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Studies on Chloride Permeability of the Skin of *Leptodactylus ocellatus*: II. Na^+ and Cl^- Effect of Inward Movements of Cl^-

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Summary. At low concentration (1 mM) of Cl^- in the outer solution, the influx of chloride through the isolated skin (J_{13}^{Cl}) of the South American frog *Leptodactylus ocellatus* (L.) seems to be carried by two mechanisms: (i) a passive one that exhibits the characteristics of an exchange diffusion process, and (ii) an active penetration. Studies of the influx and efflux of chloride (J_{13}^{Cl} and J_{31}^{Cl}) indicate that the presence of a high (107 mM) concentration of Cl^- in the outer solution activates the translocation of this ion through the cells. Studies of the unidirectional flux of Cl^- across the outer barrier (J_{12}^{Cl}) indicate that Na^+ out stimulates the penetration of Cl^- at this level. Cl^- out, in turn, stimulates the J_{12}^{Na}, but this effect is only detected at low concentrations of Na^+ out.

Several lines of evidence indicate that Na^+ and Cl^- influence—and might control—each other's permeability: (i) electroneutrality requirements make net Na^+ transport *in vivo* (i.e., in open circuit) dependent on Cl^- movement (Linderholm, 1952). Passive Cl^- fluxes *in vitro* are driven by the electrical potential difference created by Na^+ transport (Ussing & Zerahn 1951); (ii) Na^+ net flux depends on the nature of the anion (Lindley & Hoshiko, 1964; Gil-Ferreira, 1968); (iii) Chloride permeability is enhanced by Na^+ (Macey & Meyers, 1963); (iv) The net transport of Na^+ is stimulated by $(Cl^-)_i$ and depressed by $(Cl^-)_o$ (Huf, 1972). This mutual influence might be a key factor allowing frogs to take up NaCl when it is available in the bathing medium, but avoid losses when it is absent. This is a second of a series of three articles (Ques-von Petery, Rotunno & Cereijido, 1978, and Rodríguez Boulan *et al.*, 1978) devoted to study the influence of Na^+ and Cl^- on chloride

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movements. In the previous paper it was found that at low concentration of Cl^- on the outside, $(Cl^-)_o$, passive fluxes of Cl^- have the characteristics of an exchange diffusion and are not affected by the $(Na^+)_o$. The outflux of Cl^- (J_{31}^{Cl}) is increased fivefold by the addition of Cl^- as choline chloride in the outer solution, and 17-fold by the addition of NaCl to the outside. Na^+, by itself, does not affect J_{31}^{Cl}. This would suggest that the mechanism operating the translocation of Cl^- at high NaCl concentrations outside is also an exchange diffusion. Yet concomitant increases of the electrical conductance discarded this second exchange diffusion mechanism. At high $(Cl^-)_o$, chloride would use a transcellular route whose permeability is markedly enhanced by Na^+ outside.

The addition of choline chloride or NaCl (107 mM) to the outer solution produces an increase in the total conductance (G_T) and in the specific chloride conductance (G_{Cl}) calculated on the basis of J_{31}^{Cl}. The curve relating G_T and G_{Cl} in skins bathed with Ringer's with 107 mM NaCl on both sides has a slope greater than 1.0. This indicates that Cl^- and Na^+ on the outside activate some other process besides the one carrying the J_{31}^{Cl}. Since the skin of the South American frog *Leptodactylus ocellatus* is known to have an active influx of Cl^- (Zadunaisky & Candia, 1962; Zadunaisky, Candia & Chiarandini, 1963) it is suspected that Na^+ and Cl^- on the outside may influence the active transport of Cl^- as well as its passive fluxes. Therefore, in this paper we investigate the influences of Cl^- and Na^+ on the influx of chloride J_{13}^{Cl}.

Materials and Methods

All studies were done on the abdominal skin of the South American frog *Leptodactylus ocellatus* (L.) mounted as a flat sheet between two Lucite chambers. Experiments were carried out at room temperature (20–22 °C). Fluxes were measured under short-circuit conditions.

Unidirectional Sodium and Chloride Flux across the Outer Border of the Epithelium

When one adds tracer sodium or chloride to the outer bathing solution, the unidirectional flux J_{12}^{Na} or J_{12}^{Cl} across the outer border of the epithelium can be calculated by measuring the initial rate of net uptake (Fig. 1). The technique to measure J_{12}^{Na} was designed and described in detail by Rotunno *et al.*, (1970) and modified by Cereijido *et al.*, (1972). In the case of ^{22}Na, the samples of skin were counted directly in a well-type scintillation counter (Nuclear Chicago Auto Gamma). In order to obtain the activity of ^{36}Cl, the samples of skin were collected in plastic tubes with 1 ml 0.1 N-H NO_3. The tubes were

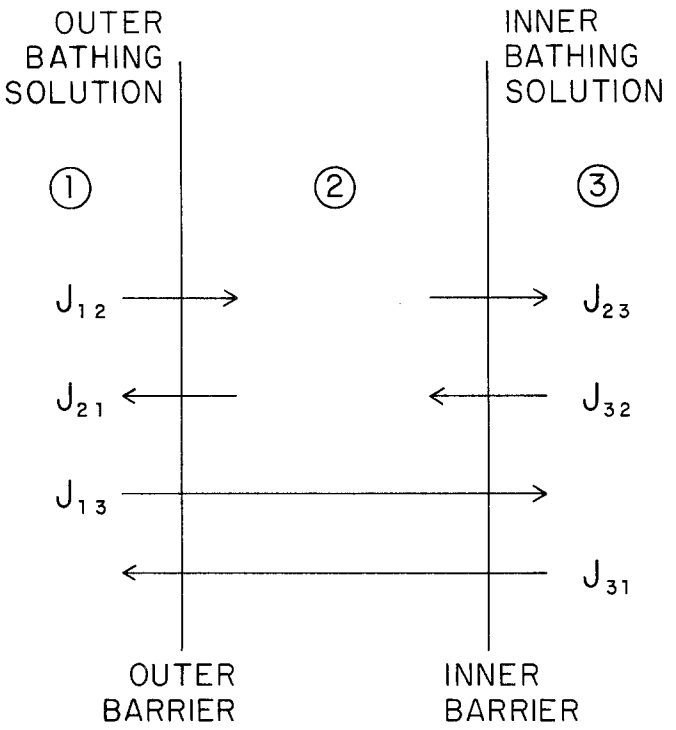

Fig. 1. Kinetic model used to analyze ionic movements through the frog skin

stoppered with Parafilm (Morathon) and left overnight to be extracted. Aliquots were diluted in Bray's mixture and counted in a liquid scintillation counter (Nuclear Chicago ISOCAP 300).

Influx of Chloride

The influx of chloride was measured with ^{36}Cl added to the outer solution. After an equilibration period of 30 min, duplicate samples of 200 μl were taken from the inside and the volume replaced with fresh Ringer's solution, at the end of the experiment, duplicate samples of 200 μl were taken from the outer bathing solution and conveniently diluted for counting. The samples were collected in Bray's solution and counted as described above.

Solutions

Chloride internal solution (mM): 107 Cl^-; 115 Na^+; 10 pyruvate; 2.4 HCO_3^- 2.4 K^+; 1 Ca^{++}. Sulphate internal solution (mM): 52.5 $SO_4^=$; 115 Na^+; 10 pyruvate; 2.4 HCO_3^-; 2.4 K^+; 1 Ca^{++} and 53.5 sucrose. The Ringer's solutions used on the outside had no calcium.

The outflux of chloride and the electrical and chemical methods used were described in detail in the previous manuscript (Ques-von Petery *et al.*, 1978).

Results are expressed as mean ± SE (number of observations).

Results

The increase of the concentration of chloride in the outer bathing solution $(Cl^-)_o$ elicits a significant increase in its *outflux* J_{31}^{Cl} (Ques-von Petery *et al.*, 1978). As mentioned in the introduction, the fact that J_{31}^{Cl} is increased by Cl^- on the *trans* side suggested that the passive flux was carried by an exchange diffusion process, but later, studies of the electrical conductivity led to the opinion that Cl^- on the outside opens a route that allows the net passage of ions under the effect of an electrical potential. Therefore, a more direct demonstration that the change in Cl-permeability by Cl^- on the outside is carried by a mechanism other than exchange diffusion would be a test of the symmetry of the effect. In other words, the fact that exchange diffusion is a passive mechanism requires not only that J_{31}^{Cl} be increased by chloride in the outer bathing solution but also that J_{13}^{Cl} be influenced by changes in the concentration of Cl^- in the inner bathing solution.

Table 1 shows J_{13}^{Cl} measured from an outer bathing solution with 107 mM NaCl under two different experimental conditions: with and without Cl^- in the inner bathing Ringer's. In the second case, Cl^- was replaced by an equivalent amount of $So_4^=$ and the osmolarity was adjusted by adding sucrose. No effect of Cl^- on the *trans* side is observed. We may conclude that Cl^- on the outside increases the passive chloride permeability of the skin as it was shown in the previous study (Ques-von Petery *et al.*, 1978) with the increase in the value of J_{31}^{Cl}. This would mean that the passive component of J_{13}^{Cl} must also increase by a mechanism which is not an exchange diffusion. A small exchange diffusion component of the kind discussed in the previous paper might not be detected in the presence of the large unidirectional fluxes in Table 1. Since in the two sets of observations described in Table 1 the concentration of Cl^- on the outside was high (107 mM), the mechanism was opera-

Table 1. Influx of Cl^- in the presence or absence of Cl^- in the inner bathing Ringer's[a]

$(Cl)_i$ (mM)	J_{13}^{Cl} (μmole hr^{-1} cm^{-2})
107	1.89 ± 0.25 $(n=12)$
0	1.89 ± 0.36 $(n=12)$

[a] *First line:* Chloride internal solution. *Second line:* sulphate internal solution. Ringer's outside (mM): 107 Cl^-; 107 Na^+; 18 sucrose; 2.4 HCO_3^-; 2.4 K^+.

Table 2. Influx (J_{13}^{Cl}) and permeability (P_{Cl}) to chloride as a function of its concentration in the outer bathing solutions $(Cl^-)_o$[a]

$(Cl^-)_o$ (mM)	J_{13}^{Cl} (μmole hr^{-1} cm^{-2})	P_{Cl} (cm hr^{-1})
0.2	0.025 ± 0.002 $(n=24)$	0.125
2.4	0.12 ± 0.01 $(n=39)$	0.050
9.3	0.29 ± 0.03 $(n=27)$	0.031
23	0.67 ± 0.008 $(n=14)$	0.029
40	1.10 ± 0.12 $(n=33)$	0.027

[a] $(Na^+)_o$ was kept constant at 90 mM. The sulphate internal solution was used inside.

tive in both of them, and the presence or absence of Cl^- inside made no difference. The aim of the next series of experiments in this paper, was then to study the effect of increasing $(Cl^-)_o$ on the *influx* J_{13}^{Cl} and permeability (P_{Cl}) of chloride. The permeability P_{Cl} can be calculated as $P_{Cl} = J_{13}^{Cl}/(Cl^-)_o$.

The experiments were performed at high concentration of Na^+ on the outside because in the previous paper it was observed that Na^+ out increases the activation of J_{31}^{Cl} elicited by $(Cl^-)_o$. The inner bathing solution contained no Cl^-. Table 2 shows that at low $(Cl^-)_o$ the value of P_{Cl} is 0.125 cm hr^{-1} and decreases to 0.05 cm hr^{-1} when $(Cl^-)_o$ is raised from 0.2 to 2.4 mM. Further increases of $(Cl^-)_o$ produce a further decrease of the permeability up to a near constant value. This indicates that J_{13}^{Cl} occurs through a mechanism that saturates as the outside concentration of Cl^- increases. This fact, together with the observation made in the previous paper (Ques-von Petery *et al.*, 1978) that $(Cl^-)_o$ increases the value of P_{Cl} measured with J_{31}^{Cl} suggests the existence of an active transport of chloride dependent on the outside chloride concentration.

Active transport of Cl^- has been demonstrated in *in vivo* and *in vitro* preparations and at both low and high concentrations of NaCl on the outside (Krogh, 1937; Jørgensen, Levi & Zerahn, 1954; Zadunaisky *et al.*, 1963; García-Romeu, Salibián & Pezzani-Hernández, 1969; Huf, 1972; Kristensen, 1972). The purpose of the experiments that follow is to study the effect of Na^+ and Cl^- on such transport and compare their influences on J_{13}^{Cl} their effects on J_{31}^{Cl}.

Figure 2 shows the relationship between the influx J_{13}^{Cl} and the outflux J_{31}^{Cl} obtained in paired skins from the same frogs, in short-circuit condi-

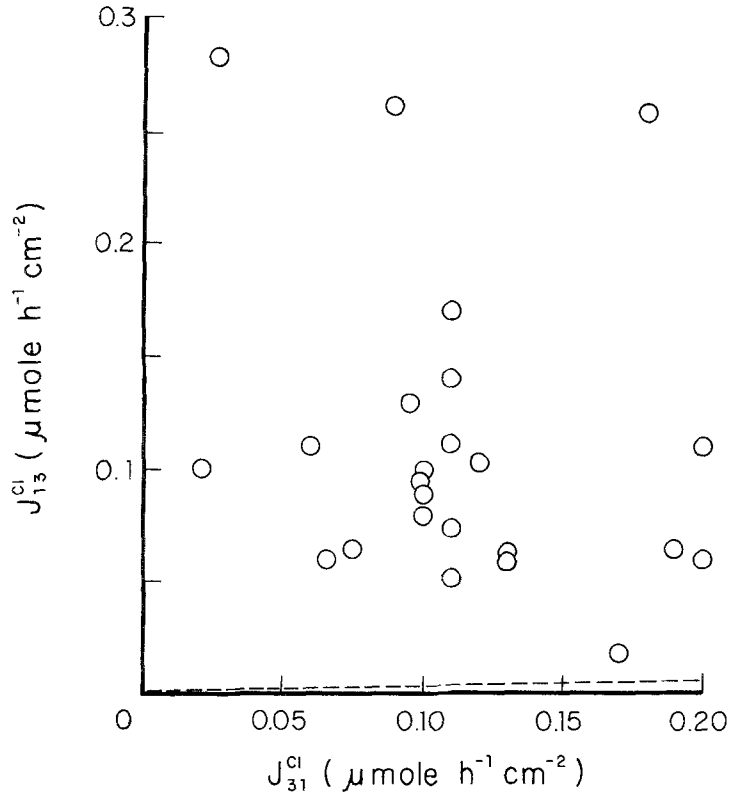

Fig. 2. Influx (J_{13}^{Cl}) *vs.* outflux (J_{31}^{Cl}) of chloride in frog skin mounted with chloride internal solution on the inside and 1 mM NaCl; 2.4 mM $KHCO_3$ and 231 mM sucrose on the outside. Each circle represents a series of measurements made in two halves of the same skin under short-circuit conditions. The dotted line represents the ration J_{13}^{Cl}/J_{31}^{Cl} expected in the absence of active transport of chloride. The mean value for J_{13}^{Cl} is 0.111 ± 0.014 ($n=24$)

tions. The skins were mounted with the chloride internal solution inside, and Ringer's containing 1 mM NaCl on the outside. The Ringer's on the outside was made isotonic with sucrose. The broken line indicates the relationship expected on the basis of the outer/inner ratio of Cl^- concentrations (0.01) if J_{13}^{Cl} and J_{31}^{Cl} were due to simple diffusion. All points fall well above this line, indicating that the inward permeability is much higher than the permeability in the outward direction. This could have, in principle, two possible explanations: (i) that J_{13}^{Cl} and J_{31}^{Cl} are carried exclusively by an exchange diffusion mechanism that saturates around 1 mM; in this way the mechanism will carry the same amount of Cl^- in both directions in spite of the fact that the concentration of Cl^- on the inside is 100 times higher than the outisde; (ii) that there

is a powerful transport of Cl^- directed inward. Although the information reported in the previous paper indicates that there is in fact an exchange diffusion mechanism, it could not fulfill the requirement of being the only mechanism translocating Cl^- and working at saturation at 1 mM so as to carry an identical amount of Cl^- in both directions. This is demonstrated by the fact that while J_{13}^{Cl} from an outer solution with 1 mM Cl^- has an average value of 0.111 ± 0.014 ($n=24$) μmole hr^{-1} cm^{-2}, J_{31}^{Cl} from an inner solution with 1 mM Cl^- is only 0.014 μmole hr^{-1} cm^{-2}. This value of J_{31}^{Cl} is computed with the value of P_{Cl} given in Table 1 of the previous paper (Ques-von Petery *et al.*, 1978): 0.014 ± 0.004 ($n=11$) cm hr^{-1}. Therefore, the 0.111 μmole hr^{-1} cm^{-2} carried by J_{13}^{Cl} is composed of 0.014 μmole hr^{-1} cm^{-2} translocated passively and 0.097 μmole hr^{-1} cm^{-2} actively pumped.

The next step is to study the influence of Cl^- and Na^+ on these components of the influx of Cl^-. Figure 3 shows the relationship between J_{13}^{Cl} and J_{31}^{Cl} obtained this time at the same concentration of Cl^- on both sides (107 mM). The unidirectional fluxes were again measured in paired halves of the same skin under short-circuit conditions. Most points fall above the identity line indicating that there is an inward active transport of Cl^-. There are two sets of points. Open circles illustrate the effect of raising Cl^- to 107 mM while keeping Na^+ at 1 mM. The mean value of J_{31}^{Cl} is 0.77 ± 0.07 ($n=25$) μmole hr^{-1} cm^{-2}, i.e., much higher than the 0.113 reported in Fig. 2. This increase of the passive flux J_{31}^{Cl} by the addition of Cl^- on the *trans* side was already described in the previous paper (Ques-von Petery *et al.*, 1978). The mean value of J_{13}^{Cl} is 1.28 ± 0.13 ($n=25$) μmole hr^{-1} cm^{-2}. Since in this case the concentration of Cl^- is identical on both sides, the net active flux can be obtained simply by subtraction of J_{31}^{Cl} from J_{13}^{Cl}: 0.51 ± 0.13 (25)μmole $\cdot hr^{-1} \cdot cm^{-2}$. This indicates that the addition of Cl^- on the outside increases the passive (from 0.113 to 0.77 μmole $\cdot hr^{-1} \cdot cm^{-2}$) as well as the active transport of Cl^- (from 0.097 to 0.51 μmole $\cdot hr^{-1} \cdot cm^{-2}$). The other set of points (filled circles) were obtained at both high Cl^- and high Na^+ concentration on the outside (107 mM). J_{31}^{Cl} increases to 2.80 ± 0.14 ($n=10$) μmole $hr^{-1} \cdot cm^{-2}$. This effect of Na^+ on J_{31}^{Cl} was also already described in the previous paper. J_{13}^{Cl} rises to 4.34 ± 0.22 ($n=10$) μmole $hr^{-1} \cdot cm^{-2}$. Therefore the active influx of Cl^- is 1.54 ± 0.16 ($n=10$) μmole $hr^{-1} \cdot cm^{-2}$. Thus, Na^+ increases the passive chloride flux from 0.77 to 2.80 μmole $hr^{-1} \cdot cm^{-2}$ and also the active net chloride transport from 0.51 to 1.54 μmole $hr^{-1} \cdot cm^{-2}$.

Since the activation of J_{13}^{Cl} and J_{31}^{Cl} were elicited by Cl^- and Na^+

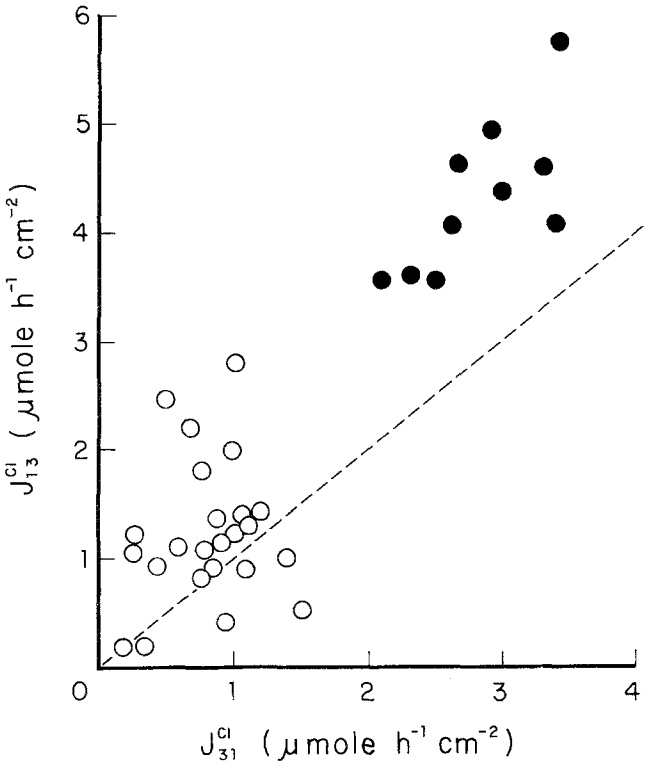

Fig. 3. Influx *vs.* outflux of chloride in skins mounted with the (chloride internal solution) on the inside and 107 mM Cl^-, 107 mM Na^+ or choline; 2.4 mM KCO_3H, and 18 mM sucrose on the outside. Each circle represents a determination of J_{13}^{Cl} and J_{31}^{Cl} in paired halves of the same skin under short-circuit conditions. *Open circles:* Skins with 107 mM Cl^- on the outside (added as a choline salt). Filled circles: skins with 107 mM NaCl on the outside

acting on the outside, it was suspected that they acted on a mechanism located at the outer barrier. To test this hypothesis we studied J_{12}^{Cl} in the presence of low (6 mM) and high (100 mM) concentration of Na^+ outside. Figure 4 shows a representative experiment. The uptake of Cl^- is shown at 6 mM (open circles) and 100 mM of Na^+ out (filled circles). The flux J_{12}^{Cl} is given by the slope of the lines. Table 3 summarizes all the measurements made as in Figure 4. It may be concluded that Na^+ out activates the penetration of chloride at the outer barrier.

The activation of the flux of a substance by the presence of another is a common situation in membrane biology. In most instances, when both substances are permeable, the typical finding is that they activate each other's flux. Since Na^+ activates J_{12}^{Cl}, it was expected that Cl^-

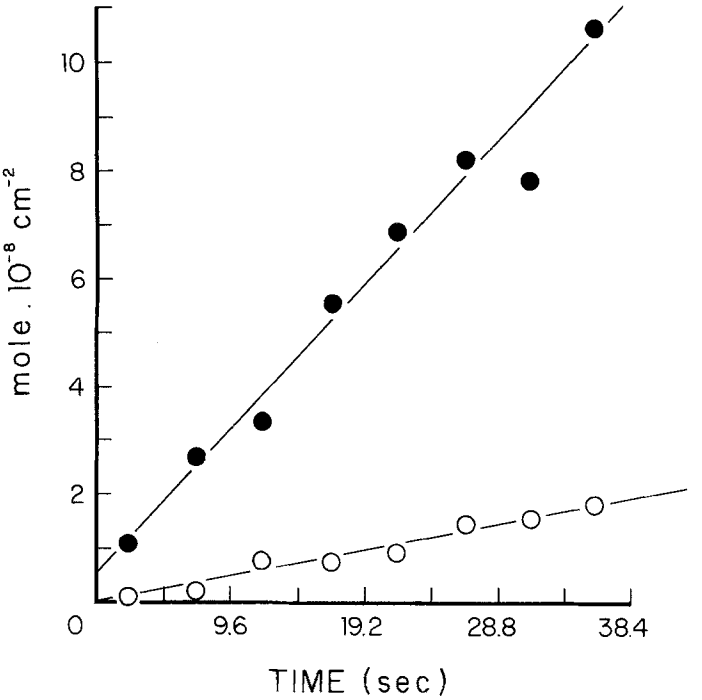

Fig. 4. The uptake of Cl^- as a function of time determined with the method described in detail by Cereijido *et al.* (1972). Each line corresponds to a single experiment. Inside: chloride internal solution; Outside: 107 mM Cl^-, (107) (Na^+) mM choline, 2.4 mM $KHCO_3$ and sucrose to make it isotonic. *Open circles:* uptake in the presence of 6 mM Na^+ on the outside. *Filled circles:* uptake in the presence of 100 mM Na^+ on the outside

Table 3. Effect of $(Na^+)_o$ on the penetration of chloride across the outer barrier of the epithelium[a]

$(Na^+)_o$ (mM)	J_{12}^{Cl} (μmole hr^{-1} cm^{-2})
6	1.77 ± 0.24 ($n=14$)
100	3.55 ± 0.55 ($n=15$)

[a] The Ringer's used are specified in Fig. 4.

would activate J_{12}^{Na}. The effect of Cl^- on J_{12}^{Na} was tested at high and low concentration of Na^+ out. When the concentration of Na^+ out is high (115 mM), the flux J_{12}^{Na} is not affected by the presence or absence of Cl^- out (3.68 *vs.* 3.56 μmoles hr^{-1} cm^{-2} (Table 4)). When the concentration of Na^+ out is low, its flux is increased by Cl^- (2.20 *vs.* 3.53 μmoles

Table 4. Effect of Cl^- on the penetration of Na^+ across the outer barrier of the epithelium[a]

$(Na)_o$ (mM)	$(Cl)_o$ (mM)	J_{12}^{Na} (μmole hr^{-1} cm^{-2})
115	0	3.68 ± 0.16 $(n=8)$
115	115	3.56 ± 0.27 $(n=9)$
10	0	2.20 ± 0.47 $(n=7)$
10	115	3.53 ± 0.37 $(n=7)$

[a] Ringer's inside: chloride internal solution.

Ringer's outside (mM): Cl^- 115; Na^+ 115; K^+ 2.4; HCO_3^- 2.4. Na^+ was replaced by choline to give $(Na^+)=10$ mM and $(Cl^-)=115$ mM. Cl^- was replaced by $SO_4=$ to give $(Cl^-)=0$ mM and $(Na^+)=10$ or 115 mM. Sucrose was added to make the Ringer's isotonic where needed.

hr^{-1} cm^{-2}). The saturation of J_{12}^{Na} at high $(Na^+)_o$ was described by Moreno *et al.*, (1973). This suggests that chloride out increases the affinity of the sodium sites without affecting their maximal capacity.

Discussion

In 1937 Krogh reported that living frogs can take up a net amount of Cl^- from very diluted outer solutions. His observations were later extended to several frog species and to *in vitro* preparations (Salibian, Pezzani-Hernández & García Romeu, *et al.*, 1968; García-Romeu, *et al.*, 1969; Martin, 1964; Martin & Curran, 1966). These observations were made with outer solutions having very low concentrations of NaCl (usually 1 mM or less). This active transport of Cl^- was not observed when the skins were mounted between two identical Ringer's with high (115 mM) concentrations of NaCl (Ussing & Zerahn, 1951; Koefoed-Johnson, Levi & Ussing, 1952). García-Romeu (1971) pointed out that the fact that the active transport of chloride was observed *in vivo*, but was absent *in vitro*, indicates that it depends on a frail mechanism which does not withstand the maneuvers necessary to mount it on a chamber plus the high saline concentration in contact with the outer face. Particularly pertinent is the observation of Watlington and Jessee (1975) that a large proportion of frog skins conserves an important inward transport of Cl^- even at high NaCl and that the skins that exhibit this transport have low electrical potential ($\Delta\psi$) and low short-circuit current (I). This means that the common practice of accepting a frog skin to work with

when it has "good" (high $\Delta\psi$ and I, is just a discrimination that discards all skins with Cl-transport. Zadunaisky *et al.* (1962, 1963) took advantage of the fact that the South American frog *Leptodactylus ocellatus* (L.) has a more enduring chloride transport to perform pioneering *in vitro* studies of this phenomenon. It is also probable that, now that Watlington and Jessee have warned against discrimination of skins of *R. pipiens*, *R. esculenta* and *R. temporaria* with Cl^- transport, Zadunaisky's descriptions will also be confirmed in these species. It is therefore important to study in some detail the mechanisms which perform the active and passive translocation of Cl^-. This is precisely the aim of the present series of papers (Ques- von Petery *et al.*, 1978, and Rodríguez Boulan *et al.*, 1978).

One must always have in mind the importance of studying skins which are in contact with ambient solutions that resemble their natural environment: chloride internal Ringer's on the inside and Ringer's with low salinity on the outside. Under these conditions we found here and in the previous paper (Ques-von Petery et al., 1978) that Cl^- is translocated mainly through two mechanisms: (i) a mechanism of exchange diffusion which is not affected by the presence or absence of Na^+ outside and (ii) an inward active transport (Fig. 2).

Considering that: (i) this transport is observed at 1 mM $(Cl^-)_o$, (ii) that the overall concentration of Cl^- in the epithelium is 50 mM (Rotunno, Zylber & Cereijido *et al.*, 1973; Zylber, Rotunno & Cereijido, 1973) and (iii) that under short-circuit conditions the cells have a negative electrical potential with respect to the outer solution (Cereijido & Curran, 1965), we conclude that the Cl pump must be located at the outer barrier. Zylber *et al.*, (1973) have also found that isolated epithelial cells of the frog skin have their Cl^- content at the concentration (50 mM) expected on the basis of a passive distribution and the intracellular electrical potential. Therefore the epithelial cells need not have pumps to extrude Cl^- *from* the cytoplasm. This is another indication that the pumps that translocate Cl^- across the frog skin might not be located at the inner barrier, but must be at the outer one.

This has the underlying assumption that the chloride concentration is uniform throughout the epithelium and equal to 50 mM. The same conclusion was obtained by Kristensen (1972) working in *Rana temporaria*. Therefore, one of the pictures of chloride movements that emerges from the data presented is that at low (physiological) concentrations, Cl^- exchanges through an electrically neutral mechanism (Ques-von Petery *et al.*, 1978), and penetrates also through an active mechanism, as

is already well known (Zadunaisky *et al.*, 1963), which could be located at the outer barrier.

Since Cl^- and Na^+ out increase both J_{13}^{Cl} and J_{31}^{Cl} (Figs. 2 and 3), one may conclude that they act on a passive movement of chloride. However, several results indicate that the effects of choline chloride and NaCl are not limited to passive movements of Cl^-: (i) variations in passive fluxes of chloride (J_{31}^{Cl}) are accompanied by relatively larger variations of the total skin conductance, indicating that the factor that has changed J_{31}^{Cl} has thereby changed the movements of some other ion, or the active movement of Cl^-, or both. (ii) the increase of $(Cl^-)_o$ and of $(NaCl)_o$ not only changes J_{31}^{Cl} but enhances also the active component of J_{13}^{Cl} (Fig. 3). (iii) the increase of $(Cl^-)_o$ helps Na^+ to cross the outer barrier and vice versa (Fig. 4 and Table 4). The fact that Na^+ out increases the flux J_{12}^{Cl} across the outer barrier (Table 3) indicates that the mechanism stimulated by this ion is also located at the outer barrier.

Although in the experiments described in Figs. 2 and 3 the net influx of chloride is increased when adding choline chloride or NaCl outside, the efficiency of the active transport measured as the relation of inward over outward permeability decrease markedly.

In summary, the results obtained in this paper demonstrate that Na^+ only affects Cl^- movement at high $(Cl^-)_o$ and explains the fact that the active transport of chloride was easily found at low saline concentrations outside.

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References

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

Cereijido, M., Moreno, J.H., Rodríguez Boulán, E., Rotunno, C.A. 1972. On the evaluation of fluxes across the outer border of the epithelium. *In:* Role of Membranes in Secretory Processes. L. Bolis, R.D. Keynes, and W. Wilbrandt, editors. pp. 279–283. North Holland, Amsterdam

García Romeu, F. 1971. Anionic and cationic exchange mechanisms in the skin of anurans, with special reference to *Leptodactylidae in vivo. Phil. Trans. R. Soc. London* B, **262**:163

García Romeu, F., Salibián, A., Pezzani-Hernández, S. 1969. The nature of the *in vivo*

sodium and chloride uptake mechanisms through the epithelium of the Chilean frog *Calyptocephalella gayi* (Dum. et Bibr., 1941). Exchanges of hydrogen against sodium and of bicarbonate against chloride. *J. Gen. Physiol.* **53**:816

Gil-Ferreira, K.T. 1968. Anionic dependence of sodium transport in the frog skin. *Biochim. Biophys. Acta* **150**:587

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

Jørgensen, C.B., Levi, H., Zerahn, K. 1954. On active uptake of sodium and chloride ions in anurans. *Acta Physiol. Scand.* **30**:178

Koefoed-Johnson, V., Levi, H., Ussing, H.H. 1952. The mode of passage of chloride ions through the isolated frog skin. *Acta. Physiol. Scand.* **25**:150

Kristensen, P. 1972. Chloride transport across isolated frog skin. *Acta Physiol. Scand.* **84**:338

Krogh, A. 1937. Osmotic regulation in the frog (*R. esculenta*) by active absorption of chloride ions. *Scand. Arch. Physiol.* **76**:60

Linderholm, H. 1952. Active transport of ions through frog skin with special reference to the action of certain diuretics. *Acta Physiol. Scand.* **27 (suppl.)**:97

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

Macey, R.I., Meyers, S. 1963. Dependence of chloride permeability on sodium in the isolated frog skin. *Am. J. Physiol.* **204**:1095

Martin, D.W. 1964. Reserved potentials of isolated frog skin. *J. Cell. Physiol.* **63**:245

Martin, D.W., Curran, P.F. 1966. Reserved potentials in isolated frog skin. II. Active transport of chloride. *J. Cell. Comp. Physiol.* **67**:367

Moreno, J.H., Reisin, I., Rodríguez Boulán, E., Rotunno, C.A., Cereijido, M. 1973. Barrier 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 movement of Cl^-. *J. Membrane Biol.* **42**:317

Rodríguez Boulán, E., Ques-von Petery, M.V., Rotunno, C.A., Cereijido, M. 1978. Studies on chloride permeabilities of the skin of *Leptodactylus ocellatus:* III. Na^+ and Cl^- Effect on Electrical Phenomena. *J. Membrane Biol.* **42**:345

Rotunno, C.A., Vilallonga, F., Fernández, M., Cereijido, M. 1970. The penetration of sodium into the epithelium of the frog skin. *J. Gen. Physiol.* **55**:716

Rotunno, C.A., Zylber, E.A., Cereijido, M. 1973. Ion and water balance in the epithelium of the abdominal skin of the frog *Leptodactylus ocellatus. J. Membrane Biol.* **13**:217

Salibian, A., Pezzani-Hernández, S., García Romeu, F. 1968. *In vivo* ionic exchange through the skin of the South American frog *Leptodactylus ocellatus. Comp. Biochim. Physiol.* **25**:311

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**:110

Watlington, C.O., Jessee, F., Jr. 1975. Net Cl^- flux in short-circuited skin of *Rana pipiens*: Ouabain sensitivity and $Na^+ + K^+$ dependence. *Biochim. Biophys. Acta* **382**:204

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-circuited current in the isolated skin of the South American frog *Leptodactylus ocellatus. J. Gen. Physiol.* **47**:393

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