Pulmonary Artery Remodeling and Pulmonary Hypertension After Exposure to Hyperoxia for 7 Days

A Morphometric and Hemodynamic Study

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This study shows by morphometric and hemodynamic techniques that exposure to hyperoxia at normobaric pressure causes rapid structural remodeling of rat pulmonary arteries and pulmonary hypertension. After 7 days of 90% O_2, pulmonary artery cross-sectional area is reduced by a striking loss of intraacinar arteries (control, 13 ± 1 sq mm; exposed, 8 ± 1 sq mm; $P < 0.001$), the ratio of arteries to alveoli being 4:100 in control rats and 2.5:100 after hyperoxia. The lumen of preacinar and intraacinar arteries is narrowed by a reduction of vessel external diameter (ED) and an increased medial wall thickness (MT). There is a significant reduction in the percent medial thickness ([2 × 100 × MT]/ED) in both regions. The proportion of muscular and partially muscular intraacinar arteries increases at the expense of nonmuscular ones (P [χ^2] < 0.01), and fully muscular arteries appear in the alveolar wall where they are not normally found. Intimal thickening occurs in 19% of alveolar duct and 34% of alveolar wall nonmuscular arteries. Right ventricular hypertrophy occurs, the ratio of the left ventricle plus the septum to the right ventricle being significantly reduced (control, 4.07 ± 0.26; exposed, 3.23 ± 0.10; $P < 0.02$). After 3 days of 87% O_2, pulmonary artery pressure is still normal (17.0 ± 0.9 mmHg) but after 7 days it is significantly increased (26.2 ± 0.9 mmHg; $P < 0.01$), as is pulmonary vascular resistance (control, 0.033 ± 0.003; exposed, 0.065 ± 0.015 U/kg; $P < 0.05$). Return to air breathing (after 7 days at 87% O_2) causes pulmonary vasoconstriction and a further rise of the pulmonary artery pressure (to 38.3 ± 3.3 mmHg after 60 minutes). (Am J Pathol 1984, 117:273–285)

THE ADULT respiratory distress syndrome (ARDS) in man is commonly accompanied by pulmonary artery hypertension due to an elevated pulmonary vascular resistance.[1] Morphologic changes in the lungs of patients dying of ARDS include loss of capillaries,[2,3] increased muscularization, and dilatation of some precapillary arteries and loss of others.[3–5] An Fi_{O_2} greater then 0.5 is common treatment for patients with ARDS because of the ventilatory maldistribution and hypoxemia. At high alveolar tensions oxygen is known to induce lung injury and has been suggested as a factor contributing to the pathologic changes in ARDS[6] or at least in causing additional lung injury.[7] The alveolar wall changes associated with breathing hyperoxia include damage to both capillary endothelial cells and Type I pneumonocytes, increase in the number of Type II pneumonocytes, alveolar edema, formation of hyaline membranes, cellular infiltration, and fibrosis.[8] Similar morphologic changes have been reported in human lungs after oxygen treatment, including those from patients without underlying pulmonary disease[7,9–12] and in a variety of species in experimental studies, including the mouse, rat, guinea pig, rabbit, dog, lamb, and monkey.[13]

Morphologic techniques have clearly established that hyperoxia causes extensive damage to the pulmonary capillary bed.[14,15] Less well established is the effect of breathing hyperoxia on the structure of pulmonary arteries. In rats breathing 4 atm compressed air for 30 days (P_{AO_2} = 618 mmHg) Smith et al[16] reported

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thickening of small pulmonary arteries after 30 days and of large arteries after 45 days. The number of capillaries appeared reduced; whereas small pulmonary arteries appeared more numerous and showed endothelial cell proliferation, irregular medial thickening with hyaline cartilage formation and calcification, and hyalization of the vessel wall typical of nephrosclerosis. In rats exposed to normobaric hyperoxia (100% O_2) for 35 days, Brooksby et al[17] reported alveolar changes typical of emphysema (with formation of extensive bullae) and the presence of enlarged and tortuous pulmonary arteries. Kydd[18] mentioned hypertrophy and described streaming of the media into the adventitia in the pulmonary arteries of rats exposed to hypobaric hyperoxia (100% O_2 at 500 mmHg) for 30 days, whereas Schaffner et al[19] found no changes in the pulmonary arteries or veins of rats exposed to subatmospheric hyperoxia (100% O_2 at 700 mmHg) for 10 days.

The purpose of this study was to establish by quantitative techniques the structural changes present in proximal and distal regions of the pulmonary vascular bed after 1–2 weeks of exposure to normobaric hyperoxia, and to assess their effect on pulmonary hemodynamics. In excised lungs, after the pulmonary arteries are distended at a constant injection pressure, we assess their density, external diameter and medial thickness, wall structure, and position in the arterial branching pattern. Hemodynamic changes are monitored in rats, awake, with indwelling catheters placed in the pulmonary artery and in the abdominal aorta.

Materials and Methods

Seventy-one male cesarean-derived specific-pathogen-free Sprague–Dawley rats were used in this study (Charles River Breeding Laboratories, Portage, Mich, and LaPrairie, Quebec). In the experiments reported here our aim was to expose rats to a level of hyperoxia that would allow survival for 1–2 weeks and produce pulmonary microvascular changes. Several concentrations of oxygen were examined to establish the best for our purpose: three are included in the experiments reported here (80% O_2, 90% O_2, and 87% O_2). Breathing 80% O_2 was well tolerated for the first 10 days, but less so for the next 4. Since this level of oxygen did not produce striking vascular changes, we next used 90% O_2. This concentration was tolerated only for 7 days, but structural changes were evident and assessed by morphometric techniques. In later studies, however, 90% O_2 proved to be a concentration that few rats survived beyond the first few days. Thereafter, to study pulmonary hemodynamics, we used 87% O_2, and we have since found that rats tolerate up to 28 days of exposure to this concentration.[20,21] The experiments described here are Experiment A, 80% O_2 for up to 14 days; Experiment B, 90% O_2 for up to 7 days; and Experiment C, 87% O_2 for up to 7 days (see Table 1 for details). Control rats breathed air for equivalent periods.

At the start of each experiment the lungs of 2 animals from each group were examined histologically: a minimal degree of airway cuffing and the absence of infiltration of the surface epithelium by lymphocytes indicated that the lungs of animals in each group were satisfactory.[22] The rats received food (Purina Formulab Chow #5008) and water *ad libitum*, and their bedding of dust-free absorbent white pine shavings was changed every other day.

Vascular Catheterization

In 20 anesthetized rats catheters were placed in the main pulmonary artery via the right external jugular vein (silicone rubber, outside diameter [OD] 0.64 mm, inside diameter [ID] 0.32 mm) and the abdominal aorta, just above the iliac bifurcation (PE-10, OD 0.61 mm, ID 0.28 mm; and PE-20, OD 1.09 mm, ID 0.38 mm fused—the PE-10 tubing being placed in the artery and the PE-20 sutured to the psoas muscle). Both catheters were exteriorized through the back of the neck, heparinized, and sealed with a blunt wire plug.[23] The animals were catheterized 48 hours before the start of exposure to hyperoxia to allow recovery from anesthesia and surgery; during this time all animals lost weight (4% of body weight for both groups). One control and one exposed rat were removed from the study because a hematoma developed where the catheter entered the aorta.

Exposure to Hyperoxia

Cages containing rats that were to be exposed to hyperoxia were placed inside a perspex box (10 × 8 × 13 cm). The appropriate oxygen concentration was obtained by mixing 100% O_2 with compressed air (each from an on-line supply). Gas flow through the box (20–25 l/min) was regulated to maintain appropriate temperature (22 ± 1 C) and humidity so that there was no condensation. The oxygen concentration in the box (±1%) was calibrated twice daily with an Instrumentation Lab Analyzer (#407), and the carbon dioxide concentration was monitored by mass spectrometry (Beckman, DB-9) and found never to rise above 0.05%. Effluent gas from the box was taken to the outside of the building via a duct. Control rats were kept in the same room as rats breathing hyperoxia.

Table 1—The Effect of Hyperoxia on Body Weight*

	O_2 Concentration	Number of days of exposure	Exposed group n	Exposed group Body weight (g)	Control group n	Control group Body weight (g)
Experiment A	80%	1	3	231.3 ± 2.7	3	229.3 ± 2.2
		2	3	228.7 ± 2.7	–	
		3	3	221.7 ± 5.4	–	
		7	3	211.0 ± 15.3†	3	282.3 ± 5.2‡
		10	3	201.7 ± 7.2	–	
		14	5	194.0 ± 10.7†	8	311.1 ± 5.4‡
Experiment B	90%	0‖	10	240.3 ± 2.4	7	237.2 ± 3.3
		3	3	242.2 ± 3.3†	3	275.7 ± 5.1§
		7	7	225.1 ± 5.5†§	4	303.8 ± 5.5§
Experiment C	87%	0	10	216.8 ± 3.9	10	276.6 ± 5.5
		7	9	200.4 ± 5.0§	9	310.9 ± 7.3§
Total number of animals			40		31	

* All data mean ± SEM, n, number of animals.
† Value differs significantly from the corresponding control value, $P < 0.01$.
‡ Value differs significantly from Day 1 control value, $P < 0.001$.
§ Value differs significantly from the Day 0 value of each group, $P < 0.05$.
‖ Day 0 = just before the start of exposure.

Hemodynamic Measurements

Hemodynamic measurements were obtained in air just before the start of exposure to hyperoxia, and then 15 minutes and 3 and 7 days after the start of exposure. Measurements in control rats were made at similar times. In 3 rats exposed to hyperoxia for 7 days pulmonary artery pressure was also measured 15, 30, and 60 minutes after the return to breathing air. Oxygen consumption was measured (in 100% O_2) in 4 control rats and in 3 rats exposed to hyperoxia for 7 days.

For hemodynamic measurements control or exposed rats, awake, were placed inside small perspex chambers. With a nose cone connected to a source of 87% O_2 over their snout, single rats were transferred from the main exposure chamber to a modified isolette, where they continued to breathe 87% O_2 while being placed in the small chamber (being handled through portholes in the Isolette); the chamber was then connected to a source of 87% O_2 and removed from the Isolette to allow pulmonary hemodynamics to be measured. These were obtained with a pressure transducer (Ailtech, MS10) connected to the rat's catheter (exteriorized through portholes at the top of the chamber) and a Hewlett-Packard recorder (#1308A).

The following were measured: pulmonary artery pressure (PAP, mmHg), blood pressure (BP, mmHg), heart rate (HR, beats/min), respiratory rate (RR, breaths/min), arterial blood gas tensions (Pa_{O_2}, mmHg; Pa_{CO_2}, mmHg), blood pH (pH_a) and hematocrit (Hct, %), arterial (Sa_{O_2}, %) and venous oxygen saturations, (Sv_{O_2}, %) blood bicarbonate (HCO_3, mEq/l), and oxygen consumption ($\dot{V}_{O_2}$, ml/min kg). The following were calculated by standard formulas: cardiac index (CI, ml/min/kg), stroke volume index (SVI, ml/kg), hemoglobin oxygen-carrying capacity (Hb capacity), pulmonary vascular resistance index (PVRI, U/kg), and systemic vascular resistance index (SVRI, U/kg). The left and right ventricular end diastolic pressures (LVEDP and RVEDP) were taken as 3.5 mmHg, a value obtained in studies of normal rats when awake (Aronovitz, personal communication). After measuring gas tensions, blood was returned to the animal through its aortic catheter. When measurements were complete, animals breathing 87% O_2 were returned to the main chamber via the isolette and nose cone: control rats were returned to their cages.

Preparation of Tissue

All rats were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight)—the exposed animals first being transferred to the isolette, where they continued to breathe a high oxygen tension until they were fully anesthetized. The trachea and esophagus were transected immediately below the larynx, and the thoracic block (heart, lungs, and thymus with the trachea and esophagus attached) was quickly excised from each animal: the esophagus and thymus were removed. The tissue block was weighed and stored frozen (at −15 C): before injection into the pulmonary arteries it was thawed at room temperature for 30 minutes, and a catheter (PE-205, ID 1.57 mm, OD 2.08 mm; Clay Adams, Parsippany, NJ) was inserted through the right ventricle and

tied into the main pulmonary artery. The tissue was then warmed at 37 C for 15 minutes, and the pulmonary arteries were given 2-minute injections of barium–sulfate gelatin suspension at 60 C and 100 cm H_2O pressure. By this technique all patent pulmonary arteries are recruited and fully distended. The suspension contained 400 ml Micropaque powder (Nicholas Picker Co., Stoughton, Mass), 50 g of gelatin (Bloom 8-G, Fisher Scientific Co., Fairlawn, NJ) and 500 ml distilled water with a few drops of liquefied phenol as a preservative. The lungs were distended by intratracheal instillation of neutral buffered formol saline at 23 cm H_2O pressure until the lung margins were defined: the trachea was ligated to maintain distension of the lungs and pulmonary arteriograms were prepared immediately.

Pulmonary arteriograms were obtained on Kodak films (X-OMAT TL2) with a Faxitron (#43805N, Hewlett Packard Co., McMinnville, Ore). The exposure factors were 40 kv and 2.5 mA for 0.2 minute, with a distance of 58 cm between the focal spot and the specimen. These factors were constant for all rat lungs. Films were developed in an automatic X-ray film processor. On the arteriogram, arterial lumen diameter was measured at the level of the bifurcation of the axial pathway with its first, second, and third lateral branches (Levels 1–3), and 2 mm along the third lateral branch (Level 4). In the normal rat most intraacinar arteries are smaller than 160 μ in lumen diameter, and these vessels appear as a background "haze" in the arteriogram; larger vessels are discerned as separate lines.[24]

After 48 hours' fixation three slices of tissue were taken for microscopy from the cardiac and diaphragmatic lobes of the right lung and from the single-lobed left lung. All tissue was processed for paraffin wax embedding, 4-μ sections were cut and stained with hematoxylin and eosin, or stained to demonstrate elastic fibres[25] and counterstained with van Gieson's stain (MEVG). The heart was fixed for 7 days and the right ventricle (RV) and left ventricle plus the septum (LV+S) were weighed.[26] The ratio of the RV to LV+S was calculated to assess right ventricular hypertrophy (RVH).

Morphometry

Pulmonary artery structure and position was assessed in tissue sections stained with MEVG. Along each arterial pathway from the hilum to the lung periphery the structure of the pulmonary artery changes from muscular to partially muscular to nonmuscular. In the rat, while transition from a muscular to partially muscular wall sometimes occurs in the preacinar region (at the level of the terminal bronchiolus), it more often occurs within the acinus (at the level of the respiratory bronchiolus): the transition to a nonmuscular artery is intraacinar and usually within the alveolar wall. The wall structure of each artery seen in cross-section was noted, as was the accompanying airway (bronchiolus, terminal bronchiolus, respiratory bronchiolus, alveolar duct or alveolar wall). In the rat, bronchiolus describes all intrapulmonary preacinar airways proximal to the terminal bronchiolus, since cartilage is present within the lung only in the first lateral branch of the axial pathway. Using an eyepiece reticle, the external diameter (ED) of each vessel was measured as the distance between the two edges of the elastic lamina (the external elastic lamina being used for muscular and partially muscular vessels). In true cross-section all radial axes of a vessel are equal: in a tangential cut, where the vessel appears as an ellipse, the shortest axis, which is perpendicular to the longest axis of the vessel, is taken to equal its true cross-sectional diameter. For each muscular and partially muscular artery the medial thickness (MT) was measured as the thickness of the muscle layer between the internal and external elastic laminae (along the same axis as the measurement of ED), and the percent medial thickness (%MT) was calculated ([2 $\times$ MT $\times$ 100]/ED). In each lung section, at least 25 arteries associated with terminal bronchioli or lying within the acinus, were analyzed (a total of at least 75 arteries per animal). In each section, the first microscopy field (0.42 mm in diameter) was randomly selected, and all other fields were consecutive, all arteries in each field being included in the count. Arteries running with bronchioli were analyzed separately, and because of their small number, all vessels in each lung section were included (a total of 18–25 arteries per animal). Arteries showing intimal thickening were noted.

For each field the number of arteries and alveoli was counted, and the total in 20 fields per section was determined (ie, a total of 60 fields per animal). The field area was calculated (each field being 0.42 mm in diameter), and the total number of arteries and alveoli counted was related to it: the number of arteries and alveoli per unit area (square millimeter) were calculated and expressed as a ratio, this value being relatively independent of the degree of lung inflation.

Statistical Analysis

Levels of significance were calculated either by Students unpaired t test or by chi-square analysis.[27]

Results

Body Weight (Experiments A, B, and C)

Hyperoxia caused loss of weight in all rats (Table 1). The mean weight loss at the end of Experiment A (14

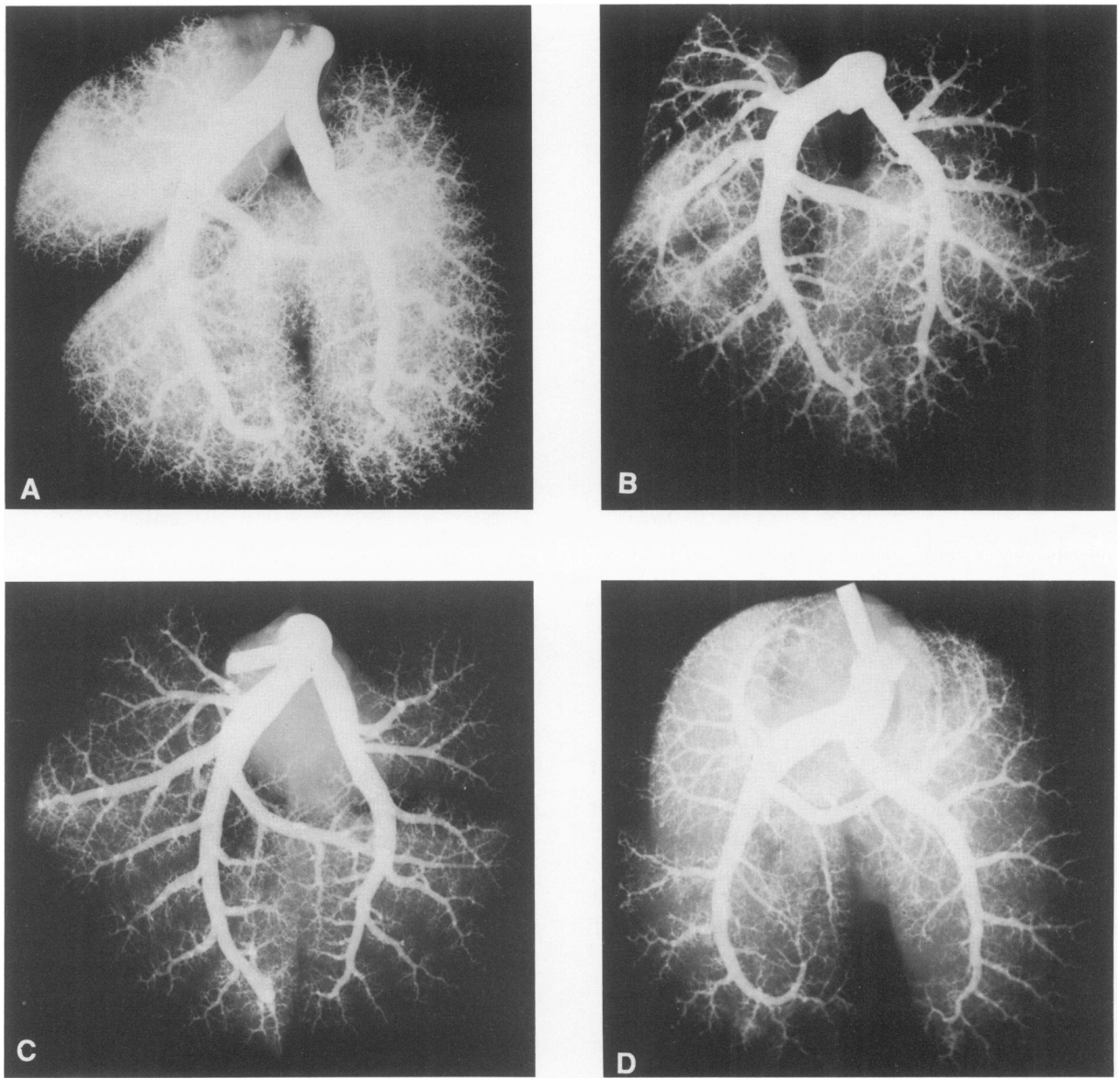

Figure 1—Normal rat pulmonary arteriogram showing fine background haze formed by filled arteries less than 160 μ ED contrasted with the loss of haze and narrowing of main arteries after hyperoxia (×2): control (**A**), 80% O_2 for 14 days (**B**), 87% O_2 for 7 days (**C**), and 90% O_2 for 7 days (**D**).

days—80% O_2) was 16%, and at the end of Experiments B and C (7 days—87% and 90% O_2, respectively) 6% and 8%. Control rats steadily gained weight.

Macroscopic and Microscopic Appearance of Lungs (Experiments A, B, and C)

After hyperoxia there was no evidence of lung hemorrhage or of petechiae. The lungs were not obviously enlarged. The absolute weight of the thoracic block was similar to the control; but because the exposed rats lost body weight, there was a small relative increase in the weight of the thoracic block (values not given).

Histologic changes were similar after exposure to 80%, 87%, or 90% O_2, being most marked in the lungs of rats breathing 90% O_2 for 7 days. The cellularity of the alveolar wall increased mainly by hyperplasia of Type II pneumonocytes and a mononuclear cell infiltrate. In the alveolar space mononuclear cells were seen either singly or clumped with occasional binucleate cells: hyaline membranes were not seen. Polymorphonuclear leukocytes were not increased in number within the interstitial or alveolar space, there being only 1–2 of these in any lung field (0.42 mm in diameter). In some lungs a few foci of proliferating fibroblasts were present within the interstitium, but alveolar wall or

Table 2—Pulmonary Arterial Lumen Diameter (mm) and Its Percentage Reduction (%↓) in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2, Experiment C—87% O_2)*

	Right lung			Left lung		
	CON (n = 4)	90% O_2 (n = 7)	%↓	CON (n = 4)	90% O_2 (n = 7)	%↓
Experiment B						
Level 1	3.6 ± 0.20	2.7 ± 0.17†	26	2.9 ± 0.12	2.0 ± 0.05†	30
Level 2	2.8 ± 0.10	1.9 ± 0.07†	32	2.1 ± 0.12	1.6 ± 0.12‡	23
Level 3	1.9 ± 0.07	1.1 ± 0.05†	41	1.8 ± 0.06	1.0 †	45
Level 4	0.9 ± 0.06	0.06 ± 0.07†	31	0.8 ± 0.06	0.5 ± 0.03‡	34
		Mean	33		Mean	33
	Right lung			Left lung		
	CON (n = 9)	87% O_2 (n = 7)		CON (n = 9)	87% O_2 (n = 7)	
Experiment C						
Level 1	3.5 ± 0.10	2.4 ± 0.05†	31	2.7 ± 0.09	2.1 ± 0.04†	22
Level 2	3.3 ± 0.10	2.6 ± 0.05†	21	2.2 ± 0.04	1.8 ± 0.04†	18
Level 3	2.0 ± 0.10	1.3 ± 0.05†	35	1.7 ± 0.07	1.1 ± 0.04†	35
Level 4	1.0 ± 0.01	0.7 ± 0.05†	30	1.0 ± 0.01	0.8 ± 0.02†	20
		Mean	29		Mean	24

* All data mean ± SEM; n, number of animals.
† Value differs significantly from the corresponding control value; $P < 0.01$.
‡ Value differs significantly from the corresponding control value; $P < 0.02$.

space fibrosis was absent. There was moderate perivenous edema.

Pulmonary Arteriograms (Experiments A, B, and C)

Pulmonary arteriograms were obtained from all animals. The arteriograms of control rats showed uniform filling (Figure 1A), whereas hyperoxia caused narrowing of the lumen of large pulmonary arteries and loss of the background haze that represented filling of small arteries (Figures 1B–D).

After 1 day of breathing 80% O_2 (Experiment A), although loss of haze was obvious on the arteriogram, it was patchy and limited to the edge of the lung: the loss was similar after 2 and 3 days but greater after 7, 10, and 14 days, being most marked after 14 days, when the lumen of the main axial artery was also narrowed (Figure 1B). Seven days of breathing 87% or 90% O_2 (Experiments B and C) caused lumen narrowing of the main axial artery and its lateral branches and loss of background haze (Figures 1C and D). These changes at 7 days were similar but more uniform than those produced by 14 days' exposure to 80% O_2. After 7 days the lumen diameter of large pulmonary arteries was reduced by about one-third after 90% and one-quarter after 87% O_2 (Table 2).

Morphometric Findings (Experiment B, 7 Days—90% O_2)

Values for the external diameter, medial thickness, and percent medial thickness of intraacinar and preacinar arteries by wall structure and location are given in Tables 3 and 4.

Intraacinar Arteries

Density

Hyperoxia reduced the density of intraacinar arteries by one-third (control, 13 ± 1 sq mm; exposed, 8 ± 1 sq mm; $P < 0.001$) but did not change alveolar concentration (control, 341 ± 16 sq mm; exposed, 340 ± 24 sq mm). The ratio of arteries to alveoli was 4:100 in control rats and 2.5:100 in exposed rats.

Wall Structure

At the level of the alveolar duct and alveolar wall hyperoxia caused a significant shift in the distribution of arteries by their wall structure (Table 5, $P[\chi^2] < 0.01$ and <0.001 for alveolar duct and alveolar wall vessels, respectively), increasing the relative number of muscular and partially muscular arteries at the expense of nonmuscular ones: a similar shift did not occur at the level of the respiratory bronchiolus, because there are few nonmuscular arteries in this region. Muscular arteries were present in the alveolar wall, where they are not normally found. Hyperoxia significantly shifted the distribution of arteries by structure within a given size range (Table 6). Within the population of vessels 15–50 μ in ED more were partially muscular and fewer nonmuscular: there were no muscular arteries in this size range ($P[\chi^2] < 0.01$). The proportion of muscular and partially muscular arteries 51–100 μ in ED also increased ($P[\chi^2] < 0.001$) at the expense of nonmuscular arteries

Table 3—Intraacinar Arteries: External Diameter, Medial Thickness, and Percent Medial Thickness of Muscular (M), Partially Muscular (PM) and Nonmuscular (NM) Vessels Associated With Respiratory Bronchioli (RB), Alveolar Ducts (AD), and Alveolar Walls (AW) in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

			External diameter (μ)				Medial thickness (μ)				% Medial thickness (2 × 100 × MT/ED)			
		n	Mean	SD	Med	Range	Mean	SD	Med	Range	Mean	SD	Med	Range
RB														
M	CON	13	150.2	24.9	160	118-202	1.72	0.44	1.6	1.6-3.2	2.38	0.89	2.00	1.59-5.13
	90% O_2	21	103.8	38.9	99	51-186	2.59	1.28	1.6	1.6-6.4	5.60	3.74	4.55	2.00-18.18
PM	CON	14	125.1	41.8	114	70-218	1.71	0.42	1.6	1.6-3.2	3.05	1.30	2.80	1.47-6.25
	90% O_2	17	95.9	33.6	102	51-163	1.97	0.70	1.6	1.5-3.2	4.64	2.57	4.35	1.96-12.5
NM	CON	2	21.5	9.2	22	15-28	–	–	–	–	–	–	–	–
	90% O_2	2	67.2	59.0	67	26-109	–	–	–	–	–	–	–	–
AD														
M	CON	17	104.1	23.1	96	70-147	1.69	0.38	1.6	1.6-3.2	3.41	1.09	3.33	2.17-6.67
	90% O_2	38	100.5	31.9	91	51-163	2.48	0.96	2.4	1.6-4.8	5.54	2.87	4.90	1.96-12.5
PM	CON	49	87.4	25.2	83	38-140	1.60	0	1.6	0	3.99	1.28	3.85	2.27-8.33
	90% O_2	68	77.6	30.5	70	32-221	1.95	0.72	1.6	1.5-4.8	5.70	3.09	4.76	1.44-16.67
NM	CON	88	64.1	21.8	58	29-128	–	–	–	–	–	–	–	–
	90% O_2	67	49.1	15.2	48	22-102	–	–	–	–	–	–	–	–
AW														
M	CON	0	–	–	–	–	–	–	–	–	–	–	–	–
	90% O_2	23	86.0	18.7	90	48-122	2.71	1.1	3.2	1.6-6.4	6.46	3.03	6.89	2.70-13.33
PM	CON	9	77.2	26.8	70	42-125	1.60	0	1.6	0	4.62	1.62	4.54	2.56-7.69
	90% O_2	110	71.8	26.3	70	29-163	1.83	0.72	1.6	0.75-6.4	5.78	3.13	5.00	1.96-25.00
NM	CON	114	54.9	18.9	51	22-131	–	–	–	–	–	–	–	–
	90% O_2	146	49.4	24.5	45	19-246	–	–	–	–	–	–	–	–

* n, number of arteries; Med, median.

in this size range. There was no significant change in the distribution of vessels 100-200 μ in ED. Intimal thickening occurred only in nonmuscular arteries, being present in 19% of vessels associated with alveolar ducts and in 34% of vessels in the alveolar wall.

Medial Thickness

The percent medial thickness of all intraacinar muscular arteries and partially muscular arteries (Table 7) increased after hyperoxia ($P < 0.01$ for both). Both decrease in mean external diameter ($P < 0.01$ for each vessel type) and increase in mean medial thickness ($P < 0.01$ and 0.05 for each vessel type) contributed to this change in percent medial thickness. The mean external diameter of all nonmuscular intraacinar arteries was also reduced but not significantly so. Analysis of arteries ≤200 μ in Ed by region showed narrowing—more arteries associated with respiratory bronchioli being

Table 4—Preacinar Arteries: External Diameter, Medial Thickness and Percent Medial Thickness of Muscular (M) and Partially Muscular (PM) Vessels Associated With Bronchioli (B) and Terminal Bronchioli (TB) in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

			External diameter (μ)				Medial thickness (μ)				% Medial thickness (2 × 100 × MT/ED)			
		n	Mean	SD	Med	Range	Mean	SD	Med	Range	Mean	SD	Med	Range
B														
M	CON	86	544.3	470.3	360.0	132-2040	4.82	4.04	3.2	1.6-22.4	2.12	1.83	1.78	0.23-14.54
	90% O_2	106	478.9	446.1	280.0	51-1840	9.21	8.16	6.4	1.6-41.6	4.61	2.49	4.08	1.45-14.4
PM	CON	5	172.94	34.3	204.8	128-210	2.56	0.87	1.6	1.6-3.2	3.21	1.57	3.87	1.56-5.00
	90% O_2	15	172.00	62.2	163.5	99-278	3.00	1.36	2.4	1.6-4.8	3.53	1.43	3.14	1.78-6.47
TB														
M	CON	12	172.3	38.4	171.2	118-237	2.26	0.82	1.6	1.6-3.2	2.73	1.37	2.51	1.63-4.76
	90% O_2	14	142.2	29.8	137.6	106-198	2.97	0.85	3.2	1.6-4.8	4.28	1.49	4.12	2.38-8.33
PM	CON	6	147.3	33.3	143.5	106-192	1.86	0.65	1.6	1.6-3.2	2.55	0.65	2.50	1.67-3.50
	90% O_2	9	122.7	37.8	112.0	77-198	2.48	1.62	1.6	1.6-4.8	3.91	1.49	3.33	2.50-6.45

* n, number of arteries.

Table 5—Chi-Square Analysis of the Distribution of Muscular (M), Partially Muscular (PM), and Nonmuscular (NM), Alveolar Duct (AD) and Alveolar Wall (AW) Arteries in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

	90% O_2		CON		Total
AD					
M	38	(21%)	17	(11%)	55
PM	68	(39%)	49	(32%)	117
NM	67	(40%)	88	(57%)	155
Total	173		154		327
	$\chi^2 = 12.91$		$P < 0.01$		
AW					
M	23	(8%)	0	(0)	23
PM	110	(40%)	9	(7%)	119
NM	146	(52%)	114	(93%)	260
Total	279		123		402
	$\chi^2 = 54.35$		$P < 0.001$		

* Observed values.

51–100 μ in ED, and more alveolar duct arteries being 15–100 μ in ED, at the expense of larger vessels (Table 8, P [χ^2] < 0.01 and 0.05 for each region, respectively).

An increase in the maximum ED of partially muscular and nonmuscular (but not muscular) intraacinar arteries indicates dilatation (see Table 3). The external diameter of the largest partially muscular artery associated with an alveolar duct was 140 μ in control and 221 μ in exposed rats, and the corresponding values were 125 μ and 163 μ for partially muscular arteries in the alveolar wall (Table 3). The ED of the largest nonmuscular artery in the alveolar wall was 109 μ in control and 246 μ in exposed rats.

Table 6—Chi-Square Analysis of the Distribution of Intraacinar Muscular (M), Partially Muscular (PM), and Nonmuscular (NM), Arteries 15–50 μ and 51–100 μ in External Diameter in Control Rats (CON), and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

	90% O_2		CON		Total
15–50 μ					
M	1	(0.6%)	0	(0)	1
PM	38	(22.6%)	4	(5%)	42
NM	129	(76.8%)	78	(95%)	207
Total	168		82		250
	$\chi^2 = 12.71$		$P < 0.01$		
51–100 μ					
M	49	(19%)	9	(5%)	58
PM	124	(49%)	46	(27%)	170
NM	81	(32%)	117	(68%)	198
Total	254		172		426
	$\chi^2 = 56.26$		$P < 0.001$		

* Observed values.

Table 7—External Diameter, Medial Thickness, and Percent Medial Thickness of All Intraacinar and Preacinar Muscular (M), Partially Muscular (PM), and Nonmuscular (NM) Arteries in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

	External diameter (μ)	Medial thickness (μ)	% Medial thickness
Intraacinar			
Muscular			
CON	126.1 ± 3.2	1.7 ± 0.04	2.8 ± 0.1
90% O_2	96.9 ± 7.3†	2.7 ± 0.2†	6.1 ± 0.8†
Partially muscular			
CON	98.7 ± 3.8	1.6 ± 0.04	3.8 ± 0.3
90% O_2	79.8 ± 3.9†	2.1 ± 0.2‡	5.6 ± 0.4†
Nonmuscular			
CON	60.7 ± 4.4	–	–
90% O_2	54.9 ± 4.0	–	–
Preacinar			
Muscular			
CON	419.6 ± 39.9	4.0 ± 0.3	2.4 ± 0.1
90% O_2	368.7 ± 34.8	7.1 ± 0.6†	4.5 ± 0.4§
Partially muscular			
CON	155.2 ± 9.4	2.1 ± 0.2	2.5 ± 0.1
90% O_2	136.2 ± 8.9	2.7 ± 0.2‖	4.1 ± 0.3§
Nonmuscular			
CON	–	–	–
90% O_2	–	–	–

* Number of animals: control, 4; hyperoxia, 7. All data mean ± SEM.
† $P < 0.01$.
‡ $P < 0.02$.
§ $P < 0.001$.
‖ $P < 0.05$.

Preacinar Arteries

Medial Thickness and External Diameter

Hyperoxia increased the percent medial thickness of all preacinar arteries—both muscular and partially muscular (Table 7, $P < 0.001$ for each, respectively). The mean ED of muscular and partially muscular preacinar arteries was reduced, although not significantly so, whereas their mean medial thickness was significantly increased ($P < 0.01$ and 0.05, respectively, for each vessel type).

Pulmonary Veins

The pulmonary veins were not analyzed morphometrically, but whereas thickening of the artery wall was obvious, the vein walls appeared normal.

Right Ventricular Hypertrophy

Hyperoxia did not alter the absolute weight of the RV after 3 days (Table 9), whereas that of the LV + S was reduced, but not significantly so. Because the body

Table 8—Chi-Square Analysis of the Distribution of Respiratory Bronchioli (RB) and Alveolar Duct (AD) Arteries 15-50 μ, 51-100 μ, 101-150 μ, and 151-200 μ in ED in Control Rats (CON) and Rats Exposed to Hyperoxia for 7 Days (Experiment B—90% O_2)*

	90% O_2		CON		Total
RB					
15-50	0	(0)	0	(0)	0
51-100	19	(51%)	4	(15%)	23
101-150	13	(35%)	11	(41%)	24
151-200	5	(14%)	12	(44%)	17
Total	37		27		64
	$\chi^2 = 11.55$		$P < 0.01$		
AD					
15-50	51	(29%)	29	(19%)	80
51-100	97	(56%)	102	(66%)	199
101-150	20	(12%)	23	(15%)	43
151-200	5	(3%)	0	(0)	5
Total	37		154		327
	$\chi^2 = 7.88$		$P < 0.05$		

* Observed values.

Table 10—Pulmonary Artery (PAP) and Systemic Blood (BP) Pressure of Control Rats, Rats Exposed to Hyperoxia, and Rats Breathing Air for Various Periods After Hyperoxia (Experiment C—87% O_2)*

	n	PAP (mmHg)	BP (mmHg)
Control group			
Air—baseline	9	22.1 ± 0.7	126.0 ± 3.6
Air—3 days	9	22.5 ± 0.5	115.5 ± 2.4
Air—7 days	9	20.2 ± 0.7	116.4 ± 2.9
Exposed group			
Air—baseline	10	17.0 ± 0.7	104.1 ± 1.8
87% O_2—15 minutes	10	16.6 ± 0.7	108.8 ± 1.9
87% O_2—3 days	9	17.0 ± 0.9	101.4 ± 2.5
87% O_2—7 days	9	26.2 ± 0.9†‡	97.3 ± 3.2†
15 minutes air	3	31.3 ± 3.3†	95.1 ± 3.8†
30 minutes air	3	32.6 ± 2.3†	93.7 ± 4.9†
60 minutes air	2	38.3 ± 3.3†§	—

* All data mean ± SEM; n, number of animals.
† Value differs significantly from the control values at baseline and after 7 days; $P < 0.01$.
‡ Value differs significantly from the 87% O_2 value at baseline; $P < 0.01$.
§ Value differs significantly from the 87% O_2 value after 7 days; $P < 0.01$.

weight of rats exposed to hyperoxia fell, the relative weight of the RV was increased ($P < 0.05$), while that of the LV + S was unchanged. After 7 days, the absolute weight of the LV + S was less than the control value ($P < 0.001$), and that of the RV was reduced, but not significantly so. Because the body weight of exposed rats continued to decrease, the relative weight of the LV + S was slightly increased and that of the RV, significantly so ($P < 0.001$). Relative RVH was present, the ratio of LV + S/RV being significantly reduced from 4.07 ± 0.26 in control rats to 3.23 ± 0.10 in exposed rats ($P < 0.02$).

Pulmonary Hemodynamics (Experiment C—87% O_2)

The PAP was not increased after 15 minutes or 3 days of hyperoxia (Table 10) but was after 7 days, when it was significantly above the baseline value before the start of exposure (Δ + 9 mmHg) and the control value (Δ + 6 mmHg; see Table 10 for levels of significance). The PVRI rose from 0.033 ± 0.003 U/kg to 0.065 ± 0.015 U/kg ($P < 0.05$). The mean CI fell from 520.23 ± 29.90 ml/min/kg in the controls to 373.93 ± 70.81 ml/min/kg in the exposed rats, although this difference was not significant. The $\dot{V}_{O_2}$ also fell from 34.28 ± 1.64 ml/min/kg to 16.16 ± 1.88 ml/min/kg ($P < 0.01$). The HR was significantly reduced (control, 443 ± 15 minutes; exposed, 313 ± 11 minutes; $P < 0.01$) as was the RR (control, 146 ± 18 minutes; exposed, 102 ± 7 minutes; $P < 0.05$). The SVI was 1.13 ± 0.067 ml/kg in control and 0.981 ± 0.139 ml/kg in exposed rats. The BP was significantly less than the control value (Δ − 19 mmHg) and the SVRI was similar in the two groups (0.244 ± 0.001 and 0.255 ± 0.048 U/kg in control and exposed rats, respectively). The Hct of exposed rats was not significantly different from the control (Ta-

Table 9—Absolute and Relative Weight of the Left Ventricle and Septum (LV + S) and Right Ventricle (RV) in Control Rats (CON) and in Rats Exposed to Hyperoxia (Experiment B—90% O_2)*

	Absolute weight (g)			Relative weight (as % body weight)		
	n	LV + S	RV	n	LV + S	RV
3 Days						
CON	3	0.58 ± 0.045	0.16 ± 0.009	3	0.21 ± 0.014	0.06 ± 0.002
90% O_2	3	0.51 ± 0.024	0.16 ± 0.018	3	0.21 ± 0.006	0.07 ± 0.006†
7 Days						
CON	4	0.64 ± 0.026	0.16 ± 0.003	4	0.21 ± 0.006	0.05 ± 0.001
90% O_2	7	0.49 ± 0.017‡	0.15 ± 0.005	7	0.22 ± 0.005	0.07 ± 0.002‡

* All data mean ± SEM; n, number of animals.
† Value differs significantly from corresponding control value, $P < 0.05$.
‡ Value differs significantly from corresponding control value, $P < 0.001$.

ble 11). During exposure to hyperoxia the Pa_{O_2} was significantly higher than normal – being higher on Day 3 than on Day 7; the Sv_{O_2} increased significantly after 3 and 7 days of exposure.

On Day 7, within 15 minutes of return to air, the PAP of exposed animals was significantly increased, and it increased progressively with time (see Table 10): 60 minutes after return to air PAP was significantly greater than both the control value (Δ+ 18 mmHg) and the baseline value for rats before the start of exposure (Δ+ 21 mmHg); it was also greater than the pressure measured at the end of oxygen exposure (Δ+ 12 mmHg). On return to air the BP fell slightly, and the Hct and pH_a were unchanged. The Pa_{O_2} was significantly reduced, and the Pa_{CO_2} was unchanged. The blood HCO_3 remained low.

Discussion

Our study shows that hyperoxia causes marked structural alterations in rat pulmonary arteries and altered pulmonary hemodynamics. We discuss here the structural changes after exposure to 90% O_2 for 7 days, these being the most significant, and our hemodynamic findings. Both preacinar and intraacinar regions of the pulmonary artery wall are remodeled, although most change occurs within the acinus, ie, within the pulmonary microcirculation. The density of functional arteries and arterial lumen size are both reduced, while in remaining arteries medial muscle thickness increases and muscle is present in previously nonmuscular segments of the vessel wall. Relative right ventricular hypertrophy is present. After 7 days of breathing 87% O_2 the pulmonary artery pressure and pulmonary vascular resistance are significantly increased.

The first days of exposure to hyperoxia are marked by extensive necrosis of lung cells and adaptation by surviving cells.[14,28] Necrosis results from intracellular generation of toxic free radicals and their products,[29] in particular, superoxide, the hydroxyl radical, and hydrogen peroxide. The generation of these metabolites above basal rates raises cellular levels of protective enzymes (eg, superoxide dismutase, catalase, or glutathione peroxidase) and free radical scavengers (eg, α-tocopherol or ascorbate), allowing cell survival and adaptation.[13,30,31] After the first days of hyperoxia, pulmonary edema diminishes as the integrity of the alveolar wall is restored by cell hypertrophy and hyperplasia, with a change in the distribution of the various cell types.[15] Within small pulmonary arteries also, endothelial cell injury and necrosis is extensive during the first days of hyperoxia (unpublished findings); endothelial cell hypertrophy and hyperplasia also occur.[32] The widespread remodeling evident after 7 days is probably the result of acute as well as persistent but sublethal injury of cells of the vessel wall by free radicals, including cells of the intima, media, and adventitia. In addition, it is likely that cell injury activates the inflammatory response to compound the damage; early during exposure to hyperoxia (100% O_2) polymorphonuclear leukocyte (PMNs) are recruited to the lung,[33] possibly by the local generation of chemotactic mediators from injured alveolar macrophages[34,35] and by complement activation.[36] During phagocytosis of cell debris free radicals and proteases and lysosomal hydrolases are released into the extracellular space,[37] causing additional injury to cell membranes and structural components of the extracellular matrix. Although PMNs are absent after 7 days' exposure, the presence of many monocytes at this time in the lungs of rats included in our study indicates the persistence of the inflammatory response and possibly secondary lung injury.

Loss of Functional Precapillary Units

Both the arteriogram and the density count of alveolar duct and alveolar wall arteries filled with injection medium reveal a reduction in the number of patent peripheral arterial units. This could arise from 1) obstruction of vessels by thrombi, by fibrin, or by aggregates of erythrocytes, leukocytes, or platelets; 2) functional closure of vessels, by encroachment on the lumen of wall structures, as from endothelial cell swelling; or 3) obliteration of vessels, caused by loss of integrity of the endothelium and fragmentation and resorption of the vessel wall. By morphometric techniques reduction in density is as much as one-third. The features typical of obstruction are not seen frequently enough in our animals to explain this degree of reduced filling of small vessels, indicating that functional closure or obliteration contribute. Evidence for functional closure, as after hypoxia in the rat, is the rapid increase during recovery in the density of filled vessels identified by arteriography and microscopy[38]; evidence of obliteration is the presence of artery remnants and persistence during recovery of an arterial density less than normal, including filled and unfilled vessels. Because in this experiment recovery has not been studied, the contribution of functional closure or obliteration cannot be assessed quantitatively. The presence of some vessel remnants after hyperoxia, albeit rarely, indicates that obliteration contributes to the reduction in vessel density.

Muscularization of the Pulmonary Artery Wall

After hyperoxia, a muscular and partially muscular structure is found in more distal pulmonary arteries than in the normal lung. Landmarking of arteries

confirms that the shift that occurs from nonmuscular to muscularized vessels represents the structural remodeling of artery walls at a given branching level. A clear example of this is the appearance of muscular arteries in the alveolar wall, because vessels with this wall structure are not normally found here.

The new muscle present in pulmonary artery walls after hyperoxia probably represents a phenotypic change to smooth-muscle cells by precursor cells, ie, the intermediate cell and pericyte. These cells lie within the intima of the vessel wall and internal to the elastic lamina: the intermediate cell lies external to the basement membrane of the endothelial cell; and the pericyte, internal to it. While rare in the normal rat lung,[39] in response to hypoxia or monocrotaline, these cells undergo hypertrophy and hyperplasia.[40,41] In a recent morphometric study (of 1-μ thick tissue sections) we found that hyperoxia (87% O_2) stimulates these cells: 4 days after the start of exposure increased numbers of hypertrophied precursor cells were identified both in the nonmuscular region of partially muscularized arteries and in nonmuscular arteries, their number increasing as exposure continued.[32] Electron microscopy confirm that most have the structure of intermediate cells.[32] The intimal thickening reported in the present study, in about one-fifth of alveolar duct and one-third of alveolar wall nonmuscular arteries, doubtless represents hypertrophy and hyperplasia of these cells.

The phenotypic change of precursor cells to smooth-muscle cells may be a response to acute or continuing injury of the vessel wall by oxygen metabolites; it may be the response to a raised transmural pressure produced by higher blood flow in a restricted arterial bed; or it may be in response to specific growth factors. Thrombocytopenia, sequestration of platelets in the lung, and diffuse intravascular coagulation early in the exposure period[42] may lead to release of platelet-derived growth factor (PDGF) from platelet α-granules, stimulating the growth of medial smooth-muscle cells[43] and perhaps of precursor cells. Serotonin released from platelets or mast cells will enhance this effect,[44] and granulocytes can also release growth factors.[45,46]

Narrowing of Pulmonary Artery Lumen

In the present study hyperoxia decreases the external diameter of arteries both with and without muscle in their wall. In pulmonary hypertension, narrowing of muscular arteries even in the absence of increase in medial muscle mass has been attributed to a shortening of smooth-muscle cells, with increase in adventitial collagen maintaining the constriction to produce contracture.[47] If nonmuscular pulmonary arteries constrict *in vivo*, a similar process may lead to a decrease in their external diameter. Constriction of rat nonmuscular alveolar wall arteries has recently been reported *in vitro*,[48] presumably by constriction of the endothelial cell and pericyte, as in capillaries.[49,50] Medial thickening is also present after hyperoxia, but the contribution to this of any contracture, or of smooth-muscle cell hypertrophy and hyperplasia, awaits future ^{3}H-thymidine labeling studies.

Either a decrease in external diameter or an increase in medial thickness can narrow the vessel lumen. In preacinar and intraacinar regions, as the cross-sectional area of the bed is reduced, the hydraulic resistance will increase per unit length of vessel. While Poiseuille's Law may not apply to arteries less than 100 μ in external diameter,[51] the hydraulic resistance certainly rises in these vessels. As peripheral vessel narrowing and loss increases the pulmonary vascular resistance and causes pulmonary hypertension, the latter has a secondary mechanical effect on the walls of preacinar arteries because they are subjected to increased transmural pressure, and pulse-wave reflections will change along the wall.

Dilatation of Pulmonary Arteries

Dilatation is a feature of the pulmonary vascular changes in ARDS[4,5] but is not typical of other types of early human or experimentally induced pulmonary hypertension. Dilatation may represent a passive response to severe inflammation and collagenolysis leading to loss of supporting tissue within the vessel wall. In regions where pulmonary blood flow is increased because of adjacent vessel block and maximum recruitment and distension of vessels is maintained, it may represent severe injury with loss of endothelial and precursor smooth-muscle cells preventing neomuscularization. While hyperoxia causes a significant increase in plasma levels of 6-keto-$PGF_{1\alpha}$ in the rat (unpublished findings), the focal distribution of the dilated vessels we report here suggests change in the mechanical properties of the wall rather than a response to local generation of a vasodilator.

Pulmonary Hemodynamic Changes

Within 7 days of breathing 87% O_2 we found a significant but moderate rise in the pulmonary vascular resistance and pulmonary artery pressure and evidence of RVH. The increased PAP is probably based upon the structural remodeling, because it occurs in the absence of systemic hypoxemia, and we found no evidence of polycythemia (see Table 11). Vessel loss and excessive muscularization of residual arteries each will contribute to the increased pulmonary vascular resistance, causing a rise in the pulmonary artery pressure and subsequent RVH. However, we cannot exclude the contribution of diffuse release of vasoconstrictor agents

Table 11—Arterial Hematocrit, pHa, Arterial Blood Gas Tensions, Bicarbonate, and Arterial and Mixed Venous Oxygen Saturation in Control Rats, in Rats Exposed to Hyperoxia, and in Rats Breathing Air After Hyperoxia (Experiment C—87% O_2)*

	n	Hct (%)	n	pHa	n	Pa_{O_2} (mmHg)	n	$PaCO_2$ (mmHg)	n	HCO_3^- (mEq/l)	n	Sa_{O_2} (%)	n	Sv_{O_2} (%)
Control group														
Air—baseline	9	38.67 ± 1.49	9	7.52 ± 0.01	9	83.81 ± 1.25	9	30.32 ± 0.91	9	25.71 ± 1.02	9	88.92 ± 0.43	9	60.24 ± 0.89
Air—Day 3	9	43.50 ± 0.23	8	7.47 ± 0.01	8	76.84 ± 1.86	8	37.80 ± 0.49	8	27.14 ± 0.73	8	89.91 ± 0.37	9	60.69 ± 1.09
Air—Day 7	9	43.17 ± 0.22	7	7.52 ± 0.01	7	77.42 ± 2.23	7	28.56 ± 0.82	7	24.95 ± 0.86	7	89.57 ± 0.29	9	61.77 ± 1.29
Exposed group														
Air—baseline	7	35.50 ± 1.69	9	7.45 ± 0.01	9	89.16 ± 2.31	9	30.67 ± 0.73	9	21.71 ± 0.73	3	89.20 ± 0.30	7	58.96 ± 7.67
87% O_2—15 minutes	4	35.75 ± 2.28	9	7.43 ± 0.01	9	231.33 ± 4.72	9	33.06 ± 0.86	9	22.17 ± 0.71	2	95.00 ± 0	8	75.04 ± 4.63
87% O_2—Day 3	9	34.22 ± 1.74	8	7.47 ± 0.02	8	250.30 ± 12.32†	8	26.17 ± 1.51	8	26.17 ± 1.51	2	95.00 ± 0.10	7	75.04 ± 4.63†
87% O_2—Day 7	9	42.56 ± 2.00	9	7.27 ± 0.01†	9	192.58 ± 2.34	9	30.24 ± 1.37	8	18.14 ± 0.36	3	96.33 ± 0.48	8	75.69 ± 2.42†
87% O_2—Day 7 + 15 minutes air	9	38.78 ± 1.93	9	7.25 ± 0.01†	9	59.39 ± 5.46†‡	9	28.38 ± 0.66	9	17.53 ± 0.27		—	9	53.50 ± 2.55‡
87% O_2—Day 7 + 30 minutes air		—	6	7.25 ± 0.01†	6	55.76 ± 3.67†‡	6	30.62 ± 1.00	6	17.80 ± 0.27		—	5	59.24 ± 4.35‡
87% O_2—Day 7 + 60 minutes air		—	3	7.26 ± 0.01†	3	48.47 ± 8.03†‡	3	32.00 ± 1.70	3	17.30 ± 0.21		—	3	51.33 ± 4.63†‡

* All data mean ± SEM; n, number of animals.
† Value differs significantly from the 87% O_2 baseline value; $P < 0.01$.
‡ Value differs significantly from the 87% O_2 value after 7 days; $P < 0.01$.

to the increased pulmonary vascular resistance. Our findings confirm the report of Bennett and Smith[52] of pulmonary hypertension in rats breathing 4 atm compressed air ($P_{A_{O_2}}$ = 618 mmHg) for 18 days, and indicate that increased PAP can occur earlier—doubtless the effect of more extensive remodeling of the pulmonary artery walls in the rats included in our study. On return to breathing air, and to a reduced $P_{A_{O_2}}$, it is likely that diffuse hypoxic pulmonary vasoconstriction (Pa_{O_2} = 48 mmHg at 60 minutes) produces the sustained and further rise of pulmonary artery pressure.

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