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Pulmonary Artery Structural Changes in Two Colonies of Rats With Different Sensitivity to Chronic Hypoxia

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Chronic hypoxia causes more severe pulmonary hypertension in the Hilltop colony of Sprague–Dawley rats than in the Madison colony and also greater polycythemia and vasoconstriction. This study examines the structural features of the pulmonary artery bed, another contributing factor to hypoxic hypertension. After 14 days of hypobaric hypoxia, in Hilltop rats, more of the intraacinar arteries became muscular, and the medial thickness of intraacinar and preacinar arteries was greater. In Hilltop control rats, muscle was found in more intraacinar arteries, but, paradoxically, acute hypoxic vasoconstriction was less. Thus, while in chronic hypoxia increased muscle correlates with pulmonary hypertension, in control rats the reserve seems to be true. The increased muscle in control Hilltop rats could, however, predispose to the greater muscularization seen after chronic hypoxia. (Am J Pathol 1987, 128:61–66)

THE PULMONARY HYPERTENSION of chronic hypoxia is determined by three main factors: structural remodeling of walls of pulmonary arteries, polycythemia, and the hypoxic vasoconstrictive response.[1] Ou and his colleagues have identified that the severity of pulmonary hypertension produced by chronic hypoxia is different in two colonies of Sprague–Dawley rats.[2,3] With similar exposure to chronic hypoxia, the hypertension is more severe in Hilltop rats than in Madison rats, as is the polycythemia.[4] Also, after exposure to chronic hypoxia, vasoconstriction represents a greater part of the pulmonary hypertension in Hilltop rats than in Madison rats.[4] However, even when vasoconstriction is reversed on return to room air, the chronically hypoxic Hilltop rats have a higher pulmonary artery pressure than the Madison rats.[4] If structural remodeling were to be more severe in the Hilltop rats, it could contribute to the higher pressure. We undertook the present study to examine differences between the two groups in the extent and nature of the vascular remodeling after chronic hypoxia, and to look at pulmonary artery structure in normal rats from both colonies. Paradoxically, acute hypoxic vasoconstriction causes less pulmonary artery pressure rise in normoxic Hilltop rats than in Madison rats.[4] We therefore measured pulmonary artery pressures during both acute and chronic hypoxia and hematocrit and found them to be similar to those previously reported for these colonies.[2]

Materials and Methods

Nine Hilltop (hypoxia-hypersensitive colony, Hilltop Laboratories, Scottsdale, Pa) and 9 Madison (Madison Farms, Madison, Wis) rats (male, 150–170 g) were used, and took food and water *ad libitum*. Three rats from each group served as room air controls; the other 6 were placed in hypobaric hypoxic chambers (380 mm Hg) for 14 days. The chambers

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were opened once daily for 10 minutes for changing drinking water and cage shavings. On Day 13, 3 control and 3 hypoxic rats from each group were anesthetized with intraperitoneal pentobarbital while breathing air or 10% oxygen, respectively, and indwelling pulmonary arterial catheters were inserted via the right jugular vein as previously described.[5] Hypoxic rats were returned to the chamber. On Day 14, all rats were weighed. In the catheterized hypoxic rats pulmonary artery pressure (PAP) was measured during hypoxia and then 15 minutes after return to air. In control rats PAP was measured in air, then after breathing 10% O_2 for 15 minutes. Blood for hematocrit was taken at the time of catheterization.

All animals were then killed with intraperitoneal injection of pentobarbital. The heart and lungs were immediately removed *en bloc,* and the pulmonary artery was cannulated with a polyethylene catheter. The heart was dissected away from the cannula and fixed in formalin for 1 week. The pulmonary artery was perfused with barium-gelatin mixture (60 C, 76 mm Hg, 5 minutes). The airways were then distended with 10% formalin at 25 cm H_2O, and the pressure was maintained for 1 week. From each animal blocks were taken from the right diaphragmatic, right cardiac, and left lung lobes and embedded in paraffin. Microscopic sections (4 μ) were then cut and stained with Miller elastic–Van Gieson stain. By means of a light microscope with a calibrated eyepiece reticle, 75 intraacinar and 15 preacinar arteries were examined for each rat. Features recorded included 1) external diameter (measured from external elastic lamina to external elastic lamina), 2) medial thickness from the external edge of the internal elastic lamina to the internal edge of the external elastic lamina, 3) artery structure (fully muscular—with a complete circumferential medial muscle coat; partially muscular—with an incomplete noncircumferential muscular coat; nonmuscular—without any medial muscle), and 4) accompanying airway landmark (preacinar, respiratory bronchiolus, alveolar duct, or alveolar wall).[6]

For detection of right ventricular hypertrophy (RVH), the right ventricular (RV) free wall was dissected from the left ventricle plus septum (LV + S). Both were weighed, and the ratio RV/(LV + S) was calculated.

Values are expressed as the mean ± standard error of the mean (SEM). Data were examined by means of analysis of variance. To then detect which groups differed significantly, we performed Duncan's New Multiple Range Test. In instances where unequal treatment sizes existed, Gabriel's Simultaneous Test procedure was used.[7] When only the two hypoxic groups could be compared (eg, Figure 4—the percent medial thickness of muscular alveolar wall arteries), the Student t test was used. To detect shifts between the groups in the proportions of structural types of arteries at a given airway landmark (Table 2 and Figure 3), a chi-square analysis was performed. In all instances, P values <0.05 were taken to be significant.

Results

Animal Growth (Table 1)

Final body weight of control rats from both groups was similar. Chronic hypoxia depressed final body weight in both groups, but not significantly more in the Madison rats.

Pulmonary Artery Pressure (Figure 1)

In control rats of both groups, baseline PAP values were similar. On exposure to acute hypoxia, PAP rose in both groups; but as an absolute value and as a percentage of baseline, the rise was significantly greater in madison rats than in Hilltop rats.

Chronic hypoxia caused pulmonary hypertension in both groups, the pressure in Hilltop rats being greater than in madison rats. After 15 minutes of breathing air, the fall in PAP in Hilltop rats was greater than in Madison rats, both as an absolute value and as a percentage of room-air baseline.

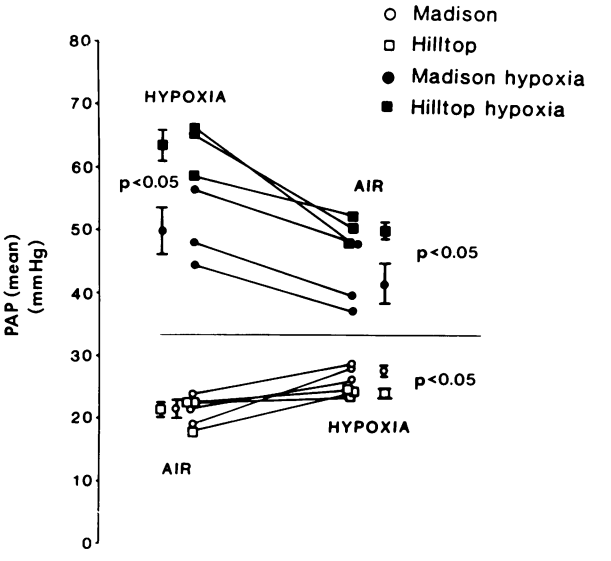

Figure 1—PAP (mm Hg) of control and chronically hypoxic Hilltop and Madison rats, both in hypoxia and air. In controls, the vasoconstriction caused by hypoxia was measured as the acute hypoxic pressor response, resulting in a rise in PAP. Madison rats had greater constriction than Hilltop rats. The vasoconstrictive component of PAP in chronically hypoxic rats was measured as the acute normoxic vasodilator response, resulting in a fall in PAP. Chronically hypoxic Hilltop rats had a higher PAP than Madison rats, and the fall in PAP on exposure to air was greater in Hilltop rats, representing greater hypoxic vasoconstriction. *Bar,* group mean ± SEM.

Right Ventricular Hypertrophy (Figure 2)

RV/(LV + S) was similar in both control groups. Chronic hypoxia caused a rise in RV/(LV + S) that correlated closely with the rise in PAP for individual animals from both groups ($r = 0.96$. $P < 0.001$, data not shown). In Hilltop hypoxia rats a higher RV/(LV + S) developed than in Madison hypoxia rats, representing more RVH.

LV/body weight ratios were similar in all four groups (data not shown). The RV/body weight ratio was the same for both control groups. After chronic hypoxia it rose in both groups, but was higher in the Hilltop group (0.00160 ± 0.000196 mean ± SEM) than in the Madison group (0.00115 ± 0.00007, $P < 0.05$), demonstrating that in the Hilltop group more RVH developed.

Hematocrit (Table 1)

The hematocrit was similar in both control groups. With chronic hypoxia it rose in both groups, significantly more so in Hilltop rats.

Table 1—Final Body Weight and Hematocrit

	Madison	Hilltop	Madison hypoxia	Hilltop hypoxia
Body weight (g)	317 ± 5.1 (n = 3)	325 ± 2.0 (n = 3)	220.4 ± 5.3 (n = 6)	245.7 ± 8.7 (n = 6)
Hematocrit	43.7 ± 1.9 (n = 3)	45 ± 0.6 (n = 3)	56.2 ± 0.2 (n = 6)	60.7 ± 1.2* (n = 6)

Values are means ± SEM.
*$P < 0.05$ versus Madison hypoxia group.

Arterial Morphometry

Intraacinar Arteries

Structure (Figure 3)

In the alveolar wall of control rats, almost all arteries were nonmuscular, there being no difference between Hilltop and Madison rats. In both groups, chronic hypoxia caused many of these arteries to become fully or partially muscular. The percentage of arteries restructured to fully muscular was higher in Hilltop rats.

Normally, some alveolar duct arteries are partially muscular. In Hilltop controls, significantly more showed this structure than in Madison controls. After chronic hypoxia, there was marked muscularization of arteries in both groups, only a few arteries remaining nonmuscular. Again, the percentage of arteries restructured to fully muscular was higher in Hilltop rats.

At respiratory bronchiolar level in controls rats, almost all arteries had either partially or fully muscular walls. With chronic hypoxic exposure, and to a similar degree in both groups, most partially muscular arteries became fully muscular.

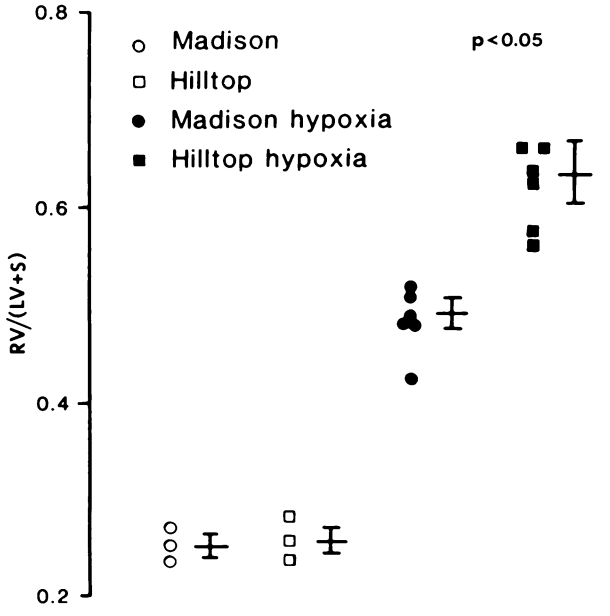

Figure 2—RV/(LV + S). Values for control groups were similar. After chronic hypoxia, RV/(LV + S) rose in both groups, representing development of RV hypertrophy. Hilltop rats developed more hypertrophy than Madison rats ($P < 0.05$ versus Madison hypoxia). *Bar*, group mean ± SEM.

Medial Thickness (Figure 4)

In control rats no muscular arteries were detected at the alveolar wall level, and only one muscular alveolar duct artery was found—in a Hilltop rat. Medial thickness data can, therefore, not be presented for muscular arteries at these landmarks. Partially muscular arteries were, however, found at all airway levels. There were no significant differences between the control groups in percent medial thickness of either muscular or partially muscular intraacinar arteries.

Where comparison with controls was possible, chronic hypoxia caused a marked rise in medial thickness in both groups. After chronic hypoxia, muscular arteries at alveolar wall and respiratory bronchiolar levels in Hilltop rats had a significantly higher percent medial thickness than those of Madison rats. For partially muscular arteries, when examined at each landmark, there were no significant differences in percent medial thickness between the two groups; but when all intraacinar arteries were examined together, the difference was significant ($P = 0.04$).

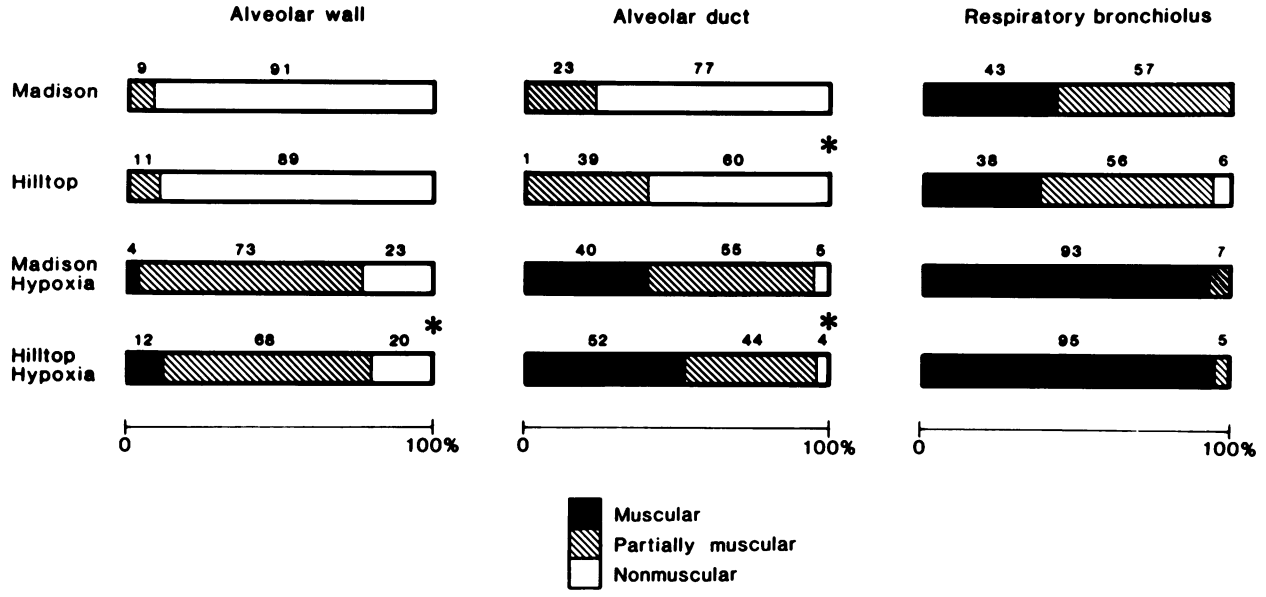

Figure 3—Structure of arteries. *Bars* are the percentage of arteries at an airway landmark (alveolar wall, alveolar duct, respiratory bronchiolus) which are of a given structure (muscular, partially muscular, nonmuscular). *Asterisks* present significant differences ($P < 0.05$) in the structure distribution between respective Hilltop and Madison groups.

External Diameter (Table 2)

For intraacinar arteries with a given wall structure and found at a given airway landmark, there were no differences in external diameter between control groups. Chronic hypoxia in Madison rats, as compared with control Madison rats, reduced the external diameter of nonmuscular alveolar wall and alveolar duct arteries and partially muscular alveolar duct arteries. In Hilltop rats, as compared with controls, chronic hypoxia caused a reduction in the external diameter of nonmuscular alveolar wall and alveolar duct arteries, partially muscular alveolar duct arteries, and muscular respiratory bronchiolar arteries. However, for any given artery population, after chronic hypoxia there was no difference in external diameter between the Hilltop and Madison groups.

Preacinar Arteries

Structure and Medial Thickness

All preacinar arteries were fully muscular, both in control and hypoxic rats.

Preacinar arteries of both control groups had similar percent medial thicknesses (Madison mean 1.4 ± 0.1 versus Hilltop 1.4 ± 0.2 [SEM]). Chronic hypoxia caused an increase in percent medial thickness in both groups, but the rise was greater in Hilltop rats (Madison hypoxia mean percent medial thickness, 3.8 ± 0.3, versus Hilltop hypoxia, 4.8 ± 0.4 [SEM], $P <$ 0.05).

External Diameter (Table 2)

The external diameter of preacinar arteries was similar in control groups. Chronic hypoxia caused a reduction in external diameter in Madison rats, as compared with controls. After chronic hypoxia, there was no difference in preacinar artery external diameter between Hilltop and Madison groups.

Lung Histology

Rats from neither colony were viral antigen-free (VAF) certified. Examination of hematoxylin and eosin-stained lung sections from the rats used in these experiments and other rats from the same colonies revealed a slight increase in wall and air-space cellularity (mononuclear) and some perivascular peribronchial mononuclear infiltrates in both colonies. In several rats (not used in this study) pulmonary abscesses were found.

Discussion

Although only 3 animals in each group underwent hemodynamic study, the response patterns and levels of vasoconstriction, hematocrit, pulmonary artery pressure, and right ventricular hypertrophy observed in control and hypoxic rats were similar to those previously reported for the Hilltop and Madison colonies. We have thus established that the hypoxic pul-

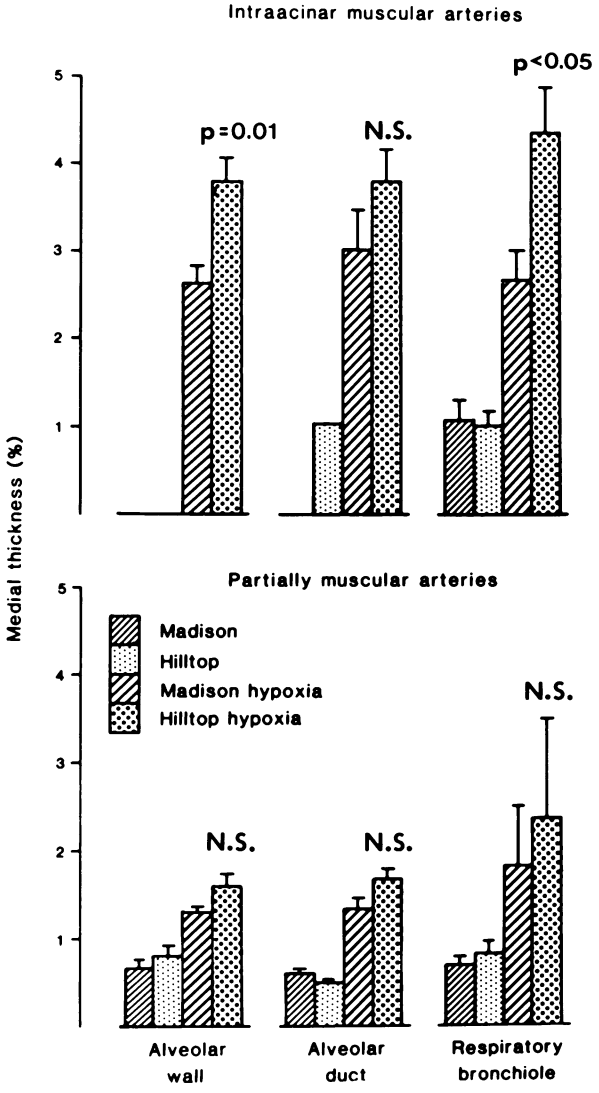

Figure 4—Percentage medial thickness of muscular and partially muscular arteries at alveolar wall, alveolar duct, and respiratory bronchiolus levels. *P* values represent differences between Madison hypoxia and Hilltop hypoxia groups. *Bar*, group mean ± SEM, calculated from individual animal mean percent medial thickness. n = 3 for control groups and n = 6 for hypoxia groups.

monary hypertension model using these colonies is consistent in different laboratories. Therefore, the structural findings in the present study can be applied to previous and future studies.

Our study shows differences between the Hilltop and Madison colonies in the degree of vascular structural response caused by chronic hypoxia. Muscularization is greater in Hilltop rats, judged either from the increase in the proportion of arteries that develop a muscular structure or from the greater medial thickness. Because the arterial external diameter is similar in both hypoxic groups, such intraacinar muscularization, quite apart from any vasoconstriction, decreases the cross-sectional area of the bed and, by restriction, contributes to increased pulmonary vascular resistance and pressure. Greater medial thickness of preacinar arteries of hypoxic Hilltop rats, as compared with Madison rats, could be a response to increased wall stress from greater pulmonary artery pressure and greater pulmonary blood volume, or it could reflect in Hilltop rats a heightened sensitivity to hypoxia of muscle cells of preacinar arteries.

RV/(LV + S) correlates well with the level of PAP.[5] We confirmed[2-4] that Hilltop rats respond to 14 days' hypoxia with higher PAP and degree of RVH, slightly higher hematocrit, and with a greater degree of vasoconstriction as compared with Madison rats. While the latter two differences contribute to the higher PAP, the relatively greater increase in muscularized arteries in Hilltop rats in itself also contributes to the pulmonary artery pressure difference between the groups.

In control animals, the only hemodynamic difference between the colonies was the greater acute pressor response in Madison rats, as previously reported.[4] After chronic hypoxia, this changed paradoxically, in that it was the Hilltop rats that had the greater vasodilator response to air. In controls, it was the Hilltop rats that had the slightly greater number of partially muscular arteries, which suggests that the degree of acute hypoxic response is determined by something other than the amount of muscle present in alveolar duct arteries. The presence of more partially muscular arteries is unlikely to cause any hemodynamically significant narrowing, but it may represent more muscle substrate, which allows the greater muscularization seen in Hilltop rats after chronic hypoxia. Muscularization of nonmuscular arteries in response to hypoxia occurs when relatively undifferentiated precursor smooth muscle cells (pericytes and intermediate cells) hypertrophy, proliferate, and differentiate.[8,9] Whether the presence of more partially muscular arteries in a group of control rats also implies a greater number of precursor smooth muscle cells in nonmuscular arteries has not yet been established.

Different susceptibility to chronic hypoxia has been studied both within a species (cattle)[10] and between species.[11] The medial thickness or percentage medial thickness of undistended arteries in sea level controls is considered to predict well the degree of pulmonary hypertension at high altitude and the response to vasoconstrictors.[10,11] The percent thickness of medial smooth muscle was the same in both control groups in our study and so did not predict the degree of pulmonary hypertension that ultimately developed. The slight increase in number of partially muscular arteries in Hilltop control rats found in this study does

Table 2—Artery External Diameter and Population Distribution

Group	Artery structure	Intraacinar: Alveolar wall ED	Alveolar wall n	Alveolar duct ED	Alveolar duct n	Respiratory bronchiole ED	Respiratory bronchiole n	Preacinar ED	Preacinar n
Madison	M	—	0	—	0	117.7 ± 8.1	6	346.1 ± 24.0	45
(n = 3)	PM	47.1 ± 5.8	9	77.9 ± 2.9	25	120.1 ± 9.7	8	—	0
	NM	39.7 ± 0.7	94	66.1 ± 3.5	83	—	0	—	0
Hilltop	M	—	0	90.5	1	125.3 ± 13.0	6	393.5 ± 33.6	45
(n = 3)	PM	48.4 ± 4.6	12	74.8 ± 4.7	40	93.2 ± 8.2	9	—	0
	NM	41.8 ± 0.7	94	64.5 ± 1.3	62	82.2	1	—	0
Madison	M	36.5 ± 1.7	9	59.3 ± 1.8	86	97.9 ± 7.8	26	271.8 ± 8.7*	78
hypoxia	PM	31.7 ± 1.2	151	46.9 ± 2.1*	119	77.7 ± 2.3	2	—	0
(n = 6)	NM	30.3 ± 1.3*	46	43.1 ± 1.8*	11	—	0	—	0
Hilltop	M	34.3 ± 2.8	27	57.5 ± 2.3	100	83.7 ± 4.7*	40	315.4 ± 21.5	87
hypoxia	PM	34.1 ± 0.8	147	47.1 ± 1.7*	84	61.6 ± 14.5	2	—	0
(n = 6)	NM	30.5 ± 1.2*	43	39.8 ± 3.5*	7	—	0	—	0

M, fully muscular; PM, partially muscular; NM, nonmuscular; ED, group external diameter in μm (mean ± SEM), calculated from individual animal mean external diameter; N, within a treatment group, the total number of arteries at a given airway landmark which are of a given structure (see Figure 3 for percentages derived from these numbers).

*$P < 0.05$ versus respective room air control group value.

not predispose to greater constrictor responses to either acute hypoxia or angiotensin II.[12]

Macek and Hakim[13] have studied rats that have acute and chronic hypoxic pressor responses similar to those of Madison rats. In their animals, the deformability of red blood cells after acute hypoxia is low but returns to normal after chronic hypoxia. Similar changes in red blood cell rheologic characteristics, were they to occur in Madison rats, could contribute to the decreased vasoconstrictive component seen after chronic hypoxia.

We have demonstrated that Hilltop rats have greater muscularization of their pulmonary artery bed, both in control rats and after chronic hypoxia. These studies have demonstrated further that the response to hypoxia is complex, and that colony differences must be considered in assessing the relative importance of factors contributing to the degree of pulmonary hypertension.

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