

5. Monod, J., Wollman, E., *Ann. Inst. Pasteur*, 1947, v73, 937.
6. Kun, E., Fanshier, D. W., *Fed. Proc.*, 1958, v17, 259.
7. Kun, E., *Biochim. Biophys. Acta*, 1957, v25, 130.
8. Friedmann, T. E., Haugen, G. E., *J. Biol. Chem.*, 1943, v147, 415.
9. Goldstein, F., *ibid.*, 1950, v187, 523.
10. Sorbo, B., *Acta Chem. Scand.*, 1951, v5, 1218.

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Enhancement of Hematopoietic Action of Hydrocortisone by Cobalt in Adrenalectomized Rats. (24561)

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We have recently reported that adrenalectomy interferes with the hematologic response to cobalt in the rat although rats subjected to a sham operation respond to cobalt as readily as normal rats(1). Further analysis of this effect seemed to require experiments in which adrenalectomized rats were treated with both cobalt and adrenal hormones. Several reports indicating that adrenocortical hormones stimulate erythropoiesis in the bone marrow of the adrenalectomized and intact rat(2-5) and induce an elevation in total circulating red cell volume in the normal rat(6) suggested that the hormones of the adrenal cortex should be investigated in this way. The present report describes the results of concurrent administration of cobalt and hydrocortisone to adrenalectomized rats in which it was found that these substances together stimulate erythropoiesis considerably more effectively than hydrocortisone alone.

Materials and methods. Male albino rats of the Wistar (Purdue) strain were studied in 2 experiments: (I), 8 control rats, 8 adrenalectomized rats receiving 2 mg hydrocortisone per kg daily and 8 adrenalectomized rats receiving a combination of 2 mg hydrocortisone and 2.5 mg elemental cobalt per kg daily for a period of 28 days after operation. (II) 8 control rats, 8 adrenalectomized rats receiving

1 mg hydrocortisone per kg daily, and 8 adrenalectomized rats receiving a combination of 1 mg hydrocortisone and 2.5 mg elemental cobalt per kg daily for a period of 28 days after operation. Erythrocyte counts, hemoglobin and hematocrit determinations were made at 10, 20 and 28 days. The hydrocortisone and cobalt injections were started on the day following the operation. Total red cell volumes were determined at 28 days. Cobalt chloride[†] was given as an intraperitoneal injection daily at a dosage of 2.5 mg elemental cobalt (10 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) per kg of body weight. The hydrocortisone acetate[‡] was suspended in 0.9% NaCl solution and injected subcutaneously daily at various sites under the skin of the back. Blood was obtained for erythrocyte, hemoglobin and hematocrit determination by anesthetizing the rat with ether and clipping the tail. Erythrocyte counts were done with U.S. Certified blood pipettes and the improved Neubauer counting chamber. Hematocrit determinations were made with Van Allen hematocrit tubes (1.6% aqueous sodium oxalate as diluent) which were spun for 30 minutes at 3,000 rpm with a radius of 17 cm. Hemoglobin determinations were made on the Klett-Summerson colorimeter by the acid hematin method(7). Body weights were determined at the time of the hematologic studies. Red cell volume was

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[†]Analytical grade $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ with maximum limits of impurity for lead of 0.005%.

[‡]Hydrocortisone acetate supplied through courtesy of Upjohn Co., Kalamazoo, Mich.

TABLE I. Effects of Combined Injections of Hydrocortisone and Cobalt on Blood Picture of Adrenalectomized Rat.

Day	No. rats	B.W.	RBC	Hemat	HB	MCV	MCH	MCHC	Red cell vol
EXP. I									
Nonadrenalectomized—No treatment									
0	8	357.4 ± 23.4	9.5 ± .78	44.4 ± 2.7	16.0 ± 1.3	47.7 ± 1.7	16.8 ± .95	35.8 ± 1.1	
10	8	362.7 ± 22.7	9.5 ± 1.1	44.5 ± 2.1	16.7 ± 1.0	45.7 ± 7.0	17.6 ± 1.3	37.4 ± 1.6	
20	8	389.5 ± 22.5	9.4 ± 1.0	43.1 ± 1.7	15.4 ± 1.3	45.5 ± 4.1	16.6 ± 2.0	35.8 ± 3.2	
28	8	391.0 ± 30.6	8.0 ± 1.8	47.0 ± 1.4	15.0 ± .78	49.3 ± 4.7	18.0 ± 5.0	31.1 ± 1.4	2.68 ± .45
Adrenalectomized—hydrocortisone—2 mg/kg/day									
0	8	335.4 ± 26.9	8.2 ± .97	43.6 ± 1.6	15.1 ± .81	54.0 ± 8.2	18.7 ± 2.9	34.6 ± 1.5	
10	8	274.3 ± 28.7	13.5 ± 1.2	47.3 ± 3.3	17.2 ± 1.9	35.3 ± 2.6	13.4 ± .60	38.4 ± 2.2	
20	7	281.0 ± 33.6	11.4 ± 1.4	45.5 ± 3.4	17.3 ± 1.7	39.4 ± 5.3	15.3 ± 1.2	38.5 ± 1.9	
28	5	258.0 ± 36.7	11.4 ± 2.0	48.0 ± 5.5	16.6 ± 1.7	42.5 ± 4.2	14.8 ± 2.1	34.7 ± 2.6	2.40 ± .46
Adrenalectomized—cobalt—hydrocortisone—2 mg/kg/day									
0	8	325.4 ± 36.5	9.5 ± 1.1	44.1 ± 2.8	16.5 ± 1.2	46.9 ± 6.5	17.7 ± 2.5	37.5 ± 1.4	
10	8	270.0 ± 24.1	11.4 ± 1.4	50.3 ± 3.8	19.2 ± .94	44.4 ± 4.4	17.0 ± 2.0	38.3 ± 2.9	
20	7	276.0 ± 28.0	13.5 ± 1.4	55.0 ± 4.6	19.6 ± 2.4	41.1 ± 6.4	14.6 ± 1.8	35.6 ± 3.5	
28	5	223.0 ± 25.3	14.7 ± 1.8	65.6 ± 3.8	19.3 ± 1.7	45.0 ± 4.5	13.2 ± 1.4	29.4 ± 1.4	9.06 ± 1.08
EXP. II									
Nonadrenalectomized—No treatment									
0	8	320.6 ± 31.6	8.7 ± .87	41.2 ± 2.0	14.6 ± .60	47.5 ± 5.4	16.9 ± 1.5	35.4 ± 1.3	
10	8	340.6 ± 32.5	9.6 ± 1.3	43.2 ± 1.0	14.7 ± .45	45.7 ± 6.9	15.6 ± 2.3	34.4 ± 1.6	
20	8	357.8 ± 28.4	9.2 ± .96	41.8 ± 2.4	14.7 ± .80	45.5 ± 4.0	16.2 ± 1.9	35.3 ± 1.2	
28	8	379.0 ± 29.8	9.6 ± 1.1	47.0 ± 2.0	15.0 ± .96	49.3 ± 4.8	15.8 ± 3.5	32.0 ± 2.0	2.61 ± .10
Adrenalectomized—hydrocortisone—1 mg/kg/day									
0	8	321.4 ± 20.9	8.4 ± 1.5	41.8 ± 2.2	14.3 ± .93	50.6 ± 8.9	17.2 ± 2.7	34.0 ± 2.3	
10	8	282.1 ± 24.2	10.0 ± 2.0	40.4 ± 4.5	13.9 ± 2.0	41.6 ± 8.6	14.1 ± 3.3	32.8 ± 4.0	
20	8	260.8 ± 15.7	8.8 ± 2.5	42.0 ± 3.5	14.4 ± .98	53.1 ± 11.9	16.1 ± 4.0	35.5 ± 1.2	
28	8	252.0 ± 14.6	9.0 ± 1.9	48.0 ± 2.0	13.5 ± .79	52.5 ± 10.7	15.7 ± 3.5	28.2 ± 2.2	3.58 ± .28
Adrenalectomized—cobalt—hydrocortisone—1 mg/kg/day									
0	8	299.3 ± 24.1	9.0 ± 1.7	40.8 ± 1.2	14.4 ± .99	48.7 ± 9.4	16.6 ± 3.7	35.3 ± 1.7	
10	8	274.0 ± 20.5	10.6 ± 1.4	43.7 ± 3.7	14.7 ± 1.1	41.8 ± 5.4	14.2 ± 2.4	33.8 ± 2.2	
20	7	262.0 ± 47.5	11.9 ± 1.6	47.6 ± 5.5	17.3 ± 1.9	40.3 ± 4.5	14.7 ± 1.9	36.4 ± 2.6	
28	5	241.0 ± 51.0	12.5 ± 1.5	60.0 ± 4.9	18.6 ± 2.1	48.9 ± 4.0	15.0 ± 1.9	32.1 ± 3.6	6.66 ± .66

Day = Day treatment; B.W. = Body wt in g; RBC = Red cell count in millions/mm³; Hemat = Hematocrit in %; HB = Hemoglobin in g/100 ml; MCV = Mean corpuscular vol in μ³; MCH = Mean corpuscular hemoglobin in μg; MCHC = Mean corpuscular hemoglobin conc. in %; ± = Stand. dev. and red cell vol = ml red cells/100 g B.W.

determined with P³² tagged erythrocytes by the method of Hevesy and Zerahn (8) as modified by Berlin *et al.* (9). The tagged cells were injected into a vein, exposed by an incision on the inner aspect of the thigh, and a blood sample removed *via* heart puncture for counting after 10 minutes. The blood was dried on aluminum planchets and counted with an end window Geiger-Mueller counter. Bilateral adrenalectomy was performed under ether anesthesia by a short transverse incision through the skin and lumbar muscles just below the last rib on each side. In order to insure against leaving behind any closely adhering accessory adrenal tissue, the fat surrounding the gland to a distance of 1/2 to 1 cm was

removed on each side. Each animal adrenalectomized was given 10,000 units of Procaine Penicillin intramuscularly at time of operation and on the following day. The adrenalectomized rats were maintained on 0.9% NaCl. At the end of the study, the adrenalectomized animals were examined for evidence of accessory adrenal tissue. P values were determined by Fisher's "t" test.

Results. The data presented in Table I indicate that the anemia of adrenalectomy was prevented, for the most part, by administration of hydrocortisone at a dose of 1 mg/kg/day. After 28 days of treatment with 1 mg hydrocortisone there was an increase of 15% in hematocrit, 7% in rbc count and a 6% de-

crease in hemoglobin. The MCV was markedly decreased at 10 days but was slightly above normal at 20 and 28 days. The red cell volume was 3.58 ml per 100 g body weight.

Hydrocortisone at a dose of 1 mg/kg per day given concurrently with cobalt at a dose of 2.5 mg/kg daily induced a marked elevation in hematocrit (47%), rbc count (39%) and hemoglobin (29%). The red cell volume was 6.66 ml/100 g at 28 days. MCV was decreased at 10 and 20 days but returned to normal at 28 days. The adrenalectomized rats progressively lost weight when treated with 1 mg hydrocortisone with or without cobalt.

The 2 mg/kg dose of hydrocortisone in adrenalectomized rats resulted in 10% increase in hematocrit, 39% increase in rbc count, and 10% increase in hemoglobin at 28 days. However, total red cell volume was only 2.4 ml per 100 g. The combination of 2 mg hydrocortisone and cobalt induced a pronounced rise in hematocrit (49%), rbc count (55%) and hemoglobin (17%). The red cell volume was elevated to the extremely high value of 9.1 ml per 100 g. Adrenalectomized rats treated with either 2 mg hydrocortisone or the combination progressively lost weight, the former decreasing 30% and the latter 46% in 28 days. Autopsies at 28 days revealed no evidence of accessory adrenals in any adrenalectomized animal receiving either hydrocortisone alone or the combinations.

Discussion. The remarkable increases in hematocrit and red cell volume evoked by the combination at 20 and 28 days suggest a synergistic (more than additive) action between cobalt and hydrocortisone. A thorough analysis of this synergism becomes quite involved because (1) adrenalectomy alone produces temporary anemia(1.4); (2) cobalt in

the adrenalectomized animal may correct the anemia during the first 20 days, although satisfactory statistical evidence for this is lacking(1); (3) cobalt clearly stimulates the adrenalectomized rat during the second month after adrenalectomy(1); (4) the complete dose-response curve for hydrocortisone in the adrenalectomized rat is not available; (5) loss of weight in certain groups introduces an extraneous variable and (6) the large number of different groups needed makes it quite difficult to carry out a complete experiment in which all variables are studied simultaneously.

Summary. In the rat, hydrocortisone prevented the anemia of adrenalectomy and in one experiment caused a moderate increase in total red cell volume. Hydrocortisone and cobalt injected concurrently in adrenalectomized rats produced a marked elevation in hematocrit, rbc count, hemoglobin and total red cell volume. This joint action of cobalt and hydrocortisone at least equalled the sum of the separate effects.

1. Fisher, J. W., *Endocrinol.*, 1958, v62, 547.
2. Fruhman, G. J., Gordon, A. S., *Proc. Soc. Exp. Biol. and Med.*, 1955, v88, 130.
3. ———, *Endocrinol.*, 1955, v57, 711.
4. Gordon, A. S., Piliero, S. J., Landau, D., *ibid.*, 1951, v49, 497.
5. Hayes, M. A., Baker, B. L., *Univ. Mich. Med. Bull.*, 1952, v18, 109.
6. Fisher, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1958, v97, 502.
7. Wintrobe, M. M., *Clinical Hematology*, 4th Edit., Lea & Febiger, Phila., 1956.
8. Hevesy, G., Zerahn, K., *Acta Physiol. Scand.*, 1942, v4, 376.
9. Berlin, N. I., Huff, R. L., Van Dyke, D. C., Hennessy, T. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 176.

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