plants, although chlorophyll itself does not contain this metal. δ-Aminolævulic acid, however, is an intermediate of this biosynthetic process[8]. Further work on these observations is in progress and will be reported in full elsewhere.

I wish to thank Prof. C. Rimington for his interest and advice and Miss B. Knight for technical assistance during the course of this work, which was carried out during the tenure of an I.C.I. Research Fellowship.

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Effect of Copper Deficiency on Synthesis of Hæm

Although copper is essential for the formation of normal hæmoglobin, it has not been demonstrated that this element functions directly in the synthesis of hæm. We have demonstrated an increased synthesis of hæm *in vitro* from glycine labelled with carbon-14, by means of adding inorganic copper to copper-deficient chicken blood.

Cross-bred chicks from a commercial hatchery were rendered deficient in copper in two weeks by feeding the following diet: non-fat milk solids, 55 per cent; glucose-monohydrate, 40 per cent; cotton-seed oil, 2 per cent; glycine, 0·6 per cent; L-arginine monohydrochloride, 0·5 per cent; L-cystine, 0·2 per cent; choline chloride, 0·6 per cent; and sodium chloride, 0·5 per cent. In addition, each 100 gm. of diet contained 2 mgm. of nicotinic acid, 240 I.U. of vitamin A-palmatate, 12·4 mgm. magnesium sulphate, 1·9 mgm. manganese sulphate and the equivalent of 14 mgm. carbonyl iron (General Aniline Works, Grasselli, New Jersey). The iron was fed in the carbonyl form in experiments 1, 2 and 3 and in the chloride form in experiments 4, 5 and 6. The control diets were supplemented with 1·6 mgm. of copper sulphate per 100 gm. of diet. The criteria for deficiency in copper were reduced growth-rate and hæmoglobin concentration.

To determine the effect of a deficiency of copper on synthesis of hæm, 0·1 ml. of a solution of glycine containing 1 μc. methylene-labelled glycine and sufficient non-labelled glycine to yield a final concentration of $1·33 \times 10^{-2}$ *M* was added to either 3 or 5 ml. of heparinized whole blood. In addition, antibiotics[1] and 0·1 ml. of a ferrous sulphate solution were added to yield a final concentration of 2×10^{-4} *M*, as recommended by Dresel and Falk[2]. All samples were incubated at 37·5° C. under an oxygen atmosphere in a Warburg apparatus for 24 hr. Hæm was isolated and recrystallized according to the method of Shemin *et al.*[1], except that strontium chloride was used instead of sodium chloride. A large excess of carrier hæm was added in experiments 2, 3, 4 and 6 but not in experiments 1 and 5. In each experiment blood samples from 4–10 chicks were pooled and incubated either with or without supplemental copper sulphate to a final concentration of $1·6 \times 10^{-5}$ *M*.

Table 1. Effect of Copper on Synthesis of Hæm in Blood deficient in Copper

Experiment*	Treatment†	Weight gain‡ (gm.)	Hb (gm. per cent)	Specific activity of hæm −Cu	Specific activity of hæm +Cu	Standard error of the mean $S_{\bar{x}}$	Significant level of the copper effect
1	Control	68·8	7·35	37·8	37·7	±3·5	0
	Cu-def.	34·5	3·70	12·1	29·0		0·01
2	Control	62 0	6·52	9·4	7·9	±0·6	0
	Cu-def.	36·4	4·25	6·7	9·3		0·05
3	Control	62·0	5·78	2·3	3·3	±0·2	0·05
	Cu-def.	36·4	4·25	1·9	2·5		0·05
4	Control	69·8	8·39	8·0	7·9	±0·6	0
	Cu-def.	39·9	5·18	8·7	11·1		0·01
5	Control	95·0	6·33	39·1	27·9	±0·8	0·01
	Cu-def.	66·0	3·57	24·9	23·7		0
6	Control	69·8	8·39	4·3	1·6	±0·4	0·01
	Cu-def.	39·9	5·18	3·8	3·6		0

* Results in experiments 1, 2 and 3 are from chicks fed carbonyl iron. Results in experiments 4, 5 and 6 are from chicks fed iron chloride.
† Control diet contained 1·6 mgm. copper sulphate per 100 gm. of diet.
‡ The weight gains in experiment 5 are for four weeks and all others are for two weeks.

The results in Table 1 show that in blood from chicks deficient in copper and fed the diet containing carbonyl iron, synthesis of hæm was reduced, but the addition of copper to this blood consistently increased this synthesis. Blood obtained from chicks deficient in copper and fed iron chloride, however, gave an increased synthesis of hæm in the presence of copper only in experiment 4. With the exception of experiment 3, the addition of copper to the blood from control chicks failed to increase the synthesis of hæm. This exception may possibly be related to the low concentration of hæmoglobin in the control chicks of this experiment.

The results obtained in these experiments demonstrate that copper has a direct function in the synthesis of hæm. From the fact that the copper effect was obtained consistently only when carbonyl iron—a source of iron of low availability—was used in the diet, it appears that a dual copper–iron deficiency may be essential to demonstrate the effect of copper on the *in vitro* synthesis of hæm.

Experiments are in progress to determine the site(s) of copper activity in the synthesis of hæm. Recently, Iodice, Richert and Schulman[3] have demonstrated that δ-aminolævulinic acid dehydrase contains copper and that its activity is reduced in copper-deficiency. It is possible, however, that copper has functions in the synthesis of hæm other than in connexion with this enzyme and the system described here should prove beneficial in elucidating the role of copper in the synthesis of hæm.

We wish to thank Dr. C. H. Hill for providing the chicks used in these experiments and for his aid and advice in the care and feeding of them. This project was partially supported by a grant from the Frasch Foundation.

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