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POTASSIUM FLUX AND SODIUM TRANSPORT IN THE ISOLATED FROG SKIN

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SUMMARY

The K^+ fluxes at the inside surface, and the short-circuit current (s.c.c.), were measured in a 20-cm^2 piece of the abdominal skin of the frogs, *Leptodactylus ocellatus* and *Rana catesbeiana*. The inside of the skin was perfused with Ringer's solution containing $^{42}K^+$ circulating in a closed system including a scintillation detector. The disappearance of radioactive material from the Ringer's solution was measured during a period greater than 3 h. A compartmental analysis was used to find out the transfer constants and unidirectional fluxes. Most of the experiments were performed under steady-state conditions. Despite the fact that the skin of *L. ocellatus* shows an active transport of Cl^- not present in the skin of the *R. catesbeiana*, results in both skins were similar. The K^+ content of the skins was found to be in the order of 1 μequiv/cm^2 of skin surface. K^+ influx at the inside surface was of the order of 0.3 μequiv./cm^2 per h. The K^+ flux did not vary when the short-circuit condition was changed to open circuit and *vice versa*, or upon removal of Cl^- from the Ringer. However, the Na^+ flux, or s.c.c., was significantly larger than the K^+ flux. The permeability properties for K^+ at the inside surface seem to be relatively independent of external electrical or ionic changes. K^+ flux is not directly related to the mechanism of Na^+ transport.

INTRODUCTION

Na^+ transport across the isolated frog skin is dependent on the presence of K^+ in the inner bathing solution. Consistent with this observation, KOEFOED-JOHNSEN AND USSING[1] proposed in 1958 their classical model of transport that included a 1:1 Na^+-K^+ pump located in the inner barrier of the transporting cells. Since then, evidence has been presented that contradicted some of the predictions of the model. CEREIJIDO AND CURRAN[2] found, by impelling microelectrodes in the skin, that changes in the K^+ concentration in the inside bathing solution produced a change in the potential difference (PD) of the outer surface. However, the outer surface PD responds only partially to changes in Na^+ concentration. To account for the new observations, modifications of the original model were made by USSING AND WINDHAGER[3]. In spite

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Abbreviations: s.c.c., short-circuit current; PD, potential difference.

of the abundant work done pertaining to ionic transport in frog skin, the measurement of the K^+ influx across the inside barrier was done only once (CURRAN AND CEREIJIDO[4]), using the isolated sking of *Rana pipiens*.

CURRAN AND CEREIJIDO[4] reported an average K^+ influx of 1.04 μequiv./h per cm^2 and an average net Na^+ transport of 0.91 μequiv./h per cm^2. They reasoned, however, that the K^+ influx may be an overestimation of the K^+ influx coupled to the Na^+ pump and that the net Na^+ transport is a minimal measure of the Na^+ pumped at the inner barrier. Based on this, they suggested that a hypothetical Na^+–K^+ exchange system would have a Na^+/K^+ ratio of 6/1. Furthermore, they found no correlation between net Na^+ transport and the K^+ influx at the inner barrier.

Very recently, BIBER *et al.*[5] reported results of K^+ influx across the inner barrier of the epithelium of a split frog skin. They found a net Na^+/K^+ influx ratio of 3.37 and a proportionality between the two fluxes.

The measurement of K^+ influx presents some technical difficulties, and the validity of the results may be questioned on the basis that the connective tissue may restrict the K^+ uptake by the skin so that the computed K^+ influx will be lower than the real flux coupled to the pump.

In 1964, CANDIA AND ZADUNAISKY[6] reported preliminary results on K^+ influx measured in the South American frog *Leptodactylus ocellatus*. Average K^+ influx was 0.30 μequiv/h per cm^2. This value was only 1/9 of the net Na^+ transport found in this frog skin. Since skin of *L. ocellatus* shows an active transport of Cl^- (ZADUNAISKY *et al.*[7]) it was assumed that the large difference between Na^+ and K^+ fluxes may be due to basic differences between the mechanisms of Na^+ transport in skins with or without Cl^- transport.

However, the values of K^+ influx and Na^+–K^+ correlation found by us in the whole skin of *L. ocellatus* are in accord with those recently reported by BIBER *et al.*[5] in the corium-free skin of *R. pipiens*. This suggests that the connective tissue may not represent a difficulty in comparing Na^+–K^+ influx at the inner barrier of the frog skin. This assumption is further supported by the fact that Na^+ fluxes measured in the isolated epithelium by ACEVES AND ERLIJ[8,9] are similar to those commonly observed in whole skins. Because of this, it seemed worthwhile to investigate in detail and compare the relationship between Na^+ transport and K^+ influx in whole skins with and without active Cl^- transport such as the skins of *L. ocellatus* and *Rana catesbeiana*.

METHODS

General

The experimental design was based on the observation that K^+ uptake by the skin from the outside bathing solution and unidirectional K^+ fluxes across the skin are negligible. The skin and the inside bathing solution can then be treated as a closed system.

Large specimens of *L. ocellatus* and *R. catesbeiana* (about 400 g) were selected so that more than 20 cm^2 of white abdominal skin could be used. The isolated skin was mounted with the inside up on the bottom half of a lucite chamber (Fig. 1). The skin was held in place by a set of pins. The top half was then screwed onto the bottom with the skin held between them. The exposed area of the skin was 20 cm^2.

Each side of the chamber had outlets for PD measuring and current sending electrodes. In addition, the top half was connected to both ends of a loop of polyethylene tubing through which the fluid in the top half of the chamber was circulated by means of a peristaltic pump. The polyethylene loop included a coiled section that could be placed in a well scintillation detector. The inside diameter of the tubing was 1.9 mm.

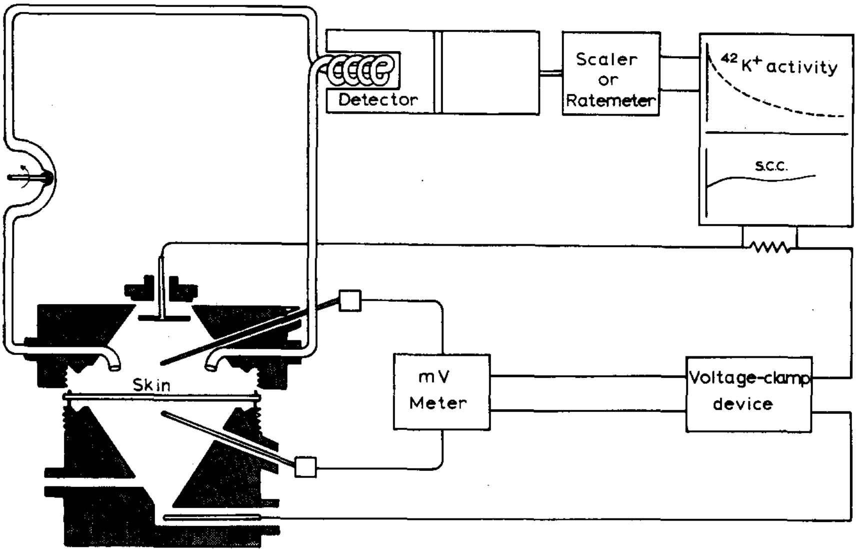

Fig. 1. Diagram of the experimental arrangement for measuring K^+ uptake by frog skin. The inside of the skin is in contact with the solution in the top-side of the chamber.

The volume of the loop, including the pump and the coil, was about 8 cm^3. The equivalent volume of the coil, as detected by the scintillation detector, was about 1 cm^3. The total volume bathing the inside of the skin (loop + top chamber) was 20 cm^3. Because of the conical shape of the top half of the chamber, the depth of the Ringer above the inside of the skin was about 7 mm. The flow in the loop was about 1 cm^3/sec. The $T_1/_2$ for the mixture between chamber and loop was then 14 sec (0.693/0.05 per sec). There was no aeration or circulation in the bottom half of the chamber. However, it was found in preliminary experiments that this did not affect PD or short-circuit current (s.s.c.). The Ringer's solution had the following composition (in mM): Na^+, 104; K^+, 3.0; Ca^{2+}, 1.0; Mg^{2+}, 1.2; Cl^-, 75.0; HCO_3^-, 25; SO_4^{2-}, 1.8; HPO_4^{2-}, 2.9; gluconate^{2-}, 1.0; glucose, 26. In the Cl^--free solutions, SO_4^{2-} replaced Cl^-, and osmolarity was compensated for with sucrose. PD was measured through agar–Ringer bridges and calomel electrodes with a Keithley 200 B millivoltmeter. s.c.c. was controlled either manually or with an automatic voltage-clamping device.

Determination of K^+ concentration in skin and bathing solution

K^+ concentration was determined in the bathing solutions before and after the experiments by flame photometry (Eppendorf 700). At the end of the experiment, the part of the skin exposed to the solutions was gently dryed for about 2 sec on filter paper, weighed, counted for $^{42}K^+$ activity and digested in concentrated HNO_3. The HNO_3 was evaporated at low temperature. The remaining solids were diluted in a known volume, and the K^+ concentration measured by flame photometry. $^{42}K^+$ activity was also measured again in the fluid dilution.

Determination of K^+ influx at the inner side of the skin

The skin was mounted in the previously described chamber. Fluid in the top half of the chamber was continuously circulated. After a 30-min period of stabilization, $^{42}K^+$ was added to the inside solution. About 5 μl of $^{42}KCO_3$ solution was added to obtain a specific activity of at least 30 000 counts/min per μequiv. Activity in the inside solution was continuously monitored after the addition of $^{42}K^+$ and throughout the rest of the experiment (5–8 h). Maximum activity was detected about 30–45 sec after the addition of $^{42}K^+$. This was probably due to the mixing time of the system. After the maximum 30-sec peak, the activity decreased continuously toward equilibrium as the skin was taking up $^{42}K^+$. At the end of the experiment, a sample was taken from the inside solution and counted in the same well detector used for the continuous monitoring during the experiments. This activity was compared with the activity recorded in the coil loop at the time of sampling. Computations and graphs were made following correction for decay of $^{42}K^+$.

Determination of extracellular space

Extracellular space was estimated by determining the inulin and $^{35}SO_4^{2-}$ space. Skins were incubated in a Ringer's solution containing $^{35}SO_4^{2-}$ (100 μC/l) and/or inulin (1 %) for a period of 2 h or more. The skins were then rinsed, blotted, and transferred to an unloading solution.

Inulin was measured by a colorimetric method[10]. ^{35}S was measured with a Packard scintillation detector.

RESULTS

Inulin and sulfate space

The K^+ concentration in the extracellular space, after 30 min of equilibration, was assumed to be the same as in the Ringer (3 μequiv/ml). In order to determine the K^+ pool in the extracellular space, its volume was determined with inulin in the skin of *L. ocellatus*. Inulin and $^{35}SO_4^{2-}$ was used in the skin of *R. catesbeiana*. The results are shown in Table I.

TABLE I

EXTRACELLULAR SPACE OF FROG SKIN

As a fraction of wet weight *minus* dry weight. Figures represent mean ± S.E. (number of experiments in parentheses).

	Inulin	*Sulfate*
L. ocellatus	0.237 ± 0.012 (29)	—
R. catesbeiana	0.165 ± 0.009 (15)	0.490 ± 0.013 (15)

SO_4^{2-} space is larger than the inulin space. It is uncertain whether the inulin or the SO_4^{2-} space gives the best estimation of the extracellular K^+ space. In any case, the K^+ pool in the extracellular space can not be larger than 1.47 μequiv/g. This amount represents only 4 % of the average K^+ in the skin (27.39 μequiv/g).

K^+ uptake

Total K^+ in a 20-cm² area skin (av. wt. 767 mg) was about 21 μequiv. Total K^+ in the inside bathing solution was 60 μequiv. (3 μequiv/ml·20 ml). With a K^+ concentration of 3 μequiv/ml, the skin did not gain or lose K^+ from the bathing solutions for a period of up to 10 h. If all K^+ in the skin is free to interchange with the K^+ in the inside bathing solution, $^{42}K^+$ added to the inside bathing solution will distribute, at infinite time, with the same ratio as the non-labeled K^+ (about 3–1 in our system). Consequently, about 25 % decrease in the original activity of the inside solution should be observed during the uptake of $^{42}K^+$ by the skin. If the uptake can be described by a single exponential, this will be an indication that most of the K^+ in the inside solution interchanges with a single compartment in the skin. A typical record of K^+ uptake by frog skin is shown in Figs. 2a and 2b.

In accordance with a treatment of a closed two-compartment system described by SOLOMON[11], the ordinate in Fig. 2a is P, the amount of tracer in the inside solution (compartment A) at a given time. Fig. 2b shows the logarithm of $(P/P_\infty - 1)$ plotted

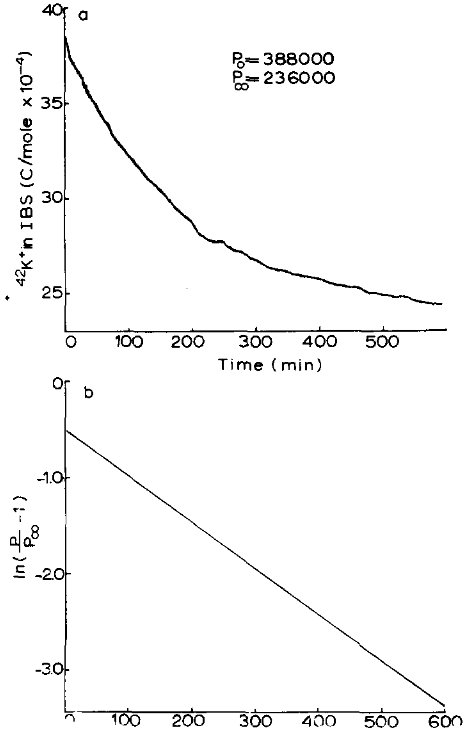

Fig. 2. a. Continuous record of $^{42}K^+$ decline in inside bathing solution (IBS) produced by skin uptake as a function of time in *R. catesbeiana*. Open circuit (Experiment R-15 in Table III). b. ln $(P_0/P_\infty - 1)$ as a function of time (least squares fit from curve in Fig. 2a).

against time. P_∞ is the stable amount of tracer in compartment A at infinite time. The line in Fig. 2b can be adequately described by a single exponential equation of the form:

$$\ln (P/P_\infty - 1) = -(k_{ba} + k_{ab})t - \ln k_{ba}/k_{ab} \tag{1}$$

where: k_{ab} is the coefficient of transfer from compartment A to B; k_{ba} is the coefficient of transfer from compartment B to A and t is time.

In order to have a quantitative indication of how accurately Eqn. 1 can describe the K+ uptake by the skin, the linear correlation coefficient R was determined for the values of ln $(P/P_\infty - 1)$ in all experiments. A correlation ranging between 0.87 and 0.99 was found.

Also

$$P_0/P_\infty = \frac{k_{ab} + k_{ba}}{k_{ba}} \tag{2}$$

and

$$\frac{P_0 - P_\infty}{P_\infty} = k_{ab}/k_{ba} \tag{3}$$

where P_0 is the total amount of labeled K+ added to compartment A at time zero. From Eqns. 1, 2 and 3

$$k_{ba} = \frac{-\ln (P/P_\infty - 1) + \ln (P_\infty/P_0 - P_\infty)P_\infty}{tP_0} \tag{4}$$

The coefficient of transfer from compartment B to A (k_{ba}) times the amount of K+ in B is equal to the unidirectional K+ flux (K^+_{out}) from the skin to the inside solution.

$$K^+_{out} = k_{ba} \cdot K^+ \text{ pool in skin} \tag{5}$$

Since the amount of K+ in both compartments is constant (no net interchange) the unidirectional flux of K+ from the skin to the inside solution K^+_{out} is equal to K^+_{in}, or

$$K^+_{in} = K^+_{out} = k_{ab} \cdot 60\ \mu\text{equiv.} \tag{6}$$

It is clear that P_∞ must be determined accurately in order to solve Eqn. 4. P_∞ can be determined in three different ways:

(a) the ordinate at zero time in Fig. 2b is ln k_{ab}/k_{ba}. Knowing this ratio and provided the system can be described by Eqn. 1, Eqn. 3 allows P_∞ to be calculated.

$$P_\infty = \frac{P_0}{(k_{ab}/k_{ba}) + 1}$$

(b) P_∞ can be predicted from values of P measured during the experiment. This is done by using a program for a least square's fit to an exponential function.

(c) In about 6–9 h, the decreasing value of P reaches steady state to remain stable for several hours. This value of P can be considered as P_∞ for practical purposes.

In some experiments the value of P_∞ was determined by the three methods and, in others, by Methods (a) and (b). Very good agreement was found between the three values of P_∞.

The other factor influencing the final computation of K^+_{out} is the determination of the K^+ pool in the skin. Total K^+ in the skin can be readily determined. Extracellular K^+ is a very small fraction of this total. Total activity of $^{42}K^+$ in skin at infinite time (9–12 h) was determined in many experiments. Specific activity in the skin was found to be equal to, or slightly smaller than that in the inside bathing solution at infinite time. This equality suggests that the K^+ measured in the skin by flame photometry is the pool participating in the uptake of $^{42}K^+$.

CURRAN AND CEREIJIDO[4] considered in great detail the effect of the connective tissue on the K^+ uptake by the skin. They reasoned that the initial slope of the uptake curve may not represent the unidirectional K^+ flux, if the diffusion across the connective tissue is not fast enough to insure specific activity equal to that in the bathing solution. However, results from our experiments are not based on the initial slope but rather on uptake curves of at least 4 h. The amount of K^+ in the connective tissue is rather small. The connective tissue will not act as a compartment but rather as a barrier. This barrier will present the same restriction to the non-labeled, as to the labeled K^+. A more detailed analysis of this point will be presented in the discussion section.

Tables II and III show, respectively, a series of experiments done in *L. ocellatus* and *R. catesbeiana* to determine the ratios of labeled and non-labeled K^+ between the inside bathing solution and the skin at infinite time. Also, the linear correlation coefficient R between ln $(P/P_\infty - 1)$ and time is shown in Table II. For the *L. ocellatus*, the ratio for the labeled K^+ (column 5) is somewhat larger than the ratio between the amount of K^+ in the inside bathing solution and the skin (Column 6) indicating that K^+ in the skin is free to interchange. The values of R strongly suggest that about 90 % of the exchange can be described by a single exponential for a closed two-compartment system. For the *R. catesbeiana*, the corresponding ratios for the labeled and non-labeled K^+ are equal. Although not computed, very good correlation was obvious from the plotting of ln $(P/P_\infty - 1)$ against time.

TABLE II

LABELED AND STABLE K^+ RATIOS BETWEEN SOLUTION AND SKIN OF *L. ocellatus*

Expt.	*Total K in skin (μequiv)*	P_0 *(counts/min)*	P_∞ *(counts/min)*	P_∞/Q_∞	K_{sol}/K_{skin}	R
L-1	13.16	59 248	45 000	3.16	4.58	−0.99
L-2	23.19	24 759	19 500	3.71	2.61	−0.88
L-4	21.14	25 751	19 200	2.93	2.85	−0.93
L-5	16.90	36 556	27 700	3.13	3.58	−0.91
L-6	13.41	25 931	21 000	4.26	4.50	−0.97
L-10	25.68	115 358	84 800	2.77	2.36	−0.99
L-11	21.08	81 600	65 300	4.00	2.87	−0.98
L-12	14.46	67 418	56 000	4.90	4.17	−0.98
L-13	20.16	47 529	39 000	4.57	3.00	−0.97
L-14	22.30	40 998	31 000	3.10	2.71	−0.99
L-15	25.27	58 785	43 000	2.72	2.40	−0.96
Mean	19.70			3.57	3.24	
S.E.	1.37			0.23	0.25	

TABLE III

LABELED AND STABLE K^+ RATIOS BETWEEN SOLUTION AND SKIN OF *R. catesbeiana*

Expt.	*Total K in skin (μequiv)*	P_0 *(counts/min)*	P_∞ *(counts/min)*	P_∞/Q_∞	K_{sol}/K_{skin}
R-11	17.73	257 200	180 000	2.33	3.38
R-12	20.91	229 000	160 000	3.31	2.87
R-13	18.00	207 200	150 000	2.62	3.33
R-14	22.01	246 600	173 000	2.35	2.73
R-15	41.82	388 000	236 000	1.55	1.43
R-21	27.16	70 800	49 600	2.34	2.21
R-22	26.54	130 700	100 000	3.26	2.26
R-23	17.24	65 600	50 500	3.34	3.48
R-24	23.86	139 000	107 000	3.34	2.51
R-31	22.32	56 800	42 400	2.94	2.69
R-41	16.22	172 000	133 000	3.41	3.70
R-42	18.32	236 000	164 500	2.30	3.27
R-43	24.67	225 300	176 000	3.57	2.43
R-44	23.88	146 500	105 000	2.53	2.51
R-53	24.52	136 500	95 000	2.29	2.45
Mean	23.01			2.76	2.75
S.E.	1.62			0.15	0.15

TABLE IV

K^+ INFLUX AT THE INSIDE FACE AND S.C.C. IN THE ISOLATED SKIN OF *L. ocellatus*

In Na_2SO_4 and NaCl solutions. Paired values.

	s.c.c. (μequiv/h per cm²)	*K^+ influx (μequiv/h per cm²)*	$k_{ab}+k_{ba}$ (h^{-1})	*R*	k_{ab}/k_{ba}
SO_4^{2-}	2.05	0.14	0.297	−0.81	0.194
SO_4^{2-}	2.39	0.29	0.551	−0.89	0.211
SO_4^{2-}	3.15	0.38	0.616	−0.83	0.256
SO_4^{2-}	3.81	0.40	0.521	−0.98	0.341
SO_4^{2-}	0.74	0.14	0.196	−0.92	0.321
SO_4^{2-}	4.32	0.34	0.525	−0.80	0.275
SO_4^{2-}	2.54	0.51	0.113	−0.99	0.177
SO_4^{2-}	2.66	0.23	0.467	−0.98	0.201
Mean	2.71	0.30			
Mean of difference	2.40				
S.E.	0.36				
P	<0.001				
Cl^-	2.85	0.50	0.682	−0.99	0.322
Cl^-	2.91	0.32	0.390	−0.94	0.381
Cl^-	2.08	0.56	0.858	−0.98	0.275
Cl^-	2.32	0.38	0.750	−0.98	0.201
Mean	2.54	0.44			
Mean of difference	2.10				
S.E.	0.23				
P	<0.001				

Comparison between net Na^+ flux and K^+ flux. Effect of potential difference

In L. ocellatus. Experiments were performed in which skins were bathed in both regular Ringer's and Cl^--free Ringer's (substituted by SO_4^{2-}) solutions.

K^+ uptake by the skin was determined while the skin was either under short-circuit or open-circuit conditions. Some experiments were initiated in short circuit and after 2–4 h, the electrical condition was changed to open and *vice versa.* While the skin was short-circuited, the s.c.c. was considered as the net Na^+ transport in Cl^--free Ringer's solution and as a minimum estimation of the Na^+ transport in Cl^--rich Ringer's solution.

Table IV shows paired values for K^+ influxes and s.c.c. in Cl^--free and Cl^--rich Ringer's solution. It is clear that s.c.c. is 9 times larger than the K^+ flux in sulfate Ringer's solution, and about 6 times larger in Cl^- Ringer's solution. The smaller ratio in Cl^- Ringer's solution is probably due to the active transport of Cl^- that makes the s.c.c. smaller than the net Na^+ transport.

Table V shows paired values of K^+ fluxes under open-circuit and short-circuit conditions with the skin bathed in Na_2SO_4 Ringer's solutions. Fig. 3 shows the log of $(P/P_\infty - 1)$ plotted against time for an experiment that was alternatively kept under open and short-circuit conditions. The dashed line indicates the expected slope if the K^+ uptake was equal to the s.c.c. Not only the K^+ flux was smaller than the

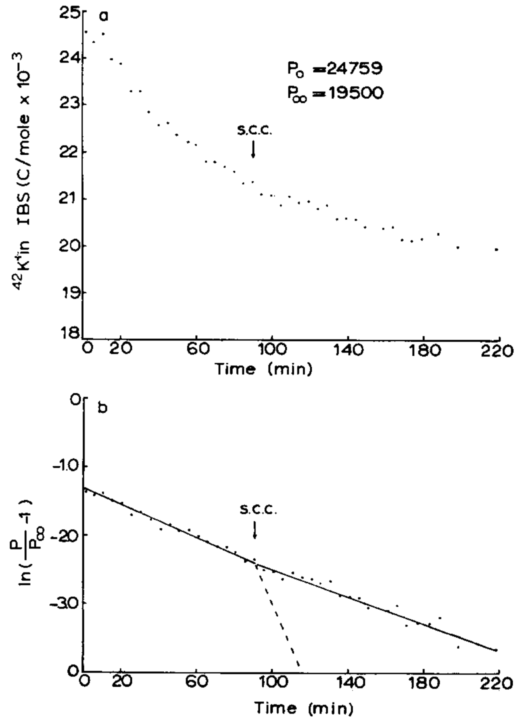

Fig. 3. a. $^{42}K^+$ activity in inside bathing solution (IBS) as a function of time. Decline is produced by uptake of $^{42}K^+$ by the skin of *L. ocellatus*. Experiment started under open-circuit condition. At 90 min the skin was short-circuited. b. ln $(P_0/P_\infty - 1)$ as a function of time from data in Fig. 3a. Solid line is a least square fit. Dashed line is the slope expected if the K^+ flux was equal to the short-circuit current.

s.c.c., but also it did not change noticeably when the PD was varied across the skin.

In R. catesbeiana. K^+ uptake by the isolated skin of *R. catesbeiana* was measured using sulfate Ringer's as bathing solutions. 15 experiments were made, 12 under open-circuit conditions, and three in which the electrical condition was alternated between open and short circuit. Fig. 2b shows a representative plotting of log $(P/P_\infty - 1)$ as a function of time for K^+ uptake by the skin of *R. catesbeiana* under open circuit conditions. Table VI shows computed values of K^+ influx for all 15 experiments. Mean K^+ influx was 0.32 μequiv/h per cm². This value was essentially the same whether the skin was under open or short circuit. It is also very similar to the K^+ influx found in the skin of *L. ocellatus*. Short-circuit current in *R. catesbeiana* is normally of the order of 1.5 μequiv/h per cm² to 2.5 μequiv/h per cm². K^+ influx is then at the most 1/5 of the s.c.c.

TABLE V

K^+ INFLUXES UNDER OPEN- AND SHORT-CIRCUIT CONDITIONS IN *L. ocellatus*

In Na_2SO_4 solution. Paired values.

	Open circuit (1) (μequiv/h per cm²)	*Short circuit (2) (μequiv/h per cm²)*	*(1) − (2)*
	0.383	0.145	0.238
	0.445	0.288	0.157
	0.215	0.397	−0.182
	0.161	0.143	0.018
	0.292	0.340	−0.048
Mean			0.037
S.E.			0.074

TABLE VI

K^+ INFLUX AT THE INSIDE FACE OF THE ISOLATED SKIN OF *R. catesbeiana*

In Na_2SO_4 solution.

	s.c.c. (μequiv/h per cm²)	*K^+ influx (μequiv/h per cm²)*	$k_{ab} + k_{ba}$ (h^{-1})	k_{ab}/k_{ba}
	—	0.220	0.244	0.429
	—	0.250	0.360	0.302
	—	0.278	0.335	0.382
	2.31	0.388	0.434	0.425
	—	0.352	0.299	0.645
	—	0.341	0.379	0.427
	—	0.213	0.303	0.308
	1.89	0.463	0.670	0.299
	—	0.328	0.474	0.299
	—	0.835	1.097	0.340
	—	0.347	0.511	0.293
	—	0.188	0.207	0.435
	—	0.238	0.363	0.280
	1.75	0.264	0.311	0.395
	—	0.117	0.129	0.437
Mean		0.321		
S.E.		0.043		

DISCUSSION

Results for K^+ influx obtained in this study are based on the assumption that the skin and the inside bathing solution behave as a closed two-compartment system for K^+. Surprisingly enough, the analysis of the K^+ uptake by the skin from the inside bathing solution seems to indicate that this assumption is valid. Our purpose was to measure the K^+ influx into the epithelial cells participating in active transport of Na^+. In measuring the K^+ uptake by the skin, cells other than the ones participating in the Na^+ transport are probably included. The K^+ pool in "non-transporting" cells is probably connected to the extracellular space. This places this K^+ pool in parallel with the K^+ pool in the "Na^+-transporting" cells. Since a single exponential was observed in the K^+ uptake, it is probable that the different cells have a similar rate of exchange. In any case, if $^{42}K^+$ is taken up from the inside bathing solution by two or more parallel K^+ compartments, and only one participates in the Na^+ transport, the K^+ influx into the Na^+-transporting compartment will be less than the measured value. In other words, if that was the case, the values for K^+ influx obtained will represent a maximum value of the K^+ influx into the transporting cells.

It has been suggested that Na^+ transport may be localized at the cellular membrane lining the intraepithelial extracellular space. Na^+–K^+ interchange may be occurring at that level. The K^+ in the intraepithelial extracellular space would act as an additional compartment interposed in the series between the inside bathing solution and the cellular compartment. If that was the case, the system could not be described by a single exponential function as it was found in our experiments. An analysis of a three-compartment system in series has recently been done by CURRAN AND SOLOMON[12].

Furthermore, it is unlikely that an appreciable restriction to the K^+ flow exists between the intraepithelial extracellular space and the corium. If changing the electrical condition from open-circuit to short-circuit produced a substained change in the K^+ flux from the intraepithelial extracellular space to the cells which lasted several hours, this change should have produced a modification in the slope of the curve representing uptake from the inside bathing solution.

Another important consideration in the interpretation of the results is the influence of the connective tissue on the movement of K^+ into the cells. It is possible that the K^+ flux into the cell from the cell's outside surface may be larger than the diffusion of tracer K^+ from the inside bathing solution across the connective tissue. If so, the measured apparent K^+ uptake will be smaller than the real K^+ uptake at the cell surface. The connective tissue could act as a barrier reducing the K^+ uptake from the inside bathing solution to the cell compartment. However, a similar effect should be expected for the movement of Na^+ from the cell compartment to the inside bathing solution. Different experimental evidence has been presented that suggests that the connective tissue does not represent a significant restriction to the movement of K^+ or Na^+ across frog skin: (a) CURRAN AND CEREIJIDO[4] derived a factor L to correct for the restriction of the connective tissue. Their evaluation of L based on direct measurement of the diffusion of K^+ on the skin's connective tissue indicated that K^+ influx was not underestimated by more than 90 %, or as little as 5 %. According to our measured Na^+/K^+ ratio of 5:1 or more, even a 90 % correction is not enough for a unity ratio. (b) Unidirectional Na^+ fluxes measured in the isolated epithelium by ACEVES AND ERLIJ[8] are in remarkable agreement with those found in whole skin. If the connective tissue is not a significant restriction to the movement of Na^+, it is unlikely that it will be to the movement of K^+. Furthermore, ACEVES AND ERLIJ studied, in the isolated epithelium, the effect of changing Na^+ concentration in the outside solution and K^+ in the inside solution. Their conclusion was that the barrier to K^+ diffusion at the internal surface is much larger than the barrier to Na^+ diffusion at the external surface. (c) The connective tissue in *R. catesbeiana* is much thicker than in *L. ocellatus* (Av. wt. for 20 cm^2 of skin: *L. ocellatus*, 0.80 g, *R. catesbeiana*, 1.80 g). However, K^+ fluxes were almost the same in both skins.

All these considerations seem to indicate that the measured K^+ influx is a fair estimation of the real K^+ flux at the inside barrier of the Na^+-transporting cells. This K^+ flux was smaller than the s.c.c. The s.c.c. is a minimum estimate of the Na^+ flux from the cells to the inside bathing solution. The difference between them is given by the amount of Na^+ leak from the inside bathing solution to the transporting cells. Na^+ outflux across the skin is usually 5 to 10 % of the s.c.c., and it is relatively insensitive to changes in concentration and PD across the skin (CANDIA[13]). Measurement of the unidirectional Na^+ flux between the outside bathing solution and the cellular compartment (ROTUNNO *et al.*[14]; CEREIJIDO AND ROTUNNO[15]) indicates that this flux is 5 times larger than the Na^+ influx across the skin. This would suggest that the Na^+ flux (between the cells and the inside bathing solution) and the Na^+ influx (across the skin) are of the same order of magnitude.

If K^+ influx to the skin from the inside bathing solution is not coupled to the Na^+ pump, it will be expected that changes in the PD between the cellular K^+ compartment and the inside bathing solution will modify the K^+ influx. Changes of up to 120 mV across the skin (from open circuit to short-circuit) had no effect on the K^+ influx. This would indicate that most of the PD change took place across the outer barrier and the PD across the inner barrier remained constant. Microelectrode studies are inconclusive about this point but, in general, the intraepithelial electrode is negative with respect to the inside bathing solution at both open circuit and short-circuit conditions, while it changes from positive to negative, with respect to the outside bathing solution, when the skin condition is changed from open circuit to short circuit. Although K^+ influx was always smaller than s.c.c., a correlation could still exist between these two parameters. This correlation could be interpreted either as a partial coupling, or simply due to the fact that the negativity of the epithelial cells is larger when the Na^+ pump is more active. The discrepancy of our results with the 1:1 ratio found by CURRAN AND CEREIJIDO[4] can only be ascribed to the different methods utilized. Nevertheless, they expressed that no significant Na^+–K^+ exchange was involved in the active transport of Na^+.

It is our conclusion that K^+ flux at the inner face of frog skin is not directly involved in the control of the transepithelial Na^+ transport.

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