counts of each specimen on semi-logarithm paper and extrapolating to zero time. The T 1/2 Cr^{51} in days was then read from the graph at 50 per cent of the zero time counts.[15] By starting the red cell survival curve 7 days prior to exposure to increased oxygen, a change in red cell survival would be reflected in a displacement of the slope of the curve.

After three weeks exposure to the higher oxygen concentration, ferrokinetic studies were performed on all subjects. The plasma obtained from 15 ml of heparinized blood was incubated at room temperature for 45 minutes with 10 μc of Fe^{59} (as $Fe^{59}\ Cl_3$). The amount of elemental Fe was calculated to be within the unsaturated iron-binding capacity of the plasma. Plasma-Fe^{59} clearance and red cell utilization of Fe^{59} were measured according to the method of Huff *et al.*[11] A single channel pulse-height gamma ray analyzer (Baird Atomic) was used to differentiate between Fe^{59} and Cr^{51}.

RESULTS

The hematocrit and reticulocyte values before, during, and immediately following the 4 weeks of exposure to elevated oxygen concentration are shown in Figures 1 and 2. Although a slight reduction in hematocrit

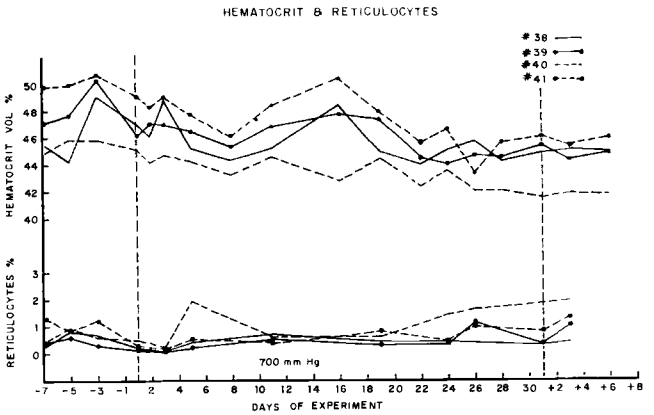

Fig. 1. Hematocrit and reticulocyte levels in 4 subjects before, during, and after exposure to 700 mm. Hg pressure, 33 per cent O_2.

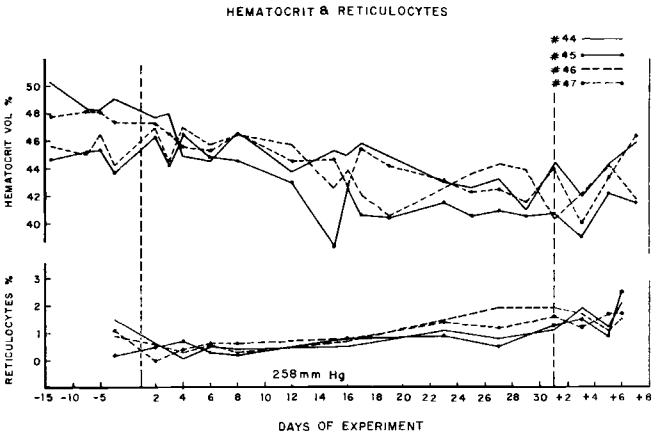

Fig. 2. Hematocrit and reticulocyte levels in 4 subjects before, during, and after exposure to 258 mm. Hg pressure, 100 per cent O_2.

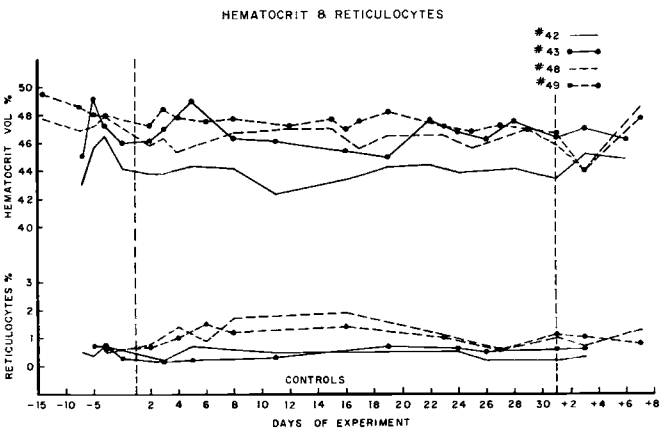

Fig. 3. Hematocrit and reticulocyte levels in 4 control subjects throughout the study.

is observed, in no instance did this reach levels of physiologic embarrassment. Comparison of average pre-oxygen exposure hematocrits with those at the end of 30 days of hyperoxia showed average reductions of 6.7 per cent and 9.1 per cent in the 700 mm. Hg group and 258 mm. Hg group, respectively. During this same period, the control subjects (Fig. 3) showed a 3.4 per cent drop in hematocrit. Although not plotted, the hemoglobin and red cell counts paralleled the changes in hematocrit. Most of the mild reduction in hematocrit took place gradually over the initial two weeks of hyperoxia, stabilizing during the final two weeks. The reticulocyte counts remained low during the period of exposure despite the modest hematocrit changes. It is of some interest to note that seven of the eight subjects in both the 700 mm. Hg and 258 mm. Hg groups showed a slight reticulocytosis for several days following return to normal atmospheric conditions. The control subjects maintained a low reticulocyte count throughout the period of study, suggesting that the repeated venepunctures (average total blood loss of 800 ml) had little influence on this parameter.

Other studies failed to indicate increased hemolysis. Serum bilirubin levels remained normal. Fecal and urinary urobilinogen excretion did not exceed normal levels (Fig. 4). Red cell osmotic fragility curves

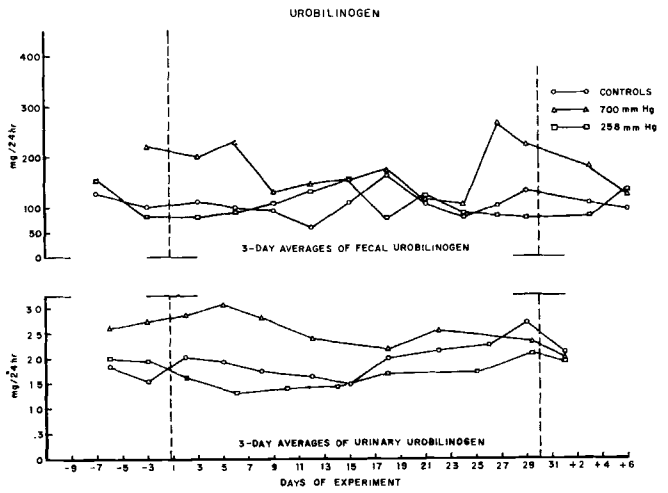

Fig. 4. Fecal and urinary urobilinogen excretion. Average values for 3-day collection periods are given.

showed no significant shifts, nor was there any increase in Heinz body formation. The apparent T 1/2 Cr^{51} red cell survival studies demonstrated no shortening of red cell life span (Table I). Pre-oxygen exposure slopes were not altered after the experimental subjects were placed in the higher oxygen environment.

Table II summarizes the results of the glucose-6-phosphate dehydrogenase assays for individual subjects as well as the mean values for pre-exposure, exposure, and post-exposure periods. Subjects in the 700 mm. Hg group showed an average decrease from 216 enzyme units before increased oxygen exposure to an average of 199.6 enzyme units during exposure. The

TABLE I. ISOTOPIC DATA

	T 1/2 Cr^{51} Survival (days)	T 1/2 Fe^{59} Plasma Clearance (minutes)	RBC Utilization Fe^{59} on Day 9 (per cent)
Control Subjects			
42	26.1	80	90.8
43	35.6	125	89.7
48	28.1	109	97.1
49	32.6	118	85.6
Means	30.6	108	90.8
700 mm. Hg Subjects			
38	32.8	114	84.8
39	35.2	71	88.2
40	25.2	80	84.4
41	31.1	88	85.4
Means	31.1	88	85.7
258 mm. Hg Subjects			
44	31.9	94	85.6
45	31.2	96	97.3
46	33.1	79	100.0
47	30.1	88	87.2
Means	31.6	89	92.5

TABLE II. GLUCOSE-6-PHOSPHATE DEHYDROGENASE (ENZYME UNITS PER 100 ml. WASHED RBC)

		CONTROL SUBJECTS				700 mm. Hg SUBJECTS				258 mm. Hg SUBJECTS			
		42	43	48	49	38	39	40	41	44	45	46	47
Pre-exposure	$\bar{x}$	195.6	199.5	200.9	215.9	222.2	197.2	233.0	211.4	194.0	223.1	201.4	207.3
	S.D.	23.9	18.9	12.5	8.9	41.4	23.4	24.5	34.6	14.9	25.7	19.8	21.1
Exposure	$\bar{x}$	192.1	168.1	203.9	229.4	212.7	194.0	216.9	174.7	212.2	232.9	236.3	202.7
	S.D.	12.3	10.4	14.2	10.5	17.8	10.4	18.7	17.6	17.2	21.5	14.2	18.9
Post-exposure	$\bar{x}$	190.8	155.0	212.2	222.9	207.5	191.6	202.3	171.7	228.4	243.8	234.6	211.5
	S.D.	18.1	12.2	13.8	5.3	7.3	7.9	20.5	7.6	13.7	3.3	24.9	9.4

GLUCOSE-6-PHOSPHATE DEHYDROGENASE MEANS FOR EACH GROUP FOR EACH PERIOD (ENZYME UNITS PER 100 ml WASHED RBC)

	PRE-EXPOSURE	EXPOSURE	POST-EXPOSURE
Controls	203.0	198.4	195.2
700 mm. Hg	216.0	199.6	193.3
258 mm. Hg	206.5	221.0	229.6

258 mm. Hg group, on the other hand, showed an average increase from 206.5 enzyme units to 221 enzyme units for these same periods. Control mean values were 203 and 198.4 enzyme units, determined at similar time intervals. Following return to normal atmospheric conditions, there was a further slight decrease in the mean value of the 700 mm. Hg group, and continued slight increase in the 258 mm. Hg group. The control subjects showed a further slight decline. However, all the observed values of G-6-PD in the different groups at the various time intervals remained well within the normal range.[22] Reduced glutathione and glutathione stability studies are summarized in Table III. Normal levels of GSH were found in all subjects regardless of atmospheric exposure. No evidence for

TABLE III. GLUTATHIONE (mg./100 ml. RBC)

	PRE-EXPOSURE		EXPOSURE		POST-EXPOSURE	
	Pre-Incub.	Post-Incub.	Pre-Incub.	Post-Incub.	Pre-Incub.	Post-Incub.
Control Subjects						
42	73.8 ± 3.6	73.2 ± 2.4	73.9 ± 6.0	62.5 ± 10.6	73.4 ± 0.9	66 9 ± 2.3
43	70.1 ± 2.6	68.0 ± 2.3	63.7 ± 3.2	64.6 ± 6.0	61 9 ± 3.3	64.5 ± 1.9
48	82.5 ± 4.6	83.8 ± 4.9	75.2 ± 4.2	78.5 ± 5 2	74.4 ± 6.2	72.9 ± 8 5
49	99.1 ± 4.9	95 5 ± 4.5	92.4 ± 3.6	89.5 ± 5.7	91.3 ± 4 9	87.2 ± 1 6
Mean	81.4	80.1	76.3	73.8	75.2	72.9
700 mm. Hg Subjects						
38	71.0 ± 1.8	69.2 ± 3.4	67.8 ± 4.0	62.6 ± 5.6	67.7 ± 1.5	67.4 ± 2.4
39	74.2 ± 3.5	67.7 ± 6.0	71.5 ± 5.3	60.0 ± 7.5	72.3 ± 4.7	62.7 ± 8.9
40	77.7 ± 4.1	73.1 ± 3.7	76.7 ± 4.9	60.2 ± 11.0	73.3 ± 5.5	61.8 ± 2.1
41	101.4 ± 5.6	91.1 ± 8.1	95.7 ± 5.6	70.4 ± 11.7	91.0 ± 5.2	72.1 ± 3.4
Mean	81.1	75.3	77.9	63.3	76.1	66.0
258 mm. Hg Subjects						
44	86.2 ± 8.8	86.3 ± 5.9	83.3 ± 5.5	79.4 ± 11.0	81.3 ± 5 1	80.8 ± 3.2
45	88.4 ± 5.1	83.1 ± 6.9	84.3 ± 4.1	76.7 ± 3.9	80.9 ± 4 8	73 6 ± 4 9
46	90.9 ± 3.4	88.7 ± 6.4	84.3 ± 3.2	82.9 ± 4.9	80.6 ± 4.0	84.1 ± 2 5
47	90.5 ± 5.7	90.3 ± 7.8	90.3 ± 2.5	89.6 ± 4.9	88.9 ± 6.9	88.6 ± 4.7
Mean	89.0	87.1	85.6	82.2	82.9	81.8

increased instability of GSH as a result of exposure to hyperoxia was apparent. LDH determinations showed no deviation from normal.

Ferrokinetic studies, as measures of red blood cell production, are shown in Table I. Plasma-Fe^{59} clearance was normal in both the 700 mm. Hg and 258 mm. Hg groups at the end of 18 days residence in the increased oxygen environment. Similarly, all subjects showed a normal Fe^{59} utilization curve while under continued oxygen exposure, reaching greater than 84 per cent red cell incorporation by the ninth day.

Morphologic study of the bone marrow showed some slight changes. Three subjects (38, 40, 41) in the 700 mm. Hg group and one subject (46) in the 258 mm. Hg group had a mildly increased myeloid : erythroid ratio at the termination of the study (Table IV).

TABLE IV. MYELOID:ERYTHROID* RATIO OF THE STERNAL BONE MARROW BEFORE AND AFTER EXPOSURE TO HYPEROXIA

	BEFORE	AFTER
700 mm. Hg Subjects		
38	3.1 : 1	6.4 : 1
39	4.3 : 1	3.9 : 1
40	2.1 : 1	5.1 : 1
41	3.7 : 1	4.9 : 1
258 mm. Hg Subjects		
44	3.2 : 1	3.1 : 1
45	4.7 : 1	3.8 : 1
46	3.8 : 1	5.3 : 1
47	2.6 : 1	2.1 : 1

*Only cells of the myelocytic and erythroid series were counted.

Because of the variations normally inherent in sampling, these changes are of questionable significance. No major alteration in the proportion of the various red cell precursors was noted. Furthermore, there was no evidence of toxic manifestations.

Although not shown, total and differential white blood cell counts remained essentially unaltered throughout the study. Red cell morphology showed no abnormalities. Estimates of platelets on the peripheral smears also appeared to lie in the normal range.

DISCUSSION

In order to evaluate any data relating the influence of increased partial pressure of oxygen to erythropoiesis, it is mandatory to consider the following conditions: 1) the concentration of oxygen in the inspired air; 2) the duration of exposure to this concentration; 3) the state of erythropoiesis at the time of the study; and finally, 4) the methods used to measure any alterations in erythropoiesis. Most investigations in the past on both animals and man were conducted with oxygen concentrations greater than 50 per cent at normal pressure. Theoretically, this would deliver oxygen partial pressures in excess of 300 mm. Hg in the alveolar air, a concentration well above the levels obtained in our subjects. In the cardiopulmonary and central nervous systems, manifestations of oxygen toxicity regularly ensue at partial pressures greater than 425 mm. Hg.[1] Duration of exposure to this abnormal gaseous environment is of especial importance in studies of the hematopoietic system. Since the normal life span of the human red blood cell is estimated to be 120 days,[2] failure of delivery of young erythrocytes into the circulation would result in a daily decrement of approximately 0.8 per cent of the red cell mass. Under these circumstances, periods of bone marrow suppression in excess of four weeks would be necessary prior to the development of overt anemia, unless some other mechanism, such as hemolysis, were operable. The more rapid the rate of red cell turnover, the earlier will a suppressive effect be apparent. On the other hand, by utilizing more sensitive indicators of erythrokinetics such as Fe^{59} studies, the physiological state of the marrow can be evaluated within a few days.[11, 13] Reinhard and co-workers[16] observed a marked reduction in reticulocytosis in patients with sickle cell anemia exposed to 70-100 per cent oxygen by mask for periods of 8-10 days.

On the basis of the studies reported here, there is little evidence that alveolar oxygen partial pressures of 177.7 and 170.3 mm. Hg exert significant effects on erythropoiesis during a 30-day exposure period. The mild decrease in hematocrit associated with a low reticulocyte count and slight diminishment of red cell precursors in half the experimental subjects at the termination of exposure might be interpreted as evidence of suppression. Cooperberg and Singer[7] reported similar though more marked findings in guinea pigs exposed to 70 per cent oxygen for periods up to 36 days. In these animals, there was evidence of increasing activity in the marrow after the second week of exposure, suggesting a compensatory phenomenon. The major component of the hematocrit reduction in our subjects occurred gradually over the first two weeks of residence in the chamber and remained reasonably constant thereafter. The normal ferrokinetic data obtained at the end of the third week may have missed the period of major bone marrow suppression. Lawrence *et al.*[13] have demonstrated the validity of using tracer amounts of Fe^{59} for acute determinations

of the influence of increased oxygen concentration on red cell production.

There is no evidence indicating increased hemolysis following exposure to hyperoxia in our subjects. Except for one report,[9] this has been the experience of several other investigators.[6-8, 16, 18, 15] In the single study purporting to observe a hemolytic process,[9] the subjects were maintained at 100 per cent oxygen at 0.5 atmospheres and showed a 2-3 gram reduction in hemoglobin over the first 2-3 days. A rise in serum bilirubin and increased reticulocytes accompanied this drop, while the subjects remained in the hyperoxic environment. No mechanism for this apparent reduction in hemoglobin was established. The normal Cr^{51} red cell survival, normal bilirubin and urobilinogen excretion, gradual and slight decrease in hematocrit, low reticulocyte counts, all fail to support a hemolytic process under the conditions of the present study.

The studies of Beutler and co-workers[4] have established an association between the intracellular reduced glutathione levels and susceptibility of red cells to injury by oxidative drugs. The energy necessary for reducing glutathione in the red cell comes primarily from the pentose phosphate pathway and any deficiency of glucose-6-phosphate dehydrogenase interferes with the ability of the red cell to regenerate GSH.[4] The possibility has been raised that hyperoxia may alter some of the metabolites comprising the pentose phosphate pathway,[9] but our findings of normal levels of GSH, GSH stability, and G-6-PD indicate that increased oxidative hemolysis was not operative. Furthermore, precipitation of methemoglobin in the red cell (Heinz bodies), which usually accompanies oxidative hemolysis,[12] was not found. Recent studies by Mengel *et al.*[14] have shown that exposure to 100 per cent oxygen at 2-3 atmospheres pressure resulted in severe hemolysis in both mice and men. Abnormal stromal lipid peroxidation along with aberrations in the glycolytic pathway of the red cell have been implicated as the mechanisms leading to hemolysis. Antoxidant drugs, such as α-tocopherol, protected against this damage. The extreme differences in the partial pressure of oxygen under these conditions as compared with ours, probably account for the absence of similar abnormalities in the present study.

Although higher concentrations of oxygen have unequivocally shown pronounced effects on erythropoiesis, the results presented here indicate that arterial oxygen partial pressures of 160-180 mm. Hg have only a minor influence over a 30-day span. For space missions not exceeding this duration, one would expect little alteration hematopoietically. Missions of longer duration would require further laboratory study since the possibility of compensatory reactivation of the bone marrow may occur, thus ameliorating the early suppressive phase. Our studies further indicate that the virtual absence of nitrogen exerts little influence on erythropoiesis.

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