

During the period of 22 days in which liver ash was given, many of the preliminary phenomena of a remission were observed. The hemoglobin rose from 26 to 37%, the erythrocytes fluctuated from 1.2 to 1.5 millions. The leucocytes increased from 2500 to 7000. The reticulocytes began to increase on the 7th day and reached a maximum of 6.5% on the 17th day, remaining at about that level until further elevated by the administration of the liver extract.

The signs of beginning regeneration just enumerated began to diminish as soon as the soluble salts of the liver ash were given. After a few days it was determined to discontinue the ash experiment. When Minot's liver extract was given a true remission of the disease began.

A third patient with pernicious anemia received the soluble salts of the liver ash for a period of a week. There was no evidence of any activity of marrow tissue observed, either in the total blood count and hemoglobin and leucocytes or in the percentage of reticulocytes.

These results are reported as a supplement to the experiments of Whipple and Robscheit-Robbins on the effects of liver ash and inorganic substances on the blood regeneration of dogs made chronically anemic by repeated bleedings.

Conclusions: 1. The administration of the ash of liver to 2 patients with typical pernicious anemia resulted in the appearance of some of the preliminary phenomena of a remission, in particular an increase in the percentage of reticulocytes. In neither case did a true remission occur until Minot's liver extract 343 was given.

2. The substance responsible for the above was apparently lost or inactivated by dissolving the ash in hydrochloric acid, neutralizing with NaOH, and evaporating the salts to dryness.

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Blood Regeneration in Severe Experimental Anemia. Influence of Inorganic Elements.

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We have recently reported in these PROCEEDINGS¹ a rather startling reaction to the inorganic ash of liver, kidney and apricots. This

ash incorporated in a standard anemia diet² given to dogs kept constantly anemic by bleeding will effect a notable increase in blood hemoglobin production above control periods. This may amount to 30 to 50 gm. hemoglobin per 2-week period.

We have made various analyses of these ashes and from such data have made various synthetic ash compounds, but such synthetic mixtures so far have not proved to be potent. These experimental data will be published in due time.

We wish to report briefly our findings when these dogs are given various simple salts added to the standard ration. It is apparent that in the doses given iron is more potent than either copper or zinc.

TABLE I.
Hemoglobin Regeneration and Inorganic Elements.

Minerals	Amount	Hemoglobin	Number of
	per day	produced per 2 weeks	
	mg.	gm.	
*Bread control		2—5	
Iron (chloride)	25	0—10	6
Iron (citrate)	60	10—70	2
Iron (citrate)	300	40—70	4
Copper (tartrate)	40	0	2
Copper (sulfate)	65	13	2
Copper (tartrate) + iron (chloride)	40 + 140	27	1
Copper (tartrate) + iron (chloride)	20 + 36	27	1
Copper (tartrate) + iron (chloride)	40 + 70	60	1
Zinc (chloride)	11	20	2
Zinc (chloride) + copper (tartrate)	24 + 40	9	1
Manganese (peptonate)	110	0	1
Aluminum (sodium sulfate)	38	0	2
Arsenic (potassium arsenite)	2	0	2
Antimony (chloride)	9	0	1
Iodide (sodium)	420	0	1

* Standard bread ration per 300 gm. contains 18 mg. iron.

Values of copper, iron, zinc, aluminum, arsenic, antimony and iodine are given in mg. of the element. The manganese is given in mg. of the peptonate.

These observations dealing with copper salts are of considerable interest when contrasted with the recent reports of Hart, Steenbock, Waddell and Elvehjem,³ who report a distinctly favorable reaction to copper salts and a negative reaction to iron salts. These distinct differences between our experiments and those of the Wis-

¹ Robscheit-Robbins, F. S., Elden, C. A., Sperry, W. M., and Whipple, G. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, xxv, 416.

² Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1925, lxxii, 395.

³ Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, lxxvii, 797.

consin investigators must be explained on the basis of animal differences or anemia differences. They use rats made anemic by faulty diets and we use dogs made anemic by constant bleeding to a standard anemia level.

Early in our anemia experiments (1923) Robscheit-Robbins and Whipple observed that an excess of a salt mixture (McCollum and Simmonds⁴ minus iron) caused a distinct increase in hemoglobin production over control periods. The control bread ration contained these same salts but a large excess added would cause an increased hemoglobin output. It could not be that these salts were incorporated in the hemoglobin molecule, so this reaction might be called "catalytic." We feel that these large doses of iron reported here, which are probably 10 times or more in excess of the body needs, cause a similar *salt reaction* of "catalytic" nature. There must be a readjustment of the internal protein metabolism so that much more hemoglobin is produced, there being an excess of iron at all times.

The doses of copper, zinc and other metals are close to the toxic dosage. We intend to extend these observations, using a variety of dosage and combinations of various salts. This is a progress report.

⁴ McCollum, E. V., and Simmonds, Nina. *J. Biol. Chem.*, 1918, xxxiii, 63.