Physiological Basis of Cystic Fibrosis: A Historical Perspective

PAUL M. QUINTON

Department of Pediatrics, University of California, San Diego, La Jolla; and Division of Biomedical Sciences, University of California, Riverside, California

I. Introduction
II. From Witches to Science
III. Research in the “Wild West”
IV. The Mucus Story
A. Pancreas
B. Airways
C. Gastrointestinal tract
D. Reproductive tract
E. Salivary glands
F. The cell
V. The Electrolyte Story
A. Sweat gland
B. Pancreas
C. Airways
D. Intestine
E. The gene and cell
VI. Which Story to Believe?

Quinton, Paul. M. Physiological Basis of Cystic Fibrosis: A Historical Perspective. Physiol. Rev. 79, Suppl.: S3–S22, 1999.—Cystic fibrosis made a relatively late entry into medical physiology, although references to conditions probably reflecting the disease can be traced back well into the Middle Ages. This review begins with the origins of recognition of the symptoms of this genetic disease and proceeds to briefly review the early period of basic research into its cause. It then presents the two apparently distinct faces of cystic fibrosis: 1) as that of a mucus abnormality and 2) as that of defects in electrolyte transport. It considers principal findings of the organ and cell pathophysiology as well as some of the apparent conflicts and enigmas still current in understanding the disease process. It is written from the perspective of the author, whose career spans back to much of the initial endeavors to explain this fatal mutation.

I. INTRODUCTION

During my career, cystic fibrosis (CF) has grown vigorously in science, medicine, and society. Scientifically, CF has advanced from innumerable speculations about its cause to a precise definition of causative mutations accompanied by accurate, quantitative descriptions of their physiological effects. Medically, the life expectancy of CF patients has increased from death in early childhood to an age over 30 years (98), with a great reduction in pediatric morbidity. Socially, it has almost become a household word, enlisting the support of millions of citizens in the struggle for its control (28). It is little wonder in view of this wide range of effects and of the remarkable progress made in our time in understanding the disease that it is now considered by some to be a paradigm for integrating scientific, medical, and social efforts in the control of a disease. But still we have no cure and no effective control of its relentless destruction of the lungs and pancreas.

This review is constrained to the events and observations that, from my viewpoint, seem to bear directly on the development of fundamentals in our understanding of the disturbances in the physiological systems and processes that result in the disease we call CF. I can in no way deal with all of the observations, insights, and efforts that have created our present understanding. An excellent recent review (266) covers a number of contemporary and
II. FROM WITCHES TO SCIENCE

Historical searches lead us to believe that at least some of the characteristic symptoms of the disease may have been recognized and been associated with its mortality in the primitive societies of eastern Europe even before the Middle Ages. Most of our information on the earliest indications of CF come from the persistent diggings of Busch (40–46). A few references uncovered in medieval folklore predict death for an infant that tastes “salty” when kissed. Such infants were thought to be “hexed” (46). In 1606, Alonso y de los Ruyzes de Fonteca, professor of medicine at Henares in Spain, wrote that it was known that the fingers taste salty after rubbing the forehead of the bewitched child (3). The modern diagnostic criterion of salty sweat seems to have been seen even then as a premonition of sickness, emaciation, and early death. Possibly the earliest accurate medical description of the pancreatic lesion in a case of CF was given in an autopsy report on a supposedly “bewitched” 11-yr-old girl in 1595 by Pieter Pauw, professor of botany and anatomy in Leiden, The Netherlands. He described the child as being meager with a swollen, hardened, gleaming white pancreas (46). Anecdotal records from the mid-1600s report conditions that must have reflected steatorrhea, presumably arising from pancreatic insufficiency, another diagnostic hallmark of the disease (40, 42, 44, 124). A few records from the 19th century and early 20th century associated steatorrhea, meconium complications, and pancreatic lesions (103, 213). Landsteiner, who also defined blood groups, published possibly the first modern description of meconium ileus associated with a defective CF pancreas (156). Meconium ileus is now considered to be almost pathognomonic of the disease (216).

The turn of the 20th century brought the first observations that began to associate lung disease with diarrhea and abnormal pancreatic function as well as reports of congenital familial steatorrhea (103, 272). Although it was still nameless, the present day terminology for CF began to evolve during the 1930s. These descriptions focused on the pancreatic involvement and steatorrhea and often related it to a form of celiac disease, although the concurrence of bronchopulmonary complications were noted as well (4, 26, 95, 119). Fanconi et al. (95), in 1936, were probably first to refer to the disease as “cystic fibromatosis with bronchiectasis” and recognized it as separate from celiac disease. Andersen in 1938 (4) used the term “cystic fibrosis of the pancreas.” Histochemical examinations of other organs revealed inspissations and concretions in the ducts and lumens of intestine and lung. By 1945 (before the abnormality in sweat was known), Farber (96) had introduced the term mucoviscidosis to challenge the fledgling nomenclature that Andersen had focused on the pancreas. He firmly believed that the cause of the disease was a generalized “state of thickened mucus.” This name is still widely used and often preferred outside the English speaking world. In retrospect, both names were misnomers reflecting the limited view of the pathology at the time. In 1946, Andersen and Hodges (5) refined the observations and presented the first compelling evidence that the disease is genetic and results from an autosomal recessive mutation. Two years later, an impressive heat wave in New York resulted in a high incidence of heat prostration among a number of her patients at Columbia Hospital (136). Fascinated by this event, di Sant’Agnese, also at Columbia, sought the source of the salt loss that had precipitated the heat prostration (73). His conclusion brought the empirical insights of primitive folklore on salty tasting kisses into the medical literature of the 20th century. di Sant’Agnese presented his findings to the American Pediatrics Society in 1953 as the last paper of the day which he noted “almost no one attended” (personal communication). General acceptance of the abnormality as a fundamental component of the disease took several years. But within 5 yr, it was established that the uniqueness of this particular abnormal physiological function afforded a method for accurate, accessible diagnosis. The sweat test quickly became and remains the most common and reliable single parameter, possibly excepting recently available genetic analysis, for differential diagnosis of CF (158).

However, these apparently unrelated findings set the stage for confusion. On the one hand, it was obvious to the pathologist that the disease was caused by abnormal mucus or proteinaceous inspissations in the lumens of pancreas, lung, and intestine. Adjectives like “thick,” “sticky,” “tenacious,” and “inspissated” were used liberally in early reports (4, 26, 95, 96, 277) and continue to be used (93, 94, 150, 184, 207, 255) to describe the microscopic changes observed in affected tissues. On the other hand, the sweat gland with its abnormally salty secretion, which produced no mentionable amount of mucus and showed no structural defects, became the clearest crite-
rion for diagnosis. Any unifying hypothesis needed to explain both.

III. RESEARCH IN THE “WILD WEST”

In the ensuing 20 years, the search for a basic defect that would unify the disparate symptoms of the disease followed numerous paths mainly based primarily on empirical observations, often completely unrelated to any known pathology. Much of the literature from the late 1960s and 1970s came from what might be viewed as a “Wild West” era of research. Few things made sense, and there seemed to be almost no guidelines. I well remember Efraim Racker’s remark in 1985 as he began an overview of CF as an address to a National Institutes of Health sponsored symposium at the Heart House with the remark that “Anyone who has read the literature in CF and says he isn’t confused, is confused.”

The first reported putative cellular defect was an offshoot of observations in glycogen storage disease in which Danes and Bearn (63, 64) reported that fibroblasts cultured from CF patients developed significantly more metachromatically stained granules than those derived from normal subjects. Later in the course of examining the effect of tobacco smoke on ciliary beating, Spock (242) reported that serum from patients with CF caused increased mucus release and induced a marked dyskinesia in the ciliary action of rabbit trachea when applied to the luminal surface in vitro. The excitement gained much momentum when Bowman et al. (34) reported that the effect could be duplicated using oyster cilia and Dogett and Harrison (83) reported that it could be reversed by heparin and that a cationically charged protein was the “factor” responsible for the effect.

The idea that a circulating humoral factor was at the heart of the pathology gained still greater attention when Mangos and co-workers reported that sweat or saliva from CF patients applied to the luminal surface of rat salivary gland ducts (172) or human sweat gland ducts (173) inhibited active Na+ absorption in these epithelia. Rao and Nadler (202) suggested that a defect in arginine esterase accounted for these effects by elevating the concentrations of cationic amino acids. A large, heat stable macroprotein with lectinlike binding properties that could be detected via agglutination of red blood cells (163), but which disappeared during menses (164), reflected the basic defect. Katz and co-workers (223) reported that intracellular Ca2+ in mononucleocytes was abnormal. Decreased protease activity (203, 261), arginine esterase (202), γ-glutamyl transpeptidase (19), galactosyl transferase (204), α-D-glucosidase (2) activities, and α-D-mannosidase enzymes from CF cells were more heat sensitive than normal (50), that intracellular Ca2+ was increased in CF fibroblasts (8, 51), and that calmodulin regulation of Ca2+-ATPase was abnormal (132).

Attention turned again to cultured cells when Breslow and co-workers (35, 37) reported that CF fibroblasts were much more resistant to dexamethasone in culture than their normal counterparts. It was reported that Tamm-Horsfall glycoprotein induced a multifold increase in alkaline phosphatase activity in CF fibroblasts (49), that acid phosphatase and α-mannosidase enzymes from CF cells were more heat sensitive than normal (50), that intracellular Ca2+ was increased in CF fibroblasts (8, 51), and that calmodulin regulation of Ca2+-ATPase was abnormal (132). Sometimes many man years of labor were lost and careers were changed in failed attempts to duplicate a result. For example, several years and literally thousands of rats (D. Dearborn, personal communication) were invested in trying to replicate the transport inhibitory factor effect reported on rat parotid glands (171, 269). None of these reports has been substantiated rigorously. Some have been refuted or seriously challenged (22, 38, 209, 258, 268). A very few have been courageously retracted (36).

Many of these reports most likely represent erroneous starts and premature conclusions that were supported by the ease of publishing in a field without a reliable, reproducible scientific point of reference. Often studies of this period rested on an uncritical faith in published results. The renowned CF physician Harry Shwachman once remarked “There have been more Chairmen of pediatrics made from CF than from any other disease because anything can be published” (personal communication). As a part of the CF story, these works may be most valuable in emphasizing the importance of setting critical experimental standards and in posting a warning of the pitfalls that must accompany attempts to tie empirical pathological observations to underlying physiological and biochemical phenomena.

IV. THE MUCUS STORY

It is now clear that abnormalities extend well beyond fibrotic cysts in the pancreas and that several target organs such as the sweat, lacrimal, and parotid salivary glands do not produce “viscid mucus” (194). Nonetheless, no doubt because of the impressive histological findings of accumulated mucus in the airways and to apparent “mucous plugs” in other organs (31, 96, 277), the idea that CF was a disease primarily caused by thick sticky mucus to this day commonly pervades the thinking of physicians, scientists, and patients alike. This perspective of the disease was entwined by observations in several exocrine organs that appeared to be based on some form of abnormal mucus secretion or metabolism.
A. Pancreas

Abnormalities in the pancreas made it easy for early investigators to conclude that the fundamental abnormality was altered and/or increased in mucus production. Overt symptoms of pancreatic insufficiency did not appear until >85–90% of the pancreas was lost (69, 99, 174). Pancreatic insufficiency developed in utero in some patients and over a period of many years in others (250). The progression has been qualitatively estimated as involving four (subjective) degrees of involvement (65): 1) eosinophilic concretions in the lumen, 2) widening of the ducts with intra- and interlobular fibrosis and microcysts appearing to be due to ductal obstruction, 3) acinar and ductal atrophy and disintegration accompanied by fatty infiltration, and 4) scar tissue surrounding isolated islets of Langerhans with total loss of acinar and ductal structures with increased incidence of insulin and glucagon insufficiency due to “strangulation” of the islets. The inspissated plugs or concretions in the ducts were reported to contain high concentrations of Ca^{2+} (151). In general, the caudal pancreas was found to deteriorate more rapidly than the head, and although there is no bacterial infection, mild leukocytic infiltration was seen even in early stages of the destruction (71). The progressive loss of function was highly variable, not an “all or none” phenomenon, and in some cases seemed to occur almost “unit by unit” over extended periods as ducts were obstructed and acini were lost to “mucus blockage”.

B. Airways

Impressions from the pancreas that CF was a disease of abnormal mucus were strongly reinforced by lung pathology. In young infants, even before significant anatomic changes occurred, the appearance of bronchial mucus hypersecretion was the first notable evidence of a pulmonary lesion (277). It remains an enigma that in contrast to most other lung diseases, the upper lobes fell prey to infection before the lower lobes, and like the pancreas, the lungs deteriorated over months or years seemingly almost unit by unit. Frequent involvement of the airways was consistently noted at autopsy as dilated bronchi with tubular bronchiectasis. Bronchial lumens were filled with purulent exudates (4, 31, 93, 94). The tracheal bronchial submucosal glands appeared normal in development but frequently hypertrophied with dilated lumens that under the microscope seemed to be filled with dense mucus (207). Histochemical studies suggested that glands in chronically infected patients produced more highly sulfated mucins, but probably not more so than non-CF, disease-related controls (154). Similarly, the airway epithelium appeared to be excessively populated with goblet cells, but probably not more so than other chronically inflamed airways (207).

Cystic fibrosis patients with infected airways generally produced sputum that was thick and viscid, but its physical properties were largely determined by the presence of cellular and bacterial debris, especially DNA, which made it impossible to define the nature of (or possible differences between) pure mucus secretions from native uninfected CF and control airways. Nonetheless, the gross appearance of the expectorant as well as analyses reporting abnormally low water content (96% for CF vs. 98% for bronchiectasis) supported the notion that airway mucus secretions were abnormally thick and “dehydrated” (137a, 185–187). Even now, the question remains as to which occurs first, increased (abnormal) mucus secretion or infection and inflammation (17, 137, 139). Undoubtedly, it is noteworthy that infections begin in the small bronchioles before detectable loss of lung function takes place (66, 184, 251). Pertinent to the question, there is very recent evidence from studies of a specially bred CF-gene knockout mouse that changes in mucus in the airway may precede disease (133). It remains significant that despite prodigious efforts to define a chemical aberration in mucins produced in CF, to date no unique compositional differences have been defined (114, 159). However, some progress may be being made (56, 220) in redefining early reports of alterations in the sulfation (29, 53) and in sialylation and fucosylation of CF mucus (79). The early recognition that DNase had marked thinning properties on crude CF sputum (52) and the recent application of this knowledge through the therapeutic use of cloned human DNase to reduce sputum viscosity in vivo (189, 201, 232, 233) underscored the fact that viscosity was not solely due to abnormal mucus. Thus the disease in the lung was frequently perceived as being caused by thick mucus, that was clearly “abnormal” as compared with normal lung secretions, but the more appropriate comparison is probably to mucus produced in other diseased lungs. Introductory sentences such as “Abnormally viscoelastic secretions in CF cause impaired mucus clearance and persistence of bacteria within the lung” were and are common in the literature of CF. That is not to say that there is no chemical abnormality in CF mucus, but it seems that it was not widely recognized that such an abnormality was still far from being established and that other diseases, asthma and especially status asthmaticus (250), bronchiectasis without CF (176), pseudohypoaldosteronism (117), Kartagener’s (immotile cilia) syndrome (221), and some cases of Sjogren’s (autoimmune destruction of exocrine tissue) syndrome (61), all produced sputum that is “thick and viscid” or “dry,” but did not result in CF lung pathology (112). Cystic fibrosis sputum was found to be almost uniquely characterized by bacterial flora containing a mucoid form of Pseudomonas aeruginosa that produced alginate. This protein, commonly used as a food
thickener, was once thought to be largely responsible for the viscosity of CF sputum (84, 85). Why the CF lung is predisposed to this form of a ubiquitous bacteria remains as another mystery. Unfortunately, the complexity of sputum, the nature of the infection, and the individual variability of the inflammatory responses (137a, 138, 196) have prevented unassailable comparisons of the composition of mucus in different diseases.

C. Gastrointestinal Tract

The biliary tree, the gallbladder, and the intestine have all been reported to show similar signs of increased mucus or “thickened” mucus production. Clearly, one of the most dramatic and compelling demonstrations that CF was a disease of “mucoviscidosis” derived from the pathognomonic association between CF and meconium ileus. During the 1960s, sufficient records were accumulated to demonstrate that ~10% of CF patients were born with this condition (75, 216). In these infants, the small bowel was blocked with a thick, dehydrated, rubbery, tarry, tenacious mucoid plug. The loss of pancreatic enzymes in utero undoubtedly gave rise to accumulations of undigested proteins (e.g., albumin) which when mixed with intestinal mucus produced the impervious, hyperviscid meconium substance. Microscopic examination of the surrounding intestinal epithelium showed dilated mucus glands with prominent mucus cells along lumens that contained a “stringy” secretion (31, 126, 255). It was taken almost as a given that the mucus glands of the colon similarly demonstrated an increased proportion of mucus goblet cells (31), but a carefully executed morphometric study of colonic biopsies showed no significant differences between goblet cell distribution in CF and normal subjects. Differences in individual cell morphology between CF and normal cells were attributed to the high lipid load associated with pancreatic insufficiency (179). A more recent ultrastructure study of the small gut suggested that cytological abnormalities were associated with crypt cells where the highest concentration of cystic fibrosis transmembrane conductance regulator (CFTR) was expressed (219).

Liver cirrhosis was not usually encountered until much later in life and then in only a few percent of patients, but a large fraction of patients at autopsy exhibited “focal biliary cirrhosis” in which focal zones of numerous bile ducts and tubules contain distinctly eosinophilic, granular secretions that appeared to form plugs that caused ductal dilation (72).

In about one-third of autopsied cases, the gallbladder was found to be hypoplastic and to contain thick or gelatinous mucus, viscous green bile, clear jelly, solid green bile mucus sometimes along with obliteration of the cystic duct. These bladders often exhibited submucosal fluid-filled cysts, but these changes apparently occurred without inflammation (92). As many as 20% of CF patients were observed to suffer cholelithiasis (gallstones), but it is most likely that this complication was secondary to depletion of the bile acid pool because of decreased digestive enzymes and increased stool (259).

D. Reproductive Tract

Observations in the female genital tract gave further evidence of a disease of viscous mucus. Insipissated mucus plugs were sometimes found obstructing the cervical canal (183). At times, the endocervix and cervix were found to exhibit polypoid masses presumed to contain insipissated mucus (86, 183). The gross quality of the mucus was found to be thick, tenacious, and of relatively low volume compared with controls (150). Cystic fibrosis women showed a significantly lower fertility rate thought to be due to the impediment the thick mucus imposed upon sperm entering the cervical canal (59).

The tissue most sensitive to CF in terms of embryological development was the epididymal duct and vas deferens. In most cases of CF, these structures were found to be incomplete (129, 181). It was speculated, but not proven, that the atresia was due to blockage of the ducts in utero (123, 155, 160). Most males with CF were found to be aspermic or hypospermic (123).

E. Salivary Glands

A few other morphological aberrations mainly in the submaxillary and minor labial salivary glands also seemed consistent with abnormal mucus. These glands were reported to contain mucous plugs and eosinophilic staining mucus in their lumen (231), but it was not possible to conclude that these observations were strictly unique to the disease (161). Figures 1 and 2 offer an example of the kinds of microscopic changes that have been observed in mucus-producing exocrine glands in the form of minor salivary glands from CF and control subjects. Even though these glands were not known to become infected or inflamed or to autodestruct by autolysis, dilated ducts with thinner walls were outstanding features of the CF specimens, but what appeared to be more darkly stained materials, thickened, were present in the lumens of the ducts of both glands.

Barbero and Sibinga (13) found that salivary glands were significantly enlarged in CF, but this result was then interpreted to be due to the chronic administration of β-adrenergic agonists (222, 230). Saliva from CF patients demonstrated an abnormal color and turbidity, presumably due to higher concentrations of Ca$^{2+}$ and phosphate...
(30, 169) that appeared to reverse upon administration of guanethidine (55). Pancreatic enzyme supplements were thought to influence salivary composition as well (247).

F. The Cell

Even though CFTR, the gene affected in CF, was cloned 10 years ago (134, 210, 215), the finding has little impact on understanding mucus abnormalities in CF. However, once it became appreciated that CFTR was a Cl− channel, Al-Awqati and co-workers (1, 11) reported that normal acidification of intracellular vesicles in CF could not be achieved and that posttranscription sialylation and fucosylation of mucous molecules would be decreased and increased, respectively, due to the effect of the altered pH on the optima of the glycosylating enzymes. Even though, however, Verkman and co-workers (25, 229) found no
differences in intraorganellar pH of CF cells and vigorously refuted this observation and speculation.

V. THE ELECTROLYTE STORY

Except for the sweat gland, most clinical disturbances have been related to abnormalities that appeared to be directly related to abnormally viscid mucus secretion. The three to five times normal elevation of NaCl in the sweat of CF patients was long viewed as something of an outlier in the etiology of the disease because the sweat gland was without significant mucus. Even Andersen was reluctant at first to believe that it could be important to the basic pathology of the disease (di Sant’Agnese, personal communication). A few investigators tried to reveal similar electrolyte abnormalities in the salivary glands (27, 74, 170, 263). In general, the Na\(^+\), Cl\(^-\), and K\(^+\) concentrations in CF salivary secretions, more so with submaxillary and labial than with parotid glands, appeared at somewhat higher concentrations than those from normal glands, but although Ca\(^{2+}\) was consistently found to be about twice normal, the results have not always been statistically significant (27, 194). If there are disturbances in tear glands, they are not large, probably because these glands secrete a relatively unmodified iso-tonic fluid (32, 74, 235). Throughout the history of CF, no exocrine secretion or physiological function has provided as clean a discriminator between normal and CF subjects as has sweat.

Undoubtedly, the overt lack of any clinically apparent renal disturbance undermined early thinking of CF as a disease of a generalized disturbance in electrolyte transport (76). The CFTR was found recently to be expressed in the human kidney (62, 178), but there was little evidence of renal compromise. No morphological abnormalities have been observed (177). There is an increased unexplained clearance of several drugs, especially aminoglyco-sides (9, 97, 128, 157, 274). A few reports of other functions such as reduced concentrating and diluting capacities (210) or altered glomerular filtration rate (121, 243) were either not followed up or remained controvers-sial (241).

A. Sweat Gland

As noted in section II, the first indication that CF extended beyond an apparently abnormal mucus appeared when di Sant’Agnese set out to determine the etiology of volume (salt) loss in Andersen’s patients afflicted by the heat wave that hit the northeast United States in the summer of 1948.\(^2\) Noting that the development of heat tolerance among troops sent to North Africa was attributed to adaptations in sweating, he pursued excessive loss of salt in the sweat as the most likely origin of volume depletion during the high heat stress (personal communication). He began collaborating with Darling, Perera, and Shea to prove it. Renal output and conservation of Na\(^+\) was within normal range. Nothing in the stool could be implicated. However, when sweat was collected and analyzed, the concentrations of both Na\(^+\) and Cl\(^-\) were greatly increased in sweat from CF patients (73). It was not possible to give strict concentration values for these ions in either CF or normal subjects simply because the fractional absorption of salt from the secreted sweat varied enormously with secretory rate, age, circulating aldosterone levels, acclimatization, drugs, and any number of less obvious factors (167, 236). For example, in salt-depleted normal individuals, fractional absorption ranged from almost 90% for sweating at low sweat rates to only a few percent in some subjects surfeiting on sodium and sweating at very high rates (personal observations; Refs. 81, 90, 91, 168). After analyzing samples from hundreds of patients (76, 236), however, it became evident that two relatively distinct zones separated normal from CF pediatric sub-jects (Table 1). In normal subjects, sweat concentrations of salt fell below ~60 mM, and in CF, sweat concentrations rose above that value with almost no overlap (76, 236).\(^3\) In an extensive study, the average sweat electrolyte values for siblings and parents of CF patients were found to be slightly, but significantly, increased over the normal population (76). A few years later, Shwachman and Mahmoodian (236) refuted these results in putative heterozy-gotes in another extensive report.

Sweat had much value as a parameter in differential diagnosis, but it was cumbersome to collect and difficult to obtain without serious evaporative losses. Some early attempts to place the subject in a body bag to obtain enough sweat for analysis proved that this approach presented a high risk for fatal hyperthermia. In the late 1950s, Gibson and Cooke (104) seized the idea of stimulating localized sweating by electrophoresing (iontophoresing)

---

\(^2\) One cannot help but wonder about the course of events had air conditioning been common then.

---

\(^3\) The overlap is much greater with adults, presumably because of the increased secretory capacity relative to its absorptive capacity.
the positively charged secretagogue pilocarpine into an area of skin of \( \sim 20 \text{ cm}^2 \). The area was then hermetically sealed under taped plastic, and after \( \sim 30 \text{ min} \), the sweat absorbed into the gauze was weighed, eluted, and analyzed for concentrations of Cl\(^-\). \(^4\) The protocol has become internationally used and accepted as almost mandatory for the diagnosis of CF (158). Only a few other very rare conditions created false-positive results. Among them were Addison’s disease (82), pseudohypoaldosteronism (117), hypothyroidism (60), and malnutrition (48, 57, 175). A few isolated cases diagnosed with CF have been reported with sweat Cl\(^-\) concentrations in the normal range (67, 244), but it is not clear whether these observations are true exceptions with an unknown physiological compensation or whether normal physiological parameters that have not been accounted for are at play.

Numerous studies were conducted over the next decade that firmly established the validity of the sweat test (70, 76, 105, 158, 236), but in the course of these studies, several observations were also noted that would form the basis of, or provide helpful insights into, the future understanding of the physiology of CF. First, almost contemporary with di Sant’Agnese’s observations, the “two-step” theory of exocrine fluid formation was published (227). The theory predicted that exocrine fluids were first secreted as an iso-osmotic fluid in the secretory or acinar structure of the gland and secondarily were modified in composition as they passed through the ductal or tubular network of the gland before being excreted. \(^5\) Direct ultra-micro sampling from the gland (224–226), histological cryo-osmometry (237, 239), and linear extrapolation (48, 80) were important approaches that verified the theory and answered the initial question of whether CF sweat was higher in concentration due to a primary secretory defect that secreted fluid at hypertonic concentrations or due to an absorptive defect in which ductal electrolyte absorption failed in CF. It was also noted that sweat Na\(^+\), but not Cl\(^-\), in CF dropped as expected in response to increased levels of circulating aldosterone (78). Slegers (238, 239) also observed that the ratio of Na\(^+\) concentration to Cl\(^-\) concentration in normal sweat was almost invariably \( >1.0 \), whereas in CF sweat, it was almost always \( <1.0 \). Both of these observations hinted that Cl\(^-\) was less effectively absorbed than Na\(^+\). Schulz and Fromter (225) noted in three patients with CF that the electrical potential associated with sweat production on the skin was more electronegative than normal and even suggested that this could only occur if Cl\(^-\) were less permeable than Na\(^+\), but these results were neglected and apparently were not mentioned again until pointed out by a reviewer of my manuscript some 14 years later (193). In 1981, Knowles et al. (140) reported that the electro-negative potential across the nasal airway in CF was significantly larger than normal. This report along with the facts that NaCl absorption was inhibited in CF sweat ducts and that Na\(^+\) was relatively more absorbed than Cl\(^-\) gave me the idea that the basic defect in the CF duct had to be due to anion impermeability (192) and not to defective anion exchange (191) (as I had been thinking earlier based on chemical measurements) or to increased Na\(^+\) absorption as was postulated for the airway (140). \(^6\) However, the fluid volumes involved in microperfusion of single ducts were far too small and the specific activity of Cl\(^-\) was too low to accurately measure isotope fluxes to prove Cl\(^-\) impermeability. In microperfused isolated segments of absorptive ducts from CF and normal subjects, I found that the average spontaneous transepithelial potential of normal sweat ducts was \( \sim 7 \text{ mV} \), whereas in CF ducts it was \( \sim 75 \text{ mV} \) (193). In the meantime, on the suggestion of Maurice Burg, I found that we could demonstrate the anion impermeability electrophysiologically by ion substitution and showed that replacing Cl\(^-\) with the relatively impermeable SO\(_4\)\(^-\) anion in normal, microperfused ducts raised the value of lumen negative potential to about equal that of the CF duct, whereas the maneuver had much less effect in CF ducts (193). Corroborating results were obtained a little later in single glands in vivo (23, 198). Because the defect was expressed in the morphologically normal sweat gland, it appeared to be independent of any secondary pathological or degenerative consequences due to infection, inflammation, or mucus disturbances that often occurred in other target tissues that secreted macro-molecules. Thus this property not only provided a physiological explanation for the high salt content in CF sweat, but it also provided the first description of a basic, cellular defect that has since proven to be uniformly inherent in CF affected cells.

Abnormalities in adrenergic control in CF had been suspected for several years (12, 246). Schwarz and Sutcliffe (228) suggested that the uptake of salt from the duct might be influenced with adrenergic agonists. But not until 1984 did Sato and Sato (217) find a fundamental corollary of the Cl\(^-\) impermeability defect in fluid secretion, also in sweat glands. They found that the secretory activity of CF sweat glands was almost completely insensitive to \( \beta \)-adrenergic stimulation. \(^7\) The insensitivity was later shown to be due to the fact that in the sweat gland \( \beta \)-adrenergically stimulated fluid secretion was coupled to the same Cl\(^-\) channel that was altered in salt absorption (195). The Cl\(^-\) conductance in absorption was also under \( \beta \)-adrenergic control.

\(^4\) The differences from normal in Cl\(^-\) concentration are larger than differences in Na\(^+\) concentration.

\(^5\) Some lower vertebrates, but not mammals, can secrete hypertonic fluids (190).

\(^6\) I felt some empathy with di Sant’Agnese because I presented this idea in 1982 at a Cystic Fibrosis symposium where I too was the last speaker of the evening, and although present, most of the audience was asleep.

\(^7\) \( \beta \)-Adrenergic innervation serves no known physiological function; thermoregulatory innervation appears to be purely cholinergic and is not abnormal in CF.
TABLE 2. Comparison of electrolyte composition and rate of pancreatic secretions collected from the duodenum of control and pancreatic sufficient CF patients

<table>
<thead>
<tr>
<th>Electrolyte Composition, mM</th>
<th>Volume, ml·kg⁻¹·h⁻¹</th>
<th>Trypsin, µg/ml</th>
<th>Protein, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻</td>
<td>HCO₃⁻</td>
<td>Na⁺</td>
<td>K⁺</td>
</tr>
<tr>
<td><strong>Hadorn’s study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects (n = 10–30)</td>
<td>60 (258)</td>
<td>75 (258)</td>
<td>107 (73)</td>
</tr>
<tr>
<td>CF patients (n = 10)</td>
<td>86 (115)</td>
<td>19 (115)</td>
<td>119 (73)</td>
</tr>
<tr>
<td><strong>Kopelman’s study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects (n = 9)</td>
<td>81</td>
<td>45</td>
<td>142</td>
</tr>
<tr>
<td>CF patients (n = 9)</td>
<td>77</td>
<td>39</td>
<td>151</td>
</tr>
</tbody>
</table>

Reference numbers are given in parentheses. Data for Kopelman’s study are from Kopelman et al. (149).

gic, cAMP-dependent control (199, 200, 206, 267). Chloride impermeability and insensitivity to activation by protein kinase A has now become the signature of expression of CF in the single cell, whether native, transfected, or cultured. Although overlap was too great to permit detection of heterozygotes on an individual basis, the average sweat rate of the heterozygote population in response to a β-adrenergic challenge still remains one of the few, perhaps the only, physiological parameter that showed intermediate expression of the gene in vivo (18, 218).

B. Pancreas

Untreated, the large majority of CF patients suffered from severe malnutrition that was attributed to the loss of pancreatic parenchyma and the concomitant loss of digestive enzymes. Some questioned whether the defect in sweat NaCl might be due to poor nutrition. About 10% of diagnosed patients escaped pancreatic insufficiency (71), which we now know is due to different genotypes (135, 152). Although the gross and microanatomy of pathological changes in the pancreas were obvious from the outset, it was not until the late 1960s that Beat Hadorn recognized that fluid and electrolyte secretion, like electrolyte absorption in sweat, was also abnormal in CF. In 10 patients who did not exhibit overt pancreatic insufficiency, secreted volumes and HCO₃⁻ content were markedly decreased in CF while Cl⁻ and digestive enzyme concentrations were increased (115, 116, 125) (Table 2). Most CF patients were relatively refractory to secretin stimulation but responded to pancreozymin. These early observations were the first evidence that like the sweat gland, only one of at least two mechanisms of fluid secretion involved in pancreas was seriously impaired.

About 15 years later, Kopelman et al. (149) used an enteric double-ballooned catheter to isolate the ampulla of Vater and collect relatively pure pancreatic secretions also during in vivo pharmacological stimulation with intravenous pancreozymin/secretin. Although Kopelman et al. (148) did not find significant differences in electrolyte concentrations (Table 2), both studies concluded that macromolecules were abnormally concentrated and that HCO₃⁻ and volume outputs were abnormally low in CF patients. The fact that secretin-stimulated HCO₃⁻ secretion was mediated by cAMP-dependent protein kinase and that the pancreas was so adversely affected in CF revealed that Cl⁻ impermeability has a pronounced negative effect on exocrine HCO₃⁻ transport. This effect still remains as one of the most important but neglected and poorly understood aspects of pathophysiology in CF. With the recognition that fluid secretion was inhibited, the explanation for pancreatic deterioration no longer required ductal blockage by abnormally viscid mucus. Without sufficient fluid and HCO₃⁻, digestive proenzymes stagnated and activated prematurely in the pancreatic ducts. The results, nonetheless, were the same: microautolysis, focal injury, inflammation, infiltration, calcification, plugged ducts, fibrosis, and scarring until the entire parenchyma of the pancreas, like the lung, but from a seemingly different cause, was destroyed.

The first fundamental work on the molecular properties of the Cl⁻ conductance affected in the disease also arose from the pancreas. The earliest attempts (101, 264) to use patch-clamp techniques to show the molecular defect through Cl⁻ single-channel activity encountered severe controversy. Several investigators, most of whom did not publish (J. Hanrahan, J. Wine, J. Bijman, and R. Greger), were unable to repeat the published results that a nonlinear rectifying Cl⁻ channel could be activated by β-adrenergic agonists, as required for the affected channel in CF (153). After 2 years of controversy, using an organ culture system of human pancreatic ducts developed by Ann Harris, Argent et al. (109) found two types of Cl⁻ channel activity: one with outwardly rectifying chord conductances ranging from ~19 to 53 pS was seen rarely and

* Protein kinase A is the most established intracellular mediator of CFTR Cl⁻ conductance, although other pathways may be involved.
was activated by strong depolarization and another with 
~4–7 pS linear conductance was abundant and activated 
by cAMP/protein kinase A. These results were rapidly con-
firmed (21, 58, 120, 273), and the rectifying channel was 
shown not to be correlated with CF (262).

C. Airways

While searching for evidence that electrolyte trans-
port in the airways was important for host defense, 
Knowles et al. (140) included a few CF patients in his 
cohort of asthmatic and heart disease subjects. He discov-
ered that the electrical potential associated with the nasal 
mucosa of CF patients was significantly more electronegative 
with respect to blood than with all other subjects. 
This finding in 1981 provided the first physiological link 
between the lung, the pancreas, and the sweat gland (140).
The common link was not mucus, but electrolytes. Al-
though it was not rapidly appreciated, the finding allowed 
the possibility that problems with mucus were due not to 
abnormalities with its synthesis or composition, but to 
the fluid environment into which mucus was secreted 
(195, 196). In CF subjects, the spontaneous electrical po-
tentials measured across respiratory mucosa were about 
twice normal (53 mV in CF vs. 25 mV in healthy control 
subjects) with no overlap in values. The difference in elec-
trical potential between CF patients and normal subjects 
persisted down the airways as far as the airway lumen 
could be accessed, although the absolute values became 
smaller so that in CF segmental bronchi the average po-
tential difference was 32 mV (CF) vs. 15 mV (normal) in 
bronchi (145), lumen negative relative to blood. In the 
presence of amiloride, a diuretic known to block epithelial 
Na\(^+\) conductance, the transepithelial electrical potential fell in both groups to about the same level (~5 mV).

Knowles et al. (140) postulated that in the airways, the 
increased potential found in CF was due to significantly 
accelerated active Na\(^+\) absorption from the airway surface 
fluids in the lumen. This hypothesis was appealing be-
cause it presented a mechanism (dehydration by exces-
sive fluid absorption) by which abnormally thick mucus 
could be formed in the airways (33). The same Cl\(^-\) imper-
meability defect previously described in the sweat duct 
(192, 193) was then found in the airways (142, 144). So-
dium absorption was not enhanced but clearly decreased 
in the sweat duct, and it is not clear that Na\(^+\) transport 
is affected in the pancreas or intestine. Neither the mecha-
nism of enhanced Na\(^+\) absorption in CF airways nor its 
link to the genetic defect has yet been explained.

From these initial observations, a new clinical proce-
dure using the potential differences measured across the 
nasal mucosa was developed to assist in the diagnosis of 
CF (141). The potential was measured while the nasal 
mucosa was perfused with different solutions. After the

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>CF patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joris' study</td>
<td>Na(^+)</td>
<td>121</td>
</tr>
<tr>
<td>K(^+)</td>
<td>23</td>
<td>29</td>
</tr>
<tr>
<td>Knowles' study</td>
<td>Na(^+)</td>
<td>76</td>
</tr>
<tr>
<td>K(^+)</td>
<td>24</td>
<td>22</td>
</tr>
</tbody>
</table>

TABLE 3. Concentrations of electrolytes in airway surface fluids from two studies

The spontaneous potential was read, Na\(^+\) conductance was 
blocked with amiloride, the mucosa was challenged with 
isoproterenol, a \(\beta\)-sympathomimetic agonist, and Cl\(^-\) was 
removed from the perfusate. The response of the electrical 
potential to amiloride was two- to threefold larger, but 
though it was not rapidly appreciated, the finding allowed 
the possibility that problems with mucus were due not to 
abnormalities with its synthesis or composition, but to 
the fluid environment into which mucus was secreted 
(195, 196). In CF subjects, the spontaneous electrical po-
tentials measured across respiratory mucosa were about 
twice normal (53 mV in CF vs. 25 mV in healthy control 
subjects) with no overlap in values. The difference in elec-
trical potential between CF patients and normal subjects 
persisted down the airways as far as the airway lumen 
could be accessed, although the absolute values became 
smaller so that in CF segmental bronchi the average po-
tential difference was 32 mV (CF) vs. 15 mV (normal) in 
bronchi (145), lumen negative relative to blood. In the 
presence of amiloride, a diuretic known to block epithelial 
Na\(^+\) conductance, the transepithelial electrical potential fell in both groups to about the same level (~5 mV).

Knowles et al. (140) postulated that in the airways, the 
increased potential found in CF was due to significantly 
accelerated active Na\(^+\) absorption from the airway surface 
fluids in the lumen. This hypothesis was appealing be-
cause it presented a mechanism (dehydration by exces-
sive fluid absorption) by which abnormally thick mucus 
could be formed in the airways (33). The same Cl\(^-\) imper-
meability defect previously described in the sweat duct 
(192, 193) was then found in the airways (142, 144). So-
dium absorption was not enhanced but clearly decreased 
in the sweat duct, and it is not clear that Na\(^+\) transport 
is affected in the pancreas or intestine. Neither the mecha-
nism of enhanced Na\(^+\) absorption in CF airways nor its 
link to the genetic defect has yet been explained.

From these initial observations, a new clinical proce-
dure using the potential differences measured across the 
nasal mucosa was developed to assist in the diagnosis of 
CF (141). The potential was measured while the nasal 
mucosa was perfused with different solutions. After the
cidal activity and raising the salt concentration of the fluid collected from control epithelia removed its bactericidal activity. Similar results were reported for CF and control cells grown on tracheal xenografts in immunodeficient mice (107). Analysis of the salt concentrations in the ASP over primary cultures supported the conclusion that, like the sweat gland, the NaCl concentration in CF ASP was significantly elevated (276). These results may strengthen the possibility that abnormalities in the composition of the airway fluid as opposed to inherent abnormalities in the composition of airway mucus was the component that predisposes the CF lung to airway disease.

D. Intestine

Work on the electrolyte abnormality in the gut was slow to evolve probably because of an early prevalence of the idea that malabsorption in CF was essentially due to pancreatic dysfunction. In 1985, the first transport abnormality in the CF intestine was reported as an enhanced rate of glucose uptake in vivo (100), which was proposed to be due to smaller diffusional barriers. The finding was later confirmed in vitro and shown to be associated with significantly higher nutrient-stimulated short-circuit currents in CF (15, 118, 254). Additional electrolyte transport disturbances in the CF intestine arose from observations that jejunal biopsies from CF patients in contrast to normal subjects failed to respond with a significant increase in lumen to serosal positive current when stimulated with PGE$_2$, dibutyryl cAMP, acetylcholine (252, 253), or the Ca$^{2+}$ ionophore A-23187 (180). Consistent results were obtained in the ileum, colon, and rectum (20, 24). The in vivo intraluminal potential in the jejunum when perfused with Cl$^-$-free solution was significantly more positive in CF than in control subjects, whereas theophylline stimulated net fluid secretion in control, but not CF, subjects (254). Both the increased Na$^+$-dependent nutrient uptake and the depressed secretory responses of the CF gut were compatible with an inherent loss of Cl$^-$ conductance. The absence of a normal anion shunt in the apical membrane should increase the apical membrane Na$^+$ gradient of the enterocyte in CF, thereby producing larger currents when nutrients were present. As for diminished secretion, any agonist that required the CFTR Cl$^-$ channel in the apical membrane would necessarily fail to elicit a response if the channel were missing or nonfunctional.

There was accompanying evidence of pH aberrations in the CF intestine. Postprandial contents in the early gut remained at a lower pH (<6.0) for significantly longer periods of time than in that of control subjects (106, 211, 275). These effects may be simply the result of diminished pancreatic HCO$_3^-$ secretion but could also, like the pancreas, reflect a compromised ability of the intestine to secrete HCO$_3^-$ as a function of lost Cl$^-$ permeability in the enterocyte cells. Several other alterations in the gastrointestinal tract have been reported, but clear abnormalities have been difficult to establish (110). Ironically, it now seems that these abnormalities in the gut may be the most likely cause for the high incidence of some CF mutations, notably AF508. Earlier, the selective pressures thought to be responsible for the high gene frequency of CF were numerous and almost completely speculative (147, 188, 245). When it became clear that all three target organs, the sweat gland, the pancreas, and the lung, were products of electrolyte transport disturbances, I suggested altogether too narrowly that the selective advantage of the CF heterozygote might be in the intestine and related it to surviving cholera infections (192). I did not realize that cholera did not strike Europe until the 19th century, obviating this plague as a selective pressure. However, I later learned that that diarrheal diseases caused by ubiquitous organisms such as enterotoxic *Escherichia coli* (197) have affected humans for long enough period to conceivably constitute the selective pressure. Proof of the notion has not been forthcoming, but CF patients have been found to be refractory to acute exposure to Sta (heat-stable *E. coli* enterotoxin) in vivo (108). The survival of transgenic CF mice exposed to cholera toxin perhaps provided the strongest support of the hypothesis (102).

E. The Gene and Cell

In addition to being a dramatic achievement in science, the search for the CF gene was one of the greatest successes in CF research. Because there was no known gene product and only Cl$^-$ impermeability, a membrane function property, was known to be characteristically altered, the gene had to be found without a chemical marker. The alternative approach was positional cloning, but only the assumption that the mutated gene had to be expressed in the sweat gland, where Cl$^-$ impermeability had been demonstrated, linked the patient phenotype to the positional cloning effort. Before 1985, the location of the CF gene in the human genome was completely unknown. Eiberg et al. (89) observed that a polymorphic serum paroxonase was inherited with high frequency (linked) with the CF gene. In the same year, Tsui and co-workers (146, 257) used another restriction fragment linked polymorphism (RFLP) marker to localize the gene to the long arm of chromosome 7. Thereafter, the race was on to find the gene. Its localization was narrowed to within 1–2 × 10$^6$ base pairs using RFLP. The most vigorous competition was between Williamson’s group in London and Tsui’s group in Toronto. It was rumored that Bob Williamson had the gene, but the lead did not hold. In 1985, in a stunning trilogy of papers published simultaneously (134, 210, 215), the Toronto group including Jack
Riordan’s laboratory supported by Francis Collins’ group at Michigan reported the cloning of the gene for CF. The gene was conserved throughout numerous species, indicating its evolutionary importance; it was predominantly expressed in the target organs affected by the disease including cells from the sweat gland. The cDNA hybridizing to the candidate gene sequences from the CF locus were isolated from libraries constructed from sweat gland mRNA from CF patients and unaffected subjects. Sequence comparisons between initial partial cDNA from the two sources revealed the absence of three nucleotides in the patients even before the full-length sequence was obtained, which was ultimately necessary to predict the protein product structure. The subsequent finding that this ΔF508 deletion was present in almost 70% of the chromosomes from patients diagnosed with the disease provided assurance that the correct gene had been found. They named its protein product CFTR, suggesting that it might not be a Cl⁻ channel itself, but that it might regulate Cl⁻ channel activity.

The next important step was to express the gene in living cells. At first, great difficulty was encountered in creating a full-length cDNA that could be propagated in bacteria and expressed in cells. Gregory et al. (111) cleverly surmised and validated that a cryptic region in exon 6 of the CFTR cDNA induced a transcript that was toxic to the host bacteria. When they inactivated the promoter, the bacteria were able to carry plasmids containing CFTR cDNA. This important conclusion was fundamental to developing cell lines expressing the gene and its mutants. Using a low copy number plasmid, Welsh and co-workers (208) first corrected CF in cells ex vivo by transfecting a CF cell line with normal cDNA. The approach was quickly improved upon by using mutated E. coli in which production of the toxic component was suppressed (54, 87). With the advent of cell lines capable of expressing CFTR and its mutants came Seng Cheng’s startling discovery that not only did CFTR deliver Cl⁻ ions to the cell membrane, it exhibited reasonably good function as a Cl⁻ channel (88, 162). Shortly later, Welsh and co-workers (68) also discovered that these mutants were thermosensitive, and growing cells at reduced temperature enhanced processing and allowed expression of CFTR Cl⁻ channels. These findings generated new thinking about therapeutic approaches. They suggested that correcting the defect would require enhancing expression and delivery of the mutant protein to the membrane. Still, direct proof that CFTR was a Cl⁻ channel was lacking. It remained possible that it was a regulator, not a channel itself. The first evidence that CFTR was a channel was presented by mutating lysine at position 95 and 335 to glutamate and showing that this change reversed the anion selectivity sequence of the channel (7). Although Greger and co-workers (122) refuted these results, it is difficult to imagine how such alterations would cause changes in permeability if they were not directly involved with conducting the anion.

We pass over an avalanche of data provided subsequently by patch-clamp and whole cell electrical recording techniques. The principal results of which described an ion channel with an anion selectivity of NO₃⁻ > Br⁻ ≫ Cl⁻ > I⁻ > gluconate and a nonrectifying conductance of ~6–8 pS (58, 109, 131, 273). The channel was primarily activated by cAMP-dependent protein kinase (58, 214, 248, 265). Activity was ATP dependent (6, 200), and ATP hydrolysis was required for channel opening and for channel closing as well as a nonhydrolyzed ligand (14, 113, 205, 256).

VI. WHICH STORY TO BELIEVE?

What does CF history show us in the past half century? It is clear that CF involves electrolyte transport abnormalities in all affected epithelial tissues and that mucus secretion is or becomes enhanced in some tissues but not in others. It is clear that mutations in CFTR directly affect Cl⁻ permeability and likely have other effects either directly or indirectly on Na⁺, HCO₃⁻, and probably other ion distributions. However, the effect on mucus remains unclear. Admittedly, I have been biased for many years that the basic defect probably does not directly involve both electrolytes and mucus, largely because it seemed superficially, at least, incompatible with my obsolete education in the “one gene, one protein” concept. The sweat gland offers no support for a defect in mucus production or synthesis. The luminal blocks and concretions of the pancreas can probably be explained as the result of focal stagnation of autolytic enzymes as discussed in section IVB. It has been tempting to say that the “abnormality” of mucus in the airways is simply the result of infection and that the apparently abnormal increase in viscosity is due to the presence of DNA or to relative dehydration. Certainly, a number of related anecdotal observations made on very young infants as well as on adults dying of nonres-
piratory causes report the presence of multiple casts and plugs in very small airways that conceivably could precede infection. The lack of normal, cyclical changes in the viscosity of vaginal fluids during the menstrual cycle and the occasional mucus plugs encountered in the female CF cannot be easily explained by infection either. In addition, the submucosal glands of the large airways and the intestine have been described repetitively as containing thick, stringy, or viscous mucus. It also seems unlikely that these results are directly due to infection. Still, the evidence that the mucus per se in these glands is abnormal in CF is weak. We may surmise that the “apparently” abnormal mucus might well be a normal response to an underlying challenge, but it is not possible to dismiss the mucus condition in CF as simply due to chronic infection.

There are fundamental tissue phenomena that may help in integrating these events, at least from my armchair. First, one of the most primitive defenses that epithelia offer to challenges from the outside world is mucus release. Irritation of an epithelium results in hypersecretion of mucus. If mucus secretion is increased in CF and not inherently constitutively increased or a function of infection, some form of “irritation” of the epithelium seems likely to be present. Changes in the composition (and/or volume) of the epithelial surface or luminal milieu should inherently accompany the physiological defect in electrolyte transport in CF. Because epithelia are skilled in detecting surface or luminal changes, I ask if the defective changes in themselves could constitute a chronic irritation to target epithelia. The defect would be even more aggravating if CFTR has functions that extend beyond two reasons: changes in themselves could constitute a chronic irritant. Irritation of an epithelium results in hypersecretion, some form of “irritation” of the epithelium seems likely to be present. Changes in the composition (and/or volume) of the epithelial surface or luminal milieu should inherently accompany the physiological defect in electrolyte transport in CF. Because epithelia are skilled in detecting surface or luminal changes, I ask if the defective changes in themselves could constitute a chronic irritation to target epithelia. The defect would be even more aggravating if CFTR has functions that extend beyond creating a Cl⁻ conductance, especially if it either directly or indirectly impedes the movement of other ions such as HCO₃⁻, where the pancreas has tried so emphatically to teach us for years that the transport of the two ions must be coupled. In addition to potentially constituting a chronic irritation and provoking increased mucus secretion, alterations in composition also present an abnormal environment into which mucus secretions are released, leading to a second, related phenomenon: physical properties of mucus are intrinsically dependent on their environment. The viscoelastic properties of macromolecular polyelectrolytes such as mucus are critically sensitive to ionic strength and pH (165, 166, 249). Alterations in the ionic strength, divalent ion concentration, or pH are certain to affect the physical status of the mucus, which, again, in itself, may contribute to irritation to create a vicious cycle. The facts that in nature mucus and HCO₃⁻ secretion are often coupled and that there is clearly a defect in HCO₃⁻ movements in CF suggest that this aspect of the basic pathophysiology may be as important, if not more important, to the clinical disease than Cl⁻ impermeability, if indeed the two can be separated.

The possibility that this inherent weakness in the epithelial system can be provoked or at least exacerbated by sporadic events seems to fit well with the disease process; that is, neither of the two organs that completely fail in CF, the lungs and the pancreas, do so suddenly. Rather, there is progressive destruction (sometimes enduring decades) of the organ unit by unit as it were. In the airways, the sporadic insults that continually occur with debris that is deposited from inhaled air push isolated units beyond the threshold of homeostasis to produce a localized, destructive spiral of mucus release, accumulation of debris, and inspissation of ineffective mucus with subsequent loss of function. We know too little about variations in acinar and lobular functions in the pancreas to be able to suggest what precipitating factors initiate focal stagnation in ductules, but it seems likely that such variations exist and that beyond a point, localized flow and probably pH, becomes too low to provide proper clearance of the unit before proteolytic enzyme activation and autolysis occurs. Because a few percent of CF males are fertile, the vas deferens may follow the same pattern, possibly again due to problems in HCO₃⁻ and pH. Without further knowledge of the processes incumbent with its development, we cannot know which potentially destructive elements might be present, but stagnant lytic enzymes in the seminal fluid seem likely to lead to the same course seen in the pancreas. The intestine and other organs largely escape this tide of deterioration because there seems to be few, if any, destructive elements such as highly concentrated digestive enzymes in the pancreas or intensely inflammatory agents in the airway to damage the epithelia. The sweat gland escapes structural alterations for at least two reasons: 1) physiological sweating is driven by a secretory mechanism that is not affected in CF so that there is no reduction in flow, and 2) the composition of its product, sweat, normally varies over a wide range that includes the composition of sweat produced in CF so that the composition in the CF gland lumen is still within a well-tolerated physiological range.

Which story is correct? Neither a consideration of mucus nor of electrolytes alone is likely to present the whole truth. Both must be interdependent and interrelated. History has not yet resolved where and why they overlap to produce the complex path that witches dubbed as a curse and that we in science still struggle to find. Until we chart that path, we may wander the Wild West with the stark possibility that even now we have not yet considered the true reason that CF is a disease.

**REFERENCES**


33. CEDER, O., P. HOSLI, E. Vogt, AND I. KOLLBERG. Diagnosis of cystic fibrosis homozygotes and heterozygotes from plasma and


105. **GIBSON, L. E., AND P. A. DI SANT’AGNESE.** Studies of salt electrolyte movement in exocrine tissues.

106. **GIBSON, L. E. AND R. E. COOKE.** A test for the concentration of vasoactive intestinal peptide and abnormal mucus accumulation in Cystic Fibrosis.


270. YAFFE, S. J., L. M. MOSOVICH, M. E. MAT- 
TAR, M. DANISH, AND W. J. JUSKO. Pharmacokinetics of methi- 
831, 1977.

Cystic fibrosis ciliary dyskinesia substances and pulmonary dis- 

272. WILSON, J. R., AND R. O. DUBOIS. Report of a fatal case of kerato- 
malacia in an infant, with postmortem examination. Am. J. Dis. 

273. WINE, J. J., D. J. BRAYDEN, G. HAGIWARA, M. E. KROUSE, T. C. 
LAW, U. J. MULLER, C. K. SOLC, C. L. WARD, J. H. WIDDICOMBE, 
AND Y. XIA. Cystic fibrosis, the CFTR, and rectifying Cl⁻ channels. 

274. YAFFE, S. J., L. M. GERBRACHT, L. L. MOSOVICH, M. E. MAT- 
TAR, M. DANISH, AND W. J. JUSKO. Pharmacokinetics of methi- 
831, 1977.

275. YOUNGBERG, C. A., R. R. BERARDI, W. F. HOWATT, M. L. HY- 
NECK, G. L. AMIDON, J. H. MEYER, AND J. B. DRESSMAN. Comparison of gastrointestinal pH in cystic fibrosis and healthy sub- 

276. ZABNER, J., J. J. SMITH, P. H. KARP, J. H. WIDDICOMBE, AND 
M. J. WELSH. Loss of CFTR chloride channels alters salt ab- 

277. ZEULER, W. W., AND W. A. NEWTON. The pathogenesis of fibrocy- 
sic disease of the pancreas. A study of 36 cases with special refer- 