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Cobalt Polycythemia. II. Relative Effects of Oral and Subcutaneous Administration of Cobaltous Chloride.

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(Introduced by Arthur A. Hellbaum.)

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In spite of the fact that the efficacy of cobalt salts in stimulating erythropoiesis is well established, cobalt therapy is little used in the treatment of anemia.[1] This is probably due to the fact that many experimenters have given cobalt salts to animals by subcutaneous injection, whereas oral administration should be preferred for human use. Also, there are few data concerning relative toxicity. No data have been recorded concerning the relative effects of cobalt salts on erythrocyte and hemoglobin production when administered orally, in contrast to subcutaneous injection. It has therefore been impossible to evaluate the effects of subcutaneous injection in terms of effective oral dosage. Approximately 4 times as much cobalt is excreted in the urine within the first 72 hours following subcutaneous administration as when the same amount is given orally.[2,3] This difference is apparently related to the difference in rates of absorption.

In order to determine the effects of cobalt on erythrocyte and hemoglobin production under conditions of oral versus parenteral administration, and to establish further the optimal and minimal toxic dosage of this substance, the following experiment was devised. Of 30 albino rats, Sprague-Dawley strain, weighing approximately 250 g each, 24 received cobaltous chloride;* 6, untreated, served as controls. The group of animals to be treated was subdivided into 6 groups of 4 each. Group I received 2.5 mg/kg $CoCl_2 \cdot 6H_2O$ daily by mouth. Group 2 received 2.5 mg/kg daily by subcutaneous injection. Groups 3 and 4 received 10 mg/kg daily, orally and by subcutaneous injection, respectively. Groups 5 and 6 received 40 mg/kg daily, orally and by subcutaneous injection, respectively.

Each dose of cobalt salt was injected subcutaneously in a solution of 0.2ml of distilled water. For oral administration, a solution of the cobalt salt was mixed with equal parts of wheat flour and powdered sugar to form a stiff paste and packed in No. 5 gelatin capsules. Just before administration the capsules were moistened and then inserted far back in the animal's pharynx. This method allowed a careful control of the oral dosage both as to amount and time of administration.

The experiment was continued for 8 weeks. Blood counts and hemoglobin determinations were made at the beginning of the experiment and at 2-week intervals. These data are summarized in Table I. It is apparent that 2.5 mg $CoCl_2 \cdot 6H_2O$/kg/day, given subcutaneously, effected an increase of 30% in the number of erythrocytes per cu mm of blood in 6 weeks. It required approximately 40 mg/kg/day of cobaltous chloride by mouth, or 16 times as much to produce a comparable increase in erythrocytes. Animals which received 10 mg/kg of cobaltous chloride daily by the subcutaneous route presented a more prompt erythrocyte and hemoglobin response. A daily dose of 2.5 mg/kg produced an essentially

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[1] Stanley, A. J., Hopps, H. C., and Hellbaum, A. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 130.

[2] Greenberg, D. M., Copp, D. H., and Cuthbertson, E. M., *J. Biol. Chem.*, 1943, **147**, 749.

[3] Sheline, G. E., Chaikoff, I. L., and Montgomery, M. L., *Am. J. Physiol.*, 1946, **145**, 285.

* Preparations of $CoCl_2 \cdot 6H_2O$ labeled "Reagent" vary considerably in their content of impurities. At least one such preparation contained sufficient lead to produce lead intoxication if given in amounts described here. We have restricted our use to the preparation marketed by Eimer and Amend and labeled "Cobalt Chloride C. P. $CoCl_2 \cdot 6H_2O$ Mol. wt. 237.95 Cat. no. C-371."

TABLE I.
Relative Effects of Cobaltous Chloride ($CoCl_2 \cdot 6H_2O$) on Production of Erythrocytes and Hemoglobin in Rats when Administered Orally and by Subcutaneous Injection.

No. animals	Daily dose mg/kg Oral	Daily dose mg/kg Subcut.	Erythrocytes in millions/cu mm. Time in weeks 0	2	4	6	8	Hemoglobin in g per 100 ml Time in weeks 0	2	4	6	8
4	2.5		8.84	9.07	8.90	9.56	8.64	16.2	16.2	17.0	17.0	17.2
4		2.5	8.65	9.44	10.33	11.38	11.65	16.1	16.4	19.4	21.8	22.5
4	10.0		9.03	9.76	10.28	10.58	10.40	16.2	16.6	18.2	20.0	20.0
4		10.0	9.36	10.07	12.59	11.98	11.73	16.4	18.2	22.2	22.9	22.4
4	40.0		8.83	9.25	10.05	11.17	11.42	15.9	17.0	18.6	20.8	21.7
4*		40.0	8.84	10.01	—	—	—	15.8	17.8	—	—	—
6	Controls		8.72	9.01	9.51	8.82	9.06	16.0	16.5	16.7	16.8	16.2

* None of these 4 animals survived beyond 8 days due to the toxicity of cobalt in such massive doses. Erythrocyte and hemoglobin determinations were made at the end of 6 days rather than at 2 weeks. The animals receiving the same dose orally survived.

similar effect, but required an additional 2 weeks.

Rats which received 2.5 mg/kg of cobaltous chloride subcutaneously or 40 mg/kg by mouth did not manifest significant toxic effects. Their body weight remained essentially constant, as did that of the controls. Rats which received 10 mg/kg of cobaltous chloride subcutaneously lost an average of 24% of their body weight by the end of 6 weeks. Those animals which received 40 mg/kg daily, subcutaneously, survived 5, 7, 8, and 8 days, respectively.

Summary. The relative effect of parenteral versus oral administration of cobaltous chloride on erythrocyte and hemoglobin formation in rats has been determined. Daily subcutaneous injection of 2.5 mg/kg of body weight, over a period of 6 weeks, resulted in an increase of more than 30% in the number of erythrocytes per cu mm and in grams of hemoglobin per 100 ml of blood. To produce a similar effect from oral administration of cobaltous chloride, within the same period of time, 40 mg/kg of body weight was required. These dosages are without significant toxic effects. Rats which received 10 mg cobaltous chloride/kg daily, subcutaneously, averaged 24% weight loss by the end of 6 weeks. Rats which received 40 mg/kg body weight, subcutaneously, did not survive beyond 8 days.

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Inhibition of the Hirst Haemagglutination Reaction by Pneumococcal Extract, Normal Serums, and Blood Cell Esterases.*

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During the course of other studies it was observed that culture filtrates of Type I and Type V pneumococci would inhibit the agglutination of chicken red cells by influenza virus. Stone[1,2] found that lecithinase would

* While the final draft of this paper was being prepared several studies[3-5] were reported before the Society of American Bacteriologists, in Philadelphia, dealing with the nature of agglutinin-inhibiting substances.

1 Stone, J. D., *Australian J. Exp. Biol. and Med. Sci.*, 1946, **24**, 191.

2 Stone, J. D., *Australian J. Exp. Biol. and Med. Sci.*, 1946, **24**, 197.