JCI The Journal of Clinical Investigation

Cystic fibrosis.

M J Welsh, R B Fick

J Clin Invest. 1987;80(6):1523-1526. https://doi.org/10.1172/JCI113237.

Research Article

Cystic Fibrosis

Michael J. Welsh and Robert B. Fick
Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

The last few years have brought considerable progress toward understanding the metabolic abnormality in cystic fibrosis (CF),[1] principally because of advances in two different areas: studies in several CF organ systems show decreased epithelial chloride (Cl^-) permeability, and the CF gene has been localized to a small part of chromosome 7. In this article we will focus on the cellular defect and describe some of the work that has led to the current understanding of abnormalities in the CF cell. There are several sources that describe the localization of the CF gene by restriction fragment length polymorphisms (1, 2).

Clinical aspects

CF is an autosomal recessive disorder, the most common lethal genetic disease in Caucasians. The disease was initially called cystic fibrosis of the pancreas because pancreatic failure was a major clinical manifestation. However, with oral pancreatic enzyme replacement therapy, pancreatic failure has become less of a problem, and respiratory disease is the major cause of morbidity and mortality. Indeed, 95% of CF patients die of respiratory failure. The lung disease develops because thick, dehydrated mucus impairs airway mucociliary clearance and predisposes the patient to recurrent bronchial infections. CF airways show a particular propensity to colonization and infection with the opportunistic bacterium *Pseudomonas aeruginosa.* The recurrent infections and pneumonia progressively destroy the lung and lead to respiratory failure. Abnormalities of sweat gland function were discovered early in the history of the disease and led to the classical clinical test for CF: an increased concentration of Cl^- (and Na^+) in the sweat. Other affected organs include salivary glands (hypertrophy and abnormal secretory function), intestine (meconium ileus), epididymis (obstruction and maldevelopment), and liver (focal biliary cirrhosis).

These clinical manifestations have been important in guiding investigators to perform the basic research studies that have increased our knowledge of the metabolic abnormalities.

Decreased epithelial chloride permeability in CF

Three organs classically involved in CF: sweat glands, airways, and pancreas are each composed of epithelia. In CF, each of these epithelia have a decreased anion permeability. These observations provide the basis for a unifying hypothesis about the cellular defect in epithelia and link basic research observations to the pathophysiology.

Sweat gland duct. The sweat gland is composed of two regions, the secretory coil and the reabsorptive duct. Fig. 1 shows a schematic representation. The secretory coil produces nearly isotonic sweat. Then, as the sweat passes up through the water-impermeable duct, NaCl is absorbed and a hypotonic fluid emerges at the surface of the skin. In the coil, active Cl^- transport drives fluid secretion; in the duct, active Na^+ transport drives electrolyte absorption. In each case the counter ion appears to follow passively.

The clinical observation that Cl^- and Na^+ concentrations are increased in CF sweat led Quinton and Bijman to examine the ion transport properties of the sweat duct (3). They found that both in vivo and in vitro CF sweat ducts had a higher transepithelial electrical potential difference than normal ducts. Isolated, perfused ducts from normal subjects had a voltage of about -7 mV whereas ducts from CF patients had a voltage of -76 mV (lumen voltage with respect to bath). When the lumen Cl^- (or NaCl) concentration was reduced in normal ducts, lumen voltage became more negative; this finding indicated that Cl^- transport is electrically conductive. In contrast, when the lumen Cl^- concentration was reduced in CF ducts, transepithelial voltage became more positive (4, 5). These results indicate that CF sweat ducts have a decreased Cl^- permeability and suggest that the increased transepithelial voltage results from an intact Na^+-absorptive mechanism in the presence of Cl^- impermeability. This abnormality also explains the pathophysiology: because Cl^- cannot follow active Na^+ absorption, net absorption is blocked and the NaCl concentration in the sweat increases.

Recent studies of transepithelial electrical conductance confirm and extend the conclusion that the CF sweat duct is Cl^- impermeable (6, 7). Normal sweat ducts have a transepithelial electrical conductance of $\sim$ 100–125 mS/cm^2, 90% of which results from the Cl^- conductance. Current data also suggest that conductive Cl^- flow is predominantly through the cells (transcellular) rather than between the cells through the tight junctions (paracellular).[2] In contrast to normal ducts, CF ducts have a conductance of 10–20 mS/cm^2; Cl^- conductance is low or absent in CF, possibly because of an abnormality in the intracellular regulatory events that lead to Cl^- channel opening. This explanation is consistent with recent preliminary work suggesting that CF sweat duct cells contain the same

Address all correspondence to Dr. M. J. Welsh, Pulmonary Div., Dept. of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242.

Received for publication 21 September 1987.

1. *Abbreviations used in this paper:* CF, cystic fibrosis.

2. The high value of conductance is in the same range as reported for renal proximal tubules. It was originally unsuspected because of the ability of the duct to generate large transepithelial voltages and osmotic gradients, properties usually associated with low conductance epithelia. Thus, the sweat duct would appear to be a high conductance epithelium because of a high conductance cellular pathway and a lower conductance paracellular (tight junction) pathway. A similar situation occurs in the submandibular duct but contrasts with the renal proximal tubule, where the paracellular pathway provides the high conductance.

J. Clin. Invest.

0021-9738/87/12/1523/04 $2.00
Volume 80, December 1987, 1523–1526

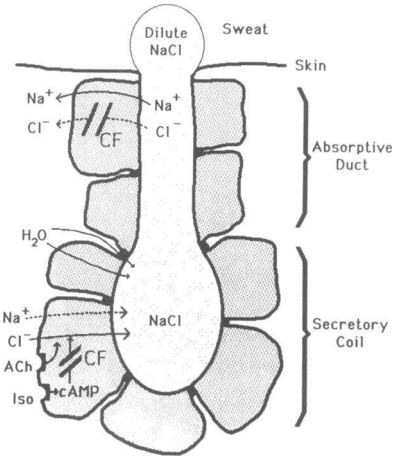

Figure 1. Schematic representation of electrolyte transport by the sweat gland.

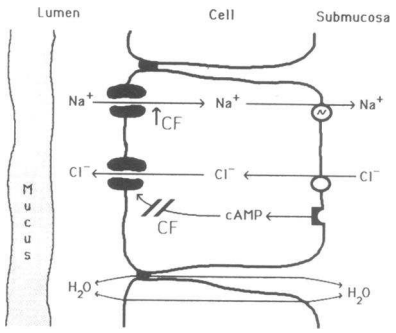

Figure 2. Schematic representation of electrolyte transport by airway epithelium.

number of Cl^- channels as normal cells (8). However, substantial uncertainty remains about the mechanism and regulation of ion transport by normal sweat duct cells, so it is not yet possible to define the specific abnormality in CF.

Sweat gland secretory coil. Sato and Sato have shown that the secretory coil of CF sweat gland also transports electrolytes abnormally (9). In normal sweat glands, either cholinergic or β-adrenergic agonists stimulates sweat production, although β-adrenergic–induced sweat production is one-fifth the rate of cholinergic-induced sweating. Methacholine stimulates similar rates of sweat production in both normal and CF secretory coil. In contrast, the β-adrenergic agonist isoproterenol and theophylline stimulate secretion in normal but not in CF glands. The abnormality in β-adrenergic regulation was observed both in vivo and in vitro, and it does not appear to result from an abnormal interaction between hormone and receptor since isoproterenol-induced cAMP accumulation was normal in CF coils. These results suggest a defect in the cAMP-mediated regulation of Cl^- secretion. At present it is not clear whether β-adrenergic agonists and muscarinic-cholinergic agonists stimulate different cells in the secretory coil, different secretory mechanisms in the same cell, or the same secretory mechanism via different second messenger pathways.

Thus, the sweat gland shows two abnormalities in CF: in the duct, decreased Cl^- transport inhibits fluid absorption; in the secretory coil, decreased Cl^- transport inhibits cAMP-mediated fluid secretion. Both abnormalities could be explained by abnormal regulation of Cl^- transport.

Airway epithelia. Fig. 2 schematically represents the airway epithelium. Transepithelial electrolyte transport controls the quantity and composition of the respiratory tract fluid; thus, it is important in effecting normal mucociliary clearance (10). The epithelium can actively transport Cl^- from the submucosal to mucosal surface, thereby driving fluid secretion. The epithelium can also actively absorb Na^+, driving fluid in the opposite direction. In both cases, the counter ion is thought to move passively through the paracellular pathway. The relative magnitude of the two active transport processes is determined by the airway region under study and the neurohumoral environment. Cl^- secretion is acutely stimulated by agents such as β-adrenergic agonists and prostaglandins.

The clinical observation that CF airway secretions are thick and dehydrated led Knowles and colleagues to study electrolyte transport by CF respiratory epithelium (11). They found that in vivo the voltage across upper (nasal) and lower (tracheal and bronchial) airway epithelia is higher in CF patients (−50 mV, lumen with respect to submucosa) than in normal subjects or disease controls (−20 to −25 mV). This finding suggested some defect in electrolyte transport. The results of subsequent in vivo Cl^- substitution studies (12) and the observation that transepithelial Cl^- fluxes are decreased in excised nasal polyps (13) indicate that CF airway epithelium is relatively impermeable to Cl^-.

Airway cells maintained in primary culture on permeable supports retain the properties of the native epithelium. These cultured cells develop tight junctions, polarize with the submucosal surface against the filter and the mucosal (lumenal) surface facing the media, and retain the capacity for hormonally stimulated transepithelial ion transport. Primary cultures of CF airway epithelium also retain the properties of the native epithelium: they are Cl^- impermeable and fail to secrete Cl^- when stimulated with isoproterenol (14–16). These results indicate that the Cl^- impermeability is an intrinsic property of the epithelial cells and that a circulating "factor" is not the cause of the ion transport defect. Intracellular microelectrode measurements combined with ion substitutions localize the CF Cl^- impermeability to the cellular pathway and specifically to the apical membrane (14, 17).

To understand the reason for the decreased apical membrane Cl^- permeability, investigators have applied the single-channel patch-clamp technique to primary cultures of airway cells. The patch clamp is a microelectrode technique in which a high resistance seal is formed between a glass pipet and the cell membrane (18). It thereby allows one to record the opening and closing of single ion channels and measure the current (ions) flowing through them. Airway cells studied with this technique contain Cl^- channels; the properties of these Cl^- channels indicate that they are responsible for the hormonally sensitive apical membrane Cl^- permeability (19, 20). In recordings from cell-attached patches (in which the membrane under the pipet is still attached to the cell), isoproterenol, which increases cellular levels of cAMP, opened Cl^- channels in normal cells. Importantly, isoproterenol failed to open Cl^- channels in CF cells (20, 21).

The failure of beta agonists to open Cl^- channels in CF cells does not result from the absence of Cl^- channels; we know that CF cells contain Cl^- channels and that these channels have conductive properties identical to those of normal cells since Cl^- channels in CF cells as well as normal cells open spontaneously when patches of membrane are excised from the cell (21). Cl^- channels in CF cells can also be induced to open while attached to the cell by a manipulation of cell Ca^{++}. The Ca^{++}-ionophore A-23187 added to the bath solution of CF cells induces Cl^- secretion and Cl^- channel opening, even though cytosolic Ca^{++} does not appear to be a primary regula-

tor of the Cl^- channel (20, 22). Abnormal regulation of Cl^- channels does not appear to result from a defective interaction between hormone and receptor: the secretagogue isoproterenol increases cellular levels of cAMP appropriately in CF airway cells and addition of secretagogue opens a Ca^{++}-activated K^+ channel in CF cells (21). Even when the receptor is bypassed by addition of 8-bromo-cAMP or forskolin, CF Cl^- channels fail to open. Because the effects of cAMP result from the activation of a cAMP-dependent protein kinase in a variety of cells, we asked whether the cAMP kinase could directly activate the Cl^- channel. Purified catalytic subunit of cAMP-dependent protein kinase, added to the cytosolic surface of excised, cell-free patches, opened Cl^- channels from normal cells, but failed to open Cl^- channels from CF cells (unpublished observations).

These studies clearly point to defective regulation of Cl^- channels in CF airway cells. But where does the defect lie? There are several possibilities: an abnormal regulatory site on the Cl^- channel (or channel complex), an abnormal interaction between the cAMP regulatory pathway and the channel, or a defect in some other pathway that also regulates the Cl^- channel.

Increased sodium absorption in CF airway epithelia. The increased transepithelial voltage in CF airway epithelia and the dehydrated airway secretions raise the possibility that Na^+ absorption might also be abnormal in CF. In excised, short-circuited nasal epithelium, the rate of active Na^+ absorption in CF epithelium averages twice the rate in non-CF epithelium (23). Of greater interest, β-adrenergic agonists and forskolin, both of which increase cellular concentrations of cAMP, stimulate Na^+ absorption in CF epithelia. The opposite is found in normal epithelia: cAMP either does not change or decreases the rate of Na^+ absorption (10). What explains this paradoxic regulation of Na^+ absorption in CF? It may be a direct result of the genetic abnormality or it may be secondary to the decreased Cl^- conductance. cAMP also paradoxically stimulates Na^+ absorption when Cl^- conductance is decreased in normal canine airways by bathing them in Cl^--free media (24).

Pancreas. The clinical observation that CF patients develop pancreatic failure led to studies of ion transport function in that organ. To date the most important studies have been conducted in vivo because the pancreatic architecture is complex and viable CF pancreas is difficult to obtain for in vitro studies. Kopelman and colleagues (25) studied pancreatic secretions from CF patients before total organ failure developed and studied control subjects with similar levels of pancreatic acinar function. They found higher concentrations of protein in CF secretions than in control secretions. The difference results from a decreased rate of fluid secretion from CF pancreas at all levels of pancreatic function. Because anion secretion appears to be important in the production of pancreatic fluid, the results suggest that anion secretion is decreased in the CF pancreas.

Thus, CF pancreas may have an anion secretory abnormality similar to that observed in CF airways and CF sweat gland secretory coil. Defective anion secretion would also explain the pathology of CF pancreatic disease: dehydrated secretions obstruct the ducts and eventually destroy the gland.

Abnormal protein secretion and accumulation in CF epithelia. The conclusion that CF involves a defect in a postreceptor regulatory step is supported by two other observations in epithelia. In CF submandibular acinar cells, β-adrenergic receptor stimulation produces less secretion of amylase and mucin than in normal glands, even though cAMP accumulation is normal (26). In CF sweat glands β-adrenergic receptor stimulation of serine protease accumulation is decreased (27).

Other abnormalities

Although numerous other abnormalities have been observed in CF, it is difficult to know whether they are a direct result of the genetic defect or a secondary effect of the disease. It has also been difficult to tie together disparate observations into a unifying hypothesis about the basic defect. These uncertainties point to the fact that we still do not completely understand the basic abnormality. We will not try to list the numerous abnormalities; we would certainly miss some important points and it is difficult to rank them by priority. Nor will we try to constrain the observations into some general or unifying hypothesis; given our current knowledge it would appear to be artificial and awkward. However, we will address a few recent observations.

Two serum protein abnormalities in CF have attracted attention. One abnormality has been described in the pattern of proteins in CF serum separated by isoelectric focusing on polyacrylamide thin layers (28). Researchers find a doublet band with pI of 8.41 in CF serum. This cationic protein has a low molecular mass (11,000–13,700 D) and appears as a complex with IgG in serum. Although the protein has been called "CF protein" or "CF antigen," this is somewhat of a misnomer because it is present at low concentrations in the serum of some normal individuals and is a product of several normal and CF cell types. Using an antiserum against CF protein, some investigators have been able to distinguish homozygotes from heterozygotes and normals (29). Using somatic cell hybrids, van Heyningen and co-workers (30) mapped the CF protein to chromosome 1. Because the CF gene has been assigned to chromosome 7, accumulation of CF protein is likely not to be the direct result of the genetic mutation. The predicted sequence of the CF protein, obtained by complementary DNA cloning (31), has been compared with other protein sequences and found to be significantly homologous (43% identity over a stretch of 72 amino acids), with a bovine S100a subunit of brain-associated calcium-binding protein and its pig and rat homologues. Another serum abnormality that has been observed is the underglycosylation of CF IgG with respect to galactose and sialic acid (32).

One possible way of tying these two serum protein abnormalities together and possibly relating them to other findings, such as defective regulation of Cl^- channels, is to suggest that the involved proteins share a common step in posttranslational processing, which is defective in CF. A defect in posttranslational processing could account for the variety of defects observed in CF; it could produce a defect in the function of regulatory or membrane proteins, such as channels; it could also produce abnormalities in other cellular or serum proteins. I-cell disease is an example of a disease in which defective posttranslational modification of a glycoprotein increases extracellular levels of an otherwise intracellular protein (33). The possibility that posttranslational glycosylation is abnormal in CF has received some support from the observation that the fucose to sialic acid ratio in membrane glycoproteins is decreased in CF skin fibroblasts (34).

Conclusion

We have described how clinical observations of CF led investigators to the basic research laboratory and particularly to the

study of CF epithelia. Studies of epithelia led to a unifying hypothesis about the physiologic defect: several CF epithelia are Cl^- impermeable. CF airway epithelia, sweat gland secretory coils, and probably pancreas have decreased Cl^- secretion; CF sweat gland ducts have decreased Cl^- absorption. In airway epithelia, the abnormality has been localized to defective regulation of an apical membrane Cl^- channel. Defective regulation of Cl^- channels in the other involved epithelia would also explain the abnormalities. The defect appears to lie close to the channel, either in the channel itself or in a regulatory pathway controlling the channel. These abnormalities in electrolyte transport provide a plausible explanation for the major clinical manifestations of CF: the dehydrated obstructing secretions that lead to organ destruction.

How do these findings in epithelia fit together with other abnormalities in CF? It is difficult to know. There are suggestions that the sequence of the CF gene may appear very soon. At that time the function of the gene product may be immediately apparent, or we may still have to discover how the genetic defect produces the pathophysiology. In either case, we must be ready to apply this knowledge at the point where the inquiry began, at the bedside and care of patients.

References

1. Newmark, P. 1985. Testing for cystic fibrosis. *Nature (Lond.).* 318:309.

2. Goodfellow, P.N. 1987. Cystic fibrosis: Classical and reverse genetics. *Nature (Lond.).* 326:824.

3. Quinton, P. M., and J. Bijman. 1983. Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. *N. Engl. J. Med.* 308:1185–1189.

4. Quinton, P. M. 1983. Chloride impermeability in cystic fibrosis. *Nature (Lond.).* 301:421–422.

5. Bijman, J., and P. Quinton. 1987. Permeability properties of cell membranes and tight junctions of normal and cystic fibrosis sweat ducts. *Pflügers Arch.* 408:505–510.

6. Quinton, P. M. 1986. Missing Cl conductance in cystic fibrosis. *Am. J. Physiol.* 251(Cell Physiol. 20):C649–C652.

7. Bijman, J., and E. Fromter. 1986. Direct demonstration of high transepithelial chloride-conductance in normal human sweat duct which is absent in cystic fibrosis. *Pflügers Arch.* 407:S123–S127.

8. Welsh, M. J., J. D. McCann, and D. G. Dearborn. 1987. Chloride and sodium channel currents in normal and cystic fibrosis sweat duct epithelium. *Fed. Proc.,* 46:1271.

9. Sato, K., and F. Sato. 1984. Defective beta adrenergic response of cystic fibrosis sweat glands in vivo and in vitro. *J. Clin. Invest.* 73:1763–1771.

10. Welsh, M. J. 1987. Electrolyte transport by airway epithelia. *Physiol. Rev.* 67:1143–1184.

11. Knowles, M., J. Gatzy, and R. Boucher. 1981. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N. Engl. J. Med.* 305:1489–1495.

12. Knowles, M., J. Gatzy, and R. Boucher. 1983. Relative ion permeability of normal and cystic fibrosis nasal epithelium. *J. Clin. Invest.* 71:1410–1417.

13. Knowles, M. R., M. J. Stutts, A. Spock, N. L. Fischer, J. T. Gatzy, and R. C. Boucher. 1983. Abnormal ion permeation through cystic fibrosis respiratory epithelium. *Science (Wash. DC).* 221:1067–1070.

14. Widdicombe, J. H., M. J. Welsh, and W. E. Finkbeiner. 1985. Cystic fibrosis decreases the apical membrane chloride permeability of monolayers cultured from cells of tracheal epithelium. *Proc. Natl. Acad. Sci. USA.* 82:6167–6171.

15. Yankaskas, J. R., C. U. Cotton, M. R. Knowles, J. T. Gatzy, and R. C. Boucher. 1985. Culture of human nasal epithelial cells on collagen matrix supports: a comparison of bioelectric properties of normal and cystic fibrosis epithelia. *Am. Rev. Respir. Dis.* 132:1281–1287.

16. Yankaskas, J. R., J. T. Gatzy, M. R. Knowles, and R. C. Boucher. 1985. Persistence of abnormal chloride ion permeability in cystic fibrosis nasal epithelial cells in heterologous culture. *Lancet.* 8435:954–956.

17. Cotton, C. U., M. J. Stutts, M. R. Knowles, J. T. Gatzy, and R. C. Boucher. 1987. Abnormal apical cell membrane in cystic fibrosis respiratory epithelium. An in vitro electrophysiologic analysis. *J. Clin. Invest.* 79:30–85.

18. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391:85–100.

19. Welsh, M. J. 1986. An apical membrane chloride channel in human tracheal epithelium. *Science (Wash. DC).* 232:1648–1650.

20. Frizzell, R. A., G. Rechkemmer, and R. L. Shoemaker. 1986. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science (Wash. DC).* 233:558–560.

21. Welsh, M. J., and C. M. Liedtke. 1986. Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature (Lond.).* 322:467–470.

22. Widdicombe, J. H. 1986. Cystic fibrosis and β-adrenergic response of airway epithelial cell cultures. *Am. J. Physiol.* 251:R818–R822.

23. Boucher, R. C., M. J. Stutts, M. R. Knowles, L. Cantley, and J. T. Gatzy. 1986. Na^+ transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J. Clin. Invest.* 78:1245–1252.

24. Cullen, J. J., and M. J. Welsh. 1987. Regulation of sodium absorption by canine tracheal epithelium. *J. Clin. Invest.* 79:1–7.

25. Kopelman, H., P. Durie, K. Gaskin, Z. Weizman, and G. Forstner. 1985. Pancreatic fluid secretion and protein hyperconcentration in cystic fibrosis. *N. Engl. J. Med.* 312(6):329–334.

26. McPherson, M. A., R. L. Dormer, J. A. Dodge, and M. C. Goodchild. 1985. Adrenergic secretory responses of submandibular tissues from control subject and cystic fibrosis patients. *Clin. Chim. Acta.* 148:229–237.

27. Sato, K., H. Yokozeki, K. Saga, and F. Sato. 1987. Some characteristics of beta adrenergic dysfunction in cystic fibrosis sweat glands. *In* Epithelial Ion Transport in Cystic Fibrosis. J. R. Martinez, editor. University of Missouri, Columbia. 45–60.

28. Wilson, G. B., T. C. Johl, and J. R. Fonseca. 1973. Demonstration of serum protein differences in cystic fibrosis by isoelectric focusing in thin-layer polyacrylamide gels. *Clin. Chim. Acta,* 49:79–91.

29. Manson, J. C., and D. J. H. Brock. 1980. Development of a quantitative immunoassay for the cystic fibrosis gene. *Lancet.* 16:330–331.

30. van Heyningen, V., C. Hayward, J. Fletcher, and C. McAuley. 1985. Tissue localization and chromosomal assignment of a serum protein that tracks the cystic fibrosis gene. *Nature (Lond.).* 315:513–515.

31. Dorin, J. R., M. Novak, R. E. Hill, D. H. J. Brock, D. S. Secher, and V. van Heyningen. 1987. A clue to the basic defect in cystic fibrosis from cloning the CF antigen gene. *Nature (Lond.).* 326:614–617.

32. Margolies, R., and T. F. Boat. 1983. The carbohydrate content of IgG from patients with cystic fibrosis. *Pediatr. Res.* 17:931–935.

33. Reitman, M. L., A. Varki, and S. Kornfeld. 1981. Fibroblasts from patients with I-cell disease and pseudo-hurler polydystrophy are deficient in uridine 5'-Diphosphate-*N*-Acetylglucosamine. Glycoprotein *N*-Acetylglucosaminylphosphotransferase activity. *J. Clin. Invest,* 67:1574–1579.

34. Scanlin, T. F., Y. Wang, and M. C. Glick. 1985. Altered fucosylation of membrane glycoproteins from cystic fibrosis fibroblasts. *Pediatr. Res.* 19:368–374.