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Cystic Fibrosis: Does CFTR Malfunction Alter pH Regulation?

Douglas B. Luckie and Mauri E. Krouse

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1. Introduction

“Woe to the child kissed on the forehead who tastes salty, for it is cursed and soon must die,” is a form of diagnosis for cystic fibrosis that dates back to the Middle Ages in Eastern Europe [1]. This observation was rediscovered and made relevant in 1948 during a heat wave in New York City. Paul di Sant’Agnese at Columbia University noticed that CF patients had a larger incidence of heat prostration than others, due to excessive salt loss [2]. This ultimately led to the use of the “sweat test” (measurement of sweat electrolytes) as the definitive test for diagnosing cystic fibrosis. Originally, Dorothy Andersen in 1938 created the term cystic fibrosis [4] in describing the appearance of the pancreas from certain individuals. Later she went on to show that CF was a genetic disease [5].

Cystic fibrosis is the most common lethal genetic disease in the Caucasian population. As first postulated by Paul Quinton it is now known that it is due to a lack of chloride flux, and hence salt movement, in all affected organs [3]. For instance, in the coiled tube of the sweat gland, fluid initially has roughly the same salt concentration as blood plasma (high Na⁺ and Cl⁻). This fluid passes upward toward the skin surface through the sweat duct. Ions are reabsorbed into the body through the Na⁺ and Cl⁻ (CFTR) channels. Thus salt is normally reabsorbed out of the sweat and the concentration falls to about 1/5 of blood plasma. But in the disease cystic fibrosis the Cl⁻ channels are either absent or non-functioning and the salt concentration in the sweat remains high as it reaches the skin surface, which one can taste on the forehead of a child with CF.

The gene itself was discovered through a remarkable collaboration by the lab of Francis Collins at the University of Michigan and those of Jack Riordan and Lap-Chee Tsui at the University of Toronto in 1989 [6-8]. It was expressed and found to code for a small linear chloride channel by Christine Bear and others [9].

But the question remained, how did a missing chloride channel cause CF airway lung disease? In many organs (pancreas, lungs, and reproductive organs) it was not understood how the lack of salt movement led to the blockage of the pancreas, the disappearance of the vas deferens and bacterial infection in the lung. But the link between genotype and phenotype might have been more difficult to understand perhaps because we, the CF research field, were initially focused too narrowly on just chloride.

It is easy to think only about sodium and chloride, as they are the main salt component of plasma. But the second most common anion is bicarbonate. The CFTR channel is permeable to bicarbonate [10] thus whenever it opens both chloride and bicarbonate can move through it. With bicarbonate movement you can get corresponding changes in pH which have broad effects. In CF, while the disease pathology in many organs is attributed primarily to impaired Cl^- conductance [11], manifestations in some organs have been difficult to attribute solely to a defect in Cl^- conductance [12]. Since CFTR is permeable to HCO_3^- and has also been shown to regulate other membrane proteins [13,14] its absence in CF may lead to significant changes in intracellular or extracellular fluid composition in impacted organs. It is the investigation into changes in pH and bicarbonate that are associated with the disease cystic fibrosis, which we hope to summarize in this review.

2. Context #1: Organized by organ systems

During many years of research, scientists develop a mental encyclopedia for their field. These expert mental databases often have the same knowledge flexibly organized in different manners. Much like a software database, an expert can re-conceptualize data in different contexts. In the field of cystic fibrosis, an expert researcher tends to think about findings in several context-dependent ways like: impacted organs, research groups, animal models and chronology. We will organize several sections of this review in a similar format and then conclude with a brief discussion of the latest findings.

It all began with the pancreas. The diagnosis and naming of the disease, cystic fibrosis, resulted when Dorothy Andersen saw a pattern of fibrotic cysts that formed on the pancreas of a group of patients. That organ was first in the understanding of the disease and that organ was also first in detecting abnormalities related to pH. In the 1980s the *in vivo* pathology that ravages the pancreas in cystic fibrosis was directly tied the CFTR malfunction altering fluid and bicarbonate secretion in the pancreatic ducts [15,16]. Lost regulation of pH is a problem caused by cystic fibrosis in that organ.

2.1. The pancreas

The pancreas is both an endocrine organ, releasing hormones such as insulin and glucagon into the blood, and an exocrine organ, releasing digestive enzymes in a bicarbonate rich fluid (pancreatic juice) into the intestines and eventually out of the body. Both of these systems often become defective in individuals with cystic fibrosis. In healthy humans the pancreas can produce 2 liters of concentrated sodium bicarbonate solution (140 mM) per day [17]. The

exact mechanism for this remarkable feat is still controversial and certainly depends on the exact species under investigation (the bicarbonate concentration in mice is only 70 mM).

Simply put, the model for a healthy functioning human pancreas requires an active apical anion channel, that has some bicarbonate permeability, to be present in pancreatic duct cells, which turns out to be CFTR. The other proteins involved are a basolaterally located sodium bicarbonate cotransporter (NBC) needed to move bicarbonate into the duct cells and a Na-K pump to maintain the sodium gradient and intracellular voltage. During secretion the intracellular chloride concentration becomes so low that chloride secretion cannot be maintained and all the secretion is bicarbonate. This model was developed by many people [18] but was put to an elegant test in a paper by Whitcomb & Ermentrout [19] where they mathematically modeled the ductal secretion with predicted results that aligned exactly with those found by organ studies. The controversy in this field is simply explained as whether another protein, an apical chloride-bicarbonate exchanger, exists or is needed to produce the very high bicarbonate concentrations detected. Muallem and others [20] have shown that there is an apical membrane located chloride-bicarbonate exchanger found in mice. The activity of this exchanger appears to be coupled somehow to CFTR. But it is not certain whether this same system also is also normally active in the human pancreas.

On the other hand, the model for a poorly or non-functioning human pancreas impacted by the disease cystic fibrosis involves chloride, bicarbonate and pH. When the critical apical anion channel needed in duct cells, CFTR, is broken or missing, the system breaks down and the pancreas cannot produce the alkaline solution it normally would. Likely the lack of enough pancreatic juice to help dilute the enzymes secreted by the pancreatic acinar cells leads to pancreatic insufficiency. There is also evidence from measurements in the duodenum that support the idea that liquid secreted from the CF pancreas has a much more acidic pH [21]. The changed environment in the fluid secreted into the CF pancreatic duct leads the pancreatic enzymes to form protein plugs and block the pancreatic ducts [17]. In addition the secreted digestive enzymes, which are normally activated by acidic pH in the duodenum, may activate early and start to destroy the individual ducts. Eventually, with the help of an abnormal immune response, enough ducts are destroyed or blocked that very few enzymes reach the intestines to help digest fats and proteins, leading to the characteristic steatorrhea seen in cystic fibrosis patients. Roughly 10% of CF individual are born without a functioning pancreas. Over time this number climbs to 85% as more and more pancreatic ducts are destroyed. Those 10-15% of CF patients with pancreatic sufficiency are associated with mild CFTR mutations, or mutations that preserve some channel function.

It was work by Choi et al in 2001 [22] that brought the topic of the pancreas back to the forefront of CF research. The amazing finding was that when they compared different mutations of CFTR, they found that those channels with measureable residual bicarbonate conductance were associated with the mild form of CF in pancreatic sufficient patients and the mutations of CFTR where bicarbonate secretion was lost were always associated with the more severe version of CF, pancreatic insufficient. Later that year Jeff Wine [23] wrote a paper bringing into question some of their findings. While there may be a correlation between bicarbonate conductance and pancreatic sufficiency in cells in culture artificially expressing

CFTR, in the sweat gland under normal conditions all the patients with pancreatic insufficiency had high chloride concentration in their sweat gland secretions (the definitive test of CF is the sweat chloride level as mentioned above) and those with pancreatic sufficiency had intermediate sweat chloride levels, indicative of mild mutations that allowed for residual chloride conductance. While these sweat chloride measures supported that the chloride conductance is important in the pancreas, bicarbonate has not since left the spotlight.

The destruction of the pancreas does not stop with just damaging the exocrine function but eventually spreads to the endocrine pancreas. Cystic fibrosis related diabetes (CFRD) develops when the ducts and cells responsible for delivering insulin to the blood supply are finally destroyed and the patient develops diabetes and needs insulin. CFRD is rare at birth and develops over time. Patients with CFRD have a 6-fold higher mortality rate. It is unfortunate that the loss of an anion channel in the pancreas can lead to such devastation. Fortunately there is at least enzyme replacement therapy and insulin treatment for CF patients. This has circumvented the mortality associated with the digestive system and failure-to-thrive so that now 90% of all CF deaths are due to lung disease.

2.2. The lungs

In the 1940's cystic fibrosis transitioned from a disease of the pancreas to a disease of the airway. Many doctors were involved in clinical research at this time and it was Dr. Harry Schwachman who tried to further the idea that what was known then as "cystic fibrosis of the pancreas" was in fact a disease of many organs. At the same time Dr. Paul Di Sant'Agnese was using the newly discovered drug penicillin to treat lung infections associated with cystic fibrosis. In the 1950's Hans Ussing invented a method for investigating epithelium named the Ussing chamber. The method helped standardize experiments on epithelia and led to an increase in the scientific investigation of the organs affected in cystic fibrosis. It wasn't until the 1980s that the basic defect that leads to cystic fibrosis in the airways was found.

Initially, Drs. Knowles, Gatsy and Boucher [24] measured the difference in voltage across the nasal epithelium in 24 CF patients. They reported a much larger voltage in CF individuals and this voltage could be greatly reduced by blocking the epithelial sodium channel with amiloride. They speculated that excess sodium absorption could lead to excess salt and liquid absorption in the airway, leading to CF lung disease. Two years later they published another paper [25] "excessive active Na^+ transport can account for the abnormalities" seen in CF airway. And today excessive sodium absorption is still the leading hypothesis of how CF lung disease develops.

To review what was mentioned earlier, in 1983 Paul Quinton published the seminal paper, which correctly identified the CF defect as a loss of chloride permeability [3]. He focused his studies on the sweat gland where any abnormality found could be attributed to the CF defect. It wasn't until 1989 that Collins, Riordan and Tsui discovered the gene. They called it the cystic fibrosis transmembrane conductance regulator (CFTR) because it was not yet certain as to its function. In 1991 Bear and others expressed the CFTR gene and discovered a

small linear anion channel as the gene product. It is with this background that bicarbonate and pH became important in the airway.

One of the first reports of bicarbonate secretion in the airway was in the Ussing chamber. A special method of studying epithelial cells as a monolayer in which only the electrical currents moving into or out of the cells is measured. When cells are bathed in a solution that lacks chloride the other major anion in airway fluid and blood plasma, bicarbonate, takes its place. Smith and Welsh [26] reported that in airway epithelium under these special circumstances an elevation of cyclic AMP (cAMP) in the cell opened a channel, which could carry bicarbonate in normal tissue but was absent in CF tissue. The same thing happened when the calcium inside the cells was increased except a different set of anion channels were opened and bicarbonate can pass through in both normal and CF tissues. The following year Smith and Welsh [27] published a paper studying electrolyte transport in cultured airway epithelia cells. It was found that both normal and CF tissue secreted H⁺ in exchange for K⁺. They discovered an apical hydrogen-potassium ATPase that works to absorb potassium while secreting H⁺. It was noted that the H-K ATPase did not account for all the luminal acidification and other mechanisms of H⁺ secretions may be present. In normal airway cells the addition of forskolin to raise intracellular cAMP inhibited luminal acidification probably by stimulating bicarbonate secretion through the activated CFTR channel. Poulsen [28] then showed the same thing in transfected cells expressing the CFTR gene. C127 (mammary epithelia) and NIH-3T3 (fibroblasts) expressing normal wild-type CFTR could be made acidic inside under certain conditions and the acidity was reduced only in healthy cells when forskolin was added to the solution raising cAMP and opening CFTR channels. The alkalization was not seen either in cells transfected with the mutant $\Delta F508$ CFTR or non-transfected cells that expressed no CFTR. They went on to measure the ratio of chloride to bicarbonate permeability through the CFTR channel to be near 4:1 (3.9). Linsdell [10] then found the same result of chloride to bicarbonate permeability of 4:1 (4.0) using single channel studies. Hence 1 million bicarbonate ions could accompany the 4 million chloride ions that moved through the CFTR channel every few seconds.

A second group later repeated the experiments on airway epithelium [29]. In a different culture system both the apical culture liquid pH and the K⁺ concentration were measured. In unstimulated culture both the pH (or bicarbonate concentration) and the potassium concentration fell over 24 hours. Activating CFTR resulted in alkalization of the surface liquid in normal cultures while CF tissues were unaffected and just continued becoming more acidic. They also went on to conclude the CFTR could conduct bicarbonate under normal conditions.

A seminal paper in 1992 was published that led a few of scientist to consider a different origin of CF lung disease. The paper by Englehardt et al [30] showed by antibody staining of CFTR channels was concentrated in the submucosal glands of the human bronchus. The new paradigm was that since most of the CFTR was in the glands it was likely that CF lung disease starts in the glands. Two scientists then led the investigation of airway submucosal glands, Steve Ballard at the University of South Alabama studied intact glands in pig airway and Jeffrey Wine at Stanford University initially studied a cell line model of gland cerous cells (Calu-3) and later went on to study intact human glands. Lee et al in [31] and Devor et

al in [32] had previously established the fact that Calu-3 cells secrete bicarbonate and we, Krouse et al [33], went on to study acid and base secretion in Calu-3 cells. The cells secreted base (bicarbonate) via CFTR and secreted acid via an H-K ATPase. We quickly became very interested in the study of abnormal properties of the mucus secreted into the lungs from the glands. In cystic fibrosis this demonstrated that even before newly made mucus reached to the airway surface there was a mechanism to control the mucus pH that was altered dramatically in cystic fibrosis.

3. Context #2: Organized by research groups

Dorothy Andersen and her student Paul di Sant'Agnese at Columbia University led the first CF research groups. To gain an understanding of the work done to date, we now present a sample of the researchers in the United States who have helped make great strides in the understanding of how bicarbonate movement and pH changes may influence the pathophysiology of cystic fibrosis.

3.1. Michael Welsh and Jeffrey Smith, University of Iowa, Iowa City, IA

Dr. Michael J. Welsh is one of the most productive scientists in the field of cystic fibrosis and at the University of Iowa Medical Center has led one of the most respected CF research programs in the US. His work with Dr. Jeffrey J. Smith has been significant for scientists interested in examining pH abnormalities in CF. While the relevance of pH for the CF pancreas is accepted and understood, the thought that pH might also be important in the pathology of the human CF airway has only recently gained wide interest. The Welsh group takes advantage of a variety of methods in their studies but cell culture studied by electrophysiology, and in particular use of the Ussing Chamber, is a common technique they apply.

As early as 1992, in the *Journal of Clinical Investigation*, Smith and Welsh [26] reported that in studies with cultured canine and human epithelial cells cAMP stimulation of the CFTR channel increases bicarbonate secretion across normal, but not cystic fibrosis, airway epithelia. In this paper they posed the question, might HCO_3^- may play role in CF? A year later Welsh and Smith [27] reported their findings on how human epithelia cells control the quantity and composition of respiratory tract fluid. To do so they measured fluid and electrolyte transport by cultured human nasal epithelia and found that elevated cAMP stimulated fluid secretion across some epithelia, but for others the reverse, cAMP stimulated fluid absorption. They also reported "The finding that cAMP agonists inhibited luminal acidification may be explained by the recent finding that cAMP increases apical HCO_3^- conductance." These results provided new insights into how the intact airway epithelium may actively modify the composition of the respiratory tract fluid which drew colleagues' interest to the very thin film of liquid lining the interior of the lung, the airway surface liquid (ASL).

In 1996 Michael Welsh and Jeffrey Smith [34] published a widely read paper in the journal *Cell* where they reported a finding that cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Their evidence pointed to an abnormally high con-

centration of salt, NaCl, in the ASL that inhibits the killing of bacteria. This finding was reminiscent of the high salt seen in CF sweat glands. This paper led to a very exciting time in recent CF research. Numerous labs tried to measure the salt concentration in the lung. Some found low salt in normal lung but most found the same salt concentration in the ASL of the CF lung. The alternative hypothesis put forward, of how lung disease develops, was the model of Richard Boucher and others that the hyper absorption of the sodium leads to a depletion of the airway surface liquid, as mentioned earlier. Many people tried to find this depleted ASL in CF lungs but it has yet to be found in vivo but does exist in cultures of airway cells. In 2001 Smith and Welsh returned [35] to canine and human cells to test for the presence of HCO₃⁻ transport in epithelial cells. They reported that their data suggest that cAMP and Ca²⁺ stimulate HCO₃⁻ secretion across airway epithelium, and suggest that HCO₃⁻ leaves the cell across the apical membrane via conductive pathways. They found the cAMP-induced secretory response was absent in cystic fibrosis (CF) airway epithelial cells, although Ca²⁺-stimulated secretion was intact. This result suggested that HCO₃⁻ exit at the apical membrane is through the Cl⁻ channel that is defectively regulated in CF epithelia. These results also raise the possibility that a defect in HCO₃⁻ secretion may contribute to the pathophysiology of CF pulmonary disease.

Most recently in 2012 [36] the Welsh group reported a dramatic finding, that their data suggests in the CF pig model that reduced airway surface liquid pH impairs bacterial killing in the cystic fibrosis lung. It was found that the ASL pH is more acidic in pigs with cystic fibrosis, so in the end increasing pH in ASL restored the bactericidal activity to the ASL from the CF pigs. This finding will draw much attention in the years to come.

3.2. Robert Bridges, Rosalind Franklin University & University of Chicago, Chicago, IL

Robert Bridges joined the CF research group at the University of Alabama (UAB), Birmingham in the mid 1980s. Dr. Krouse, an author, remembers meeting Dr. Bridges at a meeting organized by the Cystic Fibrosis Foundation, where a number of researchers were discussing the purported rectifying anion channel as the CF channel. He was complaining prophetically that this small linear channel kept getting in the way of his single channel recordings. In 1992 he teamed with Bear et al [9] to show that this small linear channel was in fact the correct gene product of the CF gene (CFTR). Bob Bridges teamed with many different CF scientists at UAB. With Neil Bradbury he helped explore the role of CFTR in endo- and exocytosis. In their Science paper from [37] they concluded, "CFTR is critical for cAMP-dependent regulation of membrane recycling in epithelial tissues, and this function of CFTR could explain in part the pleiotropic nature of cystic fibrosis." Simply put, stimulation of CFTR not only opens the channel but causes insertion of CFTR containing vesicles into the membrane and slowing removal of CFTR from the membrane. Thus increasing the number of CFTR channels in the membrane.

In the mid 90's Dr. Bridges moved to the University of Pittsburgh in Pennsylvania. There he continued working on cystic fibrosis. His work with Dan Devor resulted in two papers in 1996 [38,39] that helped formulate the idea that chloride and bicarbonate secretion is dependent on the driving force for each anion. This work culminated in another paper in 1999

[32] where they showed that Calu-3 cells secreted bicarbonate and this secretion is dependent upon the mode of stimulation. They ended their abstract with possibly the understatement of the year: "If these results with Calu-3 cells accurately reflect the transport properties of native submucosal gland serous cells, then HCO_3^- secretion in the human airways warrants greater attention." In early 2000 Dr Bridges teamed with Martin Hug to investigate intact submucosal glands [40]. They found that the gland cells secrete bicarbonate in response to stimulation. In order for cells to transport bicarbonate there must be a way for bicarbonate to enter the cells. In collaboration with Kriendler et al [41] they identified two sodium bicarbonate cotransporters on the basolateral side of Calu-3 cells.

Dr Bridges' other passion, like most CF scientists, is for finding a cure for cystic fibrosis. He has collaborated with a variety of people testing various compounds for CFTR corrector activity. His papers are too numerous to mention here. After moving to Rosalind Franklin University and Chicago Medical School he continued his interest in CFTR correctors. He is head of the Cystic Fibrosis Foundation Therapeutics CFTR Modulator Chemical Compound program. Each year he gives out compounds for others scientists to use and screens thousands of new possible compounds that may "cure" CF.

3.3. Shmuel Muallem, University of Texas Southwestern, Dallas, TX

Dr. Shmuel Muallem is the leader of a significant research group at the University of Texas Southwestern and now at the NIH. One thread of his work relates bicarbonate transport to the disease cystic fibrosis and has carefully examined and identified every potential element in the mechanism of bicarbonate secretion by epithelial cells. He has done work using a variety of methodologies centered on electrophysiology and patch clamp supported by molecular genetic approaches in his group's studies to determine which genes are important for bicarbonate secretion. His research group also has published considerable research on other proteins which are essential for bicarbonate secretion, focusing on bicarbonate-secreting exchanger proteins. His work has greatly contributed to understanding the molecular mechanisms that explain cellular bicarbonate secretion and its role in the disease cystic fibrosis.

If we limit ourselves to this decade, Muallem's first papers regarding the relationship between bicarbonate transport and CF was published in 2001 [42]. It examined at the relationship between CFTR and a Na/H^+ exchanger (NHE3) in pancreatic duct cells. His group found evidence to support that CFTR indirectly regulated and inhibited Na/H^+ exchange activity at the apical membrane via PDZ binding domains, which inhibited NHE3 activity. In another study published in Nature [22] his research group found evidence that CFTR mutations associated with pancreatic insufficiency, the more severe version of cystic fibrosis, had no HCO_3^- transport. And some of them still had good Cl^- conductance. From a pH perspective they found "alkaline fluids are secreted by normal tissues, whereas acidic fluids are secreted by mutant CFTR-expressing tissues, indicating the importance of this activity." This Nature paper impacted the field in its suggestion that CF is not a result of Cl^- transport problems but rather it was all about CFTR's conductance of bicarbonate ions. However, this view did not exist long unchallenged as Jeff Wine published a paper establishing that the

measurements of chloride conductance in these experiments did not agree with the data from the sweat gland.

Muallem's group followed a significant paper in *Nature* with another in 2002 in the *Journal of Biological Chemistry* [20], which presented evidence that CFTR, and NBC3 (sodium bicarbonate cotransporter) are part of the same HCO₃-transporting complex assembled with the aid of PDZ domain-containing scaffolds, and this interaction is how CFTR regulates NBC3 activity. A few years later in 2006 [43] Muallem hypothesized that the Slc26a6 gene product (a Cl/HCO₃- exchanger) regulates and primarily inhibits CFTR activity. He followed that up by adding that the SLC26 family of transporters may be part of a transporter complex with CFTR where all regulate each other. The complex could be organized in PDZ domain scaffolds (with NHE3 and NBC3) and each transporter protein has a specific role and all in the apical membrane of epithelia. When taken altogether, the bicarbonate transport chain is formed and in cystic fibrosis it does not function and leads to the disease state. Their model supports that HCO₃- secretion is vital to epithelia cells and is mediated by a cotransporter pNBC1 at the basolateral membrane and exits the luminal side via the CFTR. Since 2009 his group has published several papers identifying the mechanism by which this CFTR-NHE-NBC complex is regulated by IRBIT and WNK/SPAK kinase pathways in the process of HCO₃- secretion.

3.4. Alan Verkman, University of California San Francisco, San Francisco, CA

Alan Verkman's research group is a force of nature with an incredible variety of productivity. Dr. Verkman's research group works at the University of California San Francisco; and his group uses microscopy and fluorescent dyes to enable the detection of ion concentrations, pH, and even precise measurements of the volume of the smallest intracellular compartment. Their findings have greatly informed the field.

In the recent decade their work has focused on whether there are changes in Na, Cl, pH and salts in general in the pathophysiology of cystic fibrosis. They often tested controversial findings from researchers in the field and either supported or refuted their work. In the early years of this decade [44] they tested and refuted the "high salt" hypothesis of the Welsh and Smith group. They found a similar ASL tonicity and pH in cystic fibrosis (CFTR-null) mice as well as human bronchi. Their findings regarding Na and Cl levels being unchanged led them to conclude this provided "direct evidence that the ASL is approximately isotonic and not saltier in cystic fibrosis." In 2001 they also published a report that measured the ionic composition and viscosity of fluid from airway submucosal glands. Neither Na⁺ nor pH differed in gland fluid from CF airways versus controls but mucus viscosity was significantly elevated compared to normal airways [45]. In 2002 they reported that Cl⁻ flux is important to support the normal acidification process that occurs in the cell's endosomes but CFTR does not really change the acidification of endosomes because other Cl⁻ channels, ClC-5, are present, at least in mice [46]. This refuted a variety of hypotheses that endosome pH was abnormal in cystic fibrosis, which perhaps altered the characteristics of mucus produced in the airway.

In a *Journal of General Physiology* paper in 2003 [47] they reported findings with a novel lung preparation designed to measure ASL composition and depth in small distal airways. Distal ASL was studied with ion- or pH-sensitive dyes. The ASL pH was found to be 7.28 and not affected when CFTR was inhibited by the drug CFTRinh-172.

In 2004 they started seeing pH differences. They tested airway submucosal gland fluid by using a selective CFTR inhibitor (CFTRinh-172) in pig and human airways. Gland fluid pH was 7.1 and was reduced by 0.4 units after CFTR inhibition [48].

In 2006 they investigated whether gland fluid pH is abnormal in early CF, using nasal biopsies from pediatric subjects having minimal CF lung disease [49]. Having minimal disease is very important as Fischer and Widdecombe published a paper showing the airway surface pH changes with the longitudinal progression of different disease states. Gland fluid pH, was 6.57 in biopsies from six CF subjects, much lower than the recorded pH of 7.18 in eight non-CF biopsies. In pig trachea and human bronchi, gland fluid pH was also reduced by up to 0.45 units by CFTR inhibitors. They found evidence for CFTR-dependent bicarbonate transport by the tracheal epithelium and stated: "These results provide evidence for intrinsic hyperacidity in CF gland fluid secretions, which may contribute to CF airway pathology." Their group's recent papers have focused again on intracellular pH in the lysosomes of macrophages and epithelia from humans and mice and found no evidence it is altered in CF. "We conclude that biologically significant involvement of CFTR in organellar acidification is unlikely." [50].

3.5. Paul Quinton and Malla Reddy, University of California, La Jolla, CA

In a publication in 1983 Paul Quinton [3] was the first investigator to make the connection that cystic fibrosis might be caused by a broken Cl⁻ channel. He also may have been the first CF researcher to have the disease. When he was 19 he diagnosed himself, "I started reading up on bronchitis and bronchiectasis out of curiosity about my own lung problems. When I noticed a footnote that referenced cystic fibrosis, the definition of the disease gave me chills because everything seemed to fit with what I was already experiencing." Paul Quinton works at the University of California-San Diego; his research with Dr. Malla Reddy has used human cells and electrophysiological techniques to explore the problems in CF.

In the recent decade their work has focused on how changes in pH and bicarbonate ion concentrations could lead in the pathophysiology of cystic fibrosis through changes in mucus. In the year 2000 Quinton and Reddy [51] reported that they found the CFTR anion channel is somewhat permeable to bicarbonate anions. At the time Quinton was unsure that this new fact was relevant to the pathogenesis of cystic fibrosis at normal physiological levels. In a 2001 paper in the *Journal of the Pancreas* [52], Quinton and Reddy found evidence that the presence of mutated CFTR correlated to a decrease in cell membrane permeability to bicarbonate ions. They also found that the severity of disease was related to the phenotypic ability of a mutant CFTR to express a bicarbonate conductance and different CF mutations may have differing level of bicarbonate conductance, this is very similar to the views expressed by Shmuel Muallem about the loss of bicarbonate conductance being the most important

this in the pancreas. They surmised that the severity of the pathogenesis in CF might be closely related to the phenotypic ability of a mutant CFTR to express a HCO₃⁻ conductance.

Very recently, in 2010, Paul Quinton reported in the *Journal of Physiology* [53] that CF epithelial cells in the cervix could secrete fluid normally, but unlike wild type cells, they could not secrete bicarbonate. And furthermore that mucus released in CF epithelial was severely impaired even though stimulated fluid secretion was similar to healthy cells. They reported “Mucus release was severely inhibited in the absence of serosal HCO₃⁻, HCO₃⁻ transport, or functional CFTR.” In the same year in an *American Journal of Physiology* report the [54] Quinton group found that extracellular bicarbonate is necessary for mucoviscosity. They hypothesized that extracellular bicarbonate could chelate calcium from the mucins at the moment of secretion to allow the mucins to fully expand. In CF disease the mucins don’t fully expand and appear as “dehydrated mucus.” Lastly in a 2010 review, Quinton [55] put forward the possibility that mucus build up in cystic fibrosis patients may largely be caused by bicarbonate disruption, not by salt/fluid imbalance as traditionally explained. This now has supplemented an existing hypothesis of how lung disease develops, that is that the mucus from the submucosal glands is already defective as it emerges and adheres to the glands and airway surface providing an island for bacterial growth. In this case though, Paul Quinton has put forward a mechanism (mentioned above) whereby lack of bicarbonate leads to this sticky mucus. This will lead to many more experiments as it is tested.

3.6. Steve Ballard, University of South Alabama, Mobile, AL

Steve Ballard started his career in cystic fibrosis research working with Richard Boucher and John Gatzky at the University of North Carolina at Chapel Hill. His first paper [56] helped form the basis for one of the leading model of CF lung disease. That is that alveolar cells secrete fluid and the rest of the lung absorbs that fluid. After moving to the University of South Alabama in Mobile, Ballard and others [57] showed in distal bronchi that most of the secretion comes from submucosal glands. This helped form the basis for a second model of CF lung disease. That is that the disease pathophysiology of cystic fibrosis originates in the submucosal glands. In subsequent papers [58,59] the Ballard lab showed that bronchial secretion was due to both chloride secretion and bicarbonate secretion.

In 1997 Steve Ballard and others [60] published a paper on the visualization of bronchial submucosal glands from pigs. Dr. Krouse, an author, remembers watching their movies of submucosal glands secretion, with the gland orifice opening, mucus streaming out and cilia beating, showing that glands were an exciting dynamic part of airway fluid secretion. In the same year they discovered that “inhibition of the anion (chloride and bicarbonate) secretion response to acetylcholine leads to mucus obstruction of submucosal gland ducts that resembles the early pathological changes observed in CF.” This result provided another link between cystic fibrosis and submucosal gland function. In the following paper [61] they also noted that the mucus (after inhibition of anion secretion) on the airway surface was different, having less water and altered rheology, similar to CF.

By the turn of the century the Ballard lab had established that most of the secretion in the upper airway was due to chloride and bicarbonate secretion through CFTR in the submu-

cosal glands. That inhibition of anion secretion leads to impacted mucus within glands and defective mucus on the airway surface. In a paper by Trout et al. [62] they showed that much more of the airway is covered by defective mucus when the glands are stimulated to secrete and there was no measureable goblet cell secretion. They concluded that the source of the defective mucus on the airway surface was from the submucosal glands. They concluded, "that inhibition of anion and liquid secretion in porcine lungs disrupts the normal morphology of airway surface mucus, providing further evidence that impaired anion secretion alone could account for critical aspects of CF lung disease." In 2004 Ballard and Inglis [63] published an excellent review summarizing their findings.

3.7. Terry Machen and Horst Fischer, University of California Berkeley, Berkeley CA

Drs. Terry Machen and Horst Fischer have been collaborators at the University of California Berkeley for two decades and were perhaps the first group to put forward the idea that pH and HCO₃⁻ may be aberrant in the disease cystic fibrosis. They most frequently use the methods of electrophysiology, including transepithelial short-circuit current measurements by Ussing Chamber (sometimes with pH-stat technique), whole cell and single cell patch clamp techniques, and nasal potential difference (PD) measurements from living subjects. An early PNAS paper [28] directed the field's attention to the topic HCO₃⁻ permeability through the CFTR channel. Their study assessed intracellular pH and channel activity of mouse wild type or mutant CFTR-expressing epithelial cells. They found evidence that HCO₃⁻ could move into cells through CFTR channels and hypothesized since the electrochemical gradients of both Cl⁻ and HCO₃⁻ are physiologically directed outward, "HCO₃⁻ secretion may be important for controlling pH of the luminal, but probably not the cytoplasmic, fluid in CFTR-containing epithelia. In CF, a decreased secretion of HCO₃⁻ may lead to decreased pH of the luminal fluid."

In a 1996 Pflugers Archives paper [64] this research group studied intracellular pH regulation using a fluorescent dye, BCECF, and cultured bovine epithelial cells. They again found evidence that HCO₃⁻-dependent, Na and Cl⁻ independent, pHi recovery may be due largely to an influx of HCO₃⁻ via CFTR Cl⁻ channels. They suggested that CFTR may mediate HCO₃⁻ secretion and contribute to regulation of pH in periciliary fluid. In the following American Journal of Physiology paper [65] they extended this line of inquiry in Ussing Chambers and using a variety of channel blockers. They found "Blocker effects were absent in human CF tracheal cells homozygous for the delta F508 mutation of CFTR (CFT1); Cl⁻ and HCO₃⁻ currents were rescued in CFT1 cells recombinantly expressing wild-type CFTR. Thus CFTR functions as a HCO₃⁻ and Cl⁻ conductor, and genistein and bromotetramisole maximize CFTR activity in airway epithelial cells." In 1999 in Pflugers Archives [66] they evaluated a new cell model for airway submucosal gland cells, Calu-3, and reported data elucidating the ion selectivity of the CFTR channel.

Their research group also explored another question related to pH, whether the intracellular organelles like the endosome, lysosome or golgi which acidify themselves are altered in cystic fibrosis. In a Chandy et al [67] paper they used pH-sensitive dyes targeted to the golgi apparatus and reported "Comparison of genetically matched DeltaF508 and wt-CFTR cells

showed that the absence of CFTR statistically increased Golgi acidity by 0.2 pH units, though this small difference was unlikely to be physiologically important." In 2006 Fischer co-authored a review paper with Jonathan Widdicombe [68] which directed the fields attention to mechanisms that might explain pH changes in the lung's airway surface liquid, ASL, could alter the function of antimicrobial factors that mean to keep the lung sterile. They also point out: "CFTR Cl⁻ channel conducts HCO₃⁻ and, therefore, may contribute to ASL pH. However, the acidity of the ASL indicated parallel mechanisms for H⁺ secretion. Recent investigations identified several H(+) transporters in the apical membrane of the airway epithelium." Thus we should not ignore the role proton channels will also play in modifying pH in the airway surface liquid. In a recent publication [69] this research group explored human nasal mucosa pH and rate of acid and base secretion using Ussing chambers and the pH-stat technique. They found the pH of nasal epithelia from CF patients was pH = 7.08 while that in normal subjects was pH = 7.34. They conclude with "Our data suggests that CF patients exhibited significantly lower base secretion by the nasal airway epithelium. It is possible that improper regulation of ASL pH in CF may negatively impact the innate host defense system."

3.8. Lane Clarke, University of Missouri, Columbia, MO

Lane Clarke started studying cystic fibrosis at the University of North Carolina, Chapel Hill, under the guidance of Richard Boucher and Mike Knowles. Their first paper together [70] showed that cystic fibrosis patients responded to extracellular ATP and UTP, suggesting the presence of functional P2 receptors in the airway. In 1992 Clarke et al [71] showed that in the newly engineered CF mice that there was a lack of chloride transport in the mouse intestine. In 1993 they [72] explained one of many CF mysteries. They found evidence that the channel originally claimed to be the CF channel (the outwardly rectifying anion channel) was regulated by CFTR. This could explain the earlier findings that the rectifying channel was not gated properly by cAMP in CF tissues.

Upon moving to the University of Missouri, Lane Clarke continued his work on epithelia, especially the intestine. With Harline in [73] he published a paper that CFTR was responsible for both chloride and bicarbonate secretion in the mouse duodenum. He also found an chloride/bicarbonate exchanger in the intestine that was regulated by CFTR. He also described an apical H-K ATPase in the mouse colon [74]. It is interesting in two systems, the lungs and the intestine, there exists a mechanism for secreting both base (bicarbonate) and acid (H⁺). Two years later the Clarke group found yet another mechanism by which CFTR controls the apical pH [75], they found evidence that CFTR regulates a Na⁺/H⁺ exchanger.

In 2004 Dr. Clarke published a paper [76] that changed the thinking on how CF disease develops and added a new word, bioavailable, to our vocabulary. They showed that "Paneth cell granules undergo limited dissolution and accumulate within the intestinal crypts of cystic fibrosis (CF) mice." These granules should carry the antimicrobials from the Paneth cells to the intestinal surface. In CF the antimicrobials are secreted into the crypts but are not bioavailable to help kill bacteria. This result influenced people like Jeff Wine where it was hypothesized that the antimicrobials secreted by the airway submucosal glands are not bio-

vailable on the airway surface where they are needed. In 2009 [77] the Clarke lab identified the anion exchanger that is regulated by CFTR. It was named "Down-regulated in adenoma" (DRA later changed to be SLC26A3) and was responsible for all the basal bicarbonate secretion and $\frac{1}{2}$ of the cAMP stimulated current in the mouse intestine.

Lane Clarke has shown that in the intestine, the CFTR is a member of a large family of proteins that can regulate the pH within the organ. Describing the huge variety of bicarbonate secretors and acid secretors involved in intestinal pH regulation the Clarke's research group unearthed would take a review of its own and will not be attempted here.

3.9. Mike Knowles and Richard Boucher, University of North Carolina, Chapel Hill, NC

Mike Knowles and Ric Boucher first teamed up at the University of North Carolina, Chapel Hill in 1981 [78,79] and adapted technique of measuring the difference in voltage across the nasal epithelium to humans. They quickly applied this new technique to cystic fibrosis where the potential difference in CF was much higher than in normal individuals. Much of the potential difference could be inhibited by amiloride, which blocked the apical sodium channel (ENaC). This led them to conclude, "the greater reduction in potential difference in response to amiloride suggests that absorption of excess salt and perhaps liquid from respiratory epithelial surfaces contributes to the pathogenesis of lung disease in cystic fibrosis." This was the basis for the leading theory of how CF lung disease develops. The airway absorbs too much liquid via increased sodium absorption leading to dehydration of the mucus. The dehydrated mucus becomes plastered to the airway surface and provides a breeding ground for bacteria. This was the theory in the 1980's and is still the theory today, with minor modifications. In another paper [80] they agreed with Paul Quinton that there is loss of chloride permeability in CF, but the cause of CF lungs disease was still the excess fluid absorption. The link between the loss of an anion channel and the activation of a sodium channel was not clear at that point and many researches in the field searched for the link.

In collaboration with Jim Yankaskas [81] they discovered that CF epithelial cells retained the characteristic chloride permeability loss and sodium absorption increase in culture. In 1986 they published a review [82] which is a good summary of the early history of CF. To test the theory that excess sodium absorption was the problem, they did a pilot study [83] of the effect of amiloride on 14 CF patients over 1 year. The loss of forced vital capacity over that year was slowed and the rheology of the sputum improved. Thus it appeared that blocking ENaC might provide a way to help cure or prevent CF lung disease.

In the mid 90's, thanks to a paper published by Smith et al [34], there was a big debate whether the salt concentration in the surface liquid was different between normal and CF cells. Simply put, was the airway like the sweat gland and CF individuals had salty airway fluid? In 1997 Knowles and Boucher [84] published a paper where they measured the ion concentrations. They used filter paper to sample the airway liquid and found no difference in any concentration between normal and CF. Interestingly, they found very little anion gap (supposedly bicarbonate) in CF or normal airways. The only thing odd was the very high concentration of potassium in the fluid (between 15-30 mM).

In 2002 Knowles and Boucher [85] published an excellent review of their view on how CF lung disease develops. The work included many scientists at their institution and other places, too many to cover here. In their model a healthy lung's alveoli secrete large amounts of fluid that the rest of the lung absorbs via sodium absorption. Yet in CF the increased ENaC activity in the airway leads to a dehydration of the airway surface until the fluid level drops below that of the height of the cilia. The mucus becomes dehydrated and stationary, a stable island more easily colonized by bacteria. This does not happen across the entire lung in general as the act of breathing and walking releases ATP onto the surface in many places and opens a second chloride channel, which supports rehydration. However the hydration balance of the CF airway is very precarious. Insults to the airway such as noxious fumes or viruses upset the balance and lead to a patchy distribution of bacterial infection in the lung.

3.10. Jeffrey Wine and Mauri Krouse, Stanford University, Stanford, CA

Dr. Jeffrey Wine switched the whole direction of his research in 1986. Turning from a successful career in crayfish behavior to trying to find the cause and cure for cystic fibrosis. Jeff came to Stanford in the early 1970's as an assistant professor of psychology. In 1981, doctors diagnosed his first daughter Nina with CF, forever changing his personal and professional life. "My daughter could have been born with many diseases and I wouldn't have decided to work on them. But I saw a paper that said CF is an ion channel disease and I knew about ion channels," Wine recalled. Moving from the science of neurons to the function of CFTR in ion channels marked a change in focus that led to 30+ years of contributions to understanding the basic defect in CF. After a sabbatical in Paul Quinton's research lab, just one year, he published his first paper with Behm et al [86] expanding on the previous work of Sato and Quinton. His paper is still important today as it measured the secretion rate in sweat glands and found that CF heterozygotes had $\frac{1}{2}$ the secretion rate of normals whereas CF sweat glands have no secretion to beta-adenergetic stimulation.

Some 24 of those years he worked in collaboration with Dr. Mauri Krouse. I joined the Wine lab at the very beginning of its transition to CF work, in 1986, which also was the year when two papers (see section on Mike Welsh) were published claiming to have discovered the chloride channel (an outwardly rectify chloride channel) that was defective in cystic fibrosis. The laboratory of Prof. Wine could not repeat these experiments and found it likely CF was caused by another channel. Jeff Wine led a 5-year campaign to discover the correct anion channel that was the cause of cystic fibrosis. In a meeting of the North American Cystic Fibrosis society in 1990 in Arlington Virginia, Wine and Krouse, with collaborators from Stanford University, submitted an abstract on a small linear chloride channel that was activated by cAMP in canine airway cells. This was followed up with a paper the next year [87] where they showed the small linear anion channel and finding no correlation between the claimed outwardly rectifying chloride channel and levels of CFTR expression. Others at that 1990 meeting had also noted the small linear chloride channel such a M.A. Gray and B. E. Argent from the UK and Dr. Grygorczyk from Canada. In 1991 the CFTR channel was confirmed as a small linear chloride channel by Bear et al when they expressed recombinant CFTR in cells [9]. Once the small CFTR channels identity was confirmed Wine and Krouse teamed with

Christine Haws to be the first to describe a kinetic model for the gating of CFTR [88]. This model has since been modified and expanded, by researchers such as David Sheppard in the UK and TC Hwang in Missouri, but it was the first. This same year a very influential paper from Engelhard et al [30] was published using “in situ hybridization and immunocytochemistry to characterize the cellular distribution of cystic fibrosis (CF) gene expression in human bronchus.” They discovered that most of the CFTR in the lung was in the submucosal glands. After reading this paper Jeffrey Wine again changed the course of his lab to almost exclusively study submucosal glands. Within two years he helped discover the now famous submucosal gland cell line (Calu-3) with the labs of Drs. Walt Finkbeiner and Jonathan Widdicombe at UCSF [89]. Again Christine Haws worked to carefully measure the channel properties of CFTR in Calu-3 cells.

Unfortunately the Calu-3 cells represented healthy tissue and there was no CF version of the cell (even though many people have tried to make some), thus Haws, Krouse and Wine [90] used a stably transfected mouse mammary cell line to measure the properties of $\Delta F508$ CFTR. The values they found over 16 years ago are still true today. The channel density of $\Delta F508$ CFTR on the surface is less than 5% of wild type and the open probability is $\sim 1/3$ of normal CFTR. With the realization that the main form of cystic fibrosis was due to the loss of channels, Prof Wine abandoned patch clamping single cells and concentrated on sheets of cells in the Ussing chamber. Monolayers of Calu-3 cells secreted bicarbonate [31] and most of the unstimulated resting secretion was bicarbonate-dependent [91]. Work with Luckie et al [92] found evidence that in a variety of cells expressing CFTR that bicarbonate may be secreted because mutant non functional CFTR altered the pH surrounding cells.

Using an ingenious method Irokawa [93], and Wine designed a chamber where all the secretion of a sheet of epithelial cells was secreted out a tiny orifice, they called the apparatus the “virtual gland.” When Calu-3 cells were tested in the virtual gland the bicarbonate concentration of the fluid secreted to carbachol (a Ca^{2+} elevating agonist) was the same as the bathing solution (25 mM), but when the cells were stimulated with forskolin (a cAMP elevating agent that opens CFTR) the bicarbonate concentration rose to ~ 80 mM. Roughly half the secretion was due to bicarbonate secretion in the virtual gland. This was confirmed by Krouse et al [33] in the Ussing chamber with the addition that there existed a H-K ATPase to help neutralize some of the bicarbonate which was secreted. Such a mechanism had been previously reported by Smith and Welsh [27] in airway cells and again by Coakley et al [29] in primary airway cell cultures. In the same year Wu et al [94] published a paper showing an inwardly rectifying potassium channel (Kir4.2) in the apical membrane of Calu-3 cells and also in the apical membrane of freshly dissected airway submucosal glands. This channel may supply the potassium needed by the H-K ATPase.

In recent years the Wine research group has been in the middle of the most exciting topics in the field, new studies on mucus [95], new drugs developed to correct the disease [96] and the hottest topic in the research field today, the CF Pig [97,98].

4. Final context: What's new, what's next?

Everything new these days in the field of cystic fibrosis research seems to have something to do with a pig. The birth announcement for the first CF pig occurred in 2008 [99]. It was a collaboration of over 20 people at the University of Iowa and the University of Missouri. They noted that “these pigs should be of value in producing new models of CF” and they certainly have. While CF mice do not develop lung disease, CF pigs do. The pathology of the newborn pig mirrors that of human infants [100]. They have involvement of the pancreas, intestines (including meconium ileus), liver and gallbladder. In the pig lungs have no initial inflammation or infection. Within months the CF piglets develop infection, inflammation, remodeling and mucus accumulation [101] just like human lungs. The piglets failed to clear/eradicate bacteria in the lungs suggesting a defective defense system that was apparent within hours after birth. An unexpected finding was reduced levels of insulin-like growth factor were reduced in the CF pig [102]. When human CF infants were tested they found a reduced IGF1 level. This finding might explain why some patients fail to reach their full growth potential even under the best clinical care. It also suggests a new IGF1 supplement therapy for newborns with CF.

A closer look at the lung of CF piglets revealed that there was a loss of both chloride and bicarbonate secretion [103]. They found no increase of sodium or liquid absorption and no change in the depth of the periciliary liquid depth. The defect in sodium transport is the hallmark of the leading hypothesis of how airway lung disease develops. But the authors point out that these neither are not mature pigs nor are they $\Delta F508$ pigs, but rather piglets lacking CFTR. In 2011 a study of $\Delta F508$ pigs was published [104], even though about 6% (compared to normal) CFTR makes it to the cell surface the results are the piglets are almost identical with the earlier knockout experiments. The lungs still display a greatly reduced chloride and bicarbonate secretion. A second unexpected finding was that the teeth of CF piglets are hypomineralized [105]. Both CFTR and an anion exchanger (AE2) expression went up during the enamel maturation stage. They proposed that there is an increased demand for chloride and/or bicarbonate during the development of the teeth.

Just this year the CF pig revealed another important clue to the pathogenesis of CF airway disease. At birth CF lungs are sterile and the infection is due to a defect in a basic defense mechanism so that the bacteria are not eradicated. In a recent Nature paper [36] they found that the pH of the airway surface liquid determines the killing properties of the lungs. Airway surface liquid from normal piglets effectively killed bacteria and if the pH was made more acidic the killing was reduced. The airway surface liquid from CF pigs did not kill bacteria until the pH was made more alkaline. To quote from their abstract “these results directly link the initial host defense defect to the loss of CFTR, an anion channel that facilitates HCO_3^- transport.” This paper just might be a new paradigm shift for how a loss of an anion channel leads to CF lung infection.

5. Conclusions

The future is bright for the CF pig. And perhaps the most dramatic finding for those who study pH is the one by Pezzulo et al [36]. They found evidence in CF pig model that reduced airway surface liquid pH impairs bacterial killing in the cystic fibrosis lung. This stands upon an earlier finding by Verkman's group when they found that gland fluid pH is abnormal in early CF, using nasal biopsies from pediatric subjects having minimal CF lung disease before bacteria and immune response could disturb the system [49]. Thus the reduced pH in the thin layer of fluid that lines the lungs may play an important role in the ability of pathogenic bacteria to colonize the airway in cystic fibrosis.

Beyond the alteration of pH, bicarbonate itself may play an important role in the abnormal behavior of mucus in cystic fibrosis. Quinton's new hypothesis [55], that in a healthy person the extracellular bicarbonate functions to chelate calcium from the mucins at the moment of secretion to allow the mucins to fully expand but in CF the mucins don't fully expand and appear as "dehydrated." The possibility that mucus build up in cystic fibrosis patients may largely be caused by bicarbonate disruption, not by salt and fluid imbalance, is intriguing idea from a man who has been correct before.

These results discussed in this review provide evidence for an intrinsic defect in the ability of CF tissues to secrete base [29] or regulate pH, and they come closer to conclusively demonstrating that CF airway surface liquid is abnormally acidic. More data needs to be collected concerning the steady-state pH of the airway surface liquid of humans under physiological conditions. If such a pH difference is eventually established, it will then be necessary to determine if attempts to correct it might improve the health of patients.

Author details

Douglas B. Luckie¹ and Mauri E. Krouse²

¹ Department of Physiology, Michigan State University, East Lansing, Michigan, USA

² Flatley Discovery Lab, The Schrafft Center, Charlestown, MA, USA

References

- [1] Busch R. On the history of cystic fibrosis. *Acta Univ. Carol.* 1990;36:13-15.
- [2] Di Sant'Agnese, PA, Darling RC, Perrera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. *Pediatrics* 1953;12: 549-563,
- [3] Quinton PM. Chloride impermeability in cystic fibrosis. *Nature* 1983;301:421-422.

- [4] Andersen DH. Cystic Fibrosis of the pancreas and its relation to celiac disease. *Am J. Dis. Child.* 1938;56: 344-399.
- [5] Andersen DH, Hodges G. Celiac syndrome V. Genetics of cystic fibrosis of the pancreas with a consideration of the etiology. *Am. J. Dis. Child.* 1946;72: 62-80.
- [6] 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;245:1073-80.
- [7] Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;245:1066-73.
- [8] Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989;245:1059-65.
- [9] Bear CE, Li CH, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, Riordan JR. Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell.* 1992;68(4):809-18.
- [10] 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.
- [11] Fuller CM, Benos DJ. Cftr! *Am J Physiol* 1992;263:C267-86.
- [12] Quinton PM. Physiological basis of cystic fibrosis: a historical perspective. *Physiol. Rev.* 1999;79:S3-S22.
- [13] Donaldson SH, Poligone EG, Stutts, MJ. CFTR regulation of ENAC. *Methods in Molecular Medicine-Cystic Fibrosis Methods and Protocols II*, 2002;70(3)343-364.
- [14] 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;274:14670-7.
- [15] Ahmed N, Corey M, Forstner G, Zielenski J, Tsui L-C, 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.
- [16] Kopelman H, Corey M, Gaskin K, Durie P, Weizman Z, Forstner G. Impaired chloride secretion, as well as bicarbonate secretion, underlies the fluid secretory defect in the cystic fibrosis pancreas. *Gastroenterology* 1988;95: 349-355.
- [17] Ishiguro H, Steward M, Naruse S. Cystic fibrosis transmembrane conductance regulator and SLC26 transporters in HCO₃⁻ secretion by pancreatic duct cells. *Acta Physiologica Sinica* 2007;59(4): 465-476.

- [18] 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(2 Pt 1):C240-51.
- [19] Whitcomb DC, Ermentrout GB. A mathematical model of the pancreatic duct cell generating high bicarbonate concentrations in pancreatic juice. *Pancreas* 2004;29(2):e30-40.
- [20] Park M, Ko SB, Choi JY, Muallem G, Thomas PJ, Pushkin A, et al. The cystic fibrosis transmembrane conductance regulator interacts with and regulates the activity of the HCO₃⁻ salvage transporter human Na⁺-HCO₃⁻ cotransport isoform 3. *J Biol Chem*. 2002;Dec 27;277(52):50503-9.
- [21] Kaur S, Norkina O, Ziemer D, Samuelson LC, De Lisle RC. Acidic duodenal pH alters gene expression in the cystic fibrosis mouse pancreas. *Am J Physiol Gastrointest Liver Physiol*. 2004;287(2):G480-90.
- [22] Choi JY, Muallem D, Kiselyov K, Lee MG, Thomas PJ, Muallem S. Aberrant CFTR-dependent HCO₃⁻ transport in mutations associated with cystic fibrosis *Nature*. 2001;Mar 1;410(6824):94-7.
- [23] Wine JJ. Cystic fibrosis: the 'bicarbonate before chloride' hypothesis. *Current Biology* 2001;Vol 11 No 12 R463-466.
- [24] Knowles M, Gatz J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N. Engl J Med*. 1981;305(25): 1489-95.
- [25] Knowles M, Gatz J, Boucher R. Relative ion permeability of normal and cystic fibrosis nasal epithelium. *J. Clin Invest*. 1983;71(5):1410-7.
- [26] Smith JJ, Welsh MJ. cAMP stimulates bicarbonate secretion across normal, but not cystic fibrosis airway epithelia. *J Clin Invest*. 1992;89: 1148-1153.
- [27] Smith JJ, Welsh MJ. Fluid and electrolyte transport by cultured human airway epithelia. *J Clin Invest*. 1993;91(4): 1590-1597.
- [28] Poulsen JH, Fischer H, Illek B, Machen TE. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci USA*. 1994;91:5340-4.
- [29] Coakley RD, Grubb BR, Paradiso AM, Gatz 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(26):16083-8.
- [30] Engelhardt JF, Yankaskas JR, Ernst SA, Yang Y, Marino CR, Boucher RC, Cohn JA, Wilson JM. Submucosal glands are the predominant site of CFTR expression in human bronchus. *Nat. Genet*. 1992;2: 240-247.
- [31] Lee MC, Penland CM, Widdicombe JH, Wine JJ. Evidence that Calu-3 human airway cells secrete bicarbonate. *Am J Physiol*. 1998;274(3 Pt 1):L450-3.

- [32] 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(5):743-60.
- [33] Krouse ME, Talbott JF, Lee MM, Joo NS, Wine JJ. Acid and base secretion in the Calu-3 model of human serous cells. *Am J Physiol Lung Cell Mol Physiol.* 2004;287(6):L1274-83.
- [34] 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(2):229-36.
- [35] Welsh MJ, Smith JJ. Stimulation of HCO₃⁻ secretion across airway epithelia. *JOP.* 2001;2(4 Suppl):291-3.
- [36] Pezzulo AA, Tang XX, Hoegger MJ, Alaiwa MH, Ramachandran S, Moninger TO, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung *Nature* 2012;487 (7405) 109–113.
- [37] Bradbury NA, Jilling T, Berta G, Sorscher EJ, Bridges RJ, Kirk KL. 1992 Regulation of plasma membrane recycling by CFTR. *Science.* 256(5056): 530-2.
- [38] Devor DC, Singh AK, Frizzell RA, Bridges RJ. 1996 Modulation of Cl⁻ secretion by benzimidazolones. I. Direct activation of a Ca(2+)-dependent K⁺ channel. *Am J Physiol.* Nov;271(5 Pt 1):L775-84.
- [39] Devor DC, Singh AK, Bridges RJ, Frizzell RA. 1996. Modulation of Cl⁻ secretion by benzimidazolones. II. Coordinate regulation of apical GCl and basolateral GK. *Am J Physiol.* 271(5 Pt 1):L785-95.
- [40] Hug MJ, Bridges RJ. pH regulation and bicarbonate transport of isolated porcine submucosal glands. *JOP.* 2001;2(4 Suppl):274-9.
- [41] Kreindler JL, Peters KW, Frizzell RA, Bridges RJ. Identification and membrane localization of electrogenic sodium bicarbonate cotransporters in Calu-3 cells. *Biochim Biophys Acta.* 2006;1762(7):704-10.
- [42] Lee MG, Ahn W, Lee JA, Kim JY, Choi JY, Moe OW, Milgram SL, Muallem S, Kim KH. Coordination of pancreatic HCO₃⁻ secretion by protein-protein interaction between membrane transporters. *JOP.* 2001;2(4 Suppl): 203-6.
- [43] 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 HCO₃⁻ secretion: relevance to cystic fibrosis *EMBO J.* 2006;25(21):5049-57.
- [44] Jayaraman S, Joo NS, Reitz B, Wine JJ, Verkman AS. Submucosal gland secretions in airways from cystic fibrosis patients have normal [Na(+)] and pH but elevated viscosity. *Proc Natl Acad Sci USA.* 2001;98(14):8119-23.
- [45] Jayaraman S, Song Y, Verkman AS. Airway surface liquid pH in well-differentiated airway epithelial cell cultures and mouse trachea. *Am J Physiol Cell Physiol.* 2001;281(5):C1504-11.

- [46] Hara-Chikuma M, Wang Y, Guggino SE, Guggino WB, Verkman AS. Impaired acidification in early endosomes of ClC-5 deficient proximal tubule. *Biochem Biophys Res Commun.* 2005;329(3):941-6.
- [47] 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(5):511-9.
- [48] Thiagarajah JR, Song Y, Haggie PM, Verkman AS. A small molecule CFTR inhibitor produces cystic fibrosis-like submucosal gland fluid secretions in normal airways. *FASEB J.* 2004;18(7): 875-7.
- [49] Song Y, Salinas D, Nielson DW, Verkman AS. Hyperacidity of secreted fluid from submucosal glands in early cystic fibrosis. *Am J Physiol Cell Physiol.* 2006;290(3):C741-9.
- [50] Haggie PM, Verkman AS. Defective organellar acidification as a cause of cystic fibrosis lung disease: reexamination of a recurring hypothesis. *Am J Physiol Lung Cell Mol Physiol.* 2009;296(6):L859-67.
- [51] Quinton PM, Reddy MM. CFTR, a Rectifying, Non-Rectifying Anion Channel? *J Korean Med Sci.* 2000;15(Supp):S17-20.
- [52] Reddy MM, Quinton PM. Selective activation of cystic fibrosis transmembrane conductance regulator Cl⁻ and HCO₃⁻ conductances. *JOP.* 2001;2(4 Suppl):212-8.
- [53] Muchekehr RW, Quinton PM. A new role for bicarbonate secretion in cervico-uterine mucus release. *J. Physiol.* 2010;588(Pt 13):2329-42.
- [54] Chen EY, Yang N, Quinton PM, Chin WC. A new role for bicarbonate in mucus formation. *Am J Physiol Lung Cell Mol Physiol.* 2010;299(4):L542-9.
- [55] Quinton PM. Role of epithelial HCO₃⁻ transport in mucin secretion: lessons from cystic fibrosis. *Am Journal of Cell Physiol.* 2010;299(6):C1222-33.
- [56] Krochmal EM, Ballard ST, Yankaskas JR, Boucher RC, Gatzky JT. Volume and ion transport by fetal rat alveolar and tracheal epithelia in submersion culture. *Am J Physiol.* 1989;256(3 Pt 2):F397-407.
- [57] Ballard ST, Fountain JD, Inglis SK, Corboz MR, Taylor AE. Chloride secretion across distal airway epithelium: relationship to submucosal gland distribution. *Am J Physiol.* 1995;268(3 Pt 1):L526-31.
- [58] Inglis SK, Corboz MR, Taylor AE, Ballard ST. Regulation of ion transport across porcine distal bronchi. *Am J Physiol.* 1996;270(2 Pt 1):L289-97.
- [59] Inglis SK, Corboz MR, Ballard ST. Effect of anion secretion inhibitors on mucin content of airway submucosal gland ducts. *Am J Physiol.* 1998;274(5 Pt 1):L762-6.
- [60] Inglis SK, Corboz MR, Taylor AE, Ballard ST. Effect of anion transport inhibition on mucus secretion by airway submucosal glands. *Am J Physiol.* 1997;272(2 Pt 1):L372-7.

- [61] Trout L, Gatzky JT, Ballard ST. Acetylcholine-induced liquid secretion by bronchial epithelium: role of Cl⁻ and HCO₃⁻ transport. *Am J Physiol.* 1998;275(6 Pt 1):L1095-9.
- [62] 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(Pt 3):845-53.
- [63] Ballard ST, Inglis SK. Liquid secretion properties of airway submucosal glands. *J Physiol.* 2004;556(Pt 1):1-10.
- [64] Poulsen JH, Machen TE. HCO₃⁻-dependent pH_i regulation in tracheal epithelial cells. *Pflugers Arch* 1996;432:546-54.
- [65] Illek B, Yankaskas JR, Machen TE. cAMP and genistein stimulate HCO₃⁻ conductance through CFTR in human airway epithelia. *Am J Physiol.* 1997;272(4 Pt 1):L752-61.
- [66] Illek B, Tam AW, Fischer H, Machen TE. Anion selectivity of apical membrane conductance of Calu 3 human airway epithelium. *Pflugers Arch.* 1999;437(6):812-22.
- [67] Chandy G, Grabe M, Moore HP, Machen TE. Proton leak and CFTR in regulation of Golgi pH in respiratory epithelial cells. *Am J Physiol Cell Physiol.* 2001;281(3):C908-21.
- [68] Fischer H, Widdicombe JH. Mechanisms of acid and base secretion by the airway epithelium. *J Membr Biol.* 2006;211(3):139-50.
- [69] Cho DY, Hwang PH, Illek B, Fischer H. Acid and base secretion in freshly excised nasal tissue from cystic fibrosis patients with $\Delta F508$ mutation. *Int Forum Allergy Rhinol.* 2011;1(2):123-7.
- [70] Knowles MR, Clarke LL, Boucher RC. Activation by extracellular nucleotides of chloride secretion in the airway epithelia of patients with cystic fibrosis. *N Engl J Med.* 1991;325(8):533-8.
- [71] Clarke LL, Grubb BR, Gabriel SE, Smithies O, Koller BH, Boucher RC. Defective epithelial chloride transport in a gene-targeted mouse model of cystic fibrosis. *Science.* 1992;257(5073):1125-8.
- [72] Gabriel SE, Clarke LL, Boucher RC, Stutts MJ. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature.* 1993;363(6426):263-8.
- [73] Clarke LL, Harline MC. Dual role of CFTR in cAMP-stimulated HCO₃⁻ secretion across murine duodenum. *Am J Physiol.* 1998;274(4 Pt 1):G718-26.
- [74] Spicer Z, Clarke LL, Gawenis LR, Shull GE. Colonic H⁽⁺⁾-K⁽⁺⁾-ATPase in K⁽⁺⁾ conservation and electrogenic Na⁽⁺⁾ absorption during Na⁽⁺⁾ restriction. *Am J Physiol Gastrointest Liver Physiol.* 2001;281(6):G1369-77.

- [75] Gawenis LR, Franklin CL, Simpson JE, Palmer BA, Walker NM, Wiggins TM, Clarke LL. cAMP inhibition of murine intestinal Na/H exchange requires CFTR-mediated cell shrinkage of villus epithelium. *Gastroenterology*. 2003;125(4):1148-63.
- [76] Clarke LL, Gawenis LR, Bradford EM, Judd LM, Boyle KT, Simpson JE, Shull GE, Tanabe H, Ouellette AJ, Franklin CL, Walker NM. Abnormal Paneth cell granule dissolution and compromised resistance to bacterial colonization in the intestine of CF mice. *Am J Physiol Gastrointest Liver Physiol*. 2004;286(6):G1050-8.
- [77] Walker NM, Simpson JE, Brazill JM, Gill RK, Dudeja PK, Schweinfest CW, Clarke LL. Role of down-regulated in adenoma anion exchanger in HCO₃⁻ secretion across murine duodenum. *Gastroenterology*. 2009;136(3):893-901.
- [78] Knowles MR, Carson JL, Collier AM, Gatzky JT, Boucher RC. Measurements of nasal transepithelial electric potential differences in normal human subjects in vivo. *Am Rev Respir Dis*. 1981;124(4):484-90.
- [79] Knowles M, Gatzky J, Boucher R. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. *N Engl J Med*. 1981;305(25):1489-95.
- [80] Knowles MR, Stutts MJ, Spock A, Fischer N, Gatzky JT, Boucher RC. Abnormal ion permeation through cystic fibrosis respiratory epithelium. *Science*. 1983;221(4615):1067-70.
- [81] Yankaskas JR, Knowles MR, Gatzky JT, Boucher RC. Persistence of abnormal chloride ion permeability in cystic fibrosis nasal epithelial cells in heterologous culture. *Lancet*. 1985;1(8435):954-6.
- [82] Knowles MR, Stutts MJ, Yankaskas JR, Gatzky JT, Boucher RC Jr. Abnormal respiratory epithelial ion transport in cystic fibrosis. *Clin Chest Med*. 1986;7(2):285-97.
- [83] Knowles MR, Church NL, Waltner WE, Yankaskas JR, Gilligan P, King M, Edwards LJ, Helms RW, Boucher RC. A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. *N Engl J Med*. 1990;322(17):1189-94.
- [84] Knowles MR, Robinson JM, Wood RE, Pue CA, Mentz WM, Wager GC, Gatzky 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(10):2588-95.
- [85] Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest*. 2002;109(5):571-7.
- [86] Behm JK, Hagiwara G, Lewiston NJ, Quinton PM, Wine JJ. Hyposecretion of beta-adrenergically induced sweating in cystic fibrosis heterozygotes. *Pediatr Res*. 1987;22(3):271-6.
- [87] Ward CL, Krouse ME, Gruenert DC, Kopito RR, Wine JJ. Cystic fibrosis gene expression is not correlated with rectifying Cl⁻ channels. *Proc Natl Acad Sci USA*. 1991;88(12):5277-81.

- [88] Haws C, Krouse ME, Xia Y, Gruenert DC, Wine JJ. CFTR channels in immortalized human airway cells. *Am J Physiol.* 1992;263(6 Pt 1):L692-707.
- [89] Shen BQ, Finkbeiner WE, Wine JJ, Mrsny RJ, Widdicombe JH. Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl⁻ secretion. *Am J Physiol.* 1994;266(5 Pt 1):L493-501.
- [90] Haws CM, Nepomuceno IB, Krouse ME, Wakelee H, Law T, Xia Y, Nguyen H, Wine JJ. Delta F508-CFTR channels: kinetics, activation by forskolin, and potentiation by xanthines. *Am J Physiol.* 1996;270(5 Pt 1):C1544-55.
- [91] Singh M, Krouse M, Moon S, Wine JJ. Most basal I(SC) in Calu-3 human airway cells is bicarbonate-dependent Cl⁻ secretion. *Am J Physiol.* 1997;272(4 Pt 1):L690-8.
- [92] Luckie DB, Singh CN, Wine JJ, Wilterding JH. CFTR activation raises extracellular pH of NIH/3T3 mouse fibroblasts and C127 epithelial cells. *J Membr Biol.* 2001;179(3):275-84.
- [93] Irokawa T, Krouse ME, Joo NS, Wu JV, Wine JJ. A "virtual gland" method for quantifying epithelial fluid secretion. *Am J Physiol Lung Cell Mol Physiol.* 2004;287(4):L784-93.
- [94] Wu JV, Krouse ME, Rustagi A, Joo NS, Wine JJ. An inwardly rectifying potassium channel in apical membrane of Calu-3 cells. *J Biol Chem.* 2004;279(45):46558-65.
- [95] Khansaheb M, Choi JY, Joo NS, Yang YM, Krouse M, Wine JJ. Properties of substance P-stimulated mucus secretion from porcine tracheal submucosal glands. *Am J Physiol Lung Cell Mol Physiol.* 2011;300(3):L370-9.
- [96] Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, Wine JJ, Frizzell RA, Ashlock M, Negulescu PA. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc Natl Acad Sci USA.* 2011;108(46):18843-8.
- [97] Joo NS, Cho HJ, Khansaheb M, Wine JJ. Hyposecretion of fluid from tracheal submucosal glands of CFTR-deficient pigs. *J Clin Invest.* 2010;120(9):3161-6.
- [98] Cho HJ, Joo NS, Wine JJ. Defective fluid secretion from submucosal glands of nasal turbinates from CFTR^{-/-} and CFTR (Δ F508/ Δ F508) pigs. *PLoS One.* 2011;6(8):e24424.
- [99] Rogers C.S., Hao Y., Rokhlina T., Samuel M., Stoltz D.A., Li Y., et al . Production of CFTR-null and CFTR- Δ F508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. *J Clin Invest.* 2008; 118(4): 1571–1577.
- [100] Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, et al Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science* 2008;321: 1837–1841.

- [101] Stoltz DA, Meyerholz DK, Pezzulo AA, Ramachandran S, Rogan MP, Davis GJ, et al. Sci Transl Med. Cystic fibrosis pigs develop lung disease and exhibit defective bacterial eradication at birth. 2010;2(29):29-31.
- [102] Rogan MP, Reznikov LR, Pezzulo AA, Gansemer ND, Samuel M, Prather RS, et al. Pigs and humans with cystic fibrosis have reduced insulin-like growth factor 1 (IGF1) levels at birth. Proc Natl Acad Sci USA. 2010;107(47): 20571-5.
- [103] Chen JH, Stoltz DA, Karp PH, Ernst SE, Pezzulo AA, Moninger TO, et al. Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell. 2010 ;143(6):911-23.
- [104] Ostedgaard LS, Meyerholz DK, Chen JH, Pezzulo AA, Karp PH, Rokhlina T, et al. The $\Delta F508$ Mutation Causes CFTR Misprocessing and Cystic Fibrosis-Like Disease in Pigs. Sci Transl Med. 2011;3(74): 74ra24.
- [105] Chang EH, Lacruz RS, Bromage TG, Bringas P Jr, Welsh MJ, Zabner J, Paine ML. Enamel pathology resulting from loss of function in the cystic fibrosis transmembrane conductance regulator in a porcine animal model. Cells Tissues Organs. 2011;194(2-4):249-54.