0031-3998/92/3102-0157$03.00/0
PEDIATRIC RESEARCH

Vol. 31, No. 2, 1992
Printed in U.S.A.

Structure-Function of Airway Generations 0 to 4 in the Preterm Lamb

SUSAN P. GAUTHIER, MARLA R. WOLFSON, KIRAN S. DEORAS, AND THOMAS H. SHAFFER

Department of Physiology and Pediatrics, Temple University, School of Medicine, Philadelphia, Pennsylvania 19140

ABSTRACT. Five generations of airways from 15 preterm lambs of 130–137 d (90% term) gestation were studied to investigate the effect of generation on structure-function of preterm airways. Airway rings were measured to determine the internal radius (r), and wall thickness (t). The ratio r/t was then calculated as a morphometric index used in the determination of wall stress. Airway rings from each generation were placed in tissue baths to compare passive, active, and total force development. Contraction via membrane depolarization (KCl) and muscarinic receptor stimulation (acetylcholine) were evaluated. As r and t decreased, r/t declined by a factor of 3.48 down the generations. At the optimal length for active force development, the passive, active, and total stresses decreased significantly as a function of generation. The receptor-mediated response to acetylcholine was significantly less in generations 0, 1, and 2 than in generations 3 and 4. No differences were found among the various generations in contractility as measured by the response to KCl. These data suggest that based on the interrelationship between airway morphometry and force development the trachea is exposed to greater wall stress than the lower airways during continuous positive airway pressure. Taken together, these data may help to explain the structural changes, such as tracheomegaly, as well as the physiologic changes in airway reactivity seen in the premature infant after mechanical ventilation. (*Pediatr Res* 31: 157–162, 1992)

Abbreviations

ACh, acetylcholine
ANOVA, analysis of variance
ASM, airway smooth muscle
AS, active stress
AS_m, maximal active force generation (at L_O)
EC_{50}, concentration producing one-half the maximal response
KBS, Krebs buffered solution
L_O, optimal length for active force development
PS, passive stress
PS_m, passive stress at L_O
PC, pars cartilaginous
PM, pars membranacea
r/t, internal radius to wall thickness ratio
TS, total stress
TS_m, total stress at L_O

Received January 8, 1991; accepted September 17, 1991.
Correspondence: Susan Gauthier, Dept. of Physiology, Temple University, School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140.
Reprint requests: Susan Gauthier, M.S.N., c/o Thomas H. Shaffer, Ph.D., Dept. of Physiology, Temple University, School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140.
Supported by NIH Fellowship 5 F31 NR06141-03.

Premature airways are highly compliant structures compared with those of the mature newborn or adult (1–4). This increased compliance can cause airway collapse at negative airway pressures, which results in an increase in resistance to airflow, flow limitation, hyperinflation, poor gas exchange, and ultimately an increased work of breathing (1, 5, 6). The immature airway is especially susceptible to the barotrauma associated with mechanical ventilation (7–11).

The decreased compliance seen with maturation may be due to changes in the structural and/or functional components of the airways. For the trachea, it was demonstrated that the main structural component, the C-ring cartilage, becomes stiffer with maturation (12) and that the cartilage type changes with age (13). Furthermore, even brief periods of ventilation induce marked changes in the structure of the preterm trachea (9). The functional force-generating component of the trachea, the tracheal smooth muscle, reduces compliance and therefore collapsibility of the immature trachea by developing tone (5, 6, 14). Both contractility and receptor sensitivity to ACh have been shown to rise with maturity in the lamb (15).

An abundance of data exists regarding the structure-function of the adult (16–18), newborn (2, 5, 19), and, more recently, the preterm (6, 11, 12, 15) trachea. Although data regarding the trachea are often used to explain the function of all large conducting airways, the structure of the trachea is markedly different from that of the more distal airways. This implies that the functional properties of the trachea may also vary from those of the lower airways. Although several studies have examined contractile responses of adult (20–24) and newborn (3, 25) bronchi, only one has examined the function of preterm airway generations (25).

The purpose of this study was to compare regional morphometry, length-stress relationships, and pharmacologic responsiveness of the trachea and more distal airways in the preterm animal. To estimate wall stress at the different generational levels, the morphometric parameter r/t, based on Laplace relationships and modified from the study of Ebina *et al.* (26), was calculated. To evaluate passive, active, and total force development as a function of airway generation, length-stress relationships were determined using an airway ring preparation and *in vitro* tissue baths. To evaluate force development as a function of receptor-dependent *versus* receptor-independent mechanisms among the airway generations, the responses to both ACh and KCl were measured and compared.

MATERIALS AND METHODS

In this study, airway rings were obtained from preterm lambs ranging in age from 130 to 137 d (88–93% gestation). The protocol for this study was approved by the Institutional Animal Care and Use Committee of Temple University, Philadelphia.

Determination of airway generation. Trachea was labeled generation 0; mainstem bronchus, generation 1; lobar bronchus,

generation 2; segmental bronchus, generation 3; and subsegmental bronchus, generation 4 (Fig. 1).

Specimen preparation. Adult pregnant ewes were sedated with ketamine HCl (5 mg/kg intramuscularly) and then received an epidural anesthesia with 0.75% bupivicaine HCl (0.5–1.0 mg/kg). After these procedures, preterm lambs were delivered via uterotomy and were killed by a lethal dose of T-61 (Hoechst-Roussel Agri-Vet, Somerville, NJ) (0.3 mg/kg). Upon death, the lungs were removed, weighed, and then placed in KBS (composition in mM: 120 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 11.5 glucose, 22 $NaHCO_3$, 1.2 NaH_2PO_4), pH 7.4 ± 0.05, at 13°C.

The airway generations were exposed immediately and within 2 h were dissected free as rings. Beyond generation 1, rings were chosen randomly from either the right or left lung. In generation 1 it was only possible to collect rings from the left lung because the right mainstem bronchus branched so abruptly that it was impossible to secure rings of sufficient width. For fine dissection, rings were placed in a Sylgard (Dow-Corning, Midland, MI)-lined petri dish filled with KBS at room temperature. Using a dissecting microscope, the bronchial rings were carefully stripped of external lung parenchymal tissue and then trimmed to uniform widths (2 mm). Care was taken to prevent damage to the epithelium. Morphometric data was gathered at this time via a graduated eyepiece micrometer.

Muscle baths. Within 24 h, each airway ring was placed in an individual tissue bath filled with 37°C KBS, through which a gas mixture (95% O_2–5% CO_2) was continuously bubbled to maintain physiologic pH. Each airway ring was supported by two triangles. One triangle was attached to a metal rod which, when lowered by means of an attached micrometer (Mitutoya no. 153–203), would increase the length of the smooth muscle in the ring. The other triangle, formed at the end of a 10-cm noncompliant wire, was attached to a strain gauge (Grass no. FT03C, Quincy, MA) to measure isometric forces (28). Before the study, each airway ring was equilibrated under zero load for a 1-h period.

To ensure that the storage of tissues (for up to 24 h) did not alter contractile or receptor responsiveness, some tissues were used immediately upon dissection, others, after storage in 13°C KBS for a 24-h period. No obvious differences were noted in maximum force generation between the two groups, as previously reported (15).

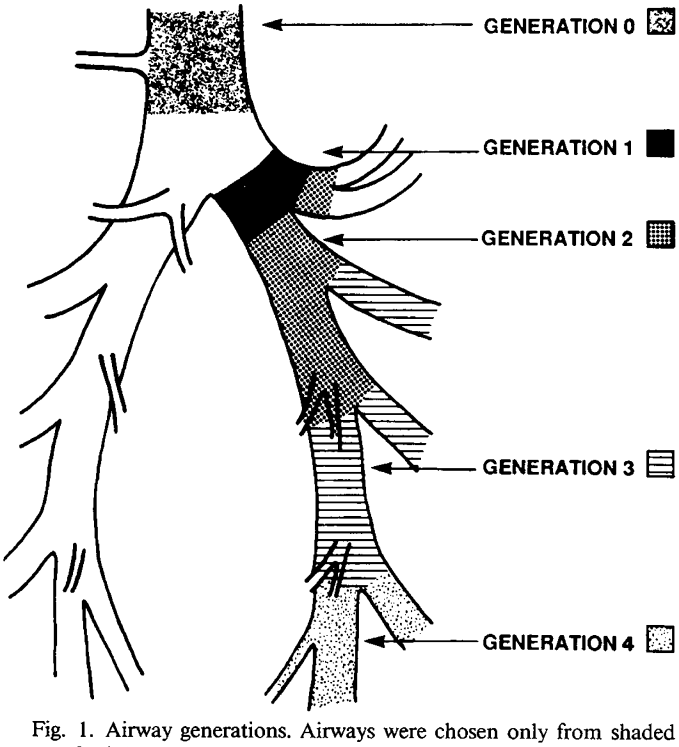

Fig. 1. Airway generations. Airways were chosen only from shaded areas of left lung and comparable areas of right lung in above diagram (except generations 0 and 1; see Materials and Methods). The generations are defined as: trachea, generation 0; mainstem bronchus, generation 1; lobar bronchus, generation 2; segmental bronchus, generation 3; and subsegmental bronchus, generation 4. Figure adapted from Hare (27).

Morphometry/histology. A total of 125 tracheal and bronchial rings from 11 preterm lambs were studied. Morphometric values, which included the internal radius (r) and wall thickness (t), were collected for each fresh airway ring. The morphometric parameter r/t was then calculated as an index of wall stress. For trachea, the thickness was measured perpendicular to the plane of the PM, across both the PM and the PC of the ring. Histologic sections were used to confirm the presence of an intact epithelium.

Length-stress studies. A total of 70 tracheal and bronchial rings from six preterm lambs were studied. PS and TS were recorded on a multichannel recorder (Grass 7P1). Each ring was stretched until an initial resting (passive) stress was first observed and then was stretched in stepped increments. At each length, the baseline was allowed to reach a steady state PS and then ACh was added to the bath for a final concentration equal to 10^{-5} M. This dose was previously shown to produce an approximately 70% maximal response in a preterm trachealis preparation (15). The ring was allowed to contract isometrically until it reached a steady state TS. AS was calculated as the difference between the steady state TS and PS, at the highest point of contraction. The ring was then washed with KBS until stress returned to baseline PS.

The rings were stretched in small increments until they showed a maximum active stress (AS_m), followed by a decline, in force development. After the decline in force development, the rings were returned to the L_O. This length was measured as the distance between the two triangular supports using a graduated eyepiece micrometer. To normalize for different initial ring lengths, all lengths were reported as a percentage of L_O, and intermediate values were calculated by linear interpolation for statistical analysis and graph clarity. Next, each ring was blotted dry on a paper towel and the wet weight was recorded. As previously described (15, 29, 30), all stresses were normalized to the cross-sectional area (A) of tissue by the equation A = M/pL, where M is mass (g), p is density (g/cm^3, assumed to be 1.05 g/cm^3), and L is the length (L_O) of the tissue (cm). Length-stress curves were constructed for each airway generation (0–4) using meaned data and then plotted (Sigmaplot; Jandel Scientific, Sausalito, CA).

To validate the length and cross-sectional area of the PM at L_O, a small number of tracheae (n = 7) were fixed at this length in 2% glutaraldehyde. Measurements were taken of the length and cross-sectional areas of the PM and PC using the dissecting microscope.

Concentration-effect studies. KCl concentration-effect curves were obtained on 42 airway rings from four preterm lambs. With each airway set to its L_O before study, KCl concentrations from 5 to 100 mM were delivered. The desired concentration of KCl was achieved by replacing an isosmotic equivalent of NaCl in the buffer with KCl. Each new concentration was delivered by replacing the entire contents of the tissue bath with the next highest concentration of KCl. Normal KBS rinses were applied between new concentrations of KCl until baseline PS was achieved.

In several pilot experiments, atropine (10^{-3} M) was added to the baths to test whether the response to KCl was due to depolarization of the smooth muscle membrane or stimulation of intramural nerve endings. After the KCl studies, airways were treated with ACh (10^{-5} M) to assess muscarinic receptor blockade.

ACh concentration-effect curves were obtained on 55 airway rings from five preterm lambs. With each ring set to its L_O, ACh final concentrations ranging from 10^{-8} to 10^{-3} M were delivered in a cumulative fashion. After the study, each ring was washed with KBS until baseline PS was achieved. Concentrations greater than 10^{-3} M were not used because they had previously been

shown to prevent the return to baseline PS in a preterm trachealis preparation (15).

Drugs and suppliers. The following drugs were used in this study: ketamine HCl (Ketalar; Parke-Davis, Morris Plains, NJ); bupivicaine HCl (Marcaine; Winthrop-Breon, New York, NY); T-61 (Hoechst-Roussel Agri-Vet); and acetylcholine and atropine sulphate (Sigma Chemical Co., St. Louis, MO).

Data analysis. For morphometric studies, a one-way ANOVA was used to determine differences in the r/t as a function of generation. For length-stress studies, all forces were normalized for cross-sectional area of tissue, and then a one-way ANOVA was used to determine if the PS_m, AS_m, or TS_m values varied with respect to generation. For concentration-effect studies, the contractile responses of the rings at L_O to each concentration were expressed as a percentage of the maximum response, averaged, and plotted against concentration. The EC_{50} was calculated from individual concentration-effect curves, and then a one-way ANOVA was used to determine if the EC_{50} values differed as a function of generation. Statistical significance was accepted at the $p < 0.05$ level.

RESULTS

Morphometry/histology. Morphometric analysis showed that as the internal radius and wall thickness decreased, the r/t value decreased progressively as generation number increased; r/t was 348% greater in generation 0 than in generation 4 (Table 1). Airway rings used in muscle bath studies showed an intact epithelium upon histologic examination.

Length-stress studies. PS in each generation of airway increased as the length of the ring was increased (Fig. 2*A*). As shown in Table 2, PS_m of generation(s) 0, 1, and 2 was greater than that of generation(s) 3 and 4; generation 0 was greater than generation 4 by approximately 54% ($p < 0.05$).

The AS curve displayed a length dependency such that as the ring, and therefore the smooth muscle of the ring, was stretched active force development increased until the L_O was reached (Fig. 2*B*). Stretching the ring beyond L_O resulted in a decrease in active force development. The AS_m of generation 0 differed significantly ($p < 0.01$) from that of generation(s) 1–4, whereas the AS_m of generation(s) 1–4 did not differ from each other (Table 2). The AS_m of generation 0 was 42% greater than that of generation 4. Similarly, it was shown the TS_m of generation 0 was greater than that of generation(s) 1–4 ($p < 0.01$). The TS_m of generation 0 was 36% greater than that of generation 4 ($p < 0.01$).

Results of the glutaraldehyde fixation studies revealed that there was no significant difference in the length of the PM at L_O when measured as the difference between the triangular supports or when measured under the microscope in the fixed state. Direct measurements of the cross-sectional areas of the PM and PC fixed at L_O varied by less than 5% from those calculated with the formula described in Materials and Methods. Also, the cross-sectional area values for the PM and PC were within 10% of each other.

Table 1. *Morphometry of live airways**

Gen	*n*	r (cm)†	t (cm)‡	r/t§
0	20	0.246 ± 0.011	0.136 ± 0.008	2.077 ± 0.187
1	12	0.132 ± 0.007	0.131 ± 0.009	0.971 ± 0.109
2	26	0.085 ± 0.004	0.112 ± 0.005	0.783 ± 0.042
3	36	0.056 ± 0.002	0.085 ± 0.004	0.701 ± 0.037
4	31	0.042 ± 0.002	0.070 ± 0.003	0.597 ± 0.041

* Values are means ± SEM. Gen, generation; r, internal radius; and t, wall thickness.

† r of all airways differed at $p < 0.01$, except that generations 3 and 4 did not differ from each other.

‡ t of all airways differed at $p < 0.01$, except that generations 3 and 4 differed at $p < 0.05$.

§ r/t of generation 0 was greater than generations 1–4 ($p < 0.01$); generation 1 was greater than generation 3 ($p < 0.05$) and generation 4 ($p < 0.01$).

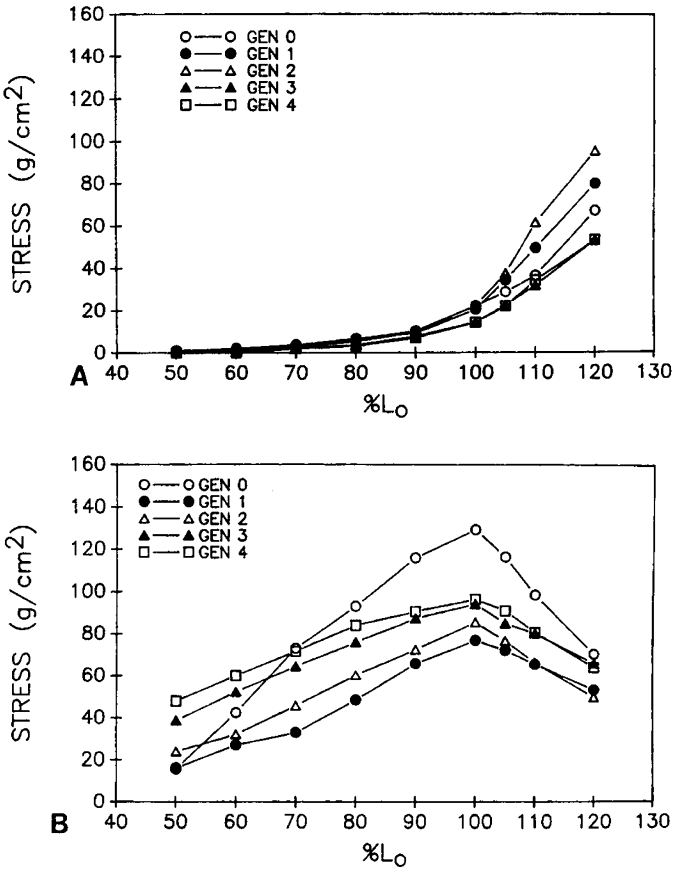

Fig. 2. *A,* PS curves for preterm airway generation(s) 0–4. Values are means at each length (n = 9, 6, 11, 17, and 14 for generation(s) 0–4, respectively). SEM bars excluded for clarity. *B,* AS curves for preterm airway generation(s) 0–4. Values are means at each length (n = 9, 6, 11, 17, and 14 for generation(s) 0–4, respectively). SEM bars excluded for clarity.

Table 2. *PS_m, AS_m, and TS_m**

Gen	*n*	PS_m	AS_m	TS
0	9	22.13 ± 2.32†	131.98 ± 6.59‡	149.76 ± 6.70‡
1	6	20.51 ± 2.50	77.08 ± 7.59	97.59 ± 9.43
2	11	22.51 ± 2.49†	85.90 ± 5.73	107.17 ± 7.85
3	17	14.24 ± 1.45	97.25 ± 6.17	108.18 ± 6.16
4	14	14.33 ± 2.04	93.11 ± 5.79	110.19 ± 7.01

* Values are means ± SEM (g/cm²). Gen, generation.

† PS_m of generations 0 and 2 was greater than generations 3 and 4 ($p < 0.05$).

‡ AS_m and TS_m of generation 0 was greater than generations 1–4 ($p < 0.01$).

Concentration-effect studies. For all airway generations set to L_O, increasing the concentration of KCl or ACh resulted in an increase in active force development (Fig. 3*A* and *B*).

As shown in Figure 3*A*, none of the airways responded to 10 mM KCl; the initial response occurred in all airway generations when 20 mM was delivered. A plateau value was reached between 40 and 80 mM; beyond 80 mM, a slight decline in force development usually occurred. There were no differences in developed forces across airway generations for the tested range of KCl concentrations. Also, no differences were noted in those airways pretreated with atropine and those that were not.

After the KCl studies above, all airways were treated with a 10^{-5}-M dose of ACh. All airways in the atropine-free group

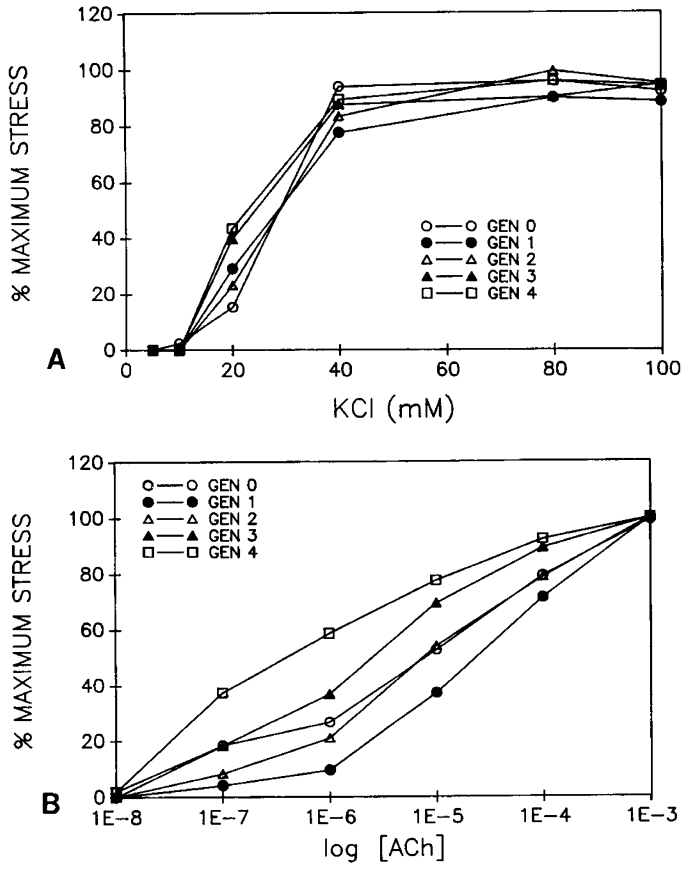

Fig. 3. *A*, Effect of KCl stimulation on preterm airway generations 0–4. Values are means for each concentration (n = 4, 4, 10, 16, and 9 for generation(s) 0–4, respectively). SEM bars excluded for clarity. *B*, Effect of ACh stimulation on preterm airway generations 0–4. Values are means for each concentration (n = 9, 5, 12, 17, and 12 for generation(s) 0–4, respectively). SEM bars excluded for clarity.

Table 3. *EC_{50} values for ACh**

Gen	n	EC_{50}	±SEM
0	9	10.43†	3.32
1	5	31.48‡	9.86
2	12	9.53†	1.88
3	17	3.75	0.83
4	12	0.50	0.15

* Values are means ($\times 10^{-6}$ M). Gen, generation.

† Generations 0, 1, and 2 were greater than generation 4 ($p < 0.05$).

‡ Generation 1 was greater than all other generations ($p < 0.01$).

responded to ACh, whereas this response was totally abolished in the atropine-treated group.

As shown in Figure 3*B*, no force was developed in any airway in response to ACh 10^{-8} M; the initial response for all airway generations occurred with the delivery of 10^{-7} M. The EC_{50} values of generation(s) 0, 1, and 2 were all significantly greater than that of generation 4 (Table 3).

No differences were found, in any measured parameter, between the right and left lungs.

DISCUSSION

The trachea has been used as a model of airway function in adult (16, 17, 31, 32), newborn (5, 19, 33), preterm (6, 11, 14), and developmental (2, 4, 8, 12, 15, 18) studies in a variety of animal species. The trachea has a unique anatomical position and structure that may qualify it to perform a unique function. Differences in anatomical position and structure of lower airways may confer unique functional differences to the airway generations as compared to the trachea. Several investigators have studied the function of airway generations in adult animals (20–24, 28, 34), but very little is known regarding the function of preterm airway generations (25).

The morphometric parameter r/t was used in this study to compare the relative stresses imposed upon the various airway generations during specific moments in time when airway pressures are equal, such as with continuous positive airway pressure ventilation. The law of Laplace states that the wall stress (WS) of a cylinder is equal to the transmural pressure (P) times the internal radius (r) divided by the wall thickness (t), or:

$$WS = P\ (r/t)$$

If the transmural pressure of all intrathoracic airways within the bronchial tree is equal, as it is during certain phases of continuous positive airway pressure ventilation, then at those times wall stress is directly proportional to the parameter r/t, or:

$$WS\ \alpha\ (r/t)$$

In this study, it was shown that the r/t values of the uppermost airways were greater than those of the lower airways and that there was a steady decline in r/t down the airway generations. Overall, there was a 348% decline in r/t from generation 0 to generation 4. These differences imply that higher airways experience greater wall stress during positive pressure ventilation. To offset this effect, the upper airways would require a mechanism to protect their walls from the possibility of strain. Such a mechanism could be either a wall with inherently stronger structural components or one that could produce a physiologic response capable of supporting the wall against such stress. A structurally stronger wall should be less compliant and as such should display greater PS when applied forces attempt to stretch it. Similarly, an increased ability to generate active stress could also lower compliance and therefore serve to fortify the wall.

PS developed by generation(s) 0, 1, and 2 were similar to each other but significantly greater than those of generation(s) 3 and 4. This decreased compliance may be due to several factors: a greater proportion of cartilage, less compliant cartilage or intervening connective tissue, a type of smooth muscle that is intrinsically less compliant at rest, or a geometric arrangement that causes similar tissues to behave differently. Although the present study did show that the PS_m was greater in generation(s) 0, 1, and 2 than in generation(s) 3 and 4, it was only higher by a factor of 1.54 (generation 0 *versus* 4) and thus was disproportionate to the 3.48-fold increase in r/t reported above. Based on this evidence, it seems unlikely that the largest airways rely solely on inherent structural support to protect against pressure-induced strain.

The active stress curves for each generation of airway revealed that the trachea was able to generate more AS_m than any other airway generation and that generation(s) 1–4 were similar to each other in AS_m development. This greater force development may result from a larger proportion of smooth muscle in the wall or to a more favorable geometric arrangement of muscle bundles. It may also be due to unique intracellular features such as a greater amount and/or more efficient type of actomyosin. Although the AS_m of generation 0 was 1.42 times greater than generation 4, this higher force development is not proportional to the increased r/t values seen proximally. These data indicate that even when structural and physiologic wall stresses are generated in the preterm airways, the larger airways are still at a mechanical disadvantage and are more likely to deform because of their structure-function relationships.

All airway generations in this study reacted to KCl stimulation in a similar fashion, indicating that electrophysiologic mechanisms are similar (Fig. 3*A*). KCl elicits a contraction by depolarizing the membrane and is not dependent on a receptor-mediated mechanism (35). It has been reported previously that KCl may

stimulate intramural nerve endings to release ACh (36). However, there was no difference in AS development between airways pretreated with atropine and those that were untreated.

ACh elicited a response in all atropine-free airways and in none of the atropine-treated airways, indicating the presence of functional ACh receptors in airway generations 0–4. Analysis of concentration-effect curves (Fig. 3*B*) and EC_{50} values (Table 3) shows that the EC_{50} values were greatest in generation(s) 0, 1, and 2 and least in generation(s) 3 and 4. This may represent differences in the type, density, or distribution of receptors within the smooth muscle or a diffusion-limitation phenomenon. A diffusion barrier effect is not likely, inasmuch as all airway generations were given ample time to contract and no generation displayed an increase in AS development over time, even when time periods were lengthy. Ebina *et al.* (26) recently detailed the distribution of ASM in the adult human bronchial tree and found that the relative amount of ASM increased down the airway generations. If there is a preponderance of ASM in the more distal airways and these airways are more sensitive to ACh, they may be more able than the upper airways to stabilize their walls against pressure-induced deformity, especially in light of their more favorable morphometry.

Airway rings were chosen for these studies because the exact intraairway geometric arrangement of epithelium, smooth muscle, cartilage, and other connective tissues remained intact. The rings were studied *in vitro* so that the responses of the tissues were freed from any systemic neural or humoral influences that might alter the responses of the tissues via potential reflex pathways. The procedure of normalizing the force production to the cross-sectional area of tissue that was used in this study is a standard one in smooth muscle research (15, 29, 30). Although the precision of this method could be challenged, so could any normalization process, inasmuch as the definitive factor responsible for force generation remains unclear. For example, normalization for smooth muscle cross-sectional area may not be accurate if muscle bundle geometry is not taken into consideration or if the relative amount of actomyosin within smooth muscle cells varies at different locations within the bronchial tree. Similarly, normalization for actomyosin cross-sectional area would be no more reliable if the actin and/or myosin isoforms varied with airway generation. In the present study, the net radial force generation of airways whose geometry was preserved was of primary interest; therefore, force generation was normalized for the cross-sectional area of the entire airway ring.

Changes in pulmonary structure and function occur with development and are especially prominent during the late prenatal and early neonatal periods (1, 2, 4, 12). Developmental changes in airway mechanics may result from geometric remodeling and/or alterations of the passive or active tissue properties. It is well known that preterm airways are more compliant than those of the newborn or adult (1, 3, 6, 12, 19). This increased compliance may result in airway collapse during ventilation and is especially problematic during assisted ventilation because the immature airways are subjected to high pressures that increase wall stress and may strain the airway wall (8, 11). The high pressures associated with assisted ventilation are known to produce a distention deformity that may cause permanent airway damage (7–9). The increased radii of damaged airways increase pulmonary dead space and resistance to airflow and, therefore, increase the work of breathing (11). ASM contraction has been shown to decrease compliance and resistance and to increase flow in the immature trachea (6). Although increasing ASM tone may ameliorate barotraumatic injury to the airways, based on structure-function results presented here, the larger airways appear to be at an inherent mechanical disadvantage in resisting such injury.

Finally, the present study shows that, although there were significant regional differences in the receptor-mediated ACh response such that the lowest airways were much more responsive, contractility via membrane depolarization with KCl was the same for all airway generations. These findings raise important questions regarding the use of pharmacologic agents to modulate smooth muscle tone in preterm airways.

Acknowledgments. The authors thank Steven P. Driska, Ph.D., Howard B. Panitch, M.D., and James R. Ryan, Ph.D., for their suggestions and advice in planning and organizing the collection of this data and in the preparation of this manuscript. We also thank Robert Roache for the technical assistance provided throughout this investigation.

REFERENCES

1. Burnard ED, Grattan-Smith P, Picton-Warlow CG, Grauaug A 1965 Pulmonary insufficiency in prematurity. Aust Paediatr J 1:12–38
2. Bhutani VK, Rubenstein SD, Shaffer TH 1981 Pressure-volume relationships of tracheae in fetal newborn and adult rabbits. Respir Physiol 43:221–231
3. Croteau JR, Cook CD 1961 Volume-pressure and length-tension measurements in human tracheal and bronchial segments. J Appl Physiol 16:170–172
4. Shaffer TH, Bhutani VK, Wolfson MR, Penn RB, Tran AA 1989 *In vivo* mechanical properties of the developing airway. Pediatr Res 25:143–146
5. Bhutani VK, Koslo RJ, Shaffer TH 1986 The effect of tracheal smooth muscle tone on neonatal airway collapsibility. Pediatr Res 20:492–405
6. Penn RB, Wolfson MR, Shaffer TH 1988 Effect of tracheal smooth muscle tone on collapsibility of immature airways. J Appl Physiol 65:863–869
7. Bhutani VK, Ritchie WG, Shaffer TH 1986 Acquired tracheomegaly in very preterm neonates. Am J Dis Child 140:449–452
8. Bhutani VK, Rubenstein D, Shaffer TH 1981 Pressure-induced deformation in immature airways. Pediatr Res 15:829–832
9. Deoras KS, Wolfson MR, Bhutani VK, Shaffer TH 1989 Structural changes in the tracheae of preterm lambs induced by ventilation. Pediatr Res 26:434–437
10. O'Brodovich HM, Mellins RB 1985 Bronchopulmonary dysplasia (state of the art). Am Rev Respir Dis 132:694–709
11. Penn RB, Wolfson MR, Shaffer TH 1988 Effect of ventilation on mechanical properties and pressure-flow relationships of immature airways. Pediatr Res 23:519–524
12. Penn RB, Wolfson MR, Shaffer TH 1989 Developmental differences in tracheal cartilage mechanics. Pediatr Res 26:429–433
13. Deoras KS, Wolfson MR, Searls RL, Hilfer SR, Sheffield JB, Shaffer TH 1990 The use of a touch sensitive screen and computer assisted image analysis for quantitation of developmental changes in pulmonary structure. Pediatr Pulmonol 9:109–118
14. Penn RB, Wolfson MR, Shaffer TH 1988 Influence of smooth muscle tone and longitudinal tension on the collapsibility of immature airways. Pediatr Pulmonol 5:132–138
15. Panitch HB, Allen JL, Ryan JP, Wolfson MR, Shaffer TH 1989 A comparison of preterm and adult airway smooth muscle mechanics. J Appl Physiol 66:1760–1765
16. Coburn RF, Palombini B 1972 Time-dependent pressure-volume relationships of the *in vivo* canine trachea. Respir Physiol 16:282–289
17. Olsen CR, Stevens AE, Pride NB, Staub NC 1967 Structural basis for decreased compressibility of constricted tracheae and bronchi. J Appl Physiol 23:35–39
18. Hayashi S, Toda N 1980 Age-related alterations in the response of rabbit tracheal smooth muscle to agents. J Pharmacol Exp Ther 214:675–681
19. Koslo RJ, Bhutani VK, Shaffer TH 1986 The role of tracheal smooth muscle contraction on neonatal tracheal mechanics. Pediatr Res 20:1216–1220
20. Shioya T, Solway J, Munoz NM, Mack M, Leff AR 1987 Distribution of airway contractile responses within the major diameter bronchi during exogenous bronchoconstriction. Am Rev Respir Dis 135:1105–1111
21. Shioya T, Munoz NM, Leff AR 1987 Effect of resting smooth muscle length on contractile response in resistance airways. J Appl Physiol 62:711–717
22. Shioya T, Pollack ER, Munoz NM, Leff AR 1987 Distribution of airway contractile responses in major resistance airways of the dog. Am J Pathol 129:102–117
23. Stuart-Smith K, Vanhoutte PM 1987 Heterogeneity in the effects of epithelium removal in the canine bronchial tree. J Appl Physiol 63:2510–2515
24. Stuart-Smith K, Vanhoutte PM 1988 Airway epithelium modulates the responsiveness of porcine bronchial smooth muscle. J Appl Physiol 65:721–727
25. Sparrow MP, Mitchell HW 1990 Contraction of smooth muscle of pig airway tissues from before birth to maturity. J Appl Physiol 68:468–477
26. Ebina M, Yaegashi H, Takahashi T, Motomiya M, Tanemura M 1990 Distribution of smooth muscles along the bronchial tree. A morphometric study of ordinary autopsy lungs. Am Rev Respir Dis 141:1322–1326
27. Hare WCD 1955 The bronchopulmonary segments of the sheep. J Anat 89:387–402
28. Flavahan NA, Aarhus LL, Rimele TJ, Vanhoutte PM 1985 Respiratory epithelium inhibits bronchial smooth muscle tone. J Appl Physiol 58:834–838
29. Denehy CM, Ryan JR 1986 Development of gallbladder contractility in the guinea pig. Pediatr Res 20:214–217
30. Herlihy JT, Murphy RA 1973 Length-tension relationship of smooth muscle of the hog carotid artery. Circ Res 33:275–283

31. Coburn RF, Thornton D, Arts R 1972 Effect of trachealis contraction on tracheal resistance to airflow. J Appl Physiol 32:398–403
32. Knudson RJ, Knudson DE 1975 Effect of muscle constriction on flow-limiting collapse of isolated canine trachea. J Appl Physiol 38:125–131
33. Murphy TM, Mitchell RW, Blake JS, Mack MM, Kelly EA, Munoz NM, Leff AR 1989 Expression of airway contractile properties and acetylcholinesterase activity in swine. J Appl Physiol 67:174–180
34. Martin HB, Proctor DF 1958 Pressure-volume measurements on dog bronchi. J Appl Physiol 13:337–343
35. Bolton TB 1979 Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 59:607–718
36. Vanhoutte PM, Verbeuren TJ, Lorenz RR 1975 Effects of potassium ions on nerve endings and effector cells in adrenergically innervated vascular smooth muscle. Colloq INSERM 50:425–442