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

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 Estudios Avanzados del IPN of México, Apartado Postal 14-740, México 14, D.F.

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Summary. The outflux of chloride through the isolated skin (J_{31}^{Cl}) of the South American frog *Leptodactylus ocellatus* (L.) is carried by a mechanism that saturates at high concentration of chloride on the inside, and is stimulated by the presence of Cl^- in the outer solution (*trans* side). The presence of Na^+ on the outside, by itself, does not increase J_{31}^{Cl}. However, when J_{31}^{Cl} is already increased by chloride on the *trans* side, the addition of Na^+ produces a significant further increase. At low concentration of Cl^- on the outside J_{31}^{Cl} is carried by an exchange diffusion mechanism. At high concentrations of Cl^- outside, J_{31}^{Cl} proceeds through a route which involves changes in electrical parameters. The results suggest that both mechanisms are located on the cell membranes and, therefore, that the fluxes would cross through the cytoplasm of the cells. Na^+ stimulates the second mechanism only.

A considerable amount of information on Na^+ transport across epithelia has been obtained in frog skin mounted between two identical solutions under short-circuit conditions. Because of this short circuiting, the movement of Na^+, although influenced, does not depend on other cations crossing in the opposite direction, nor on anions travelling along with Na^+. Yet *in vivo* the skin is not short circuited and, therefore, the preservation of electroneutrality makes Na^+ transport dependent on the movement of other ions (García Romeu, Salibian & Pezzani-Hernandez, 1969; García Romeu, 1971). In this way the movement of these ions becomes a sort of control of the movement of Na^+ and vice versa. However, requirements of electroneutrality might not be the only mutual

* *Mailing Address and to whom reprint requests should be made:* Dept. of Fisiología, Centro de Investigaciones del IPN, Apartado Postal 14-740, México 14, D.F., Mexico.

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influence between the movement of Na^+ and other ions. Thus, Na^+ net flux depends on the nature of the anion even under short circuit (Lindley & Hoshiko, 1964; Gil Ferreira, 1968; Huf, 1972). Conversely, Cl^- movement is influenced by Na^+, not just because of the electrical potential difference developed by Na^+ transport (Ussing & Zerahn, 1951; Linderholm, 1952; Koefoed-Johnsen, Levi & Ussing, 1952), but also because its permeability is increased by Na^+ (Macey & Meyers, 1963). It is therefore interesting that Na^+ facilitates the movement of Cl^- which, in turn, allows Na^+ to cross the frog skin in a net amount. This mutual influence between Na^+ and Cl^- might be a key factor in allowing frogs to take up NaCl when it is available in the bathing medium, but to avoid losses when it is absent. This and the following papers (Rodriguez Boulan *et al.*, 1978; Rotunno, Ques-von Petery & Cereijido, 1978) are devoted to the study of this interrelationship. This first one is concerned with passive movements of chloride. From the pioneer work of Zadunaisky and coworkers it is known that the skin of *Leptodactylus ocellatus* used in this study may exhibit an appreciable inward active transport of chloride (Zadunaisky & Candia, 1962; Zadunaisky, Candia & Chiarandini, 1963; Zadunaisky & Fisch, 1964; Fischbarg, Zadunaisky & Fisch, 1967). Therefore, in order to study the passive movements of Cl^-, we measured the outflux of this ion.

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 °C. The skins were mounted as a flat sheet in a Lucite chamber with 5 ml of volume. The area of skin exposed to the Ringer's solution was 3.14 cm^2. The edges of the chamber in contact with the skin were greased beforehand, and the compression of the tissue was minimal. In studies of the effect of changes in the composition of the bathing solutions, it is a common practice to change both solutions symmetrically so as to avoid chemical gradients. However, Rabito, Rodríguez Boulan and Cereijido (1973) have shown that changes in the inner Ringer's elicit electrical phenomena that are frequently ascribed to events on the outer side. Therefore, in this study, when possible (with the exception of experiments in Table 1), we decided to keep the composition of the inner solution constant. The Ringer's solution used on the inside contained either (mM): 52.5 Na_2SO_4, 2.4 $KHCO_3$, 1.0 $CaSO_4$, 10 sodium pyruvate and 53.5 sucrose or 105 NaCl, 2.4 $KHCO_3$, 1 $CaCl_2$ and 10 Na pyruvate. Several Ringer's solutions were used on the outside. Their composition is specified in *Results*. They never contained Ca^{++} nor pyruvate. The osmolarity of all Ringer's was adjusted with sucrose. Two agar-3 M KCl bridges connected the outer and the inner solutions to calomel half cells. These, as well as the terminals in contact with the silver wires that serve to pass current through the skin, were connected to a voltage clamp apparatus. When sulfate Ringer's was used, the current was passed through agar

Table 1. The permeability of urea measured varying the NaCl concentration in the outside Ringer's solution[a]

T (min)	$(NaCl)_o$ (mM)	$P_{urea} \cdot 10^2$ (cm sec^{-1})
15	1	12.6±2.3
30	1	14.1±2.8
45	1	15.7±2.9
60	115	16.4±3.0
75	115	15.8±2.3
90	115	15.4±3.0

($n=10$)

[a] Ringer's inside (mM): 52.5 Na_2SO_4; 2.4 $KHCO_3$; 1 (mM): 1 NaCl; 2.4 $KHCO_3$; 228 sucrose. Ringer's outside 115 (mM): 115 NaCl; 2.4 $KHCO_3$.

bridges. Unless otherwise stated, all flux measurements were performed under short-circuit conditions. The electrical potential difference ($\Delta\psi$) was measured with a Keithley model 200B DC electrometer, or an Orion Research voltmeter (input independance, 10^{14}).

The values of $\Delta\Psi$ in a given condition were quite reproducible in the skin of the same frog, and show little scattering among frogs of the same shipment. However, $\Delta\psi$ as well as other parameters measured in this and the following papers show significant seasonal variations. To avoid this inconvenience, each study described by a given figure or table was performed on frogs of the same shipment.

The solutions were continuously gassed and stirred by bubbling moistened air.

The outflux of chloride was measured with ^{36}Cl added to the inner solution. After an initial equilibration period of at least 30 min, 200 µl samples of the outer bathing solution were taken immediately before and after a 15-min flux period and these volumes were replaced with fresh Ringer's. Duplicate samples of 100 µl from the inner bathing solution were taken at the end of the experiment and conveniently diluted for counting. All samples were diluted in Bray mixture and counted in a liquid scintillation counter (Nuclear Chicago, ISOCAP 300). The Chloride concentrations were measured with the method of Schales and Schales (1941).

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 causes a significant reduction of the measured potential difference as well as the electrical resistance, in particular in small (0.64 cm^2) chambers. These authors have also found that the electromotive force of the sodium battery (E_{Na}), as determined by the ratio of the unidirectional fluxes, appears to be markedly reduced by the presence of edge damage. Accordingly, the E_{Na} was measured on the basis of the short-circuit current (I) and the outflux of Na. The Ringer's inside was the sulphate solution described above. In the outside the following Ringer's was used (mM): 55 Na_2SO_4, 2.4 $KHCO_3$, and 68 sucrose. The outflux of Na was measured with ^{22}Na with the same protocol described above for the Cl^- flux. ^{22}Na was measured in a Nuclear Chicago Auto Gamma scintillation counter set as spectrometer in the ^{22}Na peak.

Mandel and Curran (1972) have found that in the frog skin the permeability of the

shunt pathway is proportional to the unidirectional flux of urea. In the present study the outflux of urea was measured with ^{14}C-urea: 30 μCi of ^{14}C-urea were added to the inside (The Radiochemical Center, Amersham). After an initial equilibrating period of at least 30 min, consecutive samples were collected thereafter at 15-min intervals.

The K and Na concentration was measured with an EEL flame photometer.

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

Results

When an epithelial membrane is studied as a flat sheet between two chambers, the edge of the membrane is compressed and damaged thus creating an artifactual shunt pathway in parallel with the undamaged epithelium. This shunt causes significant reduction of the measured electrical potential and resistance (Dobson & Kidder, 1968; Helman & Miller, 1973).

Helman and Miller (1974) found that the values of the permeability of urea, the passive permeability of sodium, and the electromotive force of the sodium battery (E_{Na}) are strongly dependent on the extent of the edge damage. On the basis of their observation, we evaluated, in the present study, the extent of the edge damage by measuring these parameters. In the case of urea, the value obtained in the present study was 15×10^{-8} cm sec^{-1} (mean value, Table 1). The permeability obtained with the outflux of sodium (J^{Na}) under control conditions (minimal compression) was $2.6 \times 10^{-8} \pm 0.6$ ($n=6$) cm sec^{-1}. E_{Na} was evaluated according to $E_{Na}=(RT/F) \ln J^{Na}_{31}/J^{Na}_{13}$. The value of the influx was computed according to J^{Na}_{31}. The E_{Na} found was 123.9 ± 3.5 ($n=16$) mV. All these values are similar to the ones obtained by Helman and Miller (1974) in the absence of edge damage. Once the value of the E_{Na} was measured under control conditions, the chambers were gently tightened. This procedure decreased the E_{Na} to 89.0 ± 2.2 ($n=17$) mV. The fact that the edge of the skin was "damageable" is an indirect but further evidence that it was not severely damaged under the control conditions.

The Influence of the Composition of the Inner Solution

The permeability to chloride (P_{Cl}) under short-circuit conditions and constant concentration of chloride on the outside can be obtained as the ratio between the unidirectional flux J^{Cl}_{31} and the concentration of chloride on the inside $P_{Cl}=J^{Cl}_{31}/[Cl^-]_i$.

Table 2. Chloride permeability (P_{Cl}) of frog skin as measured by the outflux of chloride under short-circuit conditions[a]

$[Na^+]_i$ (mM)	$[Cl^-]_i$ (mM)	P_{Cl} (cm hr^{-1})
115	1	0.0140 ± 0.0040 ($n=11$)
115	13	0.0043 ± 0.0003 ($n=16$)
115	100	0.0017 ± 0.0003 ($n=12$)

[a] Internal Ringer's had the following composition (mM): 1 Ca^{++}; 2.4 K^+; 2.4 H^-CO_3; 115 Na^+; X=(1, 13 or 100) Cl; $(107-X)/2$ SO_4; 10 pyruvate and sucrose to keep the osmolarity constant. The outside Ringer's contained (mM): 1 Cl^-; 115 Na^+; 57 SO_4; 2.4 K^+; 2.4 HCO_3 and 60 sucrose.

The skins were mounted under the conditions specified in Table 2 and allowed to equilibrate for half and hour before adding the isotope. Each value was obtained with a different skin. Table 2 shows that by raising the internal concentration of chloride from 1 to 100 mM, P_{Cl} drops by a factor of 8. One may thus conclude that the outflux of Cl^- does not occur through a process of simple diffusion. This observation may at first appear at variance with Fischbarg *et al.* (1967) conclusion that the passive permeability of Cl^- is constant. Yet their measurements of the outflux as a function of the concentration of Cl^- were done by changing not only $[Cl^-]_i$—as in the present study—but $[Cl^-]_o$, as well. As demonstrated below and in the next paper (Rotunno *et al.*, 1978), changes in $[Cl^-]_o$ elicit marked changes of P_{Cl} of its own, so that when one changes both $[Cl]_i$ and $[Cl]_o$, one may observe a sum of two different effects.

The Influence of the Composition of the Outer Solution

These experiments were performed at a constant concentration of Na^+ (115 mM) and Cl^- (107 mM) on the inside but varying the composition of the outer Ringer's. The experiments were performed by changing the outside Ringer's from one of the conditions stated in the first three columns into NaCl 115 mM or vice versa. The outside chamber was rinsed three times with the new solution. The skins were allowed to equilibrate 10 min and then three samples were taken every fifteen min. The NaCl 115 mM condition was taken as control condition. The first

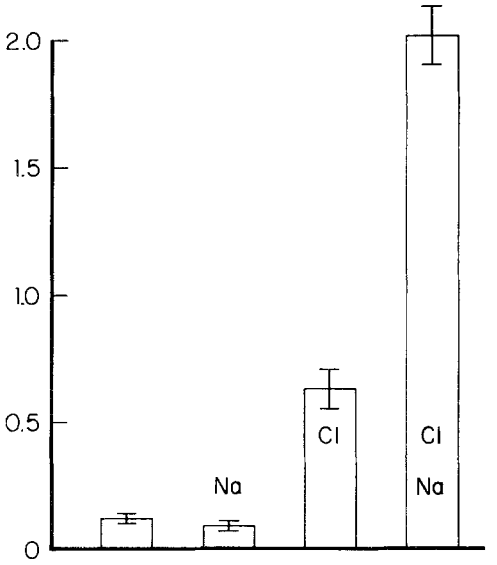

Fig. 1. Outflux of chloride from a constant inside Ringer's solution to different outside solutions with constant osmolarity. Ringer's inside (mM): 115 Na^+; 107 Cl^-; 2.4 HCO_3^-; 2.4 K^+; 1 Ca^{++}; 10 pyruvate. The composition of the external solutions used were (mM): *First column:* 1 Cl^-; 1 Na^+; 2.4 HCO_3^-; 2.4 K^+. *Second column:* 1 Cl^-; 115 Na^+; 2.4 HCO_3^-; 2.4 K^+; 57 SO_4. *Third column:* 115 Cl^-; 1 Na^+; 2.4 HCO_3^-; 2.4 K^+; 114 choline. *Fourth column:* 115 Cl^-; 115 Na^+; 2.4 HCO_3^-; 2.4 K^+. In all Ringer's sucrose was added to give constant osmolarity. The values of n were: 13, 16, 16 and 30, respectively

column in Fig. 1 corresponds to the outflux in the presence of low (1 mM) NaCl: $J_{31}^{Cl} = 0.128 + 0.022$ ($n = 13$) μmole hr^{-1} cm^{-2}. The second shows the outflux in the presence of 115 mM Na^+ added as sulphate salt: $J_{31}^{Cl} = 0.10 + 0.02$ ($n = 16$). Na^+, by itself, does not increase the value of J_{31}^{Cl}. Chloride, added as choline salt (third column), increases the outflux from 0.128 ± 0.022 ($n = 13$) to 0.64 ± 0.08 ($n = 16$). The enhancement of a flux by the addition of the same molecular species on the contralateral side is generally taken as an indication of exchange diffusion. A mechanism of exchange diffusion of Cl^- in the frog skin was suggested by Mandel and Curran (1972) in the skin of *Rana pipiens*. The addition of Na^+ to an outer solution which already had Cl^- (fourth column) elicits a considerable increase of J_{31}^{Cl}, 2.05 ± 0.12 ($n = 30$). The fact that Na^+ by itself does not increase the outflux (second column), but it does in the presence of Cl^-, suggests that it activates the mechanism of exchange diffusion.

A mechanism of exchange diffusion has two main characteristics. The first one is the already-mentioned property of enhancing the flux by the

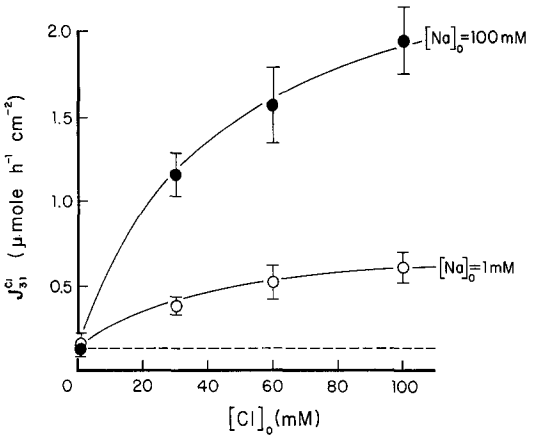

Fig. 2. Outflux of chloride from the chloride internal Ringer's solution described in *Materials and Methods*. The lower curve (○) was obtained with outside Ringer's solutions containing (mM): 1 NaCl; 2.4 $KHCO_3$; 0, 29, 59 or 99 choline chloride to give the different chloride concentrations. Osmolarity was kept constant by adding sucrose. In the upper curve (●) the composition of the outside Ringer's solutions was (mM): 100 Na^+; 2.4 HCO_3^-; 2.4 K^+; x Cl^-; $(100-x)/2$ $SO_4^=$. As before, sucrose was used to give a constant osmolarity ($n=8$)

addition of the same substance on the opposite side. The second is that the mechanism is electrically neutral, as it moves no net amount of charges. The experiments which follow were designed to study these two properties.

Perhaps the simplest kinetic model to account for a process of exchange diffusion, is that of a carrier that only travels across a barrier when loaded with Cl^-.

Figure 2 shows J_{31}^{Cl} as a function of the concentration of Cl^- in the outer solution. The dotted horizontal line would represent the portion of J_{31}^{Cl} which does not cross via the carrier: 0.14 μmole hr^{-1} cm^{-2}. The lower curve (open circles) was obtained at 1 mM Na^+ on the outside. It was drawn on the basis of the following equation:

$$J_{31}^{Cl} = 0.14 + J_{max}^{Cl} \frac{[Cl^-]_o}{[Cl^-]_o + K_m}. \quad (1)$$

where $J_{max}^{Cl} = 0.70$ μmole hr^{-1} cm^{-2} refer to the maximal flux that could be handled by the carrier, and K_m refers to the value of $[Cl]_o$ at which J_{31}^{Cl} is one half that of J_{max}^{Cl}. The upper curve, which fits the outflux obtained at high (100 mM) Na^+ on the outside, was obtained with the same Eq. (1), except that J_{31}^{Cl} was 2.65 instead of 0.70. Since J_{max}^{Cl} reflects

both the number of carriers operating the exchange and their mobility in the membranes, the increase from 0.70 to 2.65 elicited by Na^+ suggests that its activation may be due to a considerable increase in one or both of these parameters.

The other characteristic of mechanisms of exchange diffusion is that they are electrically neutral. By moving an identical amount of charges in opposite directions they do not contribute to the electrical current. The total conductance of the skin (G_T) is defined as:

$$G_T = I/\Delta\psi, \tag{2}$$

where I is the short-circuit current and $\Delta\psi$ is the spontaneous electrical potential taken as the mean value of the $\Delta\psi$ recorded before and after the measurement of J_{31}^{Cl} under short-circuit conditions. A fraction of this conductance is due to chloride movement (G_{Cl}). When the skin is mounted between two solutions of the same composition under equilibrium conditions, G_{Cl} is given by:

$$G_{Cl} = \frac{F^2}{RT} J_{31}^{Cl} \tag{3}$$

where F is the Faraday constant, R the gas constant, and T is the absolute temperature.

Figure 3 shows a plot of total conductance *vs.* chloride conductance obtained in skins mounted between two NaCl 115 moles Ringer's. The values were obtained in different frogs and represent the normal scattering between animals. The regression line, obtained by the least squares method, has a correlation coefficient of 0.85 and is described by the equation:

$$G_T = 1.51\, G_{Cl} + 0.73. \tag{4}$$

The intercept (0.73 mmho · cm^2) represents the conductance given by components other than Cl^-. It seems justifiable to assume that it is a Na^+ conductance. Since skins with high values of G_{Cl} have high values of total conductance in the condition tested, J_{31}^{Cl} must contribute to the electrical current. The slope of the line is 1.51 and not just 1.0, indicating that another parameter probably associated with J_{31}^{Cl} is also increasing the total conductance. The fact that this preparation has an inward Cl-pump, and that this pump as well as J_{31}^{Cl} are influenced by changes in the concentration of Cl^-, requires a detailed study of the role of the active mechanism for Cl^- transport in the phenomena described here. This study is reported in the next paper (Rotunno *et al.*, 1978).

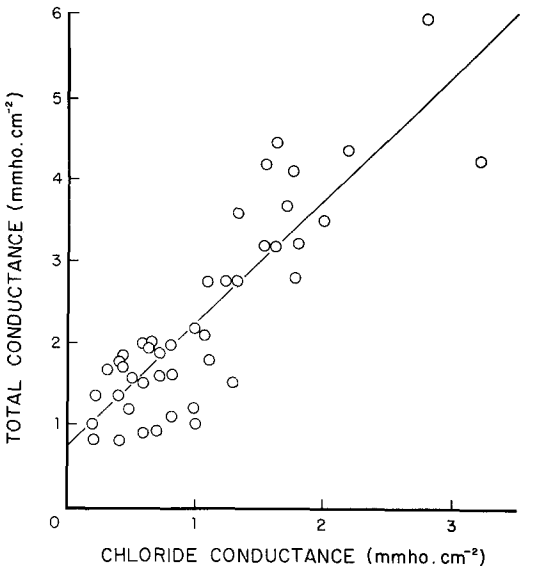

Fig. 3. Total *vs.* specific chloride conductance in skins mounted in Ringer's 115 mM NaCl on both sides under short-circuit conditions. Total conductance is defined by Eq. (2) and chloride specific conductance by Eq. (3). These values were obtained in different frogs and represent the normal scattering between different skins. The straight line found by the least squares method follows Eq. (4)

Since Eq. (3) holds for equilibrium conditions, it was only used when the skin was mounted between two 115 mM NaCl Ringer's and was not applied to analyze the cases when the skin was bathed with low NaCl, low Cl^-, or low Na^+ on the outside (the three first columns in Fig. 1). Therefore, Fig. 3 only demonstrates that when a skin mounted in Ringer's 115 mM NaCl has a high G_T, it will also have a high G_{Cl} and vice versa. Yet it does not demonstrate that when J_{31}^{Cl} is varied by changing the concentration of Cl^- of NaCl these variations are accompanied by changes of the electrical conductance. Therefore in Fig. 4 the G_T is plotted as a function of the value of J_{31}^{Cl} found in the different conditions tested. In this plot an exception was made with the points at low $[Cl^-]_o$ (open circles). Instead of making just a single group, they were split in four subgroups, because a graph (not included in this paper) showing all the individual points obtained at 1 mM Cl^- outside (26 measurements) indicates that G_T did not vary with J_{31}^{Cl}. The mean values of $\Delta\psi$ and I sec in this condition were 85 ± 4 mV ($n=26$) and 52 ± 3 $\mu A\, cm^{-2}$ ($n=26$). The effect is now clearly noticed in the representation chosen: (i) at low Cl^- on the outside, changes in J_{31}^{Cl} do not

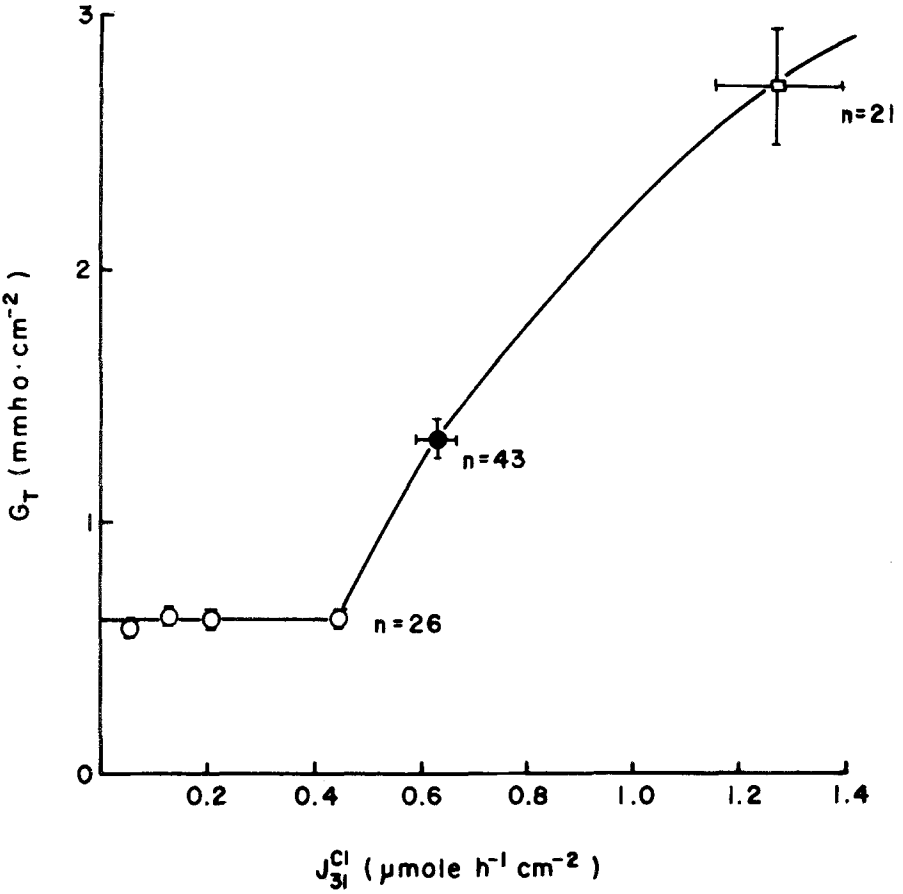

Fig. 4. Total conductance *vs.* outflux of chloride. The inner solution was the NaCl Ringer's described in *Materials and Methods.* The outer solution was (mM): (○) 1 NaCl; 2.4 $KHCO_3$; 57 Na_2SO_4; (●) 1 NaCl; 2.4 $KHCO_3$; 114 choline Cl^-; (□) 115 NaCl; 2.4 $KHCO_3$. Sucrose was added to give constant osmolarity. The four values corresponding to 1 mM 115 mM Na outside (open circles) represent the normal scattering between different animals in this condition

involve changes in G_T, suggesting that an exchange diffusion is operating. Yet (ii) the addition of Cl^- to the outer solution (full circle) raises both J_{31}^{Cl} and G_T, indicating that Cl^- may not be activating a mechanism of exchange diffusion, but turns on some other process, and (iii) that Na^+ (open square) further enhances the mechanism activated by Cl^-.

Mandel and Curran (1972) have shown that several procedures which increase passive chloride fluxes (e.g., changes in ionic strength and osmolarity) increase also the unidirectional flux of urea. Table 1 shows

the permeability of urea (P_{urea}) measured under short-circuit conditions at low and high concentration of NaCl outside. No significant change is observed, indicating that the effects of J_{31}^{Cl} are not due to the opening of an unspecific leak.

Discussion

The outflux of chloride (J_{31}^{Cl}) occurs through a passive process which saturates at high concentration of internal chloride (Table 2), is sensitive to the concentration of Cl^- and Na^+ on the outside (Fig. 1), and, at high external concentration of Cl^-, it correlates with the total conductances (Figs. 3 and 4). Mandel and Curran (1972) found that Cl^- outflux depends on external Cl^- concentration (*trans* side), a condition typically interpreted as evidence for the presence of an exchange diffusion mechanism. Yet, even when the kinetics of J_{31}^{Cl} can be described by a rather simple mechanism of exchange diffusion (Fig. 2), the stimulation of J_{31}^{Cl} by Cl^- on the *trans* side is accompanied by an increase in the conductance. The present evidence would thus indicate that the presence of Cl^- on the outside is required to open a channel, but not to replace the site left in a carrier by an outgoing Cl^-.

The present results also show that chloride permeability depends on sodium on the outside (Figs. 1 and 2). This was already demonstrated by Macey and Meyers (1963). Yet we found that Na^+ cannot do it directly, but by enhancing the effect that chloride exerts on the *trans* side.

García Romeu and Ehrenfeld (1975) studied the *influx* of Cl^- in live frogs. They found that Na^+ does not affect the influx at low concentrations of Cl^-, but that it does stimulate it at high concentrations of Cl^-. This leads them to suggest that there must be two different mechanisms which act at low and high concentrations. Watlington and Jessee (1975) also have shown that the mechanisms operating at high concentrations may be different from the ones which act at low Cl^-. In this respect it is worth noticing that changes in the passive outflux J_{31}^{Cl} observed at low values of J_{31}^{Cl} (which in turn were obtained by lowering the Cl^- concentration outside) are not reflected in changes of the electrical conductance (Fig. 4). Higher values of J_{31}^{Cl} instead, are correlated with G_T. Then the present observations would be consistent with an exchange diffusion operating at low – physiological – concentrations of chloride on the outer solution, and a conducting channel activated at high concentrations of chloride outside. An exchange diffusion mecha-

nism could be operated either by an ion exchanger or by a carrier. Fixed sites in the tight junctions could constitute an ion exchanger. However, a change in J_{31}^{Cl} through this exchanger would be accompanied by a change in G_T, which is an expectancy at variance with the results in Fig. 4 (open circles). On the other hand, when the unidirectional fluxes translocated by a carrier in opposite directions achieve a near maximal value, the net flux becomes negligible, the permeability decreases, and the phenomenon appears as an exchange diffusion. Table 1 shows that with 1 mM Cl outside and 100 mM NaCl inside the mechanism operating J_{31}^{Cl} is near saturation as the permeability is decreased some eightfold. This, together with the lack of correlation between J_{31}^{Cl} and G_T observed in this condition would therefore agree with the presence of a carrier working at near saturation. Thus it indicates that the flux follows a transcellular route, as it would require that the carrier be placed in the plasma membrane of an epithelial cell.

Under short-circuit conditions the electrical potential inside the transporting cells is negative with respect to both the outer and the inner bathing solutions (Cereijido & Curran, 1965; Nagel, 1975). The removal of Na^+ from the outer bathing solution produces a considerable increase in the negativity of the cells. Candia (1975) has suggested that this increase in negativity represents a powerful energy barrier to passive chloride movements. The effect of Na^+ on the intracellular electrical potential would thus constitute the key factor by which Na^+ on the outside controls the permeability to chloride at higher concentrations.

Nonelectrolytes of small molecular size seem to transverse the skin through extracellular routes (Mandel & Curran, 1972). The lack of effect of NaCl on urea movements (Table 1) would support the view that paracellular routes may not be involved in the phenomena described in this paper.

The discussion above has the underlying assumption that there is only one kind of transporting epithelial cell, and that Cl^- would cross either through these cells or else through the intercellular junctions. However, it might not be necessarily so, and one may be cautioned to keep in mind that the different permeation routes of Cl^- could perhaps represent translocation through different types of cells.

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