

Influence of Transepithelial Potential Difference on the Sodium Uptake at the Outer Surface of the Isolated Frog Skin

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ABSTRACT The unidirectional uptake of sodium across the outer surface of the isolated frog skin (J_{12}^{Na}) was measured in the presence of transepithelial potential difference ($\Delta\psi$) ranging from +100 to -100 mV. With a sodium concentration of 115 mM in the bathing solutions J_{12}^{Na} increases significantly when the spontaneous $\Delta\psi$ is reduced to zero by short-circuiting the skin. With an Na concentration of 6 mM a progressive increase J_{12}^{Na} can be observed when $\Delta\psi$ is decreased in several steps from +100 to -100 mV (serosal side positive and negative, respectively). The observed change J_{12}^{Na} amounts to a fraction only of that predicted from the shift in $\Delta\psi$. The results suggest that under open circuit conditions the potential step across the outside surface is at most one half of $\Delta\psi$ and that the resistance across the outside and inside barrier of the skin is ohmic. This is in agreement with measurements of intracellular potentials in the frog skin and with resistance measurements carried out in the toad skin. The data strongly support the view that the saturating component of J_{12}^{Na} proceeds via a charged carrier system. Exposure to negative values of $\Delta\psi$ of 50 mV or more for times of 24 min or more result in a marked reduction of J_{12}^{Na} which shows only partial or no reversibility.

Recent experiments from different laboratories (1-6) have shown that the Na uptake across the outside surface of the frog skin involves an interaction with the membrane or some component in the membrane. These findings contradict the classical concept that the uptake of Na across the mucosal surface of epithelial cells in general proceeds by simple diffusion. We found that the uptake of Na across the outside barrier of the frog skin as measured by the unidirectional influx of Na is not a linear function of the Na concentration in the bathing solution and that it is competitively inhibited by the presence of Li ions in the bathing solution (1). Furthermore, we observed that the Na influx is inhibited by lack of oxygen, ouabain, and amiloride (2).

All our previous measurements were done under short-circuit conditions.

The fact that one can observe sizeable potential differences across the isolated frog skin under open circuit conditions prompted us to investigate the extent to which the sodium uptake across the outer surface of the skin is influenced by changes in transepithelial potential differences. A study of this kind may not only provide us with additional information about the mechanism of the Na uptake, but may also give us valuable estimates about the size of the "apparent" potential step across the outside border of the skin. This question is also of special interest in connection with a number of recent publications on Na transport by the frog skin. In three very recent investigations (5-7) the unidirectional uptake across the outer surface of the frog skin was studied under open circuit conditions with varying Na concentrations in the bathing solutions. Under these conditions the spontaneous potential difference across the skin (8) and across the outer surface (9) may vary considerably (anywhere from 0 to over 100 mV) since it is affected by the Na concentration in the bathing solutions. However, in these particular studies the effect of changes in transepithelial potential difference per se on Na uptake was not examined. Several other workers have investigated the effect of changes in transepithelial potential difference on oxygen consumption (10) and on unidirectional transepithelial sodium fluxes (11) in the frog skin and urinary bladder of the toad (12). A more precise knowledge about the relationship between this potential difference and Na uptake may enable us to evaluate what role this phase of the Na transport system plays in these overall processes.

GLOSSARY

J_{12}^{Na}	unidirectional influx of Na across outer surface of frog skin (= Na influx or zero time rate of Na uptake or simply Na uptake)
J_{21}^{Na}	unidirectional efflux of Na across outer surface of frog skin (= Na efflux)
J_{13}^{Na}	unidirectional influx of Na across entire frog skin (= transepithelial influx = J^{IN})
J_{31}^{Na}	unidirectional efflux of Na across entire frog skin (= transepithelial efflux = J^{EFF})
$J_{12}^{\text{Na}} \text{ net}$	net flux of Na across outer surface of frog skin
$J_{13}^{\text{Na}} \text{ net}$	net flux of Na across entire frog skin
$\Delta\psi_{13}$ or simply $\Delta\psi$	potential difference across entire frog skin (serosal side with respect to mucosal or outside)
$\Delta\psi_{12}$	potential difference across outer surface of frog skin
I	electrical current density across total frog skin
I_0	electrical current density under short-circuit conditions ($\Delta\psi = 0$)
R	total resistance across entire frog skin

METHODS

The method used for measuring J_{12}^{Na} has been described in detail previously (1, 2, 13). In brief, circular pieces of the abdominal skin of *Rana pipiens* were mounted between two chambers (exposed area 0.42 cm^2)¹ which, with the exception of tracers and drugs, always contained solutions of the same composition. The chambers were connected via bridges, calomel half-cells, and silver wires to an automatic clamping device which maintained the skins in clamped conditions, anywhere from -200 to $+200$ mV, or in open circuit conditions. Before the Na influx determination, the skins were allowed to equilibrate and were subjected to the various experimental procedures outlined below. Then, at time zero, the solution bathing the outside surface of the skin was withdrawn and rapidly replaced by a test solution which was identical to it except for the addition of D-[1-³H]mannitol ($10-12 \text{ } \mu\text{Ci/ml}$), [¹⁴C]inulin ($1-2 \text{ } \mu\text{Ci/ml}$) and either ²⁴Na ($5-15 \text{ } \mu\text{Ci/ml}$) or ²²Na ($5-7 \text{ } \mu\text{Ci/ml}$). In some experiments no [¹⁴C]inulin was added. After the outside surface of the skins had been exposed to the test solution for an appropriate time (30 s unless otherwise indicated), a number of steps which are indicated in the schematic drawings on Fig. 1 were carried out in rapid succession. The skin was removed from the tracer containing test solution and blotted on several layers of Whatman no. 1 filter paper. Then the solution bathing the serosal side was sucked out of the inside chamber. The section of skin which had been exposed to tracer was punched out of the holder, dropped into a test tube containing 2 ml of 0.1 N nitric acid and shaken for 2 h. At the end of this time the eluate was assayed for radioactivity by counting in a liquid scintillation spectrophotometer. The activity levels of [³H]mannitol and of [¹⁴C]inulin served as an indicator for the amount of radioactive test solution remaining on the outside surface of the skin after blotting.

The skins obtained from each frog were assigned to different groups (experimental, controls) by matching the values of I_o , spontaneous $\Delta\psi$, and R recorded after the initial equilibration period. It proved possible to match the skins in such a way that in any given experiment the average values of these electrical parameters did not differ more than 20% (usually 5-10%) between the groups. In the experimental period which followed, the various groups of skins were maintained at different values of $\Delta\psi$ either by clamping or by leaving them under open circuit conditions. The influx determinations were then carried out at the level of $\Delta\psi$ maintained or observed during the experimental period or with $\Delta\psi = 0$ mV. In the experiments in which $\Delta\psi$ was different from the open circuit value the skin preparations were stabilized at the experimental value of $\Delta\psi$ for at least 6 min before determining J_{12}^{Na} because we noted regularly that the rapid change in current observed during the first

¹ It may be worth mentioning that in spite of the small area used in these chambers no signs of edge damage could be detected. Dobson and Kidder (14) have described pronounced effects when the area of skins mounted in conventional chambers is decreased. However, the method for mounting the tissue is different here, since we use very flexible material with a large contact area. Spontaneous $\Delta\psi$, R , I_o are not significantly different when separate areas of the same abdominal skin are placed in our chamber or in much larger conventional chambers. In addition, J_{31}^{Na}/J_{13}^{Na} net obtained in our chambers is not different from the corresponding ratio obtained in chambers with a surface area of 7 cm^2 .

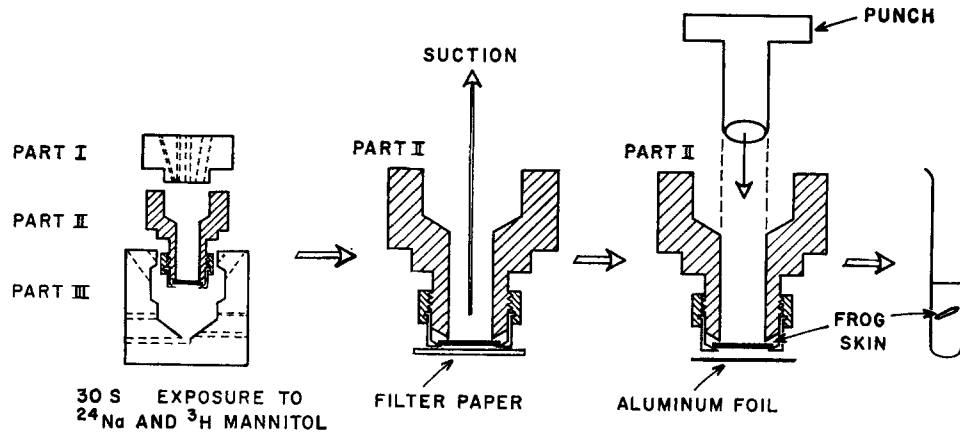


FIGURE 1. Schematic diagram of sequential steps performed during influx determination.

few minutes of clamping was followed by a much more stable condition after several minutes.

In those experiments in which a comparison was made between the effect of open and short-circuit conditions on Na uptake all tissues were clamped at $\Delta\psi = 0$ mV for 6 min immediately preceding the 6 min experimental period and the subsequent influx determination. Two different Na concentrations were used for these experiments. In the first series of experiments, the Ringer solutions contained (in millimoles/liter): 115 NaCl, 2.5 KHCO_3 , and 1 CaCl_2 . In the second series of experiments and in all other experiments reported here, all but 6 mM NaCl was replaced by choline chloride.

The determinations of J_{12}^{Na} at values of $\Delta\psi$ ranging from -100 to $+100$ mV were made in two separate sets of experiments which were carried out alternately. In one set J_{12}^{Na} was measured in three groups of skins at a $\Delta\psi$ of $+20$, $+60$, and $+100$ mV (serosal side positive) and in the other set J_{12}^{Na} was determined in another three groups of skins at a $\Delta\psi$ of 0 , -50 , and -100 mV. All skins were first observed for 6 min at a $\Delta\psi$ of 0 mV. Then they were clamped for 6 min at the experimental value of $\Delta\psi$ (i.e. $+100$, $+60$, $+20$, 0 , -50 , and -100 mV) at which the influx determination was made. For this series of experiments we chose 6–8 min as the upper time limit for exposure to different values of $\Delta\psi$ because pilot experiments showed that longer exposure times may result in irreversible changes in the spontaneous values of $\Delta\psi$, I_o , and R , at negative values of $\Delta\psi$ of 50 mV or more. After 24 min these changes occurred regularly.

In another series of four experiments the J_{13}^{Na} was compared to the J_{12}^{Na} obtained in the same skins. In each experiment, two matched pieces of skin obtained from the same frog were used as a pair. After an initial equilibration period with a value of $\Delta\psi$ of 0 mV, the two skins were subjected to the same protocol except that one skin was clamped at a $\Delta\psi$ of 0 and the other skin was maintained at a $\Delta\psi$ of 100 mV. To determine the transepithelial influx, J_{13}^{Na} , the outside surface of the skin was ex-

posed to a test solution containing ^{24}Na and the rate of appearance of the isotope in the inside bathing solution was measured by sampling and replacing the inside bathing solution every 2 min. During this procedure the current flow through the chamber and the skin had to be interrupted briefly (<5 s). Immediately after the 10th collection, i.e. after 20 min exposure to the ^{24}Na , the part of the chamber on which the skin was mounted was separated from the other part of the chamber in which the outside bathing solution was present. The outside surface of the skin was blotted and the mounted skin transferred to another set-up in which the J_{12}^{Na} was measured in the usual way using ^{22}Na and [^3H]mannitol as tracers. The radioactivity levels of ^{22}Na and [^3H]mannitol were determined 2 wk later. Standards of ^{24}Na indicated that this tracer had decayed to background levels by this time.

The determinations of J_{12}^{Na} were alternated between the experimental and control group whenever there was a possibility that aging of the preparation might affect one group more than another. Regression lines were calculated by the least squares method. One frog was used per experiment unless stated otherwise.

RESULTS

A. Accumulation of Radioactive Na as a Function of Time

The uptake of radioactive Na by the frog skin is a linear function of time under short-circuit conditions at exposure times ranging from 8 to 32 s (1, 2, 13). In Fig. 2 the uptake of radioactive Na under open circuit conditions is plotted against the time of exposure to the tracer. The data were obtained from one frog with a Na concentration of 6 mM in the bathing solutions and indicate that the tracer accumulation in the tissue increases linearly with time. The regression line has an intercept which is close to and not significantly different from zero which suggests that the amount of tracer

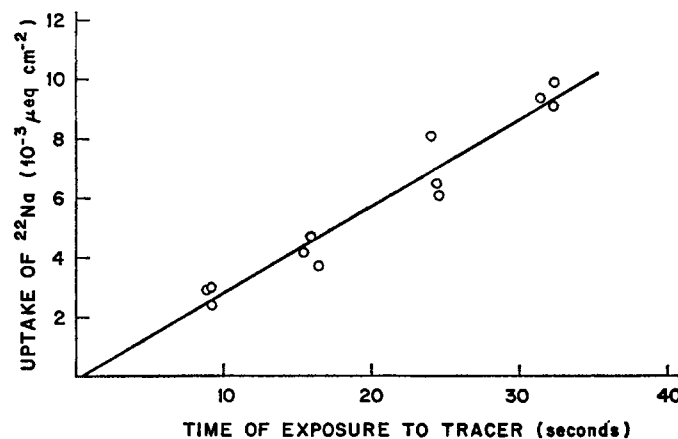


FIGURE 2. Uptake of radioactive Na as a function of exposure time to tracer. Individual points indicate single flux determinations. Skins obtained from one frog. Na concentration 6 mM. Regression line $0.29 (\pm 0.02) \times -0.14 (\pm 0.49)$, $r = 0.973$.

remaining on the outside surface of the skin after blotting has been adequately accounted for by subtracting from the total tissue activity the tracer located in the [³H]mannitol space. A corresponding correlation between tracer uptake and exposure time was obtained under open circuit conditions using a Na concentration of 115 mM.

In order to test whether the uptake of tracer is linear with time under the other experimental conditions used in this study, J_{12}^{Na} was determined employing exposure times to the radioactive Na of 16 and 32 s with $\Delta\psi$ set at +100 mV (six determinations at each point) and -100 mV (seven determinations at each point). At both potentials, the fluxes calculated from the 16 s exposure time were close to and not significantly different from those obtained with a 32 s exposure time. This suggests that within a time period of 32 s the accumulation of tracer is not large enough to raise the specific activity of Na in the tissue to levels at which backflux of tracer into the outside bathing solution or extrusion of the isotope into the inside bathing solution play a significant role. Indeed, with the short exposure times which were employed in these experiments we have never observed that radioactive Na crosses the epithelium and appears in the inside bathing solution. All this indicates that, under these conditions, the initial rate of accumulation of radioactive Na determined with this method is an appropriate measure for the unidirectional influx of Na across the outside surface of the frog skin.

B. Comparison between J_{12}^{Na} Obtained under Open Circuit and Short-Circuit Conditions

Four experiments were carried out with a Na concentration of 115 mM in the bathing solutions in order to compare the J_{12}^{Na} obtained under open circuit conditions with the one measured with $\Delta\psi = 0$ mV. The results of these experiments are summarized in Table I. They show that there is a significant

TABLE I
COMPARISON BETWEEN Na INFLUX UNDER OPEN CIRCUIT (x) AND SHORT-CIRCUIT (s) CONDITIONS

Experiment	Number of observations		Average PD x only	Na influx x	I_o^*x
	x	s		Na influx s	I_o^*s
1	3	3	41.8 ± 6.2	0.808	0.99
2	4	5	55.6 ± 3.2	0.758	0.88
3	4	4	55.9 ± 5.0	0.815	1.08
4	5	4	36.0 ± 3.7	0.766	0.86
1-4	16	16	47.3 ± 5.0	0.79 ± 0.01	0.95 ± 0.05

* Short-circuit current measured before the Na influx determination. Na concentration 115 mM. The average values for Na influx under open and short-circuit conditions were 2.76 ± 0.23 (16) and 3.62 ± 0.27 (16) $\mu\text{eq h}^{-1} \text{cm}^{-2}$, respectively ($P < 0.025$). The difference between the average values listed on the last line of the second last and last column are significantly different.

increase in J_{12}^{Na} when the spontaneous $\Delta\psi$ across the frog skin averaging 47 mV is reduced to 0. The open circuit and short-circuit groups were reasonably well matched as indicated by a ratio of 0.95 ± 0.05 for the value of I_o measured initially.

J_{12}^{Na} was measured in five experiments at a Na concentration of 6 mM in open and short-circuited preparations. The data are shown in Fig. 3 as a function of the I_o measured before the influx determination. Only a few determinations were made in short-circuited skins since the relationship between I_o and J_{12}^{Na} is already well known under these conditions (2, 13).

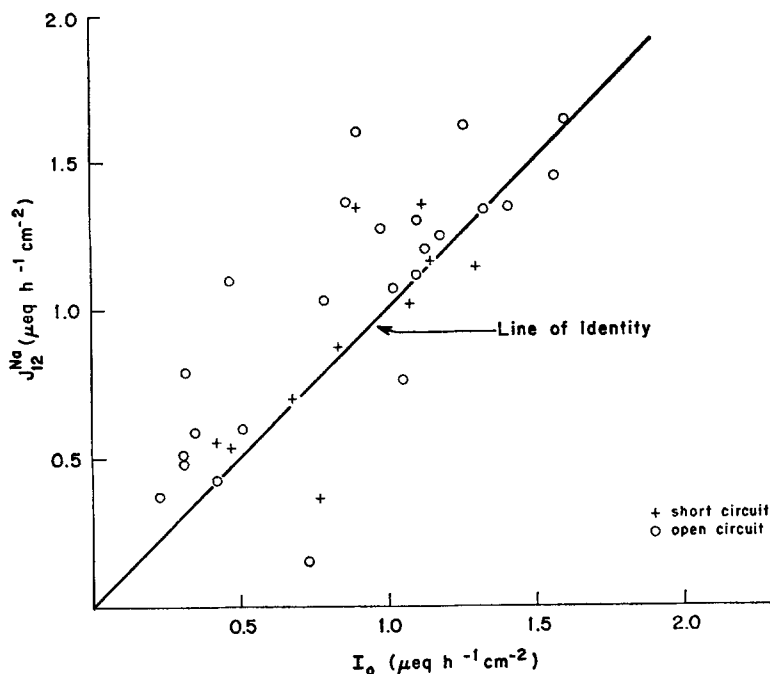


FIGURE 3. Plot of J_{12}^{Na} vs. I_o recorded before the influx measurement. J_{12}^{Na} measured under short-circuit (+) or open circuit (O) conditions. Na concentration 6m M.

There is a considerable amount of scatter in the influx values obtained in both groups of skins, but this can be ascribed to the different levels of sodium transport in individual skins. This can be seen from Fig. 3 as there is a highly significant correlation between the I_o recorded before the Na influx determination and the Na influx measured subsequently. This applies not only for influx values obtained in short-circuited skins but also for J_{12}^{Na} obtained in open circuit skins. The regression line is $0.82 (\pm 0.13) \times + 0.29 (\pm 0.12)$, $r = 0.79$ for the 26 measurements obtained under open circuit conditions. The correlation coefficient for the short-circuited skins is less significant for this series of measurements ($r = 0.78$ for nine measurements) but has been shown to be highly significant in previous studies (2, 13). From Fig. 3 it is

quite clear that the J_{12}^{Na} measured under open circuit conditions is very similar to the one measured under short-circuit conditions. The spontaneous $\Delta\psi$ of the 26 skins used for the open circuit measurements was 14.0 ± 1.1 mV.

C. J_{12}^{Na} Measurements at $\Delta\psi$ Ranging from -100 to $+100$ mV

165 flux measurements were made in 13 experiments in order to study the effect of different levels of $\Delta\psi$ on the J_{12}^{Na} at a Na concentration of 6 mM. In six experiments J_{12}^{Na} was measured at -100 mV, -50 mV, and 0 mV and in seven experiments carried out alternately J_{12}^{Na} was determined at $+20$, $+60$, and $+100$ mV. The results of the 165 influx determinations are shown in Table II. A shift of $\Delta\psi$ from 0 to -100 mV causes an increase in J_{12}^{Na} of some 80% and a change in $\Delta\psi$ from 0 to $+100$ mV is accompanied

TABLE II
EFFECT OF CHANGES IN $\Delta\psi$ ON J_{12}^{Na}

$\Delta\psi$ during influx	J_{12}^{Na}	SE of normalized J_{12}^{Na}	I_o before experiment	Number of observations	I
mV	$\mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^2$		$\mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-1}$		$\mu\text{eq} \cdot \text{h}^{-1} \cdot \text{cm}^{-2}$
+100	0.431 ± 0.039	± 0.028	0.972 ± 0.100	29	6.024 ± 0.532
+60	0.608 ± 0.062	± 0.033	0.904 ± 0.090	28	3.179 ± 0.520
+20	0.881 ± 0.096	± 0.027	0.870 ± 0.084	30	0.566 ± 0.174
0	1.406 ± 0.104	± 0.045	0.997 ± 0.069	25	-1.079 ± 0.086
-50	2.011 ± 0.146	± 0.077	0.959 ± 0.076	29	-4.015 ± 0.217
-100	2.555 ± 0.245	± 0.160	0.979 ± 0.078	24	-8.492 ± 0.584

by a decrease in the value of J_{12}^{Na} to about 30% of the value obtained under short-circuit conditions. Part of the scatter in the data is related to the different rates at which Na is actively transported, which one may observe in individual skin preparations at the beginning of the experiments before the skins are clamped at various values of $\Delta\psi$. A plot of the I_o obtained in a given skin at the end of the equilibration period (i.e. before the experimental $\Delta\psi$ is employed) against the J_{12}^{Na} measured in the same skin at a given $\Delta\psi$ reveals a highly significant correlation between J_{12}^{Na} and I_o ($P < 0.001$) for each value of $\Delta\psi$ (-100 , -50 , 0 , $+20$, $+60$, and $+100$ mV). The regression lines for the different levels of $\Delta\psi$ are shown in Fig. 4. These relationships can be used to reduce the scatter of J_{12}^{Na} by taking into account differences in transport rates in individual skins observed before the experimental procedures and we may obtain the "normalized" value of SE shown in column 3 of Table II simply by using the following relationship:

$$J_{12(\text{norm})}^{Na} = J_{12}^{Na} - m(I_o - I_{o(\text{av})}), \quad (1)$$

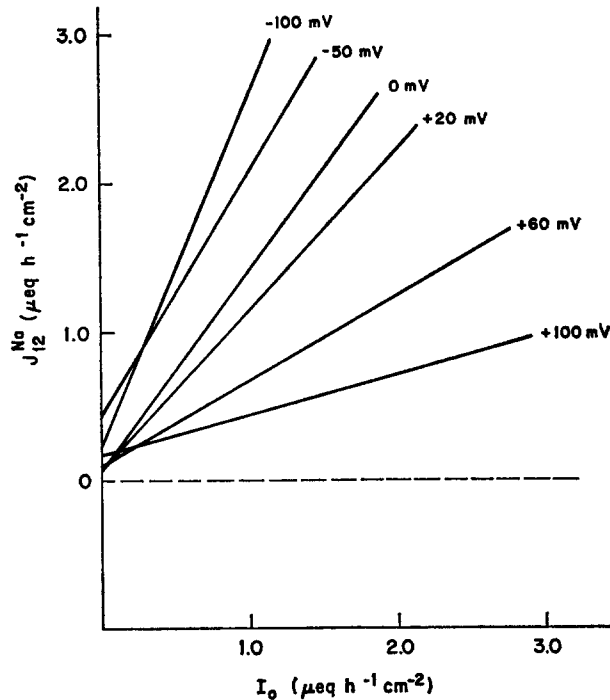


FIGURE 4. Regression lines obtained from Na influx determined at various levels of $\Delta\psi_{13}$. I_o measured during the initial equilibration period. See text for details.

where $J_{12(\text{norm})}^{\text{Na}}$ is the normalized Na uptake, J_{12}^{Na} the experimentally determined Na uptake. $I_{o(\text{av})}$ represents the average short-circuit current and m the average slope of the regression line in a plot of I_o vs. J_{12}^{Na} (see Fig. 4) for all skins used at a given value of $\Delta\psi$.

The effect of $\Delta\psi$ on J_{12}^{Na} in amiloride-treated skins was tested in two frogs in the following way. After an initial equilibration, amiloride (final concentration 10^{-4} M) was added to the outside bathing solution of all tissues. After 6 min observation under short-circuit conditions the control and experimental skins obtained from a single frog were kept for a further 6 min under short-circuit conditions and at an experimental value of $\Delta\psi$ of -100 mV, respectively. Then, the J_{12}^{Na} measured at a $\Delta\psi$ of -100 mV in the presence of amiloride was tested against the J_{12}^{Na} measured under short-circuit conditions also in the presence of amiloride. The 13 flux measurements obtained at a $\Delta\psi$ of -100 mV averaged $0.13 \pm 0.02 \mu\text{eq h}^{-1} \text{cm}^2$. This value is not significantly different from the average influx determined in 11 matched control skins at a $\Delta\psi$ of 0 mV ($0.11 \pm 0.04 \mu\text{eq h}^{-1} \text{cm}^2$).

D. Relationship between J_{12}^{Na} and J_{13}^{Na} at $\Delta\psi$ of 0 and $+100$ mV

In five pairs of skins the transepithelial influx (J_{13}^{Na}) was compared to the

influx across the outer surface of the skin (J_{12}^{Na}). In each pair, one skin was kept continuously at a $\Delta\psi$ of 0 and the other skin was maintained at a $\Delta\psi$ of +100 mV. The $\Delta\psi$ of +100 mV was chosen because we found in pilot experiments that skins could be exposed to a $\Delta\psi$ of +100 mV for up to 25 min without irreversible effects on spontaneous $\Delta\psi$, I_o , and R . In each skin, the approach to a steady state for the ^{24}Na fluxes could be observed. The rate of appearance of the tracer in the inside bathing solution reached stable values 6–12 min after the addition of ^{24}Na to the outside solution. The average of the last three samples taken 16–20 min after the introduction of the tracer was used for the calculation of J_{13}^{Na} . The J_{12}^{Na} was determined in the same skin no later than 2 min after the last sample was removed from the inside bathing fluid. The results of the five experiments are listed in Table III. It

TABLE III
COMPARISON BETWEEN J_{12}^{Na} AND J_{13}^{Na} IN PAIRS OF SKINS AT $\Delta\psi$ OF 100 mV (E) AND 0 mV (C)

Pair	J_{12}^{Na} E/C	J_{13}^{Na} E/C	J_{12}^{Na} C	J_{13}^{Na} C	I_{o2} C	$J_{13}^{\text{Na}}/I_{o1}$ C	I_{o1} E/C
1	0.301	0.081	1.726	1.557	1.531	1.02	0.94
2	0.411	0.129	0.454	0.405	0.385	1.05	0.97
3	0.421	0.130	0.963	1.045	0.823	1.27	1.10
4	0.388	0.124	0.874	1.031	0.942	1.09	1.00
5	0.344	0.095	0.833	0.569	0.612	0.93	1.21
Average	0.37±0.02	0.11±0.01	0.97±0.21	0.92±0.20	0.86±0.19	1.07±0.06	1.04±0.05

J_{12}^{Na} and I_o are expressed in $\mu\text{eq h}^{-1} \text{cm}^{-2}$. I_{o1} and I_{o2} are the short-circuit currents after equilibration and during the measurement of J_{13}^{Na} , respectively. Na concentration 6 mM.

can be seen in columns 4–7 that in the control skins ($\Delta\psi = 0$ mV) J_{12}^{Na} and J_{13}^{Na} are quite close together, and that although they are on the average somewhat higher than the I_o they reflect the marked differences of I_o observed in individual skins remarkably well. The last column documents the fact that in each pair the initial I_o was comparable. The results shown in columns 1 and 2 indicate that at a $\Delta\psi$ of +100 mV J_{13}^{Na} is reduced to about 11% of the value obtained at a $\Delta\psi$ of 0 mV whereas J_{12}^{Na} is decreased to about 37% of the control value. The difference between the two average ratios listed in column 1 and 2 is highly significant.

E. Effect of Exposure to Larger Negative Values of $\Delta\psi$

In one experiment carried out at a Na concentration of 6 mM one group of six skins was exposed to a $\Delta\psi$ of -100 mV for 6 min and another group of six matched skins was clamped for the same time at a $\Delta\psi$ of 0 mV. After this, the J_{12}^{Na} was determined in both groups at a $\Delta\psi$ of 0 mV and I_o , spon-

taneous $\Delta\psi$, and R were measured. None of the measured parameters was significantly different in the two groups. This suggests that maintaining the skin at a $\Delta\psi$ of -100 mV for 6 min does not produce any changes in the skin which are large or prolonged enough to result in significant changes in the values of I_o , spontaneous $\Delta\psi$, R , or of the J_{12}^{Na} measured immediately afterwards at $\Delta\psi$ or 0 mV.

However, a longer exposure to a $\Delta\psi$ of -50 mV or more may lead to marked decrease J_{12}^{Na} measured at a $\Delta\psi$ of 0 mV. Two protocols were followed to allow a study to be made of the effect of a larger or more prolonged depolarization of the skin on Na uptake. In the first protocol, a depolarization with a value of $\Delta\psi$ of -125 mV was applied to the experimental group for 40 min; the control group was left under short-circuit conditions for the same time. J_{12}^{Na} was then determined at $\Delta\psi = 0$ mV. The result of such an experiment can be seen in Fig. 5. This figure demonstrates clearly that I_o and J_{12}^{Na} were proportionately decreased after an exposure of 40 min to a $\Delta\psi$ of -125 mV to about one-third of the values obtained for the control group. In the second protocol, three skins (triplets) obtained from a single

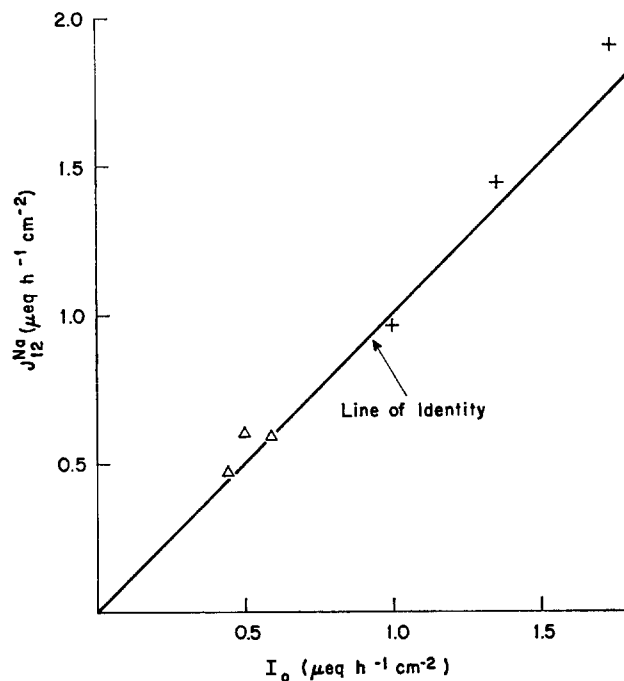


FIGURE 5. Comparison of Na influx determined at $\Delta\psi_{13} = 0$ mV with I_o recorded during flux determinations. During experimental period of 40 min controls (+) were maintained at $\Delta\psi_{13} = 0$ mV whereas experimentals (Δ) were exposed to $\Delta\psi_{13} = -125$ mV. All skins were obtained from same frog. At end of initial equilibration period I_o averaged 1.19 and 1.32 $\mu\text{eq h}^{-1} \text{cm}^{-2}$ in control and experimental skins, respectively.

frog were matched according to their initial values of I_o , in order to test whether such a decrease in J_{12}^{Na} and I_o is at least partly reversible. One skin was used as control and was maintained under short-circuit conditions for 30 min at which time the J_{12}^{Na} was determined at a $\Delta\psi$ of 0 mV. The two experimental skins were clamped at a value of $\Delta\psi$ of -200 mV for 15 min. At the end of this time the value of I_o was determined for both skins. J_{12}^{Na} was then measured immediately in one skin at $\Delta\psi = 0$ mV. The other experimental skin was used to investigate the possibility of a recovery and was clamped at $\Delta\psi = 0$ mV for a further 15 min before making the determination of J_{12}^{Na} at $\Delta\psi = 0$ mV. The results of these experiments are shown in Table IV. Three groups of skins were obtained from two frogs. The initial

TABLE IV
EFFECT OF 15 MIN EXPOSURE TO $\Delta\psi_{12}$ OF -200 mV ON J_{12}^{Na} AND I_o

Experiment	Group	Initial I_o	After 15 min		After 30 min	
			I_o	J_{12}^{Na}	I_o	J_{12}^{Na}
I	Control	1.22	1.19		1.18	1.91
	Experimental	1.40	0.28	0.64		
	Recovery	1.25	0.22		0.81	0.92
II	Control	0.80	0.79		0.78	0.97
	Experimental	0.99	0.26	0.29		
	Recovery	0.90	0.22		0.27	0.34
III	Control	0.89	0.88		0.83	0.84
	Experimental	0.73	0.22	0.26		
	Recovery	0.67	0.15		0.30	0.31

I_o and J_{12}^{Na} are given in $\mu\text{eq h}^{-1} \text{cm}^{-2}$.

values of I_o are listed in the table. The control skins were maintained at a $\Delta\psi$ of 0 mV for 30 min over which time there was only a slight decrease of I_o . The J_{12}^{Na} measured at a $\Delta\psi$ of 0 mV after 30 min was at least as great as I_o . The experimental skins started off with a comparable value for I_o , but exhibited a dramatic decrease of I_o after a 15 min exposure to a $\Delta\psi$ of -200 mV. The J_{12}^{Na} determined at a $\Delta\psi$ of 0 mV immediately after this exposure was only a fraction of the J_{12}^{Na} measured in the control skins. The skins which were used to test the recovery were treated like the experimental group except that a recovery period of 15 min was added after the exposure to a value of $\Delta\psi$ of -200 mV. During this recovery period $\Delta\psi$ was maintained at 0 mV and at the end of this period J_{12}^{Na} was determined at $\Delta\psi = 0$ mV. It can be seen that in the recovery skins I_o dropped after the exposure to -200 mV just as much as it did in the experimental skins. Then, I_o recovered to between one-third and two-thirds of the original values. Our records show that all the recovery of I_o occurred in the first 3–6 min. The J_{12}^{Na} measured in the

recovery skins was less than half of the corresponding value determined in the control skins and was much closer to and not significantly different from the J_{12}^{Na} determined in the experimental skins.

DISCUSSION

The results listed in Table II show that the Na influx is substantially influenced by changes in the transepithelial potential. The Na influx increases progressively as $\Delta\psi_{13}$ becomes more negative and decreases as $\Delta\psi_{13}$ is made more positive. The values for Na influx listed in Table II can be used to make predictions about the electrical driving force, $\Delta\psi_x$, needed to cause such changes in Na influx. We can then compare such a value for $\Delta\psi_x$ which is calculated from a given change in Na influx with the actually observed change in $\Delta\psi_{13}$. This is important since $\Delta\psi_x$ may give us information about $\Delta\psi_{12}$, the "effective" potential step across the outer surface of the frog skin and its relationship to $\Delta\psi_{13}$. However, for this approach we will have to define the relationship between ion flux and electrical forces quantitatively.

The next flux of an ion i across a membrane can be characterized by the following equation which is derived from the relationship described by Nernst (15, 16) and Planck (17) with the additional assumption of a constant field across the barrier (18):

$$J_i^{net} = p_i z_i \chi E \left[\frac{(a'_i - a''_i) \exp(z_i \chi E)}{1 - \exp(z_i \chi E)} \right], \quad (2)$$

where p_i , z_i , and a_i are the permeability coefficient, valency, and chemical activity of the ion in question, χ refers to F/RT , i.e. Faraday's number divided by the gas constant times the absolute temperature, E is the potential, and prime and double prime superscripts indicate the outside and inside bathing solutions, respectively. The net flux across the membrane can be considered as the result of two independent unidirectional fluxes, an influx which proceeds from the outside bathing solution to the inside bathing solution

$$J_i^{IN} = p_i z_i a'_i \left[\frac{-\chi E}{1 - \exp(z_i \chi E)} \right], \quad (3)$$

and an efflux which moves from the inside to the outside solution

$$J_i^{EFF} = p_i z_i a''_i \left[\frac{\chi E}{1 - \exp(-z_i \chi E)} \right]. \quad (4)$$

We can use Eq. 3 (or, as a matter of fact Eq. 4 since only the sign of the voltage differs in these two equations) to describe the Na influx, J_{12}^{Na} , across the outer surface of the frog skin. The different values for J_{12}^{Na} listed in Table

II represent changes in influx which can be expressed as ratios. Eq. 3 can be employed to calculate the ratio of E necessary to obtain an equivalent ratio for J_{12}^{Na} . χ , z_i , and a'_i , are constant in these experiments and the only assumption we have to make for such a computation is that p_i remains constant as $\Delta\psi_{13}$ is changed. As pointed out above, the ratio of E gives us information about the force $\Delta\psi_x$ which is necessary to cause the observed change in J_{12}^{Na} and this force $\Delta\psi_x$ may be used as an estimate for the potential step across the outside surface of the frog skin, $\Delta\psi_{12}$. Fig. 6 represents a plot of such values of $\Delta\psi_{12}$ calculated from the changes in J_{12}^{Na} against the $\Delta\psi_{13}$ which was used during the measurement of J_{12}^{Na} . Earlier measurements with microelectrodes have shown that the intracellular potential obtained under short-circuit conditions is about -15 mV (1, 9). The value of -15 mV was therefore chosen to represent $\Delta\psi_{12}$ at a $\Delta\psi$ of 0 mV in Fig. 6. The regression line drawn in this figure represents a remarkably good fit for the experimental data if one considers the possible errors involved in the experimental techniques.

Two points of particular interest emerge from the plot shown in Fig. 6. First, $\Delta\psi_{12}$ represents one-half or less of the total transepithelial potential observed at a Na concentration of 115 mM under open circuit conditions (30–90 mV). This is consistent with observations of intracellular potentials made by Ussing and Windhager in a study in which the position of the microelectrode was localized histologically by iontophoresis of lithium carmine (19). They noticed stable potentials with a value for $\Delta\psi_{12}$ of about one-third of the $\Delta\psi_{13}$ in epithelial cells which were farthest removed from the pig-

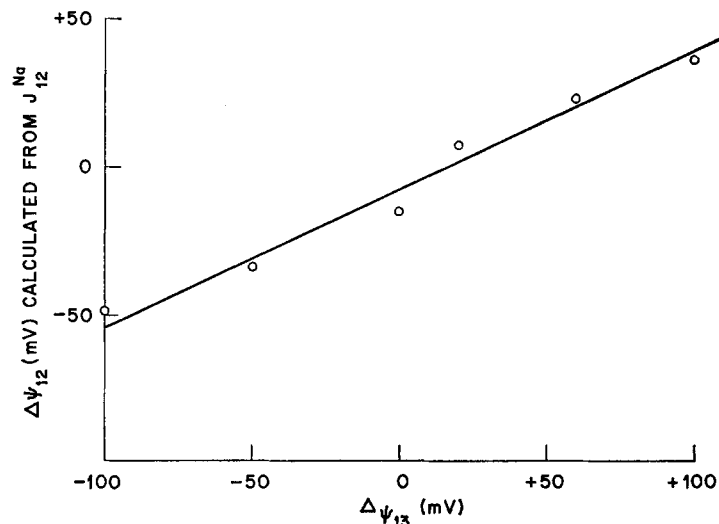


FIGURE 6. Average $\Delta\psi_{12}$ plotted against $\Delta\psi_{13}$. $\Delta\psi_{12}$ calculated from change in Na influx. See text for details.

ment layer, i.e. closest to the outside surface (Fig. 9 in ref. 19). In all the other studies of potentials in the epithelial cells of the frog skin no attempt was made to localize the position of the microelectrode histologically (9, 20, 21). Second, the plot in Fig. 6 suggests further that $\Delta\psi_{12}$ is a linear function of $\Delta\psi_{13}$. The slope of the regression line suggests that the change of $\Delta\psi_{12}$ is about one-half of the change in $\Delta\psi_{13}$. This, together with the observation that $\Delta\psi_{13}$ is a linear function of I (Table II and Fig. 7) indicates that the outside and inside barrier, as well as the whole frog skin behave like resistors which are not affected by the passage of current. This is in agreement with

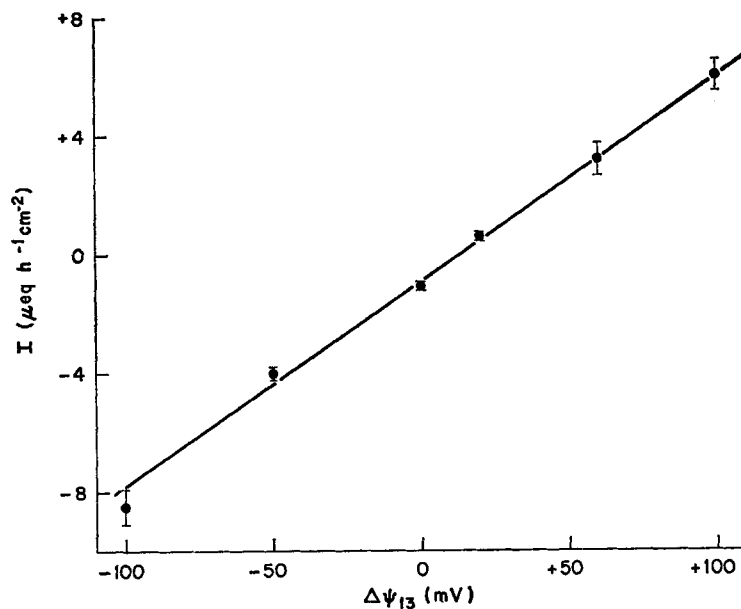


FIGURE 7. Relationship between current (I) and transepithelial potential difference ($\Delta\psi_{13}$). Values were obtained during determinations of Na influx (Table II).

the study carried out by Whittembury who measured intracellular potentials in epithelial cells of the toad skin as a function of current applied across the tissue (22). He located the puncture site histologically. Clamping the skin at different levels of $\Delta\psi_{13}$ ranging from +100 to -50 mV resulted in a linear relationship between I on one hand and $\Delta\psi_{13}$ as well as intracellular potentials on the other hand (Fig. 4 in ref. 22). Unfortunately we cannot compare the intracellular potentials obtained in Whittembury's study with the values of $\Delta\psi_{12}$ presented in Fig. 6, since he reported potential measurements from stratum germinativum cells but not from the cell layer which is most likely much more relevant to our measurements, namely the cell layer located between the stratum germinativum and the stratum corneum.

At a Na concentration of 6 mM we could not observe a significant differ-

ence between J_{12}^{Na} measured under open circuit conditions and the one determined under short-circuit conditions. This is not surprising when we consider the scatter of the J_{12}^{Na} determination (Fig. 3 and Table III) and in view of the fact that the average $\Delta\psi_{13}$ for the open circuit skins differed only by 14 mV from the $\Delta\psi_{13}$ maintained in the short-circuited skins.

At a Na concentration of 115 mM we can observe a significant difference between the J_{12}^{Na} measured under open circuit conditions and the one determined under short-circuit conditions. However, considering that the $\Delta\psi$ of the open circuit skins averaged 47 mV, the difference is much smaller than expected from the relationship shown in Fig. 6 for fluxes obtained at a Na concentration of 6 mM. This may be due to the possibility that the linear component of J_{12}^{Na} , which makes up for some $1.73 \mu\text{eq h}^{-1} \text{cm}^2$ at this Na concentration (13), is not affected by changes in $\Delta\psi_{13}$. In fact, the experiments carried out in this study with amiloride-treated skins seem to support this possibility. Previous experiments have demonstrated that if skins are treated with amiloride the linear component remains unchanged (2). Hence, amiloride-treated skins can be used to investigate the linear component. The amiloride experiments reported here, in agreement with earlier determinations (13), show that the linear component is at most about $0.11 \mu\text{eq h}^{-1} \text{cm}^2$ at a Na concentration of 6 mM. Since we found that this remaining component of J_{12}^{Na} is not significantly affected by a change of $\Delta\psi_{13}$ from 0 to -100 mV we may conclude that changes in $\Delta\psi_{13}$ have no effect on the linear component of Na uptake. Therefore, it seems likely, that at a Na concentration of 115 mM the linear component of J_{12}^{Na} also remains unchanged when $\Delta\psi_{13}$ is shifted from 47 mV under open circuit conditions to zero under short-circuit conditions. If we subtract the linear component from the average values listed in the legend of Table I we obtain a corrected J_{12}^{Na} of 1.03 and $1.89 \mu\text{eq h}^{-1} \text{cm}^2$ for open and short-circuit skins, respectively. In other words at a Na concentration of 115 mM the saturating component of J_{12}^{Na} measured under open circuit conditions was about 54% of the corresponding value obtained under short-circuit conditions. This inhibition is about halfway between the inhibition observed for $+20$ mV and $+60$ mV at Na concentration of 6 mM (Table III).

The experiments presented here establish clearly that J_{12}^{Na} is a function of $\Delta\psi_{13}$. This finding is of particular interest in view of the fact that in three recent studies J_{12}^{Na} was measured under conditions in which one would expect large changes of $\Delta\psi_{13}$ (5-7). However, a possible effect of changes in $\Delta\psi_{13}$ was not considered by these authors.

Erlj and Smith (5) determined J_{12}^{Na} under open circuit conditions as a function of the outside Na concentration with the technique described by Schultz et al. (23). By using radioactive inulin as extracellular marker they

confirmed the finding of Biber and Curran (1) that the Na influx is made up of a saturating and a linear component and that it is given by

$$J_{12}^{\text{Na}} = \frac{J^m[\text{Na}]_o}{K_{\text{Na}} + [\text{Na}]_o} + \alpha[\text{Na}]_o, \quad (5)$$

in which J^m is the maximal influx for a saturating component, K_{Na} is an "apparent" Michaelis constant, α is a permeability coefficient, and $[\text{Na}]_o$ is the Na concentration in the outside bathing solution. However, Erlij and Smith obtained much smaller values for J^m and α (J^m was 0.6 vs. 4.0 and α was 0.020 vs. 0.037). Moreover, α became zero when they used mannitol as extracellular marker. Although these authors did not publish any values for $\Delta\psi_{13}$ it is clear that the increase in Na concentration in the outside bathing solution must have been accompanied by an increase in $\Delta\psi_{13}$ (and $\Delta\psi_{12}$). This must have caused a substantial decrease in J_{12}^{Na} at higher Na concentration. Hence, it is not surprising that the values for J^m and for α (since J^m and α are obtained with the same measurement) are lower than those obtained under short-circuit conditions (1, 2).

Rotunno et al. (6) and Cerejido and Rotunno (7) have published determinations of J_{12}^{Na} which were made with an entirely new technique. The measurements were carried out under open circuit conditions and involved the use of a wide range of Na concentrations (1–115 mM) and also of anti-diuretic hormone. Under such conditions $\Delta\psi_{13}$ must have reached very different values. A comparison of their data with those presented here is not easy since no information is given about the values of $\Delta\psi_{13}$ during the influx determination. Additionally, a comparison of the influx values is made even more difficult by significant differences in procedures used in these methods. For example, in our experiments we aimed to reduce problems arising from the distribution of tracers in unstirred layers on the surface of the frog skin by vigorous stirring of the tracer solution (13). The method employed by Cerejido and coworkers (6, 7) precludes such rapid stirring since the level of tracer solution bathing the skin has to rise at a constant rate without disturbing the surface of the fluid level. Nevertheless it is possible to say that the value of J_{12}^{Na} obtained by Cerejido and collaborators must have been reduced by the presence of substantial potential differences across the skin when the outside bathing solution contained a Na concentration of 115 mM. Ellory et al. determined J_{12}^{Na} in another tissue, the goldfish intestine (24). For these measurements they used different Na concentrations in the bathing solutions and so it is likely that substantial changes in $\Delta\psi_{13}$ occurred. However, it is possible that, similar to other epithelia in the intestine (25, 26) a shunt pathway influences decisively the potentials across the cell borders and across the whole epithelium.

The disparity between J_{12}^{Na} and J_{13}^{Na} which one can observe at a $\Delta\psi$ of 100 mV (Table III) demonstrates that under special conditions J_{12}^{Na} may become substantially larger than J_{13}^{Na} . At first glance one might think that these observations prove that in this case the Na influx across the outside surface of the skin is no longer rate limiting for the transepithelial influx of this ion. Although this may be the case, another explanation must be considered. An increase in $\Delta\psi_{13}$ will be followed by an increase in $\Delta\psi_{12}$. As a consequence, the Na efflux across the outside surface of the skin (J_{21}^{Na}) which is normally only a small fraction of J_{12}^{Na} will become larger. Hence, under these circumstances the net flux of Na across the outside surface of the skin ($J_{12}^{\text{Na}}_{\text{net}}$) may not be larger than the net flux across the inside surface of the skin or the net flux across the whole skin ($J_{13}^{\text{Na}}_{\text{net}}$). Whatever the explanation, these experiments show clearly that one cannot draw conclusions about the size of J_{12}^{Na} from measurements of transepithelial fluxes ($J_{13}^{\text{Na}}_{\text{net}}$ or J_{13}^{Na}) alone.

Any study in which negative values of $\Delta\psi$ are employed should take into account that detrimental tissue damage may occur under these conditions. Voûte and Ussing observed irreversible cell damage including cell necrosis after 20–30 min exposure to twice the value of the short-circuit current (27). During such a procedure $\Delta\psi$ averaged in their experiments from about –50 to –110 mV. Apart from reversible swelling of epithelial cells they noted no damage to the tissue when the skins were maintained under short-circuit conditions for as long as 50 min. In pilot experiments we could confirm that changes with only partial or no reversibility take place after an exposure of 24 min to a negative value of $\Delta\psi$ of 50 mV or more. However, after shorter exposure times not exceeding 8 min to negative $\Delta\psi$ values of up to –100 mV, we were unable to observe such changes. The results presented here support these findings and demonstrate that, on one hand there is no change in J_{12}^{Na} after such short exposure times and on the other hand changes in J_{12}^{Na} with only partial or no reversibility appear after longer (i.e. 15–40 min) exposures to negative values of $\Delta\psi$. These experiments with prolonged exposure to negative potentials lend themselves to the prediction that the voltage dependence of J_{12}^{Na} shown in Table II changes when these potentials are maintained for longer than 8 min and that under such circumstances J_{12}^{Na} and with that J_{13}^{Na} may reach saturation as $\Delta\psi$ becomes more negative.² The cause for these changes is not known and one may speculate whether excessive heat damages the cells or whether a polarization of a carrier system occurs.

In view of the description of shunt pathways in the frog skin (19, 28, 29),

² Indeed, when J_{12}^{Na} was determined in three frogs at a $\Delta\psi_{13}$ of –100 mV after 40 min (experimental) and 6 min (control) exposure to this potential the ratio of experimental over control averaged 0.66, 0.68, and 0.74 in these frogs (total 12 experimental and 12 control values).

it would be interesting to know to what extent these pathways contribute to the Na influx under the conditions of the presented experiments. This question becomes even more important in view of the recent observation of Mandel and Curran (11) that the shunt pathway increases markedly at large negative values of $\Delta\psi_{13}$. It should be pointed out, however, that Mandel and Curran made their measurements under conditions which differed substantially from those present in this study. The main differences were the following: the exposure of their skins to applied potentials was many times as long, lateral and dorsal parts of the frog skin were used besides abdominal parts, and the skins used to measure movements of Na, K, Cl, and mannitol were treated with 10^{-4} M ouabain. It is known that ouabain, even at a lower dose of 10^{-5} M may increase sodium fluxes by a factor of two (30). In spite of these differences, we may average the values for J_{13}^{Na} measured by Mandel and Curran at a $\Delta\psi$ of -100 , -50 , and 0 mV (Fig. 2 in ref. 11) in order to get a rough estimate for the magnitude of the shunt pathway in our experiments. If we do this and if we consider that Mandel and Curran obtained their values at a Na concentration of 115 mM and that except for one series of experiments all the data presented in this study were collected at a Na concentration of 6 mM we can estimate that the shunt pathway contributes roughly 0.13, 0.06, and 0.020 $\mu\text{eq h}^{-1} \text{cm}^{-2}$ at a $\Delta\psi_{13}$ of -100 , -50 , and 0 mV, respectively. At positive values of $\Delta\psi_{13}$ this shunt pathway component will be even smaller. Hence, it seems that the flux through the shunt pathway did not contribute much to the results presented in this study.

Earlier experiments (1, 2) have shown that at a Na concentration of 6 mM the saturating component makes up for some 90% of the Na uptake and that this portion of the Na influx can be described by the first term on the right hand side of Eq. 5:

$$J_{12(\text{sat})}^{\text{Na}} = \frac{J^m[\text{Na}]_o}{K_{\text{Na}} + [\text{Na}]_o} \quad (6)$$

In addition, this component of J_{12}^{Na} is inhibited by Li ions (1). Hence, the finding that the saturating component of J_{12}^{Na} is sensitive to potential changes can be discussed in terms of a carrier system as shown in Fig. 8. Of particular interest are two models which exhibit different characteristics when the membrane potential is changed. Na combines with a carrier in the outward facing cell membrane of the first living cell layer of the frog skin epithelium and forms an ion-carrier complex (step 1) which then moves across the membrane (step 2), and releases the Na ion towards the cytoplasm (step 3). According to the first model the free carrier either returns unchanged as X (step 4) or transformed (metabolism?) as Y (steps 5, 6, and 7) toward the

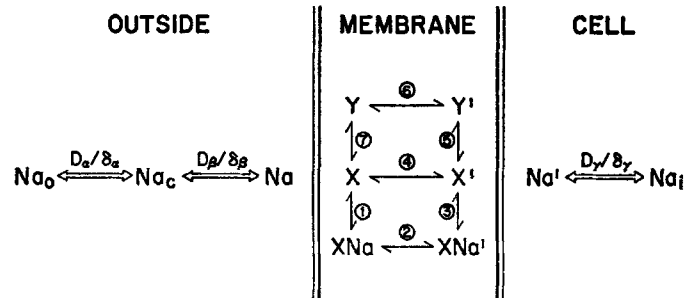


FIGURE 8. Hypothetical model for carrier-mediated Na movement across outward facing cell membrane of the first living cell layer of the skin. Na_o , Na_c , Na , Na' , and Na_i denote Na concentrations in outside bathing solution, at outside surface of cornified cells, at outside and inside surface of the outward facing cell membrane of first living epithelial cell layer, and in the cell cytoplasm, respectively. D and δ are diffusion coefficients and thickness for layers characterized by the subscripts α , β , and γ which refer to an unstirred layer in the outside bathing solution, the cornified cell layer, and to an unstirred layer at the inward facing surface of the outer cell membrane, respectively. Steps 2, 4, and 6 are translocation steps of a mobile carrier across the membrane. X and XNa refer to a free carrier and to a Na-carrier complex, respectively. Y designates a modified carrier X' (metabolism?) which uses steps which are alternative to step 4.

outside of the membrane to pick up another Na ion. Accordingly, this system will be sensitive to potential changes. A second possibility is that the free carrier combines with another cation (H^+ or NH_4^+) and forms an ion-carrier complex Y^* (step 5*), which then translocates across the membrane (step 6*) and releases this cation towards the outside bathing solution (step 7*). In such a system Na exchanges in a one-to-one relationship with the other cation and potential changes across the membrane should have no effect. Our experiments are compatible with the first model and seem to rule out a one-to-one exchange for the saturation portion of J_{12}^{Na} . On the other hand, an exchange with a ratio deviating from unity (for example 3 Na^+ to 2 H^+) cannot be ruled out. We would like to point out that we have no knowledge which of the steps depicted in these models is rate limiting. We have added to the model an indication of unstirred layers through which Na transport must proceed. In the cornified layer, the diffusion coefficient for Na (D_β) is far from the one observed in free solution (13) and it is possible that the Na movement across this layer is carrier mediated.

The two barriers (especially the cornified cell layer) drawn in Fig. 8 between the outside bathing solution and the outer membrane of the first living cell layer may shift the "effective" Na concentration at the outside surface of this cell layer to a value which is different from the activity of Na in the bulk solution. Hence, one can argue that under the experimental conditions described in this paper the mechanism of Na uptake per se is not

affected by changes in $\Delta\psi_{13}$ but that the "effective" Na concentration at the outside surface has changed sufficiently to cause the changes in Na influx which we observed in these experiments. We can use the kinetic analysis between Na concentration and Na influx described previously for short-circuit conditions (1, 13) in order to calculate the "effective" Na concentration necessary to increase or to reduce the Na influx to the values observed in our experiments. At a $\Delta\psi_{13}$ of -100 mV the Na concentration would have to increase to 18 mM and at a $\Delta\psi_{13}$ of $+100$ mV the Na concentration would have to decrease to 1.6 mM. This amounts to a three- to fourfold change from the Na concentration measured in the outside bathing solution and is equivalent to a potential change of some ± 35 mV across these outside barriers. This seems highly unlikely in view of two observations. First, Whittembury (22) during his measurements of the potential profile across the toad skin determined also the DC resistance between the microelectrode located in different parts of the skin and the two (inner and outer) bathing solutions. He could not locate any significant resistance in the cornified layer and the slope $\Delta V/\Delta I$ (change of potential per change of current) recorded for the whole skin over a wide range of $\Delta\psi_{13}$ (from $+100$ to -50 mV) was identical with the corresponding slope $\Delta V/\Delta I$ measured between the inside bathing solution and the microelectrode located in the cornified layer. Furthermore, he added that "the skin potentials and profiles recorded by impaling an area where the stratum corneum had been removed were not different from those recorded when this stratum was present." Second, values for three frogs in which the cornified layer had been removed are included in Table II since they showed no significant difference from the other values.

The effect of changes in $\Delta\psi_{13}$ on transepithelial fluxes of Na has been studied by several investigators previously. Ussing and Zerahn (31) observed a marked decrease in J_{13}^{Na} and an increase in J_{31}^{Na} when the inner solution short-circuited conditions were compared with conditions where the inner solution was electrically positive with respect to the outer solution. Linderholm (32) observed in one experiment a 2.8-fold increase in J_{13}^{Na} when the spontaneous $\Delta\psi_{13}$ of about 30 mV was reduced to 0. In another experiment the same procedure caused a decrease in J_{31}^{Na} to about 70% of the open circuit value. Biber et al. (30) measured bidirectional sodium fluxes in the presence of a low sodium concentration in the outside bathing solution. They noticed that J_{13}^{Na} measured in skins under open circuit conditions (average $\Delta\psi_{13}$ was 49 mV) was 37% of the J_{13}^{Na} determined in control skins maintained under short-circuit conditions. Walser (12) has recently summarized similar studies carried out in the toad and turtle bladder when he reported his own experiments in which he studied the effect of the transepithelial potential difference on transepithelial sodium and chloride fluxes across the toad bladder wall. The present study provides at least a partial

explanation for the finding in these earlier studies that J_{13}^{Na} changes when $\Delta\psi_{13}$ changes. Most investigators who carried out these previous studies have interpreted the effect of the transepithelial potential difference on J_{13}^{Na} exclusively in terms of the characteristics of the sodium pump presumed to be located at the serosal or intercellular boundary of the transporting cells. However, J_{13}^{Na} on its way across the epithelial cells must proceed at least via two boundaries arranged in series, the outward facing cell boundary and the serosal or intercellular boundary. Hence, any attempt at relating the transepithelial potential to the electromotive force of the pump or E_{Na} must take into account the effect of this potential difference not only on the fluxes across the serosal (intercellular) surface but also on the fluxes across the outer membrane. This study demonstrates clearly that the movement of Na across the outside boundary must be considered whenever such computations are made.

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