



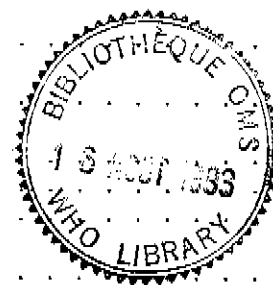
HEREDITARY DISEASES PROGRAMMES
 DIVISION OF NONCOMMUNICABLE DISEASES AND HEALTH TECHNOLOGY

Washington, D.C., USA, 14 October 1992

REPORT OF A JOINT WHO/ICF(M)A MEETING ON THE
 POSSIBILITIES FOR THE TREATMENT OF CYSTIC FIBROSIS

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1. INTRODUCTION¹⁻⁸

The geographical distribution, control, and screening of cystic fibrosis (CF) have been the subject of several joint meetings between WHO and the International Cystic fibrosis (Mucoviscidosis) Association (ICF(M)A).¹⁻⁵ This disease is one of the commonest genetic diseases of the Caucasian race and represents an important chronic cause of suffering and death in childhood and adults. In the past two decades marked prolongation of life has occurred in this disease as the result of improved case-finding and an extensive regimen of therapy. More recently a variety of new approaches in therapy have been developed or proposed as the result of the development of cell physiology and molecular biology.

In 1989, the CF gene was discovered and opened the vision of possible genetic modification of the disease. Since that time considerable progress in understanding the pathogenesis in cystic fibrosis has emerged.⁶⁻⁸

The WHO Advisory Meeting in Hereditary Diseases on consideration of these recent developments in possible therapy of the disease proposed a meeting to review the current status of possible therapies for cystic fibrosis with their implications for treatment in various countries of the world. This report summarizes the presentation and discussions of this meeting.

2. INCREASED SURVIVAL IN CYSTIC FIBROSIS⁹⁻¹⁴

Life expectancy in cystic fibrosis has increased in many nations probably as the result of increased availability of medication, overall vigorous care, and diagnosis of milder forms of the disease. Some uncertainty exists as to whether there is a difference in survival rate between males and females. Early treatment of newborns screened for cystic fibrosis and well-organized cystic fibrosis centres have also appeared to be important components in the care of the CF patient in certain countries. Frequent regular visits to the CF centre have been found to lead to more coordinated and careful care and seem to be correlated with better outcome. It would, thus, appear that an important component of the care of the CF patient with current and projected new approaches to treatment is a coordinated and supportive system of medical care in well-organized cystic fibrosis centres, where the current median survival is about 30 years.

3. THE BACTERIAL INFECTION¹⁵⁻²²

(a) Antibiotic treatment

The patients with cystic fibrosis frequently have repeated or chronic lung infections which produce the major morbidity and mortality of the disease. Intensive antibacterial therapy has contributed to the improved prognosis.

The CF patient has a propensity to develop colonization of the respiratory tract with *Staphylococcus aureus* in the earlier stages, and later with *Pseudomonas aeruginosa*. Careful long-term controlled studies with antibiotics have been difficult to carry out; therefore, many of the antibiotic regimens currently in use have resulted from clinical experience and observations on the longer preservation of lung function, improved clinical status and extension of life.

The use of antibiotics is related to understanding of the course of the lung disease in cystic fibrosis. During infancy or beyond it is often quiescent with CF children experiencing the usual respiratory problems of others in their local community. As they get older they have an increase in cough and respiratory signs and culture of the upper respiratory tract may show *Staphylococcus aureus*. At some point this usually changes to intermittent or chronic colonization with *Pseudomonas aeruginosa*, which has been virtually impossible to eradicate with intensive antibiotic therapy. The microbial population may markedly increase during acute respiratory exacerbations frequently arising from viral infections. The antibiotic therapeutic approach is usually related to these periods of respiratory exacerbation and increased bacterial flora.

A number of key principles for antibiotic therapy have been widely observed to be important in the pulmonary infection of the CF patient, which differ from the treatment of other short-term respiratory infections among normal individuals (Table 1). Emphasis should be placed on microbial diagnosis, high dosage and prolonged courses of antibiotics in the CF patient. The pharmacokinetics for some antibiotics is altered, particularly from increased renal excretion. Inhaled antibiotics also have some clinical benefit as an adjunct or replacement of systemic chemotherapy. Table 2 shows an illustrated programme of antibiotic administration as it relates to sputum bacteriology.

The treatment of *Staphylococcus aureus* infection should be by the oral route. It is not always eradicated even with intensive therapy. It is thought that some of the early respiratory damage in the CF infant is caused by the *Staphylococcus aureus* as a basis for subsequent *Pseudomonas aeruginosa* colonization.

Haemophilus influenzae infection may become chronic in some patients and is found with moderate frequency during acute exacerbations. A combination of amoxicillin with beta-lactamase inhibitors, or ciproflaxacin, are useful in treatment of this infection.

The treatment of *Pseudomonas aeruginosa* in the lungs of CF patients is an intensive process usually involving the combination of two intravenous antibiotics most commonly tobramycin with ticarcillin or piperacillin, in high dosage to reach significant blood levels for penetration into the intraluminal respiratory tract of the lungs. The Danish centre has pioneered an intensive preventive approach for chronic *Pseudomonas* persistence by treating their patients every three months. This pattern of management has markedly changed the process of pulmonary progression in the Danish CF patients. In severe chronic illness aerosol tobramycin or colistin have been found to help stabilise the clinical state and decrease the frequency of exacerbations requiring hospitalizations.

Although some studies have shown little difference between antibiotic and placebo treated groups, such studies have not been convincing enough to alter the vigorous therapeutic intervention against the *Pseudomonas aeruginosa* in most centres. The value of antibiotics is reinforced by the improved mortality seen in those centres with more aggressive antipseudomonal treatment.

In recent years *Pseudomonas cepacia* has appeared in some centres and has been accompanied by considerably increased deterioration in lung function and patient status. *Pseudomonas cepacia* develops antibiotic resistance more easily

than *Pseudomonas aeruginosa*: chronic suppression with doxycycline in high dosage may diminish some symptoms.

The approach to *Pseudomonas cepacia* in some centres has been to cohort the patients with *Pseudomonas cepacia* from regular contact with the other patients with *Pseudomonas aeruginosa*. This step has been found to stem the increased recovery of *Pseudomonas cepacia* in the patient population and suggests that cross infection occurs. As a preventive approach the Danish centre has separated its patient population into (1) those without *Pseudomonas* infections, (2) those with *Pseudomonas aeruginosa*, (3) those with multiple resistant *Pseudomonas aeruginosa*, and (4) those with *Pseudomonas cepacia* in the hope to minimize cross-infection. Some preliminary data suggests that such measures may help prevent the development of more severe *Pseudomonas* infection. At least it clearly appears that direct skin contact or kissing should be avoided in these groups.

Other organisms such as atypical *Mycobacteria*, *Klebsiella*, and *Proteus* may be found and warrant treatment when present. *Aspergillus fumigatus* is not uncommonly grown from the sputum of CF patients and allergic bronchopulmonary aspergillosis develops in some. This can be treated with prednisone.

The cost of antimicrobial chemotherapy can be high in the CF patient and is a major problem in the presence of limited resources (Table 3). Such costs usually exceed normal familial resources and require support from other public or private systems.

The high cost of the antibiotic programme as well as the intensive demands on the patients highlight the need for newer approaches to diminish the vulnerability of the CF patient to pulmonary bacterial colonization. Early preventive efforts, the use of aerosols, oral agents and lower costs for drugs by arrangements with drug companies may be areas for development.

(b) Immunology²³⁻²⁵

The CF immune system in CF responds to the chronic bacterial lung infections with the production of specific antibodies against many bacterial antigens, immune complex formation, the rapid recruitment of neutrophils from the bloodstream and cytokine production. During this type III hypersensitivity reaction, lysosomal enzymes are released which are held responsible for tissue damage. Neutrophil elastase (NE) is by far the best-studied of these enzymes. NE may reach concentrations of more than 100 μg per ml of sputum. About 90% of the endogenous inhibitor for NE, α_1 -proteinase inhibitor, is locally inactivated by NE and probably also by oxidative attack. Thus, a high imbalance between proteinases and proteinase inhibitors is present in the inflamed lungs of these patients. The pathological effects of free NE, demonstrated in a number of *in vitro* and *in vivo* investigations, include cleavage of fibronectin, lung elastin, immunoglobulins and immune complexes, complement, complement receptors on neutrophils, and other receptors on T-cells and B-cells. Furthermore, NE inhibits ciliary beating and stimulates mucus production from goblet cells and facilitates *P. aeruginosa* adherence. Finally, by cleaving receptors for interleukin (IL) 1 and IL 2 or the T-cell antigen receptor, it may hypothetically inhibit message transmission and immune recognition. Thus, besides destruction, chronic infection may lead to a state of acquired immune suppression. These notions have to be investigated in

greater detail in order to design more specific treatment strategies to protect the lung tissues.

(c) Prevention²⁶

Prevention of bacterial colonization in the lungs is another strategy for early treatment of the CF host. Active immunization against *P. aeruginosa* with an exotoxin A-polysaccharide conjugate is currently being studied in non-infected CF children at an early age. Additionally, two passive immunization studies were recently carried out using intravenous gammaglobulin preparations. In both studies transient lung function improvement was noticed in the treatment group and larger studies should be carried out to validate these data.

Bacterial transmission routes in general and especially within the hospital between patients, or between patients and healthy carriers (e.g., hospital personnel) and patients and environmental sources have been investigated using reliable and highly discriminatory typing methods for *P. aeruginosa* and other bacteria. It was shown that normal hand washing without appropriate disinfection thereafter may lead in some cases to contamination with microorganisms, particularly *P. aeruginosa*. Therefore, hygienic measures to decontaminate washing basins and toilets have been recommended as well as improved hygienic measures for hand disinfection. Such approaches may include the installation of heating devices in hospital sink drains. An improved heating device has recently been developed which is currently under clinical investigation. However, improvements in the methods of dispensing and use of disinfectants, isolation of infected patients, and improvements in aseptic techniques, notably the use of gloves for many nursing procedures, have also been proven to be successful in reducing *P. aeruginosa* infections in hospitals.

4. RESPIRATORY DISEASE - NEW THERAPIES²⁷⁻²⁹

The manifestations of CF in the lung are complex and change during the evolution of the lung disease. The primary problem is one of airway epithelial electrolyte and fluid balance which reduces clearance of airway secretions and leads to abnormalities in the airway surface micro-environment. This is probably most important early in life, whilst later the epithelium is injured, perhaps by viral infection, leading to inflammation which predisposes to a vicious cycle of bacterial infection and continuing host defence-based injury. Current policies of antibiotic treatment for the three main bacteria causing injury, *Staphylococcus aureus*, *Hemophilus influenzae* and *Pseudomonas aeruginosa* have only a small impact on halting the progression of lung destruction in this disorder.

A range of treatments have been proposed to address the two important aspects which contribute to lung destruction in CF:

- (a) Agents able to improve clearance of secretions from the lung.
- (b) anti-inflammatory or anti-injury mediator therapy.

With regard to clearance mechanisms, there are a variety of therapeutic agents which aim to normalize defective electrolyte transport. However, in patients with established airways infection one of the major factors leading to decreased secretion clearance is the presence of host derived DNA. Although the thickened secretions consist of those secretions normally produced by patients with CF and host derived

products of plasma exudation in response to infection, a major factor is DNA derived almost entirely from disintegrated inflammatory cells, such as neutrophils. It is an inherent property of DNA to form thick viscous gels and interaction with mucus already present in the airways leads to extremely adherent secretions. DNA may reach high concentrations (up to 15gms/l) in sputum. Studies with aerosolized recombinant human DNase have suggested that cleaving DNA in purulent lung secretions may help patients with CF to improve their airways clearance with safety. The patients taking part in these studies noticed improvement in their breathing ability while on the rh-DNase and had objective improvement in both FVC and FEV₁ compared with baseline values. *In vivo* biological activity of the aerosolized DNase product was demonstrated by detection of cleaved DNA in the sputum.

A variety of other agents such as ibuprofen, a broad spectrum anti-inflammatory agent, and corticosteroids, are of interest in suppressing the host inflammatory response in CF. Recently, more specific compounds capable of antagonizing proteases, such as α_1 -antitrypsin, serum leucocyte protease inhibitor (SLPI) and ICI 200,800 have been studied in patients with CF. Of further interest would be the use of agents such as specific anti-cytokines, such as anti-tumor necrosis factor (TNF α) or anti-IL2 or IL8 which may have more profound effects on the inflammatory process. These agents may be specifically designed as receptor blocking agents, or possibly humanized monoclonals capable of stopping the action of cytokines, or other agents already in existence, e.g. pentoxifylline.

Corticosteroids remain to be proven of value following an earlier report of a study in children suggesting that there was preservation of lung function and a reduction of immunoglobulin levels. Recent studies with a higher dose have had to be stopped because of unacceptable side effects, while a lower dose study continues. Pentoxifylline is promising as an anti-cytokine agent capable *in vivo* of inhibiting the transcription of the TNF α gene. Hence, this may go some way to reducing the inflammatory process within the lungs, but more work is needed. A side effect of such an agent may be better control of the nutritional state of patients with CF as TNF α may be a contributory factor to some of the cachexia associated with infection.

The use of anti-elastases is of special interest in preventing destruction of lung tissue. Nebulized human α_1 -antitrypsin has been delivered to the airways in an attempt to correct the imbalance of the protease-anti-protease components in epithelial lining fluid. A preliminary study demonstrated that α_1 -antitrypsin could be safely delivered to the airways and produce biologically active anti-elastase function in the airways. A side effect noted in the initial study is that neutralizing neutrophil elastase in the environment around neutrophils enhanced their ability to kill *Pseudomonas aeruginosa in vitro*.

Therapy with oxygen radical scavengers is another potential way to reduce inflammation. A combination of superoxide dismutase and catalase is needed to completely reduce the superoxide anion radical. Markers for oxidative lung damage have to be developed.

At an early phase, it would seem worthwhile to tackle the airways of those patients who are already infected and undergoing continuous lung injury both from the point of view of improving clearance and of dealing with the consequences of the host inflammatory response in addition to traditional anti-bacterial therapy and physiotherapy. By these means it may be possible to enhance protection of the lung and possibly affect long-term survival. Larger and longer studies need to be carried out in order to define inhibitor deposition after aerosolization, the degree of

proteinase inhibition, aerosolization time and other parameters. Particularly, markers for lung tissue damage by neutrophil elastase or other proteinases have to be developed.

5. PHYSIOTHERAPY³⁰⁻³⁵

Pulmonary hypersecretion blocking airways and paving the way for chronic bacterial colonization with frequent exacerbations is one of the main problems in CF, as it causes airway obstruction and destruction of lung parenchyma. Eventually breathing muscles become over-exerted due to gradually increased pulmonary obstruction. Malnutrition, inactivity, frequent infections and heavy work of breathing lead to muscle wasting and poor endurance. Without an optimal treatment including efficient chest physiotherapy, each infection results in further physical impairment.

Regular chest physiotherapy aims at making leading a normal life possible by counteracting exacerbations and optimizing physical function of the body. The frequent pulmonary exacerbations are counteracted by improving airway clearance with ensuing maintenance or improvement of lung function and diminution of airway destruction due to removal of secretions containing proteolytic enzymes. Physical function of the body is optimized by physical exercises of different kinds. Chest physiotherapy is considered an integral part of the treatment.

Different techniques to improve airway clearance have been developed throughout the world. Results of short and long term studies of mucus clearance techniques have been somewhat controversial. Current techniques to loosen, transport and evacuate pulmonary secretions are:

- Active cycle of breathing techniques. A cycle consists of postural drainage, thoracic expansion exercises, forced expirations and breathing controls. It can be carried out with or without percussion.
- Autogenic drainage. Different modified ways exist.
- Positive expiratory pressure (PEP) used in different ways:
 - PEP combined with forced expirations and breathing controls.
 - High pressure PEP.
 - Oscillating PEP.
- Physical exercise interspersed or combined with forced expirations and breathing controls.

Evaluation of different physiotherapy methods show that a highly individualized approach is necessary and that there is not one universally accepted optimal method. Combined use of the methods is the rule. All the different techniques can be used either exclusively, combined or mixed. Factors such as age, condition of the lungs, mucosal swelling, airway reactivity, airway stability, treatment motivation, culture, individual interest, surroundings, time of the day, and more, affect the content of each chest physiotherapy programme. Over time the most suitable techniques usually change depending on, e.g., disease stage. Only by jointly involving parents, partners, patients and therapists can chest physiotherapy be established as a set routine of daily life and thereby of the treatment. The programme made for each individual must be designed in a way that it is likely to be carried out. All

youngsters are educated to perform the daily treatment independently in an efficient way. Independence is to be looked upon as a possibility where the programme performed and techniques used are checked at regular intervals and changed whenever needed.

Nebulizing therapy with bronchodilators and/or mucolytics should precede or intersperse each chest physiotherapy session, if prescribed and of benefit for the patient. If nebulized cortico-steroids or antibiotics are prescribed they should be taken after chest physiotherapy. Choice of nebulizer and training of inhalation technique are essential for the result of the treatment. Most chest physiotherapy techniques and physical exercises can be carried out without a special device. However, a device or a system made for the patient can be of value. There are a number of these products commercially available but most of them can be custom made. In a few cases it is difficult to obtain expected results without the specific device with which the technique has been developed.

Success of physiotherapy should be monitored by regular pulmonary function tests along with oxygen measurements. Untoward side effects caused by chest physiotherapy are infrequent. All techniques and devices used are continuously being evaluated and developed. New techniques and devices are occasionally introduced. However, designing and performing controlled studies within this field is difficult. Better objective methods to evaluate current treatment is needed. Comparative data are scarce and many questions remain to be answered. Questions such as:

- Is there a logical chronologic order for introduction to various physiotherapy techniques according to patients' ages?
- What are the comparative short and long term benefits of physiotherapy according to patients' characteristics?
- What type of physiotherapy suits best under special circumstances (malnutrition, corpulmonale, pneumothorax, etc.)?
- What are the optimal criteria for successful physiotherapy?
- How to improve acceptance of and compliance with physiotherapy?

6. LUNG TRANSPLANTATION³⁶⁻⁴³

The first successful heart-lung transplant for cystic fibrosis (CF) was performed in the United Kingdom in 1984. Since then approximately 180 CF patients have been transplanted world-wide with encouraging results. During this time other options for lung transplantation have become available. Single-lung transplantation is unsuitable as the native lung would be a source of infection. One block double-lung transplantation with revascularization requires further evaluation. The actuarial survival for 79 patients after heart-lung transplantation at Harefield Hospital in England was 69% to one year. The Papworth English series of 34 patients who were more selected had an actuarial survival of 79% at one year and 66% at two years. A North American series had an actuarial survival to one year of 42%. The Toronto group reported 17 patients with 58% one year survival after bilateral single-lung transplantation. The University of North Carolina has performed bilateral lung transplants in 27 CF patients (100% operative survival) with a 90% one year survival.

The demand for transplantation is far greater than the available organs. It is essential that patients are properly assessed so that no one is transplanted without a proper trial of maximal medical treatment. Indications for transplantation are deteriorating chronic respiratory failure in spite of maximum medical treatment, a severely impaired quality of life and a life expectancy of less than 18 months. The patients must positively want a transplant. Some contra-indications include high dose corticosteroids, psychosocial instability, infection with mycobacteria or aspergillus, other end-organ failure, and gross malnutrition. Factors known to increase the risks are previous thoracic surgery, pleurodesis, ventilation and severe liver insufficiency. The major matching criteria are donor size, patient size, blood group and cytomegaloviral status. Patients require a very detailed medical and psychological assessment before surgery. Routine immunosuppression is cyclosporin and azathioprine with steroids used for acute episodes of rejection. Major post-operative problems are hemorrhage, multi-organ failure, infection, rejection and obliterative bronchiolitis. The reported incidence for obliterative bronchiolitis is about 30% increasing with time after surgery. There are specific challenges when a CF patient is transplanted which the surgical team does not meet with other patients requiring transplantation. All transplant teams treating CF patients should include a physician experienced in the medical treatment of CF.

Lung transplantation is now an accepted technique for management of patients with end-stage CF. The problems that should be addressed in the future are the following:

- (a) At present there is no adequate way of predicting how sick patients are and when they should be transplanted. One individual parameter such as FEV1 less than 30% predicted, for example, cannot be relied upon solely to determine candidacy of the patient for transplantation. Currently CF patients who have evidence of progressive decline in status despite maximum medical and nutritional support is a common criterion. As long-term results of lung transplantation are not satisfactory, it is preferable to delay transplantation of patients rather than accepting them too early. The logistics of organ allocations in each particular country and region must be put into consideration in deciding when a patient should be put on the transplantation list.
- (b) There are particular groups of patients in which we are not certain whether lung transplantation is contra-indicated or not e.g., patients with *Pseudomonas cepacia*, or mycobacterial infections. While some programmes would accept patients with mycobacterial infections, as well as fungal infections, there are concerns about accepting patients with *Pseudomonas cepacia*.
- (c) The incidence of bronchiolitis obliterans is quite alarming and results in the demise or dysfunction of a significant number of transplanted patients on long-term follow-up. Emphasis should be on identifying strategies for the prevention and treatment of bronchiolitis obliterans.

These issues cannot be answered through individual centre approaches. In France the Cystic Fibrosis Transplant Study Group has been developed with the specific purpose of organizing clinical research which would address these problems. A transplant registry with detailed patient information has been set up and could be expanded for the purpose of retrospective analysis as well as for the design of prospective clinical trials.

It is clear, that CF transplants should be carried out only in a limited number of centres where special expertise can be available. It may be more appropriate that poorer countries concentrate their resources on good nutrition, enzyme replacement,

physiotherapy and antibiotics. We should aim to improve medical treatment so less CF patients need transplantation. Malnutrition, upper respiratory tract infection, malabsorption of cyclosporin, diabetes mellitus, salt loss, bowel obstruction, and liver disease can all cause problems.

7. CELL PHYSIOLOGY⁴⁴⁻⁵⁸

(a) Possible approaches to pharmacological treatment of CF

During the past few years, knowledge regarding the basic abnormality in CF at the level of cell function has advanced very rapidly. Despite these advances, it is not yet possible to advocate specific therapies for direct intervention in the disease process. Nonetheless, several cellular defects which may become principal targets for drug therapy are now clearly recognized. Current views of the problems of CF are consolidating around the notion that the gene product of CF is a protein (CFTR) which forms a channel for the passive, conductive movement of chloride through the cell membrane. It is well established that the normal opening or activation of this chloride-specific channel is dependent upon phosphorylation of the protein via a phosphorylating enzyme, protein kinase A, whose enzymatic activity depends upon the concentration of AMP in the cell. In numerous fluid-secreting systems, the concentration of cyclic AMP, and hence the opening of the chloride channel, depends upon stimulating the cell with a β -adrenergic agonist. In CF affected tissues, β -adrenergic stimulation fails to open chloride channels. Even though there is no direct link between the failure to open chloride channels and the pathology expressed in patients with CF, the most prevalent, but perhaps still too simplistic, underlying assumption is that correcting the defect in chloride permeability will markedly ameliorate the disease process.

Three potential approaches to drug therapy are (1) protein synthesis and processing, (2) chloride channel activation, and (3) collateral compensation. As regards the synthesis and processing of CFTR, soon after the gene product was expressed *in vitro*, it was noted that CFTR in CF cells, failed to undergo complete glycosylation. Further immunocytochemical localizations with antibodies to the protein demonstrated that in CF, the protein appeared to accumulate in the cytoplasm in contrast to normal cells where it is primarily associated with the apical membrane. However, it is doubtful that failure to process the protein is the only, and absolute, defect. Insect and amphibian cells induced to synthesize mutant CFTR proteins from corresponding messenger RNA templates, clearly show that mutant CFTR is processed to the cell membrane even though it exhibits different kinetics with respect to stimulation and function after activation. Very recent studies have demonstrated that the failure to process is temperature sensitive, that is, recombinant mammalian cells grown a few degrees below body temperature appear to process mutant CFTR proteins much more efficiently than those grown at normal temperatures. These observations suggest that drugs, which affect the manner in which proteins are folded and processed in order to reach the cell membrane, could have an important role in therapeutic intervention. The fact that at least some chloride conductance can be elicited from mutant chloride channel proteins by certain drugs, may suggest an effect on protein processing. The action of these drugs will be a subject for further study.

Regarding activation of the chloride channel, the observation that extraordinarily high doses of phosphorylating agonists seems to restore at least

some function to mutant CFTR chloride channels suggests that drugs which enhance phosphorylation or inhibit dephosphorylation could potentially be used to "hyperphosphorylate" mutant proteins in order to ameliorate the effect of the basic molecular defect. The success of this approach will depend upon complete processing of at least some portion of the mutant CFTR to the membrane and upon at least some functional activity of the mutant once it is in place in the appropriate cell membrane.

The most common mutation in CF, $\Delta F508$, occurs in a region of the CFTR protein which should bind nucleotides. It has recently been suggested that binding of ATP at this site is an additional requirement for opening the chloride channel. Mutations in this region may very well interfere with normal ATP binding, phosphorylation, or function. Consequently, chemical analogues of ATP that may bind more readily to this region and potentiate the activation of mutant CFTR chloride channels may present a class of drugs for study.

The third approach would be one which does not target defects directly involved with the mutant CFTR protein, but which would treat the expression of the defect in affected systems. The object is to compensate for abnormal or deleterious functions caused by the mutant channel. For example, it is now well established that other types of chloride channels exist in many cell membranes. Drugs which might affect the function of these channels in such a way so as to compensate for the loss of the CFTR chloride channel could lessen the impact of a defective physiological expression. Along these same lines, but from a different perspective, CFTR may have functions other than chloride conductance or be closely associated with other cellular functions that require chloride conductance. In this regard, the CFTR protein may conduct chloride under some types of stimulation and carry out additional function(s) which are as yet unknown. In view of the tissues that are affected most prevalently in CF, it seems most likely that such a function may involve hydrogen ions and therefore fluid pH because the normal movement of bicarbonate in the pancreatic duct is thought to be highly dependent upon an open chloride channel. Failure of this system is almost certain to alter the pH of secreted fluid and may be closely tied to the pathology of the disease. Drugs which inhibit or stimulate hydrogen or bicarbonate transport might conceivably be used to compensate for, and at least partially correct, a defect in physiological expression precipitated by loss of CFTR chloride channel function.

These considerations should not be taken as being all inclusive. It is almost certain that numerous other avenues will be discovered that may provide much better approaches to controlling CF. It is more than encouraging to note, however, that within this year for the first time in the history of this disease, it is possible to discuss potential drug therapy in terms of basic scientific observations and fundamental knowledge regarding the biochemical and physiological processes which are affected by mutations in the disease. Thus, research in CF has apparently entered a new era which has every possibility of being characterized by the discovery and application of therapeutic drugs.

(b) Aerosolized amiloride and triphosphate nucleotides

(i) Normal airway epithelial physiology: Conducting airway epithelia absorb surface liquid as it is moved by the mucociliary escalator from distal regions to proximal conducting airways. Active Na^+ absorption is the dominant basal ion flow, and provides the driving force for reabsorption of airway surface liquid. The absorption of Na^+ occurs in a two step process; Na^+ enters the cell down an electrochemical gradient through an amiloride-sensitive Na^+ channel on the

apical membrane, and is pumped from the cell by the basolateral membrane $\text{Na}^+/\text{K}^+/\text{ATPase}$. The accompanying movement of chloride ion occurs through cellular and/or paracellular paths.

There is no net liquid (i.e., Cl^-) secretion under resting conditions, because electrochemical driving forces do not favor movement of Cl^- across the apical membrane. However, airway epithelia have the capability to secrete liquid across the apical membrane into the airway lumen by active Cl^- secretion across several types of Cl^- channels. For example, Cl^- secretion can occur via AMP-dependent (CFTR) or calcium-mediated pathways. Thus, airway epithelia have the capability for either salt and water absorption (driven by active Na^+ transport), or liquid secretion (driven by active Cl^- transport).

Abnormal ion transport in CF airway epithelia: CF airway epithelia exhibit two defects in ion transport that contribute to abnormal airway secretions. The predominant transport dysfunction is excessive absorption of Na^+ (and liquid). CF airway epithelia also have limited ability to secrete Cl^- via the CFTR protein (Cl^- channel) in response to CAMP-mediated stimulation. Taken together, excessive Na^+ absorption and limited Cl^- secretion contribute to dehydration of airway surface liquid and impaired mucociliary clearance.

(ii) Treatment of excessive Na^+ (and liquid) absorption in CF airways:

Amiloride, a Na^+ channel blocker, inhibits Na^+ absorption across normal and CF airway epithelia. Thus, amiloride delivered to the surface of CF airways might limit excessive Na^+ absorption and prevent dehydration of airway surface liquid. Acute studies demonstrated that amiloride aerosol improved mucociliary clearance compared to vehicle. A double-blinded crossover study of chronic amiloride aerosol in 14 adult CF patients suggested clinical benefit. This study tested the effect of amiloride aerosol on pulmonary function over a six-month period after parenteral antibiotic to normalize lung function and provide for optimal aerosol delivery. The decrement in forced vital capacity (FVC) during the amiloride aerosol was only approximately 40% of the decrement in FVC during the control vehicle. The safety and efficacy of amiloride aerosol in patients with CF is being explored in a multicentre placebo-controlled trial in CF patients down to the age of 12 years. An important concept of amiloride aerosol in CF relates to its "protective effect" from mucus impaction, airway damage, and prevention of chronic bacterial infection. Under optimal circumstances, aerosolized amiloride instituted in early childhood might retard or prevent airways disease.

(iii) Approach to treatment of abnormal Cl^- secretion in CF airways: Recent efforts have targeted the abnormality in Cl^- (liquid) secretion. Triphosphate nucleotides [adenosine triphosphate (ATP) and uridine triphosphate (UTP)] induce Cl^- secretion across human airway epithelia *in vitro* and *in vivo* via interaction with extracellular purinergic (P_2) receptors. *In vivo*, superfusion of ATP and UTP onto amiloride pretreated nasal epithelium induced Cl^- secretion in normal subjects and CF patients. The induction of Cl^- secretion by these agents was dose-related, and the nucleotides were equipotent (approximately $3\text{-}5 \times 10^{-6}$ M) in both groups (maximal effective concentration approximately 10^{-6} M). Interestingly, the efficacy of these compounds was greater in CF patients than in normal subjects.

The mechanism of nucleotide-induced Cl^- secretion was explored *in vitro* and *in vivo*. Primary cultures of airway epithelial cells were studied with double-barrelled Cl^- -selective microelectrodes; the tissues were pretreated with

amiloride on the luminal membrane to induce a favourable driving force for Cl^- secretion. Both ATP and UTP induced greater Cl^- secretion in epithelial cells from CF patients as compared to normal subjects. The greater Cl^- secretory response in CF epithelia reflected a change from a lower basal rate of Cl^- secretion in CF to a level of Cl^- secretion similar to that of normal epithelia after nucleotide application. These observations are consistent with activation of an apical membrane Cl^- conductance and Cl^- secretion.

The relatively prolonged duration of Cl^- secretion in response to these nucleotides *in vivo* suggests that more than one mechanism may be operative. One mechanism clearly involves P_2 receptor activation, phospholipase C and inositol triphosphate generation, and an increase in cytosolic calcium. This type of activation is usually short-lived, and may contribute only to the initial phase of the Cl^- secretory response. The duration of action suggests that a more direct gating effect on the Cl^- channel may be present, and that this mode of action may sustain the secretory response.

These nucleotides also stimulate ciliary beat frequency and goblet cell degranulation of canine and human airway epithelia, also probably via P_2 receptor mechanisms. These effects, coupled to the Cl^- secretory effect of luminal nucleotides, suggest a "physiological" and/or integrative effect of these compounds to assist in the clearance of airway secretions as a defense mechanism in the airways. For example, a coordinated response to an inhaled irritant or infectious agent might be amplified by luminal nucleotides to stimulate ciliary beat frequency and induce goblet cell degranulation and liquid secretion in the airway lumen, thereby assisting in mucociliary clearance.

(iv) *Summary for future directions.* In CF, defined abnormalities of ion transport by airway epithelia provide the rationale for therapeutic intervention with aerosolized pharmacologic agents that modulate ion transport. In CF patients, aerosolized amiloride inhibits excessive Na^+ absorption, improves biorheology and mucociliary clearance of airway secretions, and may retard the loss of lung function in adults. Recent studies suggest that one may also be able to attack the other limb of abnormal ion transport i.e., the defect in Cl^- secretion. Aerosolized nucleotides hold promise for inducing Cl^- secretion in CF airway epithelia. The pyrimidine compounds, such as UTP, may be preferable to ATP because metabolic products of UTP (uridine) may have less effect on airway epithelia than ATP metabolites.

8. GENE THERAPY⁵⁹⁻⁶²

New research developments may permit the introduction of a normal copy of the CFTR gene into affected tissues of those with cystic fibrosis. Somatic genetic therapy provides a great hope for the future, and raises no ethical problems. It should be noted that there are no proposals currently to carry out germ line gene therapy, which would raise completely different issues.

Because CF is a recessive disease, a single copy of the normal gene is sufficient for correct physiological function, a hypothesis which has been confirmed using cells from CF patients in culture. There is now an accurate CF mouse model, which should permit more rapid assessment of the efficacy and safety of gene treatment. Vectors based on a disabled adenovirus which appears to meet stringent safety criteria can place the gene into epithelial cells, such as those lining the lungs. The lung is relatively accessible to genetic constructs. However, it must be

noted that it is not certain that correction of the ion defect will be equivalent to correction of the lung pathology.

The major questions raised by gene therapy are whether the new forms of treatment will differ in a revolutionary way from existing treatments, whether they will be available within a reasonable time frame, and whether they will be sufficiently easy to apply and inexpensive to provide the possibility of application to all those who could benefit. These are difficult questions.

It is apparent that the first clinical trials will start in 1993. These studies will address specific questions concerning the entry and expression of the gene in human respiratory epithelium, and also questions of safety, all of which will also be studied in the CF mouse model. It may be prudent to assume such trials will advance quickly with support from governmental agencies, biotechnology companies, and the CF medical charities. If clinical benefit is evident, early approval by regulatory agencies will result in a product entering trials to assess clinical benefit as early as 1995.

However, it should not be forgotten that the genetic therapies which are being studied at present will only treat cells lining the lungs, and that gut and liver pathologies and infertility are not yet the subjects of investigations in this field. Clinical benefit will not be evident to all with the disease; other systemic problems will arise in time. Genetic therapy may be an improvement on existing therapy rather than a "cure" for CF. It should also be remembered that there has only been one successful clinical trial of gene therapy to date involving a handful of patients with severe combined immunodeficiency.

Finally, current models predict that genetic therapy will be easy to deliver in any country which has a modern medical system; therefore, there are no reasons in principle why any patient should be deprived of the benefit of advances once efficacy and safety are proven. However, the development costs of these therapies are considerable and private companies will expect to recoup these costs when treatments are introduced.

Thus:

- (a) Somatic genetic therapy for CF should be encouraged.
- (b) The time scale for this development is being shortened by the provision of adequate funding, and the availability of a mouse model. Safety and efficacy studies may be well under way in the next two years.
- (c) Genetic therapies which are presently under consideration will only improve lung function, and other systems will still be affected by CF; therefore it is important to regard this therapy as improved treatment rather than as a cure.
- (d) The WHO/ICF(M)A should maintain contact with the major CF charities, government agencies and the biotechnology companies involved to ensure that new therapies will be available as quickly as possible to those with CF at reasonable cost, so that treatment is not limited solely by finance.

9. NUTRITIONAL MANAGEMENT⁶³⁻⁶⁶

Growth retardation affects a significant number of patients and may affect progression and survival of the disease. A wide range of nutritional problems are present in cystic fibrosis. Height and weight are displaced to lower percentile levels, as the patient grows older. Body cell mass and fat are deficient as well as diminished lean and muscle mass and an increase of extracellular water. Diminished body cell mass can appear in the first months of life. Specific deficits of essential fatty acids, fat soluble vitamins, some water soluble vitamins, and some micronutrients have been demonstrated. Excessive sodium chloride loss in sweat, particular in young infants, leads to significant collapse in some patients. The nutritional problems arise from a combination of factors, among which are increased nutrient loss in the stool from maldigestion, diminished intake during acute illness, and increased energy utilization to sustain the breathing of diminished lung function. Some evidence has been supportive for a maladaptation to undernutrition as a basic defect in cystic fibrosis, although the multiple variables leading to malnutrition in cystic fibrosis make it very difficult to consider anything more than the recognition that multiple factors are involved in the nutritional and growth deficiency.

The major consequences of the malnutrition in cystic fibrosis are reflected in growth retardation, delayed puberty, problems for the pulmonary disorder, malabsorption, immune status, and finally adverse progression of the disease.

An improved nutritional status has been noted in patients who have both milder pulmonary disease and pancreatic sufficiency.

Nutritional rehabilitation of malnourished patients has been observed to improve the course of the pulmonary disease. Aggressive nutritional surveillance and intervention is clearly indicated at all stages of the disease.

To achieve adequate protein and energy intakes, daily intakes of more than 130% of recommended dietary intake may be necessary to compensate for losses. Fat intake of 40% of total energy intake are tolerated well with adequate pancreatic enzyme support. Dietary supplements, vitamin and mineral supplements are also required to replace deficiencies. Dietary support and counselling are of central importance to maintain adequate nutrition. Significant failure to grow or weight loss may require nocturnal intragastric feedings.

It is also apparent that pancreatic enzyme supplements do not fully correct the maldigestion but there is considerable subject variation. In some patients significant maldigestion still persists in spite of high pancreatic enzyme ingestion. Adjuncts such as H₂ receptor antagonist, prostaglandin analogues (misoprostol) have sometimes been found to be beneficial. The key to the problem of nutrition in cystic fibrosis is to place its surveillance and management at a high priority in the patient care programme from the time of diagnosis.

10. SOCIAL AND EMOTIONAL ADAPTATION⁶⁷⁻⁷²

The psychosocial aspects of cystic fibrosis are related specifically to the nature of the disease, and non-specifically to the problems of all chronic disease. CF has the following elements of importance to adaptation and acceptance of the illness.

- (a) A varying degree of progressive or perceived disability limiting normal life vocational aspirations in about 20% of the patients.
- (b) A rigorous regimen of therapy requiring time, knowledge, and commitment to maintain or attain a functional state of well-being.
- (c) A perception of defect arising from the genetic state and some other visible symptomatic defects such as cough and activity limitations not easily concealed.
- (d) A psychological coping mechanism with denial and avoidance of indicated therapeutic interventions to attain a sense of normality sometimes at some biologic cost to the person with cystic fibrosis, observed in approximately 20% of the CF population.
- (e) Moderate to high economic cost to sustain the therapeutic programme in about 40-50% of the CF population usually requiring significant input from familial and public support systems.

The problems related to chronicity are as follows:

- (a) Significant potential and actual acute short or long interruption of life activities in at least 25% of the CF population.
- (b) A sustained process of chronic care and daily effort to maintain health.
- (c) A perceived or realistic fear of progressive morbidity or a shortened trajectory of life duration.
- (d) Perception by others and guilt in the family of the CF host.

In the past, dysfunctional maladaptive emotional states were thought to be very common. However, recent studies have shown a remarkable high level of functional adaptation and strength in this patient group. Similarly, it has been observed that many families have been strengthened by the demands of the disease. Conversely, it does appear that at least 20% of the patients and families have borderline or serious maladaptation to life, some of whom might benefit from formal psychological treatment. All families or patients may have periods of psychologic maladaptation and readjustment requiring ongoing counselling and advocacy by the cystic fibrosis care providers through the stressful periods of diagnosis, maintenance, acute exacerbations and finally the grief surrounding the death of the patient.

Of special importance is compliance with a complicated regimen requiring major organizational skill to manage and integrate into the regular living day. Many individuals achieve a kind of survival non-compliance as they try to blend their treatment needs with their life priorities. A significant degree of flexibility in the treatment programme allowing self decisions shared with the care provider is vital. The care providers require understanding of the problems of chronicity, psychologic adaptation, and counselling to assist families and patients.

The life crises of family breakdown, divorce, deaths, and other significant life events can have significant impact on the maintenance of the therapeutic programme, the biologic reaction of the disease, and the utilization of health resources. As in all chronic disease, whether genetic or not, a biopsychosocial approach is a major model orientation in helping the patient and family with cystic fibrosis. Although

the degree of disease certainly influences the psychosocial aspects of the disease, it is of interest to note that some mild cases of cystic fibrosis have marked insecurity and emotional immaturity prolonging adolescence into the adult years; thus, limitation of life activities may be observed in a small number of relatively well individuals with cystic fibrosis based on a perception of significant potential threat from their disease.

One can conclude that a major requirement of the CF individual and family is dedicated, compassionate care providers providing continuity and advocacy. It is also clear that human life problems are as important a component in the disease as its specific biologic aspects and may take as much care, time and commitment.

11. THE ROLE OF WHO AND ICF(M)A IN THE DEVELOPMENT OF THERAPIES FOR CYSTIC FIBROSIS

The WHO, with its focus on world health, has an interest to develop strategies in conjunction with its Member States to improve the status of patients with CF. The ICF(M)A has a focused international commitment to carry out the same mission by bringing together lay leaders, physicians, scientists, and patients with CF for collective efforts in each of its member nations. These two organizations function well together to stimulate the development and availability of better care for patients with CF throughout the world.

12. CONCLUSIONS AND RECOMMENDATIONS

This report highlights a variety of therapeutic approaches in different stages of planning and development. Clearly the various therapies have increased the survival and improved the morbidity in the person with CF. It is now possible to identify the majority of the genetic distribution of the population and apply current and soon-to-be available treatment when the health system of a nation has organized itself to achieve this goal. New genetic therapies appear ready to be studied and carry promise to alter the pattern of CF.

It is recommended that WHO and ICF(M)A continue their collaborative efforts to develop the following areas:

- (a) Issue regular reports to countries of the status of therapeutic developments in cystic fibrosis.
- (b) Bring together scientists and medical leaders to plan and share technical knowledge which will be made available to all countries.
- (c) Assist in the development of collaborative efforts which can improve and facilitate the more rapid development of effective therapeutic modalities.
- (d) Help in the development of financial support for such scientific programmes and research.
- (e) Plan an educational campaign with countries interested in sharing information on care and research.
- (f) Work on trade agreements with countries and pharmaceutical companies to improve the wider availability, at the least cost, of medications for the treatment of cystic fibrosis.

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TABLE 1

PRINCIPLES OF CHEMOTHERAPY OF
LUNG INFECTIONS IN CYSTIC FIBROSIS PATIENTS

1. Microbial diagnosis based on secretions from the lower respiratory tract is required before chemotherapy is initiated.
 2. High doses of preferably bactericidal antibiotics for 14 days is used.
 3. Preferably use of antibiotics with rare occurrence of resistant variants, or use of combinations of antibiotics.
 4. Avoid prophylactic chemotherapy.
 5. Be aware of cumulative side effects due to frequent use of antibiotics.
 6. Be aware of changed pharmacokinetics of some antibiotics in CF patients especially as regards increased renal excretion.
 7. Inhalation of antibiotics may be useful as a support of systemic chemotherapy or as replacement of systemic treatment.
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TABLE 2

ANTIBIOTICS USED TO TREAT LUNG
INFECTIONS IN CYSTIC FIBROSIS PATIENTS

- S. aureus*: Dicloxacillin 25 mg/kg/24 h orally
+ Fusidic acid 50 mg/kg/24 h;
alternative drugs which can replace one of the above
mentioned: Flucloxacillin, Rifampicin and Clindamycin.
- H. influenzae*: Pivampicillin 35 mg/kg/24 h orally
or Amoxicillin 25-50 mg/kg/24 h;
alternative drugs: Amoxicillin + Clavulanate or Rifampicin in
combination with Erythromycin.
- P. aeruginosa**: Ciprofloxacin 20-30 mg/kg/24 h orally
+ Colistin 2-3 mill. units/24 h aerosolized.
- P. aeruginosa***
Tobramycin 10-20(-30) mg/kg/24 h i.v. (or aerosolised)
+ Piperacillin 300 mg/kg/24 h
or + Cefsulodin 100-150 mg/kg/24 h
or + Ceftazidime 150-250 mg/kg/24 h
or + Aztreonam 150-250 mg/kg/24 h
or + Thienamycin 50-75 mg/kg/24 h
and + Colistin 2-4 mill. units/24 h aerosolized
and/or + Ciprofloxacin 20-40 mg/kg/24 h.

Probenecid is given orally to all patients receiving beta-lactam antibiotics
eliminated by tubular excretion.

* Intermittently colonized.

** Chronically colonized.

TABLE 3
COST OF ANTIMICROBIAL CHEMOTHERAPY IN DENMARK
(DANISH CYSTIC FIBROSIS CENTRE) IN US\$ PER YEAR

<u>Regimen</u>	<u>Cost/Patient/Year</u>
Anti-pseudomonas treatment intravenous (4 courses/yr)	\$ 7,400
Anti-pseudomonas aerosol (colistin - 365 days/yr)	2,500
Anti-staphylococcal treatment (oral)	640
Anti-haemophilus treatment (oral)	60
Probenecid (oral)	10
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Total	\$10,610
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