diovascular disease in the diabetic. In: Berger M, Wahren J, eds. Diabetes and exercise. Bern, Switzerland: Huber. (in press).
33. Wong HYC, David SN, Orimilikwe SO. Effect of exercise on collagen and elastin in cockerels with induced atherosclerosis. In: Schettler G, Weizel A, eds. Atherosclerosis III. New York: Springer-Verlag, 1974:362-5.
34. Heikkinen E, Vuori I. Effect of physical activity on the metabolism of collagen in aged mice. Acta Physiol Scand. 1972; 84:543-9.
35. Tomanek RJ, Taunton CA, Liskop KS. Relationship between age, chronic exercise and connective tissue of the heart. J Gerontol. 1972; 27:33-8.
36. Wolinsky H, Goldfischer S, Katz D, et al. Hydrolase activity in the rat aorta. III. Effects of regular swimming activity and its cessation. Circ Res. 1979; 45:546-53.
37. Stevenson JAF, Feleki V, Rechnitzer P, Beaton JR. Effect of exercise on coronary tree size in the rat. Circ Res. 1964; 15:265-9.
38. Fischell T. Running and the primary prevention of coronary heart disease. Cardiovasc Rev Rep. 1981; 2:238-44.
39. Simko V, Kelley RE. Effect of physical exercise on bile and red blood cell lipids in humans. Atherosclerosis. 1979; 32:423-34.
40. Leung DYM, Glagov S, Mathews MB. Elastin and collagen accumulation in rabbit ascending aorta and pulmonary trunc during postnatal growth: correlation of cellular synthetic response with medial tension. Circ Res. 1977; 41:316-23.

INCREASED BIOELECTRIC POTENTIAL DIFFERENCE ACROSS RESPIRATORY EPITHELIA IN CYSTIC FIBROSIS

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Abstract To investigate respiratory epithelial function in cystic fibrosis, we measured the transepithelial electrical potential difference across the upper and lower respiratory mucosa in patients with cystic fibrosis and control subjects. The nasal potential difference in the 24 patients with cystic fibrosis exceeded by more than 3 standard deviations the mean voltage in healthy controls, subjects with other diseases, and subjects heterozygous for cystic fibrosis. Potential differences in lower airways were measured in four patients and were significantly greater than in controls ($P<0.05$). Superfusion of the luminal surface with amiloride, an inhibitor of active sodium absorption, induced greater reductions in both nasal and airway potential differences in patients than in controls. We conclude that the increased respiratory-epithelial potential differences appear to be a specific abnormality in homozygotes for cystic fibrosis. The greater reduction in potential difference in response to amiloride suggests that absorption of excess salt and perhaps liquid from respiratory epithelial surfaces contributes to the pathogenesis of lung disease in cystic fibrosis. (N Engl J Med. 1981; 305:1489-95.)

ABNORMALITIES in the electrolyte and water content of luminal liquid or "secretions" of the airways, sweat glands, intestinal and reproductive tracts, and pancreas suggest that cystic fibrosis is characterized by a generalized epithelial dysfunction.[1,2] Airway secretions are tenacious and excessively thick. Although a search for the cause of this abnormal surface liquid has failed to identify unique mucus glycoproteins,[3] altered electrolyte composition and lower water content ("dehydration") of airway secretions from patients with cystic fibrosis have been reported.[4,5]

The volume and composition of airway secretions is regulated at least in part by active ion-transport mechanisms located within the respiratory epithelium.[6-8] Active sodium transport appears to be a dominant driving force for salt absorption across excised human airways.[9,10] This flow, combined with passive hydrostatic and oncotic forces, probably contributes to liquid absorption across respiratory epithelia. Transport also contributes to bioelectric properties (e.g., potential difference, short-circuit current, and conductance) that can be measured in excised tissues.[6,7] Transepithelial potential differences across airways of animals and human beings have been measured in vivo, are always lumen negative, and are similar to the voltages measured in vitro.[11,12]

An earlier study demonstrated parallel behavior of nasal and airway potential difference in healthy subjects.[13] Because the nasal mucosa is accessible, and because in patients with cystic fibrosis it is relatively free of infection as compared with bronchi, we measured potential differences across the nasal mucosa in 24 patients and compared these voltages with those in healthy control subjects. Since the nasal potential difference was increased in the patients, potential difference was also measured in their parents (obligate heterozygote subjects) and siblings and in subjects with diseases that mimic some aspects of cystic fibrosis. We attempted to evaluate the genesis of the potential difference by measuring the voltage responses to superfusion of relatively selective inhibitors of the flow of sodium (amiloride) and chloride (indomethacin) across respiratory epithelia. The link between abnormalities in potential difference of the upper and lower airways in cystic fibrosis was evaluated by measuring potential difference and the amiloride response in the lower airways of a small number of patients.

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METHODS

Subjects

Patients

We studied 23 patients with cystic fibrosis (14 female and nine male patients, three months to 32 years old) from among 44 pa-

tients who attended the Cystic Fibrosis Center of the North Carolina Memorial Hospital. The diagnosis of cystic fibrosis was established by clinical criteria[1] and by the presence of increased concentrations of chloride in sweat. Clinical criteria had no role in the selection of patients for the measurement of nasal potential difference. In general, these patients were taking pancreatic enzymes and antibiotics; three were taking methylxanthines. Four were known to have normal serum vitamin E concentrations. Of the seven youngest patients (under three years), three were clinically free of disease and required only vitamins and pancreatic enzymes. We also studied a 29-year-old woman who had clinical cystic fibrosis with consistently normal sweat concentrations of sodium and chloride.[14] Many of the parents of these patients (nine fathers and 15 mothers, 19 to 62 years old) were studied, as were some siblings (seven sisters and seven brothers, six months to 31 years old).

Controls

The "diseased" control group contained 32 subjects with the following conditions: chronic respiratory disease — postinfectious bronchiectasis (three subjects), immotile-cilia syndromes (three), asthma (four, including two who were receiving aminophylline and inhaled beta-adrenergic agents), chronic obstructive pulmonary disease (two), and sarcoidosis (one); pancreatic insufficiency and malabsorption — carcinoma of the pancreas with diabetes (one subject) and amyloid (one); fluid and electrolyte disorders — chronic liver disease with ascites (two subjects), chronic renal failure treated with furosemide (240 mg per day) (one), congestive heart failure treated with digoxin and furosemide (100 mg per day) (one), essential hypertension (one), and primary hyperaldosteronism treated with captopril (150 mg per day) (one); and miscellaneous conditions — allergic or perennial rhinitis (four subjects), pregnancy (one), lactation (one), Sjögren's syndrome (one), and cigarette smoking (four).

The healthy control group contained 54 subjects without a history of atopy, cigarette smoking, medication use, or symptoms of acute rhinitis.

All procedures were approved by the Committee on the Rights of Human Subjects at the University of North Carolina, and informed consent was obtained from the patients and controls or their parents.

Measurement of Electrical Potential Difference across Nasal Epithelia

Nasal potential difference was measured between a fluid-filled exploring bridge and a reference bridge in the subcutaneous space of the forearm, as previously described.[13] Potential differences were recorded from the anterior tip, medial surface, and five sites under the inferior turbinate, as well as from the nasal septum and floor. Measurements of potential difference of the medial surface were repeated in some of the 24 patients with cystic fibrosis after abrasion of the mucosa was performed.[13] Because subjects under five years of age had smaller turbinates, and because measurement of potential difference at sites comparable to those in older subjects was difficult, only the maximal potential difference under the turbinate was recorded in these younger subjects.

Effects of Application of Pharmacologic Agents on Nasal Potential Difference

A double-barreled exploring bridge delivered either drug-free or drug-containing Ringer solution onto the nasal surface as previously described.[13] Base-line measurements of potential difference were recorded for one minute under the inferior turbinate. Because the actions of amiloride on airway epithelia are rapid (under two minutes),[15] the effects of superfusion of this drug (10^{-7} to 10^{-3} M) on potential difference were monitored for three minutes. Since the time course of indomethacin's action on bioelectric properties of respiratory epithelia is not well documented,[16] perfusion of the drug (10^{-6} M) onto nasal surfaces was monitored for at least six minutes, and in some subjects measurements of potential difference were repeated 20 minutes after perfusion.

Measurement of Lower-Airway Potential Difference and Effects of Amiloride

Older Subjects (≥5 Years Old)

Three patients with cystic fibrosis (18 to 20 years old) were selected on the basis of clinical condition (stable), forced expiratory volume in one second (>40 per cent of predicted level), and arterial-blood gas levels (partial pressure of oxygen, >60 torr; partial pressure of carbon dioxide, normal). Sputum cultures from one patient grew *Pseudomonas aeruginosa*, and those from another grew *Staphylococcus aureus*. After the patients were premedicated with atropine, promethazine, and meperidine and their vocal cords were anesthetized with lidocaine, a fiberoptic bronchoscope was positioned in the trachea; the potential difference was recorded from a perfused (0.3 ml per minute) double-lumen exploring bridge. After a stable (>30 seconds) base line had been recorded, the effect of amiloride (10^{-4} M) was measured during its perfusion (45 seconds). Next, potential differences were recorded at two to four sites in main-stem bronchi, the bronchus intermedius, the upper and lower lobar bronchi, and segmental and subsegmental bronchi. The effect of amiloride (10^{-4} M) on segmental-bronchi potential difference was recorded during a 45-second superfusion. Because general anesthesia does not affect airway potential difference in dogs[11] or human beings (unpublished data), airway potential difference in patients with cystic fibrosis was compared with that in seven subjects without respiratory disease who were anesthetized and intubated for minor surgical or diagnostic procedures. The trachea and a segmental bronchus of four of these subjects were exposed to amiloride.

Younger Subjects

The tracheal potential difference was measured in a three-month-old patient with cystic fibrosis who was undergoing elective herniorrhaphy; this patient had previously had a positive sputum culture for *P. maltophilia*. After anesthesia, intubation, and insertion of a reference bridge, a perfused (0.1 ml per minute) exploring bridge was positioned on the tracheal surface, and the potential difference was recorded for 10 seconds at three sites 0.5 cm apart. For comparison, tracheal potential difference was recorded in five controls (six weeks to two years old) without respiratory disease, who were undergoing minor urologic procedures or plastic surgery under general anesthesia.

Histologic Studies

Biopsy specimens of nasal mucosa from the sites of measurement of potential difference were obtained to determine the surface cell populations in the patients and controls as previously described.[13]

Data Analysis

In older subjects the mean and maximal potential differences of the inferior surface of the turbinate, as well as the potential differences of the medial surface, septum, and floor, were calculated as previously described.[13] Although both measures of inferior-turbinate potential difference showed the same pattern, the mean potential difference had a smaller variance.[13] In each younger subject the maximum potential differences under each inferior surface were averaged.

The potential differences of all study subjects were compared by the t-statistic for independent means. Changes induced by drugs were assessed by paired t-analysis. All values, unless otherwise indicated, represent means ±S.E.M., and $P<0.05$ was considered significant.

Results

All airway potential differences were lumen negative with respect to the reference bridge. The pattern of potential difference in a healthy control (Fig. 1*A*) was typical of patterns that we have reported pre-

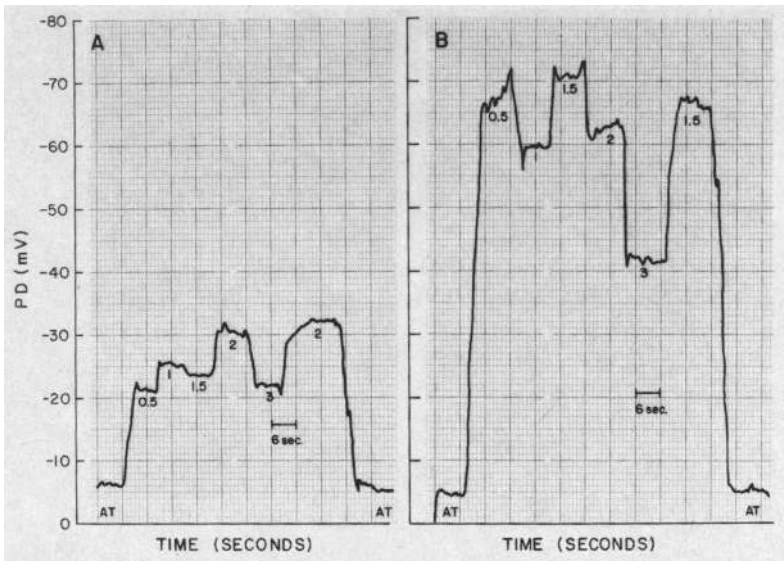

Figure 1. Continuous Tracing of Nasal Potential Difference (PD) from the Anterior (AT) and Inferior Surface of the Inferior Turbinate.

Panel A shows a tracing from a healthy control, and Panel B shows one from a patient with cystic fibrosis. The numbers under the tracing refer to the distance (in centimeters) under the turbinate.

viously.[13] The tracing in a patient with cystic fibrosis (Fig. 1*B*) was characterized by a similar potential difference at the anterior tip but by greater voltages at all sites on the inferior surface. There was little mucus or inflammation of the inferior turbinate of the patients, and nasal cultures from eight patients grew no pathogens. Prolonged perfusion at a single site did not alter the voltage (see below), nor did relocation of mucus alter any voltage, suggesting that surface mucus did not contribute to the measured voltages. In contrast, abrasion of four patients' epithelium abolished the potential difference (−0.1±1.6 mV).

None of the potential differences in the patients overlapped those in the healthy or diseased control groups (Fig. 2). The mean potential difference in the older patients (53.0±1.8 mV) was different from that in healthy controls (24.7±0.9 mV) or diseased controls (20.5±1.3 mV) (Fig. 2*A*). The mean potential differences among patients did not differ with regard to the patients' sex. Increased potential differences in

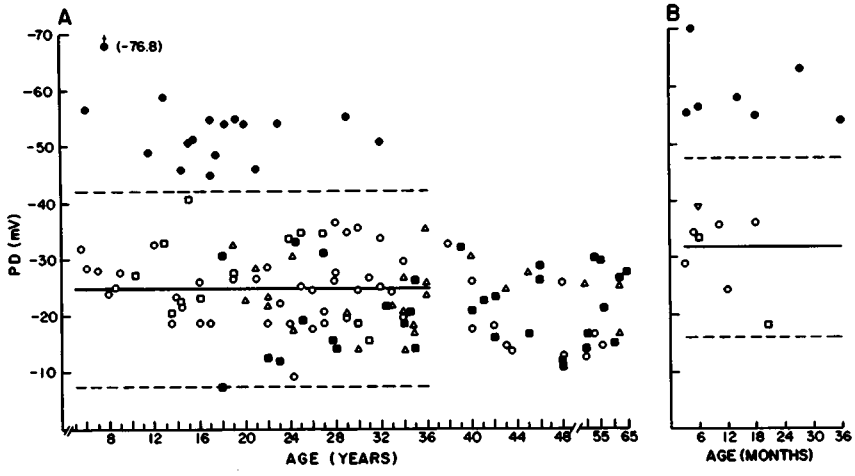

Figure 2. Potential Difference as a Function of Age.

Panel A shows the mean nasal potential difference for the inferior surface in the older subjects. Panel B shows the maximal potential difference in the younger subjects.

Patients with cystic fibrosis are represented by ●, their parents by △, their siblings by □, and a child of one patient by ▽. Healthy control subjects are represented by ○, and diseased control subjects by ■; the solid lines denote mean values for healthy controls, and the dashed lines ±3 S.D. Symbols representing patients in whom measurements of the mean potential difference were repeated (see text) denote the mean of all measurements.

six patients persisted when measurements were repeated up to one year later (+8.5±7.0 per cent change). The chronology of the increased potential differences in patients with cystic fibrosis was investigated by measuring maximal potential difference in the younger subjects (Fig. 2*B*). In each of seven patients this potential difference exceeded the normal range.

Nasal potential differences for obligate heterozygotes (parents of patients) and subjects of whom two thirds would be expected to be heterozygotes (siblings of patients) were not different from those of control subjects. No differences in mean potential difference were found when either the parents of the patients or only the 15 mothers (23.0±1.5 mV) were compared with healthy controls. Because only the maximal potential difference was measured in the younger subjects, we compared this value in 10 healthy siblings of the patients and 31 controls. The values were not significantly different (36.3±3.2 vs. 32.3±1.2 mV).

Surface cell populations within the nasal cavity of patients with cystic fibrosis were not different from those previously reported for healthy subjects[13] (cell populations in six patients: inferior surface, 67±8.1 per cent ciliated cells and 33±4.3 per cent goblet and villous cells; medial surface, 28±8.9 per cent ciliated cells and 72±7.9 per cent goblet and villous cells; anterior tip, 100 per cent squamous epithelium). The nasal potential difference in the patients as compared with controls was increased approximately twofold at sites populated by ciliated epithelium (inferior surface, medial surface, floor, and septum) but not at a site lined by squamous epithelium (anterior tip).

The effect of amiloride on nasal potential difference was dose dependent, and the drug was as potent in the healthy controls as in the patients (data not shown). However, despite the greater nasal potential difference in the patients, a maximal amiloride concentration reduced the potential difference to a similar absolute voltage in both patients and healthy controls (Fig. 3). The mean fall in potential difference in the patients (73±3 per cent) was greater than the mean fall in the healthy subjects (53±4 per cent).

Indomethacin superfusion for six minutes decreased the potential difference by 1.4±6.0 per cent in five patients and by 1.3±2.0 per cent in six controls. Neither change was significantly different from zero or from the 0.9±3.0 per cent decrease induced in four control subjects by a six-minute superfusion with the drug-free solution. In three patients potential differences that were measured 20 minutes after indomethacin superfusion were not different from base-line potential differences.

The pattern of potential difference in airways of normal persons (Table 1) was similar to that observed in other mammals.[11] The mean potential difference in each region of the airways of the patients with cystic fibrosis significantly exceeded that in healthy controls ($P<0.05$). In addition, the tracheal potential difference in a three-month-old patient studied under general anesthesia was 56 mV, whereas the mean tracheal potential difference in five controls of similar ages was 19.5±1.8 mV. The effect of amiloride on airway potential difference is summarized in Table 2. Amiloride inhibited the tracheal and bronchial potential difference of both healthy controls and patients but was more efficacious in the latter group (Table 2).

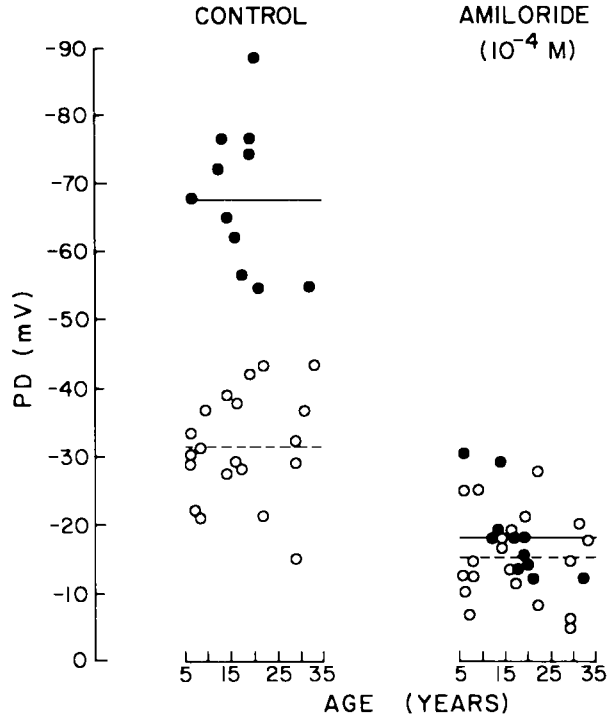

Figure 3. Nasal Potential Difference before Drug Superfusion (CONTROL) and afterward (AMILORIDE).

Patients with cystic fibrosis are represented by ● and healthy control subjects by ○. The mean value in the patients is denoted by a solid line, and in the controls by a dashed line.

Discussion

The increased potential difference in the patients with cystic fibrosis probably reflects an inherent epithelial abnormality rather than a consequence of chronic disease. The increased nasal voltage preceded the development of clinically detectable chronic effects in several younger patients. Moreover, the tracheal potential difference was increased in the three-month-old patient who was clinically free of disease at the time of study. We also found no association between the magnitude of nasal potential difference and typical sequelae associated with cystic fibrosis. *(1)* The increased nasal potential differences do not appear to be a consequence of chronic respiratory infections, because the nasal mucosa of the patients was not infected, and the control subjects with other diseases characterized by chronic pulmonary infection did not have increased nasal potential differences. *(2)* High concentrations of circulating aldosterone, a hormone that affects ion transport and

bioelectric properties of target epithelia,[17] have been noted in patients with cystic fibrosis.[18] However, these changes occur mainly in older patients.[19] The raised nasal potential difference in both the young and older patients, as well as the "normal" potential difference in control subjects who probably had increased circulating aldosterone levels, argues against the participation of increased concentrations of aldosterone in inducing the abnormal voltage. *(3)* Pancreatic insufficiency not due to cystic fibrosis was not associated with increased nasal potential differences.

The increased respiratory-tract potential differences in the patients may provide some insight into the pathophysiology of the lung disease of cystic fibrosis. Respiratory epithelia behave in vitro like an ohmic resistor.[7,20] Hence, the transepithelial potential difference reflects the relation between the magnitude of the ion current and the passive ion conductance. The ion current is linked to active ion transport, thus allowing the magnitude of the potential difference to help identify the direction or magnitude (or both) of ion transport. For example, a direct correlation between the rate of sodium absorption and potential difference has been noted in some sodium-absorbing epithelia,[21-23] and we have observed a similar relation in respiratory epithelia.[7] Consequently, because active sodium absorption is a major ion flow across human nasal[9] and airway epithelia,[10] the increased potential difference in patients with cystic fibrosis may denote a greater rate of sodium transport.

The response of the potential difference to amiloride, a selective pharmacologic antagonist of sodium absorption, supports this hypothesis. Recent studies have shown that active sodium absorption in airway epithelia is inhibited by amiloride.[15,24] By analogy with amiloride's action on other epithelia, the effect probably results from interaction with receptors on the luminal membrane of the surface cells, inhibiting the passage of sodium across this barrier.[25] The topical application of amiloride to normal nasal and airway epithelium in vivo reduced potential difference about 50 per cent (Fig. 3) and 25 per cent (Table 2), respectively. In the patients, amiloride induced a greater absolute and proportional reduction in both nasal (Fig. 3) and airway potential difference (Table 2). A simple reduction in passive ionic conductance of the barrier in epithelia affected by cystic fibrosis probably cannot account for this finding, because according to Ohm's law, the response of the potential difference to amiloride in patients should be proportionate to that in controls if equivalent decreases in active flow are induced in both groups. Consequently, since amiloride selectively inhibits sodium transport, and because a direct relation between the magnitude of net sodium transport and amiloride efficacy has been reported in a number of epithelia,[23,26,27] the increased action of amiloride on respiratory epithelia in cystic fibrosis implicates the participation of enhanced net sodium absorption in the genesis of the increased potential difference.*

Chloride secretion probably does not contribute to the raised potential difference in patients with cystic fibrosis. Active sodium absorption is the dominant ion translocation in excised "normal" human bronchi, whereas chloride secretion has not been detected.[10] Furthermore, indomethacin, an agent that inhibits chloride secretion in canine trachea,[16] has failed to affect potential difference in the nasal epithelium.

Since the potential differences in the patients lay outside the distribution for a healthy population (Fig.

Table 2. Effect of Superfusion of Amiloride (10^{-4} M) on Airway Potential Difference in Controls and Patients with Cystic Fibrosis.

Group; Age *	Airway Region	Potential Difference * (Lumen Negative)		
		Before Drug	After Drug	Per Cent Change
		millivolts		
Controls (n = 4); 24.8±2.3	Trachea	21.9±3.8	16.5±5.8	−24.7±4.8 †
	Segmental bronchus	19.0±2.6	14.4±1.8	−24.2±6.4 †
Patients (n = 3); 19.0±0.6	Trachea	47.1±2.7	7.3±3.1	−84.5±4.0 †‡
	Segmental bronchus	38.9±2.5	3.8±2.9	−90.2±8.0 †‡

*Mean ±S.E. †Significantly different from zero change ($P<0.05$).
‡Significantly different from value in controls ($P<0.05$).

*Increased net sodium absorption could reflect an increased driving force (greater rate of active transport) or decreased passive sodium backleak. The pattern of amiloride action and conventional electrical modeling of sodium paths through and between cells of airway epithelia indicate that the sodium leak through the paracellular path is probably not defective in cystic fibrosis. The abnormality appears to reside in the transcellular path, but there is insufficient information to resolve the nature and site of the dysfunction.

Table 1. Tracheobronchial Potential Difference in Controls and Patients with Cystic Fibrosis.

Group	Age *	Potential Difference *† (Lumen Negative)				
		Trachea	Main-Stem Bronchus	Bronchus Intermedius	Lobar Bronchus	Segmental Bronchus
	yr	*millivolts*				
Controls (n = 7)	22.0±2.1	27.7±6.4	19.1±5.0	20.1±4.7	18.7±5.3	13.5±4.3
Patients (n = 3)	19.0±0.6	44.0±2.6	41.0±2.5	45.2±15.0	32.3±3.5	36.8±5.4

*Mean ±S.E. †All values in patients were significantly different from the values in controls ($P<0.05$).

2 and Table 1), the increased sodium absorption that we suggest is indicated by this clustering may be "excessive." Since the potential differences in the heterozygous subjects did not range between those of the healthy controls and the patients, the postulated defect in sodium transport appears to be linked with the clinical expression of cystic fibrosis. If we assume that excessive sodium absorption (accompanied by a counter ion) drives excessive water absorption from the surface liquid on the airways, our observations are consistent with the decrease in water content observed in respiratory-tract secretions in cystic fibrosis.[4,5] However, dehydration of mucus, along with reductions in mucociliary clearance, is probably not the sole cause of the specific microbiologic flora[1] associated with the lung disease of cystic fibrosis, because other diseases (e.g., immotile-cilia syndromes) that selectively depress mucociliary clearance are characterized by a microbiologic flora similar to that seen in chronic bronchitis.[28] Perhaps another transport process linked to the rate of sodium transport (e.g., proton exchange) creates other abnormalities in the airway microenvironment.

It is not obvious that excessive sodium absorption can explain the dysfunction of all epithelia that are affected in cystic fibrosis. The changes in volume of pancreatic liquid, cervical mucus, and meconium are compatible with increased salt and water absorption, whereas the defect in the sweat ducts implicates decreased salt absorption.[1,2]

Measurements of nasal potential difference could not identify "carriers" of cystic fibrosis because the voltages of the subjects heterozygous for cystic fibrosis were not different from those of control subjects. However, nasal potential differences may provide a diagnostic adjunct to the sweat test in the diagnosis of cystic fibrosis. Although the sweat chloride test is reliable and specific in confirming the diagnosis in clinically suspected cases, 1 to 2 per cent of patients with clinical criteria for cystic fibrosis have intermediate (or normal) concentrations of chloride in sweat.[14,29] The nasal potential difference in one patient with normal sweat electrolyte concentrations was increased (Fig. 2*A*, 29-year-old woman). Similarly, diagnosis may be assisted by measurement of the potential difference in neonates, in whom the sweat test is technically difficult to perform, or in older subjects, in whom the normal range of sweat chloride concentrations is increased.[29]

If excessive epithelial sodium absorption is important in the pathogenesis of lung disease in cystic fibrosis, then amiloride's action may have therapeutic implications. A number of major issues must be addressed before the drug's use in cystic fibrosis is contemplated. *(1)* The quantitative relation between the rate of net active ion transport and the volume of airway surface liquid in the normal lung and the lung affected by cystic fibrosis is not known. Consequently, maintenance of a proper surface liquid volume after drug administration may be difficult. *(2)* Neither the presence of sodium-linked translocations in airways nor the effectiveness of amiloride blockade of such processes have been assessed. *(3)* Amiloride is most potent after applications to the luminal surface of sensitive epithelia.[25] Consequently, systemic administration would probably be ineffective and aerosolization would be necessary. Aside from the erratic nature of aerosol delivery, cephalad movement of drug in airway surface liquid by ciliomotion and concentration of the agent by airway liquid absorption in more central regions could lead to concentration gradients along the airways. *(4)* The pharmacokinetics, pharmacodynamics, and toxicity of aerosolized amiloride in healthy animals and in animal models of cystic fibrosis have not been explored. Finally, the clinical abnormality in the lungs of patients is often not evident for years. Accordingly, evaluation of the effectiveness of a putative therapeutic agent may be expected to take a similarly long time.

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References

1. Wood RE, Boat TF, Doershuk CF. State of the art: cystic fibrosis. Am Rev Respir Dis. 1976; 113:833-78.
2. Dearborn DG. Water and electrolytes of exocrine secretions. In: Mangos JA, Talamo RC, eds. Cystic fibrosis: projections into the future. New York: Symposia Specialists, 1976:179-91.
3. Davis PB, di Sant'Agnese PA. A review. Cystic fibrosis at forty — quo vadis? Pediatr Res. 1980; 14:83-7.
4. Chernick WS, Barbero GJ. Composition of tracheobronchial secretions in cystic fibrosis of the pancreas and bronchiectasis. Pediatrics. 1959; 24:739-45.
5. Potter JL, Matthews LW, Spector S, Lemm J. Studies on pulmonary secretions. II. Osmolality and the ionic environment of pulmonary secretions from patients with cystic fibrosis, bronchiectasis, and laryngectomy. Am Rev Respir Dis. 1967; 96:83-7.
6. Olver RE, Davis B, Marin MG, Nadel JA. Active transport of Na^+ and Cl^- across the canine tracheal epithelium *in vitro*. Am Rev Respir Dis. 1975; 112:811-5.
7. Boucher RC, Stutts MJ, Gatzy JT. Regional differences in canine airway epithelial ion transport. J Appl Physiol. 1981; 51:706-14.
8. Boucher RC, Stutts MJ, Bromberg PA, Gatzy JT. Regional differences in airway surface liquid composition. J Appl Physiol. 1981; 50:613-20.
9. Melon J. Activite secretorie de la muqueuse nasale. Acta Otorhinolaryngol Belg. 1968; 22:5-244.
10. Knowles MR, Murray GF, Shallal JA, Gatzy JT, Boucher RC. Bioelectric properties (BEP) and ion transport (IT) in excised human bronchi. Physiologist. 1980; 23(4):100. abstract.
11. Boucher RC Jr, Bromberg PA, Gatzy JT. Airway transepithelial electric potential in vivo: species and regional differences. J Appl Physiol. 1980; 48:169-76.
12. Bromberg P, Buntin W, Gatzy J, Boucher R. In vivo measurements of tracheal bioelectric potential differences in normal human subjects. Physiologist. 1979; 22(4):15. abstract.
13. Knowles MR, Carson JL, Collier AM, Gatzy JT, Boucher RC. Measurements of nasal transepithelial electric potential differences in normal human subjects in vivo. Am Rev Respir Dis. 1981; 124:484-90.
14. Davis PB, Hubbard VS, di Sant'Agnese PA. Low sweat electrolytes in a patient with cystic fibrosis. Am J Med. 1980; 69:643-6.
15. Boucher RC, Gatzy JT. Effect of amiloride (Am) and mucosal sodium removal on canine bronchial ion transport. Fed Proc. 1981; 40:447. abstract.

16. Welsh MJ, Smith PL, Frizzell RA. Electrophysiology of Cl secretion by the canine tracheal epithelium. J Gen Physiol. 1980; 76:27a.
17. Frizzell RA, Schultz SG. Effect of aldosterone on ion transport by rabbit colon *in vitro*. J Membrane Biol. 1978; 39:1-26.
18. Simopoulos AP, Lapey A, Boat TF, di Sant'Agnese PA, Bartter FC. The renin-angiotensin-aldosterone system in patients with cystic fibrosis of the pancreas. Pediatr Res. 1971; 5:626-32.
19. Montalvo JM, McCaa CS, Cole WQ. Aldosterone metabolism in cystic fibrosis. J Clin Endocrinol Metab. 1968; 28:582-5.
20. Al-Bazzaz FJ, Al-Awqati Q. Interaction between sodium and chloride transport in canine tracheal mucosa. J Appl Physiol. 1979; 46:111-9.
21. Frizzell RA, Koch MJ, Schultz SG. Ion transport by rabbit colon. I. Active and passive components. J Membrane Biol. 1976; 27:297-316.
22. Lewis SA, Diamond JM. Na^+ transport by rabbit urinary bladder, a tight epithelium. J Membrane Biol. 1976; 28:1-40.
23. Will PC, Lebowitz JL, Hopfer U. Induction of amiloride-sensitive sodium transport in the rat colon by mineralocorticoids. Am J Physiol. 1980; 238:F261-8.
24. Widdicombe JH, Welsh MJ. Ion transport by dog tracheal epithelium. Fed Proc. 1980; 39:3062-6.
25. Cuthbert AW. Characteristics of sodium channels in transporting epithelia. In: Callingham BA, ed. Drugs and transport processes. Baltimore: University Park Press, 1974:173-84.
26. Gatzy JT. The effect of K^+-sparing diuretics on ion transport across the excised toad bladder. J Pharmacol Exp Ther. 1971; 176:580-94.
27. Frizzell RA, Turnheim K. Ion transport by rabbit colon: II. Unidirectional sodium influx and the effects of amphotericin B and amiloride. J Membrane Biol. 1978; 40:193-211.
28. Miller RD, Divertie MB. Kartagener's syndrome. Chest. 1972; 62:130-5.
29. Report of the Committee for a Study for Evaluation of Testing for Cystic Fibrosis. J Pediatr. 1976; 88:711-50.

DIET, FAT ACCRETION, AND GROWTH IN PREMATURE INFANTS

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Abstract To compare the growth and accumulation of protein, fat, and carbohydrate in the formula-fed premature infant and in the fetus of a similar postconceptional age, we performed 22 metabolic studies in 13 infants of very low birth weight (1155±39 g [mean ±S.E.]). Measurements combining nutritional balance and indirect calorimetry demonstrated the deposition rates of protein and fat. We found that the formula-fed, very-low-birth-weight infant who gained weight comparably to the fetus retained the same amount of protein (1.92±0.1 g per kilogram of body weight per day) but accumulated fat at a rate of 5.4±0.3 g per kilogram per day — about three times that in the fetus, as confirmed by increased skin-fold thickness. How this change in body composition affects the future growth of formula-fed premature infants, and how body composition is altered by other dietary regimens such as the provision of human milk, remain to be determined. (N Engl J Med. 1981; 305:1495-500.)

THE Committee on Nutrition of the American Academy of Pediatrics suggests that the "optimal diet for the low-birth-weight infant may be defined as one that supports a rate of growth approximating that of the third trimester of intrauterine life." [1] This rate of growth is commonly equated with the rate of weight gain, without reference to the ingredients of this gain and consequent body composition. Since there is currently no better clinical criterion for determining the nutritional needs of premature infants, the ability to match intrauterine growth rates "remains a widely used target of achievement." [2] Special formulas have been designed to provide adequate intake of the calories, fluids, and solid nutrients necessary to support the rapid cell division and synthesis of new tissue in the infant of very low birth weight. These formulas attempt to meet fetal accretion rates, according to the belief that the postnatal changes in body composition of the premature infant should resemble those of the normal fetus.[3] The composition of the fetus and the accretion rates of the three principal nutrients (carbohydrate, protein, and fat), as well as minerals and water, has been determined predominantly by chemical analysis of stillborn fetuses.[3-5] Although Fomon has calculated the body composition of the term male reference infant during the first year of life,[6] this information is not available for the growing premature infant. The composition of weight gain of "the male reference infant" has been shown to change markedly after birth,[6] with increasing fat and decreasing water components. Despite these different characteristics of postnatal body composition, fetal growth pattern still forms the basis for designing the optimal nutrition for the growing preterm infant during this vulnerable period of development.

We show that fat accretion in the growing formula-fed premature infant is markedly increased, as compared with fat accretion in the fetus of similar weight, postconceptional age, and rate of weight gain, suggesting that the composition of weight gain and subsequent body composition of the premature infant will differ from that of the comparable placentally nourished fetus.

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METHODS

Twenty-two studies were undertaken in 13 infants (seven boys and six girls). Their clinical characteristics are shown in Table 1. The following characteristics were our criteria for inclusion: birth weight ≤1300 g; appropriate size for gestational age; growth, shown by increasing weight, length, and head circumference; and formula feeding (SMA 20 and 24, Wyeth Laboratories, Philadel-