0031-3998/91/3002-0170$03.00/0
PEDIATRIC RESEARCH

Vol. 30, No. 2, 1991
Printed in U.S.A.

Developmental Changes in Tracheal Structure

KIRAN S. DEORAS, MARLA R. WOLFSON, ROBERT L. SEARLS, S. ROBERT HILFER, AND THOMAS H. SHAFFER

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

ABSTRACT. Mechanical properties of the proximal airways are known to change with development; the highly compliant airways of the immature animal become stiffer and less collapsible with increasing age. Although the relationship between tracheobronchial architecture and function has been described for adult physiology, little is known regarding this relationship during early development. This study was, therefore, designed to test the hypothesis that alterations in tracheal morphometry parallel developmental differences in tracheal functional properties. Tracheal segments obtained from 29 lambs ranging in age from 70% of gestation to full-term newborn lambs up to 6 d old were examined using anatomic, morphometric, and histochemical techniques. The results showed *1)* progressive increases in the dimensions of the trachea and the tracheal wall components, *2)* alterations in the geometric arrangement of the tracheal ring, and *3)* changes in the compositional characteristics of the tracheal cartilage with maturation. These findings demonstrate alterations in tracheal architecture, each of which contribute to the greater stiffness of the trachea, in older animals. When considered together, these factors help explain the differences in tracheal functional characteristics with development. (*Pediatr Res* 30: 170–175, 1991)

Abbreviations

GAG, glycosaminoglycan

Several investigators have examined the mechanical properties of the developing airways, both in humans and in various animal species (1–6). These studies indicate that the airways from preterm neonates are highly compliant and collapsible structures that become stiffer and better able to withstand pressure-induced deformation with increasing maturity. The greater collapsibility of the immature airways predisposes premature infants to subsequent abnormalities of pulmonary function such as increased dead space with gas trapping, poor gas exchange, increased airway resistance, and increased work of breathing (5, 7).

The functional properties of the adult tracheobronchial tree are known to reflect variations in the anatomical structure of the tracheobronchial wall (2, 3, 8–10). There is, however, little information regarding the structural basis for the differences in the functional properties of the airways during early development.

Mechanical properties of the trachea have been extensively evaluated (1–5, 8, 11–15). To test the hypothesis that alterations in tracheal structure parallel developmental differences in tracheal functional characteristics, we examined tracheal structure over a range of development from 70% of gestation to the neonatal period. Anatomic, morphometric, and histochemical techniques were used to study and quantify tracheal structural characteristics from preterm and full-term newborn lambs. Correlation of these findings with existing information on tracheal mechanics may aid in understanding how the premature infant differs from the full-term neonate with regard to airway structure function.

Received May 3, 1990; accepted April 22, 1991.
Correspondence: Kiran S. Deoras, M.B.B.S., Ph.D., c/o Thomas H. Shaffer, Ph.D., Department of Physiology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140.
Supported in part by the National Institutes of Health Public Health Service Grant 32031.

MATERIALS AND METHODS

Tracheal segments (2–3 cm in length) were obtained from 21 preterm lambs, 105 to 145 d gestational age (term, 147 ± 3 d) and eight full-term newborn lambs, 1 to 6 d postnatal age (developmental age range, 105–153 d). All segments were obtained within 0.5 h of killing the animal, and care was taken to ensure that segments were excised from the same region and the same tracheal rings were studied (i.e. the 2nd and 3rd rings below the cricoid) from each animal, to avoid differences due to regional variation. This study was a part of other investigations regarding pulmonary function (*in vivo* and *in vitro* studies) in preterm and newborn lambs (4, 5, 16), and the protocols for these studies had been approved by the Institutional Animal Care and Use Committee, Temple University, Philadelphia.

Morphology. Tracheal segments were uniformly fixed in 2% glutaraldehyde in phosphate buffer; no transmural pressure was applied to prevent architectural distortion. Routine histologic techniques, as described below, were then used to prepare the segments for light microscopic examination (17). The segments were dehydrated in increasing concentrations of ethyl alcohol, cleared in cedarwood oil, and embedded in paraffin. Transverse sections (7 μm thick) cut serially with a microtome, throughout the thickness of at least one tracheal ring (*i.e.* between two adjacent intercartilagenous spaces), were mounted on glass slides preparatory to staining. Milligan's Trichrome (17) was used to stain the tracheal sections, coloring the muscle magenta and cartilage green, thus enhancing the contrast between the principal tracheal wall components. The sections were examined by light microscopy and used to conduct morphometric measurements.

Morphometry. Airway dimensions were quantified using a computerized image analysis system (18–20) in conjunction with a light microscope. This system uses contour tracing and computes both morphometric and densitometric parameters of user-selected regions of histologic slides. The slides were placed under the microscope, presented via a camera to an image processing board in the computer system, digitized, and displayed on a video monitor coupled to a touch screen. Selected areas were traced onto the touch screen for analysis of morphometric data. The luminal diameter, cross-sectional area, and internal and external circumference of the tracheal section were measured along with the length, area, and thickness of tracheal cartilage and muscle. Several measurements were made in each case and average values taken. Because both cartilage and muscle thick-

ness varied along their lengths, measurements were made at different points and an average value determined. The radius was calculated from the average diameter.

Because the fibers of the trachealis stained with a different color and intensity compared to the surrounding and intervening connective tissue, they were outlined using the densitometric function of the image analysis system before computation of their dimensions (18). By measuring only the dimensions of the muscle fibers, this procedure enabled the determination of muscle mass. The percentage of muscle fibers within the trachealis bundle was also calculated (area of muscle fibers per unit area of muscle bundle).

The dimensions of every 5th section in one ring of the trachea were similarly computed (preliminary studies of sections through the entire tracheal ring indicated that tracheal dimensions remained relatively unchanged through at least five adjacent 7-μm sections). The amounts of muscle and cartilage per tracheal ring were then determined from the area of muscle or cartilage per section and the number of sections per ring.

Histochemistry. To identify any differences in the compositional characteristics of tracheal cartilage, tracheal segments were obtained from two groups of lambs representing the preterm (n = 5; 114–119 d gestational age) and full-term newborn animals (n = 5; 3–6 d postnatal age) and were processed for paraffin embedding. The paraffin blocks were carefully trimmed to include only the trachael cartilage and 7-μm sections prepared as described previously (25 sections per animal). These sections were investigated histochemically using the Alcian Blue 8GX (pH 2.5) staining technique to investigate the presence and relative amount (as reflected by the staining intensity, compared with background) of sulfated and carboxylated mucosubstances (GAG) in the paraffin sections of tracheal cartilage from the two groups of animals. This technique is based on the electrostatic binding of polycationic functional groups on a pthalocyanine ring to anionic functional groups in tissues (21, 22). The densitometric function of the image analysis system was used to measure the relative staining intensity of Alcian Blue as follows: the slides were placed under the microscope and focused on the video monitor such that half the screen was blank to serve as background, and half was occupied by the image of the tracheal cartilage. The color intensity of the pixels, per unit area of cartilage, was determined relative to the intensity of the pixels in the background portion of the screen. All sections were examined in the same manner while ensuring a constant background intensity. Selected sections from both groups of preterm and term animals were pretreated with bovine testicular hyaluronidase (1 mg/mL in acetate buffer; incubation period 1 h) to remove hyaluronate, chondroitin, and chondroitin sulfate from the tissue sections before staining (23, 24). A reduction in the intensity of Alcian Blue staining in enzyme-treated adjacent sections when compared with untreated (n = 10) or control (n = 10) sections would confirm the presence of GAG in the tissues.

Data analysis. Linear regression analyses were used to correlate the measured tracheal dimensions (radius, cross-sectional area, internal and external circumference, cartilage length, muscle length, cartilage area, muscle area, cartilage thickness, muscle thickness, amount of cartilage, and amount of muscle) to developmental age.

The following ratios were calculated: cartilage length/section:muscle length/section, cartilage area/section:muscle area/section, and amount of cartilage/tracheal ring:amount of muscle/tracheal ring. To assess age-related changes in the dimensions of tracheal cartilage relative to muscle, the above-mentioned ratios were correlated with developmental age using linear regression analyses.

To evaluate developmental alterations in tracheal wall thickness in relation to luminal size, the ratios of cartilage thickness:tracheal radius and muscle thickness:radius were determined and plotted against developmental age.

The percentage of muscle fibers within the muscle bundle were also correlated with developmental age to evaluate any changes in functional muscle mass with maturity.

The above linear regression coefficients were also tested for statistical significance, and significance was accepted at the $p < 0.05$ level.

Differences in the relative amounts of GAG and collagen in the tracheal cartilage between the preterm and full-term newborn lamb tracheal sections (*i.e.* differences in color intensity, relative to a constant background, with Alcian Blue) were analyzed for significance using an unpaired, two-tailed t test. p values of less than 0.05 were considered significant.

RESULTS

Morphology. We examined tracheal segments from a total of 29 lambs ranging in age from 105 d of gestation to 6 d postnatal (*i.e.* 105 to 153 d developmental age). The increase in developmental age was accompanied by a linear increase in birth weight, from 1.05 kg in the youngest animal to 10.23 kg in the oldest animal ($r = 0.81$; $p < 0.05$).

Upon visual inspection, the tracheal segments appeared cylindrical, and in the preterm animals, the posterior wall demonstrated an overlap of the ends of cartilage. Light microscopic examination of transverse sections of the trachea from all animals showed roughly circular outlines, with the tracheal walls composed mainly of cartilage and muscle and lined internally by ciliated columnar epithelium. The posterior tracheal wall contained the smooth muscle fibers of the trachealis, which bridged the gap between the free ends of cartilage and inserted along the inner perichondrium. A change in the geometric arrangement of the tracheal cartilage occurred with development. In animals less than 145 d gestational age, the posterior free ends of cartilage, beyond the smooth muscle-cartilage junction, overlapped each other in a highly deformed manner and adopted different configurations in all of the segments examined (Fig. 1). In the older animals, the overlap was greatly decreased or absent with the free ends of cartilage abutting each other, and the configuration of cartilaginous ends was seen to be similar in all of the segments studied (Fig. 2). (Note: To present similar views of the posterior tracheal wall in both the younger and older animals, it was necessary to use different magnifications to photograph the sections.) No differences were noted in the manner of muscle insertion.

Morphometry. Dimensions of the tracheal section, the radius, cross-sectional area, and internal and external circumference, increased with increases in developmental age. As shown in Figure 3, correlation analyses demonstrated a significant linear

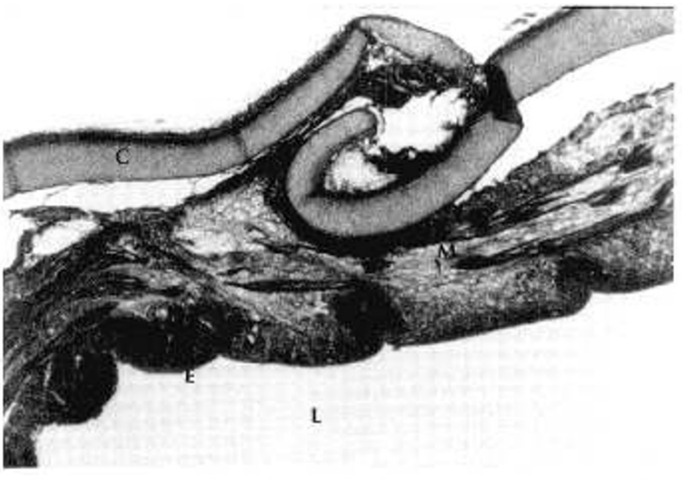

Fig. 1. Representative micrograph of a transverse tracheal section from a preterm lamb, 107 d developmental age. *E*, epithelium; *C*, cartilage; *L*, lumen; and *M*, muscle. Note the deformability of the free ends of overlapped cartilage in the posterior wall (magnification ×60; stain: Milligan's trichrome).

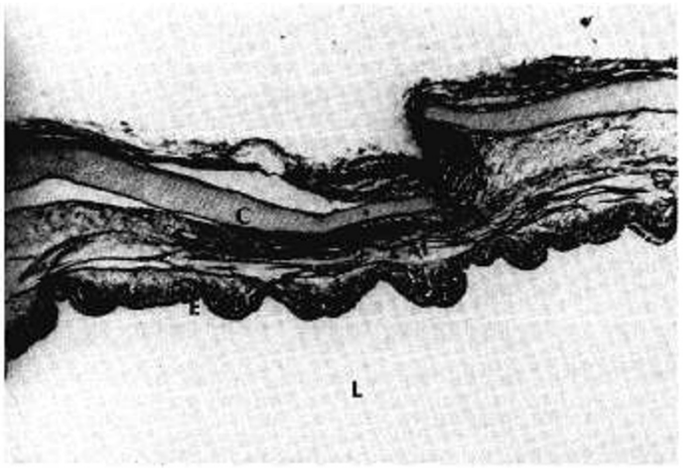

Fig. 2. Typical transverse section of the trachea from a full-term newborn lamb, 153 d developmental age, showing the posterior tracheal wall with no overlap of the free ends of cartilage (magnification ×30; stain: Milligan's trichrome). *E*, epithelium; *C*, cartilage; *L*, lumen; and *M*, muscle.

correlation between each of these parameters and developmental age ($p < 0.05$).

Tracheal cartilage and trachealis muscle sections increased linearly with developmental age ($p < 0.05$), and growth occurred proportionately, with the ratio of cartilage to trachealis muscle length remaining relatively unchanged over the age range studied (Fig. 4). Similarly, the observed linear increases in the area of tracheal cartilage and muscle cartilage per tracheal section (area = 0.26 developmental age −21.57, $r = 0.79$, $p < 0.05$; and trachealis area = 0.03 developmental age −2.72, $r = 0.79$, $p < 0.05$) were proportional to each other and the ratio of cartilage to smooth muscle area was relatively constant with development.

The amounts of cartilage and muscle per tracheal ring calculated as described before correlated significantly ($p < 0.05$) with developmental age. Both components showed a 7- to 8-fold increase from the youngest to the oldest animal studied, resulting in no significant difference in the ratio of the amounts of cartilage to muscle with development (Fig. 5).

Regression analyses also indicated significant ($p < 0.05$) linear correlation between both cartilage thickness and developmental age, and muscle thickness and developmental age. To obtain the relationship between wall thickness and luminal size as a function of age, ratios of cartilage thickness to radius and muscle thickness to radius were determined, and each ratio was plotted against developmental age (Fig. 6). These ratios were observed to remain unchanged during the period of development studied.

Within the trachealis muscle bundle, smooth fibers were separated by a space containing an amorphous matrix. The proportion of fibers in the muscle bundle did not change significantly with development, nor did the proportion of intervening connective tissue. The muscle fiber:muscle bundle ratio was 72.85% in the youngest animal compared with 72.69% in the oldest animal studied (Fig. 7).

Histochemistry. Table 1 details the relative staining intensity of the tracheal cartilage and provides a semiquantitative assessment of the GAG content of tracheal cartilage.

Studies using the Alcian Blue 8GX (pH 2.5) staining technique for GAG showed a 20% greater staining intensity ($p < 0.05$) of tracheal cartilage in the group of older animals compared with the younger animals. After enzymatic digestion by testicular hyaluronidase, the staining reaction was absent in the sections from the group of younger animals and was greatly decreased in the group of older animals.

DISCUSSION

Various changes in pulmonary function are known to occur with development, especially in the perinatal and neonatal periods (1, 6, 7). These include an increase in the volume, compliance, and conductance of the lung and a decrease in the resistance, specific conductance, and compliance of the airways with maturity. The increase in lung volume, which occurs as a function of both age and body weight, is necessary to support the functional and metabolic requirements associated with growth and is attributed to alveolarization as well as an increase in airway dimensions (6, 7). The greater lung volume in older neonates also contributes to the improved lung compliance seen with maturity, which in turn depends mainly on the anatomical and biochemical maturation of the respiratory apparatus. The increase in lung conductance seen with development may be explained on the basis of increasing airway dimensions and airway branching (6).

Developmental differences in airway mechanics may be affected by alterations in airway caliber, as well as changes in the active and passive tissue components of their walls. For example, in the case of the trachea, the major components of the tracheal wall that contribute to its mechanical properties are the tracheal

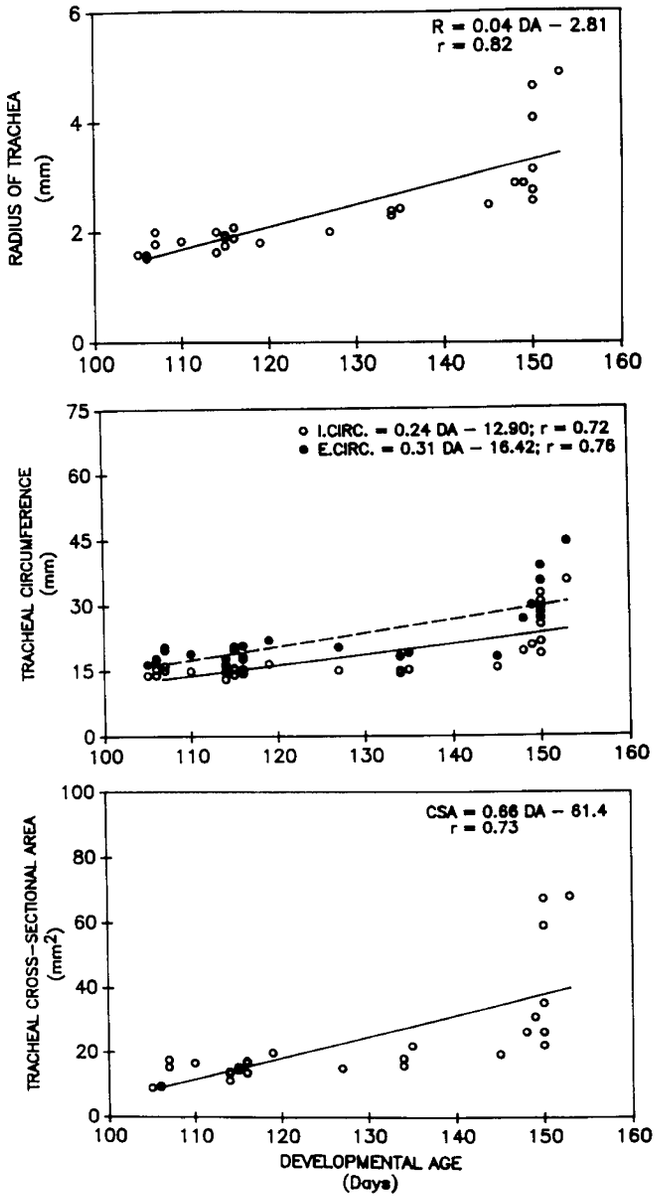

Fig. 3. The figure shows significant linear relationships ($p < 0.05$) of (*top*) tracheal radius (*R*); (*middle*) internal (*I. CIRC*) and external (*E. CIRC*) circumference; and (*bottom*) tracheal cross-sectional area (*CSA*) with developmental age (*DA*).

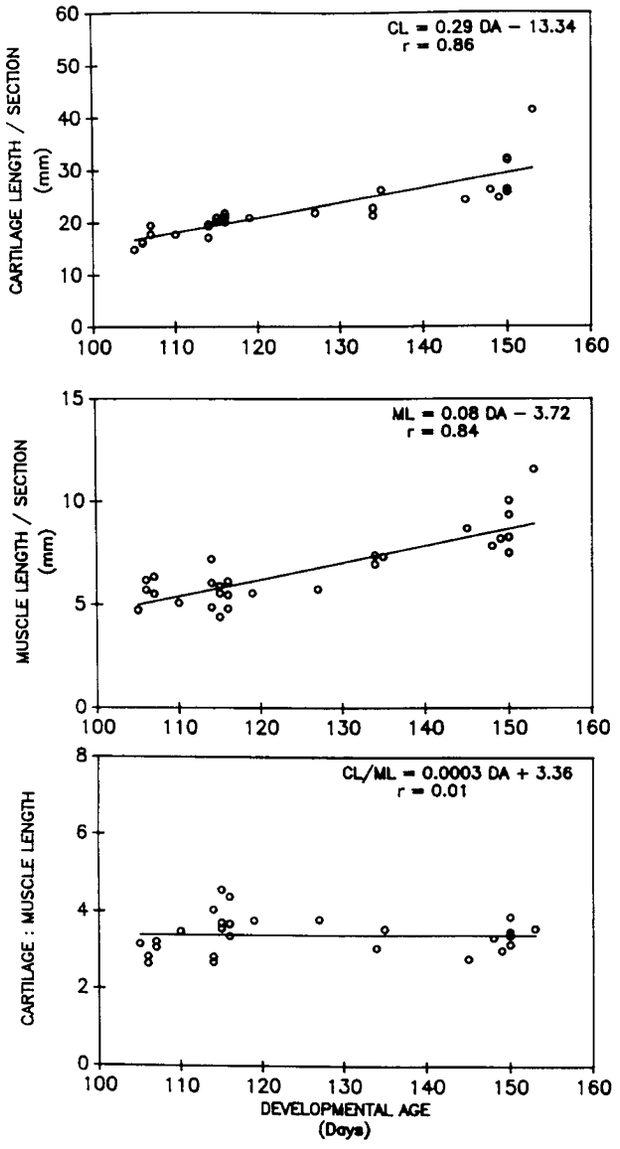

Fig. 4. Significant linear correlation ($p < 0.05$) was seen between (*top*) cartilage length/section (*CL*) and developmental age (*DA*) and (*middle*) muscle length/section (*ML*) and developmental age, whereas (*bottom*) the ratio of cartilage to muscle length per section (*CL/ML*) remained relatively unchanged with development.

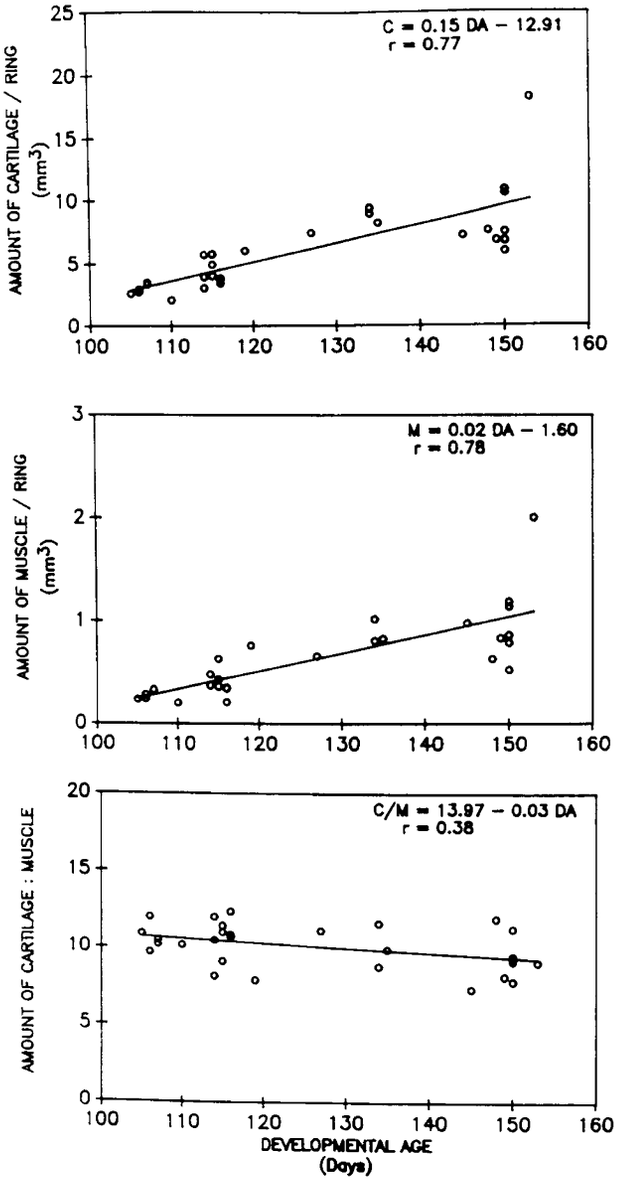

Fig. 5. The figure depicts the relationships between (*top*) the amount of cartilage/tracheal ring (*C*) and developmental age (*DA*) ($p < 0.05$); (*middle*) the amount of muscle/tracheal ring (*M*) and developmental age ($p < 0.05$); and (*bottom*) the ratio of the amount of cartilage to the amount of muscle per tracheal ring (*C/M*) and developmental age.

cartilage and the tracheal smooth muscle, and changes in the geometry and integrity of these tissues, as well as their relationship with each other, may affect mechanical function of the organ.

This research project was, therefore, designed to examine the tracheal structure at various stages of development during the prenatal and neonatal periods and to evaluate the role of tracheal morphology in determining tracheal physiology. We chose to study the lamb trachea because of its similarity to human tracheal structure, particularly in the manner of insertion of the trachealis muscle (both species demonstrating an insertion on the internal perichondrium). In addition, several studies regarding developmental changes in tracheal physiology have been performed in the lamb model (4, 12, 14, 15), allowing for better correlation of structure and function. Airway compliance is known to decrease with increasing maturity (1, 3, 5), immature animals demonstrating extremely compliant and collapsible airways. Application of transmural pressure would cause differing degrees of architectural distortion depending on airway compliance, which in turn is affected by developmental age. In view of these facts, we chose to standardize fixation procedures under conditions of zero transmural pressure.

Effect of maturation on tracheal dimensions. The results of this study demonstrate significant increases in the tracheal radius, cross-sectional area, and circumference with maturity. This increase in tracheal caliber contributes to the greater lung volume and decreased airway resistance seen with development. The associated decrease in specific airway conductance is believed to be due to the disproportionate increase in lung volume compared with the increase in airway dimensions (6).

Tracheae from preterm animals are known to be extremely compliant structures, which become stiffer with increasing maturity (1, 2, 5–7). Simplistically, one mechanism for the greater rigidity of the trachea with development could be alterations in wall thickness compared with luminal size, a thick-walled structure being more rigid than a thin-walled structure, all other factors remaining unchanged. And, inasmuch as the tracheal wall is not a homogeneous structure, both components, tracheal cartilage and muscle, must be considered. However, our results

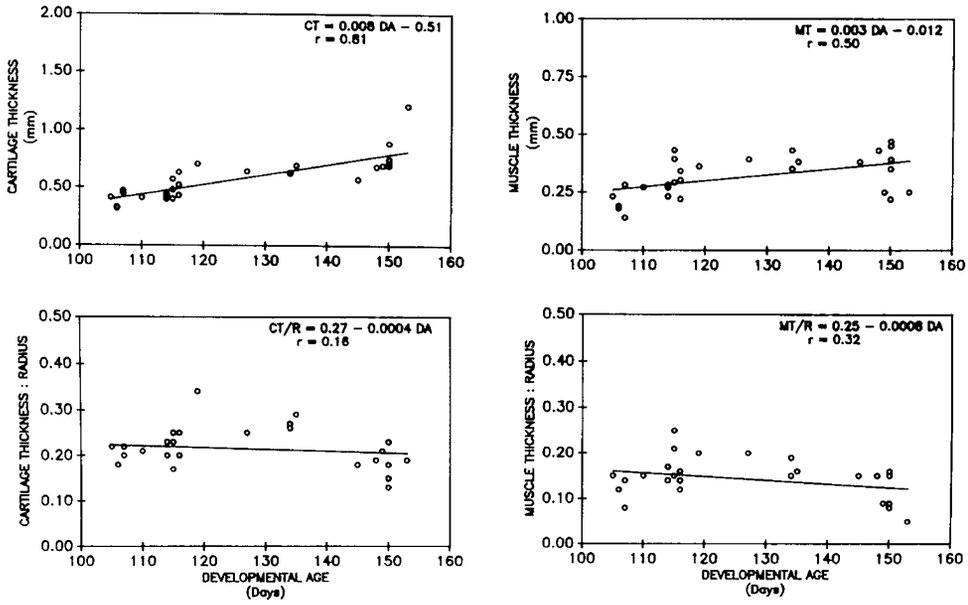

Fig. 6. The relationships between cartilage (*CT*), muscle thickness (*MT*), and developmental age (*DA*) are shown in the *upper left and upper right panels* of the figure ($p < 0.05$). The *lower left and lower right panels* depict the relationships between the ratios of cartilage thickness:radius (*CT/R*) and muscle thickness:radius (*MT/R*), respectively, and developmental age.

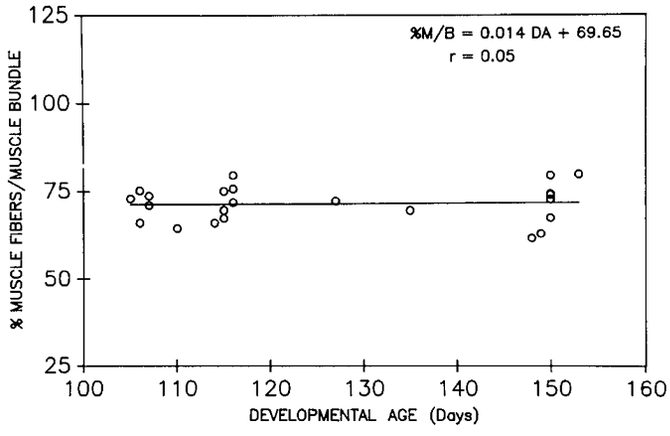

Fig. 7. Correlation between muscle mass in the trachealis muscle bundle (%*M/B*) (expressed as a percentage) and developmental age (*DA*).

Table 1. *Semiquantitative estimation of the GAG of tracheal cartilage**

Treatment	Preterm ($x \pm$ SEM)	Full-term ($x \pm$ SEM)
AB only	105.5 ± 0.8	138.7 ± 4.7†
AB after t. hyase	7.8 ± 0.2	26.1 ± 0.6†

* Values indicate the degree of stain absorption measured as the staining intensity of tracheal cartilage (per unit area) relative to the background, in arbitrary units, expressed as mean ± SEM. AB, Alcian Blue; t. hyase, testicular hyaluronidase.

† $p < 0.05$.

demonstrate that the thickness of both the cartilage and muscle increased proportionately with the tracheal radius, and the ratios of wall thickness to radius (cartilage:radius and muscle:radius) did not change significantly over the period of development studied, indicating the presence of a different mechanism for the increased stiffness of the trachea with maturity.

Effects of maturation on tracheal cartilage. Our results show progressive, gradual increases in the length, area, thickness, and amount of tracheal cartilage with developmental age, and growth occurring proportionately, with similar increases in the dimensions of the trachealis muscle with development. Tracheal growth in the prenatal and neonatal period thus appears to be similar to growth occurring postnatally as demonstrated by Wailoo and Emery (25) and Matsuba and Thurlbeck (26), with no differences in the relative proportions of muscle and cartilage of the trachea and large bronchi in children and adults.

Light microscopic examination revealed a marked difference in the geometric arrangement of tracheal cartilage in the posterior tracheal wall with development. In the very preterm animals, the thin, deformable, free ends of cartilage may be indicative of decreased support of the posterior tracheal wall and may cause it to be a "weak link" in the tracheal ring. Distortion, *i.e.* evagination or invagination, of this portion of the trachea may contribute to uneven air distribution and ineffective ventilation.

In the older animals, the posterior tracheal wall assumed a more stable configuration with the free ends of cartilage butting each other. With such an arrangement, even a small degree of muscle shortening would be adequate to form a rigid, completely cartilaginous ring that is better able to resist collapsing and distending transmural pressures. This arrangement of the cartilage also helps in the formation of a more rigid structure by placing the trachealis muscle at a better mechanical advantage and limiting muscle shortening (8) and could explain the greater rigidity of the trachea in the older animals.

The differences in the posterior wall configuration between the older and younger animals do not appear to be merely an effect of postnatal breathing, inasmuch as the unventilated trachea from a preterm lamb, 145 d gestational age, also showed a similar arrangement as the term newborn animals.

Tracheal rigidity is also affected by the stiffness of its cartilage, which in turn depends on the components of the extracellular ground substance, mainly the GAG, and collagen (13, 24, 27, 28). It is the proteoglycans in cartilage that are responsible for its ability to withstand compressive forces (8, 13), and the load (both preload and afterload) related to airway cartilage can be affected by alterations in cartilage stiffness. Enzymatic degradation of proteoglycans has been shown to cause a marked reduction in the stability of particular cartilage to withstand compressive stresses (28). Moreno *et al.* (13) studied papain-treated rabbit tracheae and demonstrated an increase in tracheal collapsibility after enzymatic softening of the cartilage. in this study, we examined tracheal segments from two groups of lambs representative of the preterm and term newborn animals for altera-

tions in compositional characteristics of tracheal cartilage with development. The relative staining intensity of Alcian Blue provided a semiquantitative index of GAG content. Alcian Blue was used to stain the GAG in the tracheal cartilage (as explained earlier, Alcian Blue can be used to stain chondroitin sulfate, hyaluronic acid, heparin, and the acid glycoproteins in tissues); the increase in the staining intensity of Alcian Blue (relative to background) in the group of older animals indicates the increased content of GAG in the tracheal cartilage. These findings are in agreement with those of Bucher and Reid (29), who have reported increased basophilia (which also depends on the presence of chondroitin sulfate) of airway cartilage in a 16-wk-old human fetus *versus* a term infant. Upon enzymatic digestion of the proteoglycans by testicular hyaluronidase, the results showed a slightly positive staining reaction with Alcian Blue 8GX in the group of older animals compared with absence of staining in the group of younger animals. These findings suggest the presence of proteoglycans resistant to digestion by testicular hyaluronidase (23) in the tracheal cartilage of the older animals, which may contribute to the increased rigidity of their tracheae.

These differences in the compositional characteristics of tracheal cartilage parallel the age-related alterations in the mechanics of the tracheal cartilage recently demonstrated by Penn *et al.* (15), which indicate a much greater compliance of the tracheal cartilage in preterm lambs as compared with that from adult sheep.

Effect of maturation on tracheal smooth muscle. As aforementioned, tracheal stability, circumference, and caliber can be affected by the tracheal smooth muscle. The contribution of the smooth muscle to these parameters may be dependent on various factors (4, 10): proportion of tracheal smooth muscle in the tracheal wall, the smooth muscle's contractility (which may be related to smooth muscle mass), and the length-tension and dose-response characteristics of the muscle. Our findings demonstrate significant linear increases in the length, thickness, and area of tracheal smooth muscle per tracheal section, as well as an increase in the volume of muscle per tracheal ring with development. However, the proportion of muscle in the tracheal wall remained relatively unchanged during this period. In this study, we also measured muscle mass by quantifying the dimensions of the muscle fibers in the trachealis muscle bundle. The results demonstrate that the proportion of muscle fibers in the muscle bundle remains relatively unchanged over the age range studied, indicating a proportionate increase in muscle mass.

Force generation properties of the tracheal smooth muscle have been shown to vary between preterm and adult sheep (14). Studies by various authors (4, 11, 12) have indicated that the compressibility of the trachea can be finely controlled by the contraction of the trachealis muscle and that differences in force generation capabilities would play an important role in determining the differences in tracheal functional properties. As shown by our results, changes in tracheal smooth muscle force generation capabilities cannot be attributed to increases in relative amounts of muscle mass. Therefore, intrinsic differences in the smooth muscle cells or connective tissue components of the muscle bundle are implicated. Biochemical investigations of nonairway smooth muscle cells in various animal species have demonstrated clear differences between embryonic, neonatal, and adult myosin isoforms (30, 31), as well as changes in the proteoglycan content of the intervening connective tissue (32).

In summary, there are a number of changes that occur in the tracheal structure with development. All of these factors—increases in the dimensions of the major tracheal wall components (cartilage and muscle), alterations in their geometric arrangement, and differences in their material characteristics—considered together help explain the developmental changes in functional properties of the trachea.

Acknowledgments. The authors thank Dr. J. B. Sheffield for his assistance in the use of the computerized image analysis system and Dr. V. K. Bhutani for his advice in the earlier portions of this research.

REFERENCES

1. Bhutani VK, Rubenstein SD, Shaffer TH 1981 Pressure-volume relationships of the trachea in fetal, neonatal and adult rabbits. Respir Physiol 43:221–231
2. Bhutani VK, Rubenstein SD, Shaffer TH 1981 Pressure induced deformation in immature airways. Pediatr Res 15:829–832
3. Croteau JR, Cook CD 1961 Volume-pressure and length-tension measurements in human tracheobronchial segments. J Appl Physiol 16:170–172
4. Penn RB, Wolfson MR, Shaffer TH 1988 Effect of tracheal smooth muscle tone on the collapsibility of extremely immature airways. J Appl Physiol 65:863–869
5. Shaffer TH, Bhutani BK, Wolfson MR, Tran NN 1989 *In vivo* mechanical properties of the developing airway. Pediatr Res 25:143–146
6. Stocks J, Godfrey S 1977 Specific airway conductance in relation to postconceptional age during infancy. J Appl Physiol 43:144–154
7. Shaffer TH, Wolfson MR, Forman D 1985 Developmental alterations in pulmonary function of the lamb. Respiration 47:129–137
8. James AL, Pare PD, Moreno RH, Hogg JC 1987 Quantitative measurement of smooth muscle shortening in isolated pig trachea. J Appl Physiol 63:1360–1365
9. Martin HB, Proctor DF 1958 Pressure-volume measurements on dog bronchi. J Appl Physiol 134:337
10. Moreno RH, Hogg JC, Pare PD 1986 Mechanics of airway narrowing. Am Rev Respir Dis 133:1171–1180
11. Coburn RF, Thornton D, Arts R 1972 Effect of trachealis muscle contraction on tracheal resistance to airflow. J Appl Physiol 16:170–172
12. Koslo RJ, Bhutani VK, Shaffer TH 1986 The role of tracheal smooth muscle contraction on neonatal tracheal mechanics. Pediatr Res 20:1216–1220
13. Moreno RH, McCormack GS, Mullen JBM, Hogg JC, Bert J, Pare PD 1986 Effect of intravenous papain on tracheal pressure-volume curves in rabbits. J Appl Physiol 60:247–252
14. 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
15. Penn RB, Wolfson MR, Shaffer TH 1989 Developmental differences in tracheal cartilage mechanics. Pediatr Res 26:429–433
16. Wolfson MR, Shaffer TH 1990 The differential effects of pancuronium bromide on cardiopulmonary function in the neonatal lamb. Pediatr Pulmonol 8:233–239
17. Humanson GL 1979 Animal Tissue Techniques. WH Freeman, San Francisco
18. Deoras KS, Wolfson MR, Searls RL, Hilfer SR, Sheffield JB, Shaffer TH 1990 Use of a touch sensitive screen and computer assisted image analysis for quantification of developmental changes in pulmonary structure. Pediatr Pulmonol 9:109–118
19. Sheffield JB, Hilfer SR, Silage D 1987 A simple image analysis system using a microcomputer and video grabber board. J Cell Biol 105:513a(abstr)
20. Silage DA, Gil J 1984 The use of touch sensitive screen in interactive morphometry. J Microsc 134:315–321
21. Bancroft JD, Cook HC 1984 Manual of Histologic Techniques. Churchill-Livingstone, New York, pp 109–110
22. Scott JE, Quintarelli G, Dellovo MC 1964 The chemical and histochemical properties of Alcian Blue. I. The mechanism of AB staining. Histochemie 4:73–85
23. Meir S, Hay ED 1973 Synthesis of sulphated glycosaminoglycans by embryonic corneal epithelium. Dev Biol 35:318–331
24. Webster EH, Searls RL, Hilfer SR, Zwaan J 1987 Accumulation and distribution of sulfated materials in the maturing mouse lens capsule. Anat Rec 218:329–337
25. Wailoo MP, Emery JL 1982 Normal growth and development of the trachea. Thorax 37:584–587
26. Matsuba K, Thurlbeck WM 1972 A morphometric study of bronchial and bronchiolar walls in children. Am Rev Respir Dis 105:908–915
27. Greep RO, Weiss L 1973 Histology. McGraw Hill, New York, pp 145–146
28. Kempson GE, Muir H, Swanson SAV, Freeman MAP 1970 Correlations between stiffness and the chemical constituents of cartilage on the human femoral head. Biochim Biophys Acta 215:70–77
29. Bucher U, Reid L 1961 Development of the intrasegmental bronchial tree: the pattern of branching and development of cartilage at various stages of intrauterine life. Thorax 16:201–218
30. Kawamoto S, Adelstein RS 1987 Characterization of myosin heavy chains in cultured aorta smooth muscle cells. J Biol 262:7282–7288
31. Masaki T, Takano-Ohmuro H, Suzuki R, Obinata T, Hamada Y, Mikawa T, Tanaka T, Yoshikawa A, Katoch S 1986 Changes in the smooth muscle myosin isoforms during development of chicken gizzard. UCLA Symposia on Molecular and Cellular Biology, Vol, 29, Molecular Biology of Muscle Development. Alan R Liss Inc, New York, pp 323–336
32. Carrino DA, Caplan AI 1986 Proteoglycan synthesis during skeletal muscle development. In: UCLA Symposia on Molecular and Cellular Biology, Vol 29, Molecular Biology of Muscle Development. Alan R Liss Inc, New York, pp 323–336