Na Transport across Frog Skin at Low External Na Concentrations

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ABSTRACT Isolated frog skin was bathed with a dilute solution containing 1 mM NaCl on the outside and with normal Ringer's solution on the inner surface. Net Na flux was determined by simultaneous measurement of unidirectional fluxes with Na^{22} and Na^{24} and intracellular electrical potentials were examined with microelectrodes. There was a net inward transport of Na under both open-circuit and short-circuit conditions. The short-circuit current was approximately 15% greater than the net Na flux; the discrepancy could be accounted for by a small outward flux of Cl. The electrical potential profile did not differ greatly from that observed in skins bathed on the outside with normal Ringer's solution. Under open-circuit conditions, there were usually several potential steps and under short-circuit conditions the cells were negative relative to the bathing solutions. Estimates of epithelial Na concentrations utilizing radioactive Na suggested that if all epithelial Na were in a single compartment, an active entry step would be necessary to allow a net inward Na transport. The results could also be explained by a series arrangement of Na compartments without necessarily postulating an active Na entry. The behavior of the potential profile suggested that this latter alternative was more likely.

The active transport of Na across isolated frog skin has been examined extensively, but in the majority of studies the outer surface of the skin has been bathed with solutions containing relatively high (115 mM) concentrations of Na. Variation in rates of Na transport and in electrical properties of the skin with changes in Na concentration has also been studied, but in most cases constant anion concentration was maintained by using other cations to substitute for Na. However, the early data of Krogh (1) indicated that intact frogs could take up Na through the skin from solutions containing only 10^{-5} M NaCl. Since the model of the Na transport system proposed by Koefoed-Johnsen and Ussing (2) suggests that Na entry into the skin cells is a

passive process, such uptake would require that cell Na concentration be less than 10^{-5} M or that the cell interior be electrically negative to the external solution by a substantial amount. No data are available on these points under appropriate conditions. Experiments on both frog skin (3) and toad bladder (4) have indicated that the pool of Na in the cells decreases as external Na concentration is lowered. However, in skin the lowest external Na concentration studied was 7 mM and very few experiments were carried out at lower concentrations in toad bladder. In both these studies, choline was used to replace Na. In the work on skin, Na concentration in the inside solution was lowered concomitantly with that in the outside solution while in the bladder studies, the inside solution contained no Na. Thus, in neither study did conditions correspond to those obtaining in Krogh's experiments and the question of whether Na entry into the skin is purely a passive process when the outside solution is low in Na (and other ions) and the inside solution is high in Na remains unanswered. Further, there are suggestions in the experiments of Koblick (5) and of Lindley and Hoshiko (6) that the skin may have somewhat different properties when bathed with hypotonic solutions on the outer surface.

These considerations have led us to examine further both Na transport and electrical phenomena in frog skins bathed on the outer surface with a dilute solution containing 1 mM NaCl and on the inner surface with normal Ringer's solution. Net Na flux has been measured in both open- and short-circuit conditions and the profile of electrical potential was determined by microelectrodes. Attempts have also been made to obtain information on Na concentration in the epithelial cells.

METHODS

Undirectional Na Fluxes

The abdominal skin of *Rana pipiens* was mounted in a chamber similar to that used by Ussing and Zerahn (7) in which electrical potential difference (PD) and short-circuit current were measured as previously described (8). The area of skin exposed to the bathing solutions was 7.1 cm^2. The inside of the skin was bathed with a Ringer solution containing 115 mM NaCl, 2.5 mM $KHCO_3$, and 1.0 mM $CaCl_2$. The outside solution contained only 1.0 mM NaCl, 2.5 mM $KHCO_3$, and 1.0 mM $CaCl_2$. The agar bridges used for electrical measurements had the same ionic composition as the bathing solution with which they made contact. In order to measure Na influx and outflux simultaneously, Na^{22} (0.4 μc) and Na^{24} (100 μc) were added to the outside and inside solutions respectively, and both solutions were sampled every 30 min. The outside chamber was emptied completely and refilled with new solution each hour in order to minimize changes in Na^{22} and total Na concentrations.

Samples were dried on aluminum planchettes and counted using a procedure similar to that described by Ussing and Zerahn (7). Samples of the outside solution were

counted at the end of the experiment with an end window Geiger-Mueller counter using an aluminum absorber of 110 mg/cm². Ten days later these planchettes were recounted under the same conditions in order to measure the background due to Na^{22} and the amount of Na^{24} originally present was determined by difference. The amount of Na^{22} in the inside samples was determined by counting with a windowless gas flow counter after allowing Na^{24} to decay to an insignificant level. Fluxes were calculated from the amount of activity appearing on the "cold" side and the specific activity of the particular isotope on the "hot" side. Na concentrations of the solutions were determined by flame photometry after each experiment.

Microelectrode Experiments

Intracellular electrical potentials were determined with microelectrodes using the technique and apparatus described in detail by Cereijido and Curran (9). Skins were mounted in the same bathing solutions used in the flux experiments. A number of punctures were made in each skin and during each puncture, sufficient current was passed through the skin to reduce the total skin potential to zero. In some experiments, the ion composition of the outside bathing solution was altered with a microelectrode in place in the skin using the technique previously described (9). Na concentration was reduced by replacing NaCl by KCl and Cl concentration was increased by addition of choline Cl. The Na concentration of the solutions was determined by flame photometry and the Cl concentration by potentiometric titration with $AgNO_3$.

Cell Na Concentration

An attempt was made to estimate cell Na concentration indirectly by assuming that Na involved in the transport process is in a single compartment in the skin. Under the conditions of this assumption Na movement across the skin will involve two steps, an entrance into the tissue at the outside and an exit at the inside. If tracer Na is placed in the outside solution and the inside solution contains no tracer, it can be shown (see Appendix) that when the skin reaches a steady state with respect to tracer

$$\frac{p_o}{[\mathrm{Na}]_o} = \left[1 + \frac{\Phi_{\mathrm{out}}\Phi_{si}}{\Phi_{\mathrm{in}}\Phi_{so}}\right]\frac{p_s}{[\mathrm{Na}]_s} \tag{1}$$

in which p is tracer concentration (CPM/ml) and [Na] is Na concentration. The subscripts o and s refer to outside solution and skin compartment respectively. The symbols Φ_{out} and Φ_{in} refer to unidirectional outflux and influx of Na across the whole skin, and Φ_{si} and Φ_{so} are unidirectional fluxes from skin to inside and skin to outside respectively. Equation 1 has been used to estimate $[\mathrm{Na}]_s$ using the following method.

Skins were mounted in small chambers (3.14 cm²) (10) in the bathing solutions used for flux measurements, Na^{22} was added to the outside solution, and the system allowed to equilibrate for 1 hr. During this period samples were removed from the inside solution to determine Na influx (Φ_{in}) and from the outside to determine p_o and $[\mathrm{Na}]_o$. After 1 hr, the bathing solutions were removed and both chambers were rapidly rinsed twice. New solutions were introduced, left for 1 or 2 min intervals,

removed by aspiration into tubes, and replaced. Aliquots were counted in a scintillation counter to determine Na^{22} washout rates at the two sides of the skin and the mean ratio of these rates during the first 8 to 10 min of washout was taken as a measure of Φ_{si}/Φ_{so}. In experiments in which the skin was short-circuited, the ratio of Φ_{out}/Φ_{in} was evaluated from influx measured directly during the equilibration period and the mean outflux determined in double labeling experiments with adjustment for the different surface areas of the chambers. In open-circuit experiments, Φ_{out}/Φ_{in} was taken as the mean value observed in double labeling experiments (Table I). At the end of each experiment, the exposed portion of skin was removed and its wet and dry weights and the amount of Na^{22} remaining in it were determined. The total Na^{22} in the skin at the start of washout was evaluated by adding the total tracer washed out to that remaining in the skin. This total Na^{22} was corrected for that in the epithelial extracellular space and in the connective tissue using the methods described by Cereijido et al. (3). To evaluate the concentration of Na^{22} in the epithelium (P_s) from the total amount of Na^{22} present, we have taken the epithelial water content as 25% of total tissue water.[1] Thus all quantities in equation 1 except $[Na]_s$ have been estimated and $[Na]_s$ can be calculated directly.

RESULTS

Flux Measurements

Skins bathed with the solutions described above had potential differences of approximately 50 mv, inside solution positive, and short-circuit currents[2] of the order of 100 μa for 7.1 cm^2. Double labeling experiments were carried out on 10 skins, 8 under short-circuit conditions and 2 with open circuit. A total of 40 half-hour periods was obtained with short-circuited skins under control conditions. The results are summarized in the first row of Table I. There was a mean net inward Na flux of 3.09 μeq/hr even though the Na concentration of the inside solution was approximately 100 times greater than that in the outside solution. The mean observed short-circuit current expressed as movement of positive charge in the inward direction was 3.59 $\pm$ 0.25 μeq/hr. On the basis of paired data, the difference of 0.50 $\pm$ 0.08 μeq/hr between the observed current and the net Na flux is statistically significant ($p < 0.01$). Such a discrepancy between current and Na flux

[1] In 8 experiments in which H^3-inulin was placed in the inside solution for 1 to 1.5 hr, a mean inulin space of 52% of total tissue water was obtained. In 3 experiments utilizing connective tissue from which the epithelium was removed (11), approximately 70% of the connective tissue water had equilibrated with inulin in this same time interval. Thus total connective tissue water would comprise about 75% and epithelial water 25% of total skin water. This figure is in agreement with Huf's statement that the cross-sectional area of the epithelium is about 25% of the total skin cross-sectional area.

[2] With different solutions on the two sides of the skin, the diffusion of ions along their concentration differences may contribute to the short-circuit current. Thus the current does not represent exclusively net active transport of ions as it does with identical bathing solutions but it is a measure of the total net flow of ions. The actual source of the current can be determined by measurement of the net ionic flows.

might be expected as a result of net outward movement of the negative Cl ion down its large concentration gradient. This net Cl outflux would lead to a short-circuit current larger than the net Na flux as observed, but the discrepancy seems unexpectedly small. In order to estimate the contribution of net Cl flux to total current, 3 experiments were carried out in which Cl *outflux* was measured with Cl^{36} under the same conditions as the Na flux measurements (1 mM NaCl outside, short-circuited). A mean Cl outflux of 0.64 μeq/hr was observed. Martin and Curran (unpublished observations) have measured Cl influx under conditions nearly identical to those used here and obtained a mean value of 0.27 μeq/hr. These two unidirectional fluxes yield a net outflux of 0.37 μeq/hr. Thus, these calculations indicate that the discrepancy between net Na flux and short-circuit current can almost certainly be accounted for by Cl outflux. Since Cl outflux with the skin bathed on both sides with 115 mM NaCl Ringer's is approximately 6

TABLE I

Na FLUXES UNDER CONTROL CONDITIONS*

Influx	Outflux	Net	Short-circuit current	Potential difference
μeq/hr	*μeq/hr*	*μeq/hr*	*μeq/hr*	*mv*
3.55±0.27	0.46±0.09	3.09±0.25	3.59±0.25	—
1.33±0.08	0.67±0.08	0.56±0.08	—	49±1

* Errors are given as ± one standard error of the mean. Fluxes were measured for 30 min periods. The data are averages of 40 periods for short-circuit and 10 periods for open-circuit conditions.

μeq/hr (3), the use of a hypotonic outside solution of low NaCl concentration causes a marked reduction in Cl permeability.

The Na fluxes observed in 10 half-hour periods under open-circuit conditions are summarized in the second row of Table I. A mean net inward Na flux of 0.56 ± 0.07 μeq/hr was observed; this net transport takes place against an electrical potential difference of approximately 50 mv in addition to the Na concentration difference. The observed flux ratio of 1.99 was approximately 1300 times greater than that predicted (12) for passive Na movement (Φ_{in}/Φ_{out} = .00135).

Since an appreciable osmotic pressure exists across the skin in these experiments, the observed net Na flux could be due to solvent drag caused by an osmotic water flow. To test this possibility, 2 experiments were performed in which 220 mM sucrose was introduced into the outside solution after 1.5 hr in order to minimize water flow. The results are summarized in Table II. In one experiment, the net Na flux remained constant while in the other it increased. Thus, there is no evidence for an effect of solvent drag on net Na transport.

The effect of the inhibitor ouabain (1×10^{-5} M) was tested in 3 short-

circuit experiments and in the 2 open-circuit experiments. The results are given in Table III. Under both conditions treatment with ouabain abolished net Na flux. In short-circuited skins there was a marked decrease in influx and a significant increase in outflux. Similar but less marked changes occurred in open-circuit skins.

TABLE II

EFFECT OF OSMOTIC GRADIENT ON Na FLUXES

Experiment	Control*			220 mM sucrose outside*		
	Influx	Outflux	Net	Influx	Outflux	Net
		μeq/hr			*μeq/hr*	
A	2.31	0.33	1.98	2.55	0.40	2.15
B	1.07	0.31	0.67	1.96	0.45	1.51

* Control fluxes are average values for three 30 min periods in each experiment. Fluxes with sucrose are averages of four periods.

TABLE III

EFFECTS OF OUABAIN ON Na FLUXES

	Influx	Outflux	Net	Short-circuit current
		μeq/hr		*μeq/hr*
		Short-circuit		
Control*	4.33±0.25	0.78±0.09	3.55±0.20	4.22±0.25
Ouabain (10^{-5} M)	1.75±0.37	1.57±0.29	0.20±0.33	0.60±0.22
		Open-circuit		
Control*	1.33±0.07	0.77±0.08	0.56±0.07	—
Ouabain (10^{-5} M)	1.05±0.27	1.14±0.15	−0.09±0.39	—

* Control values are from the experiments in which ouabain was used. Number of experimental periods, short-circuit, control, 11, ouabain 8; open-circuit, control 10, ouabain 4.

Microelectrode Experiments

The model of the frog skin proposed by Koefoed-Johnsen and Ussing (2) suggests that Na entry into the cells is a passive process. Under the present conditions, passive entry of Na into the skin requires a very low cell Na concentration unless the cells are electrically negative relative to the outside solution. The latter possibility was explored by examining the electrical potential profile of 15 skins bathed with normal Ringer's on the inside and the hypotonic Ringer on the outside. The profile of electrical potential obtained with the outside of the skin bathed in dilute solution containing 1.0 to 1.2 mM NaCl was relatively similar to that observed when normal Cl Ringer's is used (9). The first deflection observed was negative relative to

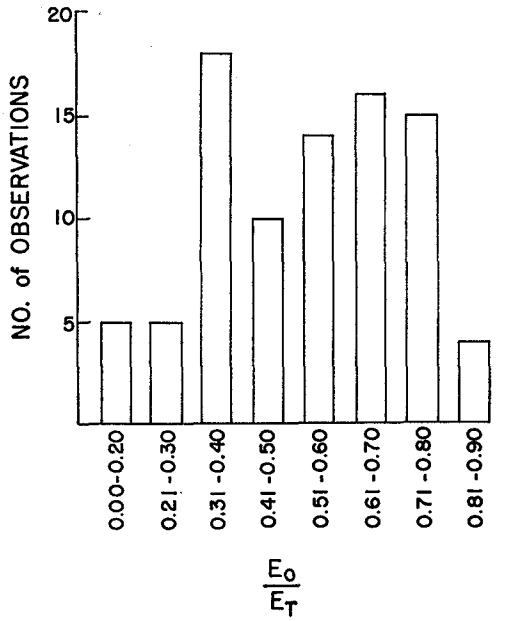

FIGURE 1. Intracellular electrical potentials in frog skin bathed with 1 mM Na on the outside. Number of observations in various ranges of E_o/E_t is shown. E_o is potential difference between the microelectrode and the outside solution; E_t is total potential difference across the skin.

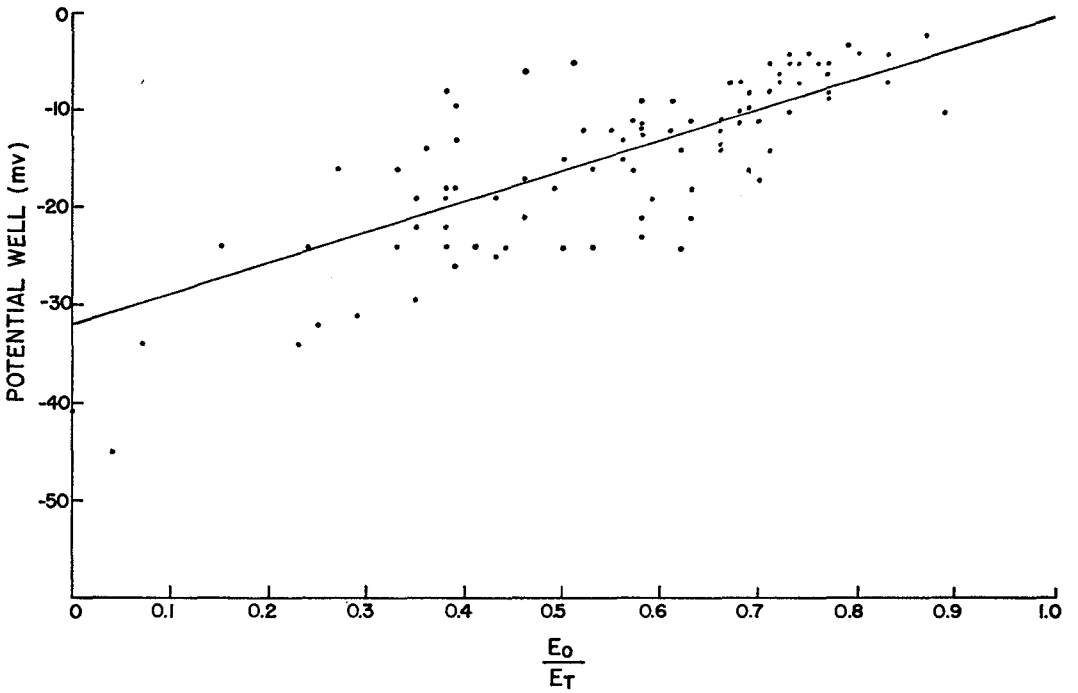

FIGURE 2. Electrical potential difference between microelectrode and outside solution under short-circuit conditions as a function of E_o/E_t observed before passing current. Each point represents a separate puncture. The line was determined by least squares.

the outside solution by 10 to 40 mv. This phenomenon was observed in nearly all punctures and in over half the cases there appear to be two or more distinct potential changes in this region. Further advancement of the electrode gave a step to a potential positive to the outside solution. In all skins there appeared to be at least two levels of positive potential within the skin. In 20 punctures, clear observation of two or more potential steps was obtained by repeated advances of the electrode. In the remaining cases no

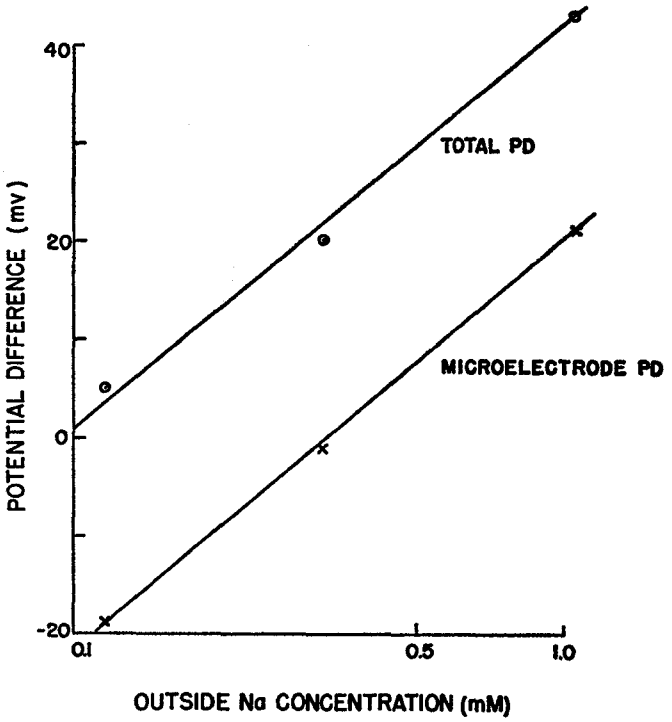

FIG. 3 *a*

FIGURE 3. Total PD and PD between microelectrode and outside solution as functions of log $[Na]_o$ and log $[Cl]_o$. Na concentration was lowered by replacing NaCl by KCl and Cl concentration was increased by addition of choline Cl.

attempt was made to obtain a complete profile. The distribution of observed potential levels is summarized in Fig. 1. The potential is expressed as the ratio of the PD between outside solution and microelectrode to total skin PD (E_o/E_t) and the number of observations of each level is shown. Although there is a relatively uniform distribution of observations in the range of 0.2 to 0.8 for E_o/E_t, there appear to be tendencies toward two peaks around one-third and two-thirds of the total PD.[3]

[3] Although the distribution shown in Fig. 1 appears relatively uniform, we have not observed in a single skin the continuous change in potential reported by Chowdhury and Snell (13). Under our

When the skin was short-circuited with the microelectrode in place, the potential in the skin always became negative to the bathing solutions which were then at equal potentials. The mean value of this potential well was -15 ± 2 mv. However, as illustrated in Fig. 2, there was a tendency for the well to become smaller as the fraction of total PD passed by the microelectrode (E_o/E_t) became larger. Thus, the mean value of the well may have little meaning in terms of properties of the tissue. Such a correlation between size

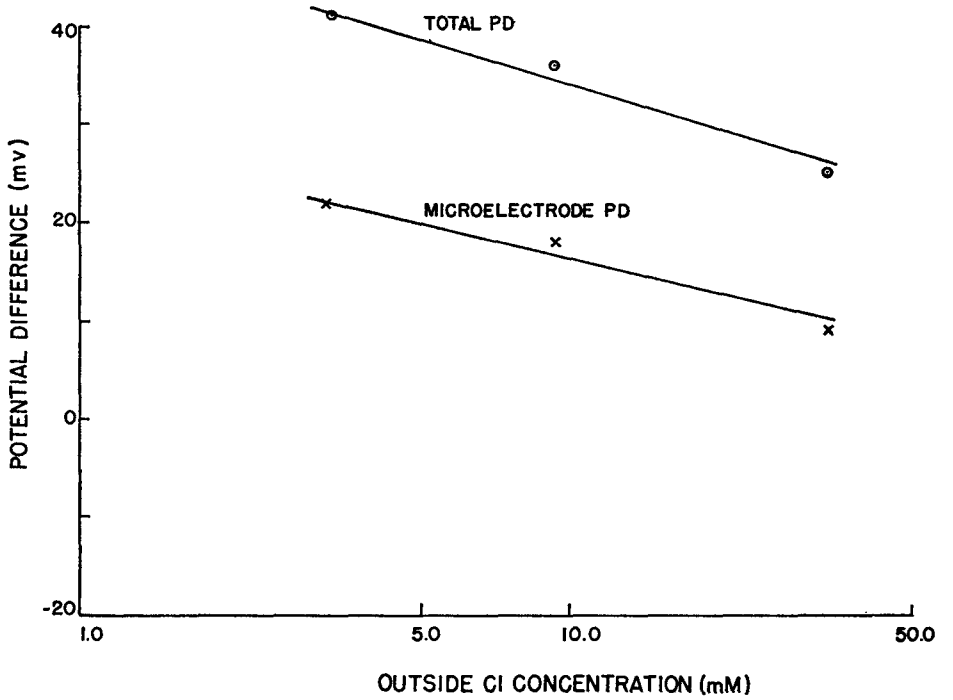

FIG. 3 *b*

of the potential well and E_o/E_t was not observed by Cereijido and Curran (9) in experiments with normal Cl Ringer's as the outside bathing solution.

Under present conditions, most of the skin resistance appeared to be located near the outer surface of the skin in a region external to the first positive step in potential. Thus in 90% of the punctures the resistance between the microelectrode and the outside solution was over 70% of the total

conditions, the potential changes with advancement of the electrode appear as distinct steps. The reasons for this difference are unknown but may relate to the fact that the electrodes used by Chowdhury and Snell are much smaller than ours. A very small electrode might advance much more cleanly than a larger one with little or no tissue distortion and could produce a more uniform profile. On the other hand, very small electrodes may have a relatively small resistance through the glass and this factor could contribute to the observation of a more uniform profile. A careful investigation into the reasons for and the meaning of these very different types of profiles is, however, essential.

skin resistance with a mean value of 90%. There was no consistent variation of resistance with the ratio E_o/E_t. Reexamination of the data obtained by Cereijido and Curran indicated that with Cl Ringer's outside, the resistance between the microelectrode and the outside solution was greater the greater value of E_o/E_t. It seems likely that the data shown in Fig. 2 are a result of the fact that with 1 mM Na outside skin resistance is not distributed in the same manner as it is with 115 mM Na outside.

TABLE IV

ESTIMATION OF CELL Na CONCENTRATION

Experiment	Total water	Cell water	Cell Na^{24}	External Na^{24}	$\Phi si/\Phi so$	$[Na]_o$
	μl	μl	$cpm/\mu l$	$cpm/\mu l$		mM
			Open-circuit			
1	66.9	16.7	499	309	10.0	10.9
2	71.5	17.9	634	305	7.0	10.5
4	81.0	20.2	339	243	8.9	9.2
6	61.4	15.4	311	204	6.7	7.6
7	58.5	14.8	327	234	4.5	5.5
Mean ± SE						8.7±1.0
			Short-circuit			
3	72.0	18.3	1104	246	17.5	15.1
5	58.0	14.5	954	213	19.4	21.9
8	63.5	15.9	328	180	17.5	12.1
9	62.9	15.7	504	225	13.5	13.8
10	48.9	12.2	1237	255	15.5	23.4
Mean ± SE						17.3±2.2

Several experiments were carried out to investigate the source of the PD across the skin under the present conditions. This question is of some interest since the PD, usually 40 to 60 mv, is not different from that observed in *R. pipiens* when the outside bathing solution contains 115 mM NaCl. In these experiments, the Na or Cl concentration of the outside bathing solution was altered with a microelectrode in place in the skin using the technique described by Cereijido and Curran (9). The results are shown in Fig. 3. Changes in both Na and Cl caused changes in the total PD and in the PD between the microelectrode and the outside solution. In both cases the major change in PD occurred between the microelectrode and the outside solution. When the total PD was plotted against log $[Na]_o$ or log $[Cl]_o$, straight lines were obtained having slopes of 40 mv per decade for Na and 16 mv per decade for Cl. On the average, the PD across the outer barrier became zero when outside Na concentration was 0.34 mM.

Cell Na Concentration

An understanding of the nature of the Na entrance into the skin requires a knowledge of Na concentration in the cells or in the compartment through which actively transported Na moves. An attempt has been made to obtain information on this point using the approach outlined in the Methods section. The results of experiments on open-circuited and short-circuited skins are given in Table IV. Under both conditions, the value of $[Na]_s$ calculated from equation 1 is appreciably greater than the Na concentration in the outside solution. The value of $[Na]_s$ is greater for short-circuited than for open-circuited skins as might be expected on the basis of the differences in electrical potential profile under the two conditions.

DISCUSSION

The data summarized in Table I show that net Na transport occurs across isolated frog skin when the outside solution contains 1 mM Na and the inside solution 115 mM Na. The main question considered here is whether Na enters the epithelial cells from the outside by active or passive transport. If this entry step is a passive process, the electrochemical potential of Na ($\tilde{\mu}_{Na}$) must be lower in the cells than in the outside solution. If the Na in the epithelial cells is assumed to be in a single compartment, the present data indicate that Na entry is against an electrochemical potential difference and that an active process is required. This conclusion is based on the data shown in Figs. 1 and 2 and Table IV which can be analyzed using the following equation

$$\tilde{\mu}_{Na(o)} - \tilde{\mu}_{Na(s)} = RT \ln \frac{[Na]_o}{[Na]_s} + F(\psi_o - \psi_s) \qquad (2)$$

in which ψ is electrical potential, R the gas constant, T absolute temperature, and F the Faraday. The subscripts o and s refer to outside solution and skin compartment. We have assumed that the activity coefficients of Na are identical in the outside solution and in the cell. This assumption is questionable, particularly under the present conditions, but no reasonable alternative is available.[4]

In open-circuited skins, $[Na]_o/[Na]_s = 0.092$ and $\psi_o - \psi_s$ varies from -5 to -40 mv; equation 1 shows that $\tilde{\mu}_{Na_{(s)}} > \tilde{\mu}_{Na_{(o)}}$. Under short-circuit conditions $[Na]_o/[Na]_s = 0.061$ while $\psi_o - \psi_s$ varies from $+5$ to $+40$ mv. Again, $\tilde{\mu}_{Na_{(s)}} > \tilde{\mu}_{Na_{(o)}}$; this concentration ratio requires a $\Delta\psi$ greater than $+70$ mv to give $\tilde{\mu}_{Na_{(o)}} > \tilde{\mu}_{Na_{(s)}}$. Since there is a net inward Na move-

[4] The ratio of activity coefficients of NaCl in free solution at 1 mM and 10 mM is 1.08. If the ratio in the skin is of this order, the conclusions will be unaffected.

ment under both open- and short-circuit conditions, these results suggest the presence of an active entry step.

This conclusion rests strongly on the assumption that the Na transported across the skin is in a single compartment since it is on this basis that cell Na concentrations were evaluated. This assumption is consistent with the original model of the skin (2) in which the stratum germinativum cells were suggested as the site of Na transport and electrical properties. The relatively high skin Na concentrations shown in Table IV are consistent with the values obtained by Hansen and Zerahn (14) in experiments involving slicing of the skin. With no Na in the outside solution, they found Na concentrations as high as 30 mM in the outer part of the epithelium (uncorrected for extra-

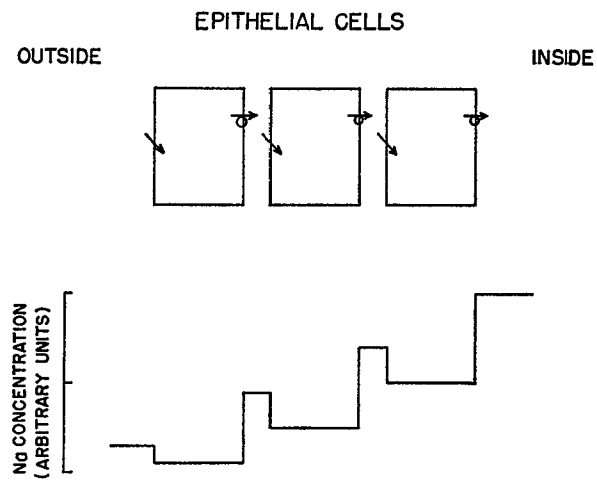

FIGURE 4. Schematic diagram of model for Na transport. Circles represent Na "pumps", straight arrows are Na diffusion. A hypothetical Na concentration profile is shown below.

cellular space). Recent observations have, however, indicated that the electrical properties of the skin are distributed over more than one cell layer and have led to the suggestion that the process of active Na transport may be similarly distributed (13, 15).

On the basis of such a concept, an alternative to active Na entry may be considered for the present case. If several cell layers are involved in the Na transport process, the outer layer may have a sufficiently low Na concentration to allow passive entry from an outside solution containing 1 mM Na. The over-all process of Na transport might then involve transport of Na through successive layers of cells with those cells deeper in the epithelium having progressively higher Na concentrations. A schematic illustration of such a system is shown in Fig. 4, together with a hypothetical Na concentra-

tion profile. The model is similar to that suggested by Ussing and Windhager (15) except that it suggests transport from one cell layer to the next. The shunt between cells suggested by the interspaces observed by Farquhar and Palade (16) and discussed by Ussing and Windhager is not included explicitly but it probably plays a role in the over-all process of Na movement. These spaces appear open but they could be blocked by material which is not visible in the electron microscope and which restricts free movement of solutes. It seems unlikely that the interspaces can lead to a complete short-circuit throughout the epithelium. If they did, an explanation of potential profiles observed in the present work and by Ussing and Windhager would be difficult. On the other hand, some of the Na transported at the first cell layer may pass toward the inside solution via this shunt path as suggested by Ussing and Windhager. A decision on the exact functional significance of these interspaces and the observed bridges between cells (16) must await further study.

Such a system is consistent with two of the electrical observations made in the present study. First, it could explain two or more jumps in potential observed when a microelectrode is advanced through the epithelium. Similar multiple potential steps under different conditions have been observed by several investigators (9, 13, 15, 17). Second, this model can explain a conclusion drawn from the behavior of the electrical profile potential as a function of Na and Cl concentrations on the outside. These data may be utilized to obtain an alternate estimate of "cell" Na concentration. The data in Fig. 3 suggest that the PD between the microelectrode and the outside solution E_o, can be described by the following expression (18):

$$E_o = 58\, t_{Na} \log \frac{[Na]_o}{[Na]_s} - 58\, t_{Cl} \log \frac{[Cl]_o}{[Cl]_s},$$

in which the t_{Na} and t_{Cl} are, respectively, transference numbers of Na and Cl for the "outer barrier." Assuming that $[Na]_o$ and $[Cl]_o$ can be altered with little change in $[Na]_s$ and $[Cl]_s$ the slopes of the lines shown in Fig. 3 indicate that $t_{Na} = 0.70$ and $t_{Cl} = 0.30$. The data of MacRobbie and Ussing (19) indicate that under the conditions employed here $[Cl]_s \cong 30$ mM, and since an increase in $[Cl]_o$ to 115 mM only increases $[Cl]_s$ to 50 mM, the value of 30 mM probably provides a reasonable estimate for the present experiments. If we now assume that $[Na]_s$ is not appreciably altered by relatively small changes in $[Na]_o$ or $[Cl]_o$, we can calculate $[Na]_s$ from the value of $[Na]_o$ for which $E_o = 0$, (0.34 mM Na). The resulting value of $[Na]_s$ is 0.9 mM, slightly below the value for external Na of 1.1 mM used in most of these flux experiments. Since several assumptions are involved in this calculation, the resulting figure cannot be used for quantitative consid-

erations, but two points can be considered in terms of the model discussed above. First, this concentration is of an order of magnitude which would allow passive Na entry if the potential step involved is of the order of +10 mv. Second, this value of $[Na]_s$ is smaller by a factor of 10 than the value estimated by tracer under open-circuit conditions. However, exactly this sort of discrepancy might be expected for the model shown in Fig. 4 if the measured E_o is determined to a large extent by events at the first cell layer. Thus, the model proposed would be consistent with the observation that virtually all the electrical resistance is at the outer side of the skin. If the outer barrier of the first cell layer is permeable mainly to Na and the concentration of this ion is low on both sides of the barrier, the contribution to total skin resistance could be relatively large. Finally, this model appears to have merit from the energetic point of view. In the open-circuit experiments, the minimum thermodynamic energy required for Na transport is approximately 4000 cal/mole. Krogh's finding of Na uptake from 10^{-5} M solutions would require a minimum of about 5000 cal/mole. This is not an impossibly high value, but it is quite large in terms of a minimum requirement. A stepwise transfer of Na across the over-all gradient would require less energy at each step and make possible net transport against much larger total gradients.

The results of the present experiments cannot be considered entirely definitive in terms of a model of the Na transport system of the skin for they may, in principle, be explained by at least two models. If we assume that "cell" Na is in a single compartment and that the transport process involves movement across only two major barriers, it appears necessary to postulate that the entry step across the outer barrier is an active process. Alternatively, if transport involves a stepwise process as suggested, the concept of passive Na entry may be preserved. This latter model is basically similar to the one proposed by Ussing and Windhager (15), and recent observations on potential profile would tend to favor it over the single compartment concept.

APPENDIX

In order to obtain an estimate of effective cell Na concentration, we have assumed that the Na involved in the transport process is in a single compartment and have used the following approach. Under the conditions of this assumption, Na movement across the skin will involve two steps, an entrance into the tissue at the outside and an exit at the inside. If tracer Na is initially placed in the outside solution, the rate of change in the amount of radioactivity in the skin (p_s) will be given by

$$\frac{dP_s}{dt} = \Phi_{os}\, p_o^* - (\Phi_{so} + \Phi_{si})\, p_s^*$$

in which Φ_{ij} is the flux from compartment i to j and p^* is specific activity (CPM/μeq).

The subscripts *o*, *s*, and *i* denote outside solution, skin compartment, and inside solution respectively. Under steady-state conditions, $dP_s/dt = 0$, and

$$p_s^* = \frac{\Phi_{os} p_o^*}{\Phi_{so} + \Phi_{si}}. \tag{1}$$

Further, net Na flux must be the same for both entrance and exit so that

$$\Phi_{os} - \Phi_{so} = \Phi_{si} - \Phi_{is}.$$

Using this expression and the definition $p_i^* = p_i/[\mathrm{Na}]_i$ in which p_i is tracer concentration (CPM/ml) in compartment *i*, equation 1 becomes

$$[\mathrm{Na}]_s = \left(1 + \frac{\Phi_{is}}{\Phi_{os}}\right) \frac{p_s}{p_o} [\mathrm{Na}]_o. \tag{2}$$

In terms of such a model of the skin, the ratio of the over-all unidirectional fluxes Φ_{in}/Φ_{out} will be given by

$$\frac{\Phi_{in}}{\Phi_{out}} = \frac{\Phi_{os}\Phi_{si}}{\Phi_{so}\Phi_{is}},$$

and equation 2 may be written as

$$[\mathrm{Na}]_s = \left[1 + \frac{\Phi_{out}\Phi_{si}}{\Phi_{in}\Phi_{so}}\right] \frac{p_s}{p_o} [\mathrm{Na}]_o. \tag{3}$$

Equation 3 has been used to estimate $[\mathrm{Na}]_s$ under the present conditions as described in the text.

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