

Cystic Fibrosis Worldwide

SIXTH

JOSEPH LEVY

MEMORIAL LECTURE

“THE SIX MEN”

John A Dodge, CBE MD FRCP FRCPCH DCH

GENOA, JUNE 2002

Some things I have said of which I am not altogether confident. But that we shall be better and braver and less helpless if we think that we ought to enquire than if we indulged in the idle fancy that there was no knowledge and no use in seeking to know what we do not know – that is a theme upon which I am ready to fight, in word and deed, to the utmost of my power.

Plato

If I say at once that when I think of the eminence and achievements of my five predecessors I feel both honoured and inadequate to give this sixth Joseph Levy Memorial Lecture, it is no more than you would expect, but it is nevertheless true. In addition, I am particularly pleased to pay tribute to Joseph Levy, whose family endow these lectures in his memory, because I believe that I am the only designated lecturer who actually met him.

JOSEPH LEVY

Who was Joe Levy? He was a businessman, who became involved with the British Cystic Fibrosis Research Trust from its foundation, having been recruited by Mr John Panchaud, another businessman, whose child had Cystic Fibrosis. Joe Levy became chairman of the Trust soon after its foundation, and served in that role for 20 years. When he retired in 1984 he was succeeded by his son, Peter. Although he had no direct family involvement with Cystic Fibrosis, Joe involved himself deeply with the Trust, its personnel and the patients it served. He not only donated generously from his own resources, but raised funds through an annual appeal to his wide circle of friends. For many years, all of the money raised for the Trust by public donations and appeals were spent directly on research, the administrative costs of the Trust being met by Joe and his friends. When the activities of the Trust expanded into welfare, Joe again dug deep into his own pocket to ensure that CF children should not lose out on life's opportunities through lack of funds, and he helped to support individual young people through University and vocational training.

Joe Levy's contribution to the conquest of Cystic Fibrosis was immense and arguably as important in its own way as that of any other individual. However, he is not one of the six men of my title.

GREAT MEN OF GENOA.

There have been many illustrious sons of Genoa and it was not difficult to find six whose greatness is universally recognised. In the Middle Ages, Genoa was a powerful independent city, almost constantly at war with Venice and torn by internal political divisions. The first native Doge after the Venetian model was Simone Boccanegra, who attempted to solve the political problems, but the arrival of the Black Death in Genoa in 1346 did little to help him. The world's most famous sailor, born in Genoa more than a century later, was Christopher Columbus, who is generally credited with the discovery of America.

Genoa subsequently came under the control of the Duke of Milan and when in turn the Duchy of Milan was claimed by the King of France, astute political leadership by Andrea Dorea restored Genoa to orderly government and made it politically a satellite of Spain, while ensuring that it would be a privileged exploiter of the vast Spanish empire. Another great political leader was Guiseppe Mazzini, an internationalist who in the 19th century dreamed of a United Italian Republic, but he subsequently accepted the idea of a United Kingdom of Italy under a liberal monarch.

Genoa also made substantial contributions to the arts and sciences. Perhaps the greatest violinist who ever lived, Niccolo Paganini, was born in Genoa in 1782. In our own time, the world's leading expert on human population genetics, Luigi Luca Cavalli-Sforza, was born in Genoa in 1922 and is still an active emeritus Professor of Genetics at Stanford University. In the words of the New York Times, "there may be no contemporary scholar who has a more detailed understanding of human diversity or a more compelling vision of its unified history." Although I shall refer to his work later, he is not one of the six men.

Nor are any of the distinguished scientists and doctors whose names are almost as familiar to us in the Cystic Fibrosis world as those of our own families. To select only six from such a galaxy would be not only invidious, but a highly personal choice. If pressed, I could no doubt choose six whose work, character and example I hold in the highest esteem, but the six men of my title were named many years ago in a simple children's poem.

I keep six honest serving-men
(They taught me all I knew);
Their names are What and Why and When
And How and Where and Who.

Rudyard Kipling

These are the trusted servants of the scientist, the doctor, the professional and the patient and if employed wisely they will serve us well, as we try to understand and overcome Cystic Fibrosis. We cannot hope to make progress without them and they epitomise the qualities and conditions needed for good research and good practice.

WHY? Signifies curiosity and asks the purpose and the justification for any scientific or clinical project.

WHAT? Suggests specificity, precision and attention to detail. (A variant, What If? introduces the qualities of imagination and speculation which are needed for developing hypotheses).

WHERE? Finds the appropriate location for a study, or for delivering treatment.

WHEN? Sets the time frame for the project and reporting of the results, or the expected duration of the treatment.

HOW? Asks for appropriate methodology.

WHO? Asks not only who will do the research, or give the treatment, but also identifies the likely beneficiaries.

We can apply these criteria to explore some of the unanswered questions which confront us as we try to understand this condition known as Cystic Fibrosis and which is the common interest we share.

“An inquiring mind and questioning attitude are central to the development of science. Received wisdom must be challenged until it is either demonstrated to be true, or else that it has outlived its usefulness as a working hypothesis”.

WHY?

This is the most basic of all questions and often the hardest to answer. The enquiry may be about causes, reasons, purposes, or mechanisms. The child asking Why?, Why?, Why? persistently may be seeking a teleological answer, as he or she tries to make sense of the world. The little boy who asked why the tide comes in, did not want to know that it is because of pull of gravity on the oceans as the moon makes an asymmetrical orbit around the earth, but wanted to know the purpose.

Some of us believe that the universe has a purpose, others follow the philosophy of Dawkins in “The Blind Watch Maker” and see the universe, the world and the human body as the results of random events, but either way a functioning watch, body, world or universe is a thing of intricate and inspirational beauty and meets a human need for order and sense in our environment.

The true scientist is like a persistent, irritating child trying to make sense of the world, who never stops asking Why? Why? Why? and is never, or rarely, satisfied with the answers. Just as each layer of onion skin removed reveals another beneath, we only have to look at the developments in physical science and the discovery of new, transient and ever smaller positive or negative particles or influences to realise that the goal of truly understanding our universe may be unobtainable.

Thus, at one level we who wish to understand CF may be satisfied with the concept of chloride, sodium, bicarbonate and potassium ions moving across cell membranes in response to stimuli in order to “maintain electro-chemical neutrality” and use this teleological goal as a working explanation for the intricacies of constant and busy ionic adjustment. Most of the time we are concerned with observing the phenomena and their disturbances in disease, rather than asking the unanswerable questions: why is it arranged, or disturbed, in this way and what alternatives could there be? The person with CF simply, but in an equally valid way, may ask “Why me?” and on being told “Because your parents passed you certain genes” may be satisfied or may go on to ask “Why?”, or “Why did they have these genes?” Going back for generations with the same question will eventually receive the answer “I don’t know”. Trying to find out “Why?” is an exciting challenge, but continually asking why ultimately leads back to fundamental questions such as “Why are we here?”; “Why is the universe like it is?”; or “Why is there disease?” about which our remote ancestors had answers which were at least as plausible and satisfactory to them as those we have today are to us. While these intriguing but unanswerable questions remain, we tend to get on with the practical business of living day to day and in the case of CF change our question from “why?” to “how?” We ask “how does the CF disorder

work?” or “how can we cope with it?” - at genetic, therapeutic, sociological and psychological levels.

WHAT?

If Why? asks a question which may be answered in different ways, What? demands a specific answer. Sometimes scientists, particularly clinical scientists, find that they cannot give precise and specific definitions of the phenomena they are discussing. Even in the case of CF there are several working definitions, none of which gives the full picture. The geneticist, cell biologist, molecular biologist, clinician and patient may each define CF differently, with equal validity for their own purposes. The naming of diseases is known as nosology and is a branch of taxonomy. Nosologists are described as either “lumpers” if they tend to group diseases together under one name according to some common feature, or “splitters” if they like to separate and name conditions differently, no matter how much they resemble each other. The development of genetics has increasingly turned us into “splitters”. In 1971, McKusick wrote

“In medical genetics there is little place for expressions such as ‘spectrum of disease’, ‘disease A is a mild form, or a variant, of disease B’ and so on. Disease A and disease B are either the same disease, if they are based on the same mutation, or they are different diseases. Phenotypic overlap is not necessarily grounds for considering them fundamentally the same or even closely related”.

That was in the early days of molecular biology. Now that we have more than a thousand mutations in the same CFTR gene, and inconsistent genotype-phenotype correlations, we are still left with a “spectrum” of distinct disorders ranging from the full-blown, classical CF syndrome to isolated unilateral atresia of the vas deferens, with all grades of the disease in between. Because clinical CF is a product of the interaction of the basic gene defect, modifying genes and the environment, even a precise genetic diagnosis does not always correlate with the clinical picture or the prognosis.

A Working Party convened jointly in 2000 by ICFMA, WHO, ECFTN and supported by ECFS and the CFF, concluded that the diagnosis of Cystic Fibrosis remains a clinical one, and some individuals with CFTR gene mutations but atypical clinical symptoms are best served by giving them an alternative “diagnosis”. They have one of a number of related, but distinctly different conditions, which do not meet the clinical criteria of CF, and which therefore requires different management. The recommendations for the classification of Cystic Fibrosis and Related

Disorders have been published by WHO and it is hoped that they will be incorporated into the next edition of the International Classification of Diseases (ICD 11).

WHAT IF?

Scientific research is usually founded on a hypothesis. Possible answers are proposed and tested. Speculation, creativeness and imagination are as much characteristics of the scientist, as they are of the artist or writer.

In a letter to Alfred Wallace, Charles Darwin wrote that he was glad that Wallace was giving attention to the distribution of species “in accordance with theoretical ideas. I am a firm believer that, without speculation, there is no good and original observation”. Wallace had written to Darwin putting forward his ideas about evolution prior to the publication of Darwin’s monumental work “On the Origin of Species” and there is still lively debate about whether or not Darwin used some of Wallace’s material in arriving at his own conclusions. However, speculation and hypotheses must be pursued with discipline and honesty and a willingness to be proved wrong. The great Italian scientist Lazzaro Spallanzani wrote “If I set out to prove something, I am no real scientist – I have to learn to follow where the facts lead me – I have to learn to whip my prejudices”. It was Spallanzani, a priest, who successfully challenged and destroyed the prevailing theory of spontaneous generation. He showed that boiled water in sealed glass flasks remained germ-free, whereas if the flasks were only closed by a porous cork they became contaminated by bacteria which must have entered from the outside air. He also carried out the first experiment of artificial insemination in the dog, in 1780. A recent commentator described it thus:

“From the outset we have an object lesson in precision, care, imagination and personal commitment to experimental science. One can only wonder to what extent his clerical responsibilities prevented him from exploring this subject further!” (Watson,1990).

The discipline shown by Spallanzani is not always in evidence. Sometimes scientists and clinicians are carried away by their hypotheses and it is often very difficult, or impossible, to disprove a false hypothesis or speculation. For example, in the United Kingdom we recently went through a period of medical and political turmoil, because the observation of some measles virus residues in the intestine of some cases of inflammatory bowel disease in children who also had features of autism, was extrapolated into a hypothesis that the combined measles, mumps

and rubella (MMR) vaccine might be a cause of infantile autism. Superficial epidemiological support came from noting that autism has become more frequently diagnosed since MMR vaccine was introduced, although changes in the definition of autism, with considerable widening of its scope, have also occurred during this period. It will be many years, if ever, before this hypothesis can be categorically disproved and in the meantime the measles immunisation rate in the UK has fallen significantly. It behoves scientists to follow the facts and go where they lead, as counselled by Spallanzani, and by Rousseau, who said “I know the truth lies in the facts, and not in the mind that judges of them, and that the less I introduce what is merely my own into the deductions I make from them, the more certain I shall be of approaching the truth”. Occasionally we are so wedded to our hypothesis that we find excuses for results which do not fit and thereby do a great disservice to our colleagues, our patients and to science in general. In a paper entitled “The Science of Things That Aren’t So”, the Nobel prize-winning chemist Irvine Langmuir listed the features of “pathological science” as: an effect of a magnitude close to the limit of detectability; theories which are fantastic and contrary to experience; criticisms met by ad hoc excuses; and effects which could only be observed by the supporters of the hypothesis and not reproduced by critics. He concluded that “the final, proper and only trustworthy arbiters are time and replication”.

WHERE?

The location of research projects, and of the delivery of clinical care, is very important. In the case of research, money for projects tends to be directed towards centres with a good track record, because they are likely to have developed the necessary infrastructure and methodology. While this makes sense, it does not always result in a satisfactory outcome. Modern medical research usually depends on collaboration between clinicians and scientists, and between scientists (and often clinicians) in different disciplines. Good interpersonal relationships between the various parties is essential and may be jeopardised if one or more of the partners feels undervalued and resentful, and they are therefore less inclined to inconvenience themselves simply in order to help the research and academic career of an ambitious colleague in another department. There is an overlap between Where? and Who? which results from the drive and ambition of the project participants, and relocation of a key individual to another city or another country can make the track record of a particular centre irrelevant. For patients, the choice of a clinic which provides and oversees their management is a central question. The range and standard of services for Cystic Fibrosis vary and depend upon teams rather than individuals. We have become accustomed to a much more critical and questioning attitude among patients than

existed in the days when there was not very much that could be done for children with Cystic Fibrosis, no matter who they consulted.

A study of European patients' rights was completed earlier this year by Herman Nys on behalf of ECFTN. It considered individual rights of patients within the European Union and found that under existing legislation patients have a right to be treated according to professional standards which apply throughout the European Union, to information on their health status, to proper information preceding consent for procedures, and protection of privacy. There are also rights of access to health services in other countries of the European Union and to general protection and promotion of health.

While not specifically relating to CF, in a key ruling on 12th July 2001, the European Court of Justice in Luxembourg decided that treatment must be given according to European, not national standards. This has profound implications for many countries including my own. In practice, Cystic Fibrosis centres learn from each other about the best ways to provide care and, as in so many matters, no single pattern suits the needs of each individual, or each population. There is no disagreement that standards and components of care should emanate from expert centres. Frequent attendance at large established clinics is not always convenient for patients who may live many kilometres away, and various patterns of outreach programmes and shared care have been developed. What is so encouraging is the evidence that wherever individuals with Cystic Fibrosis are managed, their outlook is improving year on year.

HOW?

Research and clinical services fill the gap between the desired objective and the starting point. How do we get from where we are now to the Promised Land where people with CF will have a normal lifespan and a normal quality of life? In general terms, we do so by continuing to build upon the experience we have gained, and refine and improve the familiar modalities of management: diagnosis as early as possible, nutritional care including pancreatic enzymes and vitamins, infection control including antibiotics, bronchial drainage and so forth. Even when CF complications are far advanced, life-extending measures such as lung transplants will become more widely available. But it is only when we can apply an effective treatment aimed at the primary biochemical defect in the CF cell – whether by gene replacement or drug therapy – that we are likely to reach the promised land. The How? of research, in contrast to the Why? and Where? is often tedious, repetitious and prone to disappointment. It calls for persistence, determination and ingenuity in overcoming obstacles. The true heroes are often the foot soldiers, the practical people who convert the dreams of the leaders into reality. They may not

stand behind the lectern or occupy the platform at scientific meetings, but without them, those who do so would have nothing to say.

WHEN?

The writer of Ecclesiastes wrote that “to everything there is a season ... a time to be born and a time to die ... a time to break down and a time to build up... a time to keep silence and a time to speak”, and so on. More modern writers have suggested that the whole secret of a successful life is in the timing. As a paediatrician, I am constantly reminded of the need to establish good foundations, and that mistakes made in childhood have consequences for the rest of that child’s life. My fellow Welshman, Thomas Phaire, wrote the first British text book of Paediatrics in 1545, in order “to do them good which have most need, that is to say children”. These most vulnerable members of society are also those for whom the future may hold the greatest promise. That is why we must continue to concentrate much of our effort on preventing and repairing the effects of CF in children even though in many countries they are now fewer in number than affected adults. It is unlikely that anyone with the established consequences of Cystic Fibrosis disease alive today will see them completely reversed, but there are children with Cystic Fibrosis, yet to be born, who will be enabled to live a “normal life”, subject to all of the opportunities and problems faced by everyone else in society, but with their Cystic Fibrosis brought under control from birth, or even before.

WHO?

Does it matter who finds the cure for Cystic Fibrosis? It certainly matters who we ask to undertake research projects and responsibility for clinical services. In addition to the patients, there will be many others who will benefit as and when new advances in management are achieved: the scientists who receive professional acclaim, the clinicians whose workload is (perhaps) reduced; and the pharmaceutical industry and its shareholders, who bring new products to the market. One thing we can be certain of is that there will be no shortage of people willing to take the credit.

I would now like to explore a few specific questions which have interested me in recent months, using our six serving men as starting points.

WHY IS CF SO COMMON?

In general, biological pressures act to eliminate faulty genes, particularly those which give rise to serious disease, but in some special situations carriers of a single copy of the disease mutation

enjoy a so-called “heterozygote advantage”. The carrier state then becomes widespread in that community.

A fascinating example of this principle dates from the fourteenth century. According to Fra Michele di Piazza, “In the first days of October 1347, twelve Genoese galleys fleeing before the wrath of our Lord over their wicked deeds, entered the port of Messina. The sailors brought in their bones a disease so violent that whoever spoke a word to them was infected and could in no way save himself from death...” The disease carried by the unfortunate sailors, their ship’s rats and their fleas was the plague. It spread throughout Europe so rapidly that in less than a year it had reached England. It was known as the Black Death, and it killed a quarter of Europe’s population. Affected persons died 2 or 3 days after exposure and the only defence was rigid quarantine. Outbreaks of plague continued intermittently for the next 300 years. We now know that plague is caused by an organism called *Yersinia pestis*, and may take one of two forms: bubonic or pneumonic. The bacteria enter white blood cells but are not destroyed. They are transported to the lymph nodes, which become enlarged (bubonic form). Pneumonic plague is rapidly spread by droplet infection.

In spite of its almost universal lethality, occasional persons who were quarantined in villages or homes with plague victims were miraculously preserved, either having a mild illness or none at all. The present day descendants of some of these survivors have been traced, and have been found to be carriers of a mutation called delta 32, located in a gene called CCR5, on chromosome 3. This mutation is found in up to 20% of people living in isolated villages along the route of the Black Death through Europe, but is absent from other parts of the world. The original carriers of delta 32 survived the plague and multiplied, while their neighbours died. The antiquity of this genetic explosion of delta 32 has been calculated as 700 years, coinciding with the first outbreak of plague. Of great interest is the observation that delta32 prevents multiplication of micro-organisms within lymphocytes, not only *Y. pestis* but also retroviruses such as HIV. Heterozygotes for delta 32 are about 10% of the European population, and when exposed to HIV infection they tend to acquire AIDS late and have a relatively benign course. Homozygotes, about 1 in 400, are immune.

Is there any evidence that a single copy of a CFTR mutation similarly confers a biological advantage? There are several theories and some relevant observations. One theory, with supporting physiological evidence, is that the small intestine of CF heterozygotes is relatively unaffected by the toxins of cholera, salmonella and E.coli, which were responsible for large numbers of deaths in European populations in times past. According to this theory, carriers would survive epidemics of diarrhoeal illness and a gene mutation would therefore become frequent in the surviving population. It has been convincingly shown that the intestine of both

CF subjects and heterozygotes for $\Delta F508$ does not respond to challenge by cholera or typhoid toxins with the usual outpouring of fluid and electrolytes which causes dehydration, severe illness or death in other people. However, this theory has major flaws when one looks at the epidemiology of cholera, because cholera was largely confined to the Indian sub-continent until the early 19th century, when outbreaks occurred in many other parts of the world. Carriers of Cystic Fibrosis were present in European populations long before the arrival of cholera (although the name probably included other causes of epidemic diarrhoeal illness). Furthermore, there is an almost inverse relationship between the countries where cholera occurs today and the frequency of Cystic Fibrosis. Nevertheless, protection against some form of infectious disease, analogous to the protection enjoyed by delta 32 heterozygotes against plague, is at least a theoretical possibility. We often overlook the very high mortality from infectious diseases in Europe in times past, and as recently as 1656 an epidemic of plague and associated famine wiped out up to 75% of the population of Genoa. Indeed, we often forget that the average life expectancy of people with Cystic Fibrosis today is greater than that of many European populations less than 200 years ago. For example, in 1835, one third of infants born in Ireland did not survive to their first birthday, half died before the eighth year and two thirds before the thirty eighth.

Is it possible that either a Cystic Fibrosis mutation, or the genetic background on which such mutations occur, may enhance biological fitness?

In a recent study by Milan Macek and his colleagues in Prague, a surprisingly high prevalence of SNP markers which are found in association with the CF gene (not within the gene itself), in Czech octogenarians. They found significant differences between newborn females and elderly females (but not in males), in the frequency of markers on the chromosomes on which the majority of CF mutations reside. This means that there appear to be some, yet unidentified, positive selective factors in earlier generations contributing to the increased frequency of CF carriers in the general population. These findings need to be confirmed in other European populations (Macek et al, 1997).

A second hypothesis is that CF carriers enjoy increased fertility. This is obviously not true for males, because we know that some male heterozygotes have atresia of the vas deferens and are consequently infertile. However, increased fertility would be a possibility both in females and in those males who are able to reproduce. One would expect half of the sperm of a male carrier to be bearing the CFTR mutation. At the moment of conception, the ovum has still to complete the last stage of meiosis, when it produces two nuclei, one of which will be fertilised by the head of the sperm which has successfully broken through into the cytoplasm. In order to penetrate the

tough zona pellucida around the ovum, a complex series of changes occur in the acrosome surrounding the head of the sperm. The acrosome literally fragments, releasing proteolytic enzymes which presumably help to cut a channel through the zona pellucida. There is a major influx of calcium ions, but this is preceded by a rapid influx of sodium ions which are exchanged for hydrogen ions. Thus, a sperm whose acrosome has an up-regulated sodium-hydrogen exchanger might initiate this process and burrow through the zona pellucida more effectively.

Similarly, within the ovum, influx of sodium ions into one of the two pronuclei, in exchange for hydrogen, is the initial step of the process in which the nuclear head of the sperm is incorporated to produce a new nucleus, with half its chromosomes from the ovum and half from the sperm. Expression of mutated CFTR in the nuclear membrane of the oocyte might enhance the sodium-hydrogen exchanger, and then be a conceptional advantage. It seems that CFTR expression has been shown in spermatids, but not in spermatozoa (Gong et al, 2001). Would that be enough to up-regulate the sodium-hydrogen exchanger? No expression in ova has been seen (Tizzano et al, 2001), but the critical time would be just before or during the second stage of meiosis and it might be worth another look.

Recent work by Maurice Super and his colleagues in Manchester suggested such a meiotic drive. They found that about 57% of the offspring of CF heterozygotes, ascertained through extended family studies, had inherited carrier status, compared with the 50% which would have been expected by chance. The excess of carriers was particularly marked in the offspring of female carriers (Super et al, 2001).

It is quite possible that the heterozygote advantage is not conferred by the CF mutation itself, but by association of the CFTR gene with another nearby gene which consistently segregates with it. The $\Delta F508$ mutation, and other mutations of the CFTR gene, originated on the so-called "B Haplotype". This is defined by various markers such as KM 19 and CS 7 which are found upstream of the CFTR locus, and so frequently associated with CF that a gene in that region was prematurely reported as a possible candidate for the CF gene in 1987. (Estivill et al 1987)

The study of a stable population in the Czech Republic already referred to showed a higher incidence of the "B Haplotype" in elderly females than among new born or young adult females, suggesting that post-natal selective factors (so far unidentified), operated in the early years of the 20th century which conferred greater fitness (that is the ability to withstand and survive infectious diseases), and that these survival factors may no longer be relevant. The advantage, however, lay in the "B haplotype" rather than in the CFTR gene (Macek et al, 1997).

WHERE DID CF ORIGINATE?

It is generally agreed that modern man came out of Africa and migrated through the Middle East to the other continents. Human colonisation of Europe therefore proceeded from South East to North West. Secondary migrations from the Basque region of Spain northwards also occurred. The development of agriculture, and to some extent of languages, followed the same general directions. It should therefore come as no surprise to learn that by using various mathematical processes which I only vaguely understand, population geneticists have concluded that Cystic Fibrosis was found earlier in Greece, Italy and Spain than in middle and northern Europe. A recent paper from Barcelona examined the non-coding regions of the CFTR gene itself, and found them to be remarkably stable. It appears that the gene mutations occurred in a distinct population with different intra-genic haplotype backgrounds from those of the larger population in which they are found. Furthermore, three of the most common mutations ($\Delta F508$, G542X and N1303K) occur on a similar background, which gives support to the hypothesis that they arose in a single population in which this haplotype background was frequent. It is a haplotype which is either extremely infrequent or quite absent from other parts of the world, suggesting that it may have a very ancient origin. The two other frequent mutations studied (G551D and W1282X) are found on different but closely related haplotypes which are widespread in African, Asian and European populations. Cystic Fibrosis resulting from these mutations is most frequently found around the Mediterranean, the W1282X accounting for 36% of Israeli mutations (Mateu et al, 2002).

HOW OLD IS CYSTIC FIBROSIS?

There have been various estimates of the age of Cystic Fibrosis mutations, using a variety of methodologies, and the consensus appears to be that the $\Delta F508$ mutation is about 200 generations old. It seems that the G542X mutation, which occurs on the same genetic background, is considerably older, perhaps 500 generations (Guo & Xiong, 1997). However, the most recent paper draws attention to a wide range of estimates for the age of $\Delta F508$, ranging from 3000 years ago to more than 40,000 years ago, and on the basis of the overall frequency of CF alleles it has been proposed that $\Delta F508$ may pre-date the emergence of anatomically modern humans. The mutation certainly appears to be pre-Neolithic, and is an ancient mutation in human history. (Mateu et al, 2002; Reich & Lander, 2001) It may therefore be meaningless to speculate about either the place or population of origin of $\Delta F508$ and the other common CF

mutations, because they may be older than the ethnogenesis process that originated present European populations (Cavalli-Sforza et al, 1994).

WHERE DOES CF OCCUR?

In view of its ancient origins, it is not surprising that Cystic Fibrosis due to the $\Delta F508$ mutation is widespread throughout Europe, although less common mutations have local areas of high frequency. In general the incidence of Cystic Fibrosis in the community is roughly proportionate to the incidence of the $\Delta F508$ mutation: where it is frequently found there will be a high incidence of CF. In parts of the world where classical CF is rare, such as South East Asia, other diseases caused by mutations in the same gene may take its place, an example being infective pan-bronchiolitis found in China and Japan. However, it must be remembered that until relatively recently Cystic Fibrosis was thought to be far less common than it really is in countries such as Argentina and Brazil, where although it was present it was frequently overlooked. If medical students and young doctors are told that Cystic Fibrosis does not occur in their community, they will not think of it and consequently not diagnose it even when it presents in their clinics and hospitals. We need a great deal more information about the gene frequency in different parts of the world, so that we can estimate the extent to which under-diagnosis, rather than absence of Cystic Fibrosis is the real problem.

So much for the gene, but why do individuals with CF, who have the same mutation, have a different clinical course and outcome?

WHAT OTHER FACTORS MODIFY CYSTIC FIBROSIS?

In general terms, CF is produced by a combination of genetic and environmental factors. There are possibilities of intervention in both components. Some of the modifying factors are listed below (Table 1).

- Socio-economic status
- Other genes, e.g.
 - “Meconium ileus” gene (chromosome 19)
 - Mannose-binding lectin
 - Polymorphisms for anti-oxidising enzymes
- Infection - organisms, strains & genomovars
- Nutrition –
 - General
 - Specific, e.g. Iron, Zinc, Selenium

Table 1. Factors modifying severity of Cystic Fibrosis.

Every paediatrician knows that children from poor or disadvantaged backgrounds seem to have more frequent and severe chest infections than children from more privileged homes. In a recent study from the United States CF Registry, records of more than 20,000 children were analysed. The investigators found that poverty adversely affected growth, lung function and survival, but access to health care was not involved. They suggested that greater exposure to tobacco smoke, poorer nutrition and more respiratory virus infections in the poorer children may be contributory factors, and concluded that “socio-economic status is far and away the strongest known predictor of disease course and severity in cystic fibrosis” (Schechter et al, 2001).

Other genes, inherited independently of the CFTR gene, also play an important part in determining the clinical picture. One well known example is the gene on chromosome 19 which seems to be related to the occurrence of meconium ileus in CF babies with a “severe” CFTR genotype such as ΔF 508. Another is the gene for mannose-binding lectin: this protein binds all kinds of infective agents including viruses, bacteria and yeasts, and low levels correlate with reduced survival (Garrod et al, 1999), perhaps related to infection with *Burkholderia cepacia* (Davies et al, 2000). Much of the lung injury in CF and other conditions is attributed to peroxidation of cell membrane fatty acids by free oxygen radicals, produced from neutrophils. This oxidative stress depletes glutathione levels (Hammerschmidt et al, 2002), which vary between individuals according to genetic polymorphisms. The severity of cystic fibrosis, as measured by the rate of deterioration of lung function, is associated with gene polymorphism of anti-oxidising enzymes, specifically those involving glutathione (Henrion et al, 2001).

Certain strains and variants of the common bacteria which colonise the lungs of people with CF are more pathogenic than others. This is the reason why many advocate strict segregation of patients known to harbour these organisms, showing that quarantine still has a place in modern medicine.

The importance of good nutrition in the management of CF has often been stressed, but the role of specific nutrients is again attracting attention. Iron deficiency is associated with more severe lung disease, but this may be because bacteria such as *Pseudomonas aeruginosa* need iron for their own metabolism, and may deplete the body of its iron stores in the course of suppurative infection (Reid et al, 2002). Another trace element which is worth re-examining is selenium, an essential component of glutathione.

HOW DOES ALTERED CFTR CAUSE DISEASE?

Everyone knows that CFTR is a chloride channel, but it has a number of other functions which may contribute to the clinical features of CF (Table 2)

- A chloride channel
- Regulates other channels for sodium and chloride
- Glutathione transport
- Affects membrane recycling, exocytosis / endocytosis
- Affects fatty acid metabolism
- Affects bacterial binding, e.g. *Pseudomonas aeruginosa*

Table 2. Functions of CFTR

Disturbance of any or all of these functions can be used to explain many of the clinical features of CF. It is possible that the mechanisms of damage to different organs may result from different disturbances of function. For example, lung lesions may result from a combination of abnormal concentrations of ions and water in the airways, increased binding of bacteria, and uncontrolled oxidation of fatty acids in the cell membranes. Clearly, infection has no role in the pancreatic lesion; instead, like other forms of pancreatitis which it closely resembles, oxidative damage may be the predominant mechanism. I would therefore like to consider the importance of glutathione (GSH), which is a major antioxidant in the epithelial lung lining fluid. GSH is decreased in the apical fluid of CF airway epithelia, and this appears to be secondary to the chloride channel function of CFTR. In a human CF airway epithelial cell line, glutathione secretion was restored by a synthetic chloride channel-forming peptide (Gao et al, 2001) Because GSH is particularly effective in reducing hypochlorous acid, the major protein-damaging agent in CF sputum, the defective secretion of GSH could have a profound effect on the course of the disease. There is increasing evidence that irreversible consumption of glutathione in the pancreas is a central factor in chronic pancreatitis (Wallig, 1998). By implication, failure of glutathione transport into the pancreatic duct in Cystic Fibrosis might account for the characteristic pancreatic damage. What, then, is glutathione and how does it work? It is a sulphhydryl-containing tripeptide that efficiently scavenges reactive oxygen species, including hydrogen peroxide in its reduced form. The enzyme glutathione peroxidase (GSH-Px) oxidises reduced GSH to the oxidised form, thereby converting hydrogen peroxide to water and fatty acid hydroperoxides to hydroxy fatty acids. Oxidised GSH is reduced back to GSH by another enzyme, glutathione reductase.

The active site of GSH-Px is selenocysteine, and selenium status is closely correlated with GSH-Px activity. Could selenium status in Cystic Fibrosis therefore help play a role in the natural history of the disease? We are now entering the realms of speculation.

WHAT IF.....?

More than 20 years ago an American veterinary pathologist, Dr Joel Wallach, suggested that Cystic Fibrosis is a disease caused by nutritional deficiency of selenium. He based this hypothesis on autopsy findings in a rhesus monkey which in many respects resembled those of CF, and he reported this in the lay press rather than the medical literature. As a result, at least one child died from selenium toxicity after selenium supplements were given enthusiastically by her parents. But what if there were some underlying correlation between selenium status, glutathione metabolism and oxidative damage in the lungs and pancreas? Various authors have found low levels of plasma selenium in CF patients compared with controls (van Caillie-Bertrand et al, 1982; Neve et al, 1983; Dworkin et al, 1987) but in some parts of the world, notably New Zealand and some parts of China, healthy children have selenium levels similar to those found in Cystic Fibrosis children in Europe and North America. Others have found that plasma selenium levels in CF patients may be similar to those in healthy controls, even though erythrocyte SeGSH-Peroxidase activity was significantly lower in the patients. The SeGSH-Px activity increased in response to increased oral selenium, achieved by increasing pancreatic enzyme intake (Winklhofer-Roob et al, 1998). Selenium availability could thus still be a limiting factor for GSH-Px synthesis and levels which suffice for healthy children may be inadequate in situations of oxidative stress (Hammerschmidt et al, 2002). It is likely that it is the failure of CF epithelia to secrete glutathione which is most important, rather than the availability of selenium, but increased activity of glutathione peroxidase within leucocytes may also outstrip selenium availability. I have already referred to the correlation between the severity of lung disease and genetic variation in levels of antioxidizing enzymes, particularly those related to glutathione metabolism (Henrion et al, 2001; McKone et al, 2001)

There is another way in which selenium deficiency may affect the course of Cystic Fibrosis. The relationship between dietary selenium deficiency and an endemic cardiomyopathy with seasonal variations in China (Keshan disease) suggested that an enteral viral infection, together with selenium deficiency, may be required for the development of disease. Selenium-deficient mice proved to be much more susceptible to a benign Coxsackie virus-induced myocarditis than mice with adequate selenium. The authors of that study have now developed their hypothesis further, and shown that selenium deficiency increases the pathology of an influenza virus infection. (Nelson et al, 2001)

The principal findings of the study were that replication of a mild strain of influenza virus in selenium deficient mice resulted in a novel virulent strain that caused severe lung pathology

even when passed into selenium adequate mice. Changes in the viral genome had occurred in the selenium deficient mice, which increased the pathogenicity of the virus. The same team are now considering extending their studies to *Pseudomonas aeruginosa*.

Perhaps then there was some substance behind the hypothesis that some clinical manifestations of Cystic Fibrosis, if not its fundamental cause, might be due to a deficiency or unavailability of selenium. It is likely that if the speculation had been presented a medical journal it would have been rejected out of hand, because it was not in line with current thinking and fashion.

Releasing it to the lay press caused at least one personal tragedy. Had our Six Men become involved and questions like Why? What? What if? and How? been asked, it may not have taken two decades for interest in selenium to again become respectable. We need to be able to think laterally and to speculate, and it is the maverick ideas, perhaps originating from a chance observation, which often lead to new insights. But they must be kept under firm control, and tested rigorously before being accepted.

WHERE ARE WE GOING?

The Promised Land which is our destination is a normal life span, with a good quality of life, for people with Cystic Fibrosis. There may be more than one possible route to that destination. Some believe that the best way is through gene therapy. There are scientifically exciting new technological approaches to gene therapy, and it must be pursued, but a recent Editorial pointed out that intervention with gene therapy once a disease process has started may well have no effect on its course. Real, corrective gene therapy – probably many years from now – will require germ-line modification (Grainger, 2001).

Others put their hope in a pharmacological approach. For example, Dormer and his colleagues have recently shown that a new substance, MPB-07, developed by Dr Frederic Becq of Poitiers, can move ΔF 508 CFTR to the apical membrane of CF nasal epithelial cells in vitro, and in vivo studies are planned (Dormer et al, 2001).

If facilitating glutathione secretion in the CF airway epithelium can be shown to prevent most of the consequences of Cystic Fibrosis, we have already seen that other chloride channels may work just as well as CFTR (Gao et al, 2001) and alternative chloride channels may be activated by various drugs. We also need to know whether the availability of trace nutrients such as selenium is adequate for the demands of chronic suppurative lung disease, and, if not, whether it is possible to give supplements safely.

The importance of antimicrobial treatment is so obvious, and my expertise in microbiology so limited, that I have not attempted to discuss it today. Relevant questions in this field are more likely to begin with How? than Why?

A review of current progress in CF therapy is beyond the scope of this lecture, but exciting new developments will be presented at this and subsequent CF conferences. Such international meetings are valuable above all because they generate new questions to ask, new hypotheses to be tested, and new ideas for research.

CONCLUSION

We will only overcome Cystic Fibrosis with the aid of Kipling's six serving-men. The questions they frame need to be answered and the attitudes they typify must be accommodated. You may recognise some of them amongst your colleagues.

We need irritating Mr Wye, the nagging questioner, who challenges all our assumptions. We need pedantic Dr Watt, the obsessional who keeps us on our toes and insists on precise definitions and inflexible standards, and we certainly need his distant, imaginative and speculative cousin Dr Watif. The ambitious Professor Ware wants his Department to take a major share of the credit, and we need his drive. We all pay tribute to the doers, the workers and the practical Nurse Howe, who translates science into medicine, theory into practice, teaching into healing. We hear the anxiety of impatient Mrs Wenn with Cystic Fibrosis, who feels that time is running out for her, and stimulates us to increase our efforts. Those add up to six names, but we have left out one, perhaps the most important of all. Who will lead us to our Promised Land just over the horizon, or open the door to a better world?

“If we're the ones who can imagine it,
If we're the ones who dream about it,
If we're the ones who need it most,
Then no one else can do it ...
We're the ones”

Aurora Levins Morales, Puerto Rico.

Each of us at this conference has a unique contribution to our great adventure. By working together, and supporting each other, we shall reach our objective.

Acknowledgements.

Peter Levy and Sandra Kennedy provided information about Joseph Levy. I have consulted many people while preparing this lecture, including Melinda Beck, Manuel Buchwald, Jane Davies, Bob Dormer, Milan Macek, Maurice Super, and Billy Thompson. Some of the slides I have used were theirs, but any mistakes are likely to be my own.

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