THE INTERRELATIONSHIP OF DIETARY COPPER AND AMINE OXIDASE IN THE FORMATION OF ELASTIN[1,2]

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Recent investigations have revealed that copper deficiency results in abnormal elastin formation (O'Dell et al., 1961; Coulson and Carnes, 1963; Shields et al., 1962; Starcher et al., 1964). The abnormality is characterized by both a decrease in elastin synthesis and an increase in the lysine content of the elastin that is synthesized. Partridge et al. (1964) reported that the cross linkage groups of elastin are synthesized from lysine residues and proposed that the epsilon amino group of lysine was oxidized to an aldehyde prior to condensation to the cross-linkage groups, desmosine and isodesmosine. Such a reaction would be catalyzed by an amine oxidase. The fact that those amine oxidases which have been isolated contain copper (Yamada and Yasunobu, 1962; Hill and Mann, 1962; Buffoni and Blaschko, 1964) led to the speculation that the role of copper in elastin formation is a reflection of its role in amine oxidase activity. The investigations presented in this report were undertaken to examine this hypothesis.

Methods

Chicks were fed either a copper deficient or a copper supplemented diet (Starcher et al., 1964) from the day of hatching. At various times chicks were killed and a segment of the aortas, including the common carotids

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to the point of division into the subclavian arteries and the main aorta from the base of the heart to a point on the aortic arch equal in length to the carotids, was removed for amine oxidase activity determinations. The method used for enzymatic assay was essentially that of Gorkin et al. (1962) with Cutscum[3] used as the detergent. Twenty aorta segments were pooled for the first day's assay, fifteen from each group for the third day's, ten for the fifth day's, and five thereafter.

For tissue culture studies this segment of the aorta was removed from 2 to 3-week old chicks fed either the copper deficient or the copper supplemented diets. Slices of an individual aorta, 0.5 to 1.0 mm thick, were placed together on a fibrin clot in a petri dish. Four to five aortas were used from chicks in each experimental group.

Five ml. of tissue culture medium (TC 199 with $NaHCO_3$ and glutamine[4]) containing 400 units of penicillin, 400 mcg. of streptomycin, and 50 units of mycostatin per ml. was placed in each petri dish.

For the first 24 hours the tissues were incubated with 1 uc/ml of uniformly labeled lysine-^{14}C. After this time the medium was removed and 5 ml of fresh medium added to each petri dish. In those groups treated with amine oxidase, the fresh medium contained in addition to the radioactive lysine, .4 ml of a suspension of diamine oxidase from hog kidney[5], assaying 20 to 30 Δ O.D. (250 mu)/min./gr. protein of oxidase activity. In the first experiment no additions were made to the control tissues, while in the second the same amount of diamine oxidase which had been boiled was added to the controls. After 12 hours the medium was removed and the tissue washed once with fresh medium containing neither radioactive lysine nor diamine oxidase. The tissue was then incubated for an additional 24 hours with medium containing only the diamine oxidase. In order to insure the presence of active enzyme over this period, the medium was changed every 2 to 3 hours.

3. Fisher Scientific Co., New York, N. Y.
4. Grand Island Biological Co., Grand Island, N. Y.
5. Sigma Chemical Co., St. Louis, Mo.

After a total of 60 hours of incubation the aorta slices were removed from the medium and elastin isolated by extraction with 0.1N NaOH for 90 minutes at 90° C. The residue was washed 3 times with distilled water, dried under vacuum and weighed. The elastin was then hydrolyzed with 6 N HCl for 72 hours at 110° C under vacuum. After removal of the HCl, the lysine and desmosines were separated by thin layer chromatography in a single dimension using a solvent system of butanol-acetic acid-H_2O (3-1-1) followed by 75% phenol. Lysine and desmosines[6] standards were also developed in the same solvent systems. The supporting medium for chromatography was silica gel H.

After chromatography the chromatogram was sprayed with ninhydrin and the lysine and desmosines area located. These areas were removed from the chromatogram, placed in a vial with 0.25 ml of H_2O and 15 ml of toluene-ethanol scintillation mixture. The counting time was 50 minutes in the first experiment and 100 minutes in the second. Efficiency was determined by external standardization.

Results

The results of the amine oxidase determinations are presented in Table 1. The newly hatched chick has no detectable amine oxidase in the aorta or liver. When the diet contained copper the activity in both organs reached a level in three days which was essentially maintained throughout the experimental period of 26 days. On the other hand, when the diet was deficient in copper, enzymatic activity remained undetectable in the aorta until the 26th day and the activity in the liver was consistently lower than that of the control livers.

These results are consistent with the premise that the role of copper in elastin synthesis is mediated by the effect of the element on amine oxidase.

In the next experiment tissue culture studies were conducted to measure the effect of amine oxidase on desmosine formation directly. The results of

6. The authors are indebted to Dr. S. M. Partridge for supplying the samples of desmosine and isodesmosine used in these studies.

Table 1

Amine Oxidase Activity in Liver and Aorta in Relation to Dietary Copper.

	Diet	days of age 1[a]	3[b]	5[c]	10[d]	17[d]	26[d]
		Δ O.D. 250 mu/min./gr. protein					
Aorta	- Cu	0	0	0	0	0	4
	+ Cu	0	15.3	20.7	19.4	14.8	22.2
Liver	- Cu	0	0	1.5	5.6	0	1.9
	+ Cu	0	9.6	8.8	7.3	8.4	8.2

a. Pool of 20 aortas
b. Pool of 15 aortas per group
c. Pool of 10 aortas per group
d. Pool of 5 aortas per group

Table 2

Effect of Amine Oxidase on the Incorporation of Lysine in Desmosines.

Diet	Amine Oxidase	Desmosine dpm (D)	Lysine dpm (L)	D/L
	Exp. 1			
- Cu	-	4	52	.083
- Cu	+	12	74	.157
	Exp. 2			
- Cu	-*	12	210	.058
- Cu	+	44	227	.193
+ Cu	-*	47	280	.168
+ Cu	+	65	227	.284

*Boiled enzyme present at same levels as enzyme treated groups.

these experiments are presented in Table 2. In both experiments the presence of amine oxidase in the culture medium resulted in a greater incorporation of radioactive lysine into the desmosines as indicated by the increased radioactivity of the desmosine with relatively little change in the amount of labeled lysine. In the second experiment the ratio of the radioactivity of the desmosine to lysine was increased by amine oxidase added to the copper deficient aortas to a level commensurate with that observed in the untreated aortas from copper supplemented chicks.

Discussion

The basic premise behind these investigations was that dietary copper affects elastin synthesis by decreasing the activity of an enzyme which is essential for the conversion of lysine to the cross-linkage groups, the desmosines. The most logical type of enzyme to consider in this regard was an amine oxidase since the epsilon amino groups of three of the lysine which condense to form the desmosines are removed and this removal seems likely to be an oxidative one.

If such an hypothesis were correct there were at least two logical consequences. First, in copper deficiency the amine oxidase activity of the aorta would be reduced. The results of the first experiment indicate that this is true. Second, the addition of amine oxidase to a copper deficient system synthesizing elastin would stimulate the formation of the desmosines from lysine. The tissue culture studies indicate that this, also, is true.

With these results it is possible to reconstruct the lesion in elastin formation which occurs when copper deficient diets are fed. The primary lesion is a reduction in amine oxidase activity in the aorta. This reduction results, in turn, in reduced oxidative deamination of the epsilon amino groups of elastin lysine so that fewer lysine residues condense to form desmosine. This would account for the greater amount of lysine and lesser

amount of desmosine found in elastin from copper deficient animals (Miller et al., 1965). The fewer cross-linkages present are probably the cause of the loss of elasticity of the elastin. It is also possible that the fewer cross linkages tend to make this protein more labile and may be the basis for the histological pictures of copper deficient elastin showing discontinuous and fragmented elastin fibrils. Aneurysms, which are the immediate cause of death of copper deficient animals, are probably caused by the lack of elasticity of the vessel.

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