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THE EFFECT OF NON-ELECTROLYTE OSMOLARITY ON FROG OOCYTES

I. VOLUME CHANGES

KAREL SIGLER AND KAREL JANÁČEK

Laboratory for Cell Membrane Transport, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

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SUMMARY

The time course of oocyte volume in mannitol hypertonic media is characterized by a biphasic curve; shrinkage of the cells leads to a volume minimum and this is followed by a slower swelling. Behaviour of this type may be explained by a mathematical model in which the inflow of mannitol is followed by osmotically driven uptake of water. The permeability of the oocyte surface towards mannitol appears to increase with increasing the mannitol concentration in the medium. In hypotonic saline the time course of the oocyte volume is again biphasic; from chemical analysis it may be concluded that the initial swelling is followed by a slower shrinkage mostly due to a loss of osmotically active potassium chloride from the cells. This process may be described by a model taking into account the non-zero hydrostatic pressure inside the oocytes.

INTRODUCTION

Because of their relatively large volume (of the order of mm^3) and spherical shape which simplifies volume estimation from microscopic data, frog oocytes represent an attractive model for the study of various factors involved in cell volume regulation. Simple experimental conditions for the investigation of these factors are obtained when the osmolarity of the external media is varied by an addition of a non-electrolyte, while the ionic composition of the medium remains constant. In the preceding series of experiments[1] poorly permeating lactose was used for the purpose and an attempt was made to draw the attention to the non-zero hydrostatic pressure inside the oocytes, resulting presumably from the gel properties of the oocyte cytoplasm and playing apparently a rather important role in the water relations of the oocytes. The aim of the present investigation was to establish the importance of other factors influencing the cell volume and its changes, *viz.* the transfer of osmotically active solutes across the surface of the oocyte. For this reason mannitol, penetrating the oocyte surface more readily than lactose, was used to vary the osmolarity of experimental media and attention was focussed on the time courses of the volume with the aim to describe the kinetics of oocyte swelling and shrinkage by simple physico chemical model.

MATERIALS AND METHODS

Ovarian oocytes of *Rana temporaria* were obtained from female frogs kept at 5° or 20° in the dark. Before the experiment, the animals were decapitated, pithed, the ovaries excised from the body and, with the aid of a forceps, divided into groups of several oocytes. These were rinsed quickly with distilled water and single oocytes were separated with the forceps. The ovarian membranes were removed. For the experiments we selected only large spherical oocytes showing no visible damage of the surface. DETTLAFF'S[2] technique was used to check the developmental stage of the oocytes. In one set of experiments, groups of 150–180 oocytes were incubated in experimental media in Perspex vessels with occasional shaking for 3–6 h at 20°. For determining the time course of cell swelling and shrinking groups of 5 oocytes were withdrawn from the medium at given time intervals (10–20 min) and their volume or water content assayed as shown below. The incubation solutions were replaced every 45 min. The composition of the incubation medium (modified frog Ringer) was 50 mM NaCl, 1.88 mM KCl, 0.89 mM $CaCl_2$, 2.38 mM $NaHCO_3$, the osmolarity was approx. 110–120 mosM. Higher osmolarities were achieved by adding D-mannitol to the medium. After removal from the incubation media the oocytes were again rinsed with water, blotted with a piece of tissue paper on a Perspex plate and weighed on a torsion balance. The content of water was determined by drying overnight at 95° and by subsequent weighing. The sodium and potassium contents were determined by flame photometry, the content of chloride anions by potentiometric titration[3], the samples for analysis being obtained by extracting dry solids with dilute H_2SO_4. The osmolarity of the media was determined from the freezing point depression. The osmolarity of the cell homogenate was determined by the following procedure: after incubation in media of different osmolarities groups of oocytes (total weight 150–300 mg) were homogenized for 1 min with a glass or Teflon homogenizer at 0°. Before the homogenization 2.0 ml of ice-cold water were added to each sample in order to eliminate the effect of excessive viscosity of the homogenate. The osmolarity was determined using an Advanced Instruments Osmometer.

The uptake of mannitol by the oocytes was determined chromatographically following the procedure of CERBULIS[4]. Quantitative determination of the mannitol concentration was carried out after elution using oxidation with sodium periodate[5]. The resulting formic acid was titrated with NaOH. In the calculation 1 molecule of mannitol was assumed to yield 4 molecules of formic acid.

The oocyte volume was calculated from their diameter measured in a cytoplast microscope provided with an eyepiece scale, magnifying 40 times. The individual oocytes were placed in small pits in a Perspex plate filled with the experimental medium. Misreading of the oocyte diameter due to the meniscus of the fluid was eliminated by covering the surface of the fluid with a cover glass.

RESULTS

Freshly isolated frog oocytes used in our experiments had an average water content of 1.0–1.2 kg water per kg of dry solids. The "isotonic" medium in which the water content remained constant after 3 h of incubation was usually about 200 mosM (ref. 1). The dependence of the oocyte water content on the osmolarity of the external

medium, varied by mannitol in the range of 100–600 mosM, after 3 h of incubation is shown in Fig. 1. For convenience the whole range of osmolarities is divided into three sections: A, the hypertonic region; B, the nearly isotonic region; C, the hypotonic region. Whereas in the isotonic region the behaviour of the cells appears to approximate that of a simple osmometer, both in hypotonic and hypertonic regions the water content is seen to be markedly less dependent on the external osmolarity.

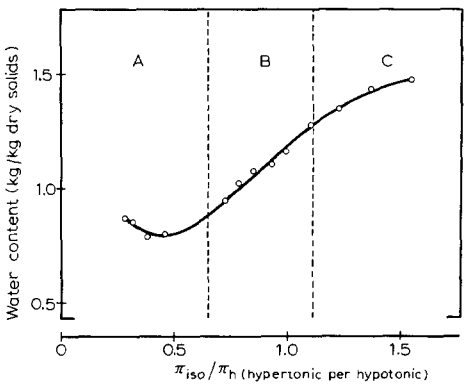

Fig. 1. Water content of *Rana temporaria* oocytes at various external osmolarities. The oocytes were incubated for 3 h in modified Ringer solution, the osmolarity was adjusted with mannitol. A, hypertonic region; B, nearly isotonic region; C, hypotonic region.

TABLE I

THE UPTAKE OF MANNITOL BY *Rana temporaria* OOCYTES IN HYPERTONIC MEDIA AFTER 3 h OF INCUBATION AT 17°

Medium osmolarity (mosM)	*External mannitol osmolarity (mosM)*	*Mannitol uptake (μg/mg dry solids)*	*Intracellular mannitol osmolarity* (mosmoles/kg cell water)*
106	—	—	—
221	115	6.3	28.1
480	374	42.8	290.3
627	521	65.5	412.0

* The values were calculated assuming an osmotic coefficient equal to 1.

Qualitatively, the curve resembles that obtained when the medium osmolarity was varied by the considerably less permeant lactose[9]. The uptake of mannitol by the oocytes in μg/mg dry solids after 3 h of incubation is shown in Table I. The content of the principal ions, sodium, potassium and chloride, was determined under the same conditions and is given in Table II. It may be seen that the changes in sodium and chloride contents proceed in a very similar manner and that there is a distinct increase in the content of these ions in the hypertonic region. The content of potassium, on the other hand, reaches its maximum when the external medium is nearly isotonic; the osmolarities outside this region bring about a loss (in the case of hypertonicity very substantial) of this ion from the oocytes.

The determined osmolarity of the cell homogenate served for the calculation of the approximate intracellular osmolarities of oocytes incubated under different

TABLE II

INTRACELLULAR CONTENTS OF PRINCIPAL IONS IN *Rana temporaria* OOCYTES IN A TYPICAL EXPERIMENT AFTER 3 h OF INCUBATION IN MEDIA OF DIFFERENT OSMOLARITIES

Medium osmolarity was varied by D-mannitol.

Medium osmolarity (mosM)	*mmoles/kg dry solids*		
	Na^+	K^+	Cl^-
Fresh oocytes	65.1	118.9	130.6
130	61.8	93.6	102.9
196	52.9	109.5	98.7
287	57.2	112.2	104.2
391	54.5	93.8	99.7
627	79.2	12.7	127.5

external osmolarities. The interval between the removal of the cells from incubation media and the measurement was maintained as short as possible (3–5 min); during this period the homogenate was kept at 0° to minimize the effect of the decomposition of cell components on the intracellular osmolarity observed[6]. The dependence of intracellular hydrostatic pressure (the difference between the internal and the external osmolarity) on the osmolarity of the bathing medium is shown in Fig. 2.

When the time courses of the volume changes occurring in media of different osmolarity are examined either by direct measurement of the oocyte diameter or by determing the water content of the cells, biphasic curves shown in Fig. 3 are obtained.

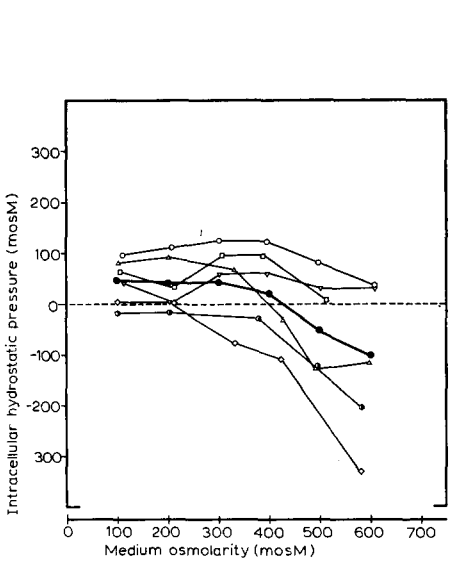

Fig. 2. Dependence of intracellular hydrostatic pressure on medium osmolarity. Each curve represents the result of a single experiment, heavy line representing their arithmetic mean.

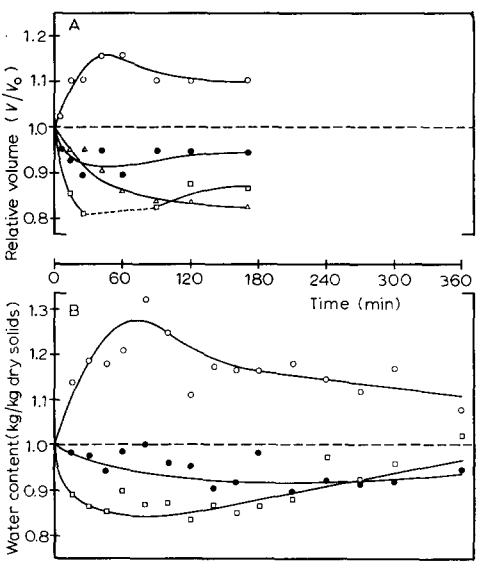

Fig. 3. The time course of the relative oocyte volume (A) and water content (B) at various osmolarities. A. ○—○, 113 mosM; ●—●, 208 mosM; △—△, 395 mosM; □—□, 607 mosM; B. ○—○, 126 mosM; ●—●, 226 mosM; □—□, 675 mosM.

Volume maxima and minima observed in hypotonic and hypertonic media, respectively, show that transient swelling and transient shrinking take place in the two types of experiments. The curves obtained for the oocyte swelling in hypotonic media show a relatively rapid intake of water by the cells. The maximum volume as well as the water content, amounting to 1.15–1.30 of the initial value, is reached after 60–90 min. After this stage the two quantities decrease slowly, approaching their original values. A similar situation is seen to prevail in the case of oocyte shrinkage in strongly hypertonic media (600–700 mosM); the minimum volume and the minimum water content, attained after 60–90 min, are approx. 0.8 of the initial values. The curve describing cell shrinkage in slightly hypertonic media (200–400 mosM) displays two distinct features: the depth of the minimum is less than that found in more concentrated media and it appears to occur only after 2–3 h of incubation.

DISCUSSION

For the biphasic character of the curves depicting oocyte swelling and shrinking the explanation was offered by BERNTSSON *et al.*[7] in that the phenomenon is due to the tension of vitelline membrane formed around the oocyte deprived of epithelial layers in their experiments. In our opinion, the shape of the curves is likely to be associated with flows of osmotically active solutes across the cell surface. Thus, in hypotonic media, the oocyte swells due to osmotic inflow of water, but then the osmolarity gradient, diminished already by the dilution of intracellular solutes, is actually reversed by the outflow of potassium chloride from the fresh oocyte observed under these conditions (Table II) and shrinkage, rate-limited by the KCl efflux, takes place. The height of the maximum on the curve depicting this course of events is determined not only by the rate of osmotic gradient changes but also by other factors, *e.g.* mechanical tension of the cell surface layers or by gel properties of the cytoplasm[1], giving rise to a non-zero hydrostatic pressure inside the cells. Similarly, the time course of the curves describing the oocyte behaviour in hypertonic media containing mannitol may be considered to reflect a rapid osmotic loss of water followed by swelling due to the reversal of the osmotic gradient resulting from the slower penetration of mannitol.

An attempt was made to describe the course of the cell volume and the water content in media of various osmolarities by means of a simple model which would take into account the above factors.

Behaviour in hypertonic solutions

In the present model the oocyte is assumed to correspond to a system, a part of which behaves like an osmometer surrounded by a membrane permeable to mannitol. For the sake of simplicity the osmotic changes caused by the flows of electrolytes across the cell surface of the oocyte are neglected; mannitol is considered to be the only osmotically active substance permeating across the surface. This is only an approximation since at extremely high osmolarities the outflow of K^+ exceeds considerably Na^+ uptake (Table II), this fact being likely to produce some osmotic effects. Also in another respect the model is to be considered an oversimplification: it neglects entirely the intracellular hydrostatic pressure of the real oocyte. As shown in Fig. 2, a whole range of both positive and negative hydrostatic pressures (determined as differences between the internal and external osmotic pressure) may be found in media with osmolarities extending from isotonicity to extreme hypertonicity. The

model neglecting the hydrostatic pressure may thus be considered as approximating the behaviour of an oocyte whose internal hydrostatic pressure would not be too far from zero for volumes corresponding to the whole osmolarity range. If it were required to construct a model simulating the behaviour of an oocyte with a more distinct pattern of dependence of intracellular hydrostatic pressure on oocyte volume, some empirical function, corresponding to the mean relationship between the two parameters, could be used.

The model is described mathematically by two simultaneous differential equations, the first of which describes the volume changes of the osmometer, the second the changes of the intracellular mannitol concentrations:

$$\frac{dV}{dt} = k_w\left[c_i - \frac{\gamma}{V} - c_0 - \frac{\gamma}{V_0}\right] \tag{1}$$

$$\frac{dc_i}{dt} = k_c c_0 - k_c c_i - \frac{c_i}{V}\frac{dV}{dt} \tag{2}$$

where V is the volume of the osmometer (a part of the oocyte), c_i and c_0 are the internal and external mannitol concentration, respectively (c_i is a variable, c_0 is a constant owing to the large volume of the medium). γ is the amount of salts in mequiv in the osmometer so that γ/V is the salt osmolarity; γ/V_0, its value at the beginning of the experiment, is equal to the constant osmolarity of the "isotonic" medium. The relation of the coefficients k_w and k_c to the conventional coefficients used for the description of the membrane permeability is obtained on comparing Eqn. 1 with Eqn. 3 describing the volume flow J_V:

$$J_V = \frac{1}{A}\frac{dV}{dt} = L_p RT \Delta c \tag{3}$$

and comparing Eqn. 2 with Eqn. 4 expressing the penetration of a non-electrolyte across a membrane:

$$\Phi_c = \frac{1}{A}\frac{dn}{dt} = \frac{1}{A} V \frac{dc}{dt} = P_c \Delta c \tag{4}$$

Hence

$$k_w = A \cdot L_p \cdot RT \tag{5}$$

where A is the surface area of the oocyte and L_p stands for the hydraulic conductivity of the membrane. Further

$$k_c = P_c \frac{A}{V} \tag{6}$$

where P_c is the permeability of the surface of the cell towards mannitol (*e.g.* in $cm \cdot sec^{-1}$) and A/V is the surface volume ratio for the oocyte. Considering the shape of the oocyte as permanently spherical $A/V = 3/r$ where r is the oocyte radius.

The last term in Eqn. 2 refers to the changes in the mannitol concentration brought about by the volume changes of the oocyte, *i.e.* the concentration changes not related to the ability of the substance to penetrate the oocyte. For a non-penetrating solute it holds that $c_i V_i$ = constant; on differentiating with respect to time

$$\frac{dc_i}{dt} V_i + c_i \frac{dV_i}{dt} = 0, \text{ i.e. } \frac{dc_i}{dt} = -\frac{c_i}{V_i}\frac{dV_i}{dt} \tag{7}$$

is obtained and by superposition of this concentration change on the change caused by mannitol permeation Eqn. 2 results.

Eqns. 1 and 2 are equivalent to those derived by JACOBS[8], except that in Eqn. 2 changes in the concentration of the solute rather than those of its content are included. JACOBS' equations were recently modified by JOHNSON AND WILSON[9] for solutes with a reflection coefficient lower than 1 and solved by methods of perturbation analysis. Such modification is warranted when solutes with small molecules are used (glycols of low molecular weight were used in their study) but does not seem to be necessary when mannitol with much higher molecular weight is used.

In the present paper no attempt was made to achieve the utmost accuracy in the solution of Eqns. 1 and 2 as the equations themselves are a mere approximation. Computation of the polygons approximating the time course of the volume and concentration was considered to be the most suitable solution, both for its explicitness and simplicity. The following numerical procedure was used: the first value of dV/dt, considered to be valid for the first 10 min of the volume changes, was calculated from Eqn. 1 by inserting the initial intracellular concentrations, corresponding to those found in fresh oocytes. The k_w value used was derived from the initial slope of the experimental curves. Inserting this value of dV/dt into Eqn. 2 together with the initial intracellular mannitol concentration (equal to zero), its constant external concentration and a tentative k_c value yielded the corresponding dc_i/dt. The initial parts of the curves describing the time courses of the volume and concentration changes were thus approximated by linear segments; the values of V and c_i corresponding to the end points of these segments were inserted again into Eqns. 1 and 2 and the slopes valid for subsequent 10 min were calculated.

By repeating this operation it was possible to draw polygons approximating the time course of the functions sought (Fig. 4). The values of the coefficients k_c used to obtain a fair agreement between the theoretical polygons and experimental curves together with other quantities used in the calculation are given in Table III.

The close resemblance of the theoretical and experimental curves does not only suggest that the function of the model approximates reasonably well the actual mechanism of the volume changes in hypertonic media but allows also to estimate, from k_c, the permeability of the cell surface towards mannitol at two different external mannitol concentrations. The shift of the minimum on the volume curve thus reflects the dependence of mannitol permeability on its external concentration; as seen from Table III, the higher the concentration of mannitol in the medium the higher the permeability. A certain confirmation of this permeability change may be inferred from Table I, describing mannitol uptake.

The extent of the relative volume changes predicted by the theoretical polygons exceeds considerably that found in the experiments. From this it may be concluded that only part of the oocyte behaves like an osmometer as discussed above, the rest of the cell representing a non-compressible volume. The proportion of the oocyte occupied by the non-compressible volume may be estimated in the following way: from the volume of the oocyte in the isotonic medium (calculated from their diameter measured under the microscope) and from their weight the oocyte density of 1.089 ± 0.021 was calculated; the oocytes contained 1.166 kg water per kg dry solids[1]. The wet weight corresponding to 1 kg of dry solids was therefore 2.166 kg; dividing this value by the overall density of the oocyte yields the volume taken up by this wet weight

(1.989 l). On subtracting the volume of the oocyte water (numerically equal to the water content) the volume of 1 kg of dry solids is obtained (0.823 l). Thus the volume percentages of water (compressible volume) and of dry solids (non-compressible volume) are 58.6 and 41.4 %, respectively.

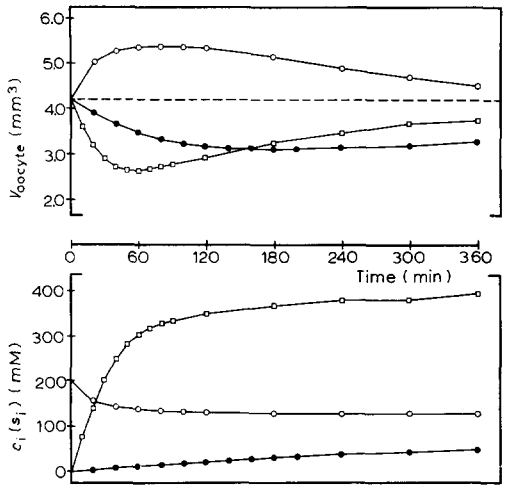

Fig. 4. The time course of the oocyte volume and of the intracellular concentration of the permeating solute calculated on the basis of the mathematical model. ○—○, hypotonic medium, permeating solute KCl (concentration s_i); ●—●, slightly hypertonic medium, permeating solute mannitol (concentration c_i); □—□, strongly hypertonic medium, permeating solute mannitol (concentration c_i).

TABLE III

NUMERICAL PARAMETERS USED IN THE CALCULATION OF THE THEORETICAL CURVES DESCRIBING VOLUME CHANGES

The curves were calculated for a spherical osmometer of the following dimensions: $r = 0.1$ cm, V_0 (initial) $= 4/3 \cdot \pi r^3 = 4.2 \cdot 10^{-3}$ cm^3, $A = 1.3 \cdot 10^{-1}$ cm^2, $A/V = 3/r = 30.0$ cm^{-1}.

Medium	*External concentration of permeating solutes*		*Permeability coefficients*		*Intracellular content of impermeant salts*	*Hydrostatic pressure*
	$c_{(mannitol)}$ ($moles \cdot cm^{-3} \times 10^4$)	$c_{(salts)}$ ($moles \cdot cm^{-3} \times 10^4$)	$k_c(k_s)$ ($sec^{-1} \times 10^4$)	k_w ($moles \cdot sec \times 10^3$)	($moles \times 10^7$)	P_i ($osM \cdot cm^{-3} \times 10^4$)
Hypertonic (600 mosM)	4.0*, †	—	3.0**	2.5***	8.4	—
Hypertonic (300 mosM)	1.0*, †	—	0.3**	2.5***	8.4	—
Hypotonic (110 mosM)	—	1.06*, †	16.1(i) 0.6(o)	10.0***	8.4	0.4

* $c_{(mannitol)i} = 0$ for $t = 0$; $s_{(salt)o} \approx s_{(K)o} \approx 0$ (Na^+ assumed to be impermeant and $s_{(K)o}$ negligibly low).

** Tentative values.

*** Calculated from initial rates derived from experimental curves.

† Approximated from the experimental figures.

Hence an additional non-compressible volume of $2.97 \cdot 10^{-3}$ cm³ must be added to the fully compressible spherical osmometer assumed by the above model (volume $4.2 \cdot 10^{-3}$ cm³, Table III). This absolute value of the non-compressible volume is not likely to be affected appreciably by the medium osmolarity and thus the corrections of this type for any point of the theoretical curve can be calculated. Hence the minimum volume of the theoretical osmometer in the hypertonic medium (520 mosM), $2.64 \cdot 10^{-3}$ cm³, corresponds to a volume of $5.61 \cdot 10^{-3}$ cm³ of the "real" oocyte. The relative volume change at the minimum is then $5.61 : 7.17 = 0.78$, a value very close to experimental values of about 0.8 (Fig. 4).

Behaviour in hypotonic solutions

Whereas in the model describing the behaviour of oocytes in hypertonic media the volume changes resulting solely from movements of the osmotically active non-electrolyte mannitol were considered, in hypotonic media a considerable outflow of KCl from fresh oocytes takes place, this being obviously the cause of water outflow following the initial swelling of the oocyte. While the influx and outflux of a non-electrolyte are related to its concentration at the corresponding side of the membrane by the same coefficient, the ionic fluxes are generally not such simple functions of the concentrations but depend also on the potential difference across the membrane and on the presence of ionic pumps in the membrane. If the membrane potential is constant and the active flux is directly proportional to the concentration of the ion (in the case of a non-saturated pump) the two fluxes are again directly proportional to the respective concentrations, the proportionality coefficients, however, being different for each flux.

The membrane potential indeed becomes steady a few minutes after the osmolarity change[10]; therefore k_i and k_o ($k_i \neq k_o$), relating the influx and the efflux of K^+ to its respective concentrations were considered a feasible approximation. The ratio of the constants was chosen so as to correspond to the distribution of K^+ in a hypotonic medium.

As can be seen from Fig. 2 the values of the intracellular hydrostatic pressure in the hypotonic region are mostly positive, covering a range from -20 to $+100$ mosM. For this reason a mean value of $+40$ mosM of the intracellular hydrostatic pressure was used in the model. The model is then characterized by two differential equations:

$$\frac{dV}{dt} = k_w s_i + k_w \frac{\gamma}{V} - k_w(s_o + S_o + P_i) \tag{8}$$

$$\frac{ds_{(K)i}}{dt} = k_i s_{(K)o} - k_o s_{(K)i} - \frac{s_{(K)i}}{V}\frac{dV}{dt} \tag{9}$$

where S_o is the external concentration of non-permeating solutes (NaCl), s_i and s_o in Eqn. 8 are the osmolarities of KCl in the internal and external medium, respectively, $s_{(K)i}$ and $s_{(K)o}$ in Eqn. 9 are the concentrations of K^+ in the two media.

Eqn. 9 describes the movement of K^+ for which the constants k_i and k_o are defined. It is obvious that Cl^- accompanying K^+ in an equivalent amount will double the changes in the content of osmotically active solutes so that the resulting change of KCl osmolarity in Eqn. 8 will be twice that predicted by Eqn. 9 for K^+ osmolarity changes (thus $s = 2s_{(K)}$).

P_i is the hydrostatic intracellular pressure in mosM. As mentioned above, the value of +40 mosM was chosen for the calculation.

The condition for a transient swelling is that the oocyte volume decreases while the permeating solute still moves out of the oocyte, *i.e.*

$$\frac{dV}{dt} < 0 \tag{10}$$

when still

$$k_i s_{(k)0} - k_0 s_{(k)i} < 0 \tag{11}$$

From the condition (10) and Eqn. 8 it follows that

$$s_i + \frac{\gamma}{V} - s_0 - S_0 - P_i < 0$$

hence

$$s_i - s_0 < -\frac{\gamma}{V} + S_0 + P_i \tag{12}$$

From the inequality (11) it can be deduced that

$$s_{(k)i} > \frac{k_i}{k_0} s_{(k)0}$$

which can be written as

$$s_i = \alpha \frac{k_i}{k_0} s_0, \text{ where } \alpha > 1 \tag{13}$$

Combining Eqns. 12 and 13 we obtain

$$s_0 \left(\alpha \frac{k_i}{k_0} - 1\right) < -\frac{\gamma}{V} + S_0 - P_i \tag{14}$$

The proportionality constants relating the K^+ fluxes to the respective concentrations were calculated as follows:

The exchange of a tracer across a membrane may be described by the equation

$$\frac{dc_i^*}{dt} = \frac{\Phi A}{V} \frac{c_0^*}{c_0} - \frac{\Phi A}{V} \frac{c_i^*}{c_i}$$

where Φ is the unidirectional steady-state flux and c^*'s are the concentrations of the labelled ionic species.

In our case, one can write for the rate of the concentration change of non-labelled K^+ as an approximation

$$\frac{dc_i}{dt} = k_i c_0 - k_0 c_i$$

On comparing the two equations we obtain

$$k_i = \frac{\Phi A}{V} \frac{1}{c_0} \qquad k_0 = \frac{\Phi A}{V} \frac{1}{c_i}$$

The experimental value of Φ for *Rana temporaria* oocytes in a hypotonic medium[10] is $1.074 \cdot 10^{-10}$ mole·cm^{-2}·sec^{-1}. From this value and from parameters given in Table III $k_i = 1.611 \cdot 10^{-3}$ sec^{-1} was calculated. In the steady-state $s_{(K)i} \approx 50$ mM, $s_{(K)o} \approx 2$ mM; hence $k_o = k_i/25 = 6.44 \cdot 10^{-5}$ sec^{-1}. On inserting into 14 the following values: $k_i/k_o = 25$; $\gamma = 8.4 \cdot 10^{-8}$ mole ($0.2 \cdot 10^{-4}$ mole·cm$^{-3} \cdot V_O$, the intracellular concentration of free NaCl (ref. 10) multiplied by the initial volume); $s_o = 0.04 \cdot 10^{-4}$ mole·cm^{-3}; $S_o = 1.06 \cdot 10^{-4}$ mole·cm^{-3}, and for α the minimum value of this coefficient (practically equal to 1), it may be seen that for $V = V_O$ the inequality 14 is satisfied for P_i higher than 0.1 osmole·cm^{-3}. This is a minimum value, since, in reality, $V > V_O$. Our value $P_i = 0.4 \cdot 10^{-4}$ osmole·cm^{-3} complies well with this condition.

The solution of Eqns. 8 and 9 was again carried out by the polygon approximation and is shown in Fig. 4, the parameters used for the calculation being summarized in Table III.

The similarity of the curve thus obtained with that shown in Fig. 3 suggests that the model explains reasonably well the behaviour of the oocytes in hypotonic media. The loss of KCl from the cells under these conditions is accepted in the model as an experimental fact without discussing the underlying mechanism. As will be shown in the forthcoming communication[10] a single postulate about an increase of the passive permeability (leak) of the cell surface towards K^+ by the hypotonicity of the external medium permits to explain not only this experimental finding but also the independent observation that the intracellular potential of the oocyte increases after transfer into a hypotonic medium.

The correction for the non-compressible space, carried out analogously as in the case of hypertonic media yields for the maximum relative volume the value of $V/V_O = 1.16$ which is in good agreement with values found experimentally (Fig. 3).

REFERENCES

1 K. SIGLER AND K. JANÁČEK, *Physiol. Bohemoslov.*, 18 (1969) 147.
2 T. A. DETTLAFF, *J. Embryol. Exptl. Morphol.*, 16 (1966) 183.
3 J. A. RAMSAY, R. H. J. BROWN AND P. C. CROGHAN, *J. Exptl. Biol.*, 32 (1955) 822.
4 J. CERBULIS, *Anal. Chem.*, 27 (1955) 1400.
5 E. L. HIRST AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 1278.
6 E. J. CONWAY, H. GEOGHEGAN AND J. I. MCCORMACK, *J. Physiol.*, 130 (1955) 427.
7 K. E. BERNTSSON, B. HAGLUND AND S. LØVTRUP, *J. Cellular Comp. Physiol.*, 65 (1965) 101.
8 M. H. JACOBS, in E. S. GUZMAN BARRON, *Modern Trends in Physiology and Biochemistry*, Academic Press, New York, 1952, p. 149.
9 J. A. JOHNSON AND T. A. WILSON, *J. Theoret. Biol.*, 117 (1967) 304.
10 K. SIGLER AND K. JANÁČEK, *Biochim. Biophys. Acta*, 241 (1971) 539.