Response of the Frog Skin to Steady-State Voltage Clamping

I. *The shunt pathway*

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ABSTRACT Properties of the shunt pathway (a pathway in parallel to the Na transport system) in frog skin have been examined. The permeability of this shunt to urea increases markedly when the skin is depolarized to −100 mv (inside negative) but hyperpolarization to +100 mv produces no change in urea permeability compared to short-circuit conditions. The permeability increase at depolarizing potentials is dependent on the external solute concentration and is considerably reduced by the presence of external Ca. Neither urea permeability nor its response to changes in potential difference are affected by complete inhibition of Na transport by ouabain. In ouabain-poisoned skins, movements of Na, K, Cl, and mannitol through the shunt change in parallel with urea movements. Ion fluxes under these conditions and their response to potential can be described by the constant field equation. The selectivity of the shunt is in the order Cl > urea > K > Na > mannitol and this order does not appear to be affected by the absolute magnitude of the shunt permeability. Arguments are presented suggesting that the pathway is mainly between cells and that its permeability may be affected by cell swelling.

INTRODUCTION

Ussing and Windhager (1) and Ussing (2, 3) have described the movement of various solutes through the frog skin in terms of a pathway parallel to the active Na transport path and have denoted this parallel pathway a shunt (see, for example, Kedem and Essig [4] for a general discussion of such parallel systems). The conductance of the shunt pathway of skins bathed in sodium sulfate Ringer was estimated from Na outflux and SO_4 fluxes through the skin (1). The shunt conductance increased when the outside solution was made hypertonic with permeable solutes such as urea (1, 2). Little additional information is available on properties of the shunt in the skin and its morphological location is not known. Such a shunt has been studied in the proximal tubule of the kidney (5), in gallbladder (6), and in ileum (7); in all three

tissues there is evidence indicating that the primary pathway is between adjacent cells.

In the present study, the permeability of the shunt pathway in the skin to various electrolytes and nonelectrolytes was examined as a function of an applied potential. The permeability to all solutes was found to increase markedly at high depolarizing potentials, while maintaining a predictable relationship between electrolytes and nonelectrolytes. In addition, the solute selectivity of the shunt as a function of over-all permeability changes was utilized to determine some of its physical properties.

METHODS

The skin of *Rana pipiens* was mounted as a flat sheet (3.14 cm^2) between Lucite chambers equipped with solution reservoirs similar to those described by Schultz and Zalusky (8). Many of the experiments involved paired skins obtained from the same frog by splitting the skin longitudinally along back and abdomen to yield two pieces as nearly symmetrical as possible. The solutions in each chamber (10 ml each) were stirred and oxygenated by bubbling with air. The method used to measure the potential difference (PD) across the skin and that used to pass current through the skin have been previously described (8). In some of the studies, an automatic voltage clamp that compensated for the resistance of the solution between the agar bridges was used to pass the appropriate current through the skin to maintain a preset PD value.

To measure unidirectional fluxes, the skins were allowed to equilibrate until the open circuit PD reached a steady value and were then voltage clamped at the PD desired for the first flux period. The appropriate isotopes, ^{22}Na (1 μCi) or, ^{24}Na (2–10 μCi), ^{36}Cl (1 μCi), urea-^{14}C (4.2 μCi), and mannitol-^{3}H (10 μCi), were added to one bathing solution and, after a 30 min equilibration period, 1-ml samples were withdrawn from the opposite solution at 20-min intervals and replaced with nonradioactive solution. Three such samples were taken at each PD, after which the skin was equilibrated at a new level of PD and the procedure was repeated. At the end of the experiment, a 1 ml sample was withdrawn from the side to which the isotopes were added. Samples were assayed for their respective isotopes in Bray's solution using a liquid scintillation spectrometer (Model Mark I; Nuclear-Chicago, Des Plaines, Ill.). Unidirectional fluxes were calculated from the rate of tracer appearance on the "cold" side and the specific activity of the "hot" side.

The composition of regular NaCl Ringer solution was 112 mM NaCl, 2.5 mM $KHCO_3$, and 1 mM $CaCl_2$. In all experiments, the inside solution was regular NaCl Ringer. The NaCl concentration of the outside solution was reduced in some experiments leaving the remaining ionic concentrations constant; these hypotonic solutions are denoted as either dilute 20 mM NaCl or dilute 2–3 mM NaCl Ringer. Choline or potassium Ringer were made by substituting for all the NaCl with identical concentrations of choline or potassium chloride. In one series of experiments, the $CaCl_2$ concentration was varied to obtain no-Ca^{++} Ringer (no $CaCl_2$ plus 0.5 mM ethylenediaminetetraacetate [EDTA]) and 10 mM Ca^{++} Ringer (10 mM $CaCl_2$). When urea or mannitol fluxes were measured, both bathing solutions contained a 2 mM concentration of the appropriate nonelectrolyte.

RESULTS

Effects of Applied PD on Urea Permeability

Unidirectional influxes of urea were measured as a function of potential with results summarized in Fig. 1. The urea influx displays no significant change with applied potential at hyperpolarizing PD's (positive inside), whereas there is a significant rise in flux at depolarizing potentials. This relationship between flux and applied PD is also observed for the urea outflux. The in-

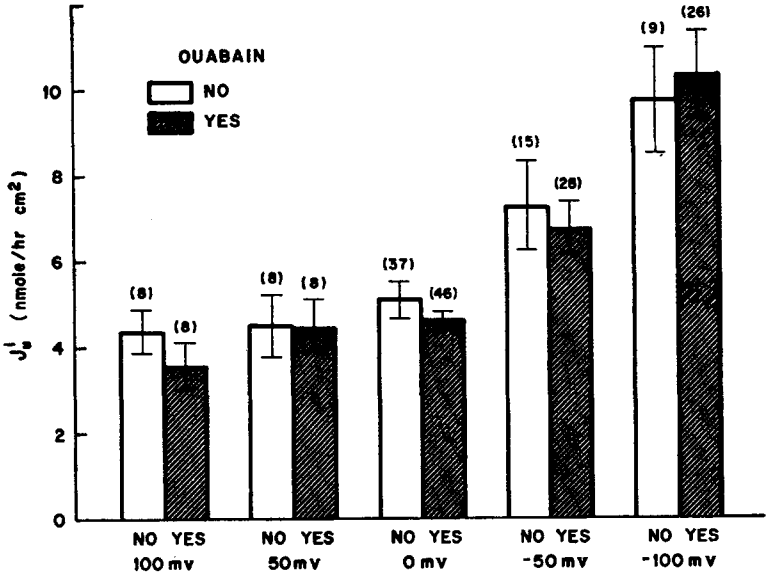

FIGURE 1. Unidirectional influxes of urea (J_U^i) as a function of applied potential for ouabain-poisoned and unpoisoned skins.

crease in urea fluxes in both directions indicates that the applied potential causes a true permeability change rather than solvent drag or solute-solute coupling, since both of those effects should lead to an increase in one unidirectional flux and a concomitant decrease in the other. Inhibition of the Na transporting system with 10^{-4} M ouabain (virtually 100% inhibition of short-circuit current) caused no change in the urea permeability, or its increase with applied potential, as shown in Fig. 1. This result demonstrates that urea moves passively through a shunt pathway, without coupling to active Na transport.[1] Ouabain was used in all subsequent experiments to provide

[1] Experiments were performed in two skins to test whether edge damage contributed to the increase in shunt permeability. The skins were tied like balloons and their urea permeabilities were measured through an area not encompassing the tied portion in an experimental setup similar to that described by Walser (9). The results, averaged over the approximate area of fluid contact, demonstrated an increase in permeability as a function of potential similar to that of skins mounted in a Lucite chamber. We conclude, therefore, that edge damage does not contribute significantly to the shunt permeability increase of frog skin.

virtually complete inhibition of the active Na transport system in an effort to obtain the simplest possible system for studying properties of the shunt.

Relationship between the Shunt Permeabilities to Ions and Urea

(A) NA FLUXES THROUGH THE SHUNT The shunt permeability to Na was measured concurrently with that to urea in ouabain-treated skins. As indicated in Fig. 1, ouabain does not affect the shunt pathway as measured

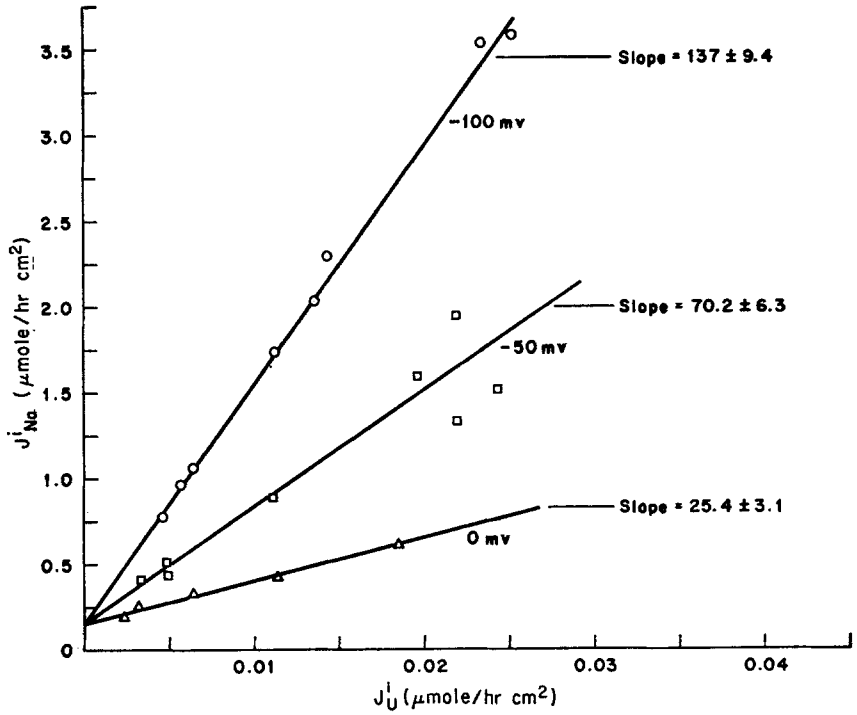

FIGURE 2. Unidirectional influxes of Na vs. urea at three applied potentials in ouabain-poisoned skins. The points appearing in the figure are representative only; the lines were calculated by the least squares method using results from 17 skins.

by urea fluxes. Influxes were measured simultaneously using ^{22}Na and urea-^{14}C because any increase in Na permeability at large depolarizing potentials would be easily detected in this direction. The experimental results with 112 mM NaCl Ringer are summarized in Fig. 2, in which unidirectional Na influx is plotted against the unidirectional urea influx at three experimental PD's. There appears to be a linear relationship between the influxes of urea and of Na at each PD with the ratio of Na to urea influxes increasing with increased depolarization. Regression lines were calculated at each PD and the slopes obtained are shown in Table I (first row).

The relationship between Na and urea permeabilities shown in Fig. 2 can

be quantitatively described using the expression for ionic flux derived from the assumption of a constant electric field (10). As shown in the Appendix, the resulting expression relating Na and urea fluxes is

$$\frac{J_{Na}^{i(s)}}{J_U^i} = \frac{\alpha_{Na} C_{Na}^o}{C_U^o}\left[\frac{-FV/RT}{1-\exp(FV/RT)}\right] \quad (1)$$

in which $J_{Na}^{i(s)}$ and J_U^i are the Na and urea influxes through the shunt, $\alpha_{Na} =$

TABLE I

LEAST SQUARES SLOPE OF J_i VS. J_U AT DIFFERENT POTENTIALS

Ion i	Direction	No. of skins	Slopes (± SE)			
			50 mv	0	−50 mv	−100 mv
Na^+	$o \rightarrow i$	17		25.4 ± 3.1	70.2 ± 6.3	137.0 ± 9.4
Na^+	$i \rightarrow o$	5	90.5 ± 12.9			
K^+	$o \rightarrow i$	4		48.3 ± 14.8	94.4 ± 33.3	133.0 ± 13.4
Cl^-	$o \rightarrow i$	6	366.0 ± 23.0	126.0 ± 7.4	44.0 ± 12.0	10.1 ± 5.2
Cl^-	$i \rightarrow o$	5			321.0 ± 139	

TABLE II

CALCULATED VALUES OF α FOR Na, K, Cl, AND MANNITOL AT DIFFERENT APPLIED POTENTIALS

Applied potential	α(± SE)			
	Na^+	K^+	Cl^-	Mannitol
mv				
50			2.79±0.18	
0	0.45±0.06	0.84±0.29	2.21±0.13	0.38±0.03
−50	0.55±0.05	0.73±0.27	2.49±0.68	0.45±0.04
−100	0.61±0.04	0.60±0.06	2.15±1.11	
Average	0.54	0.72	2.41	0.42

(P_{Na}/P_{urea}) where P is permeability, C_{Na}^o and C_U^o are the external Na and urea concentrations, F is the Faraday, V is the applied potential, R is the gas constant, and T is the absolute temperature. In the derivation, α_{Na} is assumed to be a constant independent of PD.

Equation 1 predicts that J_{Na}^{is} should be a linear function of J_U^i at each PD with the ratio of slopes of the regression lines given by the term in brackets. The expected ratios of the slopes for 0, −50, and −100 mv is 1:2.3:4.0; the observed values of 1:(2.8 ± 0.4):(5.4 ± 0.4) do not differ markedly from those expected. Values of α_{Na} were calculated at each potential from equation 1, utilizing the slopes shown in Table I; the results, shown in Table II, indicate no marked variation of α with potential. The average value of $\alpha_{Na} =$

0.54 indicates that the effective permeability of Na through the shunt pathway is about one-half that of urea. This value is significantly different from the ratio of Na to urea mobilities in free solution, where it is close to unity. As indicated in Table I (second line) the effect of a PD of +50 mv on Na outflux is essentially the same as the effect of −50 mv on Na influx; the slopes of the respective lines do not differ significantly. The value of α_{Na} for Na outflux at +50 mv is 0.68. Thus the behavior of Na movement in this pathway seems to be essentially symmetrical.

The intercepts of the lines in Fig. 2 on the ordinate are not statistically different from zero, except for the one at short circuit (0.13 ± 0.03 μeq/hr cm²). The exact significance of this intercept is difficult to assess. It would represent a minimal amount of Na still passing through the pump, although under the conditions of the experiments most of the Na appears to move through the same pathway as urea.

(B) K FLUXES THROUGH THE SHUNT The movement of K through the shunt pathway can also be described by equation 1. Values for slopes of regression lines calculated for the relationship between K fluxes and urea fluxes are shown in Table I and values of α_K calculated from the slopes at the three experimental potentials are given in Table II. The values of α for K are higher on the average than those of Na, although not statistically different. The ratio of the average α for Na to that for K is approximately equal to the ratio of their mobilities in free solution. The intercepts on the ordinate are, again, not statistically different from zero.

(C) Cl FLUXES THROUGH THE SHUNT The relationship between urea influx and Cl influx could also be described by equation 1 as illustrated in Fig. 3. The calculated slopes of the lines at each potential are given in Table I. The ratios of the slopes for Cl influx at 50, 0, −50, and −100 mv are 2.9:1:0.35:0.08, in good agreement with the expected values of 2.3:1:0.31:0.076. The symmetric nature of the system is again suggested by similar slopes for Cl influx at 50 mv and Cl outflux at −50 mv (although the latter data showed a large degree of scatter). The values of α_{Cl} are given in Table II; they appear to be independent of applied potential and significantly higher than α_{Na} or α_K.

(D) MANNITOL FLUXES The effect of PD on permeability of the skin to mannitol was compared to the effect on urea in experiments carried out at 0 and −50 mv. The average mannitol influx increased from 1.6 ± 0.2 nmole/hr cm² at 0 mv to 2.1 ± 0.3 nmole/hr cm² at −50 mv. The increase paralleled the increase in urea fluxes measured simultaneously and the values of α obtained for mannitol are included in Table II. From the data given in Table II, the shunt pathway appears to display a selectivity in the order Cl > urea > K > Na > mannitol.

(E) NONMEASURABLE IONIC FLUXES A series of six experiments was performed in which unidirectional fluxes of Na, Cl, and urea as functions of potential were measured concurrently in opposite directions in matched halves of frog skin. The sum of the net fluxes of Na (inward) and Cl (outward) calculated from these experiments was consistently lower than the total electric current. The average difference (ΔJ) between electric current and the sum of Na and Cl fluxes is shown in Table III, together with the average urea flux. The data in this Table indicate that ΔJ increases roughly propor-

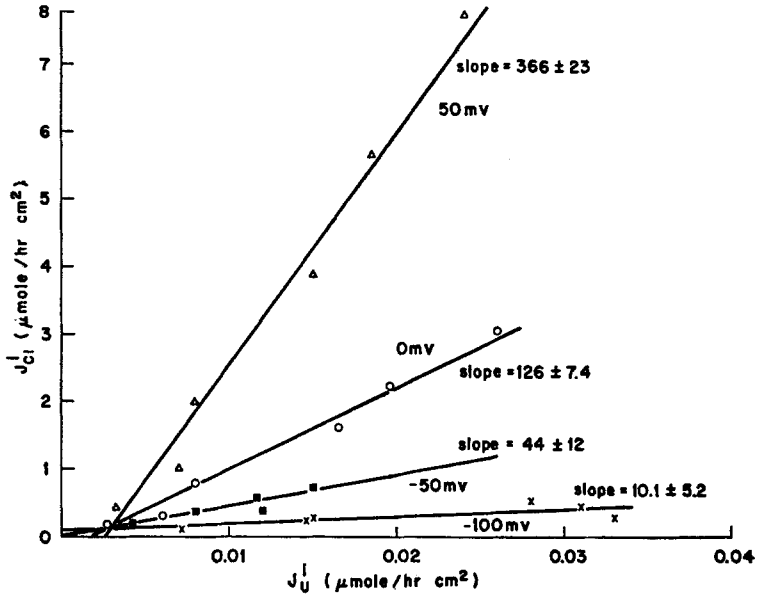

FIGURE 3. Unidirectional influxes of Cl vs. urea at four applied potentials in ouabain-poisoned skins. The lines were calculated by the least squares method.

TABLE III

VALUES OF ΔJ, J_U AND THEIR RATIO AS A FUNCTION OF PD

$\Delta J = I - J_{Na} - J_{Cl}$	J_U	PD
μeq/hr cm²	*nmol/hr cm²*	*mv*
(*a*) *At different applied potentials*		
0.7	8.6	50
1.0	10.1	−50
2.4	16.2	−100
(*b*) *Four experiments at the same potential (spontaneous differences averaged over the first hour after PD application)*		
0.35	3.6	−50
0.72	5.7	−50
0.83	5.6	−50
1.16	10.2	−50

tionately to J_U. The relatively large magnitude of ΔJ precludes the possibility of its being due to a K flux, since the K concentration was only 2.5 mM.

A series of four experiments was performed to test whether ΔJ could arise as a result of nonsteady-state conditions within the epithelium. Unidirectional fluxes of Na, Cl, and urea were measured concurrently in opposite directions in matched skins at −50 mv over a 2 hr period. The results, shown in Fig. 4, indicate that ΔJ decreases more or less linearly over the entire course of the experiment. This change is due almost entirely to a decrease in the current necessary to maintain −50 mv, since the average net fluxes of Na and Cl remain essentially constant (J_{Na} was 0.52 μeq/hr cm² during the first hour, and

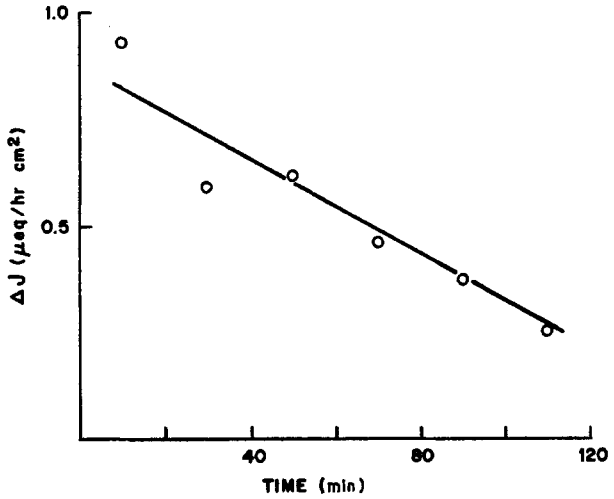

FIGURE 4. Average difference between electric current and the sum of net Na and Cl fluxes plotted as a function of time. Points are average values from four experiments. The straight line has been fitted by eye.

0.59 μeq/hr cm² during the second hour. Corresponding values of J_{Cl} were −1.09 and −1.03 μeq/hr cm²). Thus, the tissue as a whole appears to approach a final steady state rather slowly while fluxes through the shunt pathway stabilize much more rapidly.

Factors Affecting the Increase in Urea Permeability with PD

The increase in urea permeability with PD is dependent on the external NaCl and Ca concentrations. The dependence on NaCl is shown in the first three rows of Table IV. There is no significant difference between hypotonic outside solutions containing 3 and 20 mM NaCl, but skins bathed in 112 mM NaCl Ringer outside displayed a higher urea permeability at −50 and −100 mv than the skins exposed to the lower NaCl concentrations. This dependence

on external salt concentration does not appear to be specific for NaCl, since the urea permeability at all three depolarizing potentials is the same for identical concentrations (112 mM) of NaCl, KCl, and choline chloride. The effect of external Ca concentration is shown on the last two rows of Table IV. Urea permeability increases when no Ca is present in the external medium and increasing the Ca concentration to 10 mM decreases somewhat the response of urea permeability to PD. The action of Ca appears to be limited to the control of permeability increase with PD, since the external Ca concentration does not appear to affect the properties of the shunt. As illustrated in

TABLE IV
EFFECT OF IONIC COMPOSITION ON UREA PERMEABILITY

Solution	Urea influx (nmoles/hr cm²)		
	0 mv	−50 mv	−100 mv
Control			
112 mM NaCl	4.6±0.2 (46)*	6.8±0.6 (28)	10.3±1.0 (26)
20 mM NaCl	3.8±0.2 (12)	4.6±0.3 (12)	5.9±0.6 (12)
3 mM NaCl	4.6±0.4 (19)	5.2±0.4 (20)	6.7±0.6 (20)
112 mM KCl	5.2±0.6 (8)	7.3±0.8 (8)	11.1±1.2 (8)
112 mM choline Cl	6.1±1.1 (12)	7.8±1.1 (12)	10.3±1.7 (12)
"Zero" Ca	—	7.7±0.8 (26)	17.1±2.1 (12)
10 mM Ca	—	5.5±0.5 (16)	8.2±0.7 (16)

* Number of observations.

Fig. 5, essentially the same value of α_{Na} is obtained at −50 mv with no Ca (plus EDTA) and with 1 and 10 mM external Ca.

The permeability of the shunt pathway can also be influenced by changing the osmolarity of the inside solution. 10 paired experiments were carried out in which the inside solution for the control skin was normal Ringer while the inside solution of the experimental skin was Ringer plus 112 mM sucrose. At 0, −50, and −100 mv, the urea flux was lower in the skins exposed to a hypertonic inside solution. The effect was the same at each PD and the average value for all experiments of the ratio J_v (sucrose)/J_v(control) was 0.84 ± 0.03.

The increase in permeability observed at high depolarizing potentials has an onset with time measured in minutes as shown in Fig. 6. Once the increase

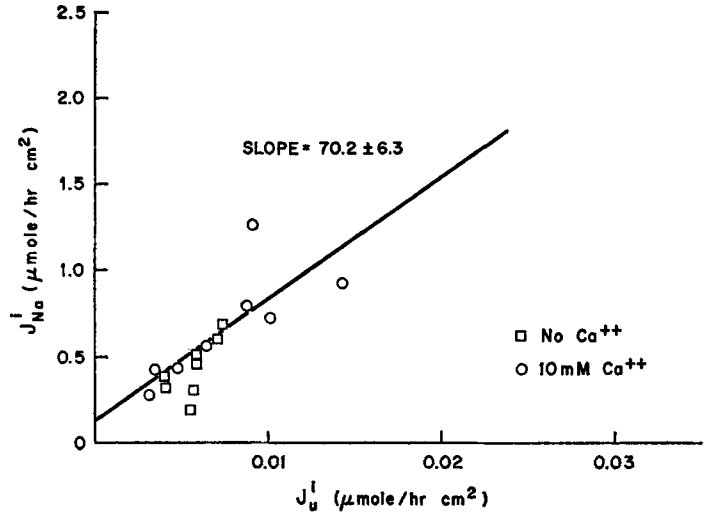

FIGURE 5. Unidirectional influxes of Na vs. urea at −50 mv and two external Ca concentrations. Notice that the regression line calculated for 1 mM Ca fits the data for no Ca and 10 mM Ca.

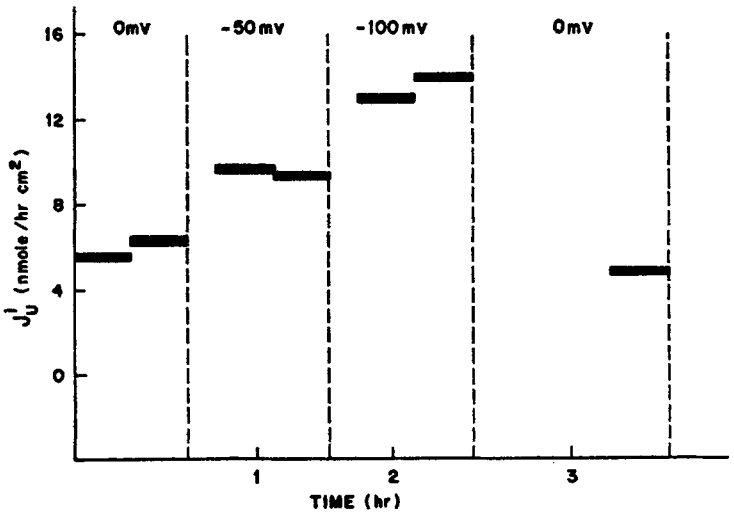

FIGURE 6. Typical experiment showing the time-course of the urea influx as a function of time; the frog skin is subjected to a full applied potential cycle from 0 to −100 mv and returned to 0 mv. Dark bars represent the urea influx over 20-min flux periods.

in permeability has been effected by exposing a skin to a depolarization of −100 mv for about 1 hr, removal of the applied potential usually leads to a decrease of the shunt permeability to its initial value. This reversible process is a slow one, full recovery of the skin occurring only after about 30 min.

DISCUSSION

The results presented in Fig. 1 indicate that the movement of urea across the frog skin is independent of the operation of the Na pump. Thus, this nonelectrolyte appears to cross the skin via a shunt pathway in parallel with the Na pump. In ouabain-poisoned skins, there is a consistent relationship between unidirectional fluxes of ions and urea (described by equation 1), suggesting that the passive movement of ions occurs through the same shunt pathway as that of urea. The permeability of this pathway increases with applied potential in the depolarizing direction. The location of this shunt cannot be specified exactly because of the complex morphology of the skin but the major barrier involved is probably located near the outer surface. Most of the electrical resistance of the skin is in a barrier or barriers near the outer surface (11, 12). Furthermore, tight junctions between cells are localized in the stratum corneum and in the outermost living cell layer (outer layer of the stratum granulosum [13, 14]). The recent observations of Bracho et al. (15) that lanthanum penetrates readily through cells of the cornified layer suggests that the site of major resistance to solute movement is probably in the outermost living cell layer. Therefore, the shunt pathway should pass either through the cells of the outermost cellular layer, or extracellularly through the tight junctions between adjacent cells, or possibly through both. Beyond this cellular layer the pathway is probably extracellular, through the intercellular canals.

Recent observations on three other epithelia, gall bladder (6), proximal tubules of the kidney (5), and ileum (7), indicate the presence of a significant extracellular pathway for transmural solute movement. Two observations are consistent with a similar possibility in the frog skin. First, the movements of ions through the shunt can be described fairly accurately by a form of the Goldman constant field equation. This observation suggests that the path has characteristics of a single barrier rather than of two (or more) barriers of different properties in series, as could be expected for a transcellular pathway. Second, mannitol appears to pass through the shunt pathway and its permeability is altered by PD in much the same way as the other solutes tested. Biber et al.[2] have recently obtained evidence suggesting that mannitol penetrates the cells of frog skin epithelium very slowly, if at all, so that the behavior of mannitol is consistent with an extracellular location for the shunt. However, the evidence is certainly not sufficient to exclude participation of a transcellular path.

The selectivity of the shunt pathway to the solutes shown in Table II would

[2] Biber, T. U. L., L. Cruz, and P. F. Curran. 1972. Sodium influx at the outer surface of frog skin. Evaluation of different extracellular markers. *J. Membrane Biol.* In press.

be consistent with a rate-determining barrier to solute movement through the shunt that is a porous structure lined with charges that are probably dipoles (16). The over-all solute permeability through the shunt increases at depolarizing potentials, but fairly constant permeability ratios between Na, K, Cl, and urea are maintained. This effect could probably be ascribed to an increase in channel radius (providing the channels are fairly large initially), although alternate mechanisms involving increased number of pores (17) or decreased length of pores cannot be ignored. A sample calculation (18) shows that a doubling in pore radius from 20 to 40 A causes only a 20% change in Na:K selectivity and the present experimental data are not sufficiently accurate to detect a change of that magnitude with certainty.

The shunt pathway displays a selectivity to solutes in the order Cl > urea > K > Na > mannitol (Table II). The shunt does not appear to have great cation selectivity, since the ratio of the α's for Na and K is close to the ratio of their diffusion coefficients in free solution. The movements of mannitol and urea also appear to follow the ratio of their diffusion coefficients. These observations again suggest that the channel is initially fairly large in radius. The cationic and anionic movements, however, differ from those predicted by their diffusion coefficients since the relative Cl permeability is appreciably greater than that of Na or K. Ionic discrimination of this kind could be produced by positive charges of the dipoles facing the pore opening (16) or by fixed positive charges lining the channel. A very similar situation but with opposite selectivity has been recently reported for the shunt pathways of kidney proximal tubules (5), gallbladder (6), and ileum (7).

The effect of Ca in the external solution appears to be limited only to the nonspecific permeability increase. The selectivity of the shunt pathway does not seem to be affected by the presence of Ca (Fig. 5). Thus, if Ca impedes the movement of Na into the shunt, it appears to impede the movement of urea to the same extent to maintain a constant α. This observation is consistent with the known mode of action of Ca on external surface sites (19), whereas the selectivity of the shunt is probably dominated by processes within the shunt pathway, such as dipole orientation.

The permeability of the shunt path is virtually unaffected by hyperpolarizing potentials but it increases markedly at depolarizing potentials. If the properties of the shunt are determined mainly by the outermost living cell layer as proposed above, depolarizing potentials might be expected to cause changes in these cells. Voute and Ussing (14) observed swelling of the outer cellular layer at high depolarizing potentials which was usually reversible, although occasionally patches of necrotic tissue occurred. The swelling of the outermost living cell layer may be related at least in part to the nonsteady-state flux, denoted ΔJ, observed in the present studies. This flux indicates the existence of transient ionic displacements within the tissue, a portion of which

could lead to cell swelling if cations and anions accumulated in the cells by entering from opposite sides under the influence of a depolarizing potential. The ionic accumulation would contribute to the net electric current but not to net ionic fluxes measurable across the whole tissue.

The information available at present is not sufficient to establish that cell swelling and an increase in shunt permeability are causally related. However, the observation that J_v is influenced by the osmolarity of the internal solution is consistent with such a hypothesis since a hyperosmolar inside solution will cause the cells to shrink (20). In addition, the time-course of swelling and recovery (14) is similar to that of increased shunt permeability and recovery (Fig. 6), and the occasional tissue necrosis (14) could account for those cases in which the effects of PD on the shunt are not reversible. Finally, the transient flux ΔJ, which may provide a measure for the degree of swelling, is roughly proportional to J_v (Table III). However, in the absence of direct evidence demonstrating causality between swelling and urea permeability, the possibility that these two phenomena could be unrelated to one another cannot be ignored. Both processes could occur independently as a response to the same stimulus; that is, an applied potential could cause cellular swelling by the mechanism discussed earlier, and it could lead to an increased shunt permeability by a direct action of the applied electric field on an extracellular pathway.

The change in shunt permeability with applied potential is quite similar in solutions containing Na, K, or choline as major cation. Thus, whatever the mechanism of the permeability increase, it is not strongly dependent on ionic composition of the outside bathing solution. In this respect, it is of interest to note that Lindemann (21) has recently proposed two parallel ionic channels at the outer barrier of frog skin, one mainly permeable to Na and one with approximately equal permeabilities to Na and K. It is tempting to suggest that this second channel is the one associated with the shunt and with the effects of applied potential on it. Clearly, additional information regarding choline is necessary to test this possibility.

It would be interesting to compare some of the properties of the shunt pathway described herein with those obtained when the skin is exposed to external hyperosmolar urea. The latter condition results in a shunt permeability increase (1) without any visible swelling of the outermost cellular layer (H. Ussing, private communication). Studies on the ionic selectivity of the shunt pathway obtained in hyperosmolar external urea might clarify whether or not this shunt permeability increase utilizes the same pathway as the shunt obtained at high depolarizing potentials and whether the pathway is cellular, or extracellular, or both.

In frog skin the shunt pathway usually makes up a relatively small fraction of the inward Na flux under short-circuited conditions. However, its perme-

ability will clearly play an important role in the balance of Na movements under in vivo conditions when the outside is bathed in solutions of low Na concentration. The fact that the permeability of this pathway can change significantly under certain conditions will be important in any attempts to use imposed PD's to study properties of the skin. The present study indicates that a solute such as urea can be used to detect such changes in shunt permeability since its movement across the skin appears to be independent of changes in cellular ionic composition occurring under applied potentials and ouabain poisoning. The consistent pattern observed between urea movements and those of other solutes should make possible corrections for changes in shunt permeability.

APPENDIX

Derivation of the Equation Relating Na and Urea Permeabilities Through the Shunt

The relationship between Na and urea permeabilities shown in Fig. 2 can be quantitatively described as follows. Starting with the Nernst-Planck equation describing the net flux of an ion i under the influence of a concentration gradient and an electric field, we write

$$J_i = -D_i\left[\frac{dC_i}{dx} + Z_iC_i\frac{F}{RT}\frac{dV}{dx}\right] \qquad (1\,a)$$

where the subscript i referes to the properties of the ion in question, D is the diffusion coefficient, C is the concentration, Z is the valence, V is the potential, and F, R, and T are, respectively, the Faraday, the gas constant, and the absolute temperature. If equation 1 a is integrated across a uniform barrier of thickness d, assuming that (dV/dx) is constant across the barrier (constant electric field assumption [10]), this results in

$$J_i = -\frac{D_i}{d}\frac{FVZ_i}{RT}\left[\frac{C_i^o - C_i' \exp\left(Z_iFV/RT\right)}{1 - \exp\left(Z_iFV/RT\right)}\right] \qquad (2\,a)$$

where the o superscript refers to the outside solution, whereas the prime superscript refers to the inside solution.

The net flux J_i could be interpreted as arising from two independent unidirectional fluxes,

$$J_i\ (\text{forward}) = P_iZ_iC_i^o\left[\frac{-FV/RT}{1 - \exp\left(Z_iFV/RT\right)}\right] \qquad (3\,a)$$

and

$$J_i \text{ (reverse)} = P_i Z_i C_i' \left[\frac{-(FV/RT) \exp (Z_i FV/RT)}{1 - \exp (Z_i FV/RT} \right] = P_i Z_i C_i' \left[\frac{(FV/RT)}{1 - \exp (-Z_i FV/RT)} \right], \quad (4\,a)$$

where $P_i = D_i/d$ = permeability of i.

The unidirectional fluxes of equations 3 *a* and 4 *a* are completely symmetrical, differing only in the sign of the voltage; thus, denoting a flux as forward or reverse is completely arbitrary, depending on the arbitrary designation of the sign of applied potential. Equation 3 *a* describes the predicted variation of unidirectional ionic flux with potential; in the case of the shunt pathway, however, it appears that the permeability term varies with potential in an unpredictable manner. An independent measure of the change in P_i with potential would be obtained if the urea permeability changed in a proportional manner. Thus, the urea influx is

$$J_U^i = P_{\text{urea}}\, C_{\text{urea}} \quad (5\,a)$$

where $P_{\text{urea}} = f(V, C_{\text{NaCl}}, C_{\text{Ca}})$; it could be assumed that

$$P_{\text{Na}} = \alpha_{\text{Na}}\, P_{\text{urea}} \quad (6\,a)$$

where P_{Na} is the Na permeability and α_{Na} is a constant given by the ratio of Na to urea permeabilities through the shunt pathway. From equation 3 *a*, the influx of Na through the shunt could be expressed as

$$J_{\text{Na}}^{i(s)} = \alpha_{\text{NA}} P_{\text{urea}} C_{\text{Na}}^o \left[\frac{-FV/RT}{1 - \exp (FV/RT)} \right]. \quad (7\,a)$$

Combining equations 5 *a*, 6 *a*, and 7 *a*, we obtain

$$\frac{J_{\text{Na}}^{i(s)}}{J_U^i} = \frac{\alpha_{\text{Na}} C_{\text{Na}}^o}{C_{\text{urea}}^o} \left[\frac{-FV/RT}{1 - \exp (FV/RT)} \right]. \quad (8\,a)$$

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