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Copper Deficiency and Elastin Metabolism in Avian Lung (41066)[1]

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Abstract. Copper deficiency in the chick causes anatomical changes in lung that are characterized by an apparent thinning of the air–blood capillary network of tertiary bronchi. Although the net content of the elastin in lung was not changed significantly by nutritional copper deficiency, it was possible to demonstrate an increase in the lysine content and a decrease in the desmosine content of lung elastin from copper-deficient chicks compared to that from controls. Further, the content of soluble elastin was also increased two- to fourfold in lung from copper-deficient chicks. Subsequently, nutritional copper deficiency was used to facilitate the isolation of chick lung tropoelastin. This lung protein appeared to be similar in selected properties to tropoelastin isolated from aorta of copper-deficient chicks. In addition, data are presented that indicate elastin in avian lung is possibly subject to only limited turnover.

Elastin is responsible in part for the elastic properties of lung, blood vessels, and skin (1). In the chick, morphologically distinct elastin fibers first appear in embryonic lung at Day 14 (2). By Day 20 of embryogenesis, these fibers are almost fully developed. The elastin is most predominantly observed in the walls of the tertiary bronchi, the pulmonary vasculature, and in the pleural covering. Jones and Barron (2) have suggested that the function of elastin in the tertiary bronchi is to provide tension against which smooth muscle may contract. Smooth muscle lines the lumen of the tertiary bronchi and appears to control air flow through tertiary bronchi. Although elastic fibers do not directly extend into the air exchange areas of the avian lung (2, 3), their presence in the septa separating each tertiary bronchi suggest that they may also be important in maintenance of air–blood capillary patency by providing tension on the outer wall of tertiary bronchi.

Subsequently, it was considered of interest to examine selected features of elastin metabolism in avian lung. Herein are reported estimates for the content of mature lung elastin in young growing chicks. Nutritional copper deficiency was used as a technique to effect changes in the degree of crosslinking of elastin as well as an approach to condition chicks for the isolation of a soluble precursor to mature elastin, tropoelastin. Data are also presented on the estimation of elastin turnover in avian lung.

Material and Methods. *Supplies.* The White Leghorn chicks were purchased from a local hatchery. The Japanese quail were obtained from the Department of Avian Sciences, University of California, Davis, California. L-[^{14}C]lysine was purchased from ICN Chemical and Radioisotope Division (Irvine, Calif.). Reagents used in the determination of radioactivity were purchased from New England Nuclear (Boston, Mass.). Protein standards and supplies for polyacrylamide gel electrophoresis or chromatography were purchased from either Bio Rad (Richmond, Calif.) or Pharmacia (Piscataway, N.J.). For all other an-

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alytical procedures, the reagents were of analytical grade and were purchased from either Mallinckrodt (St. Louis, Mo.), Fisher Chemical Company (Pittsburgh, Pa.), or Sigma Chemical Company (St. Louis, Mo.). Dietary ingredients were products of Nutritional Biochemicals (Cleveland, Ohio), Crystal Dairy (Sacramento, Calif.) or Ralston Purina Company (St. Louis, Mo.).

Animals. Day-old White Leghorn cockerels were fed semipurified diets based on spray-dried skim milk either sufficient (25 ppm Cu as $CuSO_4$) or deficient (<1 ppm) in copper for 21 or 35 days (Table I). Japanese quail were used in experiments to estimate lung elastin turnover. The quail were fed from hatching a commercial ration (Ralston Purina Turkey Starter). The chicks and quail were housed in stainless-steel brooders with free access to feed and deionized water.

Lung morphology. The lungs from chicks were examined histochemically at 21 and 35 days. In addition, scanning electron microscopy was performed using lungs from 21-day-old chicks. To prepare the chicks for histochemistry they were first anesthesized with an ip injection of sodium pentobarbital (2 mg/100 g body wt). The pulmonary artery was cannulated (23-gauge needle) and the lungs were perfused (1 ml/min) with 10 ml of heparinized saline (120 units/100 ml) to remove as much blood as possible from the pulmonary vasculature. Following this step, an equal volume of 10% neutral Formalin was perfused (1 ml/min). The tissue comprising the thoracic cavity was then cut away from the body in one piece in order to prevent mechanical damage to the lungs and the tissue was fixed in Formalin for an additional 14 days.

TABLE I. COMPOSITION OF BASAL DIET[a]

Ingredient	g/kg
Dried skim milk	600.0
DL-Methionine	3.0
L-Arginine HCL	5.0
Glycine	5.0
Corn Oil	50.0
Vitamin mixture[b]	10.0
Choline chloride	1.1
NaCl	5.0
$FeSO_4$ $7H_2O$	0.6
$MnSO_4$ H_2O	0.3
Glucose	320.0

[a] The copper-supplemented diet contained 25 ppm copper as copper sulfate.

[b] Supplied the following: (mg/kg diet) thiamin HCl, 16.0; riboflavin, 10.0; nicotinic acid, 50; *d*-calcium pantothenate, 20.0; pyridoxine HCl, 6.0; biotin, 0.6; vitamin B_{12}, 0.030; inositol, 250; menadione sodium bisulfite, 1.52; and vitamin A palmitate, 5000 IU; *dl*-α-tocopheryl acetate, 110 IU; and vitamin D_3, 1500 IU. The vitamins were mixed with glucose so that the mixture could be added at 10% in the diet.

The Formalin-fixed lungs were cut parallel to the posterior surface at 3- to 4-mm intervals. This manner of cutting produced five to six transverse sections that were in turn cut longitudinally from the tapered lateral margin of the lung to the flat medial surface, thereby exposing cross sections of the tertiary bronchi. Following routine processing, the lung slices were stained with acid orecein in 70% ethanol (4). Light green (SF yellowish) was used as a counter stain. In addition, selected lungs were removed immediately and frozen in isopentane (−70°) and sliced using a cryostat (10–20 μm). These sections were examined directly or following the staining procedure primarily to confirm that the methods of fixation did not result in tissue damage and hemorrhage.

To prepare samples for scanning electron microscopy, the lungs were perfused with heparinized saline and the thoracic cavity was excised from the carcass. The lungs were then perfused via the trachea with Karnovsky's fixative (5). A reservoir containing Karnovsky's fixative with a constant fluid height of 5 cm was used to allow an uninterrupted flow of fixative for 4–6 hr. After perfusion the lungs were removed from the thoracic cavity and sectioned with a razor blade to obtain 10 × 2-mm slices of lung. They were cut so that the tertiary bronchi were sectioned longitudinally. The blocks were then processed for scanning electron microscopy (accelerating voltage, 4 to 9 kV) as described by Nowell *et al.* (5).

Isolation and estimation of aorta and lung insoluble elastin. The net amount of elastin in the thoracic aorta (trimmed of tissue adhering to the adventia) and whole lung (trimmed of extra parenchymal tracheobronchial and vascular tissue) was

estimated by the method of Lansing *et al.* (6) as modified by O'Dell *et al.* (7) or the method outlined by Paz *et al.* (8). The insoluble residue that was obtained from lungs following the use of either procedure was defined operationally as elastin. Following dehydration *in vacuo* or lyophilization, the residue was weighed and expressed per unit of fresh or dried aorta or lung. The purity of selected samples was also estimated from amino acid analysis using criteria established by Sandberg (9).

Amino acid analysis. In preparation for amino acid analysis, 10 mg of material was hydrolyzed (6 *N* HCl, 40 hr, 110°). The samples were dried under vacuum and dissolved in lithium citrate buffer for amino acid analysis by using a Beckman Model 124 or Durrum amino acid analyzer.

Isolation of lung soluble elastin. Chick lung tropoelastin was isolated using a modification of procedures taken from Foster *et al.* (10) and Rucker *et al.* (11). Lung tissue (450 g) from approximately 300 copper-deficient chicks was homogenized into sodium phosphate buffer (0.05 *M*, pH 7.0) containing 0.15 *M* NaCl, 5 m*M* *N*-ethyl maleimide, 20 m*M* ethylenediaminetetracetic acid, 5 m*M* β-aminopropionitrile, and 0.1 *M* ϵ-amino hexonic acid using a high speed blender. The homogenate was further disrupted by extrusion from a Parr bomb (Parr Instrument Co., 1500 psi) using pressurized nitrogen gas. The homogenate was then centrifuged at 10,000*g* for 30 min.

Protein in the resulting supernatant fraction was first fractioned by the slow addition of NaCl to 15% (w/v) at 4°. The addition of NaCl in high concentration has been shown to precipitate soluble collagens and elastin (11). This step also results in the partial removal of surfactant and other protein constituents that interfered in subsequent isolation steps. Following centrifugation (10,000*g*, 30 min, 4°), NaCl was removed from the precipitate by dialysis against a 50 volume excess of 0.2 acetic acid (two changes, 12 hr each).

Although the recovery of soluble protein following dialysis and centrifugation (15,000*g*, 30 min) was poor (approx 20% of the material that was precipitated by NaCl), the acetic acid retentate did provide a source of tropoelastin for further purification. This was accomplished by alcohol fractionation using *n*-proponol and *n*-butanol as described previously (12). As a final step, the protein fraction that was obtained following alcohol fractionation was further purified by gel filtration using a 1 × 30-cm column of Sephadex G-100 to remove small molecular weight peptides. Pyridine acetate (0.2 *M*, pH 5.6) was used as eluent (see Results).

Determination of lung soluble elastin. Radioimmunological identification and quantitation of soluble elastin in lung and aorta extracts were accomplished using methods taken from Christner *et al.* (14) and Daynes *et al.* (15). Antiserum to a chemically derived product from insoluble elastin (α-elastin) was prepared using New Zealand white rabbits. The α-elastin was isolated from normal chick aorta as described by Abatagelo *et al.* (16). However, only α-elastin in the 70,000–80,000 molecular weight range was used for antibody preparation. This was accomplished by fractionation on a column (1.5 × 80 cm) of Sephacryl S-200 (17).

The rabbits were immunized to α-elastin fraction by subcutaneous injection with 1 ml of the antigen suspended (1–2 mg/ml) in Freund's complete adjuvant. Six injections were administered at weekly intervals. The rabbits were then boostered with three additional injections at monthly intervals and bled.

Iodinated derivatives of chick aorta tropoelastin were prepared as described by Daynes *et al.* (15) for use in standard radioimmunoassays. The aorta tropoelastin was isolated as described previously (11), and was judged to be greater than 95% pure based on amino acid composition and mobility in polyacrylamide gels using two different electrophoresis systems (11, 12). The protocol for the individual assays was similar to that described by Daynes *et al.* (15), except that Tween 80 at 1% was used in the incubation mixtures in place of Nonidet *P*-40. For the estimation of tropoelastin and other forms of soluble elastin in tissue extracts, the tissue was homogenized in extracting buffer (0.1 *M* sodium borate, pH 7.6 containing 0.4% bovine serum albumin,

0.1% α_1-proteinase inhibitor and 1% Tween 80) by using a Polytron. Ten milliliters per gram of tissue was used for extraction. The homogenate was then centrifuged (25,000*g*, 60 min; 4°) and appropriate aliquots (0.2 to 10 μl) of the supernatant fraction were assayed. The reaction mixtures contained the specific rabbit antiserum adjusted with normal rabbit serum so that the total amount of serum was constant. The iodinated antigen and then varying amounts of nonlabeled antigen or tissue extracted were added (cf. Refs. (14) and (15)). The reactions were maintained at 4° for 12 hr. This was followed by the addition of an appropriate amount of goat anti-rabbit antiserum globulin, followed by an additional incubation (6 hr, 4°) to precipitate the antibody–elastin complex. The precipitate was then washed and prepared for counting as described by Christner *et al.* (14).

Polyacrylamide gel electrophoresis. The methods were identical to those described previously (11, 12). Bovine serum albumin, ovalbumin, chick aorta tropoelastin, and cytochrome *c* were used as molecular weight markers when polyacrylamide gels containing sodium dodecyl sulfate were used.

Lung elastin turnover. It was felt essential for interpretation of some of the experiments to have an estimation of elastin turnover typical of avian lung. Thirty Japanese quail were injected intraperitoneally with L-[^{14}C]lysine (50 μCi/100 g body wt at Day 9 posthatching for the estimation of turnover. Following this injection and prior to estimation of radioactivity into elastin, the incorporation of L-[^{14}C]lysine into plasma protein and nonprotein fractions (those soluble in 10% trichloroacetic acid, TCA) was determined. This was done in order to establish the point at which the further incorporation or recycling of metabolized L-[^{14}C]lysine would be minimal (see below).

The choice of quail for these experiments was based on previous experience with quail as an experimental animal (18). Also, quail are approximately one-tenth the size of chickens at maturity. Thus, the costs of housing and isotope were reduced substantially. Their rapid growth and development (a 15-fold increase in body weight from hatching to Week 6–7) results in less chance for reutilization of labeled amino acids in long-term turnover studies because of considerable dilution. Since the turnover of plasma protein after injection was found to be approximately 72 hr (total protein fraction) and the amount of TCA soluble radioactivity was found to be negligible after 7–8 days postinjection, it was assumed recycling of metabolized L-[^{14}C]lysine would not present major difficulties in the assessment of elastin turnover.

A 21-day period was allowed to pass following the injection to further assure that recycling would not compromise the initial estimation and allow ample time for the lysine incorporated into elastin to form stable crosslinks (1). Other details regarding the sampling procedures have been published in a paper dealing with the turnover of elastin and nonelastin protein in aorta (18) or are given in Fig. 6. All data are expressed as DPMs in elastin on a whole lung basis. This was done to compensate for the dilution of isotope because of lung growth and any new elastin synthesis following the initial sampling period.

Results. *Isolation of chick lung soluble elastin.* The consistent isolation of lung tropoelastin from chicks proved to be difficult. Using the protocol described herein approximately 12 mg of protein was recovered following the *n*-proponol–*n*-butanol fractionation step. Approximately 3 mg of protein was recovered in the molecular weight range of 60,000–75,000 daltons following gel filtration. Most of the remainder was recovered as low molecular weight peptides (<20,000 daltons). These molecular weight estimates were confirmed by electrophoresis of selected samples in polyacrylamide gels containing sodium dodecyl sulfate (11, 12).

With respect to the 60,000- to 75,000-dalton fraction, two bands of protein were observed in the gels. The major component (approximately 85% of the total protein applied to each gel (100 μg) possessed the same mobility as aorta tropoelastin (apparent molecular weight, 72,000). The estimated molecular weight of the minor component was 58,000–60,000. This component was

presumed to be a degradation product derived from tropoelastin (cf. Ref. (16)).

The amino acid composition of the 60,000- to 75,000-dalton fraction is given in Table II). These data indicate that the protein in the fraction is very similar to chick aorta tropoelastin (see Discussion).

Quantitation of tropoelastin. It was also possible to demonstrate that the protein isolated from lung was antigenically similar to aorta tropoelastin. Fig. 1 shows the response of various proteins to the rabbit antiserum raised against α-elastin in a competitive binding assay. The lung elastin fraction competed similarly to aorta tropoelastin. The response is also shown for α-elastin, aorta tropoelastin degradation products (molecular weight range, 20,000–40,000), or other proteins that were selected to show antiserum specificity.

Effect of copper deficiency on the morphology and elastin content of lung. Mature elastin from lung was isolated using two different procedures in an attempt to determine the degree to which elastin content is modified by copper deficiency. It is now well recognized that copper is a cofactor for lysyl oxidase, an enzyme that regulates the initial steps in elastin crosslink formation (1). Since highly crosslinked elastin is not appreciably solubilized by extraction into alkali or solutions containing denaturants and detergents under reducing conditions (1, 6–9), the net content of residual protein after extraction is often taken as a crude measure of crosslinked elastin (1).

The values in Fig. 2 were obtained using the method of Paz *et al.* (8). Values are also given for the content of aorta elastin for comparison. The data indicate that the net content of elastin per gram of lung was not changed significantly by nutritional copper deficiency. This is perhaps due to the effects of inanition on lung growth and the observation that its elastin content appears to increase in proportion to lung weight. In growing chicks the aorta weight is less influenced by inanition and its elastin content

TABLE II. THE AMINO ACID COMPOSITION OF SOLUBLE AND INSOLUBLE LUNG ELASTIN FRACTIONS

	Tropoelastins[a]		Insoluble elastin[a,b]	
Amino acid	Lung	Aorta	Cu-Suppl.	Cu-Def.
Lys	43	40	9	15
Total desmosine (1/4 lys)	—	—	13	7
His	2	—	2	3
Arg	8	5	8	9
Hyp	12	8	16	13
Asp	8	4	12	12
Thr	13	11	10	11
Ser	12	7	7	7
Glu	18	12	20	21
Pro	120	128	147	149
Gly	313	330	317	316
Ala	180	174	170	172
1/2 Cys	—	—	0	Trace
Val	163	177	164	167
Met	Trace	—	Trace	Trace
Ile	21	18	18	18
Leu	58	52	51	65
Tyr	15	10	12	6
Phe	14	20	24	19

[a] Each value represents the average for two or three determinations. For insoluble elastin single determinations were made for three individual samples from each group.

[b] In addition to ammonia, 2–6% of the total ninhydrin positive residues (relative to norleucine) were not calculated as a part of the total, since they could not be identified and most appeared to represent less than 2 residues per 1000 total residues. If the composition of the insoluble lung elastin is the same as that in chick aorta, the values indicate approximately 10% contamination by other proteins (cf. Ref. (1)).

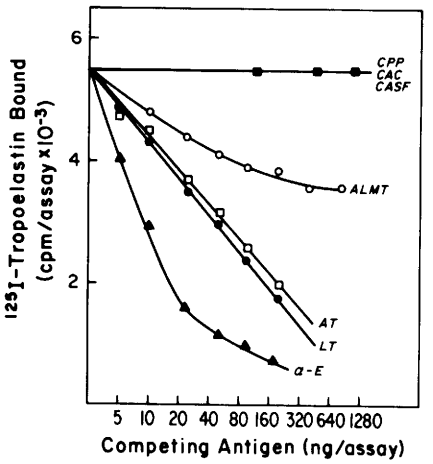

FIG. 1. Competitive binding assays for tropoelastin. Antiserum to α-elastin was incubated with ^{125}I-tropoelastin and with the unlabeled protein fractions indicated in the figure and described in the text. The antisera was used at dilutions which bound approximately 50% of the labeled antigens in the absence of unlabeled antigen (cf. Ref. (14)). Abbreviations: CPP, chick plasma protein; CAC, chick aorta collagen (cf. Refs. (10) and (11) for purification); CASF, chick aorta supernatant fraction free of tropoelastin (11); ALMT, low molecular weight peptides derived from aorta tropoelastin (11, 16); AT, aorta tropoelastin; LT, lung tropoelastin; AE, α-elastin.

per gram of aorta in normal leghorn cockerels more than doubles during the first five weeks of growth (Fig. 2).

However, it could be demonstrated that copper deficiency influences the content of soluble elastin in lung. At 7 and 35 days, there was an increase in the amount of soluble elastin recovered after extraction of lung tissue (Table III). Also, it could be demonstrated that samples of lung elastin from 21-day-old birds contained more lysine and less total desmosine than corresponding controls (Table II). The elevation in lysine and the reduction in the desmosine content was taken as a sign of decreased elastin crosslinking.

Lung morphology. Figure 3 shows sections of lung tertiary bronchi from 21-day-old copper-supplemented chicks and 21- and 35-day-old copper-deficient chicks. In many of the lung sections from copper deficient chicks, the orecein-stained fibers (presumably elastin) appeared less dense. Each tertiary bronchi in lung sections from copper-supplemented chicks was well defined and clearly separated by intertertiary bronchial septa. In all of the sections examined from copper-deficient birds the

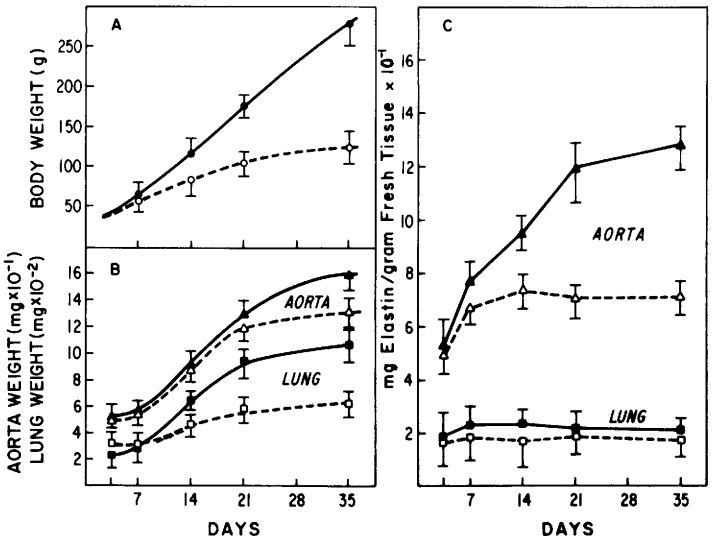

FIG. 2. Body weights (A), aorta and lung weights (B) and the elastin concentration expressed per gram of fresh aorta and lung (C). The elastin was isolated as described by Paz *et al.* (8). The solid and open circles represent body weight designations for copper-supplemented and -deficient chicks, respectively. Aorta or lung values for the copper-supplemented (solid) and -deficient birds (open) are designated by either triangles or squares, respectively. Mean values ± 1 SEM are indicated.

TABLE III. AMOUNTS OF TROPOELASTIN EXTRACTED FROM AVIAN LUNG AS MEASURED BY RADIOIMMUNOLOGICAL ASSAY[a]

Age	Copper supplemented (μg/g fresh lung)	Copper deficient (μg/g fresh lung)
7 days	41 ± 16	75 ± 28
35 days	41 ± 6	222 ± 75[b]

[a] Values represent the averages for four determinations ± 1 SEM.
[b] $P < 0.05$.

air–blood capillary network appeared compressed and appeared less cellular. Only a few of the parabronchi were separated from each other by well-defined septa. Furthermore, hemorrhage and fragmentation of various structural elements were often observed in lung sections from copper-deficient birds.

The signs of hemorrhage were related to the region of lung examined. Hemorrhage was most often observed in the central or interior of the lung from copper-deficient chicks (Fig. 4). The hemorrhage was not an artifact of the tissue preparation since hemorrhage in the same locations was also observed in lung tissue sections from copper-deficient chicks processed in liquid nitrogen–isopentane and sectioned using a cryostat.

Electron microscopic scans of copper-supplemented and -deficient chicks are shown in Fig. 5. In keeping with the observations from light microscopy, the infundibuli or air tunnels that arise from the atrial lumen of tertiary bronchi appeared well developed in the scans of lung from copper-supplemented chicks. Both the infundibuli and air capillary network of tertiary bronchi from copper-deficient chick lung appeared thinner, collapsed or less developed. The surface of the atrial cavity from copper-deficient chick lung usually appeared rougher in texture.

Estimation of lung elastin turnover. The values for the incorporation of L-[^{14}C]lysine into the alkali insoluble elastin fraction of quail lung are given in Fig. 6. These data appear to indicate that once lysine is incor-

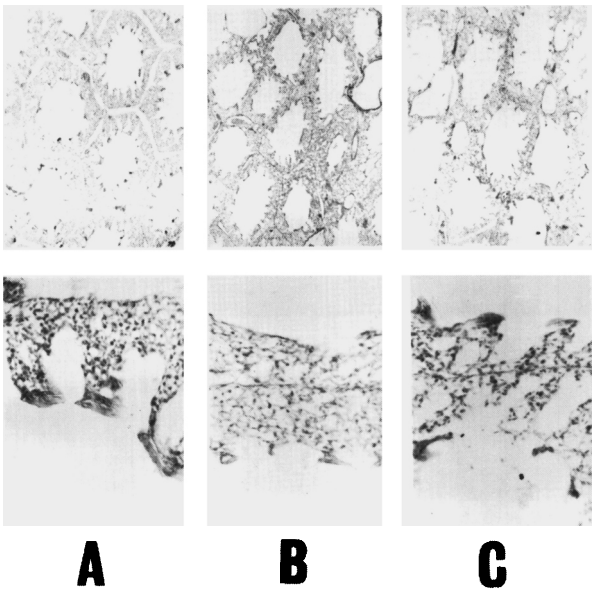

FIG. 3. Photomicrographs of orecein-stained lung sections from chicks fed a copper-supplemented (A) or -deficient (B and C) diet. Shown in (A) are tertiary bronchi located in the periphery of avian lung from a copper-supplemented chick (×40 and ×250). In contrast, note the lack of definition of the tertiary bronchial septa and the air and blood capillary network in the corresponding sections of lung from copper-deficient chicks. (B) shows sections obtained from 21-day-old copper-deficient birds (×40 and ×250). (C) sections obtained from 35-day-old copper-deficient birds (×40 and ×250).

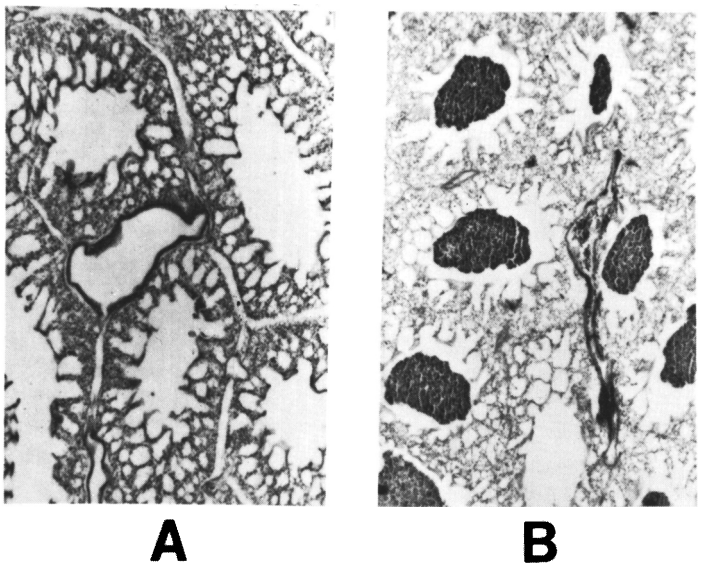

FIG. 4. Lung sections from centrally located tertiary bronchi in which hemorrhage is observed (control, (A); copper deficient (B)). The chicks were 21 days old (×75).

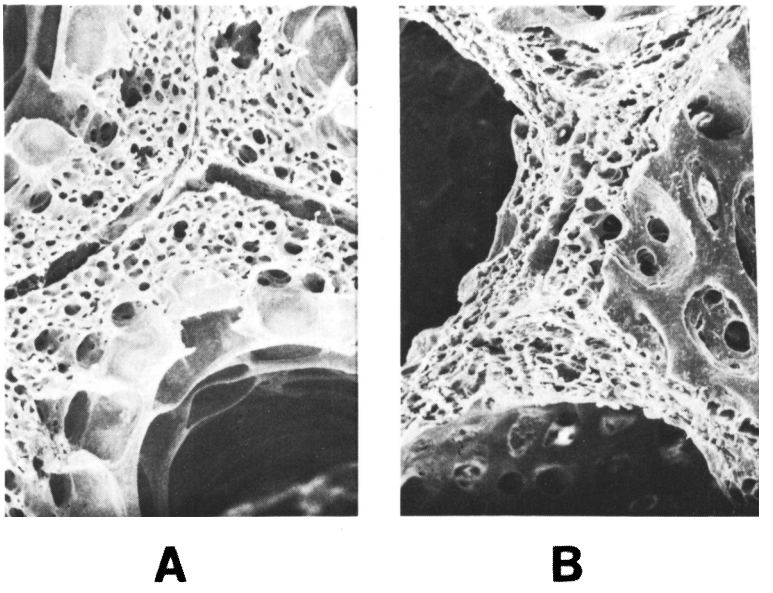

FIG. 5. Scanning electron micrographs of chick lung. In the scan of control lung sections (A) the atria and infundibuli that extend into the network of air–blood capillaries are observed. The intratertiary bronchial septi are also clearly defined. This is not the case for the scans of lung from copper-deficient birds (B) in which air capillary network appears compressed and there is little definition of the septi separating parabronchi. The surface of the atria also appears roughened.

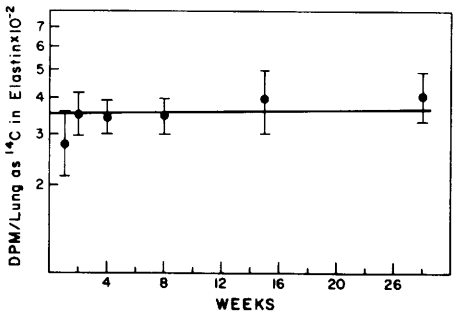

FIG. 6. Radioactivity in alkali-insoluble fractions from quail lung following the administration of [^{14}C]lysine. Values are expressed as total radioactivity in elastin per whole lung. Each value represents at least four determinations ± SEM.

porated into this fraction, there is little apparent turnover of lung elastin.

Discussion. The major thrust of this work evolved from efforts to isolate tropoelastin from a lung source. Although its degree of characterization was not substantial, our observations do provide for a limited comparison of tropoelastin from aorta and lung. It may be stated that the two elastins appear similar based on amino acid composition, electrophoretic mobility, and antigenic properties. The response of differing elastin preparations to rabbit antiserum against chick aorta α-elastin are in keeping with previously published observations by Daynes *et al.* (14) and Foster *et al.* (10). Antibodies that recognize mature elastin or α-elastin appear to cross react with tropoelastin. This was the case for the lung and aorta protein, but whether or not the lung tropoelastin was derived primarily from parenchyma or lung vascular tissue will require further clarification.

With respect to the yields for lung tropoelastin, the data given in Table III indicate that the potential pool of soluble elastin in copper-deficient chick lung is 8–20 mg/100 g of lung tissue based on the aorta tropoelastin standard. Our recovery of approximately 1 mg of tropoelastin/100 g of tissue represents 5 to 10% of this pool. For comparison, the recovery from 100 g of copper-deficient chick aorta using similar procedures is usually 100–300 mg of tropoelastin (12). The small pool of soluble elastin in lung presented a major problem in its successful isolation. Further, copper deficiency did not appear to increase substantially the amount of soluble elastin in lung. The two- to fourfold increase in lung soluble elastin indicated in Table III is approximately one-tenth the fold increase in soluble elastin observed in copper-deficient chick aorta (11, 12).

It is of interest that the net accumulation of both soluble and mature elastin in lung was less responsive to nutritional copper deficiency than that in aorta. This may be due to the differential aspects of growth of the two tissues, the apparent slow turnover of elastin, and the marked stimulation of elastin synthesis in aorta during the first 4 to 5 weeks post hatching. The impression given from the data presented in Fig. 6 is that once elastin is synthesized and assembled into mature elastin, it undergoes only limited turnover in normal birds (also see Refs. (1) and (18)). Since the percentage concentration of elastin in chick lung was constant over the first 5 weeks of growth, it was assumed that this reflected a relatively constant rate of elastin accumulation for this period independent of copper status. Apparently, the elastin in copper-deficient lung was sufficiently crosslinked to resist solubilization even though the estimates of the lysine and desmosine content of the insoluble elastin indicated impaired crosslink formation.

With respect to the effect of copper deficiency on avian lung morphology, the most significant change was the thinning and apparent collapse of the air–blood capillary network or so-called tertiary bronchial mantle. This rigid meshwork of blood and air capillaries allows for an exchange surface between blood vessels and air approximately 10 times that of mammalian lung (2, 3). A thinning of the tertiary bronchial mantle probably has the same effect as increasing alveolar size, since both processes would lead to a decrease in the gas volume to surface relationship. Although, it is not possible from the chemical data to define elastin's role in this process, it is possible to speculate that some of the change in tertiary bronchial structure may be related to elastin, since the elastin that

was isolated from copper-deficient chick lung was less mature, i.e., it contained less desmosine and more lysine than elastin from copper-supplemented birds. A problem in interpreting data related to events that influence elastin is the fact that each event is often arbitrarially and operationally defined. It would appear that in lung metabolic events related to elastin formation or destruction may have to undergo a highly significant change before simple measurements, such as net elastin content, reflect the change. This is important in that values for the net accumulation of elastin are often used in interpreting elastolytic damage in lung disease or the response of lung to pneumotoxic stress. In order to correlate morphological and chemical data as it relates to avian lung elastin, it seems clear that more sensitive chemical methods need to be developed. Further, there is also the problem of being able to distinguish parenchymal elastin from vascular elastin when chemical methods are employed.

It is also important to note that there may be some utility in understanding the factors that lead to lung defects in avian lung that also result in changes in mammalian lung. O'Dell *et al.* (4) have reported that copper deficiency results in emphysematous changes in developing rodent lung. It would be of interest to pursue further copper and elastin's role in a lung, such as the avian lung, that presumably does not undergo large volume changes during respiration. Also, an appreciation for comparative changes in avian and mammalian lung morphology and function as influenced by similar conditions (particularly in migratory birds) may be of value in the early assessment of environmental factors that may pose hazard to man and other animals.

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