J. Membrane Biol. 184, 211–217 (2001)
DOI: 10.1007/s00232-001-0096-z

The Journal of
Membrane
Biology

Ussing's Two-Membrane Hypothesis: The Model and Half a Century of Progress

L. Reuss
Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77555-0641, USA

Received: 15 August 2001

The Two-Membrane Hypothesis

The study of epithelial function during the second half of the twentieth century was patterned by the work of Hans Ussing. Among his many contributions to physiology, Ussing developed a simple and elegant mechanism to explain active Na^+ transport by the epidermis of the frog. This mechanism, named the *two-membrane hypothesis* [6] (Fig. 1), states that transepithelial active Na^+ transport occurs by two events in series: the passive (electrodiffusive) influx of the cation across the apical cell membrane and the active (pump-mediated) efflux across the basolateral membrane. The two molecules responsible for these events are the epithelial Na^+ channel (ENaC) and the Na^+, K^+-ATPase (Na^+ pump), expressed in the apical and basolateral membranes, respectively. ENaC is highly selective for Na^+ over K^+ and other "physiological" cations. The Na^+ pump exports Na^+ and imports K^+ across the cell membrane, with a stoichiometry of $3Na^+$:$2K^+$ per molecule of ATP hydrolyzed. Thus, the operation of the pump tends to reduce intracellular [Na^+] and to make the cell membrane potential (V_m) more negative. Both factors increase the electrochemical gradient for Na^+ entry at the apical membrane. Intracellular ionic contents are maintained because the Na^+ fluxes at the two membrane domains are equal, and because of the presence of a K^+ channel in the basolateral membrane, through which K^+ recycles back to the basolateral solution.

The central idea in the *two-membrane hypothesis* is this: The simplest mechanism for transcellular ion transport across an epithelial sheet is the concerted operation of an active transporter ("pump") in one membrane, in series with a passive transporter ("leak") in the other membrane. The effects of the pump on intracellular ion concentration and membrane voltage provide the driving force for ion flux across the leak.

Does the Two-Membrane Model Apply to Other Epithelia?

Electrogenic Epithelia

After the pioneering work of Ussing and coworkers, studies of other epithelia revealed other transport processes in addition to Na^+ absorption. Among these, let us consider Cl^- secretion [33, Fig. 2*A*]. The explanation is still a pump-leak system, but there are differences with the original two-membrane model: 1) Influx occurs via the pump, and efflux is via the leak. The pump, located at the basolateral membrane, raises intracellular [Cl^-], creating a driving force for passive efflux, at the apical membrane, via the Cl^- channel. 2) The "influx pump" is complex, requiring the synchronized operation of three molecules, the Na^+ pump, the Na^+, K^+, $2Cl^-$ cotransporter [12] and the K^+ channel, all expressed in the basolateral membrane. Thus, the Cl^- influx is a secondary-active, not a primary-active transport event. Nevertheless, the principle is the same: two membranes in series, one containing a pump and the other one a leak.

Another variation in the two-membrane model is the case of transcellular absorption and secretion of ions of the same charge. In the principal cells of the mammalian renal cortical collecting tubule Na^+ is absorbed and K^+ is secreted. There is a Na^+-transporting mechanism just like in the frog skin, but the presence of K^+ channels in the apical example results in K^+ secretion by primary active uptake at the basolateral membrane and passive (electrodiffusive) efflux across the apical membrane channels (Fig. 2*B*).

Electroneutral Epithelia

The notion of electroneutral transepithelial ion transport was developed from studies in epithelia of

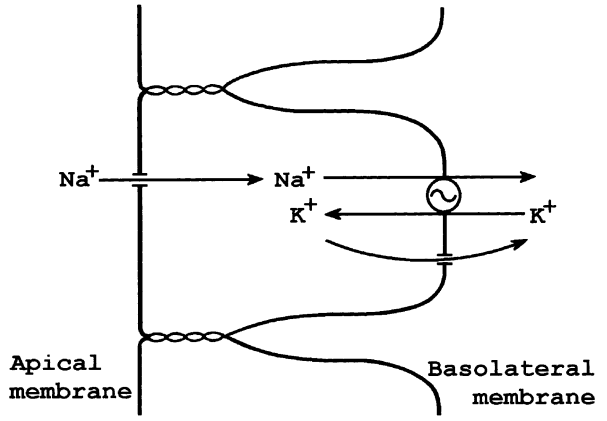

Fig. 1. The two-membrane hypothesis for Na^+-absorbing "tight" epithelia. At the apical cell membrane, Na^+ entry is via a Na^+-channel. At the basolateral cell membrane, extrusion of Na^+ is mediated by the Na^+ pump. K^+ "recycles" via a K^+ channel. From Reuss [19] with permission.

small intestine [15] and gallbladder [4]. In fact, the ion-entry step is not via ion channels, but via carriers, including Na^+-Cl^- or Na^+-K^+-$2Cl^-$ cotransporters, and Na^+/H^+ and Cl^-/HCO_3^- exchangers operating in parallel. Interestingly, these epithelia are also "leaky", i.e., have a high paracellular conductance, which helps explain why the transepithelial voltage is low, but clearly the mechanism of the low voltage is dual. Typically, transport of both Na^+ and Cl^- across the apical membrane is electroneutral and coupled: by cotransport in the same molecule or by the coordinated operation of ion exchangers (Fig. 3 and *see below*).

At the basolateral membrane of electroneutral epithelia Na^+ is transported by the Na^+ pump just like in electrogenic epithelia. In contrast, Cl^- transport is not paracellular, as in electrogenic Na^+-absorptive epithelia, but transcellular. Two pathways have been identified: Cl^- channels [16] and the K^+-Cl^- cotransporter [17]. In electroneutral epithelia, K^+ may be reabsorbed, secreted or recycled across the basolateral membrane (Fig. 3). The mechanism of active K^+ absorption (an example is the renal proximal tubule) is unclear [32].

Beyond the Pump-Leak Idea—Focus on the Paracellular Pathway

The two-membrane hypothesis explains epithelial Na^+ transport in an elegant and simple manner. However, research during the last three decades has revealed features that cannot be explained by the simple version of this hypothesis. The main two are the demonstration of the paracellular pathway and the discovery of adaptive mechanisms that coordinate

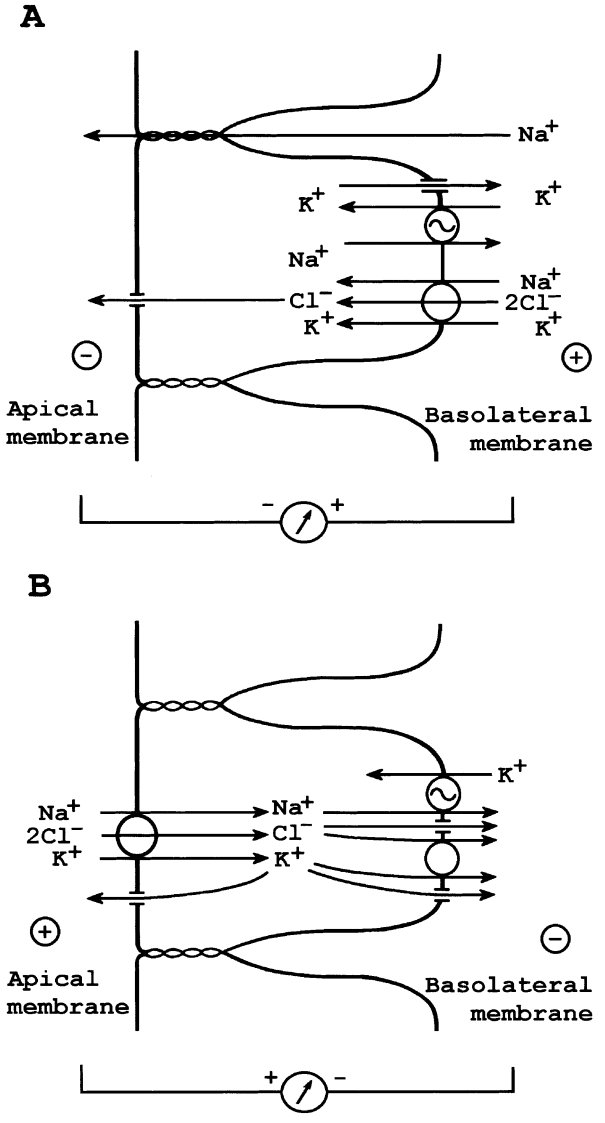

Fig. 2. Ion transport in Cl^--transporting epithelia. (*A*) Cl^--secreting epithelium. Intracellular $[Cl^-]$ is elevated above electrochemical equilibrium by Na^+-K^+-$2Cl^-$ cotransport across the basolateral cell membrane. Transepithelial secretion is induced by activation of apical-membrane Cl^- channel(s). (*B*) Cl^--absorbing epithelium. Cl^- entry across the apical membrane is mediated by Na^+-K^+-$2Cl^-$ cotransporter. Most basolateral Cl^- efflux proceeds through Cl^- channels. From Reuss [19], with permission.

the function of two or more transporters at one or both cell-membrane domains.

The Paracellular Pathway

In the seventies, it became clear that solute fluxes can be *transcellular* or *paracellular*. Transcellular transport occurs via the cells, i.e., involves transport across one membrane, distribution in the cell interior and transport across the opposite membrane. In a normal epithelium, the paracellular pathway is intercellular (junctions and lateral intercellular spaces). The idea of

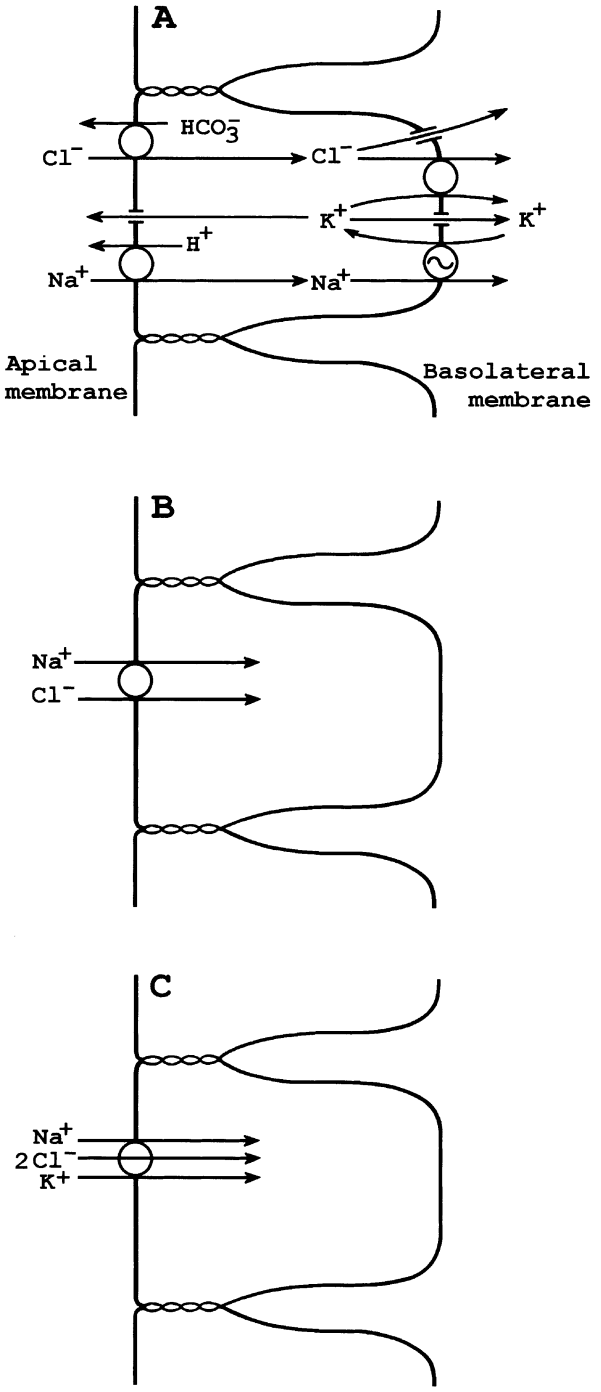

Fig. 3. Ion transport in leaky NaCl-absorbing epithelia. (*A*) Na^+ and Cl^- enter via electroneutral exchangers. Cl^- exits via channels and/or K^+-Cl^- cotransport. Na^+ extrusion is mediated by Na^+ pump. K^+ recycles via channels (basolateral or both cell membranes). This model accounts for NaCl transport in most "leaky" NaCl-absorptive epithelia. (*B*) Na^+-Cl^- cotransport (flounder urinary bladder, mammalian renal distal tubule). Basolateral transport mechanisms as in top diagram. (*C*) Na^+-K^+-$2Cl^-$ cotransport (flounder intestine). Basolateral transport mechanisms as in top diagram. From Reuss [19] with permission.

a paracellular or "shunt" pathway was first suggested by Ussing and Windhager [30] from studies in frog skin epithelia, in which the paracellular permeability was experimentally increased. Electrophysiological [31, 5] and morphological [13] studies in renal-tubule and gallbladder epithelia demonstrated a high permeability of the junctions. The classification of epithelia into "tight" and "leaky" derived from this work.

The structure of the tight junctions was debatable, until the recent work of Tsukita and his associates [29], which culminated in the discovery of the tight-junction transmembrane proteins, occludin and claudins. The latter are present in tight junctions and their expression is both necessary and sufficient to form the intramembrane strands characteristic of these structures. By itself, occludin does not form complete strands.

The paracellular pathway plays roles in the transepithelial transport of ions and water. These roles are radically different in tight and leaky epithelia. In the former, the paracellular path is mostly a barrier to ions, whereas in the latter it is a conduit.

A function of the paracellular shunt is to couple the two cell membranes electrically. In most leaky epithelia, the relative K^+ conductance of the basolateral membrane is greater than that of the apical membrane, and thus there is current flow between the two membranes that hyperpolarizes the apical membrane. This increases the driving force for entry of positively-charged solutes (e.g., Na^+ via electrogenic cotransporters). Examples of this mechanism are the proximal renal tubule and small intestine, which express Na^+-glucose and Na^+-amino acid cotransporters.

Another function of the paracellular pathway is passive ion transport, driven by electrodiffusion (electrical potential and/or concentration differences). Electrogenic transcellular transport causes a transepithelial voltage that can drive ion transport across the paracellular pathway. Examples are Cl^--transporting epithelia such as the thick ascending segment of the loop of Henle (Cl^- absorption) and the intestinal crypts (Cl^- secretion). Transcellular Cl^- transport generates a transepithelial voltage (positive on the side from which Cl^- is transported). This causes a paracellular Na^+ flux *in the same direction* as the Cl^- flux (reviewed in [27]). In these epithelia, the tight junctions are cation-selective and thus Na^+ and Cl^- move in the same direction. Interestingly, we now know that expression of specific claudins in the tight junctions is related to their ion selectivity. A dramatic example is the pathway for absorption of divalent cations (Ca^{2+} and Mg^{2+}) by the thick ascending loop of Henle. This epithelium expresses claudin-16, also named paracellin, the protein that confers the high permeability for divalent cations to the tight junctions [26]. The loss of function of this protein by mutation causes a genetic disease characterized by excessive loss of Ca^{2+} and Mg^{2+} in the urine.

Water transport may be another important function of the paracellular pathway, both in terms of solute-coupled transport and solvent drag. I mention these possibilities for completeness, but the focus of this article is ion transport.

Charge- and Mass-Balance in Transepithelial Transport

Steady-state transepithelial ion transport in the absence of external current is electroneutral, i.e., the net charge balance of the cell and the net transfer of charge between the bathing solutions are zero. These physical principles are satisfied in different ways by different epithelia.

In electrogenic epithelia (such as the frog epidermis) net charge transfer in the transcellular pathway occurs in the steady state, but there is also a paracellular charge transfer of equivalent magnitude and opposite sign. When the frog skin epithelium is incubated with physiologic salt solutions of equal composition on both sides and no current is applied, the transcellular Na^+ flux is accompanied by a paracellular Cl^- flux driven by the transepithelial voltage. This voltage can be canceled by applying transepithelial current. In this case, the driving force for paracellular Cl^- transport becomes zero and Na^+ is the only ion undergoing transepithelial transport. The balance of charge occurs in the external current circuit.

In electroneutral epithelia, electroneutrality is satisfied at each membrane by direct or indirect flux coupling. Direct or *molecular* coupling denotes cotransport or exchange, i.e., two or more ions are transported by the same protein. Indirect coupling means transport by different proteins. In the latter case, flux coupling may be: a) *thermodynamic* (e.g., transport of the primary ion changes the membrane voltage and thus the driving force for transport of the secondary ion through another protein); b) *allosteric* (e.g., primary ion transport alters the intracellular or extracellular level of a substrate, which modifies the activity of transporter(s) of the secondary ion by allosteric effects).

Under steady-state conditions, with constant cell volume and composition, there must be a balance between the transport rates at the two membranes. Also, a change in the transport rate at one cell membrane (e.g., by the effect of a hormone) must be rapidly followed by a matching change at the opposite membrane, restoring the balance. Implied in the original formulation of the two-membrane hypothesis, variations in ion-transport rate across one membrane domain would be matched by the contralateral membrane only via changes in intracellular ion concentration. In all epithelial cells at least one membrane domain is highly water permeable, and thus a change in cell solute content causes a change in cell water volume in the same direction. The existence of a mechanism of adjustment of the transport rates at the two membranes (“cross talk”) was proposed by Schultz [21].

In summary, transepithelial NaCl transport satisfies the electroneutrality principle. Further, during net transport and in the steady state, the rates of ion entry and exit must be the same if cell volume and ionic composition are to remain constant. When only one ion is transported (frog epidermis), cross-talk consists of the adjustment of the transport rates at the two membrane domains. When two (or more) ions are transported, there must be *intramembrane regulation* (equal rates of net transport at the same membrane, e.g., NaCl entry with stoichiometry Na^+:Cl^- = 1:1, as well as *cross-talk* (equal transport rates at the opposite membrane domains, e.g., apical NaCl flux = basolateral NaCl flux).

Intramembrane Regulation

Intramembrane regulation involves interactions of fluxes mediated by carriers or channels, or between pumps and other transport proteins. The particular case of adjustment between the K^+ transport rates of the Na^+ pump and the K^+ channel expressed in the basolateral membrane is called pump-leak parallelism [23].

Adjustment between Carriers Expressed in the Same Membrane

In electroneutral $NaCl^-$ absorptive epithelia, the steady-state rates of Na^+ entry and Cl^- entry are the same. This was originally interpreted as indicative of Na^+-Cl^- cotransport [4, 15], but other studies demonstrated that in most of this group of epithelia NaCl entry involves the parallel operation of Na^+/H^+ and Cl^-/HCO_3^- exchangers [11, 18]. Using intracellular ion-selective microelectrodes, I demonstrated that Na^+ and Cl^- transport across the apical membrane of amphibian-gallbladder epithelium can be *transiently* dissociated [18]. In the steady state, however, the fluxes are the same, probably because of changes in intracellular pH.

For example, if Na^+ is removed from the apical solution, the intracellular [Na^+] falls rapidly; the [Cl^-] also falls, because of the following mechanism: (a) Na^+ removal lowers the intracellular pH (pH_i), because the fluxes via the Na^+/H^+ exchange are reversed; (b) the elevation of $[H^+]_i$ reduces Cl^-/HCO_3^- exchange because the $[HCO_3^-]_i$ falls by titration with H^+ and also because the Cl^-/HCO_3^- exchanger undergoes allosteric inhibition by intracellular acidification.

Adjustment between Ion Channels Expressed in the Same Membrane

Membrane-voltage changes alter fluxes via ion channels by the change in driving force, as well as by changes in open probability (P_o) in the case of voltage-gated channels. In amphibian gallbladder epithelium, cAMP activates CFTR, thus increasing the apical-membrane Cl^- permeability. This depolarizes the membrane, thus increasing the driving force for K^+ efflux and also increases the P_o of maxi-K^+ channels expressed in the same membrane. Together, these changes cause loss of both K^+ and Cl^- and cell shrinkage [2].

Pump-Leak Parallelism

The P_o of $[ATP]_i$-sensitive K^+ channels is modulated by the intracellular [ATP] and/or related parameters. It is thought that ATP couples the rate of the pump to the basolateral membrane P_K in epithelial cells. In rabbit renal proximal-tubule cells [28] the $[ATP]_i$-sensitive K^+ channels are the main path for K^+ fluxes across the basolateral membrane and are activated by falls of cell [ATP]. $[ATP]_i$ decreases after stimulation of transepithelial Na^+ transport, and when $[ATP]_i$ is elevated experimentally, the effect of the Na^+ transport rate on the K^+ conductance of the basolateral membrane disappears.

Cross-Talk Mechanisms

Cross-talk denotes adjustment of the rates of ion transport between apical- and basolateral-membrane domains [7, 20, 21, 22]. The general mechanism is thought to operate in four steps: a) primary change in ion-transport rate at one membrane, b) signaling event in the cell, c) sensing and transduction of the signal, and d) change in transporter activity at the other membrane domain.

Cross-Talk in Tight Epithelia

The first evidence for cross-talk was obtained by MacRobbie and Ussing in frog-skin epithelium. Inhibition of the Na^+ pump with a cardiotonic steroid decreased apical-membrane P_{Na} [14]. Metabolic inhibitors or a K^+-free basolateral solution, both expected to inhibit the pump, also decreased apical-membrane P_{Na} [1, 10]. These and other experiments indicate that apical-membrane P_{Na} in tight epithelia is modulated by $[Na^+]_i$. This effect has been thought to be either direct or by changes in $[Ca^{2+}]$ and/or pH_i (reviewed in [19]). In renal collecting-duct cells, patch-clamp experiments revealed that elevated intracellular $[H^+]$ and $[Ca^{2+}]$ reduced the P_o of ENaC [3, 25].

The opposite relationship, i.e., Na^+ entry modulating the activity of the pump, was initially thought to be mediated by changes in $[Na^+]_i$. However, this is not the case, as shown in experiments in several tight epithelia (reviewed in [22] and [24]). The pump is stimulated in the absence of measurable increases in $[Na^+]_i$ [24], denoting increases in pump rate and/or number of molecules expressed in the membrane.

Cross-Talk in Leaky Epithelia

In epithelial cells from small intestine and renal proximal tubule, cotransport of Na^+ and organic solute depolarizes the apical (V_m) and reduces the ratio of cell-membrane electrical resistances (apical/basolateral = R_a/R_b). Later on, both V_a and R_a/R_b recover. The secondary rise in R_a/R_b is prevented by blocking basolateral-membrane K^+ channels with Ba^{2+}. These results can be explained by an increase in K^+ conductance secondary in response to the elevated solute influx, perhaps mediated by cell swelling [22]. Similar effects are produced by enterocyte swelling by hypotonic solutions [9]. Results in renal proximal tubule cells are similar to those described in intestine [6].

In leaky epithelia, maneuvers that increase Na^+ absorption by 2–4-fold do not elevate $[Na^+]_i$ [24], denoting Na^+ pump activation. The mechanism of this effect is unknown. Possibilities include changes in the number of operational pump molecules or in the turnover number.

Signaling Mechanisms in Epithelial Cross-Talk

As exemplified in the previous sections, cross-talk can take many forms and no mechanism explaining all these modalities has been identified. Our current knowledge is suggestive of changes in cell volume and intracellular ion concentrations as initial signals.

Changes in cell volume are thought to influence membrane-transport proteins by physical and biochemical processes, such as membrane/cytoskeleton tension and changes in mediator concentration, respectively. The transduction of the signal may involve changes in $[Ca^{2+}]_i$ and protein phosphorylation. In certain instances, pH_i, $[ATP]_i$, V_m, as well as other parameters can modulate transport rates via channels, carriers and pumps. Both signals and transduction mechanisms of cross-talk may be multiple and interactive [24].

Ussing's Legacy

Hans Ussing's two-membrane hypothesis is the definitive framework upon which the transport function of epithelial organs came to be understood. Although initially designed to explain electrogenic Na^+ transport, this elegant theory has proved appropriate to understand epithelial transport in general and remains the basic tenet of epitheliology. Major progress

has been made in comprehending epithelial structure and function in the last five decades. The epithelial black box was first broken by measurements of membrane voltages and conductances, as well as intracellular ion activities. The principal molecules involved in transepithelial transport have been identified and characterized, and our current understanding is solidly based on molecular information. The roles of cells, paracellular pathway and extracellular microdomains have been studied in depth. Finally, the principal regulatory mechanisms of epithelial transport have begun to be unraveled, also at the molecular level. Hans Ussing not only started this process, but also made fundamental contributions in several areas after the initial formulation of the two-membrane-hypothesis. Among them, the proposal of a paracellular pathway [30] the identification of the first cross-talk mechanism [14] and the recent development of the solute-recirculation model to explain isotonic transepithelial transport [8]. Hans Ussing was a scientist of great intelligence and brilliant imagination. He made a definitive contribution to physiology and inspired two generations of investigators to pursue the question at the center of his scientific life, the mechanism of ion transport by epithelia.

References

1. Chase, H.S., Jr., Al-Awqati, Q. 1981. Regulation of the sodium permeability of the luminal border of toad bladder by intracellular sodium and calcium. *J. Gen. Physiol.* **77:**693–712
2. Cotton, C.U., Reuss, L. 1991. Effects of changes in mucosal solution Cl^+ or K^+ concentration on cell water volume of *Necturus* gallbladder epithelium. *J. Gen. Physiol.* **97:**667–686
3. Frindt, G., Silver, R.B., Windhager, E.E., Palmer, L.G. 1993. Feedback regulation of Na channels in rat CCT. II. Effects of inhibition of Na entry. *Am. J. Physiol.* **264:**F565–F574
4. Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder. Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65:**769–795
5. Frömter, E., Diamond, J. 1972. Route of passive ion permeation in epithelia. *Nature New Biol.* **235:**9–13
6. Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42:**298–308
7. Lapointe, J., Garneau, L., Bell, P.D., Cardinal, J. 1990. Membrane crosstalk in the mammalian proximal tubule during alterations in transepithelial sodium transport. *Am. J. Physiol.* **258:**F339–F345
8. Larsen, E.H., Nedergaard, S., Ussing, H.H. 2000. Role of lateral intercellular space and sodium recirculation of isotonic transport in leaky epithelia. *Rev. Physiol. Biochem. Pharmacol.* **141:**153–212
9. Lau, K.R., Hudson, R.L., Schultz, S.G. 1984. Cell swelling induces a barium-inhibitable potassium conductance in the basolateral membrane of *Necturus* small intestine. *Proc. Natl. Acad. Sci. USA* **81:**3591–3594
10. Lewis, S.A., Eaton, D.C., Diamond, J.M. 1976. The mechanism of Na^+ transport by rabbit urinary bladder. *J. Membrane Biol.* **28:**41–70
11. Liedtke, C.M., Hopfer, U. 1982. Mechanism of Cl^- translocation across small intestinal brush-border membrane. I. Absence of N^+-Cl^- cotransport *Am. J. Physiol.* **242:**G263–G271
12. Lytle, C., Forbush, B., III. 1992. The Na-K-Cl cotransport protein of shark rectal gland, II. Regulation by direct phosphorylation. *J. Biol. Chem.* **267:**25438–25443
13. Machen, T.E., Erlij, D., Wooding, F.B.P. 1972. Permeable junctional complexes. The movement of lanthanum across rabbit gallbladder and intestine. *J. Cell Biol.* **54:**302–312
14. MacRobbie, E.A.C., Ussing, H.H. 1961. Osmotic behavior of the epithelial cells of frog skin. *Acta Physiol. Scand.* **53:**348-365
15. Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **225:**467–475
16. Reeves, W.B., Andreoli, T.E. 1992. Renal epithelial chloride channels. *Annu. Rev. Physiol.* **54:**29–50
17. Reuss, L. 1983. Basolateral KC1 co-transport in a NaCl-absorbing epithelium. *Nature.* **305:**723–726
18. Reuss, L. 1984. Independence of apical membrane Na^+ and Cl^- entry in *Necturus* gallbladder epithelium. *J. Gen. Physiol.* **84:**423–445
19. Reuss, L. 1997. Epithelial Transport. *In:* Handbook of Physiology. Section 14: Cell Physiology. J.F. Hoffinan, J.D. Jamieson, editors, pp. 309–388. Oxford University Press, New York
20. Reuss, L., Cotton, C.U. 1994. Volume regulation in epithelia: transcellular transport and cross-talk. *In:* Cellular and Molecular Physiology of Cell Volume Regulation. K. Strange, editor, pp. 31–48. CRC, Boca Raton
21. Schultz, S.G. 1981. Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by "flush-through". *J. Physiol.* **241:**F579–590
22. Schultz, S.G. 1992. Membrane cross-talk in sodium-absorbing epithelial cells. *In*: The Kidney: Physiology and Pathophysiology. D.W. Seldin, and G. Giebisch, editors, pp. 287–299. Raven Press, New York
23. Schultz, S.G. 1994. The "pump-leak" parallelism in *Necturus* enterocytes: some cellular and molecular insights. *Renal Physiol. Biochem.* **17:**134–137
24. Schultz, S.G., Hudson, R.L. 1991. Biology of sodium-absorbing epithelial cells: dawning of a new era. *In*: Handbook of Physiology, Section 6. The Gastrointestinal System, Vol. IV. Intestinal Absorption and Secretion. S.G. Schultz, M. Field, RA Frizzell, editors, pp. 45–81. Oxford University Press, New York
25. Silver, R.B., Frindt, G., Windhager, E.E., Palmer, L.G. 1992. Feedback regulation of Na channels in rat CCT. I. Effects of inhibition of Na pump. *Am. J. Physiol.* **264:**F557–F564
26. Simon, D.B., Lu, Y., Choate, K.A., Velazquez, H., Al-Sabban, E., Praga, M., Casari, G., Bettinelli, A., Coluissi, G., Rodriguez-Soriano, J.M.D., Milford, D., Sanjad, S., Lifton, R.P. 1999. Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science* **285:**103–106
27. Sullivan, S.K., Field, M. 1991. Ion transport across mammalian small intestine. *In*: Handbook of Physiology. The Gastrointestinal System, Vol. IV. Intestinal Absorption and Secretion. S.G. Schultz, M. Field, R.A. Frizzell, editors, pp. 287–301. Oxford University Press, New York
28. Tsuchiya, K., Wang, W., Giebisch, G., Welling, P.A. 1992. ATP is a coupling modulator of parallel Na,K-ATPase-K-channel activity in the renal proximal tubule. *Proc. Natl. Acad. Sci.* USA **89:**6418–6422

29. Tsukita, S., Furuse, M., Itoh, M. 2001. Multifunctional Strands in Tight Junctions. *Nature Reviews Molecular Cell Biology*. **2:**285–293.
30. Ussing, H.H., Windhager, E.E. 1964. Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta. Physiol. Scand.* **61:**484–504
31. Windhager, E.E., Boulpaep, E.L. Giebisch, G. 1967. Electrophysiological studies in single nephrons. In: Proceedings of the Third International Congress on Nephrology. Washington, DC, 1966, Vol. 1. G.E. Schreiner, editor, pp. 35–47. Karger, New York
32. Wright, F.S., Giebisch, G. 1992. Regulation of potassium excretion. *In:* The Kidney: Physiology and Pathophysiology. D.W. Seldin, G. Giebisch, editors, pp. 2209–2247. Raven Press, New York
33. Zadunaisky, J.A. 1966. Active transport of chloride in frog cornea. *Am. J. Physiol.* **211:**506–512