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DETERMINATION OF IONIC PERMEABILITY COEFFICIENTS OF THE PLASMA MEMBRANE OF *XENOPUS LAEVIS* OOCYTES UNDER VOLTAGE CLAMP

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SUMMARY

1. A method of estimating absolute ionic permeability coefficients which does not depend on the use of impermeant substitutes is reported.

2. The method is based on a pump leak model of the *Xenopus laevis* oocyte membrane. The procedure consists of measuring, in the same experiment, the pump current and the currents generated under voltage clamp by the partial substitution of one or two ions at a time. For each experimental condition, the measured currents are substituted in a Goldman–Hodgkin–Katz type equation with two unknowns (the permeability coefficients). The set of equations thus generated enables the computation of all the ionic permeability coefficients.

3. The *Xenopus* oocyte membrane (stages IV and V, Dumont, 1972) has been found to be permeable to conventional ion substitutes such as *N*-methyl-D-glucamine (NMG), sulphate, isethionate and gluconate.

4. The values for sodium, potassium and chloride permeability coefficients obtained from sixty-eight pooled experiments were, respectively, 5·44, 17·41 and $1{\cdot}49 \times 10^{-8}$ cm s^{-1}.

5. The diffusional currents for sodium, potassium and chloride computed from the experiments referred to above were, respectively, $-1{\cdot}16$, 0·69 and $-0{\cdot}038$ μA cm^{-2}.

6. A stoichiometry of the Na^+–K^+ pump exchange of 3/1·8 was computed.

7. The intracellular concentrations of sodium, potassium and chloride ions, as determined by ion-selective microelectrodes, were, respectively, $10{\cdot}1 \pm 0{\cdot}66$ mM ($n = 12$), $109{\cdot}5 \pm 3{\cdot}3$ mM ($n = 13$) and $37{\cdot}7 \pm 1{\cdot}18$ mM ($n = 19$), corresponding to equilibrium potentials of 61, -95 and -28 mV.

8. Since chloride is not at equilibrium across the membrane, we propose that there is an inward uphill Cl^- transport.

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INTRODUCTION

The aim of the present work was to study the control of the ionic composition of the *Xenopus* oocyte cytoplasm, which is the result of the balance between active transport and passive fluxes.

A number of studies concerning transport across the membrane of the *Xenopus* oocyte focus on the operation of the sodium pump. Richter, Jung & Passow (1984) have shown with ouabain binding studies that the membrane of the *Xenopus* oocyte contains a high density of sodium pumps which are active during the development of these cells. The pump is electrogenic as revealed by the membrane depolarization induced by ouabain or by the removal of potassium from the external solution (Vitto & Wallace, 1976; Wallace & Steinhardt, 1977; Ziegler & Morrill, 1977; Hagiwara & Jaffe, 1979; Baud, Kado & Marcher, 1982; Dascal, Landau & Lass, 1984; Lafaire & Schwarz, 1986). Lafaire & Schwarz (1986) measured the pump current and were able to study its voltage dependence. It is also possible to obtain approximate estimates of the pump fluxes from the work of O'Connor, Robinson & Smith (1977).

The passive fluxes and permeability coefficients of the membrane for the major ionic species are less well characterized. Tupper & Maloff (1973) determined ionic permeability coefficients for *Rana* oocytes through measurements of unidirectional fluxes of radioactive sodium, potassium and chloride, but they do not distinguish between diffusional and non-diffusional fluxes. The same limitation applies to the ouabain-insensitive potassium effluxes and to sodium influxes reported by O'Connor *et al.* (1977), which were measured by the same methods. Using electrophysiological methods, Dascal *et al.* (1984) published P_{Na}/P_K and P_{Cl}/P_K ratios for *Xenopus* oocytes but absolute values for ionic permeabilities were not determined.

In the present paper we describe a technique for the determination of absolute permeabilities which allows us to estimate diffusional fluxes and fluxes through the sodium pump in the same cell. The electrophysiological determination of ionic permeability coefficients is frequently based on ion substitution experiments in which the ion under study is replaced by another ion assumed to be impermeant. In the case of the *Xenopus* oocytes we found that ions that are often used as substitutes for sodium, potassium and chloride, such as *N*-methyl-D-glucamine (NMG), choline, sulphate, etc., do permeate the cell membrane. Consequently, it was necessary to develop a method which does not require the use of impermeant substitutes.

With this method we determined the sodium, potassium and chloride absolute permeability coefficients of the *Xenopus* oocyte membrane in the resting state (stages IV and V, Dumont, 1972). From these values we were able to estimate the passive ionic fluxes and the stoichiometry of the pump.

METHODS

Mature females of the species *Xenopus laevis*, obtained from Xenopus Ltd, UK, were kept in water tanks at room temperature and on a natural light cycle for at least 1 month before use. Oocytes were obtained by removing a piece of the ovary through a small abdominal incision, under anaesthesia with Tricaine (0·5 g l^{-1}). The incision was sutured and the animal allowed to recover for at least 4 weeks between operations. Ten different animals were used and the experiments were

spread throughout the year. The excised tissue was kept up to 8 days after removal at 8–10 °C in Ringer solution with added penicillin and streptomycin (50 μg ml^{-1}).

Oocytes 0·8–1·4 mm in diameter were gently separated from the surrounding tissue and allowed to equilibrate in normal Ringer solution at room temperature for 2–4 hours before the experiments.

In one set of experiments, fragments of ovary were treated with collagenase (Sigma type I, 0·6 mg ml^{-1} in Ringer solution) for 1 h at room temperature in order to remove the follicle cells.

TABLE 1. Ionic composition (in mM) of the external bathing solutions

No.	Solution	Na^+	K^+	NMG^+	Cl^-	SO_4^-	Gluconate	Isethionate	Sucrose
1	Normal Ringer	114	2·4	0	116·4	7	0	0	0
2	NMG Ringer	90	2·4	24	116·4	7	0	0	0
3	High K^+	90	26·4	0	116·4	7	0	0	0
4	Low Na^+	9	2·4	105	116·4	7	0	0	0
5	Gluconate 50%	114	2·4	0	58·2	7	66·2	0	0
6	Isethionate 50%	114	2·4	0	58·2	7	6	58·2	0
7	Sucrose	90	2·4	0	92·4	7	0	0	48

All solutions were buffered at pH 7·5 with Tris-sulphate (5 mM). Ionic calcium adjusted to 1 mM. Magnesium sulphate (1 mM) added to all solutions.

The composition of the solutions used is given in Table 1. All solutions were adjusted to an osmolality of 230 mosM; since gluconate and isethionate ions have a chelating effect on calcium, the ionic calcium concentration in the bathing solutions was always checked with a calcium electrode and adjusted to 1 mM.

Electrophysiological recording

The oocytes were laid on a stretched nylon mesh in a Perspex perfusion chamber. The solutions flowed into the chamber by gravity at a rate of 7 ml min^{-1} and a multiway rotary valve (Partridge & Thomas, 1975) was used to change the solutions. The time for 90% turnover of the solutions in the chamber was about 17 s.

Cell diameter was measured under the microscope with the aid of a graticle eyepiece and the surface area of the oocyte was calculated assuming a spherical shape (microvilli were not taken into account).

Microelectrodes filled with 2 M-KCl and with a resistance of 3–8 MΩ were used. In voltage-clamp experiments 3–5 MΩ electrodes were selected for current injection, and the preparation was clamped at the resting membrane potential.

Positive current correspond to the outward flow of positive charges. Positive or negative pulses (5–10 mV, 5 s in duration) were injected to measure membrane conductance. *I–V* plots were obtained between plus and minus 40 mV in relation to the holding potential.

Ion-selective microelectrodes

Ion-selective microelectrodes were made from filamented 1.5 mm o.d. borosilicate glass tubes. The micropipettes were silanized by vapour treatment with dimethyldichlorosilane ('simple room temperature method', Thomas, 1978) for about 1·5 min and oven-baked at 120 °C for 1 h. The shanks were filled with potassium, sodium or chloride ion exchangers (K-Lix Corning 477317; Na-Lix Fluka 71176; Cl-Lix Corning 477913); the back-filling solutions were 100 mM-KCl for potassium and chloride electrodes and 100 mM-NaCl for sodium electrodes. Electrodes were kept in dry air and were viable for a few days. An AD311K amplifier was used as an electrometer. Potassium- and chloride-sensitive microelectrodes were calibrated in KCl solutions within a concentration range from 10 to 150 mM and had slopes over 50 mV per decade. Sodium-sensitive microelectrodes were calibrated in NaCl solutions with concentrations ranging from 5 to 50 mM containing 100 mM-KCl, to take into account the interference from intracellular potassium on sodium readings. Slopes were around 45 mV per decade. The same electrodes had slopes of 50–56 mV per decade when calibrated in pure NaCl solutions.

Preliminary results showed that during sodium removal from the external solution the measured intracellular sodium concentration fell less than 15% in 30 min. Typically, experiments lasted 30–40 min and in our calculations the intracellular concentrations of sodium, potassium and chloride were assumed to be constant.

Calculations

Pump and diffusional currents. Under voltage clamp the injected current density (I_t) is given by the equation:

$$I_t = I_p + I_{Na} + I_K + I_{Cl}, \tag{1}$$

in which I_p is the pump current per unit area and I_{Na}, I_K and I_{Cl} are the sodium, potassium and chloride diffusional currents per unit area, assumed to be described by the Goldman–Hodgkin–Katz equation (Hodgkin & Katz, 1949) as follows:

$$I_i = zFP_i U(c_i \exp U - c_o)/(\exp U - 1), \tag{2}$$

where U is given by zFV/RT (dimensionlss); z is the valency (equiv mol^{-1}) of ion i; F is the Faraday constant (96500 C); P_i is the permeability (cm s^{-1}) of ion i; V is the membrane potential (V) in relation to the external solution; R is the molar gas constant (8·315 J K^{-1} mol^{-1}); T is the temperature (293·2 K); c_i is the intracellular concentration of ion i (mol cm^{-3}) and c_o is the extracellular concentration of ion i (mol cm^{-3}). When the holding potential is equal to the membrane potential under open-circuit, the total membrane current is zero ($I_t = 0$).

The pump current (I_p) was assumed to be constant under resting conditions or zero after the addition of ouabain to the external solution. All ion substitutions were made after blocking the pump with ouabain.

The change of the external concentration of a cation, C, by an amount ΔC_{oC} was performed either by replacement with the same amount of another cation (ΔC_{oD}) or by the simultaneous change of an equal amount of an anion ($-\Delta C_{oA}$).

The resulting change in current (ΔI) is given by:

$$-FP_C(U/(\exp U - 1))\Delta C_{oC} + FP_D(U/(\exp U - 1))\Delta C_{oD} = \Delta I, \tag{3a}$$

or

$$-FP_C(U/(\exp U - 1))\Delta C_{oC} + FP_A(U/(\exp U - 1))\Delta C_{oA} \exp U = \Delta I. \tag{3b}$$

Numerically,

$$\Delta C_{oC} = \Delta C_{oD} = \Delta C_{oA} = \Delta C. \tag{4}$$

Rearranging eqns (3a) and (3b)

$$-P_C + P_D = \Delta I/W, \tag{5a}$$

in the case of a cation–cation substitution, or

$$-P_C + \exp U' P_A = \Delta I/W', \tag{5b}$$

in the case of a change of the concentration of a cation–anion pair, in which $W = (F\,U/(\exp U - 1)\,\Delta C$ for cations. W takes the value of W' when the valency is negative. The same reasoning can be applied for the case of the substitution of an anion for another anion.

Resting potential. Substituting eqn (2) for each ion in eqn (1) and rearranging we obtain

$$I_t = 0 = (U''(X \exp U'' - Y)/(\exp U'' - 1)) + I_p, \tag{6}$$

where

$$X = P_{Na}[Na^+]_i + P_K[K^+]_i + P_{Cl}[Cl^-]_o,$$

$$Y = P_{Na}[Na^+]_o + P_K[K^+]_o + P_{Cl}[Cl^-]_i,$$

and

$$U'' = FV/RT.$$

Equation (6) is transcendental and implicit, but is easily solved by the Newton method.

Conductances. The membrane ionic conductance was either measured with positive or negative 10 mV pulses or computed by differentiating eqn (2) for individual ionic conductances or eqn (6) for total membrane conductance (Ferreira & Marshall, 1985).

Ion substitution procedure

The technique used consisted of maintaining constant the membrane voltage while changing the external concentrations of pairs of ions or blocking the pump current (I_p).

Once the membrane potential was constant the voltage clamp was switched on and adjusted for zero current. Ouabain was added and I_p measured. Ion substitutions were then made. As shown above, for each ion substitution an equation of type (3) or (5) in two unknowns (the permeability coefficients of the two ions) can be written. A further equation arises from the current step recorded when the sodium pump is inhibited (equation type (6)). In this way a sufficient number of equations can be generated to define the system.

In order to simplify the manipulations, both sides of each equation were divided by one of the coefficients (W) of the unknowns, so that an 'independent term' of the form I/W was obtained (Table 3). These values were individually computed taking into account the holding potential and the concentration change. The system of equations thus generated was solved by a least-squares method with subroutine FO4AMF of the NAG library.

The estimates of I_p and of the diffusional currents can be used to calculate the stoichiometry of the pump. The pump current can be expressed as:

$$I_p = I_{pNa} - I_{pK}, \tag{7}$$

where the terms on the right-hand side correspond respectively to the sodium and potassium currents through the pump.

Equation (7) can be substituted into (1) and rearranged to give:

$$I_{Na} + I_K + I_{Cl} = -I_{pNa} + I_{pK} = -I_p. \tag{7a}$$

When the oocytes are bathed in control solution under steady state I_{Cl} is very small. Thus we can write:

$$I_{pNa} = -I_{Na}$$

and

$$I_{pK} = -I_K.$$

Determination of water and ion contents

To determine the extracellular water content, fragments of ovary weighing about 50 mg were incubated for 1 h in Ringer solution containing 1 μC/ml of [^{3}H]inulin. The fragments were gently blotted with ashless filter paper, weighed in individual containers and dried to constant weight at 80 °C. Dry weight was determined and the pieces of tissue were incubated in 1 ml of 0·1 M-nitroacetic acid for 24 h with slow agitation. A fraction of the samples thus obtained was used for sodium and potassium ion determination by flame photometry (Eppendorf) and for chloride titration (Aminco-Cotlove titrator). The remaining fraction was pipetted into appropriate vials containing 10 ml scintilation fluid (Bray's solution) and monitored for radioactivity together with samples of the [^{3}H]inulin Ringer solution (Beckman LC1100 β-counter).

In a set of experiments, isolated oocytes were briefly washed with cold isotonic sucrose solution, gently blotted with ashless filter paper, weighed, placed in individual containers with 0·5 ml distilled water, and the cell membrane disrupted by sonication. The samples were centrifuged and sodium and potassium were determined in the supernatant by flame photometry.

RESULTS

Oocytes of *Xenopus laevis* in stages IV and V (Dumont, 1972) had membrane potentials up to −80 mV which were stable for several hours. As reported by Kusano, Miledi & Stinnakre (1982), most freshly collected eggs displayed brief depolarizations of 10–15 mV in amplitude for about 1 h after the impalement. These potential transients reversed polarity at membrane potentials between −20 and −30 mV and were rarely seen if the oocytes were used one or more days after collection. Except for these flutuations in membrane potential, oocytes studied immediately after removal from the animal did not differ from those studied during

the following 3 days. In a number of impalements performed 8 days or more after removal of the oocytes, the membrane potential was low (-10 mV or lower), the membrane conductance was also low (down to 3·2 μS cm^{-2} or less) and the responses to ion substitutions under voltage clamp were extremely reduced. This behaviour, which was not further characterized, must reflect a change in selectivity and a general decrease in permeability of the cell membrane and it is similar to that reported by Kado, Marcher & Ozon (1981) and by Richter *et al.* (1984) for *Xenopus* eggs treated with progesterone.

To study the effect of treating the oocytes with collagenase two sets of experiments were conducted, one using oocytes which were not subject to the action of the enzyme (hereafter called untreated oocytes), and another one using collagenase-treated oocytes.

The mean value of the membrane potential was $-39{\cdot}1 \pm 0{\cdot}99$ mV ($n = 103$) for the first group and $-43 \pm 2{\cdot}2$ mV ($n = 19$) for the second one; the difference is not statistically significant ($0{\cdot}1 < P < 0{\cdot}2$). Cells with membrane potentials less negative than -26 mV were rejected. In seventy-three untreated oocytes the mean value of membrane conductance was $34{\cdot}6 \pm 1{\cdot}62$ μS cm^{-2} as opposed to a value of $42{\cdot}7 \pm 3{\cdot}4$ μS cm^{-2} found in a sample of nineteen collagenase-treated oocytes; the difference is significant at the 5% level ($0{\cdot}02 < P < 0{\cdot}05$).

In a few cells I–V curves were obtained between plus and minus 40 mV in relation to the resting potential; the I–V plots were linear.

The intracellular ion concentrations measured with ion-sensitive microelectrodes were as follows: potassium, $109{\cdot}5 \pm 3{\cdot}3$ mM ($n = 13$); sodium, $10{\cdot}1 \pm 0{\cdot}66$ mM ($n = 12$); chloride, $37{\cdot}7 \pm 0{\cdot}18$ mM ($n = 19$). These concentrations correspond to equilibrium potentials around -95 mV (E_K), $+61$ mV (E_{Na}) and -28 mV (E_{Cl}) respectively and indicate that none of the three ions is at equilibrium across the cell membrane. Water and ion contents of the cytoplasm were also determined by chemical methods using [^{3}H]inulin as an extracellular marker. The results obtained for twelve batches of oocytes were: potassium, $-112{\cdot}7 \pm 1{\cdot}9$; sodium, $-35 \pm 1{\cdot}2$; chloride, $-56 \pm 0{\cdot}60$, all values expressed in mmoles per litre of intracellular water. The intracellular water was $1{\cdot}13 \pm 0{\cdot}01$ kg (kg dry weight)$^{-1}$. Sodium and potassium determinations from individual cells yielded similar results.

Sodium pump

In twenty-six untreated oocytes the addition of ouabain (0·05–0·1 mM) to the external solution caused a fall in membrane potential of $10{\cdot}3 \pm 1{\cdot}12$ mV, which corresponded to 27% of the resting potential. In nineteen collagenase-treated oocytes the mean potential fall after ouabain was $13 \pm 1{\cdot}3$ mV, corresponding to 32% of the resting potential. Under voltage-clamp conditions the mean pump current measured (I_p) in 104 untreated oocytes was $-414 \pm 35{\cdot}9$ nA cm^{-2} and in nineteen collagenase-treated oocytes it was $-552{\cdot}7 \pm 48{\cdot}7$ nA cm^{-2} ($0{\cdot}02 < P < 0{\cdot}05$). The pump was also blocked by removing potassium from the external solution. When the two measurements were done in the same oocyte the average ratio of the currents ($I_{K,free}/I_{ouabain}$) was $0{\cdot}93 \pm 0{\cdot}18$ ($n = 10$); this ratio is not statistically different from 1 ($P > 0{\cdot}4$).

Forty-two out of forty-four untreated oocytes showed a decrease in membrane

conductance after the addition of ouabain. The mean control value was 39·3 ± 2·92 μS cm^{-2} and after ouabain it was 31·7 ± 2·44 μS cm^{-2}; the mean difference was 7·63 μS cm^{-2} (19% of the mean control value); in nineteen collagenase-treated oocytes the membrane conductance fell from 44·2 ± 4·2 to 38·2 ± 3·6 μS cm^{-2} after the addition of ouabain.

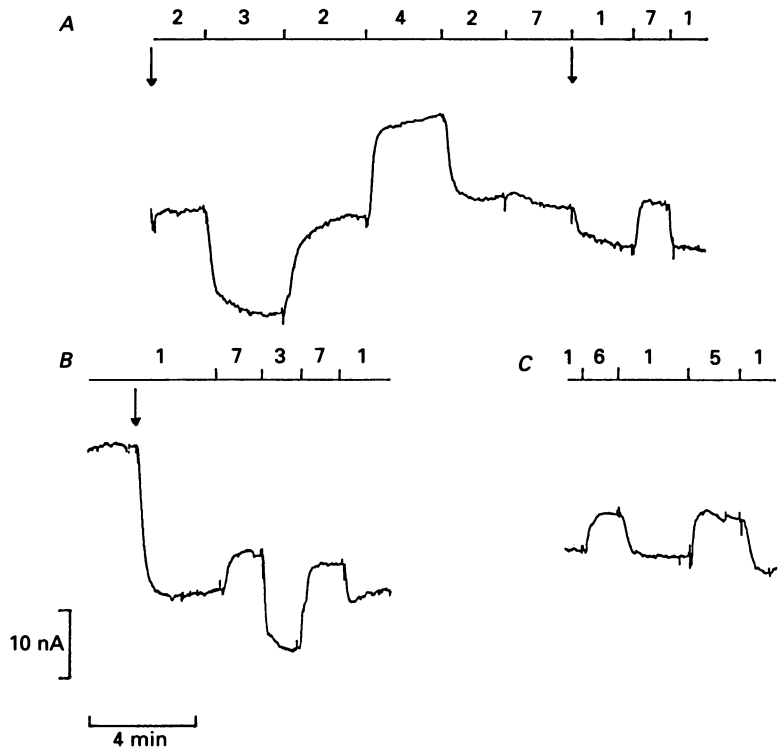

Fig. 1. Examples of the effects of ion substitutions (see Table 1 for the composition of the bathing solutions). *A*, control: solution 2 (NMG Ringer solution) + ouabain (0·1 mM); solution 3 (high-K^+ Ringer solution); solution 4 (low-Na^+ Ringer solution); solution 7 (sucrose Ringer solution); solution 1 (normal Ringer solution). *B*, control: solution 1 (normal Ringer solution); ouabain (0·1 mM) was added at the arrow; solution 7 (sucrose Ringer solution); solution 3 (high-K^+ Ringer solution). *C*, control: solution 1 (normal Ringer solution) + ouabain (0·1 mM); solution 6 (isethionate 50%); solution 5 (gluconate 50%).

Ionic permeabilities

Experiments were performed in untreated and collagenase-treated oocytes, in which ionic concentration steps (usually 24 mM) were externally applied and the resulting diffusional currents were measured. These concentration steps were applied either by substitution of one cation (or anion) by another, or by increasing (or decreasing) the concentration of an anion–cation pair while maintaining constant the osmolality and the concentration of the other ions. Typical results are shown in Fig. 1. Panel *A* shows an experiment in an oocyte previously treated with ouabain (0·1 mM), in which NMG Ringer solution was initially used as the control solution

(first arrow). When 24 mM-NMG was substituted by an equivalent amount of potassium (solution 3), there was a negative deflection in the current record of 16 nA. Substitution of sodium by NMG (solution 4) produced a positive deflection of 16 nA. At the time indicated by the second arrow, the oocyte was superfused by normal Ringer solution; the replacement of 24 mM of sodium chloride by 48 mM-sucrose

TABLE 2. Mean values and S.E. of means of the current deflections (in nA cm^{-2}) obtained in different experimental conditions. Each pair of numbers represents the control solution and the experimental solution (see Table 1) used in each case. All ion substitutions were made after addition of ouabain (0·05 mM). Values in parentheses correspond to the computed values obtained from type 3 equations, assuming the permeability coefficients given in Table 4 and the average membrane potential

	Untreated oocytes						
	1–4	2–3	1–7	1–5	1–6	7–3	1–ouabain
Mean	477	−579	185	179	—	—	−463
S.E.M.	83	81	27	17	—	—	26
n	25	23	10	12	—	—	52
	(585)	(−696)	(233)	(195)	—	—	
	Collagenase-treated oocytes						
	2–4	2–3	1–7	1–5	1–6	7–3	1–ouabain
Mean	297	−466	134	178	142	−419	−536
S.E.M.	46	43	21	10	14	50	57
n	9	9	14	5	5	5	14
	(445)	(−413)	(152)	(211)	(226)	(−434)	

(solution 7) produced a positive current deflection of 7 nA. Panel *B* shows results of substitutions of anion–cation pairs. At the time indicated by the arrow ouabain (0·1 mM) was added to the bath causing a current deflection of −23 nA. When the current reached a steady value 24 mM-sodium chloride was replaced by 48 mM-sucrose (solution 7). The current increased by 6 nA. 48 mM of sucrose was then replaced by 24 mM-potassium chloride (solution 3). There was a negative current deflection (14 nA). Panel *C* reports the results obtained when 58·2 mM of chloride was replaced by an equal amount of isethionate (solution 6) or by the same amount of gluconate (solution 5). Both substitutions produced positive current deflections of similar magnitude.

Experiments of this kind were done in untreated and in collagenase-treated oocytes. Table 2 shows the mean values of the recorded currents, normalized for a 1 cm^2 area. These values were entered into equations of type 3 (eqns (3a) and (3b), see Methods), which were rearranged into type 5 equations (eqns (5a) and (5b), see Table 3).

The values of the permeability coefficients estimated for two groups of untreated and collagenase-treated oocytes are given in Table 4. The results corresponding to the untreated oocytes were obtained from experiments in which not all the substitutes could be tested in each case. Since this group includes a very large number of oocytes, the results were pooled and the average value of the currents obtained for each substitution was used in the computations. The number of

experiments for each case is specified in Table 2. A single set of permeability coefficients is thus obtained for the whole group. For the group of collagenase-treated oocytes sufficient readings were taken in each cell for the permeability coefficients to be individually determined.

TABLE 3. Computed mean values and S.E. of means of the terms I/W (see Methods) obtained from the same experiments reported in Table 2. Each pair of numbers represents the control solution and the experimental solution (see Table 1) used in each case. All ion substitutions were made after the addition of ouabain (0·05 mM)

	1–4	2–3	1–7	1–5	1–6	7–3	1–ouabain
	Untreated oocytes						
Mean	27	−127	47	18	—	—	−63
S.E.M.	3	17	8	3	—	—	4
n	25	23	10	12	—	—	52
	Collagenase-treated oocytes						
	2–4	2–3	1–7	1–5	1–6	7–3	1–ouabain
Mean	18	−99	34	74	67	−103	−67
S.E.M.	2	10	5	17	14	16	9
n	9	9	14	5	5	5	14

TABLE 4. Ionic permeability coefficients (in 10^{-8} cm s^{-1})

	P_{Na}	P_K	P_{NMG}	P_{Cl}	$P_{gluconate}$	$P_{isethionate}$
	Untreated oocytes (pooled results)					
Mean	5·37 (0·30)*	17·65 (1)	2·45 (0·14)	1·38 (0·08)	9·78 (0·55)	—
	Collagenase-treated oocytes					
Mean	3·75	9·55	1·03	3·40	13·40	14·12
S.E.M.	0·20	0·34	0·10	0·20	0·55	0·15
n	14 (0·39)*	14 (1)	9 (0·11)	13 (0·36)	5 (1·40)	5 (1·48)

* Value in parentheses are permeability ratios in relation to P_K.

The results show that in this preparation NMG is about half as permeant as sodium, and that gluconate and isethionate are much more permeant than chloride. Two further experiments showed that the estimated permeabilities to choline and to Tris are even higher than the permeability to NMG. Experiments in which sulphate was used as a chloride substitute suggested that sulphate is more permeant than chloride. We were thus unable to identify relatively impermeant substitutes to sodium, potassium or chloride for the oocyte membrane.

DISCUSSION

The purpose of the present work was to characterize the mechanisms that regulate the ionic composition of the cytoplasm of *Xenopus* oocytes in the resting state (stages IV and V, Dumont, 1972). The membrane potential of these cells was clamped at

its resting value and the currents generated by changes in the ionic composition of the bathing solution or by the addition of ouabain (0·1 mM) were measured. By assuming that the recorded diffusional currents were adequately described by the Goldman–Hodgkin–Katz equation it was possible to estimate the permeability coefficients to sodium, potassium and chloride.

The oocyte membrane potential is known to be sensitive to several stimuli but none of these should have spuriously affected the present studies. Since the oocytes studied were voltage clamped at their resting potential and the external concentration of calcium was always 1 mM we avoided the activation of voltage- and calcium-dependent ionic channels (Tupper & Maloff, 1973; Miledi, 1982; Baud *et al.* 1982; Miledi & Parker, 1984). Some oocytes showed voltage fluctuations probably caused by the flickering of chloride channels (their reversal potential was close to E_{Cl}). To avoid errors, experiments were always done in the absence of such fluctuations. We also avoided ion substitutes, such as choline, which might be involved in the activation of chemically gated channels, since responses of *Xenopus* oocytes to a variety of chemical transmitters have been reported (Dascal & Landau, 1982; Kusano *et al.* 1982; Lotan, Dascal, Cohen & Lass, 1982; Gundersen, Miledi & Parker, 1983; Dascal, Gillo & Lass, 1985).

The sodium pump current was measured under voltage clamp by the addition of ouabain. The average figure was 414 ± 36 nA cm^{-2} for untreated oocytes and 553 ± 49 nA cm^{-2} for collagenase-treated oocytes; these figures are similar to those reported by Lafaire & Schwarz (1986). Ouabain also caused a fall in membrane potential (by almost a third of its control value) and a decrease in membrane conductance. In the region around the open-circuit voltage the total slope conductance of the cell membrane fell by about 5–7 μS cm^{-2} when the pump was blocked. The *I–V* curves obtained by Lafaire & Schwarz (1986) also show a fall in slope in the same region.

Calculation of the ionic diffusional permeabilities required the measurement of intracellular ionic concentrations. Our findings confirm the observations of Dick & McLaughlin (1969) who showed that sodium-sensitive microelectrodes measure only a fraction of the total cell sodium (as determined with flame photometry and markers of the extracellular space). Similar observations were reported by De Laat, Buwalda & Habets (1974), also in *Xenopus* oocytes, and by Palmer, Century & Civan (1978) in frog oocytes. We used the potentiometrically determined values in the calculation of ion permeability coefficients since, as suggested by Dick & McLaughlin (1969), the intracellular sodium that is not accessible to the ion-sensitive microelectrode is probably sequestered in some unidentified compartment; the same applies to cytoplasmic chloride. There was very little discrepancy between the values of cell potassium measured by the two methods.

The determination of the ionic diffusional currents was based on measurements of the membrane potential, of the intracellular ion concentrations and on a method of estimating ionic membrane permeabilities from ion substitution experiments. The method described here assumed a pump leak model of the oocyte membrane in which the leak is represented by Goldman–Hodgkin–Katz fluxes.

The results obtained from pooled data of a large number of experiments showed that in *Xenopus* oocytes (stages IV and V), the permeability of the membrane to NMG is about half the permeability to sodium. The permeability to the two anions tested (gluconate and isethionate) was much higher than that to chloride. Dascal *et al.*

(1984) published the permeability ratios P_{Na}/P_K (0·12 in untreated oocytes and 0·24 in collagenase-treated oocytes) and P_{Cl}/P_K (0·4 in both). These figures are somewhat different from those obtained in the present work. However, for the calculations of the permeability ratios they used values of intracellular sodium and potassium concentration which differ from those reported here. Furthermore, those authors used ion substitutes which are permeant according to our findings (Tris and sulphate). Tupper & Maloff (1973) published absolute ion permeability coefficients in the *Rana* oocytes membrane which are about two orders of magnitude larger than ours. These are also difficult to compare with our results, since they used tracer-flux analysis and did not distinguish between simple diffusion movements and fluxes mediated by other mechanisms. The same applies to the permeability ratio (P_{Na}/P_K of 0·1) determined by O'Connor *et al.* (1977). The differences in permeability coefficients found between collagenase-treated and untreated oocytes are difficult to explain. Since the total conductance of the cells increased 23% with the collagenase treatment it is possible that the treated oocytes were not at steady state at the time of the experiments. The following considerations apply to results obtained in untreated oocytes.

With the estimated permeability coefficients for sodium, potassium and chloride, the measured intracellular ion concentrations and the average pump current, the membrane potential can also be estimated (see Methods). The value obtained (−37 mV) is not very different from the average measured value (−39 mV). The overall membrane conductance estimated with the formula for the slope conductance is 41·9 μS cm^{-2}, which again is not very different from the average measured value (34·6 μS cm^{-2}).

Substitution of the computed permeability coefficients, average membrane potential and experimental concentration changes in the appropriate equations (types 3a and 3b, see Methods) gives figures for the current deflections obtained for each ion substitution which are close to the average experimental values (Table 2). The experimental values given in this table are averages of currents measured in cells with membrane potentials which were not necessarily identical to the average membrane potential used in the computations. Consequently, the experimental values do not match exactly the computed values.

The diffusional currents were calculated for the average open-circuit potential (−39·1 mV), by substituting in eqn (2) the computed permeability coefficients and the observed intracellular ion concentrations. The diffusional currents so determined were as followed (μA cm^{-2}): $I_{Na} = -1{\cdot}15$; $I_K = 0{\cdot}69$; $I_{Cl} = -0{\cdot}035$. Substitution of these values and the average pump current given in Table 2 into eqn (1) yields a net charge balance of 0·028 μA cm^{-2}, a figure which is within the experimental error. This implies that the currents carried by sodium, potassium and chloride account for almost all the steady-state charge movements across the oocyte membrane.

Substituting the current values in eqn (7a) and dividing I_{pNa} and I_{pK} by I_p yields a ratio of 3/1·8, which compares well with the ratio 3 Na^+/2 K^+ which has been attributed to the stoichiometry of the sodium pump in many types of cells. It has been suggested that this stoichiometry is not fixed, depending on internal sodium (Mullin & Brinley, 1969; Gorman & Marmor, 1974), on external potassium (Liberman, 1979) or on external sodium (Livengood, 1983).

Lafaire & Schwarz (1986) used dihydroouabain to inhibit the sodium pump in full-

grown oocytes of *Xenopus*. Although they did not specify the current measured per unit area, it is possible to estimate from the plot in their Fig. 5 a current of approximately 0·4 μA cm^{-2} (25 nA per oocyte of 1·4 mm in diameter), which is close to our mean value of 0·414 μA cm^{-2}. This current is equivalent to a net pump flux of 4·3 pmol cm^{-2} s^{-1}. From the values published by O'Connor *et al.* (1977) it is possible to estimate a net pump flux of 2·7–5 pmol cm^{-2} s^{-1} (assuming a cell diameter range from 1 to 1·4 mm), which compares well with our figure; this also falls within values reported in the literature for different systems (Eisner & Lederer, 1980; Cavieres, 1977; Daut & Rüdel 1982). Since the oocyte sodium content (as determined by flame photometry) is about 35 mmol (kg cell water)$^{-1}$ and the water content of each cell is about half its volume (53%), a mid-range oocyte (1·1 mm in diameter) contains about 13000 pmol of sodium. Thus, total inhibition of the pump will cause a rise in sodium content of 20% per hour. This means that, unless the cell membrane permeability is substantially increased, it is reasonable to assume that in experiments in which the bathing solutions are changed or when the sodium pump is inhibited the intracellular ion concentrations do not change much within a period of 30 min (see Methods). Furthermore, fluctuations in the pumping rate will have minor effects on the cell sodium concentration over a period of hours. Thus, in a cell such as the *Xenopus* oocyte, a small surface-to-volume ratio together with relatively low transport leads to a high stability of cytoplasmic ionic composition.

The measured chloride intracellular concentration (38 mM) is higher than the equilibrium value (24·4 mM for a membrane potential of −39·1 mV). This implies the presence of an outward diffusional flux of chloride whose magnitude can be derived from the calculated inward current. This flux must be balanced by an equal inward uphill flux which could take place through a neutral co-transport system involving also sodium or sodium and potassium.

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