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THE RELATIONSHIP OF K^+ EFFLUX AT THE INNER SURFACE OF THE ISOLATED FROG SKIN EPITHELIUM TO THE SHORT CIRCUIT CURRENT *

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Summary

Isolated frog skins (without chorion) were incubated with $^{42}K^+$ Ringer's solution, bathing the internal surface for 2 h.

All the K^+ contained in the frog skin was equilibrated in specific activity with external $^{42}K^+$.

The kinetics of the washout of $^{42}K^+$ from the internal surface of the skin exhibits one fast and one slow exponential component.

Amiloride reduces the release of $^{42}K^+$ corresponding to both components without affecting the K^+ content of the skin.

Ouabain increases the loss of $^{42}K^+$ of the slow component by 200%. Since the total K^+ in the skin decreases to 25% of its original value both compartments are affected.

The results suggest that two distinct functional compartments exist defined by two $^{42}K^+$ release ratios and that because of the large K^+ contents of these compartments both are intracellular.

The relation with the transepithelial Na^+ transport and the morphological identification of these compartments is discussed.

Introduction

Earlier studies [1—4] established that the potential difference across the internal membrane of epithelial cells was originated by an active electrogenic

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transfer of charge and not by a K^+ diffusion potential as had originally been suggested by Koefoed-Johnsen and Ussing [5].

Independent studies on the coupled exchange of internal Na^+ for external K^+ through the Na^+ pump in isolated cell systems showed a $Na^+ : K^+$ stoichiometry of about 3 : 2 with net extrusion of positive charges [6—13].

A variable coupling ratio for $Na^+ : K^+$ transport has been postulated in a variety of cells [14—16]. In epithelia, Biber et al. [17] investigated the correlation between transepithelial Na^+ transport and the 1 min uptake of $^{42}K^+$ at the internal surface of the isolated frog skin and found $Na^+ : K^+$ transport ratios ranging between 3 and 6. Robinson and Macknight [18] using one hour uptake, described at least two rate constants, only the faster being related to the transepithelial sodium transport. By comparing the K^+ fluxes obtained in these experiments (3.04 mmol $\cdot$ min^{-1} $\cdot$ kg^{-1} dry wt.) with the Na^+ fluxes obtained from the short circuit current measured in a separate group of bladders (mean 36.1 mmol $\cdot$ min^{-1} $\cdot$ kg^{-1} dry wt. ranging from 8.7 to 120) one can see that the ratio of Na^+/K^+ is always higher than unity ranging from 3 to 40 with a mean value of 12.

The main problem with the assessment of the $Na^+ : K^+$ stoichiometry in epithelial cells as opposed say, to similar estimates in single cells [13] is that of cell heterogeneity. The kinetics of $^{42}K^+$ uptake reveals a multicompartmental distribution [18,19] and the correlation between any particular compartment and the transepithelial Na^+ transport is rather arbitrary.

A further complication arises from the existence of slowly exchangeable K^+ compartments which do not equilibrate with the tracer within the experimental periods [18].

In the experiments reported here, we tried to relate the K^+ effluxes and the Na^+ transport under spontaneous conditions, and the variations introduced in both parameters by substances known to affect the transepithelial Na^+ transport namely amiloride and ouabain.

The efflux measurements were performed on isolated epithelial membranes, without basement membrane and supporting chorion, while simultaneously recording the short circuit current.

The advantage of this technique is that it avoids the use of extracellular markers, and artefacts due to large unstirred layers.

The rationale behind this experimental design is that under steady-state, active and passive K^+ influx equals the measured $^{42}K^+$ effluxes. By comparing the pharmacologically induced variations in I_{sc} and $^{42}K^+$ effluxes, the stoichiometry of the transepithelial Na^+ transport could eventually be assessed.

Methods

Frogs of the species *Rana temporaria* were used. They were kept in a cold room at 4°—6°C. Isolated skin epithelia which had been separated from the chorion, were obtained by using a modification of the technique described by Aceves and Erlij [20]. Skins were mounted on a funnel shapped perspex holder and immersed in aerated stirred Ringer at 25°C for 2 h. The chorion side was facing the inside of the funnel and Ringer with 80 U/ml of colagenase type III Sigma were used. At the end of the incubation period with colagenase a slight

pressure was exerted by blowing gently through the hole of the funnel until blisters appeared separating epithelium from chorion. The dissecation was then finished with blunt scissors and forceps.

The isolated epithelium was mounted in Ussing-type chambers with an area of 3.14 cm^2. The short circuit current was measured with an automatic voltage clamp device. When the I_{sc} reached a steady state, the Ringer containing $^{42}K^+$ was added to the internal side of the skin (chorial face of the epithelium) and was left for 2 h.

Preliminary experiments indicated that all the K^+ in the skin had reached equilibrium in the specific activity with the $^{42}K^+$ of the loading solution during that period (see also Results).

After 2 h the radioactive Ringer was removed and both sides of the chamber were quickly washed three times in 1 min and the washout of $^{42}K^+$ was started. Samples were collected every 10 min from the inside and every 30 min from the outside, for 2 to 3 h. The volume of Ringer used each time was 4 ml, measured with a calibrated syringe and collected directly into counting vials. At the end of the experiment the skin was carefully cut out of the chamber, blotted, weighed and put into a counting vial with 4 ml of 0.1 N nitric acid and counted. The labelled Ringer was diluted twice and counted as well. Samples were counted in a gamma-counter with a preset count of 10^4.

Determinations of K^+ in the Ringer and in aliquots of the nitric acid solution where the skins had been digested were made by flamephotometry, and the total K^+ content of the skin at the end of the experiments was calculated from these measurements.

The composition of the Ringer was in mM: Na^+, 112; Cl^-, 119; K^+, 3.0; Ca^{2+}, 1.0; Mg^{2+}, 1.0.

Amiloride was used at 10^{-4} M, ouabain at 10^{-2} M and pitresin at 100 mU per ml of Ringer.

The aerated Ringer's solution was titrated with Tris buffer to a pH of 8. The total amount of counts in the skin at the beginning of each collection period was calculated by adding back the counts lost in successive washout samples.

A semi-logarithmic plot of total remaining counts in the skins versus time was made and the rate constants of the various exponential components was calculated by successive linear extrapolations.

The total initial K^+ content of the skin was calculated from the total initial activity in the epithelium and the specific activity of $^{42}K^+$ in the loading solution. The initial K^+ content of each flux compartment was calculated from the extrapolated activity at zero time. The K^+ efflux from each of the compartments was obtained by multiplying the K^+ content of that compartment by the corresponding rate constant.

The extracellular water-space was determined with ^{60}Co-EDTA [21] in a parallel group of experiments. Total extracellular water was measured by adding the marker to both sides. In some experiments only the internal water space was determined. For these experiments the Ringer contained 0.5 mM EDTA and $0.35 \cdot 10^{-4}$ mM of ^{60}Co and no Ca^{2+} or Mg^{2+}, to avoid displacement of Co from EDTA. Under these conditions only about $1 \cdot 10^{-18}$ M of cobalt is free in solution [22].

After incubation, the isolated epithelia were removed from the chambers,

blotted with filter paper, weighed, dried to a constant weight at 100°C, removed into vials and counted for ^{60}Co.

The results of eight experiments expressed in kg/kg dry weight are: for total water content (3.72 ± 0.025); extracellular water (1.38 ± 0.142); extracellular water estimated by adding the marker to the inside only (0.524 ± 0.021); intracellular water (2.33 ± 0.125). These values were used to evaluate the K^+ concentrations in the total skin and in each of the different compartments.

All the results are expressed as means and standard errors of the means.

Results

Fig. 1 shows a typical result of a $^{42}K^+$ efflux experiment and of the effect of ouabain added after 2 h, and Table I shows the collected data from 23 such experiments. The results obtained with ouabain in the same experiments will be considered later. The amount of $^{42}K^+$ that is washed out to the outer side of the skin is 1.27% ± 0.22 of the total $^{42}K^+$ of the skin and probably a part of this was washed out to the internal side and moved to the external fluid via the shunt path.

Therefore we consider the results as representing the K^+ efflux from the internal face of the skin. It can be seen that we obtain two exponential rates of tracer release which are interpreted as representing the K^+ efflux from two different compartments the nature of which will be considered now.

After 2 h incubation with $^{42}K^+$ from the inside of the preparation all the K^+ in the skin is labelled, if one compares the K^+ labelled with the K^+ measured chemically (0.625 ± 0.046 versus 0.551 ± 0.035 μequiv./cm²) ($0.2 < P < 0.4$) or (365.0 ± 20 versus 314.0 ± 20 mequiv./kg dry wt.). From the values of intracellular and extracellular water described in the methods, the value of the wet weight (8.28 ± 0.45 mg/cm²) and the K^+ content the concentration of K^+ in each compartment could be calculated.

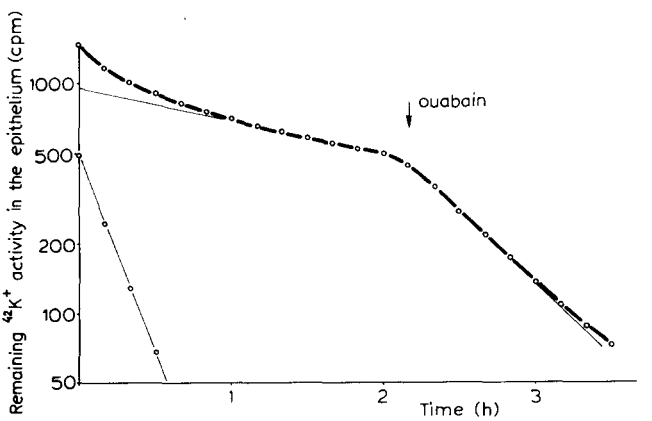

Fig. 1. Kinetics of $^{42}K^+$ release from the internal side of the isolated skin epithelium and the effect of ouabain (a typical experiment). A semi-logarithmic plot of total remaining counts in the skins versus time was made and the rate constants of the various exponential components calculated by successive linear extrapolations.

TABLE I

COMPARTMENTAL ANALYSIS OF THE K^+ EFFLUX COMPONENTS

Collected data from 23 experiments (For details of the calculations see Methods). The K^+ content of the total skin epithelium was 0.625 ± 0.046 μequiv./cm^2 or 356.1 ± 20.2 mequiv./kg dry wt.

	Compartment 1	Compartment 2
Rate constant (min^{-1})	0.054 ± 0.0016	0.0017 ± 0.0006
Potassium content in the skin (μequiv./cm^2) or mequiv./kg dry wt.	0.220 ± 0.016 125.4 ± 9.1	0.411 ± 0.024 234.2 ± 13.7
Potassium efflux from the internal face of isolated frog skin (without chorion) (μequiv. · cm^{-2} · h^{-1}) (calculated from K^+/k in each experiment)	0.707 ± 0.054	0.182 ± 0.014
Transepithelial Na^+ fluxes (μequiv. · cm^{-2} · h^{-1}) (calculated from the integrated I_{sc} measured during the 1st and 2nd hour of the washout, respectively)	1.01 ± 0.070	0.796 ± 0.057

Assuming that one compartment is extracellular and the second intracellular, their K^+ concentrations are respectively 243 ± 20.2 and 101 ± 5.0 mequiv./l. If one assumes both compartments to be intracellular the average K^+ concentration is 156 ± 8.1 mequiv./l.

The hypothesis that compartment one is extracellular leads to implausible values of extracellular K^+ concentrations. Even if we assume that there is a gradient in the intercellular spaces, and that the K^+ concentration near the cells is higher than in the bulk solution, the concentration of K^+ in this space can never be higher than that in the cells. Furthermore, the computed intracellular K^+ concentration, based on the first hypothesis, is low compared with the data from the literature, while the values obtained according to the second hypothesis fall in the range described by other authors [20,23—26].

To ensure that the two compartments are genuinely in the isolated epithelium and not just in concealed chamber compartments (i.e., agar bridges, contaminated recesses) two skins were incubated with radio isotope during the collagenase incubation, which also takes 2 h, and not in the chambers where the washout was performed. The skind were then dissected and mounted in the chambers, and the washout performed as usual.

The results obtained for two skins showed again clearly the distribution of K^+ in two compartments, namely for skin I 0.117 and 0.297 and for skin II 0.211 and 0.196 expressed in μequiv./cm^2, or for skin I 66.7 and 169.2 and for skin II 120.2 and 111.7 expressed in mequiv./kg dry wt.

The results therefore seem to suggest that there are two K^+ compartments and that both are intracellular.

We concluded therefore that all the K^+ is intracellular and if evenly distributed in the whole cell water the intracellular K^+ concentration is 156 ± 8.1 mequiv./l. The kinetic behaviour of the K^+ efflux can be due either to a compartmentalization of the K^+ within the cells themselves [27] or due to the existence of two functionally different cell populations with different K^+ permeabilities. The first hypothesis was not confirmed by Rick et al. [28] who

demonstrated identical concentrations for Na^+ and K^+ in the nucleus and cytoplasm under all experimental conditions.

The morphological identification of the two functional different cell types is difficult to do under the present circumstances.

By dividing for each skin the Na^+ fluxes obtained from the I_{sc} by the K^+ fluces both corresponding to the first period of washout one obtains a ratio of 1.39 ± 0.107.

If one assumes that the I_{sc} is an underestimation of the rate of Na^+ pumping by the Na^+/K^+ pump and that the first washout fluid may contain a small amount of isotope that remained in the chamber, the observed ratio is not incompatible with a 3/2 stoichiometry ($0.2 < P < 4$).

In order to obtain a better estimate of this stoichiometry we studied the relationship between simultaneous changes in I_{sc} and in K^+ effluxes.

We used amiloride and pitressin, two substances that act on the permeability of the external barrier to Na^+, and ouabain as a pump inhibitor.

The effect of the addition of amiloride at the beginning of the washout period and consequently on the first rate constant was investigated first. Na^+ fluxes were calculated from the I_{sc} measured immediately before amiloride was applied. The K^+ effluxes from the first compartment were calculated as described in methods. Two skins were studied. In one skin the Na^+ flux measured just before amiloride was applied was 1.9 μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$ and K^+ flux corresponding to the first rate constant was 0.169 μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$. In the second skin the Na^+ flux was 1.66 and the K flux 0.161 μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$. In both skins the I_{sc} came to zero after amiloride treatment.

Since it is not possible to measure in the same experiment values for the fast rate constant before and after amiloride, we have to compare these results with the K^+ fluxes obtained under normal conditions as presented in Table I (to a mean value of Na^+ flux of 1.01 μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$ corresponds a K^+ flux from the first compartment of 0.707 μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$).

To the above mentioned Na^+ fluxes the corresponding K^+ fluxes would be (all in μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$): to a Na^+ flux of 1.9 a K^+ flux of 1.33 and a decrease with amiloride of 1.161, and for a Na^+ flux of 1.66 a K^+ flux of 1.162 and a decrease with amiloride of 1.001.

These results showed, therefore, that amiloride strongly inhibits the K^+ fluxes from compartment one.

The results obtained when amiloride was added in the third hour of the washout are shown in Table II.

It can be seen that the K^+ efflux from compartment 2 is also decreased although much less than the efflux from compartment 1.

The results of the effect of pitressin on the I_{sc} and the K^+ efflux of the slow component can also be seen in Table II. In this case to an increase in I_{sc} corresponds as well an increase in K^+ effluxes.

This means that both compartments are involved in Na^+ transport but to different extents.

Ouabain and high potassium were only tested on the slow component of the K^+ efflux since the effect of ouabain on I_{sc} takes almost 1 h while the first compartment is washed out with a time constant of approximately 20 min.

Table III summarizes the results of two experiments (skins 1 and 2) while

TABLE II

THE EFFECT OF AMILORIDE AND PITRESSIN ON THE K^+ EFFLUX OF THE SECOND COMPARTMENT (SLOW COMPONENT)

Results expressed as μequiv. · cm^{-2} · h^{-1}. The Na^+ fluxes were calculated either from the I_{sc} measurements during the K^+ efflux periods or in the case of amiloride just before the addition of amiloride.

	Compartment fluxes in μequiv. · cm^{-2} · h^{-1}					
	Control values		Amiloride		Difference	
	ϕNa^+	ϕK^+	ϕNa^+	ϕK^+	ϕNa^+	ϕK^+
Skin 1	0.701	0.196	0.624	0.111	0.624	0.085
Skin 2	0.904	0.213	0.758	0.127	0.758	0.086
			Pitrescin			
Skin 3	0.666	0.212	1.38	0.311	0.713	0.099
Skin 4	1.48	0.315	3.57	0.464	2.08	0.149

Fig. 1 shows one complete experiment where ouabain was added in the third hour.

A considerable increase in K^+ fluxes is observed despite a decrease in I_{sc}.

In Fig. 2A we can compare the effect of ouabain on the time course of I_{sc} and the K^+ efflux. It can be seen that while the effect on K^+ was rather fast, the abolition of the I_{sc} took a longer time. These results are probably due to the fact that after the Na^+/K^+ pump is inhibited by ouabain the electrochemical gradients of Na^+ and K^+ between the intracellular compartment and the external compartments are gradually dissipated by an influx of Na^+ across the

TABLE III

THE EFFECT OF OUABAIN AND HIGH SEROSAL K^+ CONCENTRATIONS ON THE SLOW COMPONENT OF THE K^+ EFFLUX AT THE INTERNAL BARRIER OF THE ISOLATED EPITHELIUM

Values expressed in μequiv. · cm^{-2} · h^{-1}. The values for Na^+ fluxes were calculated from the values of I_{sc} measured during the period of $^{42}K^+$ washout, or in the case of ouabain just before the addition of ouabain.

	Fluxes (equiv. · cm^{-2} · h^{-1})							
	Normal		ϕNa	ϕK	Ouabain		Difference	
	ϕNa	ϕK			ϕNa	ϕK	ϕNa	ϕK
Skin 1	1.07	0.153			0.949	0.672	0.949	0.519
Skin 2	0.796	0.183			0.713	0.389	0.713	0.206
			50 mequiv./l K^+		Ouabain			
Skin 3	1.18	0.10	0.825	0.229	0.943	0.437	0.943	0.208
			20 mequiv./l K^+					
Skin 4	0.86	0.119	0.694	0.205	0.729	0.662	0.729	0.457
			10 mequiv./l K^+		20 mequiv./l K^+			
Skin 5	0.631	0.169	0.589	0.225	0.707	0.322		
					50 mequiv./l K^+			
Skin 6	0.755	0.116	0.707	0.160	1.003	0.457		

mucosal barrier and an efflux of K^+ across the serosal barrier. Under short circuit current the dissipation of these gradients produces a falling current in the external circuit. In effect, while during the first 2 h the K^+ contents of the skin remains constant and therefore the two rates of tracer efflux represent the steady state K^+ efflux from two different compartments, after addition of ouabain the total K^+ content of the skin fell from about 0.60 μequiv./cm^2 or 342 mequiv./kg dry wt. to 0.15 and 0.17 μequiv./cm^2 or 85.5 and 97 mequiv./kg dry wt. in two different experiments. The fall in tracer content of the skin after ouabain is largely due to a net K^+ loss (75%). Together with a decrease of intracellular K^+ concentrations an increase in Na^+ is described [26,28].

An alternative explanation is presented by Cala et al. [29] who where able to relate the inhibition of the I_{sc} with the binding time of ouabain. Nevertheless, it must be emphasized that they used a concentration of ouabain of 10^{-6} M.

In Fig. 2A where Na^+ fluxes and K^+ fluxes are expressed in the same units and are followed over one hour after application of ouabain the K^+ fluxes show similar values to Na^+ fluxes at 10 and 20 min and exceed Na^+ fluxes at 30 min. As the intracellular K^+ under these conditions is decreasing, the K^+ fluxes may be overestimated.

The increase in the rate of tracer efflux induced by ouabain could be due to the inhibition of $^{42}K^+$ recycling from an unstirred layer near the cells via the Na^+ pump. Insuch case, the efflux of $^{42}K^+$ is in fact equal to the difference between the efflux and the influx on $^{42}K^+$ at the inner surface of the cells due to the fact that the specific activity of K^+ near the cells is not zero. By inhibiting the pump this recycling should stop and more $^{42}K^+$ should be washed out.

To test this hypothesis, high K^+ concentrations were used in the internal Ringer together with ouabain, the idea being that an increase in the K^+ concentration would decrease the specific activity of $^{42}K^+$ near the cells and thus reduce its recirculation and unmask a direct effect on the pump. Under these circumstances, one would expect ouabain to decrease the K^+ efflux.

However, the effect of ouabain was still qualitatively the same at K^+ concentration as high as 50 mequiv./l. (See Table III (skins 3 and 4) and Fig. 2B).

Rising internal K^+ by itself also increases the $^{42}K^+$ efflux. This was clearly observed at 10 mM K^+ and saturated at a maximum efflux of 0.34 μequiv. $\cdot$ cm^{-2} $\cdot$ h^{-1}. The results of such experiments are given in Table III and Fig. 3 (number of experiments in brackets).

Both high internal K^+ and ouabain inhibition may act at least in part, through the same mechanism: namely by depolarizing the cell membrane [30,31]. In effect the electrogenicity of the Na^+ pump has been shown in a number of systems [13]. According to Cereijido and Curran [1] and Nagel [30] even with concentrations of K^+ as high as the cell concentration, the membrane is not completely depolarized. It may, therefore, be that the ouabain inhibition of the Na^+ pump has still a depolarizing effect at high K^+ concentrations. That would explain the effect of ouabain on the K^+ effluxes also at high internal K^+ concentrations.

Both amiloride and ouabain inhibit the active Na^+ transport but while one substance decreases the K^+ effluxes at the internal barrier the other increases these fluxes. It was already mentioned that ouabain depolarizes the cell mem-

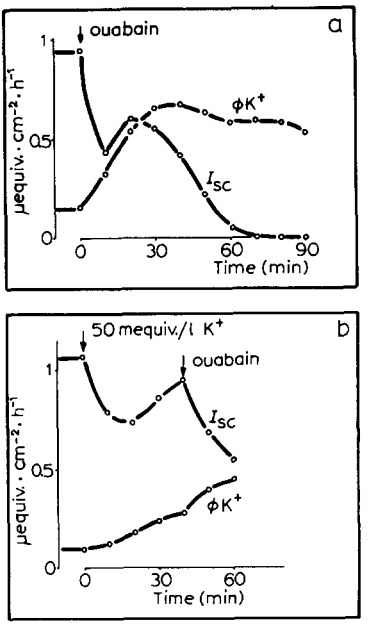

Fig. 2. (a and b) Effect of ouabain and high internal K^+ on the i_{sc} and $^{42}K^+$ efflux of the isolated frog skin epithelium. I_{sc} and $^{42}K^+$ expressed as μequiv. · cm^{-2} · h^{-1} versus time. Arrows show the moment ouabain or K^+ is introduced in the system.

brane. It is also known that amiloride hiperpolarizes the epithelial cell, mainly by a decrease in the Na^+ conductance at the external barrier as suggested by Nagel [32] and Schultz [33].

This may affect per se the K^+ effluxes across the serosal membrane. But although the electrochemical gradient is changed, under these experimental conditions there is no variation in K^+ content of the epithelial cells (0.58 ±

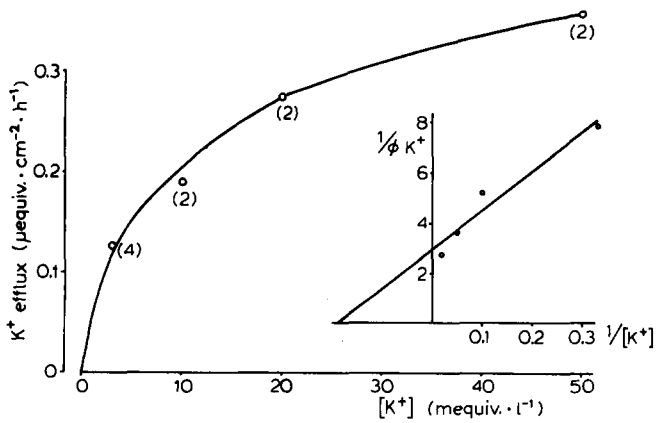

Fig. 3. Relation between potassium concentration in the Ringer bathing the internal side of the frog skin and $^{42}K^+$ effluxes from the same side. (In brackets number of experiments).

0.04 before amiloride compared with 0.63 ± 0.05 μequiv./cm^2 after amiloride; $P > 0.5$, or 330 ± 22 versus 359 ± 28 mequiv./kg dry wt.).

Furthermore, the variations in the Na^+ fluxes and in the K^+ effluxes are in the same direction, suggesting that when the supply of mucosal Na^+ for the Na^+ pump is blocked suddenly by amiloride the pump works at a much reduced rate depending then, on the serosal Na^+ leak. Hence the reduction in tracer $^{42}K^+$ efflux observed after amiloride could in fact be directly related to this effect. Similar analysis can be made for the stimulations of the short-circuit current and K^+ efflux induced by vasopressin.

Due to the considerations above we tried to compute the Na^+/K^+ stoichiometry of the Na^+ pump using the changes obtained in I_{sc} and K^+ effluxes.

This can only be regarded as an attempt since it is based on few experiments and the data were not obtained from the same skin.

Based on the experimental data that there are two functionally distinct K^+ compartments and that both respond to changes in transepithelial Na^+ fluxes with changes in K^+ effluxes, we assume:

(1) That the total transepithelial Na^+ transport is divided into two fractions, each fraction being related to one of the K^+ compartments described above

$$x + y = 1$$

(2) That the Na^+/K^+ stoichiometry has to be the same for both compartments

$$(x \cdot \Delta\phi Na_1^+)/\Delta\phi K_1^+ = (y \cdot \Delta\phi Na_2^+)/\Delta\phi K_2^+$$

were $\Delta\phi Na_1^+$ and $\Delta\phi K_1^+$ * are variations introduced simultaneously in the I_{sc} and the $^{42}K^+$ effluxes related to the first compartment and $\Delta\phi Na_2^+$ and $\Delta\phi K_2^+$ ** are simultaneous variations introduced in the I_{sc} and the $^{42}K^+$ effluxes related to the second compartment.

For the K^+ fluxes the two components are identified at least functionally.

For the Na^+ fluxes the whole I_{sc} is always measured and although all the Na^+ has to flow through the external barrier into the more superficial cells it can then be pumped either directly into the intercellular spaces, or first diffuse into the cells of the deeper layers and be pumped afterwards.

The number of Na^+ pumps of each cell or cell layer that contribute to the transepithelial Na transport is still a matter of study. Some may have more pumps or a bigger turnover involved in this transport than others.

As we have two distinct K^+ rate constants both sensitive to changes in Na^+ transport we decided to relate a fraction of the Na^+ transport to one K^+ compartment and another fraction to the second compartment. Therefore, the total Na^+ fluxes are multiplied by a factor x or y.

Table IV summarizes the computed values and the data from where the calculations were made.

It can be seen that the fraction of I_{sc} that flows through the first compartment is much bigger and that the Na^+/K^+ stoichiometry is in agreement with the value computed above and not inconsistent with a 3/2 stoichiometry.

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* See p. 18.

** See Table II.

TABLE IV

COMPUTED VALUES OF THE Na^+/K^+ STOICHIOMETRY AND OF THE FRACTION OF Na^+ THAT IS PUMPED BY COMPARTMENT 1 (x) AND COMPARTMENT 2 (y)

The fluxes, expressed in μequiv. $\cdot$ $cm^{-2} \cdot h^{-1}$, are the transcription of the results presented on p. 6, for compartment 1 ($\Delta\phi Na_1^+$, $\Delta\phi K_1^+$) and Table II for compartment 2 ($\Delta\phi Na_2'$, $\Delta\phi K_2$).

Example	$\Delta\phi Na_1^+$	$\Delta\phi K_1^+$	$\Delta\phi Na_2^+$	$\Delta\phi K_2^+$	x	y	Na^+/K^+
1	1.9	1.161	0.624	0.085	0.82	0.18	1.34
2	1.66	1.001	0.758	0.086	0.84	0.16	1.40
3	1.78 *	1.081 *	0.713	0.099	0.81	0.19	1.34
4	1.78 *	1.081 *	2.08	0.149	0.89	0.11	1.47
Means					0.84	0.16	1.39

* Means of the two values above.

Discussion and Conclusions

The aim of this work was to find a relation between the transepithelial Na^+ transport and the K^+ movements at the inner barrier of the isolated frog skin preparation.

Isolated epithelia were used. They were incubated with $^{42}K^+$ from the inside for 2 h. Efflux of $^{42}K^+$ was then measured for 2 to 3 h.

The relation between I_{sc} and K^+ effluxes were considered under spontaneous conditions and after simultaneous changes introduced in both parameters.

The results reported here show that:

(a) All the K^+ contained in the isolated frog skin epithelium equilibrates in specific activity with external $^{42}K^+$. There are no excluded K^+ compartments.

(b) Most of the $^{42}K^+$ is released to the internal bathing medium. Only 1.27% of the total $^{42}K^+$ of the skin is washed out to the external side, a fraction of this probably coming from the inside medium via the shunt path.

(c) The kinetics of tracer release exhibit one fast and one slow exponential component.

(d) We suggest that they correspond to two functionally distinct compartments both eventually cellular with a homogenous K^+ concentrations (156 mequiv./l cell water) but with different K^+ permeabilities.

(e) Both these compartments seem to be involved in the transepithelial Na^+ transport since a change introduced in this parameter affects any of the two K^+ rate constants.

(f) Amiloride reduces the release of $^{42}K^+$ corresponding to the fast component (85%) and of the slow component (58%). As the first rate constant is much faster, in absolute values the effect is much bigger. Furthermore, the K^+ content of the skin is not altered by amiloride.

(g) Ouabain accelerates the loss of $^{42}K^+$ by 200%. The loss of tracer in the presence of ouabain represents net K^+ loss from the epithelium and this affects the K^+ contained in both compartments (intracellular K^+ decreases to 25% of its original value.

(h) A tentative calculation of the Na^+/K^+ stoichiometry for the transepithelial Na^+ transport gave a value of 3/2.

(i) The morphological counterpart of the two functional compartments defined above is more difficult to evaluate.

In the last years two main hypothesis have been put forward regarding the importance of the different cells or cell layers in the transepithelial Na^+ transport in frog skin.

One is that the first reactive cell layer is the only or major layer involved in the active transport of Na^+, because this layer is the only that responds by swelling to a number of different experimental conditions (short-circuiting, reversed current injection, hydrostatic pressure to the inside of the preparation [34—36]. Alternatively they may have lost their capacity of osmoregulation.

The other is that the epithelium functions as a syncytium where all the cells are involved in the transepithelial Na^+ transport, and is based on the experimental evidence that all the cells have similar Na^+ and K^+ concentrations, change simultaneously these concentrations under experimental conditions [28], have ouabain binding sites in all their basolateral cell membranes, although more in the St. spinosium and germinatium [37] and show a considerable amount of low resistance communications between them [38].

In our case it is difficult to fit the first compartment in the first reactive cell layer (R.C.L.) as defined by Voute and Ussing [34]. If the first reactive cell layer is one cell layer out of three to five cell layers of the whole skin it could only have between 33 and 20% of the whole K^+ measured and in fact the first compartment defined by us has a mean value of 35%, unless one assumes that this layer has a higher K^+ concentration, which is unlikely.

Alternatively, [39] as the cells approach the surface of the frog skin in the process of differentiation and shedding they are first transformed from non-polar cells into asymmetric cells where the outer membrane gets more and selectively permeable to Na^+, they get linked to each other by tight junctions forming a well defined barrier in relation to the exterior, and eventually the internal facing cell membrane gets more permeable to K^+. These cells are not identified with the first R.C.L. but they may start to differentiate already at a deeper level of the epithelium. If this is correct this group of differentiated cells may be more specialized in the transepithelial Na^+ transport than the non-polar cells.

The observation of an identical behaviour of all epithelial cell layers regarding Na^+ and K^+ concentrations and ouabain binding does not mean necessarily that all epithelial cells share to exactly the same extent the transepithelial Na transport.

To that observation may contribute the fact that the homocellular regulation of Na^+ and K^+ composition and the active transcellular Na^+ transport are accomplished by the same Na^+-K^+ ouabain sensitive exchange pump located in the inner or basolateral cell membranes, and the profuse connections between the cells themselves.

Evidence for two distinctly accessible compartments for Na^+ from the external medium were described by Morel and Leblanc [40]: one rapidly accessible comprising the stratum granulosum and the other less rapidly accessible representing the deeper layers of the epithelium.

We may conclude that the syncytial functioning theory is quite well documented and convincing, although it does not allow us to exclude the

possibility of some cells being more involved in the transepithelial Na^+ transport than others.

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