Alveolar Hypoxia, Differential Effects on Pulmonary Arteries of Varied Size[1] (34259)

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Chronic alveolar hypoxia has been shown in a variety of animals to increase pulmonary vascular resistance and induce pulmonary arterial hypertension with resultant cor pulmonale (1–7). The increased vascular resistance seems related to the combined effects of vascular constriction and an increased muscle mass in small pulmonary arteries. The present study gives evidence that effects on larger pulmonary arteries may be dissimilar.

Methods. Seventy-two C-57 young adult male mice, each weighing 20–25 g were utilized in the study. Twenty-five of the mice were placed for 20 hr each day in a sea-level pressure chamber where the atmosphere was maintained at 12% oxygen and 88% nitrogen. The gas flow through the chamber was sufficient to keep the CO_2 concentration below 0.5%. Their hematocrit values reached the 65–75% range by the third week. They were killed after periods of hypoxia varying from 12 to 24 days. A second group of 21 mice was made polycythemic by nonhypoxic means; their hematocrits were maintained in the 68–80% range (mean 74%) by twice weekly intraperitoneal injections of heparinized, washed, packed erythrocytes from donor mice of the same strain (8). These mice were killed 21–24 days after induction of the polycythemia. Twenty-six mice in a third group served as controls; they remained in a nonhypoxic environment and received no transfusions. Their hematocrits were in the 42–50% range.

Following sacrifice each heart was weighed on a torsion balance. After formalin fixation, the two cardiac ventricles were separately dissected and weighed; the interventricular septum being included with the left ventricle.

Several quantitative measurements were made on pulmonary arteries and arterioles. In each case, all lung tissue was sectioned uniformly at 5 μ and separate slides were stained with hematoxylin and eosin and Verhoeff's and van Gieson's stains. Usng a camera lucida and planimeter under conditions of constant magnification, relative cross-sectional areas of intimal nuclei, media, and medial nuclei were determined in 4–6 arteries and arterioles in each of three size ranges [diameter (μ): 30–79, 80–149, and 150+] in each case. The smallest category contained vessels that some classify as arterioles. The category of greatest diameter included arteries of the largest muscular type. No elastic arteries were included and only vessels cut in cross section were measured (4). James and Thomas (7) recently reviewed the anatomy of the smaller vessels in mice.

No arterial intimal lesions or thrombi were evident in any of the sections. The mean areas of individual intimal nuclei and the total area of intimal nuclei for arteries in a given area of the lung were almost identical in animals of the various groups. Therefore, the total area of these nuclei was used as an internal standard or base line to which the area of the artery's media could be compared. The following ratio was adopted as a measure of the relative area of medial smooth muscle present in individual arteries: (Area of arterial media/total area of intimal nuclei). Since the media of arteries had no visible collagen, elastic, or other abnormal tissue, a large ratio indicates a large medial

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muscle mass and a small ratio a small muscle mass. In each group of mice, a mean ratio was determined for each category of arteries. Studies in dogs and in man indicate that this ratio is not influenced by arterial dilatation (3).

In instances where pulmonary arterial muscle mass is increased, it is useful to determine whether the increase is due to medial muscle hypertrophy or hyperplasia. The state of hypertrophy can be evaluated by determining the mean cytoplasmic mass of individual muscle cells. An index of this value can be determined by dividing an artery's medial cytoplasmic area by the number of medial nuclei present. An artery's medial cytoplasmic area was calculated in each instance by subtracting the combined area of medial nuclei from the total medial area. A mean value was calculated for each artery size in each group. This value was not influenced by gelatin dilatation of dog or infant arteries in a previous study (3).

An index reflecting the number of medial nuclei present in an artery was calculated by dividing the number of medial nuclei in a vessel by the number of intimal nuclei. This index gives a relative measure of arterial medial hyperplasia when an artery's medial muscle mass is increased. As with other calculations, a mean value was calculated for each category of artery size in each group. This ratio was not influenced by gelatin dilatation of dog or infant arteries (3). The t test was used to determine the significance of the data collected.

Results. Chronic hypoxia, but not polycythemia, led to a significant increase of muscle mass in pulmonary artieries and arterioles less than 80 μ in diameter (Table I). In all but one group, relative muscle mass increased with vessel size reflecting a thicker wall in the larger arteries (Table I). The increased muscle mass in small and medium sized arteries of the hypoxic animals was mainly related to a hyperplasia of medial fibers (Tables II, III). It is noteworthy that the relative cytoplasmic mass of individual medial muscle cells increased with artery size in all three groups (Table III).

TABLE I. Mean Relative Mass of Muscle in Pulmonary Arteries and Arterioles (area arterial media/area intimal nuclei).[a]

	(μ):	20–79	80–149	150+
Controls		8.0 ± 1.5	20.7 ± 5.7	27.3 ± 4.6
Hypoxia		18.5 ± 4.2[b]	30.2 ± 5.9[b]	23.9 ± 5.1
Polycythemia		11.2 ± 3.4	15.0 ± 4.7	23.8 ± 4.1

[a] Vessels are grouped by diameter. Values are in arbitrary planimeter units and are followed by one SD.

[b] The p value is 0.05 or less in comparison with the controls.

TABLE II. Relative Number of Medial Muscle Cells in Pulmonary Arteries and Arterioles (number of arterial nuclei/number of intimal nuclei).[a]

	(μ):	20–79	80–149	150+
Controls		0.76 ± 0.21	1.00 ± 0.36	0.99 ± 0.37
Hypoxia		1.11 ± 0.37[b]	1.41 ± 0.76[b]	1.01 ± 0.47
Polycythemia		0.85 ± 0.33	0.76 ± 0.32[b]	0.76 ± 0.20[b]

[a] Vessels are grouped by diameter. Each mean value is followed by one SD.

[b] The p value is 0.05 or less in comparison with the controls.

TABLE III. Mean Mass of Sarcoplasm in Medial Muscle Cells in Pulmonary Arteries and Arterioles (medial sarcoplasmic mass/number of sarcoplasmic nuclei).[a]

	(μ):	20–79	80–149	150+
Controls		17.5 ± 9.1	32.4 ± 19.7	49.5 ± 17.1
Hypoxia		24.0 ± 9.4	38.6 ± 16.9	45.0 ± 23.4
Polycythemia		17.1 ± 8.7	28.7 ± 16.4	44.1 ± 14.8

[a] Vessels are grouped by diameter. Values are in arbitrary planimeter units and are followed by one SD.

[b] No p values are less than 0.05 by comparison with Group I controls.

The mean weight of the right ventricle was 0.0251 g in the controls, 0.0356 in the hypoxic animals and 0.0315 in the polycythemic mice. These latter two values differed significantly from the mean value in the controls ($p<.05$).

Discussion. The study shows that in C-57 mice both chronic alveolar hypoxia and polycythemia can induce chronic cor pulmonale but only hypoxia leads to medial smooth muscle hyperplasia in small pulmonary arteries. This effect of hypoxia is not uniform

throughout the pulmonary arterial tree. It is marked in the smallest precapillary vessels, intermediate in the medium sized muscular arteries, and absent in the largest muscular arteries.

No very satisfactory explanation for this differential effect of hypoxia is available because the intimate mechanism through which hypoxia affects vascular muscle cells is unknown. Bergofsky and Holtzman (9) have shown that hypoxia induces a reversible depolarization and contraction of smooth muscle removed from pulmonary arteries but not of similar muscle taken from pulmonary veins and systemic arteries. Muscle cells in the large pulmonary muscular arteries of the C-57 mice may more closely resemble muscle cells in pulmonary veins and systemic arteries than they do muscle cells in the smaller pulmonary arteries. In any case, the present study does nothing to disturb the widely held belief that the pulmonary arterial pressor response to chronic hypoxia is based on vasoconstriction and an increased muscle mass in the small precapillary vessels. The presence of absence of vasomotor activity in the larger pulmonary arteries would be expected to have little effect on the resistance of the lesser circuit.

Summary. Mice were kept in a 12% oxygen environment for 12–24 days. The necropsy findings were compared with findings in nonhypoxic polycythemic and normovolemic controls. The 25 hypoxic and 21 polycythemic mice developed an increased right ventricular weight. Quantitative histologic analysis demonstrated that hypoxia but not polycythemia led to a marked medial hyperplasia in small pulmonary arteries, a small hyperplasia in intermediate sized arteries and no change in the largest muscular pulmonary arteries.

1. Fishman, A. P., Physiol. Rev. **41**, 214 (1961).
2. Arias-Stella, J. and Saldana, M., Med. Thorac. **19**, 484 (1962).
3. Naeye, R. L., Circulation Res. **17**, 160 (1965).
4. Naeye, R. L., Am. J. Physiol. **209**, 702 (1965).
5. Naeye, R. L., Circulation Res. **16**, 33, (1965).
6. Naeye, R. L., Am. J. Pathol. **50**, 1027 (1967).
7. James, W. R. L. and Thomas, A. J., Cardiovascular Res. **3**, 278 (1968).
8. Swigart, R. H., Circulation Res. **17**, 30 (1965).
9. Bergofsky, E. H. and Holtzman, S., Circulation Res. **20**, 506 (1967).

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