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# Kinetics of Ionic Transport Across Frog Skin: Two Concentration-Dependent Processes

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**Summary.** Sodium and chloride influxes across the nonshort-circuited isolated skin of *Rana esculenta* were measured at widely varying external ionic concentrations.

The curve describing sodium transport has two Michaelis-Menten components linked at an inflection point occurring at an external sodium concentration of about 7 meq. Chloride transport can also be represented by two saturating components. A possible explanation of these kinetics is discussed.

At sodium concentrations lower than 4 meq it is possible to define a component of the sodium transport mechanism as having a high affinity for sodium and which is independent of the nature of the external anion. A high affinity for chloride of the chloride transport system functioning at low external concentrations is also found but is significantly different from that of sodium. These systems show the physiological characteristics of the countertransports ($Na^+_{ext}/H^+_{int}$; $Cl^-_{ext}/HCO^-_{3int}$) functioning at low external concentrations.

At external concentrations higher than 4 meq a low affinity transporting system in which chloride and sodium are linked superimpose on the high affinity components.

The physiological significance of these results is discussed.

**Key words:** Frog skin, sodium transport, chloride transport, Michaelis-Menten kinetics, transport from dilute and concentrated solutions.

When the mucous face of the nonshort-circuited frog skin is bathed with dilute solutions (2 mM), ionic exchange systems assure an independence of the sodium and the chloride transports. An active proton excretion linked to the sodium transport and a tightly coupled counter-transport of chloride and bicarbonate account for these exchanges (Ehrenfeld & Garcia-Romeu, 1977, 1978). At high saline concentrations (115 mM), however, the transport of chloride and sodium seems to be largely interdependent (Watlington, 1972; Garcia-Romeu & Ehrenfeld, 1975*a–b*) since the counter-transport mechanisms, which are saturated around 2 mM (Alvarado, Dietz & Mullen, 1975; Ehrenfeld & Garcia-Romeu, 1977, 1978) can no longer maintain separate anion and cation transports, and electroneutrality has to be assured by equimolar absorption of $Na^+$ and $Cl^-$. Thus, two types of mechanism, determined by the ionic concentration of the external medium, would appear to occur, but their relationship to each other, whether, for instance, the two systems coexist or whether and at what concentrations one replaces the other, remains unknown. To study these problems, we measured the transepithelial sodium and chloride fluxes over a wide range of external concentrations of these ions. The kinetic study revealed a high affinity sodium transport system functional at low external sodium sulphate or sodium chloride concentrations (below 4 mM) and a low affinity transport dominant at high concentrations of sodium chloride. The former is independent of the accompanying anion and has all the characteristics of the $Na^+_{ext}/H^+_{int}$ exchange mechanism; the latter is a sodium transport mechanism strongly dependent on the anion. The curve representing sodium influx as a function of external sodium concentrations over the 1000-fold range of concentrations explored (0.1 to 100 mM) is a composite curve showing two levels. Chloride transport is also composed of two different mechanisms.

## Materials and Methods

Experiments were carried out on the abdominal skin of *Rana esculenta* which had been kept in the laboratory for no more than 12 days. The frogs were kept without food at a constant temperature of $15 \pm 1$ °C in aquaria containing continuously renewed dis-

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tilled water for at least 7 days before experiments. The abdominal skin (7 $cm^2$) was mounted between two Plexiglass chambers of 7 ml capacity. Before experimentation, the serosal surface of the skin was bathed in a 50% Ringer solution for 30 min. This solution was then replaced by normal Ringer and the skin left to equilibrate for 1 hr; preliminary experiments showed that this procedure induces a larger proportion of positive net fluxes stable over several hours (Ehrenfeld & Garcia-Romeu, 1977). Before any experiment, the skins were bathed in a 1-mM NaCl solution on the mucosal side and a Ringer solution on the serosal side for 20 min. Only frog skins able to maintain a net Na transport in such asymmetrical conditions $\left(\frac{Na_{int}}{Na_{ext}} > 100\right)$ were used in experiments. This procedure standardizes the different groups of animals and furthermore minimizes the problem of edge damage. In our preparations, the measured $Na^+$ effluxes were less than 90 neq·$hr^{-1}$·$cm^{-2}$, a value which is rather low considering the electrical DP[1] facilitating the Na effluxes; the value is six times smaller than that described for edge-damaged skins (Biber & Mullen, 1976).

The serosal Ringer solution had an ionic composition very similar to that of the natural internal medium (NaCl, 85 mM; $NaHCO_3$, 24 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; $MgSO_4$, 2 mM; $Na_2HPO_4$, 2.5 mM; $KH_2PO_4$, 1.2 mM; and glucose, 11 mM). It was aerated with a mixture of 5% $CO_2$, 18% $O_2$ and 77% $N_2$ (pH 7.3).

The mucosal surface of the skin was bathed with various concentrations of either NaCl solution buffered at pH 7.3 with 2 mM imidazole and $H_2SO_4$ or $Na_2SO_4$ solution buffered at 7.3 with 2 mM imidazole and $HNO_3$. The different concentrations, according to the experiment, are given in the text.

Experiments were performed in open-circuit conditions and consisted of 15–30 min periods during which the external face of the skin was bathed with successively increasing concentrations of NaCl or $Na_2SO_4$.

Preliminary experiments had shown that $Na^+$ transport was not sensitive to osmotic pressure changes; this variable was therefore not controlled. As the sodium transport system is not affected by changes in external ionic strength (Mandel & Curran, 1973), it was considered preferable not to replace $Na^+$ ions by equivalent quantities of relatively impermeant ions such as choline, $K^+$, $Ca^{++}$ or $Mg^{++}$, in order to avoid any effects these ions might themselves have on $Na^+$ transport (Curran & Gill, 1962; Macey & Koblick, 1963; Rotunno et al., 1970; Mandel & Curran, 1973).

*Flux Measurements*

When the external solution was dilute, the sodium and chloride net fluxes were calculated from the slope of the concentration of these ions in the external medium as a function of time. Sodium concentration was measured by flame photometry with an Eppendorf photometer and the chloride concentration with an Aminco Cotlove electrometric chloride titrator.

The unidirectional sodium, chloride, and sulphate fluxes were measured with the radioisotopes $^{22}Na$ (20 μCi/100 ml), $^{36}Cl$ (4 μCi/100 ml) and $^{35}S$ (30 μCi/100 ml) added to the solution bathing the mucosal skin surface. In experiments with double labeling, $^{22}Na$ was replaced by $^{24}Na$ (20 μCi/100 ml).

After a 20-min equilibration period the appearance of the isotopes in the serosal solution was followed as a function of time. The radioactivity of the $^{22}Na$ samples was measured with a Mecaserto well counter (M 13/100) and that of the $^{36}Cl$ and $^{35}S$ samples with a Nuclear Chicago scintillation counter, correction for quenching being made for the two latter isotopes.

[1] DP = difference of potential.

**Table 1.** Sodium and chloride influxes across the in vitro skin of *Rana esculenta* from external solutions of NaCl (1, 4, 29 and 115 mM)

| Concentration of NaCl solution | 1 mM | 4 mM | 29 mM | 115 mM |
|---|---|---|---|---|
| $J_iNa^+$ | 237±23 | 244±18 | 475± 77 | 742±120 |
| $J_iCl^-$ | 107±17 | 145±18 | 424±106 | 603±157 |

Internal solution: Ringer. Fluxes are expressed in neq·$hr^{-1}$·$cm^{-2}$. $J_i$ means ± SE of influx; $n=7$.

The fluxes were calculated from the quantity of radioisotope transferred in unit time and the specific radioactivity in the initial compartment. The mean ± SE of the fluxes is expressed in neq × $hr^{-1}$ × $cm^{-2}$.

Hydrogen net fluxes were obtained from the slope of the hydrogen concentration in the external buffered medium as a function of time. This concentration was determined by titration with 2 mM NaOH. Up to the highest pH value reached with this solution, there was a single inflection point (pH 8.8); the hydrogen concentration was calculated from the quantity of base added to reach the inflection point. These measurements were made on 2-ml samples with a Radiometer ATS1/TTA61 autopipetting titration system. The samples were first bubbled with air during 30 min to eliminate respiratory $CO_2$. Since the first titrations showed considerable variation, 6 blank titrations were made before the experimental ones were started. With this precaution the probable error of a single measurement was ±0.4%.

## Results

The sodium and chloride influxes were first followed with external NaCl concentrations of 1, 4, 29 and 115 meq. Table 1 summarizes the results. Influxes ($J_i$) of sodium are significantly different from those of chloride at 1 and 4 meq but not at 29 and 115 meq. Furthermore, although the relation between sodium transport and external sodium concentration is generally satisfied by a saturation curve, the kinetics of the results given in Table 1 would seem to be more complex. Thus, the $J_iNa$ are not significantly different at 1 and 4 meq, whereas with Michaelis-Menten type kinetics the $J_i$ variations should be greatest at low sodium concentration. In view of this fact, $J_iNa$ variations were studied over two external NaCl concentration ranges: one between 0.25 and 4.00 meq and the other between 4.00 and 115.00 meq; the division into two groups also allows the experiments to be carried out within a reasonable time. The values of $J_iNa^+$ found at 4 meq were slightly different in the two sets of data; the ratio of these 2 values was used to correct one set of data relative to the other. Figure 1 illustrates the results obtained. In the inset the influxes of $Na^+$ in the 0 to 4 mM region are given

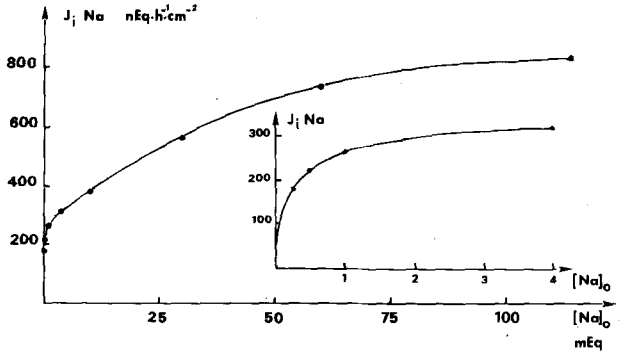


**Fig. 1.** Evolution of $J_i$Na ($n=7$) as a function of the external NaCl concentration. Internal solution, Ringer. *Inset:* Plot of $J_i$Na against $[Na]_o$ (0–4 meq).

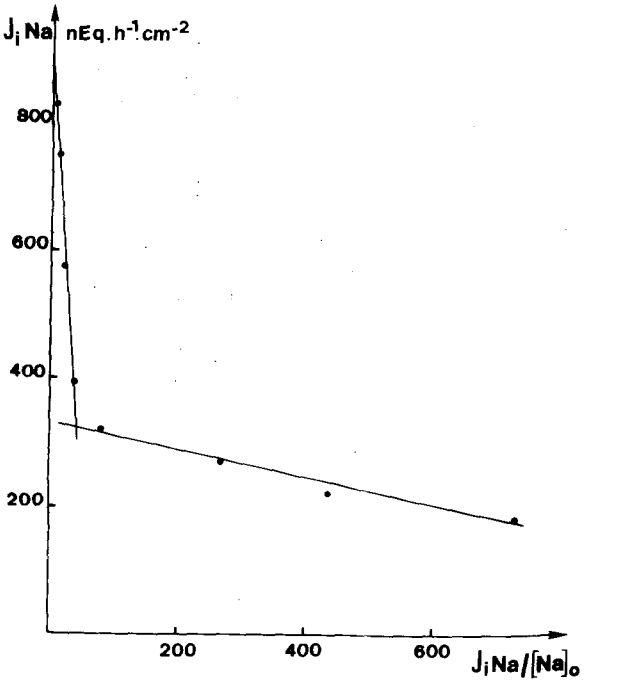


**Fig. 2.** Na influx ($J_i$Na) plotted against the Na influx divided by the sodium concentration ($J_i$Na/$[Na]_o$). The experimental points are those of Fig. 1.

on a larger scale. Figure 2 represents $J_i$Na as a function of $J_{iNa}/[Na]_{ext}$ according to the Eadie-Hofstee linear transformation magnifying departures from linearity which might be masked in a Lineweaver-Burk plot (Dowd & Riggs, 1965). This treatment reveals the occurrence of two components clearly differentiated by their kinetic parameters. The first component ($C_{I\text{-}Na}$) has a $K_{0.5}$ of $0.220 \pm 0.030$ meq and a $V_{max}$ of $331 \pm 13$ neq·hr$^{-1}$·cm$^{-2}$. The second component ($C_{II\text{-}Na}$) has an affinity 60 times less than that of component I, its $K_{0.5}$ being $13.50 \pm 2.20$ meq and its $V_{max}$ $904 \pm 52$ neq·hr$^{-1}$·cm$^{-2}$. The two representative lines intersect at an external NaCl concentration of about 7 meq. If the sodium influxes at high NaCl concentration are not normalized, component II has a $K_{0.5}$ of $13.4 \pm 2.4$ meq, as above, but a lower $V_{max}$ of $628 \pm 39$ neq·hr$^{-}$·cm$^{-2}$. The experimental curve can thus be divided into two subunits, functional at different concentration ranges, and described by two Michaelis-Menten type equations. For $C_{I\text{-}Na}$, occurring between 0.25 and 4 mM, the following equations holds:

$$J_i = \frac{331 \text{ neq} \cdot \text{hr}^{-1} \cdot \text{cm}^{-2} \cdot [Na]_{ext}}{0.220 \text{ meq} + [Na]_{ext}}$$

while for $C_{II\text{-}Na}$ it is:

$$J_i = \frac{904 \text{ neq} \cdot \text{h}^{-} \cdot \text{cm}^{-2} \cdot [Na]_{ext}}{13.5 \text{ meq} + [Na]_{ext}}.$$

These equations are purely empiric and the limitations of this treatment of the data and an interpretation will be discussed below.

Analysis of the $Cl^-$ influxes over the same concentration range also indicates two saturable components of chloride transport, one ($C_{I\text{-}Cl}$) occurring at low NaCl concentrations (0.5 to 4 mM) has a $K_{0.5}$ of $0.550 \pm 0.03$ meq and a $V_{max}$ of $353 \pm 10$ neq·hr$^{-1}$ · cm$^{-2}$ ($n=7$) and the other ($C_{II\text{-}Cl}$) at high concentrations (4 to 115 meq) has a $K_{0.5}$ of $15.4 \pm 3.6$ meq and a $V_{max}$ of $764 \pm 40$ neq·hr$^{-1}$·cm$^{-2}$ ($n=6$).

To study the possible role of the anion accompanying the $Na^+$ in the external solution on the above-defined components I and II, the chloride of the external solution was replaced by the relatively impermeant anion sulphate. Figure 3 shows the evolution of sodium, proton, and sulphate fluxes as a function of the external sodium sulphate concentration over the range 0.1–4 meq (Fig. 3*a*) and 4.0–115 meq (Fig. 3*b*). The sulphate influxes are negligible at low concentrations, and it can be seen that electroneutrality is principally maintained by exchange of protons against absorbed $Na^+$ ions, the maximum of $Na^+$ absorption and $H^+$ excretion being between 2 and 4 meq; these results confirm a previous work (Ehrenfeld & Garcia-Romeu, 1977). At high concentrations of sodium sulphate, the sulphate influxes increase slightly, and in contrast to what was found to occur over the range of high NaCl solutions, the $J_i Na^+$ between 4 and 115 meq were practically constant (the increase not being significant); the $J_n H^+$ also remained practically the same at these two concentrations. It should be noted that at the highest $Na_2SO_4$ concentrations, as the sulphate transport increases, these ions may participate to maintain electroneutrality. Figure 4 gives $J_i$Na as a function of $J_{iNa}/Na_{ext}$. A comparison of this figure with Fig. 2 shows that

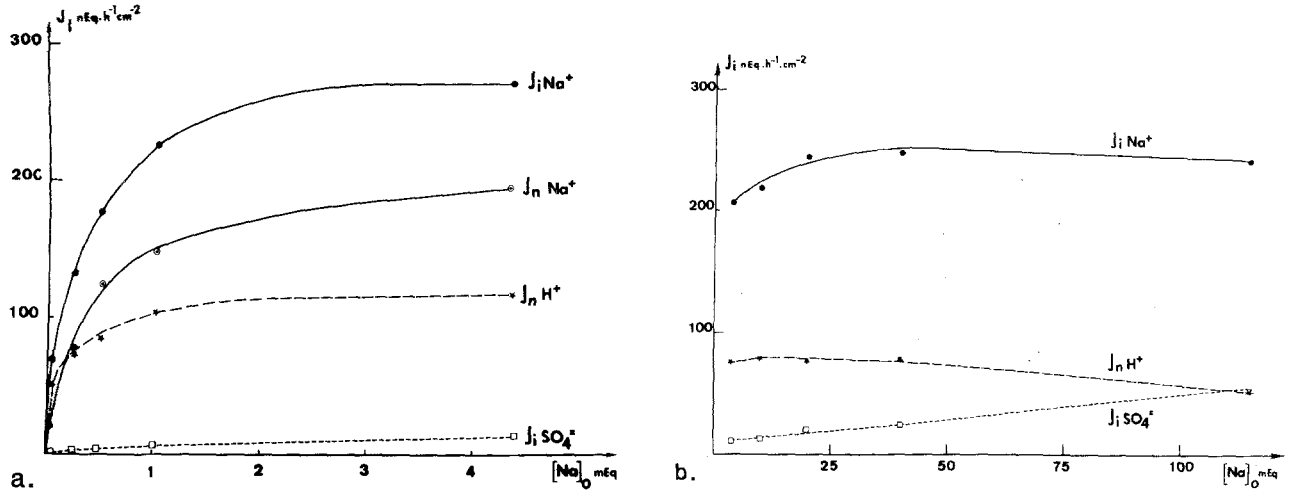


**Fig. 3.** (*A*): Evolution of $J_iNa^+$, $J_nNa^+$, $J_nH^+$ and $J_iSO_4^=$ ($n=6$) as a function of the external $Na_2SO_4$ concentration (below 4 meq). (*B*): Evolution of $J_iNa^+$, $J_nH^+$ and $J_iSO_4^=$ ($n=7$) as a function of the external $Na_2SO_4$ concentration (above 4 meq). Internal solution, Ringer. $J_iNa^+$ and $J_iSO_4^=$: influxes of sodium and sulphate, respectively; $J_nNa^+$ and $J_nH^+$: net fluxes of sodium and hydrogen, respectively. The net fluxes of sodium were positive (absorption) and the net fluxes of hydrogen were negative (excretion).

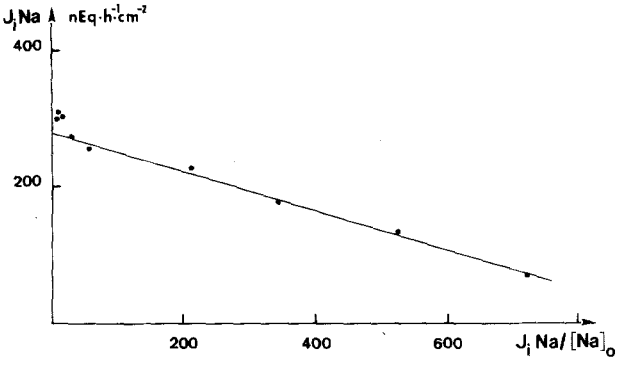


**Fig. 4.** Na influx ($J_iNa$) plotted against the Na influx divided by the sodium concentration ($J_iNa/[Na]_o$). The experimental points are those of Fig. 3.

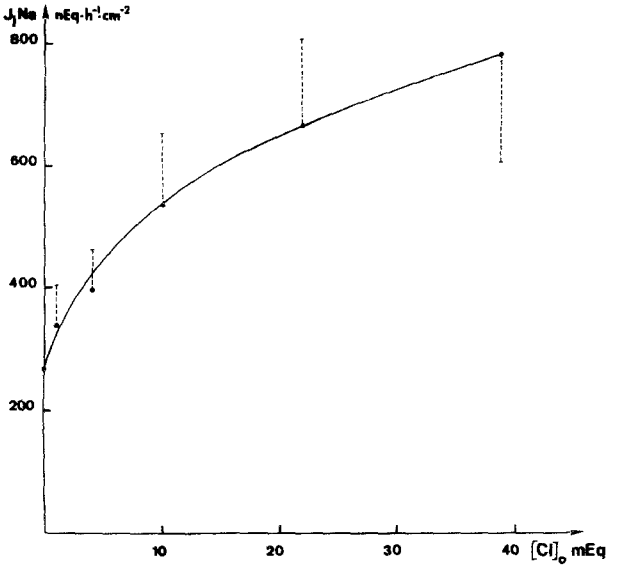


**Fig. 5.** Stimulation of $J_iNa$ by the addition of increasing concentrations of choline chloride to a 50 meq $Na_2SO_4$ external solution. Internal solution: Ringer.

component II is not present or is very small with $Na_2SO_4$ solutions. Nevertheless, at low concentrations a well-defined component persists with kinetic parameters comparable to those of component I found with NaCl solutions: $K_{0.5}=0.287\pm0.13$ meq and $V_{max}=278\pm6$ neq·hr$^{-1}$·cm$^{-2}$.

The above results indicate that component I of $Na^+$ transport is independent of the accompanying anion. Component II functions at high external $Na^+$ concentrations in the presence of $Cl^-$ but does not occur with $SO_4^=$; it would thus appear to be linked with anionic permeability. This dependence of a permeable anion was confirmed by the following experiment: at an external concentration of 50 mM $Na_2SO_4$ (at which $C_{I\text{-}Na}$ is saturated), the evolution of sodium influxes as a function of choline chloride concentration (0.1, 4, 10, 22, 40 meq $Cl^-$) was followed. The results are given in Fig. 5 and show that $Na^+$ influx is stimulated by the presence of chloride and is a function of the concentration of this anion in the external solution. This stimulation is not due to an effect of the choline ion (Macey & Koblick, 1963), since the same increase in sodium absorption is obtained with both KCl or choline chloride (Table 2).

The following experiments were performed in order to test if acetazolamide which is a known inhibi-

**Table 2.** Comparison between the effect of addition of KCl and choline chloride to the external solution on the sodium fluxes

| | Control | II | III | Control |
|---|---|---|---|---|
| $J_i$Na | 357±91 | 678±142 | 720±123 | 430±62 |

Control: external solution, 50 meq $Na_2SO_4$; II: external solution, 50 meq $Na_2SO_4$+50 meq KCl. III: external solution, 50 meq $Na_2SO_4$+50 meq choline chloride; $n=5$; $J_i$Na=influx of sodium in neq·hr$^{-1}$·cm$^{-2}$.

**Table 3.** Effect of acetazolamide ($10^{-3}$ M) on sodium influxes across *R. esculenta* skin

| | 1 mM NaCl $J_i$Na | 115 mM NaCl $J_i$Na |
|---|---|---|
| Control | 255±21 | 572±64 |
| Acetazolamide | 126±13 | 406±44 |
| Difference | 129±25[a] | 166±33[a] |
| $n$ | 14 | 38 |

External solution: 1 or 115 mM NaCl. Internal solution: Ringer; acetazolamide was added to the internal medium. Fluxes expressed in neq·hr$^{-1}$·cm$^{-2}$. $J_i$=mean±SE of influx. $n$=number of animals.
[a] $P<0.001$.

tor of the $Na^+/H^+$ exchange mechanism (Ehrenfeld & Garcia-Romeu, 1977) was affecting the Na transport with high NaCl external solutions. Table 3 summarizes the results obtained on two groups of skins: on the first group it can be confirmed that acetazolamide blocked 50% of the Na transport when the skin is bathed with a low NaCl solution; the results obtained with the second group shows that the diuretic significantly inhibits 29% of the Na transport when the skin is bathed with 115 mM of a NaCl solution. This result suggests the persistence of the high affinity component of the Na transport with high NaCl external solutions.

Amiloride ($5\times10^{-5}$ M) and ouabain ($10^{-4}$ M) inhibit $Na^+$ transport in all ranges of NaCl and $Na_2SO_4$ concentrations studied.

As it is well known that variations of Na concentrations in the mucosal solution results in modifications of the transepithelial DP (Koefoed Johnsen & Ussing, 1958) and that changes in potential gradient across the skin modify the $Na^+$ fluxes (Ussing & Zerahn, 1951), it appears of importance to follow the DP across the skin in our experimental conditions. Figure 6 reports the transepithelial DP measured as a function of the NaCl or $Na_2SO_4$ concentrations of the external medium. In both cases the DP is a linear function of the log $Na_{ext}$ for external sodium concentrations lower than 10 meq. The slopes $\frac{V_i - V_e}{\log Na_{ext}}$ found with the external side of the skin bathed with a NaCl or $SO_4Na_2$ solution are, respectively, 39.8±1.8 ($n=14$) and 38.9±1.5 ($n=7$) mV per decade concentration change. These values are not significantly different. For external sodium concentrations higher than 10 meq, the DP is practically constant as well with NaCl or $Na_2SO_4$ solutions.

## Discussion

Since transport of an ion across an epithelium is not simply the interaction of the ion with a transport site but rather the results of a series of related events, kinetic parameters are macroscopic constants, the significance of which at the molecular level remains unknown. Nevertheless, they may serve to characterize and identify different systems.

In the present work sodium and chloride influxes were measured over a wide range of external sodium ion concentrations. The external ionic concentration was the only controlled variable. Controlling other parameters such as the transepithelial difference of electric potential is often illusory since this introduces new uncontrolled variables. Thus, annulling the transepithelial potential by a short-circuit current sets up (Ussing & Windhager, 1964; Whittembury, 1964; Cereijido & Curran, 1965) or increases a negative intracellular potential (Helman & Fisher, 1977; Nagel, 1976, 1977) which is a function of the external

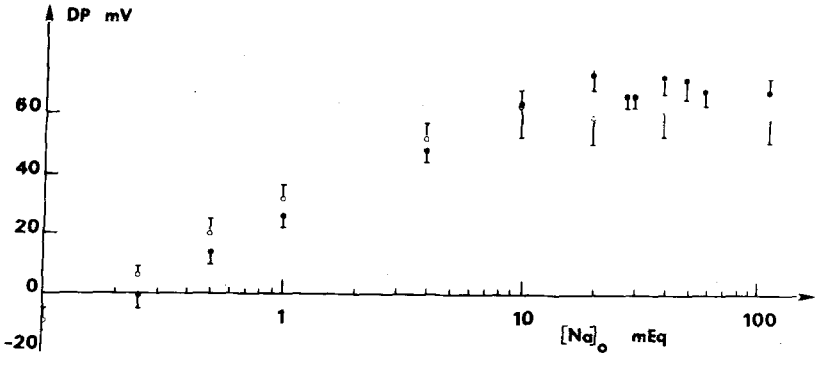


**Fig. 6.** Transepithelial potential difference (DP) across *Rana esculenta* skin, at different concentrations of NaCl (●) and $Na_2SO_4$ (○) solutions. Abscissa in logarithmic scale. DP in mV and concentrations in meq.

sodium concentration (Nagel, 1977). Furthermore, conditions which may effectively depolarize the internal and external epithelial barriers ($K^+$-Ringer and short-circuit conditions) (Rawlins et al., 1970) inhibit the active proton pump and thereby inhibit the $Na^+_{ext}/H^+_{int}$ exchange system (J. Ehrenfeld & F. Garcia-Romeu, *unpublished results*). For this reason, we decided to perform our experiments in open-circuit conditions.

### *I. Evidence for Two Sodium and Chloride Transport Components Related to External Concentration*

Under very varied experimental conditions sodium transport across the skin apparently obeys saturation kinetics of the Michaelis-Menten type (*see* Table 4). In amphibians studied in vivo in an external ionic concentration similar to that of freshwater the $K_{0.5}$ of the sodium transport mechanism varies between 0.05 meq for the most aquatic species studied (*Xenopus laevis*) and 1.1 meq for the most terrestial (*Bufo americanus*), with most species having values between 0.2 and 0.5 meq (Alvarado & Dietz, 1970; Greenwald, 1971, 1972). Similar values have been found in vivo in other freshwater animals (*see* Maetz, 1974). With the isolated skin mounted in open- or short-circuit conditions, however, the affinity of the sodium transport system is considerably lower, the $K_{0.5}$ being between 4.0 and 20 meq (Kirschner, 1955; Gil Ferreira, Guerreiro & Gil Ferreira, 1973; Mandel & Curran, 1973; Moreno et al., 1973; Zeiske & Lindemann, 1974; Mandel, 1978). These in vitro studies were carried out at external sodium concentrations between 6 and 120 meq. The differences between the results obtained in vivo and in vitro could have been due either to a true modification of the sodium transport caused by isolating the skin or to different experimental conditions, especially the concentration ranges employed in the two types of experiment. The present work confirms the second hypothesis. Thus, it shows that when the external surface of the isolated skin is bathed in dilute (0.1 to 4 meq) NaCl or $Na_2SO_4$ solutions a sodium transport system with a high affinity for this ion is predominant. The measured $K_{0.5}$, 0.220 meq in NaCl and 0.287 meq in $Na_2SO_4$, is similar to the in vivo values found by the various workers cited above. When the external surface is in contact with NaCl solutions more concentrated than 4 meq, on the other hand, the measured affinities are 60 times lower than these and comparable with the published in vitro results. It is relevant here to recall that our results were obtained from skins selected for their capacity to absorb sodium from dilute external solutions.

*a) High affinity components:* The nature of the two systems would appear to be very different. The component of the sodium transport which is saturated at low external NaCl or $Na_2SO_4$ concentration is independent of the anion accompanying the sodium and thus presents characteristics of the cationic exchange system linking proton excretion with sodium absorption (Garcia-Romeu, Salibian & Pezzani-Hernandez, 1969; Garcia-Romeu & Ehrenfeld, 1975*a–b*; Ehrenfeld & Garcia-Romeu, 1977); the high affinity of this component reflects its capacity for absorbing

**Table 4.** Kinetic parameters of Na transport across frog skin

| Animal | $J_{13}$ | | $J_{12}$ | | Experimental condition | Reference |
|---|---|---|---|---|---|---|
| | $k_m$ | $V_{max}$ | $K_m$ | $V_{max}$ | | |
| *Rana pipiens* | 0.2 | 246[a] | | | in vivo | Greenwald, 1971 |
| *Rana pipiens* | 3–10 | | | | SC | Brown, 1962 |
| European frog | 4.3 | 1,160 | | | SC | Kirschner, 1955 |
| *Rana pipiens* | 10 | 2,000 | | | SC | Cereijido et al., 1964 |
| *Rana pipiens* | | | 14.3 | 4,000 | SC | Biber & Curran, 1970 |
| *Rana temporaria* | | | 15 | 600 | O.C. | Erlij & Smith, 1971 |
| *Rana temporaria* | | | 24 | 1,280 | O.C. | Erlij & Smith, 1973 |
| *Rana ridibunda* | 25.5 | | | | SC $Na_2SO_4$ | Ferreira et al., 1973 |
| | 4.45 | | | | SC NaCl | Ferreira et al., 1973 |
| *Leptodactylus ocellatus* | 19.1 | 2,790 | 22.1 | 2,700 | O.C. | Moreno et al., 1973 |
| *Rana pipiens* | | | 6.02 | 1,680 | SC | Cruz & Biber, 1976 |
| *Rana esculenta* | 0.22 | 331 | | | O.C. low Na concentration | This work |
| *Rana esculenta* | 13.5 | 904 | | | O.C. high Na concentration | This work |

$J_{13}$ represents the transepithelial influx and $J_{12}$ the influx of Na from the external medium to the transporting compartment. $J_{13}$ and $J_{12}$ are expressed in $neq \cdot hr^{-1} \cdot cm^{-2} \cdot k_m$ in meq.

[a] $V_{max}$ expressed in $neq \cdot hr^{-1} \cdot g^{-1}$.

sodium from dilute solution such as occur in the animal's habitat. A high affinity component is also found in chloride transport from dilute solutions, its $K_{0.5}$ being significantly different from that of the sodium transport mechanism and comparable with the in vivo and in vitro kinetic parameters reported by Alvarado et al. for chloride transport from diluted solutions (Alvarado & Dietz, 1970; Alvarado, Dietz & Mullen, 1975; Alvarado, Poole & Mullen, 1975). By their level of saturation and their affinity this system may be identified with the physiological transport mechanism $Cl^-_{ext}/HCO^-_{3int}$ which is independent of the presence of sodium (Garcia-Romeu et al., 1969; Alvarado & Dietz, 1970; Alvarado et al., 1975; Alvarado et al., 1975; Garcia-Romeu & Ehrenfeld, 1975*a–b*; Ehrenfeld & Garcia-Romeu, 1978).

*b) Low affinity components:* The importance, and even the presence, of a low affinity sodium transport component is strictly determined by the permeability of the anion accompanying the sodium in the external medium. It occurs at high external concentrations of NaCl but disappears when the chloride is replaced by a relatively impermeable anion such as sulphate. In point of fact, the permeability of sulphate is variable and it is possible that this component may occur at high sulphate concentrations when the permeability of this anion is not negligible. A certain amount of evidence points out the very strong link existing between sodium and chloride transport: (i) within the range of concentrations at which the low affinity component has been shown to occur, the $K_{0.5}$ of the sodium transport mechanism ($13.5 \pm 2.2$ mM) is not significantly different from that of the chloride transport ($15.4 \pm 3.6$ mM); (ii) at high concentrations of external NaCl the sodium and chloride influxes are very similar; (iii) under these conditions the specific inhibitors of sodium transport (ouabain, amiloride) also inhibit chloride transport (Garcia-Romeu & Ehrenfeld, 1975*a–b*). The necessity of preserving electroneutrality can explain such an interdependence of the sodium and chloride transport in open circuit; other types of interaction have been proposed to explain the effect of anions on sodium transport in short-circuit conditions (Zadunaisky & De Fish, 1964; Lindley & Hoshiko, 1964; Gil Ferreira, 1968; Cuthbert, Painter & Prince, 1969; Huf, 1972; Ques-von Petery, Rotunno & Cereijido, 1978; Rotunno, Ques-von Petery & Cereijido, 1978; Ferreira & Hill, 1978) where only sodium shows a net absorption (Ussing & Zerahn, 1951).

*c) Influence of the* $DP_{Trans}$: For the reasons already mentioned at the beginning of the discussion, we decided to perform our experiments with skin mounted in open-circuit conditions. As can be seen in Fig. 6, the transepithelial DP is an increasing function of the external Na concentration and saturates at high Na concentrations. Such a transepithelial DP dependence of the outer Na concentration has been found by several authors (Steinbach, 1933; Koefeld-Johnsen & Ussing, 1958; Nagel, 1977), and a tendency of the DP to saturate at high Na concentrations has been generally reported (Steinback, 1933; Brown, 1962; Smith, Martin & Huf, 1973; Rodriguez Boulan et al., 1978). The slope that we have found at low concentrations (39 mV per decade concentration change) is much lower than the 58 mV predicted on the basis of the results of Koefoed-Johnsen and Ussing (1958) but is close to that obtained by other authors (Smith, Martin & Huf, 1973; Nagel, 1977; Rodriguez Boulan et al., 1978). In agreement with various authors (Nagel, 1977; Rodriguez Boulan et al., 1978), we did not find any difference in the slope $\frac{V_i - V_e}{\log Na}$ obtained with NaCl or $Na_2SO_4$ external solutions at low concentrations. That skins bathed with permeant and nonpermeant anions have the same transepithelial DP (maximum around 10 meq) may seem surprising. It would suggest that chloride entrance which is mainly mediated by the $Cl^-/HCO_3^-$ exchange mechanism in this range of low NaCl concentrations does not contribute to the transepithelial DP.

The question that can be raised now concerns the influence of the transepithelial DP on the two components found in this study. Figures 1 and 2 show that a Na transport mechanism is seen at high Na concentrations in a NaCl solution but either does not occur or is very small with $Na_2SO_4$ solution (Figs. 3 and 4). As the transepithelial DP is independent of the nature of the anion accompanying the $Na^+$ in the outer solution (*see* Fig. 6), the presence of this anion-dependent Na transport cannot be attributed to changes in transepithelial DP. Furthermore, the transepithelial DP is practically constant over the range of concentrations in which this Na transport occurs. For low Na concentrations in the external bath, we have found a sodium transport component independent of the anion accompanying the sodium and which presents all the characteristics of the Na/H exchange mechanism (*see* Ehrenfeld & Garcia, 1977). These authors demonstrated in *R. esculenta* skin the presence of an active excretion of protons linked to the absorption of sodium in open-circuit conditions. This $H^+$ excretion was practically unaffected (diminution of less than 10%) by the variations of the potential from 31 to 0 mV (short-circuit conditions), showing that the driving force for $H^+$ excretion is not the development of a potential linked to the

sodium transport. Thus, it appears that the coupling mechanism linking the Na absorption to proton excretion is responsible for the saturation curves obtained and not the variations of the potential by itself.

### *II. Significance of the Kinetic Parameters Characterizing the Two Components*

The type of curve which is obtained, with its two Michaelis-type components, can be interpreted as representing two saturable systems functioning in parallel. At low NaCl concentrations, the high affinity component will be predominant and for high NaCl concentrations a low affinity component will develop and coexist with the first mechanism of $Na^+$ transport, as it can be deduced from the inhibitory effect of acetazolamide persisting at high NaCl concentrations (*see* Table 3).

Curves such as described above with one or several inflection points between Michaelis-type sections are characteristic of numerous transport processes in plant tissues (Epstein, 1966; Laties, 1975; Nissen, 1974). Sometimes these processes are extraordinarily similar to those of epithelial transport in the frog (*see* Epstein, Rains & Elzam, 1963).

The results discussed in the present work confirm that in open circuit transepithelial sodium and chloride transports are brought about by very different mechanisms in dilute solutions and in concentrated solutions. In the former the counter-transports $Na^+_{ext}/H^+_{int}$ and $Cl^-_{ext}/HCO^-_{3int}$ occur, while in concentrated NaCl solutions a transport of sodium with chloride takes place. Our results also show once again that there is no essential difference between transport processes in vivo and in vitro provided that the two types of preparation are studied in identical experimental conditions.

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