Too much salt, too little soda: cystic fibrosis

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Abstract: Cystic fibrosis (CF) of the pancreas is the most widely accepted name of the most common fatal inherited single gene defect disease among Caucasians. Its incidence among other races is thought to be significantly less, but mutations in the gene have been reported in most, if not all, major populations. This review is intended to give general concepts of the molecular as well as physiological basis of the pathology that develops in the disease. First, an overview of the organ pathology and genetics is presented, followed by the molecular structure of the gene product (cystic fibrosis transmembrane conductance regulator, CFTR), its properties, functions, and controls as currently understood. Second, since mutations appear to be expressed primarily as a defect in electrolyte transport, effects and mechanisms of pathology are presented for two characteristically affected organs where the etiology is best described: the sweat gland, which excretes far too much NaCl ("salt") and the pancreas, which excretes far too little HCO$_3^-$ ("soda"). Unfortunately, morbidity and mortality in CF develop principally from refractory airway infections, the basis of which remains controversial. Consequently, we conclude by considering possible mechanisms by which defects in anion transport might predispose the CF lung to chronic infections.

Key words: sweat glands; pancreas; airways; ion transport; mucus; cystic fibrosis transmembrane conductance regulator; chloride; bicarbonate; genetic disease

1 Introduction

1.1 Cystic fibrosis (CF)
CF is a fatal inherited disease that causes high morbidity and mortality as a result of defects in exocrine tissues.
that are 3-5 times higher than in unaffected subjects\cite{3,4}. Pre-natal destruction of the pancreas associated with meconium ileus occurs in about 10% of newborn patients\cite{5}, and progressive liver failure in a small percentage (~2%) of patients, can be life threatening. Nearly all exocrine tissues show some abnormality in electrolyte and fluid transport, including salivary glands\cite{6,7}, airway submucosal glands\cite{8}, intestine \cite{9-11}, gall bladder\cite{12}, hepatic biliary tract\cite{13,14}, and the uterine cervical tract\cite{15}. In males, the vas deferens is almost always incomplete\cite{16-18}. The abnormalities associated with these latter organs are not usually life threatening. In CF, the ducts and lumens of organs that produce secretions with high mucus or protein content tend to become blocked and damaged\cite{19-22}. Thus, most of the pathology associated with CF results from apparent mucus stasis, which has yet to be explained satisfactorily, but which gave rise to its other, possibly more appropriate and yet largely abandoned name: “mucoviscidosis” (state of thick mucus).

With improved therapies, recognition of variable forms of the disease, and later in life diagnosis, the estimated life expectancy for patients has increased from just a few years when it was first recognized as a disease entity some 60 years ago\cite{23,24} to an age of about 32 years presently in the USA\cite{25}. The improved survival has paralleled improved treatments of symptoms rather than correcting underlying causes.

### 1.2 Genetics of CF

CF is inherited as a Mendelian autosomal recessive gene. The gene was cloned in 1989\cite{26-28} and named cystic fibrosis transmembrane conductance regulator (CFTR). The incidence of the disease worldwide is not accurately known, but in North America, it appears in about 1/3 200 Caucasian births and in about 1/35 000 Asian American births\cite{29}. The carrier rate estimated from these figures should be about 3.5% for Caucasians and about 1% for Asians, but heterozygotes show no clinically relevant phenotypes\cite{25}. The first mutation detected and cloned was a deletion of a three nucleotide sequence coding for a phenylalanine at the 508 position (ΔF508) of the protein now well-known as CFTR. More than 1 400 mutations have now been reported to be associated with the disease\cite{30}. The ΔF508 mutation occurs in about two thirds of all mutant chromosomes in the USA population and more frequently in Northern Europe. Of the other mutations only a few occur with a frequency greater than 1%, including G542X (2.4%), G551D (1.6%), N1303K (1.3%), and W1282X (1.2%). Although there is little data on indigenous Asian populations, ΔF508 mutations among Asians seem very rare\cite{30}. In surveys in Japan\cite{31}, Korea\cite{32}, and Vietnam\cite{33}, no ΔF508 mutations were detected, but among Koreans screened on the basis of bronchiectasis or chronic pancreatitis, three mutations, Q1352H, E217G, and IVS8-T5 appearing with a M470V allele were associated with disease\cite{32}. Similar findings were reported for Chinese in Singapore\cite{34}. One ΔF508 allele was detected in a recent study of 50 normal Malays men\cite{35}.

### 2 CFTR — the molecule

#### 2.1 Structure of CFTR

The normal CFTR is a large, complex membrane spanning protein, whose best-known cellular function is conducting anions through the plasma membrane\cite{36,37}. The molecule consists of 1 480 amino acids forming two sets of six membrane-spanning regions (Fig.1). Between the two sets of membrane-spanning regions on the cytoplasmic surface, two nucleotide-binding domains (NBDs) flank a large cytoplasmic domain called the “regulatory (R)” domain\cite{27}. The ΔF508 mutation occurs in the first NBD, but many other mutations appear throughout the protein. Mutations in CFTR may produce one of several defects: (1) mRNA is unstable or incompletely transcribed so that no or too little protein is translated, (2) the protein cannot be synthesized and is degraded before being conveyed to the cell.
membrane, (3) the protein in the membrane cannot be activated, (4) it conducts anions inadequately, or (5) its turnover is increased and its presence in the membrane decreased[38,39]. Mutations that result in any of the first three defects produce no, or almost no functional protein, while mutations that result in either of the last two abnormalities produce partial function so that the former are associated with more severe disease while the latter with less severe disease[40-42].

2.2 Molecular function

Based on the high salt concentration in the sweat of CF patients, the first cellular defect demonstrated in CF was found in the sweat duct, which proved to be impermeable to Cl-. Consistently, the most documented function of the normal CFTR protein is that of an anion conducting channel. Patch-clamp studies have established that in isotonic Cl-, the single channel exhibits a conductance of about 7-11 pS and 2-3 pS in HCO₃⁻ with an anion selectivity pattern of Cl⁻ > I⁻ > Br⁻ > NO₃⁻ > HCO₃⁻ > gluconate[43-45]. CFTR is also reported to be involved in the function or regulation of a number of other transport components and mechanisms[46,47], which exceed the scope of this writing.

3 Control of CFTR

CFTR has become known and well established as a protein kinase A (PKA)-ATP-dependent Cl⁻ channel. However, it seems to be under the control of several other mediators as well.

3.1 Phosphorylation

The second cellular defect to be demonstrated in CF was also in the sweat gland, which failed to secrete in response to β-adrenergic agonist[48,49]. The failure to respond to β-adrenergic ligands was not due to defective signal transduction[50], but simply to the inability of, or lack of, the CFTR protein to respond to stimulus.

PKA: Anion conductance is activated when the CFTR protein is exposed to activated PKA or its catalytic subunits in the presence of normal cytosolic concentrations of ATP[51-54]. That is, a signal ligand acting through a G-protein in the plasma membrane[55], activates an adenylyl cyclase to elevate levels of cAMP in the cytoplasm or more probably in a microdomain associated with CFTR (Fig. 2)[56,57].

Several questions relating to the phosphorylation process are challenging. First, there were numerous serine and threonine sites in the CFTR protein that might be phosphorylated by PKA physiologically. At least nine consensus PKA substrate sites and two consensus protein kinase C (PKC) sites are within the R domain itself[51,58,60]. However, no single phosphorylation site (or group of sites) was specifically responsible for activation of the channel, and activation may depend more on a change in net charge whose negativity increases with phosphorylation, but not critically dependent on the charge at any single site implying that CFTR may be activated in proportion to the degree of its phosphorylation[58,61,62].

PKC: Two serine sites (S686 and S790) are phosphorylated by PKC, which by themselves lead to relatively little activation. However, PKC combined with PKA potentiates PKA activation and cells pretreated with inhibitors of PKC are less responsive to PKA activation[63-67]. PKA-activated CFTR may “run down”, but adding back PKC restores activity[68]. These observations suggest that a constitutive action of membrane bound PKC may be necessary to sustain PKA activation.

The role of PKC is even more intriguing in view of a recent report that amphibian CFTR is equally activated by either PKA or PKC. Moreover, it appeared that a single site (Thr665) was essential to PKC activation of frog CFTR.
Human CFTR lacks this site, but when the equivalent site was inserted in human CFTR, it was also activated by PKC alone. 

Protein kinase G (PKG): At least in intestinal tissues and sweat ducts, cGMP-dependent kinase (PKG) activates CFTR. In the intestine, only the membrane-associated PKG II kinase, not the soluble PKG I, isoform is effective. PKG II, which phosphorylates only 5 of the same sites as PKA, is about as effective as PKA.

3.2 Kinase inhibition

Apparently not all phosphorylated sites are excitatory. Phosphorylation of S737 or S768 in the R domain may inhibit channel activity. Substitution of alanine for phosphorylatable S737A or S768A enhanced the channel activity, suggesting that phosphorylation at either of these sites may inhibit phosphorylation of other stimulatory sites.

3.3 Dephosphorylation

If kinases are responsible for activating CFTR via phosphorylation, protein phosphatases (PP) must be responsible for deactivating CFTR. Unfortunately, there is no specific phosphatase that is clearly associated with deactivation of CFTR in all cells. PP2A and PP2C (but not PP1 or PP2B) were highly effective at deactivating excised membrane patches. Okadaic acid and F1 were equally effective in blocking PP in isolated human sweat ducts indicating that PP2A may be the endogenous deactivating enzyme in this native tissue, and more recently, was found to be sensitive to cytosolic [K+] for A TP. However, neither calyculin A, FK506 (a specific blocker of calcineurin, a Ca+/calmodulin protein phosphatase), nor okadaic acid had any significant effect in preventing deactivation of cell cultures of human airway cells or of immortal colonic T-84 cells, which suggested the active phosphatase was more likely to be PP2C in these cells. In this same vein, antibodies to PP2C were co-precipitated with CFTR and vice versa in BHK cells, but no association of CFTR with PP1, PP2A, or PP2B was found. The tyrosine specific λ-phosphatase blocked the fast gating that tyrosine kinase induced with little, if any, effect on PKA activation. These results suggest that at least in some cells, CFTR and PP2C may be closely associated in a microdomain complex, while in others, PP2A may be more important. Overall, these results imply that dephosphorylation-deactivation of CFTR is likely to be tissue specific.

3.4 Other forms of stimulation

The preponderance of data showing that cAMP and ATP can activate CFTR universally has created the dogma that CFTR is a cAMP-dependent Cl channel. However, there are several indications that CFTR may be activated possibly independent of phosphorylation.

Genistein: Genistein is an isoflavone, perhaps best known as a potent tyrosine kinase (p60c-src) inhibitor. Genistein cannot activate CFTR by itself, but may enhance partially activated CFTR (but not independent of ATP). However, its stimulatory action on CFTR seems at odds with this role since tyrosine kinase Src60 stimulated CFTR when applied to the cytosolic surface of the membrane in patch-clamp experiments. The question of whether genistein acts directly on CFTR or indirectly remains controversial.

Whatever its mode of action, it is intriguing in that, genistein appeared to also directly enhance the activity of ΔF508 CFTR and other mutations when expressed to the membrane of heterologous cells. A peculiar, and perhaps unfortunate, aspect of genistein activation is that it appears to be inhibitory at concentrations above which it is maximally stimulatory.

Glutamate: Despite earlier reports that glutamate was inhibitory to single channel activity, in the native human sweat duct, CFTR was activated by cytosolic glutamate and its precursors. If either cAMP or ATP is removed form the cytosol, endogenous phosphatases deactivated CFTR within minutes, but if α-ketoglutarate, glutamate, or glutamine (at putatively normal cytoplasmic concentrations) was then added to the cytosolic medium, CFTR was activated. This effect was probably not dependent on phosphorylation since it occurred in the presence of 1×10⁻⁵ mol/L staurosporine, a potent general kinase inhibitor.

Even more intriguing, CFTR demonstrated relative permeability to HCO₃⁻ (about 15%-20% of that of Cl⁻) when activated classically with cAMP/ATP, but remained impermeable to HCO₃⁻ when activated by glutamate alone (or precursor). However, if cytosolic ATP was added in the presence of glutamate activators, CFTR developed even greater HCO₃⁻ permeability.

Curcumin: Recent reports of significant enhancement of ΔF508 CFTR function in CF mice and in HeLa cells transiently expressing the mutant suggested that this common food additive might be of therapeutic benefit in CF. The effect has not been uniformly reproducible and remains controversial.

Even though it is consistently found that PKA phosphorylation activates CFTR Cl conductance, substantial questions remain as to receptor mediated differences in CFTR functions in the types of tissue affected in CF; i.e., pancreas, airways, intestine, sweat and salivary glands, vas deferens,
uterus and cervix, etc. It seems possible, if not likely, that receptors, pathways, and mechanisms are tissue-specific. For example, recent evidence in submucosal glands indicates that vasoactive intestinal peptide (VIP) may elicit HCO$_3^-$-rich secretions through CFTR\cite{97-100} and that VIPergic and cholinergic stimulation act highly synergistically at low concentrations\cite{101}.

4 Pathophysiology in target organs of CF

Perhaps the most intriguing, long standing, and demanding challenge in understanding CF has been that of understanding how a defect in ion transport translates into thick mucus or “mucoviscidosis” that disrupts nearly all affected organs. While virtually all exocrine glands are affected in CF, the three cardinal organs of greatest clinical importance are the sweat gland (diagnosis), the pancreas (malnutrition), and the lung (morbidity/mortality). In order to establish a basis for trying to understanding the disease, we consider the absorptive defect expressed in the sweat duct (too much salt), the secretory defect in the pancreas [too little soda (with some poetic license, we take “soda” to mean “bicarbonate of soda”; that is, sodium bicarbonate)], and question whether it is “too much” or “too little” in the lung.

4.1 “Too much salt”— the sweat duct

In CF, the sweat gland loses too much salt, but it functions normally in its primary purpose of thermoregulation and does not become morphologically altered. It produces almost no mucus\cite{102,103}.

Sweating has been most highly developed in man as an efficient means to dissipate heavy heat loads quickly with minimal depletion of the extracellular and circulating volumes. The gland is a very simple structure consisting only of a single coiled, unbranched tubule. The first half of the tubule, the secretory coil, secretes an isotonic fluid, and the second half, the reabsorptive duct, hypertonically absorbs salt from the secreted fluid (Fig. 3)\cite{104,105}.

Secretion: In essence, when sweating is required for cooling, sympathetic nerves release acetylcholine in neuroglandular synapses in the gland where stimulation is mediated by a rise in intracellular Ca$^{2+}$ thought to activate Ca$^{2+}$-activated Cl$^-$ channels (CAC) in the apical pole of the secretory cell\cite{106,107}. Since the electrical potential across the apical membrane of the secretory cell is significantly more negative in the cell than in the lumen, the electrochemical potential acting on negatively charged Cl ions drives them from the cell into the lumen when apical Cl$^-$ channels open. The negative charge carried by Cl$^-$ into the lumen creates a favorable electrical gradient for paracellular Na$^+$ movement through the tight junction into the lumen thereby maintaining electroneutrality (electroneutrality requires that each charge transported be matched with the transport of a charge of opposite electrical sign; i.e., transported cationic charges must equal anionic charges transported in the same direction). The accumulation of NaCl in the lumen in isotonic proportions\cite{108} and the increased fluid volume in the lumen forces the secreted fluid through the remaining half of the tubule and onto the surface of the skin for evaporation. This Ca$^{2+}$-mediated mechanism of sweat secretion is not affected in CF\cite{109,110}. Consequently, CF patients sweat normal volumes of fluid during heat stress and are not anhydrotic.

On the other hand, in normal subjects a weak sweating response is induced by $\beta$-adrenergic, cAMP-dependent stimulation. The function of this mode of sweating is unknown, but it clearly fails in CF patients without any evident pathological consequence\cite{48}. The mechanism of $\beta$-adrenergically mediated sweating is thought to be similar to the Ca$^{2+}$-mediated mechanism of secretion mentioned above, except that it depends on a membrane-receptor
coupled trimeric G-protein that activates adenylyl kinase to elevate intracellular cAMP. Stimulated PKA then phosphorylates and specifically activates apical CFTR Cl⁻ channels, apparently independent of cholinergically mediated CAC secretion. The faulty response is not due to defects in adrenergic stimulus, but to the absence or malfunction of its target, the apical CFTR Cl⁻ channel needed to form secretion.

Absorption: Remarkably, this same CFTR anion channel normally is expressed abundantly in the luminal membrane of the second half of the gland tubule, the absorptive duct, but here it moves salt in the opposite direction, absorption [111,112]. Thus, CFTR provides for passive conductance of Cl⁻ ions during reabsorption from the lumen back into the extracellular fluid across the cell. During absorption, Na⁺ sets up the electrical driving force for the movement of Cl⁻. That is, Na⁺ passively enters the duct cell from the lumen down its electrochemical gradient through the electroconductive epithelial Na⁺ channel (ENaC) of the apical membrane [55]. The lumen to cell Na⁺ gradient is maintained by the active transport of Na⁺ out of the cell via the Na⁺,K⁺-ATPase located in the basolateral membrane [113]. Simultaneously, the transport of positive charge through the cell creates sufficient electrochemical gradients for transcellular electroconductive transport of Cl⁻ from lumen through CFTR in the apical membrane and then through CFTR in the basal membrane to the serosa. Since the sweat duct is one of the few epithelia of the body that is relatively water-impermeable, as Na⁺ and Cl⁻ leave the duct, water cannot follow, and a steep osmotic gradient develops across the duct that parallels the absorption of salt. The physiological reward is that more easy to replenish water is expended for cooling while more difficult to replenish salt is conserved (for primates, water is much more plentiful in nature than salt). Normally, sweat usually contains much less than 40 mmol/L NaCl and may be reduced significantly further under volume depletion stress to conserve salt as aldosterone rises [114,115].

Unlike secretion, absorption does not seem to be under neural control. The duct seems primed to function when the secretory load arrives. Indeed, CFTR in the duct is under the control of endogenous PKA and phosphatases, but they seem to function more in a homeostatic role for the cell during salt transport such that CFTR is modulated, but is more or less constitutively activated [76,116].

In CF the reabsorptive mechanism fails due to the lack of functioning CFTR [56,117]. NaCl in CF sweat is at least 60 mmol/L, and usually over 100 mmol/L. When the CFTR anion channel is absent or inactive, Cl⁻ cannot follow Na⁺ out of the lumen, and both Na⁺ and Cl⁻ absorption are impeded. This effect is easily understood in terms of electroneutrality, which always requires moving equal numbers of negative and positive charges when transporting salts from one compartment to another. If Cl⁻ cannot be removed from the lumen, an equivalent of Na⁺ must remain with it. Thus, in CF patients, neither Cl⁻ nor Na⁺ can be efficiently reabsorbed from the duct, and salty sweat appears on the skin surface even though the components for Na⁺ transport per se are normal. CF patients are at much greater risks for heat prostration simply because under thermal stress the excess loss of salt in the CF sweat depletes the circulatory volume much more rapidly than normal dilute sweat. In fact, the recurrence of heat prostration is a presenting complaint of many older, previously undiagnosed patients [118].

4.2 “Too little soda” — the exocrine pancreas

In CF, the exocrine pancreas produces too little soda (HCO₃⁻) and clearly illustrates that HCO₃⁻ transport also fails in this disease. CF was first recognized by its striking impact on nutrition and, in fact, was confused initially with celiac disease [23,119]. Early gross examinations of the pancreas showed extensive, progressive fibrosis of the organ with parenchyma replaced by fatty infiltrates [20,28,122]. While the nutritional status of patients is greatly improved and is generally managed well with supplemental animal pancreatic enzymes [122] (the endocrine pancreas is usually involved until the exocrine pancreas is virtually completely destroyed so that insulin-dependent diabetes in CF is increased, but not in parallel to digestive pancreatic insufficiency), more generalized defects throughout the intestinal tract also contribute to the problems of malabsorption [123,124].

But why does the pancreas fail so disastrously in most, but not all, patients with CF?

The normal pancreas secretes relatively concentrated quiescent enzymes into the lumen of the pancreatic ducts where they are diluted and kept inactive by secretion of a nearly isotonic HCO₃⁻-rich fluid [125,126]. In CF, the enzymes secreted by the acinar regions of the pancreas in the absence of ductal fluid secretion are poorly diluted, tend to stagnate in the ducts, and probably activate prematurely (Fig.4). In both normal and CF patients enzymes and fluids are secreted from acinar secreting cells, and in the normal pancreas a bicarbonate-rich fluid is added to the ducts to carry away and quiesce the pro-enzymes. In CF, however, HCO₃⁻ secretion is inadequate and macromolecules and enzymes tend to aggregate and block the small ducts so premature proteolysis and inflammation destroys individual
units until the exocrine pancreas become inadequate for normal digestion\textsuperscript{[127,128]}. That is, proteolytic enzymes such as trypsinogen are normally kept inactive or dormant by the high pH of the HCO\textsubscript{3}\textsuperscript{-}-rich juice and only become fully proteolytic after reaching the small intestine where the HCO\textsubscript{3}\textsuperscript{-} is neutralized by gastric acid secretions. The CF pancreatic duct lacks the protection of normal secretory fluid, high in [HCO\textsubscript{3}\textsuperscript{-}], and at alkaline pH, so that pro-enzymes stagnate, become active and destroy the pancreas with autolysis at the foci of stasis.

About 10\% of CF patients lose the exocrine pancreas \textit{in utero} and therefore are born, not only pancreatic insufficient, but also with acute life threatening meconium ileus, a paralyzing blockage of the intestine due to stagnated chyme. In most patients, the pancreas is lost over time, often years, in what appears to be one or a few ducts or lobules at a time. The question of why some ducts fail early and other later is fundamental, but unexplained. The same question can be asked of units of the lung and bile ducts of the liver.

Not all CF patients develop pancreatic insufficiency (PI), even when they survive for decades. Strong correlations between genotype and pancreatic sufficiency (PS)\textsuperscript{[41]} show that mutations in the gene (types 1-3 above), which yield no CFTR protein or an essentially non-functioning protein in the plasma membrane are almost always associated with eventual PI while mutations (types 4-5) that permit some expression of CFTR, even though function is compromised, are associated with PS. Mutations of the latter type have been observed in a limited Asian population\textsuperscript{[32]}. Although there is some evidence that CFTR is expressed in pancreatic acini\textsuperscript{[29,30]}, most work has focused on the ductal system for HCO\textsubscript{3}\textsuperscript{-} secretion where CFTR expression is very high in the apical membrane of the small ducts\textsuperscript{[131]}.

On a mechanistic level, the molecular functions required to excrete high concentrations of HCO\textsubscript{3}\textsuperscript{-} from the pancreas remains the subject of continued investigation\textsuperscript{[32]}. Recently, an electrogenic Na\textsuperscript{+}/nHCO\textsubscript{3}\textsuperscript{-} cotransporter (pancreatic NBC-1) has been cloned from pancreatic duct cells\textsuperscript{[133]} and reported to be responsible for HCO\textsubscript{3}\textsuperscript{-} uptake across the basilateral membrane into the cell\textsuperscript{[134-136]}. This mechanism is attractive from the point of view that it transports the HCO\textsubscript{3}\textsuperscript{-} species directly into the cell and does not require removal of H\textsuperscript{+} ions inherent to CO\textsubscript{2}/H\textsubscript{2}O/carboxylic anhydrase systems.

The problem of pancreatic HCO\textsubscript{3}\textsuperscript{-} secretion dictated by CF then becomes one of how to move HCO\textsubscript{3}\textsuperscript{-} out of the cell against a steep gradient across the apical membrane while coupling its transport dependently to the function of CFTR. Understanding this function seems paramount for understanding CF molecular pathophysiology. Initial suggestions held that HCO\textsubscript{3}\textsuperscript{-} was secreted via a chloride-bicarbonate (Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-}) exchanger. CFTR operating in parallel with the exchanger allowed apical recycling of Cl\textsuperscript{-} to balance the Cl\textsuperscript{-} influx of the Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger\textsuperscript{[137-139]}. A CFTR-dependent Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger was found in the apical membrane of native mouse pancreatic ducts, which was not present in ΔF508/ΔF508 mouse pancreatic ducts\textsuperscript{[140]}. The activity appeared to be stimulated by cAMP as well as by intracellular Ca\textsuperscript{2+}\textsuperscript{[141]}. The activity was CFTR mutation-specific and, for example, mutations such as R117H (the pancreas is spared in compound CF heterozygotes such as R117H/ΔF508) appeared to retain the ability to support exchange even though Cl\textsuperscript{-} conductance was depressed while mutations such as H620Q did not support exchange, but retained a disputed\textsuperscript{[240]} Cl\textsuperscript{-} conductance. Other mutations killed both Cl\textsuperscript{-} and HCO\textsubscript{3}\textsuperscript{-} transport. These properties showed some correlation with PS and PI phenotypes of patients with the same genotypes\textsuperscript{[142]}, suggesting that CFTR might play a role, as a Cl\textsuperscript{-} channel in Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange\textsuperscript{[143]}.

More recently the paradigm of a luminal electroneutral exchanger has been shaken. Two electrogenic exchangers, DRA (down regulated in adenoma or SCL26A3) and PAT1 (putative anion transporter or SLC26A6), are present in the pancreatic duct and dependent upon molecular interactions with CFTR via the STAS domain of the SLC26A exchangers in a scaffolding complex with EBP50. All of...
these molecules have C-terminal PDZ domains that combined with EBP50. Phosphorylation of CFTR with PKA significantly enhanced the exchanger activity\(^\text{[144,145]}\). Importantly, this mechanism depends on an apical membrane that is virtually non-conductive to HCO\(_3^-\); and probably conductive to Cl\(^-\). Recently, investigations of mice null for scl26a6 have suggested that scl6a3 may compensate for null scl26a6\(^\text{[146]}\) and that scl26a6 may exert limiting control on CFTR\(^\text{[147]}\).

Since HCO\(_3^-\) is only about 1/10 to 1/5\(^\text{[45,148]}\) as conductive as Cl\(^-\) through CFTR, intuition suggests that CFTR might not be suitable for this system. However, two recent observations suggest that CFTR may have novel properties not yet well appreciated in ion channel functions. That is, two studies have reported that, even while remaining highly conductive to Cl\(^-\), CFTR may be either relatively impermeable or relatively conductive to HCO\(_3^-\), depending on the mode of stimulation\(^\text{[94,149]}\). Further, oocytes transfected with CFTR appeared impermeable to HCO\(_3^-\) in the presence of high external Cl\(^-\), but became permeable to HCO\(_3^-\) when Cl\(^-\) was removed\(^\text{[150]}\). These recent findings make it easier to suggest not only that CFTR might be impermeable to HCO\(_3^-\) in the pancreatic duct, but that it may well alter its permeability properties according to physiological demands of transmembrane Cl\(^-\) and HCO\(_3^-\) transport.

This model however, does not easily explain why bicarbonate secretion seems to be chloride-independent or how duct cells can secrete 140 mmol/L HCO\(_3^-\) in pancreatic juice\(^\text{[124,153,155]}\). An attractive possibility might be that HCO\(_3^-\) moves conductively through CFTR\(^\text{[152,153]}\). This system would require that the apical membrane remain significantly hyperpolarized (ca. -60 mV or more negative) and that the intracellular HCO\(_3^-\) remain relatively high (possibly 15-20 mmol/L). These conditions may be obtained physiologically\(^\text{[154]}\).

Whether CFTR conducts HCO\(_3^-\) directly or critically supports HCO\(_3^-\) exchange, pancreatic failure in CF patients with severe mutations clearly demonstrate that loss of CFTR function depresses adequate HCO\(_3^-\) transport and this defect of “too little soda” seems to be present in most if not all other affected tissues.

### 4.3 “Too little, or too much”— respiratory airways

As pointed out, unlike the sweat duct and the pancreatic duct, the consequences of the defect in CFTR in the pulmonary airways are eventually fatal even with medical treatment. The inability to avoid the chronic infections and concomitant inflammation leads to progressive destruction of the small airways\(^\text{[122,155]}\), respiratory failure, cor pulmonale, heart failure, and death\(^\text{[1]}\). Despite more than twenty years of work on Cl\(^-\) impermeability and CFTR, there is no certainty as to what pathophysiological expression sets up refractory airway infections. In contrast to the sweat duct, where CF sweat is without question high in salt, and in the CF pancreas where the pancreatic juice is abnormally low in bicarbonate, we do not know with certainty what parameter(s) are critically disturbed in the airways. Since CFTR Cl\(^-\) conductance is crucial both to secretion and absorption and since the airways are thought to both secrete and absorb although via poorly understood mechanisms, it has been difficult to link a specific defect in CFTR function to respiratory disease and at best we can only speculate presently.

Some investigators however, have bypassed concern for the ion channel dysfunction and suggested the airway destruction is inherently due to hyper inflammatory responses in CF related to mutant CFTR protein and argue that inflammation precedes infection. In infants, pulmonary cytokines and inflammatory markers appeared elevated even before infection was detected in bronchial lavage\(^\text{[156-158]}\), but others have not seen such increases before infection\(^\text{[159,160]}\). Numerous observations of increased production of cytokines and related inflammatory factors have been made in culture cells that exceed the scope of this writing\(^\text{[161-168]}\), but it is at times difficult to be certain that compared cells are uniform\(^\text{[165]}\) and not all investigators have seen such inherent differences\(^\text{[166-169]}\). Intriguingly, however, growing primary cultures of normal cells with a CFTR channel blocker, CFTR-inh172, increased cytokine release, which did not occur in CF cells\(^\text{[170]}\). Somewhat similarly, even though CF cells responded more than normal airway cells, both increased IL-8 release in response to hyperosmotic NaCl\(^\text{[171]}\). Recently, perhaps the first report to link the CF impermeability defect directly to a defective immune response showed that CF macrophages fail to kill engulfed bacteria efficiently because without CFTR, they cannot properly acidify phagosomes and lysosomes\(^\text{[170]}\).

Clearly the ultimate destruction of the CF lung is mainly due to inflammation\(^\text{[172]}\) (there are apparently no abnormalities in the immune system of CF patients beyond the respiratory tract), but since CFTR is a chloride channel and disturbances in other tissues appear as an electrolyte transport disorders, most investigator have assumed, a priori, that CF airway pathology is caused primarily by a defect in electrolyte transport and probably more specifically by a defect in Cl\(^-\) transport. Overall, the most longstanding concept of pathogenesis is that the CF airway succumbs to infection due to compromised mucociliary clearance\(^\text{[173]}\).
The sputum from CF patients is thick and viscid and early pathologists held the impression that ducts of affected organs appeared plugged with “thick, inspissated” plugs and concretions of mucus. The question in understanding CF pathology may then reduce to understanding the impact of abnormal ion transport on mucus properties and macromolecule clearance.

Volume defect: Probably the most widely accepted idea for impaired airway clearance is that of hyper fluid absorption in the airways, which is thought to leave the molecular content of airway surface fluid (ASF) concentrated, “thick”, and viscous. Diminished fluid volumes presumably prevent the mucociliary escalator from removing inhaled and inherent debris rapidly enough to evade infection and inflammatory response. To absorb fluid from the CF airway, Na⁺ is absorbed through the ENaC, as in the sweat duct. However, exaggerated Na⁺ and fluid absorption putatively occurs in CF airways because the normal CFTR function inhibits the ENaC activity of these cells, but when CFTR is absent or defective, ENaC becomes unrestrained and hyperactive. One might think that without CFTR Cl⁻ channels, absorption should be impeded as it is in the CF sweat duct; however, whereas in the sweat duct Cl⁻ is absorbed transcellularly via CFTR in the plasma membrane, the airway is assumed to be different. That is, Cl⁻ is assumed to be absorbed paracellularly independent of CFTR. Since cAMP-dependent fluid secretion depends on CFTR function, its loss must have dual impacts: (1) increased absorption, and (2) decreased secretion, with the net result of more desiccated airway surfaces. This hypothesis received surprising support from transgenic mice in which the β subunit of ENaC was overexpressed to produce hyperactive Na⁺ channels. These ENaC-altered mice developed a CF-like chronic lung infection, even though CF mice with no CFTR develop little, if any, lung infection. Presumably, the null CF mice compensate for the lack of CFTR with a Ca²⁺-mediated Cl⁻ channel. Still, it is curious that if both CF and ENaC mice hyper-absorb, only the ENaC-defective mouse lungs become seriously infected, since they too should have active CAC to compensate.

Concentration defect: On the other hand, another hypothesis postulates that like the sweat glands, NaCl absorption in the lung is defective, leaving the ASF-like sweat abnormally concentrated with salt. Due to the very small volume of ASF and its inaccessibility, its exact composition remains controversial. In the face of many methodological questions, perhaps the most intriguing observation was that ASL from the primary cultures of CF airway epithelial cells in contrast to the normal cultures failed to kill inoculated bacteria. However, killing was restored by diluting and lost again by concentrating salt in the surface fluid of primary airway cell cultures. These data suggest that high concentrations of salt incapacitate bacterial killing properties of the ASF. Since there is ample evidence of bactericidal agents in the ASF that are salt-sensitive, these data imply that the normal ASF should be hypotonic and that the defect in the CF lung is “too much salt”. More recently, there is a suggestion that increased salt in itself may upregulate the expression of cytokines associated with hyper-inflammatory responses proposed in some CF cells.

Aside from the difficulties in assaying for the composition of ASF, another important difficulty resides in what we might relate to the “Heisenberg principal of uncertainty” for airway surface fluids. That is, it seems impossible to study the system without in some way disturbing it, so that there is always uncertainty as to what the normal native physiological composition of this thin layer of fluid lining the airways actually is in vivo. The airways, in order to maintain hygiene, are designed to respond to mechanical or chemical irritation with fluid secretion to facilitate clearing the contamination from the airway on the mucociliary escalator. Virtually all secreted fluids in mammals originate isotonically. Therefore, any attempt to collect surface fluid from the airway is almost certain to disturb and stimulate the surface epithelia to secrete relatively large volumes of isotonic fluid, which seems certain to obscure the actual composition of the very thin layer of fluid that normally resides on the surface of unstimulated airway epithelia.

HCO₃⁻ defect: Still, there may be another dysfunction in the airways. Reflecting on the role of HCO₃⁻ and the effect of its loss in the CF pancreas as well as in other organs, failure to secrete HCO₃⁻ may be critical in the airway as well. The physiological effects of HCO₃⁻ are therefore not merely a property of its chemistry, but also, of the H⁺ ion concentration determined by the HCO₃⁻/CO₂ buffer system. It is not hard to imagine that with respect to lung defense and inflammation, HCO₃⁻ could play at least three roles in different scenarios. HCO₃⁻ may affect: (1) viscoelastic properties of airway mucus, (2) neutrophil responses and killing capacity, and (3) bacterial colonization and viability.

Mucus secretion is generally closely associated with, possibly even tied to, HCO₃⁻ secretion. There is little in vitro data to show that HCO₃⁻ affects mucus viscosity, but compared to water, 2% NaHCO₃ reduced viscosity of...
pooled nasal discharge by about 40%. Inhaled aerosolized HCO₃⁻ is more effective than saline in reducing mucus viscosity and improving mucociliary clearance[205-208]. Likewise, pH clearly affects mucus properties. The viscosity of respiratory mucus (sputum) increased sharply with changes in pH on either side of about pH 7.4[209,210]. pH also affected conformational changes in mucus that are inherent in gel-sol transformations, which dramatically change viscoelasticity[211,212]. Closely associated with mucous properties is the ciliary activity, which in human bronchial explants appeared to function optimally between pH 7-9, but rapidly lost activity outside this range[213].

If it is possible to place these roles of HCO₃⁻ into play in the airway, it seems likely that a CF lung without control of HCO₃⁻ may be at a disadvantage from two overall abnormalities. First, in the “resting” state before a contaminating event, the lack of HCO₃⁻ secretion and the resulting unneutralized H⁺ secretion may leave the airway ASF chronically acidic[214]. Second, the CF lung is almost certain to be at a disadvantage when dynamic responses are required to suppress a contaminating event; that is, normally when debris appears in the airway upon contact with the surface, mucociliary clearance mechanisms at the location of the debris mobilize[215]. Mucin release and ciliary activity should be stimulated. HCO₃⁻-rich fluid secretion should be stimulated to out pace fluid absorption sufficiently to provide a fluid volume adequate to mobilize the contaminating debris without flooding the airway. As the ciliated escalator transports the debris orally, fluid and mucus secretion must decrease so that net reabsorption returns the volume of ASF to a nominal thickness of 3-10 μm[177,216,217]. In CF, if HCO₃⁻ is not secreted[218], the volume of fluid for displacing the debris may be compromised[203,204,219] and mucus may not be released in its normal form and be more difficult to mobilize, and clearance may slow or stagnant[193].

Recent evidence[220] indicated that mucus release from the small intestine is significantly decreased in the absence of HCO₃⁻ in both wild-type and CFTR-null mice. The results are consistent with the idea that extracellular expansion (decondensation) of mucus from exocytotic granules requires HCO₃⁻/CO₃²⁻ to complex Ca²⁺ and H⁺ from the polyanionic sites of condensed mucus. In the condensed granules these cations shield the highly negative electrostatic charges on mucin fix anions and prevent them from repelling each other and expanding the mucus. Complexing these cations with HCO₃⁻/CO₃²⁻ allows the poly anionic negative charges to rapidly expand the mucin molecule. Efficient, rapid removal of the shielding cations from condensed mucus seems critical to forming the “tangled string” network for normal mucus on epithelial surfaces. Without HCO₃⁻, to the extent that Ca²⁺ and H⁺ continue to shield the polyanionic sites, mucus tends to remain aggregated, which may contribute to the “thick” mucus in CF.

4.4 “Less may be better”

Selective advantage of CF: Where did CF come from? It is nothing less than astonishing that to date more than 1 400 different mutations have been reported to be associated with CF[221]. By far, however, the most common of all these mutation is the ΔF508. Nearly all of each of the other mutations account for much less than 1% of the genes in these populations. The fact that this disease results in impermeability to Cl⁻ ions suggested that partial expression imparted a significant selective advantage against some disease of electrolyte transport[222,223]. Clearly, one of the greatest, oldest fluid transport scourges among humans is secretory diarrhea. Almost five million children under 5 years of age succumb to enterotoxic intestinal diarrheas each year from an incidence of over a billion cases per year[224,225]. Heterozygote ancestors who carried only half the functioning genes have suppressed secretory responses to intestinal infections and less diarrheal fluid loss. The smaller loss would therefore have better enabled them to minimize the attending acute dehydration that causes circulatory collapse and death[226]. There is some proof of concept in animal studies of CFTR-null mice, that when infected with cholera toxin[227] or guanylin[228], responded with smaller volumes of intestinal secretion; in fact, heterozygote-null CF mice secreted only about half the volume as wild-type mice; the same was true for salivary secretions[229]. Further, CF mice intestines were not sensitive to the heat-stable toxin of Escherichia coli (Sta)[230]. But other studies in ΔF508 mice[230] and in human heterozygotes acutely stimulated with PGE₂ instead of cholera toxin using acute PGE₂ stimulation instead of cholera toxin[231] did not reveal diminished responses compared to normal.

These ideas and findings raise at least two puzzling questions: (1) why is the ΔF508 so imminent? and (2) why is it limited almost exclusively to Caucasians? Analysis of the frequency and origin of nearly 300 mutations in Europe showed that the ΔF508 was most common in Denmark (87%) and least common in Algeria (26%) of all chromosomes considered[232]. The data suggest a possible founder effect occurring in Northern Europe (Denmark) for this mutation within the last several thousand years, which may explain why it became prevalent in Europe and not in other parts of the world. However, since enterotoxic
diseases mainly spread as a function of contagion via water supplies, the prevalence of ΔF508 (and even its founding) may have been influenced by different types of developing communal living. One factor that should not be neglected is climate. In tropical regions where sweating provides a premium selective advantage for rapidly dissipation of heat loads, mutations in CFTR may have provided a negative pressure that outweighs the advantage of better resistance to enterotoxins because salt is a vitamin that evolutionarily requires intensive conservation. An increased loss of salt in the sweat may have placed hunter/predator heterozygotes at a significant disadvantage in eliminating heat loads without compromising extracellular and crucial circulating volumes. In northern climates, colder temperatures would reduce the pressure of sweat salt loss, allowing resistance to secretory fluid loss to perpetuate the mutation.

5 Other strange things

In closing, we have only considered three organs involved in CF, the sweat gland, the pancreas, and the lung. It should be well appreciated that the pathophysiology is not limited to these organs alone, and while all clinical aspects of the disease originate in exocrine epithelia, other tissues may be, and probably are, sub-clinically affected. Fertility is severely affected. In males, sterility arises in more than 95% of patients due to malformation or destruction of the vas deferens, and there is a high frequency of CF mutations in congenital bilateral absence of the vas deferens (CBVD) even in the absence of disease.[231]. Coincidentally, “A” cells that secrete acid and “B” which secrete HCO₃⁻ characterize this epithelium.[232]. Though not sterile, fertility among female patients is significantly reduced.[235]. The defect likely results from an inability to secrete HCO₃⁻ properly in the cervix to thin mucus and in the uterus or oviduct to affect sperm capacitation, a HCO₃⁻-dependent process by which sperm acquire their fertilizing capacity.[236]. The minor labial and submaxillary salivary glands show alterations in the electrolyte concentration of their product and mucus plugging pathology.[227]. The disturbance in the biliary tree and gall bladder, although poorly investigated, is almost certainly rooted in mismanaged HCO₃⁻.[238,239].

Lastly and regrettably, we cannot say with firm conviction whether CF mortality arises from too much salt, too little soda, both, or neither, so that CF remains an intriguing, perplexing disease in need of a cure and far more understanding.

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