The Derangement of Elastin Synthesis in Pyridoxine Deficiency[1,2]

Charles H. Hill and Chun Su Kim

Department of Poultry Science, North Carolina State University, Raleigh, N. C.

Received March 6, 1967

Kim and Hill (1966) presented evidence indicating that amine oxidase activity was essential for the conversion of lysine residues of elastin into the cross-linkage structures, desmosine and isodesmosine. The impairment of this conversion in copper deficient chicks was associated with a reduction in amine oxidase activity. In those amine oxidases which have been purified, copper has been found to be an essential component (Yamada and Yasunobu, 1962; Hill and Mann, 1962; Buffoni and Blaschko, 1964).

Blaschko and Buffoni (1965) have recently reported that pyridoxal phosphate is a constituent of benzylamine oxidase of pig plasma. If amine oxidase activity could be depressed by dietary pyridoxine deficiency, the effect on elastin should be similar to those of copper deficiency. The studies reported here support this hypothesis.

Methods

The basal diet used in these studies is presented in Table 1. In addition to the ingredients listed the positive control diet was supplemented with 25 ppm $CuSO_4 \cdot 5H_2O$, 13 ppm pyridoxine, and 0.4% KCl. In the individual deficient diets the appropriate nutrient was omitted. The diets were fed ad libitum from the day of hatching. Deionized water was used

1. Contribution from the Department of Poultry Science, North Carolina Agricultural Experiment Station. Published with the approval of the Director of the Experiment Station as paper No. 2354 of the Journal Series.
2. This research was supported in part by Grant No. AM 05651, National Institutes of Health.

throughout the experiments. At ten days of age chicks were taken randomly from each group and the amine oxidase of the aorta was determined as described previously (Kim and Hill, 1965) using the method of Gorkin et al. (1962) with Cutscum used as the detergent.

The elastin content of the aorta was determined by weighing the residue remaining after boiling a section of the aorta for 90 minutes in 0.1 N NaOH.

The conversion of lysine to desmosine was estimated by injecting the chicks with 10 microcuries (expt. 2) or 6 microcuries (expt. 3) of uniformly

Table 1

Basal Diet

	%
Casein (Vitamin Free)[1]	25.0
Glucose	62.5
Cellulose	3.0
Fat[2]	2.0
Defluorinated Rock Phosphate	4.0
Arginine HCl	0.86
Methionine	0.1
Glycine	0.58
Vitamin Mix[3]	0.2
Choline Chloride	0.15
NaCl	0.5
Mg SO_4	0.24
Mn SO_4, ppm	220
$FeSO_4 \cdot 7H_2O$, ppm	300
KI, ppm	0.5

1. Nutritional Biochemicals Corporation, Cleveland, Ohio
2. Wesson Oil, The Wesson Oil Company, New Orleans, La.
3. Supplies/Kg diet, Vitamin A, 8,800 U.S.P. units, Vitamin D, 880 I.C.U., alpha tocopherylacetate 22 mg., menadione sodium bisulfite 2.33 mg., thiamine 7.9 mg., riboflavin 15.8 mg., calcium pantothenate 44 mg., niacin 119 mg., biotin 0.4 mg., folic acid 5.3 mg., Vitamin B_{12} 40 mcg.

labelled ^{14}C Lysine, isolating the elastin as described above, hydrolyzing with 6N HCl for 72 hours, separating the lysine from the desmosine by thin-layer chromatography and determing radioactivity as described previously (Kim and Hill, 1966).

Results

The results of the experiments on the effects of pyridoxine and copper deficiencies on aortic elastin content, amine oxidase, and the proportion of lysine converted to the desmosines are presented in Table 2. Each deficiency resulted in a reduction in each of these measurements. The combined deficiencies further reduced the elastin content of the aorta and the conversion of lysine to the desmosines.

Table 2

Effect of Pyridoxine and Copper Deficiencies on Elastin Synthesis and Amine Oxidase of the Chick Aorta.

Diet	Body Wt. gm.	Elastin[1] %		Amine Oxidase[2] A250/min/gr. protein		Radioactive[3] Desmosines/Lysi
		Expt.		Expt.		Expt.
		1	2	1	2	2
-Cu $-B_6$	52	5.8	5.6	0	0	.05
-Cu $+B_6$	71	6.6	6.6	4.7	0	.19
+Cu $-B_6$	58	6.6	6.5	0	0	.08
+Cu $+B_6$	81	8.0	8.3	9.3	15.9	.25

1. Expressed as % of wet wt.
2. Assays conducted on 5 pooled aortas in each group.
3. Pooled elastin from 4 chicks in pyridoxine supplemented groups and 3 chicks in pyridoxine deficient groups.

These data indicate that the reduction of aortic amine oxidase activity brought about by pyridoxine deficiency has the same effect on the synthesis of elastin as does the reduction brought about by copper deficiency. The concept that amine oxidase activity is essential for the synthesis of normal elastin is thus strengthened.

One possible objection to this interpretation of the data is that pyridoxine deficiency results in slower growth of the animals. It has been shown by Starcher et al. (1964) and Kim and Hill (1966) that both elastin and amine oxidase increase with the age of the growing chick so that the correlations observed in these studies may have been a fortuitous association with slower growth.

In order to explore this possibility an other experiment was conducted in which potassium deficiency was imposed on one group of chicks as a means of retarding chick growth. The results are presented in Table 3.

Table 3

Effect of Pyridoxine and Potassium Deficiencies on Elastin Synthesis and Amine Oxidase of the Chick Aorta.

Diet	Body Wt. gm.	Elastin[1] %	Amine Oxidase[2] A250/min/gr. protein	Radioactive[3] Desmosines/Lysine
$-B_6$	58	6.6	3.6	0.13
-K	40	5.3	14.6	0.55
Control	76	7.8	28.5	0.21

1. % wet weight.
2. Assays conducted on 6 pooled aortas in each group.
3. Pooled elastin from 5 chicks.

The severe potassium deficiency prevented growth. The elastin content of the aorta in the potassium deficient chicks was the lowest of the groups and was approximately at the level found by Starcher et al. (1964) to be present in day old chicks. The aortic amine oxidase activity was depressed in the potassium deficient chicks but not to the extent that it was in the pyridoxine deficient animals. The conversion of lysine to desmosine, on the other hand, was not depressed but increased in the potassium deficient chicks.

Discussion

The evidence presented in this report indicates that amine oxidase activity of the aorta is sensitive to pyridoxine deficiency and would suggest that this enzyme like that present in pig plasma contains a pyridoxine derivative.

Associated with the reduced amine oxidase activity in pyridoxine deficiency is a reduced elastin content of the aorta and a reduced conversion of lysine to desmosine. It is possible that part of the reduced elastin content of the aorta is a simple association with the slower growth of the deficient animals, but the data obtained with the potassium deficient animals indicates that the reduced conversion of lysine to desmosine is not a reflection of slow growth, quite the contrary. Although growth was inhibited by the potassium deficient diet and the elastin content of the aorta remained at the hatching level, the amine oxidase activity developed and apparently was capable of catalyzing reactions leading to the conversion of lysine to desmosine. The increased conversion may be merely a reflection of the fact that little new elastin was formed under these circumstances so that the substrate of the enzyme, so to speak, remained relatively constant and the desmosines formed were not diluted with new pro-elastin. These findings lend further support to the hypothesis that amine oxidase plays an essential role in the formation of elastin.

One other aspect of these studies deserves comment. It has not been reported before that pyridoxine deficiency leads to a decreased elastin content of tissues. It is probable that this lesion has been overlooked before because severely deficient animals quickly cease growing and die from causes other than ruptured aortas. Copper deficient animals, on the other hand, survive longer and when they do die the ruptured aorta associated with the death directs attention to the tissues of this vessel.

References

Blaschko, H., and Buffoni, F., Proc. Roy Soc. B. 163, 45 (1965).
Buffoni, F., and Blaschko, H., Proc. Roy. Soc. B. 161, 153 (1964).
Gorkin, V. Z., Grindeva, L. I., Romanova, L. A., and Severina, I. S. Biokhimiya 27, 1004 (1962).
Hill, J. M., and Mann, P. J. G., Biochem. J. 85, 198 (1962).
Kim, C. S., and Hill, C. H., Biochem. Biophys. Res. Com 24, 395 (1966).
Starcher, B., Hill, C. H., and Matrone, G., J. Nutrition 82, 318 (1964).
Yamada, H., and Yasunobu, K. T., J. Biol. Chem. 237, 1511 (1962).