J. Membrane Biol. 91, 251–258 (1986)

The Journal of
Membrane Biology

Localization of Chloride Conductance to Mitochondria-Rich Cells in Frog Skin Epithelium

J.K. Foskett* and H.H. Ussing**

Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

Summary: Cell volume determinations and electrophysiological measurements have been made in an attempt to determine if mitochondria-rich (MR) cells are localized pathways for conductive movements of Cl across frog skin epithelium. Determinations of cell volume with video microscope techniques during transepithelial passage of current showed that most MR cells swell when the tissue is voltage clamped to serosa-positive voltages. Voltage-induced cell swelling was eliminated when Cl was removed from the mucosal bath solution. Using a modified vibrating probe technique, it was possible to electrically localize a conductance specifically to some MR cells in some tissues. These data are evidence supporting the idea that MR cells are pathways for conductive movements of Cl through frog skin epithelium.

Key Words chloride transport · quantitative light microscopy · cell volume · vibrating probe · extracellular current

Introduction

Studies of the isolated frog skin epithelium have provided much of our understanding of the mechanisms of electrogenic Na absorption by epithelia. However, the mechanisms underlying the absorption of Cl by this tissue have not been well understood. Cl transport in tissues bathed on both sides in Ringer's solution is conductive and predominantly passive (reviewed by Ussing (1982) and Kirschner (1983)). Thus, in open-circuit conditions the spontaneous serosa (+) voltage generated by Na transport electrically drives the absorption of Cl.

Recent electrophysiological (Nagel, 1977; Helman, Nagel & Fisher, 1979; Hudson, 1980) and tracer-flux (Stoddard & Helman, 1982) measurements suggest that there is no Cl conductance in the apical membrane of the Na-transporting stratum granulosum cells. The Cl shunt could be paracellular. Alternately, because the frog skin is a heterogeneous epithelium, the Cl conductance could be localized to the mitochondria-rich cells or glands. The density of mitochondria-rich cells correlates with the magnitude of chloride conductance (Voute & Meier, 1978; Katz & Larsen, 1984; Willumsen & Larsen, 1985), suggesting that this cell type represents the shunt. To test this hypothesis, we have employed cell volume determinations and electrophysiological meaurements to establish whether these cells are conductive. The results suggest that mitochondria-rich cells in the frog skin are localized sites of Cl conductance.

Materials and Methods

Animals and Dissection

Frogs, *Rana pipiens*, were obtained from Nasco (Riverside, WI) and maintained at room temperature in tap water which was renewed daily. Abdominal skin was excised following decapitation and pithing and was incubated in a 0.1% solution of collagenase (Worthington Biochemicals, St. Louis, MO) in frog Ringer solution for 1 to 2 hr. The epithelium was then separated from the underlying connective tissue and musculature by gentle dissection.

Experimental Solutions and Chamber

The Ringer's solution contained the following (in mM): Na, 110.5; Cl, 111.8; K, 2.0; Ca, 0.9; HCO_3, 2.5. The pH of the

* *Present address:* Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

** *Permanent Address:* Institute of Biological Chemistry A, University of Copenhagen, 13 Universitetsparken, 2100 Copenhagen Ø, Denmark.

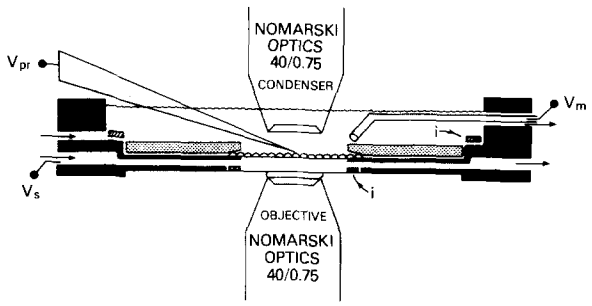


Fig. 1. Experimental set-up for modified vibrating probe (V_{pr}) measurements of extracellular current density immediately above the surface of the frog skin epithelium, and for visualization of the tissue for video-microscopic determinations of mitochondria-rich cell volume. The chamber was placed on the stage of an inverted microscope. Transepithelial voltage was measured with a pair of agar bridges (V_m, V_s); current was passed by Ag-AgCl wires (*i*). The baths were continuously perfused (arrows). For the vibrating probe experiments, the tissue was mounted with apical side in the upper bath and a reference electrode for the probe was present in the mucosal bath (not shown). Nomarski optics were not employed, and the condenser lens was replaced with a more long-working distance lamp. For cell volume determinations, the apical side of the tissue faced the bottom bath

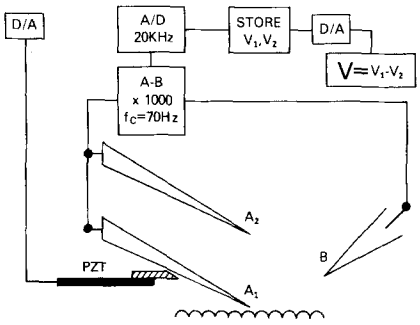


Fig. 2. Experimental set-up for modified vibrating probe measurements of extracellular current density. The electrode is shown at two positions in the bath: position A_1 is close to the epithelial surface; position A_2 is further up in the bath at the other end of the vibration excursion. At each position, the voltage at the tip with respect to a reference electrode (*B*) in the bath is recorded differentially, amplified, filtered, and sampled 1024 times by computer A/D. The average is stored in computer memory: v_1 and v_2 are the averaged voltages at positions A_1 and A_2, respectively. Computer command (*D/A*) to a pulse generator supplies a voltage to a piezoelectric bimorph (*PZT*) which causes the tip of the electrode to be moved to the other end of its vibration excursion. The difference voltages for 10 cycles were averaged to provide one measurement (V)

solution was 8.2 when gassed with air. 1 mM chloride Ringer's was made by replacement of chloride by gluconate. In these experiments, Ca was increased to 10 mM to compensate for its buffering by gluconate (Christofersen & Skibsted, 1975). Na replacement was by N-methyl-D-glucamine.

The isolated frog skin epithelia were mounted in a modified Ussing chamber, which allowed continuous perfusion of both sides of the tissues with Ringer's solution. The chamber was a composite of those previously described by Spring and Hope (1978) and Foskett and Machen (1985), and was designed to maximize visualization of the epithelium while providing the ability to voltage clamp the tissue and have access to it with microelectrodes (Fig. 1). Briefly, the tissue was stretched across a central aperture of a circular plate. A smaller annular disc was pressed into the aperture of the plate, thereby holding the tissue in position. The plate-tissue assembly was transferred to the perfusion chamber, whose aperture was covered by a nylon mesh to support the tissue. The tissue-plate assembly was secured with pins, and silicon grease was smeared between the plate and the chamber to act as a sealant. The tissue was mounted apical side up for conductance localization experiments and apical side down for measurements of cell volumes. Both baths were continuously perfused with fresh Ringer's solution by gravity-feed from overhead reservoirs. The bottom of the chamber was a coverslip; the space between the tissue and the coverslip defined the bottom compartment. Transepithelial hydrostatic pressure gradients were eliminated by manipulating the outflow resistance of the bottom bath.

Transepithelial voltage was measured by calomel electrodes connected to the tissue by agar bridges that ended near its two sides. Current was passed by chlorided silver wires which encircled the aperture on either side of the tissue. Transepithelial electrode penetrations during voltage clamp revealed that this geometry insured a uniform current density at the level of the epithelium. Average tissue resistance was 2220 $\Omega \cdot cm^2$ and transepithelial potential was 15 mV in Cl-replete media.

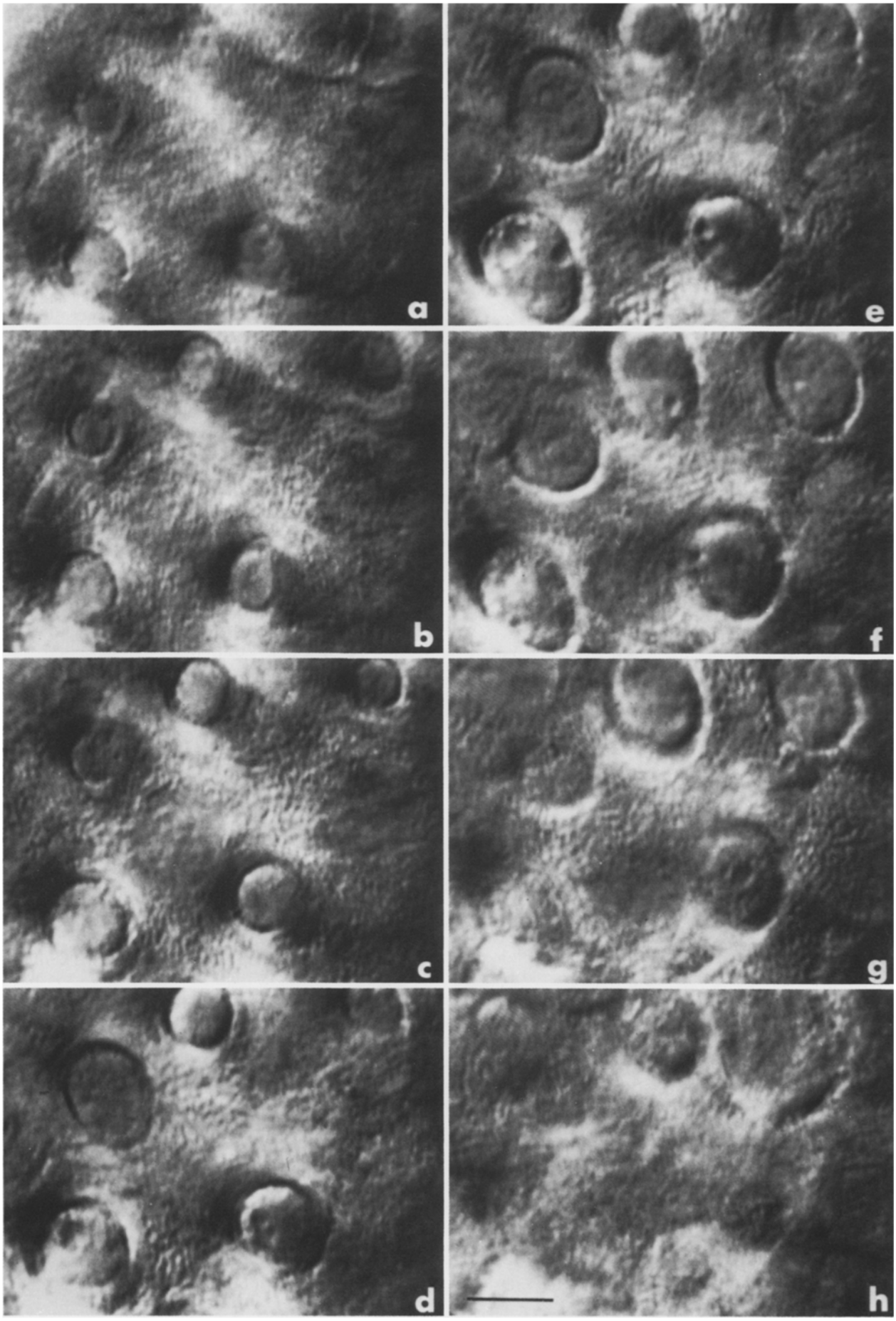
Microscopy

The perfusion chamber was mounted on the stage of an inverted microscope. For cell volume determinations, a Leitz Diavert equipped with differential interference optics was used. The standard condensor lens elements were replaced with a 40× (Zeiss; numerical aperture (n.a.) = 0.75; working distance = 1.6 mm) water immersion lens; the objective lens was also a similar 40× water immersion lens. For the conductance localization experiments, cells were visualized in brightfield, transmitted light with a fixed-stage Zeiss (Invertoscope IDO2) using a 40× (n.a. = 0.75) water immersion lens as the objective.

Cell Volume Determinations

Mitochondria-rich cells were visualized with a video system coupled to the microscope with a 25× eyepiece. Cell volume was determined by planimetry of stored video images of "optical sections" of the cells. The area and perimeter of each optical section were determined from tracings of the cell outline. Cell volume was computed from the areas and displacements of focus as previously described (Spring & Hope, 1978).

Fig. 3. A series of video-microscopic, differential interference contrast optical sections of frog skin epithelium perfused in the chamber described in Fig. 1 and voltage clamped to 0 mV. Mitochondria-rich cells are clearly defined against the backround epithelium and are round when viewed from above. Optical sections are displaced by 3 μm. Section *a* is at the surface. Bar is 10 μm


a
e
b
f
c
g
d
h

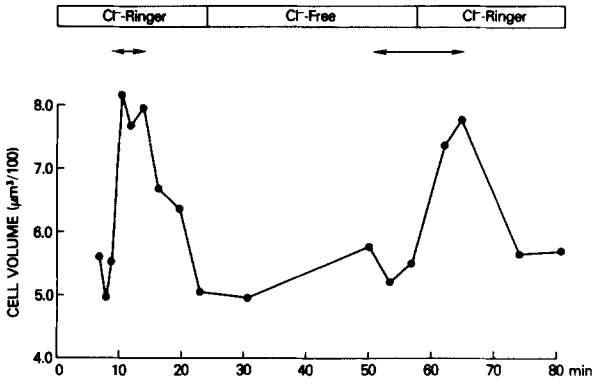


Fig. 4. Effects of voltage clamping to ~150 mV (serosa (+)) on volume of mitochondria-rich cell in the presence of normal Ringer (Cl-Ringer) or 1 mM Cl-Ringer (Cl-free) on both sides of the epithelium. Double-headed arrows denote periods when tissue was voltage clamped to serosa (+) voltage. Steady-state clamping voltage was 0 mV. *See* text for details of the experiment

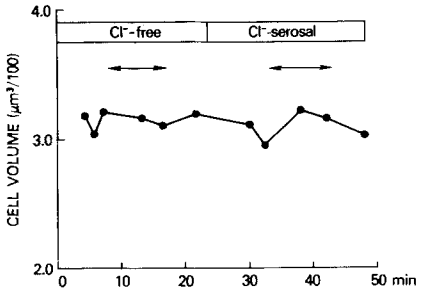


Fig. 5. Effects of voltage clamping to ~150 mV (serosa (+)) on volume of mitochondria-rich cell in the presence of 1 mM Cl on both sides (Cl-free) or with normal Cl-Ringer present on the serosal side. Details are as in Fig. 4

Conductance Localization

Conductance was localized to specific sites on the epithelium using a modified vibrating probe voltage scanning technique (Fig. 2). If localized sites of high conductance exist in the frog skin epithelium, then current flow through the tissue under voltage clamp conditions will be localized to these areas. As a result, it should be possible to measure peaks of extracellular current density immediately above these areas on the epithelium. This current density can be measured as the voltage drop at the tip of an electrode between two points in the medium. In the present experiments, these difference voltages were recorded at the tip of a vibrating KCl-filled glass microelectrode placed near the surface of the epithelium. The optics allowed unambiguous recognition of mitochondria-rich cells. Gland ducts were readily distinguished by their large size, depth, and by the specialized tripartite cell-type at the opening on the surface. The voltage of the vibrating electrode was recorded with respect to a low resistance electrode in the bath using a differential amplifier (WPI-750, New Haven, CT), and was amplified (×1000) and filtered (cut-off frequency = 70 Hz). This voltage was sampled 1024 times by computer, analog-to-digital (A/D) conversion (12 bit) at ~20 kHz, averaged, and the result stored in computer memory. The electrode was then moved vertically to a new position in the bath by displacing a glass rod (to which the electrode was attached) with a piezoelectric bimorph (PZT-5HN, Vernitron Piezoelectric Division, Bedford, OH) powered by a steady voltage from a pulse generator. The pulse generator was commanded by a trigger pulse from computer digital-to-analog converter (D/A), and provided a 10-msec ramp to the steady voltage level to minimize ringing in the bimorph (Corey & Hudspeth, 1980), allowing the electrode's position to stabilize more quickly (within 5 msec of completion of the ramp). Thus, total time required to move the tip of the electrode from one position vertically 20 μm to another was 15 msec. When the position of the electrode's tip was stable, the voltage was again sampled, averaged, and stored, as described above. The difference between the voltages recorded at the two positions is a measure of the extracellular current density and was computed and stored in computer memory. The tip of the electrode was moved back to the first position, under computer control, and the cycle repeated. Difference voltages could thus be recorded at ~8 Hz. To improve the signal/noise ratio, the difference voltages for 10 cycles were averaged to provide one measurement. The electrode was then positioned over other sites on the epithelium and the measurement was repeated, providing a current density profile immediately above the epithelial surface.

Results

Response of Cell Volume to Voltage Clamping

If the MR cell is a localized site of apical Cl conductance and has an appreciable K conductance in its basolateral membrane, as do other epithelial cells, then voltage clamping the tissue to serosa (+) voltages should drive Cl into the cell across the apical membrane and K into the cell across the basolateral membrane and the cell should swell. We tested this hypothesis by making rapid determinations of cell volume during imposition of voltage-clamp conditions.

Figure 3 shows a frog skin that has been optically sectioned while the tissue voltage was clamped to 0 mV. The mitochondria-rich cells were easily observed and were distinguished by their round shape when viewed from the apical surface. Mitochondria-rich cells are flask shaped; their necks extend towards the surface of the epithelium and appear to make contact with the external solution beneath and usually at the junction of two stratum corneum cells. The optical quality was sufficiently good to permit accurate planimetry of stored images. Figure 4 demonstrates the response of MR cell volume when the tissue was voltage clamped to a large (~150 mV) serosa (+) voltage while bathed on both sides by Cl-containing Ringer's. The epithelia were routinely voltage clamped to between 150

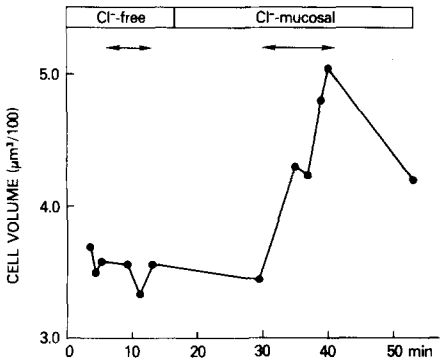


Fig. 6. Effects of voltage clamping to −150 mV (serosa (+)) on volume of mitochondria-rich cell in the presence of 1 mM Cl on both sides (Cl-free) or with normal Cl-Ringer on the mucosal side. Details are as in Fig. 4

and 200 mV to enhance salt flow to cause large enough volume changes to be easily determined. As seen in Fig. 4, voltage clamping the tissue caused a rapid reversible swelling of the cell, which averaged 51 ± 13%. When the tissue was short circuited the cell shrank back to control volume. In the presence of only 1 mM Cl no swelling was observed when the tissue was again clamped to serosa (+) voltage, but reintroduction of Cl during this second serosa (+) clamp period caused the cell to swell as before. When the tissue was again short circuited, the cell shrank back down again, demonstrating that swelling is both voltage and Cl dependent. Voltage-induced cell swelling was dependent upon Cl from the apical side only. In Fig. 5 the tissue was clamped to serosa (+) voltage in the presence of 1 mM Cl. The cell volume was not affected. The clamp was released and Cl was then introduced into the solution bathing the serosal side of the tissue. After a 10-min incubation the tissue was again clamped to serosa (+) voltage. In spite of the presence of Cl on the serosal side the MR cell did not swell. The results from a similar experiment are shown in Fig. 6, except that introduction of Cl was to the apical side only. In the presence of apical Cl, the cell swelled when the tissue was clamped to serosa (+) voltage. The Table summarizes the data and shows the number of MR cells, expressed as a percentage, which either swelled, shrank, or were unaffected as a result of clamping to serosa (+) voltage under four different Cl-conditions. When the tissue was bathed on both sides with Cl-Ringer, 64% of the cells swelled. When Cl was reduced to 1 mM on both sides or present at normal concentrations only in the solution bathing the serosal surface, however, only 10% of the cells swelled. In contrast, normal levels of Cl in the mucosal Ringer's supported cell swelling in 52% of the cells examined (average swelling 36+−6%). The data are consistent with the idea that MR cells represent localized sites of chloride conductance in frog skin epithelium.[1]

Table. Volume Responses of Mitochondria-Rich Cells to Voltage Clamping

Condition	% swell	% shrink	% unchanged[a]	*n*
Cl-Ringer's (mucosal + serosal)	64	24	12	25
1 mM Cl (mucosal + serosal)	10	12	78	41
Cl-serosal	10	5	85	21
Cl-mucosal	52	11	37	27

[a] <5% change of volume in response to voltage; *n* = number of cells.

Electrical Localization of Conductance Peaks

We attempted to electrically localize a conductance to the MR cells using a modified vibrating probe technique. To aid localization of a specific Cl conductance, the experiments were performed in the absence of mucosal Na or in the presence of 10 μM amiloride to reduce the apical Na conductance. The tissue was voltage clamped to +100 mV (serosa positive) to enhance the driving force for Cl movement. The surface of the epithelium was scanned with the electrode to localize the current flow.

In most skin epithelia it was not possible to localize a peak of conductance to mitochondria-rich cells. There were several reasons for this. In a few tissues the probe signal was within the noise level of the measurement, indicating that there was little measurable conductance anywhere on the epithelium. The probe signal obtained over most skins was above the noise levels, indicating that these skins did have an appreciable conductance, but it was still not usually possible to distinguish differences in the currents over the MR cells from those determined when the probe was moved horizontally away from the MR cells. This result, however, does not disprove that Mr cells are localized sites of conductance since the close proximity of MR cells (intercell distance < μm; Fig. 3) makes localization of conductance to a single cell difficult in this spe-

[1] We did not observe the MR cells to swell when Cl was reintroduced into either or both baths, or to shrink when Cl was removed. Although this appears to contrast with expectations for a Cl-permeable cell, it may be because these maneuvers were always under short-circuited conditions, which reduces the Cl permeability of the skin (Larsen & Rasmussen, 1982).

cies.[2] Finally, many of the gland ducts were found to be sites of extremely high conductance and contributed to the current measured over other areas of the epithelium.[3]

In two tissues from salt-adapted (tap water supplemented with 110 mM NaCl) frogs, cell density was reduced by approximately 50% and it was possible to localize peaks of current density over 13 of 23 MR cells measured. On one occasion, the skin molted while it was being perfused in the chamber, making it possible to position the electrode closer to the MR cells, and again peaks of current density were recorded from 2 of 7 MR cells examined. Figure 7 demonstrates localization of current flow to an MR cell in a skin from a salt-adapted frog.

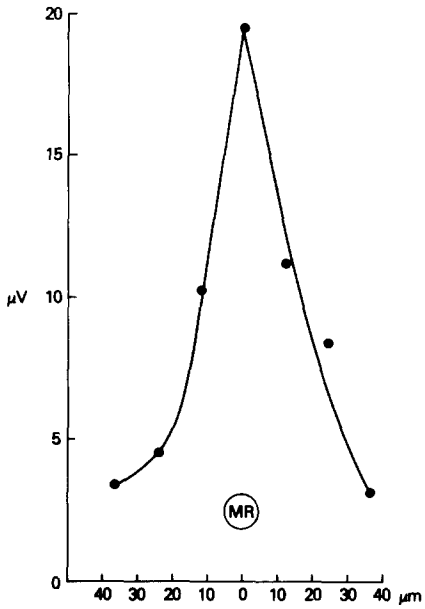


Fig. 7. Output from the modified vibrating probe, obtained as the tip of the electrode was moved from left to right at a constant height above the epithelial surface passing over a single mitochondria-rich cell voltage-clamped to 100 mV (serosa (+)). Average probe output for peaks measured over 15 MR cells was 20 ± 2 μV. Although not calibrated, assuming a solution resistivity of 1 μS/μm and a 20-μm excursion with height at 14 μm, this corresponds very roughly (since the electric field is not uniform over this distance) to 1 pA/μm^2 at the level of the center of probe excursion, or approximately 1.2 nA/cell at 100 mV. This compares favorably with estimates based on total transepithelial chloride conductance and MR cell density in toad skin (Willumsen & Larsen, 1985)

Discussion

In frog skin bathed on both sides with a Ringer's solution under open-circuit conditions, Cl permeability "shunts" the transepithelial potential generated by the Na absorptive process (Ussing & Zerahn, 1951). The present study addresses the question of the anatomical location(s) of this Cl "shunt" pathway. Recent investigations have led to the conclusion that chloride conductance in frog skin is transcellular (Kristensen, 1981). We have focused our investigations on the MR cells because a body of indirect evidence suggested that it is this cell type which is involved in Cl transport by frog skin. First, the granular cell apical membrane voltage is unaffected by changes in extracellular Cl concentration (Nagel, 1977; Helman et al., 1979; Hudson, 1980), ruling out the possibility that these cells are a pathway for conductive Cl movements. Animal-to-animal variability in the magnitude of the tissue Cl conductance is associated with a parallel variability in the number of MR cells (Voute & Meier, 1978; Willumsen & Larsen, 1985), and salt-adaptation, which reduces the Cl conductance of toad skin, causes a parallel reduction in the number of MR cells (Katz & Larsen, 1984). Further, with high K-Ringer's bathing both sides of the tissue, only the MR cells swell when Cl is introduced to the

[2] An estimate of the contribution to the electrode signal of a distant current source, expressed as a fraction of the signal the same source would produce if located immediately under the probe, is

$$[h^3\cos\theta/d^3 + h^2\sin\theta/d^2]/[1/\cos^3(\theta/2)] \quad (1)$$

where h is the height of the middle of the probe's excursion above the surface of the tissue, d is the distance on the epithelial surface from the probe to the current source, and θ is the angle from vertical of the line of vibration (Scheffey et al., 1983). In the present study the direction of electrode movement was close enough to vertical that the term can be ignored. Thus, the equation reduces to h^3/d^3. It is clear that it is necessary to minimize h and maximize d to achieve good spatial localization of current sources. The presence of a dead layer of surface cells limited the approach of the electrode tip to no closer than 2-4 μm (the thickness of the stratum corneum) to the MR cells. In addition, positioning the tip of the electrode immediately above the surface (obtained by touching the surface and then backing away until electrode noise ceased) provided spurious measurements, probably as a result of the presence of substances associated with the surface (i.e., mucus). For an electrode excursion of 20 μm $h \sim$ 14 μm Equation (1) shows that an electrode placed in the middle of a field of uniformly conductive cells to maximize its distance from any one of them ($d \sim 21$ μm) will record from the surrounding four cells ~120% of a single cell current density.

[3] The method for separating the epithelium from the underlying connective tissue results in the bases of the glands being broken off and left embedded in the connective tissue. Most gland ducts appeared to be electrically tight when measured with the probe. However, current flows could be detected over 200 μm from the opening at the apical surface of some ducts, indicating that the cells at the bases of the necks of these ducts did not form electrically tight seals.

apical side, suggesting that these cells alone have an apical Cl permeability (Voute & Meier, 1978).

We have employed both electrophysiological and optical techniques in our attempts to localize a Cl conductance to the MR cells. The vibrating probe technique has been used successfully to localize active Cl transport to mitochondria-rich cells (chloride cells) in another heterogeneous epithelium, the fish opercular membrane (Foskett & Scheffey, 1982; Scheffey, Foskett & Machen, 1983; Foskett & Machen, 1985). Although the modified version used in the present study did not have the same temporal resolution as the vibrating probe used in those previous studies, we felt that the smaller tip size of the electrode would aid in spatial resolution. However, the high density of MR cells encountered in the frog skins in the present work was sufficient to prevent localization in most instances. The effects of MR cells on the transport properties of the frog skin are generally ignored, although similar MR cell densities have been previously reported (Whitear, 1975; Ehrenfeld, Masoni & Garcia-Romeu, 1976).

The rationale for the optical determinations of cell volume was that if the MR cell has a K permeability on its basolateral membrane, as most other epithelial cells do, and a Cl permeability on its apical membrane, then the cell should swell when the transepithelial potential is serosa (+) due to an accumulation of KCl in the cell. Apical Cl-dependent swelling during transepithelial voltage clamping was observed in a majority of the MR cells, confirming this model.[4]

The responses of the MR cell volumes to voltage clamping revealed these cells to be a heterogeneous population since only 50-64% of the cells swelled. MR cells which were unaffected by current passage may be nonconductive. Although the data from the successful vibrating electrode experiments were from salt-adapted animals, they tend to support this conclusion since only 50% of the MR cells examined were conductive. These electrode experiments were performed in the absence of mucosal Na or in the presence of amiloride; thus, the peaks, if specific, were probably Cl currents. However, electrical localization of current flow, as shown here and in another recent study (Katz & Scheffey, 1984), must be interpreted with some reservation since the data only indicate that the conductance pathway is associated with the cell but do not allow localization of the pathway. For example, molting is associated with dissolution of the MR cell tight junction (Whitear, 1975) and involves shedding of some MR cells (Masoni & Garcia-Romeu, 1979). Thus, it was possible that conductive MR cells were those with dissolved tight junctions (i.e., conductance was paracellular) or "old" cells about to be shed from the epithelium. The volume experiments prove, however, that the conductance must be at least in part in the cellular pathway. Further studies will be required to resolve the bases for the apparent heterogeneity among MR cells in their responses to steady-state voltage clamping.

The results of the present study suggest that MR cells provide a shunt pathway which is required to preserve electrical neutrality during Na transport under open-circuit conditions. As such, this is the first definite proof that circular current flow through an epithelium can be through different cell types. The coupling of chloride fluxes to sodium fluxes might be assisted by the voltage gating of chloride channels (Larsen & Rasmussen, 1982) in the MR cells. An implication of this anatomical segregation of pathways is that salt transport by the tissue is the result of the coordinated activities of different cell types controlled in turn by distinct sets of regulatory mechanisms.

[4] Although not examined directly in this study, voltage clamping caused no obvious changes in the volumes of the stratum granulosum cells, which is consistent with the other data cited above which indicated that these cells do not possess an apical Cl conductance.

References

Christofersen, G.R.J., Skibsted, L.H. 1975. Calcium ion activity in physiological salt solutions: Influence of anions substituted for chloride. *Comp. Biochem. Physiol.* **52A**:317–322

Corey, D.P., Hudspeth, A.J. 1980. Mechanical stimulation and micromanipulation with piezoelectric bimorph elements. *J. Neurosci. Meth.* **3**:183–202

Ehrenfeld, J., Masoni, A., Garcia-Romeu, F. 1976. Mitochondria-rich cells of frog skin in transport mechanisms: Morphological and kinetic studies on transepithelial excretion of methylene blue. *Am. J. Physiol.* **231**:120–126

Foskett, J.K., Machen, T.E. 1985. Vibrating probe analysis of teleost opercular epithelium: Correlation between active transport and leak pathways of individual chloride cells. *J. Membrane Biol.* **85**:25–35

Foskett, J. K., Scheffey, C. 1982. The chloride cell: Definitive identification as the salt-secretory cell in teleosts. *Science* **215**:164–166

Helman, S.I., Nagel, W., Fisher, R.S. 1979. Ouabain and active transepithelial sodium transport in frog skin. *J. Gen. Physiol.* **74**:105–127

Hudson, R.L. 1980. Electrophysiology of Amphibian Skin Ph.D. thesis. Washington State University, Pullman

Katz, U., Larsen, E.H., 1984. Chloride transport in toad skin (*Bufo viridis*). The effect of salt adaptation. *J. Exp. Biol.* **109**:353–371

Katz, U., Scheffey, C. 1984. Voltage-dependent chloride conductance of toad skin: Localization by vibrating probe. *Biol. Bull.* **167**:528

Kirschner, L.B. 1983. Sodium chloride absorption across the body surface: Frog skins and other epithelia. *Am. J. Physiol.* **244:***R*429–*R*443

Kristensen, P. 1981. Is chloride transfer in frog skin localized to a special cell type? *Acta Physiol. Scand.* **113:**123–124

Larsen, E.H., Rasmussen, B.E. 1982. Chloride channels in toad skin. *Phil. Trans. R. Soc. B.* **299:**414–434

Masoni, A., Garcia-Romeu, F. 1979. Moulting in *Rana esculenta:* Development of mitochondria-rich cells, morphological changes of the epithelium and sodium transport. *Cell. Tiss. Res.* **197:**23–28

Nagel, W. 1977. The dependence of the electrical potentials across the membranes of the frog skin upon the concentration of sodium in the mucosal bath. *J. Physiol. (London)* **269:**777–796

Scheffey, C., Foskett, J.K., Machen, T.E. 1983. Localization of ionic pathways in the teleost opercular membrane by extracellular recording with a vibrating probe. *J. Membrane Biol.* **75:**193–203

Spring, K.R., Hope, A. 1978. Size and shape of the lateral intercellular spaces in a living epithelium. *Science* **200:**54–58

Stoddard, J.S., Helman, S.I. 1982. Chloride efflux from isolated epithelia of frog skin. *Fed. Proc.* **41:**1496

Ussing, H.H. 1982. Pathways for transport in epithelia. *In*: Functional Regulation at the Cellular and Molecular Levels. R.A. Corradino, editor. Elsevier North Holland, New York

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short circuited isolated frog skin. *Acta Physiol. Scand.* **23:**109–127

Voute, C.L., Meier, W. 1978. The mitochondria-rich cell of frog skin as hormone-sensitive "shunt-path." *J. Membrane Biol.* **40:**151–165

Whitear, M. 1975. Flask cells and epidermal dynamics in frog skin. *J. Zool. London* **175:**107–149

Willumsen, N.J., Larsen, E.H. 1985. Passive chloride currents in toad skin: Potential dependence and relation to mitochondria-rich cell density. *In*: Iono- and Osmoregulation. R. Gilles and M. Gilles-Baillien, editors. Springer-Verlag, Berlin-Heidelberg

Received 12 November 1985; revised 26 February 1986