Some relevant "Mega Papers" - there are many others!

**Discovery of penicillin and the start of the antibiotic era before which survival beyond infancy or early childhood was very rare.**


Alexander Fleming (1881-1955) (figure 13) was a Scottish bacteriologist, working at St Mary's Hospital, London, whose discovery of penicillin (1928) prepared the initial step towards the highly effective practice of antibiotic therapy for infectious diseases.

In 1945 Fleming shared the Nobel Prize for Physiology or Medicine with Ernst Boris Chain (1906-1979) and Howard Walter Florey (1898-1968) who both (from 1939) were responsible for carrying forward Fleming's initial observation by further isolation, purification, testing, and quantity production of penicillin.

In 1940 a report was issued describing how penicillin had been found to be a chemotherapeutic agent capable of killing sensitive germs in the living body. Thereafter great efforts were made, with government assistance, to enable sufficient quantities of the drug to be made for use in World War II to treat servicemen with war wounds.

Penicillin became available for a few patients with CF in the USA in 1943 and the results were reported by Paul di Sant'Agnese (1944 below) – prior to this virtually all children with CF died in infancy or early childhood from Staphylococcal pneumonia and malnutrition.

It is noteworthy that Fleming failed to develop his 1929 discovery – this was done over 10 years later by Florey and Chain. Nor was Fleming the first to observe the antibacterial effects of moulds. Ernest Duchesne (1874 –1912) (figure 14) was a French physician who noted that certain moulds kill bacteria. He made this discovery thirty-two years before Alexander Fleming observed the antibiotic properties of penicillin, a substance derived from those moulds, but his research went unnoticed. Duchesne entered l'Ecole du Service de Santé Militaire de Lyon (the Military Health Service School of Lyon) in 1894. Duchesne's thesis, “Contribution à l'étude de la concurrence vitale chez les micro-organismes: antagonisme entre les moisissures et les microbes” (Contribution to the study of vital competition in micro-organisms: antagonism between moulds and microbes), that he submitted in 1897 to get his doctorate degree, was the first study to consider the therapeutic capabilities of moulds resulting from their anti-microbial activity. Duchesne had made his breakthrough by observing how the Arab stable boys at the army hospital kept their saddles in a dark and damp room to encourage mould to grow on them. When he asked why, they told him that the mould helped to heal the saddle sores on the horses. Intrigued, Duchesne prepared a solution of the mould and injected it into a series of diseased guinea pigs. All recovered.

In a series of meticulous experiments, Duchesne studied the interaction between *Escherichia coli* and *Penicillium glaucum*, showing that the latter was able to completely eliminate the former in a culture containing only these two organisms. He also showed that an animal inoculated with a normally lethal dose of typhoid bacilli would be free of the disease if the animal was also inoculated with *Penicillium glaucum*. Unfortunately, as he was only 23 years old and unknown, the Institut Pasteur did not even acknowledge receipt of his dissertation! He urged more research but unfortunately his army service, after getting his doctorate degree, was the first study to consider the therapeutic capabilities of moulds resulting from their anti-microbial activity. Duchesne had made his breakthrough by observing how the Arab stable boys at the army hospital kept their saddles in a dark and damp room to encourage mould to grow on them. When he asked why, they told him that the mould helped to heal the saddle sores on the horses. Intrigued, Duchesne prepared a solution of the mould and injected it into a series of diseased guinea pigs. All recovered.

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beyond infancy.

*Discovery of insulin would eventually be important in the control of CF related diabetes mellitus that eventually would affect the majority of adults.*


The work reported in this classic paper eventually led to a Nobel Prize in 1923 for Banting and Macleod, in whose Toronto laboratory the work was done. Frederick Banting (1891-1941) (figure 11) was a Canadian orthopaedic surgeon who became an assistant in physiology in Ontario where he worked in Richard McLeod’s laboratory with Charles Best, a medical student. It was here, after many ups and downs and arguments and with the help of Bertram Collip, a highly trained biochemist (to extract the insulin) that they eventually produced and tried the insulin on a diabetic patient in 1922.

The paper begins – “The hypothesis underlying this series of experiments was first formulated by one of us in November 1920 (Banting was then assistant in Physiology at Western University, London, Ontario) while reading an article dealing with the relation of the isles of Langerhans to diabetes (Barron M: The relation of the islets of Langerhans to diabetes with special reference to cases of pancreatic lithiasis. Surg Gynec Obstetr 1920; xxxi :437-448). From the passage in the article which gives a resume of degenerative changes in the acini of the pancreas following ligation of the ducts, the idea presented itself that since the acinous but not the island tissue degenerates after this operation, advantage might be taken of this fact to prepare an active extract of the islet tissue. The subsidiary hypothesis was that trypsinogen or its derivatives was antagonistic to the internal secretion of the gland. The failures of other investigators in this much worked field were thus accounted for”.

The authors concluded from their experiments that – “Intravenous injections of extract from dog’s pancreas, removed from 7 to 10 weeks after ligation of the ducts, invariably exercises a reducing influence upon the percentage sugar of the blood and the amount of sugar excreted in the urine”.

This medical classic is a “good read” and one of the most significant medical papers of the 20th century as also is Moses Barron’s paper that gave him the idea (above). It is included here not only for its historical interest but also for its relevance to CF as the majority of those affected will eventually develop CF related diabetes in adult life. Although the Islets of Langerhans in CF are functioning adequately through childhood in most patients, despite their severe exocrine pancreatic insufficiency, they are eventually destroyed resulting in the majority of adults with CF developing diabetes mellitus. Efforts to prevent the slow destruction of the pancreas and prevent or delay the onset of CF related diabetes will surely become an area of research as more people survive to develop this complication which has an adverse effect both on their prognosis and quality of life.

Fanconi’s paper considered by many Europeans to be the first clear description of cystic fibrosis


Guido Fanconi (1892-1979), was Professor of Paediatrics in Zurich from 1929-1965 and a pioneer of modern scientific paediatrics (figure 2). Although this publication is considered by some Europeans to be the first clear description of CF, it is a brief report of only two children who died aged 10 months and three years with ‘coeliac syndrome’, purulent bronchitis and bronchiectasis. The pancreatic changes were quite typical of those later described in cystic fibrosis. “The changes in the lungs and pancreas, two vital organs, are so profound that their failure appears understandable”.

Martin Bodian quotes a 1928 publication of Fanconi’s (1928 Beih. Jb. Kinderhik 21) as noting cases of coeliac disease starting in early infancy and often associated with bronchitis but surprisingly Fanconi did not mention this in his 1936 paper which is so frequently quoted in Europe as the first description of cystic fibrosis.

Speaking in 2008, Walter Hitzig, the distinguished immunologist from Zurich, recalls (referring to this paper) that “My teacher Guido Fanconi, in his usual enthusiasm, presented a “new disease” to the Swiss paediatricians in 1936. However, one of the doctors questioned its importance in his daily practice. Fanconi’s answer: “Oh certainly it is important – I have seen already 3 cases!” of course earned him a big laughter in the audience! And today the syndrome described as Pankreas-Fibrose, now called cystic fibrosis, is considered to be the most frequent hereditary disease in the Caucasian population, and no doubt every...
In the 1930's, Margaret Harper of Sydney published a number of papers on various aspects of intestinal malabsorption and other aspects of paediatrics between 1921 and 1956 mostly in German or French. He was referred to in one article as a “Jack of all trades” — as were many of the paediatricians of the time. Reviewed objectively, none of these publications, with the possible exception of this 1936 paper, made any major or original contribution to the understanding or definition of cystic fibrosis. However, Fanconi made other very important contributions; in 1929 he described hereditary pancytopathy known as Fanconi’s anaemia, in 1941 he deduced that polio was spread via the gastrointestinal route rather than by droplets and apparently predicted that Down’s syndrome was due to a chromosome abnormality 20 years before trisomy 21 was discovered.

Second of three papers by Margaret Harper of Sydney who clearly recognised pancreatic steatorrhoea - but not clearly enough in her 1930 paper to cause others to recognise CF as a specific condition.


Further to her 1930 report of two children with steatorrhoea (Harper, 1930 above), Margaret Harper of Sydney reported a further eight children with features compatible with cystic fibrosis. Congenital steatorrhoea, abnormal glucose tolerance curves and characteristic pancreatic lesions in all the 4 who were autopsied; 8 of the 10 who died had bronchopneumonia. “The passage of fatty stools from birth (particularly “butter stools”) with failure of nutrition, the diagnosis of congenital defect of the pancreas can reasonably be made”. Subsequently she reported 42 children with pancreatic steatorrhoea from the Royal Alexandra Hospital for Children, Sydney (Harper, 1949 below). Interesting comments on Andersen’s paper in this 1949 paper (below).

The orange-yellow oil in, on and around the stools is now recognised as a typical and frequent feature of untreated cystic fibrosis. The discussion in this paper reviews previous reported cases with particular emphasis on the characteristics of the stools. A visit to the ward sluice to inspect the stools of children on the ward was a usual part of the paediatric ward round even in the Sixties and considered to be an essential part of the full evaluation of an ill child just as was chemical testing and microscopy of the urine by the ward doctor.

I am grateful to Professor John Walker-Smith for further information on Margaret Harper. He notes that she was a pioneer of women in medicine and reported to have been the first lecturer in Diseases of the Newborn to be appointed in the British Empire. She was appointed as a full time paediatrician in 1914 at the Royal Alexandra Hospital for Children in Sydney thus may be one of the first full time paediatricians.

Writing in 1979, Dr D G Hamilton described Margaret Harper (figure 2a) as - “the brilliant outstanding mind at the Royal Alexandra Hospital for Children. She continued her interest in babies and their welfare throughout her whole life. She greatly influenced the care of children with gastroenteritis with feeding problems and with chronic diarrhoea. This and with chronic diarrhoea. These latter were grouped under the title of the Coeliac Syndrome. Last century Samuel Gee in Britain had talked of offensive bread when discussing these children. Margaret Harper by patient trial of diets always seemed to achieve better results with her coeliac patients than any other physician and one of the things she did was to avoid giving them cereals. They were fed copious amounts of banana and cottage cheese. In 1945 it was recognised in Holland that one of the more common causes of chronic diarrhoea in little children is intolerance to gluten that is found in wheat, rye, barley and oats and the name coeliac disease was given to this particular condition. Margaret Harper’s diet of bananas, cottage cheese and no cereal was close to the mark. She was a very careful and thoughtful observer. Among these babies she recognised that a small group were different from the others. They were more prone to chest infections and their stools looked to her to be different and more greasy. These children were prone to die. She drew the attention of Dr Tidswell the director of pathology, to her belief that they were different from the others and at post-mortem he found characteristic changes in the pancreas. She was the first person in the world to recognise this as a separate condition and in 1930 published a report in the Medical Journal of Australia entitled “Two cases of congenital pancreatic steatorrhoea with infantilism”. Similar reports followed in 1935 by Arthur Parmalee in Chicago. In 1938 Margaret Harper published a more detailed paper on the subject in the British Archives of Diseases of Childhood entitled Congenital steatorrhoea due to Pancreatic defect. In 1954 Dr Charles May, an American expert, wrote a monograph on Cystic Fibrosis of the Pancreas (see May 1954 below) and dedicated the work to his teacher, Margaret Harper of Sydney, Australia and Arthur H Parmalee of Chicago, Illinois who recognised……and published the first papers, indicating the frequency and importance of the disease, clearly setting it apart from Coeliac disease, against prevalence practice. Margaret Harper was always a much loved figure at the hospital, tall, very helpful to those who tried hard, always prepared to be critical, but tempering her criticism with a laugh. She has splendid scientific mind. She entered happily and freely into bedside discussions and informal argument at clinical meetings where she was usually extremely stimulating, but she hated formal lectures. These she simply read from a prepared script in a monotonous tone and with her head down. She really was a dreadful lecturer. But she
was perhaps the greatest physician the hospital has known" (DG Hamilton. Hand in Hand. The Story of the Royal Alexandra Hospital for Children, Sydney. John Ferguson, Sydney. 134-136:1979)

This 1938 paper by Dorothy Andersen undoubtedly was of a size and contained sufficient detail to allow others to recognise cystic fibrosis as a definite clinical entity.


Although many infants had already been reported who, in hindsight, obviously had cystic fibrosis, this was the first clear detailed clinical and pathological description of a large series of affected infants in English. Dorothy Andersen reported 49 patients - 20 from her hospital and others from colleagues and the literature. She described the neonatal intestinal obstruction, intestinal and respiratory complications and many other features – but particularly the characteristic pancreatic histology (figures: 3 and 4). Salient points from the original description are as follows - "In 45 of the cases the pancreas presented a microscopic picture which is described by the term cystic fibrosis. The acini contain secretions of various sizes, and the acinar cells were flattened to form a thin epithelial wall around them. The smaller concretions were surrounded by relatively normal cells, which occasionally contained eosinophil granules......The size of the cysts varied in each case but large ones were not often noted in the youngest infants. Surrounding the acini and also the lobules there were moderate to large amounts of fibrous tissue, the quantity varying roughly with the age of the child.......The islets of Langerhans were usually normal in number and appearance."

Andersen likened much of the epithelial histology to that found in vitamin A deficiency which for some years she regarded as the primary cause of the systemic features of the condition; this causation was never substantiated although she continued to support this theory for some years. The paper contains an excellent tabulated review of the previously reported cases and describing her paper Martin Bodian (1952 below) writes - "such a clear account of the symptoms that it enabled many cases to be recognised that had hitherto been missed, and aroused such interest that it was followed by a shoal of case reports and confirmatory reviews" which appeared during
Dorothy Andersen (1901-1963) (figure 5) was the pathologist at the Babies Hospital at the Columbia Presbyterian Medical Center in New York. She had a wide range of interests and made contributions in many other areas of medicine. She was said to be a rugged individualist, a paediatric clinician, pathologist, a research chemist – also apparently a roofer and carpenter, happy to make her own home improvements! Eventually over 600 children with CF were referred to her in New York; she involved Dr. Paul di Sant’Agnese, eventually another leader in the CF field, to provide paediatric care for her patients as she was primarily a pathologist. Dr Phillip Farrell of Wisconsin lists 4 reasons for the referral of so many children to her in New York. First was this seminal 1938 publication, second a combination of her reputation and her arrogance (she is alleged to have said she was the only person in the world who knew about cystic fibrosis in the late Thirties and Forties!), third the desperation of parents and fourth New York was the one place people could reach easily in those days. Described as "windblown" by friends and detractors alike, Dorothy Andersen was considered quite a character. She is said to have kept a particularly untidy lab, holding semi-annual "glüg" parties there, in honour of her Scandinavian heritage. She was a niece of Hans Christian Andersen. She was a bright compassionate and sensitive scientist who was also a chain smoker and died of lung cancer at the age of 62 years.

In 1950 Milton Graub, a paediatrician who eventually became President of the US CF Foundation, suspected his two year old son had CF and describes how he called Dr Andersen on a Sunday morning and found her working in her laboratory. She listened to the medical history and saw his son the next day. The diagnosis was much more difficult and was made primarily on the clinical features plus analysis of the duodenal secretions. These were done and the diagnosis of cystic fibrosis of the pancreas was established. Since Dr Andersen was a pathologist she referred his son Lee for further clinical care to Dr Paul di Sant’Agnese. "He was a pediatric specialist and a delight – soft spoken, reassuring and supportive" (from Doershuk CF, 2001. below).

As in the case of Dorothy Andersen's 1938 paper, recognition of similar characteristic changes in the pancreas of occasional infants at autopsy suggested a specific condition.


Over a period of 15 years, examination of the pancreas in over 2,800 paediatric necropsies in Boston disclosed 35 examples of a well-defined pathologic lesion the existence of which was unsuspected during life. The lesion was characterised by inspissation of secretion, dilatation of the ducts and acini, atrophy and fibrosis. Only seven showed the stratified epithelium characteristic of vitamin A deficiency. The need for further information concerning the absorption of fat and especially nitrogen as well as pancreatic enzymes as an aid to diagnosis was suggested. The average age of death was 8 months.

Blackfan & Wolbach (1933 above) from a study of the lesion in the large amount of material available, in regard to pathogenesis, had observed "Our preliminary studies indicate that the pathogenesis of this striking pancreatic affection (figure 6) resides in the production of an abnormal secretion which inspissates and leads to distension and atrophy of the ducts and acini" (See also Blackfan & Wolbach, 1933 above; May CD 1943 below).

Dr. Charles D. May (1908 - 1992) was a paediatrician, an expert on childhood nutritional disorders and a leading figure in the United States paediatric world at the time. He qualified at Harvard Medical School in 1935. In World War II he served in the Army Medical Corps, returning to research at Harvard Medical School in 1946. May became an Associate Professor of Pediatrics at the University of Minnesota from 1947 to 1952, and subsequently held senior positions in Iowa, New York and Denver. He was succeeded as head of the Nutrition Clinic in Boston in 1947 by Harry Shwachman (of whom a great deal more later) who had worked with him.
In 1953, he was one of the clinicians to observe that abnormal sweat electrolytes in CF was one of the most important observations. It is likely that May gave the first lecture on CF in the UK at the Royal Society of Medicine during his time in the US army (May, 1943 below). Later he held the Chair of Pediatrics at the University of Iowa where later he chaired the first scientific meeting of the National CF Research Foundation in 1955 which was attended by most of the few US paediatricians involved in CF care. Also, around 1953, he was one of the clinicians to observe that *Pseudomonas aeruginosa* was usually involved when persistent pulmonary infection was present. According to Warren Warwick, May also authored a seminal work on pancreatic enzymes.

**The first report of the dramatic effect of the new antibiotic, penicillin, in children with cystic fibrosis.**

In 1943 a small quantity of penicillin had become available from the US Army to treat three children with CF and a further 2 in 1944 with intramuscular penicillin; the results were variable. Bryson and co-workers at the Carnegie Foundation were first to investigate the use nebulised penicillin (Bryson et al, Science 1944; 100:33). In 1944 Alvan Barach of the Presbyterian Hospital was using a penicillin aerosol to treat asthma and bronchiectasis (Barach et al, Ann Int Med 1945; 22:485). Similar apparatus was used by di Sant’Agnese for these children with CF; it consisted of a rubber mask with re-breathing bag and nebuliser with a flow of oxygen into the nebuliser (figure 10).

This is an interesting first report describing early treatment in CF and in particular the first use of nebulised penicillin. According to di Sant’Agnese, dramatic response occurred in 15 infants and young children with CF who eventually received the treatment but little success was achieved in infants less than a year old. From 1947 there were numerous publications on the use of the recently available penicillin in other conditions by various routes both oral, by aerosol and even powdered inhalations – no less than 18 such publications were reviewed in The 1948 Year Book of Pediatrics. (Poncher HG (ed). Year Book Publishers. Chicago). Undoubtedly a new era of treatment of infection had begun.

Di Sant’Agnese (1914 - 2005) (figure 11) wrote that “Penicillin is the first drug known to affect the course of fibrocystic disease after cyanosis has marked the existence of suppurative Staphylococcal bronchitis”. Later, in 2001, he recalled “In most patients the results (of penicillin treatment) were dramatic. From death’s door, slowly dying from chronic pulmonary disease while we watched helplessly, patients revived in a few days”. Sulphonamides were said to be useful in prophylaxis and for intercurrent infections but not after the stage of suppurative bronchitis.

Dr Lynn Taussig, who worked with di Sant’Agnese, later recalls that Paul di Sant’Agnese and Harry Shwachman were definite rivals but had considerable respect for each other. Di Sant’Agnese came from a noble family in Italy. His father was an obstetrician and radiotherapy expert who was physician to the Italian Royal Family. Paul went to Rome medical school and then came to USA in 1939 to study medicine in New York. He was chief resident in paediatrics and published his first paper on tick paralysis - as initially he worked in infectious disease and immunology; he studied the effect of immunisations in early life. He went to work with Dorothy Andersen at the Presbyterian Hospital in New York. In 1950 he described glycogen storage disease of the heart (Pompe’s disease) and as a gastroenterologist in the early 1950s he wrote on coeliac disease.

In 1953 his observation of abnormal sweat electrolytes in CF was one of the most important developments in CF research.
observations in the history of CF and provided the basis of the sweat test (Di Sant’Agnese et al, 1953 below). He wrote over 140 papers mostly on CF - physicochemical differences, mucoproteins, calcium in secretions, and with West the first report of pulmonary function tests, pneumothorax, ventilation, pancreas, duodenal contents, and measurements of absorption. He was the first to describe the liver changes - a few months before Shwachman's group. He had an interest in every aspect of the condition. But the patient always came first and he was an outstanding clinician as well as a gifted researcher. In 1955 he was involved with the medical aspects of the CF Foundation. In 1960 in Europe he met Archie Norman, David Lawson, and the International Cystic Fibrosis and Mucoviscidosis Association (ICFMA) had 28 delegates and guests from 14 countries. Di Sant’Agnese said he was most proud of training so many associates and colleagues in CF care and research. “If your plan is for one year, plant rice, if for 10 years plant trees, if for life – educate!”

In 1946 this present paper on the use of penicillin for fibrocystic disease, with special reference to aerolised penicillin, was the first publication on the use of this new antibiotic in cystic fibrosis.

The first clear demonstration that cystic fibrosis was inherited in a Mendelian recessive manner


In 1946 this present paper on the use of penicillin for fibrocystic disease, with special reference to aerolised penicillin, was the first publication on the use of this new antibiotic in cystic fibrosis.

Investigating 47 of their own families and 56 from the literature, the authors concluded that the familial incidence indicated a hereditary disorder with a recessive mode of inheritance, but which required more than one factor for its expression. Although the incidence in siblings approximates to the 25% expected of a Mendelian recessive trait, an hereditary condition requires more than one factor for its expression. This was a quite different explanation to that given by Fanconi et al, 1944 but in line with that of Bodian, 1952. Andersen continued to believe that “the pulmonary infection is the result of the nutritional deficiency” – obviously she considered this to be the additional factor required.

This is the first clear statement, backed by clinical evidence, that CF is inherited in a Mendelian recessive manner. Others, including David Lawson, would also speculate on the relative contribution of the basic defect and the secondary effects of the defect (such as chest infections and malnutrition) to the ultimate outlook for the patient. i.e. if the secondary effects could be prevented would the outlook be much better? Subsequent progress showed that this was indeed the case.

The report that eventually resulted in Paul di Sant’Agnese searching for and eventually finding the cause of salt depletion during the 1948 New York heat wave


One of the most important papers up to that time from New York. Walter Kessler, the senior resident at the time, and Dorothy Andersen reported 12 children with heat prostration. Ten were admitted during a New York heat wave in 1948 and no less than 7 were known to have cystic fibrosis. These were the days before air conditioning was generally available in New York and 1948 was a particularly hot summer. Paul di Sant’Agnese, was working with Dorothy Andersen at the time and looking after her patients, later said that he treated these particular infants as Andersen was away vacationing in Europe when they were admitted! The authors of this present report queried whether the sweat glands, as well as the glands of the pancreas and other organs, were inadequate in function or alternatively a low grade infection lowered the margin of tolerance to increased temperatures. At the time of this report there was no explanation as to why infants with CF were particularly susceptible to heat prostration and salt depletion – fortunately Paul di Sant’Agnese decided to find out! This was the first report that children with CF were particularly susceptible to heat. It was this original incident that eventually led Paul di Sant’Agnese, who was working with Dorothy Andersen, to search for the reason for salt depletion in many of these CF infants and eventually to his recognising the abnormally high sweat sodium and chloride, and to a lesser extent potassium. This was undoubtedly the first and most important major advance in the understanding of the causation of CF up to that time (see di Sant’Agnese et al, 1953 below).

The papers of Paul di Sant’Agnese and Bob Darling reporting the raised sweat electrolytes in all people with CF - the most important discovery up to that time.


The first report of elevated sweat electrolytes in cystic fibrosis. di Sant’Agnese collaborated with Bob Darling who was the head of the Rehabilitation Department of the Columbia Presbyterian Hospital. In tests department there was a constant temperature room and a method of collecting sweat. Initially two teenagers with CF and two controls were selected and although the sweating rate was similar the level of electrolytes was much higher in the patients with cystic fibrosis. Subsequently sweat from 9 CF children and 8 controls showed chloride more than three times higher in the people with CF than in the controls.

This was an unexpected finding quite unrelated to any previously recognised abnormalities in the condition (di Sant’Agnese et al, 1953 below) but it was the most important observation since the clear identification of CF as a specific entity by Dorothy Andersen in 1938.
In this study there were 50 patients with CF, 9 with other pancreatic diseases and 50 controls. All the CF patients had similar elevations in the sweat electrolytes. Their adrenal and renal function was normal. The authors considered the findings to justify abandoning the term “mucoviscidosis” and returning to the unsatisfactory term “cystic fibrosis of the pancreas” until a better one was proposed.

This is the main paper that di Sant’Agnese himself quotes as describing the sweat electrolyte abnormality expanding on the paper of Darling et al, 1953 (above). Di Sant’Agnese mentions the original report of Kessler and Andersen 1951 (above) and also that the susceptibility of patients with CF to heat in the summers was noted also during subsequent summers after 1948. Paul Quinton more recently recalls that di Sant’Agnese told him that the development of heat tolerance among troops sent to North Africa was attributed to adaptations to sweating, so di Sant’Agnese pursued excessive salt loss in the sweat as the most likely origin of volume depletion during the high heat stress (Quinton, 1999 below). Bob Darling was head of Rehabilitation at Columbia Presbyterian Hospital and had a constant temperature room. Di Sant’Agnese decided “as a shot in the dark to see if sweating function was impaired in CF patients that would lead to a smaller than normal volume of sweat, or whether there was something wrong with the sweat electrolyte concentration”. di Sant’Agnese continued - “In April 1952 two teenage children with CF and two controls were put in the constant temperature room and their sweat then analysed for electrolytes. To my surprise and excitement the answer was right there. There was a tremendous difference in the sweat electrolyte concentration between the two groups”. “In contrast the sweating rate was similar in the two groups” (Described in detail by Paul di Sant’Agnese. Experiences of a Pioneer Researcher. In: Doershuk CF (ed.) Cystic Fibrosis in the 20th Century 2001; Fanos JH. 2008; 17-35.). Sant’Agnese and colleagues showed the sweat abnormality was unrelated to renal or adrenal disease and was definitely related to sweat losses.

For this present paper they examined 43 people with CF, 9 patients with other pancreatic diseases and 50 controls in a room at 32.2 C for 1-2 hours. Sweat was collected onto dry gauze under adhesive waterproof plaster. Sweat chloride in the CF patients was 106 (60-160) meq/l, in controls and other pancreatic diseases only 32meq/l (4-80) and in CF the Na 133 (80-190), and in controls 59 (10-120).

This was undoubtedly the most important advance in the understanding of CF up to that time. However, astonishingly, di Sant’Agnese recalls the paper received a very cool reception at the 1953 meeting of the American Pediatric Society with not a single question! Also when presented before Jas Kuno, apparently a distinguished sweat physiologist, Kuno uttered one word - “impossible”- and walked out of the room! It is also said that even di Sant’Agnes’s close colleague Dorothy Andersen was at first reluctant to accept the findings. However, and perhaps predictably, Harry Shwachman soon visited di Sant’Agnese in New York; he was impressed and with his usual alacrity and energy was able to present a large supportive series by October 1954 much to di Sant’Agnese’s delight!

The Gibson and Cooke pilocarpine iontophoresis method of stimulating sweating was major advance from the potentially dangerous practice of heating infants in plastic bags etc.

This classic paper described the pilocarpine iontophoresis method for stimulating sweating to obtain sweat for analysis. It was a major advance, as prior to this these frail infants were often heated in blankets or plastic bags. The pilocarpine iontophoresis method is still used by most laboratories for stimulating localised sweating (figure 22).
Figure 22: Electrodes applied to infant’s forearm as pilocarpine is iontophoresed painlessly into the skin; later the sweat is collected from the treated area on weighed gauze and the weight and electrolyte content estimated in the laboratory. Most importantly, an experienced biochemist (here Dr Shapiro) is carrying out the procedure.

Subsequently, as more laboratories performed the test caution was expressed as when performed by inexperienced operators mistakes occurred. These mistakes often resulted in CF being over diagnosed when the sweat result was falsely high which could happen if the sweat specimen was allowed to evaporate. Also, there were reports of burns under the electrodes. The first report of such major diagnostic errors in the UK was from Birmingham (Smalley et al, Lancet 1978; ii: 415-417 below) from Prof. Charlotte Anderson’s unit but soon others followed (David TJ & Phillips BM. Lancet 1982; ii: 1204-1205). [PubMed] In Leeds we found that 7 (4%) of the first 179 children referred for Comprehensive Assessment of their CF from various parts of the UK did not have the condition (Shaw NJ & Littlewood JM. Arch Dis Child 1987; 62:1271-1273. [PubMed] The consequences of a false diagnosis of CF frequently had disastrous socio-medical consequences e.g. one mother had been sterilised to avoid having another child with CF as it was mistakenly thought her first child had CF. Many parents had avoided having further children; some parents took legal action against the paediatrician involved in the mistaken diagnosis. So the sweat test is an excellent test if carried out correctly by experienced laboratory staff but can be disastrous if performed by inexperienced staff as an occasional procedure. Also the result should not be accepted uncritically by the paediatrician but should be considered with all the other clinical and laboratory evidence when making a diagnosis of cystic fibrosis.

CF Centre care by a team of professionals who gain experience in treating many patients is regarded as the best way of providing hospital care for people with CF. The method was started by Leroy Matthews and Carl Doershuk in Cleveland and described in these two papers.


Dr Doershuk (figure 18) recalls that Dr William Wallace, Chairman of Paediatrics at the Babies and Children’s Hospital, Cleveland had been approached in 1957 by a parents’ organisation - the “Cousins Club” - one of whom had already lost a child with CF and had another deteriorating from the condition. They asked Dr Wallace to start a “research orientated treatment programme for CF” which they offered to fund. To develop this programme Dr Wallace appointed a young paediatrician, Dr Leroy Matthews (figure 19) to plan and initiate the “comprehensive and prophylactic (preventive) treatment programme” for the treatment of cystic fibrosis. The programme which developed eventually became the model for the CF Foundation CF centres programme (also Doershuk et al, 1964 & 1965 below).

Three important areas of treatment were the obstructive pulmonary lesion, the secondary infections and the pancreatic deficiency and nutritional state. Treatment was early and comprehensive even started before symptoms - where this group differed from others and as such were ahead of their time. This paper describes the “comprehensive therapeutic regimen” which so influenced CF care in N America.

Veteran CF physician Dr Warren Warwick of Minnesota has “fond memories of two great stars – Harry
Shwachman and Leroy Matthews”. Of Leroy Matthews he writes - “Leroy Matthews, the greatest genius CF has seen, single handedly established the value of Comprehensive Treatment, laid the ground work for Pediatric Pulmonology, organised and led the CF Centres as well as planning and directing excellent research. He made only two mistakes. He allowed his “Comprehensive Treatment” plan to be equated with “mist tent therapy” so when the mist tent was discredited many also felt the Comprehensive Care Program was discredited. And he tried too hard to control his diabetes and suffered hypoglycaemic brain injury and cardiovascular complications” (Warren Warwick in Doershuk CF (ed). Cystic fibrosis in the 20th Century. AM Publishing, Cleveland 2001:319)


A detailed evaluation of the results of the Cleveland comprehensive therapeutic regimen. 96 consecutive patients were followed for 18 to 60 months (average 37 months) and evaluated using a modified Shwachman score. 82% improved, 11% remained the same, and 4% showed progression beyond their initial status and only 3% died – none were less than five years of age. Patients who were regarded as having reversible pulmonary changes were reviewed separately in 1965 (Doershuk et al, Pediatrics 1965; 36:675 below).


Good results were reported in the group of children treated prophylactically with little progression over an average of 4.5 yrs. The early intervention and prophylactic approach was not the usual policy at this time and most clinicians waited until symptoms developed - even experts such as Paul di Sant’Agnese. In this study 98 consecutive patients had been followed for an average of 4.5 years and the clinical course of 49 were considered to be on prophylactic therapy was significantly different from the accepted natural course of the disease and from the 49 patients who had irreversible lung damage when first seen. No evidence of significant progression of the pulmonary state was seen in any of the prophylactic group. No deaths occurred in this group and the annual mortality rate was only 2% for the whole group. Their findings supported the need for early diagnosis and prophylactic treatment.

Sydney Gellis (always a sceptic regarding the treatment of CF!!) in the Year Book of Pediatrics questioned whether more mild cases had been included; also whether the improved survival could not have been due entirely to antibiotics and to none of the other methods of treatment described such as mist tents, aerosols, segmental postural drainage. di Sant’Agnese also observed that in one series of older patients (Shwachman et al, 1965) the diagnosis had not been made in many until teen age years suggesting they had a milder form of CF. Certainly patients with CF diagnosed later in childhood, who were included in some of the early adult series, undoubtedly more frequently would have had milder CF gene mutations as was confirmed in later studies (Gan K-H et al, 1995 below). Rather surprisingly di Sant’Agnese questioned the need to start the full prophylactic programme in all patients as soon as the diagnosis is made and agrees with most other clinicians at that time that treatment is not started “until there is indication of incipient pulmonary involvement”. This view regarding the start of treatment is interesting and in these days of neonatal screening, failure to start early microbiological monitoring, early eradication treatment of respiratory pathogens and early nutritional intervention, would be regarded as unacceptable.

The first report of the acid resistant pancreatic enzymes that revolutionised nutritional management during the Eighties.


An early report of the marked superiority of Pancrease over currently used enzymes at the time. Pancrease consisted of acid resistant micro spheres which only released their enzymes when they reached the more alkaline environment of the upper intestine, thus protecting their active enzyme
contents from destruction by the gastric acid. Viokase and Cotazym were the standard enzyme preparations in use at the time both of which were affected by gastric acid. Twelve children with CF aged eight to 14 years took Viokase four eight or 12 tablets, Cotazym two four or six capsules and Pancrease one two or three capsules per meal. All three preparations improved absorption compared with placebo but Pancrease did so at the much smaller dose. Viokase 12 capsules per meal and Pancrease 3 capsules per meal achieved 94% and 94.8 % fat absorption respectively. Similarly six Cotazym and three Pancrease capsules achieved 84.2% and 89.7% fat absorption respectively.

The acid resistant microspheres were undoubtedly one of the major nutritional advances, when gradually introduced during the early Eighties allowing most patients to take a normal fat intake and hence significantly improve their energy intake. The markedly better absorption of fat and protein was shown in all subsequent trials and the reduction in the patients’ unpleasant bowel symptoms and fat intolerance in many was quite dramatic (also Weber et al, 1979 below).

**Description of the immunoreactive trypsin test for newborn screening - eventually used in neonatal screening programmes worldwide.**


This study was from Auckland, New Zealand. Serum-immunoreactive-trypsin (IRT) was measured in children with CF and a variety of controls. In the first few months of life all the children with CF had a raised serum-IRT. A dried blood-spot assay for IRT was established using double antibody radioimmunoassay kit (Hoechst Research Laboratories) and presented as having potential as a screening test for CF in the newborn. There were none of the disadvantages of stool trypsin screening as infants with residual pancreatic function (“pancreatic sufficient” infants) also has a raised IRT (Figure 24).

In New Zealand Crossley and colleagues further evaluated the test (Crossley JR et al. Clin Chim Acta 1981; 113:111-121; Lyon IC et al. NZ Med J 1983; 96:673-675). Neonatal IRT screening was soon adopted in New Zealand and some states in Australia where Bridget Wilcken of Sydney has been a champion of neonatal screening since the early Nineties. The IRT test now forms the basis of most neonatal CF screening programmes throughout the world – now usually combined with DNA examination for CF-causing mutations when the IRT is positive. This was a really important landmark paper for neonatal CF screening. Neonatal IRT screening had the great practical advantage of using the same neonatal blood spots already collected by the heel prick in the first week of life for phenylketonuria and later hypothyroidism.

In the UK, Dr. Anthony Heeley, the biochemist in Peterborough, UK started IRT neonatal screening in East Anglia in 1981 (Heeley et al, 1982 below). In 2009 he kindly commented as follows - "Your summaries of our early papers are excellent. A point to emphasise was that Crossley & Elliott’s seminal work in 1979 required 2 x 12 mm blood spots for the assay. We realised that was unrealistic for routine screening and in our paper in the Lancet the same year (King DN. Heeley AF. Walsh MP. Kuzemko JA. Sensitive trypsin assay for dried-blood specimens as a screening procedure for early detection of cystic fibrosis. Lancet 1979; 2(8154):1217-1219) we showed that their results could be confirmed using 4 mm blood spots which was a much more practical size for routine prospective screening trials. These trials were quickly underway in the Antipodes, here and elsewhere, but we have ascertained that the first case of CF to be detected in a prospective screening trial with IRT was in East Anglia in 1980, a girl born one month before the first case detected prospectively by the New Zealanders. Even better, although homozygous Delta F 508, to my knowledge she led a robust and active life up to late teenage when I lost contact. Her paediatrician could not recall any exacerbation of her condition requiring hospital admission. I still treasure a slide of her as a healthy teenager and I was proudly able to show it at a National Neonatal Screening Seminar in Newcastle a couple of years ago (dragged out of retirement for the historical story)".

In 2009 Jeanette Crossley kindly commented as follows - "We were fortunate that our 1979 Lancet paper robustly established the potential of blood spot IRT for CF newborn screening, using the Hoechst- Behring Riaagnost Trypsin Kit. The kitset as supplied required a relatively large sample size [we used 2 x 12mm discs from a 5-day Guthrie card], so our next step was modifying that method. We achieved 10-fold greater sensitivity, enabling one 3mm disc as sample, left in the tube throughout the stages of the assay. In a retrospective study of 23 known CFs using this optimized assay [unpublished], each CF was clearly distinguishable from two controls from the same batch of Guthrie cards, despite the Guthrie cards having been stored at room temperature for up to seven years.

For several reasons, economics being a main one, we did not conduct an extensive prospective trial using the Hoechst- Behring reagents. Rather, we developed our own Auckland radioimmunoassay ‘from scratch’ [reported in detail in the Clin Chim Acta paper 1981]. Its validation included repeating the retrospective study, again clearly distinguishing all the known CFs from controls. We established that the molecular species assayed in blood is trypsinogen. Our ‘Auckland assay’ was more sensitive, and had better linearity for serially diluted samples, than our optimization of the Hoechst- Behring kitset assay. Also, knowing what was in all our reagents gave us confidence – we had been unable to obtain from Hoechst the exact make-up of their assay.

Our team effort launched dried bloodspot CF screening in NZ, first as a pilot, and then nationally – and also enabled Bridget Wilcken to get started in NSW, Australia. I’ve been told that our original purified trypsin and antiserum were used in the National CF Screening Programme until about 1990,
It had been a delight to be invited to present our experience with our 'Auckland IRT assay' and with our optimisation of the Hoechst-Behring Riaognost kitset sponsored by Hoechst France in Caen, 25 October 1980. We were very pleased to see the quick and thorough progress being made in several countries with validation of IRT as a CF newborn screening method. The first UK symposium on neonatal screening for cystic fibrosis, for example, sponsored by CIS (UK) Ltd and Sorin Biomedica SpA, was held in London 10 days after the Caen meeting. "

**The first major advance in understanding the basic defect since the discovery of the abnormal sweat electrolytes in 1953 by Sant’Agnese**


Undoubtedly this was a major landmark paper. Michael Knowles from North Carolina describes how they developed a technique that measured a single parameter of epithelial function – the transepithelial potential difference (PD); this they used to define salt (ion) transport properties of nasal and lower respiratory epithelium in normal humans *in vivo* and then in patients with CF whose transepithelial PD they showed was markedly higher than normal – a feature apparently present within hours of birth suggesting a primary genetic epithelial defect rather than due to any circulating "CF factor" or effect from infection.

Knowles and colleagues eventually identified a defect in the ability of CI to move across CF cells, the CI permeability of the airway epithelium was not activated by beta-agonists and there was a very rapid absorption of salt and water. This led to the theory that CF airway epithelium has an excessive rate of NaCl and water absorption leading to dehydration of the secretions, reduced clearance of mucus predisposing to chronic airway infection – a theory which increased in popularity and was championed particularly by Knowles’s colleague Richard Boucher. Michael Knowles (figure 1) was honoured with an award from the CF Foundation at the 2008 Annual North American CF Conference. He and Richard Boucher are certainly two of the outstanding North American CF clinicians and researchers of the era.

**The start of widespread use of inhaled antibiotics to stabilise patients with chronic Pseudomonas respiratory infection.**


Another definite landmark paper from the Adult CF Unit at the Royal Brompton Hospital, London. by Dr Margaret Hodson (figure 16), later Professor of Cystic Fibrosis there. Professor Hodson was to make many major contributions to the treatment of people with CF from her vast experience at the Brompton Hospital treating many hundreds of adult patients with CF. This early paper had a major influence on treatment in the UK although there had been many earlier concerns about increasing the incidence of bacterial resistance from nebulised antibiotics. Although nebulised penicillin had been used in the Forties when *S. aureus* was the main pathogen (di Sant'Agne, et al, 1946 above) it was undoubtedly this present paper that revived the interest in nebulised antibiotics for patients with chronic Pseudomonas infection - particularly in the UK. The nebulised anti-Pseudomonal antibiotics obviously stabilised the condition of some patients with relapsing chronic *P. aeruginosa* infection who were requiring increasingly frequent courses of IV antibiotics (figure 17) and represented a major milestone in treatment.

As a result of this paper nebulised anti-Pseudomonal antibiotics became widely used in the UK for patients with chronic Pseudomonas infection. In this respect the UK was well in advance of North America where, even in 1986, McLusky and colleagues from Toronto advised that, "until additional well-controlled trials were completed their routine use (of inhaled antibiotics) was not justified because of cost, potential side effects and the propensity to select resistant organisms" (McLusky et al, 1986 below). As it turned out, both the routine use of anti-Pseudomonal antibiotics to suppress chronic infection as recommended by Margaret Hodson in 1991 (Ramsay BW et al, 1999) and the early use of colomycin to eradicate early infection (Littlewood et al, 1985; Valerius et al, 1991) both proved to be effective, proven and eventually widely used treatments for people with CF on both sides of the Atlantic.

**Improved survival with regular courses of intravenous anti-Pseudomonal antibiotics in chronically infected patient in Copenhagen - a new concept in aggressive treatment which influenced many clinicians**


This is one of the main reports of improved survival at the Danish CF Centre since starting 3-
monthly courses of intravenous anti-Pseudomonal antibiotics for chronically infected patients. During the period 1971-75, 51 chronically infected CF patients were treated with IV antibiotics but only when their condition deteriorated. During the period 1976-80, 58 chronically infected CF patients were treated with regular courses of intravenous antibiotics every three months. The five year survival of patients from the time of the onset of their chronic *P. aeruginosa* infection increased from 54% in the first period to 82% in the second period. The authors concluded that intensive "maintenance" chemotherapy against *P. aeruginosa* improves survival and quality of life of CF patients although permanent eradication of chronic *P. aeruginosa* is not accomplished.

These results were largely ignored outside Denmark even in Europe. True, this was not a controlled trial and there were problems with the cost of the antibiotics, drug allergies, the patients' time and quality of life and there were other treatments which could have favourably affected the outcome. It is also particularly relevant that long term nebulised antibiotics, that frequently stabilise the respiratory function of chronically infected patients, were not used at the time of this study. Unfortunately, even in 2010, there is still no totally adequate controlled trial of this form of regular IV antibiotic treatment although many clinicians have increasingly adopted a policy of very early intervention – which in practice almost amounts to 3-monthly treatments for some patients. One study from the UK comparing 3-monthly elective with symptomatic treatment showed no advantage in elective treatment but even the "treat when symptomatic" group received 3 courses of IV antibiotics annually (Elborn et al, Thorax 2000; 55:355-358 below).

Perhaps the frequency of intravenous antibiotic treatments is best left to the judgement of the clinician and the CF team who know the patient – and last, but not least, to the patient’s opinion! Certainly the availability of effective inhaled antibiotic therapy has influenced the management of the patients with chronic *P. aeruginosa* infection and reduced the need for intravenous antibiotic treatments in some patients.

**Another major scientific milestone - ion impermeability rather than defective ion exchange.**


Another landmark paper in the understanding the CF defect by Paul Quinton (figure 2) , who himself has CF. He later recalls (Quinton, 1999 below) that Mike Knowles (1981 above) reported a significantly larger than normal electronegative potential across the nasal epithelium, along with the fact that NaCl absorption was inhibited in the CF sweat ducts and also that sodium was relatively more absorbed than chloride. This gave Quinton the idea that the basic defect in the CF duct had to be due to an anion impermeability and not defective anion exchange. Using a series of microperfusion experiments of sweat glands (it is said some from his own forearms) he measured the electrolytes by "energy dispersive X-ray analysis”. The chloride impermeability he had shown in sweat glands was the basis for the raised sweat electrolytes and provided a physiological explanation for the high salt content of CF sweat and also "provided the first description of a basic cellular defect that has since proven to be uniformly inherent in all CF affected cells”.

Paul Quinton holds the Nancy Olmsted Chair of Pediatric Pulmonology UC San Diego and is Professor of Biomedical Sciences at UC, Riverside.

**Cross infection with potentially dangerous pathogens - a new era in CF care. Gradually had a profound influence on the treatment and social life of people with CF and their families - cross infection with dangerous pathogens.**


The prevalence of *Pseudomonas cepacia* infection in a population of approximately 500 patients with CF in the Toronto CF centre, increased from 10% in 1971 to 18% by 1981. The carriage of *P. aeruginosa* had remained unchanged at 70% to 80% over the same period. Patients infected with *P. cepacia* had greater impairment of pulmonary function than those with *P. aeruginosa* alone. A syndrome characterized by high fever, severe progressive respiratory failure, leukocytosis, and elevated erythrocyte sedimentation rate ("cepaia syndrome") had occurred in eight patients over the previous three years, with a 62% fatality rate. Because of *P. cepacia* strains are uniformly resistant to ticarcillin, piperacillin, and aminoglycosides, and because ceftazidime is ineffective despite *in vitro* activity, treatment of these infections was very difficult. Prevention of acquisition and effective treatment of *P. cepacia* in patients with cystic fibrosis had become a major problem in the Toronto clinic.

This paper describes the devastating effect of the introduction of *B. cepacia* into a CF clinic population very clearly. The potential for spread between people with CF and the serious, often fatal, consequences (the so-called "cepaia syndrome") were not fully appreciated in the UK until the early Nineties when John Govan’s publication documented definite person to person spread (Govan et al, 1993 below). From 1993 there was a general introduction of infection control measures in CF Centres in the UK; also there was an end to the North American CF holiday camps which were shown to be an important source of acquisition of the *B. cepacia* infection.

In Leeds we had three patients who developed serious *B. cepacia* infections some months after visiting Canadian CF camps. In 1990 we published the first UK paper on *B. cepacia* in CF, having identified 11 patients with the infection over 6 years since 1984 but we found no evidence of obvious cross infection between our patients when we first identified the infection (Simmonds EJ,

**The first heart-lung transplants in CF patients by Mr Yacoub’s team in London provided hope for those with terminal cystic fibrosis.**


A major advance, for those who had reached the end stages of their disease (an FEV1 predicted of less than 30%), was the successful introduction of heart-lung transplantation in 1985, pioneered by Sir Magdi Yacoub (Figure 29) and his team in London (Yacoub et al, 1990; Scott et al, 1988 below) and Mr John Wallwork and his team in Cambridge. The possibility of successful treatment in what were previously the terminal stages of the condition had a major influence on both prognosis and the treatment of severely affected individuals. The first results of heart-lung transplants were quite remarkable and were related both to surgical skills, concentrated medical expertise in assessment and after care and also to more successful immunosuppressive therapy to prevent rejection of the transplanted organs. Later double lung transplants became more popular (Pasque et al, 1990 below) and are now the favoured operation. Living donor lung transplants have proved successful in some centres (Cohen & Starnes, 2001 below).

**The following two papers resulted in localisation of the CF gene to chromosome 7**


This was the first positive move towards narrowing down the identification of the CF gene. The linkage relationships of the serum arylesterase paraoxonase (PON) was examined in normal Danish families and in Danish and English CF families. The highest correlation was found between the inheritance of paraoxinase and cystic fibrosis. Linkage studies for PON against 64 other polymorphic marker systems did not give such a close relationship. By the present screening of about 2/3 of the genome could tentatively be excluded as the region of PON and cystic fibrosis.


Lap Chi Tsui and co-workers used a restriction fragment to localise the gene to the long arm of chromosome 7. In a set of 39 families, a polymorphic DNA marker was genetically linked to the autosomal recessive gene that causes cystic fibrosis. The DNA marker (called D0CR1-917) was also linked to the paraoxinase locus, which by independent evidence is linked to the CF locus (Eiberg et al, 1985 above). The location of the CF gene was now narrowed to about 1 percent of the human genome (about 30 million base pairs). This was the first step in molecular analysis of the CF gene. This was a major step forward localising the CF gene to chromosome 7.

Apparently Lap Chi Tsui encountered several problems when working with the pharmaceutical firm, Collaborative Research Inc., that had already invested $10 million in the project; they could see the commercial opportunities of developing markers for antenatal and carrier diagnosis. The highest correlation was found between the inheritance of paraoxinase and cystic fibrosis. Linkage studies for PON against 64 other polymorphic marker systems did not give such a close relationship. By the present screening of about 2/3 of the genome could tentatively be excluded as the region of PON and cystic fibrosis.

Essentially Collaborative Research Inc. wanted to delay publication of the location of the gene on chromosome 7 until more markers could be identified and patents could be applied for. Meanwhile other groups, Williamson’s in London (Wainwright et al, 1985 below) and Ray White’s in Utah (White et al, 1985 below) were said to know of the findings that the CF gene was on chromosome 7 and had identified other markers very close to the CF gene.

**The three papers that appeared in the historic issue of Science in 1985 describing the identification of the CF gene**


Approximately 70 percent of the mutations in cystic fibrosis patients correspond to a specific deletion of three base pairs, which results in the loss of a phenylalanine residue at amino acid position 508 of the putative product of the cystic fibrosis gene. Extended haplotype data based on DNA markers closely linked to the putative disease gene locus suggest that the remainder of the cystic fibrosis mutant gene pool consists of multiple, different mutations. A small set of these latter mutant alleles (about 8 percent) may confer residual pancreatic exocrine function in a subgroup of patients who are pancreatic sufficient. The ability to detect mutations in the cystic fibrosis gene at the DNA level had important implications for genetic diagnosis.

Overlapping complementary DNA clones were isolated from epithelial cell libraries with a genomic DNA segment containing a portion of the putative CF locus, which is on chromosome 7. Transcripts, approximately 6500 nucleotides in size, were detectable in the tissues affected in patients with CF. The predicted protein consists of two similar motifs, each with (i) a domain having properties consistent with membrane association and (ii) a domain believed to be involved in ATP (adenosine triphosphate) binding. A deletion of three base pairs that results in the omission of a phenylalanine residue at the center of the first predicted nucleotide-binding domain was detected in CF patients.


An understanding of the basic defect in the inherited disorder cystic fibrosis requires cloning of the cystic fibrosis gene and definition of its protein product. In the absence of direct functional information, chromosomal map position is a guide for locating the gene. Chromosome walking and jumping and complementary DNA hybridization were used to isolate DNA sequences, encompassing more than 500,000 base pairs, from the cystic fibrosis region on the long arm of human chromosome 7. Several transcribed sequences and conserved segments were identified in this cloned region. One of these corresponds to the cystic fibrosis gene and spans approximately 250,000 base pairs of genomic DNA.

The production of rhDNase had a major effect on the treatment of people with CF - one of the major clinical advances of the decade.


An early report of the significant effect of recombinant human DNase1 (Pulmozyme) on sputum.
viscosity in people with CF by Steve Shak (Figure 17). To evaluate the potential clinical utility of recombinant human DNase I (rhDNase) in the treatment of CF, the authors cloned, sequenced, and expressed rhDNase. Catalytic amounts of rhDNase greatly reduced the viscosity of purulent CF sputum, transforming it within minutes from a non-flowing viscous gel to a flowing liquid. The reduction in viscosity is associated with a decrease in size of the DNA in the sputum. The authors suggested that inhalation of an rhDNase aerosol may be a simple direct approach that would help individuals with CF, and other patients with pneumonia or bronchitis, to clear their airways of purulent secretions.

In 1950 Armstrong JB & White JC (Lancet 1950; 2:739-742). [PubMed] had shown that bovine pancreatic DNase1, added to viscid purulent sputum, destroyed the extra cellular fibres of DNA and reduced the viscosity. Later Elmes PC & Armstrong JB (Thorax 1953; 8:295-300). [PubMed] reported its use in chronic bronchitis, but the side effects of the bovine preparation eventually precluded its use and further development at that time (Raskin P. Am Rev Respir Dis 1968; 98:697-698). [PubMed] Subsequently the rhDNase (Pulmozyme) reported here proved to be one of, if not the, major therapeutic advance of the Nineties. By 2005 Pulmozyme was taken by almost 70% of people on the US CF Foundation patient registry; eventually it would be used earlier and by milder affected and younger patients.

The possibility of eradicating early Pseudomonas infection, first suggested in a letter to the Lancet from Leeds (Littlewood et al, 1985) was confirmed in this clinical trial from Copenhagen


A randomised controlled trial from Copenhagen confirming that early P. aeruginosa infection could be eradicated in 80% of patients with CF by three weeks treatment with oral ciprofloxacin and nebulised colistin. The infection became chronic in only 2 of 14 (14%) of treated patients but in 7 of 12 (58%) of the controls. The authors comment - "Our results thus confirm and extend the preliminary report by Littlewood et al, colleagues (1995 above) who used colistin inhalations Since chronic colonisation with Ps aeruginosa is associated with increased morbidity and mortality we recommend the use of anti-Pseudomonas treatment whenever Ps aeruginosa is isolated from the sputum of cystic fibrosis patients" . The authors refer to our letter to the Lancet in 1985 (Littlewood JM, Miller MG, Ghoneim AT, Ramsden CH. Nebulised colomycin for early Pseudomonas colonisation in cystic fibrosis. Lancet 1985;i: 865. [PubMed] Our first report of the use of nebulised colomycin to eradicate early Pseudomonas infection and, although only a modest letter, was undoubtedly the most important publication of my career!!)

Even though the Danish numbers were small and the patients not formally randomised, this was certainly one of the most important papers of the decade and confirmed our 1985 report that early Pseudomonas infection could indeed be eradicated with nebulised colomycin and oral ciprofloxacin. It is difficult to understand why this satisfactory trial from Copenhagen, which so clearly contradicted the previous widely held belief that it was impossible to eradicate Pseudomonas once cultured, was not followed by the widespread introduction of early eradication treatment for P. aeruginosa. Fortunately a few centres did introduce early eradication treatment but they were a minority. The fact that early treatment of Pseudomonas was so slow to be introduced in the UK and much of Europe (with notable exceptions) and was still not recommended in the USA over a decade after this Danish report, was surprising and still difficult to explain.

It was predictable that these varied approaches to early treatment of Pseudomonas were reflected gradually in the markedly different prevalence of chronic Pseudomonas infection in different CF centres- this difference in the prevalence of chronic infection became increasingly obvious first in paediatric patients as time progressed (Frederiksen et al, 1996; Lee et al, 2003; Lebecque et al, 2006 all below).

In 1997 I had the good fortune to interview the late Dr Christian Koch (figure 19), then the Medical Director of the Copenhagen CF centre, for a video. When I asked him at the end of the day what aspect of CF treatment he regarded as the most important, he thought for some time and then replied -

"When I look back on what we’ve done all through the years that I’ve been involved with cystic
fibrosis, I would say that the early treatment of Pseudonomas is probably the best thing that we have done for the patients. It becomes more and more clear that really what determines the long term course is whether you get Pseudomonas or not”.

It is of interest that even in 1998, reviewing the history of Pseudomonas infection in people with CF, a highly regarded US CF centre director wrote - "early administration with aerosol colistin may be done for the patients. It becomes more and more clear that really what determines the long term course is whether you get Pseudomonas or not". Though by this time early eradication of P. aeruginosa was widespread practice in Europe and already supported by many publications in addition to that of Valerius et al, 1991 from Copenhagen (Brett MM et al. Arch Dis Child 1992; 67:1066-1068; Frederiksen B et al, Pediatr Pulmonol 1997; 23:330-335; Weismann HG, et al. Pediatr Pulmonol 1998; 25:88-92; Ratjen F, et al. Lancet 2000; 358:983-984; Munck A et al. Pediatr Pulmonol 2001; 32:288-292).

Carla Colombo describes the use of ursodeoxycholic acid for CF related liver disease - a treatment subsequently used in most CF centres


The published report from Milan of data presented in 1989 at the European CF Society Meeting in Prague (1990 above) reporting the favourable effect of ursodeoxycholic acid (URSO) treatment 10-15 mg /kg day in 9 patients with CF who had chronic liver disease (presumably the same patients as reported in Prague in 1989). Liver function tests improved significantly (AST- by 34%, alanine aminotransferase – by 41%, gamma glutamyl transpeptidase – by 41% and alkaline phosphatase- by 19%).

Subsequently URSO was widely used in people with CF associated liver disease and represented one of the major advances in CF management. A further trial was published in 1996 (Colombo et al, 1996). Much information was published supporting URSO treatment at an early stage of liver involvement and eventually most people with evidence of liver involvement were treated with URSO.

However, a Cochrane Review updated in 2008 concluded "There is insufficient evidence to justify its (URSO) routine use in cystic fibrosis". In the writer's opinion this conclusion was unfortunate as it could influence clinicians to withhold treatment from people with liver involvement. The following statement would have been more appropriate - "There is a considerable amount of evidence that URSO improves liver function in people with CF particularly if used at an early stage; but, as yet, there is no clinical trial that conforms to Cochrane standards to show this”. A new and quite unexpected complication relating to the use of large doses of the recently introduced high strength pancreatic enzymes


The first report of fibrosing colopathy – a new, unexpected and serious complication subsequently shown to be related to the use of the new high strength pancreatic enzyme preparations that had been introduced during the previous 2 years (Pancrease HL, Creon 25,000, Nutrizym 22). The authors observed five children with CF, who presented over a period of two months, with meconium ileus equivalent that failed to respond to medical management. At surgery, four had a stricture in the ascending colon (figure 31 similar), and all had histopathological changes of post-ischemic ulceration repair, with mucosal and submucosal fibrosis (figure 31). The only common change in the management of these children had been a switch from conventional enteric-coated pancreatic enzymes to a high-strength product between 12 and 15 months before presentation.

A subsequent case controlled study from these authors (Smyth et al, 1995 below) showed a significant correlation with both a high enzyme dose and the make of preparation – only those enzymes containing a covering of the copolymer eudragit being implicated. A similar study from the USA confirmed the association with high enzyme dosage but not with the copolymer covering (Friedman JP, FitzSimmons SC. Colonic strictures in patients with cystic fibrosis: results of a survey of 114 cystic fibrosis care centers in the United States. J Pediatr Gastroenterol Nutr 1996; 22:153-156). [PubMed]. However, no further cases were reported or have been since reported, in patients taking the high strength Creon 25,000 preparation (which does not contain eudragit) even when given for 3 years in high doses to children (Connett et al, 1999 below).

In the UK the recommendations of the Committee on Safety of Medicines to avoid doses of enzymes in excess of 10,000 IU lipase per kg day and preparations containing the copolymer eudragit in children in the UK, abolished the condition continued to occur in the USA. The enzyme content of the various preparations are described in detail elsewhere (Littlewood JM, Wolfe SP, Conway SP. Diagnosis and treatment of malabsorption in cystic fibrosis. Pediatr Pulmonol 2006; 41:35-49)[PubMed]

The first report of serious cross infection with P. aeruginosa, proved by genomic finger printing techniques, in Liverpool


A high proportion of children attending the Liverpool paediatric CF centre were found to be
chronically infected with a \textit{P. aeruginosa} that was resistant to ceftazidime and other beta-lactam antibiotics. Two genomic fingerprinting techniques were used to see if this had arisen from epidemic spread of a single strain. 92 (76.7\%) of the 120 children attending the clinic were infected with \textit{P. aeruginosa}, and 65 (71\%) of these 92 infected infants harboured isolates that were resistant to ceftazidime; 55 of the 65 children harboured the same epidemic strain - resistant to ceftazidime, azlocillin, and imipenem, and sensitive to tobramycin and ciprofloxacin.

This study provides the first molecular evidence of a long-term “outbreak” of \textit{P. aeruginosa} in a CF centre. The authors suggested that careful surveillance of the prevalence of antibiotic resistance in CF centres should be instituted with measures to prevent cross-infection. They suggested that anti-Pseudomonal monotherapy “should be considered with caution”. This lesson had already been learned in Copenhagen in the early Eighties (Pedersen et al, 1986 above) and in the past a number of writers had already cautioned against the use of monotherapy. Most CF centres at the time already followed the recommendation to use two antibiotics when treating exacerbations of Pseudomonas infection. However, prior to this outbreak, intravenous ceftazidime monotherapy had been routine in the Liverpool CF clinic.

This was a very important paper which highlighted the risk of cross infection with \textit{Pseudomonas aeruginosa} in CF clinics now clearly identified by genomic finger printing techniques. Later this highly transmissible “Liverpool epidemic strain” of \textit{Pseudomonas aeruginosa} was reported elsewhere and subsequently spread to many other CF centres in the UK.

It is cause for concern that, even after experience such as reported here, there is still discussion as to the use of one or two antibiotics for the treatment of exacerbations – even to the extent of a Cochrane review which failed to give firm advice to use two antibiotics!! (Ephick HE, Tan A. Single versus combination intravenous antibiotic therapy for people with cystic fibrosis. Cochrane Database of Systematic Reviews. 2005).

**The first suggestions that macrolides may have a favourable effect in people with CF who had \textit{P. aeruginosa} infection**


Mark Everard (figure 41) confirmed with me that this was the first report from Sheffield UK and Perth Western Australia showing the anti-inflammatory effect of macrolides in people with cystic fibrosis. Four of six patients with CF had significant reduction in IL-8 sputum levels after one month treatment with low dose (200 mg tds) oral erythromycin. He believes the first report of the use of macrolides in CF was in a Japanese publication by Nakanishi et al in 1995 – “A 16-year-old boy was admitted to our hospital because of coughing, sputum, and exertional dyspnea. Seven months after birth cystic fibrosis had been diagnosed. The chest roentgenogram on admission showed diffuse reticulonodular shadows and overinflation. Pulmonary function tests revealed obstructive and restrictive impairment. Erythromycin and Lomefloxacin were administered by mouth, and aminoglycosides were administered by inhalation. His symptoms were alleviated, and he is now an outpatient. In Japan, cystic fibrosis is rare, and this patient is extremely rare because he has grown up to be a 16-year-old. In this case, low-dose and long-term erythromycin administration was very effective”. (Nakanishi N, Ueda N, Kitade M, Moritaka T. A case of cystic fibrosis in a Japanese student. Jpn J Thoracic Dis 1995; 33:771-774.) [PubMed]


These were important developments eventually leading to a number of large clinical trials and the widespread use of azithromycin in people with CF – undoubtedly one of the major clinical treatment advances of the Nineties and new Millennium (Equi et al, 2002 below; Saiman et al, 2003 both below). By 2005 nearly 54\% of all the patients on the CF Foundation Registry were taking azithromycin.

**The firm proof that inhaled tobramycin was an effective treatment for patients chronically infected with \textit{P. aeruginosa}**


One of the most important clinical trials of the decade showing a significant benefit of inhaled preservative free tobramycin (TOBI) given during alternate four week cycles for 24 weeks to patients chronically infected...
with *Pseudomonas aeruginosa*. Treated patients had an average increase of FEV1 of 10% predicted at 20 weeks where as those on placebo had a 2% decline; also the treated patients had 23% fewer hospital admissions.

The introduction of TOBI, supported by this excellent clinical trial, was one of the major clinical advances of the decade and the culmination of work started in the late Eighties by Arnold Smith and others (Smith AL et al. 1989 above). By 2005 nearly 60% of people with CF in the US CFF Registry were receiving nebulised TOBI. Although the cost (£10K per annum in the UK) has restricted the use of the TOBI preparation in the UK, inhaled anti-Pseudomonal antibiotics (colistin, tobramycin for injection and gentamicin) have been widely used in the UK for CF patients with chronic Pseudomonas infection since Margaret Hodson’s important 1981 paper (Hodson et al, 1981 above).

Dr Bruce Montgomery (Figure 47) now Senior Vice President and head of Respiratory Therapeutics at Gilead Sciences, was closely involved with the development and trials of this preparation and has been involved subsequently with other new treatments including, more recently, nebulised aztreonam lysine for inhalation.