# Effect of Hyperoxia on the Erythropoietic Response to Sodium-L-Triiodothyronine (33632)

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An interaction of thyroid function and hematopoiesis is evident from numerous clinical and experimental observations. Accelerated erythropoiesis has been documented in association with thyroid hyperactivity (1) and decreased erythropoiesis with thyroid hypoactivity (2). The anemia in hypothyroidism results from multiple factors, but a hyporegenerative component is the essential feature. Prior observations (3–4) have been the basis for the conclusion that the major effects of the thyroid on erythropoiesis are mediated through an alteration in oxygen requirement and oxygen utilization. Other investigators (5) have postulated an additional direct stimulatory effect not related to changes in metabolism. The present experiments tested the effects of hyperoxia on erythropoiesis and the erythropoietic response to L-triiodothyroine. Graded doses of $LT_3$ produced the expected augmentation of red blood cell mass in animals maintained in a normal environment while an atmosphere of 50% oxygen clearly attenuated this erythropoietic response to $LT_3$. These observations are consonant with the fact that a major stimulating influence of thyroid hormone on erythropoiesis is related to augmented oxygen requirement.

*Methods.* Ten-week-old female Sprague-Dawley rats weighing approximately 200 g were utilized throughout. Rodent chow and water were available at all times. The rats were divided into four groups and each animal was given either 1, 2.5, or 10 $\mu$g of sodium L-triiodothyronine[1] in saline intraperitoneally each day for 14 days; control animals were given saline alone. The four groups were further subdivided and approximately one-half of the rats of each group were maintained in plastic cages in a normal environment. The remaining animals were placed in an environment of 50% oxygen–50% nitrogen in a modified atmosphere chamber. Humidity and temperature were not selectively controlled and oxygen content was analyzed at regular intervals with a portable Beckman oxygen analyzer. Carbon dioxide was removed from the chamber by washout and absorption with calcium hydroxide. The exposure to hyperoxia was, of necessity, interrupted daily for brief periods for cage maintenance.

Initial body weights and basal metabolic rate determinations by a modification of the method of Watts and Gourley (6) were measured at the onset and again 14 days later at termination of the experiments. In addition at 14 days, final red blood cell masses, packed cell volumes, and reticulocyte counts were measured on all animals. For determination of red blood cell mass normal rats were injected intravenously with 4 $\mu$Ci $^{59}$Fe in saline and were exsanguinated at 48–72 hr when all radioiron had been cleared from the plasma and significant red blood cell incorporation had occurred. Labeled cells from several of these donors were pooled and an aliquot of the suspension of the $^{59}$Fe RBC's was injected intravenously into each of the test rats. Dilution of the tagged cells allowed calculation of the red blood cell mass (7). Measurement of radioactivity in all samples was completed by use of a well counter. Hematocrits and reticulocytes were measured by standard methods.

*Results and Discussion.* The red blood cell mass in normal rats was 2.19 $\pm$ 0.13[2] ml of cells/100 g of body weight and this compares

[1] Procured from Sigma Chemical Company, St. Louis, Mo., Lot No. 87B-2320.

[2] Standard deviation.

TABLE I. Effect of Hyperoxia on Response to L-Triiodothyronine.

| Dose ($\mu$g) | | Hematocrit (%)[b] | Red blood cell mass (ml/100 g)[b] | Reticulocytes (%)[b] |
|---|---|---|---|---|
| Control | (22)[a] | 43.7 ± 3.2 | 2.19 ± 0.13 | 1.7 ± 1.0 |
| 1.0 | (12) | 44.3 ± 2.0 | 2.42 ± 0.15[d] | 2.1 ± 1.1 |
| 2.5 | (15) | 45.8 ± 2.9 | 2.49 ± 0.14[d] | 3.7 ± 1.9 |
| 10.0 | (12) | 47.0 ± 1.4 | 2.80 ± 0.21[d] | 5.6 ± 3.4 |
| Hyperoxic control | (13) | 41.9 ± 2.5 | 1.99 ± 0.16[c] | 1.7 ± 0.9 |
| 1.0 | ( 8) | 37.9 ± 3.5 | 2.05 ± 0.23[c] | 1.8 ± 1.0 |
| 2.5 | (12) | 38.5 ± 4.2 | 2.03 ± 0.11[c] | 1.7 ± 0.7 |
| 10.0 | (11) | 40.3 ± 3.3 | 2.13 ± 0.18[c] | 2.5 ± 1.1 |

[a] Number of animals.
[b] Mean ± SD.
[c] $p < .001$ when compared to similar normal environment group.
[d] $p < .001$ when compared to normal control.

well with previously reported experiments utilizing this technique (7–8). Exposure to hyperoxia for 2 weeks produced a significant decrease in the red blood cell mass to 1.99 ± 0.16[2] ml/100 g ($p < .001$); prolongation of exposure to these conditions of hyperoxia for 3 weeks produced a more marked decrease in red blood cell mass (1.76 ± 0.16 ml of RBC/100 g). Decreased erythropoiesis following exposure to increased oxygen tension has been documented previously (9) and hyperbaric–hyperoxia (10) has been reported to result in hemolysis. In the current experiments there was apparently no significant hemolytic component to alone account for the decrease in red cell mass, since the reticulocyte counts at the end of 2-weeks exposure were not elevated above those noted in the control animals (1.7 ± 1.0 and 1.7 ± 0.9%, respectively).

Daily administration of L-triiodothyronine for 14 days produced significant increases in hematocrit and the red blood cell mass of all rats maintained in a normal room environment (Table I). Increased reticulocyte counts provide further evidence of augmented erythropoiesis secondary to the administration of thyroid hormone in these groups. Increases in both red blood cell mass and percentage of reticulocytes were dose related and the doses above 1 $\mu$g increases in oxygen consumption were clearly evident (Table II). In addition, in most groups there was failure to gain or a moderate reduction in body weight. This was more marked in the hyperoxic rats. Loss of weight or change in lean body mass might account for some of the observed differences in red cell mass between controls and $LT_3$ treated rats. This however cannot explain the fact that increases in hematocrits and red blood cell mass following $LT_3$ administration to animals in a normal environment were not noted when the rats were maintained in the hyperoxic environment. Red blood cell masses in the hyperoxic-$LT_3$ treated rats were slightly greater in each instance than the hyperoxic controls.

Evans *et al.* (3), and Waldmann *et al.* (4), administered exogenous thyroxine or triiodothyronine to dogs and rats and related the resultant erythrocythemia to the increased basal metabolic rate and tissue oxygen requirement produced by the thyroid hormone. The evident sensitivity of the humoral system which regulated erythropoiesis to variations in oxygen need provides a real basis for this formulation. Additional observations (5–11), however, militated against so simple an explanation of the effects of thyroid hormone on red blood cell production. Donati and his co-workers reported that both the metabolically inert D-triiodothyronine and L-triiodothyronine produced similar increases in erythrocyte $^{59}Fe$ incorporation in polycythemic animals (5), and in starved animals (11). In the latter comparable increases in oxygen utilization in $DT_3$ treated and $LT_3$

TABLE II. Effects of Triiodothyronine on Weight Gain and Metabolic Rate.

| | Mean wt. (g) | | | Mean BMR (ml/hr/100 g) | | |
|---|---|---|---|---|---|---|
| Dose ($\mu$g) | Initial | Final | % Difference | Initial | Final | % Difference |
| Control | 214 | 242 | +13.1 | 194.1[a] | | |
| 1.0 | 194 | 216 | +11.3 | | 192.0 | −1.1 |
| 2.5 | 201 | 222 | +10.4 | | 236.6 | +19.8 |
| 10.0 | 244 | 245 | +0.4 | | 260.7 | +34.3 |
| Hyperoxia control | 206 | 223 | +8.3 | 194.1[a] | 200.4 | +3.2 |
| 1.0 | 198 | 203 | +2.5 | | 251.3 | +29.5 |
| 2.5 | 216 | 209 | −3.2 | | 260.6 | +34.3 |
| 10.0 | 219 | 206 | −5.9 | | 305.9 | +57.6 |

[a] Mean BMR in 88 normal animals.

treated rats did not occur. It was concluded that erythropoietic acceleration following administration of triiodothyronine was not completely dependent on alteration in oxygen requirements. The current experiments clearly suggest that the change in red cell production related to increased thyroid activity is in major part the result of increased oxygen demand since this change is responsive to the influence of hyperoxia. Although this erythropoietic stimulus is apparently related to the effects of thyroid hormone on metabolism and oxygen consumption, an alternate explanation that hyperoxia inhibits red blood cell development at a site prior to the point of $LT_3$ sensitivity cannot be entirely excluded. The dynamics and interaction of these phenomena and their relation to renal production of erythropoietin are being investigated.

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