The Effect of Pyridoxine Deficiency on Aortic Elastin Biosynthesis (34219)

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An intake of dietary copper is required for the formation of desmosine and isodesmosine, the important cross-linking amino acids of elastin (1, 2). These cross-linking units arise as the result of an oxidation of lysine, at its ϵ-amino group, to α-aminoadipic semialdehyde, a step catalyzed by a copper-containing amine oxidase (3). Hill and Kim (4) suggested that this enzyme contains a pyridoxine derivative and that pyridoxine deficiency affects elastin biosynthesis in the same manner as does copper deficiency. However, information gathered in recent experiments in this laboratory was not in agreement with some of the data reported by these investigators. We decided to explore the problem by comparing quantitatively the desmosine and isodesmosine content of aortic elastin in pyridoxine-deficient and in control chicks and, with the aid of an *in vitro* ^{14}C-lysine pulse-labeling technique, the degree of oxidation of lysine, at its ϵ-amino function, in pyridoxine-deficient and in normal chick aortas.

Our results, in general, confirm the hypothesis of pyridoxine involvement in elastin biosynthesis. Discordance remains, however, between the data reported here and those reported by Hill and Kim (4) with respect to some corollary aspects.

Methods. Feeding studies were conducted with day-old Arbor Acre chicks fed a basal dried skim-milk diet, with or without copper supplement (5); or a vitamin-free casein diet (4), supplemented with all vitamins except for individual deficiencies in riboflavin, pyridoxine, and pantothenic acid. All groups were fed *ad libitum*, with the exception that a fully supplemented control group was pair fed to the pyridoxine-deficient group. After 14 days on the experimental diets, the chicks were killed, their aortas were removed and the elastin content therein was determined as described previously (5). Extraction time in 0.1 *N* NaOH was reduced to 60 min at 100°, a period previously shown to be sufficient to remove collagen and other protein (6).

A basal tissue culture medium for *in vitro* studies was prepared by a modification of SRI-8 (7) medium, with lysine, pyridoxine, and pyridoxal being omitted. Additions to the basal medium were made as 0.25-ml Millipore-filtered, aqueous solutions.

Aortas of 1–17-day-old chicks as indicated, were used in the *in vitro* experiments. The aortas were removed aseptically, freed of adhering tissue, and washed twice in culture medium. The cleaned aortas were minced and distributed into 5-cm diameter petri dishes in lots of approximately 600 mg/dish. Culture medium (2.5 ml) containing 1.5 μCi of ^{14}C-lysine was added to the tissues and the plates were incubated for 24 hr at 37°. The medium was then removed from the tissues, they were washed once with unlabeled medium, and then incubated for a further 48 hr in unlabeled medium. After a total of 72-hr incubation, the tissues were washed with water, and their elastin was purified as described above.

Amino acid analyses for determination of the desmosines and lysine were performed on 500-μg samples of 48-hr elastin hydrolysates using a Beckman model 116 analyzer (8).

For the resolution of radioactive lysine and its oxidation products, we employed chromatography on a volatile buffer column slightly modified from that described previously (9). Bio-Rad AG 50W-X8, 200-400 mesh, was

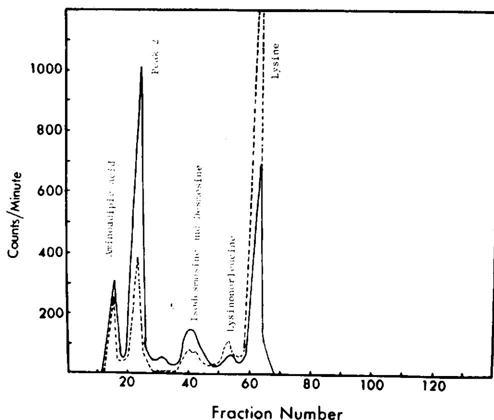

FIG. 1. Chromatogram of ^{14}C-lysine labeled elastin hydrolysates eluted from a 0.9 × 54-cm cation exchange resin column, employing a pyridine-acetic acid buffer system: (—), the hydrolysate of elastin from control chick aortas; (- - -), the hydrolysate of elastin from pyridoxine-deficient chick aortas.

packed to a resin height of 54 cm in 0.9-cm diameter columns. Flow rates with this resin enabled runs to be completed within 18 hr. The elution gradient was prepared by using 150 ml of 0.88 *N* pyridine–acetic acid buffer, pH 5.0, in the storage vessel and 150 ml of 0.2 *N* pyridine–acetic acid buffer, pH 3.0, in the mixing chamber. Fractions were collected by drop counting, with collection volumes starting at 2.5 ml and increasing to 3 ml because of changing buffer composition. Radioactivity was measured by drying 0.5 ml from each fraction on planchets and counting on a Nuclear Chicago low background counter.

Results. Pulse-labeling with ^{14}C-lysine. Aortic samples were removed from pyridoxine-deficient and pyridoxine-supplemented chicks after they had been fed the experimental diets for 10 days. The aortas were then pulsed *in vitro* with ^{14}C-lysine and the elastin hydrolysates resolved by column chromatography as desdribed in "Methods." Radioactivity measurement (Fig. 1) of the fractions showed a reduction in lysine oxidation in the aortas from pyridoxine-deficient chicks as compared to that in the controls. This was indicated by the lesser amounts of oxidation products and the greater amounts of residual lysine in the deficient aortas. Peak 2 arises from the hydrolysis of the aldol condensate formed from the α-aminoadipic semialdehydes (10).

This observed distribution of incorporated ^{14}C-lysine and the resolvable and identifiable products of lysine oxidation was exploited further in pulse-labeling experiments by using, as an index of the extent of ε-aminolysine oxidation, the ratio of the sum of the counts incorporated in peaks other than lysine to the total recovered counts including the lysine. Table I summarizes the results of several such experiments. In Expt. 1, day-old chick aortas were cultured in the presence and the absence of pyridoxine. Total incorporation was the same and there was no difference in the degree of lysine oxidation. This was not unexpected as it is very probable that enough pyridoxine is present in the day-old aortas to meet a possible cofactor requirement for the 72-hr culture period. In order to deplete the system, in Expt. II chicks were fed with pyridoxine-deficient and supplemented diets for 6 days prior to the culture of the aortas. Pyridoxine was left out of the culture medium to avoid an exogenous supply. Aortas from the chicks fed the pyridoxine-deficient diet oxidized incorporated lysine to a lesser degree than did the control aortas. Addition of pyridoxine to the culture medium did not return the oxidation rate to control values. That the reduction in lysine oxidation seen in pyridoxine deficiency is probably specific and not duplicated by other vitamin deficiencies was demonstrated in Expt. III. Chicks were fed the experimental diets for 10 days prior to culturing the aortas. Pyridoxine deficiency reduced the percentage oxidation to 63% of the controls. Pantothenic acid and riboflavin deficiencies did not show a reduction in lysine oxidation, and if anything tended to increase it.

The effect of copper deficiency on this system is shown in Table I, Expt. IV. Oxidation of lysine was reduced to 27%, a much greater reduction than that seen in pyridoxine deficiency.

Chemical composition studies. The end result of decreased lysine oxidation in elastin is a reduction in the amount of the cross-linking amino acids, desmosine and isodesmo-

TABLE I. Effect of Pyridoxine Deficiency and Copper Deficiency on the Ability of Chick Aortas to Oxidize Lysine *in Vitro*.

Expt.	Diet	Days on diet	Pyridoxine in media (mg/ml)	^{14}C-lysine (total counts)	Lysine oxidation (%)[a]	% of control
I	Control	0	2	25,742	68	100
	Unsupplemented		0	24,695	71	104
II	Control[b]	6	0	34,545	58	100
	Pyridoxine-deficient		2	36,397	49	84
	Pyridoxine-deficient		0	39,932	46	78
III	Control	10	0	12,680	60	100
	Pantothenic acid-deficient		0	14,295	66	110
	Riboflavin-deficient		0	16,377	69	115
	Pyridoxine-deficient		0	15,510	39	63
IV	Control	17	2	33,112	56	100
	Copper-deficient		2	7587	15	27

[a] Ratio of the total counts recovered from peaks other than lysine/total counts.
[b] Chicks were pair fed to the pyridoxine-deficient chicks.

sine. As these arise directly from lysine via ε-amino oxidation of lysine, perhaps the most unambiguous measure of the possible effect of pyridoxine deficiency on elastin formation would be the actual measurement by analysis of the amount of these cross-linking units compared to the amount of lysine present. Table II shows a quantitative tabulation of the effect of pyridoxine deficiency on elastin production and the synthesis of desmosine and isodesmosine. There is a highly significant reduction in the ratio of the desmosines to lysine in the pyridoxine-deficient chicks. This decrease represents approximately a 50% reduction in the amount of lysine converted to desmosine and isodesmosine. The effect of copper deficiency was again much more severe than pyridoxine deficiency, with conversion of lysine to desmosine being reduced 80% from the control values.

Contrary to the results of Hill and Kim (4), we did not find a reduction in the percentage of elastin present in aortas from pyridoxine-deficient chicks. There was also no correlation between rate of growth and elastin synthesis, as both the pair-fed and riboflavin-deficient animals grew very little, yet had normal levels of elastin. Copper-deficient chicks grew substantially more than pyridoxine-deficient chicks, yet had a much

TABLE II. Effect of Copper Deficiency and Pyridoxine Deficiency on Aortic Elastin.[a]

Diet	Expt. 1			Expt. 2		
	Body wt (g)	Elastin (%)[b]	Des + Ides/Lys[c]	Body wt (g)	Elastin (%)[b]	Des + Ides/Lys[d]
Control	96	10.3 ± 0.4	0.45 ± 0.01	—	—	—
Riboflavin-deficient	66	10.1 ± 0.3	0.48 ± 0.02	—	—	—
Pyridoxine-deficient	69	10.4 ± 0.2	0.25 ± 0.01	56	9.1 ± 0.7	0.24 ± 0.01
Pair-fed, supplemented	—	—	—	54	9.3 ± 0.5	0.47 ± 0.02
Copper-deficient	127	6.9 ± 0.3	0.11 ± 0.002	116	5.8 ± 0.2	—
Copper-supplemented	160	11.1 ± 0.3	0.47 ± 0.03	147	9.0 ± 0.3	—

[a] Chicks were fed the experimental diet for 14 days from day of hatch.
[b] Mean value of 5 individual aortas with standard error.
[c] Mean value of analysis of elastin from 3 individual aortas with standard error.
[d] Mean value of analysis of elastin from 5 individual aortas with standard error.

lower content of aortic elastin.

Discussion. The data presented here confirm the hypothesis that pyridoxine deficiency, like copper deficiency, does reduce the rate of lysine oxidation in aortic elastin with a concomitant reduction in the amount of desmosine and isodesmosine formed. This is in agreement with the results of Hill and Kim (4) and supports these authors' suggestion that there is a pyridoxine cofactor requirement for the enzymatic oxidation of lysine at its ϵ-amino position to form α-aminoadipic semialdehyde. Comparison of the amount of total lysine-oxidation products, not just of the amount of the desmosines, with the amount of lysine present in the elastin in variously treated chick aortas, leads to the further conclusion that the primary step, namely the oxidation of lysine, and not some step subsequent to it in the sequence of cross-link formation, is the one affected by the deficiencies.

Pyridoxine deficiency, however, does not affect elastin biosynthesis to the same degree as does copper deficiency. In both the tissue culture and the *in vivo* studies, the reduction in lysine oxidation and desmosine synthesis is much more marked in copper deficiency than in pyridoxine deficiency. Antimetabolites of pyridoxine might increase this reduction if used in addition to the deficiency but this possibility was not tested.

In no instance did we find that pyridoxine deficiency reduced the percentage of elastin in the aorta. Hill and Kim (4) reported such a reduction in elastin content. The reason for this discordance is somewhat puzzling unless the longer extraction times (90 min) used by these investigators began solubilizing elastin from the deficient chicks at a faster rate than from the controls. It should be recalled that the definition of an elastin preparation is operationally empirical. That the chicks used in our studies were adequately pyridoxine deficient is evidenced by their lack of growth and high mortality, reaching 50% by 14 days of age.

It was also true in these experiments that the percentage of elastin found in the aorta did not appear to be a function of growth, but rather a function of age. When measuring elastin content of aortas we have found results most reproducible when using chicks between 2 and 3 weeks of age. Control aortas from chicks younger than this have not reached plateau levels of elastin content and small differences among experimental groups can be ambiguous and sometimes misleading.

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