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Sodium–potassium pump and cell volume regulation in frog bladder

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SUMMARY

In the so-called nonpolarized preparation of frog bladder the mucosal membranes are blocked by liquid paraffin and the intracellular ionic composition is governed solely by the non-mucosal parts of the cell membranes. Results obtained with this preparation support the original idea of a coupled Na^+–K^+ pump operating in ion-transporting epithelial layers and implied in the frog skin model by Ussing and Koefoed-Johnsen. Under the physiological conditions of a normal Na^+ gradient the operation of the pump leads to a one-to-one exchange of Na^+ for K^+ and is inhibited equally by ouabain and by the absence of K^+ in the adjacent medium. Along with the coupled pump a mechanism regulating the cell volume is present which is insensitive to treatment with ouabain and to K^+-free media but is sensitive to inhibition with 2,4-dinitrophenol.

Two rather important problems concerning the active Na^+ extrusion from transporting epithelia may be solved by simple experiments with the so-called nonpolarized preparation of frog bladder[1]. In this preparation, the intracellular ion and water contents are controlled solely by the non-mucosal parts of the cell membranes, their mucosal parts being blocked by liquid paraffin (mineral oil). The problems are the following:

1. Does the Na^+ pump at the serosal border of the epithelial cells require the presence of K^+ in the serosal medium? Doubts about this assumption arose when it was shown that (a) when K^+ is absent from the serosal medium transcellular Na^+ transport inhibition might result from a decrease of the Na^+ permeability at the mucosal border of the cells[2]; (b) Na^+ transport is not inhibited when Na^+ in K^+-free serosal media is replaced with choline[3]; (c) the pump behaves as electrogenic when K^+ Ringer's solutions are used as the serosal media[4].

2. Is there, apart of the ouabain-sensitive Na^+ pump, another mode of Na^+ extrusion from epithelial cells, associated with cell volume regulation? A mechanism of this kind was discovered in kidney cortex slices[5] and may be inhibited by cold, with 2,4-dinitrophenol or with ethacrynic acid[6].

To solve the first question we examined the effect of K^+-free Ringer's solution at the serosal border of the nonpolarized preparation on the ion content of the tissue. Vesicles of the surviving bladder wall, filled with liquid paraffin, served as the nonpolarized preparation. Up to four vesicles may be prepared from a single bladder of *Rana temporaria;* the results are thus obtained in pairs and the S.E. of the differences between paired values is used in evaluating the significance by Student's *t* test. The results obtained by standard techniques[1] were expressed in kg or mequiv per kg dry solids. Control vesicles were incubated in aerated solution containing (in mequiv/l); Na^+, 114.5; Cl^-, 119.0; K^+, 5.0; HCO_3^-, 2.5; Ca^{2+}, 2.0. Another set of vesicles was incubated in a medium of analogous composition in which K^+ was replaced by an equivalent amount of Na^+. The third set was treated with 10^{-4} M ouabain in a medium otherwise identical with the control medium. The results reflecting the changes in the Na^+ and K^+ contents are shown in Table I; each difference corresponds to 11 pairs of vesicles. No significant changes were found in the Cl^- content.

TABLE I

EFFECTS OF K^+-FREE MEDIUM AND OF 10^{-4} M OUABAIN ON ION CONTENTS IN THE NONPOLARIZED PREPARATION OF FROG BLADDER AFTER 3 h OF INCUBATION

	Controls	*Difference in K^+-free medium*	*Difference with 10^{-4} M ouabain*
Na^+(mequiv/kg dry solids)	257.2	+104.2 ± 34.6 $P < 0.02$	+109.8 ± 29.4 $P < 0.01$
K^+ (mequiv/kg dry solids)	217.1	−100.5 ± 26.2 $P < 0.005$	−126.1 ± 24.8 $P < 0.001$

It may be seen that the absence of K^+ at the serosal border of the nonpolarized preparation leads to the same result as the inhibition by ouabain: part of the tissue K^+ is replaced by an equivalent amount of Na^+. It is obvious that the control preparation differs from preparations that are inhibited by ouabain or by the absence of K^+ in the operation of a coupled pump performing a net one-to-one exchange of Na^+ for K^+. The result does not imply that the coupling is so rigid that it would be preserved under conditions when the normal Na^+ gradient is abolished by leaching procedures or by using choline or K^+ solutions at the serosal side of the bladder.

Table II shows that the inhibition of the Na^+–K^+ pump is not associated with changes in water content. Each result corresponds to a set of 11 vesicles. The ouabain-sensitive Na^+ transport is thus not responsible for regulation of the cell volume. On the other hand, some mechanism sensitive to 2,4-dinitrophenol is involved in this regulation: the presence of the inhibitor at a concentration of $5 \cdot 10^{-4}$ M increases the water content of the tissue. Changes in the contents of ions related to this mechanism

TABLE II

EFFECTS OF K^+-FREE MEDIUM, 10^{-4} M OUABAIN AND $5 \cdot 10^{-4}$ M 2,4-DINITROPHENOL ON WATER CONTENT IN THE NONPOLARIZED PREPARATION OF FROG BLADDER AFTER 3 h OF INCUBATION

	Controls	*Difference in K^+-free medium*	*Difference with 10^{-4} M ouabain*	*Difference with $5 \cdot 10^{-4}$ M 2,4-dinitrophenol*
H_2O (kg/kg dry solids)	2.76	−0.06 ± 0.11	−0.04 ± 0.16	+0.45 ± 0.11
Significance		$0.60 < P < 0.70$	$0.80 < P < 0.90$	$P < 0.005$

may be viewed as an effect of 2,4-dinitrophenol above that resulting from the application of the K^+-free medium. Control vesicles were incubated in K^+-free medium, and the experimental vesicles in K^+-free medium with $5 \cdot 10^{-4}$ M dinitrophenol. The results are presented in Table III. It may be seen that the function of the dinitrophenol-sensitive mechanism, present in the bladder along with the K^+-requiring and ouabain-sensitive pump, corresponds to the exclusion of solution in which the concentrations of ions approximate those in the external medium. From the average figures for changes in ion and water contents of Table III the following concentrations (in mequiv/l) may be calculated: Na^+, 113.0; Cl^-, 118.5; and K^+, 6.6. These values agree well with those of 114.5, 119.0 and 5.0, respectively, in the extracellular medium. The two questions posed at the beginning of this paper may thus be answered in the affirmative: 1. Under the physiological conditions of a normal Na^+ pump serosal K^+ is required for its operation.

TABLE III

THE EFFECT OF $5 \cdot 10^{-4}$ M 2,4-DINITROPHENOL ON ION AND WATER CONTENTS OF THE NONPOLARIZED PREPARATION OF FROG BLADDER IN K^+-FREE MEDIUM (10 PAIRS)

	K^+-free medium	*K^+-free medium + $5 \cdot 10^{-4}$ M 2,4-dinitrophenol*	*Difference*	*Significance*
Na^+ (mequiv/kg dry solids)	326.6	393.3	+66.7 ± 27.5	$P < 0.05$
Cl^- (mequiv/kg dry solids)	304.2	374.1	+69.9 ± 16.6	$P < 0.005$
K^+ (mequiv/kg dry solids)	125.0	128.9	+3.9 ± 11.3	$0.7 < P < 0.8$
H_2O (kg/kg dry solids)	2.60	3.19	+0.59 ± 0.14	$P < 0.005$

2. There exists Na^+ extrusion associated with cell volume changes, different from the ouabain-sensitive Na^+ pump.

There are two more questions to be answered. How much are the present results influenced by the presence of various cells different from the ion-transporting epithelial cells in the preparation? Sections of bladders of *Rana* species[7] show that about one-half of the bladder wall thickness is formed by the transporting epithelial cells. Most of the rest appears to represent an extracellular space, the extent of which lies between 30 and 40% of tissue wet weight[1]. Hence the amount of heterogeneous cells is rather

small and unlikely to appreciably affect the observed large changes in the tissue ion and water content. The second question is how the two modes of Na^+ extrusion are linked with transcellular Na^+ transport. In our opinion, only the first of them, the ouabain-sensitive one, is responsible for transcellular transport. The other mechanism was shown recently[8] to be Na^+ independent and, moreover, insensitive to aldosterone[9] which, however, stimulates transcellular Na^+ transport.

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