J. Membrane Biol. 42, 345–356 (1978)

Studies on Chloride Permeability of the Skin of *Leptodactylus ocellatus:* III. Na^+ and Cl^- Effect on Electrical Phenomena

E. Rodríguez Boulan, M.V. Ques-von Petery, C.A. Rotunno*, and M. Cereijido

Department of Biophysics, Albert Einstein Center of Medical Research (CIMAE), Luis Viale 2831, Buenos Aires, Argentina and Centro de Investigación y de Estudios Avanzados del I.P.N., Apartado Postal 14-740, México 14, D.F., Mexico

Received 17 February 1978

Summary. During their flux through the skin of the frog *Leptodactylus ocellatus,* Na^+ and Cl^- interact with each other. This interaction gives rise to electrical phenomena which are studied in the present paper. The skin is mounted in Na_2SO_4 Ringer's with 115 mM Na^+ on the inside, and a variety of outer solutions. The osmolarity of all solutions is kept constant at 237.8 mosmol by adding sucrose. When the main anion used on the outside is $SO_4^=$ the electrical potential difference ($\Delta\psi$) rises steadily with the concentration of sodium $(Na^+)_o$ up to 87 mV, which is reached at about 20 mM. Thereafter $\Delta\psi$ remains constant. When the main anion is Cl^- it is observed that $\Delta\psi$ rises steadily with $(NaCl)_o$ with a slope similar to the curve obtained with $SO_4^=$ (37 mV per decade), but with a lower intercept attributed to an inward Cl pumping which is characteristic of this frog species. At 2–9 mM $(NaCl)_o$ a Cl-specific channel is activated. Further increases of $(NaCl)_o$ produce a decrease of $\Delta\psi$. The specificity of the activation of this site by monovalent cations and its use by monovalent anions is also studied.

Na^+ and Cl^- have a mutual influence on each other's penetration through the frog skin. The mechanism of this interaction was studied in two preceding papers (Ques-von Petery, Rotunno & Cereijido, 1978, and Rotunno, Ques-von Petery & Cereijido, 1978). The results of these two papers indicate that at low concentration of NaCl outside, chloride penetrates through two different mechanisms, a passive one that exhibits the characteristics of an exchange diffusion process and an active pump. Cl^- and Na^+ added to the outer solution produce a significant stimulation of the active step as well as the activation of a passive Cl channel. Most of the studies that led to this view were performed in short-circuited preparations, so we had no information on the influence that these mecha-

* *Mailing address and to whom reprint requests should be made:* Centro de Investigación del I.P.N., Apartado Postal 14-740, México 14, D.F., Mexico.

0022-2631/78/0042-0345 $02.40

nisms would have on the electrical potential difference. Since under physiological – *in vivo* – conditions the skin is not short circuited, the electrical potential difference constitutes an important driving force. Therefore, the aim of the present study is to obtain some information on the effect of changes in Na^+ and Cl^- concentrations on the electrical behavior of the skin. These changes are made in the outer bathing solution while using a sulphate Ringer's with 115 Na^+ on the inside. The reason for keeping the inner solution constant is twofold: (i) because Rabito, Rodriquez Boulan and Cereijido (1973) have shown that many effects on the electrical parameters that were observed by changing symmetrically the outer and the inner bathing solutions, and attributed to events on the outer side, were in fact phenomena due to changes on the inside; and (ii) because this resembles more closely the physiological conditions in which the epithelium is bathed with interstitial fluid on the inside and a solution with low salinity on the outside.

Materials and Methods

The studies were performed on the abdominal skin of the local frog *Leptodactylus ocellatus*. Animals of either sex were studied at 20–22 °C. The skins were mounted as a flat sheet between two Lucite chambers, 5 ml each. The area of skin exposed to the Ringer's solution was 3.14 cm^2. The Ringer's solution used on the inside contained (mM): 52.5 Na_2SO_4, 2.4 $KHCO_3$, 1.0 $CaSO_4$, 10 sodium pyruvate, and 53.5 sucrose. Several Ringer's solutions were used on the outside. They never contained Ca^{++} nor pyruvate. The osmolarity of all Ringer's was adjusted to 237.8 mosmol by adding sucrose. Two agar-3 M KCl bridges connected the outer and inner solutions to calomel half-cells. In experiments where only the measurement of the electrical potential difference ($\Delta\psi$) was intended, a Keithley model 200 B DC electrometer, or an Orion Research Voltmeter (input impedance, $10^{14}\Omega$) was used. In order to study $\Delta\psi$ as a function of the ionic composition of the outer bathing solution, the outer chamber was emptied and refilled three times with the new solution. Three minutes later the electrical potential difference was recorded. The solutions were continuously gassed and stirred by bubbling moistened air.

Studies by Dobson and Kidder (1968) and Helman and Miller (1973) have shown that the chambers commonly used in this preparation can damage the edge of the skin, thus creating an heterogeneous shunt pathway in parallel with the undamaged skin. This edge damage can seriously affect studies of the electrical behavior of the skin. As described in the first paper of this series (Ques-von Petery *et al.*, 1978) under the conditions chosen, edge damages do not seem to produce serious impairments of the observations.

Further details on experimental conditions are given in the previous two papers (Ques-von Petery *et al.*, 1978, and Rotunno *et al.*, 1978). The subscripts *o* and *i* refer to the outer and inner bathing solution, respectively. Results are expressed as mean ± SE (number observations).

Results

Figure 1 shows the electrical potential difference between the two sides of the skin ($\Delta\psi$) as a function of the Na^+ concentration of the outer bathing solution $(Na^+)_o$. The Ringer's inside was the sulphate solution described in *Materials and Methods*. In each curve the values of $\Delta\psi$ are recorded, first in a rising sequence of ion concentration, and then in a descending one. The value taken at a given concentration is the mean between the two recordings. When the main anion present on the outside is $SO_4^=$ (filled circles) the electrical potential rises gradually with the concentration of Na_2SO_4 up to 87 mV, reached at about 20 mM. Therefrom $\Delta\psi$ seems to maintain a steady value. The $\Delta\psi$ is considerably high even at 0.1 mM. The slope at low concentration is 3.5 mV per decade, i.e., much lower than the 58 mV predicated on the basis of the results of Koefoed-Johnsen and Ussing (1958), but close to that obtained by other authors in *Rana pipiens* (Cereijido & Curran, 1965; Smith, Martin & Huf, 1973).

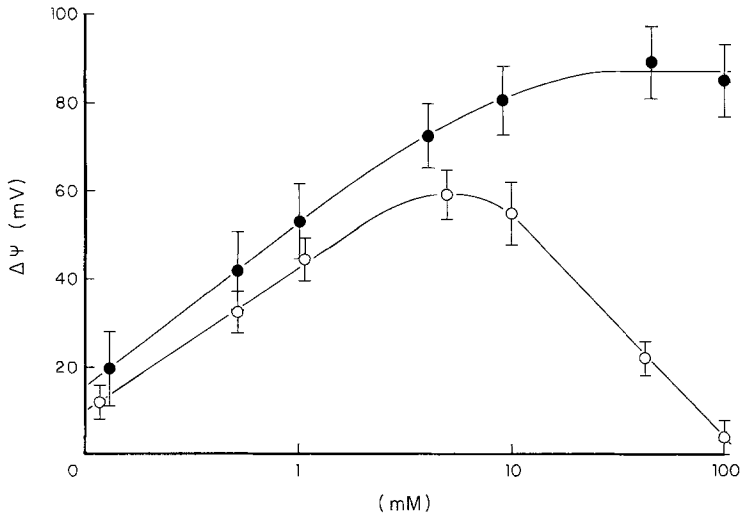

Fig. 1. Electrical potential difference ($\Delta\psi$) of the frog skin as a function of the ionic composition of the outer bathing solution. In all cases the inner solution is sulphate Ringer's with 115 mM Na^+. *Filled circles:* The outer solution is a sulphate Ringer's and the abscissa refers to its Na^+ concentration. Each point is the mean value of 14 measurements. *Open circles:* The outer solution is chloride Ringer's and the abscissa refers to its NaCl concentration. Each point is the mean value of 12 measurements. The composition of the external Ringer's is: Na_2SO_4 or NaCl at the concentration indicated, 2.4 mM, $KHCO_3$ and sucrose to keep the osmolarity constant at 237.8 mM

The departure from a slope of 58 mV could not be attributed to sulphate movement because the permeability to $SO_4^{=}$ of the frog skin is negligible: 2×10^{-9} equivalent $hr^{-1} cm^{-2}$ (Cereijido & Curran, 1965). The departure of the 58 slope and the saturation of the curve at high concentration of Na^+ can be understood on the basis of the ideas put forward by Smith *et al.*, 1973.

As in the present case, Smith *et al.* (1973) have obtained a slope of 35 mV in *Rana pipiens*, which departs from the 58 mV predicted by the model of Koefoed-Johnsen and Ussing (1958). To account for this discrepancy Smith and coworkers have assumed that the electrical potential across the "outer barrier" is a diffusion potential between the outer bathing solution and the "Na transporting compartment" that would be located on the cytoplasm of the "first reacting cell layer". The Na concentration in these cells depends on the activity of a pump and, therefore, the electrical potential developed by Na^+ becomes a function of both, its concentration in the outer solution, and of the activity of the pump. The curve described by filled cricles in Fig. 1 resembles the saturation curves of net Na^+ transport *vs.* Na^+ concentration found by Cereijido *et al.* (1964) and Moreno *et al.* (1973). Even when in the work of Moreno *et al.* it is demonstrated that, within the limits of the experimental error, there is no "Na transporting compartment", the information it affords, as well as that in the other papers quoted, suggest that the electrical potential difference developed by Na^+ added to the outer solution depends on the activity of a Na pump which is not shunted by sulphate.

At low concentration of NaCl, the curve described by open circles in Fig. 1 intercepts the ordinate at a point 6 mV lower than the sulphate curve. Three different factors may contribute to give this difference: (i) Chloride is permeable and will be driven by the $\Delta\psi$ generated by Na^+. (ii) Since the inner bathing solution has no Cl^-, this ion will diffuse from the outer to the inner solution, thus generating a negative $\Delta\psi$. (iii) This preparation has an inward active chloride transport and the negative $\Delta\psi$ that it produces lowers the total $\Delta\psi$ generated by Na^+ (Zadunaisky & Candia, 1962; Zadunaisky, Candia & Chiarandini, 1963; Zadunaisky & Fisch, 1964; Huf, 1972). Up to 5 mM Na^+ both curves rise with a very similar slope [35 (●) *vs.* 33 (○)]. The slope reflects the permselectivity of the outer barrier. Our results would thus indicate that up to 5 mM NaCl outside the permeability to chloride is close to that of sulphate, i.e., very low. This agrees with the observations made in the first paper of this series (Ques-von-Petery *et al.*, 1978) that

at low $(Cl)_o$, the translocation of Cl is operated by an exchange diffusion mechanism which does not contribute to the electrical conductance of the skin. This also suggests that the fact that the Cl curve is 6 mV lower than that of $SO_4^=$ may be due to the existence of the active transport of chloride.

But the most striking feature of the chloride curve of Fig. 1 is that, beyond 9 mM, the $\Delta\psi$ drops with a slope of 51 mV per decade. Inflection points like this were described in other species as early as 1933 by Steinbach, and found also by Greven (1944), Linderholm (1954) and Smith, Hughes & Huf (1971).

There are, in principle, two mechanisms that may account for this effect. The first mechanism is that a Cl-selective channel is opened between 2 and 10 mM $(NaCl)_o$ and a Cl^- diffusion potential sets in. As stated above, the inner bathing solution is a sulphate Ringer's and has no Cl^-. Therefore, as the concentration of Cl^- in the outside is raised, there is an increasingly greater chloride gradient between the outer and the inner solution that may create a diffusion potential. Also, Ques-von Petery *et al.* (1978) have found that at low NaCl on the outside G_T remains constant in spite of variations of J_{31}^{Cl}, but then there is a point where the concentration of NaCl outside stimulates the permeability to Cl^- and the total conductance becomes a direct function of the specific chloride conductance. The second mechanism is suggested by the observation of Fischbarg, Zadunaisky and Fisch (1967), working in *Leptodactylus ocellatus*, that the net pumping of Cl^- is not a monotonic function of the concentration of NaCl; it remains constant up to 60 mM, and thereafter rises linearly with the concentration. The concentration at which they found the inflection point (60 mM) is much higher than the one at which we find the inflection of the curve. Yet one has to take into account the fact that while we only change the outer bathing solution, Fischbarg *et al.* (1967) changed both solutions at the same time and, as mentioned above, changes in the inner solution produce variations of its own (Leb *et al.*, 1965; Rabito *et al.*, 1973). Therefore, the possibility exists that the inflection of the $\Delta\psi$ curve reflects the activation of a passive site with a concomitant increase of the rate of Cl pumping, as demonstrated in the previous paper by Rotunno *et al.*, (1978).

The two mechanisms have the common characteristics that the positive value of $\Delta\psi$ is due to Na^+, the tendency to decrease is due to Cl^-, and the activation of this effect of chloride requires Na^+. Yet there is clear evidence (Mandel & Curran, 1972) that an increase of the

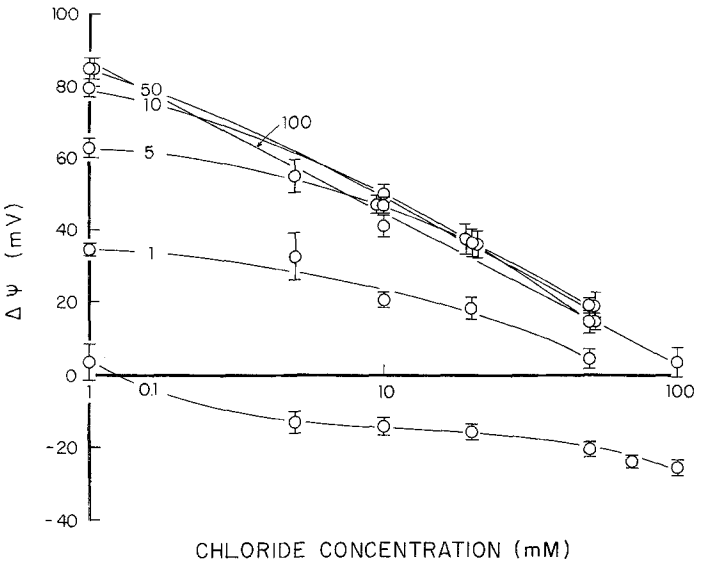

Fig. 2. Electrical potential difference ($\Delta\psi$) of the frog skin as a function of the chloride concentration of the outer bathing solution. In all cases the inner solution is a sulphate Ringer's with 115 mM Na^+. Each curve is obtained at the constant concentration of Na^+ specified on the curve. In all cases Cl^- was added as choline salt until 2 $(SO_4)_o + (Cl)_o =$ 100 mM. Higher increases of $(Cl)_o$ were obtained by substituting Na_2SO_4 with NaCl. The osmolarity was kept with sucrose. Each point represents the average of 20 determinations

ionic strength of the solution, or variations in $\Delta\psi$, may open an unspecific shunt pathway. This raises the question of whether the behavior of the $\Delta\psi$ shown in Fig. 1 is related to Na^+ and Cl^- movements and interactions, or whether the activation discussed above is just a nonspecific effect of changes in the ionic strength of the outer solution. Therefore, the experiments which follow were designed to obtain more information on the nature of the shunt and the specificity of the activation.

Figure 2 shows a series of experiments where the concentration of Cl^- is varied at constant concentration of Na^+ in the outer bathing solution. The point indicated on the ordinate corresponds to the condition in which only 1 mM Cl^- is present. As expected, the higher the concentration of $(Na)_o$, the larger the $\Delta\psi$. From 1 to 100 mM $(Na)_o$ the $\Delta\psi$ developed by this ion is decreased by the addition of $(Cl)_o$. Regardless of the mechanism involved, $(Cl)_o$ becomes more effective with increasing Na^+ concentration. Thus while in the curve of 1 mM $(Na)_o$ $\Delta\psi$ decreases with a greater slope only at $(Cl)_o$ greater than 5 mM, the curves of 10 to 100 mM $(Na)_o$ have from the beginning the same maximum slope.

The curves of 1 to 100 mM $(Na)_o$ would suggest that Na^+ opens some sort of Cl channel thus allowing this ion to shunt the $\Delta\psi$ developed by Na^+. This interpretation would not hold for the data obtained at 0.1 mM Na^+. As discussed above in relation to the slope of the Cl^- and $SO_4^=$ curves in Fig. 1, at less than 5 mM the permeability to Cl^- seems to be as low as that of $SO_4^=$. Yet in Fig. 2 (bottom curve) it may be seen that $\Delta\psi$ not only is almost zero, but that it becomes negative as the concentration of chloride is increased. This indicates that the shunt opened by Na^+ could not be the only explanation. Another possible source of the negative potential may be the following: given the low concentration of $(Na)_o$ in the bottom curve, the gradient is oriented toward the outside and may result in an outward diffusion of sodium. Thus the electrical potential arising from this gradient will be negative. However, the curve shows that, while this gradient is kept constant, $\Delta\psi$ is decreased by the addition of Cl^-. Therefore, neither the gradient of Na^+, nor the shunt of Cl^- by themselves would account for the observations in Fig. 2. A more likely interpretation comes from the observation of Fischbarg *et al.* (1967) that in the absence of Na^+ the skin exhibits a negative $\Delta\psi$ originated by an active pumping of Cl^-. While

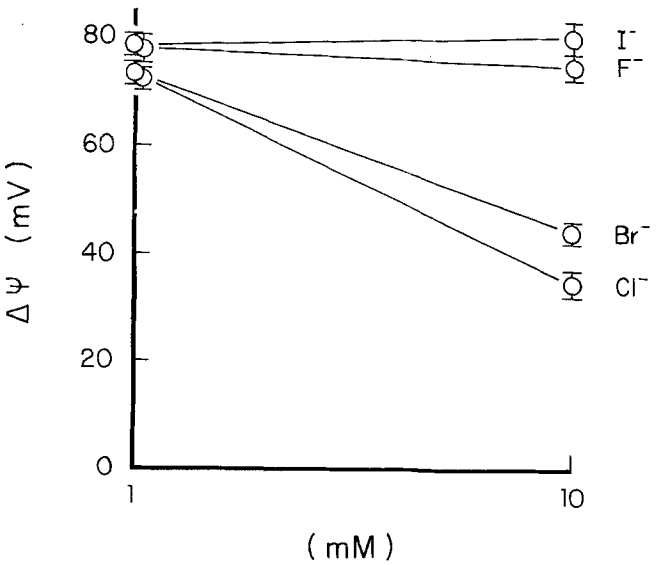

Fig. 3. Changes in $\Delta\psi$ elicited by a 10-fold increase (1 to 10 mM) of the concentration of the monovalent anions I^-, F^-, Br^- and Cl^-, in all cases the inner solution is a sulphate Ringer's with 115 mM Na^+. The concentration of Na^+ is kept constant at 100 mM by using Na_2SO_4. Osmolarity was adjusted with sucrose ($n=20$)

a Cl pump activated by high Na^+ and high Cl^- (because the slope is steeper at high Cl^-) would account for the observation in Fig. 2, a passive shunting by chloride could also be operating.

A series of experiments were designed to study the anion specificity of the activation mechanism (Fig. 3). The outside concentration of Na^+ was kept constant at 100 mM. This is a concentration which, in view of the results in Fig. 2, insures that the activation of Na^+ must be already present. As expected, under these circumstances a 10-fold increase in the concentration of chloride produces a drop of $\Delta\psi$. An equal change in the concentration of Br^- elicits a similar effect. Changes in F^- or I^- concentration fail to modify appreciably the value of $\Delta\psi$. This information does not suffice to establish an anion selectivity sequence, but does not violate those predicated by Eisenman's (1962) theory either. In fact, it is close to sequence *V*: $Cl^- > Br^- > F^- > I^-$. It involves strong

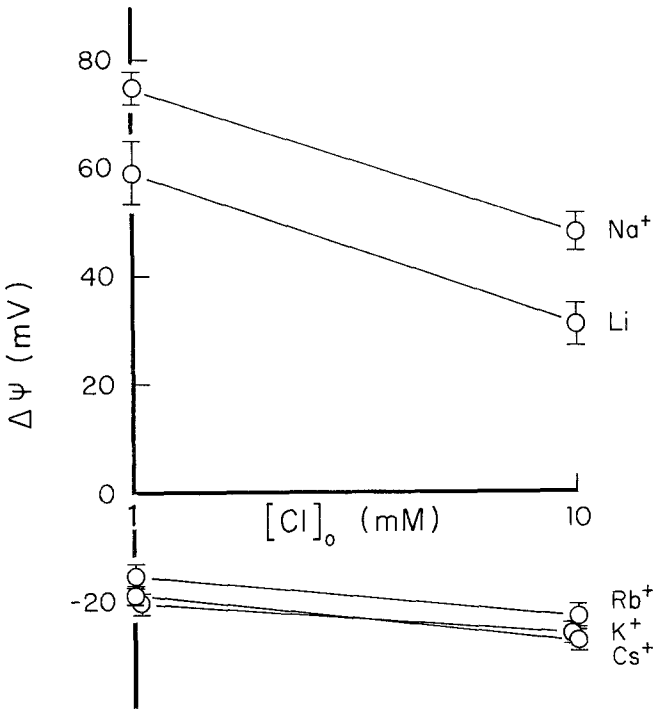

Fig. 4. Changes in $\Delta\psi$ elicited by a 10-fold increase (1 to 10 mM) of the concentration of chloride. Each curve was obtained in the presence of a different cation whose concentration was kept at 10 mM. In all cases the inner solution is a sulphate Ringer with 115 mM Na^+. At low concentration of chloride these cations were present as $SO_4^=$ salts. Osmolarity was adjusted with sucrose (n=20)

sites, but not too strong as to prefer first the smallest and most strongly hydrated anion: F^-. From the results in Fig. 3, one may conclude that the mechanism activated by Na^+, and used by Cl^-, is not a nonspecific leak, but exerts discrimination among anions.

Our next aim was to study the cation specificity of the activation elicited by Na^+. For this purpose a 10-fold increase in the concentration of Cl^- was made in the presence of 10 mM of different monovalent cations of the series 1 *A*. Figure 4 describes the experimental results. The skin has a positive value of $\Delta\psi$ only in the presence of Li^+ and Na^+. This was expected as Koefoed-Johnsen and Ussing (1958), Kidder, Cereijido and Curran (1964) and Lindley and Hoshiko (1964) have shown that the skin can maintain its $\Delta\psi$ in the presence of Li^+ as well as Na^+. With K^+, Rb^+, or Cs^+ though, $\Delta\psi$ becomes negative. This condition is analogous to the one discussed in connection with Fig. 2 (curve 0.1 mM Na^+). Here again the inward pumping of Cl^- could account for the negative $\Delta\psi$. Only in the presence of Li^+ and Na^+ can Cl^- produce its maximum effect on $\Delta\psi$. This is close to sequence *X* of selectivity and indicates that the sites involved in the activation of the Cl^- mechanism are strongly negative sites as they prefer the most strongly hydrated cations. It is the same sequence found by Lindley and Hoshiko (1964) for the $\Delta\psi$ of frog skin.

Discussion

The information collected indicates that the electrical potential difference maintained by the frog skin is not a monotonic function of the concentration of Na^+ and Cl^- in the outer bathing solution, but has a maximum between 2 and 9 mM.

On the basis of their studies of unidirectional fluxes of Na^+ and Cl^- as well as conductances, and considering that all cells have the same average concentration of chloride, Rotunno *et al.* (1978) suggested that Cl^- movement proceeds through three mechanisms. One is a Cl pump located at the outer barrier, because at this place Cl^- not only moves from the low (1 mM) concentration of the outer solution into the cells with 50 mM Cl^- (Zylber, Rotunno & Cereijido, 1973), but also, under short-circuit conditions, it must overcome an electrical potential of at least -18 mV (cell negative). The second one is a mechanism of simple diffusion. The level of $(Na^+)_o$ and $(Cl^-)_o$ can alter the rate of chloride pump and the permeability of the skin for chloride. The

third one has the characteristics of a mechanism of exchange diffusion and is not affected by Na^+. As discussed by Ques-von Petery *et al.* (1978), one of the ways in which Na^+ could affect Cl^- fluxes is through its effect on the intracellular electrical potential, which constitutes an energetic barrier to Cl^- movements.

Therefore, according to this view there are, in principle, two main possibilities to explain the behavior of the electrical potential described in the present paper. The first one is based on the activation of a Cl channel and the second on the stimulation of the Cl pump:

1) *Activation of a Cl Channel.* Several authors have shown the existence of shunt pathways that can be opened by a variety of procedures. In a tight epithelium like the frog skin these pathways can be opened by a hyperosmotic outer solution (Ussing & Windhager, 1964; Franz & Van Bruggen, 1967; Urakabe, Handler & Orloff, 1970; Di Bona & Civan, 1973; Wade, Revel & Di Scala, 1973). This opening reduces $\Delta\psi$ because it increases the influx of Cl^- and the outflux of Na^+ which short circuit the Na^+ current as if it were a leaky epithelium. Yet, hyperosmolarity may not be the factor activating the mechanism described in this paper, because in our experiments variations in $(Na^+)_o$ were at constant osmolarity (237.8 mosmol). The high concentration of Na^+ can also open the shunt path by virtue of the increase of the ionic strength (Mandel & Curran, 1972; Reuss & Finn, 1974). However, variations in the ionic strength cannot be counted among the factors operating the Cl^- mechanism described here, because the effect is not observed when it is kept constant by using K^+, Rb^+, Cs^+, I^- or F^- (Figs. 3 and 4). Also the effect of Cl^- does not seem to be the result of an unspecific decrease in the tightness of the skin, as demonstrated in the first manuscript (Ques-Von Petery *et al.*, 1978). Therefore, the activation of a Cl mechanism must be a specific property of Na^+ and Cl^-, as proposed in the hypothesis mentioned above, and not the result of an extracellular leak.

2) *Stimulation of a Cl pump.* Skins with Cl^- present at concentrations as low as 5 mM on the outside and sulphate Ringer's inside have a negative $\Delta\psi$ when $(Na^+)_o$ is below 0.1 mM (Figs. 2 and 4), and its value becomes more negative at higher concentrations of chloride outside. This fact would indicate that the effect of Cl^- might not be reduced to the shunting of a Na^+ potential. The Cl pump may also play a role. As mentioned before, the existence of Cl pumping in this preparation was demonstrated by Zadunaisky and coworkers (1962, 1963 and 1964) and in our previous paper (Rotunno *et al.*, 1978). According to Fischbarg

et al. (1967), at low concentration of Cl^- there is a pump that works at a slow rate, but this rate increases markedly at around 60 mM. The concentration at which this inflection point occurs is considerably higher than the 2–9 mM observed in Fig. 1. The discrepancy might be attributed to the somewhat different experimental procedure (Rabito *et al.*, 1973). The activation of the Cl pump by Cl^- and by Na^+ at a given concentration would account for a progressive decrease of $\Delta\psi$ above this point like the one described in this paper.

These two possibilities, the activation of the Cl pump and the stimulation of the passive penetration of Cl^- into the cells, do not necessarily exclude each other. In living frogs such mechanisms would reduce the back leak of electrolytes into the pond when the outer barrier is in contact with a low concentration of NaCl, but would enable the skin to take up NaCl when it becomes available.

Mrs. Marisa B. de González and M. Graciela A. de Carman are gratefully acknowledged for their valuable technical assistance. M. Cereijido and C.A. Rotunno were fellows of the John Simon Guggenheim Memorial Foundation. This work was supported by research grants from the U.S. Public Health Service. 5 RO5 TW00139. M.V. Ques-von Petery is a Research Fellow from the National Research Council of Argentina.

References

Cereijido, M., Curran, P.F. 1965. Intracellular electrical potentials in frog skin. *J. Gen. Physiol.* **48**:543

Cereijido, M., Herrera, F.C., Flanigan, W., Curran, P.F. 1964. The influence of Na concentration on Na transport across frog skin. *J. Gen. Physiol.* **47**:879

Di Bona, D.R., Civan, M.M. 1973. Pathways for movement of ions and water across toad urinary bladder. I. Anatomic site of transepithelial shunt pathways. *J. Membrane Biol.* **12**:101

Dobson, J.G., Jr., Kidder, G.W., III 1968. Edge damage effect "in vitro" frog skin preparations. *Am. J. Physiol.* **214**:719

Eisenman, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**:259

Finn, A.L. 1974. Transepithelial potential difference in the urinary bladder is not due to ionic diffusion. *Nature (London)* **250**:495

Fischbarg, J., Zadunaisky, J.A., Fisch, F.W. 1967. Dependence of sodium and chloride transport on chloride concentration in isolated frog skin. *Am. J. Physiol.* **213**:963

Franz, T.J., Van Bruggen, J.T. 1967. Hyperosmolarity and the net transport of nonelectrolytes in frog skin. *J. Gen. Physiol.* **50**:933

Greven, K. 1944. Ein Beitrag zum Problem des Ruhestroms der Froschhaut. *Pfluegers Arch.* **244**:365

Helman, S.I., Miller, D.A. 1973. Edge damage effect on electrical measurement of frog skin. *Am. J. Physiol.* **225**:972

Huf, E.G. 1972. The role of Cl and other anions in active Na transport in isolated frog skin. *Acta Physiol. Scand.* **84**:366

Kidder, G., Cereijido, M., Curran, P.F. 1964. Transient changes in electrical potential differences across frog skin. *Am. J. Physiol.* **207**:935

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

Leb, D.E., Edwards, C., Lindley, B.D., Hoshiko, T. 1965. Interaction between the effects of inside and outside Na and K on Bullfrog skin potential. *J. Gen. Physiol.* **49**:309

Linderholm, H. 1954. On the behaviour of the "sodium pump" in frog skin at various concentrations of Na ions in the solution on the epithelial side. *Acta Physiol. Scand.* **31**:36

Lindley, B.D., Hoshiko, T. 1964. The effects of alkali metal-cations and common anions on the frog skin potential. *J. Gen. Physiol.* **47**:749

Mandel, L.J., Curran, P.F. 1972. Chloride flux via a shunt pathway in frog skin: Apparent exchange diffusion. *Biochim. Biophys. Acta* **282**:258

Moreno, J.H., Reisin, I., Rodriguez Boulan, E., Rotunno, C.A., Cereijido, M. 1973. Barriers to Na movement across frog skin. *J. Membrane Biol.* **11**:99

Ques-von Petery, M.V., Rotunno, C.A., Cereijido, M. 1978. Studies on chloride permeabilities of the skin of *Leptodactylus ocellatus:* I. Na^+ and Cl^- effect on passive movements of Cl^-. *J. Membrane Biol.* **42**:317

Rabito, C.A., Rodriguez Boulan, E., Cereijido, M. 1973. Effect of the composition of the inner bathing solution on transport properties of the frog skin. *Biochim. Biophys. Acta* **311**:630

Reuss, L., Finn, A. 1974. Passive electrical properties of toad urinary bladder epithelium. Intercellular electrical coupling and transepithelial cellular shunt conductance. *J. Gen. Physiol.* **64**:1

Rotunno, C.A., Ques-von Petery, M.V., Cereijido, M. 1978. Studies on chloride permeabilities of the skin of *Leptodactylus ocellatus:* II. Na^+ and Cl^- effect on inward movements of Cl^-. *J. Membrane Biol.* **42**:331

Smith, T.C., Hughes, W.D., Huf, E.G. 1971. Movement of CO_2 and CO_3H^- across isolated frog skin. *Biochim. Biophys. Acta* **225**:77

Smith, T.C., Martin, J.H., Huf, E.F. 1973. Na pool and Na concentration in epidermis of frog skin. *Biochim. Biophys. Acta* **291**:465

Steinbach, H.B. 1933. The electrical potential difference across living frog skin. *J. Cell Comp. Physiol.* **3**:1

Urakabe, S., Handler, J.S., Orloff, J. 1970. Effect of hypertonicity on permeability properties of the toad bladder. *Am. J. Physiol.* **218**:1179

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

Wade, J.B., Revel, J.P., Di Scala, V.A. 1973. Effect of osmotic gradients on intercellular junctions of the toad bladder. *Am. J. Physiol.* **224**:407

Zadunaisky, J.A., Candia, O.A. 1962. Active transport of sodium and chloride by the isolated skin of the South American frog *Leptodactylus ocellatus. Nature (London)* **195**:1004

Zadunaisky, J.A., Candia, O.A., Chiarandini, D.J. 1963. The origin of the short-circuit current in the isolated skin of the South American frog *Leptodactylus ocellatus. J. Gen. Physiol.* **47**:393

Zadunaisky, J.A., Fisch, F.W. 1964. Active and passive chloride movements across isolated amphibian skin. *Am. J. Physiol.* **207**:1010

Zylber, E.A., Rotunno, C.A., Cereijido, M. 1973. Ion and water balance in isolated epithelial cells of the abdominal skin of the frog *Leptodactylus ocellatus. J. Membrane Biol.* **13**:199