J. Membrane Biol. 184, 219–223 (2001)
DOI: 10.1007/s00232-001-0101-6

The Journal of
Membrane
Biology

The Influence of Ussing's Pioneering Work on a Dutch Physiologist or How I Stopped Worrying

C.H. van Os
Department of Cell Physiology, University Medical Centre Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Received: 15 August 2001

The Rats in the Gutter

Being asked to contribute to a Hans Ussing commemorative issue provides a unique opportunity to look back in time and try to name the really important papers or events that have influenced one's scientific career. My quest in membrane biology started with a master's degree in biochemistry in 1968 at the university of Utrecht under the inspiring supervision of the late Laurens van Deenen. The international atmosphere in his laboratory and the rapid developments in the field of membrane lipid biochemistry initiated at that time my interest in biological membranes. In the same year, Hans Ussing was 57 and had already published his most significant papers when I started as a graduate student in physiology at the university of Nijmegen for a Ph.D. in membrane biology under supervision of Jan Slegers. My contribution to this volume will focus on some of Ussing's papers and ideas that were most influential at the start of my career. I have often been surprised by the fact that Hans Ussing had analyzed and discussed an issue way ahead of anyone else in the field of membrane transport and that he had also hinted at explanations that were much later proven to be correct. I didn't meet him in person before 1980 when he was already 69. This happened at the 15th Alfred Benzon symposium, which Niels Binslev organized in 1980 in Copenhagen. He impressed me by his common sense and his frankness. One expression by him at that occasion I never forgot. Sitting next to him at the symposium dinner in a fabulous castle I told him about my children and my worries about the future of our planet. He simply replied by saying: "*don't worry, even the rats in the gutter have a good life*".

Active Sodium Transport and the Two-Membrane Hypothesis

In 1968, when I started my Ph.D. work, Hans Ussing summarized at a symposium organized by D.C. Tosteson his most important experimental and theoretical work so far as follows: "*For some 20 years in my laboratory we have been using the isolated frog skin as our favorite experimental object. The isolated frog skin performs massive transport of sodium chloride from the outside to the inside bathing solution. It turned out that sodium was the active transported species since it went against its own electrochemical gradient, whereas chloride could be shown to behave exactly as a passive ion should according to the flux ratio equation.....The more detailed analysis of the nature of the frog skin potential led to the model of the epithelium that we may call the two-membrane theory. It is assumed that the outward facing epithelial boundary is permeable to chloride and selectively but passively permeable to sodium. The inward facing boundary, on the other hand, is readily permeable to potassium as well as chloride, but only slightly permeable to sodium. This ion leaves the cellular compartment only by way of an inward-directed sodium pump. Such a system would account for the net transport of sodiumchloride in the open circuit skin, for the sodium current in the short-circuited skin and also for the maintenance of the epithelial cells in a state of osmotic and ionic balance*" (Ussing, 1949;1969; Ussing & Zerahn, 1951; Koefoed-Johnsen & Ussing, 1958). Here are the essentials of active sodium transport in the kidney collecting duct and in the distal colon, summarized in a few sentences in his own prose, as we teach them today to our medical students. The beauty of this concept has not faded and the molecular properties of the epithelial Na channel, ENaC, the different K channels and the Na-K pump fit very well within this early frame work formulated by Ussing.

The observations of Jared Diamond in the early sixties on isolated rabbit gall bladders, an epithelial tissue that performs massive active NaCl transport in the absence of any transepithelial potential, must have been a big surprise to the field (Diamond, 1962). In view of the apparent contradictions with the frog

skin two-membrane hypothesis, I decided to have a closer look at the membrane permeabilities of the gall bladder epithelium. Quite surprisingly, it turned out that in the *Necturus* gall bladder the apical membrane was almost impermeable to Na and Cl ions but that its permeability to K was as high as in the basolateral membrane (van Os & Slegers, 1975). Hence, in addition to a leaky paracellular pathway, the permeability properties of the two membranes in series were very similar and these were conspicuous differences with the frog skin model of active Na transport. At the same time, but unaware of each other, Reuss and Finn (1975) arrived at similar conclusions.

Much later in my laboratory, Wim Ghijsen, Rene Bindels and Joost Hoenderop provided evidence that the Ussing model for active Na transport is also applicable to active Ca transport in the small intestine and the distal nephron (Ghijsen, & van Os, 1979; Ghijsen, de Jong & van Os, 1982; Bindels et al.,1991; Hoenderop et al., 1999.) Influx of Ca is also passive and takes place via an apical epithelial Ca channel, ECaC, whereas basolateral efflux is active and mediated by a Ca-pump or a Na-Ca exchanger.

Negative Feedback on Sodium Entry

It is now well accepted that any maneuver that brings about an increase in the cytosolic Na concentration results in a decrease in active Na transport and Na conductance (Schultz, 1992). Ussing was the first to report observations on the effect of ouabain on frog skin and hinted already at some form of negative feedback as he concluded: "*It may be that any interference with the pump mechanism is accompanied by a decrease in the passive permeabilities to ions and that the active ion transport and the passive fluxes are not entirely independent*" (MacRobbie & Ussing, 1961).

In my opinion the molecular mechanisms involved in the negative feedback of intracellular Na on ENaC are still poorly understood, in spite of extensive studies, in which either intracellular Ca, pH, cell volume or an intracellular Na receptor have been implicated (Taylor & Windhager, 1979; Harvey, Thomas & Ehrenfeld, 1988; Ishibashi et al., 1999). The story becomes now more complex because, recently, Dijkink in our laboratory was able to demonstrate also an effect of intracellular Na on the aldosterone-induced synthesis of an ENaC subunit as a clear form of negative feedback (Dijkink, 2001; Dijkink et al., 2001). She used primary cultures of immunodissected rabbit cortical collecting-duct cells and observed that stimulation of apical Na entry, by long-term short-circuiting of the monolayers, suppressed the aldosterone-stimulated benzamil-sensitive I_{sc}, while in the presence of benzamil this inhibitory effect was absent. Using an immunoprecipitation assay after labeling the β-subunit of ENaC with [^{35}S]-methionine, she could also demonstrate that the effects of modulation of apical Na entry on active Na transport were exactly mirrored in the β-ENaC subunit protein levels. Also in the absence of aldosterone, incubation of the cell layer for 18 hr with benzamil resulted in doubling of active Na transport and of the amount of β-ENaC in the cells.

To complicate matters even more, it is now becoming clear that most of the ENaC subunit protein is not present in the apical membrane but present intracellularly in a core-glycosylated Endo H-sensitive form, which is indicative of retardation in the ER or pre-Golgi compartment (Dijkink, 2001; Dijkink et al., 2001). This is not only true upon expression of ENaC in oocytes (Valentijin, Fyfe & Canessa, 1998) but also in fully differentiated distal colonic and kidney cells (Dijkink, 2001a,b). Hence, posttranslational maturation of ENaC subunits in the ER-Golgi seems the limiting step in processing functional Na channels and this is fully in agreement with a recent publication by Loffing et al. (2000) who described translocation of ENaC protein from intracellular pools to the apical plasma membrane in mice put on a low-Na diet. Forty years after the first prescient statement by Ussing hinting at feedback, we are now at the unsolved problems of posttranslational maturation of ENaC proteins and regulated trafficking of intracellular vesicles containing ENaC subunits.

The negative feedback of intracellular Ca on apical Ca influx via ECaC is, in contrast to Na influx, far more direct and faster than in the case of ENaC, and this should not really be surprising if one realizes the detrimental effects of high intracellular Ca concentrations on the viability of cells. In the actively Ca-transporting epithelia the negative feedback is realized by an intrinsic molecular property of the apical Ca channel, since it was demonstrated that ECaC is a constitutively open channel that is rapidly inactivated by Ca ions when intracellular Ca concentrations rise above the resting value. This mechanism is a perfect safeguard to prevent uncontrolled Ca influx (Vennekens et al., 2000). Another striking difference with active Na transport is the role of cytosolic high-affinity Ca-binding proteins, calbindins, which occur in high concentrations and serve as efficient Ca buffers and facilitate the diffusion of Ca^{2+} ions from the entry ports to the basolateral Ca pumps, without interfering with cellular Ca-signaling (Koster et al., 1995).

Paracellular Shunt Pathways in Epithelia

Ussing and Windhager (1964) introduced the concept of extracellular shunt pathways in epithelia when they realized that in frog skin the conductance of this pathway amounted to 40% of the conductance of the epithelium. Later, Frömter and Diamond (1972) demonstrated very elegantly and convincingly that in

Necturus gallbladder the paracellular transjunctional pathway constituted 95% of the epithelial electrical conductance and introduced the terms "tight" and "leaky" epithelia for high resistance-high potential and low resistance-low potential epithelia, respectively. In that seminal paper they also correlated water permeabilities with electrical conductances of a variety of epithelia and suggested that in "leaky" epithelia the dominant route for water could also be paracellular. This concept became generally accepted for a long period. I became skeptical about this concept when we could demonstrate that the ductal epithelium of the rabbit submaxillary salivary gland combined "tight" epithelial properties with resistance values observed in very "leaky" epithelia (Augustus et al., 1977). In addition, the water permeability of this ductal epithelium was extremely low (van Os et al., 1981). Nowadays it is not surprising anymore that water permeabilities and ionic conductances are distinct entities that can be independently regulated, however, in those days the significance of the paracellular route in isotonic water transport across "leaky" epithelia was a hotly debated issue. We now take for granted that the high density of aquaporin water channels determines the high water permeability and that the number of open ion channels in the membrane determines the ionic conductance, independent of the leakiness of the paracellular pathway or *zonula occludens*. This explains why, in the proximal tubule of an aquaporin knock-out mouse, the water permeability is greatly reduced without affecting the epithelial resistance and why the electrical conductance of the shunt pathway in the thick ascending limb of Henle's loop is extremely high, whereas the water permeability is extremely low (Schnermann et al., 1998).

At the moment it is very exciting to watch the unraveling of the molecular details of the proteins that constitute the *zonula occludens* and which determine whether the junctional route is "tight" or "leaky". It is now clear that *zonulae occludentes* are made of any one of 20 members of the claudin-encoding genes and a few other transmembrane proteins, like occludins, and many peripheral proteins (*see* Tsukita & Furuse, 1999). Indirect evidence suggests that claudins may create the selectivity properties of the paracellular pathway since mutations in the claudin-16 gene (also named paracellin-1) leads to a selective loss of the extracellular Mg pathway in the thick ascending limb and to Mg-wasting (Simon et al., 1999). More recently, Wilcox et al. (2001) reported mutations in the gene coding for claudin-14 causing autosomal recessive deafness *DFNB29*. These authors suggest that claudin-14 may play an important role in maintaining the high resting potential and the high K concentration of the endolymph in the cochlea by constituting a "tight" seal with various types of cells bordering the endolymph. Very recently, two interesting papers appeared, which described experimental modulation of the paracellular conductance and selectivity in MDCK cells. In one paper, the difference in transepithelial resistance between MDCK I and II, the high- and low-resistance forms, respectively, was demonstrated to be due to claudin-2. Induced expression in MDCK I cells of claudin-2, which is normally only expressed in the low resistance MDCK II cells, converted the MDCK I phenotype into an MDCK II phenotype (Furuse et al., 2001). In the other paper it was shown that overexpression of claudin-4 in MDCK II cells decreased the paracellular conductance through a selective decrease in Na permeability (Van Itallie et al., 2001). Taken together, it could well be that the so-called "kisses in the dark", a term coined by Moreno and Diamond for the paracellular structures that determine the cation selectivity in gallbladder epithelium, have now been identified as being members of the claudin family (Diamond, 1974).

Water Permeability of Biological Membranes

No quest for membrane channel proteins has been longer and more tortuous than the one for water channels. One reason could have been that phospholipid bilayers turned out to be already surprisingly well permeable to water, and in van Deenen's school in Utrecht everyone was convinced that water permeability of biological membranes was determined by the lipid composition and especially by the cholesterol content. By scanning the literature that reported water permeability measurements in epithelial tissues around 1970, I became more and more convinced that there must be more to it than only lipid composition and my interest in water permeability has never faded since then.

Ussing has also been a pioneer in the field of water permeability. As a student of August Krogh he studied the water permeabilty of fish eggs by using deuterated water and he concluded that the diffusional water permeability was too low to be measured accurately (Krogh & Ussing, 1937). How correct this notion was is nowadays routinely demonstrated when *Xenopus* oocytes are used as expression system for aquaporins. Noninjected oocytes will not swell even when placed into distilled water, but after expression of any one of the 10 aquaporins, they explode within a minute when put into distilled water (Preston et al., 1992).

Koefoed and Ussing (1953) were the first to report diffusional and osmotic water permeabilities in toad skin and to observe an increase in osmotic water permeability with ADH. Ussing also introduced a microscopical technique with which he was able to measure the entire thickness of the epithelial layer of the frog skin and could follow osmotically-induced swelling in time. With this technique, MacRobbie and Ussing (1961) were able to observe that the outer

barrier of the skin was much less permeable to water than the inner barrier and that the water permeability of the outer barrier was increased when the inner solution contained ADH. A year later Hays and Leaf (1962) reported a much greater effect of ADH on toad bladder epithelium. This paper as well as Ussing's papers started off a big discussion on the possible size of the water pores induced by ADH, based on the differences observed in hydraulic conductivity (L_p) and diffusional permeability (P_d). Only after correction for unstirred-layer effects on P_d measurements, Hays and Franki (1970) arrived at the, as we now know, correct conclusion that ADH increases the number and not the diameter of small aqueous pores in the apical membrane. This classical paper by Hays and Franki, together with the first morphological observation by the group of Bourguet of numerous intramembranous particle aggregates after ADH challenge of frog bladder, are in my opinion the papers that justified the search for molecular water channels (Chevalier, Bourguet & Hugon, 1974). However, most peer reviewers did not consider molecular water channels very appealing nor realistic and this delayed my search for water channels significantly.

In 1985 I was finally successful in getting a project funded, aiming at identifying water channel proteins in proximal tubular membranes. By using a stopped-flow light-scattering technique, we were able to demonstrate a Hg-sensitive water flow in rat proximal tubular brush border and basolateral membrane vesicles, a component that was completely absent from small intestinal specimens (van Heeswijk & van Os, 1986). Using a radiation-inactivation approach, van Hoek et al. (1991) were able to demonstrate that this Hg-sensitive water flow in proximal tubular membranes had a functional unit size of approximately 30 kDa. Before we were able to identify this 30 kDa protein, Peter Agre took the whole field by surprise. His group had cloned the first molecular water channel from red blood cells and proved its water-channel properties in a now classical paper (Preston et al., 1992). Agre's serendipitous discovery of the first water channel opened up a completely new field, and within a couple of years 10 aquaporins were described (*see* Deen & van Os, 1998). We were extremely fortunate that in the university hospital in Nijmegen, Knoers and Monnens were seeing and studying several patients suffering from congenital nephrogenic diabetes insipidus, NDI. In close collaboration, we were able to prove that the autosomal recessive form of NDI was caused by mutations in the gene coding for aquaporin-2, which was the ultimate proof that aquaporin-2 was the vasopressin-dependent water channel in the collecting duct (Deen et al., 1994). Up to now we are again and again surprised by new mutations in this gene, which also causes the autosomal dominant form of NDI (Mulders et al., 1998; Kamsteeg et al., 1999). However, more details about recent advances in the aquaporin field would be out of place in a tribute to Hans Ussing.

Concluding Remarks

Hopefully, I have made clear that in every aspect of my past and present work Hans Ussing has had an influence as a pioneer. He has formulated biophysical concepts that have withstood the tooth of time extremely well. He told me personally not to worry too much and since 1980 I, indeed, stopped worrying about matters that one cannot control.

Looking at the list of papers Hans Ussing produced, it is very obvious that he never retired and that he was faithful to the frog skin a long life long. I realize, however, that his most influential and original contributions to the field of biological membranes were made in the first half of his productive scientific career. So, here is another lesson to be learned from Hans Ussing's life. Don't worry when it is time to retire and make place for the young and talented ambitious young men and women! These young scientists will fully agree with the statement Hans Ussing made in 1968: "*The advances on the membrane front in experimental biology were very slow indeed until after the last world war. Then things started happening. Stimulated by the developments in electronics as well as by the availability of radioactive and stable isotopic tracers, information about the permeability properties of living cells and membranes was pouring in and was arranged in a nice pyramid of theory*". However, the new generation of membrane biologists, most likely, want to change the words "*last world war*" into "*1990*", "*electronics*" into "*genomics*" and "*theory*" into "*molecular data*"(Ussing, 1969).

Drs. Bindels, Deen, Hoenderop, Mulders, Kamsteeg and Müller are gratefully acknowledged for being such great colleagues and for suggestions for this contribution.

References

Augustus, J., Bijman, J., van Os, C.H., Slegers, J.F.G. 1977. High conductance in an epithelial membrane not due to extracellular shunting. *Nature* **268:**657–658

Bindels, R.J.M., Hartog, A., Timmermans, J., van Os, C.H. 1991. Active Ca^{2+} transport across rabbit connecting tubule cells in primary culture: stimulation by 1,25-Dihydroxyvitamin D3 and PTH. *Am. J. Physiol.* **30:**F799–F807

Chevalier, J., Bourguet, J., Hugon, J.S. 1974. Membrane associated particles: Distribution in frog urinary bladder epithelium at rest and after oxytocin treatment. *Cell. Tiss. Res.* **152:**129–140

Deen, P.M.T., van Os, C.H. 1998. Epithelial Aquaporins. *Curr. Opin. Cell Biol.* **10:**435–442

Deen, P.M.T., Verdijk, M.A.J., Knoers, N.V.A.M., Wieringa, B., Monnens, L.A.H., van Os, C.H., van Oost, B.A. 1994. Requirement of human renal water channel aquaporin-2 for vasopressin dependent concentration of urine. *Science* **264:**92–95

Diamond, J.M. 1962. The mechanism of solute transport by the gall bladder. *J. Physiol.* **161:**474–502

Diamond, J.M. 1974. Tight and leaky junctions of epithelia: a perspective on kisses in the dark. *Fed. Proc.* **33:**2220–2224

Dijkink, L. 2001 Aldosterone regulated sodium transport in the kidney. Ph.D. Thesis, University of Nijmegen

Dijkink, L. Hartog, A., van Os, L.H., Bindels, R.J.M. 2001. Modulation of aldosterone-induced stimulation of ENaC synthesis by changing the rate of apical Na entry. *Am. J. Physiol.* **281:** F687–F692

Frömter, E., Diamond, J.M. 1972. Route of passive ion permeation in epithelia. *Nature* **235:**9–13

Furuse, M., Furuse, K., Sasaki, H., Tsukita, S. 2001. Conversion of *Zonulae Occludentes* from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J. Cell Biol.* **153:**263–272

Ghijsen, W.E.J.M., van Os, C.H. 1979. Ca-stimulated. ATPase in brush border and basolateral membranes of rat duodenum with high affinity sites for Ca ions. *Nature* **279:**802–803

Ghijsen, W.E.J.M., de Jong, M.D., van Os. C.H. 1982 ATP-dependent calcium transport and its correlation with Ca-ATPase activity in basolateral plasmamembranes of rat duodenum. *Biochim. Biophys. Acta* **689:**327–336

Harvey, B.J., Thomas, S.R., Ehrenfeld, J. 1988. Intracellular pH controls cell membrane Na and K conductances and transport in frog skin epithelium. *J. Gen. Physiol.* **92:**767–791

Hays, R.M., Leaf, A. 1962. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45:**933–948

Hays, R.M., Franki, N. 1970. The role of water diffusion in the action of vasopressin. *J. Membrane Biol.* **2:**263–276

Hoenderop, J.G.J., van der Kemp, A.W.C.M., Hartog, A., van de Graaf, S.T.J., van Os, C.H., Willems, P.H.G.M., Bindels, R.J.M. 1999. Molecular identification of a Ca influx channel in 1,25-dihydroxyvitamin D3-responsive epithelia. *J. Biol. Chem.* **274:**8375–8378

Ishibashi, H., Dinudom, A, Harvey, K.F., Kumar, S., Young, J.A., Cook, D.I. 1999. Na-H exchange in salivary secretory cells is controlled by an intracellular Na receptor. *Proc. Natl. Acad. Sci.* USA **96:**9949–9953

Kamsteeg, E.J., Wormhoudt, T.A.M. van Os, C.H., Deen, P.M.T. 1999. An impaired routing of wild-type aquaporin-2 after tetramerization with an aquaporin-2 mutant explains dominant nephrogenic diabetes insipidus. *EMBO J.* **18:**2394–2400

Koefoed-Johnsen, V., Ussing, H.H. 1953. The contribution of diffusion and flow to the passage of D_2O through living membranes. *Acta Physiol. Scand.* **28:**60–76

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42:**298–308

Koster, H.P.G., Hartog, A, van Os, C.H., Bindels, RJ.M. 1995. Calbindin-D28k facilitates cytosolic Ca^{2+} transport without interfering with Ca^{2+} signalling. *Cell Calcium* **18:**187–196

Krogh, A., Ussing, H.H. 1937. A note on the permeability of trout eggs to D_2O and H_2O. *J. Exp. Biol.* **14:**35–37

Loffing, J., Pietri, L, Aregger, F., Bloch-Faure, M., Ziegler, U., Meneton, P., Rossier, B.C., Kaissling, B. 2000. Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets. *Am. J. Physiol.* **279:**F252–F258

MacRobbie, E.A.C., Ussing, H.H. 1961. Osmotic behaviour of the epithelial cells of the frog skin. *Acta Physiol. Scand.* **53:**348–365

Mulders, S.M., Bichet, D.G., Rijss, J.P., Kamsteeg, E.J., Arthus, M.F., Lonergan, M., Fujiwara, M., Morgan, K., Leijendekker, R., van der Sluijs, P., van Os, C.H., Deen, P.M. 1998. An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J. Clin. Invest.* **102:**57–66

Preston, G.M., Carroll, T.P., Guggino, W.B., Agre, P. 1992. Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* **256:**385–387

Reuss, L, Finn, A.L. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus* gall bladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25:**141–161

Schnermann, J., Chou, C.L., Ma, T., Traynor, T., Knepper, M.A., Verkman, A.S. 1998. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc. Natl. Acad. Sci.* USA. **95:**9660–9664

Schultz, S.G. 1992. Membrane cross-talk in sodium-absorbing epithelial cells. *In:* The Kidney, Physiology and Pathophysiology. Seldin, D.W., Giebisch, G., editors. pp. 287–300 Raven Press, NewYork

Simon, D.B., Lu, Y., Choate, K.A., Velazquez, H., At-Sabban, E., Praga, M., Casari, G., Bettinelli, A., Colussi, G., Rodriguez-Soriano, J., McCredie, 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

Taylor, A., Windhager, E.E. 1979. Possible role of cytosolic Ca and Na-Ca exchange in regulation of transepithelial sodium transport. *Am. J. Physiol.* **236:**F347–F352

Tsukita, S., Furuse, M. 1999. Occludin and claudins in tight-junction strands: leading or supporting players? *Trends Cell Biol.* **9:**268–273

Ussing, H.H. 1949. The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **18:**43–56

Ussing, H.H. 1969. Summary Address. *In:* The molecular basis of membrane function. Tosteson, D.C. Inc., editor. pp. 577–588. Prentice-Hall, Englewood Cliffs, NJ

Ussing, H.H., Windhager, E.E., 1964. Nature of shunt path and active sodium transport through frog skin epithelium. *Acta Physiol. Scand.* **61:**484–504

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-cicuited isolated frog skin. *Acta Physiol. Scand.* **23:**110–127

Valentijn, J.A., Fyfe, G.K., Canessa, C.M. 1998. Biosynthesis and processing of epithelial sodium channels in *Xenopus* oocytes. *J. Biol. Chem.* **273:**30344–30351

van Heeswijk, M.P.E., van Os, C.H. 1986. Osmotic water permeabilities of brush border and basolateral membrane vesicles from rat kidney cortex and small intestine. *J. Membrane Biol.* **92:**183–193

van Hoek, A.N., Hom, M.L., Luthjens, L.H., de Jong, M.D., Dempster, J.A., van Os, C.H. 1991. Functional unit of 30-kilo Daltons for proximal tubule water channels as revealed by radiation inactivation. *J. Biol. Chem.* **266:**16633–16636

van Itallie, C., Rahner, C., Anderson, J.M. 2001. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J. Clin. Invest.* **107:** 1319–1327

van Os, C.H., Slegers, J.F.G. 1975. The electrical potential profile of gall bladder epithelium. *J. Membrane Biol.* **24:** 341–363

van Os, C.H., Wiedner, G., Slegers, J.F.G., Bijman, J., Wright, E.M. 1981. Hydraulic and electrical conductivities of rabbit gall bladder and submaxillary main duct epithelium. *In:* Water transport across epithelia. Ussing, H.H., Bindslev, N., Lassen, N.A., Stenknudsen. editors. pp. 178–187. Munksgaard, Copenhagen

Vennekens, R., Hoenderop, J.G., Prenen, J., Stuiver, M., Willems, P.H., Droogmans, G., Nilius, B., Bindels, R.J. 2000. Permeation and gating properties of the novel epithelial Ca channel. *J. Biol. Chem.* **275:**3963–3969

Wilcox, E.R, Burton, Q.L, Naz, S., Riazuddin, S., Smith, T.N., Ploplis, B., Belyantseva, I., Ben-Yosef, T., Liburd, N.A., Morell, R.J., Kachar, B., Wu, D.K., Griffith, A.J., Riazuddin, S., Friedman, T.B. 2001. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. *Cell* **104:**165–172