9467

Cobalt Polycythemia in the Dog.

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The hematopoietic power of cobalt was first demonstrated on the rat by Waltner.[1] Orten and coworkers[2, 3, 4] have shown that a daily dose of 0.5 mg. cobalt, with copper and iron, produces in rats a true polycythemia involving an increase of the blood volume, and that cobalt probably acts by stimulating the hematopoietic organs. Kleinberg[5] reported that the subcutaneous injection of cobalt chloride produces in rabbits a polycythemia, and an increased reticulocyte percentage. Mascherpa[6] reported that the oral administration of cobalt to dogs produces an increased erythrocyte number. Brewer,[7] however, reported that the daily feeding of cobalt chloride in doses of 0.8 to 4 mg. of cobalt per kg. failed to produce a significant polycythemia in his dogs. He asserts that the erythrocyte number in normal dogs may vary by 15 to 20% as observed on consecutive days.

In view of these conflicting reports on the effect of cobalt upon the erythrocyte number of the dog, I decided to reinvestigate the question.

Four dogs used in this investigation were fed a uniform daily diet of Purina dog chow. Two of these dogs were normal females, while the other 2 (males) were polycythemic due to the fact that they had finished a prolonged period of daily treadmill running just 3 weeks before the cobalt feeding was commenced.

Cobalt was administered in the form of cobaltous chloride in 1:1000 solution (cobalt equivalent) by stomach tube each day just before feeding. It was found that the salt could not be given in gelatin capsules, as this form of administration caused the dogs to vomit. During the first period of cobalt feeding, 2 of the dogs were given 2 mg. cobalt per kg. daily, while the other 2 were given

1 Waltner, K., *Arch. Exp. Path. u. Pharm.*, 1929, **141**, 123.

2 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **96**, 11.

3 Orten, J. M., *J. Biol. Chem.*, 1933, **99**, 457.

4 Orten, J. M., *Am. J. Physiol.*, 1936, **114**, 414.

5 Kleinberg, W., *Am. J. Physiol.*, 1934, **108**, 545.

6 Mascherpa, P., *Arch. ital. de Biol.*, 1930, **82**, 112.

7 Brewer, G., *Am. J. Physiol.*, 1937, **118**, 207.

TABLE I.

Effect of Cobalt Feeding on Erythrocyte Number and Hemoglobin Percentage in Dogs.

Physically Trained Dogs						Normal Dogs					
Dog 1			Dog 2			Dog (6)			Dog (7)		
Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %	Date	R.B.C.s Millions	Hb Sahli %
3/24	4.76	68	3/24	5.76	87	3/27	5.02	71	3/19	5.00	72
3/25	4.76	68	3/27	5.77	85	3/31	5.14	73	3/22	5.39	72
3/30	4.66	71	3/30	5.83	89	4/6	5.12	68	3/29	5.15	68
4/6	4.74	70	4/1	5.81	86	4/9	5.19	72	4/2	5.10	68
4/14	4.90	69	4/4	5.68	82	5/3	5.25	71	4/29	5.32	71
4/16	5.21	69	4/16	Exercise discontinued		5/4	5.26	71			
4/16	Exercise discontinued		5/3	5.93	78						
4/30	4.96	69	5/4	5.93	78						
Cobaltous Chloride Administration (Commenced 5/5)											
2 mg. Cobalt per kilo daily			2 mg. Cobalt per kilo daily			6 mg. Cobalt per kilo per day					
5/8	5.71	74	5/8	6.18	82	5/7	4.87	69	5/8	5.75	69
5/11	5.28	72	5/10	6.13	79	5/11	5.29	72	5/10	5.55	68
5/14	4.83	70	5/13	5.88	78	5/13	5.29	76	5/14	6.06	73
5/17	4.91	71	5/15	6.24	80	5/15	6.08	74	5/17	6.30	69
5/19	5.14	73	5/18	5.67	81	5/19	6.58	76	5/19	5.82	71
5/21	5.14	71	5/21	5.68	76	5/21	6.51	79	5/22	5.93	73
5/24	4.88	69	5/24	5.56	78	5/24	6.24	79	5/24	5.55	70
5/25	4.96	68	5/24	Cobalt discontinued		5/29	6.16	81	5/29	Cobalt discontinued	
5/27	Cobalt discontinued		5/31	5.02	76	5/29	Cobalt discontinued		6/1	5.50	70
5/31	4.81	68	6/4	5.06	74	6/1	5.72	80	6/4	5.32	67
6/4	4.64	70	6/8	4.82	69	6/4	4.86	81	6/8	4.42	67
6/8	4.36	70	6/12	4.69	69	6/8	4.85	81	6/11	4.75	70
6/12	4.12	68	6/15	4.63	68	6/11	5.03	80	6/14	4.81	72
6/15	4.19	70				6/14	4.90	80			
6/16	Cobalt Administration: 2 mg. per kilo daily										
6/19	4.90	74	6/22	5.29	71	6/17	4.97	80	6/18	4.77	71
6/24	5.20	73	6/24	5.60	74	6/22	5.74	81	6/22	5.10	71
6/28	5.07	73	6/28	5.82	75	6/25	5.75	83	6/24	5.25	71
6/30	4.99	71	6/29	5.72	74	6/29	6.05	83	6/28	5.75	72
7/2	5.06	72	7/1	5.65	73	7/1	6.03	82	6/29	5.99	73
									6/30	5.83	74

6 mg. After 3 weeks the cobalt was discontinued for about 3 weeks, then all 4 dogs were given a uniform dose of 2 mg. cobalt per kg. daily over a period of about 2 weeks.

Red blood cell counts and hemoglobin percentages (Sahli) were determined frequently throughout the control and experimental periods. Blood was drawn from the saphenous vein while the animal was lying quietly (blindfolded) upon a table and was in a basal state with respect to previous exercise and food consumption.

Before the first period of cobalt feeding dogs 1 and 2 had erythrocyte numbers which were already 20% and 15% higher than their pre-exercise normal values. These chronic exercise polycythemias are a confirmation of previous work by the author.[5]

It will be noted in Table I that the high erythrocyte numbers of dogs 1 and 2 were maintained throughout the first 3-week period of cobalt feeding and, altogether, for 6 weeks after the cessation of daily exercise. Within 2 weeks after the discontinuation of cobalt, the cell counts dropped by 16 to 18%. On 6 mg. cobalt per kg. daily, normal dogs 6 and 7 developed significant polycythemias within 10 days. The increase in the erythrocyte numbers amounted to about 18%. Within 10 days of cessation of cobalt administration the cell counts returned to normal.

On June 16th, a second experiment (Table I) was started in which each of the 4 dogs was given 2 mg. cobalt per kg. per day. Within 4 to 12 days, the erythrocyte numbers of all 4 dogs had increased by about 20%. This increase is of approximately the same order as that observed by Mascherpa[6] in a cobalt-fed dog, and by Davis and Brewer[8] in physically trained dogs. Probably, we cannot expect to induce a large (50%) increase of erythrocytes in the dog by these methods.

My results in this and previous work force me to disagree with Brewer[7] on the range of day to day variations in the erythrocyte number of the normal dog. To obtain fairly consistent counts, it is important that blood be drawn only when the animal is quiet and unexcited. Otherwise the red cell counts will be too high, perhaps signifying an emotional polycythemia in the dog. In any event, it is known that the spleen contracts during excitement, and forces additional blood cells out into the circulation (Barcroft[9]). When all proper precautions are taken, the normal erythrocyte number of the dog should not vary by more than 7% as determined on consecutive days. This is probably a very generous allowance for the range of variability.

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8 Davis, J. E., and Brewer, N., *Am. J. Physiol.*, 1935, **113**, 586.

9 Barcroft, J., and Stephens, J., *J. Physiol.*, 1927, **64**, 1.

Throughout these experiments, the animals appeared to be in excellent condition. They did not lose any weight, and showed *no* anorexia or lethargy. Body temperatures remained quite normal.

Conclusions. The oral administration of 2 mg. cobalt (as cobalt chloride) per kg. daily to dogs produces a significant increase (about 20%) in the erythrocyte number. No toxic symptoms were observed in dogs which were fed as much as 6 mg. cobalt per kg. daily for 3 weeks.

9468

Comparative Study of Fibrinolytic and Anticoagulating Properties of *Streptococcus hemolyticus* and *Streptococcus fecalis* (Enterococcus).

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Tillett and Garner[1] made the interesting observation that hemolytic streptococci may produce a powerful fibrinolysin. Their findings were corroborated and extended by several authors, including Dart, Dennis and Berberian, Van Deventer, Hare and Colebrook, Hatfield, Magee and Perry, Lippard, Johnson and Wheeler, Madison, Meyers, Keefer and Holmes, Reich, Schmidt, Stuart-Harris, Tunnicliff and others. The fibrinolysin produced by hemolytic streptococci *in vitro* has the following main properties: It dissolves human but not animal plasma-clots, with the exception of *rhesus* plasma, as demonstrated by Van Deventer and Reich[2]; it is produced in infusion-broth in rather high concentration, and it may be neutralized by streptococcal antiserum as well as by serums of patients recovering from streptococcal infections. Besides the fibrinolysin, glucose-broth cultures of hemolytic streptococci may contain a second factor which inhibits coagulation of both human and animal plasma, as observed

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1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239; Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47; Tillett, W. S., *J. Clin. Invest.*, 1935, **14**, 276; Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

2 Van Deventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 821.