



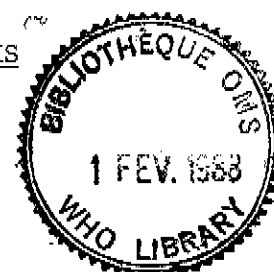
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HEREDITARY DISEASES PROGRAMME  
 DIVISION OF NONCOMMUNICABLE DISEASES

Oslo, 19 June 1987

REPORT OF A JOINT WHO/ICF(M)A MEETING  
ON PREVENTION AND CONTROL OF CYSTIC FIBROSIS



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## 1. INTRODUCTION

Following the joint WHO/ICF(M)A Meetings on Cystic Fibrosis held in Vienna, Austria, in 1983 (1) and Nicosia, Cyprus, in 1985 (2), close contact has been maintained between the two organizations.

Proposals for future collaboration were discussed and it was decided to take up the problem of determining the incidence of cystic fibrosis in parts of the world outside Europe and North America where it is likely to be overlooked or mis-diagnosed. It was known that the incidence of cystic fibrosis in most European countries and in North America varied between 1:1500 and 1:15000. However, when careful surveys or screening programmes have been carried out even in these countries, the true incidence has usually been found to be higher than was previously believed. The advent of the sweat test as a reliable diagnostic tool, and increasing awareness of cystic fibrosis among doctors, saw a rapid increase in the numbers of cases diagnosed. Available evidence points to a similar situation today in some other parts of the world. Many cases of cystic fibrosis have been found in Latin America. Sporadic cases are reported from Arab countries. There is a definite but unknown incidence among Indian and Pakistani children, and evidence from Pakistani immigrant populations in England suggests that it might be as high as in Europe.

On the other hand, negative reports from Hong Kong, where Western-trained doctors have been unable to find a single case, suggest that cystic fibrosis is rare or absent in Chinese populations. There are few, if any, documented reports of cystic fibrosis in Africa.

It therefore becomes an urgent necessity to identify those populations where cystic fibrosis may exist but be largely unrecognized, so that they may be offered whatever benefits result from further research. It was proposed to study the true incidence of cystic fibrosis in selected centres.

## 2. PROPOSED PROJECT

It was believed that the best way to do this would be by limited neonatal screening research projects. Accordingly, it was proposed that an existing laboratory already carrying out neonatal screening using the immunoreactive trypsin (IRT) method should be designated as a reference centre to which blood spots obtained from infants in the study populations would be sent. The characteristics of this screening test make it essential that good communications exist between the laboratory and the periphery so that follow-up spots can be obtained and a definitive diagnostic test (the sweat test) can be carried out on infants suspected by screening to have cystic fibrosis. The sweat test itself is reliable but requires basic equipment and experienced personnel, and even in developed countries gives rise to problems when inexpertly performed.

At the Annual General Meeting of the ICF(M)A in 1985, funding of this project was approved in principle. A revised estimate of expenditure was submitted and approved at the 1986 Annual General Meeting in Budapest, during which the WHO indicated its willingness to make a nominal contribution towards costs. The project was planned to begin in 1987. The neonatal screening laboratory in Belfast was designated as the reference laboratory, and has been screening for cystic fibrosis on a routine basis for several years. A small pilot study on blood spots sent from Argentina started in early 1987 and the feasibility of the system was demonstrated. An independent study using an ELISA method for measurement of trypsin started in Japan in 1987, which had been facilitated by the ICF(M)A but is funded locally.

The discovery that the cystic fibrosis gene is located on chromosome 7 (3) has produced a fundamental change to our plans. Since the original discovery the exact locus of the gene has been identified and work on its characterization is far advanced. This makes it highly probable that within the next two years it will be possible to use DNA technology to identify with certainty those individuals carrying the gene either in

homozygous or heterozygous form. We had assumed that it would be necessary to test about 100,000 newborns in each community before we could establish the incidence of the disease with any degree of accuracy. By application of newer genetic technology capable of identifying heterozygotes, the number of individuals to be tested would be reduced sharply to not more than 1,000, and the duration of the projects would be reduced from four years in each location to a matter of months. Moreover, there would be no need for complicated communications between the central laboratory and the periphery, nor for local follow-up and sweat testing. Even though the costs per test will inevitably be much higher than the item cost of neonatal screening, the project as a whole is likely to be much cheaper. Testing for the heterozygote incidence also avoids the ethical dilemma which results from identifying a baby with a disease for which no adequate clinical services might be available. It was therefore decided to postpone further implementation of the collaborative study until adequate discussion of the new technology could take place in Oslo.

### 3. OBJECTIVES

This joint meeting was arranged in order to:

- (a) Review the current position regarding advances in scientific research, clinical management of cystic fibrosis, and, diagnosis and management of cystic fibrosis in specific countries;
- (b) Review the joint population screening project; and
- (c) Discuss future collaboration between the WHO and the ICF(M)A.

### 4. PROCEEDINGS

#### 4.1. Review of the current position.

Scientific research on cystic fibrosis. Two important papers were presented during the meeting. The first of these was by Dr R. J. Beall, Executive Vice President for Medical Affairs at the U.S. Cystic Fibrosis Foundation. He reviewed the known physiological abnormalities in cystic fibrosis, with the evidence for an ion permeability defect affecting regulation of the chloride channel in the cell membrane. He also reviewed the principles and experience of the search for the genetic locus of cystic fibrosis on chromosome 7 before outlining the possible applications for new genetic technology resulting from recent discoveries, and emphasizing the need for international collaboration, for model screening programmes, and for awareness of ethical issues such as possible errors, confusion, stigmatization and risk/benefit.

The second paper on recent scientific advances was presented by Dr Peter Scambler of the Cystic Fibrosis Research Group at St. Mary's Hospital, London. He is part of the team which has isolated a small section of DNA from chromosome 7 which contains the cystic fibrosis gene, and described the techniques currently being used to identify the specific gene and its mutation. He also discussed currently available heterozygote detection tests using DNA probes, which can mainly be used to reduce rather than confirm the risk of carrier status. He predicted that accurate, precise information on the base change in cystic fibrosis would become available within one to two years, and that an antibody to the abnormal "cystic fibrosis" protein might become the basis for a cheap test, but this would depend on the exact sites of cystic fibrosis expression.

Clinical management of cystic fibrosis. This was reviewed by Professor J. Mangos, Chairman of the Medical/Scientific advisory committee of the ICF(M)A. He stressed the importance of the health care team in the management of cystic fibrosis and suggested that this may be the reason for better survival in countries where special cystic fibrosis services are well developed. He also pointed out that the appropriateness of providing specific health care funds and services for cystic fibrosis varies greatly between countries even when the gene frequency may be the same. However, in some developed

countries such as his own, the development of health care services for cystic fibrosis had led to the development of specialized services for other respiratory and gastrointestinal disorders which had been of benefit to thousands of children.

Diagnosis and management of cystic fibrosis in specific countries. Reports were received from several countries which highlighted the variations alluded to by Dr Mangos. Dr O. Pivetta from Argentina, speaking as General Secretary of the Latin American Group for Cystic Fibrosis and also a committee member of the ICF(M)A, produced evidence that 177 million Latin Americans (out of a total population of 280 million from Argentina, Brazil, Chile, Costa Rica, Colombia, Mexico and Uruguay) are of European descent, and calculated that approximately 1,850 children with cystic fibrosis are born each year. In contrast, only 922 diagnosed individuals with cystic fibrosis are known to be currently alive in this entire population. He made a very strong case for neonatal screening in Latin America using current and relatively cheap methods, rather than waiting for new technologies, as a means of case detection. This would be additional and complementary to studies designed to define the gene frequency.

Other reports were presented in the form of written submissions. In Belgium, prenatal diagnosis using DNA probes has already started. Ethical considerations are given high priority and were considered at an important meeting attended by the Prime Minister and other government ministers as well as by members of the board of the Belgium Cystic Fibrosis Association. If prevention of cystic fibrosis is to become a reality, the need for an educational programme directed towards the general public is evident.

Neonatal screening is not part of the programme for cystic fibrosis management in Denmark, but clinical care is highly centralized. All patients are seen monthly in a specialized clinic whether they have symptoms or not. Early and aggressive antibacterial chemotherapy has been shown to be effective, but also increases the risk of cross-infection between patients.

Greece with a population of 9,740,000 has about 130,000 births a year, with an expected incidence of about 65 cases of cystic fibrosis. Awareness of cystic fibrosis has been increasing rapidly among Greek paediatricians since a cystic fibrosis centre was opened in Athens 20 years ago, and about 45-50 cases are now being diagnosed annually, mostly during the first year of life. A neonatal screening programme would be expected to identify further patients, and also enable genetic counselling and prenatal diagnosis to be given to affected families. A programme of prenatal diagnosis has already started.

A report from Italy, referred to neonatal screening in the north-east region which started in 1974 and which has covered more than 500,000 newborns to date. The initial method used was meconium albumin testing, later replaced by the more sensitive and more specific IRT test combined with the meconium lactase test, which had now been applied to more than 120,000 newborns. Although individual variations in severity, clearly gene-dependant, were the most important factor influencing prognosis, those screened, diagnosed and treated early showed an improved clinical and nutritional state compared with patients presented with symptoms (a prospective longitudinal study is in progress on almost 400 cystic fibrosis patients who have been diagnosed since 1974).

The incidence of cystic fibrosis in Saudi Arabia is unknown, but recent clinical case reports suggest that the diagnosis is frequently missed. A neonatal screening programme using the IRT test was proposed by Dr H. Nazer from the King Faisal Specialist Hospital, Riyadh. It would be based in the metabolic laboratories of his hospital. Dr Nazer was encouraged to develop this programme, the results of which would help to define the worldwide distribution of the cystic fibrosis gene, as well as identifying patients for treatment.

#### 4.2 Review of the joint WHO/ICF(M)A screening project

In the light of the recent genetic advances referred to above, the objectives of the project were modified as follows:

- (a) To investigate the incidence of cystic fibrosis in certain populations using new methods of heterozygote detection which can be anticipated to be available in 1988, after preliminary evaluation of the new technology has been carried out in one or two countries.
- (b) To establish community-based programmes of control which may include genetic counselling, prenatal diagnosis and, where appropriate and acceptable, termination of pregnancy.
- (c) To assist in setting up neonatal diagnostic programmes in those countries with a previously unrecognized high cystic fibrosis incidence in order to provide affected infants with appropriate medical care;
- (d) To build up appropriate programmes of treatment where they do not already exist; and,
- (e) To increase awareness of cystic fibrosis in the affected communities.

The original proposal for a neonatal screening project envisaged a 5-year programme. The start of the modified project will be delayed pending development of a suitable heterozygote screening test, but its duration will be much shorter. A reference laboratory for DNA analysis will be required. It is likely that if suitable probes are made available much of the work will be possible in the countries being investigated. Results will be coordinated by WHO, who will also identify and approve reference laboratories, and facilitate exchange of information. It is necessary to assist in the evaluation of a new screening technology by coordinating a pilot study in selected countries so that the method can be available as soon as possible.

A reference laboratory for neonatal screening will also be required in respect of the third objective. Training programmes for technical and medical staff will be needed and the two Organizations may be able to assist with sponsorship in suitable cases.

#### 4.3 Future collaboration between the WHO and the ICF(M)A

Development of new genetic technology for cystic fibrosis provides an opportunity to take a rational approach to the underlying problem and to their proper use. It is important that these new tools are applied in the most efficacious way possible. Much can be learned from the experience with other diseases, such as thalassaemia, about problems arising from ad hoc development of control strategies.

When the techniques are available, pressure for their use will come from cystic fibrosis centres, parents, scientists and commercial interests. Heterozygote screening of selected populations would be an appropriate approach, but development of a protocol and suitable delivery programmes should start at once so that they are in place when the test is available, in order to avoid unsystematic application by clinicians and commercial concerns.

Decisions to set up health care systems are based on prevalence, available resources, cultural acceptability, population awareness and political factors. If decisions are made to deal with the problems of cystic fibrosis in particular countries, community control systems may include:

- (a) Genetic programmes - including heterozygote and neonatal screening, and prenatal diagnosis;
- (b) Clinical programmes - both hospital and community based; and
- (c) Educational programmes - directed to medical and paramedical personnel and the general public.

The nature of these programmes and systems will vary from country to country. However, planning can start now, and should include planning for epidemiological investigation and control systems, and propose model systems which should be subject to discussion by a wide representative range of individuals, from basic scientists to politicians. Pilot studies will be needed to determine acceptability, ethical and practical problems, and evaluation must be built into all proposed models. Heterozygote screening programmes should not be started in any country without prior consideration of these ethical and practical issues, and due note should be taken of general recommendations on the application of genetic advances which may emerge from other WHO committees.

It was generally agreed that the WHO and the ICF(M)A must work together towards control of cystic fibrosis by joint discussion and collaborative programmes. We urgently need to identify alternative strategies for different countries in the light of recent genetic advances, according to their genetic burden, health service resources and cultural differences.

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6. REFERENCES

1. Report of a Joint WHO/ICF(M)A Meeting on Cystic Fibrosis. Vienna, 6-8 October 1983. Unpublished WHO Document HMG/ICF(M)A/83.6.
2. Report of a WHO/ICF(M)A Meeting on the Distribution of Cystic Fibrosis. Nicosia, Cyprus, 30 April - 2 May 1985. Unpublished WHO document HMG/ICF(M)A/85.2.
3. Estivill X., Farrall M., Scambler P.J., Bell G.M., Hawley K.M.F., Lench N.J., Bates G.P., Kruyer H.C., Frederick P.A., Stanier P., Watson E.K., Williamson R., and Wainwright B.J. (Cystic Fibrosis Genetics Research Group, Department of Biochemistry, St Mary's Hospital Medical School, University of London, London W2 1PG, England - "A candidate for the cystic fibrosis locus isolated by selection for methylation-free islands". Nature Vol. 326, 30 April 1987.

WORKING PAPER

JOINT WHO/ICF(M)A MEETING ON PREVENTION OF CYSTIC FIBROSIS

OSLO (NORWAY), 19 JUNE 1987

BY

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In the interim between the Report of the 1983 Joint WHO/ICF(M)A Meeting<sup>1</sup> and the present, researchers have been directing their efforts toward identifying the basic defect in cystic fibrosis (CF). The evidence for a decreased permeability of chloride ions in epithelial tissue of patients with CF has recently been reviewed<sup>2,3</sup>. In addition, investigators have suggested that the basic defect in airway epithelial cells is related to increased cellular uptake of sodium ions from the airways<sup>4</sup>. The phenomenon of decreased  $\text{Cl}^-$  permeability, coupled with an enhanced  $\text{Na}^+$  uptake, has been offered as an explanation of the presumed dehydration of the respiratory mucus, which in turn is thought to lead to the apparent disruption of normal ciliary function and the resultant lung infection seen in CF patients.

The cornerstone of the current hypothesis, that is, that the basic defect in CF is related to the inability of CF cells to transport ions properly, evolved from observations in the two different kinds of tissue mainly affected in CF. Abnormal potential differences (PD) were first observed in the early 1980's in airway epithelia<sup>5</sup> and in sweat ducts<sup>6</sup>. Evidence has now accumulated that the abnormal potential difference can be attributed to the movement of  $\text{Na}^+$  and  $\text{Cl}^-$  in airway epithelial cells and to the movement of  $\text{Cl}^-$  in sweat glands. Indirect evidence has been presented that an ion permeability defect may explain the pathology associated with the pancreatic abnormality in CF patients<sup>7</sup>.

The lung is the most clinically important organ affected in cystic fibrosis and contributes to the major cause of morbidity and mortality associated with the disease. The movement of fluid across the epithelial tract depends upon the dynamic relationship between transport function on both the basolateral and apical sides of the epithelial cells. Any defect in the apical conductance is thought to impair regulation and transport of ions, and to affect water flow<sup>8</sup>.

The elevated potential differences, which are attributed in part to decreased  $\text{Cl}^-$  permeability across the apical membrane, have been recorded *in situ* in the nose, trachea, and bronchi<sup>5,9</sup>. In addition, the abnormal permeability has been demonstrated in primary cultures of nasal polyps<sup>10,11</sup> and in monolayers grown from dissociated tracheal epithelia<sup>12</sup>. A beta-adrenergic agonist (isoproterenol) failed to stimulate  $\text{Cl}^-$  secretion across CF airway mucosa<sup>11</sup>, whereas stimulation of secretion does occur in normal cells. Other studies have shown that both beta-agonists and other cAMP-dependent agonists have failed to increase  $\text{Cl}^-$  permeability in cultured CF epithelial cells<sup>12</sup>. Cyclic AMP accumulation in response to the beta-agonists was normal, suggesting that the defect in the control is distal to the accumulation of cAMP in airway epithelial cells.

With this information, investigators directed their efforts toward determining whether the defect in  $\text{Cl}^-$  permeability resided in a regulatory abnormality in the cAMP cascade or in the channel itself (conductance pathway). In a series of studies using cultured epithelial cells, investigators demonstrated that the conductance pathway (channel) in CF is normal. Thus, the defect most likely resides in the regulation of the chloride channel's activity<sup>13,14</sup>.

Using a single-channel patch clamp technique, cell-attached recordings demonstrated that beta-agonists and cAMP evoked  $\text{Cl}^-$  channel activity in membrane patches of normal airway cells. Beta-agonists and cAMP did not evoke  $\text{Cl}^-$  channel activity in CF cells. However, when the patches were removed from the CF cells (inside-out configuration), the  $\text{Cl}^-$  channel activity increased.  $\text{Ca}^{++}$  was implicated in the activation of the inside-out patches from CF cells. Excision of the patches into very low  $\text{Ca}^{++}$  bathing solutions did not result in activation of the chloride channel; however, the use of additional  $\text{Ca}^{++}$  did activate the channel. The addition of calcium ionophore A23187 activated the  $\text{Cl}^-$  channel in both CF and non-CF cells during the cell-attached recording<sup>13</sup>. The ability of  $\text{Cl}^-$  channel to function when detached from the CF cell suggests that the defect in CF resides in the regulatory cascade controlling ion movement across the cell membrane.

Similarly, a regulatory abnormality in the AMP-mediated pathways in sweat ducts has been reported<sup>15</sup>. The investigators observed that sweat stimulation by B-adrenergic

agonists is completely lacking in people with CF. However, the cells from CF persons did respond with the normal accumulation of cAMP. This data suggests that there is an analogous defect between CF airway cells and sweat gland tissue and that the defect in the stimulus secretion coupling occurs after a rise in cAMP.

Scientists are now focusing their efforts on the protein kinases, phosphatases, and on the isolation of the defective protein presumed to be involved in the  $Cl^-$  channel's regulation. A number of investigators are attempting to isolate phosphorylated intermediates that may differ between the CF and normal cell (figure 1).

The observation that  $Ca^{++}$  can cause the gating of  $Cl^-$  has also received great attention. The pathways effecting the increase in  $Ca^{++}$  content in airway cells are not well understood. Investigators are now focusing their efforts on examining other pathways, i.e. polyphosphoinositide pathways, to see what roles these pathways may play in CF cells. A better understanding of the underlying control of this  $Cl^-$ -channel gating process may provide a means of by-passing the defective step in the cAMP-mediated regulation and subsequent impaired activity of CF cells.

The observation of an abnormal  $Na^+$  transport by CF airway cells is serving as a basis for a clinical trial using the diuretic amiloride. Amiloride has been shown to inhibit the abnormal potential differences seen in CF patients and in cultured CF cells. Adults with CF are currently receiving aerosolized amiloride in conjunction with standard therapy, and an assessment is being made of the pulmonary function and other medical indices. This important study should be completed in mid-1988.

There is great optimism among CF researchers that scientists are getting closer to identifying the basic defect in CF. The biochemical and physiological studies have continued to help unravel the mysteries that have surrounded this disease for many years. Likewise, the identification of the CF gene is expected to provide important data in identifying the defective protein that may be involved in CF cell regulation.

In the early 1980's, scientists began applying the Restriction Fragment Length Polymorphism (RFLP) technology to locate the CF gene<sup>16</sup>. After intensive efforts identified families with two or more children with CF for genetic research in 1985, the first significant advances were made in this area. The first protein marker linked to CF, paraoxonase, was reported in mid-1985<sup>17</sup>. In October 1985, the first DNA marker to be linked to CF, DOCR1-917 (D7S15), was reported to be 15 million base pairs away from the CF gene<sup>18</sup> and was later shown to be linked to the long arm of chromosome 7 in the 7q 22-31 region<sup>19</sup>. Rapid progress led to identification of two additional markers on chromosome 7 closely linked to the CF gene - the met oncogene (MET)<sup>20</sup> and pJ3.11 (D7S8)<sup>21</sup>. It was ascertained that these markers resided approximately 1-2 million base pairs from the CF gene.

Subsequently, a number of additional markers have been identified, including TCRB<sup>21</sup>, COL1A2<sup>22</sup>, 7C22 (D7S16)<sup>23</sup>, B79a (D7S13)<sup>24</sup>. However, none of the latter probes have been found to be closer than met and J3.11. In January of 1987, an international collaborative group of eight centres which had been using the various probes, reported a possible linkage relation favouring the gene order met-CF-J3.11<sup>25</sup> (figure 2).

This linkage relationship provided the basis for the exciting advances reported by Drs Williamson, Wainwright, and Estivill of St Mary's Hospital in London, in which a DNA segment that is a candidate for the CF gene was identified<sup>26</sup>. The approach used by Dr Williamson's laboratory was based on the proximity between the CF gene and the met oncogene, as well as the role of the met oncogene in inducing human sarcoma.

Although the met oncogene has nothing to do with CF, and CF is not in any way related to cancer, the London group realized that with the DNA from a sarcoma cell, the met

oncogene could be used to isolate the neighbouring CF gene. Chromosomes were isolated from human cancer cells and placed in a mouse cell line. Mouse cells that incorporated the region of the chromosome containing the met oncogene began to grow in a manner similar to cancer cells. These cells also incorporated the normal equivalent of the CF gene, as a result of the proximity of the two genes. The researchers were then able to remove the DNA fragment with the met oncogene from the mouse cells and study it further.

The isolated fragment was one million base pairs long and contained both met and J3.11, and hopefully, therefore, the normal counterpart of the CF gene. If this was the case, the search for the CF gene had been reduced from an area of three million base pairs to one million base pairs.

To move even closer to the CF gene, the London group focused on the previously reported hypothesis that the "start" of a gene may be recognizable by a sequence that contains a large number of a particular sequence - guanine (G) and cytosine (C) side by side. They found such a region using specific DNA-cutting restriction enzymes. They then were able to select fragments that were start sites for coding genes. The St Mary's group used these fragments to make a new DNA probe in order to sequence human DNA from CF persons and their unaffected parents and siblings.

The piece of DNA identified to this point consists of 55,000 base pairs, in contrast to the segment of one million base pairs which was the starting point of these studies. Based on crossover and linkage disequilibrium studies<sup>26</sup>, it is believed that a portion of this fragment contains the CF gene. In addition, this piece of DNA appears to be expressed in airway epithelial cells and placenta but not expressed in brain tissue, indicating a close relationship to the CF gene.

In the months ahead, the genetic approaches and the physiological approaches will be coming together to firmly identify the CF gene and to further define the basic defect. Once the basic defect is defined, researchers will have the knowledge to determine where in the CF cell's regulatory pathway a defective protein may affect the chloride channel and alter ion transport. Then, new therapeutic approaches can be developed and studied for their ability to offset or treat the basic defect associated with CF (figure 3).

In addition, advances in understanding the basic defect will serve as a basis for the development of the technologies necessary for population screening. In the United States, the applicable population for such screening will be the three million people who enter into pregnancies annually. If a reliable, inexpensive test can be developed, 150,000 (1 in 20) of these women would be expected to be carriers of the CF gene. Of these, it would be expected that 7,500 of their husbands would be carriers, and these pregnancies would be at high risk (1 in 4) for having a child with CF.

There are a number of approaches that may be used to develop the screening tools and these will likely evolve shortly. Whatever the basis, appropriate planning must occur as was outlined in a report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioural Research<sup>27</sup>. Issues that need to be resolved relate to error and confusion, stigmatization, coercion, and risk benefit. As a screening programme for CF will involve a target population for screening which will be the largest of any disease, international collaboration for the assessment of model programmes will hopefully pave the way for the development of effective and reliable screening programmes.

FIGURE #1

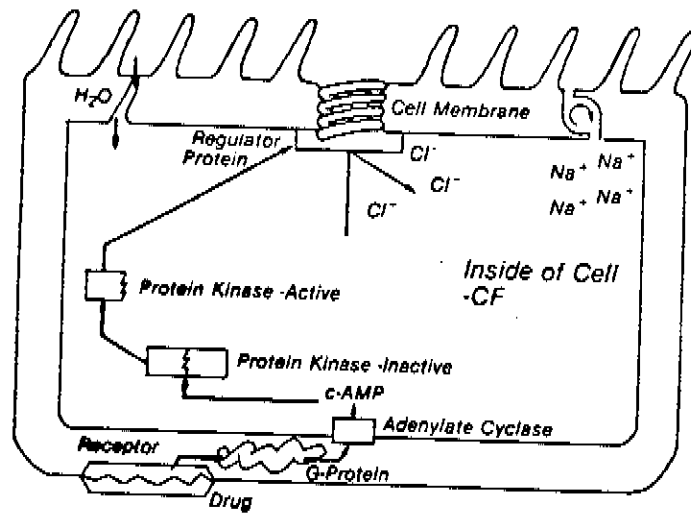


FIGURE #2

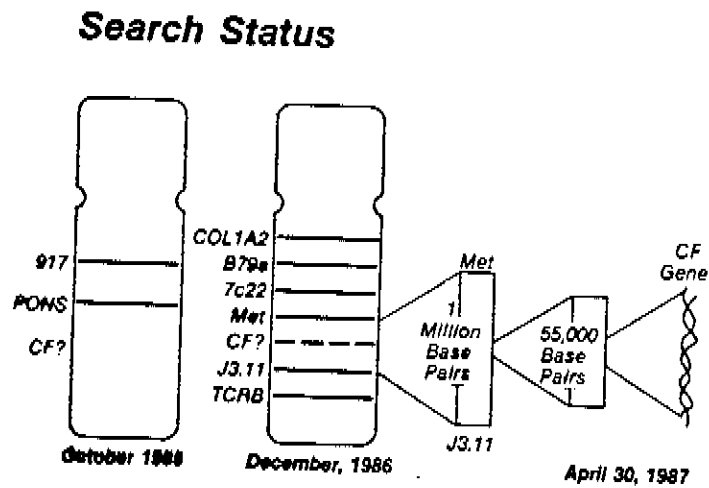
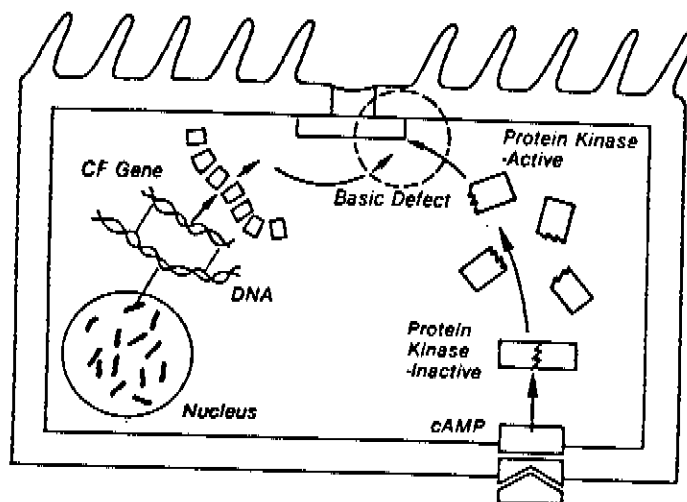


FIGURE #3



## 6. REFERENCES

1. Report of the Joint WHO/ICF(M)A Meeting on Cystic Fibrosis, Publication of the WHO Hereditary Diseases Programme, Division of Noncommunicable Diseases (Vienna, October 1983: HMG/ICF(M)A/83.8).
2. Knowles, M.R., Stutts, M.J., Yankaskas, J.R., Gatzky, J.T., and Boucher, R.C. - Clinics in Chest Medicine 1986; 7:285-297.
3. Frizzell, R.A. - Trends in Neurosciences 1987; 10:190-193.
4. Boucher, R.C., Stutts, M.J., Knowles, M.R., et al - J. Clin. Invest. 1986; 78:1245-1252.
5. Knowles, M.R., Gatzky, J.T., and Boucher, R.C. - New England J. Medicine 1981; 305:1489-1495.
6. Quinton, P.M. - Nature (London) 1983; 301:421-422.
7. Kopelman, H., Durie, P., Gaskin, K., et al - New England J. Medicine 1985; 312:329-334.
8. Welsh, M.J. - J. Clin. Invest. 1983; 71:1392-1401.
9. Knowles, M.R., Gatzky, J.T., and Boucher, R.C. - J. Clin. Invest. 1983; 71:1410-1417.
10. Stutts, M.J., Cotton, C.U., Yankaskas, J.R., et al - PNAS 1985; 82:6677-6681.
11. Yankaskas, J.R., Knowles, M.R., Gatzky, J.T., and Boucher, R.C. - Lancet 1 1985; 8435:954-956.
12. Widdicombe, J.H., Welsh, M.J., and Finkbeiner, W.E. - PNAS 1985; 82:6167-6171.
13. Frizzell, R.A., Rechkemner, G., and Shoemaker, R.L. - Science 1986; 233:558-560.
14. Welsh, M.J. and Liedtke, C.M. - Nature (London) 1986; 332:467-470.
15. Sato, K. and Sato, F. - J. Clin. Invest. 1984; 73:1763-1771.
16. Botstein, D., White, R., et al - Am. J. Hum. Genetics 1980; 32:314-331.
17. Eiberg, H., Mohr, J., Schmieglow, K., et al - Clin. Genetics 1985; 28:265-271.
18. Tsui, L.C., Buchwald, M., Barker, D., et al - Science 1985; 230:1054-1057.
19. Knowlton, R.G., Cohen-Haguenaour, O., Nguyen, V.C., et al - Nature 1985; 318:380-382.
20. White, R., Woodward, S., Leppert, M., et al - Nature 1985; 318:382-384.
21. Wainwright, B.J., Scrambler, P.J., Schmidtke, J., et al - Nature 1985; 318:384-385.
22. Scrambler, P.J., et al - Lancet II 1985; 1241-1242.
23. Scrambler, P.J., et al - Nucleic Acids Res 1986; 14:1951-1956.
24. Estivill, X., Schmidtke, J., Williamson, R., et al - Human Genetics 1986; 74:320-322.
25. Beaudet, A., et al - Am. J. Hum. Genetics 1986; 39, 681-693.
26. Estivill, X., et al - Nature 1987; 326:840-845.
27. Cystic Fibrosis: A Case Study in Screening and Counselling for Genetic Conditions: Report of President's Commission for Study of Ethical Problems in Medicine and Biomedical and Behavioural Research - 1983; 87-104. Published by U.S. Government Printing Office, Library of Congress.