ELASTIN: DIMINISHED REACTIVITY WITH ALDEHYDE REAGENTS IN COPPER DEFICIENCY AND LATHYRISM

By E. J. MILLER,* Ph.D., and HAROLD M. FULLMER, D.D.S.

(*From the National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland*)

Plates 106 and 107

(Received for publication 2 March 1966)

In order to account for the elasticity of hydrated elastin fibers as well as their insolubility and swelling properties, it has long been assumed that the individual polypeptide chains of the protein were joined together by covalent cross-links (1). Recent investigations have led to the isolation (2) of two new amino acids from bovine ligamentum nuchae elastin. The compounds were shown to be structural isomers (3) composed of a pyridinium ring with four side chains located at the 1, 3, 4, 5 positions (desmosine), and at the 1, 2, 3, 5 positions (isodesmosine). Since these amino acids were found in peptides containing multiple carboxyl- and amino-terminal groups (4), it has been suggested (3, 5) that either isomer could serve as a cross-link between as many as four different polypeptide chains.

Studies on the incorporation of radioactivity into the desmosines from lysine-U-C^{14} (5–7) as well as the age-dependent changes in the content of the desmosines and lysine in chick aorta elastin (6, 8) have indicated that lysine, in peptide linkage, is subsequently converted to the desmosines in elastin.

Defects in aortic elastin are known to occur in copper deficiency and lathyrism. The common consequence of copper deficiency observed in chicks (9–12) and in swine (13–15) was rupture of the aorta associated with histologic evidence of fragmentation and dissolution of the elastic laminae in the aortic wall. Similar degenerative changes in elastic fibers of the aorta have been observed in rats (16–25), mice (26), and turkeys (27, 28), which were maintained on diets containing β-aminopropionitrile (BAPN) or the seeds of *Lathyrus odoratus*.

The cause of these defects appears to be an interruption of the normal cross-linking. Lathyrogens such as BAPN have been shown to inhibit the biosynthesis of the desmosines (7, 29) as well as the cross-linking of collagen (30–32). Aldehydes have previously been suggested (33) as participating in collagen cross-linking and it has further been proposed (5) that the synthesis of desmosine and isodesmosine from lysine proceeds through an aldehyde intermediate.

* American Dental Association Research Associate.

Elastin isolated from aortas of copper-deficient (7, 34) and lathyritic (35) chicks had a 2- to 3-fold increase in lysine content accompanied by only a slight decrease in desmosine content suggesting the presence of an undetected intermediate in the formation of the desmosines. Thus, it would appear that in states of copper deficiency and lathyrism, lysine normally destined for conversion to the intermediate, and subsequently to the desmosines, remains as lysine.

The present report details the results of histochemical and chemical investigations designed to detect the chemical state of the intermediate in the formation of desmosine and isodesmosine from lysine. Elastin in the aortas from copper-deficient or lathyritic chicks should display little or no reactivity with a reagent specific for a chemical group carried by the intermediate. On the other hand, elastin fibers in the aortas from control chicks should react readily with such a reagent.

Materials and Methods

Rhode Island Red chicks, obtained from a local hatchery at 1 day of age, were used. Copper-deficient and control chicks (24 in each group) were raised for 3 wk on a synthetic diet as described previously (7). In these experiments, the control animals received the copper-deficient diet which had been supplemented with 25 ppm copper in the form of $CuSO_4 \cdot 5H_2O$. Lathyritic and control chicks (24 in each group) were maintained for 3 wk on a normal ration supplied by the hatchery. A state of lathyrism was promoted in the experimental animals by the addition of 1 g β-aminopropionitrile fumarate per kg of diet throughout the feeding period. At the end of 3 wk, the chicks in each group were killed by decapitation, and 0.5 cm of ascending aorta was excised from an area proximal to the point of exit of the carotid arteries.

Six aortas from each group, i.e. lathyritic and control, as well as copper-deficient and control, were studied histochemically. One half of each of the aortas was fixed for 24 hr at 25°C in Lillie's aqueous neutral calcium acetate formalin, dehydrated, embedded in paraffin, and sectioned both cross and longitudinally at 6 μ. The other half of each aorta was frozen on a block of dry ice, affixed to a chuck, and sectioned both cross and longitudinally in a cryostat at 6 μ. Frozen sections which were not used promptly were placed in a cardboard folder encased in a plastic cover to prevent dehydration and stored in a refrigerator until used (within 1 wk).

Stains used for the identification of elastic fibers were the traditional Taenzer-Unna orcein (36) and the more selective orcinol-new fuchsin (37). Aldehydes were detected histochemically by the application of Schiff's reagent which had been prepared by the cold method (36). In all experiments, comparable sections from experimental and control animals were handled and stained simultaneously in the same solutions and containers.

Elastin was isolated from some of the lathyritic and control aortas by a procedure which was designed to minimize selective hydrolysis of easily hydrolyzable peptide linkages in the insoluble product. In this procedure, the tissues were extracted at neutral pH and at 5°C for successive 48-hr periods in 3% Na_2HPO_4, 25% KCl, and 5 M guanidine hydrochloride. Following these extraction periods, the insoluble residue was incubated at 37°C for an additional 48 hr with purified collagenase (CLSPA, Worthington Biochemical Corp., Freehold, New Jersey). 1 mg of collagenase in 5.0 ml of 0.01 N tris (hydroxymethyl) aminomethane buffer (pH 7.5) was used per 10 mg of insoluble substrate. After incubation, the elastin preparations were washed several times with distilled H_2O to remove buffer, enzyme, and soluble products of the enzyme digestion. The guanidine extraction and incubation with collagenase were repeated and the elastin preparations were dried by lyophilization.

Elastin from control and lathyritic animals prepared in this manner was tested for reactivity with 2,4-dinitrophenylhydrazine (2,4-DNPH) (38), and amino acid analyses were performed on an automatic amino acid analyzer (39).

Elastin preparations were solubilized by incubating 5 mg of elastin at 37°C in 10 ml of 0.1 M glycine buffer (pH 7.0) containing 1 mg of electrophoretically purified elastase (Worthington Biochemical Corp.). Under these conditions, complete digestion of the elastin preparations was obtained within 48 hr. Aliquots of the control and lathyritic elastase digests were examined spectrophotometrically for protein content, amino acid content (39), and reactivity with the highly specific and sensitive carbonyl reagent, *N*-methyl benzothiazolone hydrazone hydrochloride (MBTH) (40). This reagent reacts with a carbonyl group to form an azine. Azines formed from aldehydes have an active hydrogen which can react with an oxidized form of MBTH to form a tetraazopentamethine cyanine dye. This second stage of the reaction provides a highly specific method for the colorimetric determination of aldehydes. As pointed out by Paz et al. (41), the azine products of the first stage of the reaction have characteristic ultraviolet absorption spectra which enable one to follow the progress of the reaction as well as distinguish between azines formed from saturated and unsaturated aldehydes. Spectrophotometric assays of the products formed by the reaction of 2,4-DNPH with insoluble elastin and by the reaction of MBTH with elastase-solubilized elastin were made in a Carey recording spectrophotometer (model 11) using an appropriate blank.

RESULTS

Histochemical Studies.—Microscopic examination of sections of aortas from lathyritic and copper-deficient animals revealed the characteristic abnormalities of elastic fibers described by previous investigators. Sections from animals in both experimental groups (Fig. 1) manifested elastic fiber fragmentation, abnormally shaped fibers, elastic fibers with bulges, and clumps of intercellular elastic material. A section illustrating the density and arrangement of elastin fibers in aortas from control and copper-supplemented animals is given in Fig. 2. Sections stained for the detection of aldehydes exhibited a considerable difference in reactivity. Elastic fibers, in either paraffin-embedded or fresh frozen sections of aortas from control chicks subjected to Schiff's reagent without a previous oxidation, stained moderately indicating the presence of indigenous aldehyde groups (Fig. 3). Elastic fibers in comparable sections from either copper-deficient or lathyritic animals stained only faintly, if at all (Fig. 4).

Chemical Studies.—The amino acid composition of elastin isolated by the nonhydrolytic method from aortas of control and lathyritic chicks is given in Table I. While the composition is similar to that previously published for chick aorta elastin which had been isolated by hot alkali extraction (6, 7), significant differences were observed in the content of acidic, basic, hydroxy- (threonine and serine), and sulfur-containing amino acid residues. It is considered unlikely that the increase in acidic and basic residues is due to incomplete removal of collagen from the insoluble residue as the hydroxyproline content of the elastin prepared in this manner is somewhat lower than that observed in the alkali-extracted preparations. The absence of hydroxylysine would also indicate that collagen was not a significant contaminant in these elastin preparations. It is likely that the differences observed between the present preparations and those

obtained by hot alkali extraction are due to a decrease in selective hydrolysis and destruction of the amino acids in the insoluble residue when the nonhydrolytic procedure is employed. Also, it was found that isolation of elastin by the nonhydrolytic procedure has the added advantage of better preservation of labile functional groups in the protein which may be destroyed during alkali

TABLE I

*Amino Acid Composition of Elastin Isolated from the Aortas of 3-wk-old Control and Lathyritic Chicks**

Amino acid	Residues/1000 total residues	
	Control	Lathyritic
Hydroxyproline	16.6	15.7
Aspartic acid	6.6	6.6
Threonine	12.8	13.4
Serine	10.0	10.2
Glutamic acid	15.7	15.7
Proline	125	123
Glycine	339	336
Alanine	171	172
Half-cystine‡	4.6	5.2
Valine	166	164
Methionine	1.0	1.1
Isoleucine	20	21
Leucine	55	55
Tyrosine	11.6	11.4
Phenylalanine	23	23
Quarter-desmosine plus isodesmosine§	7.9	8.1
Lysine	*4.8*	7.7
Histidine	1.4	1.6
Arginine	7.2	7.6

* Values in each column represent the average of 4 determinations performed on 2 separate preparations of control and lathyritic elastin.

‡ Represents sum of cysteic acid and cystine.

§ Expressed as quarter residues since each molecule is presumed to occupy four positions in the polypeptide.

extraction. This was particularly true of aldehyde groups, which are discussed below.

In the present study, control and lathyritic elastin were found to differ only with respect to lysine content. The total amount of lysine and quarter-desmosine plus isodesmosine in the lathyritic elastin equaled 15.8 residues per 1000 total residues and the corresponding value in control elastin was 12.7. This result suggests that in control elastin at least 3 lysyl residues per 1000 total amino acid residues exist in a form which is undetected after acid hydrolysis

of the protein. The actual figure is probably higher since the isolation procedure may remove a low cross-linked fraction which would be enriched in lysine.

Since the histochemical studies had shown the presence of considerably more aldehyde groups in elastic fibers of the aortas of control animals, it was reasonable to assume that the apparent loss of lysine in control elastin was due to an alteration in some of the peptide-bound lysyl residues resulting in the formation of aldehydes. Thus, additional studies were performed on elastin isolated by the nonhydrolytic method from the aortas of control and lathyritic animals in an attempt to quantitate and characterize the aldehyde groups associated with the purified protein.

5 mg each of control and lathyritic elastin were shaken at room temperature with 20 ml of a 0.5% solution of 2,4-DNPH in 1 N HCl for 20 min. At the end of this period the elastin preparations were immediately washed in fresh 1 N HCl and then dialyzed at 5°C for 1 wk against several changes of distilled H_2O. Following this treatment control elastin was stained more deeply yellow than lathyritic elastin indicating that the formation of 2,4-dinitrophenylhydrazones had been significantly greater in the case of control elastin.

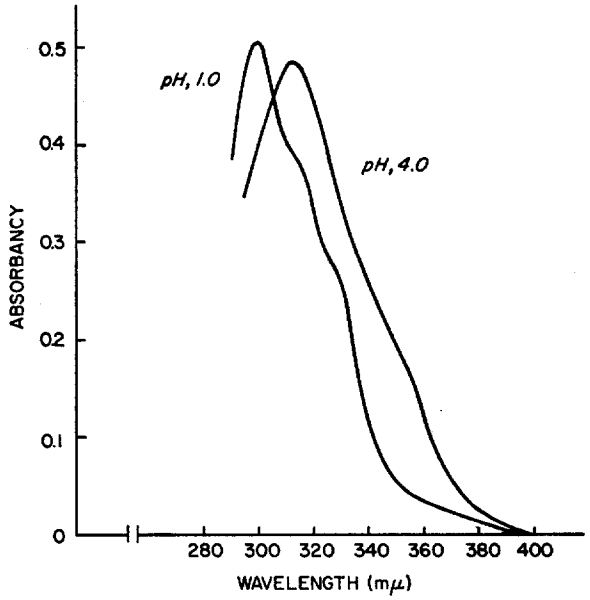
The 2,4-DNPH elastins were then solubilized by incubation in purified elastase as described in the methods section. Within an hour after the beginning of digestion with elastase the incubation solution appeared yellow. The yellow color of the solution was not apparent when similarly treated samples were incubated under the same conditions without elastase suggesting that the 2,4-DNPH was in fact bound in some manner to the elastin fibers.

When the control and lathyritic elastins had been completely dissolved in the elastase solution, absorption spectra of the incubation solutions were read from 600 to 300 mμ using blanks which consisted of elastase digests of control and lathyritic elastin which had not been reacted with 2,4-DNPH. The spectral characteristics of the control and lathyritic elastase digests were identical in that both exhibited a single peak with a maximum absorption at 380 mμ. However, the concentration of 2,4-dinitrophenylhydrazones in the control digest was approximately twice that in the lathyritic digest as indicated by measurement of the absorbance at 380 mμ. It would appear that the spectra observed in this experiment were due to the presence of 2,4-dinitrophenylhydrazones in the elastase digests since 2,4-DNPH alone absorbs maximally at 358 mμ.

Further evidence for the presence of aldehyde groups in the elastin preparations was sought by use of the Sawicki reagent, MBTH. For these experiments 5 mg each of control and lathyritic elastin were first solubilized by incubation with purified elastase as previously described. When enzyme digestion had been completed, ultraviolet absorption spectra of the incubation solutions indicated that protein concentration was equal in both digests. This was confirmed by amino acid analysis of acid-hydrolyzed aliquots of the digests. On the basis

of valine concentration, it was estimated that the concentration of enzyme-digested elastin was 0.45 mg/ml in both digests.

The pH of the digests was adjusted to 4.0 and 3.0 ml aliquots were allowed to react with 0.2 ml of an aqueous 0.1% solution of MBTH at 40°C (41). A solution of elastase, identical to that used to solubilize the elastins, was allowed to react with MBTH under the same conditions. Absorption spectra of the reaction solutions were made at hourly intervals after addition of the MBTH solution. The elastase-MBTH solution was used as a blank when spectra were

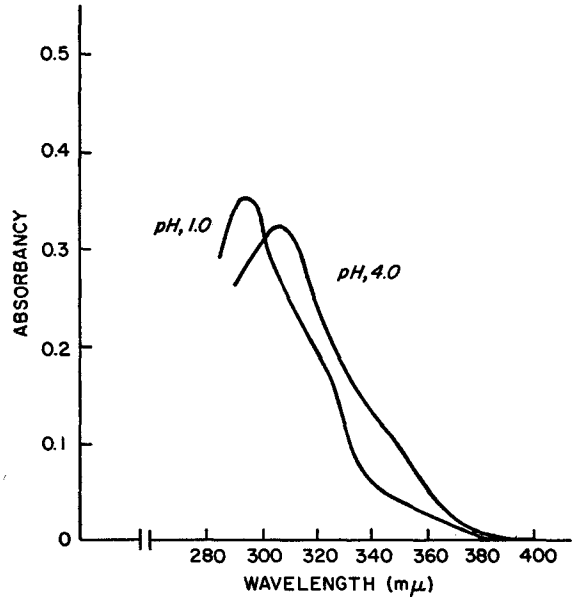


TEXT-FIG. 1. Absorption spectra read at the completion of the reaction of MBTH with the control elastin digest.

made of the control and lathyritic digests. Spectra of the elastase-MBTH solution were obtained with a blank which consisted of an aqueous solution of MBTH at the same concentration.

Approximately 16 hr were required for completion of the reactions in the control and lathyritic elastin digests and absorption spectra obtained after this period are shown in Text-figs. 1 and 2 (pH 4.0). In both instances the curves are characterized by an λ_{max} at 312 mμ and a broad shoulder at about 350 mμ. Absorption spectra of the elastase-MBTH solution throughout the course of the reaction revealed no change in absorbance over the 280 to 400 mμ range and indicated that azine formation from carbonyl groups associated with elastase were not responsible for the spectra of the digests.

Since the azine derivatives of saturated aldehydes have rather narrow absorption bands with λ_{max} from 306 to 312 mμ while the azine derivatives of α,β-unsaturated aldehydes have somewhat broader absorption bands with λ_{max} from 326 to 331 mμ (41), the wide range of wavelengths over which absorbancy was observed in these experiments suggested that the elastin digests contained a mixture of saturated and unsaturated aldehydes. This view was supported by the observation that the entire spectra could be shifted to lower wavelengths by adjusting the pH of the reaction mixtures to 1.0 as also shown



TEXT-FIG. 2. Absorption spectra read at the completion of the reaction of MBTH with the lathyritic elastin digest.

in Text-figs. 1 and 2. Spectral shifts such as these at low pH values are characteristic of azine derivatives of both saturated and unsaturated aldehydes (41).

In order to use the spectral curves as a means of estimating the number of carbonyl groups associated with the elastin preparations, solutions of model compounds at various concentrations were reacted with MBTH under the same conditions as employed in the reaction of MBTH with the elastin digests. It was found that upon completion of the reaction with MBTH, a mixture of glyceraldehyde (0.018 μmole/ml) and crotonaldehyde (0.009 μmole/ml) exhibited an absorption spectrum very similar to that obtained from the control elastin digest, Text-fig. 1. A spectral shift with maximum absorption at 300 mμ was also noted when the pH of the known mixture was adjusted to 1.0. This result indicated that in the control elastin digest, a total carbonyl concen-

tration of about 0.027 μmole/ml was associated with an elastin concentration of 0.45 mg/ml. Assuming an average amino acid residue weight of 100, the elastin concentration of 0.45 mg/ml represents an effective amino acid concentration of 4.5 μmole/ml. Thus, it would appear that approximately 6 carbonyl groups are present in control elastin for every 1000 amino acid residues. A similar consideration of the spectrum derived from the lathyritic elastin digest (Text-fig. 2) indicated that only 4 carbonyl groups were present for every 1000 amino acid residues. In general, it was found that the reaction of model compounds with MBTH was much faster than reaction with the elastin digests. The reaction in the glyceraldehyde-crotonaldehyde mixture mentioned above required only 8 hr.

1 ml aliquots of the control and lathyritic elastin digests in which azine formation had been completed were cooled to room temperature and tested colorimetrically for the presence of aldehydes (41). A bluish-green color was developed in both digests indicating the formation of tetraazopentamethine cyanine dyes and providing further evidence that the reactive groups in the elastin digests were in fact aldehydes. A standard curve for the colorimetric determinations was obtained by reacting 2:1 mixtures of glyceraldehyde and crotonaldehyde at various total concentrations with MBTH under the same conditions. The solutions were read at 670 mμ against a reagent blank. An absorbance of 0.161 was noted in the control digest while that observed in the lathyritic digest was 0.102, indicating that total aldehyde concentration in the original elastin digests was 0.031 and 0.019 μmole/ml respectively. These results for total aldehyde concentration in the elastin digests are within experimental error of those obtained by examination of the spectral curves of the azine derivatives.

DISCUSSION

In this report we have presented evidence that elastin fibers of the chick aorta possess free aldehyde groups. Inasmuch as histochemical methods do not localize at the molecular level, it cannot be categorically stated that the aldehydes identified histochemically are those detected chemically. Histochemical observations of both formalin-fixed and fresh frozen sections from aortas of control and experimental groups (lathyritic and copper-deficient) treated identically and simultaneously in the same staining and other solutions clearly revealed less staining of elastic fibers in the aortas of experimental animals in comparison to those from control animals as depicted by Figs. 3 and 4. Detection of aldehydes in comparable specimens with both histochemical and chemical methods, and alteration of their availability in response to experimentation as determined by both methods, strongly suggests that they may both be identifying the same aldehyde groups.

Chemical examination of elastin isolated from the aortas of control and lath-

yritic animals revealed that the aldehyde content varies inversely with lysine content suggesting that peptide-bound lysine is converted to an aldehyde-containing derivative in elastin. Since lysine is known to be a precursor of the desmosines in elastin (5–7) it is reasonable to presume that the aldehyde-containing derivative represents an intermediate in the formation of desmosine and isodesmosine from lysine. These results also demonstrate that lathyrogens act by inhibiting the reactions involved in the oxidation of peptide-bound lysine.

When elastase-solubilized elastin was reacted with MBTH under conditions in which the azine derivatives of aldehydes would be formed, the shape of the spectral curves obtained at the completion of the reaction was similar to that observed with a 2:1 mixture of saturated and α,β-unsaturated aldehydes. Although this is insufficient evidence to characterize the aldehydes, the results suggest the presence of more than one intermediate between lysine and the desmosines. This could occur if the reaction is step-wise involving aldol condensations of the lysine-derived aldehydes.

The results presented here substantiate the view that similar biosynthetic pathways are utilized in the cross-linking of collagen and elastin. Levene (33) has shown that purified lathyritic guinea pig collagen exhibits less reactivity with 2,4-DNPH than does normal collagen. Recently, the role which the carbonyl groups of collagen play in the cross-linking mechanism has been clarified by Bornstein et al. (42), who have demonstrated the appearance of a lysine-derived aldehyde, probably the δ-semialdehyde of α-amino adipic acid in peptides derived from the N-terminal ends of the α-chains. An intramolecular cross-link forms in this region, perhaps by an aldol type condensation of two aldehydes.

The recent identification (8) of the amino acid, N^{ϵ}-(5-amino 5-carboxypentanyl)-lysine (lysinonorleucine), in elastin hydrolysates suggests that lysine-derived aldehydes in elastin may couple with the ϵ-amino groups of unaltered lysine residues to provide yet another means of cross-linking the polypeptide chains.

SUMMARY

Elastin fibers in the aortas of control, lathyritic, copper-supplemented, and copper-deficient chicks were examined histochemically and chemically for aldehyde content. Diminished staining for aldehydes was obtained in the fibers from the aortas of lathyritic and copper-deficient chicks. Chemical studies of elastin isolated from the aortas of control and lathyritic chicks showed an apparent loss of lysine residues in control elastin to be associated with an increase in aldehyde content providing evidence that lysine is converted to an aldehyde-containing intermediate during biosynthesis of desmosine and isodesmosine. Approximately 6 aldehyde groups were present for every 1000 amino

acids in elastin isolated from the aortas of control animals, while the corresponding number in lathyritic elastin was 4. At least two types of aldehydes, saturated and α,β-unsaturated, appear to be associated with elastin, suggesting the presence of more than one intermediate between lysine and the desmosines.

The authors would like to thank Dr. P. Bornstein, Dr. G. R. Martin, Dr. K. A. Piez, and Dr. E. Schiffmann for numerous discussions during the course of this work.

BIBLIOGRAPHY

1. Partridge, S. M., and Davis, H. F., The chemistry of connective tissues. 3. Composition of the soluble proteins derived from elastin, *Biochem. J.*, 1955, **61,** 21.
2. Partridge, S. M., Elsden, D. F., and Thomas, J., Constitution of the cross-linkages in elastin, *Nature*, 1963, **197,** 1297.
3. Thomas, J., Elsden, D. F., and Partridge, S. M., Degradation products from elastin, *Nature*, 1963, **200,** 651.
4. Partridge, S. M., Elastin, *Advances Protein Chem.*, 1962, **17,** 227.
5. Partridge, S. M., Elsden, D. F., Thomas, J., Dorfman, A., Tesler, A., and Ho, P., Biosynthesis of the desmosine and isodesmosine cross-bridges in elastin, *Biochem. J.*, 1964, **93,** 30c.
6. Miller, E. J., Martin, G. R., and Piez, K. A., The utilization of lysine in the biosynthesis of elastin crosslinks, *Biochem. Biophysic. Research Commun.*, 1964, **17,** 248.
7. Miller, E. J., Martin, G. R., Mecca, C. E., and Piez, K. A., The biosynthesis of elastin crosslinks. The effect of copper deficiency and a lathyrogen, *J. Biol. Chem.*, 1965, **240,** 3623.
8. Franzblau, C., Sinex, F. M., Faris, B., and Lampidis, R., Identification of a new crosslinking amino acid in elastin, *Biochem. Biophysic. Research Commun.*, 1965, **21,** 575.
9. O'Dell, B. L., Hardwick, B. C., Reynolds, G., and Savage, J. E., Connective tissue defect in the chick resulting from copper deficiency, *Proc. Soc. Exp. Biol. and Med.*, 1961, **108,** 402.
10. Hill, C. H., Matrone, G., Payne, W. L., and Barber, C. W., *In vivo* interactions of cadmium with copper, zinc, and iron, *J. Nutr.*, 1963, **80,** 227.
11. Carlton, W. W., and Henderson, W., Cardiovascular lesions in experimental copper deficiency in chickens. *J. Nutrition*, 1963, **81,** 200.
12. Simpson, C. F., and Harms, R. H., Pathology of the aorta of chicks fed a copper-deficient diet, *Exp. Mol. Path.*, 1964, **3,** 390.
13. Shields, G. S., Carnes, W. H., Cartwright, G. E., and Wintrobe, M. M., The dietary induction of cardiovascular lesions in swine, *Clin. Research*, 1961, **9,** 62.
14. Shields, G. S., Coulson, W. F., Kimball, D. A., Carnes, W. H., Cartwright, G. E., and Wintrobe, M. M., Studies on copper metabolism, *Am. J. Path.*, 1962, **41,** 603.
15. Coulson, W. F., and Carnes, W. H., Mechanical properties of aortas from swine on a copper-deficient diet, *Lab. Inv.*, 1962, **11,** 674.

16. Ponseti, I. V., and Baird, W. A., Scoliosis and dissecting aneurism in lathyrus rats, *Am. J. Path.*, 1953, **29,** 871.
17. Bachhuber, T. E., and Lalich, J. J., Effect of sweet pea meal on the rat aorta, *Arch. Path.*, 1955, **59,** 247.
18. Bachhuber, T. E., Lalich, J. J., Angevine, D. M., Schilling, E. D., and Strong, F. M., Lathyrus factor activity of beta-aminopropionitrile and related compounds, *Proc. Soc. Exp. Biol. and Med.*, 1955, **89,** 294.
19. Churchill, D. W., Gelfant, S., Lalich, J. J., and Angevine, D. M., Alterations in the polysaccharides and elastic fibers in the aortas of rats fed toxic lathyrus factors, *Lab. Inv.*, 1955, **4,** 1.
20. Wawzonek, S., Ponseti, I. V., Shepard, R. S., and Wiedenmann, L. G., Epiphyseal plate lesions, degenerative arthritis, and dissecting aneurysm of the aorta produced by aminonitriles, *Science*, 1955, **121,** 63.
21. Walker, D. G., and Wirtschafter, Z. T., Histopathogenesis of aortic aneurysms in the lathyrus-fed rat, *Arch. Path.*, 1956, **61,** 125.
22. Walker, D. G., Elastic fiber alterations in rats treated with *Lathyrus odoratus*, *Arch. Path.*, 1957, **64,** 434.
23. Menzies, D. W., and Mills, K. W., The aortic and skeletal lesions of lathyrism in rats on a diet of sweet pea, *J. Path. and Bact.*, 1957, **73,** 223.
24. Keech, M. K., Electron microscopic study of lathyritic rat aorta, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 539.
25. Ham, K. N., The fine structure of rat aorta in experimental lathyrism, *Australian J. Exp. Biol. and Med. Sc.*, 1962, **40,** 353.
26. Dasler, W., and Milliser, R. V., Experimental lathyrism in mice fed diets containing sweet peas or β-aminopropionitrile, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 171.
27. Simpson, C. F., Prichard, W. R., Harms, R. H., and Sautter, J. H., Skeletal and cardiovascular lesions in turkeys induced by feeding β-aminoproprionitrile, *Exp. Mol. Path.*, 1962, **1,** 305.
28. Simpson, C. F., Prichard, W. R., Harms, R. H., and Sautter, J. H., Electron microscopy of the cardiovascular system of the normal and β-aminoproprionitrile fed turkey, *Exp. Mol. Path.*, 1962, **1,** 321.
29. O'Dell, B. L., Elsden, D. F., Thomas, J., Partridge, S. M., Smith, R. H., and Palmer, R., Inhibition of desmosine biosynthesis by a lathyrogen, *Biochem. J.*, 1965, **96,** 35P.
30. Levene, C. I., and Gross, J., Alterations in state of molecular aggregation of collagen induced in chick embryos by β-aminopropionitrile (lathyrus factor), *J. Exp. Med.*, 1959, **110,** 771.
31. Martin, G. R., Gross, J., Piez, K. A., and Lewis, M. S., On the intramolecular cross-linking of collagen in lathyritic rats, *Biochim. et Biophysica Acta*, 1961, **53,** 599.
32. Martin, G. R., Piez, K. A., and Lewis, M. S., The incorporation of [^{14}C] glycine into the subunits of collagens from normal and lathyritic animals, *Biochim. et Biophysica Acta*, 1963, **69,** 472.
33. Levene, C. I., Studies on the mode of action of lathyrogenic compounds, *J. Exp Med.*, 1963, **116,** 119.

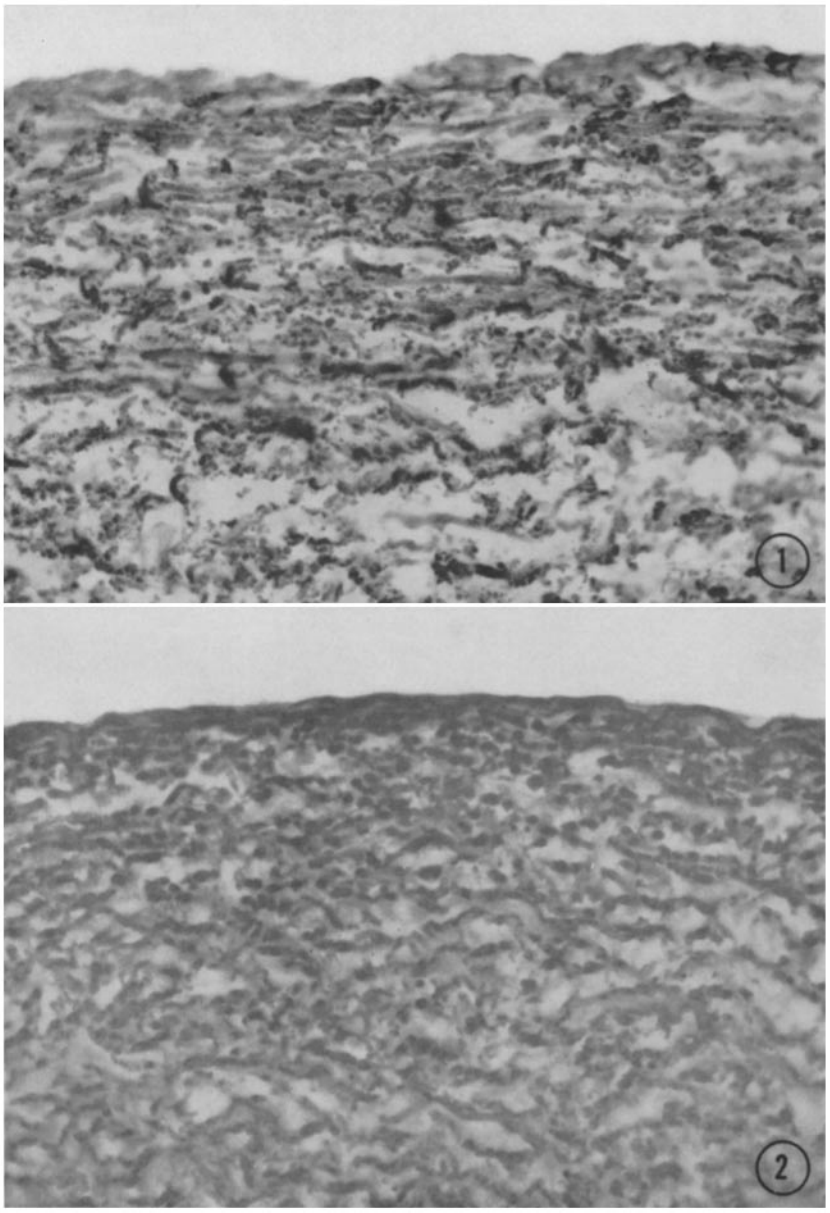
34. Starcher, B., Hill, C. H., and Matrone, G., Importance of dietary copper in the formation of aortic elastin, *J. Nutrition*, 1964, **82,** 318.
35. Partridge, S. M., Elastin, Symposium on Physiology and Biochemistry of Muscle as a Food, in press.
36. Lillie, R. D., Histophatologic Technic and Practical Histochemistry, New York, Blakiston, 2nd edition, 1954.
37. Fullmer, H. M., and Lillie, R. D., A selective stain for elastic tissue, *Stain Technol.*, 1956, **31,** 27.
38. Rojkind, M., Blumenfeld, O. O., and Gallop, P. M., Isolation of an aldehyde-containing peptide from tropocollagen, *Biochem. Biophysic. Research Commun.*, 1964, **17,** 320.
39. Piez, K. A., and Morris, L., A modified procedure for the automatic analysis of amino acids, *Anal. Biochem.*, 1960, **1,** 187.
40. Sawicki, E., Hauser, T. R., Stanley, T. W., and Elbert, W., The 3-methyl-2-benzothiazolone hydrazone test. Sensitive new methods for the detection, rapid estimation, and determination of aliphatic aldehydes, *Anal. Chem.*, 1961, **33,** 93.
41. Paz, M. A., Blumenfeld, O. O., Rojkind, M., Henson, E., Furfine, C., and Gallop, P. M., Determination of carbonyl compounds with N-methyl benzothiazolone hydrazone, *Arch. Biochem. and Biophysics*, 1965, **109,** 548.
42. Bornstein, P., Kang, A., and Piez, K. A., The nature and location of the intramolecular crosslinks in collagen, *Proc. Nat. Acad. Sc.*, 1966, **55,** 417.

EXPLANATION OF PLATES

PLATE 106

FIG. 1. Section of aorta from a copper-deficient chick stained by the Taenzer-Unna orcein method for elastic fibers. Note fragmentation of elastic fibers, and fibers with bulges and other abnormal shapes. The structure of elastic fibers, and the staining reaction of sections of aorta from lathyritic animals were indistinguishable from those of copper-deficient animals. × 520.

FIG. 2. Section of aorta from a copper-supplemented chick stained by the Taenzer-Unna orcein method for elastic fibers. Note the quantity and normal architecture of elastic fibers. Sections from control animals in the lathyrism experiment appeared identical. × 520.

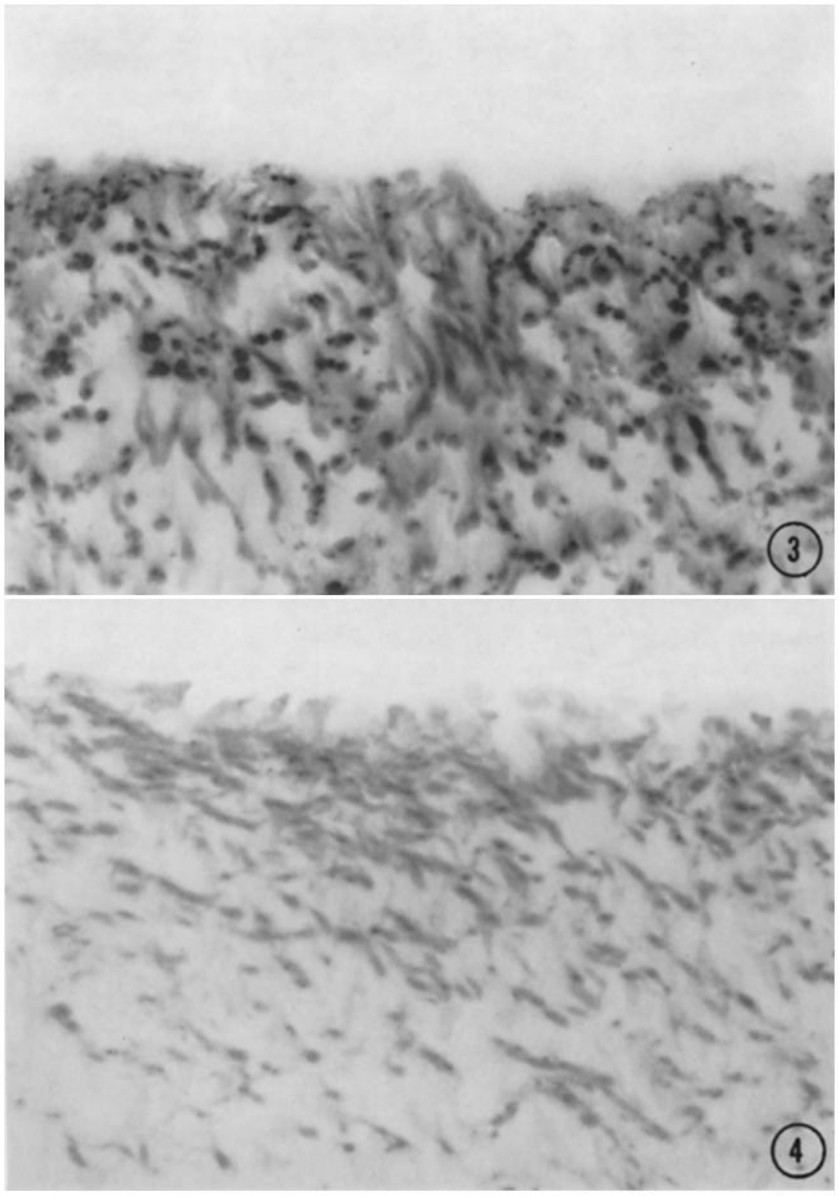


(Miller and Fullmer: Elastin)

PLATE 107

FIG. 3. Section of aorta from a copper-supplemented chick stained with Schiff's reagent for the detection of aldehydes. Note the size, structure, and staining intensity of the elastic fibers. Sections from control animals in the lathyrism experiment appeared identical. × 1030.

FIG. 4. Section of aorta from a copper-deficient chick stained with Schiff's reagent for the detection of aldehydes under conditions identical to that of Fig. 3. Note lesser stain for aldehydes, and smaller size of elastic fibers. The structure of elastic fibers, and the staining reaction of sections of aorta from lathyritic animals were indistinguishable from those of copper-deficient animals. × 1030.



(Miller and Fullmer: Elastin)