JGP 100th Anniversary

Epithelial transport in The Journal of General Physiology

Lawrence G. Palmer

Introduction

Epithelia separate the inside of the body from the outside. In multicellular organisms they enable absorption of nutrients from the environment or from ingested food, underlie extracellular volume and electrolyte homeostasis, and drive the secretion of fluids necessary for digestion, respiration, reproduction, and temperature regulation. They are, therefore, suitable subjects for the exploration of general physiology, defined by The Journal of General Physiology (JGP) to cover “basic biological, chemical, or physical mechanisms of broad physiological significance.” Although many epithelial functions obviously meet those criteria, the tissues are often difficult to study because of their complexity, generally involving two cell membranes in series with each other and in parallel with paracellular pathways. They may also include multiple cell types with different functions. Although not always a mainstay of JGP’s mission, research on epithelial function became an important component of its content in the 1950s, reaching a peak in the 1990s, before declining somewhat in recent years. This review will explore some of the most important topics in this field published in JGP. The discussion will certainly not be exhaustive; it would be impossible to cover the hundreds of relevant articles in this brief format. Instead, this review will focus on a few areas that have generated sustained coverage and interest in JGP, in many cases for several decades. This report is not meant to be a complete or unbiased review of the literature in each area. I will focus sharply on articles published in JGP, with reference to a few key papers appearing elsewhere.

Early years (1918–1950)

Epithelial biology appeared only sporadically in JGP during its early years. There were occasional articles on secretion of acid by the stomach (Teorell, 1939) and organic dyes by liver (Hober, 1939; Hober and Moore, 1939), and on the spontaneous voltage across frog skin (Amberson and Klein, 1928; Ponder and Macleod, 1937). However, even those topics did not develop sustained activity in the pages of JGP. Reasons for this include the complexities I’ve noted, as well as the lack of good experimental models. The frog skin, of course, ultimately became such a model, after the breakthrough paradigms of Ussing and Zerahn (1951); see “The Ussing model”). Stomach permeabilities could be studied to some extent in situ, although that approach obviously had its limitations. Liver function was assessed with an isolated, perfused organ from the frog, evidently not an easy preparation because that line of investigation ended after two studies. Micropuncture was one technique developed during the 1930s to study renal function in detail, but that approach did not ever gain a foothold in the studies in JGP.

Clearly, however, at least by the 1940s, JGP investigators were thinking about the basic principles underlying absorption and secretion. Winthrop Osterhout, one of the original editors of JGP and a prolific contributor to its pages, used his favorite model organism, the alga Nitella, to investigate those phenomena. Those same cells, which can grow to lengths of 5–10 cm, were also used to study bioelectric properties, including action potentials (Osterhout, 1934). In a series of experiments designed to explore trans-tissue fluid absorption and secretion, Osterhout bathed two halves of an isolated Nitella cell in separate aqueous compartments, separated by insu-
lating material, and measured water flow between the compartments (Fig. 1). To demonstrate movement of water from a solution of high osmolarity to one of lower osmolarity, as occurs in the mammalian kidney under conditions of antidiuresis, one half of the cell was immersed in 0.4 M sucrose and the other in pure H₂O to set up an osmotic gradient within the cytoplasm (Osterhout, 1949). When the concentration was suddenly reduced from 0.4 to 0.3 M, water moved toward the more-dilute compartment. This “uphill” movement of fluid was driven by osmotic forces within an intermediate compartment, namely the cell. A similar setup demonstrated osmotically driven fluid secretion (Osterhout, 1947). One side of the cell was again immersed in a sucrose solution, raising the intracellular osmolarity. When the sucrose solution was replaced with water, fluid moved from that compartment to the other, even though the two compartments had the same osmolarity. As described in "H₂O transport in epithelia," his basic idea anticipated the explanation of both fluid secretion and isotonic fluid absorption in epithelia, such as the small intestine, renal proximal tubule, and gall bladder.

Absorptive epithelia
The Ussing model. The work of Hans Ussing introduced two new paradigms for the understanding of epithelial function. The first was the recognition of active transepithelial Na⁺ transport and its quantitative assessment based on measurements of unidirectional fluxes using tracers and the short-circuit–current (voltage–clamp) technique (Ussing and Zerahn, 1951). This provided an operational definition of active transepithelial transport but revealed little about the underlying mechanism. The second was the idea that the spontaneous voltage developed across an epithelium reflects very different permeability properties of the plasma membranes facing the outside (apical or mucosal membrane) and the inside (basolateral or serosal membrane; Koefoed-Johnsen and Ussing, 1958). Thus, Ussing showed that the way to overcome the complexity of epithelial transport was to break the system down into its component parts, in this case, the two cell membranes.

In the years and decades after the appearance of this seminal work (in Acta Physiologica Scandinavica) JGP published many studies extending the basic findings to other epithelia. One of those was the toad urinary bladder (Leaf et al., 1958; Maffly and Edelman, 1963), in which the short-circuit current again was accounted for by the active transport of Na⁺. This tissue proved to be an invaluable model to study the actions of hormones, such as antidiuretic hormone and aldosterone (Leaf and Hays, 1962; Sharp and Leaf, 1968; Fig. 2). However, not all epithelia turned out to be so simple. In the skin of a South American species of frog, in contrast to that used by Ussing and Zerahn, the short-circuit current was smaller than the net flux of Na⁺, with net active absorption of Cl⁻ accounting for the difference (Zadunaisky et al., 1963). Later work with toad skin indicated that this Cl⁻ transport was powered by active H⁺ transport coupled to an apical Cl⁻/HCO₃⁻ exchange mechanism (Jensen et al., 1997). The gills of the freshwater fish also exhibited independent Na⁺ and
Cl⁻ uptake systems (Maetz and Garciaromeu, 1964). These complexities anticipated the coupled transport systems described in "Coupled transport systems."

At about the same time, the idea of active Na⁺ transport was found to apply to mammalian intestinal epithelia, studied both in vivo (Curran and Solomon, 1957) and in vitro (Curran, 1960; Schultz and Zalusky, 1964). These models also led to the understanding of the coupling of Na⁺ and solute movement and of salt and water movement (see "Coupled transport systems"). The Ussing approach was also extended to the renal proximal tubules of Necturus (Giebisch, 1961) and rat (Giebisch et al., 1964) perfused in vivo. Similar to the frog skin, the permeabilities of luminal and contraluminal membranes were asymmetric, and short-circuit current approximated the net Na⁺ flux, inferred from changes in the volume of fluid within the lumen.

Isolating the two membranes. Starting mostly in the 1970s, investigators began to use intracellular recording

Figure 2. **Epithelial Na⁺ channels in absorptive epithelia.** (A) Short-circuit current across the toad urinary bladder and its dependence on Na⁺ and oxidative metabolism are shown. The short-circuit current under normal conditions was equal to the net flux of Na measured with Na²² and Na²⁴. Transport was stimulated by oxytocin or vasopressin and was enhanced in the presence of O₂. From Leaf et al. (1958). (B) Flux-ratio analysis of Na⁺ permeation in frog skin. The value n′ = 1 is consistent with single-ion permeation through channels. From Benos et al. (1983). (C) Dependence of Na⁺ channel activity on aldosterone in rat collecting duct. From Pácha et al. (1993). (D) Conduction through WT ENaC and channels with point mutations in the putative selectivity filter. The WT channel is almost perfectly selective for Na⁺, rather than K⁺, whereas mutations in the second transmembrane domain of the α subunit confer conduction of K⁺. From Kellenberger et al. (2001).
techniques to quantify individual membrane conductances as well as intracellular ion activities in transporting epithelia. In the classic frog skin model, microelectrode recordings demonstrated a negative cell potential under most conditions and the much greater resistance of the apical membrane compared with the basolateral (Helman and Fisher, 1977; Schoen and Erlij, 1985; Harvey and Ehrenfeld, 1988). These approaches generated electrical models in the form of circuit diagrams. Circuit diagrams were also obtained for other high-resistance epithelia, including the rabbit urinary bladder (Lewis et al., 1977) and the Amphiura collecting duct (Horisberger and Giebisch, 1988), as well as “leaky” epithelia that have low-resistance paracellular pathways, exemplified by the Necturus proximal tubule (Anagnostopoulos et al., 1980) and the Necturus gall bladder (Cotton and Reuss, 1991).

That work also revealed an electrical behavior more complex than that of a simple ohmic resistance; the basolateral membrane conductance of frog skin exhibited rectification and time dependence. Detailed examination of those properties was impeded by the difficulty of controlling the membrane voltage in an intact system. Furthermore, apical and basolateral membranes interact with each other (Davis and Finn, 1982). This “cross talk” may reflect in part the sensitivity of conductances to intracellular pH (Harvey and Ehrenfeld, 1988) and intracellular Ca$^{2+}$ (Chase and Al-Awqati, 1983), both of which depend on intracellular Na$^+$ and hence on Na$^+$ transport rates.

**Recognizing individual transporters.** The next level of understanding of transport entailed more analytic descriptions of the individual components of those systems, ultimately at the level of defined transport proteins. This trend is exemplified by studies of the epithelial Na$^+$ channels that form the basis of the apical Na$^+$ permeability of frog skin, toad urinary bladder, and mammalian renal collecting duct and colon. In that case, the recognition of a very specific transport system started early with studies of saturation kinetics (Frazier et al., 1962) and block with the K-sparing diuretic amiloride (Benos et al., 1979). Advanced techniques, including fluctuation analysis, flux-ratio analysis, and single-channel recordings, showed those channels have a small, single-channel conductance that is exquisitely selective for Na$^+$ over K$^+$, slow and weakly voltage-dependent gating, minimal single filing, and control by the mineralocorticoid aldosterone (Benos et al., 1983; Helman et al., 1983; Palmer and Frindt, 1988; Pácha et al., 1993). With the cloning of the epithelial Na channel (ENaC) subunits comprising these channels, studies broadened to identify aspects of the channel important for ion selectivity (Schild et al., 1997; Kellenberger et al., 1999) and gating (Haerteis et al., 2012; Collier et al., 2014), moving the dissection of the system components to the intramolecular level. They have also included investigations of intracellular trafficking of the protein (Butterworth et al., 2005; Frindt et al., 2016).

On the other side of the cell, basolateral K$^+$ channels have proven to be more difficult to study in detail or to identify. This is due, in part, to the technical challenge of assessing the properties of that membrane and may also reflect the presence of multiple K$^+$-channel types (Germann et al., 1986). The inner membrane of the frog skin expresses low-conductance inwardly rectifying the K$^+$ channels (Urbach et al., 1994), presumably accounting for the high K$^+$ permeability of that membrane in the Koefoed-Johnsen and Usning model. Other basolateral K$^+$-channel types were identified at the single-channel level in the renal collecting duct (Wang, 1995), the proximal tubule (Mauzer et al., 1998), and the thick ascending limb of Henle’s loop (Paulais et al., 2006). In the last study, the channels were tentatively associated with the SLO2.2 gene product, but in most cases, the molecular identify of the basolateral K$^+$ channels remained uncertain.

The Na/K pump in the basolateral membrane forms the third critical component of the Na$^+$ absorbing system. Those pumps have been studied in epithelial cells (Sackin and Boulpaep, 1983). Furthermore many other articles in JGP have dealt with the properties of that transporter, but because those articles were not specific for epithelia, I will not review them here.

A final key component of absorptive epithelia, the shunt pathway, also received some attention. In the amphibian skin, mitochondria-rich cells comprise a major part of the shunt, at least with respect to the movement of Cl$^-$ that accompanies Na$^+$ uptake. That pathway includes apical membrane Cl$^-$ channels in those cells (Sørensen and Larsen, 1996). In “leaky” epithelia, paracellular transport through tight junctions becomes more important. A study of Necturus gall bladder showed that the organic cation triaminoopyrimidum selectively blocked Na$^+$ transport through that route (Moreno, 1975). Later work correlated that permeability with specific amino acid side chains in the tight-junction protein Claudin-2 (Yu et al., 2009). Rather than acting as a simple shunt, the paracellular pathway turned out to have its own complex behavior.

**Cl$^-$-secreting epithelia**

Using and colleagues were also the first to recognize active epithelial Cl$^-$ secretion (Koefoed-Johnsen et al., 1952). They applied the same measurements of tracer fluxes and short-circuit currents, and even the same frog skin preparations, but in this case, the skins were stimulated with norepinephrine to activate secretion, probably through glands embedded in the epithelium. Some of the most significant early work on Cl$^-$-secreting epithelia published in JGP involved the regulation of the process. Hokin and Hokin (1960, 1967) stimulated
secretion in the avian salt gland using acetylcholine, analogous to the stimulation of the skin by epinephrine. That prescient work, together with similar analyses of brain and pancreas, first identified changes in phospholipid metabolism in the regulation of cellular function (Fig. 3). It ultimately anticipated the role of G-protein–coupled PIP2 metabolism in the responses of cells to hormones and neurotransmitters.

In the canonical secretion process, Cl$^-$ enters the epithelial cells through secondary active transport across the basolateral membrane and exits through apical, anion-selective channels. Regulation of those channels controls secretion rates, and as such, they are analogous to the Na$^+$ channels of absorptive epithelia. This field received a huge boost in the early 1990s with the cloning of the CFTR gene and its identification as a cAMP-regulated Cl$^-$ channel. JGP provided an important forum for detailed studies of this transporter at the level of specific channel entities. Indeed, those channels mediate Cl$^-$ secretion in the glands of the frog skin (Sørensen and Larsen, 1998). This and further studies demonstrated that CFTR channels have broad selectivity for anions that follow the lyotropic series (Smith et al., 1999, 2001; Aubin and Linsdell, 2006). They are blocked by glycine hydrazide compounds (Muanprasat et al., 2004) and appear to interact with other transport systems (Tarran et al., 2006; Bertrand et al., 2009).

Two long series of publications from the laboratories of Gadsby (e.g., Vergani et al., 2003) and Hwang (e.g., Bompadre et al., 2005) elucidated some of the complex events governing the gating of CFTR by nucleotides, linking the operation of the channels with that of ATP-driven pumps.

Ca$^{2+}$-activated Cl$^-$ channels in the apical membrane offered an alternative pathway for Cl$^-$ secretion in some epithelia. Recent work has identified those channels with the TMEM16 gene family, and JGP has been a home for several detailed studies of them. Those

Figure 3. Control of epithelial fluid secretion. (A) Incorporation of $[^{32}\text{P}]$phosphatidic acid in goose nasal salt gland in response to a secretagogue. From (Hokin et al., 1960). (B) Control of airway surface liquid in cultured lung epithelial cells from healthy subjects (NL) and from patients with cystic fibrosis (CF). In CF or in the presence of bumetanide, a drug that blocks Cl$^-$ entry into the cells, the height of the surface layer is diminished. From Tarran et al. (2006). (C) Gating of CFTR by ATP. From Vergani et al. (2003). (D) Effect of specific negative charges in the outer mouth of the CFTR pore on channel conductance. From Aubin and Linsdell (2006).
proteins form dimers with independent, conducting pores (Jeng et al., 2016; Lim et al., 2016). Ca$^{2+}$ opens the channels through direct interactions that do not require calmodulin (Yu et al., 2014). The open channels conduct a range of anions, and permeation and gating are interdependent (Betto et al., 2014).

Other secretory pathways

As described in "Early years (1918–1950)," acid secretion by the stomach was a topic of early interest in JGP. Some further studies investigated the relationship between transport of H$^+$ and Cl$^-$ by the gastric mucosa (Durbin, 1964; Spencey et al., 1975), although that topic has not received much recent attention. JGP also had a role in the elucidation of H$^+$ secretion by renal epithelia, typified by the turtle urinary bladder. That tissue mimics the mechanism of acid secretion by the mammalian renal collecting duct. When active Na$^+$ transport is blocked, the short-circuit current reverses and can be accounted for by H$^+$ secretion into the urine through an active transport process tightly coupled to metabolism (Beauwens and Al-Awqati, 1976). Intracellular acidification stimulates, and mucosal acidification inhibits, H$^+$ secretion, which ultimately depends on a V-type proton pump in the luminal membrane (Cohen and Steinmetz, 1980; Andersen et al., 1985).

The kidneys and colon also secrete K$. Although JGP has not published many studies of this process at the organ or epithelial level, similar to the case for Cl$^-$ secretion, it has provided a forum for the detailed investigation of the individual channels involved. The luminal membrane of collecting-duct principal cells contains low-conductance, K$^+$-selective channels that are regulated by ATP and protein kinases (Wang and Giebisch, 1991; Lu et al., 2000). Their density increases with dietary K, supporting a role in K homeostasis (Palmer et al., 1994). The identification of those channels with the inward rectifier Kir1.1 (ROMK) facilitated structure–function studies of permeation (Choe et al., 2000; Yang et al., 2012).

Pancreatic ducts secrete HCO$_3^-$ into their luminal fluid, a process that helps to neutralize stomach acid in the duodenum. Solomon and colleagues (Swanson and Solomon, 1973, 1975) were the first to examine that transport system in JGP. Based on micropuncture measurements, they concluded that Na$^+$ and HCO$_3^-$ were both actively secreted, and that Na$^+$/H$^+$ and Cl$^-$/HCO$_3^-$ exchangers both had significant roles. A later study using isolated perfused pancreatic ducts localized Na$^+$/H$^+$ exchange to the basolateral membrane and Cl$^-$/HCO$_3^-$ exchangers to both membranes (Zhao et al., 1994). Although those anion exchangers could facilitate HCO$_3^-$ movement into the secreted fluid, CFTR could also directly conduct HCO$_3^-$ out of the cell into the lumen, analogous to the movement of Cl$^-$ in other secretory epithelia (Ishiguro et al., 2009).

Coupled transport systems

Although the frog skin and related absorptive organs use ion channels to take up Na$^+$ from the outside environment, other epithelia couple Na$^+$ influx with that of other solutes. Curran (1960) noted the strong dependence of intestinal Na$^+$ transport on luminal glucose but presumed that this reflected metabolic support for the active transport machinery. Crane (1962), whose main interest was in sugar rather than salt absorption, reinterpreted that phenomenon in terms of the simultaneous, interdependent transport of the two solutes. The cotransport concept eventually lead to the development of simple, oral rehydration solutions containing both salt and sugar to treat acute diarrheal diseases, such as cholera.

Again, JGP fostered an understanding of those systems at increasing resolution from whole tissue to intramolecular levels. Schultz and Zalusky (1964) refined the idea of coupled transport, describing the sugar specificity and kinetics of what is now known as the sodium-glucose cotransporter (Fig. 4). Subsequently the notion was extended to include the absorption of amino acids (Schultz et al., 1967).

As was the case with epithelial ion channels, the cloning of the SGLT1 gene and its expression in heterologous systems has permitted even more detailed studies of properties of the sodium-glucose cotransporter, producing a comprehensive kinetic model based on voltage-clamp and fluorescence labeling experiments (Loo et al., 2005, 2006). The availability of x-ray crystal structures of the protein, lead to further exploration of the conformational changes involved in the cotransport mechanism (Gagnon et al., 2006; Longpré et al., 2012).

Exchange with H$^+$ provides another major route for Na$^+$ entry into epithelial cells. This was demonstrated in gall bladder (Weinman and Reuss, 1982) and renal proximal tubule (Boron and Boulpaep, 1983) using intracellular pH measurements. These findings also led to the idea that parallel operation of Na$^+/H^+$ and Cl$^-$/HCO$_3^-$ exchangers could present as a coupled NaCl cotransport system (Reuss, 1984).

In the proximal tubule, Na$^+$/H$^+$ exchange serves to reabsorb HCO$_3^-$ from the renal ultrafiltrate. To complete the process, the cells transport HCO$_3^-$, formed along with H$^+$ in the cytoplasm, across the basolateral membrane. That process is electrogenic, independent of Cl$^-$ and coupled to Na$^+$ (Boron and Boulpaep, 1983a; Alpern, 1985). That cotransporter is unusual because the normal direction of Na$^+$ movement is out of the cell. Its cloning and expression in heterologous systems enabled detailed examination of its kinetics (Grichtchenko et al., 2000).

H$_2$O transport across epithelia

Transepithelial movements of fluid have intrigued the JGP community for a long time, as revealed by the early
work of Osterhout described in "Early years (1918–1950)." The discovery of active transport of salt across many epithelia suggested that the resulting ion gradients could drive water flow through osmotic forces. However, the finding that, at least in some epithelia, fluid can be absorbed without a measurable change in osmolarity (Curran and Solomon, 1957; Fig. 5) remained difficult to explain. To account for that phenomenon in the small intestine Curran (1960) proposed a restricted, intermediate compartment of increased osmolarity, very similar to the idea of Osterhout; the precise anatomic location of the compartment was not specified. Later Diamond (1964) demonstrated isotonic transport in the rabbit gall bladder and proposed that NaCl transport increased the osmolarity in the lateral spaces between cells. Osmotically driven H₂O movement increased hydrostatic pressure within those spaces, providing a driving force for its subsequent transport into the interstitium.

That basic idea has become widely accepted, but the details have been controversial. Diamond and Bossert (1967) proposed the "standing-gradient" model for isotonic fluid movement, in which the osmolarity of the interspaces increased from a closed end (the tight junction) to an open end of the paracellular channel. The model could account quantitatively for transport of fluid, at physiologically meaningful rates, with an osmolarity not measurably different from that of the source compartment. That idea inspired several experimental and theoretical tests. Sackin and Boulpaep (1975) reanalyzed the problem assuming a tight junction that was permeable to salt and water and showed that, for the proximal tubule, a hypertonic interspace could produce a nearly isotonic reabsorbate without the requirement for a gradient within the interspace. Later measurements of apical and basolateral membrane hydraulic water flow in the Necturus gall bladder showed that the water permeabilities of the cell mem-
branes were quite high (Fig. 6), suggesting that, in that epithelium, nearly isotonic fluid transport could be realized more simply with transcellular H₂O fluxes driven by osmotic gradients of <3 mOsm, which would be difficult to detect (Persson and Spring, 1982; Cotton et al., 1989).

A more recent model for fluid absorption included the idea of recirculation of Na⁺ from the serosal compartment to the interspaces. That process involves passive uptake of Na⁺ into the cell, presumably across the basal membrane, and active pumping across the lateral membranes into the interspaces (Larsen et al., 2000).

That idea accounts for the uphill movement of fluid from a higher to a lower osmolarity, anomalous solvent drag in which solutes are reabsorbed against the net flow of fluid in the opposite direction, and fluid reabsorption in the absence of net transepithelial transport.

The issue of fluid movements across epithelia also arises in the context of control of the airway surface liquid (ASL) layer mucosal surface of the lung. The depth of that layer may be reduced in cystic fibrosis, leading to impaired clearance of mucous and infective agents (Fig. 3 B). Na⁺ and Cl⁻ concentrations in the surface liquid of cultured airway cells were similar to those in plasma, implying that fluid transfer across the epithelium was nearly isotonic and that the thickness of the layer (~7 µm) was controlled by relative rates of Cl⁻ secretion and Na⁺ absorption (Tarran et al., 2001). The sensor for the regulation of the height of the ASL is thought to be a set of soluble components of the liq-

Figure 5. Isotonic fluid transport. (A) Solute and water transport in a rat ileum. The dashed line indicates the relationship for identical osmolarities of absorbed fluid and that of the luminal medium. From Curran and Solomon (1957). (B) "Standing-gradient" model to explain isotonic transport. The model postulates that interspaces between cells contain hypotonic fluid with the osmolarity decreasing from the tight junction to the interstitial space. From Diamond and Bossert (1967). (C) Simulation of isotonic fluid transport with a uniformly elevated osmolarity in the interspace. From Sackin and Boulpaep (1975). (D) Model of isotonic fluid transport using Na⁺ recirculation across the basal and lateral membranes. From Larsen et al. (2000).
uid (Tarran et al., 2006). The issue, however, is controversial. In direct studies of small airways, no effects of blocking ENaC or CFTR on the height of the ASL could be demonstrated (Song et al., 2003).

A different type of fluid movement occurs in the collecting duct of the kidney, particularly in response to antidiuretic hormone (ADH). Here, water can be absorbed from a concentrated fluid (the urine) into a more dilute fluid (the blood). This “uphill” movement also involves an intermediate compartment—in this case, the renal medullary interstitium—what has an osmolarity at least slightly higher than that of the urine, providing a driving force for reabsorption of water across the epithelium. The toad urinary bladder proved to be a good in vitro model for studying the ADH-dependent water permeability (Bentley, 1958). Hays and Leaf (1962) made a key observation that, in the presence of the hormone the hydraulic water permeability ($P_f$), assessed as bulk water flow, increased much more than the diffusional permeability ($P_d$), measured with tracers. High values of $P_f/P_d$ and the relative magnitude of changes in water and solute permeability were later interpreted to indicate that water flowed through long, aqueous pores (Finkelstein, 1976; Levine et al., 1984).

Figure 6. Measurements of epithelial water permeability. (A) Changes in cell volume of Necturus gall bladder in response to hypertonic challenge using an optical technique. From Persson and Spring (1982). (B) Similar changes measured with an intracellular microelectrode sensor. Hyper, hypertonic. From Cotton et al. (1989). (C) Measurement of $P_f/P_d$ in toad urinary bladder stimulated with ADH or cAMP, or doped with the ionophore amphotericin B. From Levine et al. (1984). (D) Measurement of $H_2O$ permeability of endosomes isolated from toad urinary bladders with different pretreatments showing high $H_2O$ permeability in these organelles. Br-cAMP, 8-bromoadenosine 3',5'-cyclic monophosphate. From Shi et al. (1990).
Eggenga (1972) had presented a similar hypothesis based on the temperature dependence of bulk water flow. That idea was eventually confirmed by the identification of the apical water channel AQP2. The mechanism underlying the control of the channels by ADH has also generated interest. Endosomes from toad bladder had very high water permeability, suggesting that water channels were inserted into the apical membrane from those vesicles in response to the hormone (Shi et al., 1990). This supported ultrastructural studies identifying putative channel proteins in both surface and tubulovesicular membranes (Muller et al., 1980). Since that time, control by transporter protein insertion into and retrieval from the plasma membrane has become an important paradigm in epithelial biology.

Conclusions

Particularly during the past 60 yr, JGP has published important work in the area of epithelial transport. As befits the mission of JGP, this research involves topics of widespread, fundamental interest, such as the mechanisms underlying absorption and secretion in a variety of epithelia. The work has progressed from a phenomenologic description of active transport to elucidation of the properties of individual cell membranes, and finally to the identification of specific molecules (and parts of molecules) conferring these properties and their regulation. Future work will likely continue this trend and, at the same time, deepen our understanding of how the various parts of the epithelia work together as a system to move solutes and water.

ACKNOWLEDGMENTS

The writing of this manuscript was supported by National Institutes of Health grants ROI-DK999284 and ROI-DK111380.

The author declares no competing financial interests.

Olaf S. Andersen served as editor.

REFERENCES

Alpern, R.J. 1985. Mechanism of basolateral membrane H+/OH−/HCO3− transport in the rat proximal convoluted tubule. A sodium-coupled electrogenic process. J. Gen. Physiol. 86:613–636. http://dx.doi.org/10.1085/jgp.86.5.613

Amberson, W.R., and H. Klein. 1928. The influence of pH upon the concentration potentials across the skin of the frog. J. Gen. Physiol. 11:823–841. http://dx.doi.org/10.1085/jgp.11.6.823

Anagnostopoulos, T., J. Teulon, and A. Edelman. 1980. Conductive properties of the proximal tubule in Necturus kidney. J. Gen. Physiol. 75:553–587. http://dx.doi.org/10.1085/jgp.75.5.553

Andersen, O.S., J.E. Silveira, and P.R. Steinmetz. 1983. Intrinsic characteristics of the proton pump in the luminal membrane of a tight urinary epithelium. The relation between transport rate and ΔφH, J. Gen. Physiol. 86:215–234. http://dx.doi.org/10.1085/jgp.86.2.215

Aubin, C.N., and P. Linsdell. 2006. Positive charges at the intracellular mouth of the pore regulate anion conduction in the CFTR chloride channel. J. Gen. Physiol. 128:535–545. http://dx.doi.org/10.1085/jgp.200609516

Beaumens, R., and Q. Al-Awqati. 1976. Active H+ transport in the turtle urinary bladder. Coupling of transport to glucose oxidation. J. Gen. Physiol. 68:421–439. http://dx.doi.org/10.1085/jgp.68.4.421

Benos, D.J., L.J. Mandel, and R.S. Balaban. 1979. On the mechanism of the amiloride–sodium entry site interaction in anuran skin epithelia. J. Gen. Physiol. 73:307–326. http://dx.doi.org/10.1085/jgp.73.3.307

Benos, D.J., B.A. Hyde, and R. Latorre. 1983. Sodium flux ratio through the amiloride-sensitive entry pathway in frog skin. J. Gen. Physiol. 81:667–685. http://dx.doi.org/10.1085/jgp.81.5.667

Bentley, P.J. 1958. The effects of neurohypophysial extracts on the flux of Na+ in the toad bladder. Studies using a fast-reaction apparatus. J. Gen. Physiol. 41:381–391. http://dx.doi.org/10.1085/jgp.41.6.381

Bertrand, C.A., R. Zhang, J.M. Pilewski, and R.A. Frizzell. 2009. SLC26A9 is a constitutively active, CFTR-regulated anion conductance in human bronchial epithelia. J. Gen. Physiol. 133:421–438. http://dx.doi.org/10.1085/jgp.200810097

Betko, G., O.L. Cherian, S. Piferi, V. Genedese, A. Boccaccio, and A. Menini. 2014. Interactions between permeation and gating in the TMEM16B/anoctamin2 calcium-activated chloride channel. J. Gen. Physiol. 143:703–718. (published erratum appears in J. Gen. Physiol. 2014: 144:125) http://dx.doi.org/10.1085/jgp.201411182

Bompadre, S.G., T. Ai, J.H. Cho, X. Wang, Y. Solma, M. Li, and T.C. Hwang. 2005. CFTR gating I: Characterization of the ATP-dependent gating of a phosphorylation-independent CFTR channel (ΔR-CFTR). J. Gen. Physiol. 125:361–375. http://dx.doi.org/10.1085/jgp.200409227

Boron, W.F., and E.L. Boulpaep. 1983a. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO3− transport. J. Gen. Physiol. 81:53–94. http://dx.doi.org/10.1085/jgp.81.1.53

Boron, W.F., and E.L. Boulpaep. 1983b. Intracellular pH regulation in the renal proximal tubule of the salamander. Na−H exchange. J. Gen. Physiol. 81:29–52. http://dx.doi.org/10.1085/jgp.81.1.29

Butterworth, M.B., R.S. Edinger, J.P. Johnson, and R.A. Frizzell. 2005. Acute ENaC stimulation by cAMP in a kidney cell line is mediated by exocytic insertion from a recycling channel pool. J. Gen. Physiol. 125:81–101. http://dx.doi.org/10.1085/jgp.200409124

Chase, H.S.J. Jr., and Q. Al-Awqati. 1983. Calcium reduces the sodium permeability of luminal membrane vesicles from toad bladder. Studies using a fast-reaction apparatus. J. Gen. Physiol. 81:643–665. http://dx.doi.org/10.1085/jgp.81.5.643

Choe, H., H. Sackin, and L.G. Palmer. 2006. Permeation properties of inward-rectifier potassium channels and their molecular determinants. J. Gen. Physiol. 115:391–404. http://dx.doi.org/10.1085/jgp.115.4.391

Cohen, L.H., and P.R. Steinmetz. 1980. Control of active proton transport in turtle urinary bladder by cell pH. J. Gen. Physiol. 76:381–393. http://dx.doi.org/10.1085/jgp.76.3.381

Collier, D.M., V.R. Tomkovicz, Z.J. Peterson, C.J. Benson, and P.M. Snyder. 2014. Intersubunit conformational changes mediate epithelial sodium channel gating. J. Gen. Physiol. 144:337–348. http://dx.doi.org/10.1085/jgp.201411208

Cotton, C.U., and L. Reuss. 1991. Electrophysiological effects of extracellular ATP on Necturus gallbladder epithelium. J. Gen. Physiol. 97:949–971. http://dx.doi.org/10.1085/jgp.97.5.949

Cotton, C.U., A.M. Weinstein, and L. Reuss. 1989. Osmotic water permeability of Necturus gallbladder epithelium. J. Gen. Physiol. 93:649–679. http://dx.doi.org/10.1085/jgp.93.4.649

Crane, R.K. 1962. Hypothesis for mechanism of intestinal active transport of sugars. Fed. Proc. 21:891–895.
Curran, P.F. 1960. Na, Cl, and water transport by rat ileum in vitro. *J. Gen. Physiol.* 43:1137–1148. http://dx.doi.org/10.1085/jgp.43.6.1137

Curran, P.F., and A.K. Solomon. 1957. Ion and water fluxes in the ileum of rats. *J. Gen. Physiol.* 41:143–168. http://dx.doi.org/10.1085/jgp.41.1.143

Davis, C.W., and A.L. Finn. 1982. Sodium transport effects on the basolateral membrane in toad urinary bladder. *J. Gen. Physiol.* 80:733–751. http://dx.doi.org/10.1085/jgp.80.5.733

Diamond, J.M. 1964. The mechanism of isotonic water transport. *J. Gen. Physiol.* 48:15–42. http://dx.doi.org/10.1085/jgp.48.1.15

Diamond, J.M., and W.H. Bossert. 1967. Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* 50:2061–2083. http://dx.doi.org/10.1085/jgp.50.8.2061

Durbin, R.P. 1964. Anion requirements for gastric acid secretion. *J. Gen. Physiol.* 47:735–748. http://dx.doi.org/10.1085/jgp.47.4.735

Eggena, P. 1972. Temperature dependence of vasopressin action on the toad bladder. *J. Gen. Physiol.* 59:519–533. http://dx.doi.org/10.1085/jgp.59.5.519

Finkelstein, A. 1976. Nature of the water permeability increase induced by antidiuretic hormone (ADH) in toad urinary bladder and related tissues. *J. Gen. Physiol.* 68:137–143. http://dx.doi.org/10.1085/jgp.68.2.137

Frazier, H.S., E.F. Dempsey, and A. Leaf. 1962. Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* 45:529–543. http://dx.doi.org/10.1085/jgp.45.5.529

Fridnt, G., D. Gravotta, and L.G. Palmer. 2016. Regulation of ENaC trafficking in rat kidney. *J. Gen. Physiol.* 147:217–227. http://dx.doi.org/10.1085/jgp.201511533

Gagnon, D.G., P. Bissonnette, and J.Y. Lapointe. 2006. Identification of a disulfide bridge linking the fourth and the seventh extracellular loops of the Na+/glucose cotransporter. *J. Gen. Physiol.* 127:145–158. http://dx.doi.org/10.1085/jgp.200609439

Germann, W.J., M.E. Lowy, S.A. Ernst, and D.C. Dawson. 1986. Differentiation of two distinct K conductances in the basolateral membrane of turtle colon. *J. Gen. Physiol.* 88:257–251. http://dx.doi.org/10.1085/jgp.88.2.257

Giebisch, G. 1961. Measurements of electrical potential differences Giebisch, G. and W.F. Boron. 2000. Extracellular HCO_3^- dependence of electrogenic Na/HCO_3 cotransporters cloned from salamander and rat kidney. *J. Gen. Physiol.* 115:533–546. http://dx.doi.org/10.1085/jgp.115.5.533

Haerteis, S., M. Krappitz, A. Diakov, A. Krappitz, R. Rauh, and C. Korbacher. 2012. Plasma and chymotrypsin have distinct preferences for channel activating cleavage sites in the γ subunit of the human epithelial sodium channel. *J. Gen. Physiol.* 140:373–389. http://dx.doi.org/10.1085/jgp.2011H0763

Harvey, B.J., and J. Ehrenfeld. 1988. Role of Na+/H+ exchange in the control of intracellular pH and cell membrane conductances in frog skin epithelium. *J. Gen. Physiol.* 92:793–810. http://dx.doi.org/10.1085/jgp.92.6.793

Hays, R.M., and A. Leaf. 1962. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* 45:905–919. http://dx.doi.org/10.1085/jgp.45.5.905

Helman, S.L., and R.S. Fisher. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* 69:571–604. http://dx.doi.org/10.1085/jgp.69.5.571

Helman, S.L., T.C. Cox, and W. Van Driesche. 1983. Hormonal control of apical membrane Na transport in epithelia. Studies with fluctuation analysis. *J. Gen. Physiol.* 82:201–220. http://dx.doi.org/10.1085/jgp.82.2.201

Hober, R. 1939. Studies concerning the nature of the secretory activity of the isolated Ringer-perfused frog liver: I. The differential secretion of pairs of dyestuffs. *J. Gen. Physiol.* 23:185–190. http://dx.doi.org/10.1085/jgp.23.2.185

Hober, R., and E. Moore. 1939. Studies concerning the nature of the secretory activity of the isolated Ringer-perfused frog liver: III. The inhibitory and the promoting influence of organic electrolytes and non-electrolytes upon the secretion of dyestuffs. *J. Gen. Physiol.* 23:191–202. http://dx.doi.org/10.1085/jgp.23.2.191

Hokin, L.E., and M.R. Hokin. 1960. Studies on the carrier function of phosphatidic acid in sodium transport. I. The turnover of phosphatidic acid and phosphoinositide in the avian salt gland on stimulation of secretion. *J. Gen. Physiol.* 44:61–85. http://dx.doi.org/10.1085/jgp.44.1.61

Hokin, M.R., and L.E. Hokin. 1967. The formation and continuous turnover of a fraction of phosphatidic acid on stimulation of NaCl secretion by acetylcholine in the salt gland. *J. Gen. Physiol.* 50:793–811. http://dx.doi.org/10.1085/jgp.50.4.793

Hokin, M.R., L.E. Hokin, and W.D. Shelp. 1960. The effects of acetylcholine on the turnover of phosphatidic acid and phosphoinositide in sympathetic ganglia, and in various parts of the central nervous system in vitro. *J. Gen. Physiol.* 44:217–226. http://dx.doi.org/10.1085/jgp.44.2.217

Hörisberger, J.D., and G. Giebisch. 1988. Intracellular Na⁺ and K⁺ activities and membrane conductances in the collecting tubule of Amphiuma. *J. Gen. Physiol.* 92:643–665. http://dx.doi.org/10.1085/jgp.92.5.643

Ishiguro, H., M.C. Steward, S. Naruse, S.B. Ko, H. Goto, R.M. Case, T. Kondo, and A. Yamamoto. 2009. CFTR functions as a bicarbonate channel in pancreatic duct cells. *J. Gen. Physiol.* 133:315–326. http://dx.doi.org/10.1085/jgp.200810122

Jeng, G., M. Aggarwal, W.P. Yu, and T.Y. Chen. 2016. Independent activation of distinct pores in dimeric TMEM16A channels. *J. Gen. Physiol.* 148:393–404. http://dx.doi.org/10.1085/jgp.201611651

Jensen, L.J., J.N. Sørensen, E.H. Larsen, and N.J. Willumsen. 1997. Proton pump activity of mitochondria-rich cells. The interpretation of external proton-concentration gradients. *J. Gen. Physiol.* 109:73–91. http://dx.doi.org/10.1085/jgp.109.1.73

Kellenberger, S., N. Hoffmann-Pochon, I. Gautschi, E. Schneeberger, and L. Schild. 1999. On the molecular basis of ion permeation in the epithelial Na⁺ channel. *J. Gen. Physiol.* 114:13–30. http://dx.doi.org/10.1085/jgp.114.1.13

Kellenberger, S., M. Auberson, I. Gautschi, E. Schneeberger, and L. Schild. 2001. Permeability properties of ENaC selectivity filter mutants. *J. Gen. Physiol.* 118:679–692. http://dx.doi.org/10.1085/jgp.118.6.679

Kofoed-Johnsen, V., and H.H. Ussing. 1958. The nature of the frog skin potential. *Acta Physiol. Scan. D.* 42:298–308. http://dx.doi.org/10.1111/j.1748-1716.1958.tb01563.x

Kofoed-Johnsen, V., H.H. Ussing, and K. Zerahn. 1952. The origin of the short-circuit current in the adrenaline stimulated frog skin. *Acta Physiol. Scand.* 27:38–48. http://dx.doi.org/10.1111/j.1748-1716.1953.tb00922.x

Larsen, E.H., J.B. Sørensen, and J.N. Sørensen. 2000. A mathematical model of solute coupled water transport in toad intestine incorporating recirculation of the actively transported
solute. J. Gen. Physiol. 116:101–124. http://dx.doi.org/10.1085/jgp.116.2.101

Leaf, A., and R.M. Hays. 1962. Permeability of the isolated toad bladder to solutes and its modification by vasopressin. J. Gen. Physiol. 45:921–932. http://dx.doi.org/10.1085/jgp.45.5.921

Leaf, A., J. Anderson, and L.B. Page. 1958. Active sodium transport by the isolated toad bladder. J. Gen. Physiol. 41:657–668. http://dx.doi.org/10.1085/jgp.41.4.657

Levine, S.D., M. Jacoby, and A. Finkelstein. 1984. The water permeability of toad urinary bladder. II. The value of Pf/Pd(w) for the antidiuretic hormone-induced water permeation pathway. J. Gen. Physiol. 83:543–561. http://dx.doi.org/10.1085/jgp.83.4.543

Lewis, S.A., D.C. Eaton, C. Clausen, and J.M. Diamond. 1977. Nystatin as a probe for investigating the electrical properties of a tight epithelium. J. Gen. Physiol. 70:427–440. http://dx.doi.org/10.1085/jgp.70.4.427

Lim, N.K., A.K. Lam, and R. Dutzler. 2016. Independent activation of ion conduction pores in the double-barreled calcium-activated chloride channel TMEM16A. J. Gen. Physiol. 148:375–392. http://dx.doi.org/10.1085/jgp.201611650

Maetz, J., and F. Garcieromeu. 1964. The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, Carassius auratus. II. Evidence for NH4/Ion/Na Ion and HCO3/Ion/CL ion exchanges. J. Gen. Physiol. 47:1209–1227. http://dx.doi.org/10.1085/jgp.47.6.1209

Maffly, R.H., and I.S. Edelman. 1963. Permeability of the isolated toad bladder. J. Gen. Physiol. 41:657–668. http://dx.doi.org/10.1085/jgp.41.4.657

Maetz, J., and F. Garciaromeu. 1964. The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, Carassius auratus. II. Evidence for NH4/Ion/Na Ion and HCO3/Ion/CL ion exchanges. J. Gen. Physiol. 47:1209–1227. http://dx.doi.org/10.1085/jgp.47.6.1209

Maffly, R.H., and I.S. Edelman. 1963. Permeability of the isolated toad bladder. J. Gen. Physiol. 41:657–668. http://dx.doi.org/10.1085/jgp.41.4.657

Mauerer, U.R., E.L. Boulpaep, and A.S. Segal. 1998. Properties of an inwardly rectifying ATP-sensitive K channel in the basolateral membrane of renal proximal tubule. J. Gen. Physiol. 111:139–160. http://dx.doi.org/10.1085/jgp.111.1.139

Moreno, J.H. 1975. Blockage of gallbladder tight junction cation-selective channels by 2,4-triaminopyrimidinium (TAP). J. Gen. Physiol. 66:97–115. http://dx.doi.org/10.1085/jgp.66.1.97

Muanprasat, C., N.D. Sonawane, D. Salinas, A. Taddei, L.J. Galietta, and A.S. Verkman. 2004. Discovery of glyacin hydrizde pore-occluding CFTR inhibitors: Mechanism, structure-activity analysis, and in vivo efficacy. J. Gen. Physiol. 124:125–157. http://dx.doi.org/10.1085/jgp.200409059

Muller, J., W.A. Kachadorian, and V.A. DiScala. 1980. Evidence that ADH-stimulated intramembrane particle aggregates are transferred from cytoplasmic to luminal membranes in toad bladder epithelial cells. J. Cell Biol. 85:83–95. http://dx.doi.org/10.1083/jcb.85.1.83

Osterhout, W.J. 1934. Nature of the action current in Nitella: I. General considerations. J. Gen. Physiol. 18:215–227. http://dx.doi.org/10.1085/jgp.18.2.215

Osterhout, W.J. 1947. Some aspects of secretion: I. Secretion of water. J. Gen. Physiol. 30:439–447. http://dx.doi.org/10.1085/jgp.30.5.439

Osterhout, W.J. 1949. Transport of water from concentrated to dilute solutions in cells of Nitella. J. Gen. Physiol. 32:559–566. http://dx.doi.org/10.1085/jgp.32.4.559

Page, J.B., and K.R. Spring. 1982. Gallbladder epithelial cell hydraulic water permeability and volume regulation. J. Gen. Physiol. 79:481–505. http://dx.doi.org/10.1085/jgp.79.3.481

Palais, M., S. Lachheb, and J. Teulon. 2006. A Na+- and Cl- activated K channel in the thick ascending limb of mouse kidney. J. Gen. Physiol. 127:205–215. http://dx.doi.org/10.1085/jgp.200509560

Peresson, B.E., and K.R. Springer. 1982. Gallbladder epithelial cell hydraulic water permeability and volume regulation. J. Gen. Physiol. 79:481–505. http://dx.doi.org/10.1085/jgp.79.3.481

Ponder, E., and J. Macleod. 1937. The potential and respiration of frog skin: I. The effect of the homologous carboxamates. II. The effect of certain lysins. J. Gen. Physiol. 20:433–447. http://dx.doi.org/10.1085/jgp.20.3.433

Poulsen, B.E., and K.R. Springer. 1982. Gallbladder epithelial cell hydraulic water permeability and volume regulation. J. Gen. Physiol. 79:481–505. http://dx.doi.org/10.1085/jgp.79.3.481

Reuss, L. 1984. Independence of apical membrane Na+ and Cl− entry in Necturus gallbladder epithelium. J. Gen. Physiol. 84:423–445. http://dx.doi.org/10.1085/jgp.84.3.423

Sackin, H., and E.L. Boulpaep. 1975. Models for coupling of salt and water transport; Proximal tubular reabsorption in Necturus kidney. J. Gen. Physiol. 66:671–733. http://dx.doi.org/10.1085/jgp.66.6.671

Sackin, H., and E.L. Boulpaep. 1983. Rheogenic transport in the renal proximal tubule. J. Gen. Physiol. 82:819–851. http://dx.doi.org/10.1085/jgp.82.6.819

Schild, L., E. Schneeberger, I. Gautchi, and D. Fiszov. 1997. Identification of amino acid residues in the α, β, and γ subunits of the epithelial sodium channel (ENaC) involved in amiloride block and ion permeation. J. Gen. Physiol. 109:15–26. http://dx.doi.org/10.1085/jgp.109.1.15

Schoen, H.F., and D. Erlij. 1985. Current-voltage relations of the apical and basolateral membranes of the frog skin. J. Gen. Physiol. 86:257–287. http://dx.doi.org/10.1085/jgp.86.2.257

Schultz, S.G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum II. Interaction between active sodium and active sugar transport. J. Gen. Physiol. 47:1043–1059. http://dx.doi.org/10.1085/jgp.47.6.1043

Schultz, S.G., P.F. Curran, R.A. Chez, and R.E. Fuisz. 1967. Alanine and sodium fluxes across mucosal border of rabbit ileum. J. Gen. Physiol. 50:1241–1260. http://dx.doi.org/10.1085/jgp.50.5.1241

Sharp, G.W., and A. Leaf. 1968. On the stimulation of sodium fluxes across mucosal border of rabbit ileum. J. Gen. Physiol. 42:433–447. http://dx.doi.org/10.1085/jgp.42.3.433

Smith, S.S., E.D. Steinle, M.E. Meyerhoff, and D.C. Dawson. 1999. Cystic fibrosis transmembrane conductance regulator. Physical
basis for lyotropic anion selectivity patterns. *J. Gen. Physiol.* 114:799–818. http://dx.doi.org/10.1085/jgp.114.6.799

Smith, S.S., X. Liu, Z.R. Zhang, F. Sun, T.E. Kriewall, N.A. McCarty, and D.C. Dawson. 2001. CFTR: Covalent and noncovalent modification suggests a role for fixed charges in anion conduction. *J. Gen. Physiol.* 118:407–432. http://dx.doi.org/10.1085/jgp.118.4.407

Song, Y., J. Thiggarajah, and A.S. Verkman. 2003. Sodium and chloride concentrations, pH, and depth of airway surface liquid in distal airways. *J. Gen. Physiol.* 122:511–519. http://dx.doi.org/10.1085/jgp.122.5.421

Sørensen, J.B., and E.H. Larsen. 1996. Heterogeneity of chloride channels in the apical membrane of isolated mitochondria-rich cells from toad skin. *J. Gen. Physiol.* 108:241–243. http://dx.doi.org/10.1085/jgp.108.5.421

Sørensen, J.B., and E.H. Larsen. 1998. Patch clamp on the luminal membrane of exocrine gland cells from frog skin (Rana esculenta) reveals the presence of cystic fibrosis transmembrane conductance regulator-like Cl− channels activated by cyclic AMP. *J. Gen. Physiol.* 112:19–31. http://dx.doi.org/10.1085/jgp.112.1.19

Spenney, J.G., G. Flemstrom, R.L. Shoemaker, and G. Sachs. 1975. Quantitation of conductance pathways in antral gastric mucosa. *J. Gen. Physiol.* 65:645–662. http://dx.doi.org/10.1085/jgp.65.5.645

Swanson, C.H., and A.K. Solomon. 1973. A micropuncture investigation of the whole tissue mechanism of electrolyte secretion by the in vitro rabbit pancreas. *J. Gen. Physiol.* 62:407–429. http://dx.doi.org/10.1085/jgp.62.4.407

Swanson, C.H., and A.K. Solomon. 1975. Micropuncture analysis of the cellular mechanisms of electrolyte secretion by the in vitro rabbit pancreas. *J. Gen. Physiol.* 65:22–45. http://dx.doi.org/10.1085/jgp.65.1.22

Tarran, R., B.R. Grubb, J.T. Gatzy, C.W. Davis, and R.C. Boucher. 2001. The relative roles of passive surface forces and active ion transport in the modulation of airway surface liquid volume and composition. *J. Gen. Physiol.* 118:223–236. http://dx.doi.org/10.1085/jgp.118.2.223

Tarran, R., L. Trout, S.H. Donaldson, and R.C. Boucher. 2006. Soluble mediators, not cilia, determine airway surface liquid volume in normal and cystic fibrosis superficial airway epithelia. *J. Gen. Physiol.* 127:391–404. http://dx.doi.org/10.1085/jgp.200509468

Teorell, T. 1939. On the permeability of the stomach mucosa for acids and some other substances. *J. Gen. Physiol.* 23:263–274. http://dx.doi.org/10.1085/jgp.23.2.263

Urbach, V., E. van Kerkhove, and B.J. Harvey. 1994. Inward-rectifier potassium channels in basolateral membranes of frog skin epithelium. *J. Gen. Physiol.* 103:583–604. http://dx.doi.org/10.1085/jgp.103.4.583

Wang, W.H. 1995. Regulation of the hyperpolarization-activated K+ channel in the lateral membrane of the cortical collecting duct. *J. Gen. Physiol.* 106:25–43. (published erratum appears in *J. Gen. Physiol.* 1995. 106:579) http://dx.doi.org/10.1085/jgp.106.1.25

Wang, W., and G. Giebish. 1991. Dual effect of ATP on the apical small conductance K+ channel of rat cortical collecting duct. *J. Gen. Physiol.* 98:35–61. http://dx.doi.org/10.1085/jgp.98.1.35

Weinman, S.A., and L. Reuss. 1982. Na+–H+ exchange at the Na+–K+–ATPase. *J. Gen. Physiol.* 79:624–639. http://dx.doi.org/10.1085/jgp.79.4.56

Wheaton, S.A., and L. Reuss. 1982. Na+ transport in the Ano1 (TMEM16A) chloride channel by calcium is not mediated by calmodulin. *J. Gen. Physiol.* 98:35–61. http://dx.doi.org/10.1085/jgp.98.1.35

Yu, K., J. Zhu, Z. Qu, Y.Y. Cui, and H.C. Hartzell. 2014. Activation of the Ano1 (TMEM16A) chloride channel by calcium is not mediated by calmodulin. *J. Gen. Physiol.* 143:253–267. http://dx.doi.org/10.1085/jgp.201311047

Zadunaisky, J.A., O.A. Cândia, and D.J. Chiardini. 1963. The origin of the short-circuit current in the isolated skin of the South American frog Leptodactylus ocellatus. *J. Gen. Physiol.* 47:393–402. http://dx.doi.org/10.1085/jgp.47.2.393

Zhao, H., R.A. Star, and S. Muallem. 1994. Membrane localization of H+ and HCO3− transporters in the rat pancreatic duct. *J. Gen. Physiol.* 104:57–85. http://dx.doi.org/10.1085/jgp.104.1.57