Quantitative Evaluation of the Development of the Exocrine Pancreas in Cystic Fibrosis and Control Infants

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The development of the exocrine pancreas has been determined quantitatively in 31 infants with cystic fibrosis (CF) both with and without meconium ileus and in 29 control infants. In the normal pancreas, the ratio of acinar to connective tissue volume is 0.5 at 32 weeks postconceptional age (PCA) and increases linearly to 2.0 at 52 weeks PCA. In cystic fibrosis infants, with or without meconium ileus, the ratio is 0.5 at 35 weeks PCA and decreases linearly to 0.3 at 52 weeks PCA. The volume of acinar and duct lumens is greater in CF than control infants but is independent of age or acinar volume. The development of the exocrine pancreas in infants with CF with and without meconium ileus diverges from the normal pattern: There is a consistent lack of exocrine tissue before or at full-term birth, which persists throughout the age range of this study. CF infants above 42 weeks PCA can be discriminated from controls on the basis of the quantitative assessment of acinar volume. (Am J Pathol 95:697–708, 1979)

CYSTIC FIBROSIS is an autosomal recessive disorder manifested by dysfunction of exocrine glands. Pancreatic changes in this disease are characterized by achylia, inspissation of secretions in the ducts and acini, and reduction of acinar tissue with increase in connective tissue.[1,2] The pancreatic lesions have been reported to vary in their severity but have not been related to the normal pattern of development or to pancreatic function.

The manifestation of meconium ileus in cystic fibrosis may result from pancreatic dysfunction *in utero*.[1] However, other predisposing factors may be involved such as the obstruction of the pancreatic duct,[3] failure in the formation or release of pancreatic enzymes,[4] or abnormal mucus production in the intestine.[5,6]

Elucidation of the early pathogenesis of cystic fibrosis is important for diagnosis in the neonatal period when the clinical manifestations may be diverse and the present histopathologic criteria, inconclusive. The purpose of the present study is as follows: 1) to define the normal development in the exocrine pancreas in control infants; 2) to compare the development of the pancreas in infants with cystic fibrosis, both with and without meconium ileus; and 3) to establish whether there are quantitative differences in the pancreas between cystic fibrosis infants dying

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with meconium ileus and those dying later with no history of meconium ileus.

Materials and Methods

Materials

The study was carried out retrospectively on 60 pancreatic autopsy specimens obtained over a period of 25 years from the archives of the Department of Pathology, The Hospital for Sick Children, Toronto. Three groups were included in this study: 1) control, 2) meconium ileus, and 3) cystic fibrosis with no history of meconium ileus. Details of the cases are summarized in Tables 1 and 2. All the subjects were less than 4 months at death, were in the normal percentiles for age, and had samples of pancreas with good histologic preservation.

Group 1 consisted of 29 control subjects with no clinical or pathologic evidence of cystic fibrosis or of any pancreatic involvement in the cause of death. Several cases of pancreatic dysmaturity, hypernatremia, and uremia were included in the control group. Group 2 consisted of 24 subjects with a history of meconium ileus and exocrine changes compatible with cystic fibrosis, based on the examination of at least three organ systems. Three cases were inconclusive. Group 3 included 7 subjects with pathologic findings compatible with cystic fibrosis and with no history of meconium ileus.

The gestational age and postnatal ages were taken from the clinical histories to calculate the postconceptional age for each patient.

Quantitative Studies

Samples of pancreas, fixed in neutral buffered formalin and embedded in paraffin wax, were sectioned and stained with either hematoxylin and eosin or Masson trichrome. Random sections from each pancreas were examined with a Reichert Visopan projection microscope and quantitative determinations were made in two stages using a point counting system.[7]

Stage 1

Acinar tissue, connective tissue (including blood vessels and nerves), interlobular ducts, and endocrine tissue were recorded from at least 20 random microscope fields, 250 × magnification using a 100-point grid. The points were separated by approximately 40 μm.

Stage 2

Lumen volume was determined from at least 40 random fields of acinar tissue, 500 × magnification, using a 400-point grid. The points were separated by approximately 5 μm.

The number of points overlying each type of component structure was calculated as a volume density and was expressed as a percentage of the total tissue volume. The sampling of tissue and determination of total number of points for estimation of the volume densities for each parameter was based on the calculations of Weibel.[7] For quantitation of acinar and connective tissue volumes (ie, Stage 1) at least 2000 points were sampled from each pancreas section, with a relative error less than 5%. For quantitation of lumen volume (ie, Stage 2) approximately 9000 points were necessary to establish confidence in the cystic fibrosis groups, but 90,000 were necessary for the same confidence level in the control group. Clearly, quantification of these numbers for the control group was impractical so that calculation of the lumen volume, which is extremely small in relation to the total tissue volume, shows a higher relative error in the control group.

Statistical Analysis

The standard error of the mean was calculated for the volume density of each component structure, for each case. The relationship between the volumes of pancreatic tissue components with age was determined by linear regression analysis and the 95% confidence limits and correlation coefficients were calculated according to standard statistical formulas.

Data were analyzed in relation to postnatal age, postconceptional age, body weight, and body length at the time of death. It was found that postconceptional age provided the best index for comparison of these cases which included neonates and infants with varying gestational and postnatal ages. Similar trends were obtained, however, when other indices of development were used.

Results

Pancreatic Acinar and Connective Tissue

The relative volumes of acinar tissue and connective tissue for each subject included in this study are summarized in Tables 1 and 2, and the ratios of acinar to connective tissue are plotted in Text-figures 1 and 2.

Control Group

The acinar to connective tissue ratio in the control group shows a significant relationship to postconceptional age (PCA) and is illustrated in Text-figure 1. The acinar to connective tissue ratio increases from approximately 0.5 at 32 weeks PCA to approximately 2.0 at 52 weeks PCA. The differentiation of the exocrine pancreas, judged by the acinar to connective tissue ratio is linear during this period ($r = 0.81$, $P < 0.001$).

Meconium Ileus Group

The acinar to connective tissue ratio for the meconium ileus group is illustrated in Text-figure 2. At 34 weeks PCA, the ratio is approximately 0.6 and then falls slightly to approximately 0.4 at 46 weeks PCA. From 34 to 42 weeks PCA, the acinar to connective tissue ratio is in the lower range of the control group. Above 42 weeks of age, the ratio is distinct from the control group. There was a negative correlation between the acinar to connective tissue ratio and the postconceptional age for cases with meconium ileus ($r = -0.52$, $P < 0.02$). Case 21 deviated greatly from the other 23 cases and was excluded from the statistical analysis but is included in Text-figure 2.

Cystic Fibrosis Group

The acinar to connective tissue ratio is plotted against postconceptional age and illustrated in Text-figure 2. The acinar to connective tissue ratio was similar in all subjects, averaging approximately 0.35 during the period

39 to 52 weeks PCA. There was no significant correlation between the acinar to connective tissue ratio and postconceptional age in this group ($r = 0.20$, $P < 0.70$).

There was no statistical difference between the regression lines for the groups with and without meconium ileus. The acinar to connective tissue ratio for both groups showed a slight negative correlation with postconceptional age ($r = -0.47$, $P < 0.01$).

Lumen Volume

The variations in lumen volume in control and cystic fibrosis infants are illustrated in Text-figures 3 and 4.

Control Group

The relative volume occupied by the acinar and duct lumens is approximately 0.2% of the pancreatic volume in all subjects included in the control group (Text-figure 3). The range is 0.1 to 0.7% and 97% of these cases have less than 0.5% lumen volume. There was no significant correlation between the lumen volume and postconceptional age in this group ($r = -0.22$, $P < 0.30$).

Cystic Fibrosis Groups

The relationship between lumen volume and postconceptional age for all cystic fibrosis subjects from Groups 2 and 3 is illustrated in Text-figure 4. The relative lumen volume varied from 0.3 to a maximum of 4.6% in the meconium ileus group (Group 2) and from 0.3 to 3.4% in the cystic fibrosis group (Group 3). The mean of Groups 2 and 3 together is 1.6%, and 83% of all these cases had lumen volumes greater than 0.5%. There was no apparent relationship between the lumen volume and postconceptional age in these groups.

Discussion

In the normal infant a clear pathway of development has been demonstrated which involves differentiation of acinar tissue and is reflected by the increased acinar to connective tissue volumes. The results from the controls dying with hypernatremia, bronchopneumonia, and uremia are indistinguishable from the normal controls. In these conditions, there is no apparent interruption of the normal maturation of pancreatic acinar tissue. Although nutritional status may contribute to some change in the number and/or size of acinar cells, there is no significant change in acinar and duct lumens.

Table 1—Group 1 Control Cases

Case	Sex	Body length* (cm)	Gestational age† (wk)	Postnatal age (days)	% Acinar tissue	% Connective tissue	% Lumen	Autopsy diagnosis
1	M	43	34	<1	31.7 ± 1.9	62.6 ± 2.0	0.1 ± .1	RDS respiratory distress syndrome
2	M	46	37	2	45.6 ± 2.7	47.5 ± 2.5	0.2 ± .1	RDS infant of a diabetic mother
3	M	43	34	<1	43.8 ± 2.4	54.5 ± 2.4	0.2 ± .1	RDS
4	M	45	36	2	32.6 ± 3.1	65.4 ± 2.9	0.7 ± .5	RDS
5	F	61	Term	105	65.3 ± 1.5	31.4 ± 1.4	0.2 ± .1	Pneumococcal meningitis; patchy pneumonia
6	F	55	Term	63	67.0 ± 2.5	30.5 ± 2.4	0.2 ± .1	Purulent meningitis; brain hemorrhage
7	M	42	32	1	33.8 ± 1.7	63.6 ± 1.6	0.4 ± .1	RDS
8	F	55	Term	46	58.5 ± 2.2	37.2 ± 2.0	0.2 ± .1	Gastroenteritis; dehydration
9	F	46	35	2	39.7 ± 1.4	52.3 ± 1.1	0.1 ± .0	RDS
10	M	47	36	1	34.2 ± 3.0	61.2 ± 2.6	0.4 ± .1	Prematurity; intrauterine pneumonia
11	M	44	32	6	33.6 ± 2.4	62.6 ± 2.8	0.1 ± .0	Prematurity; bronchopneumonia; cerebral hemorrhage
12	M	51.5	41	1	48.6 ± 1.5	50.0 ± 1.5	0.3 ± .1	RDS
13	M	50	38	<1	29.8 ± 1.9	64.1 ± 2.11	‡	Bronchopneumonia
14	F	52	39	28	55.2 ± 3.1	41.9 ± 3.5	0.2 ± .1	Intrauterine anoxia
15	F	49.5	43	1	56.1 ± 2.5	40.2 ± 2.5	‡	Meconium aspiration; bronchopneumonia
16	F	45	39	<1	47.1 ± 2.9	49.4 ± 2.3	0.2 ± .1	Intrapartum hypoxia
17	M	43.5	34	<1	41.7 ± 2.8	55.1 ± 3.0	0.2 ± .1	Prematurity; subdural hemorrhage
18	M	55	Term	46	49.8 ± 1.5	47.1 ± 2.1	0.1 ± .0	Bronchopneumonia
19	M	49	39	2	52.2 ± 2.0	41.9 ± 1.6	0.3 ± .1	Neonatal asphyxia
20	M	53	42	3	50.7 ± 3.0	44.4 ± 3.4	0.2 ± .0	Cervical medullary cord contusion
21	M	47	36	9	43.3 ± 3.1	54.0 ± 3.6	‡	Coxsackie B infection
22	M	52	41	3	50.7 ± 1.9	47.8 ± 1.9	0.3 ± .0	Perinatal asphyxia
23	M	50.5	39	2	46.6 ± 3.1	48.8 ± 2.9	0.3 ± .1	Bilateral bronchopneumonia; pulmonary hemorrhage
24	F	50	40	2	48.8 ± 2.5	48.7 ± 2.4	0.2 ± .1	Neonatal asphyxia; meconium aspiration
25	F	49	36	10	55.1 ± 2.7	39.0 ± 2.6	0.3 ± .1	Sepsis, type undetermined; congestive heart failure
26	F	46	34	4	49.5 ± 1.8	47.2 ± 1.8	0.2 ± .1	RDS
27	F	60	Term	63	51.6 ± 1.9	40.6 ± 2.1	0.1 ± .1	Purulent meningitis; disseminated intravascular coagulation
28	F	50	39	5	57.1 ± 2.5	39.4 ± 2.5	‡	Coxsackie A9 infection
29	M	53	Term	35	62.2 ± 3.2	32.1 ± 3.3	0.2 ± .1	Gastroenteritis; severe electrolyte imbalance; cerebral and pulmonary edema

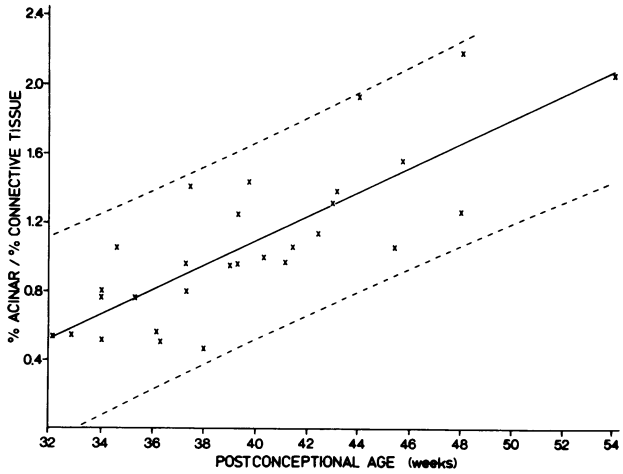
* Patients included only those with body length and/or weight in the 94% confidence limits for their age.
† For estimation of postconceptional age, deliveries recorded as "term" were assigned 39 weeks of gestation.
‡ Tissue preservation precluded Stage 2 quantitation.

Table 2—Cystic Fibrosis Cases

Case	Sex	Body length* (cm)	Gestational age† (wk)	Postnatal age (days)	% Acinar tissue	% Connective tissue	% Lumen	Autopsy diagnosis
GROUP 2—MECONIUM ILEUS								
30	F	48.5	35	11	33.2 ± 2.1	62.0 ± 2.7	2.0 ± .3	CF; meconium ileus and peritonitis; bronchopneumonia
31	F	49	Term	5	24.1 ± 2.5	67.7 ± 3.4	0.8 ± .3	CF; meconium ileus; subacute pneumonia
32	F	52	Term	21	30.9 ± 2.4	65.6 ± 2.7	2.2 ± .3	CF; meconium ileus; bronchopneumonia
33	F	52	Term	14	24.4 ± 1.5	70.2 ± 1.8	3.4 ± .4	CF; meconium ileus and peritonitis
34	M	52	Term	6	32.0 ± 1.4	65.4 ± 1.7	1.2 ± .2	CF; meconium ileus and peritonitis; ileal atresia
35	M	51	Term	28	32.2 ± 1.8	66.6 ± 2.0	2.8 ± .4	CF; meconium ileus and peritonitis
36	F	NA‡	Term	5	23.5 ± 1.8	72.3 ± 2.7	0.9 ± .2	CF; meconium ileus; micrognathia
37	F	53	40	7	34.4 ± 2.3	60.3 ± 2.9	4.6 ± 1.6	CF; meconium ileus; aspiration pneumonia; septicemia; omphalitis
38	F	NA	Term	47	21.4 ± 2.1	71.3 ± 3.5	2.6 ± .7	CF; meconium ileus; failure to thrive; dehydration
39	F	45	36	8	37.7 ± 1.7	56.7 ± 2.1	0.4 ± .2	Meconium peritonitis; ?meconium ileus; ?CF; acute laryngitis; tracheitis and esophagitis
40	M	45	37	4	34.8 ± 2.3	52.2 ± 3.2	0.8 ± .1	CF; meconium ileus; aspiration pneumonitis
41	F	51	Term	19	25.9 ± 2.1	68.4 ± 2.8	1.0 ± .2	Meconium ileus; aspiration pneumonia
42	F	NA	43	18	22.8 ± 1.8	67.9 ± 3.0	1.6 ± .2	CF; meconium ileus and peritonitis
43	M	51	40	17	29.3 ± 2.3	65.7 ± 3.0	1.7 ± .3	CF; meconium ileus; pulmonary hemorrhage and atelectasis
44	M	43	34	5	23.6 ± 2.0	73.1 ± 2.3	1.7 ± .3	CF; meconium ileus
45	M	49	Term	4	24.1 ± 2.2	69.4 ± 3.6	0.3 ± .1	CF; meconium ileus; microcolon
46	M	52	40	3	35.9 ± 3.9	62.3 ± 3.9	1.2 ± .2	CF; meconium peritonitis; perforation of colon
47	M	53	41½	11	26.1 ± 2.0	68.0 ± 2.8	1.2 ± .1	CF; meconium ileus; lung hemorrhage
48	M	44	34	3	42.0 ± 2.1	52.6 ± 2.1	0.2 ± .0	Meconium ileus and peritonitis
49	M	49	42	3	19.7 ± 2.3	73.7 ± 3.1	0.8 ± .2	CF; meconium peritonitis; intestinal atresia, pulmonary congestion and hemorrhage
50	M	47.5	39	14	44.8 ± 2.8	41.0 ± 2.9	0.4 ± .1	Meconium ileus and peritonitis; patent ductus; infant of a diabetic mother
51	M	50	38	37	30.1 ± 2.9	66.3 ± 3.1	2.2 ± .4	CF; meconium ileus and peritonitis; laryngotracheobronchitis; infant of a diabetic mother

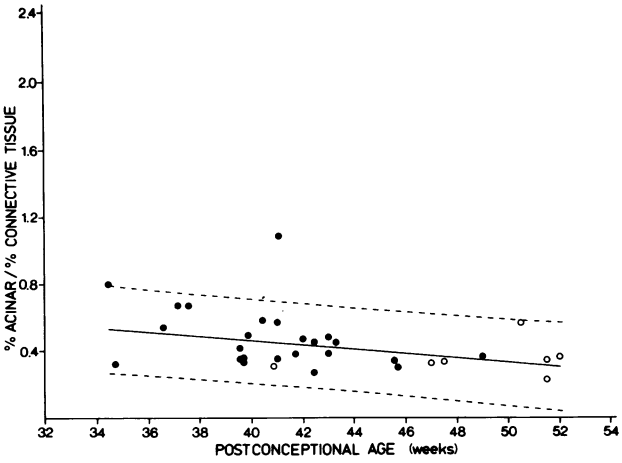
52	F	54	Term	70	25.7 ± 1.8	68.6 ± 2.2	0.7 ± .1	CF; bronchopneumonia; history of meconium ileus
53	M	50	34	39	28.2 ± 1.9	66.9 ± 2.3	0.3 ± .1	CF; bronchopneumonia; history of meconium ileus
GROUP 3—NO MECONIUM ILEUS								
54	F	57	Term	88	24.8 ± 1.9	71.0 ± 2.5	1.1 ± .4	CF; bronchitis; bronchopneumonia
55	F	56	Term	88	17.9 ± 2.1	77.1 ± 2.4	2.3 ± .9	CF; bronchopneumonia; emphysema
56	F	57	Term	91	25.1 ± 1.5	68.3 ± 2.6	1.3 ± .2	CF; bronchopneumonia; failure to thrive
57	M	59.5	Term	60	22.8 ± 1.5	67.4 ± 2.5	0.3 ± .1	CF; pneumonitis
58	F	54.5	Term	81	35.5 ± 2.1	62.3 ± 2.3	3.4 ± 1.1	CF; pneumonia; failure to thrive
59	M	52	40	49	21.8 ± 1.7	66.1 ± 3.1	3.4 ± .9	CF; pneumonia; pneumothorax
60	M	48	38	20	23.1 ± 1.8	75.3 ± 2.1	1.2 ± .3	CF; bronchopneumonia; Arnold-Chiari syndrome

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

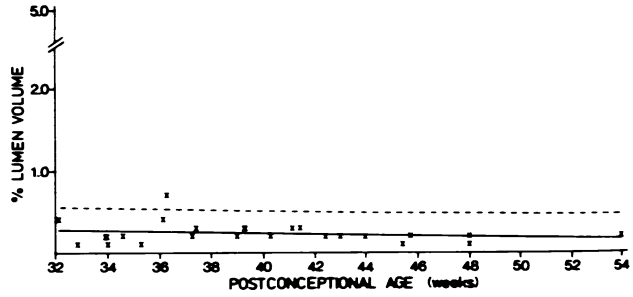


TEXT-FIGURE 1—Relationship between acinar/connective tissue ratio and postconceptional age of control infants. Each point represents the value for 1 case. The solid line represents the regression line; broken lines represent the 95% confidence limits ($r = 0.81$, $P < 0.001$).

In contrast, patients with meconium ileus and with cystic fibrosis show a time-related pattern which clearly deviates from that of the normal. At 32 to 38 weeks PCA, most meconium ileus and cystic fibrosis patients show acinar to connective tissue ratios in the lower range of the control values (Text-figure 5). This suggests that there is a lack of early normal maturation or a persistence of the fetal pattern in pancreatic exocrine tissue with the degenerative process supervening after birth. Approximately 80% of all cystic fibrosis infants, with and without meconium ileus, have lumen volumes quite distinct from the control ranges. The increase in lumen volume in these infants does not correlate with the ratio of acinar tissue to



TEXT-FIGURE 2—Relationship between the acinar/connective tissue ratio and postconceptional age of CF infants. *Closed circles*, infants with a history of meconium ileus ($r = -0.52$, $P < 0.20$); *solid circles*, infants without a history of meconium ileus ($r = 0.20$, $P < 0.70$). The relationship between acinar/connective tissue ratio is similar in both groups (combined data: $r = -0.47$, $P < 0.01$).

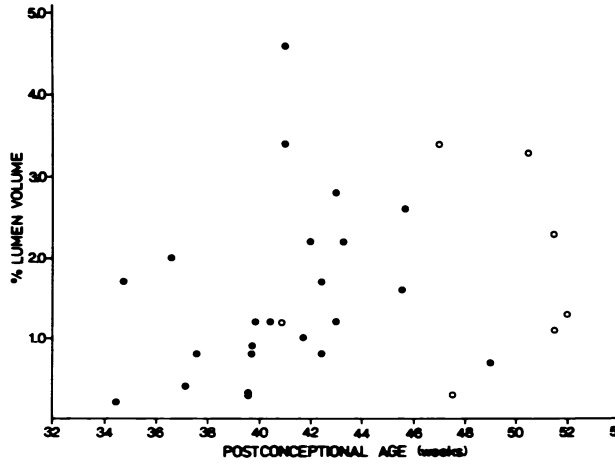


TEXT-FIGURE 3—Relationship between lumen volume and postconceptional age of control infants ($r = -0.22$, $P < 0.30$).

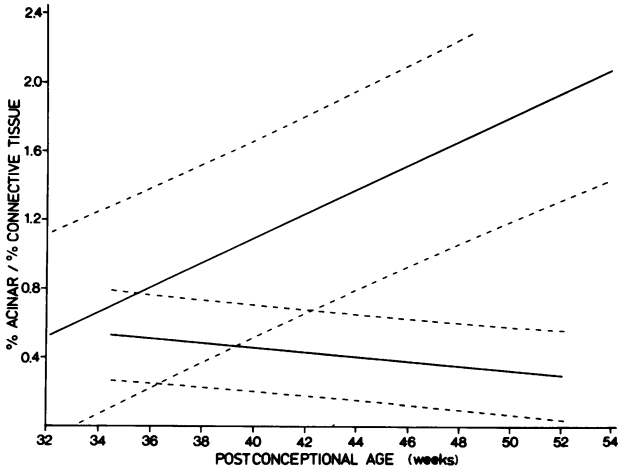
connective tissue nor with the age of the infants with cystic fibrosis (Text-figure 6).

At the present time the criteria for diagnosis of cystic fibrosis within the first few months of life are subjective. Clinical features are very variable and histologic signs have been reported as equivocal or absent. At autopsy, Esterly and Oppenheimer [8,9] have indicated that pancreatic changes are difficult to diagnose in early life on the basis of microscopic examination. By quantitative microscopy, we have the basis of an objective method for diagnosing cystic fibrosis in infants at autopsy by the examination of the pancreas. In the present study, only 2 of 30 cystic fibrosis cases (7%) defied differentiation on the basis of the pancreas alone. This approach may prove to be very valuable in confirming the diagnosis of cystic fibrosis at early ages, which is particularly important in relation to genetic counseling of parents.

Meconium ileus is an early manifestation of cystic fibrosis occurring in

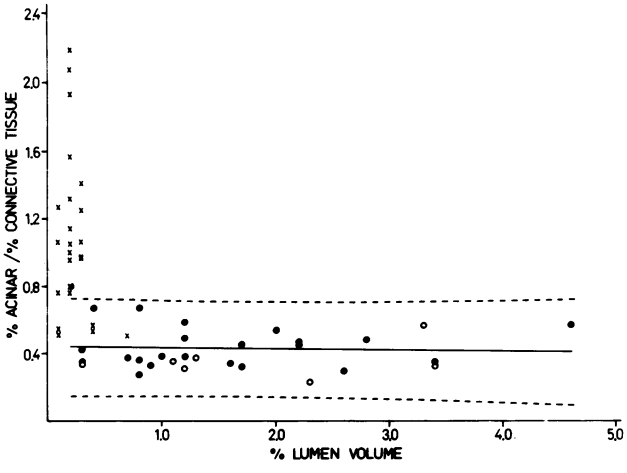


TEXT-FIGURE 4—Relationship between lumen volume and postconceptional age of cystic fibrosis infants with (*closed circles*) and without (*open circles*) a history of meconium ileus.



TEXT-FIGURE 5—Comparison of the regression lines and confidence limits of change in acinar/connective tissue ratio with age in control and cystic fibrosis infants: composite from Text-figures 1 and 2.

about 15% of cystic fibrosis patients.[9,10] In most studies it has been implied that meconium ileus is almost exclusively a symptom of cystic fibrosis, although this has been questioned recently by Rickham et al,[11] who suggested that up to 25% of patients presenting with meconium ileus do not have cystic fibrosis judged by clinical criteria. In the present study, the pancreatic changes appear similar in all infants with cystic fibrosis including those with and without a history of meconium ileus. All cases except one (Case 50) show the lack of maturation of exocrine tissue and replacement of acinar tissue by fibrous connective tissue. Two further cases with meconium ileus (Cases 39 and 48) have acinar/connective tissue ratios in



TEXT-FIGURE 6—Relationship between the acinar/connective tissue ratio and lumen volume of control infants and of cystic fibrosis infants with (*closed circles*) and without (*open circles*) a history of meconium ileus.

the lower percentiles of normal but show no increase in lumen volume so that they could not be discriminated from the controls. In these infants, changes in the pancreas and other organs are not conclusive for diagnosis of cystic fibrosis. Such observations may indicate meconium ileus from a cause other than cystic fibrosis or may preclude the diagnosis of cystic fibrosis before 37 weeks PCA according to conventional histologic criteria. Although pancreatic dysfunction may be a contributing factor to meconium ileus, the findings indicate that this is not the sole determining factor but must be accompanied by other predisposing factors in the affected infants. These observations and the observations of the greater incidence of meconium ileus in some families may reflect genetic heterogeneity in this disease.

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