Title

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Journal

Physiology, 32(5)

ISSN

1548-9213

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Publication Date

2017-09-01

DOI

10.1152/physiol.00013.2017

Peer reviewed

REVIEW
PHYSIOLOGY 32: 380–390, 2017. Published August 16, 2017; doi:10.1152/physiol.00013.2017

Both Ways at Once: Keeping Small Airways Clean

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The small airways of the lungs are under constant assault from the pathogens and debris in the air that they must conduct to alveoli. Although hygiene is of paramount importance for respiratory health, the underlying principles of airway clearance have not been well integrated or established. Newly emerging concepts of simultaneous absorption and secretion of airway surface liquid (ASL) and the role of HCO_3^- in the maturation of mucins have advanced from experimental evidence as well as observations from the congenital disease cystic fibrosis (CF) to present a novel model that integrates microanatomy with organ physiology to meet the constant challenge of cleaning small airways.

The Airway Surface Liquid Challenge

The small airways or bronchioles normally constitute most (85–90%) of the epithelial surface lining the conducting airways (104) and are perhaps the most vulnerable components of the respiratory system (49, 50, 87, 92, 94). They are vulnerable because their small size (diameter of <2 mm in humans) places them at constant risk for blockage by accumulated mucus and debris formed in response to continuous exposure to particulates and pathogens in each breath of ambient air (18, 114). The continued patency and normal hygiene of these delicate tubes are assured not by air but by a layer of liquid and mucins that must constantly wash the luminal surfaces of all conductive airways via a "ciliated escalator" that constantly sweeps debris orally to be expelled in the pharynx (56, 111). The depth (volume) of this layer of airway surface liquid (ASL) is critical, but reports of thickness vary from ~10 μm (106) in cultures of bronchial cells to ≥50 μm in vivo in native trachea (29, 54). Disturbances in the properties of either the liquid or the mucins are invitations for infection, inflammation, and pathology.

The functional challenge for both large and small airways is to maintain an ASL volume (depth) that does not leave the lumen flooded or desiccated, either of which will acutely or eventually compromise the bronchiole. Meeting the challenge for small airways differs from large airways (bronchi) in that, first, they lack supporting cartilaginous rings that limit the constriction and prevent closure of larger airway lumens; patency of small airways depends on intrathoracic elastic retractile forces to "pull" them open, which are decreased or lost at low lung volumes. Second, and more importantly for maintaining ASL, unlike large airways, bronchioles lack submucosal glands as a source for secreting and replenishing surface fluid. How small airways meet the challenge of maintaining just the "right" depth of ASL in vivo is not established, but perhaps because the airway microanatomical surface is usually conceptualized as a flat epithelium (91), it has been widely accepted that the luminal epithelial cells alternate in unison between uniformly absorbing fluid when ASL is excessive and uniformly secreting fluid when ASL is depleted in a manner that might be seen as a "drain and flood" model (15, 16, 21, 32) (see *Supplemental Figure S1* at *Physiology* website).

Aside from the issue of unknown signals to reverse fluid transport directions, it is difficult to support such a model of alternating fluid transport with physiological examples of native tissues that routinely acutely reverse directions of fluid transport. That is, most, if not all, native secretory and absorptive epithelial cells differentiate to either permanently absorb or secrete but are not known to be capable of physiologically cycling acutely between secreting and absorbing fluid and electrolytes (41, 53). Even more, all mammalian fluids are secreted isotonically but are not secreted from flat surfaces. Instead, they are secreted into closed water-permeable compartments, i.e., tubule lumens, crypts, or plicated surfaces (e.g., ciliary body, choroid plexus), presumably to accommodate iso-osmotic equilibration (79). Nonetheless, it is clear that intact small airways must have both secretory as well as absorptive capacities.

Because the tissue is so small and friable, attempts to understand the properties and nature of fluid and electrolyte transport across native small airway epithelia have relied almost exclusively on electrophysiological analyses of segments of isolated tubules of bronchioles. Active fluid-coupled electrolyte transport generates a transepithelial

electrical current proportional to the total transport activity across the epithelium (97). It may be helpful to recall that absorption in the airway is predominately driven by active Na^+ absorption from the lumen, whereas secretion is driven by active Cl^- or HCO_3^- secretion into the lumen. The currents from each are additive, since cations with a positive electrical charge absorbed in one direction generate a current that is in the same direction as anions with a negative charge secreted in the opposite direction. Thus the contributions from absorptive and secretory currents can be extracted and defined by the effects of selective inhibitors and agonists on the total transepithelial current. Since the transepithelial voltage is usually directly proportional to the equivalent current (I_{eq}), all other things being equal, and since it is technically less complicated to measure, the magnitude of the voltage or transepithelial potential (TEP) is taken at times as an indirect measure of the transport activity.

Absorption

Despite very small TEPs and the difficulties of handling a fragile tissue, heroic early efforts revealed that both secretory and absorptive transport are expressed in isolated segments of native bronchioles. Amiloride is a highly selective inhibitor of the epithelial Na^+ channel (ENaC; a trimer of gene subunits SCNN1A,B,G,D) that is essential for electroconductive Na^+ uptake across a number of absorptive epithelia (38, 48). In early microperfusion studies of sheep and pig bronchioles, amiloride consistently depolarized (reduced) the TEP by 40–60% (2, 4, 9, 10, 51). Amiloride depolarized the TEP and reduced the I_{eq} by ~50% in isolated sheep bronchioles and by 30–40% in pig bronchioles that were microperfused (9, 10) or mounted in a small-aperture Ussing chamber (85, 86). Although no significant effect of amiloride was observed on the constitutive TEP of undissected bronchioles microperfused in small blocks of pig lung tissue, amiloride did markedly depolarize the TEP of an imposed Cl^- gradient into the lumen (103). Parenthetically, application of phloridzin to inhibit Na^+-dependent glucose absorption had no significant effect on TEP (2); however, we observed that the inhibitor consistently reduced total TEP by ~10%.

Most significantly, although amiloride was inhibitory in all of these studies, it only partially inhibited the I_{eq} and/or TEP in any study, which indicates that a significant portion of the active ion transport is due to something other than amiloride-sensitive Na^+ absorption.

Secretion

The findings that inhibiting absorption with amiloride does not inhibit all, or even most, of the I_{eq}, and therefore fluid transport, indicates that the residual transport current is most likely due to secretory activity. Characteristically, epithelial fluid secretion depends on very high expression of the sodium-potassium-2-chloride-cotransporter 1 (NKCC1; gene *SLC12A1*) in the basolateral membrane of fluid-secreting cells to drive Cl^- secretion across the cell (43, 64, 110).

Bumetanide is a well-established selective inhibitor of NKCC1 commonly used to block fluid secretion (44, 45). The earliest study of microperfused sheep airways reported a small inhibitory effect of bumetanide on TEP, but only after incubating the tissue in indomethacin and isoproterenol (4). Somewhat later, the same investigators found that this inhibitor depolarized the constitutive TEP of these bronchioles by ~20% (2). However, in pig bronchioles, bumetanide inhibited >50% of the amiloride-insensitive I_{eq} but showed little

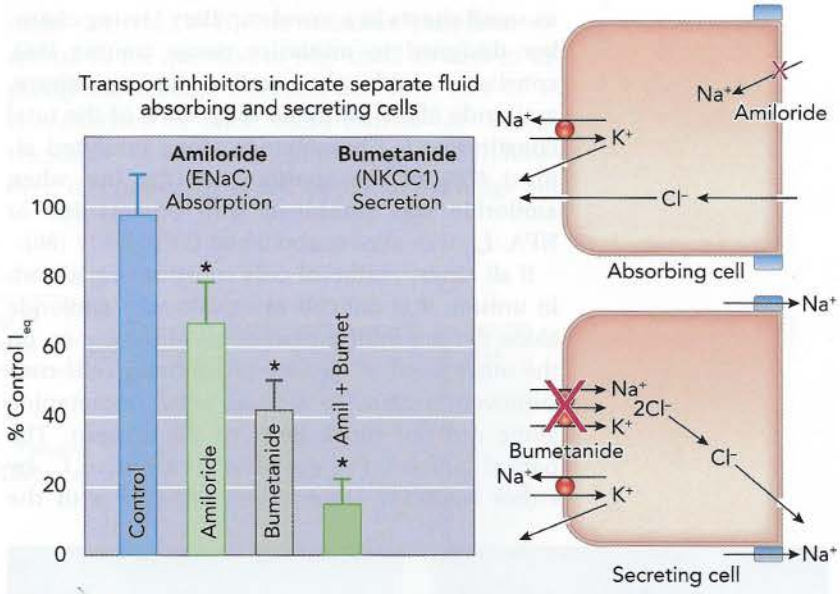

FIGURE 1. **Effect of specific inhibitors on constitutive ion transport current of pig small airways**
The effects of selectively inhibiting absorption with amiloride (amil), secretion with bumetanide (bumet), and both absorption and secretion with the two inhibitors combined are presented as the fraction of the control I_{eq} (equivalent short-circuit current) taken as 100% for each paired condition (n = >6; $P < 0.03$, for all conditions). Neither inhibitor alone inhibits all current, but most of the I_{eq} is inhibited when inhibitors are combined, indicating concurrent absorptive and secretory transport. Diagrams on the right of an absorptive (*top*) and a fluid-secretory (*bottom*) cell show the essential components and conductances with inhibited components in each cell type. During absorption, Na^+ moves down its electrochemical gradient from the lumen into the cell through the amiloride inhibitable Na^+-selective channel (ENaC), as Cl^- follows electrochemically through an anion-selective conductance in the apical membrane conductance. During secretion, two Cl^- anions are electroneutrally coupled to the transport of a Na^+ and a K^+ cation into the cell on the bumetanide-inhibitable NKCC1(Na-K-2Cl carrier 1) in the basolateral membrane as Cl^- is secreted through an anion conductance in the apical membrane into the lumen. Na^+ moves paracellularly through the cation-selective tight junction. Both transports are driven by energy from Na^+-K^+-ATPase in the basal membrane. Large red *Xs* indicate the components inhibited that block transport.

inhibitory effect in the absence of amiloride, leading to the suggestion that amiloride may induce a secretory phenotype (9, 10). When examined as epithelial sheets of opened airways in a small-aperture Ussing chamber, serosal bumetanide did not reduce the TEP or I_{eq} of sheep bronchioles until the tissue was stimulated by acetylcholine (Ach) or by elevating intracellular Ca^{2+} with the Ca^{2+} ionophore A-23187 (3). Likewise, bumetanide sharply reduced the rate of Ach-stimulated fluid-volume secretion in pigs by ~70% (95). Although more variable than the overall results for amiloride, all of these studies found bumetanide inhibition of a component of the I_{eq} or TEP under given experimental conditions.

Absorption + Secretion

From the above studies, it is clear that small airways express properties of both absorption and secretion, but the results do not afford an interpretation of how these distinct functions are related or distributed in the tissue. More recently, when opened bronchioles were mounted as small sheets in a novel capillary Ussing chamber designed to minimize tissue trauma (86), consistent with the earlier finding above, amiloride alone inhibited only ~35% of the total constitutive I_{eq}; bumetanide alone inhibited almost 60% of the spontaneous I_{eq}; but when amiloride was combined with bumetanide or NFA, I_{eq} was almost abolished (FIGURE 1) (86).

If all airway epithelial cells constitutively absorb in unison, it is difficult to explain why amiloride alone did not inhibit most or all of the I_{eq}, or, on the other hand, if the airway epithelial cells constitutively secrete in unison, why bumetanide alone did not block most of the current. The partial inhibition of the total constitutive I_{eq} by either inhibitor alone taken together with the almost total inhibition of I_{eq} when the inhibitor of absorption was combined with the inhibitor of secretion yields strong evidence that both constitutive fluid secretion and constitutive fluid absorption occur simultaneously in native small airways.

Since it is impossible for the same cell to transport solutes and fluid in opposite directions at the same time, separate groups of fluid-secretory and -absorptive cells in the epithelial surface must transport constitutively and concurrently in opposite directions. The conclusion begs the questions of which cells in the luminal epithelium are secretory, which are absorptive, and how they are distributed microanatomically.

What is Where?

The microanatomy of the small airway offers insights into the distribution of transport functions in the luminal epithelium. In cross section, the luminal surface of the small airway is highly plicated (FIGURE 2). The plications in the luminal surface run like "furrows" between "ridges" along the longitudinal axis of the airway, distal to proximal (aboral to oral). Historically, the plications have been assumed to exist to accommodate maintaining a constant luminal surface area during the breathing cycle. That is, dynamic changes in the luminal diameter of the airway continually reoccur with exhalations that constrict followed by inhalations that expand the small airways as the lung deflates and inflates. Thus the luminal plications widen and narrow accordion-like as the airway diameter changes with little or no effect on the plicated surface area.

However, beyond preserving a constant surface area, the plicated microanatomy of the lumen suggests, by analogy with the crypts and villi of the intestine (90), distinct transport functions for subgroups of cells in the plications in the airway, as suggested above in the functional data (FIGURE 1).

Immunocytochemistry offers an approach to localize and identify different cell phenotypes in the epithelium with biomarkers for specific fluid-transport components. As noted, NKCC1 is well known to be uniquely and highly expressed in the basolateral membranes of fluid-secreting cells. The T84 antibody specific for NKCC1 (64, 110) localized to cells in the contraluminal region of the furrows of the plications of the luminal surface, but not in the cells in the region of the luminal ridges of the plications (FIGURE 3) (35). This result indicates that the cells in the furrows secrete fluid and implies that the remaining unlabeled cells of the more luminal region of the ridges are absorptive epithelium.

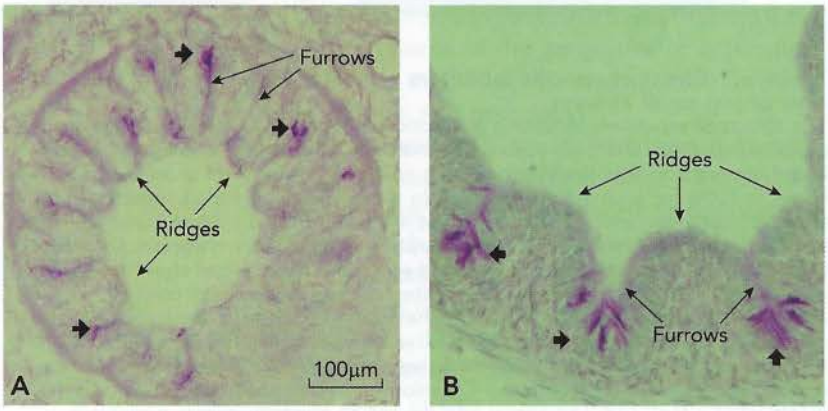

FIGURE 2. **Microanatomy of small airway**
A: the highly plicated luminal surface defines the entire circumference of the lumen with furrows between each plication that give rise to ridges in the lumen. *B*: mucus or goblet cells (blunt arrows) are generally more concentrated at the fundus of the furrows. Stain: hematoxylin and periodic acid-Schiff.

Autonomy

The distribution of distinct transport functions within the airway microanatomy projects a mechanism for autoregulation of the ASL volume. As long as the transport capacity of absorptive cells exceeds the secretory activity of secreting cells, the small airway lumen cannot flood or desiccate. That is, continuous constitutive secretion of ASL by fluid-secretory cells in the furrows of the pleats ensures that the furrows of the pleats always retain liquid and remain "wet" (FIGURE 4). Constitutive fluid secretion into the furrow concurrent with constitutive uptake of the secreted fluid by the absorptive cells of the luminal ridges prevents luminal flooding. Thus this plicated structure-function locally "recycles" ASL secreted from the contraluminal furrows of the pleats back via reabsorption through the luminal ridges into the serosal fluid compartment from which the ASL of the furrow is replenished to be reabsorbed again (FIGURE 4). The design for recycling exists all along the longitudinal axis of the plicated airways, and thereby establishes a normal steady state by which the absorptive rates obligatorily match secretory rates, leaving the furrows of the pleats wet, but not too wet, and the airways never dehydrated without fluid—or flooded with excessive ASL.

This design, at least at the level of constitutive transport, also implies little need for moment-to-moment regulation of the ASL depth for which monitoring and signaling mechanisms (62) seem difficult to put in place. This is not to say that the airway is not subject to intrinsic or neuronal regulation that may acutely increase (or decrease) the volume of ASL in response to normal or pathological stimuli that uncouple this balance as may be required locally at particular sites to clear any incipient or accumulated debris by either stimulating secretion or possibly inhibiting absorption.

Mucins → Mucoviscidosis

Mucins are intrinsic to, and crucial for, maintaining airway hygiene because these macromolecules form the conveyor belt for moving fluid, mucus, and debris along the airway surface for removal from the lung. The conveyor with its debris is propelled orally by underlying ciliary activity, often termed the "ciliated escalator" (FIGURE 5). Cystic fibrosis (CF) is a genetic disease that is usually fatal due to progressive respiratory infections that arise when the conveyor fails to clear the airway normally (14, 102, 114). The common simplistic response to the question of why small airways in CF precipitate chronic infection has been that CF is characterized by thick, sticky mucus—or "mucoviscidosis" of the bronchial tree (14, 33, 69); however, this pathology is not limited to the lungs but

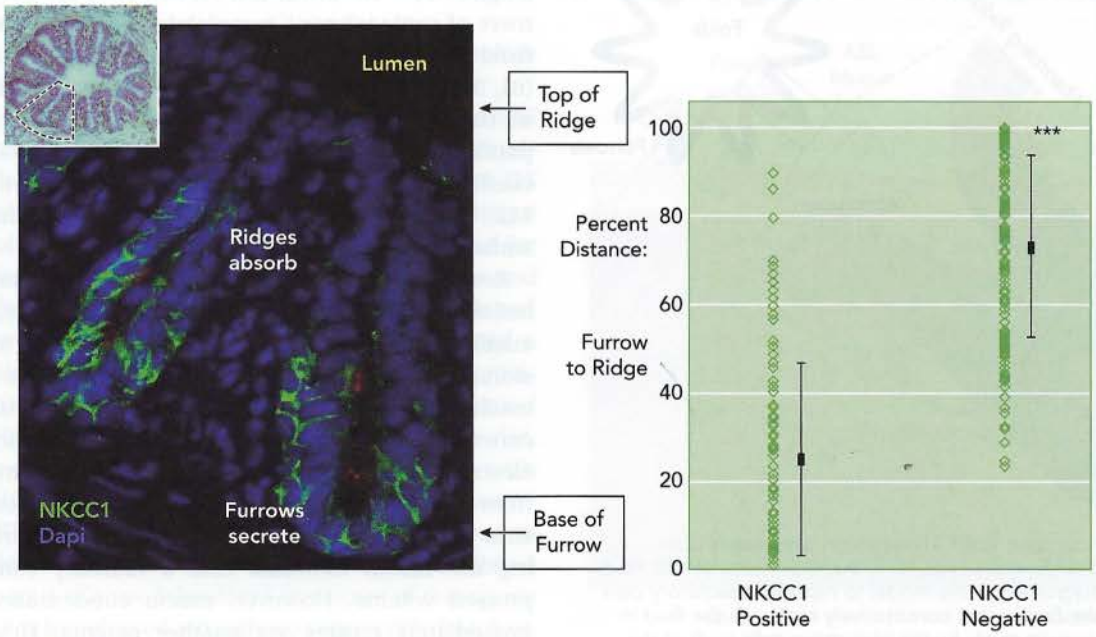

FIGURE 3. **Distribution of secretory cells**
Immunocytochemical localization of the T84 antibody for NKCC1 identifies secretory cells in the epithelial surface. NKCC1-labeled cells are located predominately within the fundal zone of the furrow of the plications. The plot shows the distance of each labeled cell (left plot, mean 25%) and of each unlabeled cell (right plot, mean 72%) from the base of the furrow to the top of the ridges. Most of the labeled secretory cells form the fundal zone of the furrow, whereas most of the unlabeled, presumably absorptive, cells are in the luminal zone forming the ridge of the plications. *Inset*: cross sections of small airway illustrating cutout of plications as shown in immuno-stained image (blue: DAPI nuclear stain). Image taken from Ref. 35 and used with permission from *American Journal of Respiratory Cell and Molecular Biology*.

occurs characteristically in other mucus-secreting organs throughout the body as well (8, 46).

It has become widely accepted, based largely on observations in cultured airway tracheobronchial cells, that the airways of the CF lung are abnormally depleted of ASL because Na^+ absorption with its osmotically coupled fluid absorption is unregulated and excessive, presumably due to dysfunctional CFTR (cystic fibrosis transmembrane regulator, the mutated protein that causes the disease). That is, in the aforementioned model of alternating secretion-absorption for ASL management (see *Supplemental Figure S1* on the *Physiology* website), pathogenic mucus in CF results because the epithelium is in a predominantly hyperactive state of absorption so that, once secreted, there is insufficient fluid to disaggregate and thin mucins normally. Consequently, mucus appears thickened and "desiccated"; i.e., as "mucoviscidosis" or thick sticky mucus that is difficult to transport along the luminal surfaces and clear from the lung (19, 26, 58, 60, 65, 66, 93, 107).

However, the concept that desiccated mucus is due to hyper-absorption of Na^+ and fluid in CF is difficult to reconcile with the fact that there is little, if any, evidence of ENaC expression or ENaC-dependent Na^+ absorption in most of the major CF organs affected by mucoviscidosis (small bowel, pancreas, gall bladder, and liver), so that excessive electro-conductive Na^+-dependent fluid absorption cannot be a generalized defect causing mucoviscidosis in this disease (68, 78). Neither is it consistent with the fact that mucins (excepting airways) are secreted into fluid-filled, water-permeable compartments or lumens where there is no apparent lack of hydrating fluid as the basis for generalized mucoviscidosis in all CF target organs. Perhaps it should be noted also that hypersecretion of thick mucus is not simply the result of inflammatory infections either, since, among all the target organs in CF, only the airways are chronically infected.

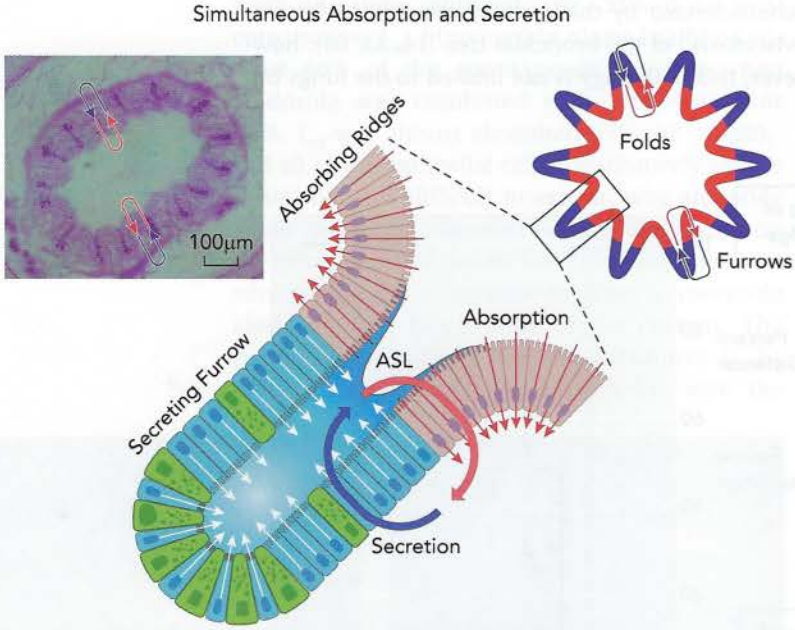

FIGURE 4. **Model of concurrent fluid absorption and secretion**
Based on the cell distributions shown in FIGURE 3, the movement of ASL fluid within a single plication is diagramed in the model to represent secretory cells (blue) in the fundal half of the furrow that constitutively replenish the fluid in the furrow as it is taken up constitutively by the absorptive cells (red) of the luminal ridges. ASL is continuously recycled such that fluid is always present within the furrow over the secretory cells, but as ASL emerges toward the lumen it is constitutively absorbed by the cells of the ridges that prevent secreted fluid from excessively filling the lumen. Thus the airway can never normally desiccate or flood. *Top left inset*: a micrograph of a cross section of the plicated airway from native tissue (diagramed for simplicity in the *top right inset*). In both insets, curved red lines indicate the path of absorbed fluid flow, and curved blue lines indicate the path of secreted fluid flow to illustrate the process of local ASL recycling.

The Mucus Mix

Mucus originates from highly condensed mucin molecules stored mainly in small, cytoplasmic granules in goblet (mucin) cells that populate mucus-secreting epithelia. Upon exocytosis and release from the cells, the condensed mucin granules normally expand ("explode") within ~1 s to volumes that are two to three orders of magnitude larger than the original granule (100, 101).

This astonishing event can occur because mucin molecules are among the longest, most negatively charged macromolecules in nature (~1- to ≥5-μm length; ~10^6–10^8 mwu) (20, 57, 88). They are polymers of proteoglycans completely decorated with fixed anionic sites, mainly sialic acid and sulfate (61, 99). The density of the electro-repulsive fields on the fixed negative charges endows mature, expanded mucus molecules with among the lowest coefficients of friction of known materials (6, 61, 112), effecting its ease of transfer along the luminal surface.

Recognition of these electro-repulsive properties insists on a means for compacting such huge, negatively charged molecules into dense granules for storage in goblet cells in the first place. The issue is resolved by the presence of very high intra-granule concentrations of Ca^{2+} (~500 mM) (52, 100), which electrostatically shield the mucin-negative sites from each other and at the same time attract these sites to their divalent electropositive centers, forcing the mucin molecule into a radically compressed volume. However, mucin condensation immediately creates yet another enigma: How, upon release of the granule, is Ca^{2+} displaced from the fixed negative sites so that mucin molecules can electrostatically self-repel and expand almost instantaneously? Previously, extracellular Na^+ and K^+ cations were presumed to displace the Ca^{2+} by mass action (98, 101), but it is unlikely that physiological concentrations of these monovalent

cations are sufficient to compete away the bound divalent calcium. Indeed, in the light of results described below, they are not.

No HCO_3^- = Cystic Fibrosis

The pathology of CF renders clues to the puzzle. Since the earliest descriptions of CF, the loss of pancreatic function either in utero or within a few years of birth is a highly consistent, but not invariable, pathogenic event in CF (30); hence, the name, "cystic fibrosis of the pancreas" (7, 33). But despite the fact that an essential and critical function of the pancreas is to secrete a HCO_3^- (not Cl^-)-rich pancreatic juice, and despite the fact that patients surviving with pancreatic sufficiency still produced a pancreatic juice with enzyme competence but with greatly diminished HCO_3^- secretion (47, 59), a basic role for HCO_3^- loss in CF pathogenesis was not advanced (70, 76). However, some evidence linked the variability in disease severity to the level of HCO_3^- conductance expressed by distinct mutations in CFTR (23). A survey of target organs in CF reveals that destructive lesions due to luminal mucus obstruction occur in all affected organs (14, 34) except the sweat gland, which secretes almost no mucins. Thus, since these organs also fail to secrete HCO_3^- (77, 78), the disease pathology strongly implicates defective CFTR HCO_3^- conductance as the basic defect underlying the secondary, but potentially lethal, defect in mucus; i.e., mucoviscidosis in CF (75).

Then, does a lack of HCO_3^- conductance result in abnormal mucus?

Paired segments of excised wild-type mouse ileal intestine were incubated with and without physiological concentrations of HCO_3^- conductance (25 mM) and perfused luminally with isotonic Ringer solution. The collected perfusates were assayed for the presence of mucus proteoglycans before and after stimulation with PGE_2, 5-HT, carbachol, and isoproterenol. Both PGE_2 and 5-HT stimulated a release of mucus that was enhanced by approximately twofold in the presence of HCO_3^- compared with no HCO_3^- in the incubation media. Likewise, and consistent with these results, inhibition with Gly-H 101 to block CFTR anion conductance or DIDS to inhibit NBC (Na^+ / HCO_3^- cotransporter) to block HCO_3^- secretion both effectively inhibited mucus release. Stimulated intestines of transgenic CF mice released virtually no mucus (37) (see *Supplemental Figure S3* at *Physiology* website). High concentrations (~100 mM) of luminal media HCO_3^- normalized mucus consistency in vivo (42). These effects were not tissue specific since almost identical results for mucus release were obtained when the mouse uterine cervix was examined similarly (67). Histological examination revealed that, in the absence of HCO_3^- conductance, mucin granule exocytosis occurred, but release and dispersion of mucins were apparently repressed (113).

As more direct evidence of the impact of the loss of HCO_3^- conductance in airways, the mucociliary transport (MCT) rate of particles on native mouse trachea stimulated in vitro with Ach was ~35% lower when HCO_3^- conductance was blocked than in control preparations (24), which was consistent with earlier results from inhibiting anion transporters that induced thickened mucus discharge (96). Overall, these results present compelling evidence that mucin secretion must be accompanied by concurrent HCO_3^- secretion if normal mucin expansion and maturation is to proceed physiologically (see *Supplemental Figure S2* at *Physiology* website).

Defects in Cystic Fibrosis

Looking at CF as a special case of obstructive small-airway diseases in the light of the plicated structure-function model for pulmonary hygiene admits several possible, if not probable, levels of pathogenesis in CF lungs. That is, given the fact that CFTR is the defective molecule central to fluid transport in both absorptive and fluid-secretory cells that express it (74), CFTR-dependent fluid secretion and absorption must be diminished in small airways as they are in other CF-affected organs (13, 39, 74, 108). To wit, NaCl is diagnostically

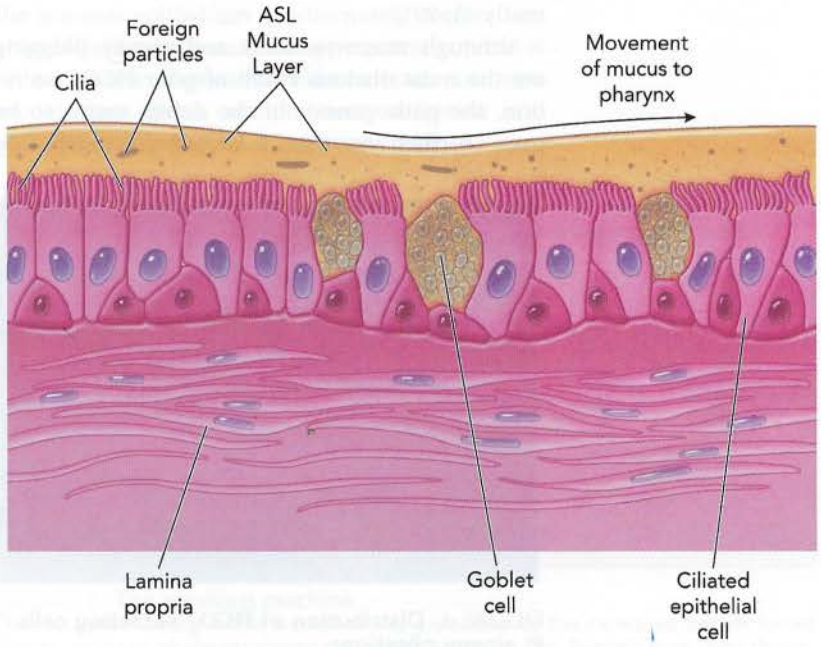

FIGURE 5. **Rendition of airway surface ciliated escalator**
The ciliated cells of the apical surface of the airway epithelium normally propel a thin mucus blanket of the ASL like a conveyor belt orally to dispose of entrapped foreign materials and intrinsic debris. The mucins of the mucus belt must be appropriately expanded and thinned to avoid aggregation and stasis as it is transported for removal. Lack of secreted HCO_3^- causes "mucoviscidosis" and stagnation in CF, which promotes chronic infection and inflammation of small airways.

elevated in CF sweat due to the loss of CFTR-dependent Cl^- conductance in the sweat gland reabsorptive duct, which not only blocks Cl^- uptake but concomitantly inhibits Na^+ absorption due to the demands of electroneutrality in ion transport (73). At the same time, β-adrenergic sweat secretion, which is also CFTR dependent, is essentially absent in CF subjects (11, 12, 28, 72, 82, 83, 109). It follows that CFTR-dependent ASL secretion in pleats of CF small airways should be depressed, but even so, the constitutive volume of ASL may not be completely depleted, irrespective of ASL absorption, and, especially if constitutive, spontaneous secretion is not completely CFTR dependent (86).

Still, in CF, the loss of CFTR-dependent HCO_3^- secretion seems to be the most likely cause of mucoviscidosis (75). Accordingly, although mucus production is enhanced in the presence of inflammation (55), without adequate HCO_3^- in the ASL media, secreted mucins remain severely condensed, thick, aggregated, and especially problematic for plugging small airways. In addition to the major deleterious effects of losing the ability to sequester Ca^{2+} away from premature mucins for optimal expansion (see *Supplemental Figure S3* at *Physiology* website), the loss of HCO_3^- forces a reduction in pH, which also impedes mucin expansion due to protonation of the fixed negative charges on the mucins. That is, the anionic sites become more protonated and do not repel neighboring sites to extend the molecules normally (5, 22).

Although mucoviscidosis and airway plugging are the most obvious result of poor HCO_3^- secretion, the pathogenesis of the defect seems to be even further nuanced. At physiological CO_2 tensions, loss of HCO_3^- inherently lowers the ASL pH, but in the airway the defect is likely exaggerated significantly by the presence of recently reported active proton secretion into airways, which further impedes the immune defense of the airway (63, 84). HCO_3^- is required to render some bacteria susceptible to certain antimicrobial peptide (AMP) killing, e.g., cathelicidins and defensins (27), but the unbuffered effects of active proton secretion markedly lowers the pH of ASL that was recently shown to compromise native airway immune defenses as well (1). Recently, studies of the intact native tracheal surface of genetically modified CF pigs show that low ASL pH inhibits the native bacterial killing capacity of CF trachea (71) and cultured small airway epithelial cells (63).

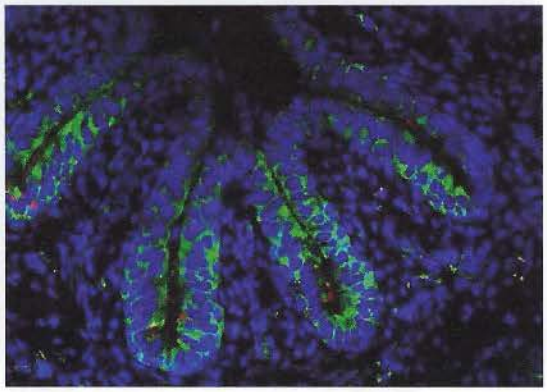

FIGURE 6. **Distribution of HCO_3^- secreting cells in airway plications**
The Na^+/HCO_3^- cotransporter, NBC1, that drives HCO_3^- secretion is localized by immunocytochemistry to the epithelial cells in the fundal zone of the plications of the epithelium. These cells likely provide active HCO_3^- secretion into the ASL that is required for normal mucin expansion and thinning. Green, KiaNBC antibody; blue, DAPI nuclear stain.

Is Airway Pancreas?

Airways are not usually thought to have much in common with the pancreas, the mother of HCO_3^--secreting organs, but if HCO_3^- is essential for normal mucin release, expansion, and maturation to support airway clearance, small airways must be capable of secreting HCO_3^-, even though they are not usually suspected of doing so. HCO_3^- secretion is generally dependent on a Na^+/HCO_3^- cotransporter (NBC; gene *SLC4A4*, variant pNBC1), which is highly expressed in the basolateral membrane of HCO_3^--secreting cells (81). NBC couples the inward Na^+ gradient to HCO_3^- uptake into the secreting cell, where, intracellularly, the electrochemical gradient across the apical membrane forces HCO_3^- into the lumen through an anion channel, e.g., CFTR or CaCC (17, 80), as in Cl^--dependent fluid secretion.

To evaluate the HCO_3^- transport capacity in bronchioles, specimens of epithelial sheets from pig and human small airways were isolated and mounted in a capillary Ussing chamber (85) to measure the effects of agonist and inhibitors on constitutive HCO_3^--dependent I_{eq}. Ringer solutions were modified to ensure that HCO_3^- was the only major transportable ion present (89, 105). That is, gluconate was substituted for Cl^- to obviate active Cl^- transport, and amiloride was added to block Na^+-absorptive currents. The bronchiole exhibited a spontaneous constitutive HCO_3^- current that approximately doubled when stimulated by adding cAMP-elevating agonists [forskolin (FSK) plus IBMX or Isoproterenol (IPR)] or Ca^{2+}-elevating agonists (UTP or PGE_2). The separate cAMP and the Ca^{2+}-mediated responses were additive. An inhibitor of the CFTR anion channel, Gly-H 101, inhibited the FSK/IBMX, and IPR stimulated HCO_3^- I_{eq}. An inhibitor of the Ca^{2+}-activated anion channels (CaCC), niflumic acid (NFA), but not Gly-H 101, blocked the UTP-stimulated increases in I_{eq}.

Both NFA and Gly-101 combined were required to block the response to PGE_2. These findings strongly indicate inherent, constitutive HCO_3^- secretions that can be regulated substantially by apparently independent cAMP-mediated and Ca^{2+}-mediated pathways that likely control distinct mechanisms for secreting HCO_3^- in the small airways (85).

Preliminary immunocytological staining with the K1A antibody for NBCe1 (courtesy W. Boron) localized the cotransporter at the basolateral membrane of epithelial cells generally distributed within the furrows of the luminal surface (FIGURE 6) (36). The presence of mucus goblet cells, usually much more concentrated in the base of the furrows of the plications (FIGURE 2), could argue that this distribution is another illustration of efficient structure-function coupling since mucin granules should be released into a HCO_3^--rich fluid environment essential for immediate mucin expansion and maturation (see *Supplemental Figure S4* at *Physiology* website). If the secreted fluid is isotonic (composition presently is unknown), HCO_3^- concentrations in this confined space might conceivably approach those of the pancreas (40).

Since mucin-secreting goblet and Clara cells undergo hyperplasia and broader distribution over the epithelium with increasing inflammatory response (25, 31), a wider distribution of more cells capable of HCO_3^- secretion may increase with inflammation.

Washing Machines

Maintaining the hygiene of the small airways presents a striking example of integrating microanatomical structure and tissue function with macro-organ level structure-function to overcome vital physiological challenges. The plicated structure of the airway surface ensures a continuous local flux of ASL throughout the length of the bronchioles that inherently evades stagnation (FIGURE 4). Concurrent secretion of mucin granules into bicarbonate replete ASL produces expanded mucins to form the "conveyor belt" of the ciliated escalator that constantly carries debris orally for expulsion (FIGURE 5).

The organ-level mechanics of the breathing cycle that force dynamic changes in airway diameters serve to amplify the lavage effect of fluid flow within the plications of the luminal surface. That is, as recalled above, during inhalation as the airway diameter expands toward total lung volume (vital capacity), the furrows spread and the volume between the walls of the plications increases (FIGURE 7A). Always followed by exhalation, the tractile forces around the airways relax, compressing luminal diameters and narrowing the furrows, which reduces the volume within the plications to virtually zero (FIGURE 7B) with airway closure (residual lung volume), thereby squeezing the ASL out of the furrow toward or over the luminal ridges. These repetitive changes in the furrow volume, even without complete expansion or closure, agitate the ASL contents in and out from the fundus of the furrows to the tips of the ridges, which in effect continuously washes the entire bronchiolar surface driven simply by the mechanics of breathing (FIGURE 7, A AND B). This system might be seen as endogenous little "washing machines" that ensure a continual lavage of the small-airway luminal surfaces. The washing machine agitation not only supports spreading of the aqueous fluid and mucins but also ensures clearance by impeding stasis, aggregation, and consolidation of mucins, pathogens, and debris that constantly challenge airway hygiene before colonization, infection, inflammation, and/or luminal plugging occur.

The enhanced agitation of the ASL during deep, rapid breathing may justify exercise as therapy in cystic fibrosis and perhaps other obstructive diseases of the airways.

Conclusions

Control of the thickness and volume of the ASL in small airways is imperative to maintain respiratory hygiene without drying or flooding the airway lumen. In contrast to the earlier model that requires the luminal epithelium to alternately secrete and absorb fluid to maintain an appropriate volume of ASL, a review of present evidence leads to a new model that allows simultaneous secretion and absorption by virtue of the uniquely plicated

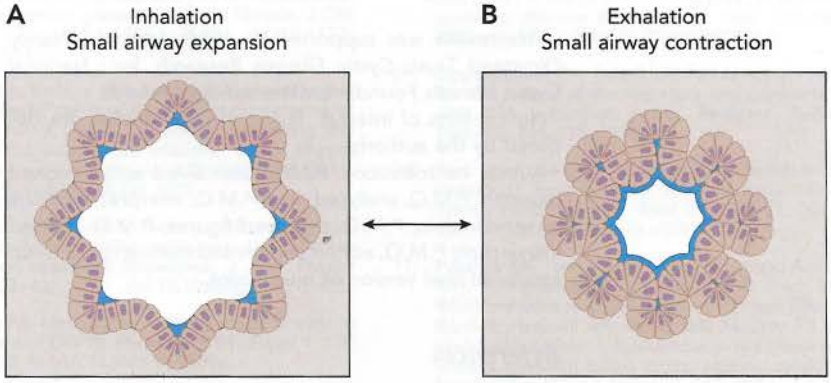

FIGURE 7. **The washing machine**
The illustration at *left* shows a small airway expanded by the increased tractile forces that accompany inhalation during the breathing cycle. The illustration at *right* shows the contraction of the airway that accompanies exhalation. During inhalation, ASL remains in the fundus of the furrows over secreting cells where it cannot be absorbed. In contrast, with exhalation, the walls of the furrows compress and squeeze ASL out of the furrow toward and over the zone of absorptive cells of the ridges of the plications. The repetitive extrusion from and refilling of the furrows continuously agitates the ASL, like a washing machine, continuously stirring the fluid of the ciliated escalator to prevent aggregation and stagnation of the ASL and mucus.

structure of the luminal surface of small airways. In the new model, ASL volume is constantly recycled, i.e., replenished and reabsorbed by constitutively secreting cells distributed within the furrows and constitutively absorbing cells distributed along the ridges of the plications in the luminal surface. Provided absorption is not rate limiting or impeded, secretion and absorption rates are obligatorily matched so that ASL is autoregulated, permanently secreting and retaining fluid in the furrows, while preventing excessive fluid accumulations in the lumen by constant absorption at the ridges.

Mucins are essential to hygiene and must be secreted into the ASL for transport with entrapped debris over the ciliated surface of the luminal epithelium for expulsion by the ciliated escalator. However, experimental evidence along with the disease CF that arises from the defective anion channel CFTR further informs us that, without secreted HCO_3^-, mucus expansion and thinning are impeded and mucoviscidosis ensues, which is consistent with new evidence that small airways constitutively secrete HCO_3^-. Possibly in contrast to prior notions that mucoviscidosis in CF is due dehydrated mucus, more recent evidence indicates that lack of HCO_3^- secretion is the more likely defect underlying abnormal CF mucous properties.

Intriguingly, the model projects that the microanatomical plications are physiologically coupled to the act of breathing to provide continual agitation of the luminal ASL that thwarts stagnation and aggregation of luminal contents as the plications spread and narrow when the airway diameter expands and contracts with each cycle of inhalation and exhalation, respectively.

The small airway is an elegant display of anatomical structures integrated with physiological functions. ■

This review was supported by funds from the Nancy Olmstead Trust; Cystic Fibrosis Research, Inc.; National Cystic Fibrosis Foundation Therapeutics Awards.

No conflicts of interest, financial or otherwise, are declared by the author(s).

Author contributions: P.M.Q. conceived and designed research; P.M.Q. analyzed data; P.M.Q. interpreted results of experiments; P.M.Q. prepared figures; P.M.Q. drafted manuscript; P.M.Q. edited and revised manuscript; P.M.Q. approved final version of manuscript.

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