Acta Physiologica Sinica, August 25, 2007, 59 (4): 397-415
http://www.actaps.com.cn

Review

Too much salt, too little soda: cystic fibrosis

QUINTON Paul M.*
Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093-0831, USA

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

囊性纤维化：太多 NaCl，太少 HCO_3^-

QUINTON Paul M.*
加利福尼亚大学圣地亚哥分校儿科，拉乔拉，加利福尼亚 92093-0831，美国

摘 要：胰腺囊性纤维化(cystic fibrosis, CF)是一种单基因缺陷导致的致死性遗传疾病，在高加索人种中广泛分布。这种疾病在其它人种的发生率非常低，但据报道大部分人种中发现有该基因的突变。本文对CF发生的分子和病理生理学基本概念进行阐述。首先，阐述了CF的病理学和遗传特征，其基因产物囊性纤维化跨膜电导调节体(cystic fibrosis transmembrane conductance regulator, CFTR)的分子结构、特征、功能和调控。其次，由于突变的主要表现是电解质转运失调，其病理学效应和机制在两个典型受累器官中得到了很好的阐明，一个是汗腺，其病理发生是由于分泌过多NaCl，另一个是胰腺，其病理发生是由于分泌太少HCO_3^-。然而，CF的发病率和死亡率主要来自难治性呼吸道感染，其发生机制存在争议，我们推断可能的机制为阴离子转运失调导致CF肺部慢性感染。

关键词：汗腺；胰腺；呼吸道；离子转运；黏液；囊性纤维化跨膜电导调节体；Cl^-；HCO_3^-；遗传疾病
中图分类号：R657.5；R758.74；

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. Almost all patients affected by the disease now die from respiratory failure due to chronic, prolonged, and refractory infections of the airways[1]. About 90% of all CF patients suffer from pancreatic insufficiency[2], and virtually all patients exhibit salt concentrations in their sweat

Received 2007-05-02 Accepted 2007-07-05
This work was supported by grants from the USPHS-NIH (No. R01 HL084942, R01 DE14352, R01 DK51899).
*Corresponding author. Tel: +1-619-5432884; Fax: +1-619-5435681; E-mail: pquinton@ucsd.edu

that are 3-5 times higher that in unaffected subjects[3,4]. Pre-natal destruction of the pancreas associated with meconium ileus occurs in about 10% of newborn patients[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[6,7], airway submucosal glands[8], intestine[9-11], gall bladder[12], hepatic biliary tract[13,14], and the uterine cervical tract[15]. In males, the vas deferens is almost always incomplete[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[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[23,24] to an age of about 32 years presently in the USA[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[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[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[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[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[30]. In surveys in Japan[31], Korea[32], and Vietnam[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[32]. Similar findings were reported for Chinese in Singapore[34]. One ΔF508 allele was detected in a recent study of 50 normal Malays men[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[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[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

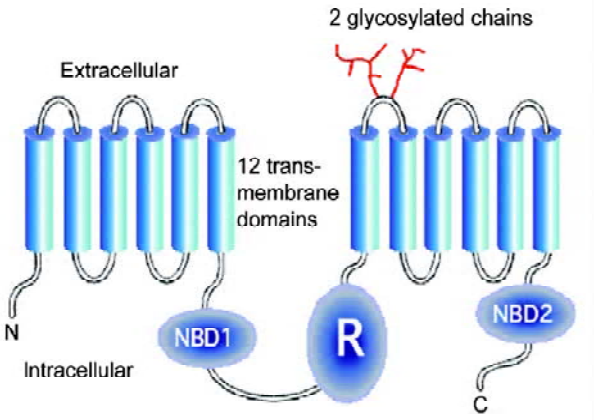

Fig. 1. Component model of the CFTR Cl^- channel. The channel is anchored through the membrane with 12 linked membrane spanning domains interrupted between the sixth and seventh domains by an intracellular nucleotide binding domain (NBD1) and a putative "regulatory (R)" domain. A second intracellular nucleotide-binding domain (NBD2) occurs near the C terminus. During processing the protein, two glycosylated side chains are added to the mature protein to the extracellular loop between transmembrane domains seven and eight.

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 HCO_3^- with an anion selectivity pattern of $Cl^- > I^- > Br^- > NO_3^- > HCO_3^- >$ 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

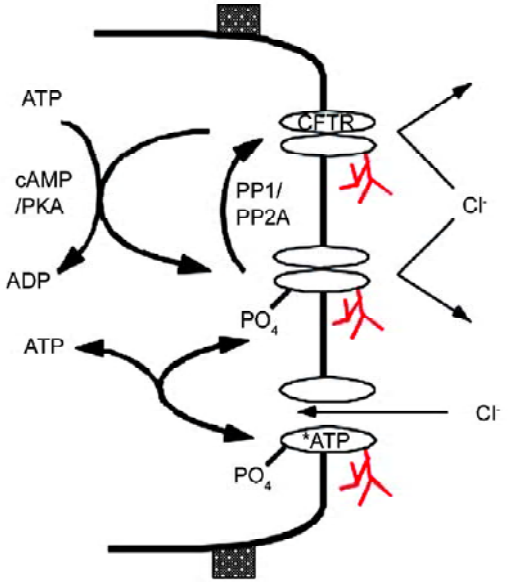

Fig. 2. Model of principal mode of activating CFTR in cell membrane. In every system known, the Cl^- channel can be activated to conduct chloride by protein kinase A (PKA) phosphorylation if ATP is also present for binding (lower channel). The channel deactivates if ATP is removed (middle channel) or if phosphatases (probably PP1 or PP2A) dephosphorylate it (upper channel). Generally, a β-adrenergic receptor coupled to a G-protein stimulates adenylyl cyclase (not shown) to elevate cAMP. Nonetheless, other mechanisms of activation are present at least in some systems (see text).

(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[63]. 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[68].

Protein kinase G (PKG): At least in intestinal tissues[69] and sweat ducts[70], 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[69,71].

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[72].

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[73,74]. Okadiac acid and Fl^- were equally effective in blocking PP in isolated human sweat ducts[75] indicating that PP2A may be the endogenous deactivating enzyme in this native tissue, and more recently, was found to be sensitive to cytosolic $[K^+]$[76]. However, neither calyculin A, FK506 (a specific blocker of calcineurin, a Ca^{2+}/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[73]. 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[77]. The tyrosine specific λ-phosphatase blocked the fast gating that tyrosine kinase induced with little, if any, effect on PKA activation[78]. These results suggest that at least in some cells, CFTR and PP2C may be closely associated in a microdomain complex[60], 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[78]. The question of whether genistein acts directly on CFTR or indirectly remains controversial[79-83]. 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[83-85]. A peculiar, and perhaps unfortunate, aspect of genistein activation is that it appears to be inhibitory at concentrations above which it is maximally stimulatory[82,86,87].

Glutamate: Despite earlier reports that glutamate was inhibitory to single channel activity[88], 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[89,90], endogenous phosphatases deactivated CFTR within minutes[52], but if α-ketoglutarate, glutamate, or glutamine (at putatively normal cytoplasmic concentrations[91,92] 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[93,94].

Even more intriguing, CFTR demonstrated relative permeability to HCO_3^- (about 15%-20% of that of Cl^-) when activated classically with cAMP/ATP, but remained impermeable to HCO_3^- when activated by glutamate alone (or precursor). However, if cytosolic ATP was added in the presence of glutamate activators, CFTR developed even greater HCO_3^- permeability[94].

Curcumin: Recent reports of significant enhancement of ΔF508 CFTR function in CF mice[95] and in HeLa cells transiently expressing the mutant[85] suggested that this common food additive might be of therapeutic benefit in CF. The effect has not been uniformly reproducible and remains controversial[96].

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 indicate that vasoactive intestinal peptide (VIP) may elicit HCO_3^--rich secretions through CFTR[97-100] and that VIPergic and cholinergic stimulation act highly synergistically at low concentrations[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[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)[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[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^- anions 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

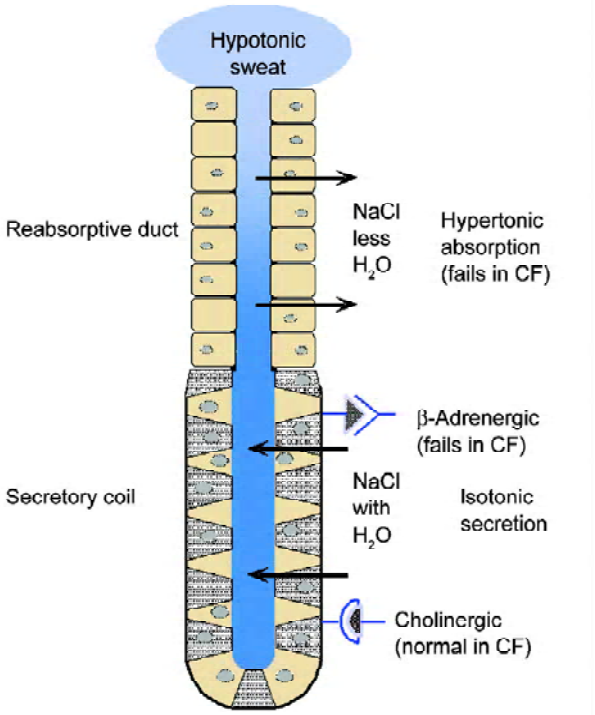

Fig. 3. Simplified drawing of the normal function of a single eccrine sweat gland. During thermoregulatory sweating, cholinergically mediated secretion functions equally well in CF and normal glands. However, due to the lack of CFTR Cl^- channels β-adrenergically stimulated secretion fails in CF as well as hypertonic absorption of NaCl in excess of water. Consequently, sweat emerging from the CF gland contains 3-5 times more NaCl than normal sweat.

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[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[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 β-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[48]. The mechanism of β-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[36,117]. 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 (HCO_3^-) and clearly illustrates that HCO_3^- 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[20,120,121]. 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 not 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 HCO_3^--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, HCO_3^- secretion is inadequate and macromolecules and enzymes tend to aggregate and block the small ducts so premature proteolysis and inflammation destroys individual

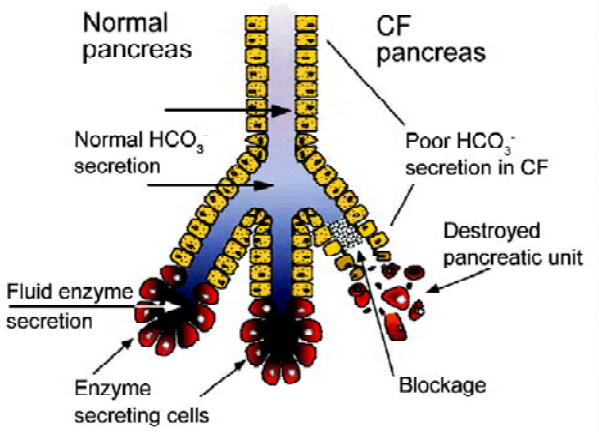

Fig. 4. Illustration of basic functions and abnormalities in the normal (left) and CF (right) pancreas. In both normal and CF patients enzymes and fluids are secreted from acinar secreting cells, and in the normal pancreas bicarbonate-rich fluid is added to the ducts to carry away and quiesce the pro-enzymes. In CF, however, HCO_3^- 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.

units until the exocrine pancreas become inadequate for normal digestion[127,128]. That is, proteolytic enzymes such as trypsinogen are normally kept inactive or dormant by the high pH of the HCO_3^--rich juice and only become fully proteolytic after reaching the small intestine where the HCO_3^- is neutralized by gastric acid secretions. The CF pancreatic duct lacks the protection of normal secretory fluid, high in [HCO_3^-], 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 *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)[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[32]. Although there is some evidence that CFTR is expressed in pancreatic acini[129,130], most work has focused on the ductal system for HCO_3^- secretion where CFTR expression is very high in the apical membrane of the small ducts[131] .

On a mechanistic level, the molecular functions required to excrete high concentrations of HCO_3^- from the pancreas remains the subject of continued investigation[132]. Recently, an electrogenic $Na^+/nHCO_3^-$ cotransporter (pancreatic NBC-1) has been cloned from pancreatic duct cells[133] and reported to be responsible for HCO_3^- uptake across the basilateral membrane into the cell[134-136]. This mechanism is attractive from the point of view that it transports the HCO_3^- species directly into the cell and does not require removal of H^+ ions inherent to CO_2/H_2O/carbonic anhydrase systems.

The problem of pancreatic HCO_3^- secretion dictated by CF then becomes one of how to move HCO_3^- 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_3^- was secreted via a chloride-bicarbonate (Cl^-/HCO_3^-) exchanger. CFTR operating in parallel with the exchanger allowed apical recycling of Cl^- to balance the Cl^- influx of the Cl^-/HCO_3^- exchanger[137-139]. A CFTR-dependent Cl^-/HCO_3^- exchanger was found in the apical membrane of native mouse pancreatic ducts, which was not present in ΔF508/ΔF508 mouse pancreatic ducts[140]. The activity appeared to be stimulated by cAMP as well as by intracellular Ca^{2+}[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^- conductance was depressed while mutations such as H620Q did not support exchange, but retained a disputed[240] Cl^- conductance. Other mutations killed both Cl^- and HCO_3^- transport. These properties showed some correlation with PS and PI phenotypes of patients with the same genotypes[142], suggesting that CFTR might play a role, as a Cl^- channel in Cl^-/HCO_3^- exchange[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[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[146] and that scl26a6 may exert limiting control on CFTR[147].

Since HCO_3^- is only about 1/10 to 1/5[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[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[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[134,135,151]. An attractive possibility might be that HCO_3^- moves conductively through CFTR[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[154].

Whether CFTR conducts HCO_3^- directly or critically supports HCO_3^- exchange, pancratic 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[22,155], respiratory failure, cor pulmonale, heart failure, and death[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 bronchiolar lavage[156-158], but others have not seen such increases before infection[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[161-164], but it is at times difficult to be certain that compared cells are uniform[165] and not all investigators have seen such inherent differences[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[170]. Somewhat similarly, even though CF cells responded more than normal airway cells, both increased IL-8 release in response to hyperosmotic NaCl[171]. Recently, perhaps the first report to link the Cl^- 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[170].

Clearly the ultimate destruction of the CF lung is mainly due to inflammation[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[173].

The sputum from CF patients is thick and viscid[174,175] and early pathologists held the impression that ducts of affected organs appeared plugged with "thick, inspissated" plugs and concretions of mucus[9,20,22,176]. 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 macromolecular content of airway surface fluid (ASF) concentrated, "thick", and viscous[177]. Diminished fluid volumes presumably prevent the mucociliary escalator from removing inhaled and inherent debris rapidly enough to evade infection and inflammatory response[178]. 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[179,180]. 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[181]. 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[182], even though CF mice with no CFTR develop little, if any, lung infection[183]. Presumably, the null CF mice compensate for the lack of CFTR with a Ca^{2+}-mediated Cl^- channel[184]. 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[185]. Due to the very small volume of ASF and its inaccessibility, its exact composition remains controversial[186-194]. 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[195]. 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[196-198], 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[166,171].

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[101] to facilitate clearing the contamination from the airway on the mucociliary escalator. Virtually all secreted fluids in mammals originate isotonically[199]. 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.

HCO_3^- defect: Still, there may be another dysfunction in the airways[200]. Reflecting on the role of HCO_3^- and the effect of its loss in the CF pancreas as well as in other organs, failure to secrete HCO_3^- may be critical in the airway as well. The physiological effects of HCO_3^- are therefore not merely a property of its chemistry, but also, of the H^+ ion concentration determined by the HCO_3^-/CO_2 buffer system. It is not hard to imagine that with respect to lung defense and inflammation, HCO_3^- could play at least three roles in different scenarios. HCO_3^- 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, HCO_3^- secretion[201-204]. There is little *in vitro* data to show that HCO_3^- affects mucus viscosity, but compared to water, 2% $NaHCO_3$ reduced viscosity of

pooled nasal discharge by about 40%. Inhaled aerolized HCO_3^- 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 effected conformational changes in mucins that are inherent in gel-sol transformations, which dramatically change viscoelasticity[211,212]. Closely associated with mucus 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 HCO_3^- into play in the airway, it seems likely that a CF lung without control of HCO_3^- may be at a disadvantage from two overall abnormalities. First, in the "resting" state before a contaminating event, the lack of HCO_3^- 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. HCO_3^--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 HCO_3^- 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 HCO_3^- 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 HCO_3^-/CO_3^{2-} to complex Ca^{2+} 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 HCO_3^-/CO_3^{2-} allows the poly anionic negative charges to rapidly expand the mucin molecule. Efficient, rapid removal of the shielding cations from condensed mucins seems critical to forming the "tangled string" network for normal mucus on epithelial surfaces. Without HCO_3^-, to the extent that Ca^{2+} and H^+ continue to shield the polyanionic sites, mucins tend 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, heterogygote-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)[10]. But other studies in ΔF508 mice[230] and in human heterozygotes acutely stimulated with PGE_2 instead of cholera toxin using acute PGE_2 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 (CBAVD) even in the absence of disease[233]. Coincidentally, "A" cells that secrete acid and "B" which secrete HCO_3^- characterize this epithelium[234]. Though not sterile, fertility among female patients is significantly reduced[235]. The defect likely results from an inability to secrete HCO_3^- properly in the cervix to thin mucus and in the uterus or oviduct to affect sperm capacitation, a HCO_3^--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[237]. The disturbance in the biliary tree and gall bladder, although poorly investigated, is almost certainly rooted in mismanaged HCO_3^- [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.

REFERENCES

1 Fink RJ, Doershuk CF, Tucker AS, Stern RC, Boat TF, Matthews LW. Pulmonary function and morbidity in 40 adult patients with cystic fibrosis. Chest 1978; 74: 643-647.

2 Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui LC, Durie P. Genetic determination of exocrine pancreatic function in cystic fibrosis. Am J Hum Genet 1992; 50: 1178-1184.

3 Di Sant'Agnese PA, Powell GF. The eccrine sweat defect in cystic fibrosis of the pancreas (mucoviscidosis). Ann NY Acad Sci 1962; 93: 555-599.

4 Shwachman H, Antonowicz I. Sweat test in cystic fibrosis. Ann NY Aca Sci 1962; 93: 600.

5 Munck A, Gerardin M, Alberti C, Ajzenman C, Lebourgeois M, Aigrain Y, Navarro J. Clinical outcome of cystic fibrosis presenting with or without meconium ileus: a matched cohort study. J Pediatr Surg 2006; 41: 1556-1560.

6 Wiesmann UN, Boat TF, Di Sant'Agnese PA. Flow-rates and electrolytes in minor-salivary-gland saliva in normal subjects and patients with cystic fibrosis. Lancet 1972; 2: 510-512.

7 Warwick WJ, Bernard B, Meskin LH. The involvement of the labial mucous salivary gland in patients with cystic fibrosis. Pediatrics 1964; 34: 621-628.

8 Joo NS, Irokawa T, Robbins RC, Wine JJ. Hyposecretion, not hyperabsorption, is the basic defect of cystic fibrosis airway glands. J Biol Chem 2006; 281: 7392-7398.

9 Farber S. Pancreatic function and disease in early life. V. Pathological changes associated with pancreatic insufficiency in early life. Arch Pathol 1944; 37: 238-250.

10 Pratha VS, Hogan DL, Martensson BA, Bernard J, Zhou R, Isenberg JI. Identification of transport abnormalities in duodenal mucosa and duodenal enterocytes from patients with cystic fibrosis. Gastroenterology 2000; 118: 1051-1060.

11 Russo MA, Hogenauer C, Coates SW Jr, Santa Ana CA, Porter JL, Rosenblatt RL, Emmett M, Fordtran JS. Abnormal passive chloride absorption in cystic fibrosis jejunum functionally opposes the classic chloride secretory defect. J Clin Invest 2003; 112: 118-125.

12 Esterly JR, Oppenheimer EH. Observations in cystic fibrosis of the pancreas. I. The gallbladder. Bull Johns Hopkins Hosp 1962; 110: 247-255.

13 Blanc WA, Di Sant'Agnese PA. A distinctive type of biliary cirrhosis of the liver associated with cystic fibrosis of the pancreas. Pediatrics 1956; 18: 387-409.

14 Lindblad A, Hultcrantz R, Strandvik B. Bile-duct destruction and collagen deposition: a prominent ultrastructural feature of the liver in cystic fibrosis. Hepatology 1992; 16: 372-381.

15 Kopito LE, Kosasky HJ, Shwachman H. Water and electrolytes in cervical mucus from patients with cystic fibrosis. Fertil Steril 1973; 24: 512-516.

16 Kaplan E, Shwachman H, Perlmutter AD, Rule A, Khaw KT, Holsclaw DS. Reproductive failure in males with cystic fibrosis. N Engl J Med 1968; 279: 65-69.

17 Rule AH, Kopito L, Shwachman H. Chemical analysis of ejaculates from patients with cystic fibrosis. Fertil Steril 1970; 21: 515-520.
18 Holsclaw DS, Perlmutter AD, Jockin H, Shwachman H. Genital abnormalities in male patients with cystic fibrosis. J Urol 1971; 106: 568-574.
19 Zuelzer WW, Newton WA. The pathogenesis of fibrocystic disease of the pancreas. A study of 36 cases with special reference to the pulmonary lesions. Pediatrics 1949; 4: 53-69.
20 Bodian M. Fibrocystic Disease of the Pancreas: A Congenital Disorder of Mucus Production—Mucosis. New York: Grune and Stratton, Inc., 1953, 1-244.
21 Gugler E, Pallavicini CJ, Swerdlow H, Di Sant'Agnese PA. The role of calcium in submaxillary saliva of patients with cystic fibrosis. J Pediatr 1967; 71: 585-588.
22 Burgel PR, Montani D, Danel C, Dusser DJ, Nadel JA. A morphometric study of mucins and small airway plugging in cystic fibrosis. Thorax 2007; 62: 153-161.
23 Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease. Am J Dis Child 1938; 56: 344-399.
24 Andersen DH, Hodges RG. Celiac syndrome. V. Genetics of cystic fibrosis of the pancreas with a consideration of the etiology. Am J Dis Child 1946; 72: 62-80.
25 Statistics. Cystic Fibrosis Statistics. http://personalnbnetnbca/normap/cfstatshtm
26 Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245: 1073-1080.
27 Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, *et al*. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245: 1066-1073.
28 Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Collins FS, *et al*. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989; 245: 1059-1065.
29 Palomaki GE, FitzSimmons SC, Haddow JE. Clinical sensitivity of prenatal screening for cystic fibrosis via CFTR carrier testing in a United States panethnic population. Genet Med 2004; 6: 405-414.
30 Database. Cystic Fibrosis Mutation Database. In: http://www.genet.sickkids.on.ca/cftr/
31 Wang W, Okayama H, Shirato K. Genotypes of cystic fibrosis (CF) reported in the world and polymorphisms of cystic fibrosis transmembrane conductance regulator (CFTR) gene in Japanese. Nippon Rinsho 1996; 54: 525-532.
32 Lee JH, Choi JH, Namkung W, Hanrahan JW, Chang J, Song SY, Park SW, Kim DS, Yoon JH, Suh Y, Jang IJ, Nam JH, Kim SJ, Cho MO, Lee JE, Kim KH, Lee MG. A haplotype-based molecular analysis of CFTR mutations associated with respiratory and pancreatic diseases. Hum Mol Genet 2003; 12: 2321-2332.
33 Nam MH, Hijikata M, Tuan le A, Lien LT, Shojima J, Horie T, Nakata K, Matsushita I, Ohashi J, Tokunaga K, Keicho N. Variations of the CFTR gene in the Hanoi-Vietnamese. Am J Med Genet A 2005; 136: 249-253.
34 Ngiam NS, Chong SS, Shek LP, Goh DL, Ong KC, Chng SY, Yeo GH, Goh DY. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in Asians with chronic pulmonary disease: A pilot study. J Cyst Fibros 2006; 5: 159-164.
35 Zilfalil BA, Sarina S, Liza-Sharmini AT, Oldfield NJ, Stenhouse SA. Detection of F508del mutation in cystic fibrosis transmembrane conductance regulator gene mutation among Malays. Singapore Med J 2006; 47: 129-133.
36 Quinton PM. Chloride impermeability in cystic fibrosis. Nature 1983; 301: 421-422.
37 Sheppard DN, Welsh MJ. Structure and function of the CFTR chloride channel. Physiol Rev 1999; 79: S23-S45.
38 Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993; 73: 1251-1254.
39 Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med 2005; 352: 1992-2001.
40 Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration 2000; 67(2): 117-133.
41 Ahmed N, Corey M, Forstner G, Zielenski J, Tsui LC, Ellis L, Tullis E, Durie P. Molecular consequences of cystic fibrosis transmembrane regulator (CFTR) gene mutations in the exocrine pancreas. Gut 2003; 52: 1159-1164.
42 Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet 2003; 67: 471-485.
43 Gray MA, Harris A, Coleman L, Greenwell JR, Argent BE. Two types of chloride channel on duct cells cultured from human fetal pancreas. Am J Physiol 1989; 257: C240-C251.
44 Berger HA, Anderson MP, Gregory RJ, Thompson S, Howard PW, Maurer RA, Mulligan R, Smith AE, Welsh MJ. Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel. J Clin Invest 1991; 88: 1422-1431.
45 Linsdell P, Tabcharani JA, Rommens JM, Hou YX, Chang XB, Tsui LC, Riordan JR, Hanrahan JW. Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. J Gen Physiol 1997; 110: 355-364.
46 Greger R, Mall M, Bleich M, Ecke D, Warth R, Riedemann N, Kunzelmann K. Regulation of epithelial ion channels by the cystic fibrosis transmembrane conductance regulator. J Mol Med 1996; 74: 527-534.
47 Kunzelmann K. CFTR: interacting with everything? News Physiol Sci 2001; 16: 167-170.

48 Sato K, Sato F. Defective beta adrenergic response of cystic fibrosis sweat glands *in vivo* and *in vitro*. J Clin Invest 1984; 73: 1763-1771.

49 Sato K, Sato F. Variable reduction in beta-adrenergic sweat secretion in cystic fibrosis heterozygotes. J Lab Clin Med 1988; 111: 511-518.

50 Hitchin BW, Dobson PR, Brown BL, Hardcastle J, Hardcastle PT, Taylor CJ. Measurement of intracellular mediators in enterocytes isolated from jejunal biopsy specimens of control and cystic fibrosis patients. Gut 1991; 32: 893-899.

51 Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. Cell 1991; 66: 1027-1036.

52 Quinton PM, Reddy MM. Regulation of absorption by phosphorylation of CFTR. Jpn J Physiol 1994; 44 (Suppl 2): S207-S213.

53 Li C, Ramjeesingh M, Wang W, Garami E, Hewryk M, Lee D, Rommens JM, Galley K, Bear CE. ATPase activity of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 1996; 271: 28463-28468.

54 Gadsby DC, Dousmanis AG, Nairn AC. ATP hydrolysis cycles and the gating of CFTR Cl^- channels. Acta Physiol Scand (Suppl) 1998; 643: 247-256.

55 Quinton PM. Effects of some ion transport inhibitors on secretion and reabsorption in intact and perfused single human sweat glands. Pflugers Arch 1981; 391: 309-313.

56 Schwencke C, Yamamoto M, Okumura S, Toya Y, Kim SJ, Ishikawa Y. Compartmentation of cyclic adenosine 3',5'-monophosphate signaling in caveolae. Mol Endocrinol 1999; 13: 1061-1070.

57 Ostrom RS, Violin JD, Coleman S, Insel PA. Selective enhancement of beta-adrenergic receptor signaling by overexpression of adenylyl cyclase type 6: colocalization of receptor and adenylyl cyclase in caveolae of cardiac myocytes. Mol Pharmacol 2000; 57(5): 1075-1079.

58 Rich DP, Berger HA, Cheng SH, Travis SM, Saxena M, Smith AE, Welsh MJ. Regulation of the cystic fibrosis transmembrane conductance regulator Cl^- channel by negative charge in the R domain. J Biol Chem 1993; 268: 20259-20267.

59 Gadsby DC, Nairn AC. Regulation of CFTR channel gating. Trends Biochem Sci 1994; 19: 513-518.

60 Dahan D, Evagelidis A, Hanrahan JW, Hinkson DA, Jia Y, Luo J, Zhu T. Regulation of the CFTR channel by phosphorylation. Pflugers Arch 2001; 443 (Suppl 1): S92-S96.

61 Chang XB, Tabcharani JA, Hou YX, Jensen TJ, Kartner N, Alon N, Hanrahan JW, Riordan JR. Protein kinase A (PKA) still activates CFTR chloride channel after mutagenesis of all 10 PKA consensus phosphorylation sites. J Biol Chem 1993; 268: 11304-11311.

62 Seibert FS, Chang XB, Aleksandrov AA, Clarke DM, Hanrahan JW, Riordan JR. Influence of phosphorylation by protein kinase A on CFTR at the cell surface and endoplasmic reticulum. Biochim Biophys Acta 1999; 1461: 275-283.

63 Jia Y, Mathews CJ, Hanrahan JW. Phosphorylation by protein kinase C is required for acute activation of cystic fibrosis transmembrane conductance regulator by protein kinase A. J Biol Chem 1997; 272: 4978-4984.

64 Liedtke CM, Cole TS. Antisense oligonucleotide to PKC-epsilon alters cAMP-dependent stimulation of CFTR in Calu-3 cells. Am J Physiol 1998; 275: C1357-C1364.

65 Middleton LM, Harvey RD. PKC regulation of cardiac CFTR Cl^- channel function in guinea pig ventricular myocytes. Am J Physiol 1998; 275: C293-C302.

66 Bajnath RB, Groot JA, De Jonge HR, Kansen M, Bijman J. Synergistic activation of non-rectifying small-conductance chloride channels by forskolin and phorbol esters in cell-attached patches of the human colon carcinoma cell line HT-29cl.19A. Pflugers Arch 1993; 425: 100-108.

67 Tabcharani JA, Chang XB, Riordan JR, Hanrahan JW. Phosphorylation-regulated Cl^- channel in CHO cells stably expressing the cystic fibrosis gene. Nature 1991; 352: 628-631.

68 Button B, Reuss L, Altenberg GA. PKC-mediated stimulation of amphibian CFTR depends on a single phosphorylation consensus site. insertion of this site confers PKC sensitivity to human CFTR. J Gen Physiol 2001; 117: 457-468.

69 Vaandrager AB, Ehlert EM, Jarchau T, Lohmann SM, de Jonge HR. N-terminal myristoylation is required for membrane localization of cGMP-dependent protein kinase type II. J Biol Chem 1996; 271: 7025-7029.

70 Reddy MM, Quinton PM. Functional interaction of CFTR and ENaC in sweat glands. Pflugers Arch 2003; 445: 499-503.

71 Vaandrager AB, Smolenski A, Tilly BC, Houtsmuller AB, Ehlert EM, Bot AG, Edixhoven M, Boomaars WE, Lohmann SM, de Jonge HR. Membrane targeting of cGMP-dependent protein kinase is required for cystic fibrosis transmembrane conductance regulator Cl^- channel activation. Proc Natl Acad Sci USA 1998; 95: 1466-1471.

72 Csanady L, Chan KW, Angel BB, Nairn AC, Gadsby DC. Negative regulation of CFTR chloride channel gating through R-domain serine 768. Ped Pulmon 1998; 17: 205.

73 Travis SM, Berger HA, Welsh MJ. Protein phosphatase 2C dephosphorylates and inactivates cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci USA 1997; 94: 11055-11060.

74 Nairn AC, Qin J, Chait BT, Gadsby DC. Indentification of sites in the R domain of CFTR phosphorylated by cAMP-dependent protein kinase and dephosphorylated by protein phosphatase 2A and 2C. Ped Pulmon 1996(Suppl 13): 21.

75 Reddy MM, Quinton PM. Deactivation of CFTR-Cl conductance by endogenous phosphatases in the native sweat duct. Am J Physiol 1996; 270: C474-C480.

76 Reddy MM, Quinton PM. Cytosolic potassium controls CFTR deactivation in human sweat duct. Am J Physiol Cell Physiol 2006; 291: C122-C129.

77 Zhu T, Dahan D, Evagelidis A, Zheng S, Luo J, Hanrahan JW. Association of cystic fibrosis transmembrane conductance regulator and protein phosphatase 2C. J Biol Chem 1999; 274: 29102-29107.

78 Fischer H, Machen TE. The tyrosine kinase p60c-src regulates the fast gate of the cystic fibrosis transmembrane conductance regulator chloride channel. Biophys J 1996; 71: 3073-3082.

79 Nguyen TD, Canada AT, Heintz GG, Gettys TW, Cohn JA. Stimulation of secretion by the T84 colonic epithelial cell line with dietary flavonols. Biochem Pharmacol 1991; 41: 1879-1886.

80 Sears CL, Firoozmand F, Mellander A, Chambers FG, Eromar IG, Bot AG, Scholte B, De Jonge HR, Donowitz M. Genistein and tyrphostin 47 stimulate CFTR-mediated Cl^- secretion in T84 cell monolayers. Am J Physiol 1995; 269: G874-G882.

81 French PJ, Bijman J, Bot AG, Boomaars WE, Scholte BJ, de Jonge HR. Genistein activates CFTR Cl^- channels via a tyrosine kinase- and protein phosphatase-independent mechanism. Am J Physiol 1997; 273: C747-C753.

82 Wang F, Zeltwanger S, Yang IC, Nairn AC, Hwang TC. Actions of genistein on cystic fibrosis transmembrane conductance regulator channel gating. Evidence for two binding sites with opposite effects. J Gen Physiol 1998; 111: 477-490.

83 Bulteau-Pignoux L, Derand R, Metaye T, Joffre M, Becq F. Genistein modifies the activation kinetics and magnitude of phosphorylated wild-type and G551D-CFTR chloride currents. J Membr Biol 2002; 188: 175-182.

84 Schultz BD, Singh AK, Devor DC, Bridges RJ. Pharmacology of CFTR chloride channel activity. Physiol Rev 1999; 79: S109-S144.

85 Berger AL, Randak CO, Ostedgaard LS, Karp PH, Vermeer DW, Welsh MJ. Curcumin stimulates cystic fibrosis transmembrane conductance regulator Cl^- channel activity. J Biol Chem 2005; 280: 5221-5226.

86 Lansdell KA, Cai Z, Kidd JF, Sheppard DN. Two mechanisms of genistein inhibition of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in murine cell line. J Physiol 2000; 524 (Pt 2): 317-330.

87 Moran O, Zegarra-Moran O. A quantitative description of the activation and inhibition of CFTR by potentiators: Genistein. FEBS Lett 2005; 579: 3979-3983.

88 Linsdell P, Hanrahan JW. Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. Am J Physiol 1996; 271: C628-C634.

89 Quinton PM, Reddy MM. Control of CFTR chloride conductance by ATP levels through non-hydrolytic binding. Nature 1992; 360: 79-81.

90 Bell CL, Quinton PM. Regulation of CFTR Cl^- conductance in secretion by cellular energy levels. Am J Physiol 1993; 264: C925-C931.

91 Raj D, Langford M, Krueger S, Shelton M, Welbourne T. Regulatory responses to an oral *D*-glutamate load: formation of *D*-pyrrolidone carboxylic acid in humans. Am J Physiol Endocrinol Metab 2001; 280: E214-E220.

92 Dall'Asta V, Bussolati O, Sala R, Parolari A, Alamanni F, Biglioli P, Gazzola GC. Amino acids are compatible osmolytes for volume recovery after hypertonic shrinkage in vascular endothelial cells. Am J Physiol 1999; 276: C865-C872.

93 Reddy MM, Quinton PM. Selective activation of cystic fibrosis transmembrane conductance regulator Cl^- and HCO_3^- conductances. J Pancr (Online) 2001; 2: 212-218.

94 Reddy MM, Quinton PM. Control of dynamic CFTR selectivity by glutamate and ATP in epithelial cells. Nature 2003; 423: 756-760.

95 Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Glockner-Pagel J, Canny S, Du K, Lukacs GL, Caplan MJ. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. Science 2004; 304: 600-602.

96 Mall M, Kunzelmann K. Correction of the CF defect by curcumin: hypes and disappointments. Bioessays 2005; 27: 9-13.

97 Seidler U, Blumenstein I, Kretz A, Viellard-Baron D, Rossmann H, Colledge WH, Evans M, Ratcliff R, Gregor M. A functional CFTR protein is required for mouse intestinal cAMP-, cGMP- and Ca^{2+}-dependent HCO_3^- secretion. J Physiol 1997; 505 (Pt 2): 411-423.

98 Martin LC, Hickman ME, Curtis CM, MacVinish LJ, Cuthbert AW. Electrogenic bicarbonate secretion in mouse gallbladder. Am J Physiol 1998; 274: G1045-G1052.

99 Ianowski JP, Choi JY, Wine JJ, Hanrahan JW. Mucus secretion by single tracheal submucosal glands from normal and CFTR knock-out mice. J Physiol 2007; 580(Pt 1): 7-8.

100 Wu JV, Krouse ME, Wine JJ. Acinar origin of CFTR-dependent airway submucosal gland fluid secretion. Am J Physiol Lung Cell Mol Physiol 2007; 292: L304-L311.

101 Choi JY, Joo NS, Krouse ME, Wu JV, Robbins RC, Ianowski JP, Hanrahan JW, Wine JJ. VIP and carbachol cause synergistic airway gland secretion that is lost in cystic fibrosis. J Clin Invest (In Press).

102 Jenkinson DM, Mabon RM, Manson W. Sweat proteins. Br J Dermatol 1974; 90: 175-181.

103 Jirka M, Kotas J. The occurrence of mucoproteins in human sweat. Clinica Chimica Acta 1957; 2: 292-296.

104 Sato K. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. Rev Physiol Biochem Pharmacol 1977; 79: 51-131.

105 Quinton PM. Structure and Function of Eccrine Sweat Glands in Humans. In: Antiperspirants and Deodorants. Laden K, Felger CB. eds. New York and Basel: Marcel Dekker, Inc., 1988, 57-

82.

106 Prompt CA, Quinton PM. Functions of calcium in sweat secretion. Nature 1978; 272: 171-172.

107 Kidd JF, Thorn P. Intracellular Ca^{2+} and Cl^- channel activation in secretory cells. Annu Rev Physiol 2000; 62: 493-513.

108 Silva P, Stoff J, Field M, Fine L, Forrest JN, Epstein FH. Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. Am J Physiol 1977; 233(4): F298-F306.

109 Harper S, Quinton PM. Adrenergic and cholinergic stimulation of CF sweat glands. In: Cystic Fibrosis: Horizons Proceedings of the 9th International Cystic Fibrosis Congress. 1st ed. Lawson D. ed. Brighton, England: John Wiley & Sons, 1984, 178.

110 Bijman J, Quinton PM. Influence of abnormal Cl^- impermeability on sweating in cystic fibrosis. Am J Physiol 1984; 247: C3-C9.

111 Cohn JA, Melhus O, Page LJ, Dittrich KL, Vigna SR. CFTR: development of high-affinity antibodies and localization in sweat gland. Biochem Biophys Res Commun 1991; 181: 36-43.

112 Kartner N, Augustinas O, Jensen TJ, Naismith AL, Riordan JR. Mislocalization of delta F508 CFTR in cystic fibrosis sweat gland. Nat Genet 1992; 1: 321-327.

113 Quinton PM, Tormey JM. Localization of Na/K-ATPase sites in the secretory and reabsorptive epithelia of perfused eccrine sweat glands: a question to the role of the enzyme in secretion. J Membr Biol 1976; 29 383-399.

114 Conn JW. Electrolyte composition of sweat. Arch Intern Med 1949; 83: 416-428.

115 Lobeck CC, McSherry N. Response of sweat electrolyte concentrations to 9 alpha-fluorohydrocortisone in patients with cystic fibrosis and their families. J Pediatr 1963; 62(3): 393-398.

116 Reddy MM, Quinton PM. cAMP activation of CF-affected Cl^- conductance in both cell membranes of an absorptive epithelium. J Membr Biol 1992; 130: 49-62.

117 Quinton PM, Bijman J. Higher bioelectric potentials due to decreased chloride absorption in the sweat glands of patients with cystic fibrosis. N Engl J Med 1983; 308: 1185-1189.

118 Ballestero Y, Hernandez MI, Rojo P, Manzanares J, Nebreda V, Carbajosa H, Infante E, Baro M. Hyponatremic dehydration as a presentation of cystic fibrosis. Pediatr Emerg Care 2006; 22: 725-727.

119 Di Sant'Agnese PE, Andersen DH. Celiac syndrome. Am J Dis Child 1946; 72: 17-61.

120 Farber S. Some organic digestive disturbances in early life. J Mich State Med Soc1945; 44: 587-594.

121 Oppenheimer EH, Esterly JR. Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases. Perspect Pediatr Pathol 1975; 2: 241-278.

122 Marcus MS, Sondel SA, Farrell PM, Laxova A, Carey PM, Langhough R, Mischler EH. Nutritional status of infants with cystic fibrosis associated with early diagnosis and intervention. Am J Clin Nutr 1991; 54: 578-585.

123 Sinaasappel M. Relationship between intestinal function and chloride secretion in patients with cystic fibrosis. Neth J Med 1992; 41: 110-114.

124 Pencharz PB, Durie PR. Pathogenesis of malnutrition in cystic fibrosis, and its treatment. Clin Nutr 2000; 19: 387-394.

125 Domschke S, Domschke W, Rosch W, Konturek SJ, Wunsch E, Demling L. Bicarbonate and cyclic AMP content of pure human pancreatic juice in response to graded doses of synthetic secretin. Gastroenterology 1976; 70: 533-536.

126 Wada K, Yamadera K, Yokoyama K, Goto M, Makino I. Application of pure pancreatic juice collection to the pancreatic exocrine function test. Pancreas 1998; 16: 124-128.

127 Hadorn B, Johansen PG, Anderson CM. Pancreozymin secretin test of exocrine pancreatic function in cystic fibrosis and the significance of the result for the pathogenesis of the disease. Can Med Assoc J 1968; 98: 377-385.

128 Johansen PG, Anderson CM, Hadorn B. Cystic fibrosis of the pancreas. A generalised disturbance of water and electrolyte movement in exocrine tissues. Lancet 1968; 1: 455-460.

129 Kopelman H, Ferretti E, Gauthier C, Goodyer PR. Rabbit pancreatic acini express CFTR as a cAMP-activated chloride efflux pathway. Am J Physiol 1995; 269: C626-C631.

130 Zeng W, Lee MG, Yan M, Diaz J, Benjamin I, Marino CR, Kopito R, Freedman S, Cotton C, Muallem S, Thomas P. Immuno and functional characterization of CFTR in submandibular and pancreatic acinar and duct cells. Am J Physiol 1997; 273: C442-C455.

131 Marino CR, Matovcik LM, Gorelick FS, Cohn JA. Localization of the cystic fibrosis transmembrane conductance regulator in pancreas. J Clin Invest 1991; 88: 712-716.

132 Whitcomb DC. Pancreatic bicarbonate secretion: role of CFTR and the sodium-bicarbonate cotransporter. Gastroenterology 1999; 117: 275-277.

133 Abuladze N, Lee I, Newman D, Hwang J, Boorer K, Pushkin A, Kurtz I. Molecular cloning, chromosomal localization, tissue distribution, and functional expression of the human pancreatic sodium bicarbonate cotransporter. J Biol Chem 1998; 273: 17689-17695.

134 Ishiguro H, Steward MC, Wilson RW, Case RM. Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495 (Pt 1): 179-191.

135 Ishiguro H, Steward MC, Lindsay AR, Case RM. Accumulation of intracellular HCO_3^- by Na^+-HCO_3^- cotransport in interlobular ducts from guinea-pig pancreas. J Physiol 1996; 495 (Pt 1): 169-178.

136 Shumaker H, Amlal H, Frizzell R, Ulrich CD, 2nd, Soleimani M. CFTR drives Na^+-$nHCO_3^-$ cotransport in pancreatic duct cells: a basis for defective HCO_3^- secretion in CF. Am J Physiol 1999;

276: C16-C25.

137 Novak I, Greger R. Electrophysiological study of transport systems in isolated perfused pancreatic ducts: properties of the basolateral membrane. Pflugers Arch 1988; 411: 58-68.

138 Novak I, Greger R. Properties of the luminal membrane of isolated perfused rat pancreatic ducts. Effect of cyclic AMP and blockers of chloride transport. Pflugers Arch 1988; 411: 546-553.

139 Sohma Y, Gray MA, Imai Y, Argent BE. HCO_3^- transport in a mathematical model of the pancreatic ductal epithelium. J Membr Biol 2000; 176: 77-100.

140 Lee MG, Choi JY, Luo X, Strickland E, Thomas PJ, Muallem S. Cystic fibrosis transmembrane conductance regulator regulates luminal $Cl^-/HCO3^-$ exchange in mouse submandibular and pancreatic ducts. J Biol Chem 1999; 274: 14670-14677.

141 Namkung W, Lee JA, Ahn W, Han W, Kwon SW, Ahn DS, Kim KH, Lee MG. Ca^{2+} activates cystic fibrosis transmembrane conductance regulator- and Cl^--dependent HCO_3^- transport in pancreatic duct cells. J Biol Chem 2003; 278: 200-207.

142 Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO_3^- transport in mutations associated with cystic fibrosis. Nature 2001; 410: 94-97.

143 Choi JY, Lee MG, Ko S, Muallem S. Cl^--dependent HCO_3^- transport by cystic fibrosis transmembrane conductance regulator. JOP 2001; 2: 243-246.

144 Ko SB, Zeng W, Dorwart MR, Luo X, Kim KH, Millen L, Goto H, Naruse S, Soyombo A, Thomas PJ, Muallem S. Gating of CFTR by the STAS domain of SLC26 transporters. Nat Cell Biol 2004; 6: 343-350.

145 Shcheynikov N, Wang Y, Park M, Ko SB, Dorwart M, Naruse S, Thomas PJ, Muallem S. Coupling modes and stoichiometry of Cl^-/HCO_3^- exchange by slc26a3 and slc26a6. J Gen Physiol 2006; 127: 511-524.

146 Ishiguro H, Namkung W, Yamamoto A, Wang Z, Worrell RT, Xu J, Lee MG, Soleimani M. Effect of Slc26a6 deletion on apical Cl^-/HCO_3^- exchanger activity and cAMP-stimulated bicarbonate secretion in pancreatic duct. Am J Physiol Gastrointest Liver Physiol 2007; 292: G447-G455.

147 Wang Y, Soyombo AA, Shcheynikov N, Zeng W, Dorwart M, Marino CR, Thomas PJ, Muallem S. Slc26a6 regulates CFTR activity *in vivo* to determine pancreatic duct HCO_3^- secretion: relevance to cystic fibrosis. EMBO J 2006; 25: 5049-5057.

148 Illek B, Tam AW, Fischer H, Machen TE. Anion selectivity of apical membrane conductance of Calu 3 human airway epithelium. Pflugers Arch 1999; 437: 812-822.

149 Ko SB, Shcheynikov N, Choi JY, Luo X, Ishibashi K, Thomas PJ, Kim JY, Kim KH, Lee MG, Naruse S, Muallem S. A molecular mechanism for aberrant CFTR-dependent HCO_3^- transport in cystic fibrosis. EMBO J 2002; 21: 5662-5672.

150 Shcheynikov N, Kim KH, Kim KM, Dorwart MR, Ko SB, Goto H, Naruse S, Thomas PJ, Muallem S. Dynamic control of cystic fibrosis transmembrane conductance regulator Cl^-/HCO_3^- selectivity by external Cl^-. J Biol Chem 2004; 279: 21857-21865.

151 Steward MC, Ishiguro H, Case RM. Mechanisms of bicarbonate secretion in the pancreatic duct. Annu Rev Physiol 2005; 67: 377-409.

152 Ishiguro H, Naruse S, San Roman JI, Case M, Steward MC. Pancreatic ductal bicarbonate secretion: past, present and future. JOP 2001; 2: 192-197.

153 Devor DC, Singh AK, Lambert LC, DeLuca A, Frizzell RA, Bridges RJ. Bicarbonate and chloride secretion in Calu-3 human airway epithelial cells. J Gen Physiol 1999; 113: 743-760.

154 Ishiguro H, Steward MC, Sohma Y, Kubota T, Kitagawa M, Kondo T, Case RM, Hayakawa T, Naruse S. Membrane potential and bicarbonate secretion in isolated interlobular ducts from guinea-pig pancreas. J Gen Physiol 2002; 120: 617-628.

155 Esterly JR, Oppenheimer EH. Cystic fibrosis of the pancreas: structural changes in peripheral airways. Thorax 1968; 23: 670-675.

156 Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med 1995; 151: 1075-1082.

157 Noah TL, Black HR, Cheng PW, Wood RE, Leigh MW. Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. J Infect Dis 1997; 175: 638-647.

158 Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. Am J Respir Crit Care Med 1999; 160: 186-191.

159 Armstrong DS, Grimwood K, Carlin JB, Carzino R, Gutierrez JP, Hull J, Olinsky A, Phelan EM, Robertson CF, Phelan PD. Lower airway inflammation in infants and young children with cystic fibrosis. Am J Respir Crit Care Med 1997; 156: 1197-1204.

160 Armstrong DS, Hook SM, Jamsen KM, Nixon GM, Carzino R, Carlin JB, Robertson CF, Grimwood K. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol 2005; 40: 500-510.

161 Srivastava M, Eidelman O, Zhang J, Paweletz C, Caohuy H, Yang Q, Jacobson KA, Heldman E, Huang W, Jozwik C, Pollard BS, Pollard HB. Digitoxin mimics gene therapy with CFTR and suppresses hypersecretion of IL-8 from cystic fibrosis lung epithelial cells. Proc Natl Acad Sci USA 2004; 101: 7693-7698.

162 Hajj R, Lesimple P, Nawrocki-Raby B, Birembaut P, Puchelle E, Coraux C. Human airway surface epithelial regeneration is delayed and abnormal in cystic fibrosis. J Pathol 2007; 211: 340-350.

163 Eidelman O, Srivastava M, Zhang J, Leighton X, Murtie J, Jozwik C, Jacobson K, Weinstein DL, Metcalf EL, Pollard

HB. Control of the proinflammatory state in cystic fibrosis lung epithelial cells by genes from the TNF-alphaR/NFkappaB pathway. Mol Med 2001; 7: 523-534.

164 Joseph T, Look D, Ferkol T. NF-kappaB activation and sustained IL-8 gene expression in primary cultures of cystic fibrosis airway epithelial cells stimulated with *Pseudomonas aeruginosa*. Am J Physiol Lung Cell Mol Physiol 2005; 288: L471-L479.

165 Rubin BK. CFTR is a modulator of airway inflammation. Am J Physiol Lung Cell Mol Physiol 2007; 292: L381-L382.

166 Machen TE. Innate immune response in CF airway epithelia: hyperinflammatory? Am J Physiol Cell Physiol 2006; 291: C218-C230.

167 Aldallal N, McNaughton EE, Manzel LJ, Richards AM, Zabner J, Ferkol TW, Look DC. Inflammatory response in airway epithelial cells isolated from patients with cystic fibrosis. Am J Respir Crit Care Med 2002; 166: 1248-1256.

168 Pizurki L, Morris MA, Chanson M, Solomon M, Pavirani A, Bouchardy I, Suter S. Cystic fibrosis transmembrane conductance regulator does not affect neutrophil migration across cystic fibrosis airway epithelial monolayers. Am J Pathol 2000; 156: 1407-1416.

169 Becker MN, Sauer MS, Muhlebach MS, Hirsh AJ, Wu Q, Verghese MW, Randell SH. Cytokine secretion by cystic fibrosis airway epithelial cells. Am J Respir Crit Care Med 2004; 169: 645-653.

170 Perez A, Issler AC, Cotton CU, Kelley TJ, Verkman AS, Davis PB. CFTR inhibition mimics the cystic fibrosis inflammatory profile. Am J Physiol Lung Cell Mol Physiol 2007; 292: L383-L395.

171 Chan MM, Chmura K, Chan ED. Increased NaCl-induced interleukin-8 production by human bronchial epithelial cells is enhanced by the deltaF508/W1282X mutation of the cystic fibrosis transmembrane conductance regulator gene. Cytokine 2006; 33(6): 309-316.

172 Chmiel JF, Davis PB. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can't they clear the infection? Respir Res 2003; 4: 8.

173 Regnis JA, Robinson M, Bailey DL, Cook P, Hooper P, Chan HK, Gonda I, Bautovich G, Bye PT. Mucociliary clearance in patients with cystic fibrosis and in normal subjects. Am J Respir Crit Care Med 1994; 150: 66-71.

174 Chace KV, Naziruddin B, Desai VC, Flux M, Sachdev GP. Physical properties of purified human respiratory mucus glycoproteins: effects of sodium chloride concentration on the aggregation properties and shape. Exp Lung Res 1989; 15: 721-737.

175 Chace KV, Flux M, Sachdev GP. Comparison of physicochemical properties of purified mucus glycoproteins isolated from respiratory secretions of cystic fibrosis and asthmatic patients. Biochemistry 1985; 24: 7334-7341.

176 Oppenheimer EH, Esterly JR. Observations in cystic fibrosis of the pancreas. II. Neonatal intestinal obstruction. Bull Johns Hopkins Hosp 1962; 111: 1-13.

177 Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med 2007; 58: 157-170.

178 Boucher RC. Pathogenesis of cystic fibrosis airways disease. Trans Am Clin Climatol Assoc 2001; 112: 99-107.

179 Knowles M, Gatzy J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N Engl J Med 1981; 305: 1489-1495.

180 Stutts MJ, Canessa CM, Olsen JC, Hamrick M, Cohn JA, Rossier BC, Boucher RC. CFTR as a cAMP-dependent regulator of sodium channels. Science 1995; 269: 847-850.

181 Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. J Intern Med 2007; 261: 5-16.

182 Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na^+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med 2004; 10: 487-493.

183 Grubb BR, Boucher RC. Pathophysiology of gene-targeted mouse models for cystic fibrosis. Physiol Rev 1999; 79: S193-S214.

184 de Jonge HR. Cystic fibrosis mice rehabilitated for studies of airway gland dysfunction. J Physiol 2007; 580(Pt 1): 301-314.

185 Quinton PM. Viscosity *versus* composition in airway pathology. Am J Respir Crit Care Med 1994; 149: 6-7.

186 Cowley EA, Govindaraju K, Lloyd DK, Eidelman DH. Airway surface fluid composition in the rat determined by capillary electrophoresis. Am J Physiol 1997; 273: L895-L899.

187 Zabner J, Smith JJ, Karp PH, Widdicombe JH, Welsh MJ. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia *in vitro*. Mol Cell 1998; 2: 397-403.

188 Joris L, Quinton PM. Filter paper equilibration as a novel technique for *in vitro* studies of the composition of airway surface fluid. Am J Physiol 1992; 263: L243-L248.

189 Widdicombe JG. Airway Surface Liquids: Concepts and Measurements. In: Airway Mucus: Basic Mechanisms and Clinical Perspectives. Rodgers DF, Lethem MI. eds,. Basel: Birkhauser, 1997, 1-17.

190 Hull J, Skinner W, Robertson C, Phelan P. Elemental content of airway surface liquid from infants with cystic fibrosis. Am J Respir Crit Care Med 1998; 157: 10-14.

191 Song Y, Thiagarajah J, Verkman AS. Sodium and chloride concentrations, pH, and depth of airway surface liquid in distal airways. J Gen Physiol 2003; 122: 511-519.

192 Verkman AS. Lung disease in cystic fibrosis: is airway surface liquid composition abnormal? Am J Physiol Lung Cell Mol Physiol 2001; 281: L306-L308.

193 Matsui H, Grubb BR, Tarran R, Randell SH, Gatzy JT, Davis W, Boucher RC. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic

fibrosis airway disease. Cell 1998; 95: 1005-1015.

194 Knowles MR, Robinson JM, Wood RE, Pue CA, Mentz WM, Wager GC, Gatzy JT, Boucher RC. Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects. J Clin Invest 1997; 100: 2588-2595.

195 Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell 1996; 85: 229-236.

196 Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, Greenberg EP, Valore EV, Welsh MJ, Ganz T, Tack BF, McCray PB Jr. Production of beta-defensins by human airway epithelia. Proc Natl Acad Sci USA 1998; 95: 14961-14966.

197 Singh PK, Tack BF, McCray PB Jr., Welsh MJ. Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. Am J Physiol Lung Cell Mol Physiol 2000; 279: L799-L805.

198 Travis SM, Conway BA, Zabner J, Smith JJ, Anderson NN, Singh PK, Greenberg EP, Welsh MJ. Activity of abundant antimicrobials of the human airway. Am J Respir Cell Mol Biol 1999; 20: 872-879.

199 Quinton PM. Water Metabolism: Protozoa to man. In: Comparative Animal Nutrition. 3rd ed. Recighl M. ed. Basel: S. Karger, 1979, 100-231.

200 Quinton PM. The neglected ion: HCO_3^-. Nat Med 2001; 7: 292-293.

201 Livingston EH, Miller J, Engel E. Bicarbonate diffusion through mucus. Am J Physiol 1995; 269: G453-G457.

202 Allen A, Flemstrom G, Garner A, Kivilaakso E. Gastroduodenal mucosal protection. Physiol Rev 1993; 73: 823-857.

203 Trout L, King M, Feng W, Inglis SK, Ballard ST. Inhibition of airway liquid secretion and its effect on the physical properties of airway mucus. Am J Physiol 1998; 274: L258-L263.

204 Joo NS, Krouse ME, Wu JV, Saenz Y, Jayaraman S, Verkman AS, Wine JJ. HCO_3^- transport in relation to mucus secretion from submucosal glands. JOP 2001; 2: 280-284.

205 App EM, Danzl G, King M. *In vitro* sputum rheology changes in cystic fibrosis, chronic bronchitis and asthma lung diseases following the application of different salt solutions. Am J Crit Care Med 1996; 153: A824.

206 Cheng HF, Lin HC, Yu CT, Kuo HP. Effect of aerosolized alkaline solution on patients with bronchiectasis with sputum hypersecretion. Eur Respir J 1996; 8: 390s.

207 Rhee CS, Majima Y, Cho JS, Arima S, Min YG, Sakakura Y. Effects of mucokinetic drugs on rheological properties of reconstituted human nasal mucus. Arch Otolaryngol Head Neck Surg 1999; 125: 101-105.

208 Haidl P, Schonhofer B, Siemon K, Kohler D. Inhaled isotonic alkaline *versus* saline solution and radioaerosol clearance in chronic cough. Eur Respir J 2000; 16: 1102-1108.

209 Holma B. Influence of buffer capacity and pH-dependent rheological properties of respiratory mucus on health effects due to acidic pollution. Sci Total Environ 1985; 41: 101-123.

210 Holma B, Hegg PO. pH- and protein-dependent buffer capacity and viscosity of respiratory mucus. Their interrelationships and influence on health. Sci Total Environ 1989; 84: 71-82.

211 Bhaskar KR, Gong DH, Bansil R, Pajevic S, Hamilton JA, Turner BS, LaMont JT. Profound increase in viscosity and aggregation of pig gastric mucin at low pH. Am J Physiol 1991; 261: G827-G832.

212 Cao X, Bansil R, Bhaskar KR, Turner BS, LaMont JT, Niu N, Afdhal NH. pH-dependent conformational change of gastric mucin leads to sol-gel transition. Biophys J 1999; 76: 1250-1258.

213 Luk CK, Dulfano MJ. Effect of pH, viscosity and ionic-strength changes on ciliary beating frequency of human bronchial explants. Clin Sci (Lond) 1983; 64: 449-451.

214 Coakley RD, Grubb BR, Paradiso AM, Gatzy JT, Johnson LG, Kreda SM, O'Neal WK, Boucher RC. Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium. Proc Natl Acad Sci USA 2003; 100: 16083-16088.

215 Sanderson MJ, Dirksen ER. Mechanosensitive and beta-adrenergic control of the ciliary beat frequency of mammalian respiratory tract cells in culture. Am Rev Respir Dis 1989; 139: 432-440.

216 Duneclift S, Wells U, Widdicombe J. Estimation of thickness of airway surface liquid in ferret trachea *in vitro*. J Appl Physiol 1997; 83: 761-767.

217 Widdicombe JH, Bastacky SJ, Wu DX, Lee CY. Regulation of depth and composition of airway surface liquid. Eur Respir J 1997; 10: 2892-2897.

218 Smith JJ, Welsh MJ. cAMP stimulates bicarbonate secretion across normal, but not cystic fibrosis airway epithelia. J Clin Invest 1992; 89: 1148-1153.

219 Trout L, Townsley MI, Bowden AL, Ballard ST. Disruptive effects of anion secretion inhibitors on airway mucus morphology in isolated perfused pig lung. J Physiol 2003; 549: 845-853.

220 Garcia AB, Quinton P. HCO_3^- enhances mucus release in mouse small intestine. Pediatr Pulmonol 2006; 21(Suppl): 243.

221 Cystic Fibrosis Mutation Database. Cystic Fibrosis Genetic Analysis Consortium. 2003.

222 Quinton PM. ed. Fluid and Electrolyte Abnormalities in Exocrine Glands in Cystic Fibrosis. San Francisco: San Francisco Press, Inc., 1982, 298.

223 Quinton PM. Cystic fibrosis: a disease in electrolyte transport. Faseb J 1990; 4: 2709-2717.

224 Snyder JD, Merson MH. The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. Bull World Health Organ 1982; 60: 605-613.

225 Thapar N, Sanderson IR. Diarrhoea in children: an interface between developing and developed countries. Lancet 2004; 363:

641-653.

226 Quinton PM. Human genetics. What is good about cystic fibrosis? Curr Biol 1994; 4: 742-743.

227 Gabriel SE, Brigman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. Science 1994; 266: 107-109.

228 Cuthbert AW, Hickman ME, MacVinish LJ, Evans MJ, Colledge WH, Ratcliff R, Seale PW, Humphrey PP. Chloride secretion in response to guanylin in colonic epithelial from normal and transgenic cystic fibrosis mice. Br J Pharmacol 1994; 112: 31-36.

229 Best JA, Quinton PM. Simple salivary secretion assay for drug efficacy for cystic fibrosis. Exp Physiol 2005; 90(2): 189-193.

230 Cuthbert AW, Halstead J, Ratcliff R, Colledge WH, Evans MJ. The genetic advantage hypothesis in cystic fibrosis heterozygotes: a murine study. J Physiol 1995; 482 (Pt 2): 449-454.

231 Hogenauer C, Santa Ana CA, Porter JL, Millard M, Gelfand A, Rosenblatt RL, Prestidge CB, Fordtran JS. Active intestinal chloride secretion in human carriers of cystic fibrosis mutations: an evaluation of the hypothesis that heterozygotes have subnormal active intestinal chloride secretion. Am J Hum Genet 2000; 67: 1422-1427.

232 Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. The Biomed CF Mutation Analysis Consortium. Hum Mutat 1997; 10: 135-154.

233 Dumur V, Gervais R, Rigot JM, Delomel-Vinner E, Decaestecker B, Lafitte JJ, Roussel P. Congenital bilateral absence of the vas deferens (CBAVD) and cystic fibrosis transmembrane regulator (CFTR): correlation between genotype and phenotype. Hum Genet 1996; 97: 7-10.

234 Brown D, Breton S. H^+V-ATPase-dependent luminal acidification in the kidney collecting duct and the epididymis/vas deferens: vesicle recycling and transcytotic pathways. J Exp Biol 2000; 203 (Pt 1): 137-145.

235 Oppenheimer EA, Case AL, Esterly JR, Rothberg RM. Cervical mucus in cystic fibrosis: a possible cause of infertility. Am J Obstet Gynecol 1970; 108: 673-674.

236 Wang XF, Zhou CX, Shi QX, Yuan YY, Yu MK, Ajonuma LC, Ho LS, Lo PS, Tsang LL, Liu Y, Lam SY, Chan LN, Zhao WC, Chung YW, Chan HC. Involvement of CFTR in uterine bicarbonate secretion and the fertilizing capacity of sperm. Nat Cell Biol 2003; 5: 902-906.

237 Davis PB. Pathophysiology of cystic fibrosis with emphasis on salivary gland involvement. J Dent Res 1987; 66: 667-671.

238 Cohn JA, Strong TV, Picciotto MR, Nairn AC, Collins FS, Fitz JG. Localization of the cystic fibrosis transmembrane conductance regulator in human bile duct epithelial cells. Gastroenterology 1993; 105: 1857-1864.

239 Curtis CM, Martin LC, Higgins CF, Colledge WH, Hickman ME, Evans MJ, MacVinish LJ, Cuthbert AW. Restoration by intratracheal gene transfer of bicarbonate secretion in cystic fibrosis mouse gallbladder. Am J Physiol 1998; 274: G1053-G1060.

240 Wine JJ. Cystic fibrosis: the “bicarbonate before chloride” hypothesis. Curr Biol 2001; 11: R463-R466.

* * * * *

Paul M. Quinton, Ph.D.

Paul M. Quinton holds the Nancy Olmsted Endowed Chair as Professor in Pediatric Pulmonary Medicine at UC San Diego and is Professor of Biomedical Sciences at UC Riverside. He received his doctorate from Rice University in 1967 and postgraduate training in cell physiology at UCLA where he joined the faculty in 1975. He transferred to UC Riverside in 1980 and then received a concurrent appointment in Pediatrics at UCSD in 1997. Dr. Quinton is recognized internationally for his work in discovering that tissues affected in cystic fibrosis (CF) cannot properly transport chloride ions. This work provided the first description of a basic defect in cells expressing mutations in the CF gene and was fundamental to understanding this illness as a disease of abnormal electrolyte transport. His interests are now focused on understanding the role of the defective anion channel protein, CFTR, in mucus and infections in CF. He has received several awards for his work including the Nancy Olmsted Chair in Pediatric Pulmonary Medicine, the Joseph Levy Memorial Lecture from the International Cystic Fibrosis Foundation, the Paul di Sant’Agnese Distinguished Scientific Achievement Award from the National Cystic Fibrosis Foundation, the Doris F. Tulcin Cystic Fibrosis Research Award. He is an inducted Fellow in Medicine of the American Association for the Advancement of Science.