lipoproteins and chylomicra, there is less need to place its site of activity at the cell membrane. It appears that plasma lipoprotein lipase is stimulated by fat intake(2,12), is destroyed by passage through the liver(13), and is inactivated in the process of lipolysis. Thus the quantity of demonstrable circulating enzyme at a given moment is a resultant of its stimulation and release on the one hand, and its destruction and inactivation on the other. It is therefore not surprising that lipoprotein lipase can only occasionally be demonstrated in the blood since physiologically it is probably slowly released in moderate amounts in response to lipemia, and it dissociates as lipolysis occurs.

Summary. Evidence has been obtained by *in vitro* studies that human post-heparin and endogenous plasma lipolytic activity (lipoprotein lipase) are inactivated during the process of triglyceride lipolysis. Addition of heparin to supernatant plasma after removal of lipoprotein lipase activity by $Ca_3(PO_4)_2$ adsorption occasionally restores its lipemia clearing activity. This indicates that heparin is a component of the enzyme, and that its dissociation from the apoenzyme readily occurs.

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Experimental Production of Polycythemia in Humans by Administration of Cobalt Chloride. (24395)

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The erythropoietic stimulating action of cobalt was first demonstrated by Waltner and Waltner(1) who produced polycythemia in rats by administration of this element and its chloride. Since that time, polycythemia has been induced in many animals such as rabbit (2), dog(3,4), duck(5), chicken(6) and other animals(7), by continued administration of cobalt salts. Although it is logical to presume that cobalt can produce polycythemia in normal human beings, we have been unable to find data in the literature which actually demonstrate ability of cobalt to cause this condition in healthy people. Berk, Burchenal and Castle(8) have shown that cobalt exerted erythropoietic stimulating effect in some hospitalized patients, most of whom were anemic, but very seldom did their erythrocyte numbers approach polycythemic levels. We believe that the results of the investigation herein described constitute the first clear-cut demonstration of experimental production of polycythemia in normal humans by daily administration of a cobalt salt.

Procedure. Six apparently normal men, ranging 20 to 47 years in age, served as subjects. Red blood cell counts, hemoglobin percentages, leukocyte counts, reticulocyte percentages and thrombocyte counts were determined during pre-experimental control period as well as during experimental period of cobalt medication. Hemoglobin was estimated by the Sahli method. Reticulocyte and thrombocyte counts were made on wet mount preparations in which a drop of fresh blood on slide was covered with cover slip coated with film of brilliant cresyl blue stain. Erythrocyte counts were made with a Spencer

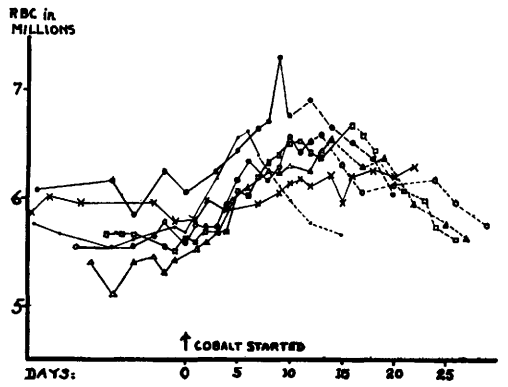

FIG. 1. Erythrocyte numbers in 6 subjects before, during and after periods of daily cobalt administration. Dashed lines indicate recovery periods following cessation of medication.

bright line hemacytometer. Counts were made in duplicate and averaged. If the variation in numbers was great, further counts were made, and entered into the average. Observations on blood were made during pre-experimental control periods of about 2 weeks, after which each subject was given daily oral doses of cobaltous chloride for variable periods of time during which observations on blood were continued. Five subjects received 150 mg of cobalt chloride daily, while one was started on 120 mg/day, which later was increased to 150 mg. The daily dose was given in divided doses at meal times. A 2% solution of cobalt chloride was either ingested and followed by water or milk, or diluted prior to ingestion. Blood samples were obtained from free-flowing punctures of fingertips at approximately the same hour of day for each individual. In all cases, blood samples were taken at least 2 hours after eating, and at least 15 hours subsequent to last dosage with cobalt.

Results. Fig. 1 shows red blood cell counts on 6 subjects before, during and after the experimental periods of cobalt medication. It will be seen that cobalt feeding caused development of polycythemia in all 6 subjects within 7 to 22 days. Increases in red cell numbers ranged from 0.5 to 1.19 million, with average increase of 0.96 million. Polycythemic erythrocyte counts returned to normal within 9 to 15 days after cessation of cobalt administration.

Fig. 2 illustrates development of cobalt polycythemia in one of our human subjects. In addition to erythrocyte numbers, this graph also shows hemoglobin percentages (100% equals 14.5 g hemoglobin/100 cc of blood), total leukocyte counts and reticulocyte percentages.

The initial, control reticulocyte values ranged between 0.8 and 1.9%, with average of 1% for the 6 subjects. Following commencement of daily cobalt feeding, reticulocyte percentages began climbing and reached a peak value in all subjects between fifth and ninth experimental days. Highest reticulocyte peak value was 2.7%; lowest was 1.9%. In 5 of the 6 subjects, reticulocyte percentages reached at least twice the pre-experimental values.

Hemoglobin percentages were increased to a lesser extent than erythrocyte numbers by cobalt chloride administration. They were increased by 6 to 11%, which is in disproportion to the increases of 16 to 21% which occurred in erythrocyte numbers of 5 of our 6 cases.

Total leukocyte counts, which were followed frequently, remained relatively constant throughout experimental period without deviating significantly from pre-experimental counts.

Although thrombocyte counts fluctuated widely, no consistent or uniform changes in their values occurred in our subjects.

Discussion. We believe that our results show that cobalt chloride administration can cause a true experimental polycythemia. The early mild reticulocytosis, the slow develop-

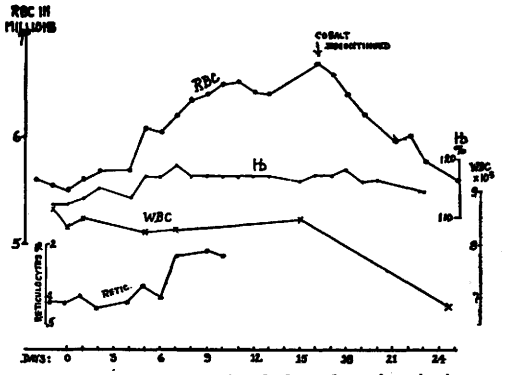

FIG. 2. Development of cobalt polycythemia in one human subject. Daily administration of cobalt chloride was started at zero time.

ment of increased erythrocyte numbers during cobalt medication, and gradual recovery to normal after cessation of cobalt chloride feeding, indicate that we are not dealing with a simple concentration of blood, but with stimulation of bone marrow activity. The fact that leukocyte counts remained relatively constant throughout the experiment also supports the concept that this is a true polycythemia. Although blood volume determinations were not made, we have previously shown that cobalt chloride feeding increases the blood volume in dogs(9).

One subject (designated by ×'s in Fig. 1) was given only 120 mg of cobalt chloride daily for the first 15 days. He showed a good reticulocyte response, but a rather mild increase in erythrocytes. The daily dose was then raised to 150 mg for the next 7 days but this caused little additional increase. We did not continue cobalt administration longer than necessary because of the possibility that prolonged use of high doses might ultimately prove deleterious to healthy people. For example, we have shown in ducks(5) that cobalt can first cause hyperplasia of bone marrow, but may finally cause hypoplasia. In the present experiment, the only untoward effects noted during cobalt administration, were occasional headache and vague abdominal discomfort. In one subject the occurrence of pimples on the face may have been due to cobalt. This condition was mistaken for acne rosacea by a dermatologist.

Weissbecker and Maurer(10) briefly stated that cobalt can produce polycythemia in people, but did not publish data to substantiate this claim.

Berk, Burchenal and Castle(8) showed an erythropoietic stimulating effect of cobalt in 17 hospitalized patients who were allegedly hematologically normal people. However a close look at initial erythrocyte counts of these patients reveals that most of them were anemic, at least mildly so. Administration of cobalt exerted an erythropoietic stimulating action in most of these patients, but 8 of 17 patients *did not* reach erythrocyte numbers of 5 million, while only 3 achieved counts of 6 million or more.

All of our subjects were healthy and hematologically normal, and developed quite definite polycythemias in response to cobalt chloride.

Conclusion. Daily oral administration of 150 mg of cobaltous chloride to 6 normal human subjects caused development of increased erythropoiesis characterized by erythrocyte increases ranging from 0.5 to 1.19 M (average 0.96 million) cells/mm^3 of blood within 7 to 22 days. The high red blood cell counts returned to normal within 9 to 15 days after cessation of cobalt administration.

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