The American Journal of PATHOLOGY

JUNE 1978 • VOLUME 91, NUMBER 3

The Lung of the Copper-Deficient Rat

A Model for Developmental Pulmonary Emphysema

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Based on the hypothesis that cross-linked elastin is critical for normal lung structure, lung tissue from copper-deficient rats was studied. Copper deficiency was induced in the second generation by feeding dams a milk-based diet low in copper (< 1 ppm) during gestation and lactation. The weanlings were fed the same diet until they showed severe signs of deficiency between 6 and 10 weeks of age. Controls animals received the basal diet supplemented with 10 ppm copper. Liver cytochrome oxidase activity, which served as the chief index of deficiency, decreased from a normal level of approximately 80 to 15 μmole/min/g. The lungs of the deficient animals contained 17% less elastin and had 35% larger alveolar spaces (34.7 vs 47.7 intercepts), as determined by the mean alveolar intercept method. The ultrastructure of elastin in the bronchi, arterioles, and alveolar ducts had a "washed out" appearance. To determine the reversibility of the pathology, deficient animals, 5 to 10 weeks of age, were repleted by feeding a copper-supplemented diet for 1, 2, and 3 months. During this period growth resumed, anemia disappeared, and liver cytochrome oxidase returned to normal. There was no improvement in lung structure with regard to alveolar size (28.4 intercepts compared with 43.6 in controls and 35.1 in deficient littermates killed at the start of repletion). The ultrastructure and electron density of pulmonary elastin was restored to near normal. The lung of the copper-deficient rat is proposed as a model for developmental pulmonary emphysema. (Am J Pathol 91:413–432, 1978)

ALTHOUGH THE PATHOGENESIS of human pulmonary emphysema is not understood, it has been defined as permanent abnormal enlargement of distal air spaces due to destruction of their walls. Since the

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This is a contribution of the Missouri Agricultural Experiment Station, Journal Series No. 7843. Supported in part by Grants HL-11614 and OH-00511 from the Public Health Service.

Accepted for publication February 13, 1978.

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0002-9440/78/0608-0413$01.00

structural and elastic properties of the lung depend primarily on the connective tissue proteins collagen and elastin, the concentrations of these proteins in the lung have received increasing attention in recent years.[1] Most animal models of emphysema depend on destruction of the integrity of these proteins by proteolytic enzymes, such as papain [2,3] or elastase,[4] or by administration of lathyrogens [5,6] or cadmium.[7] Cross-linking of collagen and elastin is impaired by lathyrogens and penicillamine [8] and is naturally defective in the lungs of the blotchy mouse.[9] In all of these cases, lung structure and function are abnormal. Observations made on lungs treated with proteolytic enzymes show that increased compliance correlates more closely with decreased elastin than with decreased collagen content.[10]

The cross-linking of collagen and elastin is initiated by oxidative deamination of lysine or hydroxylysine residues in the precursor proteins, giving rise to the corresponding aldehyde residue, allysine.[11] This reaction is catalyzed by lysyl oxidase,[12] which is a copper-dependent enzyme.[13] There is abundant evidence that the activity of lysyl oxidase is decreased in arteries [13] and cartilage [14] of copper-deficient animals. The structural integrity of arteries is greatly impaired by copper deficiency.[15,16]

On the basis of the literature cited, one would predict that the connective tissue proteins in the copper-deficient lung would not be properly cross-linked. This paper is concerned with the morphology and elastin content of lung tissue from severely copper-deficient rats before and after copper repletion. It entertains the thesis that some emphysema may be a developmental defect of the lung.

Materials and Methods

Animals

Immature albino rats of both sexes whose copper status was adequate or deficient were used. They came from an inbred (closed) colony of Wistar rats which were cesarean-section–derived and maintained for over 20 years. The copper-deficient rats were the offspring of dams fed a low (<1 ppm) copper diet* [17] from the day of breeding, ie, first caging with the male rat. Thus, the dams were on the low-copper milk–sucrose diet during gestation and lactation. Controls were produced of dams fed the same diet supplemented with 10 ppm of copper as $CuSO_4$. After weaning at 4 weeks of age, the offspring were fed the same diet as their dams until they were killed for biochemical or morphologic studies. There was a relatively high mortality soon after birth and also beginning 2 weeks after

* The percentage composition of the basal diet was as follows: nonfat dry milk, 60.0; sucrose, 34.1; DL-methionine, 0.3; soybean oil, 5.0; NaCl, 0.5; choline chloride, 0.1. The diet was supplemented with trace elements and vitamins (ppm): Mn, 100; Fe, 100; Mg, 300; Zn, 50; I, 0.2; thiamine HCl, 16; riboflavin, 16; pyridoxine HCl, 16; niacin, 16; Ca pantothenate, 40; biotin, 0.2; folacin, 5; cyanocobalamin, 0.05; menadione, 10; α-tocopherol, 50; retinol palmitate, 11; cholecalciferol, 0.07. The diet always contained less than 1 ppm of copper and averaged approximately 0.7 ppm.

weaning, ie, at approximately 6 weeks of age. The nutritional status after weaning was monitored by the rate of weight gain and hematocrit values. During this 2-week period the control males gained an average of 5 g/day and had hematocrits of approximately 40%. The deficient males gained at one fifth this rate or less and had hematocrits of one half the control value. Females developed anemia and other signs of deficiency more slowly than males. Final assessment of copper status was made by determinination of liver cytochrome c oxidase activity.[17] The lungs of all animals were examined and found to be free of edema, infection, and other disease.

To determine the effect of copper repletion on lung morphology, severely depleted rats, 5 to 10 weeks of age, were given copper supplementation for one, two, and three months. Littermates were killed at the beginning of the repletion period to establish the degree of deficiency; animals given copper throughout life served as positive controls. Lungs were fixed for sectioning, and livers were analyzed for cytochrome oxidase. Five deficient littermates killed at the beginning had an average liver cytochrome oxidase activity of 15, compared with a control value (7 animals) of 50 μg of Cu^{2+} intraperitoneally on the first day of repletion and then were fed the copper-supplemented diet (10 ppm) until they were killed. During repletion, they gained weight and eventually reached mature size. Their hematocrits returned to normal as did the liver cytochrome oxidase activity (72 μmole/min/g).

Cytochrome c Oxidase Assay

Cytochrome oxidase was determined at 37 C by the method of Smith,[18] in which the rate of oxidation of ferrocytochrome c is followed spectrophotometrically at 550 nm. The rate constant was determined using 3.0 ml of solution which had an absorbance of 0.5 and contained liver homogenate equivalent to 0.33 mg. From the ferrocytochrome c concentration and the rate constant, enzyme activity was calculated as μmole per minute per gram of liver tissue.

Determination of Lung Elastin

Rats were killed by guillotine and allowed to bleed freely. The whole lung was dissected clean and the trachea was removed flush with the organ. Following excision, the lungs were rinsed free of blood, blotted on filter paper, and weighed. The tissue was dried to constant weight in a vacuum oven at 60 C to determine dry weight. Elastin was determined by the Lansing procedure.[19] A dehydrated lung was allowed to stand at least 3 hours in 5 ml of 0.1 *N* NaOH and was then heated for 1 hour in a boiling water bath. After cooling, it was centrifuged and the supernatant solution was removed. The residue was heated again with 0.1 *N* NaOH for 15 min. This residue was washed twice with water and twice with acetone and was dried in the vacuum oven. Since sodium hydroxide erodes glass and the weight of residue is small, tare weight was determined after (rather than before) the extraction.

Morphology and Ultrastructure

The rats used for morphologic and morphometric studies were anesthetized with pentobarbital intraperitoneally, and their tracheas were intubated. Lungs were excised, inflated, examined, and measured or fixed *in situ*. Thus, two somewhat different methods were used to fix the lungs in a state of inflation: a) excised lungs were inflated to 25 cm H_2O with 1.5% osmium tetroxide in fluorocarbon (FC–80) for 15 minutes [20] with abdomen open. The sternum was split and the thorax was opened during the last 12 minutes. Although both methods "ironed out" the pleura to a smooth contour on histologic sections, the second method was preferable and was used exclusively for electron microscopy. Sections of fixed lungs were cut sagittally on the left and parallel to the diaphragm on the right side through both the lower and the upper lobes.

These large sections were infiltrated with paraffin, sectioned, and stained with hematoxylin and eosin. After 15 minutes lungs fixed in osmium FC-80 were dissected into cubes approximately 2 to 2.5 mm on a side and postfixed in 3% glutaraldehyde followed by reosmification. They were dehydrated in graded concentrations of ethanol, cleared in propylene oxide, and embedded in Epon 812. After curing, 1-μ sections were cut with glass knives on an ultramicrotome and stained with basic fuschin and toluidine blue. From these sections, areas were selected to include arterioles, bronchi, and alveolar ducts for thin sectioning with diamond knives. The thin sections were stained with uranyl acetate and lead citrate and photographed with a Philips 300 electron microscope at 80 kV accelerating voltage.

The number of alveoli in 10 randomly selected fields at 100 × magnification were estimated in 6-μ histologic sections using an adaptation of the mean linear intercept method.[21] A Howard Mold reticle with a square grid was mounted in a 10 × wide-field eyepiece, and the alveolar structures crossing the 20 horizontal grid lines were counted in each field. This technique was applied to lungs from copper-deficient rats; to deficient rats which were repleted with copper for 1, 2, and 3 months; and to controls supplemented with copper throughout life. In addition, rats fed a stock diet and weighing 50 and 200 g were studied to determine the correlation of body weight to alveolar size. Counts were made by three investigators without knowledge of the tissue source. The average counts for each group are presented as means and standard deviations.

Results

Copper deficiency caused growth retardation, anemia, and depressed levels of liver cytochrome oxidase activity. As shown in Table 1, at approximately 7 weeks of age, the average weight of copper-deficient rats was 57 g, compared with 104 g for controls. The average liver cytochrome oxidase activity, 15.1, was less than 20% of the control level, 84.2 μmole/min/g. Although the wet weights of the copper-deficient lungs were reduced (357 vs 521 mg for controls), they represented a greater percentage of total body weight than in controls. The deficient lungs averaged 680 mg/100 g body weight; the control lungs averaged 500 mg/100 g body weight. There was no difference in water content of the lungs, but the deficient lungs contained significantly less elastin.

Table 1—Dietary Status and Lung Elastin

Copper status	Body weight (g)	Age (days)	Liver cytochrone oxidase (μmole/min/g)	Lung		
				Weight (mg)	Dry matter (%)	Elastin (% dry weight)
+ (16)*	104 ± 6.7†	51 ± 3.2	84.2 ± 8.3	521 ± 28	20.9 ± 0.18	4.30 ± 0.16
− (16)	57 ± 4.0	54 ± 4.7	15.1 ± 2.1	357 ± 24	20.0 ± 0.31	3.56 ± 0.24
P‡	<0.001		<0.001	<0.001		<0.02

* No. of animals in group
† Mean ± standard error
‡ Statistical significance of the difference as determined by Student *t* test

The copper-deficient rat lungs were larger and occupied more volume compared with body size than did the controls. Displacement of water by lungs from deficient rats after inflation to 15 cm H_2O was 6.2 ± 0.4 ml compared with 5.8 ± 0.3 ml for controls. Pleural surfaces appeared to be slightly knobby. Pleural leaks were so frequent in copper-deficient lungs that pressure–volume studies were impossible. The hematoxylin–eosin-stained histologic sections of copper-deficient rat lungs appeared paler than controls probably due to less dense tissue. The pattern of lung parenchyma, alveolar ducts, and alveoli was coarser in copper-deficient lungs which had fewer alveoli (Figure 1). Other pathologic evidence was absent: none of the lungs from deficient, control, or copper-refed rats showed signs of edema, pneumonia, or airway infection. Leukocytic or lymphocytic alveolar infiltrations, goblet cell hyperplasia, and peribronchial lymphocytic infiltrations were absent in all rats; the pleuras were thin and all vessels were normal.

The apparent differences in alveolar architecture were confirmed by a reduction of 25% in the numbers of alveolar elements crossing the lines of a grid (Table 2). Lungs from copper-deficient rats averaged 34.7 ± 7.5 intercepts compared with 47.7 ± 4.5 in controls. At a given age the copper-deficient animals had lower body weights than controls; therefore, matching was done for body size (weight) rather than for age. Age and body weight had no appreciable effect on the number of alveolar intercepts in normal rat lung. This is shown by the data on rats fed a commercial stock diet. These animals ranged from weanling age (50 g) to near maturity at approximately 8 to 11 weeks (200 g).

Deficient rats repleted with copper gained in body weight, and their liver cytochrome oxidase activity and hemoglobin increased to normal levels. Nevertheless, the number of alveolar intercepts remained low (Table 3). There were no significant differences after repletion for 30, 63,

Table 2—Relation of Copper Status and Alveolar Size*

Diet	Body weight (g)		Age (days)		Alveolar mean linear intercept
	Mean	Range	Mean	Range	
Copper-deficient (6)†	72	50–100	73	50–100	34.7 ± 7.5‡
Copper-supplemented controls (6)	88	55–135	43	25–76	47.7 ± 4.5
Lab chow (3)	50	45–55		21–28	52.0 ± 2.9
Lab chow (4)	200	190–210		56–78	48.4 ± 2.6

* The lungs were fixed with glutaraldehyde, as described in *Morphology and Ultrastructure.*
† No. of animals is indicated in parentheses.
‡ Mean ± standard deviation. Compared with control group by the Student *t* test ($P < 0.001$).

Table 3—Failure of Copper Repletion to Reverse Morphologic Change*

Diet	No. of animals	Body weight (g) Range	Body weight (g) Mean	Mean age (days)	Alveolar mean linear intercept Mean	Alveolar mean linear intercept Group mean
Deficient	5	47-134	81	56	35.1 ± 5.0	*35.1* ± 5.0†
Repleted (10 ppm Cu)						
30 days	2	92-101	96	95	31.3 ± 5.1	
63 days	4	200-252	233	126	26.3 ± 6.0	
98 days	5	180-320	261	141	28.9 ± 3.6	*28.4* ± 4.2
Controls (10 ppm Cu)						
	2	80-105	92	46	40.8	
	2	150-180	165	113	40.3	
	3	305-320	314	133	47.4	43.6 ± 5.6

* Lungs were fixed with osmium tetroxide, as described in *Morphology and Ultrastructure.*
† Standard deviation of the mean. Statistical significance of the difference of means was determined by the Student *t* test.
Difference from control mean, $P < 0.001$, indicated by italics.

and 98 days. The alveolar intercepts were 31.3 ± 5.1, 26.3 ± 6.0, and 28.9 ± 3.6, respectively, compared with 35.1 ± 5.0 for littermates killed at the beginning of repletion. Thus, the larger repleted animals continued to have fewer alveolar intercepts and, therefore, larger alveolar ducts and alveoli than controls that received copper throughout life. The differences were apparent by inspection of the sections of the lungs at low magnification. They did not differ from the deficient lungs, as illustrated in Figures 1B and 1D. Males and females were equally affected. In paraffin sections the morphologic features of the bronchial tree and vessels of the lungs of deficient rats could not be distinguished from those of controls. However, in the 1-μ plastic-embedded sections, the fragmentation and abnormal staining of elastin in vessels and bronchi were apparent. The ultrastructure of elastin at its usual sites showed large differences.

In the copper-deficient lungs there was a decrease in the electron density of amorphous elastin observed in the lamina propria of bronchi, the elastin of alveolar ducts, and the inner and outer elastic laminae of arterioles. The elastin of airways beneath the basal lamina was uniformly pale and devoid of internal structure, although the area normally occupied by elastin was not apparently reduced. Compare Figure 2B with the specimen for copper-fed controls in Figure 2A. High magnification of such areas (Figure 4B) revealed almost no structural features, compared with the control (Figure 4A) which showed variable density and peripheral microfibrils.

Considering the morphologic changes observed, the most striking contrast in ultrastructure was in the tips of alveolar ducts where the helical

elastin is wound spirally and maintains the three-dimensional form of the alveolar ducts.[22,23] As shown in Figures 3A and 3B, the tips of the alveolar ducts are rich in connective tissue. Higher magnification of these areas (Figures 4C and 4D) allows visualization of elastin and microfibrils in the control tissue (Figure 4C), but only in the deficient tissue were clear zones seen where amorphous elastin is normally found. Collagen has maintained its normal periodicity. The ultrastructure of elastin in the walls of small blood vessels was markedly affected by copper deficiency. The inner and outer elastin laminae of a control arteriole are shown in Figure 5A. Comparable areas of a deficient arteriole are shown in Figure 5B. Note the electron lucency of the laminal areas.

Repletion of deficient rats by feeding a copper-supplemented diet for 60 days generally restored the elastin ultrastructure to normal. Key features of the airway, arteriole, and alveolar tip in a repleted animal are shown in Figure 6. The elastin in the airway (Figures 6A and 6B), the arteriole (Figure 6C), and the alveolar tip (Figure 6D) appear normal or near normal. The elastic laminae of the arteriole, particularly the outer lamina, may be only partially restored.

Comparison of sections at 80,000 to 90,000 × magnification from copper-repleted rats and from controls showed similar density of amorphous elastin and numbers, distribution, and density of microfibrils in airways (Figures 7A and 7B) and, at 36,000 × magnification, in vessels (Figures 8A and 8B). Sections of alveolar duct tips were similarly normal and contrasted sharply with sections from copper-deficient rats. Despite the restored ultrastructure of elastin, the repleted animals still had lungs with large distal airspaces.

Discussion

This study shows that copper-deficient rats born of dams fed a low-copper diet during gestation had enlarged alveolar ducts and alveoli. Repletion by feeding copper to severely deficient young rats cured the usual metabolic features of deficiency, including anemia, low levels of hepatic cytochrome oxidase, and growth retardation but did not restore the alveolar size to normal. The numbers of alveolar intercepts remained 30% below normal. Elastin, as a percentage of dry weight, was decreased in the lungs of deficient rats. Although this difference was only 17% it may be greater than this in parenchymal tissue; such a loss may be crucial for the alveolar zone. Only a small proportion of pulmonary elastin is in the alveolar zone, as revealed by light and electron microscopy. Tips of alveolar ducts are the major site of elastin aggregates in the alveoli of rat lungs.

Mice of the blotchy allele, one of several mutations of the mottled locus on the x-chromosome, have connective tissue abnormalities, similar to copper-deficient and lathyritic animals. Besides aortic aneurysms and weak skin, their lungs have been characterized as having large volumes, decreased mean linear intercepts, and diminished internal alveolar surface area.[9] Scanning electron microscopy shows a coarse pattern of the distal lung with enlarged alveolar ducts and diminished alveolar septums.

Deficiency of copper induces growth retardation and failure to thrive as judged by poor weight gain, which is difficult to match in control animals. Controls matched for weight were studied, but neither pair fed nor starvation controls were produced. Thus, the remaining possibility that relative starvation during postnatal development could have reduced the numbers of alveolar intercepts has been eliminated. However, in view of the stable numbers of alveolar intercepts in rats from 50 to over 300 g, it seems unlikely.

The elastin of the pulmonary connective tissue in vessels, bronchioles, and alveolar ducts of copper-deficient rats lacks the usual ultrastructural features. There is a decrease in microfibrils and a marked loss of electron density, producing a pale, washed-out appearance in deficient animals. The electron density was restored, for the most part, in animals subsequently repleted with copper for 1 to 3 months. Although such repletion largely restored the appearance of elastin, it did not affect the morphology of the alveolar ducts and alveoli. This is interpreted to mean that the elastin skeleton of the lung encompassing the alveolar ductile, vascular, and bronchial elastin networks of Orsos [23] has developed defectively, resulting in an abnormal morphology. Little is known about elastin turnover, but in all tissues studied it is slow, especially in lung and bone.[24] In this experiment, copper repletion restored the ultrastructure and electron density of elastin in the lung. Nevertheless, the enlarged air spaces persisted, suggesting that the defect of the lungs' alveolar architecture induced early in life was not reversed by feeding copper.

Emphysema is usually regarded as a disease resulting from destruction of alveolar septums and alveoli. Exceptions, such as congenital lobar emphysema, have been ignored or regarded as minor variants of the primary etiology. However, several hundred cases of congenital emphysema have been recognized, and many patients have been treated by surgical excision of lobes since 1949.[25] Observers of the condition suggest that less severe cases may escape recognition in infancy and childhood [25,26] and continue into adulthood. The data from the lungs of copper-deficient rats and from the lungs of blotchy mice [9] suggest that when the cross-

linking of connective tissue proteins, especially elastin, is defective, the lungs develop fewer alveolar ducts and alveoli so that those present are abnormally large.

Copper is an essential component of lysyl oxidase,[13] the enzyme which catalyses the oxidative deamination of lysine residues in the precursor proteins of elastin and collagen. Lysyl oxidase plays a key role in the cross-linking of collagen and elastin; thus, one would expect a deficiency of copper to result in a metabolic defect such as the decrease in elastin concentration and alteration in the staining properties of elastin, as observed, which would occur if lysyl oxidase were deficient. Lysyl oxidase activity is decreased in the blotchy mouse.[27] The structural abnormalities which resemble pulmonary emphysema may arise from the failure to cross-link the connective tissue proteins. Since copper repletion appeared to restore the density and ultrastructure of parenchymal elastin but did not reverse the structural (architectural) abnormality, one must conclude that copper exerts a critical metabolic function during development of the lung. Emphysema, or at least some types of emphysema, may be a developmental disease resulting from failure of normal remodeling during late embryonic, fetal, or neonatal development.[26,28] Quantitative studies of lungs or lobes removed for congenital emphysema show four patterns, including a) atresia of bronchial branches, b) reduced number of bronchial generations with reduced number of alveoli, c) normal bronchial generations with normal alveoli, and d) normal bronchial generations and increased number of alveoli.[28] Cross-linking of elastin may be crucial in the further partitioning of the primitive alveolar sacs during the alveolarization of the canalicular lung.

The alveolar zones of lungs from all human subjects may not be equal in complexity or extent of partitioning at birth.[29] The various congenital defects of bifurcation,[30] perhaps including unilateral radiolucent lung, Swyer-James [31] and MacLeod's [32] syndromes, and even idiopathic honeycomb lung,[33] could represent the manifestations of unilateral or generalized metabolic defects in the lung. Thus, the lung may be one of the more sensitive organs to failure of elastin cross-linking and a normal alveolar zone morphogenesis.

We submit evidence for a model of a type of emphysema which results from a developmental abnormality induced by copper deficiency in the rat. This concept should stimulate investigators to examine patients during the maternal (embryonic), neonatal, and early postnatal periods for toxic materials which could affect the lungs' development of connective tissue, particularly elastin, as do lathyrogens and copper deficiency.

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[*Illustrations follow*]

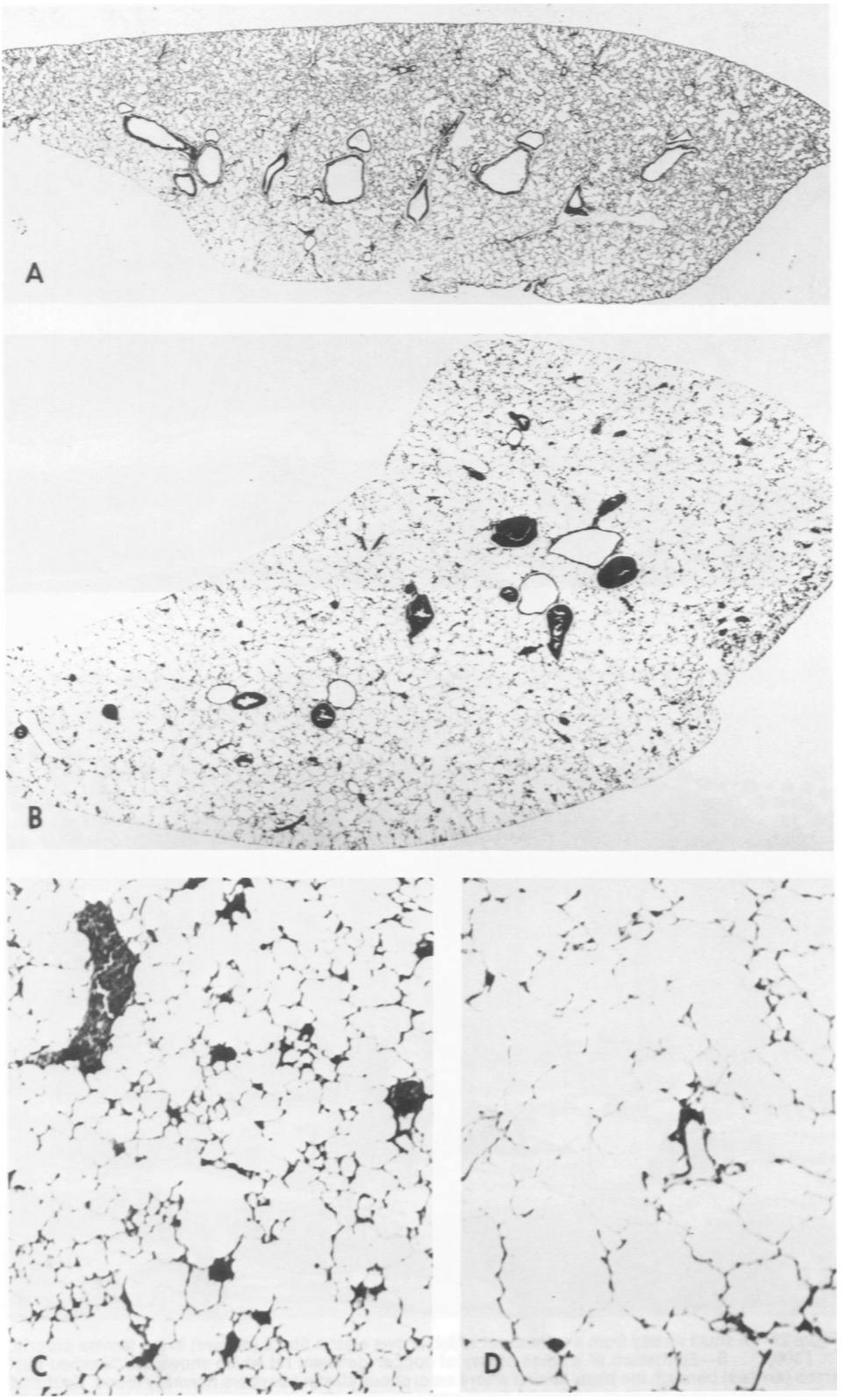


Figure 1A—A sagittal lung section for a normal rat showing the parenchyma with alveoli and alveolar ducts. **B**—A similar section from lung of copper-deficient rat, which shows a coarse alveolar pattern with many large alveolar ducts. The paraffin-embedded lung tissues shown in Figures A and B were fixed in inflation at a pressure of 25 cm H_2O. (H&E, × 3.5) **C**—Enlargement of **A** showing normal alveolar ducts and alveoli. (× 25) **D**—Enlargement of B showing course, large irregular alveolar ducts and few normal alveoli. (× 25)

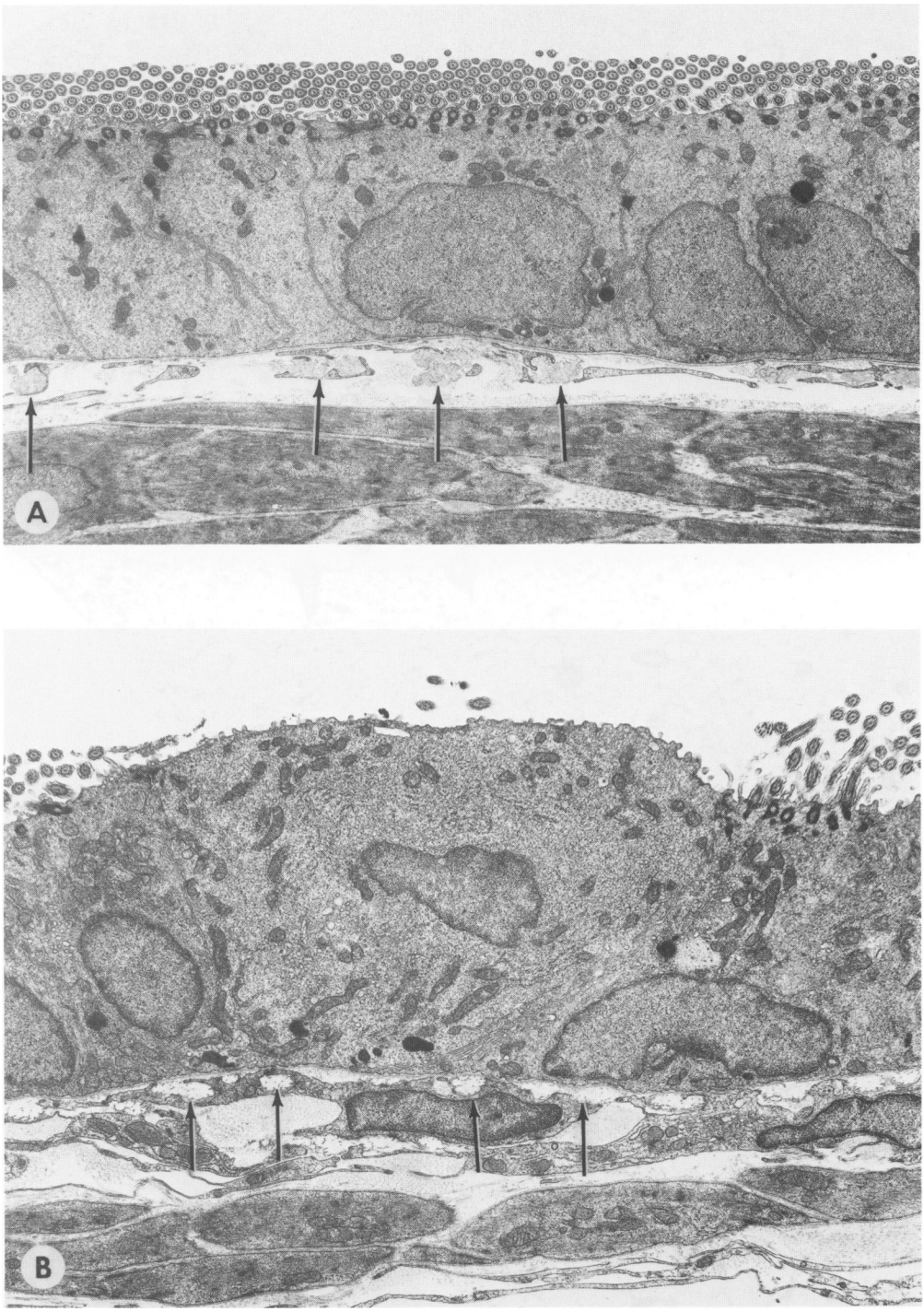
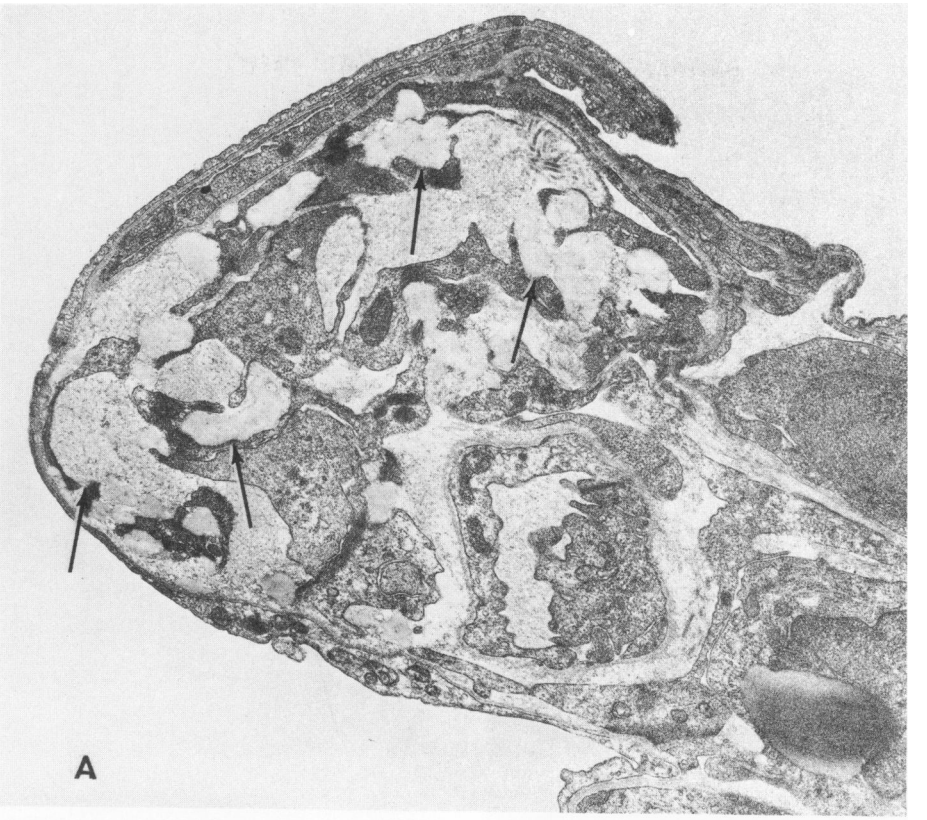


Figure 2A—A small airway from a normal rat which shows elastin fibers (*arrows*) in the lamina propria. (× 7300) **B**—Epithelium of a small airway of copper-deficient rat which shows the "washed out" areas (*arrows*) beneath the basal lamina where amorphous elastin fibers are normally found. Epithelial cells appear normal. Osmium-tetroxide–fluorcarbon fixation. (Uranyl acetate and lead citrate, × 7300)



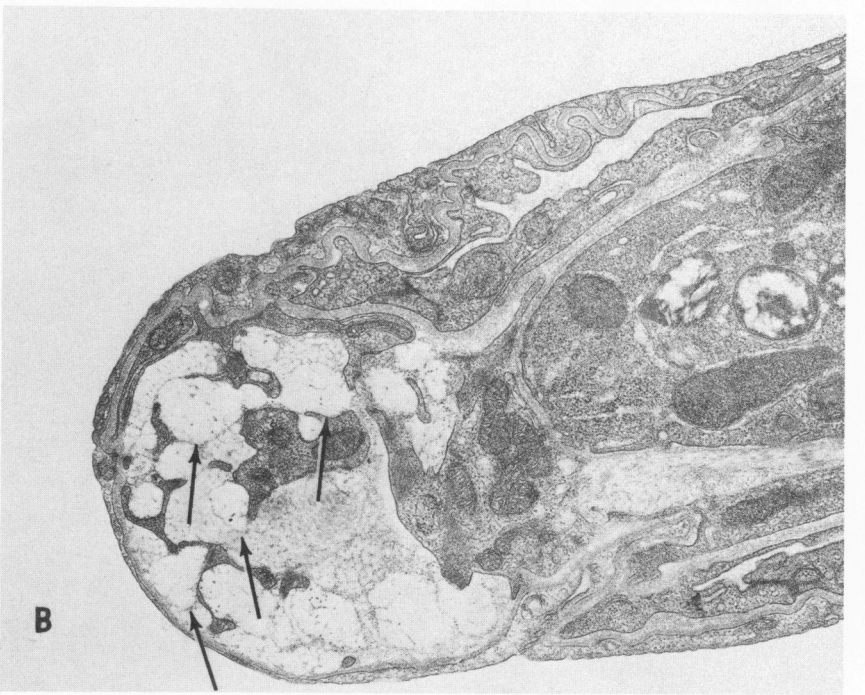


Figure 3A—Tip of alveolar duct from control rat. **B**—Tip of alveolar duct from copper-deficient rat. Elastin fibers are indicated by *arrows*. Higher magnifications showing the areas with elastin are shown in Figures 4C and D. (**A**, × 14,000; **B**, × 17,000)

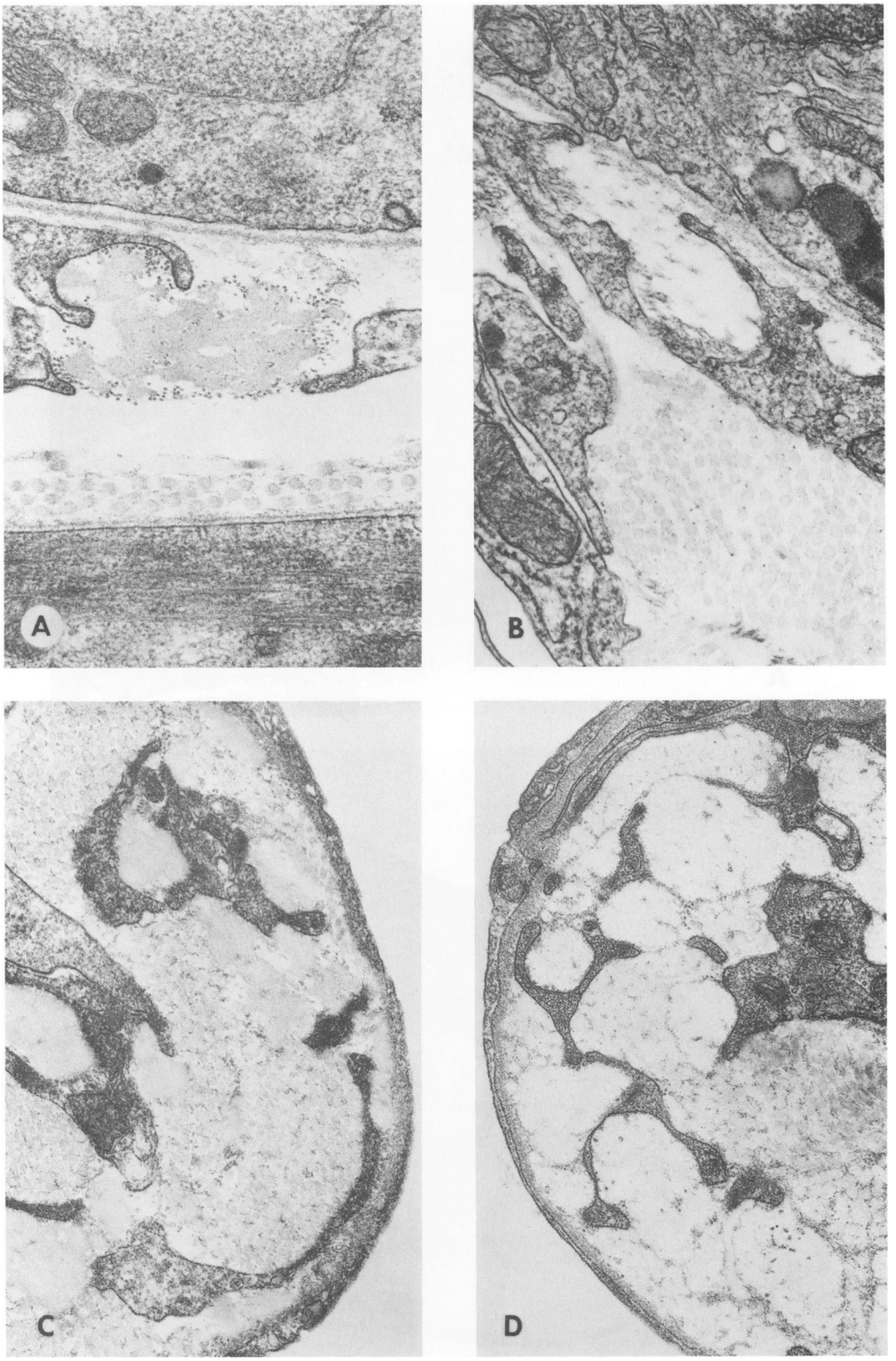


Figure 4A—A high-power view of the normal rat airway depicted in Figure 2A, which shows the elastin with mottled density and associated microfibrils beneath the basal lamina. (× 38,000) **B**—A high-power view of the airway of the copper-deficient rat depicted in Figure 2B, showing decreased density and lack of structure of elastin beneath the basal lamina. (× 38,000) **C**—Higher power view of Figure 3A, a normal alveolar duct tip, showing areas of deposition of amorphous elastin. (× 32,000) **D**—Higher power of Figure 3B, tip of an alveolar duct from a copper-deficient rat, showing "washed out" elastin. Normally, elastin at this site forms the spiral helical skeleton of the alveolar duct. (× 34,000)

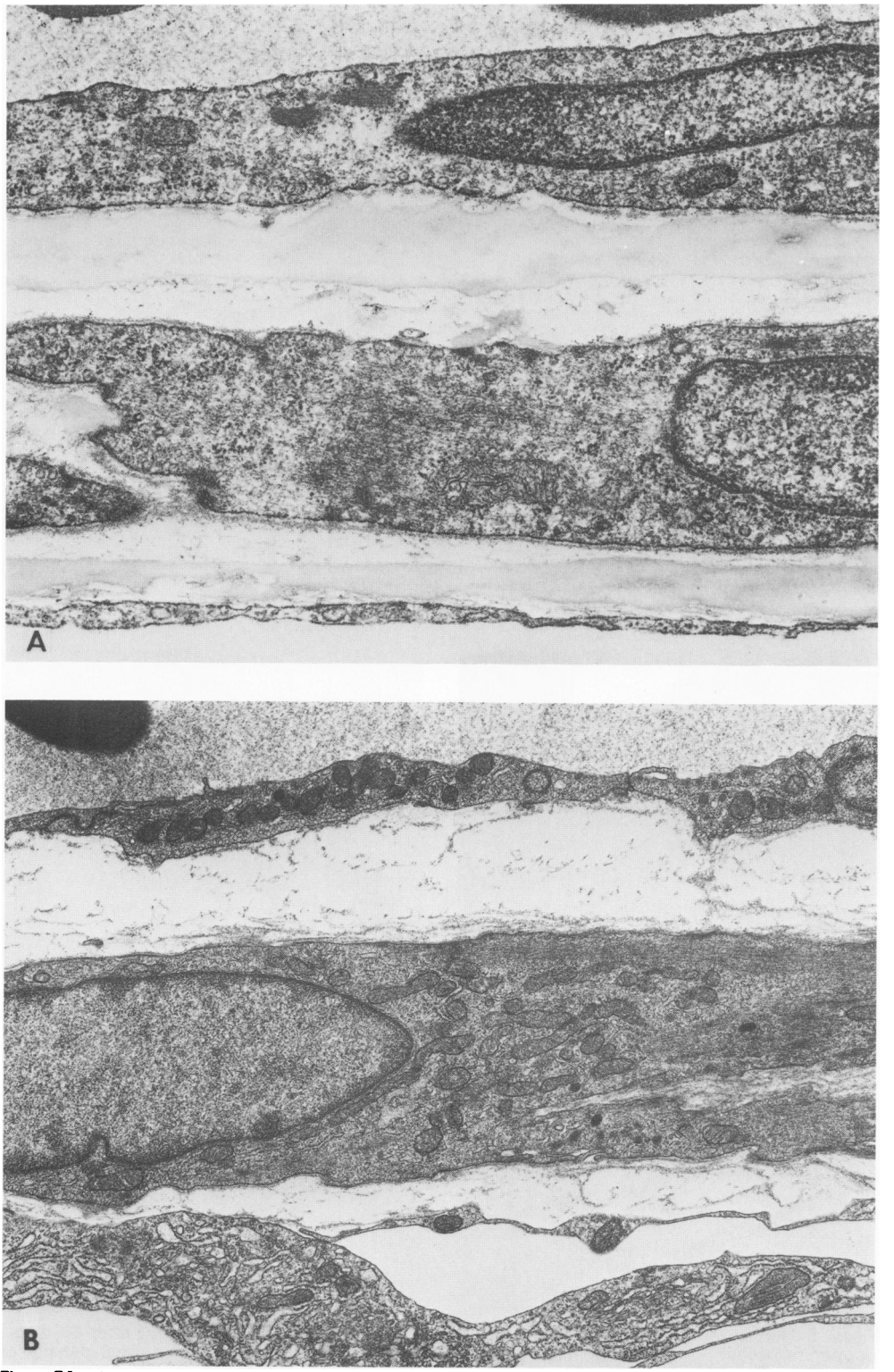


Figure 5A—A pulmonary arteriole from a control rat showing the inner (*top*) and outer elastic laminae. Note amorphous elastin. (× 32,000) **B**—Small pulmonary arteriole from a copper-deficient rat showing "washed out" appearance of elastic laminae. Somewhat better retention of the fibrillar component is apparent next to smooth muscle. The smooth muscle between the elastic laminae appears normal. (× 14,500)

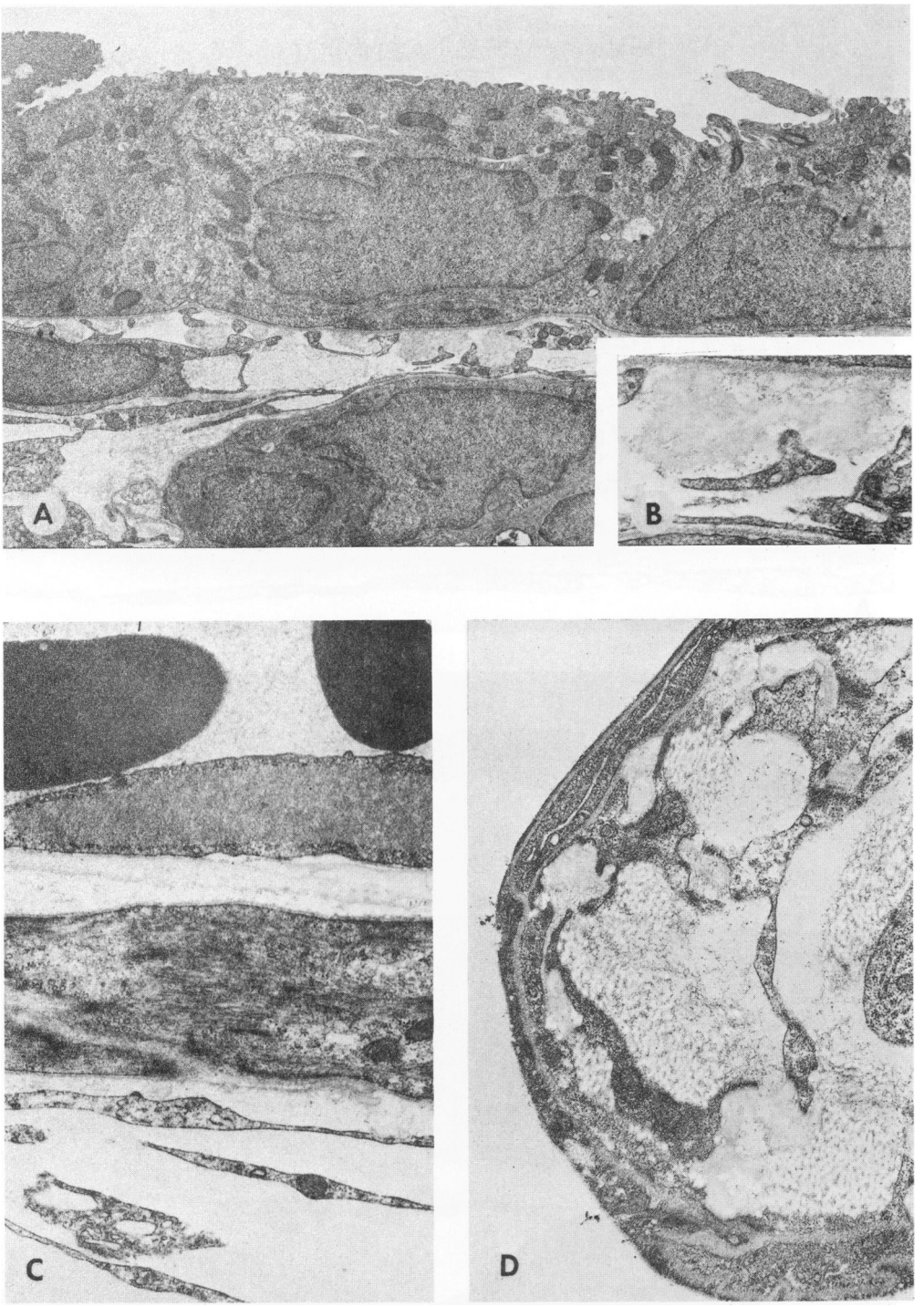


Figure 6—Airway arteriole and alveolar tip from a previously copper-deficient rat after 60 days of copper repletion. **A**—Airway from copper-repleted rat showing elastin of normal density and pattern. **B**—Higher magnification of portion of elastin beneath airway in Figure 6A, showing details of reconstituted normal-appearing airway elastin. **C**—Arteriole of copper-repleted rat, although smaller than those in Figure 5 shows distinct thin elastic laminae. **D**—Alveolar duct tip showing elastin restoration of normal or near normal density and morphology in a copper-repleted rat. (**A** × 8000; **B**, × 28,000; **C**, × 15,500; **D**, × 14,500)

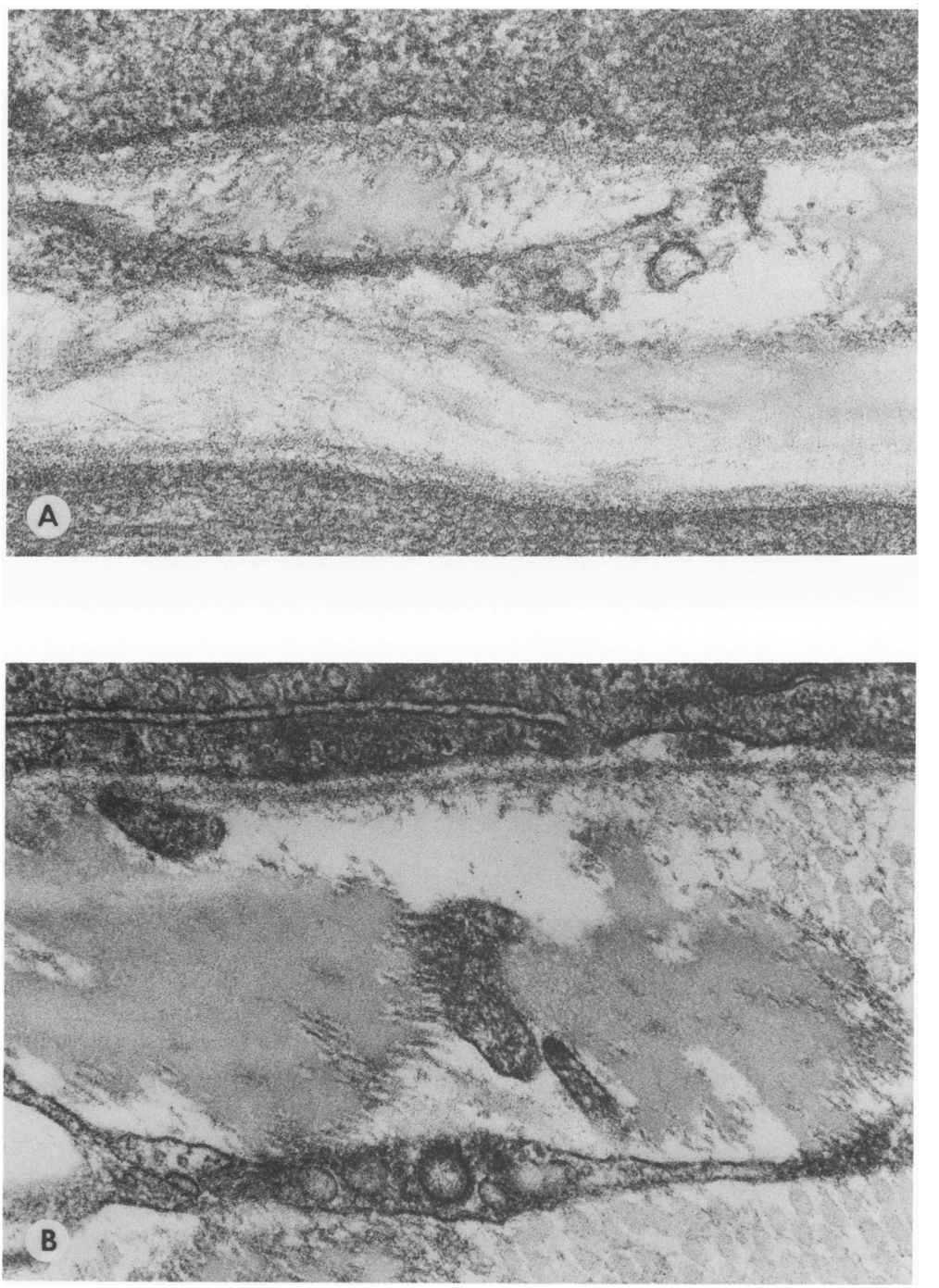


Figure 7A—Detail of elastin from airway of copper-repleted rat (60 days), showing many microfibrils and electron density of amorphous elastin. B—Comparison of detail of elastin from airway of control rat showing normal abundant microfibrils and electron density of amorphous elastin. (× 81,000)

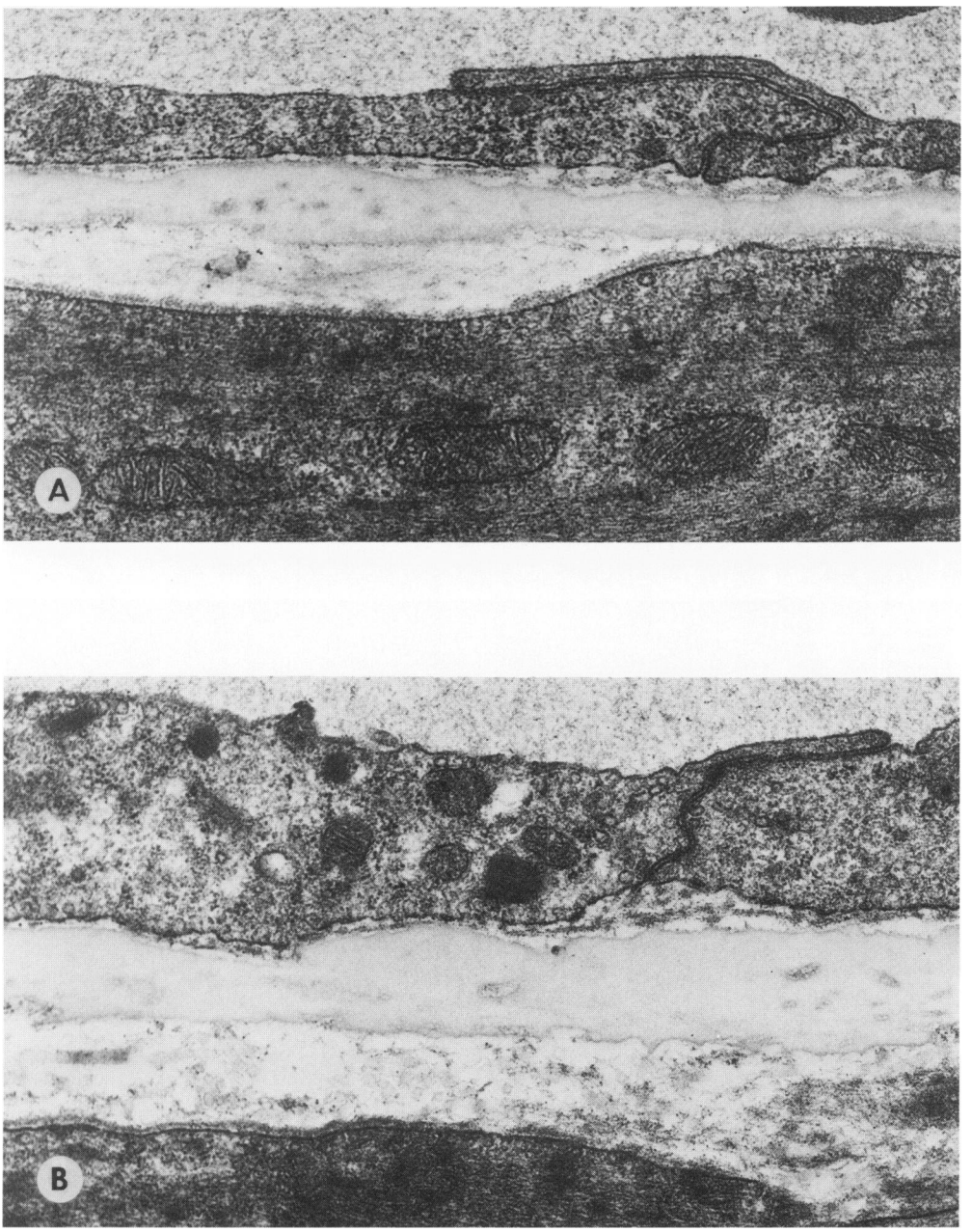


Figure 8A—Detail of elastin in vascular lamina from copper-repleted rat, showing many microfibrils and electron-dense amorphous elastin. **B**—Comparison of elastin from control rat showing normal microfibrils and amorphous elastin. Not distinguishable from 8A. (× 36,000)