JGP 100th Anniversary

Epithelial transport in *The Journal of General Physiology*

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Epithelia define the boundaries of the body and often transfer solutes and water from outside to inside (absorption) or from inside to outside (secretion). Those processes involve dual plasma membranes with different transport components that interact with each other. Understanding those functions has entailed breaking down the problem to analyze properties of individual membranes (apical vs. basolateral) and individual transport proteins. It also requires understanding of how those components interact and how they are regulated. This article outlines the modern history of this research as reflected by publications in *The Journal of General Physiology*.

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-

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Abbreviations used: ADH, antidiuretic hormone; ASL, airway surface liquid; ENaC, epithelial Na channel; P_d, diffusional water permeability; P_f, hydraulic water permeability.

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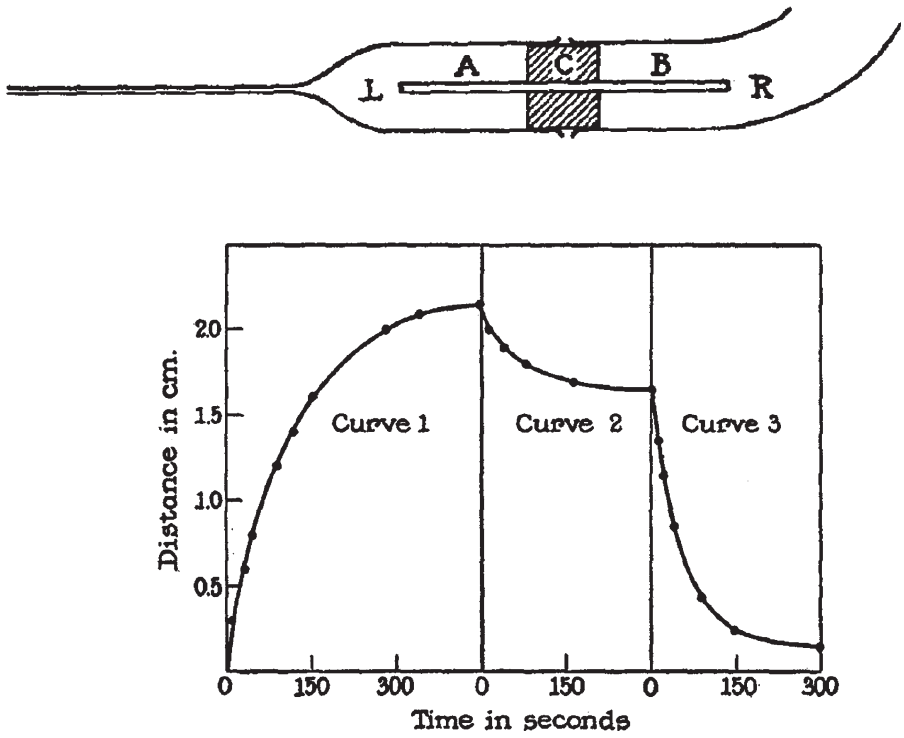

Figure 1. **H_2O movement through cells of *Nitella*.** (Top) Device for measuring fluid movement. A, Half of a single cell immersed in L; B, half of a single cell immersed in R; C, seal separating L and R; L, left aqueous compartment; R, right aqueous compartment. The compartments were insulated with a rubber pencil eraser or a piece of cork, anticipating later development of sucrose–gap preparations. (Bottom) Time course of movement of water from compartment L to R. The distance on the y axis indicates the movement of the water meniscus in the narrow neck of the capillary in compartment L. Curve 1 shows movement from L to R when the fluid in R is switched from H_2O to 0.4 M sucrose with distilled H_2O in L. Curve 2 shows movement in the reverse direction when solution R is replaced with 0.3 M sucrose, showing water flows from a more concentrated compartment to a more dilute compartment. In curve 3, compartment R is replaced with distilled water, bringing the system back to its original state. From Osterhout (1949).

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 H_2O 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 "H_2O 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^-/HCO_3^- exchange mechanism (Jensen et al., 1997). The gills of the freshwater fish also exhibited independent Na^+ and

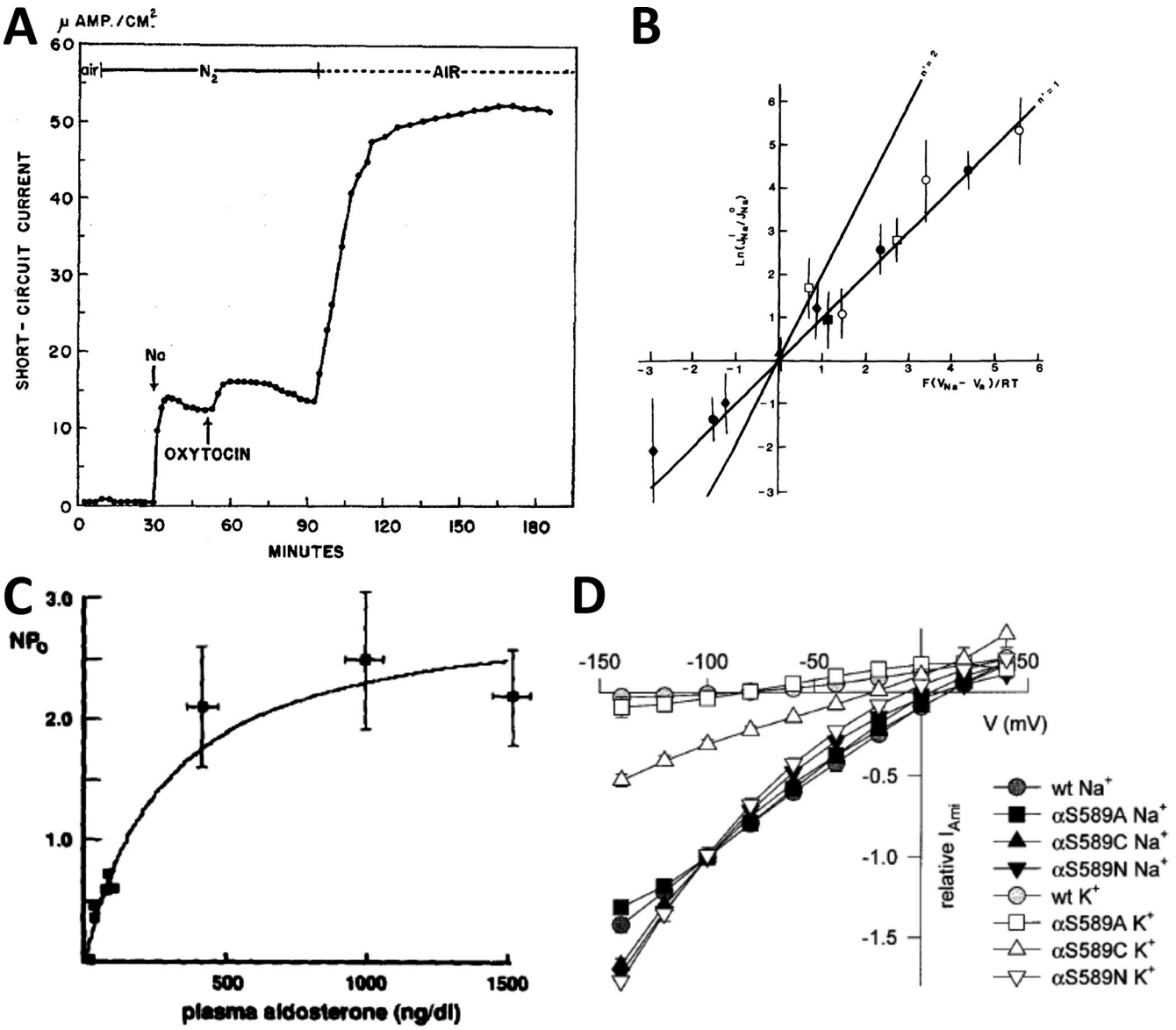

Figure 2. **Epithelial Na^+ channels in absorptive epithelia.** (A) Short-circuit current across the toad urinary bladder and its dependence on Na^+ and oxidative metabolism are shown. The short-circuit current under normal conditions was equal to the net flux of Na measured with Na^{22} and Na^{24}. Transport was stimulated by oxytocin or vasopressin and was enhanced in the presence of O_2. 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).

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

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 *Amphiuma* 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 Ussing model. Other basolateral K^+-channel types were identified at the single-channel level in the renal collecting duct (Wang, 1995), the proximal tubule (Mauerer 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 triaminopyridium 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

Ussing 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

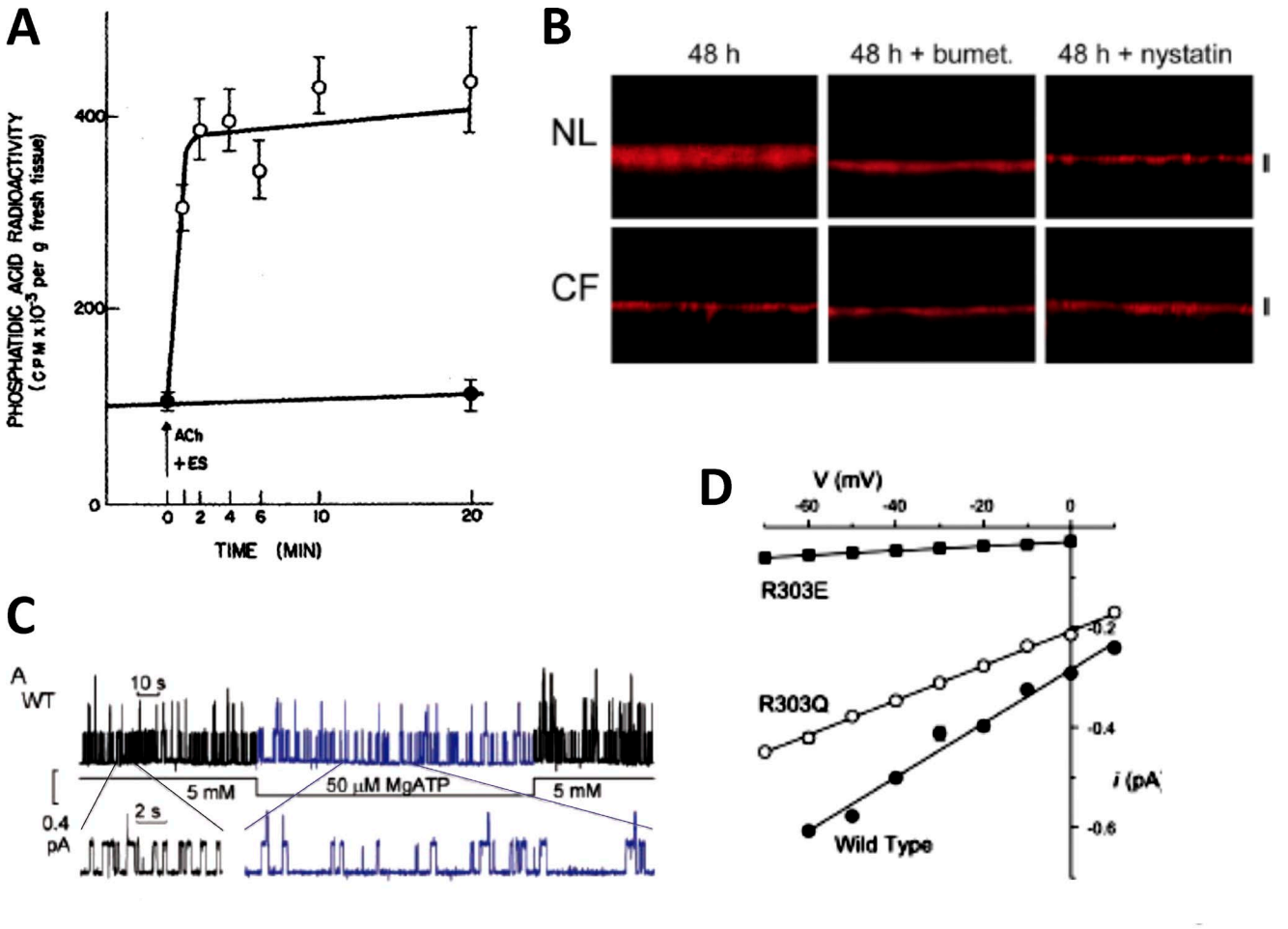

Figure 3. **Control of epithelial fluid secretion.** (A) Incorporation of [^{32}P]phosphatidic acid in goose nasal salt gland in response to a secretagogue. From (Hokin et al., 1960). (B) Control of airway surface liquid in cultured lung epithelial cells from healthy subjects (NL) and from patients with 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).

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

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; Spenney 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, 1983b) 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_2O transport across epithelia

Transepithelial movements of fluid have intrigued the JGP community for a long time, as revealed by the early

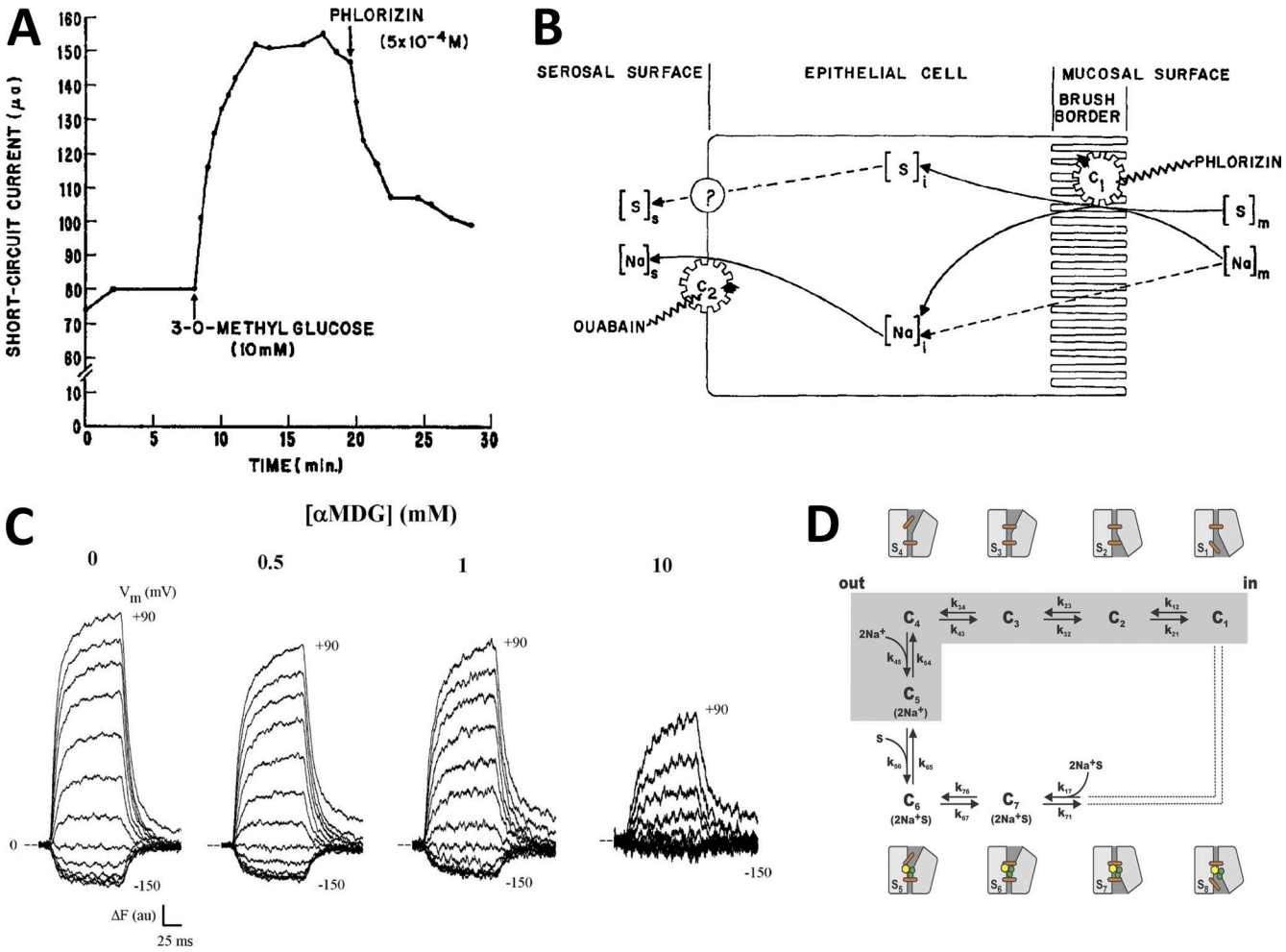

Figure 4. **Sodium-glucose cotransport.** (A) The effect of a nonmetabolizable glucose analogue on Na^+ transport (short-circuit current) by rabbit ileum. (B) Cell model of Na-dependent glucose transport. From Schultz and Zalusky (1964). (C) Effect of sugar on voltage-dependent protein conformational changes in SGLT1, measured with a fluorescent label. αMDG, α-methyl-D-glucopyranoside. From Loo et al. (2006). (D) Structure-based kinetic model of sodium-glucose transport. From Longpré et al. (2012).

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 H_2O 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 differently 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-

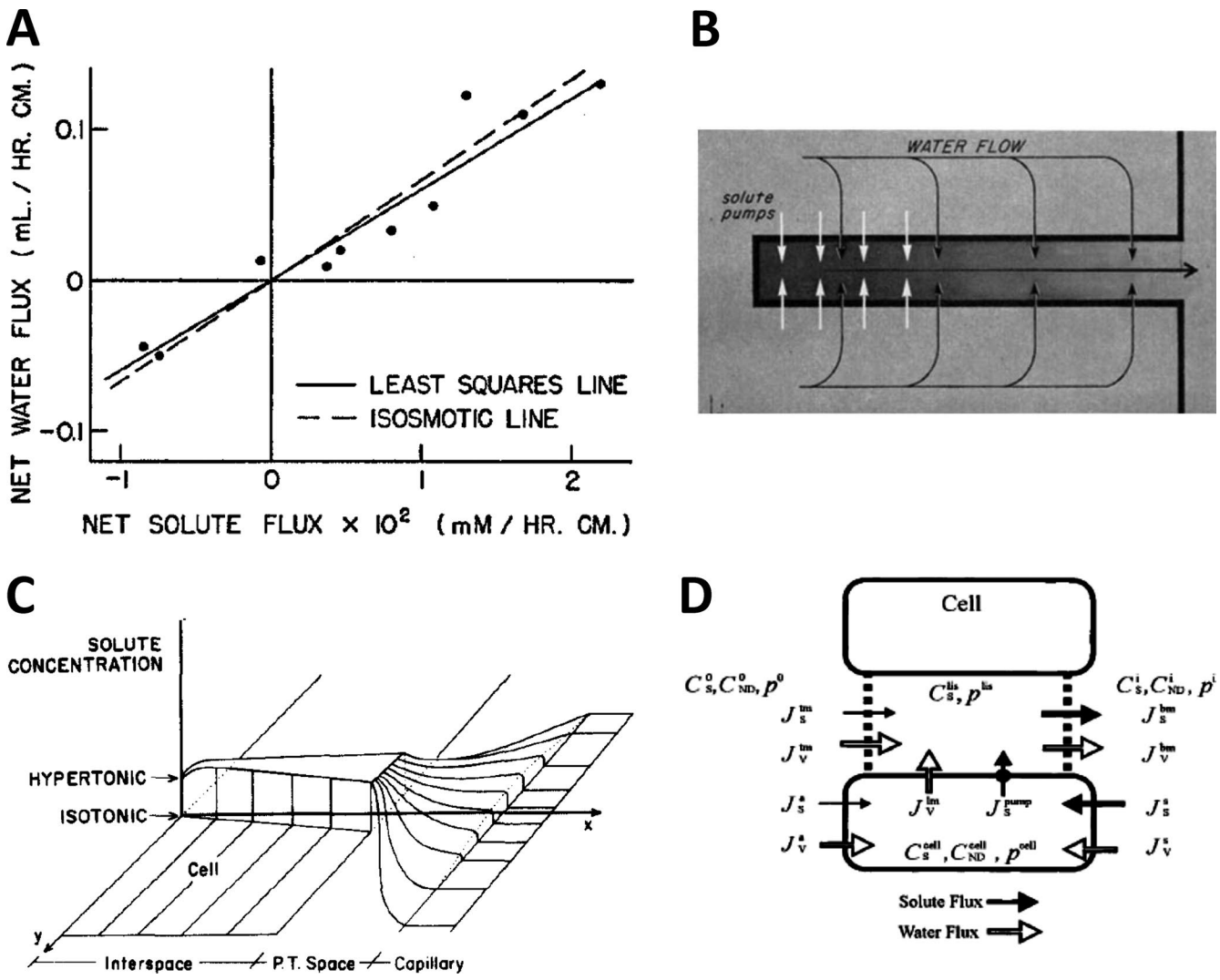

Figure 5. **Isotonic fluid transport.** (A) Solute and water transport in a rat ileum. The dashed line indicates the relationship for identical osmolarities of absorbed fluid and that of the luminal medium. From Curran and Solomon (1957). (B) "Standing-gradient" model to explain isotonic transport. The model postulates that interspaces between cells contain hypertonic 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).

branes were quite high (Fig. 6), suggesting that, in that epithelium, nearly isotonic fluid transport could be realized more simply with transcellular H_2O 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-

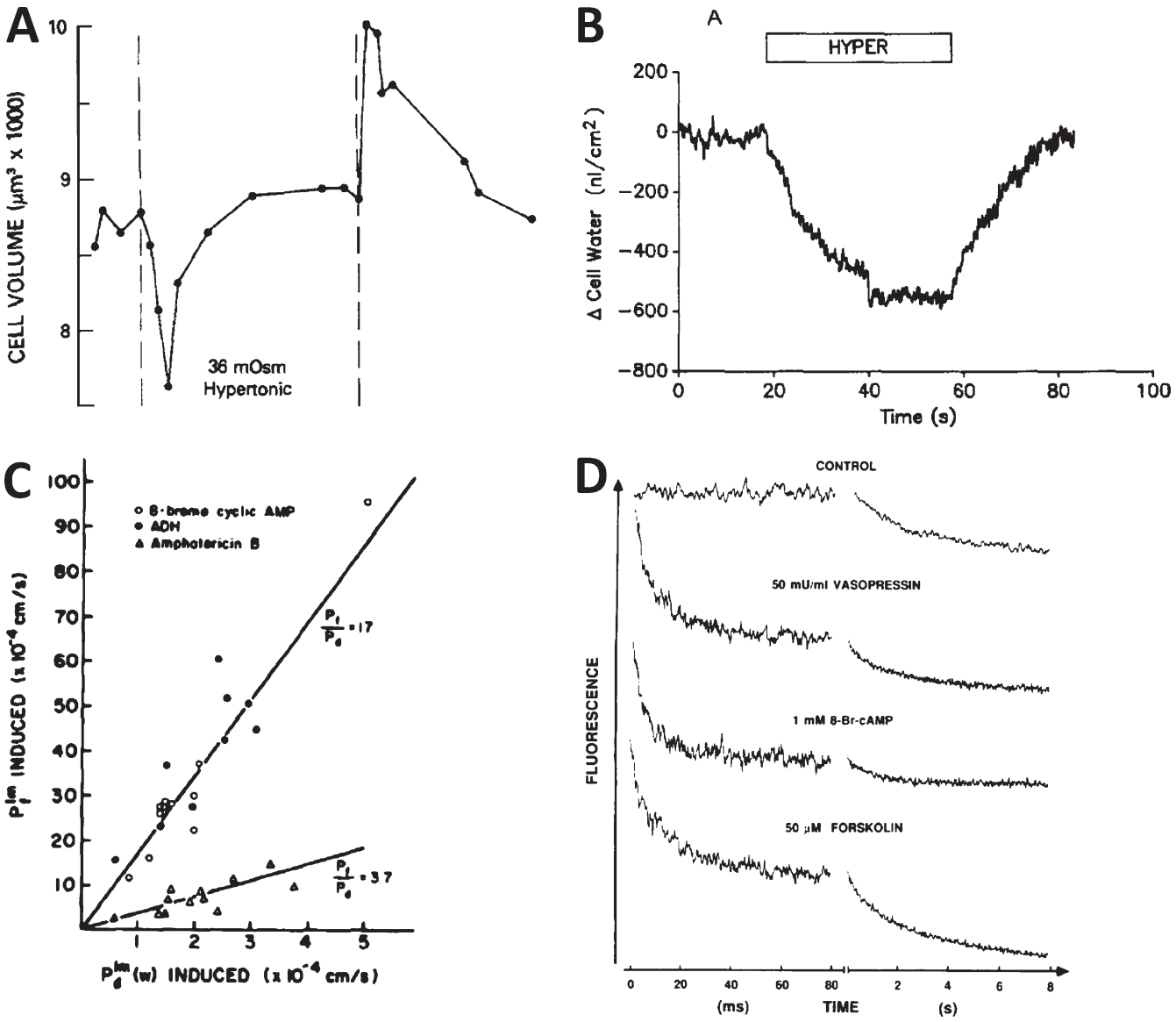

Figure 6. **Measurements of epithelial water permeability.** (A) Changes in cell volume of *Necturus* gall bladder in response to hypertonic challenge using an optical technique. From Persson and Spring (1982). (B) Similar changes measured with an intracellular microelectrode sensor. Hyper, hypertonic. From Cotton et al. (1989). (C) Measurement of Pf/Pd 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).

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). Eggena (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.

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