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Effect of Copper and Iron on Hemoglobin of the Rat in Nutritional and Hemorrhagic Anemias.

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Following Hart, Steenbock¹ et al. on the influence of minute amounts of copper in improving the hemoglobin in rats with nutritional anemia it seemed desirable to determine whether or not this same thing could be obtained in the anemia of the rat induced by repeated bleedings.

We have compared two series of rats, (1) a group made anemic by long continued milk diet; (2) a group of normal animals made anemic by repeated bleedings. In both groups the animals were of the same age and weight, and treated exactly alike in every other particular.

Group I. Nutritional Anemia Group. These rats were kept on a diet of milk until they showed a definite anemia, and then divided into 3 sub-groups. The first sub-group was kept on milk only. The second sub-group was kept on milk with the addition of iron and copper. The third sub-group was kept on milk with iron only. The amount of iron used was 3 mg. per rat per day given in the form of ferric ammonium citrate. The amount of copper used was one-tenth milligram per rat per day, in the form of copper sulphate. We had on hand a considerable amount of the iron salt from our former experiments, and this was carefully analysed for the presence of copper as an impurity, and was found to contain only very slight traces of the latter metal.

After a preliminary examination of the blood of a few trial animals had shown that they had become sufficiently anemic the hemoglobin of all the animals was determined spectrophotometrically. Where relative results are wanted this is the most accurate method at our disposal for the determination of hemoglobin. These figures are recorded in each sub-group under the caption "First determination." On the twenty-fourth and fifty-fourth days afterwards other determinations are made, which are recorded in the corresponding columns of each sub-group.

Table I summarizes results for 54 days; in those fed on milk only

¹ Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

SUMMARY OF TABLE I.
Nutritional Anemia.

	1st Determination		2nd Determination		3rd Determination		No. of Rats
Diet of milk only	0.829	Var 0.729	0.785	Var 0.696	0.662	Var 0.535	26
Milk plus copper plus iron	0.785	Var 0.667	0.984	Var 0.918	0.937	Var 0.842	23
Milk plus iron	0.821	Var 0.715	0.874	Var 0.735	0.692	Var 0.589	24

NOTE: The figures given are averages. Such determinations as were greater than the average were again averaged, and this result is the numerator of the fraction. In the same way, the denominator is the result of treating those determinations below the average in the same way.

the hemoglobin was lowered from an average of 0.829 to 0.662. In the sub-group on milk with iron only added the hemoglobin went down to approximately the same extent, from 0.821 to 0.692.

It is interesting to note in this group in the determination made 24 days after that there is a slight rise, namely to 0.874, hardly more than a normal variation, and this moreover is not maintained.

In the sub-group on milk with the addition of both iron and copper there is a very striking improvement. Beginning with a concentration of 0.785 there is an immediate increase in the hemoglobin to 0.984 which remains up to 0.937 on the fifty-fourth day.

To any one thoroughly familiar with the spectrophotometer the difference is much more striking than these figures would indicate, and it was almost possible to pick out the rats which had copper at the first glance through the instrument.

This confirms the work of Hart and Steenbock that in the nutritional anemia of the rat the addition of a minute amount of copper to the diet produces a prompt and satisfactory increase in the hemoglobin, whereas the addition of iron alone not only fails to produce an increase in the hemoglobin but fails even to check the progressive development of anemia.

Hemorrhagic Anemia. The second set of experiments concerned itself with the anemia produced by abstraction of blood. A hemoglobin determination was made on a large group of healthy animals. They were then placed on the standard iron-free (iron-poor) diet used in our previous work and then divided into 2 similar groups and the following day all were bled one-third of their blood volume. A similar amount was removed again 7 days later. Subsequent hemoglobin determinations were made on the twenty-fourth and

seventy-first days after bleeding. The results are summarized in Table II.

SUMMARY OF TABLE II.
Hemorrhagic Anemia.

	Before Bleeding		24 Days After Bleeding				No. of Rats
Control Group (Practically iron-free)	0.821	Var 0.731	0.787	Var 0.627	0.817	Var 0.723	49
Copper and iron group	0.803	Var 0.725	0.809	Var 0.633	0.791	Var 0.641	51

The results are very different from those obtained in the nutritional group. The rats in the sub-group receiving copper and iron in addition to the diet had a concentration of 0.803 before and 0.791 on the seventy-first day after bleeding. An intermediate reading was taken to determine the rate of change. In other words, in this group the hemoglobin was almost exactly the same on the seventy-first day as in the beginning, whereas in the nutritional group, a marked improvement occurred as early as the twenty-fourth day, which remained at substantially the same level on the fifty-fourth day. In the sub-group used as control, we find a concentration of 0.821 before and 0.817 on the seventy-first day after bleeding. Expressed in another way, the group treated with the addition of iron and copper to a standard diet did not improve either more rapidly or to a greater degree than those on a standard control diet alone.

Conclusions: In rats with nutritional anemia the addition of iron and copper to the diet produces definite and prompt improvement in the hemoglobin concentration. In rats with anemia produced by repeated abstraction of blood the addition of iron and copper to the diet produces no perceptible effect.

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Fermentation of Glucosamine Hydrochloride by Bacteria.

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With the exception of a few observations on glucosamine the fermentation reactions of micro-organisms on the hexosamines have not been studied. Inasmuch as these nitrogenous sugars contain a carbohydrate and an amino group, both of which are necessary for the growth of bacteria, an investigation of the metabolic activities