THE EFFECT OF COBALT ON HEME SYNTHESIS BY BONE MARROW IN VITRO*

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Since the demonstration by Waltner and Waltner (1) that cobalt will produce a polycythemia in rats, this effect has been produced in various other laboratory animals and human beings. However, the mechanism by which cobalt produces erythroid hyperplasia of the bone marrow remains obscure. This unique action of cobalt continues to be of interest because of its importance in leading to a better understanding of erythropoiesis and because of the possible therapeutic role of cobalt in various anemias.

There have been a number of attempts to explain cobalt polycythemia in terms of an anoxic mechanism (2). Barron and Barron (3) observed an inhibition of the respiration of immature red cells by small amounts of cobalt. They concluded that this inhibition led to premature release of young red cells into the circulation and hence to polycythemia. However, Warren et al. (4) were not able to confirm these findings. They observed that cobalt in concentrations of $10^{-4}$ to $10^{-2}$ M did not interfere with the respiration of bone marrow or reticulocytes in vitro unless the concentration was as high as 0.01 M.

Attempts have been made to link cobalt with the cytochrome system of biological oxidation. Schultze (5) noted a close relationship between the cytochrome oxidase activity of the bone marrow and the ability to form hemoglobin. Levey (6) gave intravenous injections of cytochrome c to cobalt-polycythemiac rats, but failed to abolish the polycythemia. However, it was pointed out that cytochrome c administered intravenously does not appear in tissue cells.

Although the hypothesis that cobalt produces cellular anoxia is an attractive one, the experimental evidence does not justify such an assumption. Attempts to explain cobalt polycythemia in terms of cellular bone marrow anoxia are made difficult by the fact that anoxia itself has not been shown to stimulate bone marrow directly (7).

The development of a method for measuring hemoglobin synthesis by bone marrow in vitro by measuring the rate of incorporation of radio-

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active glycine into heme has provided a new technique for studying the mechanism of action of cobalt (8). The rate of heme synthesis is only one part of the process of erythropoiesis, but it is a specific function of erythroid cells. This approach is more indicative of erythropoiesis than studies based on oxygen measurements alone, as it has been noted that heme synthesis and oxygen consumption are not always parallel (8). In this investigation the effect of cobalt on bone marrow in vitro was studied by the simultaneous measurement of heme synthesis and oxygen consumption.

Method

Rabbits weighing 4 to 5 pounds were used. The technique for preparing the marrow, incubating it with radioactive glycine, and determining the radioactivity of the hemin has already been described (8).

Cobaltous chloride\(^1\) was dissolved in an aliquot of bicarbonate-free serum, and progressive dilutions were made with the same serum to give the final molarity desired. The pH of the serum was adjusted to 7.35 to 7.40 just before use. Each experiment lasted 10 hours, and the oxygen consumption was measured for the first 3 hours.

The glycine-2-\(^{14}\)C\(^\text{U}\) had a specific activity of 0.2 mc. per mmole and a final concentration of \(1.4 \times 10^{-3}\) M. In the experiment with \(\alpha\)-\(^{14}\)C\(^\text{C}\) acetate the specific activity was 0.02 mc. per mmole, and the final concentration was \(12.5 \times 10^{-3}\) M.

Oxygen consumption and hemin radioactivity were determined in duplicate and averaged. The results were expressed as the per cent of the control without cobalt.

Results

Five experiments were carried out on normal bone marrows which contained 26 to 52 per cent erythroid cells (Fig. 1). There was no effect of cobalt on heme synthesis until concentrations of approximately \(10^{-4}\) M were reached, at which point inhibition began and was complete at \(10^{-2}\) M. There was no significant effect of cobalt on oxygen consumption at concentrations up to \(10^{-2}\) M. At no cobalt concentration was there a significant stimulation of oxygen consumption or heme synthesis.

Cobalt is known to form complexes with many substances, including amino acids (9). The observed inhibition of heme synthesis by cobalt could be an artifact produced by complex formation between cobalt and the radioactive glycine. Acetate, as well as glycine, is a heme precursor (10), but does not form complexes with cobalt. Therefore, two experiments with radioactive acetate rather than glycine were carried out.

\(^1\) Courtesy of Lloyd Brothers, Inc., Cincinnati 3, Ohio.
The results obtained with acetate were very similar to those in which glycine was used (Fig. 1).

The dissociation of oxygen consumption and heme synthesis produced by cobalt could be due to a differential effect on the erythroid as compared to the myeloid cells. Three experiments were performed on marrows containing 70 to 78 per cent erythroid cells obtained from rabbits given acetylphenylhydrazine. The results still showed a dissociation of oxygen consumption and heme synthesis essentially identical with those of the normal marrow (Fig. 1), except at a cobalt concentration of $10^{-2}$ M, at which there was perhaps a greater inhibition of oxygen consumption.

**DISCUSSION**

It has been reported that the normal level of cobalt in human serum is approximately $10^{-9}$ M (11). The serum level of cobalt in cobalt polycythemia has not been measured with accuracy, but is probably $10^{-5}$ M or less (12). If cobalt stimulates bone marrow directly, a zone of stimulation should be found between $10^{-5}$ and $10^{-9}$ M. Our experiments in the range from $10^{-2}$ to $10^{-9}$ M failed to show stimulation of heme synthesis or oxygen consumption. Such a failure suggests that cobalt does not have a direct effect on the bone marrow. However, these experiments do not
exclude this possibility. It may be that cobalt stimulates some phase of erythropoiesis other than heme synthesis. For instance, stimulation of cell proliferation would not be detected in experiments of 10 hours duration, such as these. It may be that cobalt in vivo is converted to a biologically active complex which acts directly on the bone marrow.

The most important observation here is that cobalt at concentrations greater than $10^{-4}$ M inhibits heme synthesis, but does not interfere with oxygen consumption until concentrations of $10^{-2}$ M are reached. The fact that cobalt "poisons" hemoglobin synthesis at concentrations well below those which inhibit oxygen consumption would seem to exclude the possibility that cobalt can cause polycythemia by a mechanism of bone marrow anoxia.

**SUMMARY**

The effect of cobalt in vitro on bone marrow was studied by measuring oxygen consumption and heme synthesis simultaneously.

Cobalt in concentrations of $10^{-2}$ to $10^{-8}$ M did not stimulate either oxygen consumption or heme synthesis.

Cobalt at concentrations greater than $10^{-4}$ M inhibits heme synthesis, but does not interfere with oxygen consumption until concentrations of $10^{-2}$ M are reached. This is interpreted to mean that cobalt does not produce polycythemia by a mechanism of bone marrow anoxia.

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