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Effect of Cobalt Sulfate on Erythrocyte Count of the Splenectomized Albino Rat.*

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Prior to 1929 little was known of the biological behavior of cobalt. Waltner and Waltner¹ showed that inorganic salts of cobalt when fed or injected into normal albino rats produced a remarkable polycythemia. Orten, Underhill, Mugrage and Lewis² obtained similar results. The literature of cobalt polycythemia has now become quite extensive.

Our experiments were undertaken to determine if polycythemia can be produced by cobalt in splenectomized albino rats.

Orten, Underhill, Mugrage and Lewis² ashed the organs, finding the largest amounts of cobalt in the liver, pancreas and spleen and minute quantities in the bone marrow.

Since it is not possible to remove the liver or pancreas in recovery experiments, cobalt was tried on splenectomized rats, in view of the long disputed question of the hematopoietic spleen function.

Fourteen healthy, adult, white rats, mostly of the Wistar strain were used, 8 being splenectomized under sodium pentobarbital (40 mg./kg.) and morphine sulfate (10 mg./kg.) anesthesia. An incision 2.5 cm. long was made just below the left costal margin. The lienorenal, gastrosplenic and splenic vessels were tied and the spleen removed aseptically; the animals then placed for an hour in oxygen 90%, carbon dioxide, 10%.

After 3 weeks, cobalt injections were started. Duplicate erythrocyte counts were made routinely before and after injections; at first, weekly; later, fortnightly, using Max Levy counting chambers and Yankee Certified mixing pipettes, and Hayem's diluting fluid. Two control splenectomized rats were used. The remaining 6 operated

* This research was aided by a grant from the Hendricks Research Fund.

¹ Waltner, K., and Waltner, K., *Klin. Wochenschr.*, 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.

animals and 6 unoperated animals received cobalt daily. Blood was taken from the projecting tail, the animal being confined in a closely fitting box, to avoid excitement. Animals' weights were charted at each count; and at the height of the polycythemia in the normal animals, specific gravity tests on all of the rats were made by the chloroform-benzene method without significant variations from the normal.

TABLE I.
Erythrocyte Counts on Various Groups.

Group	Count in millions/mm. ³ before injection	Count in millions/mm. ³ days after injection	Difference	Daily Dose in mg.
Normal plus cobalt	8.42	40 12.78	4.36	2
	7.65	40 12.20	4.55	2
	9.31	55 12.75	3.44	1
	10.03	55 13.01	2.98	1
	8.15	10 10.95	2.80	3
	8.35	10 11.08	2.73	3
Splenectomized plus cobalt	8.20	40 9.90	1.70	2
	7.85	40 8.24	0.39	2
	8.63	10 7.56	—1.07	3
	9.62	10 10.49	0.87	3
	8.45	55 4.14	—4.31	1
	9.45	55 2.63	—6.82	1
Splenectomized with no cobalt	8.71	40 9.64	0.93	2
	7.29	40 7.77	0.48	2

Results. Table I shows cobalt polycythemia graphically in 6 normal animals; beginning from the minimum daily dose (1 mg.) in about 3 weeks; from the maximum (3 mg.) after one week; average increase in erythrocytes being nearly 50%. Two treated operated animals showed extreme anemia probably due to *Bartonella muris* infection, common following splenectomy in the albino rat. Four treated, operated animals maintained normal counts as well as 2 untreated operated animals. Splenectomy in non-infected (*Bartonella muris*) animals produced no variations in blood count except for a slight anemia the second or third day after operation. All animals gained in weight during the experiment. Six weeks after injections were stopped previously polycythemic rats had normal erythrocyte counts.