Summary and Conclusions. Priscol (benzyl-imidazoline) is sympatholytic as well as adrenolytic in reference to the sympathetic neural control of blood pressure and salivation. Adrenolysis invariably precedes sympatholysis with any dose that is adequate to produce both effects. These pharmacodynamic actions persist during priscol hypotension, pituitrin hypertension and in the presence of atropine.

Although sympathetic depression of salivation and vasoconstriction can be produced with variable doses of priscol, up to 15 mg, they are without sympatholytic or adrenolytic effect upon the cervical sympathetic neural components controlling mydriasis, indicating that a differential sympathetic depression characterizes this antisympathetic agent.

15249

Observations on Cobalt Polycythemia. I. Studies on the Peripheral Blood of Rats.*

ALLAN J. STANLEY, HOWARD C. HOPPS, AND ARTHUR A. HELLBAUM.

From the Departments of Physiology, Pathology, and Pharmacology, University of Oklahoma School of Medicine, Oklahoma City, Okla.

The experimental production of polycythemia by the administration of cobalt has been demonstrated by a number of investigators since its discovery by the Waltners.[1-9] This effect of cobalt has also received application in the treatment of various anemias in both animals and human beings.[10,11]

The mechanism of action of cobalt remains obscure. Several theories have been advanced, however none has been fully agreed upon as a satisfactory explanation by those who have investigated the problem. During the course of some experiments relating to factors influencing various types of anemia, certain new observations concerning the action of cobalt came to our attention.

Male rats of the Sprague-Dawley strain, 4 to 5 months of age, weighing approximately 200 g were given cobaltous chloride, $CoCl_2 \cdot 6(H_2O)$, 0.4% solution, by subcutaneous injection in the back and shoulder region 6 times weekly throughout a period of 8 months. Nine animals, divided into 3 groups of 3 each, received 2.5 mg, 5.0 mg and 10.0 mg per kg of body weight respectively. Other animals of the same age, weight, and sex served as controls. The rats were fed dog chow only (Friskies, Albers Milling Co.)

Erythrocyte, reticulocyte, leucocyte and differential counts as well as hemoglobin determinations were made every 2 weeks. Care was taken to draw only the amount of blood necessary for the determinations, the puncture wound in the tail vein being promptly closed after each bleeding. A Haden-Hausser hemoglobinometer (Improved Clinical Model) was used for the hemoglobin determinations.

At the end of 32 weeks, blood volumes

* This work was supported by a grant from the University of Oklahoma School of Medicine.

1 Waltner, K., and Waltner, K., *Klin. Wchnschr.*, 1929, **8**, 313.

2 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 174.

3 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **99**, 457.

4 Davis, J. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 96.

5 Barron, A. G., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 407.

6 Mascherpa, P., *Arch. ital. biol.*, 1930, **82**, 112; *Chem. Abstr.*, 1930, **24**, 3280.

7 Sutter, J., *C. R. Soc. de biol.*, 1934, **116**, 994.

8 Stare, F. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1933, **99**, 473.

9 Davis, J. E., McCullough, A. W., and Rigdon, R. H., *J. Lab. and Clin. Med.*, 1945, **30**, 327.

10 Kleinberg, W., Gordon, A. S., and Charipper, H. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 119.

11 Kato, K., *J. Ped.*, 1937, **11**, 385.

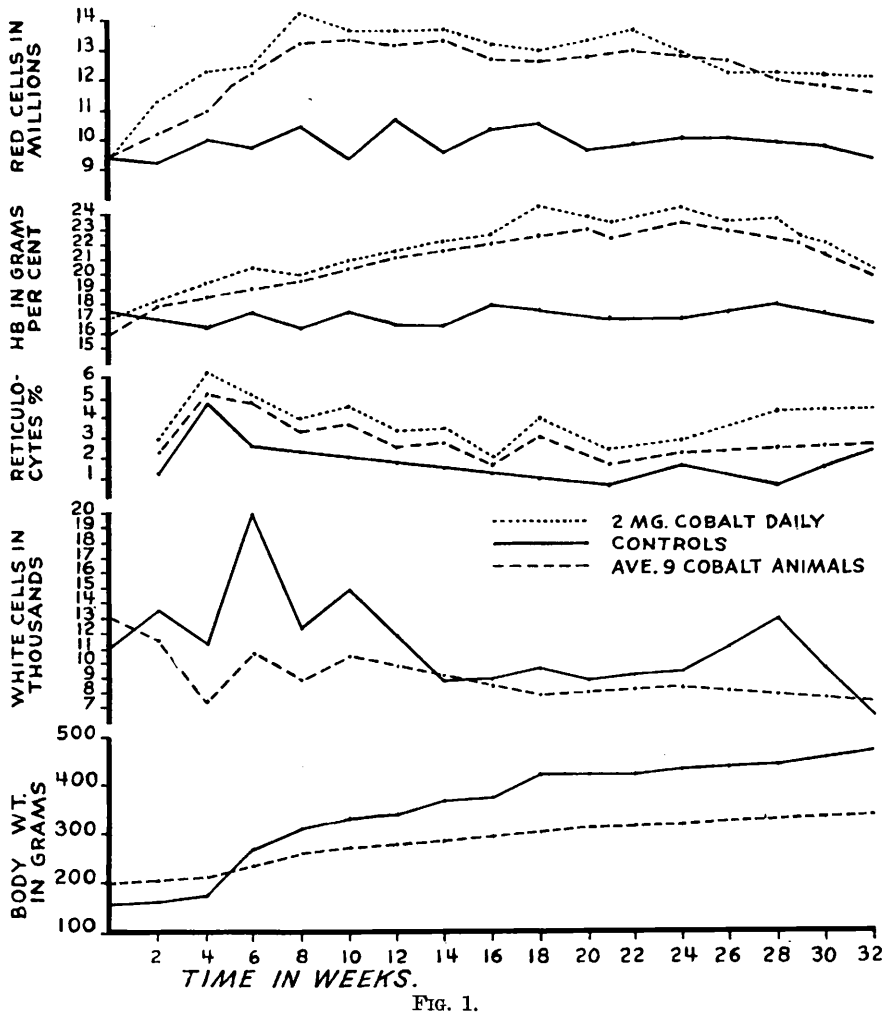

FIG. 1.

The course of polycythemia produced by cobalt in rats. The dotted line in each of the 3 upper charts shows the maximal values in red cells, hemoglobin, and reticulocytes produced by 2 mg of cobaltous chloride daily.

were determined in the following manner. The dye T-1824 (Evans blue) in a solution of 1.0 mg per ml was injected into a tail vein in amounts of 1.5 mg per kg of body weight. Twenty-five minutes±30 seconds were allowed for the dye to mix with the circulating blood. The animals were lightly anaesthetized with ether for one to 1½ minutes prior to drawing 2.0 ml of blood by heart puncture. A precise amount of dry ammonium and potassium oxalate was used as an anticoagulant. Samples for blood counts were taken from the non-oxalated blood while smears for differential leucocyte and reticulocyte counts were made from the oxalated blood. The mean cell diameters of the red cells were determined by means of a Haden-Hausser erythrocytometer using a fresh solution of 1.4% sodium oxalate as the diluent. Hematocrit determinations were made and the supernatant plasma was used for plasma volume calculation. A Klett-Summerson Photoelectric colorimeter employing a Wratten filter F-29 was used to determine the dye concentration. In order to limit blood loss to a minimum, the plasma of each animal was diluted from 1:4 to 1:7 with saline and centrifuged immediately prior to reading.

TABLE I.
Blood Values of Cobalt-Injected Rats.

Rat No.	Body wt, g	Hematocrit	Blood vol., cc	Blood vol., cc/kg	Plasma vol., cc	Plasma vol., cc/kg	Red cell vol., cc	Red cell vol., cc/kg	Blood/body wt	Red cells/body wt	Plasma/body wt.
1	340	76	44.46	130.8	10.67	31.4	33.79	99.4	13.07	9.94	3.14
2	345	Data lost									
3	360	72	49.51	137.6	13.86	38.6	35.65	99.0	13.76	9.90	3.86
4	264	81	43.00	162.8	8.17	30.9	34.83	131.9	16.28	13.19	3.09
5	343	65	41.60	121.2	14.56	42.4	27.04	78.9	12.12	7.88	4.24
6	338	75	59.24	175.2	14.81	43.8	44.43	131.4	17.52	13.14	4.38
7	296	76	47.84	161.6	11.48	38.7	36.36	122.9	16.16	12.29	3.87
8	332	77	54.04	162.7	12.43	37.4	41.61	125.3	16.27	12.53	3.74
9	321	74	44.56	138.8	11.58	36.0	32.98	102.8	13.88	10.28	3.60
Avg	324	74.5	48.03	148.8	12.19	37.4	35.83	111.4	14.88	11.14	3.74
					Controls.						
10	457	43.5	34.12	74.6	19.38	42.4	14.74	32.2	7.46	3.22	4.24
11	487	48	38.00	78.0	19.76	40.5	18.24	37.4	7.79	3.74	4.05
12	315	49	29.6	94.0	15.1	47.9	14.5	46.0	9.40	4.60	4.79
13	312	44	26.4	84.6	14.8	47.5	11.6	37.1	8.46	3.71	4.75
Avg	393	46.1	32.03	82.8	17.26	44.5	14.79	38.2	8.28	3.82	4.45
					Difference between controls and experimental animals.						
		28.4	16.00	66.0	5.07	7.1	21.04	73.2	6.60	7.32	.71
					% increase or decrease experimental over controls.						
		+61	+50	+80	—29.3	—16	+142	+192	+80	+192	—16

Data derived from hematocrit and plasma volume determinations at the end of 32 weeks of cobalt injections. Rats 1, 2, and 3 received 0.5 mg cobalt daily; rats 4, 5, and 6 received 1.0 mg cobalt daily; rats 7, 8, and 9 received 2.0 mg cobalt daily.

TABLE II.
Data Relative to Shape, Size, and Hemoglobin Content of Red Cells of Rats After Injections of Cobalt.

	Controls	Cobalt	% increase
Mean cell diameter in μ	6.45	6.55	—
Mean red cell volume in μ^3	47.0	66.5	41.0
Mean red cell thickness in μ	1.44	1.97	36.8
Mean red cell hemoglobin in $\mu\mu g$	17.0	17.0	—
Mean red cell hemoglobin conc. in %	36.4	26.5	—27.4

Data relative to the red cells of rats injected with cobalt. The mean corpuscular volume and mean corpuscular thickness are derived from red cell counts, hematocrit determinations and mean cell diameters. The hemoglobin values are calculated from hemoglobin determinations, red cell counts and total red cell mass.

Calibration curves were made on rat plasma containing known amounts of T-1824 and also diluted 1:4 to 1:7. Dilution of rat plasma with as much as 7 parts of saline did not alter the absorption characteristics of T-1824 as determined by the photoelectric colorimeter. At the time the blood volume determinations were made, blood counts also were made using blood taken from a tail vein and from the heart of each animal. The differences between the two counts for each animal were within the tolerated limits of technical error.

Fig. 1 summarizes the data obtained at

regular 2-week intervals. It is apparent that the response of reticulocytes, red cells, and hemoglobin is influenced slightly by the dosage of cobalt but that the average figure for the 3 groups is close to that elicited by the maximum dose of 10.0 mg per kg of body weight. The curves for body weight and leucocyte count similarly show no marked differences as affected by the 3 doses given. While the experimental animals failed to gain as much weight as the controls, no other deleterious effects were noted, which indicates a low systemic toxicity from cobaltous chloride in adult animals in the dosage administered.

Table I presents the numerical data for each animal relative to the blood volume at the end of 32 weeks. The striking increase in total red cell mass and the slight but consistently lower plasma volumes are worthy of note.

Table II contains data derived from cell counts, measurements of the mean cell diameter, hematocrit and hemoglobin content. The cell size, cell volume, cell thickness, absolute hemoglobin content and hemoglobin concentration were calculated according to Wintrobe.[12] Regarding the size, shape and hemoglobin content of erythrocytes from animals with cobalt polycythemia the following facts have come to light as a result of this study: 1. Although the mean cell diameter remains normal the cell volume increases by about one-third, a change which is proportionate to the increase in cell thickness. 2. The absolute amount of hemoglobin per red cell remains unchanged.

The blood volume per kilogram of body weight of these animals compares favorably with that found in the more extreme cases of polycythemia vera in man.[13]

Detailed histopathologic studies are in progress as well as further work on the components of red cells and plasma.

Conclusions. 1. Injections of cobaltous chloride continued over a period of 8 months without apparent serious toxicity has resulted in persistent polycythemia during this period of a degree comparable to that occurring in polycythemia vera in man. 2. The average increases in blood volume and total erythrocyte mass as compared with controls was 80% and 192% respectively, due entirely to increase in erythrocytes since plasma volume decreased 16% on the average. 3. The erythrocyte of polycythemic animals as compared with the normal increased in volume 41% due almost entirely to an increase in the thickness of the cell. The hemoglobin content remains essentially unchanged.

[12] Wintrobe, M. M., *Clinical Hematology*, Lea & Febiger, 1942.

[13] Gibson, J. B., 2d, Harris, A. W., and Swigert, V. W., *J. Clin. Invest.*, 1939, **18**, 621.

15250

Influence of Dimethylaminoethylbenzhydryl Ether Hydrochloride Upon Histamine Flare Reactions.

HERBERT ELIAS, AND THOMAS H. MCGAVACK. (Introduced by Israel S. Kleiner).

From the New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York City.

The alleviation of histamine induced anaphylaxis in guinea pigs has been described following the use of a number of benzhydryl alkamine ethers of the general formula, where R represents a carbon chain carrying a primary, secondary or, in most cases, a tertiary nitrogen atom.[1,2,3] In one of the

[1] Loew, E. R., Kaiser, M. E., and Moore, V., *J. Pharm. and Exp. Therap.*, 1945, **83**, 120.

[2] Loew, E. R., and Kaiser, M. E., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 235.

[3] Sharp, E. A., personal communication.