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Role of Mitochondria-rich Cells for Passive Chloride Transport, with a Discussion of Ussing's Contribution to Our Understanding of Shunt Pathways in Epithelia

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Abstract. A non-invasive method is applied for studying ion transport by single isolated epidermal mitochondria-rich (MR) cells. MR cells of toad skin (*Bufo bufo*) were prepared by trypsin (or pronase) treatment of the isolated epithelium bathed in Ca^{2+}-free Ringer. Glass pipettes were pulled and heat-polished to obtain a tip of 2–4 μm with parallel walls and low tip resistances. The neck of an MR-cell was sucked into the tip of the pipette for being 'clamped mechanically' by the heat-polished glass wall. In this configuration the apical cell membrane faces the pipette solution while the major neck region and the cell body are in the electrically grounded bath. With Ringer in bath and pipette, transcellular voltage clamp currents were composed of an ohmic (I_{leak}) and a dynamic ($I_{dynamic}$) component. The dynamic component was studied by stepping the transcellular potential (V_p) from a holding value of +50 mV to the hyperpolarizing region ($50 > V_p \geq -100$ mV). The steady state $I_{dynamic}$–V_p relationship was strongly outward rectified with $I_{dynamic}$ being practically zero for $V_p > 0$ mV. At $V_p = -100$ mV, MR cells isolated by trypsin or pronase generated a steady-state $I_{dynamic}$ of, -2.72 ± 0.40 nA/cell ($N = 21$ MR cells). Continuous superfusion of the MR cell during recording increased the current to -7.99 ± 1.48 nA/cell ($N = 10$ MR cells). The time course of the reversible activation of $G_{dynamic}$ varied among cells, but was usually sigmoid with $T_{1/2}$ decreasing with V_p ($-25 \geq V_p \geq -100$ mV). $T_{1/2}$ was in the order of 10 sec at $V_p = -100$ mV. The single-MR-cell currents recorded in this study are fully compatible with Cl^- currents estimated by relating density of MR cells to transepithelial I_{Cl} or by measurements with the self-referencing ('vibrating') probe technique.

In the discussion, Ussing's work on epithelial shunt pathways is considered. His thinking and experiments leading to his theory of isotonic transport in leaky epithelia is emphasized. It is our thesis that the understanding of the physiology of epithelia owes as much to Ussing's studies of shunt pathways as to his studies of the active sodium pathway.

Key words: Toad skin — Mitochondria-rich cells — Chloride channels — Epithelial shunt pathways — Leaky epithelia — Recirculation theory of isotonic transport

Introduction

The Ussing-Zerahn paper from 1951 introduced the short-circuit technique, proved the existence of active sodium transport, and suggested an electric circuit analogue of frog skin with a shunt path in parallel with the active sodium path [27]. With these methods, results, and concepts the paper founded a new era of membrane physiology.

Our paper will concentrate on shunt pathways of epithelia. When introduced [21, 27] and further considered by Koefoed-Johnsen and Ussing [5], it was assumed that the shunt was cellular like the active sodium transport. The study by Ussing and Windhager [26] introduced the concept of a paracellular shunt, and fourteen years later — in the issue of the Journal of Membrane Biology dedicated to Hans Ussing on occasion of his 65th birthday — Voûte and Meier [28] published observations showing that in the absence of active Na^+ transport there is a linear relationship between transepithelial current and density of mitochondria-rich cells. Experiments by one of us (PK) proved the currents to be carried by Cl^-. These studies in Basel and Copenhagen resulted in the hypothesis that the mitochondria-rich (MR) cells of frog skin constitute a cellular shunt path for Cl^- uptake

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[28]. Since then several techniques have been applied for studying ion transport by MR cells [9], confirming that one of their functions is to transport Cl^- [4, 6, 10, 15, 20, 30, 31]. It has never been verified, however, that the Cl^- permeability of MR cells can account quantitatively for the macroscopic Cl^- current of the intact epithelium. The aim of this study is to introduce a new non-invasive method for investigating ion transport by single MR cells. Evidence will be presented that transcellular currents generated by single voltage-clamped MR cells fully account for the macroscopic Cl^- currents generated by voltage-clamped intact epithelia. Additionally, we discuss other functions of shunt pathways in epithelia, demonstrating how this concept has been influenced profoundly by Ussing's studies.

Materials and Methods

Isolation of Mitochondria-Rich Cells

Isolated skin epithelia of toads (*Bufo bufo*) were obtained by exposing the serosal side of the skin for about 2 hrs to Ringer's solution containing 2 mg/ml collagenase (Type 2, Worthington Chemical, NJ). Subsequently, the isolated epithelium was bathed in trypsin-free collagenase-Ringer for 1 hr (2 mg/ml, C-9891, EC 3.4.24.3, Sigma, St. Louis, MO), washed in Ca^{2+}-free Ringer, transferred to a Ca^{2+}-free trypsin-containing Ringer for 3 min (0.1 mg/ml, T-4665, EC 3.4.21.4, Sigma), and centrifuged at 800 rpm for 5 min. Trypsin treatment was repeated 3–6 times until a sufficient number of isolated cells was obtained. In some experiments pronase (1 mg/ml, P-5147 Type XIV, EC 3.24.31, Sigma) was used instead of trypsin. The isolated cells were washed thoroughly before transfer to the recording chamber mounted on the stage of an inverted microscope (Nikon TMS, Tokyo, Japan) with long focus distance objective (CF Achromat LWD DL 40 × N.A. = 0.55, Nikon). The neck of an MR cell was sucked into a pipette for transcellular voltage clamping with Ringer in both pipette and bath. A similar method was used for studying light-sensitive currents in retinal rods [32].

The recording chamber was modified during the study. At the beginning (and in the majority of experiments reported here) a Petri dish was used with a bath volume of ~1 ml (4 cm o.d., Nunc, Roskilde, Denmark). To avoid flushing the few MR cells away, either we did not perfuse the chamber at all or, alternatively, between recordings the bath was renewed very slowly. This summer a chamber designed in our own workshop was applied with a bath volume of about 300 μl, which is renewed by gravity feeding with a half time of ~3 sec from an external well-aerated reservoir.

Single-Cell Voltage-Clamping

Suction pipettes were pulled from borosilicate glass tubes with internal filament (Vitrex, Modulohm, A/S, Herlev, Denmark) on a two-step vertical puller (Hans Ochotzki, Homburg, Germany). They were pulled with a fairly open tip and subsequently heat-polished on a microforge (MF-90, Narishige, Tokyo, Japan) to obtain a tip with 2–4 μm of parallel walls and a resistance of 1–3 MΩ (measured with Ringer in bath and pipette), but pipettes of significantly higher (5–7 MΩ), or smaller (800 KΩ), resistance were used in some experiments. Cells were gently sucked into the 'mouth' of the pipette such that the heat-polished glass wall mechanically clamped the apical region of the neck. In this configuration the apical cell membrane is facing the pipette solution while the cell body is in the electrically grounded bath. The method presupposes that the cell has the shape of a bottle. Some MR cells are slender with no distinct neck and body regions and they could not be used. However, even the morphology of bottle-shaped MR cells is quite variable with respect to length and diameter of the neck. Ideally, the suction pipette should be made-to-measure and this was attempted. However, in several cases the cell slipped into the pipette or did not seal well for electrical recordings. Failing attempts exceeded by a fair number the cells that were successfully studied. Voltage clamping was performed either with an RK-300 (Biologic, Claix, France) or an Axopatch-200 amplifier (Axon Instruments, Foster City, CA) using the CED Patch and Voltage Clamp software (Cambridge Electronic Design, Cambridge, UK) or the Clampex/Clampfit software package from Axon, respectively. Currents were recorded at low gain (0.05–1 mV/pA) and digitized at rates of 20–50 Hz, with faster rates used occasionally (100–150 Hz).

Solutions

The Ringer solution used in all steps during isolation of MR cells contained (in mM): 115.2 Na^+, 2 K^+, 1 Ca^{2+}, 0.5 Mg^{2+}, 112 Cl^-, 0.4 H_2PO_4), 2.4 HPO_4^{2-}, 3 acetate, adjusted to pH = 7.4 and equilibrated with 100% O_2. Ca^{2+} was omitted during trypsin treatment. In a majority of experiments this solution was also used in bath and pipette in the voltage-clamp experiments. In some experiments, voltage clamping was performed with a Ringer solution in both bath and pipette having the following composition (in mM) 116.5 Na^+ 1.9 K^+, 1 Ca^+, 114.8 Cl^-, 2.4 HCO_3^-, 3 acetate, pH = 8.2 when equilibrated with atmospheric air.

Data Presentation

Traces shown in figures stem from on-line digitized recordings transferred to a graphic software program (Origin version 6.1) for further analysis and presentation. Mean ± standard errors of the mean are given for pooled data with N indicating the number of cells studied.

Results

Transcellular voltage clamp of single MR cells was initiated 5 years ago, the experiments continued during the summer months of 1996, 1998, and late spring and summer 2001. During this period the method underwent improvements with respect to design of suction pipettes and superfusion of cells. The same cell can now be studied for more than an hour without signs of 'run-down'.

Steady-state Transcellular Current-Voltage Curves Contain a Strongly Outwardly Rectified Component

Figure 1*A* shows results of an experiment in which the transcellular potential (V_p) was shifted respectively between +40 and −100 mV. The transcellular

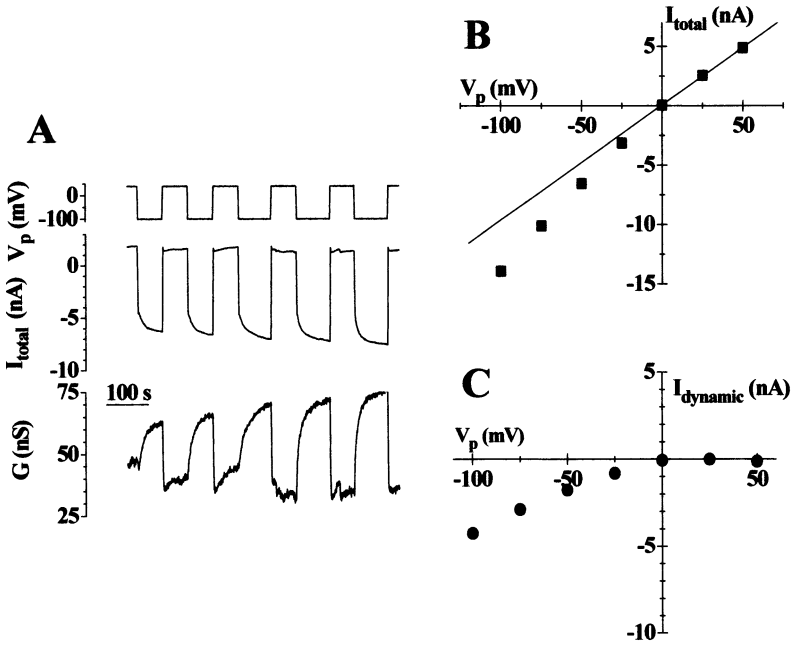

Fig. 1. The dynamic conductance of single mitochondria-rich cells studied by "nano Ussing-chamber technique" with Ringer's solution of identical composition in pipette and bath. (*A*) The conductance of the cell increases reversibly in response to a step change of the transcellular potential (V_p) here illustrated by repeated shifts of V_p from +40 to −100 mV. (*B*) Steady-state voltage clamp currents (I_{total}) as function of pipette potential. For $V_p > 0$ mV the relationship is simple ohmic (linear, I_{leak}). (*C*) Subtraction of the linear component from I_{total} results in a current–voltage relationship that is strongly outwardly rectified, $I_{dynamic} = I_{total} - I_{leak}$.

hyperpolarization ($V_p < 0$) resulted in slow reversible activations of the clamping current (I_{total} of Fig. 1*A*) caused by reversible conductance activations (*G*, lower panel). In this experiment the activated conductance increased about twice from the first to the last activation illustrating a 'run up'. Following this kind of exercise the voltage response of the cell becomes quite reproducible, so that a steady-state current–voltage curve covering a considerable range of pipette potentials can be obtained. In Fig. 1*B* is shown the steady-state I_{total} as a function of the pipette potential at which the cell was clamped. The recorded steady-state currents can be resolved in a linear ohmic component, I_{leak}, and a non linear dynamic component $I_{dynamic}$. The voltage dependence of the dynamic component is depicted in Fig. 1*C*. It is strongly outwardly rectified with fairly large currents in the hyperpolarizing region.

The dynamic component varied somewhat among MR cells and appeared to be dependent on superfusion of the cell during the recordings, but not on the enzyme used for separating the cells. In non-perfused chambers (*see* Methods), cells isolated with pronase generated a steady-state dynamic current at $V_p = -100$ mV that varied from −0.98 to −4.2 nA/cell with an average of -2.14 ± 0.72 nA/cell ($N = 5$). Cells isolated with trypsin and studied in the same chamber exhibited a variation from −1.0 to −7.6 nA/cell with a mean of -2.90 ± 0.48 nA/cell ($N = 16$). The two means are not statistically different ($p > 0.36$, Student's *t*-test of unpaired data) and the overall mean of the steady-state $I_{dynamic}$ recorded at −100 mV was -2.71 ± 0.40 nA/cell ($N = 21$ cells). In more recent experiments a recording chamber was used with fast exchange of the bath solution (*see* Methods), which turned out to increase the dynamic currents. With this chamber and cells isolated by trypsin treatment, steady-state dynamic currents were recorded at $V_p = -100$ mV that varied between −3.25 and −14.4 nA/cell with an average of -7.99 ± 1.48 nA/cell ($N = 10$ cells). The family of current activations shown in Fig. 2*A* was obtained from a cell continuously superfused with well-aerated Ringer and is typical for this type of experiments. From a holding potential of +50 mV, V_p was varied in steps of 25 mV between −100 and +50 mV. The resulting outwardly rectified steady-state current–voltage relationship depicted in Fig. 2*B* was calculated as explained in the legend of Fig. 1. The associated steady-state conductance-voltage relationship is sigmoid, *see* Fig. 2*C*. It shows that the MR cell expresses a membrane conductance, which is saturating at a transcellular potential near −100 mV. The rate at which the conductance was activated increased as the transcellular potential was clamped to more negative values (Fig. 2*D*). In this experiment, $T_{1/2}$ of current activations decreased from 8.6 to 3.9 sec when V_p was decreased from −25 to −100 mV.

Evidence that the Dynamic Current is Carried by Chloride

Also the intact epithelium displays a conductance that is activated slowly by transepithelial hyperpolarizations [11]. Fig. 3*A* shows the striking similarity between the time course of the transepithelial current and the single-MR-cell current, respectively. In the intact epithelium, the deactivation often proceeds

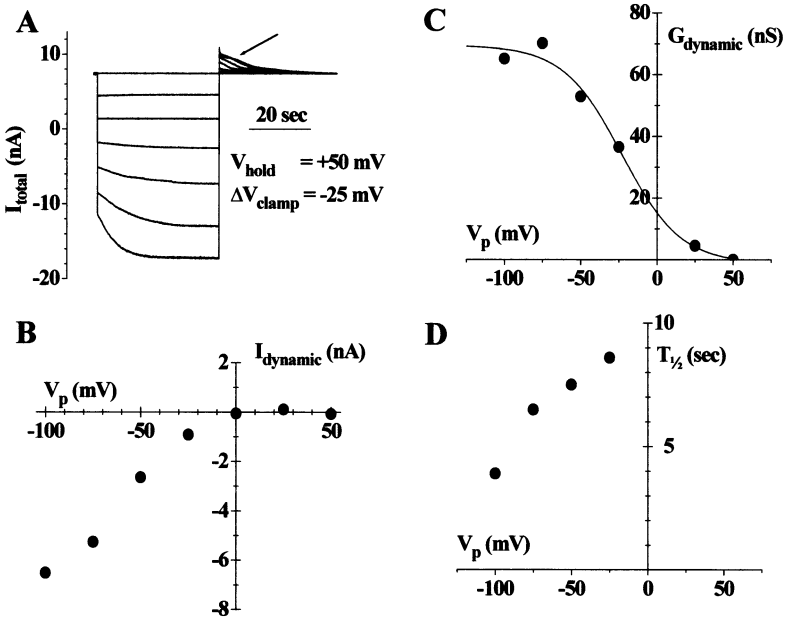

Fig. 2. (*A*) Family of voltage-clamp currents of a single MR cell obtained from a holding potential of +50 mV. Note the constancy of the steady-state current recorded at the holding potential, indicating literally no change of the leak conductance during the fairly long experiment. (*B*) Steady-state *I-V* relationship of the dynamic component. (*C*) Steady-state conductance–voltage relationship of the MR cell, $G_{dynamic} = I_{dynamic}/V_p$. (*D*) Half time ($T_{1/2}$) of current activation decreases with V_p in the hyperpolarizing region of the current–voltage curve.

with a 'shoulder' on the otherwise exponential-like current decay, *see* e.g., Figs. 12 and 14 in [15]. Such a time course of conductance deactivation was also seen in experiments with isolated cells as indicated by arrows of Fig. 2*A*. The range of transcellular potentials where the conductance is activated is similar for intact epithelia and single MR cells. This can be seen by comparing Fig. 2*C* and Fig. 3*B* showing similar position of the *G*/*V*-curves on the *V*-axis. Based on unidirectional $^{36}Cl^-$ flux measurements the dynamic current of the intact epithelium could be identified unequivocally as a Cl^- current with no contributions from other ion species, *see* Fig. 3*C*. Taken together, the above observations provide compelling evidence that the dynamic current component of single MR cells is carried by a transcellular inward flux of Cl^-.

This conclusion is further confirmed by considering previous estimates of the voltage-activated Cl^- currents of single MR cells in situ. Studies with the self-referencing probe technique and of the relationship between the density of MR cells of intact toad skin epithelium and chloride currents led to the hypothesis that single-MR cell Cl^- currents would have to be impressively large, i.e., in the order of a few nA/cells at a transcellular potential of −100 mV [2, 4, 30]. Transcellular currents of this magnitude were recorded in the present study of identified single MR cells (Figs. 1 and 2, and text above). Finally, previous conventional 'whole-cell patch-clamp' studies of isolated MR cells showed that a conductance specific for Cl^- could be reversibly activated by cell depolarization [10]. The dynamic range of cell potentials in 'whole-cell patch-clamp' experiments, and the range of transcellular potentials of which the dynamic conductance was activated in the present study, agree reasonably well with an intracellular potential (zero membrane current) of isolated MR-cells, $V_{cell} = -32.5 \pm 2.5$ mV, $N = 9$ [20].

Discussion

The Cellular Shunt of Amphibian Skin

This study applies a novel technique for investigating ion transport by epithelial mitochondria-rich cells, which constitute a minority cell type of heterocellular high-resistance renal and extra-renal epithelia. The result of greatest interest based on 31 identified toad skin MR cells is that the currents generated by single MR cells account for the voltage-activated Cl^- currents of the intact epithelium. Our investigations of the regulation of the 'chloride shunt' of anuran skin can now definitively be associated with this cell type. The physiological significance is that the passive, voltage-activated Cl^- uptake by MR cells can be regulated by the transepithelial electrical potential difference generated by the active uptake of Na^+ by principal cells, but in such an intriguing way that MR cell Cl^- channels are activated only if this ion is present in a sufficient concentration in the fluid bathing the external side of the epithelium [7]. A similar 'knock on the door regulation' has been described for CIC-0 channels of *Torpedo* electric organ [17].

Single-Channel versus Single-MR-Cell Studies

Analysis of stationary fluctuations of currents flowing through depolarization-activated Cl^- channels of isolated MR cells indicated a large single-channel

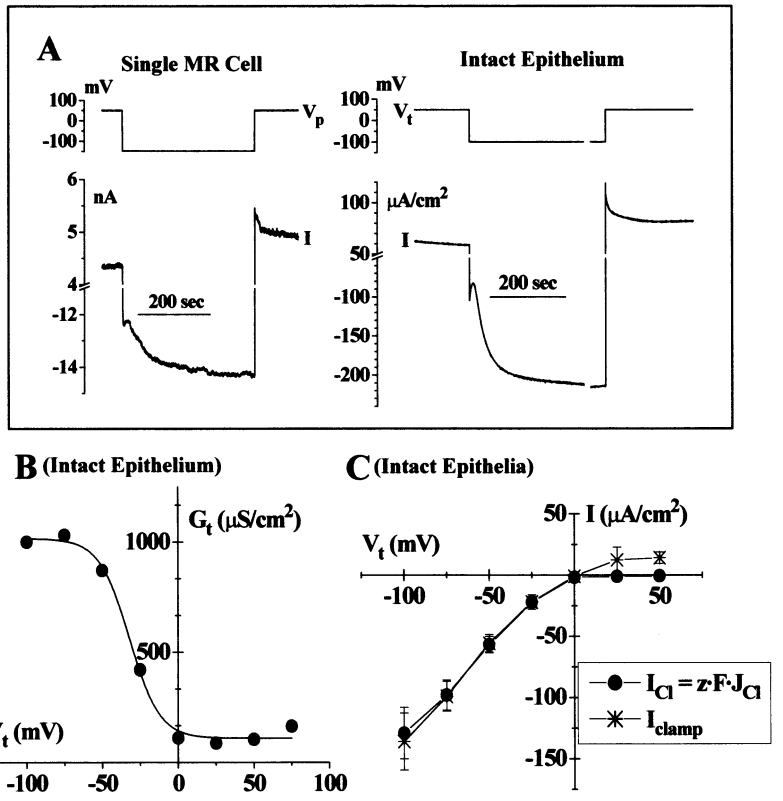

Fig. 3. (*A*) Voltage-activated currents of single MR cell (*left*) and intact isolated toad skin epithelium (*right*). The time course of the current is similar in the two preparations, but the dynamic current of the latter is larger by about ×50,000. The number of MR cells/cm^2 is of a similar order of magnitude [30]. (*B*) The conductance-voltage curve of intact epithelium (active sodium transport eliminated by replacing Na^+ in the outside solution with K^+) resembles that of single MR cells with respect to shape and position on voltage-axis (*conf.* Fig. 2*C*). (*C*) The dynamic current of the intact epithelium with active sodium uptake eliminated (outside Na^+ replaced by K^+) is carried exclusively by a net inward flux of chloride ions. The Cl^- currents were calculated from unidirectional $^{36}Cl^-$ fluxes of paired skin preparations. Redrawn from [30].

conductance of 200–300 pS [10]. Channels of similar large conductance were observed in apical membrane patches of single MR cells [20], but the frequency with which they occurred is much too low to account for the transcellular currents recorded in the present experiments. Studies published elsewhere [1] demonstrate transcription of the CFTR gene in toad skin epidermal cells, verify specific expression of CFTR in the MR cells, and present the sequence of cloned *Bufo*CFTR DNA (GenBank accession #AYO26761). Also in this case there is a very significant discrepancy between the halide ion-specific epithelial anion conductance activated by β-adrenergic receptor occupation [1] and the occurrence in apical membrane patches of the small CFTR-like Cl^- channel [20]. Results of the present study show that the above-mentioned quantitative discrepancies between transepithelial currents and single-channel currents cannot be ascribed to enzymatic or other protocol-dependent destructions of apical Cl^- channels. Other explanations would be that membrane patches of high-density Cl^- channels might have been too noisy for analysis or judged to be governed by a leaky seal. Although this may seem a logical explanation, the problem needs to be explored in more detail before it is satisfactorily solved.

Time Constants of the Dynamic Current

While previous studies have shown that MR cells express Cl^- permeabilities in apical and basolateral membranes [4, 10, 15, 20, 31], the present study is the first that provides evidence that the voltage-activated Cl^- currents of amphibian skin are generated entirely by MR cells. This said, it should be mentioned that there are differences that should not be overlooked between features of Cl^- currents of the intact epithelium and the voltage-activated outward currents of isolated MR cells. Although the dynamic current of most cells exhibited time constants resembling those of transepithelial Cl^- currents of the intact epithelium (Fig. 3*A*), in some cases single MR cells activated 4–5 times faster (Fig. 2), with a few isolated MR cells exhibiting even faster current activations ($T_{1/2} < 1$ sec has been observed at $V_p = -100$ mV, *not shown*). These observations should be compared with the fast voltage-activation of outward Cl^- currents in the 'whole-cell patch-clamp' mode [10]. With this configuration it was observed that the stationary current fluctuations resulting from opening and closure of the large-conductance depolarization-activated Cl^- channels were governed by a very small time constant (~5 msec). This observation, together with the above mentioned discordance between the time constant of some isolated MR cells and the time constants of MR cells in situ, constitute challenges for future studies.

The Paracellular Shunt

Under some circumstances tight junctions constitute the physiologically important shunt for passive ion

fluxes crossing the epithelium. Ussing and Windhager first showed this in their studies of frog skin exposed to a hyperosmotic external solution [26]. Ussing explored the paracellular transport further in studies resulting in his concept of 'anomalous solvent drag' [22]. More recently the toad urinary bladder has been used as experimental model for studying reversible permeability regulation of tight junctions [8]. In what follows we will restrict the discussion of the physiological role of paracellular pathways to epithelia that have capacity of transporting water and salt in isotonic proportions.

The same year Ussing and Windhager published their milestone paper indicating that a pathway between the epithelial cells constitutes a physiological shunt, Whitlock and Wheeler [29] suggested that the lateral intercellular space constitutes a compartment coupling the transepithelial active sodium flux and water flow across the gallbladder epithelium. Thus, Whitlock and Wheeler adopted the theoretical model of Curran [3], developed in his studies of isotonic transport in small intestine, by associating the compartments of his model with macroscopic structures of the epithelium. The subsequent establishment of the concept of 'leaky epithelia' *pari passu* with that of 'isotonic transport' owes a lot to these three papers.

In our discussions in Copenhagen during the early 90s Ussing maintained that the problem of isotonic transport could not be solved unless it was realized that this type of transport constitutes a paradox. He would not accept as an explanation that the water permeability of the epithelium is so high that the osmotic force driving water through the epithelium would be below the limit of experimental resolution. The scientific challenge, he insisted, is to explain how water and salt are transported in truly isotonic proportions under truly transepithelial thermodynamic equilibrium conditions, that is, in the absence of any transepithelial chemical and electrical potential difference, or difference in hydrostatic pressure. This demand resulted in his Na^+-recirculation theory of isotonic transport.

Ussing's Sodium-Recirculation Theory of Isotonic Transport

Ussing's cardinal assumption was that the sodium pump in the plasma membrane lining the lateral intercellular space (*lis*) plays a dual role for generating an isotonic transportate [23, 25]. This principle is best illustrated by considering transport by small intestine. Firstly, the pump maintains a hyperosmotic and hyperbaric *lis*. With the tight junction reflection coefficient being larger than the reflection coefficient of the interspace basement membrane, water is driven by the osmotic pressure difference from the luminal solution into the space[1] and by the hydrostatic pressure difference out of the space into the serosal solution. This way of describing transepithelial fluid absorption is contained in most models of isotonic transport [18]. Secondly, to compensate for the fact that the fluid emerging from *lis* is hypertonic, by generating a steady-state flux of Na^+ from the serosal solution back into *lis* via the cells, energy consumed by the pump is also spent for maintaining an isotonic composition of the 'net transportate'. It follows that Cl^- would also have to be recirculated to maintain an overall electroneutral transport. According to the above interpretation it is the tonicity of the 'net transportate' that is studied by investigators of solute-coupled water transport.

Ussing's primary concerns were to verify recirculation of Na^+, to estimate the relative magnitude of the recirculation flux, and to provide evidence for paracellular inward convection. He initiated the experiments performed by one of us (SN) and presented the first series of results at the Alfred Benzon Meeting in Copenhagen in 1992 [25], which were supplied with further experiments during the next few years [16]. An important aspect of the study is that the cellular and paracellular components of the unidirectional fluxes were estimated without perturbing the physiological steady state of the epithelium. This was done with the pre-steady state isotope-tracer method, which was developed in a theoretical paper with Ove Sten-Knudsen [19]. Also the interpretation of the stationary fluxes represents a novel way of thinking, entirely due to Ussing. This is illustrated in Fig. 7 and associated Eqs. 6*a*, *b* of the 1999 paper [16].

By the strategy outlined above, Ussing obtained the recirculation flux of Na^+ in the physiological steady state, which by itself is a most remarkable result. Subsequent calculations indicated that 60–70% of the sodium ions pumped from the epithelial cells into *lis* is derived from the serosal bath. Further calculations showed that this would imply a NaCl concentration of the fluid emerging from the lateral space of more than 340 mM, that is, well above twice that of the bathing solutions [12]. It appeared that on the way to eliminate one paradox, another was created. The solution to this problem became evident when considering the Hertz equation for convection-diffusion processes [13]. Analysis of this equation revealed that at low rates of volume flows relative to the permeability of the dissolved solutes, the virtual concentration of the transportate emerging from *lis* would be strongly hypertonic, even if the osmotic concentration of *lis* is only slightly above that of the bathing solutions. In connection with this important

[1]Because paracellular markers are subjected to 'solvent drag', Ussing assumed that water enters the space entirely via tight junctions. The theory is more general, however, as it covers also the case of translateral water transport [13].

point it was realized that the paracellular Na^+-flux ratio is a measure of the relative significance of diffusion and convection out of *lis*. In the one limit, that of pure diffusion, the flux-ratio under equilibrium conditions is unity. In the other limit governed by pure convection, the flux-ratio is infinitely large. Recent computations, based on an extended mathematical model handling electrolytes, have confirmed that with the fluxes measured in the laboratory, including the paracellular Na^+-flux ratio of about 3.6, the recirculation would have to be ~65%, provided an isotonic net transportate is required [14].

Ussing's Na^+-recirculation theory was originally developed for dealing with fluid secretion in the exocrine glands of frog skin [23, 24]. Evidently, the principles outlined above would also apply to epithelial secretion. An additional challenge here is to understand how paracellular fluid transport in the outward (secretory) direction is accomplished by a configuration of transporters similar to the absorptive epithelium if the reflection coefficient of tight junctions is larger than that of the interspace basement membranes. A simple and logical solution to the problem would be to suggest that the osmotic concentration and the hydrostatic pressure of the lateral space are less than those of the solutions bathing the two sides of the acinar epithelium. Such a situation can develop if tight junctions are freely permeable to Na^+ but impermeable to Cl^-. Another solution would be to assume that the relative magnitudes of the reflection coefficients are reversed as compared to the absorbing epithelium. Ussing did not consider this type of problem, and both suggestions are speculative. Testing is needed for deciding which one is the most plausible.

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