Oxygen Toxicity and Restructuring of Pulmonary Arteries—A Morphometric Study

The Response to 4 Weeks' Exposure to Hyperoxia and Return to Breathing Air

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This study describes the pulmonary vascular lesions in rat pulmonary arteries and altered right ventricular weight after 1) prolonged exposure to hyperoxia (87% O_2 for 4 weeks) at ambient pressure, 2) weaning from hyperoxia to air over 7 days, and 3) return to breathing air for 2, 4, or 8 weeks. Hyperoxia for 28 days narrows the lumen of intraacinar and preacinar arteries, increasing the percent medial thickness (%MT) by reducing the external diameter and thickening medial muscle. The ratio of patent intraacinar arteries to alveoli is significantly reduced, and pulmonary vascular obstruction and obliteration is evident by electron microscopy. A higher proportion of intraacinar and preacinar arteries have muscle in their wall than in the normal lung: in alveolar wall and duct regions, the proportion of partially muscular and muscular intraacinar arteries increases at the expense of nonmuscular ones (for both regions $P_{\chi^2} \leq 0.001$); and in arteries associated with terminal bronchioli and bronchioli the proportion of muscular arteries increases at the expense of partially muscular ones (for both regions $P_{\chi^2} \leq 0.001$). Both after weaning and after return to breathing air lumen size increases; but, even after 8 weeks, the %MT remains significantly increased, and the ratio of intraacinar arteries to alveoli is less than normal. After weaning, the proportion of muscularized intraacinar and preacinar arteries is similar to that after hyperoxia. Two weeks after return to breathing air, the proportion of muscularized alveolar wall and duct arteries is greater (for both regions $P_{\chi^2} \leq 0.001$). Even 8 weeks after return to breathing air more arteries are muscularized than normal (for both alveolar wall and duct regions $P_{\chi^2} \leq 0.001$), and within the alveolar wall still more are muscularized than after hyperoxia ($P_{\chi^2} \leq 0.001$). Hyperoxia causes right ventricular hypertrophy, reducing the ratio of the weight of the left ventricle and septum to that of the right ventricle ($P_{\chi^2} \leq 0.001$). Weaning further increases the hypertrophy, the ratio being further reduced ($P_{\chi^2} \leq 0.001$, compared with both hyperoxia and control values). On return to breathing air the degree of hypertrophy is less, but it persists, and even after 8 weeks the ratio is still less than normal ($P_{\chi^2} \leq 0.01$). (Am J Pathol 1985, 121:212–223)

DURING exposure to hyperoxia the generation of oxygen metabolites, and their toxic products, injures cells of the alveolar–capillary membrane[1–5] and the pulmonary artery wall.[6] Respiratory distress is observed in animals exposed to normobaric hyperoxia: in monkeys exposed to oxygen concentrations above 90% this occurs on about the 9th day of exposure,[7] whereas in rats exposed to 87% (as in this study) it occurs earlier, on the third or fourth day. This phase of pulmonary injury is characterized by extensive edema and cell injury, particularly to Type I pneumonocytes and endothelial cells. Adaptation to breathing a high oxygen tension is associated with hypertrophy and hyperplasia of cells of the alveolar–capillary membrane[2–4,7] and rapid restructuring of the walls of pulmonary arteries in both the preacinar and intraacinar regions.[8] After 7 days of exposure (to 87–90% O_2) the vascular changes include a reduced number of patent artery segments and thickened wall muscle, associated with an increased pulmo-

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nary vascular resistance and moderate pulmonary hypertension.[8]

To establish whether vascular injury progresses, the present study examines the structure of pulmonary arteries after breathing 87% O_2 for 28 days. After hyperoxia it is necessary to wean animals to air by gradually decreasing the alveolar oxygen tension,[2,7] immediate return to air without weaning causing acute pulmonary edema and a striking rise in pulmonary artery pressure.[8] In this study we establish by morphometric techniques the effect on pulmonary artery structure of prolonged exposure to hyperoxia, the effect of weaning to air, and the extent and pattern of the vascular changes after several weeks of return to breathing air.

Materials and Methods

Forty-eight male Sprague–Dawley specific-pathogen-free rats (Charles River Breeding Laboratories, Kingston, NY), 21 control rats that breathed air, and 27 exposed to hyperoxia (87% O_2) at normobaric pressure[8] were studied. One rat died on the 27th day of exposure. After 28 days of exposure, the lungs of 8 exposed rats were removed, and the other 18 rats were weaned to air over the next 7 days. The chamber oxygen tension was reduced by 15% on each of the first 2 days and by about 10% each day during the rest of the weaning period. After weaning, the lungs of 3 rats were removed; those of another 3 rats were removed each after 2 or 4 weeks of return to breathing air; and those of 5 rats were removed after 8 weeks. Three rats did not survive weaning, and 1 rat died after 17 days in air. The lungs of control rats were removed at similar times.

At the end of exposure to hyperoxia, rats (breathing 87% O_2 via a mask) were moved from the chamber to an Isolette, where they continued to breathe 87% O_2 during anesthesia. All rats received the same dose of anesthetic (30 mg sodium pentobarbital/100 g body weight intraperitoneally). The trachea, lungs, and heart of each rat were removed *en bloc* through a sternotomy. A catheter was immediately inserted through the right ventricle and ligated in the main pulmonary artery: the pulmonary arteries were injected for 2 minutes with a barium-gelatin suspension at 60 C and 100 cm H_2O pressure.[8] The lungs were distended by intratracheal instillation (at 23 cm H_2O) of 4% formaldehyde, 1% glutaraldehyde in phosphate buffer (pH 7.4) until the lung margins were well defined, and the trachea was ligated. Pulmonary arteriograms were prepared from all control rats and all rats that survived hyperoxia, weaning, and return to breathing air (21 control and 22 exposed animals). Tissue slices were selected from the cardiac and diaphragmatic lobes of the right lung and from the single-lobed left lung.[8] The heart was fixed for 7 days, weighed, and dissected for assessment of right ventricular hypertrophy.[9] Sections of lung tissue embedded in wax (4 μ) were stained for demonstration of elastic fibres[10] and counterstained with van Gieson's or stained with hematoxylin and eosin: Epon sections (1 μ) were stained with 1% toluidine blue in 1% borax. Because vessel remnants were visible by light microscopy, Epon sections 700 Å thick were cut from tissue blocks from several rats exposed to hyperoxia. To facilitate cutting, we prepared these sections from tissue blocks trimmed to remove adjacent barium-gelatin-filled vessels. Sections were stained with 1% uranyl acetate in 100% methanol followed by lead citrate, and examined by electron microscopy at 60 kV.

Morphometric techniques were applied to tissue sections of the lungs of 5 rats selected at random after exposure to hyperoxia; from 2 rats after weaning (those of a third animal were excluded because of technical problems during vascular injection); from 3 rats each after 2, 4, or 8 weeks in air; and from 6 control rats (3 from the start and 3 from the end of the study). Sections of the other lungs were examined qualitatively. As part of morphometric analysis, the wall structure of each artery profile was noted (muscular, partially muscular, or nonmuscular), as was the associated airway region (bronchiolus, terminal bronchiolus, respiratory bronchiolus, alveolar duct, or alveolar wall). The distribution of preacinar arteries by wall structure in the normal lung (those associated with bronchioli and terminal bronchioli) and intraacinar arteries (those associated with respiratory bronchioli, alveolar ducts, or alveolar walls) is as previously described.[8] With the use of an eyepiece reticle, the external diameter (ED) of each artery and the medial thickness (MT) of muscular and partially muscular arteries was measured.[8] The percent medial thickness (%MT) was calculated as [2 × MT × 100]/ED for muscular arteries and (MT × 100]/ED for partially muscular ones. In consecutive microscopic

Table 1—Body Weight (Mean ± SEM) of Control and Exposed Rats

	Exposed group		Control group	
	n	Body weight (g)	n	Body weight (g)
A	8	255 ± 9†	8	396 ± 26
B	3	206 ± 4‡	3	388 ± 43
B + 2	3	310 ± 67	3	469 ± 35
B + 4	3	424 ± 14†	3	540 ± 14
B + 8	5	516 ± 23	4	543 ± 8

* A, 28 days 87% O_2; B, A + weaning; B + 2, 4, or 8 weeks in air. n, number of animals.
† Differs significantly from control value; $P \leq 0.01$.
‡ Differs significantly from control value; $P \leq 0.001$.

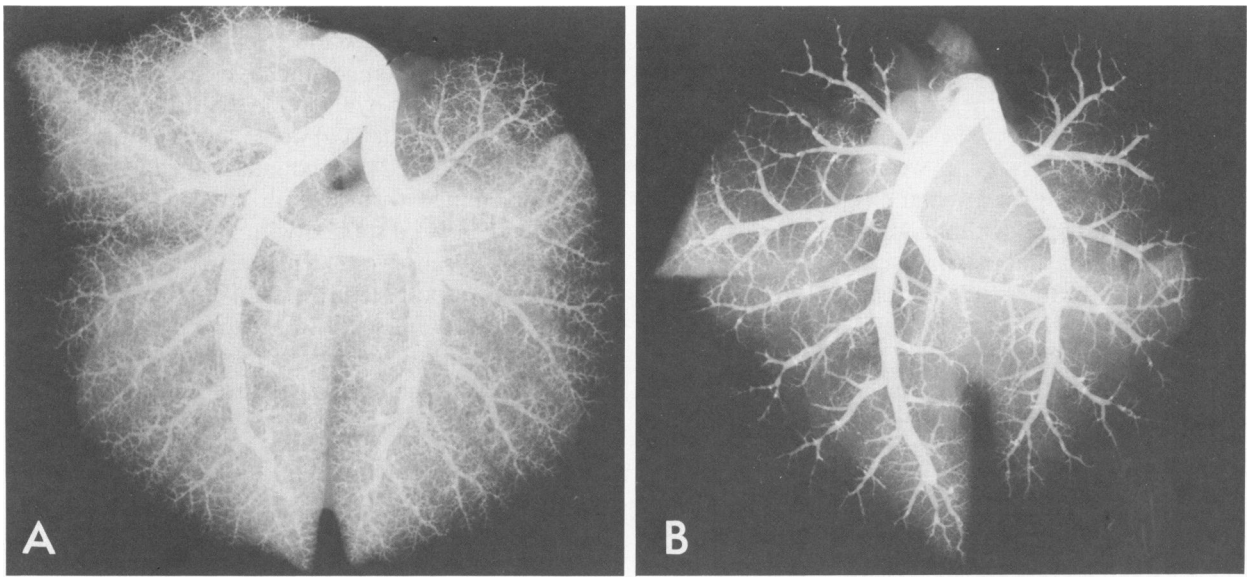

Figure 1—Rat pulmonary arteriograms (original magnification, × 2.5). **A**—Control. **B**—Eighty-seven percent O_2 for 28 days.

fields (0.42 mm in diameter) 25–26 intraacinar arteries were analyzed in each lung section (a total of 75–78 per animal) as well as all preacinar arteries (18–25 total per animal). For each animal, we counted the total number of alveoli and arteries in 20 microscopic fields (0.42 mm in diameter)[8] to establish the artery/alveolar ratio.

Levels of significance for data were calculated by the unpaired *t* test. If the variance of two compared groups was similar by the *F* test, a *t* test using pooled variance was performed. The distribution of vessels by wall structure was examined by chi-square analysis, as previously described,[8] the percent values referred to in the text being ones derived from contingency tables. To address the problem of multiple intergroup comparisons, we adopted Bonferroni's correction factor to adjust the significance level. For the purpose of this study, a result was taken to be statistically significant if the *P* value was less than 0.0125. Morphometric findings for the two groups of control animals were not significantly different, and so they were combined.

Results

Body Weight

The rats weighed between 160 and 180 g at the start of the experiment, animals of similar weight being ran-

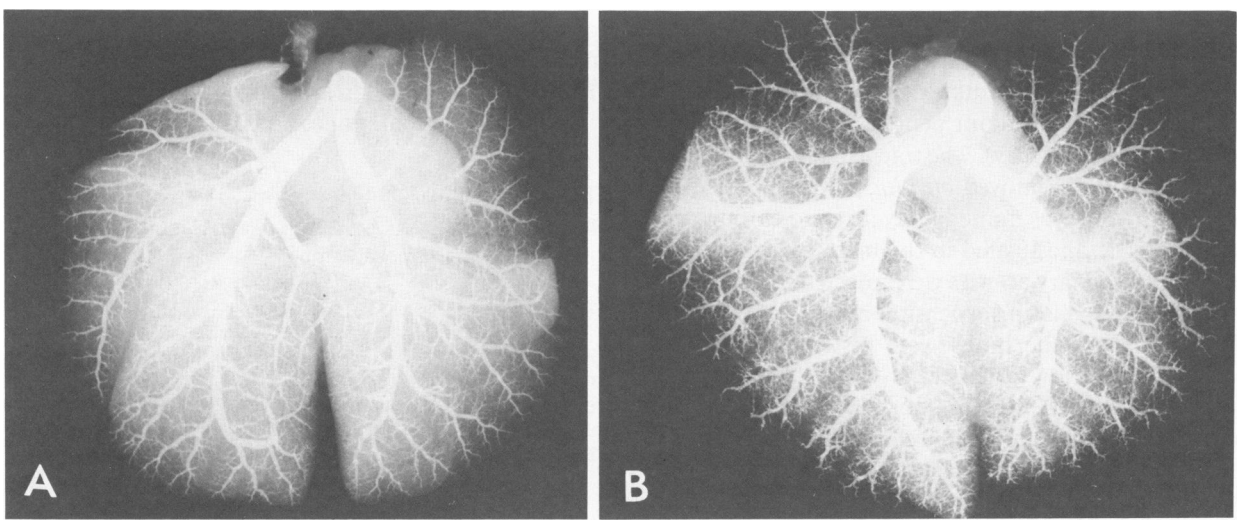

Figure 2—Rat pulmonary arteriograms (original magnification, × 2.5). **A**—Eighty-seven percent O_2 for 28 days + 7 days weaning. **B**—As for **A** + 8 weeks air.

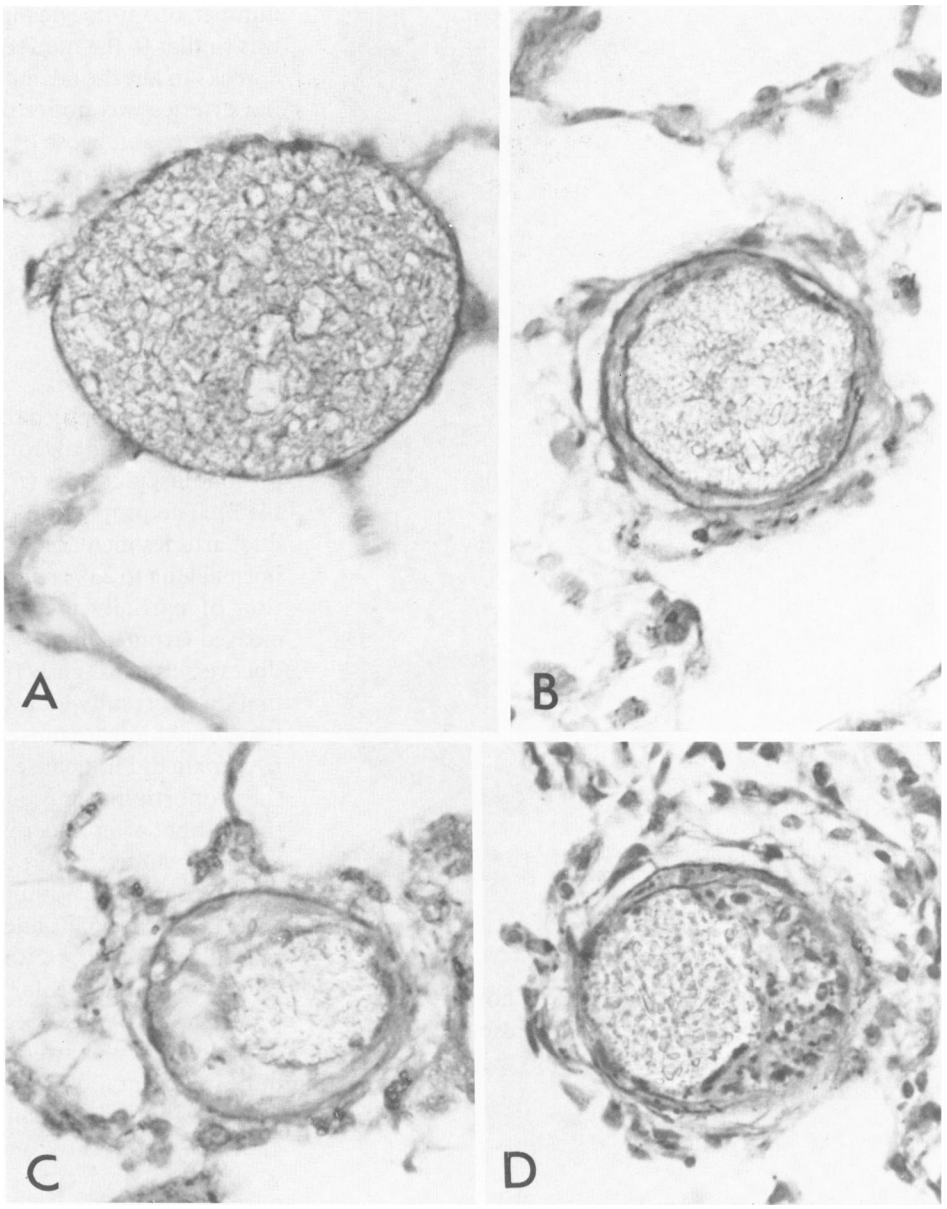

Figure 3—Rat intraacinar arteries (4 μ sections stained with MEVG); the lumen of each vessel is distended with barium–gelatin. **A**—Control, showing normal thin-walled vessel with a single elastic lamina (ED 72 μ). (×640) **B**—Eighty-seven percent O_2 for 28 days. A muscular artery with a well-developed media (that stains for muscle) between an internal and external elastic lamina (ED, 53 μ). (×640) **C**—Eighty-seven percent O_2 for 28 days. Nonmuscular alveolar wall vessel showing lumen reduction by a thickened and hyaline wall (ED, 55 μ). (×547) **D**—Eighty-seven percent O_2 for 28 days. Muscularized alveolar duct vessel showing partial occlusion of the lumen by intimal/medial cell proliferation (ED, 87 μ). (×478)

domly assigned to hyperoxia or control groups. After 28 days of hyperoxia and even further after weaning, the mean body weight of the exposed rats was significantly below the control (Table 1). It then increased and 8 weeks after return to breathing air was similar to the control value.

Macroscopic Appearance of Lungs and Pulmonary Arteriograms

After hyperoxia, weaning, and return to breathing air the lungs were white, with small petechiae on their surfaces. In the animals that did not survive weaning there were pleural effusions (2–8 ml of straw-colored or sanguinous fluid).

Vascular filling was uniform in the pulmonary arteriograms of control rats (Figure 1A). Hyperoxia for 28 days narrowed the lumen of the main pulmonary arteries and strikingly reduced the number of smaller vessels (identified as separate lines) and the diffuse background haze or opacity (these representing filled fine intraacinar arteries and general tissue fluid; Figure 1B). After weaning, the lumen of large axial arteries was more tapered than after hyperoxia, the background opacity of the arteriogram was increased, and more small arteries were seen (Figure 2A). In most pulmonary arteriograms return to breathing air increased the lumen and number of filled small arteries, but the extent varied from animal to animal—even within a single lung there was great regional variation. In all lungs

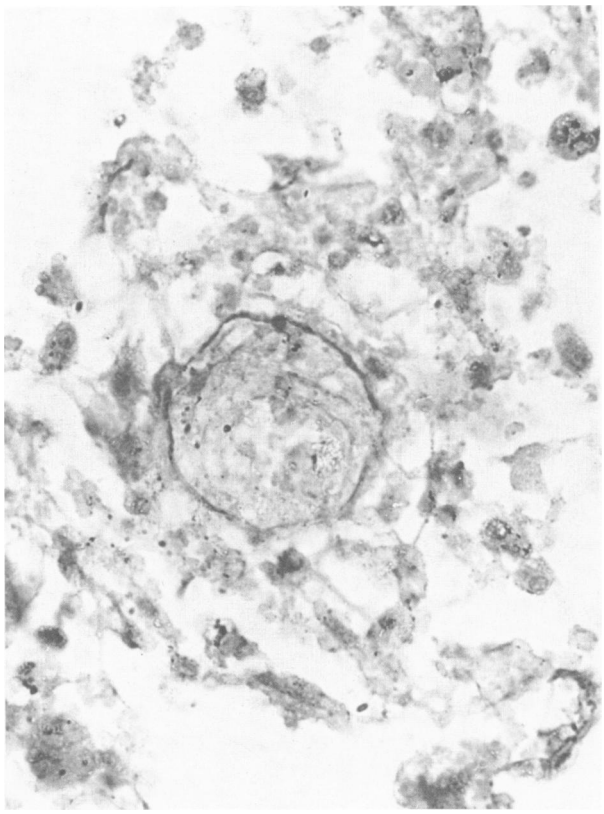

Figure 4—Eighty-seven percent O_2 for 28 days. Vessel remnant showing well-defined elastic lamina and lumen occlusion. (4-μ section stained with MEVG, ×781)

background opacity had returned to normal 2 weeks after return to breathing air. In other respects, even after 8 weeks, vascular filling in all lungs was still reduced (Figure 2B).

Histologic Features of Pulmonary Arteries

Hyperoxia visibly narrowed the lumen and thickened the wall of many pulmonary arteries and increased the number of monocytes in both the alveolar wall and space. Arteries with muscular walls were present in the alveolar wall, where they are not found in the normal lung (Figures 3A and B). The lumen of some arteries was partly occluded by hyaline thickening of the wall or by intimal/medial cell proliferation (Figures 3C and D). Vessel remnants were identified by their elastic laminae (Figure 4), and most were considered to be arteries by their position. Ultrastructurally, their walls were greatly disrupted (Figures 5A–C); in some the lumen was no longer patent (Figure 5A), and in others, while still patent, its size was greatly reduced (Figures 5B and C).

After weaning, and after return to breathing air, the number of monocytes in the alveolar wall and space was similar to the number after hyperoxia. After 4 and 8 weeks in air, the adventitia of preacinar and intraacinar arteries was noticeably thickened (Figure 6), and within the submucosa of large airways cross-filling from pulmonary to bronchial arteries was evident.

Morphometric Analysis of Pulmonary Arteries

Distribution of Pulmonary Arteries With Medial Muscle

Intraacinar Arteries

Hyperoxia increased the proportion of muscularized alveolar duct and alveolar wall arteries at the expense of nonmuscular ones ($P_{\chi^2} \leq 0.001$ for each region, Table 2). The proportion of muscular alveolar wall and duct arteries increased (from 0% in each region in the normal lung to 23% and 34%, respectively): the proportion of partially muscular alveolar wall arteries increased (from 19% to 40%), whereas that of alveolar duct vessels fell slightly (from 50% to 44%). In the normal lung virtually all arteries associated with respiratory bronchioli are muscularized, and so in this region hyperoxia did not cause a similar shift. After weaning the proportion of muscularized arteries was similar to the number after hyperoxia. Two weeks after return to breathing air even more alveolar wall and duct arteries were muscular (32% and 45%, respectively) and partially muscular (62% and 54%), at the expense of nonmuscular ones ($P_{\chi^2} \leq 0.001$, Table 2). After 8 weeks there were more alveolar wall and duct muscular arteries (20% and 24%, respectively) and more alveolar duct partially muscular arteries (52%) than normal, and still more muscular (20%) and partially muscular (59%) alveolar wall arteries than after hyperoxia ($P_{\chi^2} \leq 0.001$ Table 2).

Preacinar Arteries

Hyperoxia increased the proportion of muscular arteries associated with terminal bronchioli (from 20% to 87%) and bronchioli (from 45% to 92%) at the expense of partially muscular ones ($P_{\chi^2} \leq 0.001$ for both regions, Table 3), there being few preacinar arteries with nonmuscular walls. After weaning, the proportion of muscularized preacinar arteries was similar to the hyperoxia value, but medial muscle then regressed, and 8 weeks after return to air the distribution of vessels with muscle in their wall was normal.

Lumen Size and Alveoli/Artery Ratio

Intraacinar Arteries

Hyperoxia narrowed the lumen of intraacinar arteries, increasing the %MT of muscular and partially

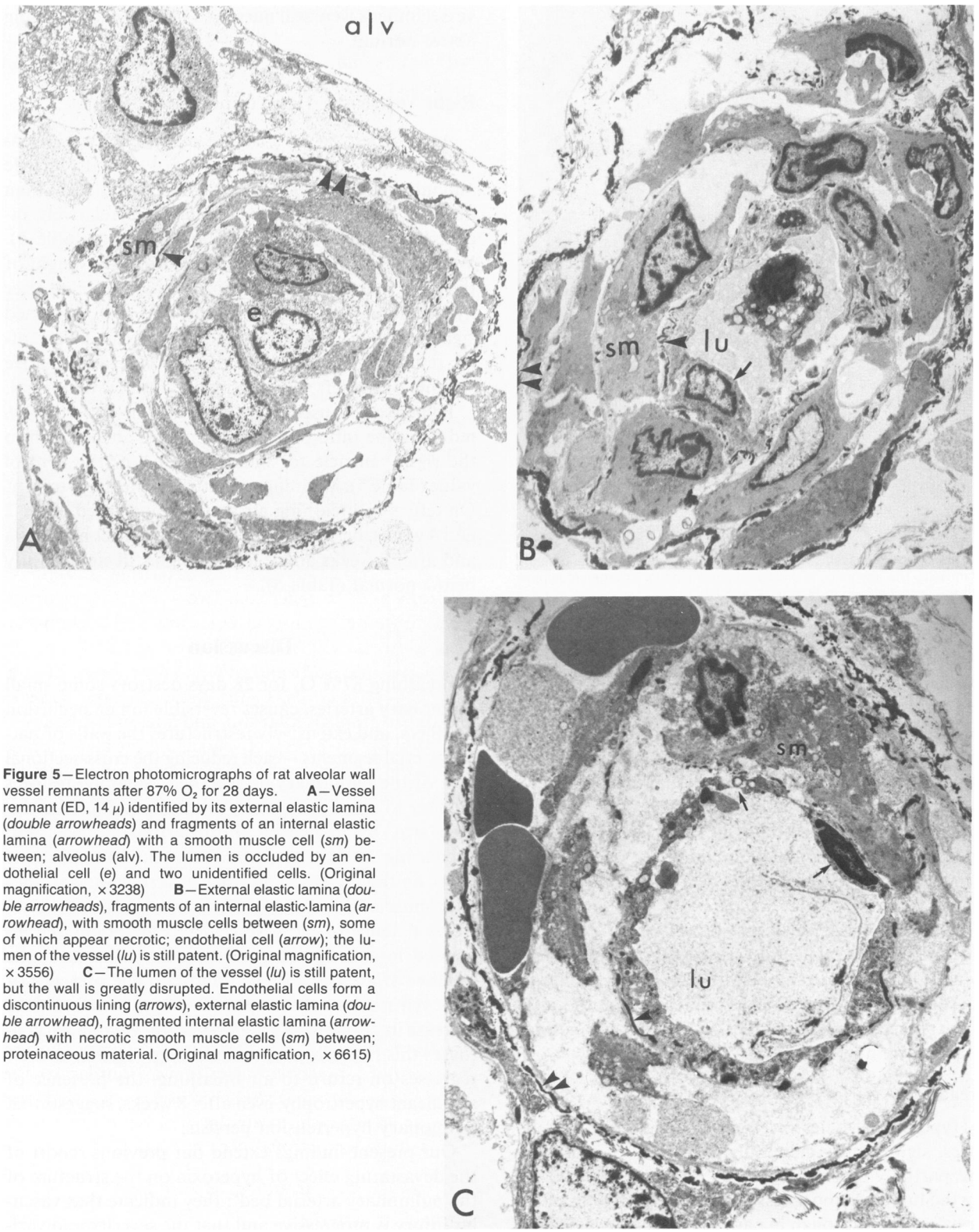

Figure 5—Electron photomicrographs of rat alveolar wall vessel remnants after 87% O_2 for 28 days. **A**—Vessel remnant (ED, 14 μ) identified by its external elastic lamina (*double arrowheads*) and fragments of an internal elastic lamina (*arrowhead*) with a smooth muscle cell (*sm*) between; alveolus (alv). The lumen is occluded by an endothelial cell (*e*) and two unidentified cells. (Original magnification, ×3238) **B**—External elastic lamina (*double arrowheads*), fragments of an internal elastic lamina (*arrowhead*), with smooth muscle cells between (*sm*), some of which appear necrotic; endothelial cell (*arrow*); the lumen of the vessel (*lu*) is still patent. (Original magnification, ×3556) **C**—The lumen of the vessel (*lu*) is still patent, but the wall is greatly disrupted. Endothelial cells form a discontinuous lining (*arrows*), external elastic lamina (*double arrowhead*), fragmented internal elastic lamina (*arrowhead*) with necrotic smooth muscle cells (*sm*) between; proteinaceous material. (Original magnification, ×6615)

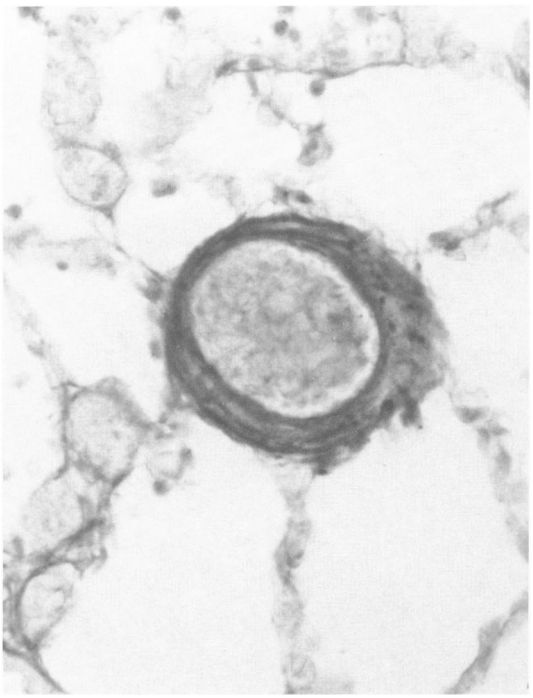

Figure 6—Rat intraacinar artery (4-μ sections stained with MEVG): 87% O_2 for 28 days + weaning (see text) + 4 weeks air (ED, 55 μ). (×530). The media of the vessel is relatively thick for the vessel size and stains for muscle. The adventitia is thickened and stains for collagen (compare with the vessel shown in Figure 3A and B).

muscular vessels (for levels of significance see Table 2), reducing their ED, and increasing their MT. Weaning and return to breathing air increased lumen size and reduced the mean %MT, but not to the normal value (Table 2). Hyperoxia significantly reduced the number of patent (ie, barium-filled) intraacinar arteries (Table 4), and their number remained reduced after weaning and after return to breathing air. Alveolar density was unchanged by hyperoxia or weaning but on return to air was reduced to significantly below the control value (Table 4).

The ratio of arteries to alveoli was significantly reduced after hyperoxia and was not restored to normal by weaning or return to breathing air: it was similar to the control value after 8 weeks of breathing air because of the reduced alveolar number, rather than any increase in artery number (Table 4).

Preacinar Arteries

Hyperoxia also narrowed the lumen of preacinar arteries, significantly increasing the %MT of muscular and partially muscular vessels (Table 3). After weaning the lumen of most vessels was less narrow than after hyperoxia, although in partially muscular vessels associated with terminal bronchioli the %MT remained significantly above the control value. As in the intraacinar region, the decreased %MT reflected an increase in ED or a decrease in MT (Table 3). On return to air, vessel lumens were still narrowed, the %MT remaining above normal.

Right Ventricular Hypertrophy

After hyperoxia the absolute weight of the right ventricle was not altered, whereas that of the left ventricle and intraventricular septum was significantly less than normal: both ventricular weights were relatively increased because of reduced body weight (Table 5). Weaning increased the absolute and relative weight of the right ventricle to significantly above the value after hyperoxia as well as the control, and each remained significantly increased thereafter. After weaning and after return to breathing air the absolute and relative weights of the left ventricle plus septum were normal.

Hyperoxia caused right ventricular hypertrophy, reducing the ratio of the left ventricle and septum to the right ventricle to significantly below the control value (Table 5). Weaning accentuated the hypertrophy. On return to breathing air the ratio increased, after 2 and 4 weeks, being restored to the value after hyperoxia and after 8 weeks above it, although still significantly below normal (Table 5).

Discussion

Breathing 87% O_2 for 28 days destroys some small pulmonary arteries, causes reversible lumen occlusion in others, and extensively restructures the walls of patent arterial segments—each reducing the cross-sectional area of the pulmonary arterial bed. Weaning restores patency to some pulmonary arteries and increases lumen size, by increasing the external diameter and decreasing the thickness of wall muscle, but it has little effect on the increased number of intraacinar arteries with muscle in their wall. Return to breathing air restores patency to more vessels and maintains the increased lumen size but, paradoxically, increases the number of arteries with wall muscle. The presence of right ventricular hypertrophy after hyperoxia indicates increase in pulmonary artery pressure. Weaning accentuates this hypertrophy; and although cardiac muscle regresses on return to air breathing, the presence of significant hypertrophy, even after 8 weeks, suggests that pulmonary hypertension persists.

Our present findings extend our previous report of the devastating effect of hyperoxia on the structure of the pulmonary arterial bed[8]: they indicate that vascular injury is progressive and that the severity and variety of the vascular lesions increase with the duration of exposure. The extent of lumen reduction and wall

Table 2—Mean (± SEM) External Diameter (ED), Medial Thickness (MT), and Percent Medial Thickness (% MT) of Muscular (M), Partially Muscular (PM), and Nonmuscular (NM) Arteries Associated with Alveolar Walls, Alveolar Ducts, and Respiratory Bronchioli in Control (Con) and Exposed Rats*

		Alveolar wall					Alveolar duct					Respiratory bronchiolus				
Group		ED	MT	% MT	n		ED	MT	% MT	n		ED	MT	% MT	n	
Con	M	–	–	–	0		–	–	–	0		156.6 ± 0	1.5 ± 0	0.9 ± 0	1	
(6)	PM	75.5 ± 4.8	1.7 ± 0.2	2.6 ± 0.3	46		88.2 ± 1.4	1.8 ± 0.1	2.1 ± 0.1	101		111.3 ± 16.9	2.1 ± 0.4	1.9 ± 0.1	11	
	NM	51.5 ± 1.8	–	–	190		64.0 ± 1.9	–	–	103		–	–	–	0	
A	M	47.2 ± 1.9	5.2 ± 0.7	12.0 ± 1.5†	45	§	51.6 ± 1.9	5.4 ± 0.6	11.2 ± 1.6	50	§	65.8 ± 9.0	6.5 ± 1.6	9.9 ± 1.8†	17	§
(5)	PM	43.4 ± 2.1	3.9 ± 0.3	10.1 ± 1.1	80		51.4 ± 2.0	4.1 ± 0.4	8.4 ± 0.7	66		55.5 ± 3.5	4.7 ± 0.9	8.9 ± 1.8†	10	
	NM	32.0 ± 1.4	–	–	73		33.0 ± 2.2	–	–	32		43.0 ± 0	–	–	1	
B	M	55.4 ± 10.6	4.4 ± 0.6	9.0 ± 2.9	11	‖	62.6 ± 1.9	3.7 ± 0.6	6.3 ± 1.2	15	‖	138.2 ± 3.1	3.8 ± 0.8	2.8 ± 0.6	2	‖
(2)	PM	55.7 ± 0.7	3.1 ± 0.6	5.9 ± 1.8	22		61.6 ± 0.7	3.0 ± 0.4	5.2 ± 0.8	40		67.9 ± 0.4	3.3 ± 0.2	4.8 ± 0.9	7	
	NM	39.7 ± 0.4	–	–	34		39.9 ± 4.5	–	–	20		–	–	–	0	
B + 2	M	58.5 ± 2.1	3.7 ± 0.1	7.4 ± 0.3	38		68.7 ± 8.6	4.0 ± 0.3	7.1 ± 1.5	38		89.6 ± 13.7	4.6 ± 0.4	5.5 ± 1.1†	13	
(3)	PM	55.5 ± 3.3	2.9 ± 0.4	5.7 ± 1.1	74		61.6 ± 6.9	3.3 ± 0.2	5.7 ± 1.3	45		77.8 ± 6.5	3.9 ± 0.5	5.7 ± 1.4	9	
	NM	41.3 ± 2.4	–	–	7		39.9 ± 0	–	–	1		–	–	–	0	
B + 4	M	62.9 ± 0.8	3.7 ± 0.2	6.4 ± 0.7	45		68.0 ± 4.2	3.9 ± 0.5	7.0 ± 2.0	38		124.5 ± 13.2	4.7 ± 0.3	4.0 ± 0.4‡	12	
(3)	PM	53.4 ± 4.8	2.4 ± 0.1	4.7 ± 0.1	62		64.0 ± 5.3	3.0 ± 0.1	4.9 ± 0.2	33		105.2 ± 13.6	4.6 ± 0.6	4.5 ± 1.0	10	
	NM	36.9 ± 2.5	–	–	22		33.8 ± 6.1	–	–	3		–	–	–	0	
B + 8	M	58.6 ± 5.4	3.6 ± 0.6	6.8 ± 1.3	31	§‖	76.2 ± 3.3	3.5 ± 0.6	4.9 ± 1.5	18	§‖	145.3 ± 2.1	5.4 ± 0.3	3.7 ± 0.2†	6	§‖
(3)	PM	65.5 ± 5.1	2.6 ± 0.3	4.1 ± 0.2†	92		74.0 ± 3.3	2.5 ± 0.1	3.4 ± 0.1	39		119.4 ± 16.0	3.1 ± 0.2	2.8 ± 0.2†	9	
	NM	51.8 ± 0.6	–	–	32		58.4 ± 2.7	–	–	18		–	–	–	0	

* A, 28 days 87% O_2; B, A + weaning; B + 2, 4, or 8 weeks in air. The number in parentheses is the number of animals; n is the number of arteries.

† Significantly different from control; $P < 0.001$.

‡ Significantly different from control; $P < 0.01$.

§ Distribution of arteries significantly different from control; $P\chi^2 < 0.001$.

‖ Distribution of arteries significantly different from hyperoxia; $P\chi^2 < 0.001$.

Table 3—Mean (± SEM) External Diameter (ED), Medial Thickness (MT) and Percent Medial Thickness (% MT) of Muscular (M), Partially Muscular (PM), and Nonmuscular (NM) Arteries Associated With Terminal Bronchioli and Bronchioli in Control (Con) and Exposed Rats*

		Terminal bronchiolus					Bronchiolus				
		ED	MT	% MT	n		ED	MT	% MT	n	
Con	M	173.7 ± 26.9	3.3 ± 1.0	2.2 ± 0.9	8		663.1 ± 114.9	13.5 ± 6.9	1.9 ± 0.5	54	
(6)	PM	166.4 ± 18.7	2.4 ± 0.3	1.6 ± 0.2	28		271.3 ± 22.1	2.7 ± 0.3	1.1 ± 0.1	66	
	NM	159.6 ± 6.1	–	–	5		–	–	–	0	
A	M	117.8 ± 7.8	8.5 ± 1.2	7.8 ± 1.4†	26	§	343.6 ± 62.0	19.5 ± 2.7	6.8 ± 1.0†	84	§
(5)	PM	94.2 ± 20.5	3.8 ± 0.8	4.2 ± 0.1‡	4		134.1 ± 27.3	6.1 ± 3.1	4.6 ± 1.7†	7	
	NM	–	–	–	0		–	–	–	0	
B	M	147.4 ± 3.1	5.4 ± 0.8	3.6 ± 0.6	3		539.8 ± 176.3	9.3 ± 0.6	3.1 ± 0.2	40	
(2)	PM	132.3 ± 18.7	4.4 ± 0.5	3.5 ± 0.1†	9		213.4 ± 76.8	5.3 ± 0.5	3.2 ± 0.9	10	
	NM	–	–	–	0		–	–	–	0	
B + 2	M	194.2 ± 3.3	6.0 ± 0.5	3.2 ± 0.3	14		398.3 ± 38.1	15.1 ± 4.1	3.6 ± 0.6	81	
(3)	PM	115.6 ± 14.3	3.1 ± 0	2.7 ± 0.3	4		175.7 ± 3.7	4.0 ± 0.2	2.5 ± 0.2†	9	
	NM	–	–	–	0		–	–	–	0	
B + 4	M	153,9 ± 28.9	7.9 ± 2.2	4.9 ± 0.7	8		420.9 ± 49.2	21.7 ± 6.0	5.1 ± 1.5	51	
(3)	PM	129.0 ± 7.7	4.3 ± 0.6	3.5 ± 0.7	12		153.3 ± 55.5	5.1 ± 0.1	3.5 ± 0‡	19	
	NM	–	–	–	0		–	–	–	0	
B + 8	M	125.9 ± 0	7.7 ± 0	6.2 ± 0†	2		551.7 ± 99.4	17.5 ± 3.0	3.2 ± 0.3	30	
(3)	PM	140.5 ± 18.6	4.3 ± 0.6	3.0 ± 0.3	8		215.7 ± 43.0	4.5 ± 0.7	2.4 ± 0.7	16	
	NM	–	–	–	0		–	–	–	0	

* A, 28 days 87% O_2; B, A + weaning; B + 2, 4, or 8 weeks in air. The number in parenthesis is the number of animals; n is the number of arteries.
† Significantly different v control; $P < 0.01$.
‡ Significantly different v control; $P < 0.001$.
§ Distribution of arteries significantly different from control; $P\ \chi^2$ 0.001.

restructuring is greater after 28 than after 7 days of exposure. Despite the progressive nature of the vascular injury, certain of the changes develop quickly. In intraacinar regions half the increase in %MT and two-thirds of the reduction in patent arteries occurs during the first week of exposure. The 28-day exposure produced lesions not seen after 7 days of exposure and typical of a chronic inflammatory response, these including hyalization of the vessel wall, intimal and medial erosion, and cell hyperplasia. More microvessels were muscularized, more muscle was present in preacinar artery segments, and injury to pulmonary veins was apparent, the lumen of many of these vessels being reduced by cell hypertrophy and hyperplasia (Figure 7).

The presence of vessel remnants after 28 days of hyperoxia and evidence of mural degeneration confirm the obliterative effect of hyperoxia on small pulmonary blood vessels. All of the remnants were intraacinar; and although all appeared to be of arteries, it was not always possible to distinguish these from small veins. The increase in remnant number as exposure continues suggests that any lumen closure by swollen or hypertrophied endothelial cells[12] is followed by extensive injury to the vessel wall. In a preliminary study[13] we reported greater increase in mean pulmonary artery pressure after exposure to hyperoxia for 28 days (+22 mmHg) than after 7 days (+9 mmHg), a difference reflected by the greater right ventricular hypertrophy reported here after longer exposure. It is likely that increase in pressure in response to hyperoxia results from the extensive restriction of the pulmonary vascular bed, demonstrated here by arteriography and morphometry.

Rats breathing 87% O_2 have an abnormally high Pa_{O_2},[8] although not as high as might be expected from the alveolar partial pressure, in part because of injury to the alveolar–capillary membrane. Breathing oxidants injures pulmonary cells and matrix components. Toxic or partially reduced species of oxygen may injure both

Table 4—Number of Intraacinar Arteries (sq mm) and Alveoli (sq mm) and ratio of Arteries to (100) Alveoli in Control and Exposed Rats*

Group	Arteries	Alveoli	Ratio
Control	14.2 ± 1.7	348 ± 33	4.0 ± 0
A	5.3 ± 1.2	366 ± 25	1.4 ± 0.3†
B	7.2 ± 0.4	385 ± 2	1.9 ± 0.1‡
B + 2	5.8 ± 0.6	301 ± 17	1.9 ± 0.2†
B + 4	7.1 ± 0.7	266 ± 6	2.7 ± 0.2
B + 8	5.7 ± 1.4	234 ± 17	2.4 ± 0.4

* A, 28 days 87% O_2; B, A + weaning; B + 2, 4, or 8 weeks in air. The number of animals in each group is as given in Table 2.
† Significantly different v control value; $P \leq 0.01$.
‡ Significantly different v control value; $P \leq 0.001$.

Table 5—Mean (± SEM) Absolute and Relative Weight (Percent of Body of the Weight) of the Right Ventricle (RV) and Left Ventricle Plus Septum (LV + S), and Ratio (LV + S/RV) in Control and Exposed Rats*

		Absolute weight		Percent weight		
	n	RV	LV + S	RV	LS + S	Ratio
Control	8	0.2 ± 0.01	0.7 ± 0.03	0.04 ± 0.002	0.17 ± 0.01	4.1 ± 0.08
A	8	0.2 ± 0.03	0.5 ± 0.03	0.10 ± 0.010†	0.22 ± 0.01†	2.3 ± 0.18‡
Control	3	0.2 ± 0.02	0.7 ± 0.08	0.04 ± 0.003	0.17 ± 0.00	4.2 ± 0.10
B	3	0.3 ± 0.02	0.5 ± 0.07	0.17 ± 0.012‡	0.25 ± 0.04	1.5 ± 0.13‡§
Control	3	0.2 ± 0.03	0.8 ± 0.05	0.04 ± 0.006	0.16 ± 0.01	4.4 ± 0.61
B + 2	3	0.3 ± 0.02	0.6 ± 0.06	0.10 ± 0.020	0.21 ± 0.03	2.2 ± 0.11†
Control	3	0.2 ± 0.01	0.8 ± 0.05	0.03 ± 0.001	0.15 ± 0.01	4.3 ± 0.22
B + 4	3	0.3 ± 0.02†	0.7 ± 0.02	0.08 ± 0.005‡	0.17 ± 0.01	2.2 ± 0.13†
Control	4	0.2 ± 0.01	0.9 ± 0.03	0.04 ± 0.001	0.16 ± 0.01	4.4 ± 0.13
B + 8	5	0.3 ± 0.01†	0.9 ± 0.01	0.06 ± 0.004†	0.17 ± 0.01	3.1 ± 0.19†

* A, 28 days 87% O_2; B, A + weaning; B + 2, 4, or 8 weeks in air; n, number of animals.
† Significantly different from control value; $P \leq 0.01$.
‡ Significantly different from control value; $P \leq 0.001$.
§ Significantly different from hyperoxia value; $P \leq 0.001$.

directly and indirectly by activation of the inflammatory response. We have previously discussed the possible mechanisms of hyperoxic damage that narrow the lumen of pulmonary arteries, as well as the mechanisms that may stimulate development of muscle in previously nonmuscular pulmonary vessel segments.[8] Development of new medial muscle is associated with hypertrophy and hyperplasia of intimal precursor smooth muscle cells.[14] These cells are stimulated by hyperoxia,[6] but the sequence of intracellular events by which new medial smooth muscle cells develop from these precursor cells is not yet established. It may be that injury

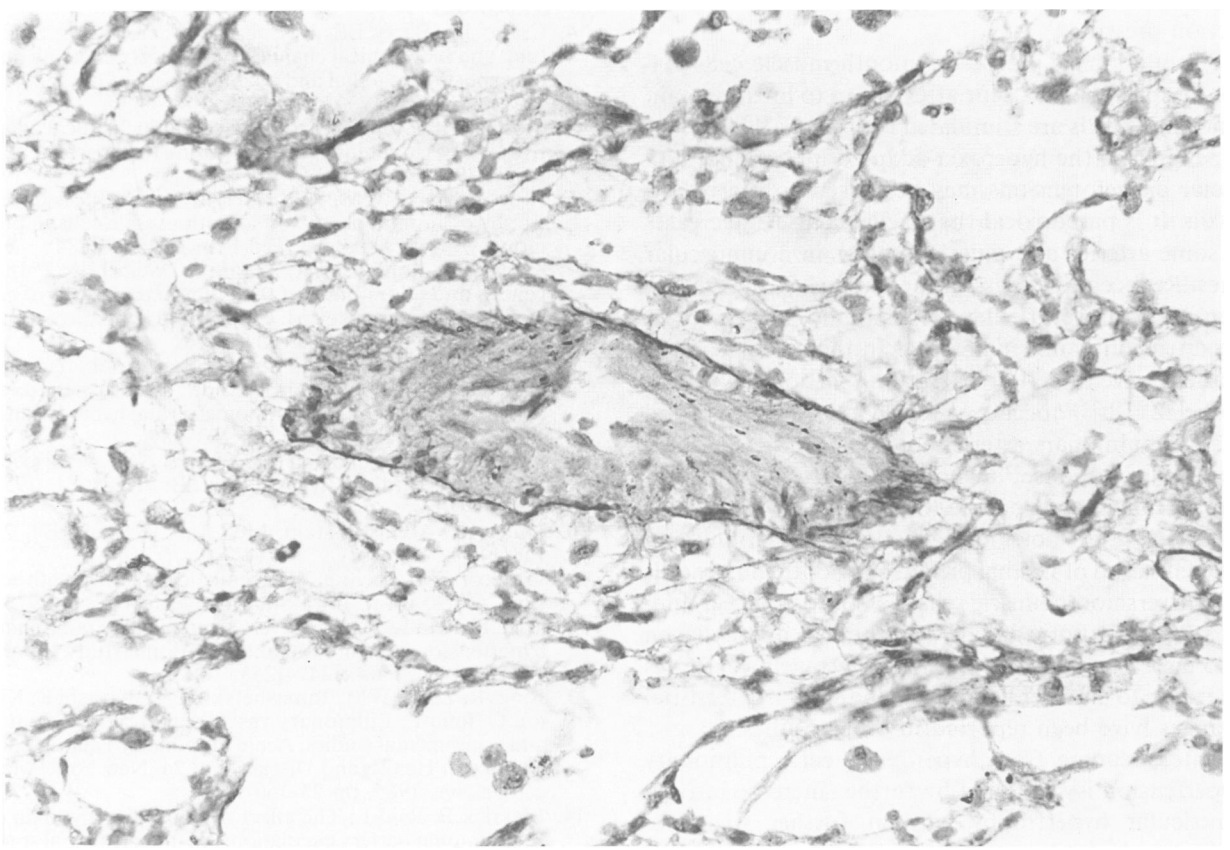

Figure 7—Rat pulmonary vein after 87% O_2 for 28 days, showing lumen reduction by intimal cell hyperplasia. (4-μ section stained with MEVG, ×488)

to endothelial cells contributes to their hypertrophy and hyperplasia by loss of the normal homeostatic inhibitory mechanisms recently demonstrated *in vitro*.[15] A change in phenotype from precursor cell to smooth muscle cell may be stimulated by specific growth factors released from platelets or granulocytes,[16,17] or perhaps by increased shear stress in response to higher blood flow in the restricted pulmonary vascular bed. Pulmonary hypertension may further restructure the walls of preacinar artery segments by increasing wall stress, although even in this region direct stimulation to cells of the vessel wall by growth mediators cannot be excluded.

The increased background opacity present in the arteriogram after weaning from hyperoxia is difficult to interpret in the presence of greater attenuation of axial pulmonary arteries and reduced numbers of small arteries. In several further studies we have confirmed that it is typical of weaning and similar to that found within hours after rapid return to air (ie, without weaning), which suggests that it represents a change in density of tissue fluid. The lumen narrowing seen on the arteriogram after weaning does not arise from the presence of clots. We know from other studies that perivascular edema can result in lumen narrowing on the arteriogram, in spite of the hypertensive vascular injection pressure.[18]

Stimulation of precursor smooth muscle cells evidently continues to occur after return to breathing air. Since these cells are stimulated by hypoxia[14] as well as hyperoxia, in the hyperoxia-adapted lung reduced alveolar oxygen tensions may be sensed as relative hypoxia. It is paradoxical that medial muscle decreases in some arteries and muscle appears in nonmuscular ones. Reduced thickness of medial muscle and reduction in right ventricular hypertrophy suggests that hypertension is not increasing. In the alveolar membrane a wave of cell proliferation, particularly of endothelial cells[19-21] occurs on return to breathing air, and in small pulmonary arteries and veins (200 μ ED) there is a similar response[22] 60 hours after return to breathing air following exposure to hyperoxia (90% O_2 for 6 days). We do not know whether hypertrophy and differentiation of intimal precursor smooth muscle cells to mature smooth muscle cells is associated with a similar burst of mitotic activity. Fibroblasts and collagen also increased in the adventitia of intraacinar arteries on return to air after hyperoxia, and similar interstitial changes have been reported in other studies.[23-25]

After weaning from hyperoxia greater pulmonary hypertension is suggested by further increase in right ventricular hypertrophy without further structural changes, which suggests that this is due to vasoconstriction, perhaps in response to relative alveolar "hypoxia." Additional hemodynamic studies will establish whether in the hyperoxia-adapted lung a reduced (but still hyperoxic) oxygen tension represents relative hypoxia, causing vasoconstriction. Thickening of the alveolar-capillary membrane may cause impaired gas diffusion and ventilation/perfusion mismatch, despite the relatively high PA_{O_2}.

Clearly, the extent and pattern of restructuring of pulmonary artery walls described here results from the oxygen tension breathed and its duration during exposure and weaning. Return to air breathing allows regression of some pulmonary vascular lesions; progression of others indicates that return to air injures the pulmonary circulation in the hyperoxia-adapted lung.

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