Quantitative Evaluation of the Development of Tracheal Submucosal Glands in Infants With Cystic Fibrosis and Control Infants

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The development of the tracheal submucosal glands has been determined quantitatively in 22 infants with cystic fibrosis and in 25 control infants, all under 4 months of age. In cross-sections of normal trachea significant relationships were found between postconceptional age (PCA) and gland area ($P < 0.001$), submucosal area ($P < 0.02$), tracheal airway diameter ($P < 0.05$), and acinar diameter ($P < 0.001$). In infants with cystic fibrosis the pattern of development was similar to that of the control infants. No statistically significant differences were found between three subgroups of infants with cystic fibrosis, which included those with meconium ileus with no lung infection, those with meconium ileus with lung infection, and those with lung infection and no history of meconium ileus. The normal pattern of development of tracheal submucosal glands in infants with cystic fibrosis was in contrast to the deficiency of normal maturation seen in the exocrine pancreas of these infants. The lumen fraction, an index of dilatation of acinar lumina, showed no significant relationship with PCA in either the control group or the group with cystic fibrosis. However, statistically significant dilatation of acini was observed in the tracheal submucosal glands of infants with cystic fibrosis (0.22) when compared with control infants (0.14, $P < 0.005$). (Am J Pathol 1982, 106:303–311)

CYSTIC FIBROSIS is the most common lethal genetic disease of Caucasians.[1] Although it causes dysfunction of exocrine glands, clinically the organs most affected are the pancreas, resulting in achylia, and the lung, resulting in mucopurulent plugging and chronic lung infection. After one month of age, pulmonary disease replaces meconium ileus and its complications as the major cause of mortality in cystic fibrosis. In recent years more effective long-term control of pulmonary disease has greatly increased life expectancy. The basic defect of the disease is still unknown, however, and the wide range of age at onset and variations in the extent of pancreatic and pulmonary involvement have yet to be explained.[2]

Although the development of bronchial submucosal glands has been investigated in the normal fetus,[3] in early childhood,[4,5] and in adults,[6-9] documentation of the changes in these mucus-secreting glands in cystic fibrosis has been limited, being based on subjective quantitative investigation[10-12] or on quantitative evaluation of only a few cases.[13] The purpose of this study is to evaluate whether there is any fundamental abnormality in the early development of the tracheobronchial submucosal glands in cystic fibrosis.

Materials and Methods

Materials

The study included 47 tracheal autopsy specimens obtained from the archives of the Hospital for Sick Children, Toronto, Canada. Four groups were included in this study: 1) the control group; 2) those with meconium ileus, with no evidence of lung infection; 3) those with meconium ileus with evidence of lung infection; and 4) those with cystic fibrosis with no history of meconium ileus. Details of all cases are summarized in Tables 1 and 2. All subjects were less than 4 months of age at death, were within normal

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Table 1—Group 1—Control Cases

Case	Body length* (cm)	Gestational age† (wks)	Postnatal age (days)	Gland area (sq mm)	Submucosal area (sq mm)	Airway lumen (sq mm)	Acinar diameter (mean ± SEM) (μ)	Lumen fraction	Autopsy diagnosis
1	57	Term	68	2.6	9.8	9.6	59.9 ± 1.0	.24	Aspiration of stomach contents
2	62	Term	94	2.8	8.8	8.1	52.9 ± 1.3	.03	Cerebral edema, pulmonary congestion; ? aspiration of stomach contents
3	57	Term	99	3.5	12.6	18.0	66.7 ± 3.1	.20	Hemorrhagic gastroenteritis
4	46	37	2	0.8	4.8	8.2	46.4 ± 1.0	.04	Respiratory distress syndrome (RDS); infant of a diabetic mother
5	43	34	<1	0.9	5.5	6.4	40.9 ± 0.9	.10	RDS
6	61	Term	105	4.5	12.9	12.7	67.8 ± 0.9	.02	Pneumococcal meningitis; patchy pneumonia
7	55	Term	46	2.3	7.7	15.8	56.7 ± 1.0	.16	Gastroenteritis; dehydration
8	51.5	41	1	2.3	7.2	8.9	56.6 ± 1.0	.13	RDS
9	45	39	<1	1.2	6.6	11.2	48.5 ± 0.8	.23	Intrapartum hypoxia
10	49	39	2	1.9	9.8	8.3	56.8 ± 1.1	.24	Neonatal asphyxia
11	53	42	3	2.8	11.5	11.6	55.1 ± 0.8	.26	Cervical medullary cord contusion
12	47	36	9	1.6	5.7	7.9	50.0 ± 1.4	§	Coxsackie B. infection
13	50	40	2	1.9	10.0	8.8	57.9 ± 1.1	.10	Neonatal asphyxia; meconium aspiration
14	49	36	10	1.0	6.3	10.9	49.4 ± 1.1	.10	Sepsis, type undertermined; congestive heart failure
15	46	34	4	1.0	5.9	8.2	50.8 ± 1.2	.12	RDS
16	60	Term	63	1.9	8.7	§	§	§	Purulent meningitis; disseminated intravascular coagulation
17	50	39	5	1.8	10.4	§	57.7 ± 1.6	.14	Coxsackie A9 infection
18	53	Term	35	3.5	11.7	12.3	61.7 ± 0.9	§	Gastroenteritis; severe electrolyte imbalance; cerebral and pulmonary edema
19	51	41	7	2.1	15.1	14.7	55.0 ± 1.0	.15	Neonatal meningitis
20	39	30	1	0.7	5.6	6.5	42.9 ± 1.1	.03	Prematurity; subependymal plate hemorrhage; treated pneumothorax; atelectasis
21	‡	34	1	0.5	5.0	12.1	45.3 ± 1.5	§	Prematurity; RDS; septicemia; subependymal plate hemorrhage; focal pneumonia
22	50.5	Term	2	4.2	18.9	11.4	60.5 ± 0.8	.23	Atelectasis of lungs; subarachnoid hemorrhage
23	42	33	1	1.6	7.7	10.9	47.2 ± 1.1	.13	Prematurity; RDS; pulmonary atelectasis and focal hemorrhage
24	40	30	1	0.8	9.3	11.4	38.5 ± 0.8	.10	Prematurity; subarachnoid hemorrhage; subependymal plate hemorrhage, pulmonary hemorrhage
25	62	‡	70	4.0	12.7	§	64.8 ± 1.4	§	Battered baby

* Patients included only those with body length and/or weight in the 94% confidence limits for their age.
† For estimation of post-conceptional age, deliveries recorded as "term" were assigned 39 weeks gestation.
‡ Data not available from patient's history.
§ Tissue preservation precluded quantitation.

Table 2—Cases of Cystic Fibrosis (CF)

Case	Body length (cm)	Gestational age† (weeks)	Postnatal age (days)	Gland area (sq mm)	Submucosal area (sq mm)	Airway lumen (sq mm)	Acinar diameter (mean ± SEM) (μ)	Lumen fraction	Autopsy diagnosis
Group 2 Meconium ileus No lung infection									
26	52	Term	14	2.9	7.9	10.6	67.0 ± 1.5	.28	CF; meconium ileus and peritonitis
27	52	Term	6	1.8	6.6	6.7	58.3 ± 1.1	†	CF; meconium ileus and peritonitis, ileal atresia
28	51	Term	28	1.4	5.8	†	45.3 ± 0.9	.17	CF; meconium ileus and peritonitis
29	*	Term	5	2.5	9.9	10.4	59.6 ± 0.9	.30	CF; meconium ileus; micrognathia
30	*	Term	47	1.6	8.6	12.2	46.6 ± 1.0	.23	CF; meconium ileus; failure to thrive; dehydration
31	45	36	8	0.9	8.3	10.5	44.3 ± 0.9	.09	Meconium peritonitis; ? meconium ileus; ? CF
32	43	34	5	1.3	5.0	7.2	52.3 ± 1.1	.12	CF; meconium ileus
33	53	41½	11	3.1	9.3	9.8	63.8 ± 1.0	.03	CF; meconium ileus; lung haemorrhages
34	47.5	39	14	2.7	8.7	12.7	52.3 ± 1.4	.22	Meconium ileus and peritonitis; patent ductus; infant of a diabetic mother
Group 3 Meconium ileus Lung infection									
35	48.5	35	11	3.2	11.4	5.4	65.9 ± 1.0	.31	CF; meconium ileus and peritonitis; bronchopneumonia
36	54.0	Term	70	4.0	12.6	12.9	50.7 ± 0.7	.15	CF; bronchopneumonia; history of meconium ileus
37	49.0	Term	5	2.7	14.2	6.6	57.8 ± 1.4	.28	CF; meconium ileus; subacute pneumonia
38	52	Term	21	1.8	5.1	†	56.7 ± 1.1	†	CF; meconium ileus; bronchopneumonia
39	53	40	7	3.2	15.2	6.7	53.7 ± 0.9	.21	CF; meconium ileus; aspiration pneumonia; septicemia, omphalitis
40	51	Term	19	2.8	9.7	†	50.2 ± 0.9	.20	Meconium ileus; aspiration pneumonia
41	50	34	39	2.4	11.4	8.5	60.7 ± 1.1	†	CF; bronchopneumonia; history of meconium ileus
42	50	38	37	1.2	7.1	12.2	50.9 ± 1.1	.23	CF; meconium ileus and peritonitis; laryngotracheobronchitis; infant of a diabetic mother
Group 4 Cystic fibrosis No meconium ileus									
43	57	Term	88	5.5	13.7	†	59.8 ± 0.8	.26	CF; bronchitis; bronchopneumonia
44	56	Term	88	2.9	17.2	7.9	66.1 ± 1.1	.29	CF; bronchopneumonia emphysema
45	57	Term	91	3.7	10.5	11.9	66.3 ± 1.2	.37	CF; bronchopneumonia; failure to thrive
46	54.5	Term	81	2.6	8.4	13.7	70.8 ± 1.4	.15	CF; pneumonia; failure to thrive
47	52	40	49	4.8	13.8	7.5	63.7 ± 0.9	.31	CF; pneumonia; pneumothorax

* Data not available from patient's history.
† Tissue preservation precluded quantitation.

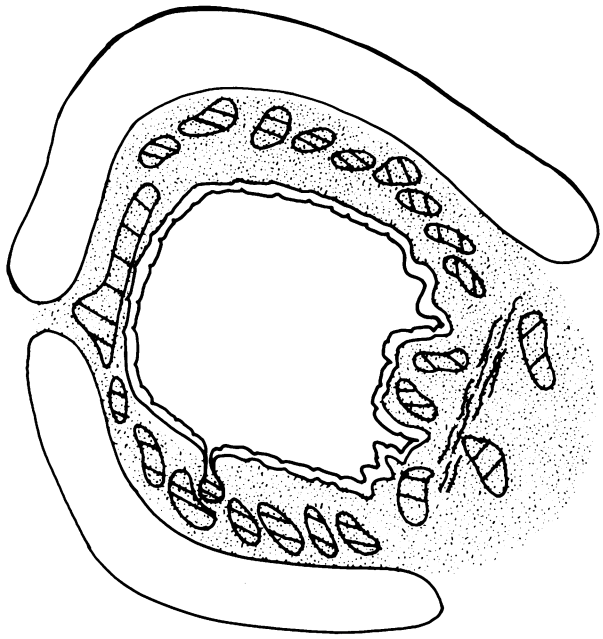

Figure 1—Diagrammatic representation of tracheal cross-section from Case 13 showing the gland (cross-hatched) and submucosal (stippled) and tracheal airway cross-sectional areas used in the quantitative studies.

percentiles for age, and had samples of trachea with acceptable histologic preservation.

Group I included 25 control subjects with no clinical or pathologic evidence of cystic fibrosis or of respiratory infection as a cause of death. Group II included 9 subjects with a history of meconium ileus and exocrine changes compatible with cystic fibrosis, based on family history and on pathologic changes on examination of at least three organ systems; in 2 of these cases the pathologic diagnosis of cystic fibrosis was inconclusive on the basis of conventional diagnostic criteria. Group III included 8 subjects with a history of meconium ileus and lung infection and exocrine changes compatible with cystic fibrosis, based on examination of at least three organ systems; 1 case was inconclusive. Group IV included 5 subjects with pathologic findings compatible with cystic fibrosis and with no history of meconium ileus. All individuals had a history of pulmonary infection, and bronchopneumonia was the cause of death.

We took the gestational and postnatal ages from the clinical histories to calculate the postconceptional age for each patient.

Quantitative Studies

Samples of trachea, fixed in neutral buffered formalin and embedded in paraffin wax, were sectioned transversely and stained with either hematoxylin and eosin (H&E) or alcian blue-periodic acid-Schiff. Most sections were from the proximal region at the level of the thyroid gland, at which site the submucosal glands are distributed uniformly and maintain a consistent volume in relationship to airway size.[5,7]

Two methods were used to quantify the tracheal sections. First, a semi-automatic image analysis system, Leitz ASM, was applied to measure *a*) the area of submucosal gland, *b*) the area of submucosal tissue (including glands but excluding respiratory epithelium), *c*) the area of tracheal lumen, and *d*) the mean diameter of secretory acini in the glands. The parameters *a*, *b*, and *c* are illustrated diagrammatically for 1 case (No. 13) in Figure 1. Preliminary studies established that in 5 cases from the control group and 5 from the cystic fibrosis group no significant differences occurred in the acinar diameters of submucosal glands situated in the anterior and posterior walls or in the length of the trachea.

Second, we applied the quantitative stereologic technique of point counting[14] to sections stained with alcian blue-periodic acid-Schiff, using a Reichert Visopan Projection Microscope at 500× magnification. A 400-point grid with points separated by approximately 5 μ was overlaid on the projected image of the submucosal tissue, and the number of points was recorded over the acinar cells or acinar lumens. The volume density of each component was used to calculate the lumen fraction, ie, lumen volume divided by the acinar cell volume plus the lumen volume.

Statistical Analyses

The relationship between the parameters measured and postconceptional age was determined by linear regression analysis. The 95% confidence limits and *P* values were calculated according to standard statistical formula. The post-conceptional age (PCA) provided the best index for comparison between these groups of infants because of their varying gestational and postnatal ages.

Results

The data obtained using the image analysis and point counting techniques for each subject are summarized in Tables 1 and 2 and illustrated in Figures 2 to 6. The development of the submucosal glands was assessed from the total gland volume, the diameter of the secretory acini and tubules, and the luminal volume. The age range for the control groups was from 30 to 54 weeks PCA and for the cystic fibrosis groups from 35 to 52 weeks PCA. Statistical analysis of the

data revealed no significant difference between parameters in the three cystic fibrosis groups; therefore, the data for all cases of cystic fibrosis were combined for comparison with that of the control group.

Gland Volume

The volumes of submucosal gland, submucosal tissue, and tracheal airway were determined from their areas in cross-sections of the trachea.

Control Group

The submucosal gland area in random cross-sections of the trachea showed a significant relationship to PCA (Figure 2A). The gland area increased from 0.7 sq mm at 30 weeks PCA, to 3.8 sq mm at 54 weeks PCA. The increase in gland volume was linear during this period (slope = 0.12, $P < 0.001$). The total submucosal area, including the glands (Figure 3A) increased from 6.5 to 12.4 sq mm from 30 to 54 weeks PCA and showed a significant linear relationship to age (slope = 0.24, $P < 0.02$ also). The area of airway lumen increased from 8.5 to 13.5 sq mm in the trachea, with a significant linear relationship to PCA (slope = 0.20, $P < 0.05$). There was a significant relationship between submucosal gland area and the total area of submucosal tissue, as shown in Figure 4A (slope = 0.27, $P < 0.001$). The ratio of gland area to submucosal area showed no significant correlation with PCA for control subjects (slope = 0.008, $P < 0.001$). Furthermore, the ratio of submucosal gland area to tracheal lumen area showed no significant correlation with PCA for the control subjects (slope = 0.009, $P < 0.001$).

Cystic Fibrosis Groups

The submucosal gland area in the trachea for the subjects with cystic fibrosis (Figure 2B) increased from 1.6 sq mm at 35 weeks PCA to 3.8 sq mm at 52 weeks PCA and showed a significant linear relationship to PCA (slope = 0.13, $P < 0.01$). The rate of submucosal gland development was similar to that of the controls, but the individual variation resulted in a wider confidence limit. The submucosal area increased linearly from 7.7 to 12.5 sq mm and showed a rate of development similar to that of the control subjects (Figure 3B). However, the wider scatter of values about the mean resulted in a lack of statistical significance (slope = 0.27, $P < 0.10$). The tracheal lumen area increased from 7.5 to 11.8 sq mm and showed a significant linear relationship to PCA (slope = 0.25, $P < 0.05$). In contrast to the control group, the ratio of gland area to area of submucosal tissue showed no significant correlation with PCA (slope = 0.015, $P < 0.20$). The same was true for the ratio of gland area to tracheal lumen area (slope = 0.002, $P < 0.80$). However, the submucosal gland area showed the significant relationship to the total area of submucosal tissue in the group with cystic fibrosis (Figure 4B) (slope = 0.23, $P < 0.001$).

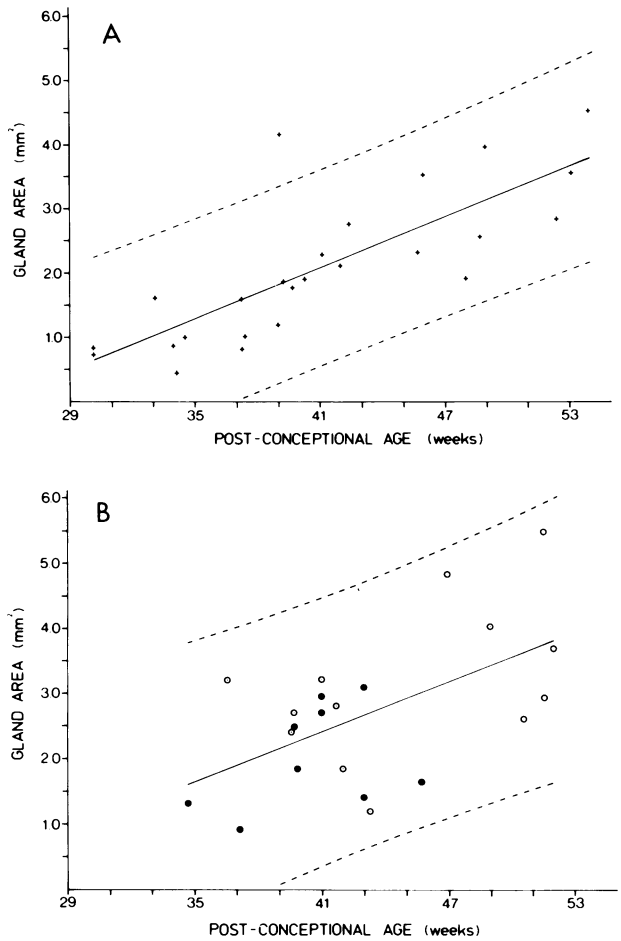

Figure 2A—Relationship between the gland area and the postconceptional age of control infants. Each point represents the value for 1 case. The solid line represents the regression line; broken lines represent the 95% confidence limits (slope = 0.13, $P < 0.001$). **B**—Relationship between the gland area and the postconceptional age of infants with cystic fibrosis. *Solid circles* = infants with a history of meconium ileus. *Dotted circles* = infants with a history of meconium ileus and lung infection. *Open circles* = infants with cystic fibrosis and no history of meconium ileus (combined data: slope = 0.13, $P < 0.01$).

Acinar Diameter

In this age group the mucous and serous acini could not be distinguished adequately even with special staining techniques for acidic and neutral glycoproteins. Therefore, no distinction was made between mucous and serous cell types for quantitation

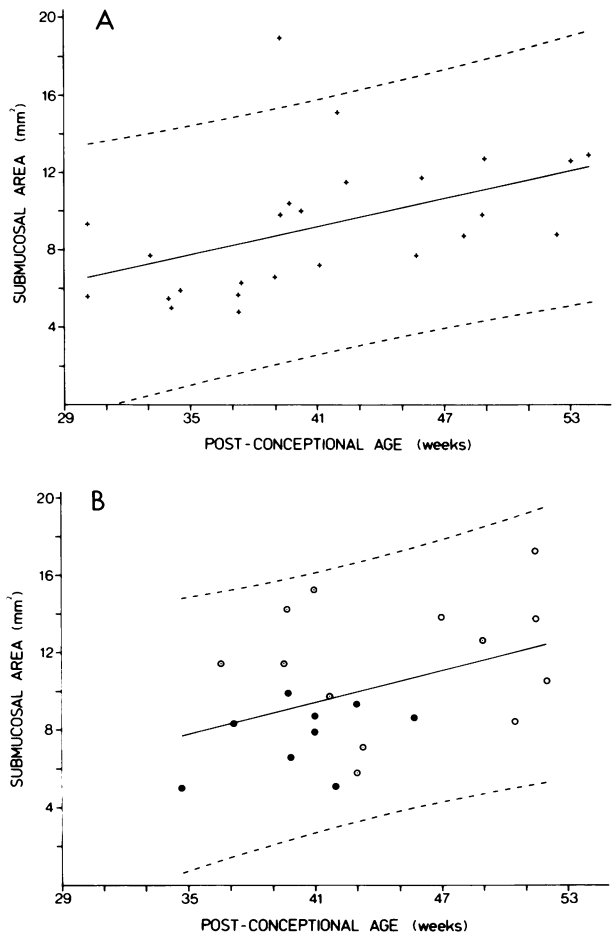

Figure 3A – Relationship between the submucosal area and the postconceptional age of control infants (slope = 0.24, $P < 0.02$). **B** – Relationship between the submucosal area and the postconceptional age of infants with cystic fibrosis (combined data: slope = 0.27, $P < 0.10$).

purposes. In both control and cystic fibrosis groups no differences were observed between the acinar diameter of the gland in the anterior and posterior wall of the trachea.

Within the glands the mean acinar diameter increased linearly with age in both the control and cystic fibrosis groups, although the variation between individuals was greater in the cystic fibrosis group.

Control Group

There was a highly significant relationship between acinar diameter and PCA in the control infants, as shown in Figure 5A (slope = 0.96, $P < 0.001$). The mean acinar diameter increased from approximately 43 μ at 30 weeks PCA to approximately 66 μ at 54 weeks PCA.

Cystic Fibrosis Groups

There was a less significant relationship between acinar diameter and PCA in the cystic fibrosis groups seen in Figure 5B, (slope = 0.51, $P < 0.02$). As in the gland area measurements, there was a wider scatter of values about the mean in the cystic fibrosis group. The acinar diameter increased linearly from approximately 53 μ at 35 weeks PCA to approximately 62 μ at 52 weeks PCA.

Lumen Fraction

The lumen fraction of the gland was an index of the dilatation of acini in the submucosal gland. As in acinar diameter, no distinction was made between serous and mucous acini. In both control and cystic fibrosis groups the values showed a random scatter when plotted against PCA (Figures 6A and B), gland

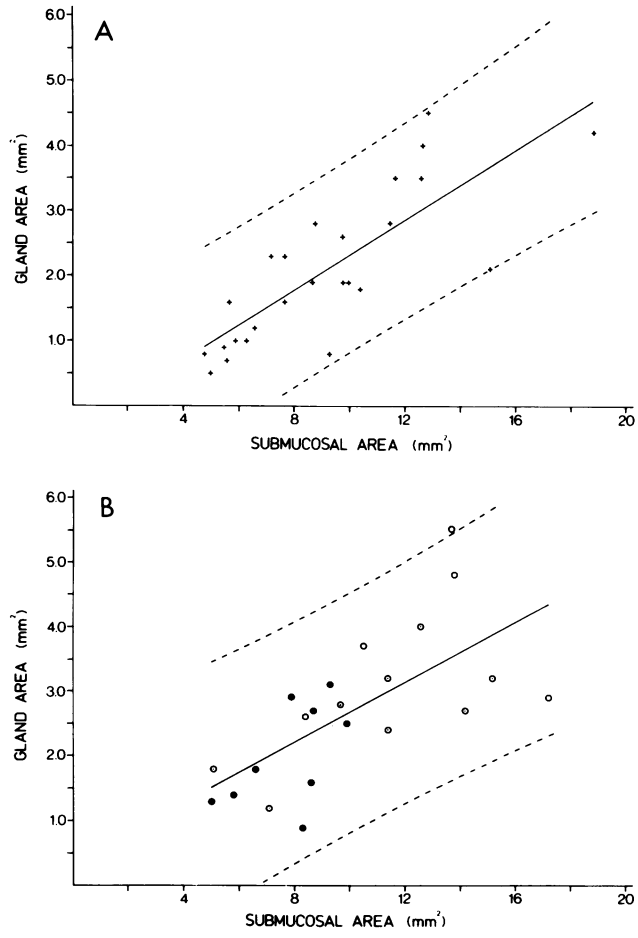

Figure 4A – Relationship between the gland area and the submucosal area for control infants (slope = 0.27, $P < 0.001$). **B** – Relationship between the gland area and the submucosal area for infants with cystic fibrosis (combined data: slope = 0.23, $P < 0.001$).

area, and acinar diameter. The mean value of the cystic fibrosis groups was higher (0.22, range 0.03–0.37) than that of controls (0.14, range = 0.02–0.26), and this difference was statistically significant ($P < 0.005$).

Discussion

The pathogenesis of cystic fibrosis has been referred to commonly as a generalized exocrinopathy with a fundamental abnormality in mucous secretions. Although mucous hypersecretion and obstruction of ducts of exocrine glands are prominent in the pathogenesis of cystic fibrosis, no specific defect has been demonstrated as yet in mucous secretions[15] or in mucus-secreting elements[16] in cystic fibrosis.

Recently, quantitative analysis of the exocrine pancreas demonstrated a significant defect in the development and maturation of the exocrine pancreas in infants with cystic fibrosis.[17] The deficiency in exocrine acinar development was clearly distinguished from normal pancreatic development at or before birth. This observation raised an important question as to whether a fundamental defect existed in the development or maturation of exocrine cells that would explain the pathogenesis of cystic fibrosis. However, defective exocrine development was not reflected by any detectable alteration in mucus-secreting glands in the respiratory tract. In the same series of subjects, the observations in the tracheal exocrine glands clearly differed from those of the pancreatic exocrine tissue. Nearly all patients with cystic fibrosis showed a lack of normal pancreatic development plus replacement fibrosis, whereas there was no significant anatomic difference in the trachea of age-matched control infants and infants with cystic fibrosis up to 3

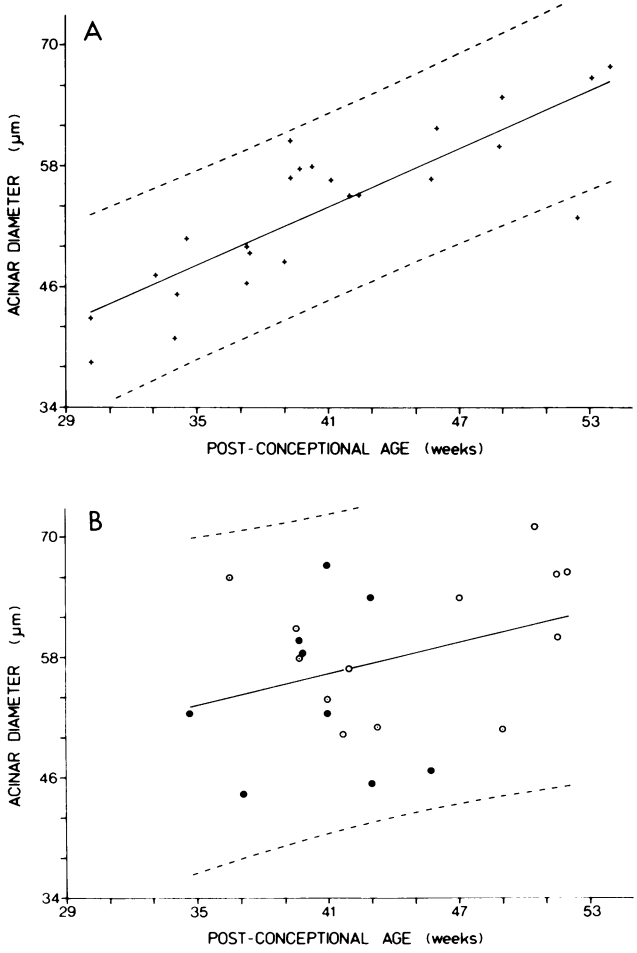

Figure 5A – Relationship between the acinar diameter and the postconceptional age for control infants (slope = 0.96, $P < 0.001$). **B** – Relationship between the acinar diameter and the postconceptional age for infants with cystic fibrosis (combined data: slope = 0.51, $P < 0.02$).

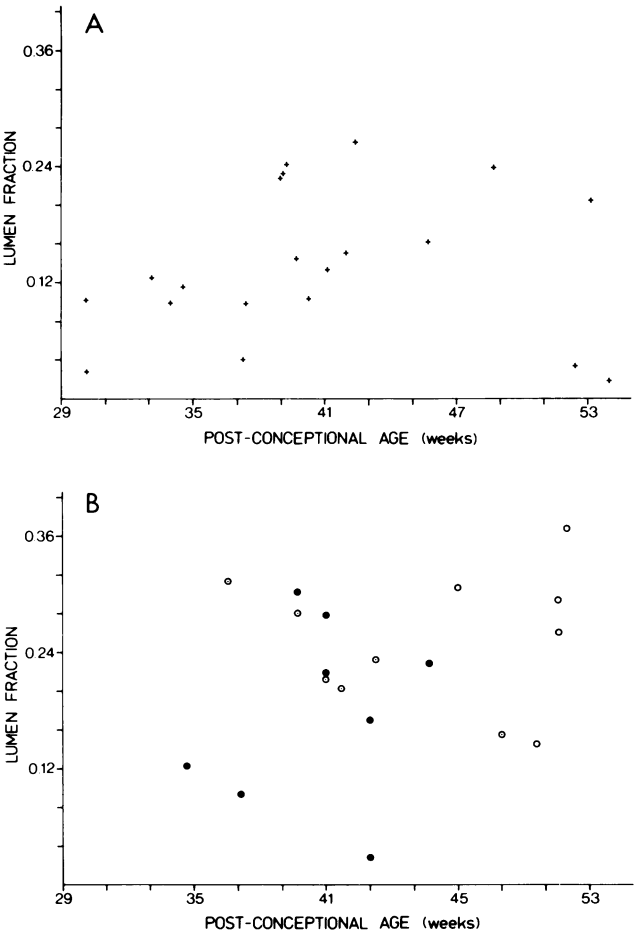

Figure 6A – Relationship between the lumen fraction and the postconceptional age for control infants ($P < 0.70$). **B** – Relationship between the lumen fraction and the postconceptional age for infants with cystic fibrosis (combined data: $P < 0.30$).

Table 3—Summary of Significant Age-Related and Cystic Fibrosis Effects in Postnatal Development of Tracheal Submucosal Glands

	Age-related effect	Cystic fibrosis-related effect*
Tracheal lumen volume	+	–
Submucosal gland volume	+	–
Submucosal volume	+	–
Acinar diameter	+	?
Lumen volume	–	+

* Compared with age-matched controls.
+, denotes statistically significant relationship.
–, no statistical significance.

months of age, judged by the lack of difference in cross-sectional areas of the airway lumen, mucosal tissues, and submucosal tissues. In the submucosal glands, the only significant difference evident between the infants with cystic fibrosis and the control infants was an increased dilatation of acinar lumens, which was not related to age or to gland enlargement (Table 3).

Quantification of the areas of glandular and submucosal tissues from random tissue sections of the trachea proved reliable and reproducible for the assessment of airways from younger infants where indices such as the gland:wall ratio[7] could not easily be applied because of the variable thickness and distribution of glands in the tracheal wall. In the control infants a clearly defined pattern of gland development was seen, with a linear increase in the gland volume from 30 to 54 weeks PCA. The development was similar for all groups of patients with cystic fibrosis, including those with and without a clinical history of meconium ileus and respiratory infection. There was a striking similarity between control and cystic fibrosis groups in the rate of increase in gland and submucosal tissue volume with age, and this presumably indicates a lack of fibrosis at this airway level. The fact that there was a highly significant linear relationship between gland area and submucosal area with a similar slope for both the control and cystic fibrosis groups suggests that there was no fundamental disturbance in the development of the tracheal submucosal glands of cystic fibrosis infants at this age.

The lumen fraction of the gland showed no relationship to age in either the control group or cystic fibrosis group. Both groups showed a wide range of values, and the higher mean in the cystic fibrosis group was not reflected in an increased acinar diameter or gland volume. The luminal changes in the absence of other anatomic or quantitative changes support better the concept of altered cellular contents in the cystic fibrosis cells, for example, a higher water content, so that shrinkage represented an artifact of the tissue processing.

The possibility that luminal changes were related to endotracheal intubation or bronchoscopic suction in these infants was considered. The majority of the patients included in the control group had been intubated, and many had been on mechanical ventilation, but rarely for more than 24 hours. In those patients without such treatment no significant difference was observed in gland volume, acinar diameter, or lumen fraction. Of the cystic fibrosis group 3 infants had been intubated, 2 infants received bronchoscopic suction, and 1 infant had a tracheotomy. None of these infants showed a significant difference in the parameters measured. This treatment was not considered, therefore, to have influenced the findings.

In the groups with cystic fibrosis there was no consistent trend to distinguish them from the control group. Clearly, individual variation occurred with hypertrophy and hyperplasia of mucus-secreting cell types. However, this inconsistent variation was not interpreted as a fundamental abnormality of glandular development or of function.

Comparison of patients with cystic fibrosis who had different clinical problems, including meconium ileus and/or pulmonary disease, showed no significant difference in the onset or severity of pulmonary changes, as judged by our measurements. The dilatation of gland lumens observed among patients with cystic fibrosis may have been caused by increased discharge or retention of secretions as a result of pulmonary infection. The 2 patients from Group II and 1 from Group III who had inconclusive diagnoses of cystic fibrosis on the basis of clinical and pathologic evaluation were not distinguishable from other infants with cystic fibrosis or from the control subjects.

The examination of tracheal submucosal gland is not a useful tool for diagnosis of cystic fibrosis in the newborn or infant, though in individual cases early changes may be seen.

The tracheobronchial submucosal glands are a compound tubulo-acinar structure with mixed cell types. The major secretory cells—mucous and serous—line the secretory tubules of the gland. At the early ages studied here, it was not possible to distinguish adequately the mucous and serous acini. The possibility of other changes in cellular populations or of secretory products in the different cell types needs further investigation. Although the older child with cystic fibrosis may show tracheobronchial gland hy-

pertrophy and hypersecretion, the results of the present study suggest these changes are secondary and do not reflect a lack of normal development, but presumably the interplay of environmental or humoral factors in the progression of the disease. This observation emphasizes the importance of aggressive prophylactic therapy to prevent and eradicate infection in the long-term care of patients with cystic fibrosis.

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