The Effect of Copper Deficiency on the Induction of Tryptophan Pyrrolase in Chick Liver by Hydrocortisone

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The induction of tryptophan pyrrolase in chick liver by hydrocortisone was studied in copper- and magnesium-deficient chicks. Magnesium deficiency did not influence the induction of the enzyme, whereas copper deficiency significantly decreased it. These results suggest that tryptophan pyrrolase of chick liver, like that in *Pseudomonas*, is a copper-containing enzyme.

Maeno & Feigelson (1965) have presented evidence indicating that copper is a component of tryptophan pyrrolase in *Pseudomonas* A.T.C.C. 11299. This enzyme is present in the livers of chicks and rats and can be induced to higher levels by the administration of hydrocortisone (Knox & Averbach, 1955; Knox & Eppenberger, 1966).

Previous work from this Laboratory (Starcher & Hill, 1965) has shown that induction of the copper-containing protein caeruloplasmin in the chick takes place under the influence of various stressors as well as adrenocorticotrophic hormone or hydrocortisone. In copper-deficient chicks, however, such induction did not occur.

In order to obtain some evidence on the role of copper in tryptophan pyrrolase of chick liver, the induction of this enzyme by hydrocortisone was studied in normal and copper-deficient animals.

MATERIALS AND METHODS

The chicks used in these studies were obtained from a commercial hatchery. In the experiments dealing with copper deficiency, they were fed on the diet described by Starcher, Hill & Matrone (1964) or the same diet supplemented with cupric sulphate to supply 25 p.p.m. of copper. In the experiment dealing with magnesium deficiency the chicks were fed on the diet given in Table 1 or the same diet supplemented with magnesium sulphate to supply either 100 or 400 p.p.m. of magnesium. The diets were fed *ad libitum* from the day of hatching.

When the chicks were 14 and 21 days old in the first copper experiment, and 7, 14 and 21 days old in the second copper experiment, and 17 days old in the magnesium experiment, three chicks from each dietary treatment were injected intraperitoneally with 10 mg. of hydrocortisone/100 g. body wt. The control chicks received an intraperitoneal injection of distilled water. At 4 hr. after the hydrocortisone was administered the chicks were killed and the livers removed.

A liver homogenate was prepared by grinding 2.5 g. of liver in 10 ml. of 0.15 M-KCl, 0.1 ml. of 40 mM-L-tryptophan,

1.0 ml. of freshly prepared 300 mM-ascorbic acid and 4 μ g. of haemin. The solutions had been adjusted to pH 7.0 and cooled before use.

After removal of the debris by centrifuging at 1000g, the homogenate was recentrifuged at 100 000g for 60 min. in the cold. The supernatant fluid from this centrifugation was used as the source of enzyme.

Tryptophan pyrrolase was determined essentially as described by Knox & Piras (1966), by measuring the increase in extinction at 360 m μ . The reaction mixture contained 0.5 ml. of the supernatant fraction, plus 2.0 ml. of 0.2 M-Na₂HPO₄ buffer (pH 7.0), 0.2 ml. of 40 mM-L-tryptophan, 0.1 ml. of 300 mM-ascorbate and 0.4 μ g. of haemin. The assays were carried out at 25°.

Table 1. Diet for magnesium-deficiency study

	Composition (%)
Casein	25
Glucose*	62.5
Cellulose	3.0
Vegetable oil†	2.0
Arginine	0.8
Glycine	0.5
Methionine	0.2
NaCl	0.5
Defluorinated rock phosphate	4.0
Vitamin mix‡	0.2
KCl	0.4
CuSO ₄	24.2 mg./kg.
FeSO ₄ ·7H ₂ O	297 mg./kg.
MnSO ₄	220 mg./kg.
KI	0.44 mg./kg.

* Cerelease (Corn Products Refining Co., New York, N.Y., U.S.A.).

† Wesson oil (Wesson Oil Co., New Orleans, La., U.S.A.).

‡ Supplies (per kg. of diet): thiamine, 3.52 mg.; riboflavin, 5.27 mg.; folic acid, 1.10 mg.; DL- α -tocopheryl acetate, 22.00 mg.; menadione sodium bisulphite, 0.792 mg.; biotin, 0.176 mg.; pantothenic acid, 18.3 mg.; nicotinic acid, 52.3 mg.; pyridoxine, 5.7 mg.; vitamin B₁₂, 8.8 μ g.; vitamin A, 11 000 U.S.P. units; vitamin D, 1980 I.C.U.

A short lag period was noted before maximal enzymic activity occurred after the reagents had been mixed in the cuvette; therefore the first reading was taken after 5 min. of incubation and the activity was followed for the next 10 min. During this time the increase in extinction was linear with time.

The data were analysed for main effects and interaction by analysis of variance.

RESULTS

The analysis of variance revealed that there was no significant difference between days in the copper experiments; therefore within each experiment the data from the 14-day and 21-day determinations in the first experiment and the 7-day, 14-day and 21-day determinations in the second experiment were combined for the overall analysis. The results are presented in Table 2.

In the first experiment the effect of hydrocortisone in increasing the enzymic activity was significant at the 5% level of confidence. Copper, alone, had no significant effect. The interaction between hydrocortisone and copper, however, was highly significant.

Table 2. *Effect of copper deficiency on the induction of tryptophan pyrrolase by hydrocortisone*

Results are given as the means of six (Expt. 1) or nine (Expt. 2) determinations for each treatment.

Hydrocortisone administered..... Dietary Cu added (p.p.m.)		Tryptophan pyrrolase (μ moles of product/min./g. of protein)	
		None	10 mg./100 g. body wt.
Expt. 1	None	10.4	13.4
	25	8.8	26.7
Expt. 2	None	6.6	10.9
	25	10.9	18.6

Table 3. *Effect of magnesium deficiency on the induction of tryptophan pyrrolase by hydrocortisone*

Results are given as the means of three determinations for each treatment.

Hydrocortisone administered..... Dietary Mg added (p.p.m.)		Tryptophan pyrrolase (μ moles of product/min./g. of protein)	
		None	10 mg./100 g. body wt.
	100	4.7	14.9
	400	6.1	15.1

In the second experiment both the feeding with copper and the administration of hydrocortisone significantly increased tryptophan-pyrrolase activity. Again, in this experiment as in the first, the interaction between the effects of dietary copper and hydrocortisone administration was highly significant.

The results of the experiment on the effects of magnesium deficiency are presented in Table 3. In spite of the fact that the deficient diet resulted in almost 100% mortality by 4 weeks of age, the administration of hydrocortisone induced tryptophan-pyrrolase activity equally in the chicks fed on either the deficient or adequate diets.

DISCUSSION

The highly significant interaction revealed by the statistical analysis of the results of the copper experiments indicates that hydrocortisone was much more effective in inducing tryptophan pyrrolase in the presence of adequate dietary copper than it was in copper deficiency. Since the interaction did not occur in an equally severe magnesium deficiency, the results obtained in the copper experiments cannot be considered as reflecting a non-specific impact of a deficiency on the induction phenomenon, but instead reflects a specificity for the element. This specificity is in accord with the hypothesis that copper is an integral component of chick-liver tryptophan pyrrolase, as found in *Pseudomonas* (Maeno & Feigelson, 1965). According to this hypothesis the induction of the enzyme by hydrocortisone would be decreased in copper deficiency because of the scarcity of this essential element to complete the enzyme synthesis.

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