THE ANEMIA OF INFECTION, VI. THE INFLUENCE OF COBALT ON THE ANEMIA ASSOCIATED WITH INFLAMMATION

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ALTHOUGH the bone marrow usually appears to be normal or even hyperplastic in cases of anemia associated with infection,¹ the anemia is refractory to therapy, being relieved only when the underlying infection is successfully eradicated. The anemia is accompanied by a profound metabolic disturbance as indicated by the finding of hypoferremia, hypercupremia, and elevated erythrocyte protoporphyrin levels.² It is recognized, also, that infection is associated with a disturbance in protein metabolism; thus the serum albumin decreases, and increased excretion of urea in the urine has been reported.³

As part of a study of the pathogenesis of the anemia associated with inflammation, and in an attempt to find a means whereby the failure to form hemoglobin might be overcome, it was considered of interest to study the effect of cobalt. By the administration of cobalt, polycythemia can be produced in many laboratory animals.⁴⁵ The increase in red cells and hemoglobin has been reported as being due to an actual increase in red cell mass,⁶⁷ and an increase in reticulocytes in the blood has been observed. The mean corpuscular volume was found increased, owing mainly to greater cell thickness,⁷ the bone marrow becomes hyperplastic,⁸⁹ and metaplasia occurs in the spleen, liver, and kidneys.⁹¹⁰ The administration of cobalt was found to overcome the anemia produced by the toxic action of benzol in rabbits¹¹ and that caused by protein deficiency in rats¹² and was even reported to relieve the physiologic and nutritional anemia of children.¹³ Kleinberg and his associates¹⁴ found that in rabbits made anemic by the injection of 0.5 to 1.0 ml. of benzene for a period of 5 weeks, the daily administration of 50 ml. of cobalt nitrate overcame the anemia despite the continued administration of benzene. The marrow of the control benzene rats was fatty and aplastic, while the marrow of the rats treated with cobalt and benzene was hyperplastic. It has been shown by Dorrance et al.¹⁰ that the work performance of rats having cobalt polycythemia is increased under conditions of anoxia, thus indicating that the increased hemoglobin is useful for oxygen carriage.

METHODS AND PROCEDURE

Rats of Sprague-Dawley strain and from the Carsworth Farms were used. Since it has been shown that sterile turpentine abscesses have the same effect in animals as chronic infection¹³¹⁴ this means was employed to produce inflammation. In the first experiment 24 rats were used, these being divided into 4 groups of 6 each. All rats were fed fox chow ad libitum. Group I were controls. Group II ("cobalt") were given 0.125 mg. cobalt (0.5 mg. cobaltous chloride, CoCl₂·6H₂O) intraperi-

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toneally daily. Group III ("cobalt and turpentine") were injected intramuscularly with 0.25 ml. turpentine (Rexall, U.S.P.), and this was repeated according to the clinical condition of the animals; cobaltous chloride, 0.5 mg., was given intraperitoneally each day as well. Group IV ("turpentine") were injected with turpentine as in group III but received no cobalt.

The second experiment represented a repetition of the first, only the following slight modifications being made: The diet was Purina dog chow; the first injection of turpentine was 0.25 ml. and the succeeding weekly doses were 0.125 ml. This was

| Group No. | Hemoglobin Gm./100 ml. Before | Hemoglobin Gm./100 ml. After | Vol. Pk. RBC ml. Before | Vol. Pk. RBC ml. After | Erythrocyte Protoporphyrin mg./100 ml. Before | Erythrocyte Protoporphyrin mg./100 ml. After | Plasma Iron mg. Before | Plasma Iron mg. After | Serum Copper mg./100 ml. Before | Serum Copper mg./100 ml. After | Protein Total Albumin Gm./100 ml. Before | Protein Total Albumin Gm./100 ml. After | Globulin Gm./100 ml. Before | Globulin Gm./100 ml. After | Serum Iron mg. Before | Serum Iron mg. After | Total A/G Ratio Before | Total A/G Ratio After | Weight, Gm. Before | Weight, Gm. After | Change per cent |
|-----------|-----------------------------|-----------------------------|-------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------|-----------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| I. "Control" | 13.4 | 16.9 | 50.0 | 33 | 354 | 231 | | | | | | | | | | | | | | | | | +45 |
| II. "Cobalt" | 14.0 | 19.3 | 35.0 | 38 | 234 | 243 | | | | | | | | | | | | | | | | | +56 |
| III. "Cobalt & Turpentine" | 13.5 | 18.7 | 51.0 | 52 | 214 | 225 | | | | | | | | | | | | | | | | | +61 |
| IV. "Turpentine" | 14.1 | 13.8 | 41.0 | 138 | 161 | 227 | | | | | | | | | | | | | | | | | +47 |

| Experiment II |
|---------------|-----------------------------|-----------------------------|-------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------|-----------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| I. "Control" | 16.3 | 15.4 | 45.0 | 52 | 281 | 225 | 6.56 | 4.56 | 2.00 | 2.32 | 172 | 202 | +37 |
| II. "Cobalt" | 16.2 | 19.3 | 56.0 | 82 | 262 | 282 | 6.32 | 4.14 | 2.18 | 1.90 | 167 | 193 | +15 |
| III. "Cobalt & Turpentine" | 15.9 | 18.2 | 52.0 | 113 | 184 | 256 | 5.47 | 3.22 | 2.25 | 1.43 | 182 | 179 | -2 |
| IV. "Turpentine" | 17.3 | 14.5 | 41.0 | 85 | 188 | 282 | 5.42 | 3.50 | 1.92 | 1.83 | 183 | 201 | +10 |

| Experiment III |
|----------------|-----------------------------|-----------------------------|-------------------------|-------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------|-----------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| I. "Control" | 16.1 | 16.9 | 49.3 | 36 | 260 | 179 | 5.95 | 3.70 | 2.25 | 1.64 | 0.162 | 161 | 208 | +29 |
| II. "Cobalt" | 16.1 | 22.1 | 62.3 | 63 | 209 | 225 | 5.32 | 3.30 | 2.02 | 1.64 | 0.174 | 163 | 202 | +24 |
| III. "Cobalt & Turpentine" | 15.6 | 20.0 | 58.0 | 77 | 125 | 247 | 5.16 | 2.48 | 2.54 | 0.98 | 0.162 | 166 | 200 | +20 |
| IV. "Turpentine" | 16.4 | 14.9 | 42.4 | 48 | 165 | --- | 4.99 | 2.67 | 2.31 | 1.15 | 0.170 | 160 | 210 | +31 |
| V. "Cobalt after Turpentine" | 15.9 | 17.0 | 47.2 | --- | --- | --- | --- | --- | --- | --- | 0.139 | 165 | 217 | +32 |

injected into the posterior muscle of the thigh. A definite abscess appeared by the third week.

In a third experiment the same procedure was followed but 14 rats were placed in each group and a fifth group was added (group V). The new group ("cobalt after turpentine") received the same preliminary treatment as group IV but, after anemia had developed, 0.125 mg. cobalt per rat was given daily. Thus the effect of cobalt on turpentine-induced anemia could be studied.

The first two experiments lasted 11 weeks. Since maximal polycythemia developed by the seventh week, the third experiment was terminated at 7 weeks.

Hemoglobin was determined by the photoelectric oxyhemoglobin method, using
an Evelyn photoelectric colorimeter. The instrument was standardized by the Van Slyke procedure and the hemin method of Clegg and King. Blood was obtained from the tail. The plasma iron was measured according to the procedure of Kitzes, Elvehjem, and Schuette in the first experiment and by the method of Barkan and

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**Fig. 1.** Hemoglobin Levels in Rats Injected with Turpentine (T) Compared with Normal Controls (N), Those Given Cobalt (C), and Those Injected with Turpentine and Given Cobalt as Well (T + C), in Each of Three Experiments

In experiment III there was a fifth group of rats which was treated with cobalt after anemia had developed following the injection of turpentine.

Note that the administration of cobalt tended in large measure to overcome the effect produced by turpentine.

Walker in experiments II and III. The method of Grinstein and Watson was followed for erythrocyte protoporphyrin determination. Serum copper determinations were made by the method of Cartwright, Jones and Wintrobe. Plasma proteins were determined by the biuret method of Kingsley as modified by Weichselbaum. The method of Van Slyke and Cullen was used to measure the urea and ammonia of the urine.

At the end of the experiment the rats were anesthetized with nembutal and blood
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was drawn from the abdominal aorta. Plasma iron, serum copper, erythrocyte protoporphyrin, and plasma proteins were determined on the pooled blood from each group of animals.

RESULTS

The data obtained are summarized in table 1 and the more significant observations are illustrated in figures 1–4.

Figure 1 represents the result of hemoglobin determinations in the various groups in all three experiments. It will be seen that the results were essentially the same throughout. The administration of cobalt led to the development of polycythemia, and the injection of turpentine was associated with the development of a moderate anemia. When cobalt was given in addition to turpentine, the tendency of the latter to produce anemia was overcome and polycythemia, somewhat less marked than that occurring in animals receiving only cobalt, developed.

The impairment of hemoglobin production which resulted from the injection of turpentine was associated with a reduction in the plasma iron level and an increase in erythrocyte protoporphyrin, just as has been observed in patients with anemia accompanying infection. The results were similar in all three experiments (fig. 2). Considering the data for all three experiments, it would not seem that the simultaneous administration of cobalt and turpentine made a significant difference in the degree of hypoferremia produced, even though the impairment in hemoglobin production was largely overcome. The degree of increase in erythrocyte protoporphyrin appeared, on the whole, to be related inversely to the degree of hypoferremia. It is of interest that the administration of cobalt alone tended to be
associated with the development of somewhat lower levels of plasma iron as compared with the controls, and there was at the same time an increase in erythrocyte protoporphyrin. Somewhat higher levels of serum copper were noted in association with the administration of cobalt and the injection of turpentine than were found in the control animals but it remains to be determined whether these differences were significant.

Weight gain tended to be impaired both by the injection of turpentine and by the administration of cobalt, but was influenced more consistently by the latter. In experiment I the injection of turpentine was associated with the most pronounced impairment of weight gain, but in experiments II and III the “turpentine” groups

![Graph showing growth curves for animals in various groups in each experiment.](image_url)
grew almost as well as the control animals while those receiving cobalt grew poorly. In all three experiments the rats receiving both turpentine and cobalt gained the least amount of weight. The data are illustrated in figure 3.

Total plasma protein levels were reduced in all the groups as compared with the controls, except for group II of experiment II. This was due to a reduction in the plasma albumin fraction. The data for experiment III, which we regard as the most reliable of those obtained in the three experiments, are illustrated in figure 4. Nitrogen excretion, as measured by the urine urea plus ammonia, was the same in all groups of rats.

**DISCUSSION**

Since the anemia associated with infection is known to be refractory to therapy, it is of great interest that the administration of cobalt appears to overcome the retardation of hemoglobin formation which is produced by inflammation. Elucidation of the mode of action of cobalt might be expected to yield valuable information concerning the pathogenesis of the anemia of infection.

As yet, however, the mode of action of cobalt in polycythemia in normal animals is not known. It has been reported that the feeding of liver or the injection of liver extract or of ascorbic acid tends to counteract or nullify the effect of cobalt. Cobalt did not produce polycythemia in splenectomized rats or when the diet was deficient in iron or copper. In fact, in the absence of copper, anemia developed. When cobalt was fed prior to the addition of iron and copper, the normal response to such supplements given to dogs made anemic by hemorrhage was inhibited. The report of Barron and Barron that small amounts of cobalt inhibit the respiration, in vitro, of various tissues, notably of reticulocytes and bone marrow, could not be corroborated by Warren et al. It was also demonstrated by the latter workers that the erythroid hyperplasia of bone marrow in cobalt-poly-
RIPIERAL INNERRATION. THEY SUGGESTED AS A MODE OF ACTION OF COBALT THAT THERE IS SOME EFFECT ON THE LIVER WHEREBY THE FORMATION THERE OF METABOLIC PRECURSORS REQUISITE FOR RED CELL PRODUCTION IS ENHANCED. THAT THE ACTION OF COBALT MAY BE BASED ON A NEURAL MECHANISM IS AN HYPOTHESIS ARISING FROM THE REPORTS OF DAVIS THAT CHOLINE AND CERTAIN OTHER VASODILATOR DRUGS DEPRESS OR PREVENT THE POLYCYTHEMIA WHICH FOLLOWS COBALT ADMINISTRATION. GRIFFITH ET AL. HAVE PROPOSED THAT INTERFERENCE WITH CELLULAR OXIDATION WITH THE FORMATION OF COMPLEXES WITH SULFHYDRYL COMPOUNDS, AS FOR EXAMPLE WITH GLUTATHIONE, MAY BE THE STIMULUS TO THE HEMOPOIETIC SYSTEM WHICH CAUSES COBALT POLYCYTHEMIA. THESE INVESTIGATORS OBSERVED THAT METHIONINE, CYSTINE, AND CYSTEINE DECREASE THE TOXICITY OF COBALT. SINCE CHOLINE IS METABOLICALLY RELATED TO THE SULFUR-CONTAINING AMINO ACIDS, ITS COUNTERACTING EFFECT ON COBALT POLYCYTHEMIA MIGHT BE EXPLAINED IN THE SAME WAY. ORTEN AND ORTEN SUGGESTED THAT COBALT OVERCOMES ANEMIA DUE TO PROTEIN DEFICIENCY IN RATS BY MAKING MORE AVAILABLE FOR HEMOGLOBIN SYNTHESIS THE PROTEINS OF THE "METABOLIC POOL.

KATO AND IOB FOUND THAT THE SPLEEN AND BONE MARROW OF DOGS AND RABBITS FED COBALT IN ADDITION TO IRON CONTAINED LESS IRON THAN THOSE OF ANIMALS GIVEN IRON ALONE. THIS WOULD SUGGEST A MORE COMPLETE UTILIZATION OF IRON FOR ERYTHROPOIESIS IN THE PRESENCE OF COBALT. WHATEVER MIGHT BE THE MEANS WHEREBY COBALT ACCOMPLISHES THIS, SUCH AN EXPLANATION IS CONSISTENT WITH AND COULD EXPLAIN OUR OWN OBSERVATIONS. STUDIES IN THIS LABORATORY HAVE SHOWN THAT THE HYPOFERREMIA ASSOCIATED WITH INFECTION IS NOT DUE TO A LACK OF IRON AND CANNOT BE CORRECTED BY INFUSING IRON INTRAVENOUSLY BUT MUST BE ASSUMED TO BE DUE TO SOME DISTURBANCE RELATED TO IRON METABOLISM WHEREBY THE UTILIZATION OF IRON IS AFFECTED. THIS FAULT WOULD APPEAR TO BE CORRECTED IN WHOLE OR IN PART BY THE ADMINISTRATION OF COBALT.

IT IS OF INTEREST THAT THE PLASMA IRON LEVEL OF RATS GIVEN COBALT (GROUP II) WAS LOWER THAN IN THE CONTROLS AND THAT THE ERYTHROCYTE PROTOPORPHYRIN WAS INCREASED. THE HYPOFERREMIA IS CONSISTENT WITH THE ASSUMPTION THAT HEMOGLOBIN SYNTHESIS IS ACCELERATED BY COBALT. A RISE IN ERYTHROCYTE PROTOPORPHYRIN IS ASSOCIATED, ACCORDING TO WATSON, GRINSTEIN, AND HAWKINSON, WITH NORMOBLASTIC ACTIVITY OF THE BONE MARROW, A FEATURE WHICH HAS BEEN OBSERVED REPEATEDLY IN COBALT POLYCYTHEMIA.

THE OBSERVATIONS CITED IN THE PRESENT REPORT WOULD INDICATE THAT NEITHER COBALT NOR TURPENTINE SEEMS TO INCREASE PROTEIN CATABOLISM, SINCE NO APPRECIABLE DIFFERENCE WAS FOUND IN URINARY NITROGEN (UREA PLUS AMMONIA) EXCRETION. THE NORMAL NITROGEN EXCRETION OF THE RATS RECEIVING ONLY COBALT (GROUP II) WOULD SUPPORT THE OPINION OF SEVERAL AUTHORS THAT COBALT IS NOT TOXIC IN THE DOSES ORDINARILY NEEDED TO PRODUCE POLYCYTHEMIA. THE LOWER GROWTH RATE MAY BE EXPLAINED BY A DECREASED FOOD INTAKE, SINCE FROST ET AL. CLAIM THAT COBALT PRODUCES ANOREXIA. UNFORTUNATELY, FOOD INTAKE WAS NOT MEASURED IN OUR ANIMALS.

IT IS PROPOSED TO REPEAT AND EXTEND IN LARGER ANIMALS THE STUDIES REPORTED HERE, SINCE THEREBY IT MAY BE POSSIBLE TO CARRY OUT MORE DETAILED OBSERVATIONS THAN ARE POSSIBLE IN THE RAT.

SUMMARY AND CONCLUSIONS

THE INFLUENCE OF COBALT ON THE ANEMIA ASSOCIATED WITH INFLAMMATION HAS BEEN STUDIED IN THREE EXPERIMENTS INVOLVING OBSERVATIONS IN 108 RATS.

IT WAS FOUND THAT BY THE SIMULTANEOUS ADMINISTRATION OF COBALT THE ANEMIA ASSOC-
associated with inflammation, as produced by the injection of turpentine, could be prevented from developing and polycythemia appeared instead.

This effect was accompanied by hypoferremia and an increase in erythrocyte protoporphyrin values similar to those encountered when anemia develops in association with inflammation.

Similar, though less marked, chemical changes were observed when only cobalt was given and polycythemia was produced.

A decrease in plasma albumin was noted in rats injected with cobalt or turpentine, or both, but this was not accompanied by an increased excretion of urinary nitrogen as measured by the urine urea and ammonia.

The observations cited are consistent with the hypothesis that cobalt favorably influences the utilization of iron for the synthesis of hemoglobin.

REFERENCES
4 Schultz, M. O.: Metallic elements and blood formation. Physiol. Rev. 20: 37, 1940.
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