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Resolution of Parameters in the Equivalent Electrical Circuit of the Sodium Transport Mechanism across Toad Skin

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Summary. In amphibian epithelia, amiloride reduces net sodium transport by hindering the entry of sodium to the active transport mechanism, that is, by increasing the series resistance (R_{ser}). Theoretically, therefore, analysis of amiloride-induced changes in potential differences and short-circuit current should yield numerical estimates of all the parameters in the equivalent electrical circuit of the sodium transport mechanism.

The concept has been explored by analysis of such changes in toad skins *(Xenopus laevis)* bathed in hypotonic sulphate Ringer's, after exposure to varying doses of amiloride, or to amphotericin, dinitrophenol or Pitressin.

The estimated values of R_{ser}, of the electromotive force of the sodium pump (E_{Na}), and of the shunt resistance (R_{sh}) were independent of the dose of amiloride employed. Skins bathed in hypotonic sulphate Ringer's exhibited a progressive rise in E_{Na}. Amphotericin produced a fall in R_{ser}, while dinitrophenol caused a fall in E_{Na}; washout of the drugs reversed these effects. Pitressin produced a fall in both R_{ser} and R_{sh}, with a rise in E_{Na}. These results are in accord with earlier suggestions regarding the site(s) of action of these agents.

Amphibian skin and urinary bladder are widely used as models for the study of sodium transport. Since the demonstration of identity between net sodium transport and short-circuit current *(SCC)* in the isolated frog skin (Ussing & Zerahn, 1951) the sodium transport mechanism has been represented by an equivalent electrical circuit (Fig. 1). Inasmuch as active sodium transport is a fundamental property of living tissues, affected by a variety of hormones, enzymes and drugs, attention has been paid by a number of workers to the problem of evaluation of each of the components of this circuit, *viz.*: The electromotive force of the sodium transporting mechanism (E_{Na}), the resistance in series with this representing the path through which the actively transported sodium ions must pass (R_{ser}), and the resistance of the shunt representing leak paths for passively

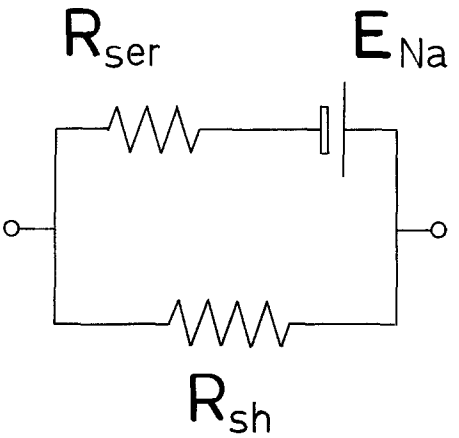

Fig. 1. The equivalent electrical circuit of the sodium transport mechanism

transported ions (R_{sh}). However, there are still no generally accepted means of determining these parameters in living tissues.

If an agent alters only R_{ser} (E_{Na} and R_{sh} remaining constant), the resulting changes in potential difference *(PD)* and *SCC* permit the algebraic evaluation of all three parameters within the equivalent electrical circuit (Yonath & Civan, 1971, and *see* below). In amphibian epithelia most of the available evidence indicates that amiloride (amipramizide hydrochloride dihydrate; Merck, Sharp and Dohme) reduces net sodium transport solely by hindrance of the entry of sodium to the active transport pathway, i.e., by increasing R_{ser} *(see Discussion)*. Theoretically, therefore, analysis of amiloride-induced changes in *PD* and *SCC* might be expected to yield numerical estimates of all three parameters of the equivalent electrical circuit of the sodium transport mechanism.

In the present investigation, the three circuit parameters in toad skin have been evaluated after administration of varying amounts of amiloride, and in the presence of agents thought to act at specific sites in the equivalent circuit. The results are consistent with the concept outlined above.

The Equivalent Electrical Circuit (Fig. 1)

By Thevenin's Theorem, any two-terminal "black-box", containing any complex network of direct-current voltage sources and resistances, can be represented by but a single voltage source in series with a single resistance. The active sodium transport mechanism can thus be represented

by a single voltage source, E_{Na} – equivalent to the electromotive force of this mechanism, and representing whatever series, parallel, or series-parallel arrangement of voltage sources that exists *in vivo* – and a single resistance, R_{ser}, the reciprocal of the sodium conductance of the pathways in series with this "pump". The equivalent electrical circuit of the isolated toad skin is completed by the addition of R_{sh}, representing the total resistance of all leak paths in parallel with the active sodium transport mechanism; these shunts may be cellular or paracellular, and may reflect the sum of several ionic conductances.

Evaluation of R_{sh}, E_{Na} and R_{ser}

The three parameters of the equivalent circuit determine the *PD* and *SCC*:

$$PD = R_{sh}\, E_{Na} (R_{sh} + R_{ser})^{-1} \tag{1}$$

and

$$SCC = E_{Na}\, R_{ser}{}^{-1}. \tag{2}$$

Inverting and substituting,

$$PD^{-1} = SCC^{-1} R_{sh}{}^{-1} + E_{Na}{}^{-1}. \tag{3}$$

That is, where only R_{ser} changes, E_{Na} and R_{sh} remaining constant, a double reciprocal plot of the resulting changes in *PD* and *SCC* yields a straight line, the slope and intersect of which equal $R_{sh}{}^{-1}$ and $E_{Na}{}^{-1}$, respectively.

Any agent capable of inducing changes in only R_{ser} might thus be used to so evaluate the parameters within the equivalent circuit. These changes, furthermore, should be large, and of rapid onset, and the resulting changes in *PD* and *SCC* observed over as short a period as possible, to ensure that such spontaneous changes in the various circuit parameters as may be occurring concomitantly, remain quantitatively insignificant. The use of amiloride is assumed here to meet these criteria *(see Discussion)*. The values of the parameters can then be calculated as follows. On the double reciprocal plot, the slope of the line equals $R_{sh}{}^{-1}$, or

$$R_{sh}{}^{-1} = PD_A{}^{-1} - PD_B{}^{-1} / SCC_A{}^{-1} - SCC_B{}^{-1} \tag{4}$$

where subscript *A* refers to the values of *PD* and *SCC* just after attainment of the full effect of the amiloride, and subscript *B* to those present immediately before its administration. Substitution of this value of $R_{sh}{}^{-1}$ into equation (3) yields E_{Na}, and R_{ser} follows from Eq. (2).

Alternatively, in those experimental situations in which spontaneous changes within the equivalent circuit can be assumed to be absent over extended periods, more accurate estimates of the various circuit parameters might be based on regression lines on observations made at several levels of R_{ser} (that is, at several concentrations of amiloride). A difficulty arises here, however, in that regression lines calculated on double reciprocal plots are peculiarly prone to error (Riggs, 1963), as minor errors in the smallest values of the raw data are exaggerated on taking reciprocals. This difficulty can be circumvented by multiplying Eq. (3) throughout by SCC, to yield

$$PD^{-1}SCC = R_{sh}^{-1} + E_{\mathrm{Na}}^{-1}SCC. \tag{5}$$

That is, where only R_{ser} changes, a plot of conductance against SCC yields a straight line of slope E_{Na}^{-1} and intercept R_{sh}^{-1} (Yonath & Civan, 1971). While otherwise exactly equivalent, Eq. (5) is in this respect preferable to Eq. (3), the arithmetical argument being identical to that invoked in the derivation of constants from other relationships involving double reciprocals (Dowd & Riggs, 1965).

Materials and Methods

The experiments were performed on isolated abdominal skins of the South African clawed toad, *Xenopus laevis*. This tissue, immersed in chloride Ringer's, exhibits occasional irregular and unpredictable fluctuations in *PD* and/or *SCC*. In sulphate Ringer's, however, the change in *PD* and *SCC* are much more consistent, each increasing gradually for some hours before reaching a sustained plateau. For this reason, sulphate rather than chloride Ringer's was used in this study. In those experiments in which paired controls were required, lateral halves of the same abdominal skin were employed. Each skin was clamped between two lucite hemichambers, the clamp being tightened just sufficiently to prevent fluid leaks; no other precaution was taken to avoid or minimize edge damage. An area of 2.85 cm^2 of skin was bathed on both sides by sulphate Ringer's. The bathing solutions were kept stirred by bubbling with air, and contained: 56 mM Na_2SO_4, 1 mM K_2SO_4, 1 mM $CaCl_2$, 2 mM $NaHCO_3$, glucose 100 mg/100 ml, and 2 mM Tris-HCl buffer, pH 7.6. The osmolality, measured by depression of freezing point, was 140 mOsm/Kg water. The *PD*, detected by saturated calomel electrodes, and the *SCC* across each skin were recorded alternately for one and three min periods, respectively, throughout the course of each experiment, by automatic apparatus constructed in these laboratories (Isaacson, Douglas & Pepler, 1971). The experiments were performed throughout the year; no seasonal differences in amplitude of *PD* or *SCC* were observed.

Commencing at 50–100 min, the outer surface of each skin was exposed to amiloride for a few minutes on several occasions during the course of each experiment. A stock solution of amiloride, 10^{-4} M, was prepared by dissolving amiloride hydrochloride, mol wt 302, in sulphate Ringer's. 0.1–0.3 ml of this, added to the 10 ml sulphate Ringer's bathing the outer surface of each skin, yielded a final concentration of $1\text{–}3 \times 10^{-6}$ M amiloride, which usually sufficed to lower the *SCC* to the desired level of half or less of its initial value (*see Discussion*).

The fall in *SCC* (and *PD*) always began almost immediately on adding the amiloride; the rate of fall was maximal within a few sec, and then slowly declined. The drug was assumed to have exerted its full effect when stable lower values of *SCC* (and *PD*) were attained, or, in those instances in which these variables had been changing slowly prior to administration of the amiloride (seldom by more than 5% in 10 min), attained rates of change no different to those present previously. This usually took 5–10 min. There was no change in pH. The amiloride was then removed by threefold washout of the outer bathing solution with fresh sulphate Ringer's.

This procedure was followed at intervals throughout the course of each experiment, on a number of control skins, and on skin exposed to amphotericin ("Fungizone"; E. R. Squibb and Sons), 2,4 dinitrophenol (British Drug Houses), or antidiuretic hormone ("Pitressin"; Parke Davis and Co.). In the latter instances, paired skins were used as controls. After addition or removal of each of these agents, sufficient time was allowed to elapse for the development of either stable, or relatively slowly changing levels of *SCC* and *PD* (seldom by more than 5% in 10 min), before again administering amiloride.

Finally, to confirm that the calculated values of R_{sh}, E_{Na} and R_{ser} were independent of the dose of amiloride employed, another group of skins were exposed to two or three successive increments of amiloride, rather than to single doses of the drug. These experiments also constituted a partial test (*see Discussion*) for the assumption of lack of effect of amiloride on R_{sh} and E_{Na}. In these, unlike the earlier experiments, the amiloride was added only after both *SCC* and *PD* had first attained completely stable levels, so as to minimize the possibility of error secondary to the occurrence of concomitant spontaneous changes within the skins. Similarly, successive increments of amiloride were *not* given to skins exposed to amphotericin,

Table 1. The effects of successive increments in amiloride dosage on *PD* and *SCC*, and upon the calculated values of R_{sh}, E_{Na}, and R_{ser} [a]

Skin	*PD* (mV)	*SCC* ($\mu A \cdot cm^{-2}$)	R_{sh} ($k\Omega \cdot cm^2$)	E_{Na} (mV)	R_{ser} ($k\Omega \cdot cm^2$)	Time (min)
1	28.1	5.37				−1
	23.3	4.04	8.41	74.6	13.91	5
	17.1	2.67	8.24	77.0	14.34	16
	12.5	1.79	8.38	74.7	13.94	24
2	24.4	4.77				−1
	21.9	3.93	9.60	52.2	10.94	9
	16.1	2.35	10.20	48.9	10.23	18
	12.1	1.65	9.52	52.7	11.03	25
3	44.0	19.37				−1
	28.8	10.53	3.62	118.4	6.10	29
	21.5	7.16	3.71	113.8	5.87	51
	15.7	4.98	3.65	117.1	6.04	70
4	12.4	4.21				−1
	7.5	2.28	3.82	54.4	12.91	12
	4.8	1.40	3.73	59.5	14.14	27
5	29.2	7.37				−2
	15.2	2.42	8.81	53.2	7.21	8
	12.2	1.93	8.01	57.8	7.84	30

[a] The time is relative to that at which amiloride was first given.

dinitrophenol or Pitressin, since each of these agents might well exert continuing effects on the various circuit parameters during the lengthy periods of time required (Table 1) to administer the multiple doses of amiloride.

Statistics: The significance of the time dependent changes in the control skins, in *PD*, *SCC*, R_{sh}, E_{Na} and R_{ser}, was assessed by the paired *t* test.

In those studies in which paired lateral halves of abdominal skins were employed, the significance of the changes in *PD*, *SCC* and the three parameters of the equivalent circuit, as induced by amphotericin, dinitrophenol, or Pitressin, was also estimated by the *t* test for paired samples within each group of experimental skins. The significance of the spontaneous time-dependent changes within each group of paired controls was similarly assessed.

Changes of $p<0.05$ were regarded as significant. Results are expressed as mean $\pm$SEM.

Results

Controls

The time-dependent changes in the values of *PD*, *SCC*, E_{Na}, R_{sh} and R_{ser}, as observed in 28 skins, over 500 min, are depicted in Fig. 2. Each skin was exposed to amiloride several times during the course of each experiment. The levels of *PD* and *SCC* shown in Fig. 2 are those which were observed immediately before each occasion on which amiloride was administered; the concomitant values of the three parameters of the equivalent circuit are those calculated from the amiloride-induced changes in these levels, as described earlier.

The actual times at which amiloride was given differed somewhat from skin to skin; the times indicated in Fig. 2 are means. The standard error of the mean time at which E_{Na} attained its maximum value was ± 13.2 min; the standard errors of the other mean times at which amiloride was given ranged between ± 2.5 and ± 4.6 min.

PD, *SCC* and E_{Na} all rose steadily ($p<0.001$) for more than two hundred min. E_{Na} reached its highest level at 248 min, whereafter it declined progressively. The fall in E_{Na} was accompanied initially by a fall in R_{ser} ($p<0.025$) which up to this time had shown no consistent change. *PD* and *SCC* were at their highest at 322 ± 2.5 min, more than an hr after E_{Na} had begun to fall; this pattern of events is consistent with the fall in R_{ser}. Finally, both *PD* and *SCC* fell ($p<0.001$), while R_{ser} increased terminally ($p<0.001$). There were no significant changes in R_{sh}.

Effects of Successive Increments in Amiloride Dosage

In 5 skins, amiloride was first added to the outer bathing solution twenty min or more after *PD* and *SCC* had risen to and finally attained

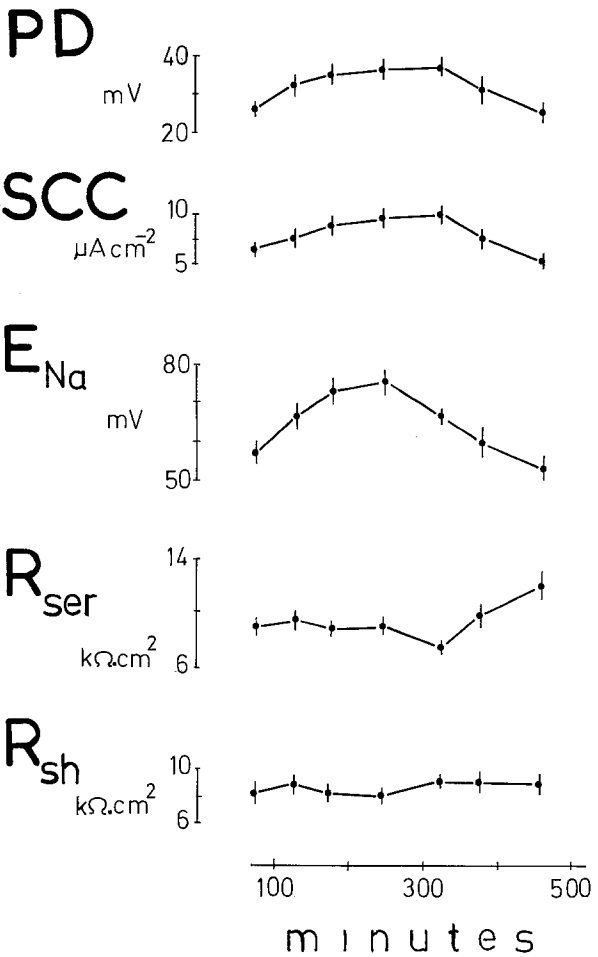

Fig. 2. Temporal changes in mean values (±SEM) of *PD*, *SCC*, E_{Na}, R_{ser} and R_{sh}, in twenty-eight isolated abdominal skins *(Xenopus laevis)*

steady levels, between 180–240 min. Two or three doses of amiloride were then added in succession to each skin. Administration of the drug was always followed by proportionately greater falls in *SCC* than in *PD*. Repeated estimates of the three circuit parameters, in any one skin, after each increment in amiloride dosage, differed by less than 10%, and were independent of the dose of amiloride employed (Table 1). The mean values of *PD*, *SCC*, and of the three circuit parameters in this group of skins did not differ significantly from those in the twenty-eight control skins (unpaired *t* test) at the same time.

The relationship between $PD^{-1} \cdot SCC$ and *SCC* [Eq. (5)] in each of these skins, as observed immediately before and then after each successive

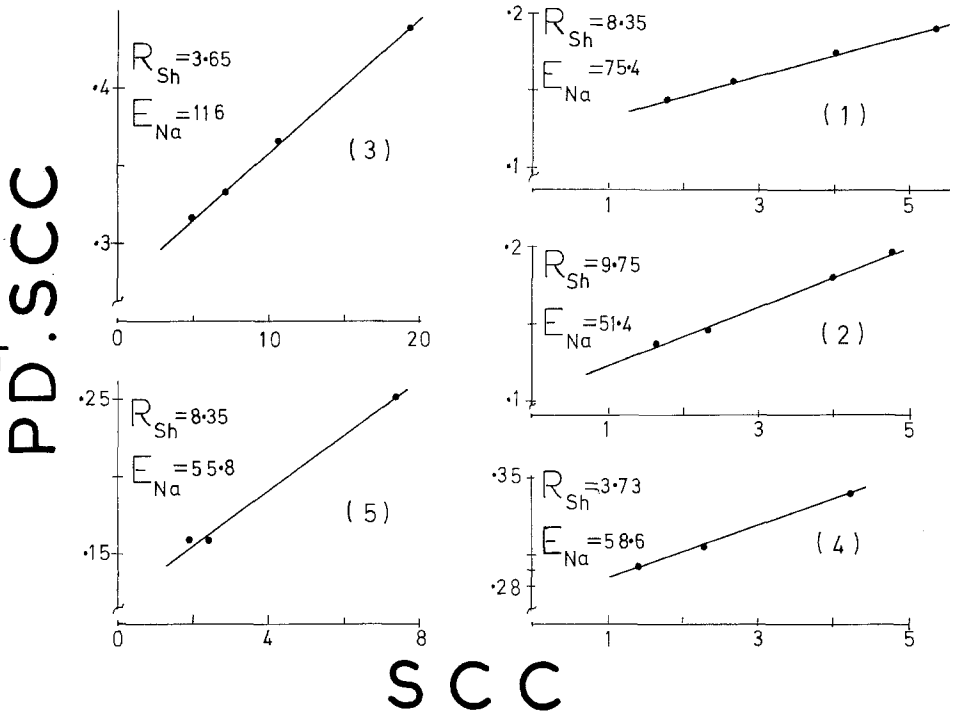

Fig. 3. Relationships between $PD^{-1} \cdot SCC$ and SCC in 5 skins, before and then after successive increments of amiloride. SCC is in $\mu A \cdot cm^{-2}$. In each case, the values shown for E_{Na} (mV) and R_{sh} (k$\Omega \cdot cm^2$) have been derived from the slope and intercept, respectively, of the regression line. Other data are from Table 1; the numbers in parentheses are the skin numbers

dose of amiloride, are depicted in Fig. 3. The regression lines, calculated by the method of least squares, and the values of E_{Na} and R_{sh} as obtained from the slope and intercept of these lines, are also shown. The latter correspond closely to those calculated in Table 1. The data points fall closely about the regression lines.

Amphotericin (Table 2)

The parameters of the equivalent electrical circuit were determined in 5 skins, 14 ± 3 min before, and 28 ± 3 min after addition of 0.25 mg amphotericin to the outer bathing solutions. Paired halves of the same skins served as controls.

In each instance administration of the drug was followed by abrupt increases in PD ($p < 0.001$) and SCC ($p < 0.05$), stable higher levels being

Table 2. The effects of administration and subsequent removal of amphotericin on *PD* and *SCC*, and upon the calculated values of R_{sh}, E_{Na}, and R_{ser}[a]

	Experimental ($n=5$)						Paired controls ($n=5$)					
	Before		With amphotericin		After ($n=4$)						($n=4$)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Time (min)	345	±55	386	±55	425	±42	345	±55	387	±55	427	±42
PD (mV)	35.1	± 6.3	42.4	± 6.3	25.2	± 4.4	32.4	± 6.3	30.0	± 5.7	20.3	± 3.6
SCC ($\mu A \cdot cm^{-2}$)	9.7	± 1.2	13.5	± 3.6	7.0	± 2.0	10.4	± 3.0	9.2	± 2.6	5.4	± 1.7
R_{sh} ($k\Omega \cdot cm^2$)	8.84	± 2.04	8.92	± 1.80	9.11	± 2.73	9.64	± 2.63	9.83	± 2.46	10.69	± 3.89
E_{Na} (mV)	77.3	± 6.2	80.0	± 5.6	65.8	± 9.6	65.7	± 7.5	60.5	± 2.2	45.2	± 7.1
R_{ser} ($k\Omega \cdot cm^2$)	10.89	± 2.45	8.30	± 1.84	12.27	± 2.47	10.60	± 3.70	10.34	± 3.38	11.13	± 2.41

[a] The time is that which elapsed after mounting the skins. The changes which occurred spontaneously during the same period in the paired controls are also shown. SE = standard error of the mean.

Table 3. The effects of administration and subsequent removal of dinitrophenol (DNP) on *PD* and *SCC*, and upon the calculated values of R_{sh}, E_{Na} and R_{ser}[a]

	Experimental ($n=5$)						Paired controls ($n=5$)					
	Before		With DNP		After							
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Time (min)	240	±17	335	±15	447	±15	237	±17	329	±13	448	±17
PD (mV)	31.8	± 7.9	14.5	± 3.4	19.6	± 2.0	32.0	± 7.0	34.9	± 5.0	30.4	± 3.2
SCC ($\mu A \cdot cm^{-}2$)	11.4	± 3.9	4.2	± 0.8	7.4	± 1.0	11.3	± 1.7	11.6	± 1.1	8.8	± 0.80
R_{sh} ($k\Omega \cdot cm^2$)	5.05	± 0.71	5.72	± 3.88	3.90	± 0.51	5.72	± 1.41	6.93	± 1.18	7.72	± 1.47
E_{Na} (mV)	81.9	± 6.4	36.2	± 6.1	80.4	± 6.5	78.8	± 7.5	70.8	± 6.6	67.1	± 5.3
R_{ser} ($k\Omega \cdot cm^2$)	7.72	± 1.35	9.50	± 1.96	12.61	± 2.70	8.07	± 1.72	6.15	± 0.46	7.70	± 0.43

[a] The changes which occurred spontaneously during the same period in the paired controls are also shown.

reached within 15–30 min. The increase in *SCC* was proportionately greater than that in *PD*. R_{ser} fell ($p<0.05$). The *SCC* in the paired control skins fell ($p<0.05$) during this period.

After exposure to amphotericin for 74–90 min, the drug was then removed from four of the experimental skins by three-fold washout of the outer bathing solution. *PD* and *SCC* fell, rapidly at first and then more slowly. Some 15–38 min later, the *PD* was lower ($p<0.01$) and R_{ser} higher ($p<0.01$) than before removal of the drug. *SCC* and E_{Na} fell too, but not significantly. The paired control skins showed a fall in E_{Na} ($p<0.02$) during this period.

In terms of the equivalent electrical circuit, the only effect of amphotericin was thus to lower R_{ser}; washout of the drug reversed the effect.

Dinitrophenol (Table 3)

The three circuit parameters were determined in 5 skins, 25 ± 8 min before, and 65 ± 18 min after replacement of the inner bathing solution with a saturated solution of dinitrophenol in sulphate Ringer's. Paired halves of the same skins served as controls.

In three instances, exposure to dinitrophenol was followed by an immediate rise, of some 5% to 25%, in both *PD* and *SCC*; this was maximal after 5 to 10 min. Thereafter both *PD* and *SCC* fell steeply, in all 5 skins. The rate of fall then gradually declined, becoming slow enough to permit the determination of the three circuit parameters 65 ± 18 min later. Both *PD* ($p<0.01$) and *SCC* ($p<0.01$) were now markedly lower than before, as was also E_{Na} ($p<0.02$). No significant change occurred in the paired control skins during this period.

After exposure to dinitrophenol for 87 ± 18 min, the drug was then removed by threefold washout of the inner bathing solution. The falls in *PD* and *SCC* were arrested, and after a variable delay, both *PD* and *SCC* increased somewhat. At 90 ± 18 min after removal of the dinitrophenol, the three circuit parameters were again determined. E_{Na} was now much greater than before ($p<0.01$). R_{ser} increased in 4 skins, but in one in which it had initially been high, was now much lower; overall, the changes in R_{ser} were not significant. The paired control skins again showed no significant changes during this period.

In terms of the equivalent electrical circuit, the only unequivocal effect of dinitrophenol was thus to lower E_{Na}; removal of the drug reversed the effect.

Table 4. The effects of administration of Pitressin on *PD* and *SCC*, and upon the calculated values of R_{sh}, E_{Na} and R_{ser}[a]

	Experimental ($n=9$)				Paired controls ($n=9$)			
	Before		With Pitressin					
	mean	SE	mean	SE	mean	SE	mean	SE
ne (min)	282	±16	391	±17	284	±16	392	±17
) (mV)	33.8	± 3.8	42.6	± 3.1	36.1	± 3.9	30.7	± 4.7
C ($\mu A \cdot cm^{-2}$)	8.5	± 0.9	14.6	± 1.0	9.1	± 0.7	7.2	± 1.2
($k\Omega \cdot cm^2$)	9.58	± 1.10	7.31	± 0.94	8.92	± 0.85	9.20	± 0.87
a (mV)	66.2	± 4.7	77.7	± 3.3	67.2	± 5.1	60.6	± 5.5
r ($k\Omega \cdot cm^2$)	8.07	± 0.63	5.56	± 0.29	7.66	± 0.67	9.58	± 0.98

[a] The changes which occurred spontaneously during the same period in the paired controls are also shown.

Pitressin (Table 4)

The three circuit parameters were determined in 9 skins, 25±10 min before, and then 78±11 min after addition of 20 mU/ml of Pitressin to the inner bathing solution. Paired halves of these skins served as controls.

In each instance, within a few min of exposure to Pitressin, both *PD* and *SCC* increased rapidly, to reach maximal values 50–143 min later. The increase in *SCC* ($p<0.001$) was proportionately greater than that in *PD* ($p<0.01$). E_{Na} became greater ($p<0.01$), and R_{sh} ($p<0.002$) and R_{ser} ($p<0.01$) lower, than before the drug was given. During this same period, the paired control skins showed a fall in both *SCC* ($p<0.05$) and *PD* ($p<0.05$); R_{ser} rose in 6, and E_{Na} fell in 7 of these skins, but overall these latter changes were not significant.

In terms of the equivalent electrical circuit, Pitressin thus increased E_{Na}, and lowered both R_{sh} and R_{ser}.

Discussion

The validity of the approach adopted here rests upon the assumption that amiloride can be used repeatedly, to induce changes in R_{ser}, without simultaneously affecting E_{Na} or R_{sh}. There is much evidence, at least in amphibian skin and bladder, to support this contention.

The ensuing fall in net sodium transport is generally agreed to be mediated very largely or solely by hindrance of sodium entry into the active transport pathway (Bentley, 1968; Crabbé & Ehrlich, 1968; Ehr-

lich & Crabbé, 1968; Dörge & Nagel, 1970; Nagel & Dörge, 1970; Salako & Smith, 1970*a*, 1970*b*; Biber, 1971; Erlij & Smith, 1973); that is, by increasing R_{ser}. The effect is of immediate onset, with a half time measured in seconds, and is gross, dose-dependent, and promptly reversible on washout of the drug. *SCC* remains a reliable expression of net sodium transport (Ehrlich & Crabbé, 1968). Apparently only sodium transport is affected; amiloride has no effect on the tissue's osmotic permeability, and extracellular fluid volume and intracellular potassium concentration remain unchanged. Carbonic anhydrase, adenyl cyclase and ATPase are not affected (Baer, Jones, Spitzer & Russo, 1967).

Some evidence that amiloride can be used repeatedly without inducing long-lasting changes in the tissues, can be found in the following. The 5 skins reported on in Table 1 had received no amiloride for the first 180–240 min, yet at this time their mean values of *PD*, *SCC*, E_{Na}, R_{sh} and R_{ser} were not significantly different from those in the control skins (Fig. 1), all of which had previously been repeatedly exposed to amiloride. Saito, Essig and Caplan (1973) have exposed frog skins, pretreated with aldosterone, to amiloride for periods of up to 4 hr; while they found that the "affinity constant"—"a measure of the negative Gibbs free energy change of the metabolic reaction driving sodium transport"—was increased by some 50% after 4 hr, no change was detectable after an hr of such exposure. These exposure times contrast sharply with those adopted here; with the exception of the skins reported on in Table 1, few of the skins in this study were exposed to amiloride for more than 10 min at any one time, or for a total of much more than an hr throughout the course of any one experiment.

Amiloride has not been shown to have a measurable effect on E_{Na}. Thus addition of ATP to the bathing solutions does not impair the action of amiloride. In both frog skin and toad bladder, the fall in *SCC* induced by amiloride is paralleled by a fall in the active sodium pool (Ehrlich & Crabbé, 1968; Dörge & Nagel, 1970), while the rate constant of sodium efflux from the tissue to the inner bathing solution, is unchanged (Salako & Smith, 1970*b*). Yonath & Civan (1971), assuming that anti-diuretic hormone alters only R_{ser}, could detect no change in E_{Na} after administration of amiloride; their methodology, however, is open to criticism (*see* below). On the other hand, Reuss and Finn (1975) have shown that amiloride produces not only an increased resistance in the outer-facing membrane, but also parallel and almost simultaneous voltage drops across both mucosal and serosal surfaces of the toad bladder; however, whatever the mechanism of these effects, the fact that they were able to

demonstrate similar effects on simply lowering the sodium concentration in the mucosal bathing solution suggests that the mode of action of amiloride here was again just to hinder the entry of sodium into the active transport pathway.

As pointed out earlier, if the only effect of amiloride is to increase R_{ser}, successive increments in amiloride dosage should yield a linear relationship between tissue conductance and SCC (Eq. (5)); the latter in fact occurs (Fig. 3). The converse, however, is not necessarily true. While concomitant irregular changes in E_{Na} and/or R_{sh} would certainly render this relationship nonlinear, changes in E_{Na} proportional to those in R_{ser}, and/or in R_{sh}^{-1} proportional to those in R_{ser}^{-1}, would still yield a linear relationship between tissue conductance and SCC. It follows that the appearance of a straight line on a conductance $-$ SCC plot confirms only that the calculated values of E_{Na} and R_{sh} are independent of the dose of amiloride employed; it is also consistent with, but does not prove, the assumption that R_{ser} was the only one of the three circuit parameters to have altered.

In short, it is generally agreed that amiloride induces large, rapid and reversible changes in R_{ser}, and there is no unquestionable evidence of effects on E_{Na} or R_{sh}. While the assumption that amiloride affects only R_{ser} cannot be proved, such data as are available thus suggest that amiloride might be a suitable probe for evaluation of the various parameters in the equivalent electrical circuit. A number of agents affecting net sodium transport across amphibian epithelia are thought to exert their effects by specifically affecting one or more of these parameters. It is of interest to compare these interpretations with the results obtained using the method described here.

The isolated abdominal skin of *X. laevis*, bathed in sulphate Ringer's, exhibits a progressive increase in both PD and SCC, each increasing gradually for 200–300 min, before reaching a sustained plateau (Fig. 2). The cause of this time-dependent behavior lies in the marked hypotonicity of sulphate Ringer's, (140 mOsm/kg water, as compared to 240 mOsm/kg water of *X. laevis* plasma). Lipton (1972) has shown in studies of shorter duration than those presented here, that a prolonged rise in SCC follows exposure of the serosal surface of toad bladder to a hypotonic medium; as the sodium content of the tissue falls concomitantly, he concluded that the effect was due to stimulation of the sodium pump, rather than to a fall in R_{ser}, an interpretation in accord with the pattern of events seen in the first four hr of the experiments presented here. However, in these experiments (Fig. 2), PD and SCC continued to rise even after E_{Na} began to decline, secondary to a concomitant fall in R_{ser}. Similar effects have been

noted by Finn and Reuss (1975); using microelectrode techniques, they found that the toad urinary bladder responds to hypotonic serosal fluid, with both increased serosal sodium pumping and an increased apical sodium conductance.

Amphotericin, added to the solution bathing the mucosal surface of toad bladder, is believed to increase net sodium transport by increasing the permeability of an outer facing barrier to the active sodium transport channel (Lichtenstein & Leaf, 1965; Finn, 1970), that is, by decreasing R_{ser}; there is no demonstrable effect on the sodium transport pump, nor for perhaps the first half hour after its administration (after which permeability to potassium and chloride ions also increases), on R_{sh}. The results obtained here are in accord with these views. In passing, it is of interest that while in other species amphotericin abolishes the response to amiloride (Bentley, 1968; Ehrlich & Crabbé, 1968), this did not occur in these experiments on *X. laevis*.

Dinitrophenol produces a reversible reduction in net sodium transport across frog skin. As judged by isotopic flux studies, the principal mechanism of its effect is a marked reduction in E_{Na}, presumably consequent upon uncoupling of oxidative phosphorylation; R_{ser} may double, particularly in skins initially of low resistance, but R_{sh} is unaffected (Fuhrman, 1952; Schoffeniels, 1955). The results obtained here agree with these observations.

The mechanism of action of antidiuretic hormone (ADH) on sodium transport, in terms of the equivalent electrical circuit, has still to be finally established. While there is considerable evidence to show that the resultant increase in net sodium transport follows a reduction in R_{ser}, there is also evidence of an induced increase in E_{Na} (Morel & Bastide, 1965; Finn, 1968; Janaceck & Rybova, 1970; Helman, Grantham & Burg, 1971). The subject has recently been exhaustively reviewed by Handler and Orloff, (1974), who point out that none of the experiments indicating either an apical permeability effect (i.e., change in R_{ser}) of the hormone, or a basal pump effect (i.e., change in E_{Na}) clearly rule out an effect at the opposite border of the cell. However, Finn (1971) has studied the rate of washout of isotopic sodium from both surfaces of prelabelled toad bladder, and found that ADH increases both rate constants of sodium efflux, so indicating a dual site of action of the hormone. The results presented here are consistent with these observations; administration of Pitressin was followed by both a fall in R_{ser} and a rise in E_{Na}. There was also a fall in R_{sh}, as might perhaps have been expected; ADH markedly increases the sulphate permeability of frog skin (Rawlins, Mateu, Fragachan & Whittembury, 1970).

The concept that changes confined to R_{ser} permit evaluation of all

three parameters within the equivalent electrical circuit has been invoked before. In a different and more complex elaboration upon this theme than that adopted here, Yonath and Civan (1971) have argued that E_{Na} might be obtained by analysis of the changes in tissue conductance and SCC (Eq. (5)) seen after administration of Pitressin. Pitressin was assumed to be without effect on E_{Na} or R_{sh}, as evidence of which they were able to demonstrate a linear relationship between tissue conductance and SCC after its administration. However, it is not generally agreed that Pitressin is without effect on the sodium "pump" (*see* above) while, as pointed out earlier, simultaneous and proportional changes in R_{ser}, E_{Na} and/or R_{sh} can also yield a linear relationship between tissue conductance and SCC. Indeed, in some of their experiments the slope of the conductance $-SCC$ curve was distinctly greater at the height than at the onset of the response to Pitressin; while the authors may have been correct in ascribing this to saturation of the sodium pump, the experiments reported here suggest that it may equally well have been due simply to a continuing fall in R_{sh}. Successive estimates of E_{Na}, following serial applications of Pitressin, were on average unchanged; such estimates in individual skins, however, occasionally differed by 30–40% (their Fig. 10*a*). The approach advocated here thus appears to compare favorably with that adopted by Yonath and Civan, in that it utilizes a less controversial agent than Pitressin to induce relatively larger and more rapid changes in PD and SCC, yields more closely reproducible results in individual skins and enables more frequent estimates of the various circuit parameters to be made during the course of a single experiment.

The procedure oulined here is not without its difficulties. The amiloride-induced falls in PD and SCC need to be sufficiently large to overshadow normally trivial errors, such as a drift of one or two millivolts in the zero potential between the voltage-measuring electrodes, or small inexactitudes in reading PD and/or SCC. (For example, in Table 1, skin 4, the values of the various parameters have been calculated (Eq. 4) by comparison of the initial values of PD and SCC with those pertaining at each of two concentrations of amiloride. The results are not dissimilar; both values of E_{Na} for example are within a few millivolts of the 58.6 mV obtained after calculation of the regression line (Fig. 3). Yet had the calculation been based upon comparison between the much lower values of PD and SCC present immediately before and after the second dose of amiloride, E_{Na} would have been estimated as 71.4 mV; it would have been 58.5 mV had the final PD been recorded as 4.845 mV rather than the barely distinguishable 4.8 mV. The discrepancy arises in that this last calculation involves small differences in PD and SCC, of magnitude similar to those in the errors of measurement). Another difficulty arises in that washing out the amiloride frequently leads to transient increases in PD and SCC, to levels higher than those pertaining immediately prior to its administration (Ehrlich & Crabbé, 1968); these transients may take 10–20 min to disappear, and so impose an upper limit on the number of times amiloride can be used in any one experiment. Finally, in Tables 2, 3 and 4, the total tissue resistance, if calculated as the reciprocal of the sum of R_{sh}^{-1} and R_{ser}^{-1},

is consistently some few score to some few hundreds of ohms higher than that obtained on dividing *PD* by *SCC*. The discrepancy is not present in Table 1. The cause of this anomaly is not obvious, but may be related to the fact that the experiments reported in Table 1 were conducted on skins with stable levels of *PD* and *SCC*, while many of those summarized in Tables 2, 3 and 4, on the other hand, were performed on skins with slowly changing levels of *PD* and SCC.

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References

Baer, J.E., Jones, C.B., Spitzer, A.S., Russo, H.F. 1967. The potassium sparing and natriuretic activity of N-amidino-3,5diamino-6-chloropyrazine carboxamide hydrochloride (amiloride hydrochloride). *J. Pharmacol. Exp. Ther.* **157**:472

Bentley, P.J. 1968. Amiloride: A potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (London)* **195**:317

Biber, T.U.L. 1971. Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. Gen. Physiol.* **58**:131

Crabbé, J., Ehrlich, E.N. 1968. Amiloride and the mode of action of aldosterone on sodium transport across toad bladder and skin. *Pfluegers Arch. Gesamte Physiol.* **304**:284

Dörge, A., Nagel, W. 1970. Effect of amiloride on sodium transport in frog skin. II. Sodium transport pool and unidirectional fluxes. *Pfluegers Arch. Gesamte Physiol.* **321**:91

Dowd, J.E., Riggs, D.S. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. *J. Biol. Chem.* **240**:863

Ehrlich, E.N., Crabbé, J. 1968. The mechanism of action of amipramizide. *Pfluegers Arch. Gesamte Physiol.* **302**:79

Erlij, D., Smith, M.W. 1973. Sodium uptake by frogskin and its modification by inhibitors of transepithelial sodium transport. *J. Physiol. (London)* **228**:221

Finn, A.L. 1968. Separate effects of sodium and vasopressin on the sodium pump in toad bladder. *Am. J. Physiol.* **215**:849

Finn, A.L. 1970. Effects of potassium and amphotericin B on ion transport in the toad bladder. *Amer. J. Physiol.* **218**:463

Finn, A.L. 1971. The kinetics of sodium transport in the toad bladder. I. Dual effects of vasopressin. *J. Gen. Physiol.* **57**:349

Finn, A.L., Reuss, L. 1975. Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol. (London)* **250**:541

Fuhrman, F. 1952. Inhibition of active sodium transport in the isolated frog skin. *Am. J. Physiol.* **171**:266

Handler, J.S., Orloff, J. 1974. Handbook of Physiology. Section 8 (Renal Physiology), p. 801. American Physiological Society, Washington

Helman, S.I., Grantham, J.J., Burg, M.B. 1971. Effect of vasopressin on electrical resistance of renal cortical collecting tubules. *Am. J. Physiol.* **220**:1825

Isaacson, L.C., Douglas, R.J., Pepler, J. 1971. Automatic measurement of voltage and short-circuit current across amphibian epithelia. *J. Appl. Physiol.* **31**:298

Janaceck, K., Rybova, R. 1970. Nonpolarized frog bladder preparation. The effects of oxytocin. *Pfluegers Arch. Gesamte Physiol.* **318**:294

Lichtenstein, N.S., Leaf, A. 1965. Effect of Amphotericin B on the permeability of the toad bladder. *J. Clin. Invest.* **44**:1328

Lipton, P. 1972. Effect of changes in osmolarity on sodium transport across isolated toad bladder. *Am. J. Physiol.* **222**:821

Morel, F., Bastide, F. 1965. Action de l'ocytocine sur la composante active du transport de sodium par la peau de grenouille. *Biochim. Biophys. Acta* **94**:609

Nagel, W., Dörge, A. 1970. Effect of amiloride on sodium transport of frog skin. I. Action on intracellular sodium content. *Pfluegers Arch. Gesamte Physiol.* **317**:84

Rawlins, F., Mateu, L., Fragachan, F., Whittembury, G. 1970. Isolated toad skin epithelium: Transport characteristics. *Pfluegers Arch.* **316**:64

Reuss, L., Finn, A.L. 1975. Dependence of serosal membrane potential on mucosal membrane potential in toad urinary bladder. *Biophys. J.* **15**:71

Riggs, D.S. 1963. The Mathematical Approach to Physiological Problems. Williams and Wilkens, Baltimore

Saito, T., Essig, A., Caplan, S.R. 1973. The effect of aldosterone on the energetics of sodium transport in the frog skin. *Biochim. Biophys. Acta* **318**:371

Salako, L.A., Smith, A.J. 1970*a*. Effects of amiloride on active sodium transport by the isolated frog skin; evidence concerning site of action. *Br. J. Pharmacol.* **38**:702

Salako, L.A., Smith, A.J. 1970*b*. Changes in sodium pool and kinetics of sodium transport in frog skin produced by amiloride. *Br. J. Pharmacol.* **39**:99

Schoffeniels, E. 1955. Action du 2-4 dinitrophenol sur le flux de sodium a travers la peau de grenouille. *Arch. Int. Physiol.* **63**:361

Ussing, H.H., Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110

Yonath, J., Civan, M.M. 1971. Determination of the driving force of the Na^+ pump in toad bladder by means of vasopressin. *J. Membrane Biol.* **5**:366