In order to fully understand and hence be able to deal with the molecular pathology of CF, the normal mechanism of action of CFTR must be elucidated. That is, its functional role in each of the tissues affected in the disease needs to be clarified. This will require the use of the tissues themselves or at least the specific epithelial cell types derived from them. However, it may first be possible to more readily establish the basic structure-function relationships of the molecule employing simpler non-epithelial cells into which CFTR can be introduced. With this in mind we have employed three such so-called heterologous expression systems, each with advantages and disadvantages. Our first aim was to generate relatively large amounts of CFTR protein suitable for structural analysis and possibly functional reconstitution. This was achieved employing the baculovirus-insect cell expression system. Despite the fact that the protein produced is minimally glycosylated (apparent Mr = 140 Kd), at least some of it is functional, imparting regulated chloride channel activity on the insect cells. However, only a small proportion of the large amount of CFTR in these cells localizes to the plasma membrane. Nevertheless, it has been possible to purify the protein to homogeneity under denaturing conditions. Efforts to renature and reconstitute this protein into synthetic lipid systems are ongoing. As a second system we have established Chinese hamster ovary (CHO) cell lines which stably express CFTR in varying amounts. The protein produced in these cells has the same molecular size (Mr = 170 Kd) as in the human epithelial cells in which it is endogenously expressed and localizes primarily to the plasma membrane. Coincident with the introduction of CFTR into these membranes, a regulated Cl⁻ permeability similar to that seen in the CFTR-infected insect cells and native epithelial cells appears. It has been possible to purify CFTR from CHO membranes under milder conditions but in much smaller quantities. Its function in lipid bilayers is also being assessed. As a third heterologous expression system, *Xenopus* oocytes which have special advantages for the study of ion channels have been used. Injection of CFTR cRNA results in the appearance of a CFTR protein of slightly larger than native size (Mr = 180 Kd), presumably because of altered glycosylation. This results in a predominantly Cl⁻ selective current constituted by low conductance channels activated by protein kinase A and has no influence on endogenous oocyte Cl⁻ channels. The finding that CFTR expression generates the same regulated low conductance Cl⁻ channel in diverse systems that do not normally have cAMP-regulated Cl⁻ permeability supports the notion that CFTR is an actively regulated anion channel.

At a practical level those systems enable a detailed dissection of CFTR structure-function as well as the development of both genetic and pharmacological means of modulating the function of defective mutant CFTR molecules.
Hybrid Arrest of CFTR and a Putative Cloned Cl Channel in Oocytes

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CFTR, the cystic fibrosis transmembrane conductance regulator, could be a chloride channel, a regulator of channels, or perhaps play a role in the cellular processing of membrane proteins (1). We report here hybrid arrest experiments that suggest at least two proteins are involved in functional expression of the cAMP-activated Cl conductance in oocytes.

Bovine H2B, a putative kidney epithelial cell Cl channel clone, was identified with antisera generated to a 64kDa indanyloxyacetic acid (IAA) affinity column eluate (2). No sequence similarity exists between CFTR and H2B. Cyclic AMP-activated Cl conductance was expressed in oocytes by injection of shark rectal gland (SRG) mRNA (3). An antisense 1443mer spanning 90% of the known open reading frame of bovine H2B was generated by asymmetric PCR, gel purified, and co-injected (10 nM) with SRG mRNA. Under these conditions, only 55±18% of the usual response to CAMP was observed. In contrast, the response to CAMP was 91±21% of control in oocytes receiving mRNA plus 10 nM of the sense 1443mer. Greater inhibition was obtained with higher concentrations of 20mer antisense H2B oligonucleotides, either as a mixture of seven oligos or as single oligos (Table 1). An equal number of non-expressors was routinely observed in all populations of oocytes; these oocytes were not excluded and contribute to the variance in average values. Random complementarity of sense DNA with endogenous or exogenous mRNA's is apparent in some experiments from the partial arrest of expression. In all cases examined, however, the same dose of antisense DNA arrested expression to a greater extent, indicating specific recognition of shark and/or Xenopus H2B mRNA (Table 1). An antisense CFTR 21mer also arrested expression in this assay, suggesting that both CFTR and H2B proteins are required for generation of cAMP-activated Cl channels by injection of SRG mRNA. Failure to observe complete arrest in these experiments might be due to interspecies sequence divergence (bovine H2B or human CFTR DNA annealed to shark or frog mRNA).

A full-length human CFTR cDNA (pKS-βglobin-CFTR 936C-polyAC) featuring a βglobin leader sequence and a poly AC tail to enhance translatability and message stability, respectively, was adapted from previous constructs (4), transcribed in vitro, capped and injected (1-5ng/oocyte). Similar to the result with SRG mRNA, a large cAMP-activated conductance with reversal potential near $E_{cl}$ and sensitivity to 200 μM NPPB was detected 2-5 days following injection. Co-injection of antisense H2B 20mer B completely prevented expression of the cAMP-activated conductance (Table 2). Subsequent experiments using human CFTR oligos gave analogous
results. The bovine H2B oligonucleotide was compared to the full-length human CFTR sequence and a similar degree of random complementarity was noted for both sense and antisense in several regions of the CFTR sequence. Complete arrest by this oligo therefore predicts an unusually low degree of sequence divergence in this domain of Xenopus H2B mRNA.

Table 1. Antisense H2B or CFTR hybrid-arrests expression of cAMP-activated Cl conductance in oocytes injected with 50ng shark rectal gland mRNA. Values (means ± 1SE) are percent of control Cl current elicited by addition of 50µM intracellular cAMP to oocytes injected one day previously with 50ng shark rectal gland mRNA alone, or in combination with sense or antisense DNA.

| DNA      | conc | sense  | anti  | n  |
|----------|------|--------|-------|----|
| H2B 1443mer | 10nM | 91 ± 21 | 55 ± 18 | 7  |
| H2B 20mers(7) | 500nM each | 55 ± 14 | 12 ± 5 | 8-9 |
| H2B 20mer A | 5µM | 61 ± 9  | 31 ± 8  | 10-11 |
| H2B 20mer B | 5µM | 80 ± 20 | 13 ± 10 | 10-11 |
| CFTR 21mer | 200nM | 68 ± 12 | 25 ± 8 | 5  |

Table 2. Antisense H2B or CFTR hybrid-arrests expression of cAMP-activated Cl conductance in oocytes injected with 2.5ng in vitro transcribed human CFTR mRNA. Values (means ± 1SE) are percent of control current elicited by addition of 50µM intracellular cAMP to oocytes injected 2-3 days previously with CFTR mRNA alone, or in combination with sense or antisense DNA.

| DNA      | conc | sense  | anti  | n  |
|----------|------|--------|-------|----|
| H2B 20mer B | 2µM | 57 ± 26 | 1 ± 1 | 4-7 |
| CFTR 21mer | 2µM | 78 ± 39 | 3 ± 5 | 6-9 |
| CFTR 24mer | 2µM | 47 ± 14 | 7 ± 5 | 5-7 |

Western analysis of nonepithelial cells including oocytes, HeLa, 3T3, CHO and Sf9 cells demonstrated the presence of anti-64kDa immunoreactivity, indicating that the H2B protein is widely expressed and is not epithelial cell-specific. The results suggest that both CFTR and H2B proteins are required for functional expression of cAMP-activated Cl conductance.

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Analysis of the Yeast STE6 Transporter, a Member of the ATP Binding Cassette Protein Superfamily

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The Saccharomyces cerevisiae STE6 protein is responsible for export of the α-factor mating pheromone, a prenylated and methylated dodecapeptide. STE6, like CFTR, is a member of the ATP binding cassette (ABC) superfamily of proteins, which share a common overall structural design and several distinct regions of homology. Another eukaryotic ABC family member is the mammalian multidrug resistance protein, MDR, whose overexpression in tumor cells facilitates drug resistance. CFTR, STE6, and MDR are comprised of two homologous halves, each encoding six predicted membrane spanning segments and a putative ATP nucleotide binding fold (NBF) domain. The considerable level of homology between these proteins suggests that they share common mechanistic features.

STE6 and most other ABC proteins likely function as transport pumps that utilize the energy of ATP hydrolysis to translocate substrates across a membrane. By contrast, CFTR appears to function as an ion channel. Since ion diffusion across a channel is energy-independent, the role of the NBF domains within CFTR is unclear. However, ATP utilization clearly appears to play a critical role for CFTR, since numerous CF mutations lie within its N-terminal and C-terminal NBF domains. In order to examine the mechanism of ATP utilization by ABC proteins, we have undertaken a structure-function analysis of STE6.

Mutations in the NBF domains of STE6

An alignment of the NBF domains of STE6, CFTR, and MDR reveals three homologous regions, designated the A, B, and Center regions (Fig 1). Homology is greatest in the A and B regions. To examine the functional significance of conserved residues among ABC proteins and to assess whether both halves of STE6 are essential for α-factor export, we generated a collection of mutations in the NBF domains of STE6, several of which are analogous to CF mutations (Fig 1). STE6 activity of the mutants was evaluated by measuring α-factor export using several assays, including mating, immunoprecipitation of extracellular α-factor, and the α-factor halo bioassay.

We found that nearly all mutations within the A and B regions inactivate STE6, although the severity of the mutations varies. Antibodies raised against a TrpE-STE6 fusion have been used to show that none of these mutations has a significant effect on the in vivo stability of STE6 polypeptide, suggesting that loss of activity is not due to degradation. The finding that a mutation in a single NBF domain is sufficient to cause inactivation indicates that both NBF domains of STE6 are critical for function, as also appears to be the case for CFTR. These results, together with the observation that
corresponding mutations in CFTR result in disease, suggest that several highly conserved ABC residues may be critical for function in all members of the ABC superfamily.

Homology among ABC proteins in the Center region is modest. We generated several deletion and substitution mutations in this region. Of particular interest is ΔL455, which corresponds to the ΔF508 mutation of CFTR. We found that neither this mutation, nor others within the Center region, has a significant impact on STE6 function. Our results suggest that this region of the NBF may not play a central role in ATP utilization for STE6. The severe consequences of the ΔF508 in CFTR, in contrast to the negligible impact of the corresponding alteration in STE6, suggest that the NBF Center region may play a very specific function for CFTR or affect its structure in a way that is not generalizable to all ABC family members.

**Half-Molecule Studies**

To further examine the functional contribution of each half of STE6, we severed the STE6 coding region and expressed the two halves of the transporter as separate polypeptides. Strikingly, co-expression of both "half-molecules" in the same cell leads to reconstitution of a functional STE6 transporter. In contrast, expression of either half-molecule alone does not provide any a-factor export function. Thus, both halves of STE6 provide an essential contribution to function. Further subdivision of STE6 is currently being carried out to provide additional insight into its domainal organization.

![Fig. 1. (Top) Model for the Structure of STE6 in the Membrane. A, B, and Center denote conserved regions of the NBF domains analyzed in this study. (Bottom) Alignment of conserved regions of the NBF domains of both halves of STE6, CFTR, and human MDR1. Highly conserved residues are shaded. The Walker A and Walker B motifs are overlined. Below are shown the alterations made in STE6 for this study. Corresponding CF mutations are also shown. Asterisk indicates the position of F508.](image-url)
The cystic fibrosis transmembrane conductance regulator (CFTR) is a 1480 amino acid protein which contains five regions with amino acid sequences that are similar to previously described functional domains: two nucleotide binding domains, two transmembrane domains, and a regulatory domain (1). To date, the functional roles of these putative domains predicted from the CFTR gene structure remain unknown. The current interest of this laboratory has been focussed on putative Nucleotide Binding Fold 1 (NBF-1) which we have referred to also as "ATP-I", because of its partial homology with ATP domains of known enzymes, particularly the mitochondrial ATP synthase and adenylate kinase (2,3). As a single amino acid deletion, namely phenylalanine 508 (F508), within the putative ATP-I domain is responsible for approximately 70% of the known cases of cystic fibrosis, we asked four fundamental questions about this region: 1) What type of secondary structure characterizes the ATP-I domain; 2) Does this putative nucleotide binding region really bind ATP? 3) Does the AF508 mutation bring about a detectable change in secondary structure of the ATP-I domain?; 4) If so, does this change alter nucleotide binding? To answer these four questions we chemically synthesized and purified two peptides corresponding to the central region of the ATP-I domain, one peptide (PP-67) which included F508 and one peptide (PP-66) in which F508 was deleted.

Results with PP-67

As the chemical synthesis and purification of long synthetic peptides is both time consuming and expensive, the wild-type peptide was first synthesized, purified, and studied in detail. Significantly, this peptide, consisting of amino residues from arginine 450 to arginine 516, was found to bind adenine nucleotides. The apparent dissociation constants (K₅') for the trinitrophenyl (TNP) adenine nucleotides, TNP-adenosine triphosphate, TNP-adenosine diphosphate, and TNP-adenosine monophosphate, were 300 nanomolar, 200 nanomolar, and greater than 1 micromolar, respectively. The K₅ for adenosine triphosphate was 300 micromolar. Circular dichroism spectroscopy was used to show that P-67 assumes a predominantly B sheet structure in solution, a finding that is consistent with secondary structure predictions. On the basis of this information, the phenylalanine at position 508, which is deleted in approximately 70 percent of individuals with cystic fibrosis, was localized to a B strand within the nucleotide binding peptide. This experimental finding was particularly intriguing, and in sharp contrast to an earlier hypothesis about CFTR (5), which placed F508 in an α-helix. The potential significance of the experimental data lies in the fact that deletion mutations are predicted to be most destructive in regions of B-sheet structure because residues within the B-sheet are oriented from one face of the sheet to the other (6). On the basis of these observations we proceeded to invest more time and limited resources into a study of a peptide PP-66 in which F508 was deleted.
Results with PP-66

Circular dichroism spectroscopy revealed that the P-66 synthetic peptide lacking F508 differed dramatically in its secondary structure from that of the parent wild type peptide, PP-67. Specifically, a significant loss in β-sheet structure was detected with a corresponding increase in random coil. Despite these marked structural changes induced by deletion of the single phenylalanine residue only very minor changes were observed in the capacity of PP-66 to bind adenine nucleotides.

Tentative Conclusion and Future Direction

These results indicate that if deletion of phenylalanine 508 does alter an adenine nucleotide-dependent function of CFTR, it does so through interactions with regions of the protein not intrinsic to the peptide. Alternatively, this deletion which is shown by our studies to bring about a profound structural change in the ATP-I region, may alter essential interactions with one or more other functional regions of CFTR (i.e., the ATP-II domain, the R-domain, or the Cl channel). To experimentally distinguish among these various possibilities it will be essential to have at hand purified regions of the CFTR protein corresponding to each of the functional domains.

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In order for aerosolized antibiotics to be an effective treatment of pulmonary infections in cystic fibrosis (CF), a sufficient dose of the drug must first reach the desired target, the infected airways of the lung. After reaching these airways, the antibiotic must then remain available on the airway surfaces for a sufficient period to allow for its bactericidal action to occur. Only a small percentage (about 10 - 20% at best) of the antibiotic placed in the nebulizer may actually be inhaled by the patient (1,2). Once inhaled the aerosol may deposit in the respiratory tract by impaction, sedimentation, or diffusion onto airway surfaces (3), or escape deposition and be exhaled. Deposition of the aerosol may occur throughout the respiratory tract, including extrathoracic (mouth, oropharynx, and larynx) and thoracic (large and small bronchi, and alveoli) sites; further diminishing the effective dose to the region of infection. Finally, the antibiotic, once deposited onto the airway surfaces, can be cleared by absorption through the airway epithelium or up the mucociliary escalator; thus, limiting the time available for its action.

The mass fraction of inhaled particles that deposits in the respiratory tract, or total deposition fraction (DF), is an important parameter in determining dose to the patient (4). To determine the DF associated with breathing liquid aerosols from a nebulizer, most investigators have used the "soup" method (1) in which a radiolabelled marker is added to the nebulizer solution (5). It has been assumed that the drug and radiolabel are equally well mixed in solution so that radioactivity measurements are a true reflection of drug mass. A number of investigators have verified this assumption (2,5), but some radiolabelled markers may be better than others (2) at achieving this goal. Using absolute filters to sample inhaled and exhaled aerosol, a measure of DF = (Mass inhaled - mass exhaled)/Mass inhaled can be made (4,5,6). This technique allows measurements of deposition that can be correlated with subsequent therapeutic effect.

A number of factors affect the measured DF in a given patient. DF has been shown to increase with increasing aerodynamic particle size above 0.5 μm (7) in normal subjects. Particle size will also influence regional deposition (discussed further below). Because the particles containing antibiotic from a nebulizer are hygroscopic, they may grow to twice their original size (measured before entering the mouth) as they traverse the lung (8). Breathing pattern has been shown to have an influence on the DF of inhaled particles in normal subjects (7); its role is less clear in patients with airways disease (5). Slower breathing and breathholds at end inhalation increase deposition by time-dependent mechanisms, i.e., sedimentation or diffusion. Increasing tidal volumes increase the depth of aerosol penetration into the lung, bringing more of the aerosol to the smallest airspaces and increasing DF. Finally, airway caliber may be an important determinant of DF, especially in these patients with airways disease (9), with DF increasing with increased airway obstruction.

Regional deposition of an antibiotic aerosol is an important determinant for achieving desired therapeutic effects. If the desired effect is relief of bronchial pneumonia, then, ideally, most of the aerosol should be delivered to the bronchial airways. Use of radiolabelled aerosols (as described above) allows gamma camera analysis of regional deposition in the respiratory tract (4). As inspiratory flow rates and particle size (> 3μm) increase, the probability for extrathoracic deposition by inertial impaction also increases (10); thus decreasing the amount of drug delivered to the lung. Rapid-shallow breathing and high inspiratory or expiratory flow rates will also enhance bronchial airway deposition (3). These breathing maneuvers are more critical for enhancing airway deposition in the normal, nonobstructed lung, where particles smaller than 3μm primarily deposit in the alveolar regions of the lung (10) during tidal
breathing. However, a significant fraction of particles as small as 1μm deposit in the bronchial airways for patients with airways obstruction (5, 11). Again, these particles were hygroscopic and may have grown some in the lung. In CF patients Ilowite et al demonstrated by gamma camera analysis a good inverse correlation between central vs. peripheral deposition (C/P) of aerosolized gentamicin and the FEV1 (%predicted) of these patients (5). They also showed a good correlation between C/P ratio and the peak sputum level of gentamicin obtained by having the patients cough after breathing the aerosol.

Clearance of the antibiotic from the airway surface may occur through two pathways, 1) transepithelial absorption into the bloodstream and 2) transport up the mucociliary escalator for subsequent clearance through the GI tract (12). The rate of disappearance from the airways can be modeled as the sum of the rate constants for these two clearance mechanisms (12). Transepithelial absorption of lipid insoluble solutes has been shown to be a function of solute molecular weight (13). Absorption is slower through the bronchial epithelium than through the alveolar surface (12). Mucociliary clearance of the inhaled solute is a function of the site of its initial deposition; with particles depositing in more proximal airways clearing much faster than those depositing more peripherally (14). Schanker et al (15) reported half-times for benzylpenicillin clearance in mouse, rat, and rabbit for both aerosolized and instilled drug. It is likely that much of this clearance was by trans-alveolar epithelial absorption. We showed, however, that the combined rate of absorptive and mucociliary clearance of DTPA, a hydrophilic solute of comparable molecular weight (492 daltons) to many antibiotics, from the bronchial airways was similar to the absorptive clearance rate alone from the alveoli (12). Thus clearance half times of 60 - 90 minutes might be expected for antibiotics delivered as an aerosol to the bronchial airways in humans. Finally, cough following the inhalation of these aerosols will also enhance the rate of clearance from the bronchial airways (5).

In summary, the disposition of aerosolized antibiotics delivered to patients with CF is characterized by the fraction of inhaled drug deposited in the respiratory tract, the regional deposition of the drug within the respiratory tract, and the clearance rate of the drug from the airway surface. Factors affecting each of these components should be considered when designing studies to evaluate effectiveness of inhaled antibiotics for treatment of airway infections.

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AEROSOL ANTIBIOTIC THERAPY

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In 1940 aerosol penicillin was used in the treatment of cystic fibrosis (CF) and some benefits were noted. Aerosols of neomycin, gentamicin, carbenicillin, colomycin and other antibiotics have been used on a rather ad hoc basis over the years. It was not until 1980 that formal trials were performed on aerosol antibiotics.

We have performed a double-blind placebo controlled study of aerosol carbenicillin 1 gram, and gentamicin 80mgs twice daily over a one-year period and showed improvement in pulmonary function as shown in the table.

| Treatment Period | Mean FEV1 (ml) | Mean FVC (ml) | Mean PEFR (l/min) |
|------------------|---------------|---------------|-------------------|
| Antibiotic aerosol* | 1,566          | 2,656         | 323               |
| Placebo aerosol† | 1,300          | 2,314         | 283               |
| Significance‡     | p<.001         | .02>p>.001    | p<.001            |

*Seventeen patients each had six monthly recordings for a total of 102 assessments.
†Sixteen patients each had six monthly recordings and one had four monthly recordings for a total of 100 assessments.
‡Student's unpaired t test.

We were satisfied that patients using aerosol antibiotics showed improvement but we were still concerned about the possible emergence of drug-resistant organisms or hypersensitivity reactions. Penketh, et al.³ reported 41 patients treated with aerosol antibiotics for a mean duration of 21 months. The frequency of hospital admissions was reduced from 1.8 to 1.03 per year and there were only minor side effects. Resistance of bacteria to gentamicin and tobramycin was not a problem. There was no hypersensitivity, bronchospasm or candidiasis. Penketh⁴ also reviewed the sputum cultures of 30 CF patients taking aerosol antibiotics and 30 control CF patients whose sputum cultures yielded Pseudomonas aeruginosa but who were not taking aerosol antibiotics over the same period of time. One hundred eighty-seven sputum cultures were received. Patients on aerosol antibiotics did not have a significantly higher incidence of organisms resistant to gentamicin or tobramycin but there was an increase in resistance to carbenicillin.

Wall, et al.⁵ reported favorable results in 1983 using ticarcillin 1 gram and tobramycin 80mgs twice daily. Since then there have been a number of encouraging reports about the use of aerosol tobramycin⁶."
Littlewood, et al. showed that children had a reduced frequency of positive sputum pseudomonal cultures after inhalation of colomycin aerosol. Jensen, et al. compared colomycin to placebo in a double-blind placebo controlled study of 40 patients. Patients in the colomycin group had superior results in terms of clinical score, pulmonary function and inflammatory mediators.

Ceftazidime has been shown by Stead, et al. to be as effective as inhaled gentamicin and carbenicillin in producing an increase in lung function and reduction in hospital admissions when compared to placebo.

With increasing use of aerosol antibiotics it became important to find the most efficient nebuliser systems for delivery of this expensive treatment. Newman et al. showed a wide variation in droplet sizes from different nebulisers. Similar studies showed weak compressors produced fewer particles in the respirable range (<5 μ) than powerful compressors. We currently use the Medic Aid CR60 air compressor with an Acorn nebuliser and System 22 antibiotic T piece so that the exhaled antibiotics are vented out of the window.

We have been using aerosol antibiotics for 12 years in adults with cystic fibrosis and are convinced that they produce marked benefits in a significant number of patients. However, there are still many issues requiring further research.

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Aerosol Aminoglycoside Administration

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Clinical resolution of exacerbations of chronic bronchitis due to pneumococci correlates with sputum amoxicillin concentrations (1). In intubated patients with nosocomial P. aeruginosa pneumonia, intratracheal administration of sisomicin was more often associated with a cure in comparison to placebo (2). Aminoglycosides do not penetrate all membranes resulting in a poor correlation between serum and sputum concentrations.

This information suggested that direct airway administration of aminoglycosides to patients with Cystic Fibrosis might be a way of treating or suppressing the chronic P. aeruginosa bronchitis characteristic of this disease. However, clinical trials treating pulmonary exacerbations of, or prophylactic administration to Cystic Fibrosis patients with aerosol antibiotics were inconclusive (3). Reasons for discrepant results included variably efficient aerosol delivery systems, wide variability in disease severity, and lack of rigorous study design. To evaluate the efficacy of inhaled antibiotics, the nebulizer must deliver the aminoglycoside to the lower respiratory tract in concentrations active against P. aeruginosa and be compared to a masked placebo.

Pus is known to antagonize the bioactivity of gentamicin (4), while CF sputum antagonizes the bactericidal activity of gentamicin and tobramycin against CF P. aeruginosa (5). This antagonism is due to binding the aminoglycosides by DNA present in purulent sputum (6) and interference with bacterial aminoglycoside uptake by sputum divalent cations and its high ionic strength (6). Due to the antagonism of aminoglycoside activity by sputum a concentration ≥ 100 µg/ml is necessary to kill a P. aeruginosa in sputum, while 4 µg/ml will kill the same bacterium in nutrient broth (6). Assuming that sputum is the target fluid, aminoglycoside concentrations need to be ≥ 100 µg/ml to eradicate P. aeruginosa. Nebulizer delivery of particles expected to impact in the medium to small airways (< 3.3 but > 1.0 µm mmd) varies twenty-fold among jet and ultrasonic nebulizers. Although there is overlap between different machines and manufacturers, the greatest drug delivery occurs with ultrasonic machines. We found that a DeVilbiss Ultraneb 99/100 would reliably deliver tobramycin to the lower respiratory tract. The peak sputum concentration was 2.72 ± 2.44 mg/gm with an elimination half-life of 58 ± 30 min; the tobramycin dissolved in a theoretical volume of 31 ± 27 ml/kg. Achieving these sputum concentrations three times daily for three months in 21 patients decreased sputum bacterial density from a mean of 10^7 to 10^4.3 cfu/gm. Local pulmonary or systemic renal or ototoxicity was not detected during or after aerosol tobramycin administration (7). 71 patients whose FVC%-predicted was within 10% of the best value in the prior 6 months were randomized to tobramycin or masked placebo for 1 month, followed by a crossover to the other regimen. All evaluations were conducted in a "blind" fashion. Aerosolized tobramycin reduced sputum bacterial density from 10^6.5 to 10^5.5 cfu/gm. While receiving placebo the mean FEV1 (%-predicted) declined 7.46 ± 16.5% while during tobramycin administration the mean increased 7.49 ± 16.3%. Similarly, the FVC (%-predicted) declined 3.52 ± 12.5% during placebo
administration, while tobramycin inhalation effected a $4.00 \pm 11.8\%$ increase. We conclude that delivery of efficacious concentrations of tobramycin to the lower respiratory tract is safe, and minimizes the gradual decay in pulmonary function.

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Delivery of drugs directly to the lungs using aerosols has been used for many years to treat a number of disease states (i.e., asthma, bronchitis, COPD). More recently, it has been demonstrated that nebulized antibiotic (1,2) and mucolytic (3) therapies improve lung function in patients with cystic fibrosis. Delivery of drug(s) directly to the lung generally allows much lower doses to be administered relative to systemic therapy and results in a lower incidence of side effects. There are three primary delivery systems capable of delivering aerosolized drug to the respiratory tract, namely the nebulizer, the metered dose inhaler and the dry powder inhaler. While each delivery system may first appear to be quite simple, there are considerable operational/delivery differences between systems and within technologies (product-to-product). The purpose of this paper is to review these differences and where appropriate, the relevant physical-chemical properties of the formulation such that drug therapy can be optimized.

**Nebulized Solutions** - Nebulization of aqueous solutions containing drug is an alternative for pulmonary delivery of drugs that have sufficient aqueous solubility and acceptable chemical stability. In principle, poorly soluble drugs can also be nebulized, although the particle size of nebulized suspensions is markedly influenced by the properties of the suspension particles.

In general, a nebulizer generates a fine mist of drug-containing aqueous solution that the patient inhales. There are basically two types of nebulizers: jet nebulizers and ultrasonic nebulizers.

Jet nebulizers utilized compressed gas to aerosolize the solution. Compressed gas is supplied by either a gas cylinder (air or oxygen), hospital air line or a mechanical compressor. The compressed gas passes through a Venturi orifice, creating a negative pressure by the Bernoulli principle. Liquid is drawn from a reservoir through a feed tube to a point where the compressed gas fragments it into droplets. Since many of the droplets are too large to be inhaled, jet nebulizers are designed with baffles to prevent the large droplets for leaving the nebulizer. These droplets return to the reservoir for renebulization. Two to eight milliliters are typically nebulized, depending on the drug product. Nebulization rate and the residual amount of drug remaining in the nebulizer is dependent on nebulizer design, and can result in significant differences in the amount of drug delivered. 'Complete' nebulization can take from 5 to 15 minutes and still have up to 60% of the drug remaining in the nebulizer, unable to be nebulized(4). The size of the emitted droplets is highly dependent on nebulizer design and compressed gas flow rate (5,6,7). Formulation variables influencing droplet size include drug/excipient concentration, viscosity and surface tension (8,9,10). Drug can concentrate in the residual nebulizer solution during nebulization due to the evaporation of bulk water caused by the high gas flow rates through the system (7,10,11).
Ultrasonic nebulizers generate high frequency (MHz) sound waves that impinge on the surface of the liquid to produce respirable size aerosol droplets. The mean size of the generated droplets is inversely proportional to the operating frequency. Although the time necessary to nebulize a given volume of liquid is generally less with ultrasonic nebulizers than with jet nebulizers, baffling of the resultant large particles is much more difficult due to the absence of a high velocity air stream. The temperature of the nebulized solution can rise in an ultrasonic nebulizer if not controlled. This may have stability implications for chemically labile drugs.

**Metered Dose Inhalers (MDI’s)** - MDI’s are pressurized oral inhalation aerosol dispensers capable of delivering multiple (up to 400) doses of finely dispersed drug to the lungs on demand. MDI’s can be formulated as solutions or suspensions. Solution MDI’s require the drug to be soluble and stable in the vehicle, which is a combination of propellant(s) and cosolvent, usually ethanol. Suspension MDI’s contain micronized drug particles suspended in propellant and usually contain a surfactant to aid in dispersing and maintaining physical stability of the formulation. A metering valve assures that a constant dose of drug is administered with each actuation. Upon actuating the metering valve, the pressurized liquid propellants provide the basic driving force for atomization, while the actuator/mouthpiece determines the spray characteristics and affects the particle/droplet size of the resultant spray. Shaking suspension MDI products prior to dosing is necessary for proper dispersal of drug and obtaining homogeneous dosing from dose to dose. Spacers can be used between the MDI and oral cavity to minimize oropharyngeal deposition.

**Dry Powder Inhalers** - Dry powder inhalers are formulated as micronized powders and usually (except for high dose drugs) require a carrier substance used to provide optimal flow and reproducible dosing characteristics. Carrier substance used to date have included lactose and glucose. Since moisture can adversely affect the particle properties of powders, it is essential to assure that these products are protected from relative humidity, including exhaling into the devices. Product is delivered to the lungs due solely to the patient's inhalation.

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CF has long been recognized as an inherited disease of exocrine gland dysfunction. Involvement of the airways, the GI tract and the sweat glands dominates the clinical picture to the extent that the disease is often seen as a triad consisting of chronic obstructive lung disease, pancreatic insufficiency and elevated sweat electrolytes. Appreciation of the marked variability in signs and symptoms and aggressive approaches to therapy have taken CF from what was originally described as a fatal disease of infancy to an illness that affects infants, children, adolescents and adults.

Isolation of the CF gene and identification of the basic defect as involving faulty regulation of chloride channels have greatly enhanced our understanding of how events at the cellular level translate to what we know about the clinical pathophysiologic processes. The manner in which the lung and gastrointestinal involvement develops and progresses will be reviewed, as will traditional and more recently developed forms of therapy. Emphasis will be placed on the role of the CF Center Physician in developing an individualized plan of medical care for patients within the framework of a team concept involving a variety of health care professionals.

Identification of a specific gene defect would logically imply that a simple diagnostic test for CF will soon be developed. Identification of over 70 different mutations of the CF gene, however, suggests the development of a simple diagnostic procedure is less likely at this time. Currently the quantitative pilocarpine iontophoresis sweat test remains the standard diagnostic procedure. This test is based on an end organ defect in which elevated sweat electrolytes in the appropriate clinical setting serve as a diagnostic marker for CF. The role of appropriate testing modalities will be reviewed.
As with most chronic illnesses, the provision of quality care requires a multidisciplinary team approach. To function well as a team, we all need a common goal - "the health and well-being of the patient." The team members integrate efforts and ideas, each discipline having a role to play in the case of the CF patient and family. Team leaders or coordinators while retaining responsibility for their team must also be willing to delegate this leadership to a member of the team more qualified to deal with a particular case. For example, when a CF patient has had significant weight loss, the dietician would coordinate team members to effectively deal with this problem.

To serve as the CF team coordinator is one of the major responsibilities of the nurse. This role of the nurse coordinator may vary depending on the size of the CF center and the number of team members. But for a team to work effectively, the nurse coordinator must promote good communication among its members, encourage support of each other and learn how to deal with a variety of team issues as well as different personalities of the team members.

The other major responsibility of the CF nurse is to provide nursing care to the patient and family. It is important to recognize that the patient and family are integral members of the team and major providers of care. Nursing care should be aimed at establishing confidence and trust with the parent and child which will help them over the forthcoming years.

Families need medical information that enables them to acknowledge the seriousness of the disease, yet at the same time gives them hope and the capacity to develop strategies permitting maximum flexibility in treatment and enjoyment of quality family life. The nurse may act as a source of information to resolve confusion. What they "hear" from the doctor may be very different from what is actually said to them.

The nurse can help patient and families frame questions to doctors to what information they seek. The nurse can advise, interpret symptoms, such as fever or cough and give patients and parents confidence to voice appropriate concerns to the doctor.

Parents need to know what to do if their child gets sick on Friday night - which symptoms are serious, which can wait and what to do about them. The nurse coordinator educates the family as to the function of each team member and how to access their help.

Education about CF - the disease process and management of it is an ongoing process. This includes education of the family
as well as the patient. The nurse develops teaching tools - computer games, "CF jeopardy", "rap sessions" - for different age groups. Education can be done in a variety of settings including the hospital, clinic, CF camp, support groups or in the home.

One of the newest role of the CF nurse is coordinating clinical research trials. The nurse must be familiar with drug study protocols, assist with choosing appropriate participants, assure protocol compliance, and coordinate follow up care. The nurse involved in research needs good communication and organizational skills as well as good nursing skills in venipunctures, medication administrations, and other special procedures.

The nursing care of the CF patient and family can not be done by the CF nurse coordinator alone. Other nurses in the health care setting that are vital to the team also include those that provide outpatient care, inpatient care, care in the community setting as well as those in the home health care setting. The nurse fulfills a unique role that enables families and patients to acquire the skills, gain the knowledge and develop the network of support necessary to establish a routine of effective regular therapy which is vital to the health of the patient. This enables families and patients to develop their own particular ways of coping while retaining quality of life and hope for the future.
Aspects of the Social Work Role in the Cystic Fibrosis Center

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The presence of a professionally trained social worker within a Cystic Fibrosis Center has been encouraged for many years. The unique perspectives that social work offers to the delivery of services to patients and families is invaluable in promoting positive adjustment throughout the lifespan of care.

A social worker's uniqueness stems from the influence of their knowledge base (systems theory, developmental theory, psychodynamic approaches, psychotherapy modalities, etc.). Regardless of the social worker's particular orientation to practice, their interventions are based on the recognition of the individual as needing to grow and succeed within a particular environment. This framework focuses the social work role on enhancing this dynamic relationship throughout all phases (diagnosis to death) and transitional stages (i.e., school, gaining independence, marriage, entering the workforce, etc.) in the patient's life. While other members of the team appreciate this holistic approach to care, it is the primary focus of the social work role to truly integrate all aspects of the patient's life in promoting care and developing treatment interventions.

One of the most important functions that social work performs is the assessment of a patient's and family's abilities to cope with this disease. Some aspects of the psychosocial assessment are obtaining a detailed family genogram, defining financial resources and work histories, exploring past and present coping styles, gaining an understanding of the social and cultural issues which might influence adjustment to care, and defining social support networks available to them. The assessment process begins upon initial introduction to the patient and family and remains a fluid and constant tool used by social workers to enhance the team's response to patient and family needs. It is important that the team recognizes the unique relationship social workers develop with the patients and families through this ongoing assessment process and respects the social worker's role as advocate for the patient and family in offering care.

In addition to assessments, social workers are aware of financial programs available to this population and are concerned about the public policies which may influence eligibility. They are aware of community resources such as support groups and many social workers initiate and facilitate support groups within their respective CF Center. Many social workers bring skills in program planning, teaching and developing educational programs, and experiences in community organization/advocacy. Maintaining the unique perspective of the individual as needing to succeed within a system, social workers can be invaluable in identifying and coordinating service delivery to patients and families thereby enhancing their reintegration to home, school, work and/or community.

Social workers also bring strong skills in communication extending beyond family work into the arena of team process. Their role as a collaborator and a facilitator not only increases more efficient access to the health care system and hospital for the patients and families but also impacts on the efficiency and effectiveness of team interventions. Issues such as compliance, sexuality, death, bereavement, the growing number of concerns of the expanding adult population, and the challenging issues of transplantation and long term survival all require close and mutually respectful team relationships. By virtue of the social worker's sensitivity to differing attitudes and philosophies, they can stimulate the team's understanding and appreciation of the stresses inherent in treating this population.
Finally, social workers can contribute significantly to the area of research. This function can be generated through their original psychosocial research or in collaboration with other professionals. It is essential that social workers be knowledgeable about the literature and advancements in medical care evolving in response to the rapid developments in CF research which can potentially affect the services we provide. With this knowledge, social workers can better assist the patients and families to be more involved in research efforts by increasing their understanding as informed consumers while helping investigators complete subject enrollment.

The mutual goal of a CF team is to provide comprehensive care to patients and families. The availability of a social worker is essential to meeting this goal and of achieving the highest level of personalized and effective care.
Nutritional Aspects of CF Care

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Nutrition plays a pivotal role in the care of individuals with cystic fibrosis (CF). Recent tremendous advances in molecular biology, with the forecast of improved therapies, focus greater attention to maintaining and promoting the optimal nutritional well-being of patients throughout the lifecycle. The majority of patients with CF are at risk for, and many patients actually exhibit some form of, chronic malnutrition. Thus, attention to the nutritional aspects of CF care and to the integral role of the nutritionist/registered dietitian (R.D.) in providing that care, are assuming higher importance in contemporary CF Centers.

Currently, parameters of nutrition practice include nutrition assessment, intervention, and follow-up of patients in an interdisciplinary manner. In combination with patients and their families/friends, the professional expertise of all CF caregivers, whether in the hospital, outpatient, home, or community settings, is crucial to the overall health status of all patients. The following rubrics and example elements comprise possible components of nutritional care in CF:

1. **Perform nutrition assessments.** Nutrition assessment may be summarized by five alphabetical categories: Anthropometric Measurements, Biochemical (Laboratory) Values, Clinical Examinations, Dietary Facts, and Extra Lifestyle Influences. When conducted at routine intervals, nutrition assessment can be the most effective method of evaluating the on-going nutritional status of patients and of evaluating the provision of nutrition care services. Nutrition assessment is classified at two levels: **screening** and comprehensive analysis. Screening is performed during routine outpatient visits; comprehensive analysis is performed at diagnosis, at the annual visit, whenever there is a change in the patient's health status, and prior to and during nutritional intervention/rehabilitation. Established protocols should be followed for both.

2. **Determine nutrition treatment goals.** In consultation with other team members and based upon interpretation of nutrition assessment data, the nutritionist recommends nutrient goals (i.e., energy, protein, fat, carbohydrate, vitamins, minerals, and fluids), pancreatic enzyme administration, supplementation (e.g., vitamin dosages, salt replacement dosages), and oral/enteral protein and kilocalorie supplements, whether by mouth or tube. Decisions to nutritionally support the patient, either by enteral or parenteral routes, are modern roles of the nutritionist in pediatric and adult CF care.

3. **Develop and implement specific nutrition care plans to achieve mutual nutrition goals.** Current theory and practice recommend patient ingestion of a wide variety of nourishing foods and beverages. Attention is concentrated on promoting the total nutritional well-being of the patient, given the constraints of the patient's dietary intolerances and disease progression, and the family's lifestyle. Depending upon the availability of CF Center staff, consultants, and resources, and coupled with the patient's needs, the strategies for intervention/rehabilitation are determined. Success-oriented, yet practical, approaches are devised. Anticipatory guidance is provided from infancy through adulthood to foster awareness of potential trouble areas, along with possible solutions. Special times of increased nutritional requirements, such as during acute pulmonary exacerbations and
pregnancy/lactation, command great attention and creativity on everyone's part. Since the patient and family have ultimate control in implementing any nutrition care plan, they and the nutritionist determine reasonable and specific suggestions.

4. **Coordinate all team members relating to the continuity of nutritional care and the nutrition educational needs of the patient and family in the hospital, outpatient, home, and community settings.** Since the inception of interdisciplinary CF care and the expansion of care settings, numerous people are involved in providing, or promoting, optimal nutritional care. For example, the patient may be involved with both the physical therapist and nutritionist in determining nutritional alterations during exercise rehabilitation. In many healthcare settings, one registered dietitian provides inpatient care, while another provides outpatient care, while a third provides community services (e.g., W.I.C., school lunch), while a fourth provides home healthcare nutritional consultation. Over the past decade, numerous educational print, audio-visual, and computer nutrition materials have been marketed. Many CF Centers have developed their own materials; some prefer to distribute proprietary materials. Each material necessitates evaluation for validity and suitability. In all these nutrition service and education program situations, the coordination is the purview of the CF Center nutritionist.

5. **Conduct basic science and clinical research studies for the delineation of the nutrition status and improved care of patients.** From the first descriptions of CF in the literature, the nutritional aspects of CF care have been noted and highlighted. In recent years, nutritionists have documented standards of care (quality assurance) in CF. A newer role for the nutritionist is participating in clinical research trials that have a nutrition component, e.g., those studying the efficacy of medications. Recently, research-trained nutritionists are assuming leadership roles in conducting clinical nutrition studies, e.g., in delineating changes in body composition of children and adults with CF, or in identifying nutritional demands of lactation by CF mothers. Research in nutritional biochemistry/physiology, as well as in food science/technology, has as the intent, enhanced quality of life and nutritional status of patients through improved understanding of the interrelationships between the science and application of nutrition theories/principles to CF.

Thus, the nutritional aspects of CF care are multi-faceted and evolving. The nutritional techniques and professionals involved in attaining optimal growth and health of each person with CF have changed dramatically over the past 60 years. Future advances in elucidating CF are expected to increase the role for nutrition research, care, and education in the eventual treatment of, and cure for, CF.

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Michel, S.H., Mueller, D.H. Practical Approaches in Nutrition Care of Patients with Cystic Fibrosis. Topics in Clinical Nutrition, 4: 46-55, 1989.
Successful medical management of respiratory disease in cystic fibrosis (CF) is based on providing effective pulmonary hygiene. Clearance of airway blockage by secretions will reduce infection, lessen parenchymal tissue damage and improve gas exchange (1). As members of the healthcare team the Respiratory Therapist (RT) and Physical Therapist (PT) contribute their skills to assess, educate and treat the CF patient to optimize their respiratory and physical condition.

The foundation of an effective pulmonary hygiene regimen is aerosol therapy and chest physiotherapy (2). These therapies continue to evolve, and are sometimes the focus of some controversy. Aerosol therapy delivers medications directly into the airways providing topical deposition. Mucolytics are often used with aerosol therapy to chemically reduce the viscosity of secretions. Use of an ultrasonic nebulizer also improves mobility of secretions, while providing more efficacious particle size and deposition. Hand held ultrasonic nebulizers can be used for delivery of aqueous solutions, such as normal saline, bronchodilators and cromolyn sodium, but are not well suited for use with mucolytics or antibiotics due to the viscid composition of these medications. Bronchodilators, most commonly beta-2 agonists, provide relief of bronchospasm, which may be induced with use of a mucolytic or ultrasonic nebulizer. Recent studies show that the addition of an anticholinergic, such as ipratropium bromide (Atrovent), enhances bronchial dilation in patients with CF (3,4). Antibiotics delivered by aerosol used as long-term therapy have been shown to decrease hospitalizations from recurrent pulmonary infections (5). Recent studies have shown that Amiloride delivered to the airways by aerosol improves mucus clearance in patients with CF, and is a promising new therapy currently under investigation (6,7). The future prospects for aerosol delivery in gene replacement therapy is a focus of current research in this field.

Clearance of secretions is aided by percussion and postural drainage, vibration, and assisted cough (8). Adjuncts to this therapy may include forced expiratory techniques (9), positive expiratory pressure (PEP) mask (10,11) or exercise. There remains some question as to the role exercise can assume in the total care regimen (12,13), but little question remains as to the benefit that exercise provides in improving working capacity and self image of the CF patient (14). Careful monitoring and assessment is crucial to the success of an exercise program, which must be tailored to the individual abilities of each patient.

Routine pulmonary function testing provides the physician with valuable information as to the chronic and acute state of the patients respiratory status. This information supports the clinical evaluation of patients and their therapies in relationship to established baselines.

Education of the patient and family is perhaps the most important service that the RT and PT can provide. The continuing care of the patient in the home is ultimately what will define the success of any established care regimen. Assuring that the care plan is well tolerated and appreciable to the patient will improve effectiveness as well as compliance. The family and patient should be able to recognize the early symptoms of infection and seek appropriate intervention. Their skills in providing therapy should be developed to support independence, and encourage participation with medical management.
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Malabsorption is one of the important factors leading to poor nutrition in cystic fibrosis, potentially causing a wide range of symptoms and signs, particularly those related to energy deficiency, essential fatty acid deficiency, fat soluble vitamin deficiency and steatorrhoea, azotorrhoea and sometimes starch intolerance. However, despite many improvements in pancreatic enzyme therapy efficacy, complete correction of malabsorption remains difficult - the result of a number of factors adversely affecting the function of exogenously administered enzymes and other intraluminal and mucosal factors contributing to malabsorption besides pancreatic insufficiency. Since the 1950s pancreatic extracts, usually of porcine origin have been used empirically to replace the pancreatic secretory abnormality and improve digestion. Unfortunately in their simple form, 90% of ingested enzymes are destroyed by gastric acid and the last 10 years have seen the development of pH-sensitive preparations of pancreatic enzymes and increasing research into adjunctive therapy and dietary methods for manipulating nutrient absorption.

_**Malabsorption in CF:**_ The pancreatic lesion in most patients with CF results in production of pancreatic secretions low in volume, enzyme, and bicarbonate concentrations. This is associated with a fall in intestinal pH caused by both relative and absolute gastric acid excess, producing lowered activity of any lipase secreted and precipitation of bile salts within the intestinal lumen. Binding of bile salts to intraluminal fat results in loss of bile salts from the enterohepatic circulation with subsequent fall in the total bile salt pool compounding the steatorrhoea. Steatorrhoea and azotorrhoea occurs when the secretion of pancreatic lipase and trypsin is reduced to less than 10% of normal. This occurs in approximately 90% of CF sufferers. Since dietary fat forms 40% of the normal caloric intake, steatorrhoea leads to significant energy deficiency. Protein malabsorption results in stool nitrogen outputs which are 2-2½ times normal tending to parallel the steatorrhoea. Reduced pancreatic amylase results in decreased starch absorption, although monosaccharide and disaccharide absorption is normal. Presumably the presence of salivary amylase and intestinal maltase prevents carbohydrate intolerance except occasionally in the very young infant. Fat malabsorption is accompanied by fatty acid malabsorption and essential fatty acid deficiency can be compounded in some children by liver disease and reduced bile acid pool. In addition, fat malabsorption is associated with malabsorption of fat soluble vitamins A,D,E, and K. Iron absorption is also reduced in some patients and may contribute to iron deficiency.

_**Pancreatic enzyme replacement:**_ Pancreatic enzyme replacement therapy should be individualised according to the amount of food eaten and the degree of malabsorption. It is calculated that, assuming there is no inactivation of ingested enzymes in the stomach, between 10 000 and 30 000 IU of lipase must be delivered to the duodenum with an average meal, depending on the age of the subject and the size of the meal. The mixing of enzymes with food in the duodenum also seems to be important. Granulated preparations or preparations with microspheres result in higher enzyme activities reaching the duodenum than with tablets. A bewildering array of porcine pancreatic enzyme preparations are available commercially but there is good evidence from several studies that preparations which are protected against peptic acid inactivation confer significant advantages over unprotected preparations. Azotorrhoea is more frequently abolished by pancreatic enzyme supplements than steatorrhoea which is often incompletely corrected, possibly because trypsin secretion is better preserved than lipase secretion in pancreatic insufficiency and because trypsin is not
inactivated by acid but only by pepsin. As mentioned, correction of steatorrhoea is usually incomplete even with enteric-coated microspheres (10-20% malabsorption compared with 2% in healthy persons). The use of acid lipases which are active at a lower pH has not been trialled as yet.

Poor response to pancreatic enzyme preparations may be due to poor compliance, inappropriate timing of administration, the presence of other conditions contributing to steatorrhoea (e.g. bacterial overgrowth, giardia, or coeliac disease), or occasionally inadequate release of enzyme from the enteric-coated spheres.

Adjuvants to pancreatic enzyme therapy: Treatment aimed at neutralising or inhibiting gastric acid and/or protecting pancreatic enzymes against inactivation may be useful in selected cases where persisting steatorrhoea and azotorrhoea persists in the absence of any of the above reasons for poor response. The addition of antacids to pancreatic supplementation to neutralize gastric acid has achieved variable results, probably because some antacids form calcium soaps and precipitate glycine conjugated bile salts. H₂ receptor antagonists are of value as an adjunct to microspheres with an additional effect on the degree of steatorrhoea. However, interaction (5) with drugs commonly used for pulmonary management limit their long-term use. Oral taurine has been reported to benefit some patients (2). We have been recently investigating a new approach to adjuvant therapy using a synthetic methylated prostaglandin E₁ analogue which decreases secretion of gastric acid and increases duodenal bicarbonate secretion (3). This therapy has inherent advantages because of the latter effect. Where steatorrhoea persists despite appropriate doses of pH sensitive microspheres, the addition of misoprostol has resulted in complete correction of the steatorrhoea (3).

Role of diet in therapy (4): An adequate caloric intake with a high protein content forms the basis of dietetic management of pancreatic insufficiency. With appropriate enzyme supplementation, normal to moderate fat intakes are quite well tolerated. In cases of severe symptomatic steatorrhoea, medium chain triglycerides may help improve fat malabsorption. Since fibre has been shown to inhibit pancreatic enzymes in vitro and in vivo, fibre enriched diets should be avoided. Adequate vitamin supplements, particularly of fat soluble vitamins should be given to all patients with pancreatic insufficiency and studies have shown that these are best administered in a water miscible form. In general, iron, zinc and vitamin B₁₂ malabsorption is corrected by the administration of pancreatic extracts. Semi-elemental formula are frequently used for aggressive enteral nutritional supplementation but may not necessarily confer an advantage over standard formulations (4).

Conclusion: Optimal therapy for malabsorption in CF includes dietary supplements with pancreatic enzyme preparations which are acid resistant and which are administered in an appropriate fashion dispersed throughout a meal. The response to this therapy should be carefully evaluated. In patients with incomplete correction of malabsorption, compliance or another underlying condition should be investigated and in the absence of these, the use of adjunctive therapy preferably with a prostaglandin analogue should be considered. It should be every practitioner's aim to maximise nutrient absorption in all cases of cystic fibrosis.

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CO-EXISTING GASTROINTESTINAL DISORDERS IN CYSTIC FIBROSIS
THE PATIENT WHOSE GI PROBLEMS PERSIST

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Introduction

Steatorrhea in CF does not normalize despite increasing pancreatic enzyme administration and efforts to reduce intestinal acidity. Furthermore, many CF patients have abdominal symptoms which are not explained by distal intestinal obstruction syndrome (meconium ileus equivalent), and are unresponsive to treatment for this problem. It has been recommended that refractory intestinal symptoms in CF merit further investigation, especially in patients who have had previous surgery (1,2). Application of upper and lower endoscopy and advances in imaging techniques (such as computerized tomography and magnetic resonance imaging) have resulted in a number of reports of Crohn’s disease in CF. Moreover, increased survival is associated with a number of late complications, including intestinal cancer. A recent editorial discussed these associations and suggested the risk of inflammatory bowel disease (IBD) complicating CF was 1% (3).

Thirteen cases of IBD and CF have been reported. Meconium ileus/equivalent was present in 46-61%. Mean age of presentation was 12 years (range 3-23 years). Arthritis was present in 31%. Laboratory abnormalities included anemia (mean Hb 10.4 g/dl), elevated ESR (mean 60 mm/hr) and lowered albumin (mean 2.4 g/dl). Ileocolitis with fistula formation was present in 69%, and 76.9% required surgical resection. Prednisone was given to 61.5%.

Several inflammatory intestinal lesions have been reported in younger children. Five out of 1100 (0.45%) CF patients were documented with Celiac disease according to ESPGN criteria (4), suggesting an abnormal linkage between these two diseases. Antigliadin antibodies are commonly elevated (IgA 15.4%, IgG 65.4%). An associated cow’s milk sensitive enteropathy has been described. Peptic ulceration was reported in 8% of autopsies and is increasingly diagnosed in clinical practice; response to H2 blockers is helpful in diagnosis.

Post-surgical (meconium ileus, atresias, intussusception, etc.) complications may occur in later years and include blind loop syndrome, anastomotic problems, small bowel contamination syndrome, and short bowel syndrome. Numerous other GI problems are covered in Comprehensive Reviews.

The reported cases of cancer of the intestine include 3 adenocarcinomas of the ileum (a rare tumor in any age group), 3 pancreatic cancers, and 2 cholangiocarcinomas of the bile duct. The mean age of presentation was 28 years (range 23-36 years)(3).

Discussion

Increased intestinal permeability, impaired protein digestion secondary to lack of proteases leading to a high antigen load to the mucosa, qualitative or quantitative abnormalities in mucous secretions, immunoglobulin deficiencies (low IgG 22%, low IgA 17%), and the presence of protease inhibitors in meconium may all contribute to the increased vulnerability of the intestine in CF to develop inflammatory lesions (Crohn’s 1%, Celiac disease 0.5%). Meconium ileus, Hirschsprung’s disease, and short bowel syndrome
all have an increased risk of IBD (3). Toxigenic C. difficile is recovered from between 22-50% of CF patients who remain asymptomatic (5), presumably reflecting chloride channel unresponsiveness. These findings are similar to those reported in response to other enterotoxins (cholera, E. coli) in vitro. Giardiasis is significantly more common than controls (28% vs 6.3%, p<0.0006), perhaps related to favorable receptor sites, and increases with age.

Numerous other biliary and intestinal alterations in CF may be involved in these associations including a primary mucosal defect of intestinal bile acid absorption, increased glycine:taurine conjugated bile acid ratio in the duodenum, decreased bile acid pool, increased fecal bile acid excretion, altered fecal bacterial flora, increased fecal amino acids, and lipid-like droplets in the surface absorptive cells of the rectum. Sclerosing cholangitis occurs in 1-4% of patients with many having evidence of colitis.

Histologically the pancreas in CF reacts strongly for carcinoembryonic antigen (CEA) and alpha-I-antitrypsin (6); these accumulations were thought to represent defects in cellular synthesis assembly or transport of glycoproteins in the ducts, and are compatible with CFTR antibody studies showing a failure of glycosylation in the endoplasmic reticulum (7). CEA is increased in serum and sputum and correlates with disease activity. The ras gene mutation is the oncogene found in 90-100% of pancreatic adenocarcinomas. The close relationship of the MET oncogene to the CF gene on chromosome 7 suggests that new mutations may be found that are associated with CF, IBD, and cancer. The increased risk of intestinal cancer (3,8) emphasizes the importance of autopsy surveillance in older patients (3).

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S4.4 Essential Fatty Acids, Eicosanoids and GI Function in Cystic Fibrosis

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Although essential fatty acid (EFA) deficiency has been a known complication in cystic fibrosis (CF) since many years, the relation to the primary symptoms have not been fully explained. The EFA deficiency can not be solely explained by malabsorption, since it has been described also in CF patients without pancreatic insufficiency (1-3). This is of special interest since many of the symptoms recognized in animals with EFA deficiency are similar to those found during the course of CF (for review see 4,5). There has been indications of an increased turnover of EFA in CF (6-8), and an impaired inhibition of arachidonic acid (AA) release in dexamethasone-stimulated lymphocytes from CF patients have been demonstrated (9). On the basis of these findings, we have formulated a hypothesis about the basic defect in CF including an increased turnover of EFA and thereby giving rise to both an increased production of eicosanoids and the subsequent development of EFA deficiency (10).

Since an increased release of AA will give an increased synthesis of eicosanoids, studies have been performed to verify if there are clinical indications of such increased synthesis. Interestingly, AA and its metabolites, prostaglandins, tromboxanes, leukotrienes and HETEs are involved in processes of basic importance in the CF disease, like the regulation of chloride transport (11), mucus production and beta-adrenerg stimulus-secretion coupling in both in vitro and in vivo studies (for review see 4,5). The large transmembraneous part of the CFTR protein makes it possible to influence the fatty acid turnover in the membranes beside a transport function, whatever this finally will be proved to be, i.e. related to chloride transport or fat metabolism.

AA, as well as prostaglandins, leukotrienes and HETEs are synthesised in the GI tract in the mucosa and smooth muscles and are highly active substances in different physiological processes (12). At least leukotrienes undergo an enterohepatic circulation but are mainly excreted in the urine (13). The eicosanoids are present in liver and probably involved in diseases of the liver, pancreas and GI tract (14). Studies in CF patients have indicated very high levels of these substances and an impaired inhibition by steroids compared to controls.

The EFA deficiency will ultimately develop as an result of an increased turnover of AA - sooner the more impaired the inhibition of the AA release is, a factor which is individually different (9) and might be genetically determined as well as influenced by other factors influencing the membrane compositions (15). The EFA deficiency in itself can eventually influence the membrane functions and this series of events will hypothetically explain both the different speed of progression of the disease in different individuals and also be a base to explain the different expression of symptoms in different patients. It would furthermore help to explain the good survival rate in Canadian and Swedish patients, who are supplied with a relatively high fat diet (16).
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S4.5

GASTROESOPHAGEAL REFLUX IN CYSTIC FIBROSIS

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Gastroesophageal Reflux (GER) in patients with Cystic Fibrosis (CF) was first reported in 1975 (1) but the significance of this association has been largely ignored. The symptoms and signs of GER bear a remarkable similarity to those ascribed to CF and seen in many CF patients (Table I).

Table I: Signs and Symptoms common to CF and GER

| CF Symptoms                        | GER Symptoms                             |
|-----------------------------------|-----------------------------------------|
| chronic cough                     | chronic pulmonary disease                |
| wheezy respiration                | bronchopneumonia                         |
| recurrent pulmonary infection      | atelectasis                              |
| vomiting/regurgitation             | bronchiectasis                           |
| abdominal pain                     | clubbing                                 |
| nutritional/growth failure         | chronic pulmonary disease                |

Regurgitation or vomiting occur readily in many CF patients, for example, after an episode of severe coughing or if chest postural percussion and drainage physiotherapy (PD) is performed too soon after a meal. This is often accepted by clinic and family as simply "part of CF" and dealt with symptomatically by measures such as scheduling PD before and not after meals, without due consideration being given to the cause or significance of the symptom. Where the presence of GER has been considered in CF patients however, it has been found to occur frequently (2).

The important question of the relationship between GER, Gastrointestinal dysfunction and Pulmonary disease in CF has not been properly addressed or resolved. A fundamental question in considering this relationship is whether the pulmonary disease of CF in itself leads to an increased incidence of GER through effects such as flattening of the diaphragm, increased sterno-vertebral angle or increased intraabdominal pressure during coughing; or whether Gastrointestinal dysfunction in CF causes reflux through altered intestinal hormonal (3) or Prostaglandin (4) synthesis or release. The corollary to this question is whether GER in CF is a significant causative factor in the development of CF pulmonary disease, due to mechanisms such as aspiration or facilitation of colonization by unusual organisms (eg, X. maltophilia) (5).

Although the interrelationship between GER, GI dysfunction and pulmonary disease in CF has not yet been clearly delineated, sufficient pieces of this puzzle are now known to begin to decipher the interaction (Figure 1) and to identify the questions which should now be addressed in order, not only to understand, but to begin to treat GER and pulmonary disease in CF more effectively.

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Figure 1: Interrelationship between GER and pulmonary disease together with gastrointestinal function in patients with CF.
Diagnosing Cystic Fibrosis: Parents' and Providers' Perspectives

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When providers first tell parents their child has cystic fibrosis (CF), the encounter marks the beginning of a life-long relationship between the family and the provider. The literature addresses what disease-related knowledge parents need (1,2,3) but not how this information is conveyed. The initial communication is central to establishing the parent-provider relationship. Very little is known about this initial encounter in terms of how the relationship begins, how either perceives the other, or what strategies they use to deal with each other. This study is a comparative analysis of parent and provider perspectives about the initial encounter. It describes what providers think is important and what parents want to know and find useful.

Sample. Eight parents (five mothers, three fathers) participated. All had at least a high school education and were married. The average age was 29 years. All parented children diagnosed with CF during the first three months of life. Data were collected within two years of diagnosis. Eight providers (six female) participated in the study. Their disciplines included four nurses, three physicians, and one social worker. All providers were members of a CF Center and all were involved with newly diagnosed families.

Method. Data were obtained through in-depth, tape-recorded interviews conducted by the investigator using Grounded Theory method. Parents were asked general questions about having a child diagnosed with CF. All focused on the time of diagnosis. A theoretical sampling was then done by interviewing providers about the issues raised by parents to facilitate a comparative analysis of the first interaction between parents and providers. A combined group of content and methodological experts conducted line-by-line dimensional analysis of the transcripts. The interviews became more focused as the categories were identified (4).

Results. Providers tended to fall into either a prescriptive, structured approach or a negotiated, responsive approach. Which approach they chose was determined by the provider's primary purpose. All providers used strategies from each approach rather than a pure form. They tended, however, to be more inclined to one or the other. These two approaches differed primarily in how directive or controlling the providers were in the interaction with parents.

The prescriptive approach was used when the provider's overriding concern was compliance with the medical regimen. They used strategies which guided parents toward information and effective responses that would increase the chances that parents would follow the prescribed interventions. The focus of information was its correctness with an emphasis on the chronic and serious nature of CF. These providers seemed to have the notion of an ideal affective response as a kind of controlled sadness. There was a concern that too much or too little emotional response would render parents unable to care for their child. Providers attempted to control parents' affective responses by sequencing information. Prognosis was discussed last because providers were concerned parents would be unable to attend to the other facts after they heard the prognosis. These strategies were based on the belief that having parents start prescribed care immediately would help parents and it would produce a healthier child. In an effort to provide parents with consistent and accurate facts, providers "gave" each family the same information from a predetermined agenda using a formal, unidirectional, didactic style.

In contrast, the negotiated approach was used when the primary goal was to ease the emotional pain of parents. Strategies focused on providing families whatever they needed to feel confident about their abilities to care for their child and themselves, both physically and emotionally. These providers "asked" parents about their perceptions and concerns about CF. Subsequent discussion
was based upon the issues identified by the parent and those the provider identified as the immediate needs of the child. Providers usually started with prognosis because parents asked about this first. The type, amount, and timing of information was individualized. The style was informal, spontaneous, and interactive. Parents were guided toward additional sources of information, including other parents' of children with CF for peer support. These providers interpreted a much wider range of affective response in parents as normal and supported the expression of feelings. In contrast to the prescriptive approach, an attempt was made to limit the tasks that parents needed to learn during the first meeting. This strategy was based upon the belief that parents needed time to deal with their emotions before engaging in new therapies.

The provider's approach to informing parents about the diagnosis was very important to parents. They wanted answers to their questions, a sense of hope, and sensitivity to their needs. Parents stated a desire for information early about the diagnosis, prognosis, and the child's immediate care needs. They did not want some of the details about the pathophysiology of CF discussed by the prescriptive providers. Instead of detailed facts, parents found comfort in seeing their own child healthy and in talking with other parents of children with CF, which was offered by providers using the negotiated approach. According to parents, matching the amount, timing, and sequence of information with a parent's needs was critical to avoiding confusion. Too much information made parents feel overwhelmed, while too little caused parents to seek out other, often inaccurate, sources resulting in misconceptions and anxiety. All parents searched out sources other than the CF Center, mostly for experiential information rather than factual information. In addition to information about CF, parents sought help with the financial burden of the disease and making the transition from being told about the illness to becoming the "teller" to family and friends. These topics were more likely to be addressed by providers using the negotiated approach.

Discussion. The issue of control of information and decision-making in caregiver style identified by this study is consistent with the literature (5). When parents described encounters with prescriptive and negotiated approaches, they expressed a preference for the negotiated approach. Not all parents needed the exact same information at the time of diagnosis. Each parent had a different reference point, depending upon the child's condition and the parent's previous knowledge or experience with the disease. This study suggests that tailoring information to the individual needs of families with a negotiated approach may lead to a more collaborative parent-provider relationship.

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The presence of chronic illness in a child is considered to be a risk factor in the child's psychosocial adjustment. Children with a medical condition are 2.4 times more likely than healthy peers to have a behaviour problem. (1) Health care providers are familiar with the "vulnerable child syndrome", (2) where parental anxiety about a child's physical health and survival disrupts parental ability to give consistent care and contributes to behavior problems. CF seems to provide especially high risk conditions for psychological development. The prognosis is for progressive deterioration and early death and parents carry heavy responsibility for daily care. However in spite of this supposedly gloomy outlook, children with CF show little evidence of adverse psychosocial adjustment when compared to a group of children whose chronic medical conditions have a seemingly better prognosis.

Since 1984, we have conducted a developmental study of children with CF diagnosed in infancy. They are followed to age 7 and compared to a group of healthy children and to children diagnosed with congenital heart disease in infancy. In contrast with the natural history of CF, in the CHD group we see children whose problems are usually corrected in the first few years of life, experience improving health and normal life span, and do not require daily treatment by parents.

The study includes 45 children with CF, 66 with CHD, and 59 in the healthy group. Of these, 39, 32, and 27 respectively have been followed to 3 years of age.

At intake, both mothers and fathers complete the Parenting Stress Index (3). Comparison of these questionnaires shows that although the three groups do not differ in significant Life Events, parents in the CHD group report higher levels of parent stress than those in the CF group, though the CF group reports more demands of childcare per se (4).

At 12-18 months, we assess quality of infant-mother attachment in a standardized laboratory paradigm (5). The highest rate of secure (optimal)
infant-mother relationships is observed in the healthy group followed by the CF and then CHD group (6).

At 2 years we observe mother and child problem-solving tasks requiring parents to assist the child while also respecting the child’s autonomy. In this task observers rate the healthy children as most persistent, enthusiastic and compliant with mothers, while children in the CF group have the least positive experience (6).

Finally, at 2 and 3 years of age mothers and fathers completed the Child Behavior Checklist (7) for their children. When we consider children whose scores place them above the clinical cutoff point (i.e. likely to have a diagnosable disorder), the CHD group rather than the CF group contributes disproportionately. (8% of the healthy group, 7% of the CF group, and 30% of the CHD group are rated in the clinical range at least once).

These data suggest that in spite of the supposedly poor psychosocial prognosis, children with CF, are psychologically healthy; marginally distinguishable from healthy children; much better off than children with CHD. Possible explanations considered are 1) better support system for CF families than CHD group; 2) parent “burden” in CF also contributes to parent “control” and “mastery”; 3) developmental timing of most serious health problems coincides with a sensitive period for CHD, but not CF; 4) uncertainty of outcome rather than illness per se is the fact or that disrupts development. Regardless of which explanation is accepted, the data point to the importance of looking for family strength as well as burdens in evaluating the psychosocial impact of CF.

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Maternal Distress, Illness Severity, and Child Adjustment in Cystic Fibrosis

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Recent studies of families of chronically ill children suggest that psychosocial dysfunction is less prevalent than originally thought and by no means inevitable (Drotar & Bush, 1985; Johnson, 1985). Thus, the current challenge for research in this area is to identify factors that influence the adjustment of the child and other family members. The goals of the present study were (a) to assess the influence of child age and illness severity on the adjustment of CF patients and their mothers, and (b) to identify the ways in which CF influences mother-child interactions involving child discipline.

Subjects included 32 mothers of CF patients in four age groups. The control group consisted of 32 mothers of well children matched by age group. Sample characteristics are described in further detail elsewhere (Walker, Ford & Donald, 1987; Walker, Ortiz, & Newbrough, 1989). Maternal adjustment was assessed with the Center for Epidemiological Studies Depression Scale (Radloff, 1977) and the Parenting Sense of Competence Scale (Gibaud-Wallston & Wandersman, 1987; Johnston & Mash, 1989). Child adjustment was assessed with the Child Behavior Checklist (Achenbach & Edelbrock, 1983). Measures of illness severity included the Schwachman Clinical Evaluation System, the Maternal Evaluation of Child Impairment (modified from Frydman, 1980), and the number of days of school absence due to illness in the previous year. Mothers also were asked to describe two recent incidents of child obedience and two incidents of disobedience. They subsequently were asked to report whether CF had affected the child's behavior and the mother's response to the child during these incidents, and if so, to describe the nature of this effect. A coding system was developed to classify maternal responses to these questions.

Results indicated that mothers of children with CF in two age groups (pre-schoolers and early adolescents) had significantly higher levels of depression than mothers of well children in these age groups. There were no differences between the CF and well groups on measures of perceived parenting competence or child adjustment. In the CF group, measures of illness severity correlated significantly with the Internalizing Scale of the Child Behavior Checklist (CBCL), indicating higher levels of anxiety and depression in children with more severe illness. Measures of illness severity were not significantly correlated with the Externalizing Scale of the CBCL, suggesting that more severe illness was not associated with conduct disorder. There was no association between measures of CF severity and maternal adjustment. The majority of mothers reported that although CF did not play a role in mother-child interactions involving child obedience, the illness was important in interactions involving disobedience. Specifically, mothers reported that CF caused the child (a) to be "spoiled" which led to misbehavior, or (b) to have symptoms of fatigue which led to misbehavior. CF was reported to affect the mother's response to child disobedience by causing her (a) to be lenient in order to avoid symptom exacerbation and child unhappiness, or (b) to be firm in cases where the child's health was involved.
Several conclusions can be drawn from these findings. First, mothers of children with CF may be particularly vulnerable to depression when their children are preschoolers or early adolescents. This may be due to difficulties adjusting to the illness when the child is young, and to difficulties managing increased demands for independence when the child enters adolescence. Second, although as a group children with CF may have levels of adjustment similar to those of well children, increases in illness severity are likely to be associated with increases in anxiety and depression. Finally, it appears that child discipline presents a dilemma for many mothers of children with CF. Specifically, many mothers try to be lenient in order to avoid causing unpleasantness in the child's life, but fear that their lenience causes the child to be "spoiled," and thus contributes to child behavior problems. The dilemma is exacerbated in interactions involving regimen compliance, when mothers feel they cannot be lenient because the child's health may be compromised if the child is noncompliant. Training in parenting skills may benefit these mothers by teaching them discipline strategies that are neither coercive nor overly lenient.

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Differential Impact of Parental Overprotection In Children with CF and Healthy Controls

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Introduction
Parental overprotection in children with a chronic condition has been long regarded as a risk factor for psychological maladjustment (1). Previous studies (2-4) found evidence of overprotection in children with CF exhibiting significant behavioural problems. The validity of these results, however, is unclear due to, i.e., reliance solely on clinical impression of overprotection, no control comparison groups, and use of small sample sizes. This study (5) re-examined the association of parental overprotection and psychosocial functioning in children with CF while attempting to overcome previous methodological problems.

Method
The sample consisted of 29 mothers, 20 fathers, and 29 patients attending the pediatric CF clinic at the Children's Hospital of Eastern Ontario matched with a randomly selected sample of control subjects obtained from the hospital's medical records. Each group was comprised of 18 males and 11 females ranging from 7-18 years of age (M=11.1, SD=2.7).

The children completed the Self-perception Profile (6) which measures perceived self-concept in the areas of scholastic competence, social acceptance, athletic competence, physical appearance, behavioural conduct, and overall self-concept. The children's behavioural and social functioning were rated by the mothers using the Child Behavior Checklist (7). The Parental Bonding Instrument (8) was used to evaluate parental overprotection (i.e. parental allowance of independence, autonomy versus overprotection, intrusion, and infantilization) and parental care (i.e. affection, emotional warmth, empathy, reciprocity versus coldness and neglect). The validity and reliability of the measures in this study have been previously demonstrated. In addition, anthropometric, pulmonary, and overall physical status scores were collected for children with CF.

Results & Discussion
No significant overall differences between the two groups were found of any of the study measures. There were, however, significant differential and interactive effects between the two groups on the impact of parental care and overprotection depending upon the sex of the parent, the sex and age of the child, and the specific psychosocial measure.

Differences between mother's and father's magnitude of care and overprotection were found within each group. In the CF group, both parents reported similar degrees of care and overprotection, whereas, in the control group, mothers scored higher on care and fathers reported more overprotection. Mothers of female children...
and older children with CF reported significantly more overprotection than any other parent group.

In the CF group but not the control group, the children's self-concept and social competence were highly sensitive to maternal care. Excessive maternal or paternal overprotection was associated with increased behavioural problems for children with CF, whereas, lack of care (i.e. neglect) was associated with increased behavioural problems for healthy children. Finally, a significant relationship was found between increased maternal overprotection and higher physical functioning scores.

Although the sample size may be considered small, the results, nonetheless, suggest that parental overprotection may a risk factor in the behavioral and emotional adjustment of children with CF. The mechanisms that may account for the development and impact of overprotection appear different for parents of healthy children and parents of children with CF. For example, the parental gender differences between the two groups on the magnitude of overprotection may reflect the impact of chronic conditions on instrumental aspects of parental roles.

In conclusion, health caregivers should be sensitive to the effects of overprotection on children. Parents could be supported in their efforts to provide appropriate care but discouraged from being excessively caring or overprotective. Finally, in view of the evidence that demonstrates the importance of overprotection on the behavioural and emotional development of the child, there is a need to better understand the mechanisms of overprotection through further research.

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Issues in clinical psychosocial research

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Purposes

The most important purpose of clinical psychosocial research is to improve patient care. Much like the investigation of the biological aspects of disease, psychosocial research aims to make the lives of patients and their families better. This may include extension of life or improvement of the quality of life. Research may be directly useful in patient care or it may be indirectly useful by expanding our knowledge base in an area.

Psychosocial research can provide guidance as to areas of potential problems in patient care and can improve efficiency or cost-effectiveness of treatment. Psychosocial research should be an integral part of the evaluation of the effects of medical interventions. The quality of life that results from a treatment is at least as important to most patients as is longevity.

Secondary effects of the research process include: questioning or changing long accepted clinical practice; fomenting institutional change. Psychosocial research may also raise the status of psychosocial concerns in an institution. Finally, research can be good for staff morale if caregivers are included in the development and execution of the projects. Research can enhance the richness and diversity of a job, can encourage development of new skills and prevent burnout.

Barriers to psychosocial research

The major barrier to psychosocial research are that there is never enough time and most of the psychosocial caregivers do not have a research background. In addition, there are often no readily available resources which can be used for research.

Some believe that ethical conflicts prohibit many types of research. Although ethical issues must be addressed in clinical research, there is an ethical imperative to do research in order to evaluate what we are doing and to improve care.

Guidelines for starting research

Both qualitative and quantitative studies are needed. Qualitative research involves the in-depth exploration of a single case or at most a few cases, using observations and interviews. Quantitative research, on the other hand, uses numbers to quantify different variables. Although it is most usual for quantitative research to examine groups of subjects, single subject designs are also used. Qualitative research is usually most important if the research problem has not been well researched and needs clarification before a quantitative study can be undertaken.
Study what you know. The most important strategy for the clinician who wishes to do research is to start with what you know best. There are usually important clinical problems that the clinician is dealing with on a day to day basis. These problems should be the foci of clinical psychosocial research.

Form a team or group around your specific project. The team should meet weekly to encourage each other and to set objectives and review progress. Most research fails because of lack of social support, not because of the inability of the investigators. Collaboration can be with more experienced researchers to "learn the ropes" or can be with a group of neophytes. Specific expertise can be brought in on a consultant basis.

Keep it simple. Start with a single narrow research question and try to keep on topic. If you start simple and try to resist the temptation to become all inclusive, you have a much better chance of success.

Programmatic research, in which one examines a specific problem from several different perspectives, is usually most productive. It often takes several studies on the same topic to understand the problem.

Case studies can be a very good place to start a research career. Case studies can be qualitative or quantitative and can be descriptive or analytic.

Be patient. Research requires a great deal of patience and will usually require about two to three times the amount of time that was originally planned.

Statistics are often a frightening quagmire for the uninitiated. In most situations, logic is much more important than the numbers. In addition, most statistical procedures are well within the grasp of most. Two excellent, very simple, introductions to statistics are Norman and Streiner (1986) and Gore and Altman (1982). In addition, Siegel and Castellan (1988) and Ferguson (1966) are particularly helpful standard texts.

Small amounts of funding can be extraordinarily useful for a project. Such funds can be obtained from local sources such as the hospital research fund or from drug companies.

Psychosocial research in cystic fibrosis has not been extensively developed. Excellent research can and should be done by a wide variety of clinicians who have the most contact with patients and their families. Although there are numerous barriers to conducting research in the care setting, there are ways of breaching these barriers.

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Back Pain and Spinal Deformity in Cystic Fibrosis

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Back pain is a common complaint of individuals with cystic fibrosis (CF) and certain musculoskeletal abnormalities associated with CF may contribute to the back pain (1,2). We investigated the prevalence of back pain and postural abnormalities in a group of patients with CF who were seen at Children's Hospital at Stanford (3). We defined back pain as recurring pain occurring in the upper, mid, or lower back of at least one year's duration having a nontraumatic onset. This was evaluated in three ways: (1) a questionnaire filled out by the patient; (2) an orthopaedic examination; (3) measurement of chest roentgenograms.

The questionnaire was completed by 50 subjects with CF and 50 age and sex matched normal subjects. CF subjects were grouped by pulmonary function. Questions addressed the frequency, severity, age of onset and location of the back pain. Examinations were performed on 21 patients with CF and 21 matched normal subjects. The postural evaluation included range of motion (R.O.M.) measurements and manual muscle tests of the shoulders, chest, abdomen and back.

Chest roentgenograms of 92 patients with CF were evaluated for thoracic kyphosis and vertebral wedging. Subjects were again grouped by age, sex, and severity of pulmonary disease. Thoracic kyphosis was measured using the Cobb method from T5-T12. Angles greater than 40 were considered abnormal. Vertebral wedging was measured by subtracting anterior from posterior vertebral body height and expressing this as a percentage of posterior vertebral height.

Forty-seven patients with CF had back pain as compared with seven subjects of the control population. This finding showed a strong correlation (P<.001) between CF and recurring, atraumatic back pain. No difference in frequency occurred with differences in age or sex. Severity of disease and incidence of back pain were related with a significance level of .90. Approximately one-half of the patients reported that the back pain interfered with their ability to cough, and one-third of the patients reported that the back pain interfered with their ability to breathe.

The postural examination revealed that all R.O.M. measurements of patients with CF averaged less than those of controls. There were three motions in which at least 45% of the patients with CF fell below the range of control measurements: shoulder retraction; trunk extension; and chest mobility. Six of the 21 patients examined had one or more muscle strength scores of 4/5 (normal, 5/5). No subjects scored lower than 4/5.

The prevalence of kyphosis was 8.7% in 92 patients with CF and was not significantly different from that in the general population (10%). Subjects 21 years and older tended to have more kyphosis than did younger subjects (r=.90). There was a high correlation between severity of disease and
increased degree of kyphosis ($r=0.95$). Previous roentgenographic studies support these findings (4,5).

Vertebral wedging in CF (68 patients, 73.9%) was higher than in the general population (0%). Correlation was high between wedging and severity of disease ($r=0.975$). Vertebral wedging correlated highly with kyphosis ($r=0.995$). Kyphosis occurred only in subjects with greater than 15% wedging and the frequency of kyphosis increased with increased wedging. Vertebral wedging and back pain seemed to be related in the 50 subjects who completed the survey. All subjects with vertebral wedging greater than 10% had back pain. Two of the three subjects without back pain did not have wedging and the third subject had 8% wedging. Two possible explanations for the vertebral wedging found in C.F. include accumulative compression fractures from osteopenia and abnormal growth of vertebral end plates. Osteopenia is well documented in C.F. probably due to malabsorption and chronic vitamin D deficiency (2,7,8,9,10).

In summary, Ninety-four percent of persons with C.F. had back pain that interfered with their ability to perform daily activities for pulmonary hygiene and for work. This pain was found to be associated with soft tissue contractures, postural changes and vertebral wedging. In the absence of kyphosis these soft tissue changes may be prevented, delayed or even reversed with appropriate exercise and postural counseling (11).

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Role of the Pectoral Girdle Muscles in Respiration: Potential implications for rehabilitation.  
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The muscles of the pectoral girdle arise in complex fashion from the upper portion of the rib cage and insert variously on the scapula, clavicle, humerus and thoracic spine. It seems likely that these muscles may apply considerable forces to the upper rib cage which may affect intrathoracic pressure, the shape of the rib cage, and even the mechanical performance of the conventional chest wall respiratory muscles. However, little is known about the role played in respiration by the extensive and complex array of skeletal muscles of the pectoral girdle (1). Recently, studies by ourselves (2-4) and others suggest (5-6) that arm posture and/or contractions of the pectoral girdle muscles alter lung volume, rib cage dimensions, diaphragm and abdominal expiratory muscle activity, and overall ventilatory capacity. For example, Celli et al (2,3) observed that vertical movement of the arms in normal subjects and in COPD patients is associated with increases in transdiaphragmatic pressure and more positive end expiratory gastric pressure at a given tidal volume, suggesting that diaphragm activation is enhanced and that the abdominal expiratory muscles are more active with the arms elevated. Furthermore, Criner et al (4) have found that arm elevation enhances maximum static inspiratory mouth and esophageal pressures at any given lung volume in normal subjects. Augmentation of maximum static inspiratory pressure was associated with enhanced outward displacement of the rib cage but no change in the diaphragm electrical activity. Although increases in maximum static pressure with arm elevation has several possible explanations, at least one possibility is that arm elevation enhances the mechanical advantage of the pectoral girdle muscles which have an inspiratory action on the rib cage. Finally, DeTroyer and Estenne (7) have observed that voluntary activation of the pectoral major muscles in patients with C5-C7 spinal cord injuries (in whom diaphragm and intercostal activation is not possible) is associated with a significant reduction in expiratory lung volume. These authors concluded that the pectoral muscles may exert an important expiratory action on the upper rib cage.

Although the above studies provide important evidence for the respiratory action of the pectoral girdle muscles in man, they are extremely indirect. In man, the need to be non-invasive limits the assessment of important variables which define pectoral girdle muscle function on the rib cage (e.g., muscle fiber length, fiber orientation with the rib cage, etc). Hence, we used a canine model to study the mechanical action of the pectoral muscles (which are anatomically arranged similar to man) in greater detail. Preliminary data indicates that the canine muscles of the pectoral girdle (at least the deep pectoral) may indeed have an important respiratory action, however, this mechanical action is complex and depends upon lung volume and the posture of the upper extremities (8). With the upper extremities elevated, isolated contraction of the deep pectoral muscle results in inspiratory changes in lung volume and intrathoracic pressure. With the upper extremities alongside the torso, contraction of the muscle results in expiratory changes in lung volume and intrathoracic pressure. Differences in the quality and magnitude of changes in intrathoracic pressure and lung volume in the different upper extremity postures appears to be due to a realignment of the pectoral muscles with the rib cage and changes in their muscle fiber length, respectively (8).

Although the above data demonstrates that the canine pectoral muscles can have a respiratory action on the rib cage, there is limited and contradictory information regarding the
Electromyographic activity of the pectoral muscles during breathing in man (9,10). Unfortunately, most of these studies were performed using surface recordings of muscle electrical activity which are limited by a variety of technical problems. Recently, we have examined the EMG of the pectoralis major muscle during eupneic breathing in 3 COPD patients with chronic respiratory failure while weaning from mechanical ventilation (11). Pectoralis major EMG activity was measured using fine wire, needle electrodes. In all 3 subjects, we found that electrical activation of the pectoralis muscle occurred spontaneously with the onset of inspiratory flow. In addition, we have measured pectoralis major EMG activity in 5 normal subjects during voluntary inspiration from FRC to TLC. As normal subjects approached 60% of their TLC, inspiratory activity of the pectoralis major became evident.

These data appear to support the notion that the pectoral girdle muscles are important agonists of the primary inspiratory and expiratory muscles when ventilatory demand is high, the mechanics of breathing are deranged, or when the primary muscles of inspiratory are placed at mechanical disadvantage by severe hyperinflation. Rehabilitative strategies that incorporate selective strength training of the pectoral girdle muscles and positioning of the arms during the activities of daily living may be beneficial in selected pulmonary patients.

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S6.3 CONCEPTS IN VENTILATORY MUSCLE TRAINING (VMT)

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The respiratory muscles, like other skeletal muscles can be trained for improved performance. The first report of specific training of the respiratory muscles for improved strength and endurance appeared in 1976. Leith and Bradley were the first to carefully demonstrate that respiratory muscle function could be improved by specific training (1). Their study was done in normal humans but paved the way for a multitude of studies which have followed in the past decade and-a-half. VMT has now been used in patients with neuromuscular disease, especially quadriplegia and chronic obstructive pulmonary diseases such as chronic bronchitis, emphysema and cystic fibrosis (2). The response to endurance training depends on several factors including intensity, duration and frequency of exercise. Final recommendations for the appropriate strategies to be used in ventilatory muscle training cannot be made as VMT is still in its infancy.

In patients with obstructive airways disease, peak exercise levels are reached at submaximal cardiac frequencies, but at ventilatory levels that are close to, or even exceed the maximal voluntary ventilation (MVV). Because of the major role of ventilatory limitation to exercise in these patients, there is strong support for the view that training directed specifically at the ventilatory muscles will improve not only ventilatory muscle function, but overall exercise capacity. This rationale has been the basis for the many studies that have been performed (2).

METHODS OF TRAINING
Three main methods of ventilatory muscle training have emerged. These are 1) resistive training; 2) hyperpneic training; and 3) threshold load training.

RESISTIVE METHOD
This utilizes inspiratory devices with small orifices through which patients breathe. During this form of training, expiration is unimpeded.

HYPERPNEIC METHOD
In this method, the patient performs voluntary hyperpnea under isocapnic conditions. Sustained ventilatory levels during this maneuver are usually in the order of 70-90% of the MVV with a breathing frequency of 40-60 breaths/minute.
**THRESHOLD LOAD METHOD**

In this method, a threshold valve is employed which opens only when a predetermined threshold inspiratory pressure is attained. Inspiration can only proceed as long as this threshold pressure is exceeded. The utility of the threshold load device in contrast to the resistive device is that the mouth pressure during inspiration remains constant and is independent of inspiratory flow rate.

**CLINICAL STUDIES OF VENTILATORY MUSCLE TRAINING**

**HYPERPNEIC TRAINING**

This method originally employed by Leith and Bradley in 1976. Subsequently, Keens et al. (3) and Belman et al. (4) studied this method in cystic fibrosis and COPD patients respectively. In both studies, ventilatory muscle function as measured by the maximal sustained ventilatory capacity was improved. Subsequently, a study by Levine and co-workers (5) compared hyperpneic training to a sham treatment and showed that although ventilatory muscle function was improved, there was no carry-over benefit in terms of treadmill or bicycle exercise, or improvement in symptoms or quality of life.

**RESISTIVE TRAINING**

Because of the simplicity of this technique, the vast majority of studies of VMT have been done using this device. Because a linear resistances are used, the mouth pressures and consequently the inspiratory load is very dependent on inspiratory flow rates (6). It became apparent after several years using these devices that even though smaller orifices were employed, the inspiratory load was not necessarily increased. In some cases, patients learned to minimize the inspiratory load by breathing more slowly and reducing inspiratory flow rates. This has reduced the value of many of the early studies in which breathing strategy was not controlled as it is not possible quantify the training stimulus. In cases where improved endurance was noted (that is, improved ability to breathe through a smaller orifice) it is not clear whether this was due to a real improvement in inspiratory muscle function, or due to a learned, more efficient breathing strategy. More recently, this point has been taken into consideration and several studies has shown improved respiratory muscle strength and endurance for loaded breathing after ventilatory muscle training (7,8,9). In two studies (8,9) the improved respiratory muscle performance was also associated with a reduction in dyspnea. Symptoms of dyspnea as recorded by a Borg scale during loaded breathing were also reduced after six weeks of ventilatory muscle training (10).

**THRESHOLD LOAD TRAINING**

Because of the difficulty alluded to above with the effect of inspiratory flow rate on inspiratory load, the threshold device was developed. Because the valve opens only at the predetermined threshold pressure, the load on the ventilatory muscles is
determined by the threshold pressure and is independent of inspiratory flow rate. Several studies utilizing this device have now been performed. While ventilatory muscle function can be improved, the two main studies did not agree on the effect of threshold load training on exercise performance and symptoms. One study found a favorable benefit, whereas the other did not. (11,12).

**VMT AND CYSTIC FIBROSIS**

Almost ten years has elapsed since the last of only two studies which examined VMT in cystic fibrosis patients. The first study published in 1977 (3) showed a greater than 50% increase in MSVC in four CF patients. Of note, was the fact that intensive whole body exercise produced a comparable improvement in MSVC. This latter effect has not been found in adult COPD patients. Whereas this first study used isocapnic hyperpnea, the second study employed an inspiratory resistance as the training device (13). Although increased strength and endurance of the ventilatory muscles were claimed in this study, the breathing strategy during testing was unmonitored (see above) and therefore, the improvements could not be documented adequately. No increase in exercise capacity was found.

Although there is much conflicting data in the literature, it appears that when one considers the studies that were well controlled using randomization of patients and employing control of the breathing strategy, that several benefits can be achieved. These include 1) improved respiratory muscle strength; 2) improved respiratory muscle endurance for loaded breathing; and 3) very probably an improvement in symptoms of dyspnea. The studies which have used controlled resistive breathing (7,8) have achieved the best results. Although hyperpneic training improves muscle endurance, no improvement in symptoms resulted. It is possible therefore, that the nature of the training load is important in providing both improved endurance and reduction in symptoms. Conceivably, a combined training load which provides both an endurance and strength stimulus is the best type of training to use. Studies are currently underway to evaluate this hypothesis. Until completion of this, it is difficult to give firm recommendations with respect to ventilatory muscle training. In the interim, it would seem however, that VMT with a resistive or high threshold load (30-50% of maximal inspiratory pressure) will improve ventilatory muscle function and may reduce symptoms in patients with chronic airflow limitation.
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INTERMITTENT NASAL VENTILATION IN CYSTIC FIBROSIS

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For the first four decades of the care of patients with cystic fibrosis, caregivers could only watch many of their young charges develop respiratory failure and die with acute respiratory insults. Most CF patients seemed unable to tolerate mechanical ventilation. Endotracheal intubation removed the ability of the patient to cough and to clear the airway of purulent material. Even those patients fortunate enough to be successfully extubated usually had a marked deterioration in their lung function.

The past decade produced two significant contributions to the ability to assist ventilation in CF individuals. The first of these was the finding that patients could actually sleep with a tightly fitted continuous positive airway pressure (CPAP) mask. The second was a growing appreciation for the role of airway collapse during expiration and the subsequent benefit of PEEP in airway toilet. As mechanical ventilators became more sophisticated, the ability to supplement respiration in an exhausted patient became possible. There now is an extensive literature on the success of intermittent nasal ventilation in patients with a wide spectrum of neuromuscular diseases and partial respiratory insufficiency. The industry has developed a number of machines which can provide oxygen, CPAP, and intermittent ventilation upon demand. The BiPaP device marketed by Respironics, Marionville, PA, is one example of these.

A number of successes with this modality have been reported in CF patients. Hodson, et al, used nearly continuous nasal ventilation in five patients for 3 - 36 days prior to transplantation. Although there was little change in the carbon dioxide, it was much easier to oxygenate the patient. More importantly, the patient could remain on the general CF ward and did not need the expense and inconvenience of the intensive care ward. Piper, et al, reported similar results in two CF patients from Australia who used nocturnal ventilation. Both of these had improvement in carbon dioxide tension. The CF studies have been relatively short-term. Benhamou, et al, reported long-term success with intermittent nocturnal ventilation in 8 patients with severe diffuse bronchiectasis and chronic respiratory failure.

There are a number of principles that are important in selecting patients and devices for successful intermittent mask ventilation. The ability to provide PEEP aides considerably in mucus clearance, since the patient still can cough or "huff." This mode of support is intended only to complement exhausted respiratory muscles, not inflate a pathologically stiffened lung. It is most successful if it can be used only during sleep or for portions of the day. Pressures of more than 20 cm of H2O and rates greater that 20/minute will usually result in the swallowing of air and uncomfortable abdominal distension. The ventilation is supposed to be delivered through the nose. A gasping patient also is more likely to swallow air. Some authors recommend a nasogastric tube to prevent distension. Higher pressures also will cause mask leaks and ineffective nasal ventilation.

Finally, it should be reserved for patients who genuinely can benefit from this mode of respiratory support. Most patients should be able to discontinue ventilation for short periods of time in order to eat, bathe, or talk to friends. It should not be used for the end-stage and extremely acidotic patient only to prolong life for a few days. The one exception...
to this is for patients who have been accepted for lung transplantation and who may avoid tracheal damage until a graft becomes available.

REQUIREMENTS OF SUCCESSFUL NASAL VENTILATION IN CF

1. A machine which can provide positive end-expiratory pressure and oxygen.
2. A pressure requirement of no more than 20 cm H2O.
3. A rate requirement of no more than 15-18/minute.
4. A patient who can tolerate a tight mask fit.
5. A patient who genuinely can benefit from and who has a realistic end-point for intermittent respiratory support.

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Salt Absorption and Secretion: Parts and Processes

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Models for Electrogenic Salt Transport

The absorption and secretion of salt by epithelial cell layers, such as those characteristic of the airways, sweat glands, distal renal tubule and the colon, are effected by electrogenic transport mechanisms which can be viewed as having two parallel limbs. The active limb is a cellular transport path which promotes either Na absorption or Cl secretion and, thereby, generates a lumen negative transepithelial potential. Equally important, however, is a transmural conductive leak path which promotes passive flow of the counter ion in response to the transmural voltage. The active path provides the free energy to drive salt flow while the role of the leak path is permissive, but both are essential to the process. The efficiency of salt transport will be strictly limited by the degree to which counter ion flow can "keep up" with active transport.

Models for electrogenic salt absorptive and secretory mechanisms can be resolved into "parts" and "processes". The parts are specific cellular proteins, particularly those which, by way of their particular distribution in the apical and basolateral membranes of polar cells, are the basic for vectorial ion transport. The processes are the dynamic cellular events which act to effect highly coordinated regulation of apical and basolateral ion flows in such a way as to permit wide swings in transcellular ion flow ("throughput") and avoid potentially lethal changes in cytosolic composition (1). Considerable progress has been made in the identification of some of the parts involved in absorptive and secretory salt transport, but our understanding of the regulatory processes remains rudimentary.

Parts: Functional versus Molecular Definitions

The recent explosion in the application of molecular cloning to transport proteins forces us to confront the fact that the molecular parts involved in any transport process can be defined in two ways: by their molecular structure or by their function. One of the challenges facing students of epithelial transport is that of matching up specific membrane proteins with specific membrane transport modalities. On the basis of functional criteria, epithelial cells appear to contain multiple subtypes of several important transport proteins, e.g. Cl channels or K channels, which may play distinctly different roles in the life of the cell. Unfortunately, however, Nature has not provided us with an equivalent multiplicity of specific inhibitory ligands (analogous to the toxins which act on voltage-dependent channels in excitable cells) which can be used to implicate individual transporters in an integrated setting. Resolving this dilemma will require, not only the application of molecular cloning techniques to a variety of transporters, but also detailed, quantitative characterization of transport properties so that a functional fingerprint can be applied to the transport process in the cell of interest and to the cloned protein expressed in another system.
Epithelial Cells Work Better in Groups

A particularly vexing property of epithelial cells is that they do not necessarily retain their differentiated transport properties if they are isolated from one another (2). This behavior compels us to develop better assays for apical, basolateral and cytosolic events in the transporting cell layer (3). One approach to this problem is the use of cell layers which have been apically permeabilized by amphotericin or digitonin so as to permit some degree of access to the cell interior while nevertheless preserving the functional integrity of the cell layer. Such preparations may permit the reconstitution of differentiated function under conditions of controlled cytosolic composition. Equally important, however, is the application of relatively noninvasive probes or "reporters" which can be used to monitor cellular composition or membrane properties during regulatory alterations in transcellular transport.

Basolateral Na/H Antiporter: A Part of a Transport Regulatory System?

The sensitivity of ion channels and the Na/K ATPase to cytosolic pH has led to the suggestion that intracellular protons could be a mediator of the coordinate regulation of apical and basolateral ion transport properties, and that basolateral Na/H exchange could be a pivotal element in this process (4). A human form of the Na/H antiporter has been cloned (5), and we used Northern Blot Analysis to identify homologous transcripts in mRNA from turtle colon, a model for electrogenic salt absorption which contains pH-sensitive ion channels. Na/H and Na/Na exchange activity was identified in the basolateral membrane of cell layers apically permeabilized by exposure to amphotericin B. The expression of Na/Na or Na/H antiport was activated by cell shrinking and exhibited some unexpected biophysical properties. Under conditions of elevated cytosolic Na the exchange cycle appeared to be associated with a Na-selective conductance. This may be an example of a functional tag which will be important for identifying the gene product in another expression system.

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Cystic fibrosis (CF) is characterized by an abnormally low Cl⁻ conductance (GCl⁻) in exocrine glands (2), but the specific secretory cell type(s) expressing this GCl⁻ is unknown. The identification and functional characterization of the cell type affected by CF is essential for understanding pathophysiology and for therapeutic intervention in CF. For example, knowledge of the defective cell type, along with its physiological role, will be required for cell targeted gene therapy. The sweat gland secretory coil (SC) is a relatively uncomplicated model for characterization of different cell types in an exocrine gland. That is, the SC is comprised of three histologically distinct cell types (myoepithelial, light and dark cells) with little connective tissue. Herein, we have described some of the properties of three distinct cell types, identified at least one of the defective cell types in CF, and discussed the physiological effects of β-adrenergic stimulation and the basis of normal cholinergic and abnormal β-adrenergic sweating in CF.

Electrophysiological identification of cells in the SC: The superficial myoepithelial cells can be identified on the basis of: a) relatively higher membrane potentials (V_m) (V_m = -67 ± 4 mV, n=21, mean ± SE, n= number of cells); b) presence of spontaneous depolarizing spike potentials (SDS), and c) a characteristic depolarization of V_m to cholinergic stimulation by mecholyl (ΔV_m = 28 ± 3 mV, n=21). The inner secretory epithelial cells are characterized by lack of SDS and a characteristic hyperpolarizing response to mecholyl. These cells can be subdivided into two physiologically distinct cell types which probably parallel the histological light and dark cells. The cells insensitive to β-adrenergic stimulation (β-I cells) with low V_m (-24.2 ± 2 mV, n=17) and the cells responsive to β-adrenergic stimulation (β-S cells) with intermediate V_m (V_m = -57 ± 3 mV, n=13). β-adrenergic agonist isoproterenol (IPR) depolarized the V_m of β-S cells by 17 ± 3 mV (n=9).

β-S cell is abnormal in CF: We have characterized the properties of SC cells as grown in primary culture to identify the defective cell type in CF and to define stimulus response coupling. Both β-S and β-I cells were identified in these cultures. In contrast to normal cells, the β-S cells in CF are insensitive to β-adrenergic stimulation, even though the cholinergic response of both β-S and β-I cells are apparently normal. To determine the physiological basis of apparently normal cholinergic but abnormal β-adrenergic responses of CF cells, we studied the ionic mechanisms underlying the β-adrenergic and cholinergic stimulations in β-S and β-I cells from normal and CF cells.

Mecholyl primarily stimulates potassium conductance (G_K⁺): Our evidence suggests that the mecholyl response of β-S and β-I cells is associated with the activation of G_K⁺. Both the resting and cholinergically activated G_K⁺ in β-I cells are inhibited by Ba²⁺ indicating that mecholyl activates G_K⁺ in β-I cells. In contrast to the β-I cells the cholinergic response of β-S cells is insensitive to Ba²⁺. However, the following evidence indicate that mecholyl mainly activates G_K⁺ in these cells also. Cholinergic response in β-S cells is dependent on the driving force for K⁺ and media Ca²⁺ (suggesting that mecholyl activates Ca²⁺ dependent G_K⁺) and is independent of media Cl⁻ (apparent E_Ach = -86 ± 2 mV, with Cl⁻, n=4 and E_Ach = -83 ± 1 mV without Cl⁻, n=4).

β-S cell is the site of expression of CF affected G_Cl⁻: The CF affected G_Cl⁻ is sensitive to cAMP (2). Further β-adrenergic stimulation of β-S cells also involves mainly the activation of cAMP-dependent G_Cl⁻ for the following reasons. The β-adrenergic
response which is mediated by cAMP is Cl⁻ dependent (removal of Cl⁻ from the medium completely abolished the depolarizing response of these cells). The depolarization of V_m induced by either IPR or cAMP is consistent with the activation of G_{Cl⁻} (2,3). These results combined with the lack of β-adrenergic sensitivity in β-S cells show that the site of expression of abnormal G_{Cl⁻} in CF is the β-S cell.

What is the role of β-adrenergic receptors in exocrine secretion? Since the β-adrenergic abnormality is associated with abnormal secretion in CF, it seems essential to understand the role of β-adrenergic receptors in the secretory process. The following results indicate that cholinergic (not β-adrenergic) stimulation is the central mechanism for fluid secretion in SC. Transepithelial studies indicated that β-adrenergic stimulation does not affect transepithelial potential (V_t) or conductance (G_t) (if anything G_t is decreased) and stimulates G_{Cl⁻} but not G_{K⁺}. In contrast, cholinergic stimulation increased V_t (up to -12 mV), G_t and G_{K⁺}. The electrophysiological events following β-adrenergic stimulation do not seem to fully compliment existing models for electrolyte fluid secretion. The principle components of the general model of stimulus response mechanism include an increase of: a) V_t as a result of increased G_t which provides driving forces for transepithelial cation and water movement, b) basolateral G_{K⁺}, and c) apical G_{Cl⁻}. However, except for the activation of G_{Cl⁻}, β-adrenergic stimulation in SC does not seem to involve several principle components in the secretory model. These findings lead us to several questions regarding the role of β-adrenergic receptors in secretion. If the activation of both β-adrenergic as well as cholinergic receptors induces electrolyte fluid secretion, what is the physiological significance of two distinct secretory mechanisms performing a single function of sweat secretion? What is the exact role of β-adrenergic receptors in SC in particular and in the pathogenesis of CF in general?

Conclusions

Three electrophysiologically distinct cell types can be identified in SC (myoepithelial, β-l, and β-S). The β-S cell is the site of expression of abnormal G_{Cl⁻} in CF.

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Pathogenesis of Cystic Fibrosis in NaCl and Volume (water) Absorbing Epithelia.

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Mutations in the cystic fibrosis transmembrane regulator (CFTR) affect electrolyte transport in many epithelia of the body. The phenotypic dysfunction generated by CFTR mutations reflects in part the native function of the epithelium. In structures like the sweat acinus and pancreatic duct, there are abnormalities in regulation of salt and water secretion. In other epithelia, there are problems of absorption. In the sweat duct the problem is the failure to absorb NaCl but not volume. In the proximal airways and the small intestine, there are abnormalities in both salt and volume absorption. The concept that hyperabsorption may be a phenotypic consequence of CFTR dysfunction in specific epithelia will be explored.

Airway epithelia exhibit both defects in Na\(^+\) absorption and Cl\(^-\) secretion. The increase in Na\(^+\) absorption appears to reflect abnormal regulation of the apical membrane Na\(^+\) conductance and does not appear to be a simple consequence of the Cl\(^-\) impermeability of the apical membrane. Measurement of Na\(^+\) fluxes indicates that excessive Na\(^+\) absorption is detected in the physiologic (open circuit) mode. Paradoxically, the absorption of Cl\(^-\) is also increased in CF airway epithelia compared to normal controls. Both the interrelations between the CFTR protein and the control of the Na\(^+\) absorptive path and the routes for Cl\(^-\) hyperabsorption across an epithelium with an impermeable cellular Cl\(^-\) conductance will be discussed.

The gut also appear to exhibit complex interrelationships between Na\(^+\) and Cl\(^-\) transport and CFTR dysfunction. Like the airways, the gut exhibits abnormal regulation of Cl\(^-\) secretion in response to Cl\(^-\) secretagogus. However, electrogenic Na\(^+\) nutrient transport appears to be raised in CF gut. Like in airway tissues, attempts to (1) bridge CFTR dysfunction to Na\(^+\) coupled solute transport across the apical membrane and (2) to integrate Cl\(^-\) flow into the pathophysiology the GI system will be attempted.

In summary, the phenotype of CFTR mutations is complex. In epithelia where Cl\(^-\) transport is dominant, it is relatively easy to assign a phenotypic role for CFTR functions and dysfunction. In tissues with complex physiologic functions with large apical membrane permeabilities for ions other than Cl\(^-\), interactions between CFTR dysfunction and pathophysiology become more complex. Because epithelia of this type, e.g., airway and intestinal epithelium, are key target tissues, integration of CFTR function/dysfunction, Cl\(^-\) channel regulation, and interrelationships to other cellular transport functions must be established.

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**Regulation of Chloride Conductance in Endocytic Vesicles and Cells Measured by Fluorescent Indicators**

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Several new chloride sensitive fluorescent indicators have been developed and applied to the measurement of chloride transport in endocytic vesicles and cultured cells. For cell staining, a concern with the use of SPQ has been the requirement of an invasive loading procedure such as hypotonic shock or microinjection (1,2). To overcome the problem of low SPQ membrane permeability, the lipophilic compound 6-methoxy-N-ethyl-1,2-dihydroquinoline (DiH-MEQ) was synthesized (3). DiH-MEQ is membrane permeable and readily oxidized in cell cytosol to the membrane impermeable and chloride sensitive indicator 6-methoxy-N-ethyl-quinolinium chloride (MEQ). In cell cytosol, MEQ has similar properties to those of SPQ. MEQ remains entrapped in cell cytosol and has been used to monitor intracellular chloride activity continuously in response to ion substitution and hormone addition.

For labeling of endocytic vesicles in intact cells, a dual-wavelength chloride sensitive dextran indicator has been synthesized (4). CNBr activated dextran was conjugated with a chloride sensitive chromophore, 6-methoxy-N-(3-aminopropyl) quinolinium, and a chloride insensitive chromophore, dansylethylenediamine. When excited at 365 nm, the emission spectrum of this indicator showed two peaks, one at 460 nm, corresponding to the chloride sensitive moiety, and a second at 550 nm, corresponding to the dansyl moiety. This indicator was used as a fluid-phase marker to label endocytic vesicles in intact cells and to measure chloride activity.

The chloride transport properties of endocytic vesicles from kidney proximal tubule were examined (5,6). Endosomes were labeled *in vivo* by intravenous infusion of SPQ into rabbits. SPQ was filtered by the glomerulus and entered the tubule lumen as a fluid-phase marker of endocytosis. A microsomal fraction, containing the labeled endocytic vesicles, was prepared by homogenization and differential centrifugation of dissected renal cortex. Chloride transport was measured by a stopped flow fluorescence technique with time resolution of 1 ms. It was found that vesicles contained a conductive chloride transporter, but no ion coupled chloride transport system. Chloride conductance was increased by phosphorylation with protein kinase A catalytic subunit (PKA) and ATP. The increase was blocked by the kinase inhibitor H-8, by removal of magnesium, or by dephosphorylation by alkaline phosphatase. The anion selectivity sequence of the chloride conductance was Cl > Br > I. Parallel phosphorylation studies using 32P-γ-ATP showed kinase independent phosphorylation bands at 13, 32 and 45 kDa, and a PKA-dependent band at 65-70 kDa. The phosphorylation at 65-70 kDa was prevented by addition of H-8 or excess unlabeled ATP, or by alkaline phosphatase. The increase in endosome chloride conductance paralleled the increase in phosphorylation of the 65-70 kDa band.

A dual-perfusion chamber was constructed to study apical and basolateral membrane chloride transport mechanisms in cultured polarized epithelial cells (7). Chloride conductance activation mechanisms were studied in primary cultures of dog tracheal epithelium and T84 cells. In tracheal epithelium, it was found that the primary mechanism of activation of chloride conductance was cAMP, with weaker activation by protein kinase C. In the presence of indomethacin to block prostaglandin synthesis, there was no activation of chloride transport by A23187 or the calcium agonist bradykinin. A mathematical model was developed to describe quantitatively the epithelial
transport experiments (8). In T84 cells, the primary activation mechanism was also cAMP, with no effect of protein kinase C (9). In addition, the compounds adenosine, AMP, ADP and ATP caused significant increase in chloride flux. The increase was blocked by 8-phenylthiophylline, and required magnesium but not hydrolysis. There was no effect of other purine or pyrimidine nucleosides on chloride conductance. A single cell microinjection technique was developed to coinject chloride sensitive dextran indicators with impermeant regulators of chloride channel activity.

Our results in endocytic vesicles demonstrate that protein kinase A regulates chloride channel activity and that the chloride conductance may modulate the kinetics of endosomal acidification. The new dextran indicators should provide a powerful approach to examine the hypothesis that endosomal acidification and chloride transport is defective in cystic fibrosis. The new cell permeable indicator of cytosolic chloride should have applications in the study of chloride transport regulation in normal and cystic fibrosis cells.

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S8.1 HEART-LUNG TRANSPLANTATION FOR CYSTIC FIBROSIS

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Patients Selection and Methods

Between September 1984 and March 1991, 79 patients with cystic fibrosis underwent heart and lung transplantation at Harefield Hospital. There were 39 males and 40 females with mean age of 23 (range 8 - 43 years). Three patients received combined heart-lung and liver transplantation. The hearts from 58 of these patients have been used for domino heart transplants. The indication for operation were severe respiratory failure with marked hypoxemia and oxygen dependence. At the time of acceptance the mean FEV1 was 22% predicted and the mean FVC was 35% predicted. All patients were judged to have a poor life expectancy and poor quality of life. Previous thoracotomy was not regarded as a contraindication to transplantation. Seven patients had previous pleurodesis and one had pleurectomy. One patient had undergone a previous double lung transplant procedure and required retransplantation because of ischaemic necrosis of the donor's trachea and main bronchi. Twelve patients who had preoperative ventilation were included as well as a patient who had received extra corporeal membrane oxygenation (ECMO) for two days before transplantation. Patients were excluded if they had active aspergillus or mycobacterial infection.

The general criteria for donor selection and organ procurement are described elsewhere (1). The Surgical methods used for heart-lung transplantation were based on those originally described (2) including those that allow for the performance of domino heart transplantation (1,3).

Results

Actuarial survival for the whole group was 69% at one year and 54% at two years. There were 30 early and late deaths due to infection (10), multiorgan failure (8), haemorrhage (6), cerebrovascular accident (2), obliterative bronchiolitis (2), anastomotic dihescence (1), hyperacute rejection (1).

Sixteen patients developed obliterative bronchiolitis. Two of them died after retransplantation and three are currently awaiting retransplantation. Two patients have successfully been retransplanted (one with a single lung) and both are very well at 27 and 9 months after surgery.

Improvement in post-operative lung function in the "well" patients was maintained with mean FEV1 of 75% of predicted at one year.
Conclusion

The initial results of combined heart-lung transplantation for treatment of CF is encouraging (4). The cost of treatment has increased because of high doses of cyclosporin required due to malabsorption and the need for repeated courses of antibiotic therapy in the initial period after transplantation (4,5).

An increased incidence of post-operative repeated infections (2.2 per patient) has been observed (4) and obliterative bronchiolitis remains a problem.

Cystic fibrosis patients provide a source of hearts which are potentially suitable for domino heart transplantation (5) which helps the overall shortage of donor organs (4).

Bilateral single lung transplantation for CF is an option for treatment but intermediate and long-term results of this procedure have not been defined and there is concern about bronchial anastomosis healing in cystic fibrosis patients when there is a high incidence of recurrent pulmonary infection.

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Outcome after Liver Transplantation for Cystic Fibrosis

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Introduction

Improvements in pulmonary care and other refinements in management allow more patients with cystic fibrosis (CF) to survive long enough for liver disease to become a limiting factor in their life expectancy (1, 2, 3). Since liver transplantation is considered the treatment of choice for patients with end-stage liver disease, it is predictable that more patients with CF and liver failure will be referred for transplantation. The results after liver transplantation in 14 patients with CF are reviewed in this report.

Clinical Material

Between January, 1981 through May, 1990, 3,019 liver transplantations were performed at the University of Pittsburgh. Eighteen of these transplants were performed in 14 patients (6 adults and 8 children) with CF who had developed end stage liver disease. The first nine patients, previously reported in 1989 (3), were done under cyclosporine, prednisone and azathioprine immunosupression. The last 5 patients were done with the new immunosuppressive drug FK506 and low dose prednisone. The patient demographics, including pre- and postoperative pulmonary function tests and current patient status, are summarized in table 1 below. One patient, who received a a double organ transplant (liver and pancreas), is not included in the present series.

Table 1. Patient Demographics, Pulmonary Function, and Current Status

| Patient | Age/Sex | FVC/FEV₁ | Preoperative | Postoperative | Current Status                  |
|---------|---------|----------|--------------|---------------|---------------------------------|
| 1       | 23y/M   | 72/81    | 109/119      |               | Alive at 7 1/2 yr, working full time |
| 2       | 30y/F   | 62/50    | 59/43        |               | Died at 27 months                |
| 3       | 24y/M   | 105/114  | 110/105      |               | Alive at 65 months, working full time |
| 4       | 15m/F   |          |              |               | Alive at 60 months, attending school |
| 5       | 3y/M    |          |              |               | Alive at 43 months, attending school |
| 6       | 21y/M   | 77/74    |              |               | Died at 2 weeks                  |
| 7       | 8y/M    | 50/38    | 109/106      |               | Alive at 36 months, attending school |
| 8       | 13y/F   | 81/68    | 114/101      |               | Alive at 31 months, attending school |
| 9       | 18y/F   | 61/50    | 81/70        |               | Alive at 31 months, attending college |
| 10      | 31y/F   | 77/66    |              |               | Died at 5 1/2 months             |
| 11      | 6y/M    | 114/94   |              |               | Died at 2 weeks                  |
| 12      | 14y/M   | 86/70    | 94/83        |               | Alive at 10 months, attending school |
| 13      | 12y/M   | 90/71    |              |               | Alive at 10 months, attending school |
| 14      | 13y/M   | 53/51    | 58/45        |               | Alive at 2 months, recuperating  |

Results

Ten of the 14 recipients are well with normal liver function tests 2 months to 7.5 years after liver transplantation. Two patients died in the early postoperative period of sepsis and multiple organ failure following retransplantation, one with hepatic artery thrombosis (patient #6) and the other (patient #11) with severe allograft rejection. A third gravely ill patient (patient #10) with advanced liver disease and severe exocrine and endocrine pancreatic
insufficiency, died with a perfectly functioning graft 6 months after transplantation. The patient, who required a femoral embolectomy on the second postoperative day, remained ventilatory dependent thereafter and died of respiratory failure. A fourth recipient (patient #2) died 27 months after transplantation from an unrecognized closed-loop obstruction and gangrenous bowel. This patient also had Friederich's ataxia and mental retardation.

Actuarial patient survival (Kaplan-Meier) for the 14 patients in this series is shown in the figure to the left and is 77.9% at one year and 68.2% at 5 years. Pulmonary function tests were done in eight patients before and after transplantation (see table 1). Forced vital capacity (FVC) and first second forced expiratory volume (FEV1) were improved in patients 1, 7, 8, 9, and 12, and essentially unchanged in patients 2, 3, and 14.

Discussion

The long-term patient survival rate of nearly 70% in this series of patients demonstrates that results for patients with cystic fibrosis are comparable to those reported for other established indications for liver transplantation (4). Nevertheless, due to the multisystemic nature of CF, these patients are more likely to develop complications in the early postoperative period. Therefore, in order to improve results in this group of patients, the following points should be stressed: 1) the need for meticulous pulmonary toilet in every patient, and for early tracheostomy in the patients who require reintubation or reoperation; 2) aggressive bowel care and early introduction of parenteral nutrition; and 3) when using cyclosporine, which is poorly absorbed in this group of patients, close monitoring of cyclosporine blood level and dosage is recommended. Our experience with liver transplantation for CF using FK 506 is still small, but encouraging.

Mild to moderate pulmonary disease is not a contraindication to liver transplantation in patients with CF. In fact, some improvement in pulmonary function was noted in 5 of the 8 patients who were tested before and after transplantation. The reason is not clear yet, although there are at least two explanations: 1) improved ventilatory mechanics secondary to disappearance of ascites, rebuilding of thoracic muscle mass, and relief of fatigue; and 2) modulation of the endobronchial inflammation by immunosuppressive agents.

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POST-OPERATIVE CARE AND FOLLOW-UP AFTER HEART-LUNG TRANSPLANTATION

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Eighty-two patients with cystic fibrosis (CF) had been transplanted in our unit by March 1991. Seventy-six received heart-lung transplants (HLT) three combined with heart-lung-liver transplants and three bilateral single-lung transplants. Patients were routinely immunosuppressed with cyclosporin A to achieve a target level of 500ng/ml (monoclonal antibody assay on whole blood), renal function permitting during the first month and thereafter 250-350ng/ml and azathioprine 2mg/kg/day. Rabbit antithymocyte globulin 100 mgs on alternate days was prescribed for the first 10 post-operative days. In the immediate post-operative period inotropic support is usually necessary and dopamine, adrenaline, isoprenaline, enoximone and nitrates are used as appropriate to achieve satisfactory tissue perfusion and renal function. Patients are extubated and mobilised as soon as possible. All CF patients receive at least 10 days anti-pseudomonal antibiotics based on the sensitivities of their immediate pre-operative sputum culture. Drains and lines are removed as soon as possible and flucloxacillin is given until this is achieved. Prophylactic oral nystatin and acyclovir are given for the first three months and inhaled colomycin indefinitely. It is essential that at all times both the patient and the relatives are kept fully informed of developments.

Following HLT, patients with CF may experience medical problems in common with all HLT recipients. These include infection, acute rejection, seizures, lymphoproliferative disorders and obliterative bronchiolitis. In the CF patients, infection is mainly due to Pseudomonas aeruginosa but this usually only causes a problem in the first post-operative month. Other bacteria and viruses especially cytomegalovirus, Aspergillus fumigatus and Pneumocystis carinii, have infected our patients and these are treated with appropriate antimicrobial agents after an accurate diagnosis is made, which usually includes fiberoptic bronchoscopy together with bronchoalveolar lavage and transbronchial lung biopsy. Acute rejection is most common in the first month (65 episodes in the first 52 patients) and this is usually confirmed by transbronchial biopsy and treated by i.v. methylprednisolone. Seizures may be due to high cyclosporin A levels, hypomagnesaemia, hypocalcaemia, hyponatraemia, hypoglycaemia, infection or intracranial bleeding. Four patients have developed lymphoproliferative disorders. These were successfully treated by a reduction in immunosuppression alone in one patient with a "T" cell disorder, and in combination with high-dose acyclovir in three patients with a "B" cell disorder. All HLT patients have home respiratory function monitors and measure their FEV1 and FVC on a daily basis. Any deterioration in respiratory function is quickly investigated. In spite of this measure 16 of our 79 HLT patients have developed obliterative bronchiolitis. Some responded to augmented immunosuppression but some continue to deteriorate and may need retransplantation.

In addition, CF-specific problems are encountered. Good nutrition is essential and patients are prescribed a high-calorie diet with pancreatic enzymes and vitamins. If the patient is debilitated an elemental feed is
given by nasogastric tube or gastrostomy. Salt loss is a particular problem and electrolytes must be carefully monitored. Some CF patients have diabetes mellitus and others become diabetic in the immediate post-operative period. An insulin infusion may be required during this time. Acetylcysteine is given to any patients who develop meconium ileus equivalent. Patients with CF require higher dose of cyclosporin than non-CF patients because of their malabsorption of this fat-soluble drug and careful monitoring of levels is essential. Many patients have coexisting liver disease, and hepatic function is monitored and hepatoxic drugs reduced as appropriate.

Lung function in general improves quickly during the first three months after transplantation. Our group of patients pre-operatively had FEV₁, 21%, FVC 35% predicted and at three months post-operatively 60% and 61% respectively. This was maintained at three years. FEV₁, and FVC being 59% and 65% respectively. Outpatient clinic monitoring of the patients clinical condition, lung function, and immunosuppression for the rest of their lives is, of course, mandatory. The majority of patients are fit to return to school, higher education or work 6/12 months after surgery. There is, for the majority, a great improvement in their quality of life⁴.

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Functional Analysis of Synthetic Analogues of Bacterial Adhesins
Specific for Mammalian Glycoconjugate Receptors

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*Pseudomonas aeruginosa* pulmonary infections continue to constitute a major source of
morbidity and mortality for patients with cystic fibrosis (1,2). *P. aeruginosa* respiratory infections
are thought to be initiated by the adhesion to and subsequent colonisation of the oral pharynx
followed by a descending infection mechanism (3,4). It is now believed that *P. aeruginosa* can
utilize three separate adhesins to bind to human respiratory epithelial cells, namely pili (5), alginate
(6) and exoenzyme S (7). The latter is an ADP-ribosylating toxin that is found both cell free and
associated with the outer surface of the *P. aeruginosa* outer membrane (8). Alginate is the principle
component of the mucoid polysaccharide capsule and is usually expressed in lungs of cystic
fibrosis patients only after initially invading as nonmucoid *P. aeruginosa* strains and subsequently
establishing a chronic infection (9). The kinetic analysis of *P. aeruginosa* adherence has revealed
that both pili and alginate function as adhesins and that they recognize receptors on the surface of
buccal and tracheal epithelial cells. The pili adhesins tend to recognize a lower number of receptors
on the surface of epithelial cells than do the alginate adhesins, but the pili adhesins have a much
higher affinity for their receptors than do the alginate adhesins (10,11).

Evan (12) has shown that many pulmonary pathogenic bacteria including *P. aeruginosa*
bind specifically to the glycosphingolipids asialo-GM1 and asialo-GM2, which contain a common
terminal or internal GalNAcβ1;1-4Gal sequence. Krivan has also shown that both purified pili
(personal communication) and exoenzyme S (7) from *P. aeruginosa* bind to asialo-GM1 and
asialo-GM2.

*P. aeruginosa* pili are long tube-like structures that extend from the poles or end of the
bacterial cell. They have been named "N-methyl-phenylalanine" or "type 4" pili because of the
novel N-terminus of the subunit protein (13). The pilus adhesin has been shown to reside in the C-
terminal region of the pilin structural protein, and this epithelial cell binding domain (ECBD) is
functional when synthesized as a synthetic peptide (14). Our studies, designed to further
characterize the *P. aeruginosa* pilin ECBD and its interaction with epithelial cell surface receptors,
have demonstrated that the ECBD is exposed at the tip of the pilus, but buried in subunit interfaces
in the remainder of the pilus. We have also shown that *P. aeruginosa* pili bind specifically and with
high affinity to asialo-GM1 and asialo-GM2, as well as synthetic disaccharide βGalNAc1-4Gal.
The pilus binding to the glycolipid or the disaccharide can be competitively inhibited with synthetic
GalNAcβ1-4Gal and with synthetic peptides that contain the *P. aeruginosa* pilus ECBD. The
adherence process can also be inhibited with mono- or polyclonal antibodies specific for the ECBD
of *P. aeruginosa* pilin.

A high degree of sequence homology between the pilin ECBD and a similar polypeptide
region within exoenzyme S was noted in the present study. An examination of the immunological
properties of these two proteins was therefore undertaken. Both mono- and polyclonal antibodies
specific for the pilin ECBD were observed to bind to exoenzyme S, while anti-exoenzyme S
antibodies bound to *P. aeruginosa* pili and the ECBD synthetic peptide. Significantly, antibodies
specific for the pilin ECBD inhibited the exoenzyme S adhesin function, while antibodies specific
for the exoenzyme S inhibited pili binding to asialo-GM1. These results suggested that the ECBD
found in pili and in exoenzyme S of *P. aeruginosa* may represent a generic binding domain existing
in adhesins of a variety of microbial pathogens. For example, adhesins with similar receptor specificities have been reported for *Helicobacter pylori* (7), *Chlamydia trachomatis* and *C. pneumoniae* (15). These organisms appear to have related adherence domains which alternately bind to GalNAcβ1-4Gal or phosphatidylethanolamine, depending on the concentration of specific divalent cations. Whether the pilin ECBD also possesses this dual adherence specificity, and what its biological significance may be remains to be determined.

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The Role of Exoenzyme-S in Adherence of *Pseudomonas aeruginosa*

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The mechanisms of colonization of the lungs of CF patients by *Pseudomonas aeruginosa* has been an intensive area of investigation because it represents an obvious step for therapeutic intervention. The bacterial components which have received the most attention as adhesins are pili and alginate. Several aspects of the studies with these ligands suggested that other adhesins must exist. Recent results from our laboratory revealed that exoenzyme-S is an important adhesin of *P. aeruginosa*.

Gangliotriosylceramide (GgO₃; GalNAcβ1-4Galβ1-4Glcβ1-1Cer), gangliotetraosylceramide (GgO₄; Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1Cer), and lactosylceramide (LacCer; Galβ1-4Glcβ1-1Cer) have been identified as possible receptors for *P. aeruginosa* (1, 3). Pili, which have been identified as an important adhesive factor for buccal cells (7), damaged tracheal epithelium (4), and mucous proteins (5), were not the adhesin for glycosphingolipids (GSL) since mutant strains which did not produce pilin retained the binding specificity of the parent strain (N. R. Baker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, B-225, p.68). Likewise, alginate could not account for this specificity since mucoid and nonmucoid strains of *P. aeruginosa* bound to the same glycolipids.

Exoenzyme-S is one of several toxic products of *P. aeruginosa* which contributes to its pathogenicity (8). This enzyme ADP-ribosylates several membrane associated eucaryotic proteins (2). Preliminary studies on the binding of exoenzyme-S to cells indicate that it may be recognizing the same carbohydrate sequences as the bacteria. Thus we have initiated a series of studies designed to characterize the binding of exoenzyme-S to glycosphingolipids and determine the role of exoenzyme-S in adherence of *P. aeruginosa* to cells.

Exoenzyme-S and the source strain DG-1 was obtained from Dr. D. Woods, University of Calgary Health Sciences Centre. The thin layer chromatography binding assay was done as described previously (1) for the purified exoenzyme-S and intact bacteria. Bound exoenzyme-S or bacteria were detected by sequential overlays with anti-exoenzyme-S and alkaline phosphatase labeled goat-anti-rabbit IgG. The plates were developed with a Fast Red/naphthol phosphate mixture. The purified exoenzyme-S and the bacteria bound to gangliotriosyl-, gangliotetraosyl-, and lactosylceramides.

Binding was quantitated using a solid phase binding assay in which the glycosphingolipids were dried to the plastic surface of microtiter plates. Exoenzyme-S was added to each well and bound exoenzyme-S was detected by sequential addition of of anti-exoenzyme-S and goat-anti-rabbit alkaline phosphatase. The plates were developed by addition of nitrophenol phosphate. The analysis of these results indicated that most of the binding of exoenzyme-S occurred within the first 15 min followed by a gradual but consistent increase in bound toxin. In addition more exoenzyme-S was detected bound to the gangliotetraosylceramide than the gangliotriosylceramide.

To prove that the results observed were not due to binding of a minor contaminant of the exoenzyme-S preparation, material which bound to glycolipids immobilized on plastic plates was eluted and detected by immunoblotting of the sample. Furthermore a crude preparation containing exoenzyme-S as a minor component was subjected to the same analysis. In both cases the exoenzyme-S could be detected.
as the ligand binding to the glycolipids. Finally, a polyclonal rabbit antiserum and a monoclonal antibody to exoenzyme-S were shown to inhibit the binding of exoenzyme-S to the glycolipids. These antibodies were shown in separate studies to inhibit the binding of whole bacteria to buccal cells. Purified exoenzyme-S also inhibited the binding of the bacteria to buccal cells.

In conclusion we have identified exoenzyme-S as an adhesin of \textit{P. aeruginosa} which mediates the binding of the bacteria to glycosphingolipids and to buccal cells. Other work has demonstrated the presence of these glycosphingolipids in extracts of buccal cells (6) indicating that they may be the receptors. Alternatively the receptors may be glycoproteins with similar carbohydrate sequences. Identification of exoenzyme-S as an important adhesin for \textit{P. aeruginosa} may lead to new approaches to prevention of lung infection of CF patients and hospitalized patients.

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Comparison of *Pseudomonas aeruginosa* binding to normal and CF respiratory epithelial cells

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Much of the morbidity and mortality associated with cystic fibrosis is due to chronic pulmonary infection caused by *Pseudomonas aeruginosa*. Although the genetic basis for the abnormalities in electrolyte transport pathognomonic for CF has been defined, and the function of the aberrant CFTR studied in detail, the molecular basis for the unusual affinity of *Pseudomonas* for the CF respiratory tract remains to be defined. The consequences of long standing *Pseudomonas* colonization and infection of the CF lung are well understood. However, the specific factors responsible for the initial colonization of the CF, but not the normal lung by environmental strains of the ubiquitous *P. aeruginosa* are unknown. One possibility is that the CF lung provides novel receptors for *Pseudomonas* binding which are not available in the normal lung. Alternatively, or in addition, specific *Pseudomonas* gene products may be expressed in the milieu of the CF, but not the normal lung, which can modify epithelial structures to facilitate binding.

To test these possibilities the binding of $^{35}$S labeled *P. aeruginosa* PAO1 to monolayers of primary cultures of respiratory epithelial cells from both normal and CF patients was compared. PAO1 bound significantly more to the CF cells than to the normal cells; the mean adherence of $5 \times 10^7$ CFU/ml/well of PAO1 was $15.09 \text{ SD } +/-. 4.25 \times 10^6$ CFU/ml/well and $7.62 \text{ SD } +/-. 2.11 \times 10^6$ CFU/ml/well respectively (Mann-Whitney U test $p < 0.0001$). The CF cells bound approximately 115 organisms/cell while the normal monolayers bound 34 organisms/epithelial cell. In addition to quantitative differences in receptors on CF and normal epithelial cells, there were also qualitative differences. Specific carbohydrates including GlcNAc, NeuAc, D-gal, and L-fuc were able to block the attachment of PAO1 to the CF cells. Sialic acid (NeuAc) blocked binding to the CF cells but not to the normal cells. Only GlcNAc significantly decreased binding to the normal cells. Alterations in sialylation of surface glycoconjugates as a result of decreased acidification in the Golgi, due to altered chloride permeability (1) might effect bacterial receptors on CF cells.

Although the difference in bacterial attachment to normal and CF cells was highly statistically significant, this numerical difference may not be sufficient to account for the biological phenomenon that *Pseudomonas* colonization of the lung occurs almost exclusively in CF patients. Several *Pseudomonas* exoproducts may be involved in facilitating colonization (2). As the production of the exopolysaccharide alginate is a phenotype seen almost exclusively in *Pseudomonas* strains isolated from CF
patients after chronic infection with *P. aeruginosa*, different phenotypes, may be associated with the initial colonization of the previously uninfected CF lung.

Based on the results of the adherence assays described above, as well as *in vitro* studies (3), it seems likely that the production of neuraminidase by *P. aeruginosa* might facilitate bacterial adherence. This enzyme would remove sialic acid residues from superficial glycoconjugates on the epithelial surface and expose asialogangliosides which serve as Pseudomonas receptors. The PA01 neuraminidase has been isolated from culture supernatants. CF epithelial monolayers exposed to this enzyme bound more organisms than controls exposed to buffer alone. The specificity of the enzyme, in exposing asialoglycolipids was further demonstrated by FITC-lectin binding studies.

The expression of the Pseudomonas neuraminidase under growth conditions expected to be present in the CF lung was tested experimentally. Just as alginate expression has been found to be triggered by hyperosmolar conditions (4), neuraminidase production was similarly increased when organisms were grown in media of high osmolarity. Thus, this Pseudomonas exoproduct, which can act to enhance bacterial adherence to CF epithelial cells, appeared to be produced in increased amounts when the organism was grown in a hyperosmolar or dehydrated milieu.

From these studies, it seems likely that factors attributable to both the host and the bacterium will be implicated in the pathogenesis of Pseudomonas infections in the CF lung. The increased number of receptors found in the CF lung, and the ability of the organisms to further expose receptors under conditions of high osmolarity contribute to the unique susceptibility of the CF lung to Pseudomonas infection. There are undoubtedly other Pseudomonas virulence factors whose expression under these specific conditions is critical for colonization. By understanding the environmental signals that trigger the genes required for Pseudomonas adherence to the CF lung, it should be possible to devise strategies to block this process and prevent the establishment of infection.

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The Adhesion-Receptor Systems of Pseudomonas aeruginosa: Where are we Now?

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The fact that P. aeruginosa is the most successful microorganism to colonize the lung in cystic fibrosis (CF) suggests two requirements. Firstly, that the organism must possess a high affinity recognition system for the milieu of the respiratory tree and secondly, it must possess well developed mechanisms to evade host defenses at this normally sterile site. While this state of colonization is most notorious in CF, it should be pointed out that in most end stage airway disease e.g. adult bronchiectasis and late Kartagener's syndrome, P. aeruginosa is also the most successful colonizer. The only similarities in these diseases are the serious alterations in mucociliary clearance and mucus hypersecretion, leading one to believe that these are important factors in P. aeruginosa colonization. It would therefore appear that if there is a specificity for CF, it may be that this colonization occurs earlier in life and that mucoid P. aeruginosa strains are the final evolutionary phenotype.

It is proposed but not certain that if the basis of this colonization is understood then means of preventing or ameliorating its sequelae may be developed. This in theory would require an in depth knowledge of the "colonizing" determinants in this disease -- the adhesin-receptor system used by this organism in CF. However, as one examines this system it must be realized that we may be dealing with two different phenomena which should be separated -- adhesion to airway cells and adhesion to airway mucus or mucins. As will be pointed out, differences are already apparent in these two systems. Most receptors on airway cells need to be unmasked whereas mucin receptors are normally exposed. In addition some receptor material found on cells are not present in mucins, e.g. lactose and asialogangliosides.

The adhesin situation in P. aeruginosa is rapidly undergoing a rethinking. The simple concept of pili and alginate as the major adhesins is no longer tenable (1). The use of molecular biology to develop genotypic pil- mutants has led to the discovery of nonpilus, nonalginate adhesins (2,3,4). In fact, there may be more than one type of nonpilus adhesin, rpoN dependent and rpoN independent (3). The current candidates for the "new" adhesins status may be an outer membrane protein, exoenzyme S and even the hemagglutinating activity of P. aeruginosa.

On the host side, the literature is perhaps even more confusing. Three disaccharide structures have achieved receptor status -- GalNAc(β1-4)Gal, Gal(β1-4)Glc (lactose), and Gal(β1-3/4)GlcNAc (5,6,7). Additionally sialic acid has been characterized as part of the receptor (6) and alternately antiadhesive (5). Some studies have suggested that both proteins and carbohydrates may be receptor material for P. aeruginosa pili (8,9).

Attempting to match even the known adhesins and receptors reveals how large a gap exists in our knowledge of this system and the discrepancies alluded to earlier, i.e. adhesion to cells versus adhesion to mucus. e.g. pili-mutants bind less to cells (2,4) but as well as or better than their pil+ parents bind to
mucins (3). In fact pilus binding to mucins may not be important at all in mucus colonization. With regards to the alginate receptor, this is a total mystery since none has been characterized. The state of affairs can best be summarized by the following table.

| Receptors                  | Cells           | Mucins         |
|----------------------------|-----------------|----------------|
| Possible adhesins          |                 |                |
| Alginate                   | ? lectin        | ?              |
| Pili                       | Carbohydrate    | ? Protein      |
| Non Pilus adhesins         |                 |                |
| OMP                        | ? Lactose       | ? Gal(β1-3/4)GlcNAc |
| exoS                       | GalNAc(β1-4)Gal | ?              |
| hemagglutinin              | ? sialic acid   | sialic acid    |
| X                          |                 |                |

Where do we go from here? Three issues require attention: 1) deciding on the relevance of cellular versus mucin adhesion in CF since the differences may decide how this problem is attacked, 2) ascertaining the true number of adhesins and the relative importance of each by the use of molecular cloning techniques, and 3) clean receptor studies using purified separated adhesins or strains which possess only one adhesin at a time. When this is all done, the availability of a relevant animal model will decide whether this approach to the prevention of *P. aeruginosa* colonization is feasible.

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Pseudomonas aeruginosa produces several extracellular products which have been proposed as virulence determinants in pulmonary infections with this organism (1,2). Included among these are exotoxin A and exoenzyme S, two distinct ADP-ribosyl transferases which differ in their structure and substrate specificity (3,4). Evidence from recent studies indicates that exoenzyme S may play a significant role in pulmonary infections in man. Phenotypic analysis of P. aeruginosa strains isolated from pulmonary infections in man showed that these strains produce significantly more exoenzyme S than strains isolated from other clinical sources such as urine or wound infections (5). In animal model studies comparing infections produced by parental P. aeruginosa strains with those produced by isogenic mutants, singly deficient in exoenzyme S, it has been shown that infections with exoenzyme S-positive strains are much more severe (6,7). Further evidence that exoenzyme S may play a role in lung injury during P. aeruginosa infections was provided by light- and electron-microscopic characterization of lung injury after intratracheal instillation of purified exoenzyme S which demonstrated that the pathological changes described for P. aeruginosa lung infections in a variety of clinical settings may be reproduced by a single P. aeruginosa exoprotein, exoenzyme S (8).

In the present studies we describe an a role for exoenzyme S in the pathogenesis of P. aeruginosa lung infections which exists in addition to its role as a cytotoxin. Exoenzyme S is present on the surface of P. aeruginosa as detected by immunogold labelling of whole organisms using antibodies to exoenzyme S. Further, exoenzyme S and monoclonal antibodies to exoenzyme S inhibit the binding of P. aeruginosa to buccal epithelial cells. Results of these studies lead to the conclusion that exoenzyme S is an important adhesin of P. aeruginosa.

In addition to serving as an adhesin of P. aeruginosa, it is possible that exoenzyme S may serve this same purpose in other respiratory pathogens. Hybridization of restriction endonuclease digests of chromosomal DNA from a number of bacterial respiratory pathogens with a 0.6-kb XhoI-SalI DNA fragment of the exoenzyme S structural gene demonstrated that these organisms may in fact carry the genetic information for exoenzyme S. Further, antibodies to exoenzyme S inhibited the binding of these organisms to respiratory epithelial cells in in vitro assays.
Analysis of the amino acid sequence of exoenzyme S demonstrated that an amino acid sequence representing the epithelial cell binding domain in *P. aeruginosa* pili is also present in the exoenzyme S protein. Thus, it is intriguing to hypothesize that a number of bacterial respiratory pathogens may adhere by similar mechanisms.

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PATIENTS CAN GAIN WEIGHT IF FED SUFFICIENT CALORIES: BY APPLYING SELF-MANAGEMENT TECHNIQUES

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Recent advances in drug therapy and medical technology have improved treatment for many chronic illnesses such as cystic fibrosis. However, patients frequently fail to receive the full benefit of the treatment because of failure to comply with the prescriptions. The rate of non-compliance with long term medications is approximately 50%, regardless of whether the intent is to prevent or alleviate symptoms. Compliance with life-style recommendations is worse. Only about 30% of patients would comply with even modest dietary advice and fewer than 10% of cigarette smokers would stop smoking on the advice of their physician (1).

Behavioral techniques have been used to increase compliance to long term regimens. One type of technique, the Social Learning Theory (2,3), acknowledges the importance of external events such as stimuli or cues and reinforcements or rewards on behavior. But they also propose that behavior change is mediated by people's thinking patterns and that these patterns may be most readily changed when people (CF patients) experience a sense of personal mastery or "self-efficacy". That is, for a patient's behavior to change, he/she should not only believe that adopting a particular behavior (e.g., taking milkshake with enzymes at 8:00 PM every day) will lead to the desired outcome (e.g., increased weight gain), but also perceive themselves as being able to successfully execute this behavior. Indeed, the strength of patients' convictions in their own effectiveness is likely to affect whether they will initiate a coping behavior, how much energy they will expend on it and how long they will persist in the face of obstacles. Counseling and education should therefore emphasize assisting patients to change their patterns of thought and to increase their self management skills (4,5). The self-efficacy model was applied in a nutrition intervention study for patients with CF (6,7). Patients were instructed on a nonrestricted fat diet emphasizing a high energy intake and the administration of an optimal pancreatic enzyme replacement dosage. The dietary plan for increasing the energy intake was based on each patient's medical history, nutritional assessment and the patient's/family's psychosocial assessment. Nutrition counseling was provided during each clinic visit. The sessions included measurements of height and weight, estimation of energy intake (24-hour recall) and discussion of an individually designed diet plan. A height and weight curve was plotted and reviewed with the patient. The growth chart and the 24-hour dietary recall were used as self-evaluation tools for the patient to determine his/her success in meeting the self-established goals. The feedback on the dietary recall was an integral part of the self-motivation process and the development of self-management skills. Self-motivation is best summoned and sustained by adopting attainable specific sub-goals that
lead to large future ones. For example, taking a 12 oz milkshake with 2 capsules of pancreatic enzyme every evening before bedtime provided a clear marker of progress towards the larger goal of higher energy intake and weight gain. Whenever necessary, changes in the diet plan were made with the patient and/or family by using problem-solving techniques and by jointly establishing new short term goals. In this study, measurements of compliance were replaced with the active participation by the patient in the decision-making process, goal setting and monitoring of the nutrition care plan.

The results showed significant increases in energy intake and body mass index values (p<.001). However, four to six counseling sessions were required until patients were able to increase the mean energy intake by approximately 25% and adjust the oral pancreatic enzyme dosage appropriately. Presently, patients are able to maintain the established behavior by receiving periodic counseling during follow-up visits. Studies have shown that none of the various intervention methods are self-sustaining; they must continue to be applied as long as compliance is required (8).

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Aggressive nutritional support has been demonstrated to improve the nutritional status and enhance growth in individuals with cystic fibrosis.\textsuperscript{1-3} Intensive nutritional counseling can have a positive effect on dietary intake.\textsuperscript{4} However, when nutrition education and oral supplementation are no longer effective, enteral feedings should be initiated rather than accept any degree of malnutrition. Studies have demonstrated short and long-term benefits to CF patients using nasogastric, jejunal, and gastrostomy feedings.\textsuperscript{5} A number of enteral routes and types of formulas are available, each with their own considerations, advantages, and disadvantages. A survey\textsuperscript{6} of 24 nutritionists at CF centers using gastrostomy feedings revealed that nearly half used semielemental formulas, 83\% administered feedings nocturnally, and 62\% administered pancreatic enzymes with feedings. The choice of the route and formula should reflect the needs of the patient and family and the expertise of the CF center.

Children and adolescents are aware of cultural stereotypes regarding personal appearance and these feelings of body image are often generalized to other parts of their lives.\textsuperscript{7} It is not known how children and adolescents with CF feel about themselves since choosing to have a G-tube, or how they have adjusted to the tube.

In 1987-89, at the University of Wisconsin, 2 males and 5 females with CF, ages 11-16 years, had gastrostomy tubes placed. These patients were all growth-retarded as evidenced by weight/height percentile less than 85\% of the median on the NCHS growth curve or dropping growth percentiles in spite of continued attempts to achieve adequate nutrition orally. They experienced pancreatic insufficiency and had chronic pulmonary disease. They received nocturnal gastrostomy feedings of Vital HN, a partially hydrolyzed formula requiring less enzyme, in a quantity to reach 120-150\% of the RDA, including their typical ad lib feedings.

Growth parameters and chest radiographs were assessed 1 year prior to G-tube insertion, at insertion, and 1 and 2 years postinsertion. These results are reported in Table I. Our results suggest that using this technique we have been able to positively impact the growth of these patients. The initial absolute weight gain the first year is most remarkable. A positive trend in weight and height/age and weight/height continues throughout the second year after insertion. Pulmonary status appeared to remain fairly stable over the 2-year period, judged by chest radiograph scores.

To assess the psychosocial effect of the G-tube, patients completed oral interviews at least 2 years after their G-tube was placed. They stated the reason they chose to get a G-tube was they did not like the way they looked and felt. They were "sick of being short and skinny." All but one responded, "yes," they were glad they got the G-tube and would tell peers to do the same if they needed it. Issues regarding maintenance, skin care, and pain with surgery and skin breakdown were cited as the hardest part of having a G-tube. Only one person said they did not want other people to notice it. When asked if they feel different now than before the G-tube, responses included: I feel better about myself, I look more like normal kids, I feel more responsible. Most responded that they feel like they do not get sick as easily. When asked if their appetites changed, most said they eat a lot better because they feel better, although many reported being unable to eat in the early morning.

The results suggest that the potential benefits of a gastrostomy tube in terms of nutritional status and psychosocial functioning are significant. The literature also supports these conclusions.

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| Pt # | Age at G-Tube | Kg Wt | Weight/Age Percentile (z Score)* | Height/Age Percentile (z Score)* | Weight/Height % of Median† | Chest X-Ray Score‡ |
|------|--------------|-------|---------------------------------|---------------------------------|---------------------------|------------------|
| 1    | 11 2/12      | 5.4   | -1 yr 0 +1 yr +2 yr             | -1 yr 0 +1 yr +2 yr             |                           |                  |
|      |              |       | 8 7 12 39 (-1.44) (-1.44) (-1.18) (-0.29) | 8 5 3 7 (-1.39) (-1.61) (-1.88) (-1.50) |                           |                  |
| 2    | 12 9/12      | 9.5   |                                 |                                 |                           |                  |
|      |              |       | 3 1 8 11 (-1.84) (-2.19) (-1.42) (-1.23) | 15 5 16 23 (-1.02) (-1.63) (-1.01) (-0.75) |                           |                  |
| 3    | 16           | 10.4  |                                 |                                 |                           |                  |
|      |              |       | 0.1 0.1 0.3 0.3 (-3.32) (-3.57) (-2.78) (-3.09) | 2 0.9 4 18 (-2.03) (-2.38) (-1.74) (-0.89) |                           |                  |
| 4    | 13 2/12      | 6.3   |                                 |                                 |                           |                  |
|      |              |       | 4 3 5 0.9 (-1.76) (-1.93) (-1.68) (-2.37) | 0.8 12 11 5 (-0.78) (-1.17) (-1.26) (-1.65) |                           |                  |
| 5    | 11 2/12      | 4.4   |                                 |                                 |                           |                  |
|      |              |       | 8 3 5 2 (-1.40) (-1.85) (-1.68) (-2.06) | 5 1 0.3 0.1 (-1.61) (-2.18) (-2.77) (-3.31) |                           |                  |
| 6    | 15 9/12      | 8.4   |                                 |                                 |                           |                  |
|      |              |       | 2 1 2 22 (-1.99) (-2.02) (-2.01) (-0.77) | 21 22 27 40 (-0.82) (-0.79) (-0.60) (-0.26) |                           |                  |
| 7    | 12           | 10.3  |                                 |                                 |                           |                  |
|      |              |       | 8 7 12 NA (-1.43) (-1.49) (-0.03) | 2 0.4 0.5 NA (-1.99) (-2.64) (-2.58) |                           |                  |
| X    |              | 7.8   |                                 |                                 |                           |                  |
|      |              |       | 4.7 3.2 6.3 12.5 (-1.88) (-2.07) (-1.54) (-1.63) | 8.25 6.6 8.8 15.9 (-1.37) (-1.77) (-1.69) (-1.39) |                           |                  |
| SD   |              | 2.3   |                                 |                                 |                           |                  |
|      |              |       | 3.0 2.6 4.2 14 (0.62) (0.66) (0.77) (0.96) | 6.7 7.3 9.1 13.5 (0.63) (0.74) (0.97) |                           |                  |

* Anthropometric Software CDC Standard Deviation-Derived Growth Reference Curves, NCHS/CDC Reference Population Series 11, No. 165, DHEW Publication No. 78-1650
† NCHS height and weight of youths 12-17 yr., U.S. Vital and Health Statistics, Series II, No. 124, Health Services & Mental Health Administration, Washington, DC, 1973
‡ Brasfield D, Hicks G, Seng-Jaw S. The chest roentgenogram in cystic fibrosis: A new scoring system. Pediatrics 1979; 63:24
Enzyme Dose – Is More Better?

Pro: Malabsorption is Effectively Treated by Providing Enough Enzyme

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The majority of patients with Cystic Fibrosis (CF) have severe pancreatic disease, with symptoms of maldigestion (pancreatic insufficiency). The degree of steatorrhea varies considerably from patient to patient. Although numerous factors likely contribute to this problem, there does appear to be a direct relationship between severity of steatorrhea and residual endogenous pancreatic enzyme secretion. Similarly, the response to enzyme replacement therapy is extremely variable as some patients correct maldigestion with small amounts of enzymes while others show little improvement on massive doses.

Factors affecting the efficacy of enzyme substitution therapy:

(A) Pancreatic Extracts
- Dose
- Potency
- Type (Capsule, Tablet, Powder, Acid-Resistant Microsphere)
- Compliance
- Timing of Ingestion

(B) Gastric
- Acid/Peptic Inactivation
- Poor Mixing with Gastric Contents
- Gastric Emptying

(C) Intestinal
- Intraluminal pH (poor dissolution, reduced enzyme activity)
- Bile Acid Deficiency/Precipitation
- Abnormal Motility?
- Bacterial Overgrowth?

Pancreatic Extracts
Most conventional enzyme preparations consist of desiccated porcine pancreatic extracts. The lipase (colipase) content of these preparations, probably the most important determinant of effectiveness, varies from approximately 3 to 20,000 units per capsule. The patient must receive adequate amounts of a potent preparation. However, too much enzyme carries the risk of hyperuricosuria and hyperuricemia due to the high purine content of pancreatic extracts. To protect the enzymes from acid/peptic inactivation, acid resistant enteric coated preparations are widely used. The early enteric coated preparations, constituted in tablet form had poor bioavailability and due to their large size were selectively retained in the
stomach. In theory, the newer microsphere preparations enter the duodenum mixed with chyme. Several groups of investigators have shown that microsphere preparations are as efficacious, or more efficacious than conventional enzymes, for treating fat maldigestion in Cystic Fibrosis. Also, there is some evidence that sequential increases in enzyme dose does improve steatorrhea, but in most studies the starting dose was extremely low. In our experience at least 30% of patients continue to have severe steatorrhea despite large doses of microsphere preparations.

Gastric

Microsphere preparations may be released in the stomach if the postprandial pH undergoes alkaline fluctuation. Furthermore, gastric emptying may be affected by the size of the microspheres -- larger spheres (2-3 mm diameter) do not leave the stomach as rapidly as small (1 mm diameter) spheres. In theory variable sized spheres (1-3 mm diameter) will empty from the stomach at different rates, thereby mixing with chyme throughout gastric emptying.

Intestinal Factors

In some patients, gastric acid hypersecretion and/or reduced pancreatic bicarbonate secretion may significantly lower intestinal pH. If intestinal pH is below 5.8 it may delay or prevent dissolution of the pH resistant coating. At lower intestinal pH (<4.0) enzyme activity may be reduced. In addition, pH-dependent bile acid precipitation will limit the efficacy of lipolysis. Proteases (trypsin and chymotrypsin) in commercial preparations may denature lipase. Consequently, protease contents of some preparations have been reduced, and the lipase content increased. There is some evidence that lipase is more susceptible to degradation in the presence of a high-carbohydrate diet but are more stable in a diet high in fat or protein.
Enzyme Dose - Is More Better  
Con: Fewer Enzymes are Needed if  
Enhancers of Enzyme Efficacy Are Used  

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The response to enzyme replacement therapy in CF patients with pancreatic insufficiency is variable and depends on a number of factors including: a) the individual's dietary intake and b) a variety of elements which define the intraluminal intestinal milieu and, thus, the availability and activity of ingested enzymes. The majority of patients maintain adequate (if not normal) digestion and absorption, provided an appropriate enzyme preparation and dose are used. There are currently no data to justify the routine administration of enhancers of enzyme efficacy to all patients in order to reduce enzyme dose.

There are, however, a small proportion of patients in whom adequate digestion and absorption are not achieved with appropriate enzyme preparation and dose. The likelihood of improving digestion in these patients by further increasing enzyme dose is negligible. Dietary manipulation to limit intake and, thus, improve absorption, is inappropriate. Success in improving enzyme efficacy in these patients can sometimes be achieved by manipulation of factors affecting the intraluminal milieu. Two factors which can affect digestion and enzyme efficacy, and are amenable to manipulation, are the intraluminal pH and bile salts.

**Intraluminal pH**

Some CF patients may have a lower proximal intestinal intraluminal pH due to inadequate HCO3 secretion and, possibly, increased gastric acid secretion. The intraluminal pH must be greater than 5.5-6.5 in order to achieve optimal dissolution of the enteric coating of most enzyme preparations. The more proximal in the GI tract this pH is achieved, the greater the time and surface area for digestion and absorption to occur. Once released from their enteric coating, enzyme activities are optimal at a more alkaline pH. In addition, bile salt precipitation, in the presence of low pH, limits availability of bile salts for digestion and absorption of lipids.

**Bile Salts**

Bile salts, in sufficient concentrations, are required for digestion and absorption of lipids. Unlike controls, bile salts in CF patients are often secreted predominantly conjugated with glycine, as CF patients are taurine deficient. Glycine-conjugated bile salts are less resistant to precipitation at low pH, thus limiting the availability of bile salts for digestion.
Enhancers of Enzyme Efficacy

Enhancers of enzyme efficacy have been used clinically and experimentally to alter the intraluminal pH and bile salts. The H2 antagonists, cimetidine and ranitidine, have been used to decrease gastric acid secretion, thereby, presumably increasing the pH in the proximal intestine. The potent inhibitor of gastric acid secretion, omeprazole, has recently been studied in CF patients. These agents, as well as a prostaglandin analogue, Misoprostil, which both decreases gastric acid secretion and enhances duodenal HC03 output, have all been shown to improve enzyme efficacy in CF.

Taurine supplementation has been able to increase the ratio of bile salts conjugated with taurine versus glycine in CF patients. Data suggests that taurine supplementation may improve absorption of a fat meal in CF patients and, thus, decrease steatorrhea. Recently, ursodeoxycholic acid has been used experimentally in the management of CF patients with liver disease. These patients showed an increase in weight which was not associated with improved digestion and absorption. While it has been suggested that urso may stimulate biliary HC03 secretion and bile flow, the mechanism responsible for the increased weight gain is unclear.
CONTROVERSY #3. WHICH FORMULA IS BEST FOR INFANTS WITH CYSTIC FIBROSIS: IS PREGESTIMIL THE BEST FORMULA FOR INFANTS WITH CF?

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High risk presentations/complications in infancy include bronchiolitis (20%), meconium ileus (10-15%), anemia and hypoproteinemia (5%). Five to ten percent undergo mechanical ventilation, and mortality remains between 4-7% (1-3). Protein calorie malnutrition, immunodeficiency, essential fatty acid (EFA), and vitamin deficiencies contribute to increased susceptibility to infection, with 20% acquiring pseudomonas by 12 months. Although complications are theoretically preventable by neonatal screening and early therapy, there is no evidence that early diagnosis improves survival. Growth in infants with CF is affected by prenatal and genetic factors, pancreatic insufficiency (PI), and pulmonary disease. The nutrient deficit results from a combination of inadequate intake, abnormal loss, and an increased requirement. Malabsorption occurs mainly 2' to PI, with 80-85% lacking trypsins (to digest protein) and lipases (to digest fat), together with excess fecal bile acids; despite enzyme replacement steatorrhea does not normalize, and malabsorption of fat soluble vitamins and some trace minerals is concurrent (4).

An ideal formula in CF should be palatable, easily concentratable (24-30 cals/oz) to give a caloric intake of 125-150 cals/kg/day, bypass protein digestion (protein hydrolysate), and have a fatty acid composition with minimal requirement for lipases. Carbohydrates are usually well absorbed unless surgery or infection is present. Breast milk and soy formulas have been associated with anemia and hypoproteinemia (4,5), and there is good evidence that protein hydrolysates (Nutramigen®, Pregestimil®, Alimentum®) are advantageous in this regard. The use of formulas containing medium chain triglycerides (MCT) as a major source of fat resulted in increased growth, but EFA status in both serum and tissues showed deterioration (6). Subsequent changes in fat composition provided other EFA and LCT in addition to the MCT. Whether MCT formulas are advantageous in long term follow up of these infants is controversial, either finding no significant difference (7), or normalization of growth parameters (2,8). Predigested formulas still require pancreatic enzymes (9).

A semi-elimental diet (Alfare-Nestle®) similar in composition to Pregestimil® was studied short term in CF infants off enzymes, and results were correlated with pancreatic function (10). Infants with a co-efficient of fat absorption (CoFa) >75% showed no improvement in fat or nitrogen absorption, but those with <75% CoFA showed significant improvement. Since pancreatic function in infancy shows wide variation and fluctuation (11), it is difficult to easily select those who would benefit from a predigested formula. There is good data that predigested formula is advantageous for infants with meconium ileus and neonatal surgical complications, due to the glucose polymer composition and the high levels of MCT. Our retrospective data (1975-85) on 52 infants treated with Pregestimil® (2) is similar to that reported by
Farrell et al. (8). Patients with meconium ileus received Pregestimil significantly longer (9.7 months ± 3.6), than patients without meconium ileus (6.7 months ± 2.9) (p<.01), and the growth records were higher at all age periods after 6 months (2). Although Australian data from neonatal screening showed no difference between breast and bottle fed infants on follow up (12), the authors excluded 7.9% for poor compliance, and all meconium ileus infants (18%).

Our prospective study (1986-91) on 30 infants treated with Pregestimil shows excellent growth parameters (better than neonatal screened infants from Colorado), rapid correction of protein and vitamin (E,A,D) deficiencies, but evidence for EFA deficiency remains. We are unsure whether the EFA deficiencies are related to the fat source ingested or some other regulatory abnormality.

Caution is advised when results are compared to retrospective data, and must take account of major advances in care including neonatal screening, meconium ileus, total parenteral nutrition, and shortened hospitalization.

Summary:

Early nutritional intervention is critical to prevent/correct malnutrition and its consequences. The use of predigested formulas can help normalize weight gain and growth of infants with CF. Predigested formulas are particularly advantageous for infants with meconium ileus and complicated neonatal surgical courses, and those with co-efficients of fat absorption <75%.

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Symposia Session Summaries

S10.6a  Which Milk is Best for Infants with Cystic Fibrosis? Breastfeeding and CF

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Almost every mother considers the option of breastfeeding for her newborn baby, a practice encouraged strongly by the American Academy of Pediatrics.1 As reviewed by Cunningham et al.,2 breastfeeding provides protection against gastrointestinal infections, significant reductions in nongastrointestinal infections (including meningitis, bacteremia, and pneumonia), decreases the risk of otitis media, and may protect against certain immune system disorders seen in later life such as celiac disease and insulin-dependent diabetes mellitus. Breast milk, however, which has only about half the protein and salt included in infant formulas frequently given to infants with CF may not be the best primary source of nutrients for these infants. Adverse effects resulting from breastfeeding CF infants who are not receiving pancreatic enzymes have included hypoproteinemia and edema due to malabsorption. Luder et al.3 surveyed the 124 CF centers in the United States CF Foundation Directory regarding breastfeeding recommendations. A total of 77% of CF centers recommend breastfeeding alone or in combination with pancreatic enzyme supplements and/or hydrolyzed formula. Most frequently cited reasons for not recommending breastfeeding were marked steatorrhea (21.5%), protein-energy malnutrition (51.1%), and meconium ileus (11.9%).

Research is needed to carefully evaluate the nutritional outcome of infants with CF who are breastfed compared to infants with CF fed standard formulas. Holliday et al.4 evaluated growth during the first two years of patients diagnosed through newborn screening in Australia and fed either human milk or formula. Of 41 breastfed infants, 30 were fed only human milk and 11 were supplemented for a duration of breastfeeding from 3 to 24 months. This group was compared to 24 formula-fed infants. No significant difference in height or weight was demonstrated between these 2 groups. The investigators concluded that human milk with pancreatic enzyme replacement appeared to provide adequate nutritional support for CF infants identified by early screening. They do not know if their conclusions would apply to infants with significant pulmonary disease.

In Wisconsin, a randomized controlled trial of effects of newborn screening on pulmonary and nutritional5 parameters is underway.6 Table I describes growth and biochemical data of 5 infants diagnosed through screening who were breast fed without other milk supplements through the first 6 months of life. Growth parameters are compared to those of 22 concurrent infants also diagnosed through screening and then fed a predigested or standard formula. All of the breastfed infants received pancreatic enzymes based on signs of malabsorption with the dose titrated to achieve adequate growth and control of steatorrhea. Weight-for-length percentile is not significantly different for the breastfed group compared to the formula-fed group. The breastfed group showed a steady decline in weight-for-length percentile over the year while the formula-fed group remained stable. Biochemical parameters were not statistically different comparing the 2 groups; all blood nutrient values were normal at 12 months for both groups other than a slightly low linoleate in the breastfed group.

Our results indicate that though breastfed babies have normal nutritional/biochemical indices, they may experience a decline in weight-for-length percentile over time. The breastfed group of babies had the same mean number of infections during the year (6) as the formula-fed babies. Their chest radiographic scores ranged from 18 to 25 at 1 year (Shwachman-Kulczycki scoring system), indicating they did not have severe lung disease.

This study underscores the importance of careful anthropometric longitudinal assessment of breastfed babies with CF. Nevertheless, these 5 infants showed acceptable growth velocity and a satisfactory nutritional status when managed exclusively with human milk during the first 6 months. Further investigation with a controlled clinical trial (that includes serial quantitation of malabsorption and attention to parameters related to respiratory infections) is needed to obtain a definitive answer to the question: Which milk is best for infants with cystic fibrosis?
Table I—Growth and Biochemical Data of Breastfed CF Infants

| MI*  | Patient | Weight/Length, % | Vitamin A, µg/dl† | Vitamin E, µg/dl‡ | Linoleate, %§ | Albumin, g/dl¶ |
|------|---------|------------------|-------------------|------------------|---------------|---------------|
|      |         | Diagnosis 6 Mo 1 Yr | Diagnosis 6 Mo 1 Yr | Diagnosis 6 Mo 1 Yr | Diagnosis 6 Mo 1 Yr | Diagnosis 6 Mo 1 Yr |
| 1    | 40 (6 wk) | 45 30          | 10 24.5 40        | 265 946 945      | 27.2 24.4 24.5 | 1.8 3.7 3.7    |
| 2    | 80 (8 wk) | 10 30          | 34 48 59          | 884 952 484      | 32.0 28.2 24.9 | 4.1 4.4 4.7    |
| 3*   | 45 (7 wk) | 20 25          | 16 26 16          | 914 1000 550     | 22.2 25.0 24.5 | 3.7 4.7 4.3    |
| 4    | 20 (4 wk) | 40 25          | 18 — 35           | 389 — 581        | 25.8 — 29.4 | 3.2 — 4.3      |
| 5*   | 25 (6 wk) | 75 40          | 9 44 38           | 433 697 510      | 16.4 23.6 22.6 | 2.8 3.4 4.0    |

Mean ± SD: 42 ± 24 38 ± 25 30 ± 6 17 ± 10 35.6 ± 10.5 38 ± 15 577 ± 301 899 ± 137 614 ± 189 24.7 ± 5.8 25 ± 2.0 25.2 ± 2.5 3.1 ± 0.9 4.0 ± 0.6 4.2 ± 0.4

Mean ± SD: 37 ± 19 32 ± 21 37 ± 22 26 ± 11 45 ± 17 44 ± 14 438 ± 317 833 ± 391 774 ± 306 26.1 ± 11.5 28.9 ± 11 27.2 ± 6.7 3.6 ± 0.7 3.7 ± 1.0 4.1 ± 0.3

* Meconium ileus (MI)
† Lower limit of normal = 20 µg/dl
‡ Lower limit of normal = 500 µg/dl
§ Lower limit of normal = 26%
¶ Mean ± SD for 22 formula-fed CF infants

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Controversy #3

S10.6b Breast-feeding by Women with CF: Effect on Mother and Infant

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Improvements in diagnostic techniques and in medical, pulmonary, and nutritional management have resulted in an increased life expectancy for person with cystic fibrosis (CF), resulting in a greater number of women with CF reaching childbearing age. Some of these women have become pregnant, delivered healthy babies, and breast-fed their infants. Little is known about the effect of lactation on the health of women with CF and about the nutrient content of breast milk produced by women with CF and its impact on the growth of their unaffected infants.

Information regarding lactation in women with CF and nutrient content of breast milk produced by women with CF is limited to case reports. Welch, et. al. (1) described one woman with advanced CF disease prior to pregnancy who did well during pregnancy. After breast-feeding for fifteen weeks she reached her lowest weight in the previous three years. At this point the baby was weaned. The woman was placed on antibiotic therapy and shortly thereafter her overall condition improved and her weight increased. Throughout the woman's exacerbation of CF the baby was described to be thriving. Analysis of the woman's breast milk revealed low fat and high protein content. Palmer, et. al (2) reported that two women breast-fed in their series of cases. They described normal growth and development in both babies, however descriptive information about these two women and their infants specific to the breast-feeding period is unavailable. Shiffman, et. al. (3) described breast-feeding by two women with CF. One woman remained stable during pregnancy and breast-feeding. The other woman had more advanced disease and experienced a difficult pregnancy, she breast-fed her infant for eight weeks. Both women delivered normal infants who were noted to have grown at the 50th percentile for weight and length at 3 and 6 months of age. Michel, et. al. (4,5) reported that five women who breast-fed their healthy infants maintained their weight and showed no change in PFTs following pregnancy and lactation. With the exception of one infant, who was exclusively breast-fed for six months, all the infants followed their expected growth curve for weight, length, and head circumference. All parameters of growth, for the one infant exclusively breast-fed for six months, fell to the fifth percentile and continued to follow this curve long past weaning.
Despite a limited number of subjects, there are reports on the nutrient composition of breast milk from women with CF. In one woman with CF an elevated sodium content in the breast milk was reported (6). However, this has not been a finding of other women with CF. Welch, et. al. (1) reported normal sodium and chloride content, but elevated protein and low total fat content in breast milk from a woman with severe CF disease. Bitman, et. al. (7) reported adequate kilocalories in breast milk of six women with CF compared to milk from women without CF. But concentration of the essential fatty acids, linoleic and arachindonic acids, were lower in the milk from women with CF. Low cholesterol and normal macronutrient and energy content in breast milk from women with mild CF disease was also reported by Mueller, et.al. (8). Shiffman, et. al. (2) described normal nutrient content of breast milk from two women with mild CF disease, but noted a reduction in the macronutrient content of colostrum when one woman experienced an exacerbation of her pulmonary disease.

In summary, women with CF are capable of breast-feeding their infants. Women with CF who wish to breast-feed should be at ideal body weight and have stable CF disease. Routine evaluation of health and weight status during the breast-feeding period is recommended. Evaluation of medications used by women during lactation is advised. Although the available case data is limited, the nutrient content of breast milk seems to be effected by exacerbation of CF disease. The fat soluble vitamin content of breast milk has been described in only one woman with CF, and there is no information regarding total vitamin content of CF breast milk. Macronutrient (fat, protein, carbohydrate) content of human milk from healthy women with CF appears to be within normal limits, but the abnormal content of specific lipid fractions raises the possibility that the infant fed only breast milk could be subjected to a diet deficient in certain essential fats. Growth and development of infants breast-fed by women with CF should monitored frequently throughout the breast-feeding period.

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The role of mycobacterial infections in cystic fibrosis pulmonary disease.

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Disease caused by nontuberculous mycobacteria is most commonly found in patients with underlying pulmonary disease or diabetes mellitus (1,2). From an epidemiologic perspective, patients with cystic fibrosis would appear to be at high risk for nontuberculous mycobacterial disease because of bronchiectasis and diabetes.

The American Thoracic Society recommends that several criteria have to be met to make a diagnosis of disease due to nontuberculous mycobacteria (3). There must be radiographic evidence of disease without other cause; at least two sputums that are smear positive and/or yield moderate growth on culture; and failure to clear the organism with pulmonary toilet. Mycobacterial disease may not be suspected in patients with cystic fibrosis in whom cough, sputum production, low grade fever, malaise, hemoptysis and an abnormal chest X-ray are ascribed to their underlying disease.

Five papers (4,5,6,7,8) and one case report (9), address the association of mycobacterial disease in cystic fibrosis. In 1976, Wood, Boat and Doershuk reported two cases of Mycobacterium Tuberculosis (MTb) and three cases of M. fortuitum in over 700 cystic fibrosis patients over an 18 year period. In 1980, Boxerbaum, reported seven patients with positive sputum cultures for nontuberculous mycobacteria, taken from a review of 430 charts. There were five cases of M Chelonei and two cases of M Fortuitum. Five patients had at least two positive cultures and one patient with M Chelonei died.

In 1984 Hodson et al reported on seven positive AFB smears in 286 patients over a 6 year period. Three had MTb. The other four were unidentified (n=1), unclassified (n=1), M Chelonei (n=1) and M fortuitum (n=1). The authors believed all were pathogenic. One patient with M fortuitum died from the infection. The authors recommend a regular AFB culture in patients with cystic fibrosis but pointed out the problem of contamination in culturing sputum from CF patients for mycobacteria. Contamination occurred in 36/62 cultures on Lowenstein-Jensen medium.

In 1990, the Stockholm CF center reported a three year prospective study of 54 patients. Four patients had positive smears and six had positive cultures. One patient had MTb. Patients had M avium complex (n=3), M avium together with M kansasii (n=1), unclassified (n=1) and one patient had M gordonae, not usually felt to be a pathogen. The authors believed that all six of these patients had a decline in FVC and FEV1 attributable to their mycobacterial disease. In 1990 a prospective study of 43 patients in Dublin found one patient with disease caused by M avium complex. The prevalence of positive skin tests to nontuberculous mycobacteria (36%) was no higher than a control population. They concluded that skin testing was a poor screening tool for nontuberculous mycobacteria and that periodic AFB sputums are necessary.

In review, by the end of 1990, a total of six cases of MTb, and 21 cases of nontuberculous mycobacteria had been described in the CF population. Recent prospective European studies suggested a higher incidence of mycobacterial disease than had been previously recognized. We wished to determine the incidence of mycobacteria disease in an adult US CF population to determine if there was any association of mycobacterial disease with longevity, with the severity of pulmonary disease, with the NIH clinical score, with CXR appearance or with the incidence of diabetes. We recognized that the prevalence of nontuberculous mycobacteria is lower in the Pacific Northwest than in other geographical locations in the US (10,11), and felt that our results may underestimate the prevalence of infection of other areas. In December 1990 we commenced a prospective
trial of all adult CF patients at the University of Washington Medical Center. PPD, mumps, candida, and trichophyton skin tests were placed, sputum was sent for AFB smear and culture, a travel history was taken, pulmonary function tests and NIH scoring were measured and CXRs were reviewed.

During the first 5 months, 39 (23 men, 16 women, aged 26.5±7(mean±SD), nine insulin dependent and four borderline diabetics) of the 65 adult CF patients have been screened. Two of 35 patients had a positive PPD (18 and 6 mm induration). The patient with an 18mm induration had known MTb contact. Five of 35 patients were anergic—two of these were taking oral prednisone. Five of 24 had positive sputum culture for nontuberculous mycobacteria, two of the five were smear positive. Four of the cultures had to be discontinued in the microbiology lab because of bacterial overgrowth. The age, spirometric values, NIH score, and CXR findings of the patients with nontuberculous mycobacteria found in their sputum were no different from the patients without nontuberculous mycobacteria in their sputum. In contrast to the Stockholm study, the spirometric indices of these five patients had remained relatively constant over the preceding two years, although two patients had rapid clinical deterioration recently. One of the five is an insulin dependent diabetic, two of the five have borderline diabetes. The CXRs of these five patients had no distinguishing characteristics. Two of the five had bullous disease. There was no significant increased travel history to more endemic parts by those with positive sputum.

Our initial conclusions are that positive sputum cultures for nontuberculous mycobacteria are not uncommon and that the prevalence rate is similar to the Swedish group. The increased prevalence of mycobacterial disease recently may reflect the longevity of the CF population or improved microbiological methods. Nontuberculous mycobacteria are found relatively commonly (21%) in adult CF patients; skin testing is unhelpful; bacterial overgrowth may be problematic. Guidelines to determine whether disease or colonization is present are yet to be determined in the CF population. Treatment for nontuberculous mycobacteria should be considered if the clinical status of the patient is deteriorating.

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Allergic Bronchopulmonary Aspergillosis: Diagnosis and Treatment

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Introduction

Allergic bronchopulmonary aspergillosis (ABPA) has been reported in children with cystic fibrosis from England (1), and several cystic fibrosis centers in the U.S.A. (2,3). It is difficult to estimate its incidence in CF. Systematic review of CF patients leads to an increased rate of diagnosis which may be as high as 5% per annum at some centers (2). However, the general incidence in CF is probably lower than this figure.

The gross pathology of ABPA reveals bronchiectasis of the central airways with a typical cylindrical configuration. These airways may become occluded by "mucoid impaction". Airway occlusion may lead to obstructive atelectasis of a segment or lobe. Saccular bronchiectasis may develop in such lesions if atelectasis persists. Microscopically, the airway walls show a heavy infiltration with plasma cells, lymphocytes and eosinophils. The airway may be occluded by mucus containing hyphal elements and inflammatory cells, particularly eosinophils.

The initial immune mechanism of ABPA is thought to be an IgE response to aspergillus colonizing the airway, leading to an allergic inflammatory reaction. This appears to lead to increased titers of IgG precipitating antibodies to the organism (4). It is likely that there is also a cellular contribution to the immunopathology of ABPA, as suggested by the presence of granulomata in pathological specimens and the observation that patients have a positive lymphocyte proliferation response to the organism (5).

Clinical Manifestations

In cystic fibrosis, ABPA may initially present as a worsening of pulmonary status. The customary diagnostic criteria include: 1) intermittent airflow obstruction, 2) transient pulmonary infiltrates, 3) proximal bronchiectasis, 4) sputum eosinophilia, 5) peripheral blood eosinophilia, 6) a positive immediate skin test by the prick method, 7) a positive delayed skin test reaction by intradermal testing, 8) the presence of precipitating antibodies to A. fumigatus, 9) an increased serum IgE value, 10) a positive specific IgE antibody test (RAST) to A. fumigatus. In CF patients, some of these abnormalities could be caused by the primary lung disease (e.g. airflow obstruction, infiltrates) so it has been difficult to establish rigid criteria.

Radiographic Features

The most common lesion in ABPA is a large homogeneous shadow in one of the upper lobes. The shadow may be triangular, lobar or patchy and it frequently moves to other sites. Tramline shadows are pairs of fine parallel lines radiating from the hila. Another characteristic finding in advanced cases is that of toothpaste shadows caused by impacted secretions in dilated bronchi (6).
Laboratory Investigation

The culture of *A. fumigatus* from the sputum provides strong circumstantial evidence of ABPA and may be one of the first clues. However, this is not a specific finding. The presence of hyphae in the sputum is more suggestive of the diagnosis. The presence of sputum or peripheral blood eosinophilia also supports the diagnosis of ABPA in the absence of other causes.

A characteristic laboratory finding in ABPA is an increased serum IgE value. Values as high as 30,000 IU/ml have been observed and the serum IgE typically is greater than 800 IU/ml in affected CF patients. The IgE level is a very useful marker of disease activity that can be used to follow outpatients for "flare ups" (4).

Serum precipitins are usually only weakly positive in CF patients with ABPA. Typically the immunoelectrophoresis shows 1-3 precipitin lines, sometimes to only one extract, whereas aspergilloma patients would show high-titer multiple precipitin reactions to all antigen extracts.

Treatment

Most cases of ABPA will require treatment with systemic corticosteroids. The treatment of choice is oral prednisone (7). The usual starting dose is 0.5 mg/kg/day, taken each morning, and this dose is maintained for two to four weeks while checking the chest radiograph for resolution of the pulmonary infiltrates. After this induction period the dose should be reduced to 0.5mg/kg given on alternate days for three months. Then the dose of prednisone should be slowly tapered over a further three months while monitoring the chest radiograph and serum IgE level for evidence of recurrence.

Prognosis

The prognosis for CF patients with ABPA is good if the disease is detected early and treatment started. However, relapses can occur many years later and it is essential to maintain long-term follow-up. Symptoms are not a reliable guide to therapy and it is important to reevaluate the serum IgE and chest radiograph at six-month intervals initially. After two years it is reasonable to schedule these tests less frequently if the disease has not recurred.

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Diagnosis of Bacterial Infections in the Lower Respiratory Tract of Young Children with Cystic Fibrosis

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Appropriate selection of antibiotic therapy for lower respiratory infection in young non-expectorating cystic fibrosis (CF) patients requires the identification of bacterial pathogens colonizing the lower airway. However, isolation of such pathogens may be problematic in this young population. To avoid exposing patients to the risk and discomfort of repeated bronchoscopic examinations, clinicians have relied upon oropharyngeal (OP) cultures or empiric antibiotic administration. Although OP cultures from children without cystic fibrosis do not accurately identify lower respiratory bacterial pathogens, OP cultures have been suggested as a useful diagnostic tool in CF patients. It has been demonstrated that buccal epithelial cells lining the oral cavity of CF patients have increased adherence to Pseudomonas aeruginosa (PA). How this increased adherence affects the sensitivity and specificity of OP cultures is not known.

There have been limited published data describing the bacteriology of the lower airway in young children with CF and no previous studies which determined the predictive value of OP bacterial cultures in these patients. For these reasons, we recently completed a two year study comparing bacterial pathogens collected simultaneously from the lower airways and oropharynx in 43 patients with CF. These patients underwent a bronchoscopic procedure at the time of optimal respiratory status.

OP cultures had higher specificity than sensitivity. In non-expectorators, specificity of quantitative cultures for PA was 93% (14/15), for Staphylococcus aureus (SA) 92% (12/13), for Hemophilus influenzae 82% (13/16), and for Klebsiella species 95% (20/21). Sensitivity of quantitative cultures in non-expectorators for PA was only 46% (5/11), for SA 77% (10/13), for H. influenzae 50% (5/10), and for Klebsiella species 80% (4/5). A total of 65 bacterial pathogens were isolated from bronchial secretions, of which 42 (65%) were correctly identified by quantitative OP culture. The remaining 23 pathogens (missed by false negative OP cultures) included: nine PA, six SA, 7 H. influenzae, and one Klebsiella species.

Predictive values are defined as the proportion of OP culture results which reflect the results of bronchial cultures. In this study, the predictive value of positive OP cultures in non-expectorating patients was relatively high, i.e. 83% (95% confidence interval: 36%-100%) for PA and 91% (59%-100%) for SA. Unfortunately, the predictive value of negative OP cultures was lower: 70% (48%-86%) for PA and 80% (52%-96%) for SA. Two previous studies comparing simultaneous upper and lower airway cultures found relatively high concordance (>90%) between sputum and OP cultures, but did not specifically look at non-expectorating, young patients and did not compare results in individual patients.

To determine whether clinical correlates could be used to improve the predictive ability of oropharyngeal cultures to correctly identify PA, the following parameters were included individually and in combination, in a logistic regression model: respiratory rate, age,
Shwachman-Kulczyki score, Brasfield score, white blood count, immature neutrophil count, and serum exotoxin A antibody titer, as well as OP culture results. Of the eight variables listed above, only two were significantly related to the presence of PA in bronchial secretions: 1) OP culture and 2) serum antipseudomonas exotoxin A antibody titer. The serum anti-exotoxin A titer was a good predictor of PA colonization when included alone in the model. However, it did not improve the predictive value of OP cultures when both were included in the regression model.

The microbiology of the non-expectorating children less than 10 years of age revealed a relatively significant proportion of PA (11/24, 46%) and Klebsiella species (5/24, 21%) in the lower airways. The remaining patients were colonized with SA or H. influenza. Subjects colonized with PA were more likely to have high titers of serum anti-exotoxin A antibodies. They also demonstrated lower Shwachman prognostic scores than patients colonized with SA or H. influenza. Subjects colonized with Klebsiella were much younger (mean age 2.3 years versus 9.2 years) and more likely to have received antistaphylococcal antibodies than patients colonized with other pathogens.

In summary, OP cultures yielding PA and SA are predictive of the presence of those pathogens in lower airways. However, negative OP cultures do not rule out the presence of these pathogens. Bronchi of young CF patients may be colonized with PA frequently preceding their ability to expectorate. These young patients may also be colonized, at least transiently, with Klebsiella species. Further prospective studies in young patients with CF are needed to determine the annual incidence of colonization with PA and other gram negative pathogens in the first years of life.

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Infants and children with cystic fibrosis are affected by the same agents that cause serious lower respiratory illnesses (LRIs) in otherwise healthy individuals. Of the viruses that are primary causes of most pediatric LRI, respiratory syncytial virus (RSV) is the most common, followed by parainfluenza virus types 1 and 3 (see table).

Table Viral agents detected at the time of illness, Tucson Children's Respiratory Study, 1980-1986 (1,2)

| Agent                  | No. identified |
|------------------------|----------------|
| RSV                    | 255            |
| Parainfluenza type 1   | 46             |
| Parainfluenza type 2   | 13             |
| Parainfluenza type 3   | 69             |
| Adenovirus             | 24             |
| Influenza A            | 19             |
| Influenza B            | 6              |
| Cytomegalovirus        | 7              |
| Nonpolio enterovirus   | 21             |
| Rhinovirus             | 8              |
| Herpes simplex         | 2              |
| **Total**              | **467**        |

Influenza C, parainfluenza type 4, human coronaviruses, and Epstein-Barr virus can also cause LRIs, but to a relatively minor extent (unpublished data).

Direct immunofluorescence (FA) (3,4) and enzyme immunoassay (EIA) of respiratory cells or secretions have been widely and successfully applied for rapid (1-2 hour) diagnosis of the common agents, with specificities of over 90% and sensitivities ranging from 76-90%. The selection and proper collection of appropriate specimens is particularly important to facilitate such diagnoses. The following is a summary of procedures that are used:

**Nasopharyngeal (NP) swabs.** A type 1 cotton- or dacron-tipped aluminum-shafted swab. The swab should be inserted deeply into the nasopharynx and left in place for 45 to 60 s. Swabs taken from infants and small children may be smeared directly on wells of slides by carefully rolling the swab on each well.

**Throat Swabs.** Swabs of the throat are of limited usefulness for the detection of most respiratory viral antigens. Pooled NP and throat swabs are helpful if influenza 2, enteroviruses, or adenoviruses are suspected.

**Nasal Aspirates.** Some investigators have found that nasal aspirates are useful specimens. This is especially true if the individuals responsible for collection are trained in the method.

**Oropharyngeal Washes.** Oropharyngeal washes often fail to yield sufficient numbers of ciliated epithelial cells for the detection of respiratory viral antigens, and are not recommended.

**Bronchoalveolar Lavage (BAL).** The diagnosis of cytomegalovirus pneumonia in immunocompromised patients is often achieved by using BAL. Although common respiratory viruses virus can be isolated from BAL specimens, the diagnosis of such infections can usually be made by less invasive techniques.

**Lung Tissue.** The presence of viral antigens in lung tissue may be evaluated by the preparation of impression smears or frozen sections.
Cells. Pleural fluid as a source of cells is of limited value for antigen detection. Often these specimens contain of erythrocytes and leukocytes which may nonspecifically bind the antisera or conjugates.

Finally, broader application of new technologies has also revealed that co-infections by other viruses, mycoplasma or chlamydiae are relatively common. For example, as many as 9.5% of RSV-positive specimens studied also had at least one other pathogen (3); more recent studies indicate that the true incidence is as high as 28% (unpublished data).

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S12.1 Retrovirally Expressed CFTR cDNAs in Airway Epithelia

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CFTR function in vivo likely reflects targeting of the protein to the apical membrane. In vitro complementation studies of the chloride permeability defect in nonpolarized cystic fibrosis (CF) epithelial cells strongly imply that exogenous CFTR is also functional (1,2). Exogenous CFTR function in differentiated or polarized epithelial monolayers has not been reported. Using an amphotropic murine retroviral vector containing the normal CFTR cDNA driven by the viral LTR and the neomycin resistance gene driven by the SV40 promoter, we assayed for functional correction of the CF phenotype in CF airway epithelial cell lines before and after differentiation into functioning epithelial monolayers.

Clonal cell lines expressing the normal CFTR cDNA and a control cell line expressing a nonfunctional reporter gene, the interleukin 2 receptor (IL2R), were generated from the infection of early passage CFT1 cells with retroviral stocks containing the above genes followed by G418 selection. In some experiments, early passage CFT1 cells were infected with high titer retroviral stocks containing either the normal CFTR cDNA (LCFSN) or high titer retroviral stocks containing the IL2R reporter gene (LISN), and pooled cell lines generated following G418 selection. These clonal and pooled cell lines, in addition to CFT1 cells not infected with retrovirus, were then plated onto coverslips or plastic for studies in dedifferentiated cells or alternatively, onto permeable collagen substrates. CFT1 cells plated on permeable collagen substrates differentiate into polarized epithelial monolayers that develop tight junctions, transepithelial resistances, and transepithelial potential differences when grown in 3T3-conditioned media. Functional correction in dedifferentiated cells was assayed on the basis of whole cell currents. Differentiated epithelial monolayers were mounted in modified Ussing chambers interfaced to electrometers and assayed for phenotypic function on basis of the following parameters: 1) the percentage amiloride inhibition of the basal short circuit current (Isc); 2) apical membrane chloride efflux with ion substitution using chloride selective microelectrodes; and 3) response of residual Isc post amiloride to cAMP mediated agonists. To assess the persistence of expression, we assayed for functional correction in both early and late passage cells (~6 months after retroviral infection).

Both dedifferentiated and differentiated CFT1 cells expressing exogenous CFTR demonstrated phenotypic correction of the CF chloride permeability defect. CFT1 cells expressing the normal CFTR cDNA plated on plastic and studied as single cells, exhibited forskolin induced Cl currents whereas noninfected CFT1 cells did not. Cells expressing the normal CFTR cDNA when differentiated on permeable collagen substrates, and studied at the time of maximal transport activity, exhibited a significantly lower %amiloride inhibition of basal Isc, a faster rate of chloride efflux across the apical membrane, and a greater frequency of cAMP mediated chloride secretion than was measured in either the control cells expressing IL2R or CFT1 cells that were not infected with retrovirus. Moreover, the %amiloride inhibition, perhaps our single best indicator of correction, appears to correlate with levels of mRNA expression in different clones. Preliminary studies also suggest a correlation between %amiloride inhibition and CFTR protein levels. Finally, phenotypic correction of the chloride permeability defect has persisted in CFT1 cells maintained in continuous culture for at least six months.

In summary, retroviral-mediated expression of the normal CFTR cDNA does lead to persistent correction of the CF chloride permeability defect in differentiated CF airway epithelial cells. The correlation of mRNA expression with %amiloride inhibition of basal
suggests that a dose effect relationship exists between mRNA, protein, and phenotypic correction. Studies are currently underway to determine the following: 1) the dose or amount of exogenous CFTR protein expression per cell required to achieve functional correction of the CF phenotype; 2) what percentage of CF airway cells must be corrected within a differentiated epithelial monolayer to achieve phenotypic correction of the monolayer; and 3) whether or not overexpression of the CFTR protein is toxic to CF airway epithelial cells.

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Primary and Recombinant CFTR Protein Expression in Airway and Intestinal Epithelia

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Cystic fibrosis (CF) is a lethal autosomal recessive disorder in which abnormal regulation of epithelial Cl⁻ channels is associated with the pathophysiology of the disease. The gene product, CFTR, contains two membrane domains each with 6 potential transmembrane segments, two nucleotide binding folds (NBFs), and a cytoplasmic highly charged domain (R domain) (1). Both the NBFs and the R domain contain multiple potential phosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC) (2). Protein kinases are important physiologic regulators of Cl⁻ secretion, and defective regulation of outwardly rectifying Cl⁻ channels by PKA and PKC is a common defect in CF patients (3,4). Since it has been suggested that CFTR is either a Cl⁻ channel (5) or a regulatory protein closely associated with the channel, we reasoned that one or more phosphorylation sites in CFTR might be important functionally and detectable immunologically. This report describes the identification of CFTR using antibodies raised against peptides containing phosphorylation sites in the CFTR NBF and R domains.

Synthetic peptides from CFTR amino acid residues 415-427 (exon 9, pre-NBF) and amino acid residues 724-746 (exon 13, R domain) were synthesized by the Applied Biosystems peptide synthesizer of the Johns Hopkins University Medical School Protein/Medical Facility, Department of Biological Chemistry. The immunogens were prepared by conjugation of the peptide to bovine serum albumin and polyclonal antibodies were raised in rabbits.

Three expression systems were used to confirm that the anti-CFTR peptide antibodies recognized CFTR in a Western blot assay: 1) recombinant CFTR cRNA was transcribed in vitro and microinjected into Xenopus laevis oocytes (6); 2) recombinant CFTR was stably transfected into COS cells (7); and 3) recombinant CFTR was stably transfected into a CF bronchial epithelial cell line previously demonstrated to retain defective cAMP-mediated regulation of Cl⁻ channels (8). In all three systems, both antisera detected a major 165 kd protein after transfection with wild type CFTR.

Three human epithelial lines known to possess the Cl⁻ secretory phenotype and CFTR mRNA were studied. All three-T84 cells (a human colon carcinoma cell line), FHT (a fetal human tracheal epithelial cell line (9)), and HT29-clone 18 (10) expressed the 165 kd protein when whole cell extracts were analysed on Western blots. To assess the relationship between changes in CFTR gene expression at the mRNA level and those at the protein level, culture conditions were manipulated to increase or decrease CFTR mRNA levels. CFTR protein expression increased or decreased appropriately (10, 11).

Extensive amino acid sequence conservation among members of the family of NBF-containing genes to which CFTR belongs suggested that these anti-CFTR antisera may react with CFTR of other species. This was investigated by PCR amplification of CFTR from rabbit lung cDNA using primers in exons 7 through 13. Sequence analysis of the amplified fragments revealed a high degree of conservation between human and rabbit in the regions of CFTR used to generate the antibodies. The antisera detected CFTR in whole cell extracts of rabbit, sheep, and rat lung and airway epithelial cells. CFTR was also identified in rabbit fetal lungs using both antisera. CFTR was present as early as day 17 of gestation (mid pseudoglandular stage) and increased thereafter in extracts of whole lung homogenates.

The most common mutation associated with CF results in the deletion of a phenylalanine at position 508 within the first NBF (exon 10). The anti-CFTR antisera in this
study easily detect native CFTR in normal and CF human tissue. Anti-CFTR antisera stain
the high molecular weight form (165 kd) of the protein in normal adult bronchial epithelial
cells and cultured nasal polyp epithelial cells from CF patients that are homozygous for the
common mutation, Δphe508. As would be expected, nasal polyp cells isolated from a CF
patient (with mild respiratory disease at age 14) carrying a missense mutation in exon 11 and a
nonsense (stop) mutation also in exon 11 (12) expressed reduced levels of the 165 kd form of
CFTR. Interestingly, an additional protein of increased mobility is visualized (with R domain
antisera only) in a patient with substantial levels of an exon 9 minus CFTR transcript (13).
Cells which would not be expected to express significant levels of CFTR protein either because
they are not epithelial (e.g. fibroblast) or because they do not manifest the secretory Cl−
phenotype, did not have detectable amounts of the 165 kd protein on Western blots.

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Regulation of Chloride Conductance in CFTR-Expressing Pancreatic Epithelial Cells

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Insufficiency of the exocrine pancreas is a prominent feature of CF. Even individuals with normal rates of enzyme secretion have reduced rates of fluid secretion, suggesting that abnormalities in ductal electrolyte secretion are a primary cause of pancreatic dysfunction (1). Microperfusion studies suggest that the mechanism for NaHCO₃ exit by the pancreatic duct depends on apical Cl/HCO₃ exchange to drive HCO₃ secretion in parallel with a Cl conductance for returning Cl to the lumen (2). A defect in the cAMP-dependent regulation of this apical Cl conductance would impair the recycling of Cl to the lumen and abolish the driving force for HCO₃ exit from the cell. Thus, improper regulation of an apical membrane Cl channel could represent a common feature of CF-associated abnormalities in ion transport in both airway and pancreatic cells.

Single channel measurements in primary cultures of human pancreatic duct cells have identified at least two types of Cl channel in the apical membrane (3). Only the small conductance Cl channel (5-10 pS) was activated by cAMP-mediated secretagogues, suggesting that it is the conductance pathway responsible for the Cl recycling step of HCO₃ secretion.

CFPAC-1 is a pancreatic adenocarcinoma cell line that is homozygous for phenylalanine deletion at position 508 (ΔF508) and expresses the appropriate CF gene product (CFTR ΔF508). Anion permeability in CFPAC-1 cells was not altered by cAMP but was increased in response to Ca ionophores, indicating that this cell line stably expresses the CF defect (4). The whole cell Cl conductance activated by Ca ionophores in CFPAC-1 was identical to the Ca-activated Cl conductance, G_{Cl(Ca)} found in a variety of normal and CF secretory epithelial cells (5).

Retrovirus-mediated transfection of cDNA for wild type CFTR (PLJ-CFTR-WT) into CFPAC-1 cells conferred cAMP-dependent regulation of Cl conductance (6). Control-transfected CFPAC-1 cells (PLJ) did not exhibit a cAMP-responsive Cl conductance. The Cl conductance evoked by cAMP, G_{Cl(cAMP)} had identical biophysical and pharmacological properties to G_{Cl(Ca)} found in normal secretory epithelial cells (5). G_{Cl(cAMP)} had a rectified current-voltage relation (IV), showed no time-dependent kinetics, was insensitive to blockade by stilbene disulfonates and exhibited the anion permselectivity Br > Cl > I. These properties differ from the G_{Cl(Ca)} which had a linear IV, showed time-dependent kinetics, was stilbene sensitive and had the halide permselectivity I > Br > Cl. G_{Cl(cAMP)} was found in CFPAC-1 PLJ and PLJ CFTR WT cells.

Forskolin or cAMP consistently activated a 9 pS Cl channel in PLJ CFTR WT cells. The single channel IV was slightly rectified in cell attached recordings (chord conductance of 10 pS vs 7 pS at +/- 80 mV) but became linear upon excision into symmetrical bath and pipette Cl concentrations. Channel activity was insensitive to disulfonic stilbenes. Anion substitution on extracellular side of the channel indicated a halide permselectivity Br = Cl > I. Channel activity
usually disappeared within seconds of patch excision even in the presence of the catalytic subunit of PKA, ATP, Mg, GTP, and cAMP. The 9 pS Cl channel was not activated by cAMP in nontransfected or control-transfected CFPAC-1 cells. The cAMP responsiveness, linear IV, stilbene insensitivity and halide permselectivity sequence suggest that the 9 pS Cl channel underlies $G_{\text{Cl(cAMP)}}$ in CFTR-expressing CFPAC-1 cells.

A distinct cAMP-activated Cl conductance, $G_{\text{Cl(cAMP)}}$, appears exclusively with expression of CFTR WT in the CF pancreatic cell line CFPAC-1. The single channel basis of $G_{\text{Cl(cAMP)}}$ in CFTR transfected cells has properties similar to the single channel evoked by cAMP in primary cultures of normal pancreatic duct cells. Whole cell and single channel studies suggest that $G_{\text{Cl(cAMP)}}$ represents the specific expression of CFTR WT in CF epithelial cells.

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Demonstration That CFTR Is A Chloride Channel And Identification Of Amino Acids That Determine Anion Selectivity.

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Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). In cystic fibrosis, cAMP fails to open apical membrane Cl- channels; as a result epithelial Cl- secretion is defective (1). Expression of wild-type CFTR corrects the Cl- channel defect in cystic fibrosis epithelia (2,3,4), however these results do not identify the function of CFTR.

To test the hypothesis that CFTR is itself a Cl- channel we expressed CFTR in cells that do not normally contain CFTR and do not have cAMP-activated Cl- channels. Expression of wild-type but not CFTR containing the AF508 mutation generated cAMP-activated Cl- current in HeLa cells, Chinese hamster ovary cells, and 3T3 fibroblasts (5). Kartner et al. (6) demonstrated that expression of CFTR also generated cAMP-activated Cl- channels in insect cells.

We found that the properties of Cl- channels generated by CFTR were similar to those of endogenous cAMP-regulated Cl- channels in the apical membrane of secretory epithelia (7). Both were blocked by the Cl- channel inhibitor diphenylamine-2-carboxylate (0.5 mM), but not by extracellular 4,4'-diisothiocyanostilbene-2,2'disulfonic acid (0.5 mM), Zn2+ (100 μM), or an indanyloxyacetic acid (IAA-94, 40 μM). For both, the majority of current lacked time-dependence at hyperpolarizing (-100 mV) and depolarizing (+100 mV) voltages. And finally, both showed the same anion permeability sequence: Br- > Cl- > I- > F-. These data suggest that CFTR functions as either a Cl- channel or a Cl- channel regulator in multiple cell types. The notion that CFTR is a Cl- channel has, however, been hard to accept, because CFTR does not resemble any known ion channels, but instead most resembles a family of energy-dependent transport proteins (8).

To test whether CFTR is a cAMP-activated Cl- channel, we mutated basic amino acids in the putative transmembrane domains of CFTR in an attempt to alter the channel properties. Mutation of lysine 95 or lysine 335 to acidic amino acids converted the selectivity sequence of CFTR from Br- > Cl- > I- > F-
to I$^-$ > Br$^-$ > Cl$^-$ > F$^-$. However other channel functions were not affected by the mutations: cAMP-dependent regulation was intact, voltage-dependence was unchanged, and selectivity for Cl$^-$ over Na$^+$ was preserved. Mutation of arginine 347 and arginine 1030 to acidic residues did not change the permeability sequence from that of wild-type CFTR, although the relative permeability of I$^-$ to Cl$^-$ increased. Based on Eisenman's equilibrium theory of ionic selectivity (9,10), the anion permeability sequence, Br$^-$ > Cl$^-$ > I$^-$ > F$^-$, suggests that CFTR forms a pore with a moderately high affinity site for anions. Our data suggest that lysine 95 and lysine 335 form at least a part of that site; when the cationic side chains of these amino acids are replaced with anionic residues, the selectivity sequence changes to that of a low affinity site: I$^-$ > Br$^-$ > Cl$^-$ > F$^-$. These data indicate that CFTR is itself a cAMP-regulated Cl$^-$ channel, and suggest that lysines 95 and 335 may interact with permeating anions.

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13.1 Folding and assembly of glycoproteins in the endoplasmic reticulum.

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Secretory proteins, plasma membrane proteins and proteins destined to the various compartments of the endocytic and secretory pathways are synthesized on membrane bound ribosomes in the endoplasmic reticulum. For most of them translocation through the endoplasmic reticulum membrane occurs cotranslationally, and they enter the lumen in an unfolded form. Folding of the ectodomains begin while the protein are still being synthesized. The cotranslational phase of folding is followed by further folding events posttranslationally which may take several minutes. For many proteins the folding process involves the formation of intrachain disulfide bonds. Many also proceed to form multisubunit oligomers while still in the endoplasmic reticulum.

While the folding and assembly processes in the ER are ultimately determined by the primary sequence of the proteins, it is increasingly clear that folding in the living cell is an assisted process in which a variety of cellular factors participate. These factors include protein disulfide isomerase which catalyzes the formation of disulfide bonds, proline isomerase which catalyzes cis-trans isomerization of peptide bonds next to proline residues, oligosaccharide transferase which induces N-linked glycosylation without which many proteins cannot fold correctly, and BiP/GRP78, a heat chock protein that associates with many folding intermediates. As a result, proteins of large molecular weight and complex multidomain structure can successfully fold in the endoplasmic reticulum, a feat which is generally not possible to reproduce during in vitro folding experiments.

Recent studies indicate that, in spite of the accessory machinery, a fraction of each protein species synthesized and translocated fails to acquire its correct three-dimensional structure. In the case of some complex oligomeric proteins the incomplete products can sometimes constitute more than half of total protein made. If glycosylation is inhibited, if mutations are expressed or if proteins are expressed in heterologous systems, all the proteins may misfold. To minimize the potential damage caused by defective protein products, cells have developed a system of quality control which ensures that only those molecules that do acquire the right tertiary and quaternary structure are transported to the Golgi complex and beyond. Misfolded, unassembled, or incompletely assembled proteins are retained and slowly degraded without becoming transport competent. Therefore, it is a commonly found that mutant proteins remain in the endoplasmic compartment and fail to be secreted. To analyze the mechanisms whereby the cells can distinguish between correct and incorrect protein structures is now under intensive study. Multiple mechanisms are evidently involved.
We are studying membrane protein folding, oligomerization and quality control using viral glycoproteins as our main model systems. They are large Type I membrane proteins and some are quite well characterized and therefore excellent as models. For Influenza hemagglutinin, which we have used most extensively, the X ray structure of the ectodomain is known. The synthesis, folding, oligomerization, and intracellular transport follow the rules and pathways of normal plasma membrane proteins. In the lecture I will go through the fate of this protein and other viral glycoproteins from co-translational folding to surface expression. I will also show what is known about the fate of misfolded viral glycoproteins: why they remain in the ER and what determines their retention and degradation. The cell biology of protein folding, oligomerization and intracellular transport is of general importance for understanding the pathology of numerous genetic diseases.

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The CFTR protein is a transmembrane protein which appears to ultimately reside at the apical surface of epithelial cells where it is responsible for chloride channel activity and/or regulation (1). As with all such membrane proteins, the CFTR protein must be processed and trafficked through the ER and Golgi complex before it is sorted and delivered to the correct, apical membrane domain. Recent studies have suggested that the mutant, Δ508CFTR protein may be improperly trafficked and sequestered in the rough ER or early Golgi complex and its subsequent absence from the cell surface results in the CF phenotype in transfected COS cells (2).

In recent studies we have explored the role of signal transduction proteins, G proteins, in the regulation of vesicular trafficking of newly-synthesized proteins to the cell surface. One of the heterotrimeric GaI subunits, αI-3 is uniquely found in the Golgi complex of epithelial cells (3). GaI-3 is a resident Golgi protein which is associated with the cytoplasmic surface of Golgi cisternae. In some epithelia, GaI-3 is also localized at the apical cell surface where it regulates Na+ channels (4). In order to study the functions of GaI subunits we have overexpressed different Ga subunits on Golgi membranes or at the cell surface by transfection into epithelial cells on inducible promoters (3). These cells have been utilized to study the effect of overexpression of GaI-3 on Golgi cisternae on the trafficking of a constitutively trafficked secretory heparan sulfate proteoglycan (HSPG) (5). We found that overexpression of the αI-3 subunit retards HSPG secretion and causes it to accumulate in the medial-trans Golgi (5). This effect is reversed by treating the cells with pertussis toxin which specifically ADP-ribosylates, and uncouples, GaI-3. Activation and inhibition of both heterotrimeric and monomeric G proteins respectively, with GTPγS, blocks intracellular trafficking at several different stages between the ER and the cell surface. These data are the first evidence that heterotrimeric G proteins are involved, not only in the regulation of ion channels and other effectors at the cell surface, but also in the trafficking of proteins to the cell surface. The possibility that GaI-3 is also coupled directly to CFTR or a related transmembrane protein on Golgi membranes is being explored.

It is becoming clear that a variety of G proteins are important in the regulation of vesicle trafficking in the exocytic and endocytic pathways. The epithelial cells effected by cystic fibrosis rely heavily on these pathways for the placement and regulation of membrane proteins and also for the secretion of mucins. Activation or inhibition of specific G proteins with nucleotides or
toxins or by genetic manipulation could be invoked as a means of altering or manipulating intracellular trafficking patterns, for instance to increase transport of Δ508CFTR to the cell surface.

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Biosynthesis, Maturation and Function of CFTR

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The gene associated with cystic fibrosis (CF) encodes a membrane associated N-linked glycoprotein called CFTR. At least three forms of CFTR have been identified, these are believed to represent the primary translation product (band A); a partially glycosylated intermediate (band B) and the fully glycosylated mature version of the protein (band C). Since band B can be synthesized in vitro in response to CFTR cDNA in the presence of added dog pancreas membranes, it is believed that this version of the protein is produced in the endoplasmic reticulum. By contrast, since addition of terminal carbohydrate residues occurs in the Golgi, it is assumed that band C is made in this organelle (1).

The most common mutation associated with CF is the ΔF508 deletion. When the synthesis of ΔF508 CFTR was studied by transient expression in COS cells, the synthesis of band B CFTR appeared normal but no band C could be detected (2). This result has been reproduced in mouse C127 cells stably transfected using BPV based vectors. Pulse-chase studies show that a high proportion of wild type nascent band B is degraded, but a small proportion matures to form band C. However, none of the nascent ΔF508 protein matures as detected by the appearance of band C. Identical results were obtained in both COS cells and C127 cells.

We interpret this result to mean that the ΔF508 mutation prevents the correct folding of nascent CFTR and that the misfolded protein is recognized by a well documented quality control mechanism that retains the protein in the endoplasmic reticulum where it is subsequently degraded. If this were the case, the ΔF508 version of CF would be caused by the failure of CFTR to traffic correctly, to mature and to appear at the correct cellular location.

One feature of the mutations associated with CF is that many of them cluster within a single domain of the protein - the first nucleotide binding domain (NBD). Thus, of 15 CF-associated missense or deletion mutations studied, 12 map to NBD 1 and 3 map to NBD2. We introduced mutations into the two NBDs of CFTR to 'mimic' changes associated with CF, to produce equivalent mutations in both NBD's or to alter highly conserved residues in either NBD within sequences thought to be associated with nucleotide binding (3). Synthesis and maturation of CFTR were studied by transient expression in COS cells. The ability of the altered proteins to generate cAMP-stimulated anion efflux was assessed using SPQ fluorescence in HeLa cells containing mutated plasmid-DNAs (4), and in some cases by patch clamp analysis (5). The results show that cells transfected with plasmids bearing CF-associated mutations commonly, but not exclusively, lack mature CFTR. Furthermore, all mutants lacking mature CFTR fail to activate CI\(^-\) channels in transfected cells. This data suggests that the absence of mature CFTR is a common feature of CF and is responsible for the defective CI\(^-\)channel activity detected in airway epithelia.
The results also show that NBD1 is much more sensitive to the effects of equivalent mutations than is NBD2. For example, 7 of 8 NBD1 mutants lack mature CFTR and all lack functional activity, whereas only 1 of 6 NBD2 mutants fails to make mature CFTR and 2 of 6 lack functional activity. Since all but one CF-associated mutations tested lack functional activity in the transient expression system studied and since these findings are consistent with the observed uneven distribution of CFTR missense mutations between NBD1 and NBD2 of CF patients, the results in HeLa cells appear to reflect events occurring in airway cells. However, the low sensitivity of methods presently available has prevented a study of the maturation of CFTR in non-recombinant human cells.

Other studies have shown that a mutant in which the two asparagine residues (892 and 900) predicted to be the glycosylation sites on CFTR were changed to glutamine resulted, as expected, exclusively in the synthesis of the band A version of the protein (2). However, this molecule retained the ability to induce cAMP activatable Cl− channels (3). We interpret this to mean that the non-glycosylated CFTR is able to fold correctly, traffic and function. This result establishes the important point that the absence of fully glycosylated CFTR is a consequence of the problem commonly associated with mutant versions of the protein not the cause of the problem. It is consistent with the earlier suggestion that most CF is caused by the failure of CFTR to traffic correctly (2). In this regard, the molecular basis of CF would mimic that causing the Z-form of hereditary emphysema, where α-1 anti-trypsin fails to traffic normally in liver cells, and that causing some forms of familial hypercholesterolemia where LDL receptor fails to reach the cell membrane.

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IDENTIFICATION AND CLONING OF A C1 CHANNEL
FROM EPITHELIAL CELLS

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Using an IAA-94 column we purified four proteins (Mr 95, 64, 40 and
27 kDa) and the column eluate was able to reconstitute C1 channels. Because
the affinity of IAA-94 was only 1 μM, it was likely that some of these
proteins were merely "drug-binding" proteins rather than C1 channels. Indeed,
N-terminal sequence of the 95 kD protein showed that it is the Na,K,ATPase
which is known to be inhibited by ethacrynic acid. The 27 kD protein turned
out to be glutathione-S-transferase an enzyme one of those whose forms is
assayed by using ethacrynic acid as a substrate. Since IAA is a derivative of
ethacrynic acid, it is not surprising that we purified these proteins. The 40
kD protein was not very consistent. We made an antibody to the 64 kD protein
which was able to deplete C1 channel activity from solubilized membranes. In
pancreatic epithelial cells from patients with cystic fibrosis, the antibody
stains the apical membrane and intracellular organelles such as golgi and
endosomes. A clone was identified from a bovine kidney cortex cDNA library
and the clone (called H2B) had a fusion protein of about 70 kD. The antiserum
was incubated with the fusion protein and eluted. The anti-H2B antibody was
also able to deplete C1 channel activity from solubilized membranes suggesting
that this protein is indeed the C1 channel or some very necessary component of
it. We have sequenced this cDNA; it does not have the 5' initiation site but
we have an open reading frame that codes ofr 480 amino acids. By primer
extension, there are about 800 more nucleotides 5' to our clone.

The protein has at least three long hydrophobic domains in the open
reading frame which are long enough to span the membrane. There is an
additional hydrophobic segment which is 17 amino acids long that is not an α
helix but is could be similar to the H5 region of shaker channels. However,
there is no homology at the amino acid or nucleotide level with any known
channel or ion transporter. Specifically not to the Torpedo C1 channel, GABA
receptor, glycine R, band 3 or shaker channels.

Sullivan and Field have expressed a cyclic AMP activated C1 channel by
injecting total mRNA from shark rectal gland. An antisense oligonucleotide to
the 5' region of H2B completely blocks the expression of this cyclic AMP
activated C1 current. More recently, they were able to obtain cyclic AMP
activated C1 channels after injection of mRNA of human CFTR. The expression
of this C1 current was also blocked by the H2B antisense oligonucleotide. H2B
protein is expressed in Xenopus oocytes and the antisense oligonucleotides
block the expression of the oocyte H2B protein. H2B protein is present in all
cells examined including SF9 insect cells, CHO, 3T3 as well as in C1 secreting
epithelial cells.
Strategies for gene therapy of cystic fibrosis in the respiratory epithelium have been forthcoming with the cloning of the cystic fibrosis gene (1,2,3) followed by cDNA reconstitution of normal, regulated, chloride conductance (4,5,6) in cell lines derived from patients with cystic fibrosis (7,8). Despite these advances, little is known about the physiologic effects of CFTR expression in the various cell types of the airway. What cell(s) of the airway, ciliated, secretory, basal, or all, are normally involved in the physiologic chloride homeostasis of the airway? Do all cell types in the airway have the ability to confer cAMP regulated currents with transgene expressed CFTR? Are there detrimental effects of ubiquitously expressing CFTR in all cells of the airway? What percentage of the airway must express CFTR to regulate normal chloride conductance? These basic questions must be answered before gene therapy for the cystic fibrosis airway will become feasible. In the absence of an animal model for cystic fibrosis, addressing several of these questions becomes more difficult. Therefore we have been studying a tracheal graft model system in which intact mucociliary epithelium can be manipulated to study the expression a CFTR transgene.

Initial studies have focused on rat tracheal graft epithelium as a model (9,10). We have achieved retroviral gene transfer into primary rat tracheal epithelial cells capable of reconstituting a fully differentiated mucociliary tracheal epithelium. Populations of retrovirally infected tracheal epithelial cells were transplanted into denuded syngeneic rat tracheas and implanted subcutaneously into the flanks of nu/nu balb c mice. Following 9, 21, and 42 days maturation, the tracheas were analyzed immunohistochemically with anti-beta-galactosidase, histochemically with Xgal, and with electron microscopy (EM) for beta-galactosidase positive cells. Following 9 days postgrafting, the epithelium consisted of a predominantly squamous monolayer of undifferentiated cells containing LacZ positive clonal populations as large as 500 cells. This observation supports the existence of a progenitor cell subpopulation with tremendous regenerative potential. Light microscopy analysis of clonal populations at 42 days indicates bacterial beta-galactosidase was present in basal cells, non-ciliated columnar, and ciliated cells, suggesting that gene transfer has been achieved into a progenitor cell(s) capable of repopulating these cell types. EM of 42 day grafts allowed for the identification of cell types not distinguishable by light microscopy such as: intermediate cells (non-ciliated columnar without secretory granules), serous cells (non-ciliated columnar with dense secretory granules), and brush cells. Additionally EM has identified gene expression, by the presence of dense X-gal precipitate, in both serous and intermediate cells. By altering the promoter driving the LacZ gene we were able to define transcriptional units with high levels of expression in ciliated and/or non-ciliated columnar cells.

Using retroviruses, we have developed an experimental system for studying 1) the expression of a transgene in differentiated tracheal epithelium and 2) the cellular dynamics and cell lineage relationships of rat tracheal epithelium during graft repopulation. In better understanding the biology of the airway epithelium, we will be able to more clearly address the questions necessary for effective gene therapy in this tissue. Namely, 1) what promoters will give high levels of expression in the targeted cell type, 2) what cell type should be targeted for prolonged expression of the gene (i.e., what is the primordial stem cell of the airway), and 3) what is the effect of overexpression and/or ubiquitous expression of the transgene on the architecture of the respiratory epithelium? This ex vivo model is currently being applied to the study of CFTR not only in rat but also in other species to which the graft model can be applied.
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Gene expression is often subjected to precise temporal, spatial and hormonal influence in specific cell types. Cystic fibrosis is characterized by abnormalities of fluid and electrolyte transport in epithelial cells related to mutations in the CFTR. Correction of the functional abnormalities in cystic fibrosis may require gene transfer to specific cellular sites within the respiratory epithelium. Cis active elements (promoter enhancers) which direct cell-specific gene expression to discrete regions are located within discrete sites, sequences of DNA, located within or close to genes. Such elements can be identified, sequenced and utilized to direct the expression of genes of interest to specific cell types in cells or transgenic animals. We have isolated the genes encoding four distinct proteins whose expression is confined to respiratory epithelial cells in mammalian lungs. A variety of techniques (DNAase hypersensitivity, gel retardation assays, transfection assays and generation of transgenic mice) have been used to characterize the cis-active elements of these genes.

Promoter enhancer elements were identified from the SP-A, SP-B, SP-C and CC10 genes. Transgenic mice were made in which high level expression of cDNA'S was directed to tracheal-bronchial or bronchial-alveolar regions of the lung. Transgenic mice were made bearing the constructs in which the lung epithelial promoter elements were designed to express the human CFTR at specific sites within the respiratory tract. It is hoped that such elements will be useful in generating new animals to assess the role of CFTR and its mutation in vivo and to generate viral or plasmid vectors which can be used to transfer the CFTR to specific pulmonary epithelial cells.
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Adeno-associated Virus Vectors and Complementation of Cystic Fibrosis

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Gene therapy with viral vectors represents a possible approach to treatment of a variety of human diseases including cystic fibrosis. There are several criteria which are important in developing useful viral vectors for gene transfer into human cells. For cystic fibrosis desirable criteria include tropism for airway epithelium, efficient integration into the host cell genome and the ability to be delivered to the airway by aerosol. One virus with these properties is adeno-associated virus serotype 2 (AAV).

AAV is a human parovirus which is defective and grows efficiently in culture only in cells which are also infected by a helper virus, usually an adenovirus or herpes virus. AAV also is defective in the normal host. In an outbreak of adenovirus respiratory infection in a pediatric population, seroconversion for adenovirus was accompanied by seroconversion for AAV2. In cell cultures AAV grows in cells derived from most human tissues including airway epithelium.

Generally, infection in the absence of helper virus results in highly efficient covalent integration of the AAV genome into host cell chromosomal DNA apparently at a site on chromosome 19. Integration of AAV does not alter cell growth or morphology and AAV has not been associated with any known disease. These properties suggest AAV as a useful vector for integration of genes into cell chromosomes.

AAV has a single-stranded, linear 4.7 Kb DNA genome. Each particle contains one single-strand but strands of either complementary sense are encapsidated with equal efficiency and are equally infectious. Replication occurs in cell nucleus via double strand DNA intermediates. Each end of both strands consists of a 145 nucleotide long, inverted terminal repeat (ITR) which comprises the cis-acting replication origins (ori). The remainder of the genome contains two major genes. The left half of the genome comprises the rep gene that is transcribed from both the p6 and p19 promoters to yield four rep proteins, Rep78, Rep68, Rep52 and Rep40. The right half comprises the cap gene and is transcribed from p40 to yield the three capsid proteins VP1, VP2 and VP3. The rep gene is required for replication of AAV DNA. The cap gene is required for packaging of the progeny genomes into viral particles.

AAV genomes cloned into prokaryotic plasmids (pAAV) are infectious when introduced into helper-infected mammalian cells. pAAV derivatives also can be used as transient expression vectors or as transducing vectors for stable integration and expression. In transient assays foreign genes can be expressed from AAV transcription promoters after transfection of the vector plasmid into mammalian cells. Also stable transfectants could be obtained after transfection of cells with pAAV derivatives which expressed reporter genes such as neo or cat from the AAV p6 or p19 promoters. pAAV derivatives deleted for rep or cap or both genes, but which retain the cis-acting ITRs, can be replicated and packaged into AAV particles after transfection into adenovirus-infected cells if the two AAV gene functions are provided by complementation with a second packaging plasmid. Infection of cells with these AAV particles (AAV transducing vectors) represents a more efficient way of introducing stably expressed genes than most transfection protocols.

A maximum of 5 kb of DNA can be packaged into AAV, but this must include one copy of the ITR (145 nucleotides) at either terminus. Since the CFTR coding region is 4440 nucleotides long, there is very little space left for transcription in any AAV-CFTR transducing vector. One approach was to use the AAV p6 promoter which forms a compact cassette together with the left hand ori (AAV
nucleotides 1-321). This cassette is only 154 bases longer than the minimal origin.

In initial experiments we investigated expression of reporter genes \textit{cat} or \textit{neo} from an ori-p\textsubscript{0} promoter cassette (pAAVp\textsubscript{0}cat and pAAVp\textsubscript{0}neo). Plasmid constructs were transfected with cationic liposomes (Lipofectin) into immortalized CF cell lines derived from the bronchial epithelium (IB3-1 cells) or pancreas (CFPAC cells) of CF patients. The maximum expression was obtained with the entire ori-p\textsubscript{0} cassette and this was about 5 to 10 fold higher than from an SV40 promoter (pSV\textit{cat}). Deletion of regions of the ori (nucleotide 1-149) decreased \textit{p\textsubscript{6}} activity. This, suggests that the AAV ori may have an enhancer effect on \textit{p\textsubscript{6}} in IB3-1 cells. Also, in IB3 cells, pAAVp\textsubscript{0}neo yielded stable transfectants expressing geneticin-resistance (\textit{gen'}) about 5 fold more efficiently than pSV\textit{neo}. These results show that, within the packaging constraints imposed by the size of the CFTR cDNA, the ori-p\textsubscript{0} cassette will be a useful promoter in AAV transducing vectors in airway epithelial cells.

To further characterize the AAV p\textsubscript{6} promoter for complementation of the CF defect in airway cells we constructed the vector plasmid pAAVp\textsubscript{6}CFTR (expressing the CFTR cDNA from p\textsubscript{6}). The pAAVp\textsubscript{6}CFTR plasmid was cotransfected (using Lipofectin) along with pAAVp\textsubscript{0}neo into IB3 cells. Control cells were transfected with pAAVp\textsubscript{0}neo alone. After geneticin selection, \textit{gen'} clones were screened for CFTR expression by immunoblotting and \textsuperscript{36}Cl\textsuperscript{-} efflux. 13 clones were characterized and showed increased expression of the 165 Kd CFTR protein (some at the level of the T84 colon carcinoma cell line). 8 of the 13 clones showed a characteristic forskolin-induced stimulation of \textsuperscript{36}Cl\textsuperscript{-} efflux. Control cells transfected with pAAVp\textsubscript{0}neo alone (N6) and the parental IB3 cells showed very low levels of CFTR and no forskolin-induced response in the \textsuperscript{36}Cl\textsuperscript{-} efflux assay. Additional studies were performed on two of the complemented clones (C38, C39) and the control N6 cells and parental IB3 cells. Both clones had a two fold increase in CFTR mRNA as measured by slot-blot assays. Immunofluorescent staining was performed using a polyclonal rabbit antibody directed against an oligopeptide on the amino terminal nucleotide side of the first nucleotide binding fold followed by fluorescein-tagged goat anti-rabbit antibody. The C38 cells showed bright cytoplasmic and perinuclear fluorescence whereas control N6 and parental IB3 cells showed only faint staining. These studies showed that the pAAVp\textsubscript{0}CFTR vector can efficiently complement the CF defect in stable transfectants of CF airway cells.

To generate AAV transducing vector particles we cotransfected the vector plasmid pAAVp\textsubscript{0}neo and a packaging plasmid (to supply \textit{rep} and \textit{cap} functions) into adenovirus-infected cells. From the resulting cell lysate AAV transducing particles containing only AAVp\textsubscript{0}neo genomes were purified by banding in CsCl density gradients. When these AAV transducing particles were infected into IB3 cells subsequent geneticin selection showed up to 60% of the cells could be transformed to the \textit{gen'} phenotype. Thus AAV transducing vectors can stably integrate and express a foreign gene at very high efficiency.

Similar packaging experiments show that the AAVp\textsubscript{6}CFTR vector can also be packaged into AAV transducing particles and purified in CsCl gradients. The packaged AAV-CFTR genome does not appear to have any major rearrangements. We are currently evaluating the efficiency of the AAV-CFTR transducing particles in correction of the CF defect in IB3 cells in culture prior to evaluating the function of such vectors in animals.
Recent results in molecular biology have demonstrated the promise of using nucleic acids as therapeutic agents. This field of study would advance more quickly with the development of efficient delivery systems. Here, we review our efforts in developing such systems.

Liposomes containing cationic lipids such as DOTMA (1) complex with DNA or RNA spontaneously and mediate the transfer of nucleic acids into cells. We have examined the toxicity and transfection activity of several cationic detergents formulated in liposomes (4). Plasmid pSV2CAT carrying a gene for chloramphenicol acetyl-transferase under the control of SV40 early promoter was used as a marker DNA. Liposome composed of 20 mol% cetyltrimethylammoniumbromide (CTAB) and 80 mol% dioleoylphosphatidylethanolamine (DOPE) was able to deliver DNA into cells in a lipid and DNA concentration dependent manner. The transfection efficiency was somewhat lower than that of lipofectin. However, the inexpensiveness and the convenience of the formulation justify its use as an alternative transfection reagent for animal cells. Recently, we have developed other non-toxic cationic liposomes which show high levels of DNA delivery activity (Zhou and Huang, Farhood et. al., unpublished results). Cationic liposomes can mediate a relatively high efficiency of DNA delivery, but the non-specific mold of action limits its in vivo usage. To overcome this problem, we have designed a vehicle which contains three components: a target-specific ligand, a polycation, and a lipophilic moiety. The last two components are supposed to possess transfection activity as those of cationic liposomes. Our results showed that lipopolylysine without the targeting ligand transfers DNA into cell efficiently and without cytotoxicity (5). The next step will be to conjugate antibodies or other ligands to the lipophilic polymer for target specificity.

There are several advantages of using anionic liposomes as a DNA carrier. For example, the lipid composition of liposome can be formulated as to allow the liposome to destabilize in an acidic pH enviroment (6). The pH-sensitive liposomes when endocytosed by cells release their contents into cytoplasm by fusing with and/or rupturing the endosome membrane. Recently we have also showed that protein kinase C activators can be incorporated into the liposome membrane to stimulate the expression of the delivered DNA in host cells (7). Furthermore, antibody can be used to coat the liposome surface and direct the liposomes to a specific target (8). However, the process of entrapping DNA into liposomes with the original protocol was somewhat complicated, time-consuming and with
low efficiency (9). We have developed a new protocol with which DNA can be entrapped into liposomes by freeze-thawing a mixture of preformed small liposomes and DNA (7). The encapsulation efficiency for DNA was up to 46% of total DNA or 18.4 ug DNA/umol lipid. To demonstrate the ability of pH-sensitive immunoliposome to deliver DNA, we have used a plasmid pPCTK-6A and Ltk- cells system (10). pPCTK-6A contains the herpes simplex virus thymidine kinase gene. Expression of the gene allows the thymidine kinase deficient Ltk- cells to survive in the HAT selection medium. Only low level of thymidine kinase activity was detected in cells treated with liposomes without antibody. In contrast, inclusion of the ligand increased the enzyme activity of the host cells by 6 fold. pH-sensitive immunoliposomes transfected cells 8 fold more efficiently than the pH-insensitive immunoliposomes. Using such a pH-sensitive immunoliposome delivery system, we had succeeded in targeting DNA to tumor cells in a nude mouse model (9,10). These studies had laid the foundation for future development of target specific vectors for gene therapy. Supported by NIH grants CA 24553 and AI 29893.

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Regulation of expression of *Pseudomonas aeruginosa* genes encoding determinants of adherence.

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Adherence of *P. aeruginosa* to epithelial cells is in part mediated by surface appendages called pili (1). The expression of the gene encoding pilin, the structural subunit of pili, is controlled by a complex regulatory network. The overall model of regulation of pilin gene expression invokes multiple interactions between positive and negative transcriptional factors. Furthermore, the newly synthesized pilin undergoes post-translational modifications before its secretion and assembly into a functional adhesin on the bacterial surface.

The alternative sigma subunit of RNA polymerase RpoN (σ^54^) is responsible for transcription of the pilin gene, as well as a number of genes encoding metabolic functions and motility (2,3). This sigma factor has been shown to specifically control the transcription of the pilin subunit gene and not the genes encoding the various secretion and assembly functions (4), because expression of the pilin gene under a strong *E. coli* promoter resulted in formation of functional pili, even in a *P. aeruginosa* mutant with a defective rpoN gene. Sequence analysis of the clone containing the rpoN gene revealed the presence of two coding sequences for proteins that have been previously implicated in negative modulation of RpoN activity (5).

Transposon Tn5 was used to obtain a library of mutagenized *P. aeruginosa*, and they were used to identify mutants which are unable to transcribe the pilin gene. Two different mutants were isolated. Using a radiolabeled probe derived from sequences flanking the transposon insertion, cosmids that complement both of the mutants were obtained. Restriction enzyme site mapping of the complementing cosmids revealed that the two genes disrupted by Tn5 are linked. Complete nucleotide sequence of the region showed that these genes encode new *P. aeruginosa* regulatory elements, however, they share significant homologies with the members of the two-component family of sensory and regulatory proteins (6). A product of one of the pilin regulatory genes, called PilR, is similar to prokaryotic transcriptional activators, such as OmpR, NtrC, DctD. The adjacent gene, pilS, encodes a homologue of a group of signal sensors, which includes EnvZ, NtrB and DctB.

The conserved amino acids in each of the pilin regulatory gene clusters suggests a model for transcriptional control of pilin gene expression involving a phosphorelay mechanism (7). PilS very likely is autophosphorylated at a histidine residue near its carboxy terminus, and then transfers the phosphate onto an aspartic acid residue near the amino terminus of PilR. Phosphorylated PilR then binds near the pilin gene promoter and plays a role in transcriptional initiation by the σ^54^ containing RNA polymerase.
The future work will involve identification of the signals that are transmitted by PilS and PilR. Interference with various steps leading to expression of virulence factors by pathogenic bacteria may provide new opportunities to modulate the pathogenic process, especially in chronic infections of patients with cystic fibrosis.

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**Control of Mucoidy in *Pseudomonas aeruginosa***

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The overproduction of the exopolysaccharide alginate causes mucoid colony appearance in *Pseudomonas aeruginosa*. Mucoidy is considered to be a critical virulence determinant expressed by this pathogen in cystic fibrosis (CF). Mucoidy is under control by bacterial signal transduction systems (1,2,3). Similar factors control virulence in other bacterial pathogens in response to environmental cues (4). Bacterial signal transduction is a relatively well understood phenomenon (5) which in the simplest rendition involves a protein kinase and a transcriptional regulator as its substrate. Mucoidy in *P. aeruginosa* is controlled by at least two such systems including AlgR (1) and AlgB (2).

The importance of these findings is several-fold: (i) It may be possible to uncover environmental signals that promote or suppress alginate production. We can already modulate mucoidy under laboratory conditions; once the environmental factors in CF are better understood it may become possible to suppress mucoidy *in vivo*. (ii) Because of the unique design of bacterial signal-transduction systems which have no direct equivalent in eukaryotic systems it may be possible to design specific inhibitors of these systems. Our laboratory is involved in investigating these possibilities.

Some of the most recent studies in our laboratory indicate that mucoidy is closely tied to global regulatory processes in *P. aeruginosa*. We have determined that the algD promoter (which has to be activated in order for cells to attain mucoidy) depends on the far upstream sites (FUS) and a peculiar organization of several AlgR binding sites [RB1, RB2 overlapping with FUS and a more proximal RB3 site(s)] (6,7). The role of FUS has been confirmed by others (8). It is possible that accessory, e.g. histone-like elements provide appropriate local nucleoid conformation to permit productive interactions of AlgR and other factors in the process of transcriptional activation of algD. One such factor is AlgP (H1,L), which has a C-terminal domain similar to eukaryotic histones H1 (9). An overall similarity has also been reported by others (10). Recent immunoelectron microscopy studies (11) showed that AlgP (H1,L) is an abundant histone-like element in *P. aeruginosa*. Because of its possible broader function, AlgP has been given an alternative name H1,L (11). Thus, apart from inputs transmitted by signal transduction systems, additional environmental factors determining global or local nucleoid conformation may be integrated at the algD promoter level.

Although a multitude of environmental factors have been proposed to induce mucoidy, there is currently no consensus opinion on this matter (3), possibly due to the strain differences. This points out a potential problem with reports based on single strain studies that lack additional verification using different CF isolates or mutational analysis. Nevertheless, it is likely that a set of environmental factors such as nitrogen availability (12), presence of nitrate (6,12), oxygen availability (13), nutrient deprivation (14), and osmolarity (12,15), may be involved, and some of them could be significant factors *in vivo*. The availability of electron acceptors may prove to be a critical factor in alginate overproduction. We recently found that alterations in genes controlling heme synthesis affect induction of mucoidy and algD transcription (S. Sonstebey, C.D. Mohr, and V. Deretic). Since synthesis of GDPmannuronic acid (a precursor for alginate) involves double oxidation of mannose, it is likely that there is a regulatory link between the state of electron transport chains and availability of terminal electron acceptors with the activity of genes controlling alginate synthesis.
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Genetic Studies of Exoenzyme S Production in *Pseudomonas aeruginosa*

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The opportunistic pathogen, *Pseudomonas aeruginosa*, produces a variety of extracellular products that aid the establishment and maintenance of chronic infections in cystic fibrosis patients (1,2). One product, exoenzyme S, may contribute to the pathogenicity of chronic infections by causing local damage and promoting the spread of the organism throughout the lung (3). In animal model systems, mutants that do not express exoenzyme S have been shown to cause little or no histological damage to pulmonary tissue (4,5). In contrast, infection with parental strains results in bronchiocentric lesions and extensive parenchymal changes. Treatment of bronchial fibroblasts in culture with as little as 100 ng of exoenzyme S results in cellular membrane damage and vacuolation (6).

Exoenzyme S is a member of the family of ADP-ribosyltransferase proteins and can be purified as an enzymatically inactive 53 kDa. form or an active 49 kDa. molecule (7,8). Amino terminal amino acid sequence analysis from several laboratories suggests that the 49 kDa. form is generated from a 4 kDa. carboxy terminal deletion of the 53 kDa. molecule. To express enzyme activity exoenzyme S requires a eukaryotic protein called FAS or factor activating S (9). The requirement of a eukaryotic protein for activity is also shared by cholera toxin (ARF) and the adenyl cyclases of *B. pertussis* and *Bacillus anthracis* (calmodulin) (9). Exoenzyme S preferentially modifies arginine residues of low molecular weight eukaryotic GTP-binding proteins (10). This subset of proteins includes members of the H-ras and K-ras families. These proteins have been implicated as playing important roles in diverse cellular processes that include growth, assembly of cytoskeletal components, and the trafficking of vesicles through exocytic and endocytic pathways (11). Although the relationship between the enzymatic activity of exoenzyme S and the toxic consequences of infection with exoenzyme S producing strains has not been adequately studied it has been speculated that ADP-ribosylation of key eukaryotic targets may disrupt host defense systems (J. Coburn, personal communication).

Production of exoenzyme S by *P. aeruginosa* is subject to strain variation and can be enhanced or suppressed by manipulating growth conditions. Exoenzyme S yields are enhanced in vitro by growing *P. aeruginosa* in medium that contains chelating agents such as nitrilotriacetic acid or EDTA (8). In contrast, subinhibitory concentrations of the antibiotics ciprofloxacin, tobramycin, and ceftazidime reduce exoenzyme S yields in vitro. Similar antibiotic treatment of rats chronically infected with *P. aeruginosa* protects animals from lung injury without affecting bacterial cell numbers (12). These observations suggest that exoenzyme S production: (1) is not constitutive, (2) responds to changes of the growth environment in vitro and in vivo, and (3) can be examined using a genetic approach.

My laboratory has focused on the genetic analysis of exoenzyme S production in *P. aeruginosa*. The goal of these studies is to develop strategies that reduce toxin expression in vivo and thereby limit the tissue damage associated with infection by exoenzyme S producing strains of *P. aeruginosa*. Transposon mutagenesis combined
with cloning and complementation analysis identified a trans-acting regulatory locus required for exoenzyme S synthesis. Determination of the nucleotide sequence suggests that at least three gene products, ExsC, ExsB, and ExsA are encoded within the trans-regulatory locus. Transcriptional fusions of predicted promoter regions indicate that all three genes are independently transcribed. These results suggest that the regulatory locus is not organized as an operon.

Peptide sequence comparisons has provided insight to the possible functional aspects of ExsC, B, and A (13). ExsC (145 residues, 16,228 Da.) shows 24% homology to the carboxy terminus of FecD. FecD is an inner membrane protein of E. coli involved in citrate dependent iron III transport (14). ExsB (137 residues, 15,026 Da.) shows 29% homology to the VirB protein of Yersinia enterocolitica. Mutants in virB show reduced expression of secreted proteins that are involved in the virulence of this pathogen (15). The most striking homology (56%) is found between ExsA (298 residues, 33,909 Da.) and the family of positive regulatory proteins that includes VirF (Y. enterocolitica), AraC, RhaS, RhaR, EnvY (E. coli) and XylS (Pseudomonas putida) (15,16). These positive regulatory proteins contain carboxy terminal domains with DNA binding characteristics (16). Thus ExsA may function to enhance transcription of the structural gene for exoenzyme S. The role of ExsC, ExsB, and ExsA in exoenzyme S synthesis is being investigated by the expression and biochemical characterization of these gene products in E. coli.

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The Role of Osmoprotectants, Oxygen and Inorganic Phosphate in the Regulation of Phospholipase C Production

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Research in our laboratory is directed at understanding the role of phospholipase C (PLC) in the basic biology and pathogenesis of *P. aeruginosa*. Using biochemical, molecular and genetic approaches we have determined that the genome of this organism carries two genes encoding PLC activity. One gene encodes a hemolytic PLC (PLC-H) while the other encodes a nonhemolytic PLC (PLC-N). PLC-H and PLC-N share significant amino acid homology, particularly in their amino terminal regions. Both enzymes hydrolyze phosphatidylcholine, a major constituent of lung surfactant, however each enzyme has different specificities for other phospholipids. PLC-H can hydrolyze sphingomyelin while PLC-N is not active on this phospholipid. In contrast to PLC-H, PLC-N hydrolyzes phosphatidylserine.

Early studies on the conditions necessary for production of PLC by *P. aeruginosa* revealed that PLC was optimally produced in media rich in glucose but low in inorganic phosphate (Pi). With the discovery that there are two genes encoding production of this potential virulence determinant, it became of greater interest to further examine their regulation. We confirmed the observations regarding regulation of PLC production by Pi and have extended them by discovering that oxygen tension, temperature and osmotic pressure have critical regulatory effects on the production of PLC as well. Thus, it is now clear that the mechanisms of regulation of PLC production in *P. aeruginosa* are considerably more complex than formerly perceived.

The genes encoding PLC production have some interesting similarities and some cardinal differences with regard to their regulation. The production of both enzymes is induced when the Pi concentration is limiting (i.e. <1mM) in the growth environment while production of both enzymes is repressed when the Pi concentration is sufficient (i.e. >10mM). However, there are quantitative and qualitative differences in the response of each gene to Pi. The gene encoding PLC-N is considerably more sensitive to environmental levels of Pi (>30X repressed by Pi) than the gene encoding PLC-H (<8X repressed). Regulation of both PLC's by Pi is at the transcriptional level and dependent upon the *phoB* gene product which is a member of the family of two component regulatory proteins in procaryotic organisms. Furthermore, salient to the potential role of PLC production in the lungs of cystic fibrosis (CF) patients, we have found that the production of both enzymes is regulated at the transcriptional level by compounds that are known to play an important role in osmoregulation in procaryotic as well as eucaryotic cells. These compounds known as osmoprotectants are derived from one of the substrate...
products of PLC cleavage of phosphatidylcholine, the major component of lung surfactant. These osmoprotective compounds include phosphorylcholine, choline, betaine, and dimethylglycine. PLC-H is produced when as little as 0.7mM choline or the other osmoprotectants are added to media that contain excess Pi (i.e. >10mM). In contrast, while the production of PLC-N is not induced by these compounds in excess Pi, its production is further induced by these compounds as much as 10-20X above the levels produced in Pi limiting conditions alone. Neither PLC is produced in a Pi sufficient environment if these osmoprotectants are not present. Also, relevant to the potential role of these enzymes in the pathogenesis of \textit{P. aeruginosa} infections in CF, is the observation that choline will completely overcome the inhibition of PLC production by concentrations of NaCl as high as 500mM. Furthermore, in contrast to the inhibition of PLC production by the high osmotic pressure and ionic strength produced by NaCl, an equivalent high osmotic pressure produced by the nonionic osmolyte sucrose, will not inhibit production of PLC. Remarkably, addition of choline to a high osmotic environment resulting from the neutral osmolyte sucrose, will enhance the production of PLC an additional 10-20X.

There are possible therapeutic benefits which could result from these basic studies on the molecular biology and genetic regulation of PLC. It may be possible to block the production of this virulence determinant by using analogues of the osmoprotective compounds described above. Such analogues could competitively inhibit induction of PLC in the lungs of CF patients thereby reducing the availability of the natural osmoprotective compounds such as choline and betaine. It is possible that by abrogating the production of PLC, the survival of \textit{P. aeruginosa} would be jeopardized in the high osmotic pressure environment of the CF lung.

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Symposia Session Summaries 199

16.1  Everything That Wheezes Is Not Cystic Fibrosis - Or Is It?

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Airway obstruction in the infant with cystic fibrosis (CF) can be caused by mucous plugging, inflammation associated with infectious or non-infectious processes, or increased bronchomotor tone. Zeulzer and Newton, Jr. studied the pathologic progression of lung disease in infants with CF (1). The earliest changes noted were plugging of bronchi and bronchioles by secretions without evidence of inflammation. Secondary inflammatory lesions with purulent exudate, and thickening and infiltration of airway walls with inflammatory cells were followed by bronchiolectasis and bronchiectasis. Bedrossian and coworkers (2) found mucopurulent plugging of airways in 67% of infants less than 4 months of age and in 75% of infants 4-24 months old. In these same two groups, bronchiectasis was noted in 20% and 75%, respectively.

Measurements of lung function may be normal at birth (3,4), but early in infancy even asymptomatic patients may develop changes consistent with small airway obstruction including increased respiratory rate, decreased compliance of the respiratory system, and increased thoracic gas volume (5,6). As symptoms progress, specific conductance decreases below normal values and dynamic compliance may also be decreased (6).

The role of bronchospasm or airway reactivity in the pathogenesis of these functional abnormalities is controversial. In older CF patients, a longitudinal study has demonstrated bronchodilator responsiveness in 95% of subjects (7), and cross-sectional studies have reported responsiveness in up to 40% of subjects (8). Similarly, a cross-sectional study of CF infants who were asymptomatic or mildly to moderately symptomatic demonstrated improvement in forced expiratory flows in 43% after bronchodilator administration (9). The combination of a bronchodilator and chest physical therapy (CPT) improved tidal mechanics and energetics of breathing in asymptomatic infants (10). These authors speculated that infants with CF have increased bronchomotor tone adding to peripheral airway obstruction, but they did not attempt to differentiate the effect of bronchodilators from that of CPT.

Others, however, have demonstrated a deleterious effect of bronchodilator agents on forced expiratory flow (11,12). These authors reasoned that bronchodilators decrease smooth muscle tone in the central airways, making them more collapsible. In the presence of peripheral obstruction by mucous plugging and inflammation, bronchodilators may show a preferential effect on central airway function. Thus, in older patients who have abnormally collapsible large airways resulting from bronchiectasis, a significant decrease in forced expiratory flow can occur after bronchodilator administration.

Likewise, the infant airway is more compliant or compressible
than its adult counterpart (13), so similar bronchodilator responses may occur in the moderately or severely ill CF infant. We have observed infants who, early in the course of a hospitalization, show worsening or no improvement in forced expiratory flows after bronchodilators, but who later demonstrate significant improvement. We speculate that as the component of airway obstruction not responsive to bronchodilators improves with antibiotic therapy and CPT, bronchodilators may have a greater effect on small airway obstruction. Anti-inflammatory drugs which do not alter smooth muscle tone may have a role in the early treatment of acute exacerbations, but no studies have been performed to evaluate their effect.

The increase in intraabdominal pressure associated with coughing or airway obstruction may predispose patients with CF to develop gastroesophageal reflux (GER). In a series of 40 infants newly diagnosed with CF, 20% had symptomatic GER (14). GER may cause lung disease by reflex bronchospasm or by aspiration of stomach contents (15). In the former situation, bronchodilators may be useful; in the latter, where small airway obstruction may result from inflammation and edema rather than from bronchospasm, they may be deleterious.

In summary, wheezing in infants with CF can result from fixed or reversible airway obstruction. CF infants may have an incidence of airway reactivity that is greater than that of the normal population. Because of the greater compressibility of the infant airway, bronchodilator therapy can cause variable results, depending on the cause of the peripheral obstruction.

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Feeding the Infant with Cystic Fibrosis: Calories and Theories

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Infants (birth to 12 months) newly diagnosed with cystic fibrosis often, but not always, present with malnutrition of varying degrees. Factors that may contribute to the severity of malnutrition include how soon after birth the patient is diagnosed, if surgical intervention is necessary, extent of resection, the degree of intestinal malabsorption, the extent of pulmonary involvement, the underlying disease process and any combination thereof. There are obviously inherent problems in studying any group of infants for the purpose of deriving recommendations for nutritional management regarding energy intake and type of formula. In addition, Cystic Fibrosis Centers may have different success rates with newly diagnosed infants achieving normal growth based on differences in aggressiveness with enzyme supplementation for those infants with pancreatic insufficiency.

Theoretically, some patients with cystic fibrosis may require a higher energy intake than normal children to ensure optimal growth. Shepherd et al. (1) studied a group nine CF children, 0.7 to 2.0 years of age, and found that, compared to 16 healthy children the CF group had a significantly higher rate of total energy expenditure (TEE) expressed as Kcalories/day. In this study, questions remain about effect of body composition on TEE and the comparability of the control to the CF group. Although there is no other comparable published study of TEE in CF infants, recent work with older children (2) indicates that there may indeed be a slight increase (5%) in resting energy expenditure between CF and control children.

Formula recommendations for newly diagnosed infants remains a controversial issue. Farrell et al. (3) recommended predigested formula based on improved growth rates in a group of prospectively studied infants fed predigested formula compared to retrospectively studied infants fed regular formula. Holliday et al. (4) compared the growth of human milk-fed and formula-fed infants with CF and concluded that human milk feeding with enzyme supplementation was at least as suitable for adequate growth. Most recently, Brennan et al.(5) report a study comparing predigested to regular infant formula in a group of infants diagnosed when less than six months of age. Growth velocity was similar in both groups.

Our experience includes 30 infants diagnosed since 1985. Formula (predigested, regular or human milk) choice that was based on an individual's presenting problems resulted in excellent growth rates 95 percent of the time.
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Gastroesophageal reflux (GER) has recently been recognized as a previously unidentified gastrointestinal manifestation of cystic fibrosis (CF), and there is a growing interest in characterizing the association and the impact of GER on the clinical course of patients with CF.

While it is not surprising that these two common disorders will occasionally occur in association (1,2), the actual prevalence of GER in the population of patients with CF is not well documented. The initial report of a high rate of radiologically identified reflux in patients with CF was by Fiegelson and Sauvegrain in 1975 (3). However, it was Bendig et al. (4) who first highlighted the significance of the complications of GER in CF patients. In their clinic, GER and its complications of peptic esophagitis, dysphagia, stricture formation, hematemesis and melena were found as frequently as cirrhosis and portal hypertension or diabetes (2-5%). A subsequent review of the records of 809 children followed in the CF clinic at the Hospital for Sick Children in Toronto between 1971-84 revealed that 9% had had a barium meal performed as part of the evaluation of suspected pathogenic GER (5). Vinocur et al. (6) reported that 35% of newly diagnosed CF patients less than 2 years of age had symptomatic GER, yet in another small prospective study Dab and Malfroot (7) reported that 24-h esophageal monitoring was abnormal in 10 successive infants diagnosed with CF. We prospectively surveyed all the children who were 5 or more years of age (n=68, age 13±7 yr) in our CF clinic and found that the prevalence of regurgitation (21%) significantly (p<0.05) more frequent than in a control group composed of their unaffected siblings (0 and 4% respectively). In fact, 7% of CF patients and none of their siblings reported occasional arousal from sleep by regurgitation or heartburn (8).

Because intrathoracic pressure is negative with respect to intra-abdominal pressure, GER would normally occur continuously were it not for the antireflux barrier present at the esophagogastric junction (9). In obstructive pulmonary disorders such as CF, the effectiveness of the antireflux barrier is reduced (10), and there is an increase in the thoracoabdominal pressure gradient favoring reflux (8). Hyperinflation and forced inspiration contribute to a greater negative intrathoracic pressure, while depression of the diaphragm and forced expiration (especially during coughing, wheezing, or chest physiotherapy (11)) cause increased intraabdominal pressure. It is probable, but not yet demonstrated that hyperinflation, depression of the diaphragm and the increased sternovertebral diameter may diminish the contribution that the crural diaphragm makes to the antireflux barrier. Both the acute administration of bronchodilators such as the xanthenes and β-adrenergic agonists, and the elevated fasting levels of cholecystokinin which have been demonstrated in patients with pancreatic insufficiency are known to reduce lower esophageal sphincter pressure (LESP). However, we found no significant association between symptomatic GER and the use of bronchodilator therapy in our CF clinic (8), and no reduction in mean LESP in 9 patients with CF (all of whom were using inhaled β-adrenergic agonists or oral methylxanthenes, and were pancreatic insufficient) compared to controls. It is not known if there is an increased incidence of transient inappropriate relaxation of the lower esophageal sphincter in patients with CF.

**Failure to Thrive.** GER may worsen the nutritional status of CF patients by contributing to reduced caloric intake through a) emesis, b) dysphagia as a result of esophagitis and stricture formation, and c) aspiration-induced exacerbation of respiratory distress which leads to feeding difficulties (4,6,7). Yet, in infants with CF and GER, aggressive treatment of the endobronchial infection will usually relieve the obstructive pulmonary symptoms, reduce the severity of GER, improve feeding and permit growth.

**Esophagitis.** The pathogenesis of reflux esophagitis is multifactorial (9). As discussed, the increased thoracoabdominal pressure gradient and the impaired effectiveness of the antireflux barrier in CF results in a significantly increased exposure of the esophagus to the corrosive effects of acid and pepsin. Prolonged intraesophageal pH monitoring is currently the best method for quantifying the frequency, duration and temporal distribution of GER. We monitored 8 patients with CF (ages 7-26 yr), and poor pulmonary function for a 24-hr interval and found that the number of reflux episodes, the % time pH was <4, the number of episodes >5 min in duration, and the duration of the longest episode were all significantly prolonged compared to published control values (8). Fiegelson et al. (12) performed incidental esophagoscopy in 49 CF patients (ages 1-38 yr) who were undergoing fibro-optic bronchoscopy - 63% had reflux esophagitis and in 51% there was frank ulceration. There was a strong positive association between the severity of respiratory disease and the occurrence of esophagitis. Cystic fibrosis has not been shown to affect gastric secretion or emptying in any fashion that would increase the volume of gastric fluid available to reflux, or the corrosive potency of that fluid. However, when it develops, esophagitis can alter motility and impair clearance of refluxed material (9). Furthermore, enlargement of the submandibular salivary glands is common in individuals with CF (13), and it is possible that their total salivary output and bicarbonate secretion is reduced. This could diminish the buffering capacity of saliva, prolong the time...
and increase the number of swallows required to neutralize and clear refluxed acid. Lastly, the tissue resistance to refluxed material may be impaired in patients with CF if malnutrition is sufficiently severe to reduce the potential for cell turnover in the esophageal mucosa.

**Pulmonary Complications.** Pulmonary function is significantly worse in CF patients with reflux as compared to those without reflux (5). GER could adversely affect pulmonary function in patients with CF through a variety of mechanisms, many of which were first characterized in patients with asthma (10, 14, 15). Firstly, esophageal acid infusion can induce a 10-20% increase in airway resistance (reflux-induced bronchoconstriction). This response is vagally mediated and the esophageal acid receptors exhibit a greater sensitivity in patients with reflux esophagitis. Furthermore, esophageal acid-induced bronchoconstriction seems to amplify the effect of other bronchoconstrictive stimuli. Secondly, very small amounts (0.05 ml) of intratracheal acid can produce dramatic (4- to 5-fold) transient and vagally mediated increases in total airway resistance. Lastly, larger aspirations of acidic solutions, with or without food particles, will result in a chemical and/or infectious pneumonitis. Recurrent chronic aspiration would significantly exacerbate the chronic pulmonary inflammatory process that is characteristic of CF, but has rarely been a problem in our CF clinic even in those patients receiving supplemental nighttime nasogastric tube feeding (8).

A variety of diagnostic methods are available to document the temporal association between reflux, and aspiration or respiratory symptoms (10). We recognize that patients with CF have an increased tendency to GER (8), that pulmonary function is significantly worse in those CF patients with, as compared to those without GER (5), and both reflux and episodes of pneumonia are reported to have been reduced after fundoplication (6). Yet there is currently little more than anecdotal and circumstantial information in the literature to suggest that GER commonly and significantly exacerbates the pulmonary dysfunction in patients with CF. However, the potential mechanism is there and controlled studies will need to be done to clarify the situation.

At the present time, it is my opinion that the pulmonary dysfunction characteristic of cystic fibrosis predisposes to GER. In its turn, GER may contribute to failure to thrive and the development of esophagitis. Although there is a mechanism for GER to adversely effect pulmonary function, there is as yet no data to indicate that this a common occurrence. Initial therapy should be directed towards optimizing treatment of the underlying chronic obstructive pulmonary disease and medical management of the reflux. Recognizing that patients with CF present with a greater than normal anaesthetic and surgical risk, fundoplication should at the present time be reserved for those infrequent patients in whom aggressive medical management fails and there is good evidence to suggest that GER is causing failure to thrive, esophagitis, or repeated aspiration.

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S17.1 Normal Stages of Emotional and Physiologic Eating Behavior

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I. Eating as a complex process
A. Medical perspective
   1. Provision of nutrients to body
   2. Impact of that process on organ systems
B. Social perspective
   1. Caring and sharing
   2. Expressing oneself, manners and choices
   3. Communicating with others
C. Developmental perspective
   1. Sequential pre-programmed changes
   2. Practice and learning to master new skill
   3. Motivation provided by medical and social perspectives

II. Acquisition of eating skills: A theoretical construct
A. Components
   1. Oral motor skills
   2. Medically conducive environment
   3. Psychosocial stages

III. Potential Pitfalls
A. Lack of motivation
   1. Little benefit
   2. High cost
B. Social stressors
   1. Family chaos
   2. Adolescence
C. Medical insult

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| ORAL MOTOR          | suckle reflex | phasic bite | munching | chewing | sucking |
|---------------------|---------------|-------------|----------|---------|---------|
| MEDICAL             | respiratory stability | hunger satiation | food tolerance |         |         |
| PSYCHOSOCIAL        | bonding       | trust       | individuation | autonomy |         |
| NUTRITIONAL         | growth needs  | growth velocity |         |         |         |

| AGE     |
|---------|
| 0 Birth | 3 6 9 12 18 24 3 6 |

Months       Years

AGE
Behavioral and Environmental Factors Affecting Nutrition in CF

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The cause of malnutrition in children with CF is a complex issue related to both organic and psychosocial factors. CF patients have increased calorie needs due to chronic respiratory infections and malabsorption secondary to pancreatic enzyme deficiencies (1,2). While a number of physiological factors may predispose the CF child to decreased hunger and more rapid satiety, little is understood about psychosocial variables that may influence calorie intake in this population.

Psychosocial research on feeding disorders have indicated a role for environmental factors in the etiology and maintenance of problems of obesity, failure to thrive and food refusal. In the area of obesity, for example, it has been found that overweight children eat at a faster rate (3) and leave less food on their plates (4) than normal weight peers. Observational data on parent-child interactions with obese children have indicated important differences between parents and their obese and nonobese children. Mothers have been found to offer less food and fewer second helpings to their normal weight children than to the obese sibling (5) and in general, parents are more likely to attend to children's food refusal than to their requests for food (6). Similarly, studies of parent-child interactions in children with feeding problems of food refusal have found that child food refusal is associated with parental use of coaxing and criticism to increase food consumption (7,8). Few studies have examined these factors in children with CF. In Stark et al. (9) parents of CF children undergoing behavioral treatment to increase calorie consumption reported that their children ate slowly and refused a variety of foods and that they attempted to deal with these behaviors through coaxing, criticism, and often giving in to the child's refusal to eat. In a case report of four CF patients Singer et al. (10) reported similar observations of four CF patients and their parents during a hospitalization for failure to thrive. Parents ceased feeding if the child began crying or showing other signs of distress.

To evaluate the role of environmental factors in poor calorie intake in children with CF, systematic assessment of child eating behaviors and parent-child mealtime interactions were conducted. CF patients and matched healthy peers, between the ages of 6 mos. and 12 years, were recruited at two sites, Rhode Island and San Diego, California. All families were videotaped during three meals which were later coded for child eating behaviors and parent-child interactions. Families also kept 4 day food diaries. To date, data for 10 CF children and their matched controls have been coded and analyzed. The results show that while the CF children were significantly below their
healthy peers in weight for age, \( t(9) = 12.82, p < .05 \), they consumed an equal or greater number of calories than their healthy peers. However, they were not consuming the 120% of RDA recommended for CF patients. Preliminary data on the child eating behaviors show that CF children above age four years take twice as long to eat their meal as control children, 30.44 mins. vs. 13.53 mins., respectively. Parents of CF children gave twice as many commands to the CF child to eat (-.57) than the parents of healthy children (.12). While the frequency of talking during the meal did not discriminate between the two groups, there was a negative correlation found between parent talk and child bites (-.57) for CF children, but not for the healthy controls (.08). There was also a negative correlation between child talk and bites for the CF children (-.61), but not for the controls (.22).

These data suggest there may be differences between the parent-child mealtime interactions in CF children and healthy peers and that these interactional patterns may be contributing to low calorie intake in CF children. In this sample the parents' strategy to increase their child's food consumption appear to be instructions to eat or eat more. However, this strategy appears to be ineffective as the CF children took twice as long to eat the same amount of food as the healthy children. Further, it appears that talking during dinner may function differently across the two groups. Talking is associated with less eating in CF children while there is no relationship between eating and talking with the healthy peers. The application of these results to clinical care of CF children will be discussed.

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Defining diabetes in CF

Diabetes mellitus is traditionally classified as insulin-dependent (IDD) and non-insulin-dependent (NIDD). Specific criteria exist for the definition of these two forms, as well as for a state of preclinical diabetes termed impaired glucose tolerance (IGT). The applicability of the notion of IGT to CF patients given standard testing is dubious, since the test is standardized for people with normal nutritional status, absence of infection, normal liver function, and normal metabolic rate (1). In patients with CF it is probably best to define diabetes on the basis of a distinctly abnormal glycosylated hemoglobin level, an elevated fasting (greater than 140 mg/dl) or post-prandial (greater than 200 mg/dl) blood glucose level or with symptoms (polyuria and polydipsia especially, since weight loss and appetite are variable in CF). Because the diagnosis of diabetes is usually made at a time of stress (illness, nutritional deterioration requiring TPN), awaiting a stable state to test is not feasible.

Importance of diabetes in CF and reasons to treat

The dubious applicability of standard glucose tolerance testing to CF patients is demonstrated by the high rates of IGT in these patients (30-75%) (2). Manifest diabetes, nonetheless, is seen in 9-10% of adolescent and adult CF patients, 30-40 times the frequency of diabetes in the non-CF population of that age group (3). Occasional instances have been reported in younger children. Whether diabetes is an indicator of greater mortality risk is uncertain, but long term complications have been reported and underscore the need for aggressive therapy with longer survival of patients with CF (4). In addition to reducing the risk of long term complications, the reasons for treating diabetes in CF include possible improvement in pulmonary function, and the prevention of acute complications, including infection and hyperosmolar coma. These patients do not develop ketoacidosis, which may be due to the absence of normal glucagon response or to the persistence of a low level of insulin secretion sufficient to prevent runaway ketogenesis but not to maintain normal glycemia, as is seen in NIDD (5).

How to treat diabetes in CF

The treatment of IDD requires the administration of insulin twice daily in a dosage of .6 to 1.0 u/kg/body weight/day total. Diabetes in CF does not meet the criteria for IDD, as noted, because of the absence of ketosis proneness and the persistence of insulin secretion. NIDD is characterized by persistent insulin secretion and a failure of development of ketoacidosis except with extreme stress. In North
America, most (80%+) patients are obese and will have significant improvement in their state of diabetes, to complete reversal, with weight loss and continued dietary restriction. This is clearly not the case for CF diabetes. CF diabetes is most comparable to NIDD occurring in thin individuals who most often require insulin for control, but can respond to oral agents (6). The third generation OHAs (glyburide, glipizide) have been effective in some clinics for maintenance of CF patients with diabetes. Insulin appears to be necessary to regulate blood glucose in times of stress (parenteral nutrition, pulmonary infection exacerbation). Insulin can be administered as a piggy-back solution along with TPN and large amounts may be required to prevent hyperglycemia. Insulin administration, twice daily intermediate-acting with additional short-acting as indicated, should be continued to maintain blood glucose levels in the 120-180 range preprandial and in the 160-220 range postprandial. With persistence of normal blood glucose levels (<120 fasting, <180 postprandial) insulin may be reduced or stopped and OHAs used. Because of the absence of glucagon responses, CF patients may be more prone to hypoglycemia and self blood-glucose monitoring is essential. This should be done initially four times daily before meals and at bedtime and subsequently twice daily. Glycohemoglobin levels should be determined quarterly.

A number of patients have reported that secretions are less viscid and expectoration facilitated when blood glucose levels are low. Whether this is due to direct effects on mucus of the glycemic level or an effect of counterregulatory hormones is unknown, but the perception may result in overzealous efforts to attain blood glucose control which can lead to dangerous hypoglycemia. In CF patients, even more than others with diabetes, the patient must be highly informed and able to make day-to-day decisions about diabetes management with the support of the treatment team.

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NON-TRADITIONAL MANAGEMENT OF DIABETES RELATED TO CYSTIC FIBROSIS

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Cystic fibrosis related diabetes mellitus (CFRDM) is of greater concern to us in the 1990's than it has been the past. This is a consequence of both the increased life expectancy of patients with CF and increased attention we are now giving to "fine tuning" medical management of all patients with CF to improve further their longevity and quality of life. However, the onset of diabetes is associated with significant difficulties in deciding the appropriate management strategies. These difficulties arise because CFRDM has different characteristics than the two main types of diabetes we are used to treating. A second reason is that the treatment program for CF has requirements that appear to be opposite those of diabetes. Finally, the addition of a second chronic illness can significantly complicate the already stressed lifestyle of the patient. Despite these problems, a logical analysis of the problems does suggest a rational approach that should lead to improved health and well being for the patient.

Cystic fibrosis related diabetes mellitus (CFRDM) is not easily classified into either type 1 or type 2 disease categories. It is not typical of type 1 because it occurs at a time in life atypical for the onset of type 1, it is almost always non-ketotic, in general, it is much more easily controlled than type 1 disease and patients with CFRDM may be more prone to hypoglycemia. At the same time, it is not typical of type 2 disease. Its onset is at an age younger than is usually seen with type 2, it is not associated with obesity whereas type 2 disease is strongly associated with obesity, and it is not characteristically associated with insulin resistance.

The classic therapies for both type 1 and type 2 diabetes are at odds with many of the therapies for CF. With diabetes we are usually interested in achieving weight loss or preventing excess weight gain, while in CF management we are frequently working for weight gain. In diabetes, insulin is used for glucose control, while in CF insulin is being used for glucose control and anabolic hormone. The diet recommendation for diabetes emphasize a low fat, low simple sugar diet as opposed to the diet for CF which often works toward increasing fat and allowing or encouraging ad-lib simple sugars in the diet as a way to promote weight gain. The diabetes diet encourages establishing a routine and tightly scheduled meal plan while in CF we tend to encourage an "eat frequently, when you have time and/or are hungry" meal plan. The approach to diabetes management over the last decade has been one of adding increasing complexity to day-to-day living schedules (frequent glucose testing, multiple insulin injections, strict meal planning, scheduled exercise) to reduce the potential for diabetes-related complications while the already very complex lifestyle of the CF patient suggests every effort should be made to simplify all elements of the therapy. The philosophy of diabetes management has been to "look ahead" and maintain strict glucose control to prevent long-term complications. The more limited longevity of CF patients tends to suggest that in respect to the non-fatal medical problems, attention to more immediate goals with corresponding less concern for 20- to 30-year complications might be a better approach. This would be particularly impor-
tant if adding additional therapies for diabetes that might decrease attention to pulmonary care programs.

Treatment of patients with CF who have glucose homeostasis problems is complicated by the many different types of glucose abnormalities seen in these patients. Experience shows that CF patients can have nothing more than an occasional abnormally elevated serum glucose to frank symptomatic diabetes. The various presentations are most likely a consequence and interaction of the size of the individuals' pancreatic beta cell population and the presence or absence of metabolic stress factors that will affect tissue insulin sensitivity, hepatic glucose output and adjunctive therapies (prednisone, i.v., hyperalimentation, etc.). In the most mild situations we might see elevations in the serum glucose values only at times of significant illness or stress. Such stress-associated glucose intolerance could be due to any one or combination of factors. The most common of these would include: 1) physiological delay in insulin release after a prolonged period of decreased caloric intake, 2) mild reduction in functional beta cell number and/or responsiveness, 3) insulin resistance from infection and stress associated increases in cortisol and catecholamine release, 4) prednisone therapy, 5) i.v. hyperalimentation with high concentrations of glucose, and 6) nocturnal enteral feedings. A more significant reduction in functional beta cell number and/or responsiveness would lead to persistent glucose intolerance. Diabetes would be the expected eventual outcome of a further loss of beta cell reserve. In addition, a certain number of individuals with CF would be expected by chance alone to develop ketotic prone type 1 DM or type 2 DM. The family history, the presence of absence of elevated insulin levels or islet cell antibodies may be of value in differentiating these types of CFRDM. While it is unlikely that such information would alter the therapy, such information may be of value for research purposes.

As with all forms of diabetes, the treatment of CFRDM will include insulin injections, diet therapy and routine monitoring of the response to these therapies. However, the unique differences of CFRDM from other forms of diabetes suggests that modifications of the traditional approaches are appropriate. Insulin therapy should include the use of the generally standard use of combinations of intermediate and short-acting insulin before breakfast and again before dinner. However, we should also consider non-traditional insulin and schedules (i.e., regular insulin only before each meal or ultralente humulin with regular before each meal). In order to accommodate the frequent meals and increase size of the meal, it is important to help the patient learn how to make small and large changes in their insulin dose to account for these diet changes. Use of insulin added to i.v. parenteral feedings or a separate insulin drip may also be necessary both in the hospital and during home therapy.

An important goal of therapy in CFRDM, second only to alleviating the classic symptoms of diabetes, is promoting normal weight gain and preventing weight loss. Tight glucose control without hypoglycemia is important but not at the expense of weight loss or decreased weight gain because of a difficult meal schedule and/or decreased palatability of the diet. Meal planning should continue to be based upon the nutritional requirements and practice needed for children with CF. Diet modifications would include more regularly scheduled meals, more consistency in meal size and composition and elimination of snacks with a high simple sugar content as primary source of calories. Moderate
amounts of such items could still be allowed into the regular meal as a dessert if given on a routine basis such that the caloric load could be offset by an appropriate adjustment in the insulin dose. Including the desert in each meal should allow for sufficient consistency that the one and two hour post-meal glucose checks can be used to determine how well the insulin is matched to the calories. Including these simple sugar items at the end of the meal would allow additional time for the regular portion of the insulin dose given 20 minutes prior to the meal to be absorbed and be available to handle the glucose load. The therapeutic goals of treatment would include the absence of symptoms of diabetes, weight gain, normal growth, and achieving post-meal glucose values within the normal range as well as normal fasting and pre-meal values. Fasting glucose values may be normal or near normal even with a marked abnormal glucose tolerance profile following a regular meal thus the post-meal checks take on a greater importance.

Since insulin has significant anabolic effects, the dose of insulin should be increased to that amount that is comfortably tolerated without hypoglycemic symptoms. Our experience shows that most patients will tolerate amounts in the range of 0.5 to 1.0 u/kg/day. This dose is often 2 to 3 times greater than the insulin dose needed for what is usually considered adequate glucose control. However, as the insulin dose is increased, the patient and support team must be careful to monitor for potentially dangerous hypoglycemia.

The addition of a second chronic illness in the day-to-day life of an individual may create serious problems that will require time and attention from support staff trained and experienced with such problems. Anticipatory counseling and monitoring of the patients' psychological health may be necessary to prevent depression or denial of the disease processes and subsequent alienation from the medical program.

There is not yet sufficient data and experience to have a clear understanding of the implications of insulin deficiency and glucose homeostasis problems or the impact of aggressive treatment of these problems on the overall health of the child with CF. There is a need to evaluate the effects of these therapeutic recommendations and alternatives, such as oral hypoglycemic agents. Important variables to study will include not only glucose control but also growth, adolescent development, progression of other elements of the CF disease complex and complications of long standing hyperglycemia and insulin deficiency as well as the effects of therapy on the general well being of the patient.

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Ciprofloxacin in the Treatment of Pseudomonas Infection in Children with Cystic Fibrosis

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Ciprofloxacin hydrochloride is a quinolone carboxylic acid derivative with bactericidal activity against both Gram-positive and Gram-negative bacteria. It is particularly active against the Enterobacteriaceae and many strains of Pseudomonas aeruginosa. Minimum inhibitory concentrations (MICs) are usually within the range of 0.01-2.0 μg/ml. Strains are considered susceptible at ≤1.0 μg/ml.

Arthropathogenic side effects have been reported in some, but not all juvenile animal species following administration of quinolone antibiotics. For this reason, experience with ciprofloxacin in children has been very limited, as there has been concern that the developing child may be more susceptible to toxic lesion in the joint cartilage.

Children with bronchopulmonary infection associated with cystic fibrosis are afflicted by frequent exacerbation of this disease, often requiring hospitalization for treatment with intravenous anti-Pseudomonas antibiotics (often a combination of an aminoglycoside and a beta-lactamase antibiotic). The use of oral ciprofloxacin for treatment of these children as an alternative mode of therapy that would avoid hospitalization seemed to be justified.

The present study describes our experience in treating children with oral ciprofloxacin for pulmonary exacerbation of cystic fibrosis. Twenty-eight children with cystic fibrosis suffering from an acute infectious pulmonary exacerbation were enrolled in this study. Fourteen of these patients received two or more therapeutic courses of ciprofloxacin for a total of 46 courses. The mean age was 10.2 years (range 2.5-17), and they were categorized as moderately to severely ill according to the clinic scoring systems of Shwachman and Kulczyk (1958) and Taussig et al. (1973) and by the x-ray scoring of Brasfield et al. (1979). Twelve of the patients were between 2 and 10 years of age.

All patients were evaluated clinically at the time of enrollment in this study and subsequently at least weekly for the duration of treatment with ciprofloxacin. At least once a week, the following laboratory tests were obtained: complete blood cell count; serum electrolytes; blood urea nitrogen; and glucose; serum creatinine; liver enzymes (SGOT and SGPT), and urinalysis. Chest roentgenogram studies were obtained at least at the beginning and end of each therapeutic course. During the past year, a determination of creatinine clearance was obtained at least once in twenty-four of the twenty-eight patients that had been treated since 1985.

A diagnosis of acute pulmonary exacerbation was indicated by the presence of four or more of the following clinical parameters: fever >37.7°C; change in appearance, volume, or color of sputum; increased respiratory rate or dyspnea; progressive physical findings on chest roentgenogram; deterioration of pulmonary function tests; and decreased appetite and weight loss. Bacteriological cultures of the sputum were obtained before initiation of therapy by the second or third week of therapy, and after completion of the therapeutic course. Pseudomonas aeruginosa was isolated from all the patients except for one in whom P. cepacia was also isolated.

Initial susceptibility to ciprofloxacin was determined for all clinical isolates by the standardized Bauer-Kirby disk method using a 5 μg ciprofloxacin disk (NCCLS, 1984). MICs were subsequently performed on 59 isolates by using the standardized broth dilutions method containing double-fold increasing concentrations of antibiotic (NCCLS, 1987). Susceptibility to other antimicrobics was determined by a commercial MIC system (SCEPTOR, BBL Microbiology Systems, Towson, MD). Serum concentration of ciprofloxacin was determined by a gas chromatographic method.

Ciprofloxacin was administered orally at a dose of 40 mg/kg/day in two equally divided doses (dose range 38.3-45.1 mg/kg/day). Mean duration of a therapeutic course was 30.5 days (range, 21-76 days).

During the first therapeutic course, the median pretreatment MIC of the strains of P. aeruginosa was 0.03 μg/ml (19 isolates). The median MIC at the end of therapy had increased to 0.13 μg/ml (20 isolates). In subsequent therapeutic courses, the pretreatment MIC median value was 0.06 μg/ml (17 isolates).
Following treatment, the median MIC value increased to 2.0 µg/ml (16 isolates). Of these, eight isolates had an MIC of ≥2.0 µg/ml. A strain of *P. cepacia* isolated from one patient had an MIC of ≥2.0 µg/ml. In all but two patients, the resistance exhibited by the isolates of *P. aeruginosa* was transient. Follow-up cultures obtained at least 2 months after completion of therapy yielded strains of *P. aeruginosa* with MICs of less than 1.0 µg/ml.

The mean peak serum concentration at 2 hours after a dose of ciprofloxacin was 3.24 µg/ml (n=29), with a range of 1.07-5.67 µg/ml (Table 1).

The results noted at the end of the 46 therapeutic courses were as follows: Most of the 28 patients improved and returned to their pretherapy state of health after completion of their first course of ciprofloxacin therapy. Clinical response to subsequent therapeutic courses has been less dramatic, although significant clinical improvement was noticed in all patients.

Bacteriologic eradication of *P. aeruginosa* from the sputum cultures was not achieved in any of the patients.

Adverse side effects attributable to the antibiotic were as follows: Two children complained of slight diarrhea, and one patient experienced abdominal pains. A skin rash developed in one patient after exposure to sunlight. One adolescent female patient, who had a previous history of intermittent arthralgia, complained of joint pain while on therapy. In an eleven year old boy ciprofloxacin had to be discontinued due to a marked skin allergic reaction that subsided within two days after discontinuation of the antibiotic and administration of an oral antihistaminic. Roentgenograms of her ankles and knee joints revealed no degenerative changes or any other abnormalities, and the joint pain disappeared promptly after cessation of therapy with ciprofloxacin. Roentgenograms of the knees and ankles were obtained in nine of the younger patients, even though they did not have any signs or symptoms of bone or joint disease. No abnormalities were detected in these studies. Likewise, no clinical or laboratory abnormalities indicative of hematologic, liver, or kidney abnormalities were noted.

The creatinine clearance determination was found to be within normal limits in all twenty-four patients tested.

Oral ciprofloxacin appears to be an effective therapy for the treatment of exacerbation of bronchopulmonary infection with *P. aeruginosa* in children with cystic fibrosis. The results of this trial are similar to those reported with intravenous aminoglycoside and beta-lactam therapy. As with conventional therapy, bacteriologic eradication of *P. aeruginosa* was not achieved with ciprofloxacin, even though there was clinical resolution of the manifestations of exacerbation of bronchopulmonary infection.

Children treated with oral ciprofloxacin experienced clinical improvement by the third to fifth day of therapy and were able to resume their usual activities and continue to attend school. They tolerated the drug very well, and in no instance was it necessary to discontinue ciprofloxacin because of excessive side effects. The most notable side effect occurred in a 16 year old girl, who experienced a moderate degree of arthralgia. This patient had a history of previous idiopathic episodes of arthralgia unrelated to the administration of ciprofloxacin. All symptoms disappeared after the therapeutic course of ciprofloxacin was completed.

Roentgenographic studies of the knees and ankles of nine of the patients in this study revealed no abnormalities. Likewise, no signs of symptoms of hematologic, liver or kidney abnormalities were detected in these children.

The development of resistant strains of *P. aeruginosa* during treatment in this study was comparable to that of other studies in the adult population. As in studies reported previously, the presence of resistant strains in the sputum culture of those patients often appeared to be transient.

The results of this study suggest that ciprofloxacin can be used safely as alternative therapy in children with cystic fibrosis suffering from bronchopulmonary infection with *P. aeruginosa*. The length of therapy should be similar to that of conventional therapy (usually 2 weeks, and possibly up to 4 weeks). If at all possible, an interval of at least 2 months should elapse before more of the resistant strains have disappeared from the sputum culture. Careful clinical, laboratory and roentgenographic monitoring of potential side effects should be planned for children treated with ciprofloxacin.
Mechanical Ventilation in the Severely Ill Adult
With Cystic Fibrosis - Pro

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With improvement in quality of life, longevity and the excitement over potential therapeutic interventions in cystic fibrosis, there is an unbridled optimism for the future of these chronically ill patients. We now have the opportunity to rationally treat patients based on the discovery of the CF gene and its gene product (CFTR) (Table 1). Biochemical interventions seem appropriate with the recent encouraging data on the use of amiloride. There is also new data on the genetically engineered use of proteases and elastases in improving lung function. These advances come on the heel of better nutritional supplementation, antibiotic usage and the national trails of corticosteroids and aerosol antibiotics. With all of these potential and real advances it is premature to lump all patients with cystic fibrosis as DNR (Do Not Resuscitate). Additionally, with the successful transplantation of CF patients on mechanical ventilation, as well as the improvement in transplantation in general (Table 2), there is cause for optimism in treating patients with CF. The use of mechanical ventilation may now have a role in a disease process that has always been typified by DO NOT RESUSCITATE.

TABLE 1
CF Treatment - Where to Now!

Genetic
1. Gene therapy
2. CFTR

Biochemical (Epithelial Cell Defect)
1. Amiloride
2. Cl transport
3. CFTR
4. DNASE

Inflammation and Immune Modulation
1. Corticosteroids
2. Antiproteases
3. Hyperimmune globulin

Antibiotics
1. Inhaled

Transplantation
1. Heart-Lung Transplantation
2. Double-Lung Transplantation

Intermittent Mechanical Ventilation
Table 2

World Experience Heart-Lung and Lung Transplants in CF 1991***

| TRANSPLANT TYPES | CASES | SURVIVING | %    |
|------------------|-------|-----------|------|
| Heart-Lungs*     | 140   | 89        | 64%  |
| Lungs**          | 58    | 40        | 68%  |
| **TOTAL**        | 198   | 129       | 65%  |

* Since 1983
** Since 1986
*** #’s approximate

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VENTILATION OF CF PATIENTS - CON

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"Desperation is a contraindication to a successful lung transplantation."
Dr. James Theodore, transplant physician

"Parents want to donate even when doctors are unwilling to do a living donor transplantation because...it would be futile or too much risk for the donor."
Dr. John Lantos, pediatrician and ethicist

There is nothing inherent about cystic fibrosis that would prevent a physician from initiating mechanical ventilation in a patient who developed respiratory failure. A recent report of an infant who was ventilated from about three weeks to six months of age with subsequent good results demonstrates that the CF lung is capable of healing\textsuperscript{1}. As was discussed in this forum last year, there still is no medical, social, or ethical justification for beginning heroic and uncomfortable therapy on someone who has no hope of recovering enough to discontinue such treatment\textsuperscript{2}. There recently has been considerable renewal of enthusiasm for the ability to perform lung transplantation on CF patients who have been ventilated for some period of time.

Any new procedure which shows promise in the therapy of previously untreatable disease will be hailed with enthusiasm by the medical community. Just as surely, the procedure will be pushed to the limits as soon as possible by physicians who feel in desperate need to do something for their patients. It was inevitable that these caregivers would try to perform lung transplantation on patients who are on ventilators, in spite of poor results from such patients in the early days of this procedure. Reports of successful transplantation of CF patients who were on mechanical ventilation now have come from a number of centers, although most of the successes have had the good fortune to find donor grafts within a few days\textsuperscript{3}. The general tenor of discussion at national meetings now is that it is quite all right to wait until a CF patient goes into respiratory failure, then ship him off to a transplantation center for definitive care.

\textit{This is not all right!} In fact, it is colossally unfair to the patient, the family, and the recipient center. The patient invariably arrives in bad shape, often febrile and poorly nourished. The recipient center has a limited number of choices. They can refuse the patient outright, receive the patient and place him in some sort of administrative limbo until he can be extubated, or utilize a graft that otherwise would have gone to a candidate who was a much better operative risk and who had been waiting patiently for several months for a new chance at life. Carried to the extreme, lung transplant recipients on life support may soon be given a UNOS Category One classification similar to heart recipients, meaning that they will bump all other candidates and receive the first available donor organ. There now is substantial experience at doing partial lung or lobar transplants, so the requirement for a perfect thoracic fit may no longer be so important. The net result of all of this is that an increasing number of grafts will be given to poor candidates, driving up the mortality from the procedure and depriving more suitable candidates of a transplant opportunity. Perhaps more importantly, most of the transportees will not obtain a graft and will therefore die on the ventilator at the new center far away from home and friends and after having incurred tremendous medical and travel expenses.
It gets worse! There now have been at least four patients who have received living donor lung tissue from a close relative. Although these were children, there was at least one instance in which a transplantation center was seriously considering using a lower lobe from each parent for a double lung transplantation for a CF teenager who was on mechanical ventilation (fortunately a cadaver donor was found before this was necessary). There have been a number of inquiries about the possibility of removal of an entire lung for transplantation into an adult relative. It is not too far-fetched to imagine a scenario in which an intubated CF patient arrives by transport with a designated tissue donor relative in tow and expects an almost immediate procedure. The Stanford Institutional Review Board pondered long and hard, then decided to permit the removal of no more than two lobes of a lung for donation. Surgeons at the University of Chicago have decided to do a living donor transplant operation only in situations where it was not an emergency in order to remove pressure from the parents to donate.

Must things inevitably get out of hand? No, not if the basic principles of successful transplantation are followed. Patients who are interested in transplantation should be referred to centers early to receive an evaluation and permit remedial procedures such as sinus surgery and the placement of gastrostomies for weight gain. Families will be able to prepare for the stress of transplantation with the guidance of center counsellors and the assistance of other transplant patients. Adequate screening of patients for potentially serious complications will permit a continued high rate of success for lung transplantation. Most centers now would intubate and ventilate a CF patient who was on their list as an active candidate, at least until that patient became septic or developed multiple organ failure. There is no reason not to ventilate a patient who has a reasonable expectation of being extubated in the near future. On the other hand, mechanical ventilation of a patient with end-stage lung disease who has no realistic hope for a lung transplant is irresponsible medicine.

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