Cystic Fibrosis: Lessons from the Sweat Gland

Lessons from the sweat gland on cystic fibrosis (CF) began long before modern medicine became a science. In European folklore, the curse that “a child that taste salty when kissed will soon die” (Alonso y de los Ruyzes de Fonteca J. Diez Previlegios para Muger es Prenadas. Henares, Spain, 1606) has been taken by many as a direct reference to cystic fibrosis [Busch R. Acta Univ Carol Med (Praha) 36: 13–15, 1990]. The high salt concentration in sweat from patients with CF is now accepted as almost pathognomonic with this fatal genetic disease, but the earliest descriptions of cystic fibrosis as a disease entity did not mention sweat or sweat glands (Andersen DH. Am J Dis Child 56: 344–399, 1938; Andersen DH, Hodges RG. Am J Dis Child 72: 62–80, 1946). Nonetheless, defective sweating soon became an inseparable, and major, component of the constellation of symptoms that diagnose “cystic fibrosis” (Davis PB. Am J Respir Crit Care Med 173: 475–482, 2006). The sweat gland has played a foremost role in diagnosing, defining pathophysiology, debunking misconceptions, and increasing our understanding of the effects of the disease on organs, tissues, cells, and molecules. The sweat gland has taught us much.

The Disease

We can surmise what the soothsayers of yesteryear knew about the fate of a child whose sweat tasted salty. They probably knew that the child would become malnourished, emaciated, and die within weeks or months due to what we now recognize as pancreatic insufficiency due to obstructed ducts and autolysis of the organ. They may very well have known that, if the child survived any length of time, it would develop a persistent cough that produced thick, sticky sputum and that he or she would soon suffocate due to infections that fatally obstruct and destroy the lungs. What they could not have known was that virtually all exocrine tissue are affected, and especially those that secrete mucus and macromolecules (118). They could not have known that if the child survived, most males would be sterile due atrophy of the vas deferens and females would have reduced fertility due to abnormal cervical fluids (91, 107). Neither could they have known that about 10–15% of the babies, who would have had salty sweat, had died as early newborns from ruptured intestines due to meconium ileus (162). They probably were keenly aware that the curse ran in families, since we now know that cystic fibrosis (CF) is inherited as an autosomal Mendelian recessive trait (4, 34) so that there was a high probability that it would have been seen more than once in the same family. They would not have known that CF would be the most common lethal inherited disease to affect their European descendents at a frequency of about 1 in 3,200 live births (75).

The curse became a recognized medical disease only about 70 years ago (3, 57). Although the pancreatic lesions from which it was then named were associated with lung disease, there was no mention or recognition in modern medicine of strangely salty sweat for more than a decade.

Sweat and salt. Lesson 1: how to diagnose CF easily

The sweat gland (FIGURE 1, A AND B) owes its recognition in CF to the weather. In the summer of 1949, an unusually intense heat wave struck New York City. Dorothy Andersen, who introduced the disease as a genetic entity (2) shortly after Fanconi observed an association of fibrocystic pancreata with lung disease (57), noted that a number of her patients were stricken with heat prostration (86). A young assistant professor, Paul di Sant’Agnese, working with Andersen at Babies Hospital (Columbia Presbyterian Medical School) assumed that the crisis resulted from accentuated dehydration due to pure salt loss. He then meticulously demonstrated that the sweat glands were the source of that loss (42, 47, 49). Over the next decade, these findings were confirmed and reconfirmed (9, 46, 94, 109, 148, 163, 183). At that time, the tests for sweat salt concentration (9, 102, 183) generally involved warming the subject enclosed up to the neck in a rubber or plastic body bag (“plastic” was very innovative at the time)
time) to obtain adequate volumes of sweat for conventional Na⁺ and/or Cl⁻ analysis (171, 180), which unfortunately posed a real risk of fatal hyperpyrexia that could occur under these circumstances (69, 103).

Fortunately, the introduction of the “Quantitative Pilocarpine Iontophoresis Sweat Test” (QPIT) removed the risk. With the QPIT, a relatively small area of 30–40 cm², usually on the volar surface of the forearm, was stimulated to secrete sweat by iontophoresing a potent sweat secretagogue (usually pilocarpine) through the skin for a few minutes, after which sweat from the stimulated area was collected hermetically for about 30 min and analyzed for Cl⁻ (65). After extensive investigations of other related conditions, Cl⁻ concentrations¹ in the sweat greater than 60 mEq/l were deemed to be characteristic of CF (50). The rule holds especially well in the pediatric population where, normally, sweat contains <40 mEq/l. The rule does not hold so well among adults, especially at high sweat rates (FIGURE 2). After nearly 50 years, the sweat test remains the foremost test in the differential diagnosis of CF to date (104). With this innovation, the sweat gland taught us that the most efficient, expedient test for diagnosing CF was to measure the concentration of Cl⁻ in a small sample of pharmacologically induced sweat.

Salt and mucus. Lesson 2: mucus is not a primary abnormality

Based almost exclusively on histochemical observations of the time, which were no doubt significantly influenced by the gross appearance of the viscous sputum coughed up by patients, CF became and remains thought of at large as the disease caused by “thick, sticky mucus.” The early pathologists focused exclusively on histopathology that showed that dilated lumina, cysts, and fibrosis were prominent features in affected tissues. That is, this destruction was associated with mucus plugs and/or concretions of macromolecules in the pancreas, the biliary tree, intestine, lung, salivary glands, and other exocrine tissues that produce “mucus” (51, 58, 59, 194). The stigma of abnormal mucus was so prevalent that most in Europe and some in the States preferred the name “mucoviscidosis” (state of viscid mucus) (163).

This is not to imply that the concept of mucoviscidosis is misplaced, but the sweat gland gave a crucial lesson in pathology and undermined the dogma. Provocatively, here was a clear abnormality, highly characteristic of the disease, expressed in a gland that did not produce mucus (92, 154).² The inescapable lesson was that there was a fundamental defect independent of the mucus abnormality (43, 48). As a corollary, since the structure and microanatomy of the CF sweat gland were normal (105, 125), unlike affected mucus-producing organs, the histopathology found in other affected mucus-secreting exocrine tissues was most likely due to secondary effects on mucus (118, 187). The central challenge for a uniform hypothesis became and frustratingly remains to understand how a fundamental defect in electrolyte transport can lead to the fatal thick, sticky mucus of CF (45).

Fate of salt. Lesson 3: salt absorption fails

As knowledge of the abnormally high salt in CF sweat was being introduced, an understanding of the mecha-

¹Cl⁻ concentration gives an empirical, best separation between patient and control populations.

²Minor concentrations of glycoproteins are present in sweat (108).
nism that determined the electrolyte concentrations of sweat and other exocrine secretions was also taking form (159, 177). In this model, two steps were postulated to be required to form the final fluid excreted from a gland. In the first, an isotonic fluid was actively secreted (never filtered as in the kidney) into the lumen by the acini or proximal tubular structures of the gland, and as secreted fluid accumulated in the gland lumen, the consequent buildup of hydrostatic pressures forced this primary secretion out of the gland through a tubule (duct) to an opening on the surface of a body cavity or the skin (FIGURE 3). During its course through the lumina of more distal ducts, a second step modified the composition of the primary isotonic fluid secretion before excretion. In the case of the sweat and salivary glands, salt was predicted to be selectively absorbed from the duct lumen in excess of water to form a final fluid that was hypotonic to the primary secretion and, of course, the extracellular fluid from which it originated. In other organs, such as the pancreas or biliary tree, ions appeared to be exchanged or secreted along the way (e.g., HCO$_3^-$ for Cl$^-$).

Proof of these concepts came later from studies by a number of investigators who used the sweat gland to show that the primary secretion was indeed isotonic and that the concentration of salt in excreted sweat depended on its rate of secretion because the modifying step (NaCl reabsorption in the sweat duct) tended to saturate at higher secretory rates (FIGURE 2) (19, 29, 55, 97, 157, 158, 165–168, 170). These theories and their proofs were crucial to understanding the emerging questions on the basic cellular defect in CF. That is, was CF sweat more concentrated because the primary fluid was secreted more rapidly than normal (hypersecretion) (150)? Or was the primary fluid abnormally concentrated with salt when initially secreted (hyperconcentration) (66)? Or was absorption of NaCl from the duct defective (28–30, 67, 158, 167)? The Thaysen-Schwartz model is now well established. In all mammalian fluid secretory systems that have been examined, the primary fluid is elaborated isotonically (or at least iso-osmotically$^3$) and then subsequently modified in the gland ducts (FIGURE 3). Primary fluid secreted in mammals is never hypertonic or hypotonic (120).

The lessons learned at the time from the sweat gland were that 1) the volume and composition (isotonic) of precursor sweat (primary fluid) from CF patients were normal but 2) the concentration of salt in final sweat excreted from CF patients was three to five times higher than normal (55, 97, 164). The large difference that began to emerge from these findings was that failure to reabsorb NaCl in the duct caused the salty sweat in CF (28, 158, 169). However, some 20 years later, these early assumptions about normal secreted volumes in CF would be found to be only partly correct.

**Genetics. Lesson 4: heterozygotes are almost normal**

The QPIT sweat test taught more than just how to test for CF easily; it provided a means of studying significantly larger numbers of patients and subjects noninvasively. Since Andersen (4) had established early on that CF was inherited and had postulated that the pattern was Mendelian recessive, it would seem logical that the sweat phenotype be used to further define genetic characteristics. Curiously, the sweat test apparently was never used to show that CF is inherited as a Mendelian recessive trait, although it has been used to show that the number of probands for Mendelian recessive traits agreed almost perfectly with predicted values for incidence in families of different sizes (34).

Nonetheless, the sweat gland provided hard evidence that the expression of the normal phenotype in salt absorption was dominant and almost, if not, completely preserved. That is, the salt concentration on average from obligate heterozygotes (parents) was, although highly variable, probably somewhat higher than normal (50, 60, 171, 183, 188). However, one of the largest studies ever performed in a single laboratory$^4$ on more than

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3 By isotonic, it is implied that the primary fluid is similar to a plasma ultrafiltrate in composition. An exception to isotonic primary secretion is the gastric juice, but it is iso-osmotic.

4 In addition to being very large, these tests had the advantage of being performed almost exclusively in the same clinic by only a few technical personnel, which should have improved the quality of the data.
5,000 subjects concluded that there is no difference in the mean concentration of Na⁺ or Cl⁻ in sweat from heterozygotes and controls (164). In any case, we learned sweat salt concentration cannot be used to predict the heterozygote status of an individual and that obligate heterozygotes seem almost, if not, normal.

On the other hand, the sweat gland came forward with a new CF phenotype that does reveal heterozygosity. After learning that CF patients do not sweat in response to β-adrenergic stimulation (153), the sweat response to isoproterenol (β-adrenergic agonist) was compared for homozygote normal, heterozygote, and homozygote disease. In this analysis, the average response of the sweat rate in heterozygotes was essentially half that of normal subjects, whereas glands from CF patients had virtually no response (12, 155).

All together, the sweat gland taught us that, for some functions, phenotypic expression in heterozygotes is partial (e.g., β-adrenergic fluid secretion), and for others it is not (salt absorption).

Selective Advantage. As strange at it may seem at first glance, the abnormality in electrolytes in sweat suggested a selective advantage for heterozygotes carrying the defective gene. Linked together, the facts that 1) the incidence of the gene seemed too high to be explained by spontaneous mutation (193), 2) fluid transport depends critically on CF permeability (114), and 3) intestinal secretory diarrheas are known to have exerted intense and widespread pressures on humans historically, all led to conjecture that heterozygotes might be more resistant to salt-loss induced circulatory collapse during bouts of secretory diarrhea than homozygotes without the defective gene (111, 116). That is, heterozygotes with an inherent limit on fluid secretion would lose smaller volumes of gastrointestinal fluids than normal homozygotes and might be better able to survive threats of fatal dehydration. The facts that heterozygotes secrete β-adrenergically stimulated sweat at a reduced rate (12, 155) and that mice genetically altered to mimic CF secreted less in response to Cholera toxin (63) and to β-adrenergic agonists (15) than normal mice seemed to support this theoretical lesson. However, two other studies of mouse intestines measuring short-circuit currents under acute stimulation (38) and of human jejunum in vivo measuring acutely PGE₂-stimulated fluid secretion (77) found no differences between WT and CF heterozygote genotypes.²

Even if correct, the hypothesis is not without uncertainties. For example, it does not explain why Caucasians, especially northern Europeans, have the highest incidence of CF-causing mutations. Nonetheless, the sweat gland may be partially responsible for this distribution. That is, in northern temperate zones inhabited by Europeans where the mutation is most frequent, thermoregulatory sweating is less vital than in southern equatorial zones where sweating is crucial to survival. If heterozygotes lose more salt in their sweat, the ancient scarcity of salt may have acted as a negative pressure to exclude the mutation in warm climates (116).

Cell Physiology

As discussed above, lessons from the sweat gland taught us that both absorptive as well as secretory fluid transport is defective in CF. In short, it taught us

²Cholera toxin usually requires hours to exert full effect.

![Diagram of the two components and two steps required in the Schwartz-Thaysen model of exocrine gland secretion to explain the final composition of excreted gland fluids](image)

**FIGURE 3.** A diagram of the two components and two steps required in the Schwartz-Thaysen model of exocrine gland secretion to explain the final composition of excreted gland fluids. In general, as shown here for the sweat gland, the secretory coil (or acinus in other glands) first secretes a primary fluid that is isotonic with the interstitial fluid and is usually stimulated mainly by cholinergic agonists that stimulate normal sweating in CF. Alternatively, adrenergic agonists can stimulate normal glands to secrete primary fluid, but generally at lower maximum rates. The β-adrenergic stimulation characteristically fails in CF patients due to absence or malfunction of its target, the CFTR Cl⁻ channel in the apical membrane of secretory cells. As the isotonic fluid is secreted, it is expressed through the remaining tubule (reabsorptive duct) to exit the gland. Along the way, the ductal cells modify the fluid, which in the case of the sweat gland absorbs NaCl hypertonically (in excess of H₂O) so that the final sweat emerges significantly hypertonic to plasma (cf. FIGURE 2). The process of NaCl absorption in CF patients is characteristically impeded by the absence or malfunction of the mutated CF gene product, the CFTR Cl⁻ channel, in the apical and basal membranes of the duct cells. Consequently, sweat emerges from these glands with the characteristically high salt concentration generally used in diagnosis (cf. FIGURE 2).
that CF is a disease of abnormal electrolyte transport. The sweat gland still offers unique classrooms for the study of the defective gene product in its native state in human tissues from normal and CF subjects. It provides the only human tissue in which the gene product affected in CF, CFTR (CF transmembrane regulator), can be examined as it functions in an intact organ as well as in its native membrane environment under the control of native cytoplasmic mediators and enzymes. That is, not only can whole body and single gland sweat functions be examined in vivo, but isolated organs (single intact glands) and isolated segments of reabsorptive duct or secretory coil and virtually isolated membranes can be examined in vitro. Perhaps no other tissue of the body offers so many settings for study. The major disadvantage is that the classrooms are small. The single gland is only about 250 μm in diameter, and the tubules of the gland measure about 30–45 μm in diameter. About half of the length of the continuous tubule (3–5 mm) is a secretory tubule (or coil) that is a purely fluid secreting, and the other half is a purely NaCl absorbing duct (FIGURES 1, A AND B, AND 3).

Reabsorption. Lesson 5: Cl– impermeability is a defect of CF epithelia

With the knowledge that the salt composition of CF sweat is characteristically 3–5 times higher than normal and that the gland was spared secondary histopathology, the sweat gland was predisposed to give lessons on the basic defect in the physiology of salt transport. Following observations that transepithelial electrical potentials (voltages) on the nasal mucosa in CF patients were hyperpolarized (lumen negative) compared with normal subjects (87), CF and normal sweat ducts came forward to confirm and explain the differences in electrical potentials. Techniques of renal tubule microperfusion (26) were adapted and applied to segments of the isolated sweat ducts from CF and control subjects (FIGURE 4, A AND B) (115). Its lessons were clear. The spontaneous transepithelial potentials of micro cannulated duct segments (bathed and microperfused with isotonic NaCl) from CF patients were hyperpolarized by about an order of magnitude more than those of non-CF subjects (–75 vs. –7 mV, respectively, lumen negative) (111, 112, 121).

Since polarization is caused by a relative separation of charge due to relative displacements of cations from anions, this dramatic lesson could have been interpreted in two different ways: 1) highly accelerated salt transport driven by increased Na+ absorption might have caused the increased luminal electronegativity or 2) Cl– absorption might have been greatly retarded relative to Na+ absorption (cf. Lesson 13). That is, in the process of salt absorption, was the positive Na+ cation abnormally accelerated relative to the negative Cl– anion or was the movement of the Cl– anion abnormally slowed relative to the Na+ ion? The first interpretation that Na+ transport was enhanced was clearly incompatible with the well established evidence that salt absorption was markedly decreased in CF sweat glands (i.e., the basis of the QPIT). Calculations based on the Thaysen/Schwartz model of isotonic secretion showed that absorption in CF, although not eliminated, was about 20% of normal (19). However, in further support of the second interpretation, if Cl– was removed from the luminal perfusate of the duct and replaced with an impermeant anion such as SO4 or gluconate, the normal duct also developed a hyperpolarized luminal potential almost the same as the CF duct perfused with Cl–. The same substitution for impermeant anions in the CF duct had almost no effect on its potential (112).

These results showed that the normal sweat duct is highly permeable to Cl– but that CF ducts are relatively impermeable to Cl–. Shortly, thereafter, relative Cl– impermeability was found to characterize CF nasal
mucosa (taken as representative of airway epithelia) as well (88). Every tissue, cell, and organelle known to be affected in CF has now been shown to exhibit some form of impermeability to Cl\textsuperscript{−}. Thus the first lesson in cell physiology from the sweat gland in electrolyte transport was that, even though salt absorption is not completely blocked, Cl\textsuperscript{−} impermeability was a defective property of the cell.

**No Humoral Factors.** In the decade of CF research preceding lessons on Cl\textsuperscript{−} permeability, there were a number of reports on the sweat duct and other in vitro systems that resulted in the once popular idea that the electrolyte defect as well as ciliary clearance of the lung was due to circulating humoral factors that inhibited cilia (25, 39, 52, 68, 172), produced other unique abnormalities (7, 21, 22, 84, 95, 179, 190, 191), and inhibited Na\textsuperscript{+} transport (100, 101). The demonstration that the defect persisted and was clearly expressed in a completely in vitro system with all systemic fluids replaced by defined media provided a compelling lesson that the defect in transport was inherent in the affected cells and not induced by external “factors,” as they were then called (112, 121). Additionally, companion studies with isolated normal ducts perfused with secretions from CF patients gave convincing lessons that such factors did not cause the electrolyte transport defect in CF tissues (18).

**Location of defect. Lesson 6: the Cl\textsuperscript{−} permeability defect is transcellular**

If the sweat gland taught us that the defect was Cl\textsuperscript{−} impermeable, would it teach us which Cl\textsuperscript{−} pathway across the epithelium was affected: apical membrane, basal membrane, both, or paracellular? To approach this problem, the Na\textsuperscript{+} and K\textsuperscript{+} permeability properties of the epithelium needed to be assessed. That is, in the normal perfused intact sweat duct, in the absence of Cl\textsuperscript{−}, if [Na\textsuperscript{+}] in the duct lumen was altered, the transepithelial potential behaved as if the apical membrane were a Na\textsuperscript{+} electrode; a behavior that was completely blocked by amiloride. This inhibitor was highly selective for the epithelial Na\textsuperscript{+} channel (ENaC), so that it was virtually certain that it was this channel that provided the Na\textsuperscript{+} conductance (GNa\textsuperscript{+}). The fact that amiloride only slightly increased Ra/Rb in normal ducts in the presence of bilateral Cl\textsuperscript{−} (4.2 vs. 5.0) but increased dramatically when Cl\textsuperscript{−} was removed (4.8 vs. 24.8) or when the maneuver was performed on CF ducts (2.7 vs. 8.1) (140) established that Cl\textsuperscript{−} conductance must be parallel to the Na\textsuperscript{+} conductance in the apical membrane and not paracellular through the tight junctions\textsuperscript{6} (16).

**Defective membranes. Lesson 7: the basolateral membrane is also impermeable to Cl\textsuperscript{−}**

Most students of CF now readily recognize that the apical membrane of affected cells is Cl\textsuperscript{−} impermeable, but the sweat duct taught us that, in absorption, both apical and basolateral membranes are defective. Creating asymmetric Cl\textsuperscript{−} gradients across the normal epithelium by replacing Cl\textsuperscript{−} with an impermeant anion (e.g., gluconate\textsuperscript{−}) on one side caused dramatic shifts in both apical and basolateral membranes consistent with a Cl\textsuperscript{−} conductance in each membrane, which did not occur in CF ducts. Having established that the Cl\textsuperscript{−} conductance pathway is transcellular, these results show that both membranes of the normal epithelium express a significant Cl\textsuperscript{−} conductance (128, 140). The fact that the Cl\textsuperscript{−} conductance in both membranes was activated by cAMP implied that both were due to CFTR (129).

Once the gene for CF was cloned and antibodies for its product were produced, immunocytochemical localization of CFTR showed that the apical membrane of the sweat duct was one of the richest specific sites for expression of the protein (37, 83). Although staining was minimally detectable at the basal membrane, the structure and function correlate well as evidence that CFTR was the principal source of Cl\textsuperscript{−} conductance in this absorptive process. The relatively low intensity of the stain at the basolateral membrane compared with the apical membrane seemed likely due to the fact that the area of the basolateral membrane in the duct epithelium is many fold greater than the area of the apical membrane so that the concentration of CFTR antigen would be much lower in the basolateral membrane, making the specific intensity of its label much lower and less visible.

Perhaps, more significantly for cystic fibrosis, a body of evidence had been growing to establish that the

\textsuperscript{6}If the paracellular shunt were permeable to either cation, the transepithelial voltage would have changed when the ion concentration was changed on either side of the epithelium. Since only Na\textsuperscript{+} on the apical and only K\textsuperscript{+} the basolateral affected the TEP, the tight junction cannot be permeable to either.

\textsuperscript{7}If the Cl\textsuperscript{−} conductance were in the tight junction, the predominant apical membrane conductance would be Na\textsuperscript{+} conductance, and Ra/Rb should have increased dramatically in response to amiloride, which it did when Cl\textsuperscript{−} was removed from normal ducts and when CF ducts were used even in the presence of CF, but not when Cl\textsuperscript{−} was present with normal ducts.
most common mutation, ΔF508 (a deletion of phenylalanine at the 508 position of the CFTR protein), did not mature and process properly (33). Immunocytochemical evidence in heterologous systems indicated that ΔF508 CFTR did not localize with the cell membrane but was diffusely distributed in the perinuclear region (41). The sweat gland provided the first demonstration that the same was true in a native tissue (83), followed shortly thereafter in nasal polyps (110), and confirmed again in bronchial submucosal glands (56).

Thus the sweat gland’s lesson on the cellular location of the defect in Cl– conductance was that CFTR is not only in the apical membrane, where it is commonly assigned, but also in the basolateral membrane of this hypertonically absorbing epithelium. The lesson also demonstrated that the most common mutation of CFTR, ΔF508, is not processed to the plasma membrane in native tissues.

**Secretion. Lesson 8: some fluid secretion is also defective**

The first lesson in Cl– transport in CF was from defects in absorption, but the gland had much more to teach about secretion. As mentioned above, it was well accepted that, when stimulated thermally or pharmacologically with analogs of the neurotransmitter acetylcholine (e.g., pilocarpine), sweat volumes from CF and normal subjects were about the same (30, 79, 158, 164). On speculation that NaCl reabsorption might be regulated (174), Sato attempted to enhance salt absorption in CF glands by stimulating with a β-adrenergic agonist. It had no detectable effect on absorption but led to a completely surprising result. In stark contrast to the normal glands, CF sweat glands refused to sweat in response to the common β-adrenergic agonist isoproterenol (153). Defective cAMP-mediated secretion in CF has now been shown to be characteristic of every exocrine secretory system affected in CF (114). This major lesson in electrolyte transport from the sweat gland quickly taught us that both CF and normal glands produce much more chloride than sodium (119, 120). It is important to note that this was not an attempt to substitute for water or sodium in the body but to balance electrolyte composition. Because of its relative stability, chloride is the major component of extracellular fluid, but in CF normal glands and the sweat gland, fluid secretion is chloride rich (121, 122).

Soon after Sato’s thrilling discovery of defective β-adrenergic sweating, a rectifying Cl– channel was identified and thought to be that involved in CF (62, 185). However, much controversy arose as to the correctness of the channel identity (192). The sweat gland provided the first demonstration that the same was true in a native tissue (83), followed shortly thereafter in nasal polyps (110), and confirmed again in bronchial submucosal glands (56).

Control of CFTR

**Cyclic nucleotides. Lesson 9: signaling is not defective**

Since the β-adrenergic stimulation of secretion was known to activate protein kinase A (PKA) through increases in intracellular cAMP levels (8) (175), the sweat gland quickly taught us that both CF and normal glands produced approximately equal levels of cAMP in response to β-stimulation (153). Later, cAMP levels were found to be similar in other CF and normal tissues as well (10, 76, 185, 186). This meant that the pathway for the G-protein receptor for β-adrenergic ligands that were linked through adenylate cyclase and phosphodiesterase were not affected and that the defect was likely due to PKA or phosphorylation of a substrate. At the same time, there was little idea of what the substrate might be. However, since the accepted model of secretion required activation of Cl– conductance in the apical membrane of the target secretory cell and since the defect in the sweat duct was Cl– impermeability, the tentative lesson was that the basic defect was somehow due to failure to activate a plasma membrane Cl– conductance. This lesson was not clear at first because attempts to support the findings by stimulating a Cl– conductance in the absorptive sweat duct met with little success. Eventually, its secret of why it seemed unresponsive to cAMP-mediated stimulation was revealed to be simply due the fact that CFTR in the normal sweat duct was constitutively active so that stimulation had little if any detectable effect (129).

When the basolateral membrane of the sweat duct cell membrane is exposed for a few minutes to α-toxin from Staphylococcus aureus, pores are formed that allow small solutes to equilibrate between cytoplasm and extracellular medium (the bathing solutions). Consequently, molecules of less than about 5,000 mwu are lost from the cytoplasm, and its small solute and electrolyte composition can be controlled by defining the composition of the extracellular medium with which it equilibrates (FIGURE 5). In the microperfused sweat duct, shortly after adding α-toxin, there was an inevitable, dramatic fall in the transepithelial conductance and a dramatic loss in the characteristic Cl– conductance that constitutively dominated the permeability properties of this epithelium. These dynamic changes were due to closing of the CFTR Cl– channels as cAMP and ATP were lost from the cytoplasm during pore formation since activity was prevented or restored by adding cAMP and ATP back to the bath. The lesson from this behavior was that CFTR in the duct is constitutively active and, unlike secretion, does not require acute stimulation to become active. There was no evidence that PKA phosphorylation of CFTR was defective in CF tissues (126).

It is not clear whether CFTR in its absorptive role is always constitutively active in other organs. To our knowledge, the sweat duct is the only human tissue
that has been investigated for these properties in its native state. However, recent evidence suggests that constitutively active Cl– conductance is characteristic of native small airways in pigs as well (184).

**CFTR and ATP. Lesson 10: CFTR Cl– conductance is ATP dependent**

When the CFTR gene was cloned and sequenced, two ATP nucleotide-binding domains similar to ABC8 transporters were indicated to be present in the gene product (85, 149, 151). Since CFTR was thought to act as a passive Cl– conductance, the purpose of ATP binding was not apparent. The enigma was confounding because CFTR expressed very little ATPase activity (90, 93, 156). Although it is now established that ATP is normally required for CFTR gating (11, 73, 181, 182), the sweat duct presented an early lesson on CFTR ATP dependence (124).

Patch-clamp studies reported that micromolar levels of ATP were sufficient to support CFTR activation in isolated membranes from NIH-3T3 cells (5), but Cl– conductance could not be activated with such low concentrations in perfused, basolateral membrane permeabilized, native sweat ducts (**FIGURE 5**). However, when cytoplasmic concentrations of ATP were adjusted to physiological levels of about 5 mM, CFTR activated immediately (113, 123), but ATP appeared not to be hydrolyzed since CFTR could be activated by adding nonhydrolyzable analogs of ATP (ATP-g-S, AMP-PNP) if micromolar concentrations of ATP were present to support PKA phosphorylation. We have since learned from other systems that ATP hydrolyzes on closing the CFTR channel so that, when a nonhydrolyzable analog binds with NBD2 (the second nucleotide binding domain in the CFTR molecule), it locks CFTR in the activated state (64). Thus the sweat gland taught us not only that ATP was essential to activate CFTR but also that normal cellular levels of ATP are required for its function in native membranes, suggesting that CFTR may govern electrolyte transport activity as a function of available cellular energy charge (5, 113, 123, 178).

Some indications arose that CFTR might conduct ATP and thereby mediate purinergic control of other ion channels via paracrine responses from the luminal membrane (31, 32, 147). Since the sweat duct is replete with CFTR in its plasma membranes, it is difficult to conceive how CFTR could serve as a conductive pathway for ATP without compromising cell energy stores. Nonetheless, to test the hypothesis, the isolated duct was microperfused with 50 mM ATP in the lumen. An ATP conductance should have depolarized the transepithelial potential; however, there was no significant change in the potential or conductance (144).

Thus this lesson from the sweat duct demonstrated that CFTR probably did not play a direct role in conducting ATP out of the cell.

**CFTR and kinases. Lesson 11: CFTR is activated not only by phosphorylation but by other mediators as well**

Few things in CF cell physiology investigations are so well established as the fact that protein kinase A activates CFTR by phosphorylation (40). However, once established for CFTR in the sweat gland (134, 141), this simple lesson quickly became more complicated due to the fact that, as in some other tissues affected in CF, other kinases or mediators also activate CFTR Cl– conductance. Thus GTP-g-S, cGMP, and Sta (heat stable Enterotoxin) activate CFTR (61, 81, 160, 161). Although genistein seems to activate CFTR in ex vivo systems (80), it appears to have no detectable effect in the sweat duct (M. M. Reddy, personal observations). But, most surprisingly (145), once CFTR in the duct was inactivated by permeabilizing the basolateral membrane, it was reactivated by simply adding millimolar concentrations of glutamate or its precursors, a-keto-glutarate or glutamine, back to the cytoplasm (132). To date, there are no reports of these agonist stimulating CFTR in other systems, but, in the sweat duct, glutamate or precursors activated CFTR–Cl– conductance even in the presence of the promiscuous kinase inhibitor staurosporine and in the apparent absence of cAMP and ATP (132). No effect was seen in sweat ducts from CF patients homozygous for ΔF508. The lesson here may be that, although the effect may be tissue specific, mediators that directly or indirectly activate CFTR may do so without the requirement for phosphorylation or ATP. These issues beg to be explained.

**CFTR and phosphatases. Lesson 12: CFTR Cl– conductance is deactivated by phosphatases**

As discussed above, when the basolateral membrane of the perfused sweat duct was permeabilized (**FIGURE 5**),

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1ABC (ATP binding cassette) transporters are a superfamily of membrane molecules with a common amino acid motif that are known to bind and hydrolyze ATP in the transport of a wide variety of substances.
the otherwise constitutively open CFTR spontaneously shut down. Since kinases activate CFTR, a phosphatase must deactivate it. Indeed, when the duct was permeabilized in the presence of a phosphatase inhibiting cocktail of fluoride, vanadate, and okadaic acid, CFTR remained active (134). In fact, okadaic acid alone at 10⁻⁸ M prevented deactivation of CFTR as did use of the phosphatase resistant ATP analog, ATP-γ-S, to phosphorylate and activate the channel. In either of these conditions, CFTR Cl⁻ conductance remained “permanently” activated but was reversibly switched off or on by simply removing or adding ATP in the complete absence of cAMP, showing that, once activated by phosphorylation, CFTR was only dependent on ATP for gating. The shutdown was insensitive to Ca²⁺ depletion, suggesting that PP2B was not involved, but the effectiveness of okadaic acid at low concentration suggested that dephosphorylation was carried out by PP2A or PP1 (133), as reported in heterologous systems (14). Thus the sweat gland instructed that native physiological deactivation of CFTR is via an inherent phosphatase.

In general, the control of phosphorylation activation of CFTR was thought to be maintained by simply controlling the activity of the kinase (PKA) in the presence of a constitutively active phosphatase, but the sweat duct had more lessons on this subject. Remarkably, just reducing the cytoplasmic K⁺ concentration (even in the presence of cAMP) deactivated CFTR; however, lowering K⁺ in the presence of okadaic acid prevented deactivation. Moreover, the degree of deactivation was proportional to cytoplasmic K⁺ concentration and independent of whether K⁺ was replaced by Na⁺, NMDG⁺, or Li⁺. Only Rb⁺, which often mimics K⁺ biologically, mimicked the K⁺ effect. Thus we have a new, complex lesson that native CFTR can be regulated indirectly by levels of cytoplasmic [K⁺] that inhibit an endogenous K⁺-sensitive phosphatase that dephosphorylates and deactivates the CFTR Cl⁻ channel.

Intracellular ion sensitivity measurements showed that, as cell Na⁺ increases, cytoplasmic [K⁺] falls (138). That is, the sum of [Na⁺] and [K⁺] tends to remain constant while their concentrations change reciprocally. By lowering cell [K⁺], Na⁺ entry, in effect, relieves the K⁺ inhibition of the phosphatase and deactivates CFTR and impedes Cl⁻ entry. Consequently, since Na⁺ cannot enter without a co-ion, salt transport slows or stops (because cations and anions must be transported in equal numbers to satisfy electroneutrality). Thus, although these findings plead for confirmation in other native tissues, the sweat gland reinforces the lesson that CFTR functions in a pivotal role to couple transport activity with transport capacity and cell energy supply on a moment-to-moment basis (113, 123, 124, 133, 137).

**CFTR and ENaC. Lesson 13: CFTR permissively controls ENaC; ENaC does not control CFTR**

Not only is the activation of CFTR well controlled by relative kinase and phosphatase activities, but CFTR itself apparently exerts further control of salt entry by controlling the activity of the only channel for Na⁺ entry (ENaC) into the duct cell. However, lessons from the native sweat gland contradict those from airway epithelia. In the original report of CF airway ion transport abnormalities in 1981 (87), transepithelial potentials across CF nasal mucosa (~50 mV) were found to be about twice as negative as normal (~25 mV). These results were interpreted as being due to abnormally increased active Na⁺ absorption (70, 87) because, with this form of electrogenic NaCl absorption, Na⁺ is the actively transported ion while Cl⁻ follows passively so that increases in Na⁺ absorption activity are paralleled by increases in the negative potential in the lumen. Sometimes it is assumed that, since amiloride inhibits a larger fraction of the CF transepithelial potential, it inhibited a larger Na⁺ absorption, but it must be kept in mind that the transepithelial potential is only a measure of separation of charge; i.e., the relative separation of Na⁺ from Cl⁻ ions during their transport, not actual transport activity. No matter what the rate, if Na⁺ is absorbed and Cl⁻ is impeded (relatively impermeable), larger negative voltages will occur even if Na⁺ absorption decreases, as it does in the CF sweat duct.

Much work over the next two decades compiled evidence in model systems that increased Na⁺ absorption in these “CF” genotype cells was due to “runaway” epithelial Na⁺ channels (ENaC) (24, 35, 72, 98, 99). That is, these reports contended that expression and/or activation of CFTR in a WT cell co-expressing ENaC normally inhibited ENaC activity so that, without CFTR activity, a “CF” cell could not inhibit ENaC activity, and Na⁺ absorption in these ENaC(CFTR) transport systems remained continuously hyperactive (54, 173). This interpretation fit well with the common notion and in vitro observations (78, 176) that CF airways seemed abnormally “dry,” leaving thickened mucus that may impede pathogen clearance and render CF airways more susceptible to infections. These results seem difficult to reconcile with decreased Na⁺ absorption in the sweat gland and in the ileum (152), and of concentrated NaCl on cultures of CF airway cells and probably in vivo in CF patients (53).

However, the lesson was the exact opposite from the sweat duct with both CFTR and ENaC in the apical membrane (37, 83, 145, 146). To wit, although the spontaneous luminal potential in CF sweat ducts (~75 mV) was like the airway much more negative than nor-
mals (~7 mV) and amiloride also blocked much more transepithelial potential in CF than in normal (112, 117, 121, 139), the CF duct clearly absorbed much less, not more, Na+ than the normal duct (19, 28, 158, 189). Thus the lesson from the sweat duct was that CFTR acts cooperatively with ENaC and does not inhibit its function in this tissue.

In fact, in the normal duct, when CFTR was deactivated by removing cAMP or ATP, ENaC also fell; however, when CFTR Cl– conductance was reactivated by adding back these mediators, amiloride inhibitable Na+ conductance also "reactivated" to a level almost equal to that of Cl– conductance (~50 mS/cm²) (130, 136). In sweat ducts from patients with CF (∆F508/∆F508), where CFTR was not present in the membrane, ENaC conductance was below normal and could not be increased (135). This lesson was something of a surprise in that previously the CF transport defect in the duct was thought to be due solely to defective Cl– (117) and that Na+ transport did not occur simply because the Cl– anion could not accompany it to satisfy electroneutrality. This new lesson dictated, however, that failed transport in CF was not only due to loss of CFTR conductance but also to an accompanying secondary loss of CFTR-dependent ENaC conductance (127).

Although it seemed clear that activating CFTR was requisite for ENaC function, it was not clear whether activating ENaC was needed for CFTR function. Again, perhaps surprisingly, the lesson from the sweat gland was that this is not the case. Direct evidence that CFTR function was independent of ENaC activity was observed in sweat ducts from pseudohypoaldosteronism (PHA) patients. In these ducts, ENaC is dysfunctional and Na+ conductance was very low; however, CFTR Cl– conductance was almost normal (146).

Thus the overriding lesson from the sweat gland on CFTR and ENaC interactions was that ENaC activity depended on CFTR activity, but CFTR function did not depend on ENaC activity.

CFTR and HCO3−. Lesson 14: HCO3− conductance is a major determinant in CF disease severity

Although HCO3− conductance is acknowledged as defective in CF, CFTR is not well appreciated for a role in HCO3− transport even though one of the principal morbilities in CF is pancreatic failure due to loss of HCO3− fluid secretory capacity. Thus, physiologically, CFTR must be integrally involved in HCO3− transport. Even though the anion gap (Na + K – Cl), which is often taken as “HCO3− + lactate,” seemed clearly larger in sweat from single glands from control subjects than from CF patients (19), significant differences in either HCO3− or lactate could not be confirmed in sweat from single glands in vivo (20). Unfortunately, the pH (<5 to >8) and HCO3− concentrations (0 to ~25 mM) of sweat normally vary with sweat rate over such wide ranges in both CF and normal glands that no striking abnormalities in HCO3− concentrations have been clearly defined in sweat. Even though low concentrations of HCO3− in CF sweat have been reported (82) and an abnormal Cl−/HCO3− exchange has been suggested (6, 119), most investigations of single gland sweat have found no significant differences in sweat HCO3− or pH (20, 55, 106).

However, when the secretory coil was isolated from the duct and stimulated cholinergically (CFTR-independent secretion), the secreted sweat contained plasma levels of HCO3− (~25 mM), but when stimulated β-adrenergically (CFTR-dependent secretion), it contained no HCO3− (131). Serendipitously in the process of investigating the permeability properties of organic anions in the sweat duct, glutamate or its immediate precursors (α-keto glutarate and glutamine) very unexpectedly activated CFTR Cl– conductance in the permeabilized duct in the absence of cAMP or ATP. Under these conditions, HCO3− was impermeant, but if ATP were added to the glutamate (or its precursor, α-keto glutarate), both Cl– and HCO3− were permeant. Neither the basis nor the role of these phenomenon are understood, but when glutamate or precursors and ATP were applied to CF ducts from patients of different genotypes, those ducts from patients with a “mild” phenotype (pancreatic sufficient: ∆F508/R117H) exhibited a significantly larger (almost normal) HCO3− conductance, whereas ducts from patients with a “severe” phenotype (pancreatic insufficient: ∆F508/∆F508) remained impermeable to HCO3− (132). There remains considerable room for debate as to whether CFTR plays a role in HCO3− management by providing a direct pathway for HCO3− ions (44) or by permissive interactions with other transport molecules such as Cl−/HCO3− exchangers (36, 89). This new lesson from the sweat gland and pancreatic duct was that at least some mutations for CF spare CFTR HCO3− conductance and perhaps thereby support a milder form of the disease (36, 142). Recent findings indicate that, although the relative ratio of HCO3− conductance/Cl– conductance is between 0.1 and .2 for single CFTR channels activated with cAMP and ATP (96), the ratio in the sweat duct can range from virtually 0 to almost 1.0, depending on conditions of stimulation. That is, combining cAMP + cGMP + α-keto glutarate can yield CFTR HCO3− conductance almost equal to that of Cl– conductance (143).

This last lesson from the sweat gland seems to be to teach something about the function of CFTR and the sweat duct that we do not yet understand for lack of knowledge about CFTR interactions with HCO3− and how the sweat gland handles HCO3−. It raises provocative questions about whether the sweat gland might parallel renal excretion by regulating HCO3− excretion as a function of acid/base status and what role CFTR plays in primary fluid secretion of sweat. No doubt, the lesson on CFTR HCO3− conductance in the duct is far from complete, and the impact of its lesson on dynamic anion selectivity intriguingly awaits explanation.
Conclusions

The miniscule sweat gland has served well in defining, diagnosing, and understanding CF in particular, and perhaps in a better disclosure and understanding of many properties of salt secretion and absorption in general. In closing, the sweat gland should impart one other lesson in that a number of its lessons herein may be unique to its on experience and disposition. Clearly, some lessons can be generalized to other tissues (faulty Cl– conductance in CF), whereas others seem at direct odds with those from other tissues (CFTR inhibition of ENaC in airways), and still others await demonstration in other systems (glutamate activation of CFTR Cl– and HCO3– conductances and indirect regulation of CFTR via K+–sensitive phosphatase).

Unfortunately, the relative inaccessibility of other native tissues may leave the sweat gland standing alone as an untested beacon into the unfamiliar for native tissues may leave the sweat gland standing alone as an untested beacon into the unfamiliar for some time to come. Nonetheless, it continues to be a captivating, provocative, and instructive little school.

References


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