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STUDIES OF EPITHELIAL ELECTROLYTE TRANSPORT BY MARKER IONS

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Abstract

The paper reviews several recent studies in which marker ions, such as Rb and Br, were used to identify ion transport pathways and membrane properties in epithelia. In the frog skin epithelium, using Rb as a substitute for K, Cl transport mechanisms across the basolateral membranes of principal cells were studied. The data suggest that intracellular Cl is maintained above electrochemical equilibrium by an Na-K-2Cl cotransport system which, under non-stimulated conditions, is normally quiescent.

In toad and frog skins, the route of transepithelial Cl movement was investigated. A subpopulation of mitochondria-rich cells demonstrated a ready exchange of Br with the apical and basal bathing media, consistent with the view that these cells constitute a transcellular anion shunt. Moreover, voltage-activation resulted in an increased Br uptake from the apical bath. Nevertheless, because of the very small number of these cells, it may be questioned whether the mitochondria-rich cell constitutes the only shuntpathway for Cl.

In other studies, Rb uptake was employed to measure the Na/K-pump activity. In principal cells of the frog skin epithelium, amiloride inhibited Rb uptake and lowered Na concentration, supporting the view that this cell type is engaged in amiloride-sensitive Na transport. In contrast, no significant changes in the Rb, Na, and Cl concentration of mitochondria-rich cells were detectable. Studies with Rb as marker ion in the rabbit urinary bladder revealed that the epithelium behaves like a functional syncytium with regard to transepithelial ion transport.

KEY WORDS: X-ray microanalysis, electron probe, epithelial transport, intracellular ion concentration, sodium, chloride, potassium, rubidium, bromide, frog skin, toad skin, rabbit urinary bladder, electrolytes.

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Introduction

Transport systems which mediate the exchange of ions across cell membranes often are not absolutely specific for only one ion species. Membrane channels or carriers may even allow the passage of ions, such as barium, bromide or rubidium, which are not normally found in biological tissues. Since these ions are transported in a manner similar to their physiological counterpart, they can be used as marker ions to study cellular transport mechanisms.

In epithelia, these marker ions may also be useful in elucidating pathways and barriers involved in transepithelial electrolyte transport. A cell type which is involved in a particular transport process should readily take up the ion from the apical or basal bath, depending on the direction of transport. A method which allows the detection of ions in individual cells or subcellular compartments, such as energy dispersive x-ray microanalysis of thin, freeze-dried cryosections (Dörge et al., 1978; Rick et al., 1982), may then be used to identify the epithelial cell that has taken up the marker ion. Moreover, from the initial uptake rates of the marker ion, membrane permeabilities or turnover rates can be calculated.

In several recent studies in different epithelial tissues, we have employed rubidium and bromide as marker ions for potassium and chloride, respectively. Bromide was used as marker ion for chloride to clarify the transepithelial route of chloride movement in toad and frog skins (Dörge et al., 1988a). Rubidium was used as marker ion for potassium to identify the sodium transport compartment in rabbit urinary bladder (Dörge et al., 1988b), to further characterize the Na-K-2Cl cotransport system in frog skin (Dörge et al., 1989), and for assessing the role of mitochondria-rich cells in transepithelial Na transport (Dörge et al., 1990).

Na-K-2Cl cotransport in principal cells of frog skins

The existence of a Na-K-2Cl cotransport system in the basolateral membrane of principal cells has been deduced from measurements of cellular volume and intracellular chloride concentration. Under control conditions, the chloride concentration in principal cells is far above the electrochemical equilibrium (Rick et al., 1978; Dörge et al., 1985; Ussing, 1985). Removal of sodium, potassium, or chloride from the inner bathing medium results in a decrease of cellular chloride concentration and cell shrinkage (Ussing 1965; Ferreira and Ferreira, 1981; Dörge et al., 1985). Moreover, the recovery of cell volume and re-uptake of chloride after chloride depletion was shown to require the presence of sodium and potassium in the inner bathing medium and to be inhibitable by

loop diuretics (Dörge et al., 1985; Ussing 1985).

To further characterize the cotransporter, the uptake of rubidium across the basolateral membrane was determined in frog skins in which the transepithelial sodium transport was abolished by addition of ouabain and amiloride. Figure 1 shows the intracellular potassium and rubidium concentrations 60 minutes after replacing potassium in the inner bath with rubidium. Compared to control, the rubidium uptake is insignificantly small when the Na/K pump is inhibited by ouabain and the apical sodium influx is simultaneously inhibited by amiloride. Application of amiloride alone also results in a drastic reduction of rubidium uptake.

The dependence of rubidium uptake on the activity of the Na/K pump suggests that most of the rubidium influx is via the Na/K-pump and that only minor amounts enter the cells via the potassium channel and/or the cotransporter. This finding is consistent with the view that the cotransporter is silent when the cell volume is not challenged (Ussing, 1982). Provided that the Na-K-2Cl cotransport system is the only mechanism for chloride uptake, we may further conclude that the chloride permeability of apical and basal membrane is very low so that the cellular chloride concentration remains above electrochemical equilibrium.

Figure 2 illustrates an experiment in which the rubidium uptake was followed during the re-uptake of chloride in chloride-depleted epithelia. Chloride-depletion was achieved by incubating the frog skin for 1 hour in a chloride-free inner bath. Ouabain and amiloride were applied throughout the experiment to prevent any rubidium uptake by the Na/K-pump and to minimize cellular sodium and potassium changes due to pump inhibition. After re-introduction of chloride and simultaneous addition of rubidium, the cellular chloride concentration increased by about 15 mmol/kg w.w. (wet weight) while rubidium increased by about 30 mmol/kg w.w.. At the same time, the cells experienced a significant volume increase as evidenced by a reduction in dry weight content.

From the changes in chloride, rubidium, and dry weight, a net cellular uptake of 80.3 mmol/kg d.w. (dry weight) of chloride and 127.6 mmol/kg d.w. of rubidium can be calculated. At first glance, the fact that the cells gained more rubidium than chloride is somewhat surprising, given that the stoichiometry of the carrier is 1Na:1K:2Cl. However, the result can be explained by assuming that the transporter is, at least in part, working in an self-exchange mode. While the exchange of potassium for rubidium would increase the apparent net uptake of rubidium, the Cl/Cl exchange would go undetected.

Transepithelial chloride pathways in amphibian skins

Amphibian epithelia such as the frog and toad skin are transporting sodium chloride from the outside to the inside. Sodium is the actively transported ion species while chloride follows passively, driven by the transepithelial electrical potential gradient created by the primary transport of sodium. While there is little doubt that Na transport follows a transcellular route involving principal cells (Rick et al., 1978), the nature of the chloride pathway is still open to debate. Since the apical membrane of principal cells is largely impermeable to chloride, it is clear that the principal cell is not part of the transepithelial chloride pathway. Chloride might take an entirely extracellular route through the tight junctions and lateral intercellular spaces or, as more recently suggested (Larsen, 1988), a transcellular route through mitochondria-rich cells.

To gain further information on the route of transepithelial chloride transport, we investigated the uptake of bromide into the different epithelial cell types of toad and frog skins. Bromide was chosen as a marker ion since Harck and Larsen (1986) demonstrated that bromide is transported similarly to chloride. In toad skins, the analyses were performed under control conditions (skin short-circuited) and after stimulating the epithelial chloride permeability by applying an inside positive electrical clamp potential. Larsen and Rasmussen (1982) showed that the large outward current observed in voltage-activated skins is mainly carried by an inwardly directed electrodiffusional flux of chloride. Substituting bromide for chloride in the apical bathing solution decreased the voltage-activated current by only 30%, suggesting that bromide shares in the same transport pathway.

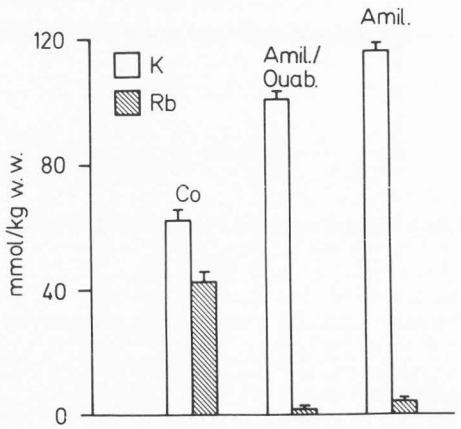

Figure 1: K and Rb concentrations in principal cells of the frog skin epithelium after 1 hour of basal incubation with rubidium Ringer's solution. Co= control conditions; Amil./Ouab.= simultaneous application of amiloride (10^{-4} M, apical side) and ouabain (10^{-4} M, serosal side); Amil.= application of amiloride alone. Mean values ± 2 standard errors of the mean (SEM) of 3 experiments.

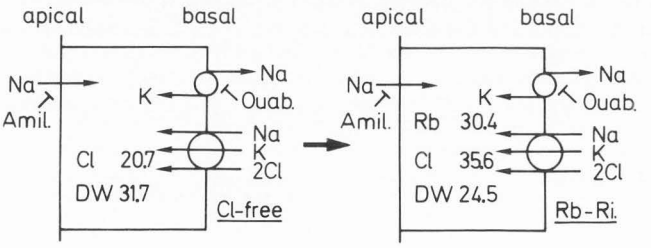

Figure 2: Rb and Cl uptake in principal cells of chloride-depleted frog skins. Skins were chloride-depleted by incubating the basal side with a chloride-free gluconate Ringer's solution for 1 hr (Cl-free). Rubidium uptake was measured during chloride re-uptake from a chloride Ringer's solution in which potassium was replaced by equimolar amounts of rubidium (Rb-Ri.). Mean values of 3 experiments.

Figure 3 depicts chloride and bromide concentrations of individual mitochondria-rich cells and mean values observed for principal cells in control and voltage-activated skins. Compared to the short-circuited control, in a subpopulation of mitochondria-rich cells a large bromide increase is observed after voltage activation. However, under control conditions and after voltage activation there are many cells that do not exchange chloride with bromide to any significant extent.

Bromide is not detectable in principal cells under control conditions and only small amounts of bromide are detectable following voltage activation. Thus, the principal cell is only minimally engaged in voltage-activated chloride movement. In fact, the small bromide increase after voltage clamping could also be explained by bromide uptake across basolateral membranes since the large influx of bromide can be expected to lead to an significant increase in bromide concentration in the spaces between the cells.

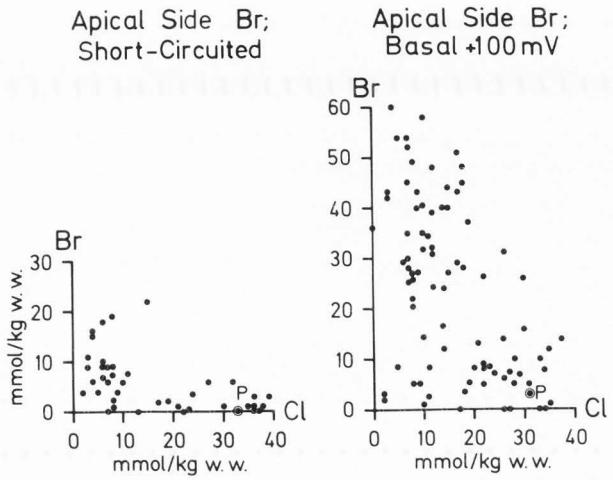

Figure 3: Br and Cl concentrations in mitochondria-rich cells of the toad skin epithelium under short-circuited conditions and after voltage clamping to 100 mV (basal side positive). P denotes mean values for principal cells. The skins were incubated with bromide Ringer's solution on the apical side for 15-30 min.(short-circuited) or 5-30 min. (voltage clamped). Combined data from 7 individual experiments.

Substituting bromide for chloride in the inner bath resulted in a Cl/Br exchange similar to that seen in Figure 3 for mitochondria-rich cells under control conditions (data not shown). The finding that mitochondria-rich cells exchanged their chloride with bromide across both apical and basal membranes supports the notion that this cell type serves as a chloride shunt pathway (Larsen, 1988). The large variability in the observed Cl/Br exchange rates and the relatively small number of cells, however, might raise some doubts about the quantitative importance of mitochondria-rich cells in transepithelial chloride movement.

Similar studies were performed in the frog skin (Rana esculenta) which, in contrast to the toad, usually exhibits only a relatively low chloride conductance that cannot be voltage activated. However, in many cases application of 25 mM procaine to the apical side induced a large chloride conductance which could be further stimulated by positive clamp potentials. Substituting bromide for chloride in the apical bathing solution led to a modest Cl/Br exchange in mitochondria-rich cells which was only slightly increased after procaine. Voltage activation did not substantially change the rate of Cl/Br exchange, neither before nor after addition of procaine.

The voltage-activated bromide flux in both toad skin and procaine-treated frog skin is so large that, if it passed through mitochondria-rich cells, the intracellular chloride should be completely exchanged within a few seconds. However, the expected, almost complete Cl/Br exchange was observed in only a small fraction of mitochondria-rich cells. Whether this subpopulation of cells can fully account for the large bromide flux observed during voltage activation or whether other transport routes are present will require further analysis.

Role of mitochondria-rich cells in transepithelial sodium transport

Cellular volume measurements performed on toad skins (Larsen et al. 1987) and electrophysiological studies combined with flux measurements performed on frog skins (Harvey and Ehrenfeld, 1988) led to the conclusion that mitochondria-rich cells are substantially involved in amiloride-sensitive transepithelial sodium transport. From the initial rate of cellular volume decrease after amiloride, the sodium transport activity of mitochondria-rich cells was calculated to be about 3 times higher than that of principal cells. This finding is in apparent contrast with our previous observation that the Na influx in mitochondria-rich cells cannot be blocked by amiloride (Rick et al., 1978).

To re-evaluate the role of mitochondria-rich cells in transepithelial sodium transport, we determined the effect of amiloride on intracellular electrolyte concentrations in principal and mitochondria-rich cells in skins of Rana esculenta. For estimation of the Na/K-pump activity, potassium in the inner bathing medium was replaced with rubidium and the uptake of rubidium was measured after 20-30 minutes.

Figure 4 shows the sodium, chloride, and rubidium concentrations of principal and mitochondria-rich cells under control conditions and after amiloride. In principal cells, amiloride significantly lowered sodium concentration and rubidium uptake, but had no effect on chloride concentration. Both the decrease in sodium and the reduced rubidium uptake after amiloride agree with the view that principal cells are involved in amiloride-sensitive sodium transport (Rick et al., 1978). Moreover, the fact that the rubidium uptake in the presence of amiloride was only a fraction of that observed under control conditions supports the notion that most of the

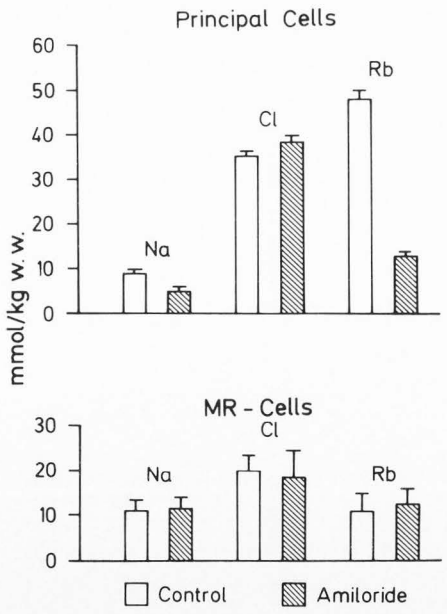

Figure 4: Na, Cl, and Rb concentrations in principal and mitochondria-rich cells of frog skin epithelium under control conditions and after apical application of amiloride (1 hr, 10^{-4} M). Mean values ±2SEM of 3 experiments.

sodium transported by the pump enters the cells across the apical sodium channel and that the back-flux of sodium across the basolateral membrane is relatively small.

In contrast to principal cells, no significant sodium or rubidium changes were detectable in mitochondria-rich cells. Also, amiloride had no effect on the chloride concentration, a finding which is in conflict with the view that the mitochondria-rich cell in the toad loses all its chloride after amiloride (Larsen et al. 1987). The minimal effect, if any, on sodium concentration and rubidium uptake, together with the small number of these cells, strongly argues against a significant contribution of of mitochondria-rich cells to amiloride-sensitive sodium transport. Whether or not in other epithelia than the frog skin epithelium this cell type may play a role in transepithelial sodium transport is yet to be established.

Localization of the sodium transport compartment in rabbit urinary bladder

The rabbit urinary bladder possesses transport features for active sodium absorption similar to those of other tight epithelia (Lewis and Hanrahan, 1985). Cellular potential and membrane capacitance measurements led to the conclusion that only the luminal cell layer is involved in transepithelial sodium transport (Lewis et al., 1976; Clausen et al., 1979). This view, however, conflicts with our previous finding that multi-layered epithelia, such as the frog skin, behave like a functional syncytium in regard to transepithelial sodium transport (Rick et al., 1978).

To investigate the question whether the different epithelial layers of the rabbit urinary bladder form a syncytial transport compartment, rubidium uptake was followed in bladders in which the apical membrane was permeabilized for anions and cations by nystatin (Lewis et al., 1977). To minimize changes in the cellular electrolyte composition, nystatin was applied in a solution which had an electrolyte composition similar to that of the epithelial cells. Insertion of nystatin channels into the apical membrane was indicated by a rapid increase of the transepithelial potential from about 15 to 50 mV. Thereafter, 50 mM potassium was replaced by equimolar amounts of rubidium and the apical uptake of rubidium was determined after 6 minutes.

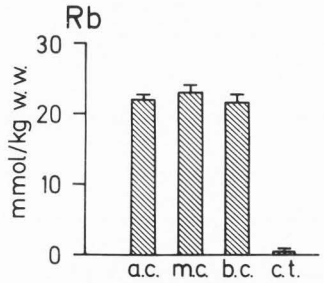

Figure 5: Uptake of Rb into the different epithelial layers and underlying connective tissue in the rabbit urinary bladder. The apical side was incubated for 6 min. with a 50 mM rubidium and 30 mg/ml nystatin containing solution. a.c.= apical cell layer; m.c.= middle cell layer; b.c.= basal cell layer; c.t.= connective tissue. Mean values ±2SEM of 2 experiments. With permission from Dörge et al. (1988b).

Figure 5 depicts the rubidium concentrations in the different cell layers of the urinary bladder and in the connective tissue. While in all 3 cell layers the rubidium concentration reaches values almost as high as in the apical bath, virtually no rubidium is detectable in the underlying connective tissue. The low rubidium concentration in the connective tissue argues against a substantial uptake of rubidium across the basolateral membranes. More likely, the cellular rubidium originates directly from the apical bath. Since the deeper epithelial layers have no access to the apical bath, the rubidium uptake can only be explained if the cell layers are connected by junctions permeable to small ions. Thus, the epithelium of rabbit urinary bladder can also be regarded as a syncytial sodium transport compartment, similar to that of the frog skin.

Acknowledgments

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Discussion with Reviewers

B. L. Gupta: For the benefit of the non-specialist, why is rubidium uptake from the basal side inhibited when only the apical Na entry is blocked with amiloride (Fig.1)? Presumably this leads to the depletion of intracellular Na and hence to the inhibition of the basolateral Na/K-pump.
G. A. J. Kuijpers: What is your explanation for the fact that Rb is excluded from the cells and the K content of the cells is increased upon application of amiloride alone? Do you take this to indicate that the Na/K-pump extrudes Na and accumulates K, mechanisms which are inhibited upon blockage of Na entry by amiloride?
Authors: Yes. A large part of the pump activity depends on the apical Na influx via the amiloride-sensitive Na channel. The reduced pump activity is reflected by the reduced Rb uptake across the basolateral membrane.

G. A. J. Kuijpers: How do you exactly conclude from your data that most of the Rb influx is via the Na/K-pump? What is the effect of ouabain alone on the intracellular cation concentrations? Even though your conclusion is in accordance with your results, without these data the alternative explanation that rubidium moves through the cotransporter or some other means is still possible.
Authors: We (Dörge et al., 1985) and others (Ussing, 1982) have shown that the cotransporter in the basolateral membrane of principal cells is normally quiescent. Ouabain alone leads to a large increase in the Na concentration and an equivalent decrease in the K concentration (Rick et al., 1978), consistent with the view that the K uptake is largely mediated by the pump. Moreover, the rate of Rb uptake under control conditions agrees well with the rate of K uptake calculated from the observed rate of transepithelial Na transport, assuming a stoichiometry of the pump of 3Na:2K.

G. A. J. Kuijpers: Which result do you refer to when you conclude that the Cl permeability of the cell membrane is very low?
Authors: As alluded to above, the cotransporter is normally quiescent. Therefore, in the presence of a significant Cl conductance, intracellular Cl should reach electrochemical equilibrium, i.e. values of 2-3 mmol/kg w.w.

G. A. J. Kuijpers: How did you measure wet weight contents? What units are the numbers for DW (dry weight) expressed in?
Authors: Concentrations are evaluated by comparison with an internal albumin standard and expressed as mmol/kg wet weight (w.w.). The dry weight content is expressed as g dry matter/ 100g wet weight.

G. A. J. Kuijpers: Why is the increase in Rb twice as much as the increase in Cl when expressed in mmol/kg w.w. (Fig.2), and only 1.59 times as much when expressed in mmol/kg d.w. (127.6 vs. 80.3)?
Authors: The different ratios are due to the different dry weights under the two conditions. After Cl-depletion, the principal cells are shrunken and, therefore, the dry weight content is increased.

G. A. J. Kuijpers: Could it be that other transport mechanisms or ion channels apart from the Na-K-2Cl cotransporter contribute to the uptake of Cl and Rb?
Authors: The re-uptake of Cl into Cl-depleted cells can be abolished by bumetanide, a highly specific blocker of the cotransport system, making it rather unlikely that other transport mechanisms are involved (Dörge et al., 1985).

B. L. Gupta: Am I not right in thinking that the primary function of mitochondria-rich cells is now believed to be proton transport?
Authors: Yes.

G. A. J. Kuijpers: Would you conclude that at least part of the Br flux in toad and frog skin is paracellular?
Authors: We cannot exclude the possibility that the subpopulation of mitochondria-rich cells which showed a significant Br increase after voltage-activation is the only transepithelial anion pathway. However, given the small number of cells, it appears likely that other pathways are involved as well.

G. A. J. Kuijpers: Do you really stimulate the epithelial Cl *permeability* or merely the electrodiffusional *flux* of Cl by applying an inside positive electric clamp potential?
Authors: It is well established that voltage-activation not only provides a larger driving force for Cl influx, but also stimulates Cl permeability (Larsen, 1988).

G. A. J. Kuijpers: How is electroneutrality maintained inside the cell when amiloride is applied and the cell looses a large part of its Rb?
Authors: The lower Rb concentration is balanced by a higher K concentration.

B. L. Gupta: Have you measured the rubidium and/or bromide concentrations in the subepithelial connective tissues in amphibian epithelia as you have done in mammalian bladder?
Authors: No bromide was detectable in the subepithelial connective tissue when the tracer was added to the apical bath. After addition of rubidium to the inner bath, the rubidium concentration in the connective tissue was about 3 mM.

G. M. Roomans: In general, it appears to be impossible to exchange all intracellular K for Rb. Would you care to speculate on a reason for this?
Authors: Apparently, this is not true for the frog skin epithelium. After 2 hours, more than 90% of all K is exchanged (own unpublished observation).

G. M. Roomans: What x-ray lines did you find most convenient for the analysis of Rb and Br?
Authors: We used the L-lines which, under the conditions applied in these studies, provided larger x-ray counts than the corresponding K-lines. The K-lines are preferable in cases

where the overlap with the K-lines of Mg and Si cannot be resolved.

G. A. J. Kuijpers: Could it be that nystatin traverses the whole bladder and makes the cells in all layers permeable to anions and cations, thereby effecting the accumulation of Rb in all cells?
Authors: Using electrophysiological techniques, Lewis et al. (1976) showed that nystatin only acts on the apical membrane when administered from the luminal side. Even if nystatin increased the permeability of the basolateral membranes, this should not have resulted in any Rb uptake since the tracer was present only in the apical bath.

G. M. Roomans: Could you provide more details of the methods applied in your studies?
Authors: The tissues were shock-frozen by plunging into liquified propane at -180 °C. From the frozen material sections of about 1000 nm thickness were cut dry with a steel knife at -80 °C in a cryoultramicrotome (Reichert, Vienna, Austria). The sections were mounted on thin collodion films and dried overnight at -80 °C and 10^{-5} mbar.

Microanalysis was performed in a scanning electron microscope (Stereoscan S150, Cambridge Instruments, Cambridge, U.K.) which was equipped with a solid-state x-ray detecting system (Link, High Wycombe, U.K.) and a special transmission stage for x-ray microanalysis. Typical measuring conditions were 20 kV acceleration voltage, 0.6 nA probe current, and 100 s analysis time. The emitted x-rays were recorded in the energy range from 0 to 10 keV, encompassing the Kα lines of Na, Mg, P, S, Cl, K, Ca, as well as the L-lines of Br and Rb.

Quantification of the cellular element concentrations (mmol/kg wet weight) was achieved by a comparison of the cellular x-ray spectra with those obtained in a peripheral standard (albumin standard). Additional informations on the quantification procedures have been given in two previous publications (Dörge et al., 1978; Rick et al., 1982).

E. H. Larsen: The hypothesis of the role of mitochondria-rich cells in transporting small ions, like Cl and Na, is based on experimental facts appearing over the past ten years. I have collected and discussed these facts, and it has been shown how previously puzzling observations are now neatly explained by our hypothesis (Larsen, 1988). I read your present paper as a challenge which, if successful, will bring everything back into a fog.

I am pleased that I get this opportunity to comment on your new data which I find important. However, rather than taking your results as evidence against our hypothesis and against our updated frog skin model, I think that your data provide useful hints for elaborating on the view on the physiological role of mr cells of high resistance epithelia.

First, mitochondria-rich cells in distal renal epithelia, i.e. urinary bladder and collecting duct of vertebrate kidney, have been shown to serve elimination of acid and base loads of the organism (reviewed by Steinmetz 1986). In these two high resistance epithelia the mr cells make up at least two subpopulations, the one being specialized for eliminating protons (α-type mr cells), and the other serving elimination of bicarbonate ions (β-type mr cells). The relative contribution of these two populations of mr cells depend on the prehistory of the organism. By acid loading the animal, α-type cells proliferate. Likewise, the density of β-type cells increases when the animal is base loaded.

Next, we should not overlook that frogs imposed with a metabolic acidosis increase the rate of cutaneous acid secretion, and that induced alkalosis results in cutaneous alkalinization of the outside bathing solution (Vanatta & Frazier, 1981). Thus the skin, like distal renal epithelia, serves excretion of body loads of acid and base. The significance of mr cells for these cutaneous transport functions is strongly indicated by the fact that mr cell density increases in response to induced metabolic acidosis (Page & Frazier, 1987).

Taken together with our studies on Cl-transport by mr cells, it follows logically that we have to consider that mr cells of amphibian epidermis belong to one of at least three different subpopulations: (i) mr cells specialized for uptake of Cl, (ii) mr cells specialized for proton secretion, and (iii) mr cells specialized for HCO_3-secretion. Moreover, we may tentatively assume the relative numbers of these three types of mr cells in a given preparation is depending on the physiological state of the animal prior to isolation of the skin.

It is the first type of mr cells that I and my colleagues have been studying. With our animals often challenged by salt depletion prior to experimentation, our skin preparations have had the Cl-transporting type of mr cells as the dominating type. This conclusion seems justified by the fact that we have proportionality between fully activated Cl-current and density of mr cells (Willumsen & Larsen, 1986). Notably, the Cl-current carried by a single mr cell thus estimated corresponded reasonably well with that estimated with a vibrating electrode probing the current density profile above the apical cell surfaces (Foskett and Ussing,1986).

Your conclusion, that the majority of mr cells of frog skin did not carry transepithelial Cl-fluxes, might well be entirely correct. I would expect then that the acid balance of your animals was unintentionally disturbed. Can you confirm this, e.g. do you see acidification of the mucosal bath? Furthermore, if I am correct, it is to be expected that the mr cell density was not correlated with the magnitude of activated Cl-currents. Am I right?

In line with this suggestion, it may not be surprising that addition of amiloride to the external bath did not decrease intracellular Na concentration, and that amiloride did not reduce Rb uptake from the serosal bath. The acid/base eliminating type of mr cells of collecting duct have been studied by electrophysiological methods (Ishibashi, Sasaki, and Takeuchi, 1986; Koeppen, 1986;1987), by quantitative light microscopy (Strange,1989), and by your own group by electron microprobe analysis (Beck et al., 1989). The results of all of these studies agree in concluding that this type of mr cells does not contain amiloride blockable channels in the apical membrane.

Results of your present study may indicate that the same applies to this particular type of mr cells in amphibian epidermis, as well. However, we need measurements of the acid secreting capacity and its cellular localization in the epidermis before this point can be finally settled. This is the way to go.

For elucidating, by x-ray analysis, the Na transporting capacity of the Cl-transporting type of mr cells, it is necessary to design a protocol that results in proliferation of this particular type of mr cell with negligible contributions from other subpopulations. It now becomes an important part of such a study to prove that this favorable condition was achieved.

Finally, I would like to correct a misunderstanding. We have never suggested that the mr cells of amphibian skin are responsible for the major active uptake of sodium. According to our estimate the specific apical Na+conductance of the mr cells is significantly larger than that of the principal cells. However, due to the small number of these cells, they contribute with less than 5% to the active Na current generated by the whole skin (Larsen et al., 1987).
Authors: We certainly do not want to bring everything back into fog that has been learned about the physiology of mitochondria-rich cells! The fact that intracellular Cl can be replaced by Br in the outer as well as in the inner bath does, indeed, support the notion that this cell type is part of a trans-

epithelial Cl shunt pathway. However, we strongly believe that other routes of transepithelial Cl movements cannot be ruled out.

We share your view that the mitochondria-rich cell could be a heterogeneous population of cells. Our results in toad skins showed that the voltage-activated Br uptake was prominent in only a subpopulation of cells and not in all cells. Given that the mitochondria-rich cell is a heterogeneous group of cells, it becomes crucial for all further research that the state of the animals is clearly defined. So far, we have tried to duplicate as close as possible the protocols that were published by you and other investigators in this field.

There has never been a misunderstanding on our part regarding your views on the role of mitochondria-rich cells in transepithelial Na transport. Indeed, because of the small number of cells, even a very high transport activity of these cells would result in only a minor contribution to transepithelial, amiloride-sensitive Na transport. The low rate of Rb uptake and lack of an effect of amiloride on intracellular Na, Rb, and Cl would argue that the majority of mitochondria-rich cells is not engaged in transepithelial Na transport.

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