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Distribution of Na^+, K^+ and Cl^- between Nucleus and Cytoplasm in *Chironomus* Salivary Gland Cells

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Summary. Previous studies of the electrochemical activity coefficients of intracellular Na^+ and K^+ have suggested that the free form of these ions may be unevenly distributed within the intracellular fluids. One possible site of such subcellular compartmentalization is the cell nucleus. In order to examine this possibility, the cells of *Chironomus* salivary glands were studied with conventional liquid ion-exchange microelectrodes sensitive to K^+ and Cl^-, with a new liquid ion-exchange microelectrode sensitive to Na^+, and with open-tipped micropipets. Both the electrochemical activities for Na^+, K^+ and Cl^-, and the electrical potential were the same on both sides of the nuclear membrane. The possibility was considered that a difference in the junction potentials within the nucleoplasm and cytoplasm might have masked a real difference in electrical potential between these two phases. To study that possibility, changes were induced in the junction potentials by altering the composition of the fluid filling the exploring micropipets. The results have suggested that the magnitudes of the junction potentials are the same on both sides of the nuclear envelope. The simplest interpretation of the data is that the chemical activities of Na^+, K^+ and Cl^- are the same within the nucleus and cytoplasm. This suggests that other subcellular structures, possibly the endoplasmic reticulum and mitochondria, are responsible for subcellular compartmentalization.

The ionic composition of the intracellular fluids is a crucial factor in many cellular processes. Similarly, the functions of subcellular organelles may depend on ionic composition, and this composition may be regulated in some cases independently of that of the cytoplasm. For example, mitochondria have been shown to possess active transport systems (Lehninger, 1965), and the sarcoplasmic reticulum of skeletal muscle is thought by some authors to exchange ions directly with the extracellular fluid (Birks & Davey, 1969; Rogus & Zierler, 1973).

The activities of Na^+ and K^+ in the cell nucleus may be important in the regulation of such cellular processes as development and metabolism. Lezzi and Gilbert (1970) found that the activity of isolated chromosomes depends on the relative activities of Na^+ and K^+ in the medium. Allfrey, Muedt, Hopkins & Mirsky (1961) showed that in isolated nuclei

the transport of amino acids and nucleotides across the nuclear membrane was stimulated by Na^+ and not by K^+ or other cations.

The regulation of nuclear electrolytes may be controlled by "pores" which perforate the nuclear envelope (Fry, 1970). Measurements of electrical resistance have demonstrated that the envelope of some cells is a significant barrier to the movement of ions (Loewenstein & Kanno, 1963; Ito & Loewenstein, 1965). In *Chironomus thummi* salivary glands the resistance is three orders of magnitude larger than is predicted from free diffusion through the "pores" if they consisted solely of simple aqueous channels (Wiener, Spiro & Loewenstein, 1965). Payne (1975) has shown that this discrepancy is smaller if the radius of these pores is taken to be 45 Å, as indicated by the movement of macromolecules across the nuclear membrane, rather than 250 Å as observed in electron microscope images.

Some investigators have, in addition, postulated the existence of a direct pathway for ion exchange between the nucleoplasm and the extracellular fluid through the endoplasmic reticulum (Watson, 1955; Epstein, 1957; Palay, 1960; Siebert & Langendorf, 1970). This pathway could provide a mechanism for the establishment of gradients in ion activity across the nuclear membrane.

Previous measurements of the ionic composition of the nucleoplasm and cytoplasm of several tissues are shown in Table 1. Agreement among the various studies is far from complete. In some cases, however, large concentration differences are indicated. Measurements of the apparent activity coefficients of Na^+ and K^+ in cells also suggest that these ions are distributed inhomogeneously in a variety of cells (Lev & Armstrong, 1975). In particular, the general finding that Na^+ activity within cells is smaller than expected from measurements of total cellular concentration implies that part of the Na^+ in the cell is either bound to macromolecules or, alternatively, is contained in some subcellular organelles at an activity higher than that in the cytoplasm. The latter phenomenon will be referred to as "compartmentalization".

The purpose of the present study was to determine the extent of compartmentalization of Na^+, K^+ and Cl^- by the nucleus, by directly measuring the activities of these ions in both nucleoplasm and cytoplasm. The salivary gland cells of *Chironomus* larvae were chosen as the model system both because the large size of the cells and nuclei permits simultaneous recording from several electrodes in a single cell, and because the nuclear membranes are known to have relatively high electrical resistance. Preliminary reports of the data obtained for K^+ (Palmer & Civan, 1975) and Cl^- (Palmer & Civan, 1976) have been presented elsewhere.

Table 1. Na^+ and K^+ contents of different tissues measured with different techniques

Preparation Method Reference	Nucleus		Cytoplasm		Ratio	
	Na^+ (mM)	K^+ (mM)	Na^+ (mM)	K^+ (mM)	Na^+	K^+
Rat liver Density gradient separation Siebert & Langendorf (1970)	121	301	11	173	11	1.7
Frog oocyte Freeze-microdissection Naora *et al.* (1962)	281	258	88	106	3.2	2.4
Frog oocyte Freeze-microdissection Century *et al.* (1970)	7.3	126	59	94	0.12	1.3
Puffer fish nerve Freeze-dry-microdissection Katzman *et al.* (1969)	86	113	102	134	0.84	0.84
Frog red blood cell Electron probe Ingram *et al.* (1974)					0.9[a]	1.8[a]
Frog skin Electron probe Dörge *et al.* (1974)					1[a]	1[a]

[a] Measurements made in units of mmoles/kg dry wt.

Materials and Methods

Animals

Chironomus larvae were obtained year-round from a local pond. The species was identified as *Chironomus Chironomus attenuatus* (Walker) by Dr. S.S. Roback. Judging from the development of the imaginal discs (Kroeger, 1964), most of the larvae were in the late fourth instar stage when the glands were isolated. Glands were exised from the larvae into an insect Ringer's solution, where they were used until the cells became swollen or opaque, or until their membrane potential fell substantially.

Solutions

Ringer's solution used in the K^+ electrode experiments had a measured osmolality of 293 mOsm and contained (in mM): NaCl, 135; KCl, 5; $MgCl_2$, 3; $CaCl_3$, 2; NaH_2PO_4, 3.0; Na_2HPO_4, 4.2; and glucose, 12; at a pH of 6.5–6.6. A more dilute solution (Robert, 1971) was used in the Na^+ and Cl^- electrode measurements, because it was found that chromosome structure was better preserved by this medium. The latter Ringer's solution had a measured osmolality of 187 mOsm, and contained (in mM): NaCl, 87; KCl, 3.2; $MgCl_2$, 1; $CaCl_2$, 1.3; NaH_2PO_4, 0.8; and Na_2HPO_4, 2.85; at a pH of 7.2. The media

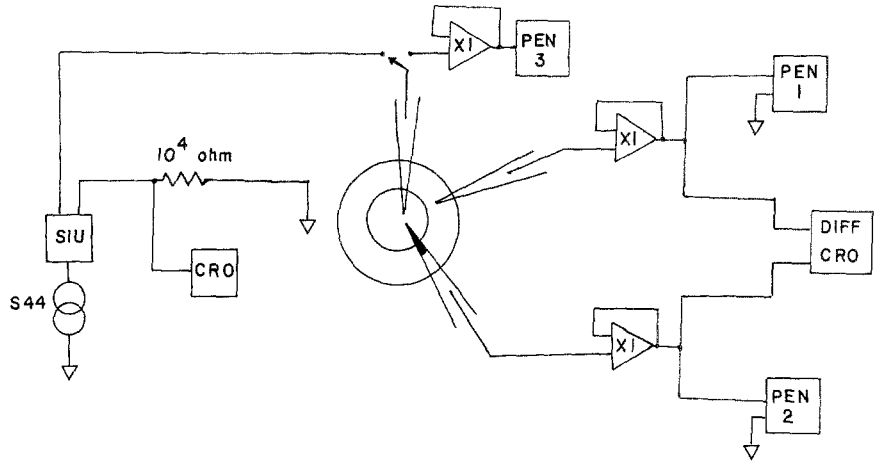


Fig. 1. Circuit used for the Cl^- and Na^+ measurements in the nucleus and cytoplasm. Experiments in which K^+ was measured were done with a similar circuit, but without the stimulus isolation unit (SIU). In this case a large (10^8 ohm) resistor was placed in series between the stimulator (S44) and the current passing electrode, to insure pulses of constant current

could usually sustain the glands for several hours. The more rapid deterioration of some glands was probably due to damage from repeated microelectrode impalements.

Optics

The glands were observed at a magnification of 250× with a Leitz compound microscope fitted with Nomarski optics. The Nomarski system improved contrast and allowed optical sectioning of the specimen. This sectioning was important in determining the vertical position of the electrodes relative to the nucleus. When the electrodes were introduced into the gland, the image was finely focused with the objective lens of the microscope, which was attached to an electromagnetic focusing device (Wild Herrbrugg, Farmingdale, N.Y.) machined to fit the Leitz objective.

Manipulation

Electrodes which were to be positioned in the nucleus were mounted on either Leitz micromanipulators, or on modified Huxley micromanipulators (Huxley, 1961). Electrodes which were to be positioned in the cytoplasm were mounted on Narashige manipulators.

Electronics

The electrode outputs were connected to preamplifiers (Instrumentation Laboratory, Watertown, Mass.) which had input impedances of at least 10^{13} ohms and bias currents of less than 10^{-12} A. The output from the preamplifiers was read on paper chart recorders and either singly or differentially on a memory oscilloscope. The circuit for the measurement of nuclear ion activities is shown in Fig. 1.

Electrodes

Micropipets were drawn from Corning 7740 glass, 0.8 mm o.d., 0.6 mm i.d., on a vertical puller (David Kopf Instruments, Tujunga, California). Micropipets used for measuring electrical potential and for passing current were then fitted with 3M KCl. Only those with resistances of 5 to 20 Mohms and tip potentials of less than 5 mV were used. Electrical contact was made between the electrolyte and the preamplifier through Ag-AgCl pellets (Martin, Wickelgren & Beránek, 1970).

Ion-selective electrodes were made from micropipets of similar dimensions. Immediately after being drawn, they were coated with dimethyldichlorosilane vapor. Best results were obtained with a 60 to 120 sec exposure to vapor from a solution of 5% silane in xylene. Immediately after coating, the pipets were baked at about 100 °C for one hr. They were used immediately, or stored in a dessicator for up to 2 days. To fill the electrodes, a drop of liquid ion exchange resin was placed in the shank of the electrode where it began to taper, and was either forced into the tip with positive pressure from a syringe, or drawn into the tip by exposing it to a vacuum.

K^+ (Corning 477317) and Cl^- (Corning 477315) liquid ion exchange resins were obtained from Corning Glass (Corning, N.Y.). Na^+ exchanger was made by dissolving $K(\phi Cl)_4B$ in triethylhexyl phosphate (K & K, Plainview, N.Y.) to make a 1.5% solution; the $K(\phi Cl)_4B$ was a generous gift from Dr. George Baum. After the electrode tips were filled with exchanger, the shanks were filled with 0.5 M KCl (K^+ and Cl^- electrodes) or 0.5 M NaCl (Na^+ electrodes). Resistances for the ion-selective electrodes were 5×10^9 to 2×10^{10} ohms, about 1000 times higher than for the micropipets filled with 3 M KCl. Electrodes were calibrated before and after each experiment using simple salt solutions. K^+ electrodes were typically calibrated in solutions containing 100, 50 and 10 mM KCl with sufficient NaCl added to maintain the total concentration constant at 100 mM. Cl^- electrodes were calibrated in 100, 50 and 10 mM KCl. Na^+ electrodes were calibrated in 100 and 10 mM NaCl, and in 0 and 50 mM NaCl in the presence of 150 mM KCl. All electrodes were also calibrated in the Ringer's solution used. Calibration curves of the voltage outputs from the K^+ and Cl^- microelectrodes were plotted as semilog functions of the ion activity. Intracellular activities of K^+ and Cl^- were calculated by subtracting the membrane potential from the potential of the ion-selective electrode, and reading the activity corresponding to that voltage directly from the calibration curve.

In the case of the Na^+ measurements, the interference from the intracellular K^+ had to be taken into account. The activity was calculated from the equation:

$$E = E_0 + 59 \log(a_{Na} + k a_K) \tag{1}$$

where k is an empirical constant calculated from the calibration data. Values of k ranged from 0.28 to 0.38.

A more flexible equation is given by:

$$E = E_0 + S \log(a_{Na} + k a_K) \tag{2}$$

where S is an additional empirical constant (Lev, 1964). Measured values of S were between 61 and 66 mV. Na^+ activities calculated from the two equations were not significantly different, the difference being at most 2 mM.

When a K^+ electrode was used simultaneously with the Na^+ electrode, a_K was measured directly and introduced into Eqs. (1) or (2) to calculate a_{Na}. In experiments where the difference in Na^+ activity across the nuclear membrane was measured, no K^+ electrode was used. Here, the apparent K^+ concentration was estimated from the relationship established in experiments where both electrodes were used:

$$C_K + C_{Na} = 120 \text{ mM}$$

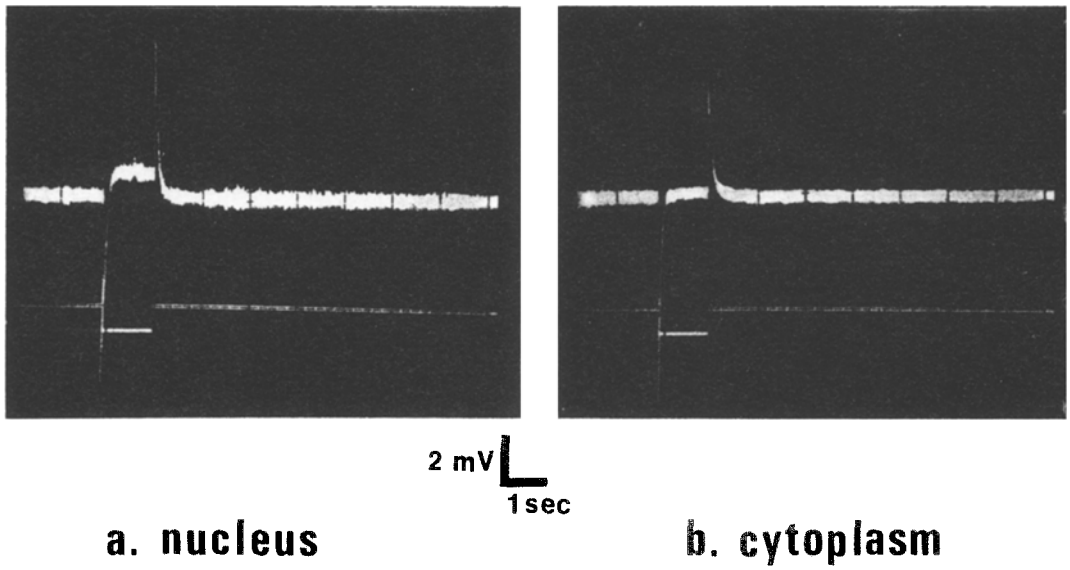


Fig. 2. Typical responses when the ion-selective electrode was in the nucleus (*a*) and in the cytoplasm (*b*). The upper trace in each picture represents the voltage difference between the ion-selective electrode and a reference micropipet in the cytoplasm of the same cell. Positive current was passed from a second micropipet in the nucleus to a grounded electrode in the external solution. The steady state voltage deflection in the upper picture is the voltage drop across the nuclear membrane. The lower trace in each picture is the voltage across a 10^4 ohm resistor in series with the nuclear and plasma membranes as shown in Fig. 1

The maximum uncertainty in the estimation of C_K by this method was about 10 mM, which corresponds to an uncertainty of about 3 mM in the calculation of Na^+ activity. Since C_K was found to be the same in nucleus and cytoplasm, this error did not affect the measurement of the difference in Na^+ activity in nucleus and cytoplasm.

For simplicity, ion activities are expressed in terms of apparent concentration. This is calculated by dividing the measured activities by 0.76, the activity coefficient of a 0.1 M KCl solution (Robinson & Stokes, 1959).

Experimental Procedure

The gland was focused in the visual field of the microscope and three electrodes were placed one at a time into the same cell. One micropipet was positioned in the nucleus of the cell, and another in the cytoplasm. The ion-selective electrode was usually positioned first in the nucleus. The positions of the electrodes were verified by focusing through the nucleus. If the electrode tip appeared and disappeared during the optical sectioning of the nucleus, it was assumed to be in the nucleus. A pulse of either inward or outward constant current, usually 10^{-7} A for 1 sec, was passed through the micropipet in the nucleus, across both the nuclear and outer cell membranes to an electrode in the Ringer's solution; the voltage difference between the ion-selective electrode (in the nucleus) and the second micropipet (in the cytoplasm) was monitored. A representative trace is shown in Fig. 2.

The steady-state change in the voltage difference divided by the magnitude of the current gives the resistance of the nuclear envelope. If there was no steady-state difference, it was assumed that the nuclear membrane had been damaged, or that the prescribed positioning was incorrect, and the measurement was rejected. If a resistance was measured, the open circuit voltages of all three electrodes were noted, before and after moving the ion-selective electrode to the cytoplasm. Then, an identical current pulse was passed, as a control, and if there was no steady-state change in the voltage difference (as in Fig. 2), the voltages were noted again and the electrodes were removed from the cell. The measurement was rejected: (1) if an apparent resistance was measured between the electrodes that were both presumed to be in the cytoplasm of the same cell, (2) if the resting potential of the cell changed substantially during the measurement, or (3) if the electrodes failed to return to their previous voltages, within 2 mV, when withdrawn from the cell. A maximum of four successful measurements was taken with one gland.

In some experiments, both a Na^+ and a K^+ electrode were used, along with a single micropipet. The three electrodes were all placed in the same cell, usually in the cytoplasm, but no current pulses for verifying the positions were used.

In a third type of experiment, three micropipets were used. One was filled with 3 M KCl and was used only to pass current. The other two were used to measure the cell potential; one was filled with 3 M KCl and the other with a different electrolyte, to study the liquid junction potential between the micropipet and the cell as a function of the electrolyte. Here, the two voltage-sensing pipets were placed either both in the cytoplasm or both in the nucleus. Pulses of current were applied; the absence of measurable resistance between the two micropipets verified that they were in the same cellular compartment. The voltage difference between these two electrodes was recorded both in the cell and in the Ringer's solution. The change in this voltage difference between the Ringer's solution and cell was used as a measurement of the difference in the liquid junction potential.

Results

The measurements of electrical potential and of the activities of Na^+, K^+ and Cl^- in the cytoplasm and the nucleoplasm are summarized in Table 2. No significant gradient in either the electrical potential or the chemical activity was observed across the nuclear membrane for any ion.

The input resistance of the glands was not routinely measured. In those experiments where it was measured, seven glands had an input resistance of $(2.6 \pm 0.6) \times 10^5$ ohms (mean $\pm$ SD).

The mean values entered in Table 2 are derived from measurements characterized by a great deal of scatter. For example, the difference in electrical potential across the plasma membrane varied from 6 to 67 mV. Therefore, although the mean values tabulated were not significantly different from zero, it was possible that one or more subpopulations of the cells studied were, in fact, characterized by ionic and electrical gradients across the nuclear membrane. For example, only cells with large membrane potentials might have been able to sustain such gradients.

Table 2. Electrolytes in nucleus and cytoplasm

	K^+	Na^+	Cl^-
C^{app} (cytoplasm) (mM)	135 ±45	16 ±16	40 ±8
ΔV (mV)	−1.2±4	−0.3±1.1	−0.5±2.4
ΔE (mV)	+0.5±1.6	−0.2±1.1	+0.5±2.4
ΔC^{app} (mM)	+11 ±29	−0.4±4.9	−1.0±2.8
n	17	30	16

Results are expressed as mean and standard deviation.

C^{app} is the apparent concentration, calculated from the measured activity divided by 0.76, the mean activity coefficient for KCl in the Ringer's. Values from the cytoplasm are given.

ΔV is the membrane potential in the nucleus minus that in the cytoplasm.

ΔE is the potential of the ion-selective electrode in the nucleus minus that in the cytoplasm.

ΔC^{app} is the apparent concentration in the nucleus minus that in the cytoplasm.

n is the number of observations. Each observation represents a different cell with a maximum of four observations from a single animal.

The K^+ data were obtained from cells bathed in a Ringer's solution with an osmolality of 293 mOsm. In obtaining the Na^+ and Cl^- data, a Ringer's solution with an osmolality of 187 mOsm was used. The absolute values of the concentrations of the three ions are, therefore, not directly comparable.

In order to examine this possibility, the correlation between the measured nuclear and cytoplasmic activities was studied, as in Fig. 3. When the output of the K^+ or Na^+ electrode in the cytoplasm (E_K(cyto) or E_{Na}(cyto)) is plotted as a function of the corresponding output in the nucleus (E_K(nuc) or E_{Na}(nuc)), the correlation is nearly perfect; the slopes (±95% confidence limits) of the linear regression lines are 0.96±0.05 and 0.99±0.02 for K^+ and Na^+, respectively. No points fall very far from the lines of identity presented in Fig. 1*A* and *B*. The correlation coefficient is 0.991 for K^+, and 0.998 for Na^+.

When the calculated apparent concentrations of the two ions in the nucleus are plotted against the corresponding values in the cytoplasm, the slopes of the regression lines are still not significantly different from unity (Fig. 1*C* and *D*). The slopes are 0.88±0.28 for K^+ and 0.90±0.10 for Na^+. The correlation coefficients are smaller, 0.81 for K^+ and 0.94 for Na^+. This increased scatter probably reflects the cumulative error associated with calculating the C^{app} values from measurements with 3 electrodes, while the values for E_K and E_{Na} were obtained with single electrodes.

Part of the variation in the measurements of membrane potential and ion activity may arise from damage inflicted by repeated impalements. In most experiments, the membrane potential obtained with consecutive impalements within a single gland fell by less than 10 mV. In some cases, however, the fall was greater than 10 mV, and the ion activities changed substantially. If these data are omitted, the apparent ion concentrations are: $C_K^{app} = 144 \pm 46$, $C_{Na}^{app} = 13 \pm 13$, $C_{Cl}^{app} = 39 \pm 7$. Here too, the mean nuclear *vs.* cytoplasmic concentration differences remain near zero: $\Delta C_K = +4 \pm 24$, $\Delta C_{Na} = +0.01 \pm 5$, $\Delta C_{Cl} = -0.4 \pm 3$ (means $\pm$ SD, where n = 12,21 and 12 for K^+, Na^+ and Cl^-, respectively).

The cytoplasmic electrochemical potential of each of the three ions studied was found to be significantly different from that in the external bathing solution. K^+ was observed to have been accumulated against an electrochemical potential difference in every cell studied. Similar electrochemical gradients have been reported for certain other epithelial tissues such as frog skin (Janáček, Morel & Bourguet, 1968) and frog intestine (Lee & Armstrong, 1972). On the other hand, Khuri *et al.* (1972) found K^+ to be near electrochemical equilibrium in the proximal tubular cells of *Necturus* kidney.

In the present study, Cl^- was also found accumulated in salivary gland cells against an electrochemical gradient. Khuri *et al.* (1975) reported similar results for Cl^- in *Necturus* proximal tubule.

In contrast to the data obtained for K^+ and Cl^-, the electrochemical activity of Na^+ within the cytoplasm was considerably lower than the equilibrium value, as is the case in most biological cells.

The mean measured resistance of the nuclear membrane was 13 kilohms. Assuming a mean nuclear diameter of about 70 μ, the resistivity of the nuclear membrane was calculated to be 2 ohm $\times$ cm^2, although considerable variation was observed. This is consistent with the results of Loewenstein (1964) and of Ito and Loewenstein (1965) who reported similar resistivities for the nuclear membrane of *Chironomus thummi* salivary glands. No consistent potential differences were seen across the nuclear membrane. Loewenstein (1964) reported nuclear potentials of 15 mV negative to the cytoplasm. Ito and Loewenstein (1965), however, found potential differences of only a few millivolts, consistent with our results.

The sources of error that are considered to have possible significant effects in this system are: (1) limited selectivity of the ion-selective microelectrodes, (2) interference with these microelectrodes from proteins and other cellular macromolecules, and (3) liquid junction potentials which

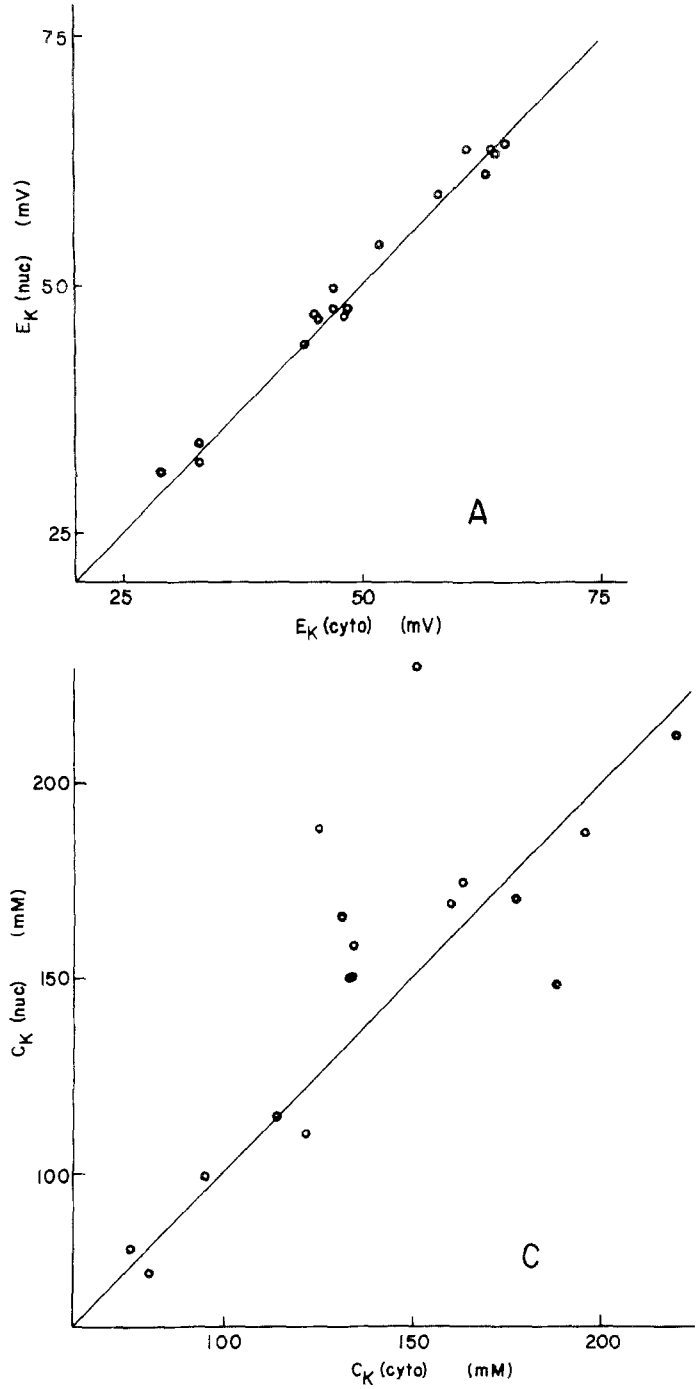
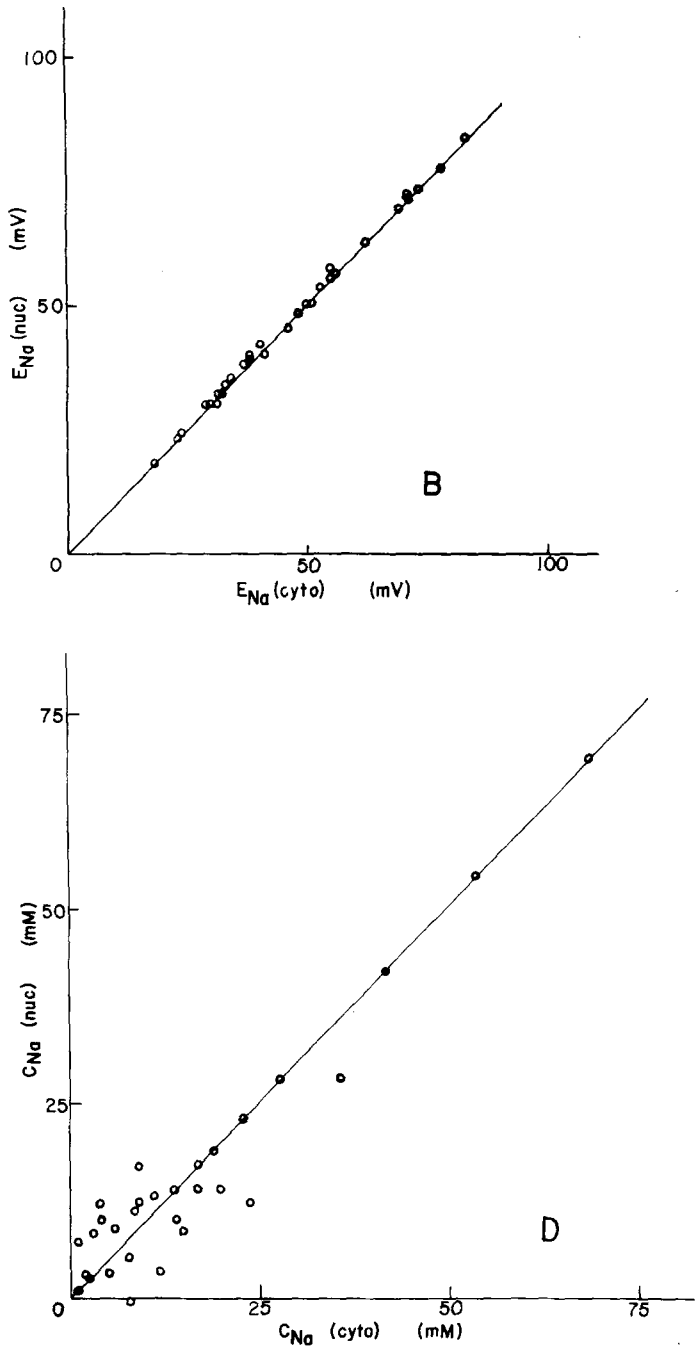


Fig. 3 (*A*) and (*B*). Correlations between measurements in the nucleus and cytoplasm with electrodes sensitive to K^+ and Na^+, respectively. E_K and E_{Na} are the electrode responses in the cell relative to those in the external solution. The absolute values of E_{Na}, which were always negative, are plotted. (*C*) and (*D*): Correlations between measurements of



apparent ion concentrations in the nucleus and cytoplasm of K^+ and Na^+, respectively. C_K and C_{Na} are the apparent concentrations of K^+ and Na^+ calculated from the ion selective electrode and micropipet measurements using calibration curves from standard solutions

Table 3. Selectivity coefficients for electrodes

Electrode	Interfering ion	k_{ij}
K^+	Choline	110
	NH_4^+	0.29
	Na^+	0.014
	Mg^{++}	0.03
	Ca^{++}	0.03
Na^+	NH_4^+	4
	Choline	0.12
	K^+	0.30
	Ca^{++}	0.6
	Mg^{++}	0.4
Cl^-	Propionate	0.5*
	Isethionate	0.2
	$H_2PO_4^-$	0.03
	HCO_3^-	0.05*
	SO_4^{--}	0.03*

Values for k_{ij} were calculated from the equation:

$$E_i = E_0 + 59 \log (a_i + k_{ij} a_j^{z_i/z_j})$$

where a_i is the activity of the principal ion, a_j is the activity of the interfering ion, z_i and z_j are their respective valences, E_i is the electrode voltage and E_0 is a constant. Values for the selectivity constants k_{ij} were obtained by replacing a solution of the principal ion with one containing only the interfering ion. A low value of k_{ij} implies a good selectivity in favor of the principal ion. All values of k_{ij} were measured during the course of the present study, except for those indicated by an asterisk (*) which were obtained from Brown *et al.* (1970).

can create errors in the measurement of membrane potentials with the micropipets.

The significance of the first effect can be determined to some extent by measurements of simple salt solutions containing the ions which are thought to be present in the cells. The selectivity ratios for a number of biologically important ions *vs.* the ions measured here are given in Table 3. In the case of the K^+ electrode, the only ion which could interfere appreciably is choline, or other quaternary ammonium ions. As little as 0.12 mM choline could create a 10% error in the K^+ measurements. Whether or not these cells have a free quaternary ammonium concentration this large is not known. For the Na^+ electrode, K^+ is the only ion which is likely to interfere. Under normal conditions, the Na^+ electrode will estimate a Na^+ activity that is too high by a factor of 2 to 3 unless K^+ activity is taken into account. To make accurate

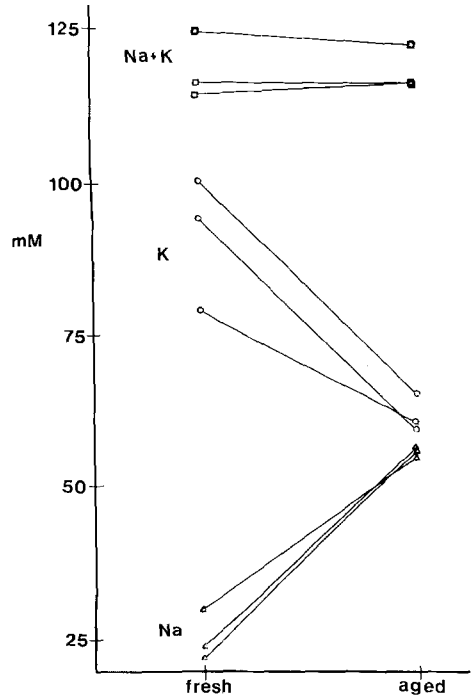


Fig. 4. Replacement of cell K^+ with Na^+ during *in vitro* aging of the glands. The ordinate represents the apparent concentrations of the two ions. Na^+ and K^+ were measured simultaneously in the same cell. Each point represents the mean of six or more measurements in different cells of the same gland. Lines connect readings from the same gland, before and after 2.5 to 3.5 hr of aging in insect Ringer's solution

measurements with these electrodes in the salivary gland or in other cells a K^+ electrode must be used to measure K^+ activity in the same preparation. The Cl^- electrode has only a modest selectivity over isethionate and propionate. We assume that these anions are at much lower concentrations in the cells studied than Cl^-, and that they do not interfere appreciably.

The electrodes used are not sensitive to the counterions that were tested. The K^+ and Na^+ electrodes showed no changes when the Cl^- in the calibration solutions was replaced by $H_2PO_4^-$ and the Cl^- electrode was similarly unaffected by replacement of K^+ by Na^+. In addition, the electrodes were insensitive to pH over the range of 6.0 to 8.2; the Na^+ electrode gave errors of several millivolts when the pH was dropped to 5.0.

Interference from macromolecules is more difficult to quantify. Neild

and Thomas (1974) reported that the Ag-AgCl electrodes which they used to measure Cl^- activity gave erroneous results which they attributed to the effects of protein at the metal surface. Brown and Kunze (1974) did a number of tests of the K^+ and Cl^- resin electrodes in *Aplysia* neurons. By using reversal potentials as independent measurements of K^+ and Cl^- activities, they concluded that these electrodes were working accurately in the cells they studied.

Similar tests cannot be performed using the salivary gland cell. However, for the purposes of this study we considered it sufficient to show that the electrodes could accurately measure changes in ion activity. After several hours of incubation *in vitro,* the ionic gradients built up by the cells' metabolic machinery begin to break down. Resting potentials usually fell by 5 to 10 mV, and Na^+ entered the cell while K^+ was lost. Changes of 20 to 30 mM could be easily detected by the ion-selective electrode, and could be made without any visible deterioration of the cells. If we assume that the cells do not change their volume, or gain or lose large or "indiffusible" ions, then the replacement of K^+ by Na^+ should be 1 for 1. This was the case in every experiment. Three experiments are shown in Fig. 4. The observation that the measured Na^+ gain equalled the measured K^+ lost, and that these changes are accompanied by an expected fall in the resting potential, suggests that the changes measured are in fact changes in ion activity, and not in the nature or the amount of the cell macromolecules, or some other artifact.

The unknown magnitude of the liquid junction potential, in this case at the tip of the micropipets measuring the membrane potential, makes the measurement of single ion activity coefficients ambiguous. This is the case not only in cells, but even in simple aqueous solutions (Frank, 1963; Garrels, 1967). In simple salt solutions, where the ion activities and mobilities are approximately known, the liquid junction potential can be estimated from the Henderson equation (MacInnes, 1961). This equation has not been shown to apply to liquid junctions between cells and micropipets, however. One potential problem is that of charged macromolecules lodging in the bore of the pipet and forming a selective barrier to the movement of ions. This could create a large junction potential as the micropipet would behave like an ion-selective electrode. Another possibility is that diffusion near the walls of the pipet would become relatively more important than diffusion at the center of the bore. The expected effect of this restriction would be to increase the "tip potential", which is included in the liquid junction

Table 4. Electrical potentials measured with different electrolytes at the liquid junction

Electrolyte	Measured (mV)	Predicted (mV)
$MgCl_2$ (2 M)	$+0.2 \pm 0.4$	$+0.3$
Na_2SO_4 (1.3 M)	$+3.3 \pm 0.2$	$+3$
KCl (0.5 M)	$+3.6 \pm 0.4$	$+4$
KCl (0.1 M)	$+8.6 \pm 0.4$	$+12$

Values of the liquid junction potential difference were measured by taking the voltage difference between the test electrode and the reference electrode filled with 3 M KCl, both in the Ringer's and in the cell. The difference represents the error in the membrane potential measurement due to the liquid junction potential of the test electrode, assuming negligible error in the measurement made with the 3 M KCl electrode. This assumption is discussed in the text. The calculated values are taken from the Henderson equation:

$$V_L = -(RT/F)\frac{\sum_i u_i(a_i'' - a_i')}{\sum_i z_i u_i(a_i'' - a_i')} \ln \frac{\sum_i z_i u_i a_i''}{\sum_i z_i u_i a_i'}$$

where i refers to the ith ion, $'$ and $''$ refer to the solutions on either side of the liquid junction, z is valence, a is activity, and u is mobility.

Activities were calculated from the concentration and the mean activity coefficients given in Robinson and Stokes (1959) p. 494–501. Values were 0.57, 0.65 and 0.77 for 3, 0.5, and 0.1 M KCl, respectively; 0.183 for Na_2SO_4 and 1.05 for $MgCl_2$. Mobilities were assumed to be proportional to the limiting equivalent conductivity, and to have the same sign as that of the ionic charge. Values were 73.50 (K^+), -76.35 (Cl^-), 50.10 (Na^+), -80.0 (SO_4^{-2}) and 53.0 (Mg^{+2}) from Robinson and Stokes (1959) p. 465.

potential in this case. This potential is thought to arise from ion-selective diffusion pathways near the glass surface (Lavallée & Szabo, 1969) which would be shunted to a greater or lesser extent by the pathway through the bore. In either case the liquid junction potential should be highly dependent on the nature of the diffusing ions. To test this possibility we measured the membrane potential in the salivary glands with micropipets filled with various electrolytes. In the absence of ion selectivity effects, the difference in the apparent membrane potentials read by the two electrodes is the difference in their liquid junction potentials in the cell, minus the same difference outside the cell, which can be estimated from the Henderson equation.

We wanted to see if the difference inside the cell could also be predicted by the Henderson equation. The results in Table 4 indicate that this is so. It was assumed that the activities of K^+ and Cl^- are as measured with the ion selective electrodes, that the mobilities of these ions are equal to their mobilities in free solution (Robinson & Stokes,

Table 5. Correction for 2 mV liquid junction potential

	K^+ (mV)	Na^+ (mV)	Cl^- (mV)
Uncorrected	100	24	39
Corrected	108	26	36

Uncorrected values were taken from two experiments, one in which Na^+ and K^+ were measured simultaneously and a second in which Cl^- was measured. Mean membrane potentials in the two experiments were -38 and -42 mV, respectively. Values in the table represent mean measured ion activities, which are converted to apparent concentrations assuming activity coefficients of 0.76. Corrected values were obtained by recalculating the data assuming membrane potentials of -40 and -44 mV, 2 mV more negative than the measured potentials.

1959) and that no other ions in the cell contribute to the junction potential. For example, when 0.1 M KCl is used to fill the micropipets, any effect of an ion-selective pathway would be greatly diminished, since the activities on both sides of the junction are similar. The junction potential predicted by the Henderson equation will increase in magnitude, however, because K^+ is at a higher activity in the cell than Cl^-. K^+ will begin to diffuse into the micropipet ahead of Cl^-, building up a positive potential there. This effect contributes a calculated error of 2 mV when 3 M KCl is used, and becomes more significant with more dilute solutions.

As pointed out by Cole (1968) measurements of membrane potential with micropipets filled with 3 M KCl can be associated with significant error. In the present case, our best estimate of the true membrane potential is 2 mV more negative than that measured with our conventional electrodes. This correction applies, to a greater or lesser extent, to all cells in which K^+ activity exceeds that of Cl^-. The results of making this correction in two typical experiments where ion activities were measured are shown in Table 5. The errors amount to about 10% for each ion. Since the correction is the same for cytoplasm and nucleus, the major conclusions of this paper are not affected.

Discussion

Descriptions of the K^+ and Cl^- sensitive microelectrodes used in the present study have been published in the past. However, the Na^+ selective electrode used has not been previously described.

Two other types of Na^+ microelectrodes have been used by investigators to measure the electrochemical activity of Na^+ within cells. Both types of electrodes are fabricated from glass. Lev (1964) used Corning 27-4 glass, which has a moderate selectivity for K^+, for the measurement of the electrochemical activities of both Na^+ and K^+. Two microelectrodes were used in each experiment, exploiting random differences in electrode sensitivity. Readings from microelectrodes possessing a relatively high selectivity for K^+ over Na^+ (mean selectivity ratio, $k_{K,Na} = 0.175$) were compared with those having a relatively lower selectivity ($k_{K,Na} = 0.303$). Armstrong and Lee (1971) and Lee and Armstrong (1972) used a similar system to measure Na^+ and K^+ activities in frog skeletal muscle and intestine, respectively. A serious limitation to this approach is the low value of $k_{K,Na}$ observed for even the most Na^+ selective 27-4 glass.

In our system, both the K^+ electrode ($k_{K,Na} \simeq 0.02$) and the Na^+ electrode ($k_{K,Na} \simeq 3$) have better selectivities for their principal ions than the corresponding electrodes in Lev's system. In both systems, a K^+ electrode must be used in conjunction with a Na^+ electrode to make accurate measurements of Na^+ activity. However, if Lev's electrodes were applied to the present preparation, using $a_K = 76$ mM and $a_{Na} = 18$ mM from a typical experiment, an error of 1 mV in the Na^+ electrode measurement would lead to an error of 43 mM in calculating the chemical activity of Na^+. Using our present electrodes, the same error leads to only a 1.7 mM error in the chemical Na^+ activity.

A second type of microelectrode system for measuring Na^+ activity was developed by Hinke (1961), using NAS 11–18 glass. These electrodes are characterized by a good selectivity for Na^+ over K^+. Dick and McLaughlin (1969) report values of $k_{Na,K}$ ranging from 0.01 to 0.002. This is considerably better than the selectivity of our resin electrodes. The disadvantage of these glass electrodes is their high electrical resistivity (Kostyuk, Sorokina & Kholodova, 1969). To be practicable, an appreciable length of Na^+ sensitive glass must be incorporated into these electrodes. A length of 50–200 μ is commonly used (Dick & McLaughlin, 1969), although it has been successfully reduced to 10–20 μ (Kostyuk *et al.*, 1969). Care must be taken that the entire length of the ion-selective glass is inserted into the cell. This is not difficult when large cells are used, such as the squid axon (Hinke, 1961), the barnacle muscle (Hinke *et al.*, 1973) or the toad oocyte (Dick & McLaughlin, 1969). The problem is more serious with smaller cells. Thomas (1970) designed an electrode made from the same NAS 11–18 glass, in which the ion-selective tip

is recessed behind the insulating glass tip. This obviates the problem of positioning a finite tip length into the cell, but introduces a dead space between the insulating and ion-selective glass which must equilibrate with the contents of the cell. This again is not a problem with cells which are much larger than the dead space (approximately 0.24×10^{-12} liters), as long as a fast electrode response is not necessary, but smaller cells can have volumes that are comparable to the dead space, and the introduction of the electrode could significantly perturb their ionic composition.

These problems can, in principle, be overcome by advanced techniques of miniaturizing the glass electrodes. However, the resin electrodes described here are relatively simple to make, and, in our hands at least, are much more consistent and reliable.

Using these liquid ion exchanger microelectrodes, we have found no significant gradients in the electrochemical activities for Na^+, K^+ or Cl^- across the nuclear membrane. Starodubov and Kurella (1972) also reported an absence of an electrochemical gradient for K^+ across the nuclear membrane of *Chironomus* salivary glands. These authors did not measure nuclear membrane resistance, which in the present study was considered important in checking for damage to the nuclear membrane, and in verifying the electrode positions. Also, this work was done with a K^+ electrode which had a selectivity for K^+ over Na^+ which was an order of magnitude less favorable than that of the K^+ electrodes reported here.

On the basis of measurements obtained with micropipets filled with 3 M KCl, we have also found no significant gradient in electrical potential. The simplest interpretation of these data is that the chemical activities of all three ions are also the same in the nucleoplasm and cytoplasm. This conclusion is subject to the intrinsic ambiguity associated with the measurement of the electrical potential. However, if the junction potentials in the nucleoplasm and cytoplasm were different, this difference must have been fortuitously equal in magnitude and opposite in sign to a true potential difference across the nuclear membrane. Furthermore, the changes in the liquid junction potential, measured as a function of the solution filling the micropipets, were in satisfactory agreement with the calculated values. This suggests that the liquid junction potential is determined by the ionic activities, which are the same in the nucleus and the cytoplasm, and not by the polyelectrolytes, which are obviously different in the two compartments.

The conclusion that Na^+, K^+ and Cl^- are not sequestered in free form within the nucleus of *Chironomus* salivary gland cells is not necessa-

rily true of other cells. However, many features of the nuclear membrane visible in electron micrographs, including the bilaminar membrane, the nuclear "pores" and the dense material subjacent to these "pores", are found throughout the animal and plant kingdoms (Fry, 1970). Although these features are not necessarily the most important factors determining ion permeability, they do suggest a general structural similarity among nuclear membranes from different cells. Furthermore, we chose to examine nuclei known to possess a relatively high electrical resistance. It is likely, then, that the data presented here can be extended to other cells.

As discussed in the Introduction, it is clear that a relatively large fraction of cell Na^+ is sequestered within certain subcellular organelles. The results of the present study show that the nucleus does not participate in this compartmentalization. It now appears that other structures, possibly the endoplasmic reticulum and mitochondria, are of much greater importance in this respect.

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