The average and minimum and maximum hemoglobin and erythrocyte values obtained on the animals of the various groups for a 20-week period are given in the accompanying table. During the entire experiment, the erythrocyte and pigment values of the milk-iron-copper controls were within the lower limits found in normal stock rats of the same age,[3] whereas those of the cobalt-treated, laparotomy controls showed the characteristic increase to high levels. During the first few weeks of the experiment, 3 of the cobalt-treated, splenectomized animals developed a severe anemia; subsequently, however, the erythrocyte and hemoglobin values of these animals, like those of the other members of the group, slowly increased and finally attained levels decidedly higher than those of the untreated controls and differing but little from those of the cobalt-treated, laparotomy controls.

It is possible that the marked decrease in erythrocytes and pigment observed during the early part of the experiment in several of the splenectomized animals may have been a manifestation of "Bartonella anemia," inasmuch as small bodies, resembling "Bartonella bodies,"[4] were found in the erythrocytes of blood smears stained with Giemsa's stain. Further studies are planned to determine the possible effect of Bartonella infection on the results herein reported.

Conclusion. From the foregoing data, the tentative conclusion is drawn that the removal of the spleen of the young rat may delay but does not prevent the production of polycythemia by cobalt.

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Relation of Calcium to Blood Formation.*

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Previous investigations in this laboratory have shown that young albino rats maintained on a diet deficient in inorganic salts fail to grow normally and develop unique hematological abnormalities, including a marked polycythemia and a concurrent, chronic anemia.[1, 2]

[4] Ford, W. W., and Eliot, C. P., *J. Exp. Med.*, 1928, **48**, 475.

* Aided by a grant-in-aid, National Research Council, 1934.

† National Research Council Fellow in Medicine, 1933-34; Alexander Brown Coxe Fellow, Yale University, 1934-35.

[1] Swanson, P. P., and Smith, A. H., *J. Biol. Chem.*, 1932, **98**, 479.

[2] Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1934, **105**, 181.

Analyses of the diet[3] have demonstrated that it is extremely low in calcium, sodium and chloride, and deficient in potassium, magnesium, phosphorus, and possibly iron.[4] Inasmuch as a lack of calcium appeared to be the most serious inorganic deficiency in the ration, a study was made of the hematological effects produced by the addition of this element to the low-ash ration.

The effect of calcium was investigated by both the curative and preventive procedures in uniform, vigorous, young male albino rats, selected and cared for as described previously.[2] In the curative procedure, the animals were placed on the basal low-ash ration[2] for an 8-week preliminary period and then were given calcium carbonate at a level of 50 mg. of calcium daily, the amount consumed by comparable normal controls. During the experimental period, the basal low-ash ration was fed in that daily amount ingested by the animal during the final 2 weeks of the preliminary period. In the preventive procedure, the young rats were placed directly on the calcium-supplemented, low-ash ration. The amount of the basal low-ash diet fed was restricted to that daily quantity ingested by unsupplemented controls. Simultaneously, 2 types of control animals were studied for each calcium-supplemented group: (a) unsupplemented controls given the low-salt diet; (b) inanition controls fed the same daily amount of the basal low-ash ration as the unsupplemented controls but containing, in addition, the quantity of salt mixture ingested daily by normal animals of the same weight. Adequate amounts of the vitamins[2] were supplied daily to all animals in equal quantities. Redistilled water was provided *ad libitum*. Each group consisted of at least 10 animals. Body weights were followed weekly; erythrocyte and hemoglobin determinations were made bi-weekly by procedures previously described.[5]

In the curative series of experiments (Table I), it was found that the animals of the inanition control group soon attained normal erythrocyte and hemoglobin levels,[5] whereas the unsupplemented control rats continued to show the typical polycythemia with a chronic anemia. The calcium-supplemented animals also showed a progressive decrease in erythrocytes to a normal value and an increase in pigment toward normal. Similar results were obtained in the series studied by the preventive procedure (Table I). The erythrocyte counts and pigment concentrations of the calcium-supplemented animals compared favorably with those of the inanition control group throughout the entire experimental period.

[3] Smith, A. H., and Smith, P. K., *J. Biol. Chem.*, 1934, **107**, 681.

[4] Brooke, R. O., personal communication.

[5] Orten, J. M., and Smith, A. H., *Am. J. Physiol.*, 1934, **108**, 66.

TABLE I.
Average Hemoglobin and Erythrocyte Values for Control and Calcium-Fed Rats.

Weeks of Exper.	Inanition Controls R.B.C. M. per cmm.	Inanition Controls Hb gm. per 100 cc.	Low-Salt Controls R.B.C. M. per cmm.	Low-Salt Controls Hb Gm. per 100 cc.	Ca Supplemented R.B.C. M. per cmm.	Ca Supplemented Hb Gm. per 100 cc.
			Curative Procedure.			
0	10.63	12.2	10.85	12.7	10.62	13.4
2	9.69	13.2	10.53	12.2	10.68	14.6
4	9.82	14.5	9.91	12.1	9.03	14.8
6	9.77	15.2	9.72	11.4	8.84	15.2
8	8.74	15.5	9.77	11.6	8.77	15.7
10	8.77	15.7	10.07	12.3	8.22	15.3
			Preventive Procedure.			
0	6.43	12.9	6.58	12.3	5.89	11.9
2	7.83	14.3	8.44	13.6	8.09	15.9
4	8.63	15.5	9.57	13.3	8.89	16.4
6	8.79	15.8	10.68	13.0	9.26	16.7
8	8.72	16.0	10.64	12.3	9.38	16.4
10	9.20	16.1	10.53	12.2	9.49	16.8
12	8.98	16.1	9.91	12.1	9.01	17.1

The foregoing results permit the conclusion that the presence of calcium in the diet deficient in certain other inorganic elements both cures and prevents the development of the expected polycythemia and concomitant chronic anemia.

The significance of these observations is, as yet, largely a matter of conjecture. It is possible that the beneficial action of calcium on the hemoglobin level may depend on the suggested ability of this element to exert a favorable effect on the economy of iron in its metabolism,[6, 7] a point under investigation at the present time. Also, the foregoing data are of importance because they show that the calcium-supplemented animals, although still consuming a ration extremely deficient in sodium and chlorine, and to a lesser degree potassium, magnesium and phosphorus, maintain a normal blood picture and, as far as can be determined by gross observations, are entirely normal.

[6] von Wendt, G., *Skand. Arch. Physiol.*, 1905, **17**, 211.

[7] Sherman, H. C., Bulletin 185, U. S. Dept. Agriculture, Office of Experiment Stations, 1907.