

SODIUM EXCHANGE IN THE CYTOPLASM AND NUCLEUS OF AMPHIBIAN OOCYTES

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SUMMARY

Oocytes of *Rana pipiens* were incubated in ^{22}Na -Ringer's solution and ^{22}Na - ^{23}Na exchange in the nucleus and cytoplasm followed by low-temperature microdissection. Cytoplasmic sodium consists of 2 kinetic fractions: the larger (88%) slowly exchanging ($t_{\frac{1}{2}} \sim 2$ days), and the smaller (12%) rapidly exchanging ($t_{\frac{1}{2}} \sim 53$ min). Nuclear sodium consists of a single fraction whose concentration is similar and whose rate constant is identical with that of the rapidly exchanging cytoplasmic fraction. Our findings are consistent with a model of compartmentalization in which rapidly exchanging sodium is nearly uniformly distributed in the water of the nucleus and cytoplasm and its exchange limited by movement through the cell surface. On the other hand, slowly exchanging sodium is restricted to the cytoplasm, sorbed to or sequestered in some as yet unidentified cytoplasmic constituent.

INTRODUCTION

In an earlier paper, we described a low-temperature microdissection technique and its application to the determination of sodium, potassium, and water in the nucleus and cytoplasm of the mature amphibian oocyte (Century, Fenichel & Horowitz, 1970). The oocytes of 4 species were studied, and all exhibited a striking nucleocytoplasmic sodium inequality. Cytoplasmic sodium averages 74.1 mequiv./l. H_2O (two-thirds the concentration in amphibian serum), while nuclear sodium is 7.3-16.8 mequiv./l. H_2O .

Analysis by ultra-low temperature autoradiography of ^{22}Na exchange in the oocytes of the salamander *Eurycea bislineata* provided an explanation for the asymmetry (Horowitz & Fenichel, 1970). Oocytic sodium consists of 2 fractions differing in exchange rate and intracellular distribution. About 15% exchanges rapidly ($t_{\frac{1}{2}} \cong 30$ min) at a rate determined by permeation of the cell surface. This 'fast' or freely exchangeable fraction, which distributes throughout the cell, is thought to be sodium in solution whose cellular level is maintained by a pump. The remaining 85% is slowly exchanging sodium ($t_{\frac{1}{2}} \cong 2.5$ days) found only in the cytoplasm and considered bound or sequestered in cytoplasmic organelles (Horowitz & Fenichel, 1970; Dick & Fry, 1973). Hence, the nucleocytoplasmic difference is due to a large,

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relatively immobile cytoplasmic fraction; the nucleus and cytoplasm sharing only a smaller, rapidly exchanging sodium fraction.

In this study, we used the low-temperature microdissection technique to investigate the ^{22}Na exchange in the nucleus and cytoplasm of *Rana pipiens* oocytes incubated in ^{22}Na -Ringer's solution. We have achieved a better quantitative comparison of nuclear and cytoplasmic exchange than is possible by autoradiography. Our results show the model proposed for *Eurycea* oocytes is also applicable to those of *Rana* and probably to amphibian oocytes in general.

MATERIALS

Oocytes

Rana pipiens from New Jersey were kept in spring water at 4 °C. Only mature oocytes from healthy females were used. Oocyte diameters and wet weights were similar to those in the previous study (Century *et al.* 1970).

Solutions

The amphibian Ringer's solution contained 92.7 mM NaCl, 2.5 mM KCl, 1.0 mM CaCl₂, 1.2 mM MgCl₂, 17.3 mM NaHCO₃, 2.0 mM NaH₂PO₄, 1.2 mM Na₂HPO₄, and 24.0 mM glucose. The pH was 7.2–7.3.

^{22}Na obtained from New England Nuclear Corporation, Boston, Massachusetts as carrier-free NaCl in 0.5 N HCl containing 2.92 mCi/ml. Portions were frozen and lyophilized, and Ringer's added to achieve a final activity of 125 $\mu\text{Ci/ml}$. Excess Na represented by ^{22}Na in the final incubation fluid was only 2.4×10^{-6} that of the Na of normal Ringer's solution and was ignored.

METHODS

Incubation

Incubations were carried out at 20.2 °C. Clusters containing 8 mature oocytes each were dissected free from an ovarian lobe in Ringer's solution at 0 °C and brought to incubation temperature. After 1 h the Ringer's solution was drained off and replaced with ^{22}Na -Ringer's. At preselected times, *t*, a cluster was removed, blotted, rinsed in ice-cold water (less than 0.05% of the fast fraction sodium was lost in this rinse), and placed on a stainless steel mounting for freezing.

Microdissection

The microdissection apparatus, preparation of oocytes for microdissection, isolation of nucleus and pieces of cytoplasm, and determination of water and sodium contents have been described in detail elsewhere (Century *et al.* 1970). Briefly, oocytes are frozen rapidly by quenching in liquid Freon-12 at -155 °C and then are dissected by hand on a -40 °C cold stage in a dry glove box. Isolated nuclei and fragments of cytoplasm are collected in tared Parafilm envelopes which serve as containers for the cell fragments during subsequent analytical procedures.

Each nuclear and cytoplasmic experimental point is derived, respectively, from the pooled nuclei or cytoplasm of the 8 oocytes of a cluster.

Analysis

Water was determined by sample drying at 50 °C over P₂O₅; sodium by flame photometry of an aqueous sample extract (with appropriate controls for contamination) as previously described (Century *et al.* 1970). Sodium-22 was measured by counting an aliquot of the extract in Bray's solution in a Packard Tri-Carb scintillation spectrometer. Counting errors were kept below 1.5 %.

Cytoplasm was taken at random locations in the oocyte. Sampling errors were apparently negligible since water and sodium in samples taken from the vegetal and animal hemispheres at opposite ends of the oocyte were not significantly different. Previously we found, by autoradiography, that fast-fraction cytoplasmic ²²Na also is uniformly distributed (Horowitz & Fenichel, 1970).

RESULTS

The concentrations of sodium in the cytoplasm, [Na]^c, and nucleus, [Na]ⁿ, were 61.8 ± 3.1 (S.E.M.) and 7.1 ± 0.3 μequiv./ml H₂O, respectively; there was no systematic variation with incubation time. These values agree well with our previous results (Century *et al.* 1970).

The time course of ²²Na-²³Na exchange is shown for cytoplasm and nucleus in Fig. 1. Two distinct kinetic fractions are discernible in the cytoplasm: a rapidly exchanging fraction, Na_f^c, which accounts for 12 % of the cytoplasmic sodium, and which completely exchanges in less than 3 h; and the remaining 88 % of the cytoplasmic sodium, Na_s^c, which exchanges extremely slowly, having a half-time of about 50 h.

Nuclear exchange, however, is described by a single exponential curve whose rate is similar to that of the cytoplasmic fast fraction (Fig. 2).

The solid lines in Fig. 1 represent the equation

$$\frac{[^{22}\text{Na}]_t}{[\text{Na}]_t} = 1 - \alpha e^{-k_f t} - \beta e^{-k_s t}, \quad (1)$$

where [²²Na]_t and [Na]_t are the concentrations of exchanged and total sodium in either nucleus or cytoplasm at time *t*. The rate constants are $k_f = 2.2 \times 10^{-4} \text{ s}^{-1}$ and $k_s = 3.9 \times 10^{-6} \text{ s}^{-1}$, and α and β the relative sizes of the fast and slow fractions, respectively. For cytoplasm, $\alpha = 0.115$ and $\beta = 0.885$; for the nucleus, $\alpha = 1.0$ and $\beta = 0$.

The rate constants for fast and slow fractions are similar in magnitude to those in *Eurycea* at 20.0 °C, which were $2.9\text{--}7.7 \times 10^{-4} \text{ s}^{-1}$ and $3.2 \times 10^{-6} \text{ s}^{-1}$, respectively (Horowitz & Fenichel, 1970). k_f for immature *Bufo* oocytes, at 17–23 °C, is $2.7 \times 10^{-4} \text{ s}^{-1}$ (Dick & Lea, 1964). The microvillous surface of oocytes makes comparison of permeabilities difficult among species and oocyte stages (see Dick, Dick & Bradbury, 1970a, and Massover, 1973).

Detailed comparison of the sodium influx into the nucleus with that into the cytoplasm provides information on the quantitative relationship of the transport barriers governing exchange in these compartments. Nuclear sodium is all fast fraction; its exchange is rapid, and data on the earliest fluxes are required for kinetic

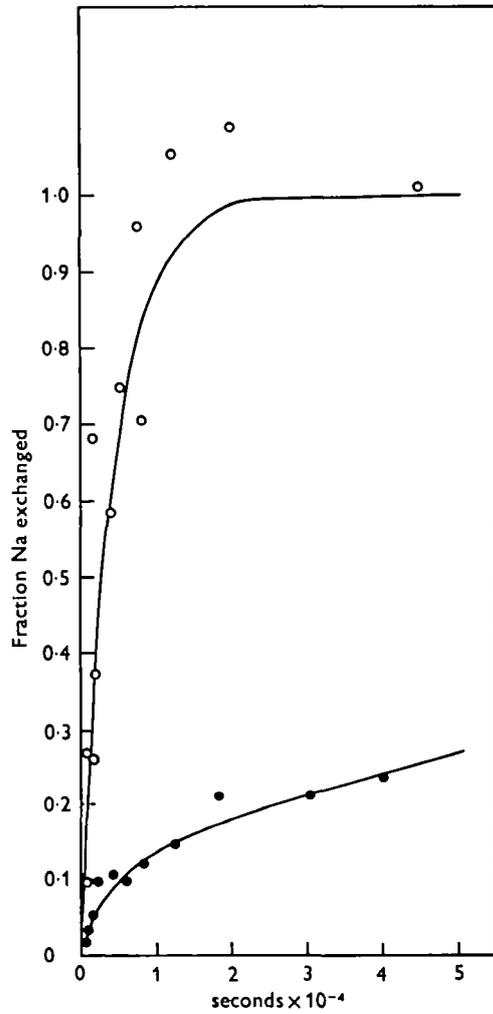


Fig. 1

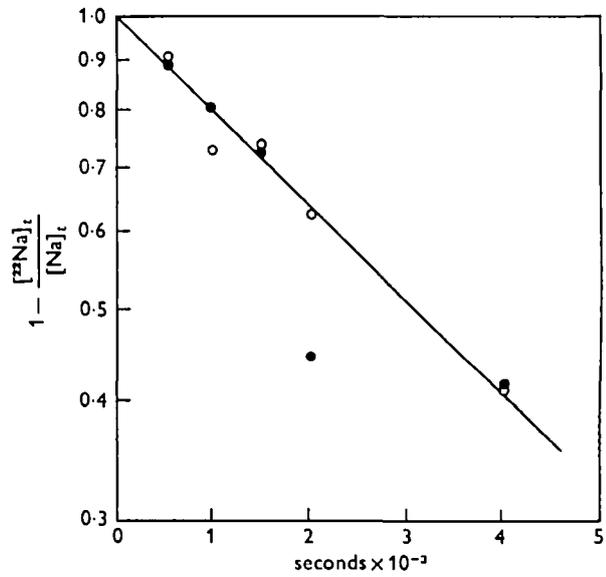


Fig. 2

Fig. 1. Time-course of sodium exchange in nucleus and cytoplasm. Points are experimental, representing the average of 3 determinations of the pooled nuclei or cytoplasmic fragments of 8 oocytes. Solid lines are theoretical, representing equation 1, for the nucleus (upper curve), $\alpha = 1.0$, $\beta = 0$, and $k_f = 2.2 \times 10^{-4} \text{ s}^{-1}$; for the cytoplasm (lower curve), $\alpha = 0.115$, $\beta = 0.885$, $k_f = 2.2 \times 10^{-4} \text{ s}^{-1}$, and $k_c = 3.9 \times 10^{-6} \text{ s}^{-1}$.

Fig. 2. Early exchange of sodium in the nucleus and rapidly exchanging cytoplasmic fraction. Points are experimental; the open circles for the nucleus and filled circles for cytoplasm. The slope of the line is $2.2 \times 10^{-4} \text{ s}^{-1}$. See text for details.

analysis. This is provided in Fig. 2, where sodium exchange in the nucleus and cytoplasm is shown for the earliest influx times. Sodium exchange here is expressed as $1 - ([^{23}\text{Na}]_t / [\text{Na}]_t)$ against time, so that the slope of the line directly yields the rate constant for the exchange. The contribution of k_c has been subtracted from the cytoplasmic data, so that the solid points in Fig. 2 represent exchange into the rapidly exchanging cytoplasmic sodium fraction. The slope of the solid line is

$2.2 \times 10^{-4} \text{ s}^{-1}$. No discernible differences appear in the rate of filling these compartments. We surmise that no diffusion or permeation barrier more restrictive than the plasma membrane lies between the nucleus and the extracellular compartment. Neither does a short-circuit route bypass the cell membrane or cytoplasm for sodium movement from the extracellular space to the nucleus.

DISCUSSION

Qualitatively and quantitatively, sodium transport in the oocyte of *Rana pipiens* is similar to that previously described for the salamander *Eurycea bislineata* (Horowitz & Fenichel, 1970). Two exponential kinetic fractions account for all the intracellular sodium in the oocytes of both species, and these fractions are alike in magnitude, kinetic behaviour, and compartmental distribution. Similarly, available data on the nucleocytoplasmic distribution of sodium in mature oocytes of other amphibian species agree at least qualitatively with the *Rana pipiens* results (cf. *Desmognathus ochrophaeus*, *Rana catesbiana* (Century *et al.* 1970), and *Triturus cristatus* (Riemann, Muir & Macgregor, 1969; Muir & Whitley, 1972)), while the extensive work of Dick and his co-workers (Dick & Lea, 1964, 1967; Dick & McLaughlin, 1969; Dick, Fry, John & Rogers, 1970*b*) on the immature oocytes of *Bufo bufo* are likewise consistent with a 2-compartment model (Horowitz & Fenichel, 1970).

At variance with this general picture is Muir & Whitley's (1972) finding that maturing *Triturus* oocytes undergo a transient rise in nuclear sodium to almost 100 mequiv./l. nuclear volume when the oocyte is about 0.5 mm in diameter. This striking observation deserves confirmation and analysis, as it alone suggests the oocyte nucleus at any stage contains sodium other than the low concentration of passively distributed fast fraction.

Recent workers agree that the rapidly exchanging fraction is in aqueous solution and its low level is maintained by a cortical sodium pump (Dick & Lea, 1967; Horowitz & Fenichel, 1970; Tupper & Maloff, 1973). This sodium diffuses freely in cytoplasm, with a rate apparently differing from that in water only because of the tortuous diffusional path caused by the presence of cytoplasmic inclusions (Horowitz & Fenichel, 1970). Fig. 2 shows that this fraction is not slowed in passing from cytoplasm to nucleus.

The nuclear:cytoplasmic ratio of fast-fraction sodium, $[\text{Na}]_n^f/[\text{Na}]_c^f$, is 1.2 as estimated from Fig. 1 and $[\text{Na}]_n$ and $[\text{Na}]_c$. In *Eurycea* oocytes, the value is 1.3. The difference between the species is probably not significant. Marked nucleocytoplasmic asymmetries have been observed in the distribution of a number of substances. They are in part attributable to the partial exclusion of solute from cytoplasmic water and may be a general property of cytoplasm (Horowitz & Moore, 1974).

The origin and significance of the slow fraction is the outstanding unresolved problem in understanding oocyte sodium. This kinetic fraction appears to account for the low sodium activity coefficient measured with sodium-sensitive electrodes in *Bufo* oocytes (Dick & McLaughlin, 1969). It clearly is analogous and may be homologous with 'bound' sodium in other cell types, as detected electrometrically

(Hinke, 1961; Lev, 1964; McLaughlin & Hinke, 1966) and by isotopic exchange methods (Troshin, 1957; Burnstock, Dewhurst & Simon, 1963; Dunham & Gainer, 1968; Keynes & Steinhardt, 1968).

Slowly exchanging sodium is restricted to oocyte cytoplasm. This has been demonstrated by microdissection in the present report and autoradiographically (Dick *et al.* 1970*b*; Horowitz & Fenichel, 1970). The fraction increases with increase in total cell sodium (Dick & Lea, 1964), which in turn increases dramatically in the later stages of maturation (Cannon & Dick, 1970), when yolk accumulation also is maximal. It decreases during embryogenesis (Slack, Warner & Warren, 1973), when yolk platelet density declines. This suggests the yolk platelet may be the site of sodium 'binding'.

In the mature oocyte, yolk is uniformly distributed throughout the cytoplasm, and so apparently is sodium. In the immature oocyte, yolk occupies a peripheral zone, although the autoradiographs of Dick *et al.* (1970*b*) do not demonstrate a comparable slow-fraction distribution. This observation may exclude yolk as a candidate for the Na-binding organelle; we believe that the autoradiographic resolution of the Dick study was insufficient to allow this conclusion to be accepted without reservation.

Dick *et al.* (1970*b*) suggested that cytoplasmic vesicles seen in electron micrographs may contain sequestered sodium. Vesicles and yolk platelets are the most prominent organelles in oocyte cytoplasm. These authors point out that, although water content of vesicles is insufficient to account for the slow fraction on a uniform distribution basis, the vesicles may accumulate sodium against a concentration gradient. (A similar idea was suggested by Merriam, 1966.) Vesicles are spherical, optically empty and have diameters up to 150–200 nm (Wischnitzer, 1966). If they accumulate sodium, their membranes must have extraordinarily low permeabilities. The ratio k_i/k_o is 56; that of the surface-to-volume ratio of vesicle to oocyte is not less than 7.5×10^3 , and greater, by a factor of 2–11 (Dick *et al.* 1970*a*), if the microvillous character of the oocyte surface at the various stages of development is considered. Hence, the vesicle membrane must be at least 4.1×10^5 times less permeable than the oocyte membrane. Similarly, were the yolk platelet the site of sequestration and its membrane limiting, the reduction in permeability would be at least 9.3×10^3 .

The low permeabilities implied by organelle-membrane models of binding can be viewed as favouring sorption models in explaining the slowly exchanging sodium fraction. Horowitz & Fenichel (1970) suggested that the crystalline array of yolk phospholipids contains Na⁺ ions as counter-ions which exchange with great difficulty. However, no direct evidence for this exists and the interpretation does not agree with autoradiographic observations on immature oocytes mentioned above.

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