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THE EFFECT OF NON-ELECTROLYTE OSMOLARITY ON FROG OOCYTES. II. INTRACELLULAR POTENTIAL

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SUMMARY

The intracellular potential of the oocytes of *Rana temporaria* and *Rana esculenta* was found to be reduced when the osmolarity of the external medium was increased by various concentrations of mannitol. A model is described in which the permeability coefficients for Na^+ and K^+ were calculated from the rates of isotopic exchange by Goldman–Hodgkin–Katz approximations and the phenomenon may be explained by a dependence of the two coefficients and of the intracellular concentrations of the two ions on osmolarity. The state of Na^+, K^+ and Cl^- inside the cells as well as the nature and magnitude of the active transport processes across the surface of the oocyte are discussed.

INTRODUCTION

The intracellular electrical potential with respect to the external medium is an important parameter in the study of ionic relations of the cell. The comparison of its steady-state value with the equilibrium (Nernst) potentials of the individual permeating ions is of assistance in discriminating between the steady distributions of ions which are in thermodynamic equilibrium and distributions which are due to a coupling of ion transport with the energy metabolism of the cell, *i.e.* due to the active transport of the ion. The passive unidirectional ion fluxes may often be related to this potential difference by the Goldman–Hodgkin–Katz approximations and the influx and efflux of an ion mutually by the equation known as Ussing–Teorell flux ratio.

The volume changes of frog oocytes brought about by the variation of the non-electrolyte osmolarity in the external medium are accompanied by profound changes in the intracellular contents of the principal ions[1], from which it may be concluded that the steady-state intracellular concentrations of ions and their unidirectional fluxes are influenced by the medium osmolarity. Measurement of the intracellular potential, carried out with microelectrodes on frog oocytes in media of various osmolarities, makes it possible to interpret the experimental data in terms of permeability coefficients and active transport fluxes of the individual ions.

MATERIALS AND METHODS

Ovarian oocytes of *Rana temporaria* and *Rana esculenta* were obtained and processed as described in the preceding communication[1]. The intracellular potential was measured with glass microelectrodes of the Ling–Gerard type with a tip diameter of approx. 1 μm, filled with 3 M KCl. The tip potential was less than 4 mV. Two experimental arrangements were used for the potential measurements:

(a) *Stationary arrangement.* Oocytes were incubated in media of different osmolarities. For technical reasons it was impossible to maintain the same incubation interval for all the oocytes. The length of incubation hence varied between 3 and 5h. After incubation, groups of about 30 oocytes were withdrawn from each medium and the potential of individual oocytes measured for 3–5 min. Usually 10–20 oocytes were measured from each medium.

(b) *Continuous-flow arrangement.* Oocytes freshly excised or kept for 20–30 min in an isotonic medium were transferred to the measuring chamber. The experimental setup made it possible to change continuously the medium flowing through the experimental chamber. In the moment of impalement the oocyte, placed in a small pit, was immobilized by drawing a part of its surface into a pipette with a polythene tip.

Measurement of tracer fluxes

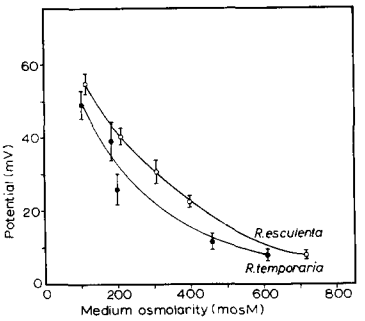
^{42}K efflux. The oocytes were labelled for 5.5 h in 100, 175, 340 and 515 mosM media. The activity of ^{42}K (in the form of ^{42}KCl) was 0.125 μC/ml. After quick rinsing with distilled water or inactive medium groups of 5 oocytes were transferred to small beakers containing 0.7 ml of inactive medium with osmolarity identical with that of the labelling medium. The washing-out was carried out for 4 h. The inactive medium was replaced every 16 min. Individual portions of the inactive medium were put on aluminium planchets, dried at 60° and measured on IDL automatic counter using an end-window GM tube.

^{22}Na influx. Media with osmolarities of 126, 226 and 675 mosM were labelled with ^{22}NaCl so that the specific activity was 0.52 μC/ml. Groups of approx. 80 oocytes were transferred to 20 ml of the radioactive medium. In intervals of several minutes, samples of 5 oocytes were taken, rinsed quickly with distilled water, blotted with a piece of tissue paper and transferred to metal planchets. After drying overnight at 95° the oocytes were crushed and Triton 200 added. The activity of dry samples was measured on Nuclear Chicago methane-flow counter.

Sodium and potassium contents were determined by flame photometry, chloride contents by potentiometric titration[2].

RESULTS

The values of the intracellular potentials found in the stationary arrangement described above are presented in Fig. 1. It may be seen that the pattern of the dependence of the intracellular potential on the external osmolarity is the same for oocytes of *Rana temporaria* (averages from two experiments, five measurements at each osmolarity per experiment, are shown) and for *Rana esculenta* (two experiments, a total of twenty measurements at each osmolarity); the potential was in all cases found to decrease with increasing osmolarity of the external medium.



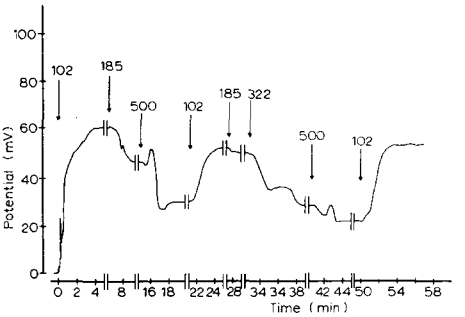


Fig. 1. The osmolarity dependence of the intracellular potentials of *Rana esculenta* and *Rana temporaria* oocytes. Vertical bars represent the standard error of the mean.

Fig. 2. The time course of the variations of intracellular potential of *Rana temporaria* oocytes brought about by the medium osmolarity changes in the continuous-flow arrangement. The numbers above the arrows indicate the osmolarity of the replacing medium.

TABLE I

OSMOLARITY DEPENDENCE OF THE INTRACELLULAR POTENTIAL OF *Rana temporaria* OOCYTES AS MEASURED IN THE CONTINUOUS-FLOW ARRANGEMENT

Measurements carried out at 20°; number of measurements indicated in parentheses.

Medium osmolarity (mosM)	*Potential (mV)*	*Average equilibration time (min)*
102	37.6 ± 1.32 (17)	2–3
185	33.0 ± 2.04 (17)	2–3
322	25.5 ± 1.93 (14)	3–5
500	24.8 ± 1.91 (12)	4–7

An analogous dependence between the potential and medium osmolarity was also found in the continuous-flow arrangement; the results are summarized in Table I. As demonstrated in Table I and in Fig. 2 the average time necessary for reaching a stable value of the potential is of the order of minutes. The dependence of the intracellular potential on the medium osmolarity is under these conditions somewhat less pronounced.

The contents of the principal ions were determined and their apparent concentrations calculated for oocytes incubated for 3 h in media of various osmolarities; the data for the anion Cl^- are given in Table II and for the cations Na^+ and K^+ in Table III.

Assuming the anion Cl^- to be in thermodynamic equilibrium (the active transport of this ion having been so far demonstrated only in rather specialized animal tissues) the concentration of the free (osmotically active) fraction of this ion can be calculated from its external concentrations and intracellular potentials, also shown in Table II. Subtracting these figures for free ions from the total apparent concentrations, the apparent concentrations of presumably immobilized Cl^- and their contents per dry solids were obtained (Table II). The contents corresponding to the immobilized

TABLE II

THE FREE AND THE IMMOBILIZED Cl^- IN *Rana temporaria* OOCYTES AT VARIOUS OSMOLARITIES

The values were calculated from the Cl^- contents found in four experiments after 3 h of incubation. The potential values marked with* were obtained by interpolation from the curve in Fig. 1. Cl^-_{eq}, equilibrium Cl^- concentration calculated as $Cl^-_{eq} = Cl^-_0 \cdot e^{FE/RT}$.

Osmolarity (mosM)	*Water content M (kg/kg dry solids)*	*Potential E (mV)*	*Ion concentrations (mmoles/kg water)*				*Content of bound Cl^- $M \cdot (Cl_i^- - Cl^-_{eq})$ (mmoles/kg dry solids)*
			Cl_i^-	Cl_o^-	Cl^-_{eq}	$(Cl_i^- - Cl^-_{eq})$	
101	1.406	−49.1	41.0	45.5	6.5	34.5	48.5
199	0.973	−25.8	75.6	43.9	15.8	59.8	58.2
610	0.822	− 7.8	116.2	56.5	41.2	75.0	61.7
130	1.477	−43.1*	64.5	64.3	11.6	52.9	78.1
196	1.077	−32.5*	98.0	64.3	18.0	80.0	86.2
287	0.868	−22.3*	118.1	63.2	25.9	92.9	80.0
391	1.010	−15.0*	110.6	65.3	35.9	74.7	75.4
627	0.955	− 7.5*	123.8	64.3	47.6	76.2	72.8
130	1.421	−43.1*	66.5	62.2	11.2	55.3	78.6
196	1.047	−32.5*	96.1	64.3	18.0	78.1	81.8
287	0.803	−22.3*	123.5	63.2	25.9	97.6	78.4
391	0.739	−15.0*	131.6	63.2	34.8	96.8	71.5
627	0.971	− 7.5*	123.2	63.2	46.8	76.4	74.2
130	1.659	−43.1*	70.6	63.8	11.5	59.1	98.0
196	1.314	−32.5*	85.8	63.8	17.9	67.9	89.2
287	1.016	−22.3*	117.8	63.8	26.2	91.6	97.1
391	0.869	−15.0*	135.2	63.8	35.1	100.1	87.0
627	1.258	− 7.5*	123.7	63.8	47.2	76.5	96.2

TABLE III

EXPERIMENTAL VALUES USED IN THE CALCULATION OF Na^+ AND K^+ PERMEABILITY COEFFICIENTS OF THE SURFACE OF *Rana temporaria* OOCYTES AT VARIOUS OSMOLARITIES

Osmolarity (mosM)	*Ion fluxes (pmoles·cm^{-2}·sec^{-1})*		*Ionic concn. (mmoles/kg water)*					*Water content (kg/kg dry solids)*	*Potential E* (mV)*
	$\Phi_{K(out)}$	$\Phi^*_{Na(in)}$	K_i^+	K_o^+	Na_i^+	Na_o^+	Na^+_{free}		
100	107.4	61.0	70.3	2.0	67.5	54.2	33.5	1.329	−49.0
175	80.7	81.5	118.3	2.0	79.6	53.7	12.6	0.985	−36.0
340	74.2	120.0	115.2	2.0	115.4	55.5	19.4	0.762	−18.5
515	35.0	151.7	13.7	2.0	61.6	53.8	0?	1.155	−10.0

* Values obtained by inter- and extrapolation of the curves in Figs. 1 and 5.

fraction of the Cl^- are plotted as a function of the medium osmolarity in Fig. 3; it may be seen that for each individual experiment the content does not vary with the external osmolarity appreciably. This finding seems to support the hypothesis about the intracellular binding of the ion and thermodynamic equilibrium of its free fraction.

Following the suggestion of DICK AND MCLAUGHLIN[3] that the immobilized Na^+ inside the toad oocyte are sequestered in some intracellular organelles the additional

assumption can be made that the cations Na^+ and anions Cl^- are immobilized in equivalent proportions, *i.e.* as NaCl, in these vesicles. From this hypothesis the approximations to the actual concentrations of free sodium can be calculated (Table III). It may be mentioned that in a nearly isotonic medium about 15 % of the total sodium appears to be in free form which is in a fair agreement with the figures given by various authors[4–6] for the exchangeable sodium content under these conditions.

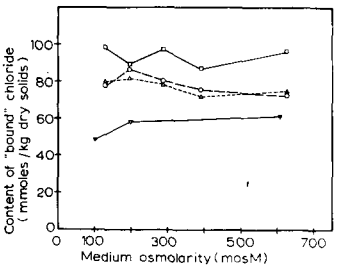
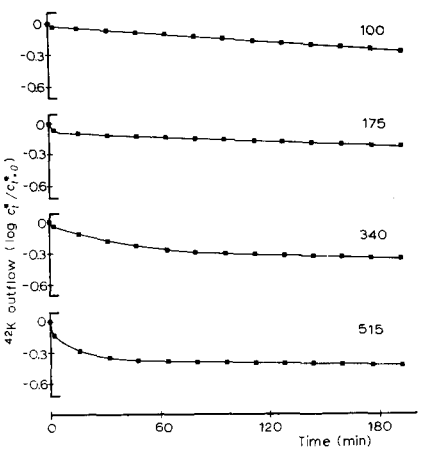


Fig. 3. The content of the immobilized Cl^- in *Rana temporaria* oocytes at different osmolarities. Each curve represents the result of a single experiment.

The unidirectional flux of potassium at various osmolarities was derived from the ^{42}K efflux curves shown in Fig. 4. The curves were analyzed graphically and from the antilogarithms of the intercepts of the resulting straight lines with the vertical axis and their slopes (rate constants) the unidirectional fluxes across the outer surface of the oocyte were calculated using the two compartments in series model according to DODD *et al.*[7]. The digital computer ICL 1900 was employed for the calculation. The values of the unidirectional sodium flux for the same osmolarities for which the potassium flux was measured were obtained by inter- and extrapolation from the curve expressing the osmolarity dependence of the unidirectional ^{22}Na influx calculated from the initial influx rates (Fig. 5). The unidirectional fluxes are summarized



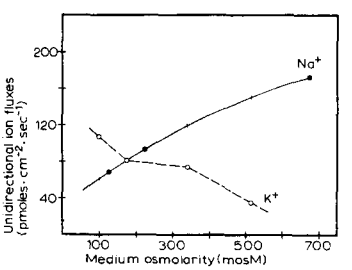


Fig. 4. The ^{42}K efflux from the oocytes of *Rana temporaria* at various osmolarities. The numbers correspond to the osmolarity of the media.

Fig. 5. The osmolarity dependence of the unidirectional ion fluxes in the oocytes of *Rana temporaria*.

in Table IV together with all other data necessary for calculation of permeability coefficients by Goldman–Hodgkin–Katz formulas. The values of the intracellular potential, corresponding to the osmolarities for which the fluxes were calculated, were obtained by inter- and extrapolation from the curve for *Rana temporaria* in Fig. 1; the intracellular concentrations of K^+ were calculated under the approximative assumption that this ion is not appreciably bound and is uniformly distributed inside the oocytes.

DISCUSSION

Assuming a passive nature of the sodium influx and potassium efflux the sodium and potassium permeability coefficients were calculated from the data of Table III, using the familiar Goldman–Hodgkin–Katz formulas for passive unidirectional fluxes, which may be written in the form:

$$P_{Na^+} = \Phi_{Na^+in} \frac{RT}{FE} \frac{1 - e^{-FE/RT}}{c_{oNa^+} e^{-FE/RT}}$$

$$P_{K^+} = \Phi_{K^+out} \frac{RT}{FE} \frac{1 - e^{-FE/RT}}{c_{iK^+}}$$

The permeability coefficients are given in Table IV and their dependence on the medium osmolarity is illustrated in Fig. 6. Using these permeability coefficients the passive Na^+ efflux and passive K^+ influx were calculated from the equations

$$\Phi_{Na^+out} = P_{Na^+} \frac{FE}{RT} \frac{c_{iNa^+}}{1 - e^{-FE/RT}}$$

$$\Phi_{K^+in} = P_{K^+} \frac{FE}{RT} \frac{c_{oK^+} e^{-FE/RT}}{1 - e^{-FE/RT}}$$

The passive fluxes are presented in Fig. 7 together with their difference for each ion, representing presumably the active flux of the ion. The difference between the active K^+ influx and active Na^+ efflux may be considered as representing the residual current through the pump mechanisms; its osmolarity dependence is given in Fig. 8. It may be seen that at osmolarities approaching isotonicity the pumping mechanisms appear to be electroneutral.

TABLE IV

Na^+ AND K^+ PERMEABILITY COEFFICIENTS AT VARIOUS OSMOLARITIES

Medium osmolarity (mosM)	P_{K^+} $(cm \cdot sec^{-1})$	P_{Na^+} $(cm \cdot sec^{-1})$
100	$4.96 \cdot 10^{-6}$	$0.50 \cdot 10^{-6}$
175	$1.52 \cdot 10^{-6}$	$0.81 \cdot 10^{-6}$
340	$0.95 \cdot 10^{-6}$	$1.54 \cdot 10^{-6}$
515	$3.13 \cdot 10^{-6}$	$2.31 \cdot 10^{-6}$

The dependence of the intracellular potential of *Rana temporaria* oocytes shown in Fig. 1 may hence be described by the modified Goldman–Hodgkin–Katz equation for membrane potential which includes a term for the residual current. This equation, resembling that of MORETON[8] with the exception that Cl^- (assumed to be in thermodynamic equilibrium) is omitted may be written as

$$E = -\frac{RT}{F} \ln \frac{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i + I\frac{RT}{F^2E}}{P_{K^+}[K^+]_o + P_{Na^+}[Na^+]_o + I\frac{RT}{F^2E}}$$

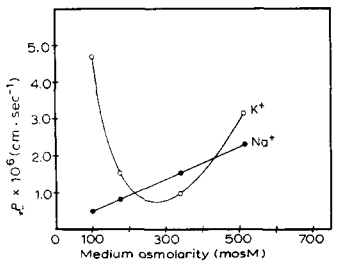
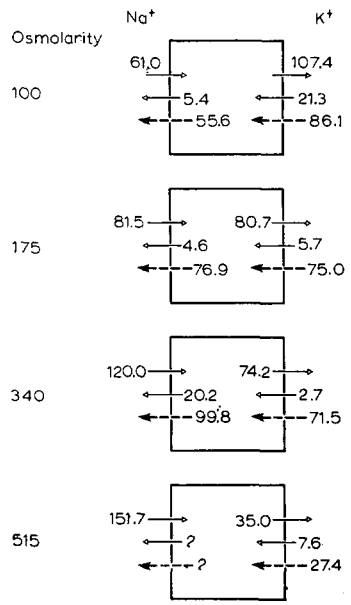


Fig. 6. The osmolarity dependence of the permeability coefficients for Na^+ and K^+ in the oocytes of *Rana temporaria*.



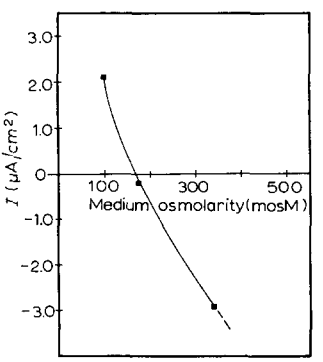


Fig. 7. A schematic representation of the Na^+ and K^+ fluxes across the surface of *Rana temporaria* oocytes at various osmolarities. Full thin arrows, passive fluxes; heavy dashed arrows, active fluxes. All fluxes in pmoles·cm^{-2}·sec^{-1}.

Fig. 8. The osmolarity dependence of the residual current through the Na^+ and K^+ pumps in the oocytes of *Rana temporaria*. The current (in electrical units) was calculated from the differences of active fluxes in Fig. 7.

As seen in Fig. 6 the K^+ permeability coefficient decreases with increasing medium osmolarity at low osmolarities while that of Na^+ increases. Hence the contribution of the unequal K^+ distribution (which, alone, would bring about a strong electronegativity of the cell interior) to the intracellular potential becomes less important as compared to that of Na^+ distribution, to which an intracellular potential of the opposite polarity would correspond. The overall result is a decrease in the intracellular potential with increasing medium osmolarity. The increased permeability of the oocyte surface to K^+ found in hypotonic media (resulting in a net outflow of KCl from the cells[1]) may serve as an independent confirmation of this concept.

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