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Activity Coefficients of Intracellular Na^+ and K^+ During Development of Frog Oocytes

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Summary. The chemical activities, (a), of Na^+ and K^+ were determined in large mature and in small immature frog oocytes, using open-tipped micropipettes and ion-selective microelectrodes. The average chemical concentrations, c, of Na^+ and K^+ were determined by spectrophotometry and by electron probe X-ray microanalysis. The apparent activity coefficient (γ^{app}) was calculated for each ion as the ratio, a/c.

With development, (a_{Na}/a_K) decreased four to fivefold and (c_{Na}/c_K) increased six to sevenfold. In the large mature oocytes, γ_{Na}^{app} was measured to be 0.08 ± 0.02 and γ_K^{app} lay within the range 1.15 ± 0.03 to 1.29 ± 0.04, constituting the smallest value for Na^+ and largest value for K^+, respectively, thus far reported. This intracellular value of γ_K^{app} was substantially greater than the activity coefficient of K^+ in the external medium (0.76). The data suggest that the inequality of γ_{Na}^{app} and γ_K^{app} in this and probably other cells reflects the development of subcellular compartmentalization of ions. Possible intracellular sites of ionic compartmentalization are considered.

Several lines of evidence suggest that intracellular Na^+ and K^+ may be important factors in the regulation of cell growth and differentiation. The activity of isolated chromosomes from *Chironomus* salivary gland cells has been reported to depend upon the relative activities of Na^+ and K^+ in the medium (Lezzi & Gilbert, 1970). Incorporation of amino acids into polypeptides has been shown to be selectively stimulated by K^+ in isolated ribosome systems (Lubin, 1963; Conway, 1964) and in intact mammalian cells (Lubin, 1967).

The processes of blast transformation (Quastel & Kaplan, 1970; Negendank & Collier, 1976) and malignant transformation (Bader, 1976) have been linked to intracellular concentrations of K^+ and Na^+, respectively. Shifts in the subcellular distribution of ions may also be important at least with respect to the process of fertilization (Tupper, 1973).

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It has become increasingly apparent that measurements of total cell Na^+ and K^+ cannot adequately describe the activities and subcellular distribution of these ions.

In characterizing the intracellular alkali cations, an apparent electrochemical activity coefficient (γ^{app}) may be defined as the ratio of the chemical activity, a, to the total average intracellular concentration, c. The activities a_{Na} and a_K are obtained from intracellular impalements with open-tipped and ion-selective microelectrodes. The average concentrations c_{Na} and c_K are calculated from measurements of the total ionic and water contents in cell extracts; due correction must be made for the Na^+, K^+ and water contents contained in the extracellular water trapped within the preparation. Despite considerable scatter from preparation to preparation, γ_{Na}^{app} has been found to be consistently smaller than γ_K^{app} for all biological cells (Lev & Armstrong, 1975). The precise basis and physiological significance of this phenomenon have been unclear.

For the further study of this problem, frog oocytes constitute a particularly favorable model system, both because of their large size and because they can be studied in varying stages of development. The intracellular activities and apparent activity coefficients for Na^+ and K^+ have already been studied in toad oocytes at a single stage of development (Dick & McLaughlin, 1969). Changes in c_{Na} and c_K have also been examined during the course of amphibian oocyte development (Riemann, Muir & MacGregor, 1969; Cannon, Dick & Ho-Yen, 1974). In the present study, we have measured a, c and γ^{app} for the Na^+ and K^+ of frog oocytes in different stages of maturation, using intracellular microelectrodes, atomic absorption and flame spectrophotometry, and electron probe X-ray microanalysis. Certain of the current findings have been presented in preliminary form elsewhere (Palmer & Civan, 1977*a*; Century, Palmer & Civan, 1977[1]).

Materials and Methods

Oocytes

Gravid frogs (*Rana pipiens*) were obtained during the months of November through April from West Jersey Biological Farm (Wenonah, New Jersey), and maintained in water at 22–23 °C on a diet of mealworms. After doubly-pithing each frog, both ovaries

1 Century, T.J., Palmer, L.G., Civan, M.M. 1977. Application of the electron microprobe to analysis of developing frog oocytes. Presented at Workshop on Biological X-ray Microanalysis by Electron Beam Excitation, Boston.

were completely excised and immersed in a Ringer's solution. Small pieces of ovary consisting of 50–100 mature and as many immature oocytes were transferred to Ringer's solution in small Petri dishes. For purposes of spectrophotometric and electron probe analysis, individual mature oocytes were then dissected free: each oocyte retained its adhering envelope of epithelial cells, constituting some 4% of the volume of the mature oocyte. Clumps of immature oocytes were collected for analysis from the remaining ovarian tissue.

For purposes of electrical measurement, smaller samples of ovarian tissue containing 30–50 immature and 30–50 mature oocytes were partially dissected into flat sheets, facilitating impalement with open-tipped micropipettes and ion-selective microelectrodes.

Solutions

The electrophysiological measurements were performed on mature and immature oocytes bathed in a Ringer's solution containing (in mM): NaCl, 108; KCl, 2.5; $CaCl_2$, 1.5; $MgCl_2$, 1.0; Na_2HPO_4, 2.4; and NaH_2PO_4, 0.4; at a pH of 7.3. A choline Ringer's solution, having the same composition except for the equimolar replacement of choline ions for Na^+, was used for rinsing the oocytes.

Mature and immature oocytes were collected for spectrophotometric and electron probe analysis from ovarian tissue immersed in a Ringer's solution consisting of (in mM): NaCl, 92.5; KCl, 2.5; $CaCl_2$, 0.7; $MgSO_4$, 1.2; $NaHCO_3$, 6.6; Na_2HPO_4, 2.0; NaH_2PO_4, 1.2; and glucose, 24; at a pH of 8.2.

Electrophysiological Measurements

The fabrication and use of the microelectrode system have been described in detail elsewhere (Palmer & Civan, 1977*b*). In brief, micropipettes with resistances of 5–10 Mohms and tip potentials of less than 5 mV were used for recording the intracellular electrical potential. The ion-selective microelectrodes were made from micropipettes of similar dimensions. Immediately after being drawn, they were coated with dimethyldichlorosilane vapor and baked at 100 °C for an hour. Potassium-selective microelectrodes were constructed by filling the micropipette tip with a K^+ liquid ion exchange resin (Corning 477317) obtained from Corning Glass (Corning, N.Y.). The Na^+-selective microelectrodes were produced by filling the micropipette tip with a 1.5% solution of $K(\phi Cl)_4B$ in triethylhexyl phosphate (K & K, Plainview, N.Y.); the $K(\phi Cl)_4B$ was a generous gift from Dr. George Baum. The shanks of the K^+- and Na^+-selective microelectrodes were filled with 0.5 M KCl and 0.5 M NaCl solutions, respectively. The microelectrodes were calibrated with standard solutions before and after each series of impalements.

The electrode outputs were connected to preamplifiers with input impedances of at least 10^{13} ohms and bias currents of less than 10^{-12} A. The outputs from the preamplifiers were read on paper chart recorders.

The output (E_K) of the K^+ microelectrode may be described empirically by the expression:

$$E_K = (E_0)_K + S \log(a_K + k_{Na,K} a_{Na}) + V \tag{1}$$

where $(E_0)_K$ is a reference constant, S is a sensitivity constant (Lev, 1964), $k_{Na,K}$ is the selectivity constant for Na^+ over K^+, and V is the electrical potential. S was measured to be 59–62 mV. Since $k_{Na,K}$ was very small, ranging from 0.017 to 0.031, the contribution of intracellular Na^+ was negligible; intracellular measurements of E_K were considered to reflect only the intracellular a_K.

The output (E_{Na}) from the Na^+-selective microelectrode may be described by an expression analogous to Eq. (1):

$$E_{Na} = (E_0)_{Na} + S \log(a_{Na} + k_{K,Na} a_K) + V. \quad (2)$$

For any given batch of micropipettes, S was measured to be the same value for the Na^+- and K^+-selective microelectrodes. Values of $k_{K,Na}$, the selectivity of K^+ over Na^+, ranged from 0.28 to 0.35, so that determination of the absolute value of a_{Na} required measurement of E_K as well as E_{Na} and V.

As a further check on the calibration of the three-electrode system, ion activities were measured in fibers of frog sartorius muscle, a tissue which has been extensively studied with glass ion-selective microelectrodes. Five to ten penetrations of different muscle fibers were made with each electrode used. In two muscles, a_K was 105 and 100 mM, while a_{Na} was 6 and 14 mM. In a third experiment, a_K was assumed to be 102.5 mM and a_{Na} was 5–11 mM, the uncertainty due to unusual electrode drift. Resting potentials were 85 to 95 mV. These values are in accord with the mean values of a_K (90 to 97 mM) and a_{Na} (5.5 to 9.6 mM) measured with glass electrode systems as reported by Lev (1964), Kostyuk, Sorokina and Kholodova (1969), Armstrong and Lee (1971) and White and Hinke (1976). We concluded that, at least in frog striated muscle, activities could be measured accurately using liquid ion-exchanger electrodes.

Chemical Analysis

In analyzing mature oocytes, 5–7 oocytes were individually rinsed for approximately 15 sec in the choline Ringer's solution described above, blotted on filter paper, and placed in small tared polypropylene containers. In analyzing immature oocytes, small samples of ovarian tissue dissected free of mature oocytes were rinsed for about 60 sec in choline Ringer's solution, and then blotted dry before being inserted in similar containers. Samples were subsequently weighed, dried for 12 hr at 60 °C, reweighed, and extracted in either 5 ml or 1 ml of deionized water overnight at 22–23 °C. The final solution and appropriate calibration standards were analyzed for Na^+ and K^+ with an EEL atomic absorption spectrophotometer or a Linear Instruments flame spectrophotometer.

Specimen Preparation for Electron Probe Microanalysis

Individual mature oocytes or clumps of immature oocytes of similar volume were mounted within concavities drilled into small brass pins. The pins carrying the freshly mounted material were rapidly frozen in Freon-12 at −155 °C. The frozen specimens were stored under liquid nitrogen prior to further processing.

Sectioning was performed with a Sorvall-JB-4 microtome modified for use as a freezing microtome. Knife and specimen temperatures were maintained at −65 °C. Sections were cut 1 μm thick, and transferred to a cold (−65 °C) beryllium block. Each section was flattened by pressing it against the block with the polished end of a small beryllium rod previously quenched in liquid nitrogen. After mounting a series of sections, the block was transferred in a cold, dry atmosphere to a specimen table (whose temperature could be regulated) within a combination freeze-drying and carbon-coating instrument (model DV 502, Denton Vacuum, Inc., Cherry Hill, N.J.) The specimen was maintained for 1 hr at −80 °C within 2–3 mm of a cold trap (−196 °C). When the specimen temperature was subsequently allowed to rise to 25 °C, no changes in system pressure (2×10^{-6} Torr) were observed. Carbon was then evaporated onto the specimen

block to a thickness of some 200 Å and the block finally transferred in a stream of dry nitrogen gas to a small dessicator, where it was stored at room temperature over P_2O_5.

Electron Probe X-ray Microanalysis

The dried carbon-coated sections were analyzed with a Cameca MBX microanalyzer (Cameca Instruments, Inc., Stamford, Conn.) equipped with three crystal spectrometers. Sodium and potassium Kα radiations were detected with TAP ($\sin A = 0.464$) and PET ($\sin A = 0.428$) crystals, respectively. Spot analyses of both mature and immature oocytes were conducted with primary electron energies of 15 keV and absorbed currents of 20 nA. Sections of mature oocytes were studied with a beam diameter of 100 μm and a counting period of 20 sec, resulting in a total irradiation of 5.1×10^{-3} C·cm^{-2}. Because of their smaller size, sections of immature oocytes were analyzed with a beam diameter of 16 μm for periods of 10 sec, resulting in a total irradiation of 9.9×10^{-2} C·cm^{-2}. The ratio of the counting rate for Na to that for K did not change significantly when a control sample of cellular material was analyzed at the two different dosages. In addition, we have found no significant changes in the Na and K signals from such specimens after periods of irradiation with the electron beam for periods as long as 7 min. Therefore, under the conditions of the present study, neither loss of specimen mass nor cumulative contamination appeared to significantly affect the measured counting rates.

Results

Electrophysiologic Measurements

In order to measure all three parameters a_{Na}, a_K and V, it was desirable to obtain simultaneous intracellular measurements with an open-tipped micropipette, and with K^+- and Na^+-selective microelectrodes within a single oocyte. This maneuver proved entirely feasible with the large mature oocytes, whose diameters were 1.5–1.6 mm. Averaging the measured values from 22 oocytes obtained from 4 frogs, the intracellular potential was -43 ± 1 mV, with respect to the external medium. The mean intracellular values of E_K and E_{Na} were 47 ± 1 mV and -62 ± 1 mV, respectively, indicating that K^+ was accumulated and Na^+ partially excluded from the cytoplasm, in comparison to their equilibrium distributions. The calculated values for a_K and a_{Na} were 120 ± 3 and 6 ± 1 mM, respectively; (a_{Na}/a_K) was 0.05 ± 0.01 (Table 1).

Despite our experience in impaling other cells with multiple microelectrodes (Palmer & Civan, 1975, 1977*b*), it proved technically difficult to introduce several microelectrodes simultaneously into a single immature oocyte, 200–300 μm in diameter, without irreversibly damaging the oocyte. In one oocyte, it did prove possible to introduce both the micropipette and 2 ion-selective microelectrodes at the same time. In 20

additional immature oocytes obtained from 4 frogs, it was possible to introduce both Na^+- and K^+-selective microelectrodes simultaneously. Another 7 oocytes from the same series of experiments were impaled with an open-tipped micropipette and an ion-selective microelectrode. In this study, we have been largely concerned with determining the ratios of the activities and concentrations of Na^+ to K^+ during the course of oocyte development. For this purpose, measurement of the intracellular electrical potential is unnecessary. From Eqs. (1) and (2), and noting that $(k_{Na,K}a_{Na}) \ll a_K$ within the cytoplasm, it is clear that (a_{Na}/a_K) can be directly calculated from measurements of E_K and E_{Na}, alone:

$$(a_{Na}/a_K)_c = \left[\frac{a_{Na} + k_{K,Na}a_K}{a_K + k_{Na,K}a_{Na}}\right]_R \cdot \left[10^{\{[(E_{Na})_c - (E_{Na})_R] - [(E_K)_c - (E_K)_R]\}/S}\right] - k_{K,Na} \quad (3)$$

Table 1. Activities, average concentrations and apparent activity coefficients of frog oocytes

Ion	Mature oocytes			Immature oocytes		
	a	c	γ^{app}	a	c	γ^{app}
	mM			mM		
Na^+	6 ±1 (22)	73 ±3 (8)	0.08 ±0.02	16 ±1 (8)	35 ±11 (3)	0.48 ±0.15
		77 ±1 (8)	0.08 ±0.02			
K^+	120 ±3 (22)	93 ±2 (8)	1.29 ±0.04	70 ±3 (8)	105 ±4 (3)	0.67 ±0.04
		104 ±1 (8)	1.15 ±0.03			
Na^+/K^+	0.05 ±0.01 (22)	0.79 ±0.04 (8)	0.06 ±0.01	0.22 ±0.02 (21)	0.3 ±0.1 (3)	0.7 ±0.2
		0.743 ±0.005 (8)	0.07 ±0.01			

The numbers of experimental determinations are entered in parentheses. For measurements of chemical activity (a), the numbers indicate the number of cells impaled. For the spectrophotometric measurements of average chemical composition (c), the numbers either indicate the number of batches of mature oocytes examined, or the number of masses of immature oocytes studied.

where the subscripts "c" and "R" refer to the cytoplasm and Ringer's solution, respectively.

Averaging the values obtained from all 28 immature oocytes, V was -15 ± 3 mV, E_K was 67 ± 1 mV, and E_{Na} was -32 ± 1 mV. The calculated intracellular values for a_{Na}, a_K, and (a_{Na}/a_K) were 16 ± 1 mM, 70 ± 3 mM, and 0.22 ± 0.02, respectively (Table 1).

Spectrophotometric Measurements

Two series of measurements were carried out of the average concentrations of Na^+ and K^+ in the mature oocytes. From the first series of 8 batches of cells, c_{Na} and c_K were calculated to be 73 ± 3 and 93 ± 2 mM; (c_{Na}/c_K) was 0.79 ± 0.04 (Table 1). From these estimates of c_{Na} and c_K, and the above measurements of a_{Na} and a_K, γ_{Na}^{app} and γ_K^{app} are calculated to be 0.08 ± 0.02 and 1.29 ± 0.04, respectively (Table 1).

A second series of measurements was performed on 8 batches of mature oocytes. The latter oocytes were obtained from the same ovarian tissue subjected to electron probe X-ray microanalysis. From the second series of results, slightly higher values were calculated for c_{Na} (77 ± 1 mM) and for c_K (104 ± 1 mM). However, the ratio (c_{Na}/c_K) was found to be 0.743 ±0.005 (Table 1), not significantly different from the first value. Presumably, a small fraction of extracellular water was retained with the first series of cells. If the electrophysiologic measurements are related to this second estimate of c_{Na} and c_K, $\gamma_{Na}^{app}=0.08\pm0.02$ and $\gamma_K^{app}=1.15\pm0.03$.

The value of (c_{Na}/c_K) of 0.74–0.77 measured in the present study is close to, but slightly higher than, the values of 0.69 reported by Morrill (1965) and of 0.67 reported by Tupper and Maloff (1973).

Measurements of the chemical composition of the immature oocytes were far less satisfactory. From analysis of 3 clumps of tissue, c_{Na} was calculated to be 24–57 mM, c_K was 98–112 mM, and the ratio ranged from 0.22 to 0.58. The mean values of c_{Na} and c_K were 35 ± 11 and 105 ± 4 mM, respectively, while that of (c_{Na}/c_K) was 0.3 ± 0.1 (Table 1). Thus, the value of (c_{Na}/c_K) characterizing the immature oocytes was significantly smaller than that (0.79 ± 0.04) characterizing the mature oocytes. However, the experimental scatter of the measurements of chemical composition for the immature oocytes was too great to permit satisfactorily precise estimation of the ratio $(\gamma_{Na}^{app}/\gamma_K^{app})$ of the apparent activity coefficients. Furthermore, the relatively small size of these immature oocytes, and their close involvement with the ovarian tissue, which includes connective tissue, blood vessels and epithelial cells, enormously complicates

the interpretation of conventional chemical analysis. In an effort to better characterize (c_{Na}/c_K) for the immature oocytes, we have turned to electron probe X-ray microanalysis; with this technique, we can analyze specific sites, known to be intracellular with a high degree of certainty.

Electron Probe X-Ray Microanalysis

After correction for background, frequency analysis indicated that the measurements of the ratio of counts per second for Na, $(cps)_{Na}$, to that for K, $(cps)_K$, in immature oocytes was not normally distributed. However, individually both $(cps)_{Na}$ and $(cps)_K$ did appear normally distributed, so that the ratio of the probe counts for Na to that for K was calculated as the ratio of the two means. 114 spot analyses were conducted of 9 immature oocytes, whose diameters ranged from 90 to 160 μm. The mean values for $(cps)_{Na}$ and $(cps)_K$ were 2.6 ± 0.2 and 83 ± 2, respectively; the ratio $(cps)_{Na}/(cps)_K$ was, therefore, 0.031 ± 0.003 (Table 2).

Similarly, 174 spot analyses were taken of 2 mature oocytes; other oocytes from the same ovary were analyzed spectrophotometrically, as described above. $(cps)_{Na}$, $(cps)_K$ and the ratio $[(cps)_{Na}/(cps)_K]$ were measured to be 7.9 ± 0.3, 37 ± 1, and 0.21 ± 0.01 (Table 2), respectively.

The molar ratio (c_{Na}/c_K) for the immature oocytes could be estimated from the probe data and from the spectrophotometrically-determined values of (c_{Na}/c_K) for the mature oocytes using the expression:

$$(c_{Na}/c_K)_{imm} = (c_{Na}/c_K)_{MAT} \frac{[(cps)_{Na}/(cps)_K]_{imm}}{[(cps)_{Na}/(cps)_K]_{MAT}}. \quad (4)$$

Table 2. Ratios of intracellular Na^+ to K^+ by electron probe microanalysis and chemical analysis

Stage of development	$\frac{(cps)_{Na}}{(cps)_K}$	$\frac{c_{Na}}{c_K}$
Mature	0.21 ± 0.01 (174)	0.743 ± 0.005 (8)
Immature	0.031 ± 0.003 (114)	0.11 ± 0.01

The ratios of the probe counts per second (cps) for Na^+ to that for K^+ are entered in the first column of data; the numbers in parentheses are the numbers of spot analyses performed. The value entered under (c_{Na}/c_K) for the mature oocytes was measured spectrophotometrically; the number of batches of eggs is entered in parentheses. The value entered under (c_{Na}/c_K) for the immature oocytes has been calculated from the other tabulated values.

The indices "imm" and "MAT" refer to the immature and mature oocytes, respectively. Using this approach, $(c_{Na}/c_K)_{imm}$ was calculated to be 0.11 ± 0.01 (Table 2). Thus, on the basis of the microprobe analysis, (c_{Na}/c_K) was found to be 6–7 times *smaller* in the immature than in the mature oocytes, in contrast to the observation that (a_{Na}/a_K) was 4–5 times *larger* in the immatures (Table 1).

The primary objective of the electron probe microanalysis was to estimate the ratio (c_{Na}/c_K) in the immature oocytes. However, it was also of interest to estimate the absolute values of c_{Na} and c_K, particularly since analysis of amphibian oocytes this small has not been previously reported. For this purpose, an appropriate calibration factor must be chosen. Calibration curves can be readily determined for sodium and potassium in the form of simple chloride standards and in the presence of other salts (Ingram & Hogben, 1967; Morel, Roinel & LeGrimellec, 1969; Lechene, 1974).

In the present study, we have taken the mature oocytes as our primary biological standard. The calibration is calculated on the assumption of a constant ratio (k) between counting rate (cps) and concentration (F) in mM · (kg wet wt)$^{-1}$:

$$k_i = \frac{(\text{cps})_i}{\text{F}_i} \tag{5}$$

where the index (i) refers either to Na or to K. If c_i is the concentration of Na or K in mM · (liter water)$^{-1}$, and w is the concentration of water in liters · (kg wet wt)$^{-1}$,

$$F_i = c_i w \tag{6}$$

for both the immature (imm) and mature (MAT) oocytes. From Eqs. (5) and (6):

$$(c_i)_{imm} = (c_i)_{MAT} \left[\frac{(\text{cps}_i)_{imm}}{(\text{cps}_i)_{MAT}}\right] \left[\frac{w_{MAT}}{w_{imm}}\right]. \tag{7}$$

From measurements of toad oocytes (Cannon *et al.*, 1974), we may estimate that the intracellular fluid probably comprises some 90% of the 1-μm sections from the frozen hydrated immature oocytes, but only 45% of the 1-μm sections from the frozen hydrated mature oocytes (Century, Fenichel & Horowitz, 1970). Thus, $(w_{imm}/w_{MAT}) \doteq 2$. Using Eq. (7) and taking $(c_{Na})_{MAT}$ and $(c_K)_{MAT}$ to be 77 ± 1 and 104 ± 1 mM, respectively, $(c_{Na})_{imm}$ and $(c_K)_{imm}$ may be calculated from the electron probe microanalysis of the immature oocytes to be 13 ± 1 mM and 116 ± 5 mM, respectively.

Discussion

The results of the present study are consistent with previous observations of oocytes from different species having different cell diameters. The microelectrode measurements have indicated that immature frog oocytes 200–300 μm in diameter contain Na^+ at a much higher, and K^+ at a much lower, activity than in the large mature oocytes. For the immature oocytes, $a_{Na} = 16 \pm 1$ mM, and $a_K = 70 \pm 3$ mM; for the mature oocytes, $a_{Na} = 6 \pm 1$ mM, and $a_K = 120 \pm 3$ mM (Table 1). Dick and McLaughlin (1969) have reported intermediate values of Na^+ and K^+ activity for toad oocytes of intermediate size, consistent with the present data.

In contrast to the fall in the ratio (a_{Na}/a_K) of intracellular ionic activities with oocytic development, the ratio (c_{Na}/c_K) of concentrations clearly increases (Tables 1 and 2). This observation in oocytes from *R. pipiens* is consistent with measurements of c_{Na} and c_K in newt oocytes over the size range 0.3–1.8 mm (Riemann *et al.*, 1969) and in oocytes from the toad (*B. bufo*) and frog (*R. temporaria*) over the size range 0.6–2.0 mm (Cannon *et al.*, 1974).

From the microelectrode and spectrophotometric measurements of mature frog oocytes, 1.5–1.6 mm in diameter, γ_{Na}^{app} may be calculated to be 0.08 ± 0.02, and γ_K^{app} lies within the range 1.15 ± 0.03 to 1.29 ± 0.04. The values for both ions are very different from 0.76, the activity coefficient ($\gamma_\pm$) of a 0.1 M KCl solution (Robinson & Stokes, 1959). In fact, these measurements appear to constitute the lowest value for γ_{Na}^{app} and highest value for γ_K^{app} ever reported for biological cells under physiologic conditions (Lev & Armstrong, 1975).

The measurement of a_{Na} is consistent with the conclusions of Horowitz and Fenichel (1970), who proposed that a small fraction of the Na^+ in the cytoplasm of the mature oocyte is free and in equilibrium with the nuclear Na^+, the latter concentration being about 7 mM (Century *et al.*, 1970). The remainder of the cell Na^+ was concluded to be sequestered by some cytoplasmic element. These authors also found a fast exchanging fraction of cytoplasmic Na^+ of similar size using freeze-microdissection (Century & Horowitz, 1974) and autoradiography (Horowitz & Fenichel, 1970).

An alternative picture of the distribution of sodium in the mature frog oocyte has recently been offered by Morrill, Ziegler and Zabrenetsky (1977); some 94% of the cell sodium is seen as being in a free, albeit slowly-exchanging, form, its rate of exchange limited solely by the

greatly reduced surface-to-volume ratio of this large, spherical cell. This concept is based upon two experimental findings; the first is the apparent lack of a rapidly-exchanging sodium fraction in oocytes which have been divested of their epithelial layers following exposure to Ca-free EDTA Ringer's solution, a treatment which has been shown to result in increased sodium permeability (Tupper & Maloff, 1973) and a 36% increase in sodium concentration in these cells (Morrill, Kaback & Robbins, 1964). In spite of this limitation, these "denuded" cells exhibit sodium influx kinetics qualitatively similar to those previously shown for oocyte cytoplasm (Century & Horowitz, 1974), at least as far as slow fraction exchange kinetics are concerned. The experimental protocol followed by Morrill *et al.*, (1977), however, would tend to mask a rapidly-exchanging fraction, since 50–75% of such a fraction would have already exchanged by the time the first point in the kinetics curves was taken. Thus, their data are still consistent with the concept of a small rapidly-exchanging cytoplasmic pool of sodium.

The second pertinent experimental result of Morrill *et al.* (1977) is the unusually large value for a_{Na} (44 mM) which they obtained. This contrasts with 6 mM in the present work, 9.3 mM in *Bufo* oocytes (Dick & McLaughlin, 1969), and 14.3 mM in *Xenopus* oocytes (Slack, Warner & Warren, 1973). Their result also differs strikingly from the value of 16 mM for the cytoplasmic concentration of readily-diffusable sodium in the oocytes of another amphibian (*Desmognathus ochrophaeus*) estimated by a different technique, microinjection and analysis of small volumes of gelatin solutions (Horowitz, Tluczek & Paine, 1977). The origin of the discrepancy is not clear. However, it should be noted that the Na^+-selective glass microelectrodes of Morrill *et al.* (1977) extended 200 μm beyond the insulating sheaths. Those investigators did make a special effort to introduce the entire ion-selective surface into the oocytes impaled. However, exposure of even part of that surface to the surrounding external Na^+-rich medium would factitiously elevate the measured values of a_{Na}. This problem is circumvented when liquid-resin ion-selective microelectrodes are used, as in the present study. Another point of difference is that the tip diameters of the present microelectrodes were approximately an order of magnitude smaller than those used by previous investigators, possibly reducing cell damage associated with the impalement procedure.

The major conclusion of our studies is that $(\gamma_{Na}^{app}/\gamma_{K}^{app})$ is 1–2 orders of magnitude larger in small oocytes than in large mature oocytes, and that γ_{Na}^{app} and γ_{K}^{app} are nearly the same in the small immature oocytes. Thus,

the common observation that $\gamma_{Na}^{app} < \gamma_{K}^{app}$ within the intracellular fluids is probably not a general characteristic of cytoplasm, but rather reflects compartmentalization of ions within subcellular organelles. This concept is strongly supported by the current datum that the apparent activity coefficient for K^+ is much larger within large mature oocytes than within the external Ringer's solution.

Although γ_{Na}^{app} and γ_{K}^{app} are similar, they are not precisely equal within the small immature oocytes. From the data of Tables 1 and 2, $(\gamma_{Na}^{app}/\gamma_{K}^{app})$ may be calculated as the quotient $(a_{Na}/a_K)/(c_{Na}/c_K) = 2.1 \pm 0.3$. If the small, immature oocytes were entirely free of subcellular compartmentalization, we would expect the quotient to equal one.

Both the uncertainty in estimating the water content of the immature oocytes and possible differential shrinkage of the mature and immature eggs could have affected the absolute values calculated for c_{Na} and c_K, but not for their ratio (c_{Na}/c_K). On the other hand, several sources of potential experimental error could have affected the ratio of apparent activity coefficients, by altering the measured values of c_{Na} and c_K in the immature oocytes. First, the extracellular fluid may have been incompletely rinsed free of Na^+ during the immersion in choline Ringer's solution, and freezing may have been too slow to prevent significant exchange of intracellular K^+ for extracellular Na^+. Second, because of the lower density of the immature than the mature oocytes, selective absorption of the Na signal may have been weaker than in the mature oocytes; thus, a larger calibration factor $(k_{Na})_{imm}$ should possibly have been used in calculating the intracellular Na^+ content of the immature oocytes. However, each of these potential artifacts would have falsely reduced the measured value of (c_{Na}/c_K), resulting in an even higher estimate of $(\gamma_{Na}^{app}/\gamma_{K}^{app})$.

It seems to us more likely that the deviation of the ratio from one reflects the fact that (a_{Na}/a_K) was measured in oocytes 200–300 μm in diameter, while (c_{Na}/c_K) was measured in oocytes 90–160 μm in diameter. Whether the compositions of these two different populations of small immature oocytes are sufficiently different to account for the observation, or whether $(\gamma_{Na}^{app}/\gamma_{K}^{app})$ is truly greater than one, can only be resolved by a detailed study of (c_{Na}/c_K) as a function of cell size over the range 90–300 μm.

The identity of the intracellular organelle responsible for compartmentalization of ions within mature oocytes is unknown. Recent studies of *Chironomus* salivary gland suggest that the nucleus is an unlikely site of ionic sequestration (Palmer & Civan, 1975, 1977); in any event, the nucleoplasm occupies only a small fraction of the total cell volume of

these oocytes. Largely on the basis of kinetic data, Dick and Fry (1975) have postulated ionic sequestration in intracellular membrane-bound channels communicating directly with the extracellular fluid; the structural correlate of these putative channels is uncertain.

Development of the oocyte is associated with the appearance of a number of well-defined intracellular elements (Wishnitzer, 1966). The yolk platelets seem a particularly likely site of compartmentalization since the small immature oocyte is free of platelets, while they occupy half the volume of the large mature oocyte. Although the yolk and cytoplasm have been reported to have similar ionic compositions, extensive redistribution of Na^+ and K^+ could have occurred during the preparation of the samples for direct chemical analysis (Riemann *et al.*, 1969). The cytoplasmic vesicles with diameters of up to 200 μm characterizing the mature oocyte (Merriam, 1966; Dick *et al.*, 1970), as well as the pigment granules, may also sequester ions.

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