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INTRACELLULAR POTASSIUM AND SODIUM ACTIVITIES OF CHICK VENTRICULAR MUSCLE DURING EMBRYONIC DEVELOPMENT

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SUMMARY

1. The basis of the resting potential of chick embryo ventricular muscle was studied by use of ion-selective micro-electrodes. Membrane resting potential hyperpolarized from $-65{\cdot}4 \pm 1{\cdot}1$ mV (mean ± S.E.) at age 4 day to $-75{\cdot}8 \pm 0{\cdot}6$ mV at age 18 day. Action potential overshoot increased from $+19{\cdot}8 \pm 0{\cdot}9$ at age 4 day to $+33{\cdot}1 \pm 0{\cdot}6$ mV at age 18 day.

2. Intracellular K^+ activity measured with ion-selective micro-electrodes increased from $71{\cdot}3 \pm 1{\cdot}9$ mM at age 4 day to $89{\cdot}9 \pm 1{\cdot}1$ mM at age 18 day. Intracellular Na^+ activity decreased from $12{\cdot}5 \pm 0{\cdot}4$ to $7{\cdot}0 \pm 0{\cdot}3$ mM during the same period. The difference between membrane resting potential and the calculated potassium equilibrium potential decreased with development. P_{Na}/P_K estimated from the constant field equation decreased from 0·012 at age 4 day to 0·005 at age 18 day.

3. The hyperpolarization of resting potential and the increased action potential overshoot during development could be explained by a rise in intracellular K^+ activity and a fall in intracellular Na^+ activity, as if the Na–K exchange pump became more active.

INTRODUCTION

The most important single factor determining the resting potential (V_m) in heart muscle cells is the transmembrane gradient of K ions (Weidmann, 1956). However, studies of developmental changes in V_m and intracellular ionic concentrations in the chick embryo heart are in conflict with this view. A progressive hyperpolarization occurs with maturation from day 2 to day 21 (hatching), but chemical analysis has shown a fall in cellular K. For example, Carmeliet, Horres, Lieberman & Vereecke (1976) reported that intracellular K concentration ($[K^+]_i$) decreases from 151 to 122 mM with maturation, in the face of a hyperpolarization of 7 mV, while intracellular Na concentration ($[Na^+]_i$) stays nearly constant at 15 mM.

The chemical measurement of intracellular ionic concentrations might be in error for several reasons, or it might not provide the information needed to interpret the resting potential. Measurement of extracellular space has been a source of error in chemical studies, especially for $[Na^+]_i$. In addition, some fraction of the cations in

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heart muscle may be bound to extracellular or intracellular components, or the intracellular ionic activity coefficients may not be the same as those in the extracellular solution (Lee & Fozzard, 1975). An alternative way to study this question is to measure intracellular K^+ activity (a^i_K) and Na^+ activity (a^i_{Na}) directly with ion-selective micro-electrodes, a technique that has been used successfully in other cardiac tissues (Walker & Ladle, 1973; Lee & Fozzard, 1975; Ellis, 1977). In these experiments we measured the potentials obtained from two types of ion-selective micro-electrodes, allowing us to calculate that a^i_K rose and a^i_{Na} fell with maturation of the embryonic heart cells. This permitted estimation of the roles of changing ionic gradients and of changing partial permeabilities on the hyperpolariztion seen with maturation. Some preliminary observations have been reported (Sheu & Fozzard, 1978).

METHODS

K^+-selective glass (K-GL) micro-electrodes were prepared from Corning $NAS_{27\text{-}04}$ glass (Corning Glass Works, Medfield, Mass.) by a method similar to that described by Lee & Armstrong (1974). The tip diameters were less than 0·5 μm and the ion-selective exposed tip lengths were less than 3 μm. The electrode resistances in Tyrode solution were about 10^8–10^9 Ω. The K^+-selective liquid ion exchanger (K-LIE) micro-electrodes were prepared with Corning K ion exchanger (no. 477317 Corning Medical, Medfield, Mass.) by a method similar to that descrbied by Walker (1971). The micropipettes had tip diameters less than 0·5 μm, and if filled with 3M-KCl, their tip resistances were 20–60 MΩ. The electrode resistances in Tyrode solution were about 10^9–10^{10} Ω.

The ion-selective micro-electrodes were calibrated before and after the membrane potential measurements with pure solutions of KCl and of NaCl, and with mixture of KCl and NaCl over a range sufficient to overlap the measured values. The basis for use of the electrodes is usually derived from the general equation describing ideal behaviour of ion-selective electrode response (E_{ij}) to activities of two species of ions a_i and a_j (Nicolsky, 1937). In these studies, the K-LIE electrodes showed nonideal behaviour, in that they responded to pure a_i and pure a_j solutions with two different slopes S_i and S_j. Eisenman (1967) found that in order to describe this nonideal response, the Nicolsky equation had to be expanded to the form

$$E_{ij} = E^i_o + nS_i \log \left[a_i^{1/n} + (k^N_{ij} a_j)^{1/n} \right], \quad (1)$$

where E^i_o is a constant for a particular electrode that inculdes the liquid junction potential and the potential at the phase boundary of the inside of ion-exchange membrane, k^N_{ij} is the selectivity coefficient for electrodes that do not behave ideally, and n is an arbitrary number. We found that the equation gave correct answers for ionic activities in mixture solutions when $n = S_i/S_j$. Pure KCl and pure NaCl activity coefficients (γ_{KCl} and γ_{NaCl}) were calculated using the equations derived by Pitzer & Mayorga (1973). For KCl + NaCl mixed solutions, γ_{KCl} and γ_{NaCl} were calculated by Guggenheim-Scatchard-Robinson generalized equations for mixed electrolyte solutions (Harned & Robinson, 1968). For the calculation of single ion activity, the MacInnes convention was used (MacInnes, 1961).

Fertilized eggs of white leghorn chickens were incubated at 37 °C with adequate humidity for 4, 7, 12, and 18 days. The entire embryo heart or a ventricular strip was pinned in a chamber and superfused with oxygenated Tyrode solution having the following composition (mM) NaCl 140, KCl 5·6, $CaCl_2$ 1·8, $MgCl_2$ 0·5, $NaHCO_3$ 12, NaH_2PO_4 1·8, glucose 5·5; pH was 7·2 and temperature was 25 ± 1 °C. The tissue remained in the chamber for 1 hr before the first impalement was undertaken. Only ventricular muscle cells were studied.

Membrane resting and action potentials were measured with conventional open-tip micro-electrodes with tip resistances of 20–60 MΩ and tip potentials less than −7 mV, using a Picometric amplifier (Instrumentation Lab., Inc., Lexington, Mass., Model 181). For a^i_K and a^i_{Na} measurements, both K-GL and K-LIE micro-electrodes were used. The electrodes were connected by reversible half cell $Hg:Hg_2Cl_2$ calomel electrodes to a differential electrometer amplifier (Keithly Instrument, Inc., Cleveland, Ohio, Model 604) with an input impedance about 10^{14} Ω.

The signals were led to a Tektronix 565 dual-beam oscilloscope (Tektronix, Inc., Beaverton-Oregon), a digital voltmeter (United System Corporation, Dayton, Ohio), and a chart recorder (Gould Inc., Cleveland, Ohio). Only penetrations that fulfilled the following criteria were taken into account; upon electrode impalement the potential appears abruptly and stays stable for at least one minute; and after the removal of electrode, the potential returns to its initial value.

The k_{KNa} for K-GL micro-electrodes were in the range of 0·1–0·4 and those for K-LIE micro-electrode were in the range of 0·01–0·04. We used average data of ΔE, the change of electrode potential upon impalement of the cell, obtained from K-GL micro-electrode measurements as one group, and the average data of ΔE obtained from K-LIE micro-electrode measurements as the other group, and solved simultaneously two equations like eqn. (2) to calculated a_K^i and a_{Na}^i (Lee & Armstrong, 1974; Lee & Fozzard, 1975).

$$a_K^{i\,1/n} + (k_{KNa}^N a_{Na}^i)^{1/n} = [a_K^{o\,1/n} + (k_{KNa}^N a_{Na}^o)^{1/n}]\, 10^{(\Delta E - V_m/n S_K)}, \quad (2)$$

where a_K^o and a_{Na}^o are the K^+ and Na^+ activities in the Tyrode solution.

RESULTS

Membrane resting and action potentials were recorded from the endocardial surface of embryonic chick ventricles. Fig. 1 shows examples of potential measurements at four stages of development. The mean values of resting potentials and overshoots of action potential at four different ages are summarized in Table 1. Average values of resting or maximal diastolic potential showed hyperpolarization with maturation from $-65{\cdot}4 \pm 1{\cdot}1$ mV at 4 days to $-75{\cdot}8 \pm 0{\cdot}6$ mV at 18 days. Action potential overshoot increased from $19{\cdot}8 \pm 0{\cdot}9$ to $33{\cdot}1 \pm 0{\cdot}6$ mV during the same period.

The calculation of intracellular ionic activities required knowledge of ionic activities in the Tyrode solution. According to the Guggenheim-Scatchard-Robinson equation, a_K^o = 4·1 mM and a_{Na}^o = 115 mM. Measurements of ionic activities were

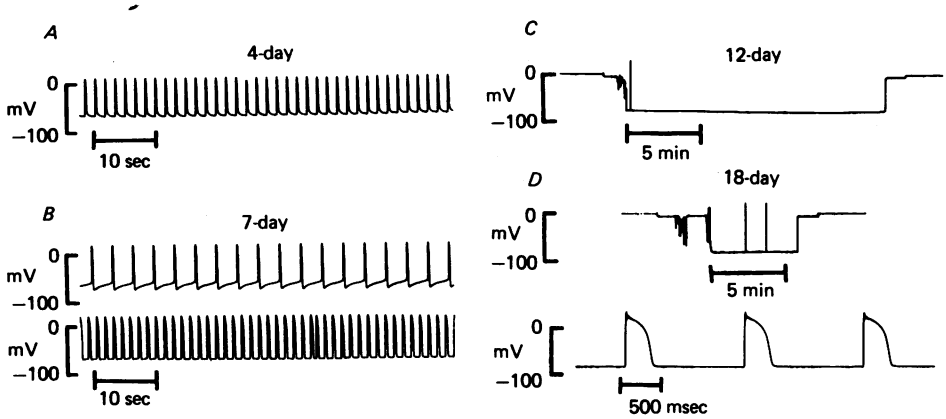

Fig. 1. Typical membrane resting and action potentials from chick embryo hearts (*A*) 4 days old (*B*) 7 days old (*C*) 12 days old (*D*) 18 days old. 4- and 7-day hearts were beating spontaneously in Tyrode solution. The action potential in these two stages sometimes showed phase four depolarization as illustrated in the top trace of (*B*). For ages 12- and 18-day, the hearts were generally quiescent, but have occasional action potentials. The vertical lines perpendicular to resting potential trace in *C* and *D* are the spontaneously firing action potential traces.

made in Tyrode solution with five pairs K-GL and K-LIE micro-electrodes, resulting in values of $a^{o}_{K} = 4{\cdot}06 \pm 0{\cdot}07$ mM (S.E.) and $a^{o}_{Na} = 114{\cdot}6 \pm 1{\cdot}9$ mM.

Examples of recordings by ion-selective micro-electrodes are shown in Fig. 2, and

TABLE 1. The mean values of resting membrane potentials (V_m), overshoots of action potentials (V_{Ov}), intracellular potassium activity (a^{i}_{K}), and intracellular Na activity (a^{i}_{Na}), at four different stages. K equilibrium potential (V_K) and Na equilibrium potential (V_{Na}) were calculated from $V_K = \frac{RT}{F} \ln \frac{a^{o}_{K}}{a^{i}_{K}}$ and $V_{Na} = \frac{RT}{F} \ln \frac{a^{o}_{Na}}{a^{i}_{Na}}$, where $\frac{RT}{F}$ is $\frac{59{\cdot}1}{2{\cdot}303}$ mV

Age (days)	4	7	12	18
V_m(mV)	$-65{\cdot}4 \pm 1{\cdot}1$* (23, 7)†	$-71{\cdot}9 \pm 0{\cdot}8$ (26, 8)	$-73{\cdot}5 \pm 0{\cdot}6$ (34, 9)	$-75{\cdot}8 \pm 0{\cdot}6$ (35, 9)
V_{Ov}(mV)	$+19{\cdot}8 \pm 0{\cdot}9$ (20, 7)	$+27{\cdot}3 \pm 0{\cdot}7$ (23, 8)	$+31{\cdot}6 \pm 0{\cdot}7$ (21, 9)	$+33{\cdot}1 \pm 0{\cdot}6$ (23, 9)
a^{i}_{K}(mM)	$71{\cdot}3 \pm 1{\cdot}9$ (12, 4)	$82{\cdot}6 \pm 0{\cdot}8$ (16, 5)	$87{\cdot}3 \pm 1{\cdot}2$ (18, 5)	$89{\cdot}9 \pm 1{\cdot}1$ (20, 5)
a^{i}_{Na}(mM)	$12{\cdot}5 \pm 0{\cdot}4$ (12, 4)	$8{\cdot}7 \pm 0{\cdot}3$ (16, 5)	$7{\cdot}3 \pm 0{\cdot}2$ (18, 5)	$7{\cdot}0 \pm 0{\cdot}3$ (20, 5)
V_K(mV)	$-73{\cdot}3$	$-77{\cdot}1$	$-78{\cdot}5$	$-79{\cdot}3$
V_{Na}(mV)	57·0	66·3	70·8	71·8

* mean ± S.E.
† (number of observations, number of hearts.)

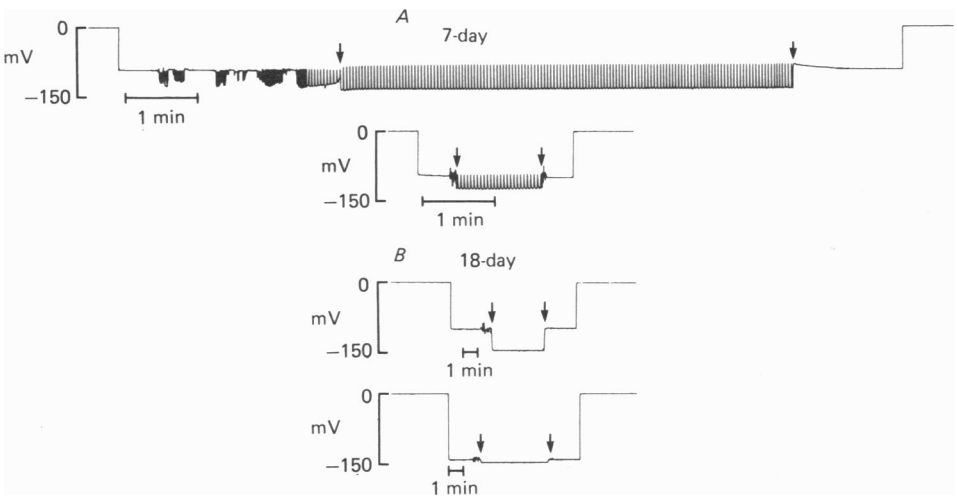

Fig. 2. Potential recordings measured with ion-selective micro-electrodes in (*A*) 7-day old and (*B*) 18-day old hearts. The top trace of each section is a K-GL micro-electrode recording and the bottom trace is a K-LIE micro-electrode recording. The first arrow of each Figure indicates impalement of the ion-selective micro-electrode. The second arrow indicates the withdrawal of the electrodes.

average values of a^{i}_{K} and a^{i}_{Na} are reported in Table 1. These activities were used to calculate the potassium equilibrium potential (V_K) and the Na equilibrium potential (V_{Na}) at each age. V_K hyperpolarized from $-73{\cdot}3$ mV at 4 days to $-79{\cdot}3$ mV at 18 days and V_{Na} increased from 57·0 to 71·8 mV. The difference between V_m and calculated V_K decreased with maturation.

The relationship between individual values of V_m and a_K^i for each heart is shown in Fig. 3*A*. This results in a scatter plot showing an apparently linear relation between V_m and log a_K^i. Calculated V_K values are also shown in this Figure for comparison. The slope of the relationship of V_m to log a_K^i is $-99{\cdot}4$ mV per tenfold change in a_K^i ($r = 0{\cdot}97$), compared to the relation to V_K, which has the predicted Nernstian slope of $-59{\cdot}1$ mV per tenfold change in a_K^i. The two curves converge with maturation. A

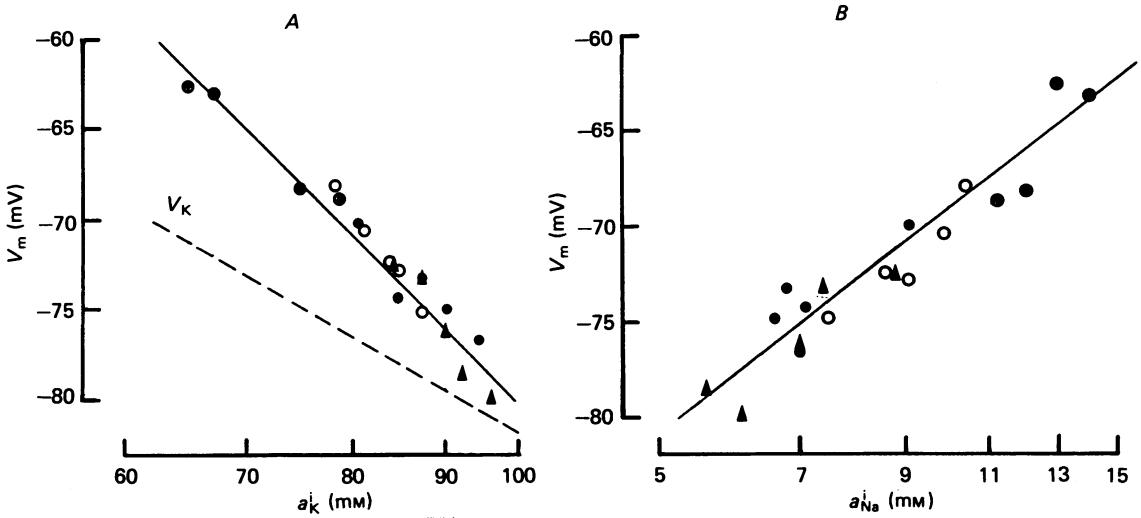

Fig. 3. *A*, the mean resting potential (V_m) obtained from nineteen separate muscles at four different stages is plotted against the value of intracellular K activity (a_K^i) observed in each fibre. The continuous line is fitted by linear regression. The correlation coefficient is 0·97 and the slope of the relation is $-99{\cdot}4$ mV per tenfold change in a_K^i. Calculated V_K values are shown in this Figure to demonstrate that the V_m, V_K difference is larger in younger stage hearts. Star circle 4-day old, open circle; 7-day old, filled circle; 12-day old, and triangle; 18-day old hearts.

B, the mean resting potential (V_m) of nineteen separate muscles is plotted as a function of the intracellular sodium (a_{Na}^i) of each fibre. The slope of continuous line, fitted by linear regression is 39 mV per 10-fold change in a_{Na}^i. The correlation coefficient is 0·96. Star circle; 4-day, open circle; 7-day, filled circle; 12-day, and triangle; 18-day.

similar relationship can be demonstrated between V_m and log a_{Na}^i (Fig. 3*B*), with a slope of 39 mV per tenfold change in a_{Na}^i ($r = 0{\cdot}96$). In the more hyperpolarized hearts a_{Na}^i was lower, approaching the value of about 6 mM that was reported for adult rabbit ventricular muscle (Lee & Fozzard, 1975).

The inverse relation of a_K^i and a_{Na}^i suggested that one was exchanged for the other during development. If we assume that the apparent intracellular activity coefficients for Na^+ and K^+ are those measured by Lee & Fozzard (1975) in adult rabbit ventricular muscle ($\gamma_K = 0{\cdot}612$, $\gamma_{Na} = 0{\cdot}175$), comparison can be made with chemical determinations. $[K^+]_i$ at 4 days is 116·5 mM and it rises to 146·9 mM at 18 days, while $[Na]_i$ is 71·4 mM at 4 days and falls to 40·0 mM. The sum of the estimated cation concentrations was constant at each age, a result that may be related to cell volume regulation.

If we assume that the membrane potential is determined by the transmembrane

activity gradients of Na^+ and K^+ and their partial permeabilities only, then we may calculate P_{Na}/P_K by use of the constant field equation. The values were 4 day 0·0124; 7 day 0·0084; 12 day 0·0072; and 18 day 0·0047. As expected from the diminishing difference between V_m and V_K during maturation, the membrane appeared to become relatively more permeable to K^+. It is important to realize, however, that these values are calculated at different V_m.

DISCUSSION

The chick embryo ventricles showed progressive hyperpolarization in resting or maximal diastolic potentials with maturation. These results agree with previously published measurements in the chick embryo heart (Shimizu & Tasaki, 1966; Sperelakis & Shigenobu, 1972; McDonald & DeHaan, 1973; Carmeliet *et al.* 1976), although our values of potential are somewhat more negative than most of these studies. In addition, the overshoot of the action potential increases with age in the chick. This property of increasing resting potential during development has also been reported for embryonic rat heart (Couch, West & Hoff, 1969; Bernard, 1975), and embryonic human heart (Tuganowski & Cekánski, 1971).

With maturation the chick ventricles showed an increase in a^i_K and a fall in a^i_{Na}. This change paralleled the hyperpolarization of the resting potential and the increase in overshoot of the action potential. V_K and V_{Na} calculated for each age also increased parallel to the change in V_m and V_{Ov}. However, these direct measurements of intracellular ionic activities during development are in conflict with the measurements of intracellular concentrations by isotopic equilibrium or chemical methods. If the activity measurements are correct, then the chemical methods must be in error or the apparent intracellular activity coefficients must change dramatically during development. For example, we can use the concentration measurements of Carmeliet *et al.* (1976) for calculation of apparent activity coefficients. For 6–8 day old hearts $\gamma^i_K = 82·6/151 = 0·55$ and $\gamma^i_{Na} = 8·7/16 = 0·54$. For 18–20 day old hearts $\gamma^i_K = 89·9/122 = 0·74$, and $\gamma^i_{Na} = 7·0/15 = 0·47$. Using the concentration measurements of McDonald & DeHaan (1973) for 4 and 18 day hearts γ^i_K is 0·46 and 0·76 and γ^i_{Na} is 0·36 and 0·20 respectively. It is certainly possible that intracellular true ionic activity coefficients do change markedly during maturation, since other constituants of the cytoplasm may be changing. On the other hand, variable amounts of Na^+ and K^+ may be bound to intracellular molecules or may be compartmentalized. Although ionic activities are the appropriate measurement for purposes of understanding cellular electrical events, the changes in content of Na^+ and K^+ could be related to important metabolic alterations during development. Complex interactions have been suggested that imply that the intracellular K^+ and/or Na^+ contents play a large role in controllowing both protein synthesis and the entire series of events responsible for cell growth and division (Kaplan, 1978).

These measurements of ionic activities permitted calculation of the ratio P_{Na}/P_K. While our values differ from previous estimates (Sperelakis & Shigenobu, 1972; McDonald & DeHaan, 1973; Carmeliet *et al.* 1976), all investigators report a decreased P_{Na}/P_K with maturation. The K^+ efflux studies of Carmeliet *et al.* (1976) indicated a twofold increase in P_K between 6–8 days and 18–20 days. This agrees with our

calculation of a twofold decrease in P_{Na}/P_K in this period. This effect of maturation on P_{Na}/P_K could be secondary either to the voltage change during maturation or to a direct membrane effect. There is a suggestion from these studies that the voltage factor may be of importance. As demonstrated in Fig. 3*A*, the slopes of the relations between V_K and a_K^i within each age group still greatly exceed the Nernst value. The change of P_{Na}/P_K on maturation, according to this interpretation, is the result of the hyperpolarization, which itself is the result of increased a_K^i. This reasoning does not, of course, exclude some changes in the resting membrane properties themselves with maturation, but it does suggest that any investigation of these properties must include control of the membrane potential.

The mechanism of increased a_K^i with maturation and concomitant fall in a_{Na}^i cannot be inferred from these studies. However, it is plausible to suggest that this is due to a maturation of the Na–K exchange pump. Klein (1963) & Sperelakis (1972) found increases in the specific activity of isolated membrane Na-K ATPase with development of the chick heart. Rosen *et al.* (1975) have reported lower sensitivity to ouabain in neonatal and young canine Purkinje fibres. An additional factor could be a metabolically determined change in intracellular 'fixed' anion content. The calculations of a relatively constant content of intracellular cations is interesting in relation to the regulation of cell volume, but the calculation should only be considered suggestive because we have no direct estimates of osmotic activity coefficients at any age.

A problem with use of ion-selective micro-electrodes was encountered during the progress of these experiments. Attempts to calibrate the electrodes with single salt solutions revealed non-parallel slopes of electrode response, making estimation of selectivity coefficients by the usual method impossible. Furthermore, calculations of ionic activities in mixed solutions such as Tyrode solutions, have suggested that the standard methods of estimating activities in mixtures was not accurate. This problem was resolved by use of an equation that describes the nonideal behaviour of electrode potentials. The equation resulted in values for ionic activities in Tyrode solution that were in agreement with the Guggenheim-Scatchard-Robinson method for calculation of ionic activities in mixtures. The *n*-factor equation may be especially important for the measurement of activities of ions in low concentration in the presence of a high concentration of interfering ion, such as the measurement of a_K^o with K^+-selective electrodes.

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