The Influence of Na Concentration on Na Transport across Frog Skin

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ABSTRACT The effects of changes in Na concentration of the bathing solutions on some transport and permeability properties of the isolated frog skin have been examined. Rate coefficients for unidirectional Na movements across the two major barriers in the skin have been estimated as functions of Na concentration. The results indicate that the "apparent Na permeability" of the outer barrier of the skin decreases markedly when Na concentration in the outer solution is increased from 7 to 115 mM. The observed saturation of rate of Na transport with increasing Na concentration can be ascribed, in part, to this permeability change rather than to saturation of the transport system itself. Unidirectional Cl flux across the short-circuited skin was not significantly altered by an increase in Na concentration from 30 to 115 mM suggesting that the changes in membrane properties are relatively specific for the Na ion. The results also suggest that the movement of Na across the outer membrane may not be due entirely to simple passive diffusion of free Na ions.

In studying the effects of Ca and antidiuretic hormone on Na transport across isolated frog skin, Curran *et al.* (1) obtained evidence indicating that the rate of active transport is strongly dependent on the effective Na permeability of the outward facing membrane of the transporting cells. On the basis of their results, the saturation of the rate of Na transport with increasing Na concentration in the outside bathing solution (2, 3) cannot be ascribed solely to saturation of the active transport mechanism, assuming it is located at the inner membrane. They suggested that this saturation phenomenon might be associated with the outer membrane, and that the properties of this barrier might be altered by changes in Na concentration. The present experiments were carried out to examine this possibility in more detail by studying the influence of Na concentration on some of the ion transport and permeability properties of the isolated frog skin. The relationship between

the effects of Na and the Ca concentration of the bathing solution has also been investigated.

METHODS

The skin of *Rana pipiens* was mounted in small chambers (3.14 cm^2 area) as previously described (1). The basic Ringer's solution contained 112.6 mM NaCl, 2.4 mM $NaHCO_3$, 2.0 mM KCl, and 1.0 mM $CaCl_2$, and was bubbled with air to give a pH of 8.1. The Na concentration of this solution was altered by replacing NaCl with choline Cl. A more limited series of experiments was also performed using a Ringer's solution containing no added Ca. The experiments were usually carried out in pairs using two pieces of skin from the same frog, each bathed in a solution of different Na concentration. Different pairs of Na concentrations were used in various experiments and no attempt was made to utilize a systematic pairing arrangement. The solutions bathing the two sides of a skin were of identical composition and the skin was kept short-circuited throughout the experiment. An equilibration period of at least 1 hour was allowed before short-circuiting.

The method to determine rate coefficients for Na movement across each of the two major barriers in the skin involves observation of the approach of Na^{24} flux to a steady value and has been described in detail by Curran *et al.* (1). In the present studies the value of $P_{2\infty}$, the amount of Na^{24} present in the transporting compartment when the tracer flux across the skin has reached a steady value, has been estimated more exactly by correcting for the Na^{24} present in the extracellular spaces at the two sides of the skin. Inulin-C^{14} was added to the outside bathing solution in each experiment in order to determine the extracellular space available from the outer side. At the end of an experiment, the skin was removed from the chamber, placed on paraffined paper ("parafilm") to prevent loss of tracer, and quickly blotted with filter paper. The skin exposed in the chamber was cut out and placed in a tube, and Na^{24} was counted in a well type scintillation counter. 1.0 ml of 0.1 N HNO_3 was then added and the tube was shaken for 48 hours to extract inulin-C^{14} (4). After allowing sufficient time for Na^{24} to decay, aliquots of this extract were counted in a windowless flow counter, and the C^{14} activity compared with that in the outside bathing solution in order to estimate the extracellular space. The total amount of Na^{24} found in the skin was then corrected for the amount in the extracellular space available from the outside solution. A correction has also been made for Na^{24} in the extracellular space or connective tissue at the inner side of the skin. Curran *et al.* (1) have derived an expression (Equation A-4 of reference 1) which can be used to estimate the amount of Na^{24} in this extracellular space from the available experimental data. The average correction for total extracellular Na^{24} amounted to 35 per cent of the Na^{24} measured in the skin. Approximately one-quarter of this correction was due to Na^{24} in the extracellular space at the inside of the skin.[1]

In spite of these corrections, the value of $P_{2\infty}$ may still be overestimated to the extent that tracer enters cells not directly involved in the transport process. To test

[1] The extracellular space available from the outer solution was approximately 1 $\mu l/cm^2$ which is larger than that previously reported. The discrepancy may be ascribed to differences in handling the skin during the cutting and blotting procedure.

this possibility, experiments were carried out to determine whether a significant part of the Na^{24} measured in the skin was present in a compartment exchanging more slowly than the compartment assumed to represent the transporting cells. The skin was exposed to Na^{24} from the outer side for 45 min., as in experiments measuring the build-up of tracer flux, and then removed from the chamber. The washout of tracer from the tissue was then followed by suspending it in a large volume of inactive Ringer's solution and removing it periodically for counting in the well counter. In each experiment, less than 5 per cent of the Na^{24} initially present in the skin had a half-time for washout greater than 2 to 5 min. The half-time for build-up of tracer flux to a steady value under similar experimental conditions was 1.5 to 4.5 min. Since the half-times for washout and build-up should depend on the same rate coefficients (6) we may conclude that there is relatively little Na^{24} in compartments which exchange significantly more slowly than the compartment concerned with the build-up of flux. The possibility remains, however, that some of the rapidly exchanging Na^{24} in the skin may be in a pool not concerned with the net flux of Na across the skin. Thus, the value of $P_{2\infty}$ may still be overestimated to some extent.

Some experiments were also carried out to test the effect of changes in Na concentration on the washout of Na^{24} from previously loaded skins. The skin was loaded with Na^{24} by brief (10 to 15 min.) exposure to Ringer's solution containing a high specific activity of the isotope and then mounted in the chamber. Washout of tracer from each side of the skin was followed separately. The entire bathing solution in each chamber was removed by rapid aspiration into tubes at 1 min. intervals and immediately replaced by fresh, non-radioactive solution. Aliquots of the solutions were taken for counting in the scintillation counter. The skin was kept short-circuited except for the brief periods during which solutions were being replaced. In these experiments, the initial bathing solution was normal Ringer's solution (115 mM Na); this was replaced, after 8 min. of washout, by a solution containing 30 mM Na. Thus, the effect of changes in Na concentration could be observed directly in each experiment.

In another series, the effect of changes in Na concentration on unidirectional Cl fluxes across the short-circuited skin was studied. Either Cl influx or outflux was measured using Cl^{36} as previously described (5). In each experiment on a single skin, Cl flux was measured first at 115 mM Na, then at 30 or 18 mM Na, and again at 115 mM Na.

RESULTS

The results of experiments using the technique of following the build-up of Na^{24} flux to its steady value may be expressed in terms similar to those used by Curran *et al.* (1). Fig. 1 illustrates the kinetic model used in these studies and serves to define the parameters. In the previous study, the unidirectional rate coefficient, k_{12}, was expressed in terms of a permeability coefficient;[2] here the rate coefficient notation will be retained in order to compare k_{12} and k_{21}.

[2] The permeability coefficient, P^o_{Na}, used in reference 1 is related to k_{12} by the expression $P^o_{Na} = V_1 k_{12}/3.14$ in which 3.14 is skin area in cm^2 and V_1 is volume of the outside solution (5.0 ml).

The values of the various parameters of the kinetic model obtained at Na concentrations varying from 7 to 115 mM are summarized in Table I for experiments carried out at 1 mM Ca. In order to illustrate clearly the effect of changes in Na concentration on k_{12} and on the Na pool in the transporting compartment, S_2, the data have been normalized. Experiments at reduced

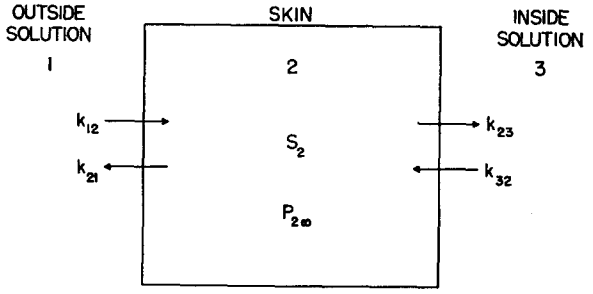

FIGURE 1. Kinetic model of skin used to analyze Na^{24} movement. The k_{ij} represent rate coefficients for Na movement from compartment i to j. S_2 is the Na pool in the skin compartment and $P_{2\infty}$ is the amount of Na^{24} in this compartment when tracer flux from 1 to 3 has reached a steady value. The unidirectional flux, Φ_{ij}, from compartment i to j is given by $\Phi_{ij} = k_{ij}S_i$.

TABLE I

EFFECT OF Na CONCENTRATION ON FROG SKIN

[Na]	No. of experiments	Net Na flux*	k_{12}* × 10²	k_{21}	k_{23}	S_2*
mM		μeq/hr. cm²	hr.⁻¹	hr.⁻¹	hr.⁻¹	(μeq/cm²)
115	14	1.13	1.0±0.1‡	4.3±0.4	12.6±0.6	0.15±0.01
73	6	1.08	1.7±0.2	6.5±1.4	12.0±1.2	0.14±0.01
58	5	1.02	2.0±0.1	6.8±1.7	14.3±1.5	0.13±0.01
30	14	0.78	3.6±0.3	8.7±0.9	13.8±1.1	0.09±0.01
18	10	0.63	5.5±0.5	10.2±1.3	12.3±1.0	0.08±0.01
7	10	0.36	7.5±0.6	10.5±1.5	13.9±1.2	0.04±0.01

* Normalized values obtained as described in the text.

‡ Errors are given as standard error of the mean.

Na concentration were not always paired with a piece of skin bathed in normal Na (115 mM). Thus, the normalization procedure is required because of the variability in net Na transport from one frog to another under similar conditions and because of the significant correlation ($p < 0.01$) between k_{12} and S_2 and the rate of net Na transport.

In order to carry out the normalization, the relationship between net Na flux and Na concentration in a single skin must be determined. Consequently,

6 experiments were carried out in which net Na transport was measured by the short-circuit current at each of the Na concentrations used in the present experiments. When the flux at each Na concentration was expressed as a fraction of the value at 115 mM Na, agreement among the various skins was excellent (Fig. 2). The mean Na flux observed in build-up experiments carried out at 115 mM was then taken as normal and normalized values of flux at other Na concentrations were then computed from the observed relationship between flux and concentration. The net fluxes given in Table I are the normalized values. These values of Na flux were then used as follows to obtain normalized values of k_{12} and S_2. For each Na concentration

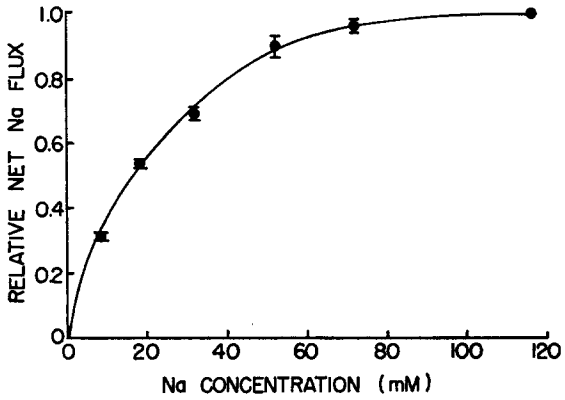

FIGURE 2. Relationship between net Na flux (short-circuit current) and Na concentration. The flux at reduced Na is given as a fraction of that at 115 mM. The points represent the average of 6 experiments and the bars indicate ± one standard error.

tested, the values of k_{12} and S_2 observed in each experiment were plotted against the corresponding observed net Na fluxes. The best straight lines through the points were determined by least squares yielding sets of equations of the form

$$k_{12} = a\,\Phi_n + b \tag{1}$$

$$S_2 = a'\,\Phi_n + b' \tag{2}$$

in which Φ_n is net Na flux and a, b, a', and b' are constants at a given Na concentration but may vary with changes in concentration. The relationships between k_{12} and Na flux obtained at two different Na concentrations are shown in Fig. 3. Normalized values of k_{12} and S_2 were then calculated at each Na concentration from Equations 1 and 2 by inserting the appropriate normalized values of Φ_n obtained as described above. The resulting values of k_{12} and S_2 are reported in Table I. Since there was no significant correla-

tion between Na flux and other parameters, the other quantities given in Table I were not normalized.

A similar series of experiments at Na concentrations ranging from 33 to 115 mM were carried out in a Ca-free solutions, and the data were normalized as described above. Although the net Na flux was higher in these solutions as expected (5, 7) (1.58 μeq/hr. cm^2 at 115 mM Na), the values of k_{23} and k_{21} were not significantly different from those given in Table I for solutions

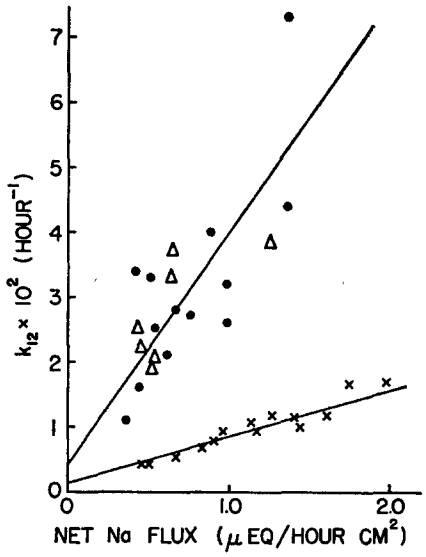

FIGURE 3. Relationship between k_{12} and net Na flux at 115 mM and 30 mM Na. Each point represents a single experiment. Filled circles, 30 mM Na–85 mM choline; open triangles, 30 mM Na–85 mM K; x, 115 mM Na. The lines have been determined by the method of least squares. For 30 mM Na only those points obtained in experiments in which choline replaced Na were used to determine the line.

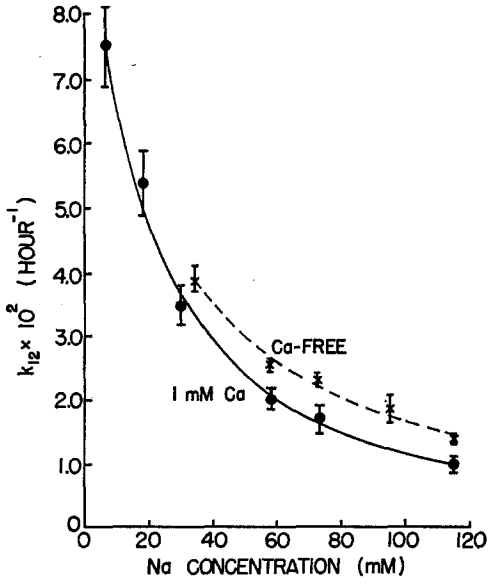

FIGURE 4. k_{12} as a function of Na concentration in the bathing solutions. The data have been normalized as explained in the text. The bars on the points indicate ± one standard error calculated from the regression lines of k_{12} as a function of net Na flux. The lines have been drawn by eye.

containing 1 mM Ca. They will not, therefore, be reported in detail. The values of k_{12} and S_2 were, however, different for the two sets of conditions as illustrated in Figs. 4 and 5.

The results given in Fig. 4 indicate that there is a marked decrease in k_{12} with increase in Na concentration of the bathing solutions. The changes followed a similar pattern in experiments carried out in the presence and absence of Ca but k_{12} was always greater in Ca-free solutions than in 1 mM Ca. As shown in Fig. 5, the Na pool in the cells changes only slightly at higher Na concentrations in the bathing solutions and then declines sharply at the

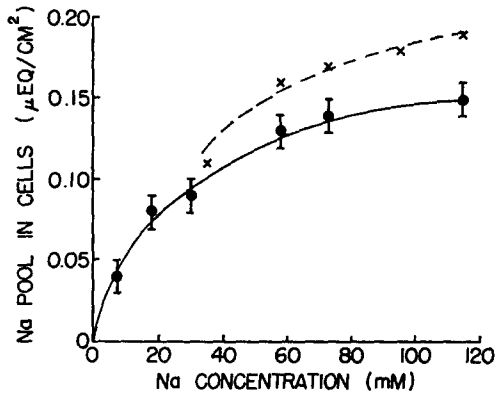

FIGURE 5. Na pool size as a function of Na concentration in the bathing solutions. Filled circles, 1 mM Ca; crosses, Ca-free. The bars on the points for 1 mM Ca indicate ± one standard error. The standard errors for Ca-free solutions ranged from ± 0.01 to 0.02; they have been omitted from the curve to avoid confusion.

lower concentrations. Again the pattern is similar for 1 mM Ca and Ca-free solutions but the Na pool was always greater in Ca-free solutions than in the corresponding solution containing 1 mM Ca, as might be expected (1), although the differences were not statistically significant in the present case. As shown in Table I there was little change in k_{23} and a definite increase in k_{21} with decreasing Na concentration. The data are presented here as averages with no attempt to compare paired skins. However, comparison of values in individual experiments indicated that the differences between paired skins at different Na concentrations were almost always in the direction indicated by the averaged data. For example, most of the experiments at 18 mM Na were paired with experiments at 30 mM Na, and in each case k_{12} was greater at the lower concentration.

Seven experiments were carried out at 30 mM Na to examine the possibility that the observed changes in k_{12} were due not to the decrease in Na concentration but to the presence of choline in the bathing solutions. In these experiments, K was used instead of choline to replace Na in the outside

solutions, but because of the effects of K at the inner side of the skin, the inside solution was normal Na Ringer's solution (115 mM Na). An accurate measure of net Na transport could not be obtained since K diffusion down its large concentration gradient contributed significantly to the measured short-circuit current. Consequently, the measured Na influx has been taken as a maximal estimate of net Na flux. The value of the rate coefficient k_{12} can, however, be estimated accurately since calculation of this quantity does not require knowledge of the net flux (1). The results of these experiments are included in Fig. 3 and, as shown, there appears to be no difference between the skins in which K and choline were used to replace Na. Since it seems unlikely that choline and K would have identical specific effects on the outer membrane, the observed change in k_{12} may be ascribed to the change in Na concentration.

As described by Curran *et al.* (1) the Na concentration in the cells can be estimated by assuming that Na diffuses freely across the outer membrane and that there is no potential difference across this barrier under conditions of short-circuiting. The present experiments cast some doubt on the first of these assumptions (see Discussion) and preliminary experiments with microelectrodes (8) have indicated that the second may be incorrect. Consequently, Na concentration in the cells will not be discussed in any detail at present. The mean values of Na concentration calculated on the basis of the above assumptions varied from 4 to 37 mM as Na concentration in the bathing solutions varied from 7 to 115 mM. In the absence of additional information, it is impossible to estimate how well these values approximate the true ones.

The data given in Table I and Fig. 4 indicate that the effective Na permeability of the outer barrier decreases as Na concentration is raised, since both k_{12} and k_{21} decrease under these conditions. However, the decrease in k_{21} is not large, and the evaluation of this parameter is somewhat less accurate than that of k_{12} because k_{21} is determined as a difference (1). Since the question of whether or not k_{21} changes is important in attempting to analyze results, an effort has been made to test directly the effect of reduction in Na concentration on the washout of Na^{24} from skins loaded with tracer. The results of one such experiment together with a control are shown in Fig. 6 in which Na^{24} efflux (in CPM/min.) from the skin to outside solution is plotted semilogarithmically against time. There was a significant increase in tracer outflux when the Na concentration of the bathing solution was lowered from 115 mM to 30 mM. Interpretation of these experiments is somewhat complicated by the fact that the points do not fall on straight lines, but the results can be expressed quantitatively. As shown in Fig. 6, straight lines were drawn through several points before and after change in Na concentration. The lines were extrapolated to the time of change and the percentage increase in tracer outflux was calculated from the difference between them.

In 8 experiments, the outflux of Na^{24} increased by 30 ± 6 per cent when Na concentration was lowered. The validity of this estimate was confirmed by carrying out 4 control experiments in which the Na concentration was not altered. When the control data were treated in the same manner, by drawing lines through the comparable points, a flux change of −4 per cent was

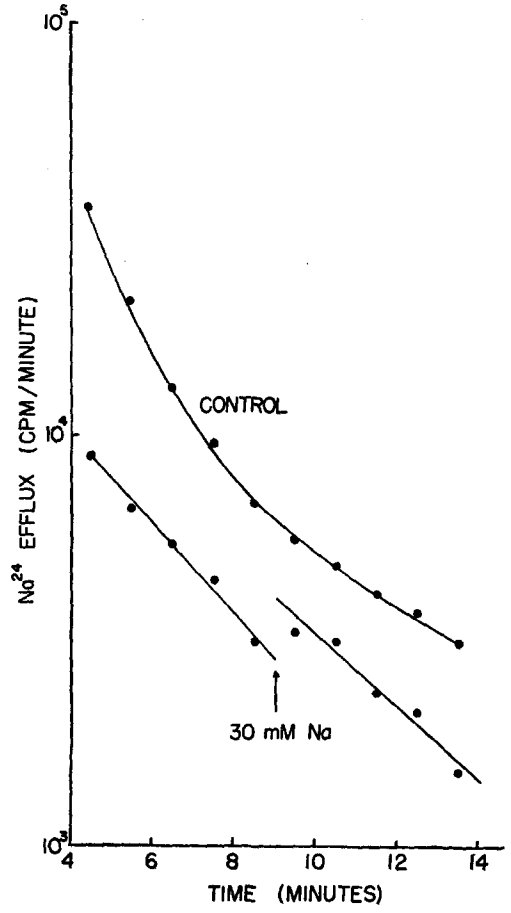

FIGURE 6. Effect of change in Na concentration on washout of Na^{24} from the skin to the outside solution. The upper curve is from a control experiment in which Na concentration was 115 mM throughout. In the experiment illustrated by the lower curve, Na concentration was reduced from 115 mM to 30 mM at the time indicated.

computed. The increase in Na^{24} outflux is considerably less than the change in k_{21} shown in Table I for a similar alteration in Na concentration. Since the washout experiments were not carried out under steady-state conditions, while those reported in Table I were, better agreement probably cannot be expected. Thus, these washout experiments appear to support the conclusion that k_{21} increases as Na concentration in the bathing solutions is lowered.

A series of 16 experiments was carried out to test the effect of changes in Na concentration on unidirectional Cl fluxes across the skin under short-circuited conditions. Since Cl crosses the skin passively (9), there is no net

flux under these conditions and the two unidirectional fluxes should be equal. In each experiment, the Cl flux measurement at either 30 or 18 mM Na was bracketed by measurements at 115 mM Na and the flux at the high Na concentration was taken as the mean of the two values. (In these experiments, the Cl flux at 115 mM Na varied from 0.12 to 1.73 μeq/hr. cm^2 with a mean of 0.85 μeq/hr. cm^2.) There was no difference between the effects obtained with 30 and 18 mM Na and similar results were obtained whether influx or outflux was measured. Therefore all experiments have been considered as a single group. In 10 experiments, Cl flux clearly decreased at the lowered Na concentration, but in 6 cases a small increase was observed. For all experiments, the average ratio of flux at 115 mM Na divided by flux at low Na was 1.25 $\pm$ 0.16 (SEM). Thus, although a decrease in Na concentration caused a decrease in unidirectional Cl flux, we have been unable to demonstrate that the change is statistically significant under the present conditions.

DISCUSSION

The Na Transport System

The earlier studies of Curran *et al.* (1) have indicated that the rate of Na entry into the transporting cells through the outer barrier plays an important role in controlling the rate of active net Na transport across the frog skin. The results of the present experiments have shown that the "apparent Na permeability" of the outer membrane varies markedly with Na concentration of the outside solution. As Na concentration is increased, permeability of the outer barrier decreases and the rate of Na entry into the cells will not increase proportionately to the concentration, nor will the net active Na flux across the skin since this flux is dependent on the rate of Na entry. Thus, the variation in Na permeability could account, at least qualitatively, for the observed saturation of net Na flux with increase in Na concentration of the outside solution (2, 3). This interpretation is also consistent with the data shown in Fig. 5 which indicate that the amount of Na in the transport pool becomes virtually independent of external Na at concentrations above 60 mM. Such a result would be expected if the saturation phenomenon is associated with the process of Na entry into the cells. If the saturation were determined by the active extrusion from the cell, the Na pool should increase with increasing Na concentration over the whole range of observation.

In these considerations of the phenomenon of saturation, the active transport system itself and its effect on the rate of net Na transport have not been discussed. In the previous study, Curran *et al.* (1) found no evidence for saturation of this system; the rate of net Na transport was approximately a linear function of the size of the Na pool in the cells. The present experiments make possible the evaluation of this relationship over a wider range of Na pools and rates of Na transport. In order to do this, the results of all experi-

ments at both Ca concentrations have been averaged to give the values shown in Fig. 7 in which rate of transport is plotted against pool size. (The relationship between flux and Na concentration cannot be evaluated adequately because of difficulties in estimating Na concentration in the cells as discussed above.) Over the range of pool sizes observed, the active transport system does not become saturated although the relationship is non-linear and there appears to be a tendency toward saturation. Thus, these results lend further support to the conclusion that the saturation effect is primarily a property of the outer barrier since the active transport system is not saturated at the highest levels of cell Na observed. However, the present data indicate

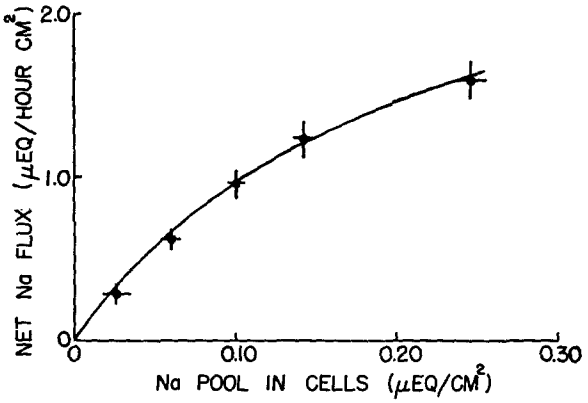

FIGURE 7. Net Na flux as a function of Na pool size, S_2. The results of all experiments have been divided into 5 groups and averaged to obtain the points shown. The crosses at each point indicate ± one standard error of the mean for both flux and pool size. The line has been drawn by eye.

that both Na permeability of the outer barrier and the active transport system, assumed to be located at the inner barrier, may influence the rate of net Na transport across the skin. In any given case, the rate of transport and the size of the Na pool in the skin are determined by a combination of the properties of these two barriers. Some observations on the over-all Na transport system may represent, at least in part, properties of the outer barrier, while others may reflect primarily the actual active step itself.

The present observations are similar, in several respects, to those reported by Frazier *et al.* (10) for toad bladder. They have found that the active transport pool of Na in this tissue does not increase linearly with Na concentration in the bathing solutions and have concluded that Na entry from the mucosal (outer) solution determines the rate of net Na transport. They have suggested that Na moves passively down a concentration gradient across the mucosal membrane but that the mechanism of Na movement is not free diffusion.

The results presented in Fig. 4 appear to offer a qualitative explanation of the rather puzzling observation of Curran and Gill (7) that the effect on net Na transport of Ca added to the outside solution is less at low than at high Na concentrations. The data suggest that the absolute magnitude of the decrease in k_{12} caused by Ca is approximately independent of Na concentration. However, this decrease is a smaller fraction of the total k_{12} at low Na concentrations due to the increase in k_{12} occurring as a result of the lowered Na concentration. Since, at a given Na concentration, the rate of net Na transport varies linearly with k_{12} (Fig. 3), the fractional decrease in Na transport caused by Ca will be less at low Na, as observed by Curran and Gill. Thus, the dependence of the Ca effect on Na does not appear to reflect an interrelationship between the two cations, and the actual effect of Ca on the outer barrier appears to be independent of Na concentration. This conclusion is further strengthened by the observation that Ca reduces Cl movement across the skin (5, 7), while decreasing Na does not have a consistent effect on Cl flux.

Properties of the Outer Barrier

The conclusion that the apparent Na permeability of the outer barrier is decreased by increased Na concentration in the bathing solutions is based on two lines of evidence: estimations of the rate coefficients for Na movement across this barrier (Table I), and direct observation of changes in Na^{24} outflux from loaded skins (Fig. 6). The exact mechanism involved in these changes in properties of the barrier cannot be entirely clarified without knowledge of the means by which Na crosses this barrier, but several alternative explanations may be considered.

One possibility is that there exists an electrical potential difference across the outer barrier under short-circuited conditions which is altered by changes in Na concentration of the bathing solutions. Since the rate coefficients, k_{12} and k_{21}, include effects of any potential difference which may exist across the barrier, they will change if the magnitude of the potential difference changes. If such a potential difference were oriented with the interior of the cell relatively negative and increased in magnitude at low Na, an increase in k_{12} would be expected. However, such a change would cause a *decrease* in k_{21}, rather than the observed *increase*, since this coefficient is related to Na movement from the cell toward the outside solution, in a direction opposite to that related to k_{12}. Further, preliminary experiments using microelectrodes (8) have indicated that the potential difference across the outer membrane when the skin is short-circuited does not vary appreciably with changes in Na concentration.

The decrease in the apparent Na permeability of the outer barrier with increasing Na concentration could be ascribed to a change in the "partition

coefficient" for Na between the bathing solution and the barrier. However, the nature of this partition coefficient depends on the nature of the barrier and on the mechanisms by which Na crosses it. Some information on this point might be obtained by considering in more detail the dependence of k_{12} on concentration in the bathing solution. The simplest empirical relationship between these quantities, fitted by the data for both 1 mM Ca and Ca-free solutions, is

$$k_{12} = 1/(A + B[\mathrm{Na}]_o) \tag{3}$$

in which A and B are constants and $[\mathrm{Na}]_o$ is Na concentration of the outside solution. Fig. 8 indicates that a linear relationship between $1/k_{12}$ and $[\mathrm{Na}]_o$ is observed as described by Equation 3.

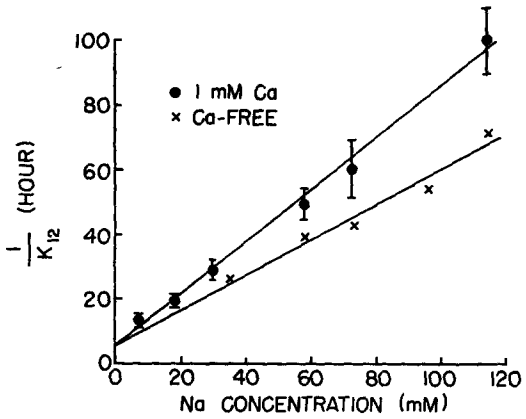

FIGURE 8. Relationship between k_{12} and $[\mathrm{Na}]_o$. The bars on the points for 1 mM Ca represent ± one standard error of the mean. The lines have been determined by the method of least squares.

This relationship is not unduly complex and there are, at present, several models which would predict such a dependence of a permeability on concentration. Two general types of mechanism may be considered briefly. If the outer barrier were a negatively charged membrane impermeable or only slightly permeable to choline, substitution of choline for Na in the outside solution would result in an increase in the partition coefficient for Na at the outer membrane surface (the Donnan ratio, r, at that surface). If the barrier were entirely impermeable to choline, the condition of electrical neutrality within the barrier would require that r be given by the expression

$$r = \frac{X}{2[\mathrm{Na}]_o} + \frac{1}{2}\left[\left(\frac{X}{[\mathrm{Na}]_o}\right)^2 + \frac{4[\mathrm{Cl}]_o}{[\mathrm{Na}]_o}\right]^{1/2} \tag{4}$$

in which X is the concentration of fixed negative charge and $[\mathrm{Cl}]_o$ is the

Cl concentration in the outside solution. At constant $[Cl]_o$, the value of r would increase with decreasing $[Na]_o$, and this change would be measured as an increase in k_{12} in the present experiments. The form of Equation 4 is not the same as that of Equation 3, but if X is sufficiently large, $1/r$ will be approximately a linear function of $[Na]_o$ over a reasonably wide range. However, an increase in the Donnan ratio at the surface of the barrier should also cause a *decrease* in Cl movement across this barrier. Such an effect could not be clearly demonstrated in the present experiments although the data do suggest that there may be a decrease in Cl movement when Na concentration is lowered to 30 mM. Macey and Meyers (11) have recently reported a significant decrease in Cl permeability of the skin caused by complete removal of Na from the bathing solution. On the basis of these considerations, the effect of a charged barrier cannot be completely ruled out, but the change in Cl permeability seems too small relative to the change in Na permeability to be explained by such a mechanism. On the other hand, Kidder *et al.* (12) have obtained evidence suggesting that the outer barrier might involve two different barriers arranged in series, one near the outer surface of the skin in addition to the membrane of the transporting cells at the base of the epithelium. As discussed by Kedem and Katchalsky (13) charged barriers in series may exhibit unexpected properties and some of the present observations might be accounted for by such an arrangement.

A more attractive possibility which could account for the present results is that a part of the Na movement across the outer barrier is carrier-mediated. The carrier-mediated flux would become independent of concentration as the system became saturated and the apparent permeability of the barrier would decrease as Na concentration increased. Such a transport system could be facilitated diffusion (14) or active transport. There is at present no evidence suggesting that Na is actively transported across the outer barrier, and it seems more likely that if carrier transport is involved, the mechanism is facilitated diffusion, which is not an energy-requiring process.

A similar behavior would be observed if the penetration of Na across the outer barrier involved an interaction of Na with sites in the membrane which are limited in number (15) or a surface absorption layer of ions as suggested by Harris and Sjodin (16). In either case the flow of Na would not be proportional to the concentration in the outside solution. The distinction between these mechanisms and facilitated diffusion is not marked and all may be considered to represent interaction of Na with the membrane during passage. Sjodin (15) has discussed in detail a kinetic model which could apply equally well to any of these cases and which indicates that the partition coefficient at the membrane surface would have the form required by Equation 3. Thus it seems likely that the observed dependence of the apparent Na permeability of the outer barrier on Na concentration arises because Na does not diffuse

freely across the barrier. The specific mechanism involved in Na movement cannot, as yet, be specified and further investigation is necessary.

In summary, the present experiments have shown clearly that the apparent Na permeability of the outer barrier of the frog skin is strongly dependent on the Na concentration of the bathing solutions. The observed saturation of the rate of net Na transport with increasing Na concentration can be explained primarily in terms of this effect on the outer barrier, rather than in terms of saturation of the active transport system itself. The specific mechanism or mechanisms which are responsible for the observed effect remain unknown, but the results can be interpreted in terms of an interaction of Na with the membrane and, therefore, suggest that this ion does not cross the barrier entirely by simple free diffusion.

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