In Vitro Studies of Erythropoiesis

II. The Effect of Anoxia on Heme Synthesis

By E. D. Thomas

SINCE the original hypothesis in 1893 by Miescher,¹ it has been generally accepted that the stimulus for the production of red blood cells by the bone marrow is anoxia. That anoxic anoxia stimulates erythropoiesis is well known from the observations of polycythemia and marrow hyperplasia at high altitudes and in various pathologic conditions producing decreased arterial oxygen tensions. Polycythemia produced by anemic anoxia and by cobalt have been explained by assuming local bone marrow anoxia and interference with bone marrow respiratory enzymes, respectively. However, direct studies of bone marrow oxygen tension have failed to show marrow anoxia after slow bleeding sufficient to produce an excellent erythropoietic response² or in chronic anemia.³

Tissue culture studies of bone marrow under various oxygen tensions have failed to demonstrate a stimulating effect on erythropoiesis by lowered oxygen tensions. Rosin and Rachmilewitz⁴ found that oxygen concentrations below 12 per cent produced degeneration of marrow cells and inhibition of mitotic activity, and that erythroid cells are more sensitive to oxygen want than myeloid cells. Magnussen⁵ studied the production of erythrocytes by bone marrow in vitro under various oxygen tensions. He found no difference in the range from 10 to 30 per cent oxygen. Below 5 per cent and above 40 per cent there was inhibition of erythrocyte production.

Warren⁶ found that the bone marrow of rabbits exposed to lowered oxygen tensions showed an increase in respiration and a reduction in glycolysis. These changes were also found in marrow with erythroid hyperplasia produced by bleeding and phenylhydrazine. Reduction of oxygen tension in vitro decreased oxygen consumption and increased glycolysis. These studies offered no support for the concept of direct erythroid stimulation by lowered oxygen tension.

In the previous paper⁷ it was shown that hemin synthesis by bone marrow in vitro does not necessarily parallel oxygen consumption. Accordingly, in the experiments described here, the ability of rabbit bone marrow to synthesize hemin under various oxygen tensions was determined.

METHODS

The details of the technic have been described in the previous paper.⁷ In the experiments involving various oxygen tensions, gas mixtures were prepared as

¹ From The Department of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, and the Children's Cancer Research Foundation, Children's Medical Center, Boston.
² This investigation was supported by a grant from the United States Public Health Service, Grant C-1721.(C2).
³ The author is indebted to Miss Charlotte Jackson for technical assistance.
⁴ Submitted November 16, 1954; accepted for publication January 1, 1955.

612
Fig. 1.—The effect of oxygen tension on hemin synthesis. The hemin radioactivity is expressed as per cent of that synthesized in air.

described by Umbreit. The Warburg cup and manometer were evacuated and filled with the gas five times, with brief shaking of the cup between each filling to allow equilibration of the gas in the serum. In the total anoxia cups, fresh pyrogallie acid was added to the center well to insure absence of oxygen.

RESULTS

The ability of bone marrow to synthesize hemin was measured under various oxygen tensions ranging from 100 per cent oxygen to total anoxia. Figure 1 is a composite of nine such experiments. The absolute number of counts per minute varied from one experiment to the next due to variations in the amount of marrow present. Therefore, in figure 1, the counts per minute synthesized in air is taken as 100 per cent, and the radioactivity at other oxygen tensions is expressed as the per cent of that in air.

It will be seen in figure 1 that there is no definite effect of oxygen tension on hemin synthesis until levels below 4 per cent oxygen are reached. There is no appreciable synthesis in the absence of oxygen. It should be noted that no level of reduced oxygen tension was found to stimulate hemin synthesis.

DISCUSSION

The data given here indicate no significant synthesis of heme in the absence of oxygen, a finding confirming the observations of others using similar methods of investigation. London et al. found no synthesis of heme by phenylhydrazine-induced rabbit reticulocytes under conditions of complete anoxia. In studying the incorporation of labeled amino acids into the proteins of rabbit reticulocytes, Borsook et al. found an extremely variable inhibition of the process by anoxia.

At low oxygen tensions considerable difficulty was encountered in getting consistent results, presumably due to oxygen still present in hemoglobin. When the
flasks were flushed with pure nitrogen, low but variable heme synthesis occurred. Complete failure of heme synthesis occurred only by the addition of pyrogallic acid to the center well. Failure to remove all oxygen from the system may account for the variable inhibition noted by Borsook et al.10

These results indicate that some oxygen is necessary for heme synthesis. This correlates well with the observations of Shemin and Kumin11 on the direct role of Krebs tricarboxylic acid cycle intermediates in heme synthesis. An absence of oxygen would cause a marked lowering of the steady state concentration of these intermediates and henceforth a lowering of heme synthesis. The pH dependent dissociation of oxygen consumption and heme synthesis demonstrated in the first paper2 may be explained in two ways. First, it suggests that the intactness of oxidative metabolism is not the only factor in the biochemistry of heme synthesis. Second, it may be that myeloid cells which constitute the majority of the bone marrow cells in these preparations continue to metabolize normally at low pHs while the erythroid cells are killed by a low pH.

The failure to observe a stimulating effect from any level of reduced oxygen tension indicates that anoxia is not a direct stimulant of the bone marrow. This is in conformity with the work of others using different methods as cited in the introduction. This data may therefore be considered as indirect evidence favoring a humoral regulator of bone marrow activity.

**SUMMARY**

The effect of various oxygen tensions on the in vitro synthesis of heme by rabbit bone marrow was measured. At levels above 4 per cent oxygen there was no effect of oxygen tension on heme synthesis. Total anoxia stopped heme synthesis completely. No level of oxygen tension was found to stimulate heme synthesis.

**SUMMARIO IN INTERLINGUA**

Esseva mesurate le effecto de varie tensiones de oxygeno super le synthese in vitro de hemo in le medulla ossee de conilios. Supra le nivello de 4 pro cento le tension de oxygene non afficeva le synthese de hemo. Anoxia total resultava in un arresto total del synthese de hemo. Le studio non revelava che il existe un tension special de oxygene che esserea capace a stimular le synthese de hemo.

**REFERENCES**


THOMAS, E. D.: In vitro studies of erythropoiesis. I. The effect of normal serum on heme synthesis and oxygen consumption by bone marrow. (See page 600 this issue.)


In Vitro Studies of Erythropoiesis: II. The Effect of Anoxia on Heme Synthesis

E. D. THOMAS