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Potassium Redistribution and Water Movement in Crayfish Muscle Fibers

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Summary. 1. After subjecting the isolated crayfish muscle fiber to a variety of external ionic conditions, the intracellular potassium concentration was measured with an ultramicro integrative flame photometer.

2. The quantity of fiber water was determined by correcting the weight of the fiber for the solid component and the adhering and extracellular water. In normal control Ringer's solution the ratio of fiber water to cell weight is 0.79.

3. The intracellular potassium concentration of a fiber bathed in normal control Ringer was determined as 130 ± 10 mM/kg-H_2O. With propionate substituted for chloride in the control solution, the final intracellular potassium concentration was 128 ± 13 mM/kg-H_2O.

4. The muscle fiber was subjected to media made hyperosmotic by the addition of K salts. When the anion was permeant (chloride) the redistribution of the intracellular potassium conformed to a Donnan system. With the impermeant ion propionate, the fiber behaved as an osmometer.

5. When the media were isosmotically changed with the addition of K salts, the fiber potassium did not conform to a Donnan redistribution with chloride, nor as an osmometer with propionate.

6. When the fiber was suddenly exposed to a propionate control solution after equilibration in chloride, the transient time course of intracellular potassium indicated a predominant water movement. This water movement was probably by electroosmosis.

A. Introduction

Volume changes in the isolated crayfish muscle fiber have been induced by variations in the media bathing the preparation and deviations from predicted osmotic behavior have been observed (Reuben, Girardier, and Grundfest, 1964). It seemed of interest to measure the internal potassium concentration with the fiber subjected to similar ionic conditions. These data could be studied to yield some correlates of potassium redistribution and water movement with electrophysiological and osmotic behavior. The potassium concentrations were determined with an integrative flame photometer that was developed to measure microquantities of salts (Katz, 1968).

B. Materials and Methods

The crayfish used in the present work were obtained chiefly from one dealer. There were all of the genus *Orconectes*, but probably of several species. No difference in the results could be discerned ascribable to the season or species.

Fibers were prepared from the flexor and extensor muscles in the meropodite of the walking limb. The dissection technique has been described by Girardier *et al.* (1963). The fibers were all from the group which comprises the largest number in the muscle having sarcomere lengths of 8–10 μ, and ranging in diameter between 100 and 400 μ (Brandt *et al.*, 1965). Analyses were made on 163 single fibers as well as on groups of up to 10 fibers. Of these preparations, 34 weighed less than 0.2 mg. There was no apparent difference in the results that could be attributed to the weight of the fibers. The experiments were carried out at room temperature.

Two standard control media of the following composition were used (meq/l):

	Na	K	Cl	Ca	Propionate	HCO_3
A. Cl saline	202.5	5	232	27	—	2.5
B. Propionate saline	202.5	5	—	27	232	2.5

These media are modifications of the Van Harreveld crayfish Ringer's solution (1936) with Mg omitted. In one of the media the major anion (Cl) was permeant, but in the second (B) Cl was replaced with propionate which is impermeant (Reuben *et al.*, 1964). The omission of Mg from the medium did not affect various parameters studied in electrophysiological experiments. Isosmotic changes in the control media were made by replacing the Na salt by equimolar amounts of the K salt; hyperosmotic changes were made by adding the K salt to the control solution. Unless otherwise noted, fibers were equilibrated in the control solution for 1 to 2 hours and if the solution was changed, the fiber was allowed to reequilibrate for another 2 to 4 hours before analysis.

A schematic representation of the integrative flame photometer used in determining the potassium is shown in Fig. 1. The sample is placed on a platinum loop, dried and inserted into the flame. The burst of characteristic luminous emission as the sample is burned is converted into an electrical signal by the photomultiplier detector, recorded and integrated. The integral is a measure of the total number of moles of the salt in the sample (Katz, 1968).

Determination of Fiber Water. The muscle fibers were removed from the chamber and weighed. The fibers were not blotted prior to the weighing procedure for fear that blotting might remove intracellular water. The quantity of intracellular water depends upon the proper estimation of the adhering or extracellular solution and the amount of solid material; or

$$W_{H_2O} = W_w - W_{ae} - W_s \tag{1}$$

where W_{H_2O} is the weight of the fiber water, W_w is the total fiber wet weight, W_{ae} is the weight of the adhering or extracellular solution, and W_s is the dry weight or weight of the solid material.

Weighing was performed on a Cahn electrobalance which was capable of detecting imbalances of 0.2 μg.

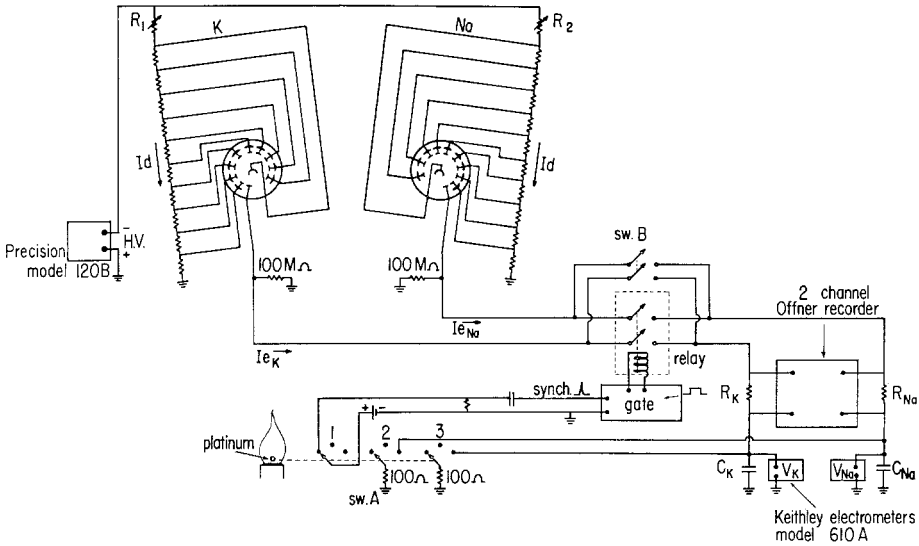

Fig. 1. Schematic diagram of the integrative flame photometer. Simultaneous with the insertion of the sample-bearing platinum loop into the flame, a synchronizing pulse initiates the relay closure for a predetermined interval. As the sample burns, the signal is recorded, and its integral (the condenser voltage) is measured by the Keithley electrometers. When the platinum is withdrawn, the condensers are discharged

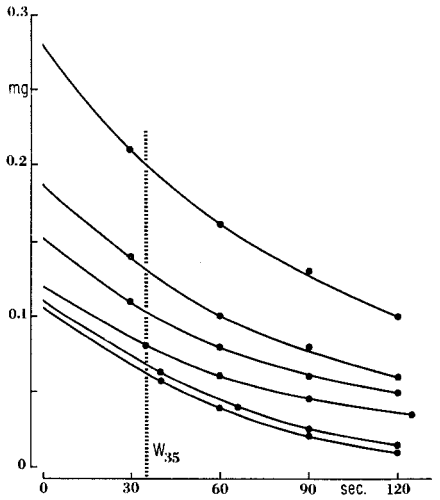

Fig. 2. Weight change in 6 small fibers due to evaporation. The fibers were not blotted and the weights so obtained ranged between 0.1 and 0.3 mg. Curves are extrapolated back to zero time. The time axis is in seconds after removal of the fiber from the solution

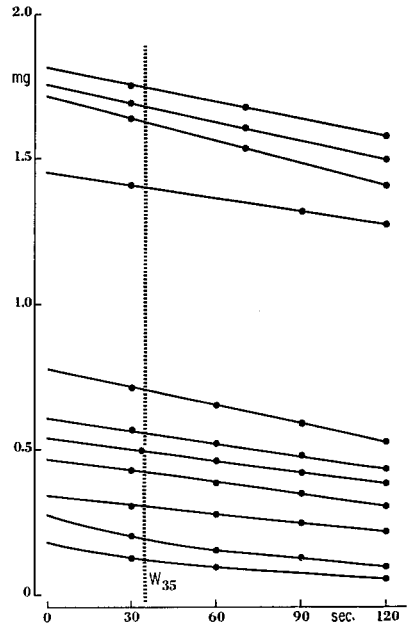

Fig. 3. Weight change in large fibers due to evaporation. The two lowest curves are for fibers within the weight range of those in Fig. 2

The fiber loses weight rapidly due to evaporation. Therefore, the time elapsed between the fiber removal from the solution and the actual time of its weighing will materially affect the determination of wet weight. A series of measurements was therefore made to determine the error introduced with variation in elapsed time. Several such weight determinations are shown in Figs. 2 and 3. The weight changes for some small fibers are plotted against time in Fig. 2 whereas in Fig. 3 the weight scale is compressed to show the weight change for large fibers or groups of fibers. It seemed permissible to extrapolate each curve back to zero time to determine the weight of the fiber at the moment of its removal from the solution. The rate of weight loss due to evaporation depends upon the ratio of surface area to volume of the muscle fiber. This is observable in the curves where the initial evaporation rate is greatest for small fibers. The correction which must be applied in order to determine the wet weight therefore varies from fiber to fiber.

In practice, the weight was measured precisely 35 seconds after removal from the solution and the proper correction applied to determine W_w. Therefore,

$$W_w\,(\text{mg}) = W_{35}\,(\text{mg}) \times \text{correction}\,(\%). \tag{2}$$

The percentage correction is based on drying tests conducted on 30 fibers and is shown in Fig. 4.

If the fiber is permitted to dry, the weight stabilizes at the weight of the solid component of the fiber, W_s. For 12 fibers the solid weight, W_s, is plotted as a percentage of the wet weight, W_w, and is shown in Fig. 5. The average ratio of $W_s/W_w = 0.177$ and is, as expected, relatively independent of the size of the individual fibers.

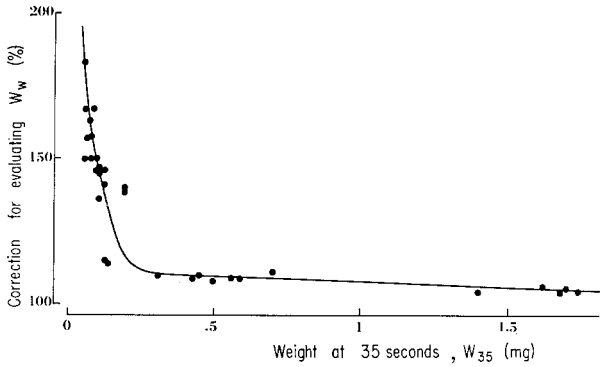

Fig. 4. Correction applied to fibers weighed 35 seconds after their removal from the solution

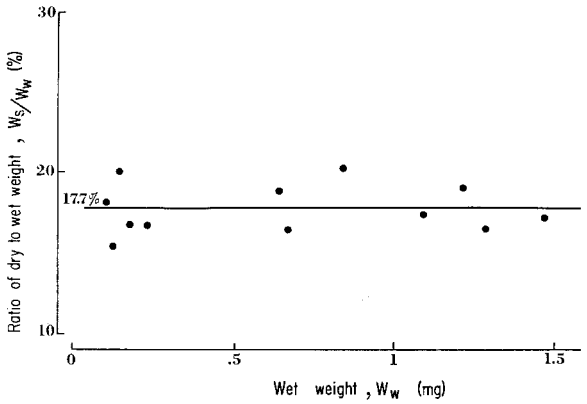

Fig. 5. Variation of the ratio, W_s/W_w, with the wet weight, W_w. The average value is 17.7%

The quantity of the adhering or extracellular solution, W_{ae}, was determined by tracer analysis. Single fibers were equilibrated in radioactive inulin solution. Using the identical technique of removing and weighing the fibers without blotting, the ratio of adhering or extracellular solution (inulin space) to total wet weight is plotted in Fig. 6. The average ratio, W_{ae}/W_w, is 0.146 and appears to be independent of fiber size.

When the values for the various components of the total fiber wet weight are substituted into Eq. (1)

$$W_{H_2O} = W_w - 0.146\, W_w - 0.177\, W_w = 0.677\, W_w. \quad (3)$$

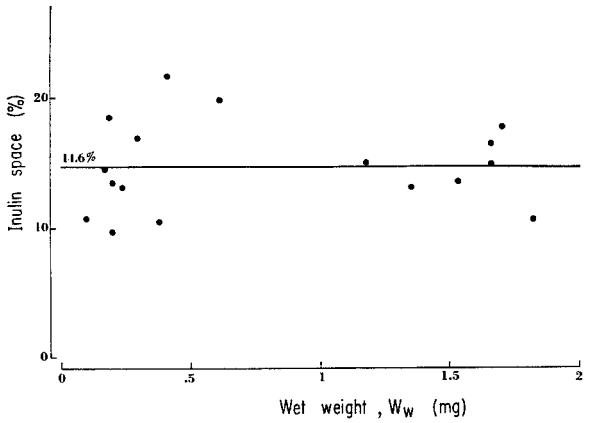

Fig. 6. Inulin space as a percentage of total wet weight. The average value is 14.6%

In order to compare the quantity of fiber water with that found by other investigators, we observe that the ratio of fiber water to cell weight (exclusive of adhering solution) is

$$\frac{W_{H_2O}}{W_{H_2O} + W_s} = 0.79.$$

This compares favorably with Boyle *et al.* (1941) value on whole frog sartorii muscle. They determined the amount of extracellular space as 0.13 l/kg of total muscle and concluded that the average fiber water was 0.67 l/kg of total muscle weight. This results in a ratio of fiber water to cell weight (exclusive of extracellular water) of 0.77.

Zachar and Hencek (1965), working on single fibers of *Astacus*, determined the ratio of fiber water to fiber weight as 0.75. However, these investigators got lower ratios (0.73) when they used a volume measurement method coupled with measurement of fiber density.

In the foregoing discussion on fiber water the component values of the total wet weight, W_w, were determined for fibers equilibrated in control solutions. When the fiber is subjected to different osmotic challenges it may shrink or swell due to ionic and water movements and the quantity of fiber water will change. If the external osmotic pressure differs (denoted by a prime on all symbols) from the control osmotic pressure, again we have

$$W'_{H_2O} = W'_w - 0.146\, W'_w - \beta'\, W'_w. \tag{4}$$

In this case $0.146\, W'_w$ is that portion of the total wet weight consisting of the adhering or extracellular solution and $\beta'\, W'_w$ is that portion of the wet weight which is the solid component of the fiber. For the fiber in control solution $\beta = 0.177$. Since the weight of the solid component is invariant under differing osmotic conditions

$$\beta'\, W'_w = \beta\, W_w = 0.177\, W_w \tag{5}$$

and by neglecting the weight of the adhering solution

$$\beta' = \frac{0.177}{W'_w/W_w} = \frac{0.177}{d'\,V'/d\,V} \tag{6}$$

where d is the fiber density, V is the total fiber volume and the prime indicates the values in media of different osmotic pressures.

The variation of volume with osmotic pressure for the crayfish muscle fiber has been investigated by Reuben *et al.* (1964). In the range of osmotic pressure that the present investigation is concerned with the regression equation is

$$V'/V = 0.74\ \pi/\pi' + 0.26 \tag{7}$$

where π is the osmotic pressure due to the impermeable ions in the external medium.

Combining Eqs. (4), (6) and (7) we get the quantity of fiber water at any osmotic pressure which is used in determining the intracellular ionic concentrations:

$$W'_{H_2O} = W'_w - 0.146\,W'_w - \frac{0.177\,W'_w}{d'/d\,(0.74\,\pi/\pi' + 0.26)}, \tag{8}$$

An error of less than $^1/_2$% is introduced in the determination of fiber water by assuming that the density of the fiber remains constant under varying osmotic pressures.

Determination of Internal Potassium. The following procedure was used to determine the internal potassium. After weighing, the fiber was digested in 500 µl of 0.3 M acetic acid. This quantity of solution permitted additional analyses to be made for other experiments. After 24 hours, quantities from 100 to 400 µl were siphoned off, $CaCl_2$ added to bring the concentration of the latter to 2 mM/l and the solution analyzed for potassium. (The purpose of adding $CaCl_2$ is detailed in Katz, 1968.)

A platinum loop capable of carrying 0.5 µl of solution was dipped into the digestion fluid and the potassium content determined with the integrative flame photometer. Since the loop utilized only a small percentage of the available sample, 3 readings were taken and averaged for each measurement. The usual precautions against contamination were taken.

Several fibers, after digestion, were removed from the digestion fluid and analyzed after ashing (Katz, 1968) to ascertain that all the potassium had been removed from the fiber into the digestion fluid. The amount that remained bound to the fiber was determined as less than 1% of the original total in the fiber.

The internal potassium concentration was calculated by

$$\mathrm{K\,(mM/kg\text{-}H_2O)} = \frac{\mathrm{K_0\,(mM/l)\,(500\times 10^{-6})\,(l) - K_e\,(mM/l)\,(0.146\,W'_w)\,(kg)}}{\left(1 - 0.146 - \dfrac{0.177}{0.74\,\pi/\pi' + 0.26}\right) W'_w\ \mathrm{(kg)}} \tag{9}$$

where: K_0 is the concentration in the digestion fluid, K_e is the concentration in the external bathing medium.

The constants are explained above and it is assumed that the density of the solution is 1.

C. Results

I. Internal Potassium; Fiber in Control Media

The internal potassium content of the crayfish muscle fiber (*Orconectes*) bathed in the chloride control medium (solution A) was determined as 130 ± 10 mM/kg-H_2O.

The internal potassium content of the crayfish muscle fiber bathed in propionate control media (solution B) was determined as 128 ± 13 mM/kg-H_2O. Within the accuracy of the methods, this may be considered as identical to the internal potassium for fibers bathed in chloride control media. There is indirect evidence to substantiate the equality of these two measurements. Zachar *et al.* (1964a) observed no long term change in resting potential which closely parallels K_i/K_0 with changing external chloride concentrations. Reuben *et al.* (1964) observe no "steady-state" volume or potential difference of the fiber in propionate or chloride control solutions. There is, however, a transient change induced in transferring a fiber from a chloride to a propionate control solution, which will be discussed subsequently.

II. Internal Potassium of Fibers in Media Made Hyperosmotic with Addition of K Salts

Increasing the concentration of the permeant potassium ion in the medium induces volume changes in the muscle fiber (Reuben *et al.*, 1964). However, the magnitude of the volume change depends upon the associated anion. When a fiber is exposed to a medium made hyperosmotic by the addition of K propionate, the fiber shrinks rapidly and maintains a smaller volume. When the fiber is exposed to a medium made hyperosmotic by the addition of KCl, there is an initial shrinkage with a gradual return to the initial volume and an overshoot to a slightly swollen state as KCl enters the cell.

The final equilibrium value of the internal potassium concentration was investigated with the fiber subjected to different hyperosmotic bathing media. Fig. 7 shows the results of these experiments. When the bathing medium is made hyperosmotic by the addition of K propionate to the propionate control medium (solution B), the fiber shrinks, and the internal concentration of potassium increases. We can determine the theoretically expected variation of internal potassium if we assume that ionic redistribution does not occur in the absence of a permeant anion. If the cell responds as a perfect osmometer to the increased osmotic pressure of the hyperosmotic solution, the fiber water compartment will decrease proportionately to the increased external concentration, and the internal concentration of potassium will likewise increase proportionately. This theoretical increase is also shown as the

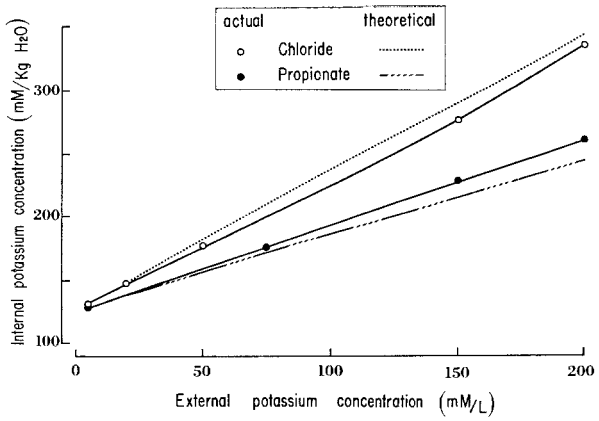

Fig. 7. The effect on internal potassium of hyperosmotic variation of external potassium concentration, as KCl (open circles) and K propionate (filled circles). The dotted and broken lines are calculated as described in text (Appendix)

broken line of Fig. 7. The observed values (filled circles) uniformly fall above the calculated ones, but fairly good agreement can be seen.

When the bathing medium is made hyperosmotic by the addition of KCl to the chloride control medium (solution A), the final ionic redistribution (by entry of KCl) results in a slight volume increase and an appreciably greater increase of the internal concentration of potassium. This is also shown in Fig. 7. Boyle and Conway (1941) analyzed this type of potassium accumulation in frog sartorii based on the consideration that the ionic distribution was described by a Donnan partial equilibrium. A theoretically expected variation of internal potassium can be calculated (appendix) if the same conditions are imposed, namely:

a) Osmotic equilibrium.

b) Electrical neutrality.

c) The Donnan equilibrium which implies in this case $[K]_i[Cl]_i = [K]_0[Cl]_0$.

d) The membrane is inelastic.

e) The activity coefficients on both side of the membrane are equal.

Again it can be seen that there is fairly good agreement with the experimental results. However, the observed values of K_i fall below the calculations.

It should be noted that in frog sartorii the value for η (representing the total nondiffusible substance) and for ε (representing the difference between the total negative and positive charges of the nondiffusible molecules) were identical and resulted in simplified theoretical equations.

In the crayfish muscle fibers η was calculated as 316 mM/l and ε was calculated as 121 meq/l of "osmotically active" fiber water which permitted no such simplifications. In the calculations it was assumed further that the value of η and ε remained constant despite the differing osmotic drives on the fibers during exposure to different solutions.

III. Internal Potassium: Fibers in Media Maintained Isosmotic with Addition of K Salts

Volume changes are also induced when fibers are subjected to media of different external potassium concentrations, but maintained isosmotic by replacing the sodium salt by equimolar amounts of the potassium salts (Reuben *et al.*, 1964). As in the hyperosmotic case, the magnitude of the volume change depends upon the anion associated with the potassium. When the fiber is exposed to an isosmotic increase of K propionate, its volume transiently decreases but stabilizes in a slightly swollen state. When exposed to isosmotic increases by KCl, the fiber gradually swells. At high concentrations of external KCl, the fiber could swell to about three times its normal value. This results from the obligatory entry of water associated with the entry of KCl.

Fig. 8 shows the final equilibrium value of the internal potassium with increasing external potassium while maintaining the bathing medium isosmotic. Despite the marked differences in volume changes (Reuben *et al.*, 1964), variable external concentrations of K propionate or KCl resulted in the same increase of internal concentration. Theoretically, based on the impermeability of the propionate ion, the internal potassium should remain constant for increases of external K propionate.

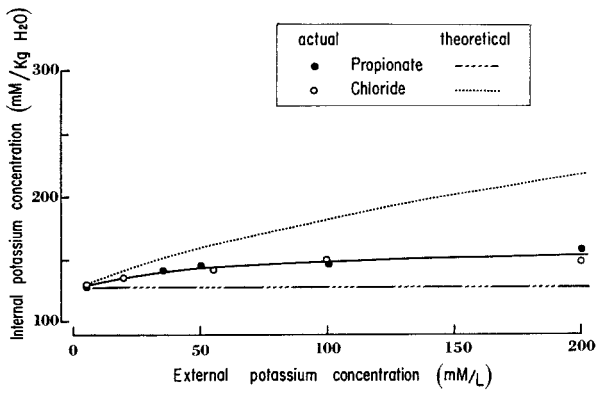

Fig. 8. The effect on internal potassium concentration of isosmotic variation of external potassium concentration. Symbols as in Fig. 7

The theoretically expected variation for the KCl case, based on the Donnan equilibrium regime and calculated in a similar manner as for the hyperosmotic solutions, shows a significant increase. In the frog sartorii, because η and ε are equal, there is no expected increase in K_i resulting from variable external KCl in isosmotic solutions. However, since η and ε are not identical in crayfish fibers, an appreciable increase in the theoretical internal potassium is expected.

In neither case was there close agreement between the calculated value of K_i and the actual observations. In the KCl case, the discrepancy is actually greater than is apparently shown by the difference between the theoretical and actual curves. The theoretical calculations indicate that for 200 mM/l of external KCl, the fiber water compartment should swell to 15 times its normal value which is obviously impossible. Reuben *et al.* (1964) have observed that the fiber swells to no more than 3 times its normal value. This restriction on size would result in the theoretical curve (dotted line) having a steeper slope and thereby exaggerating the difference between the actual and theoretical curves of Fig. 8.

IV. Transient Changes of Internal Potassium with Changes in External Chloride

When the fiber is equilibrated in the chloride control Ringer (solution A) and is suddenly exposed to the isosmotic propionate control Ringer (solution B) the fiber undergoes a simultaneous transient decrease in volume and a depolarization of the membrane (Reuben *et al.*, 1964). The fiber volume and membrane potential gradually return to their original values. A similar transient depolarization of the membrane has been observed in frog muscle fibers (Hodgkin and Horowicz, 1959). The volume response of a typical fiber from the experimental data of Reuben *et al.* (1964) but not one of the fiber used in their publication is shown in Fig. 9. With individual fibers, the transient voltage and volume changes lasted 5 to 20 minutes and the peak depolarization (not shown) varied from 15 to 25 mV.

Ten fibers were exposed to the chloride-free propionate solution and were then analyzed for internal potassium concentration at 1, 3, and 5 minutes after the immersion. The average values of K_i at the different time intervals are also shown in Fig. 9. There was a transient increase of internal potassium which gradually subsided with a return to the control value. Since individual volume variations were not determined on each of the fibers, a direct analytical treatment of the data was difficult. The average values were therefore compared with fibers which had approximately the same time course of transient volume change as the time course displayed by the internal potassium variation. For this selected fiber, the theoretical values of K_i were calculated assuming

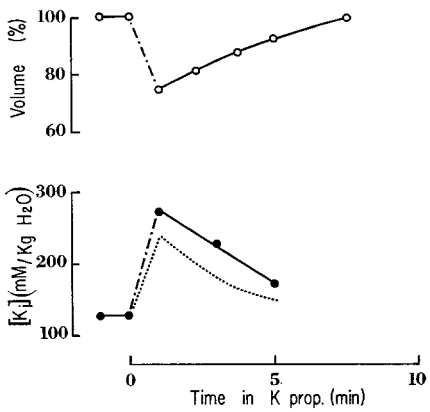

Fig. 9. Variation of volume and internal potassium with time in 200 mM/l of sodium propionate after equilibrating in 200 mM/l of NaCl. The dotted line is the theoretical variation for the transient volume change shown above. The latter measurements were from unpublished data of Reuben, Girardier, and Grundfest (1964)

that there was no exchange of ions across the membrane, but only water movement. Since a redistribution of KCl across the membrane is denoted by the transient change in the membrane potential (Hodgkin and Horowicz, 1959) these findings support the conclusion (Reuben *et al.*, 1964) that the change in membrane potential induces an electro-osmotic flow of water.

D. Discussion

Table 1 presents the internal potassium concentrations of the crayfish muscle fiber determined in this investigation as well as those of other investigators who have determined K_i in essentially the same bathing medium for muscle fibers of several genera of crayfish.

Table 1. Internal potassium concentration in crayfish muscle fibers

Genus	K (mM/kg-H_2O)	Source
Orconectes	130	This investigation
Orconectes	125	Dunham (unpublished)
Orconectes	117	Van der Kloot (1966)
Procambarus	171	Dunham (unpublished)
Astacus[a]	167	Zachar and Sajter (1965)
Orconectes[b]	128	This investigation

[a] By activation analysis on single fibers.
[b] Bathed in propionate Ringer.

Van der Kloot (1966) obtained a comparable value for K_i immediately after dissection using somatic muscle bundles from the deep flexor of the thorax. However, the value fell radically to 54.2 mM/kg-H_2O after 3 hours in Van Harreveld's solution. No such loss was observed in our preparations.

There appear to be species differences in the values obtained for *Orconectes*, which range between 117 and 130 mM/l on the one hand, and *Procambarus* and *Astacus* on the other, where K_i appears to be 171 and 167 mM, respectively. It is of interest that the electrophysiological properties of *Procambarus* muscle fibers resemble those of *Astacus* (Girardier, 1965; Zachar *et al.*, 1964b; Reuben, Girardier, Garcia, and Grundfest, unpublished).

The coincidence of transient volume changes, membrane depolarization, and increased potassium when the fiber is transferred from the normal chloride medium to one which is chloride free, substantiates the electroosmotic behavior of water flow. If the membrane contains negative fixed charges, the transient depolarization will induce a temporary outward water movement. This outward flux will result in the transient decrease in volume (and increase in internal potassium) which was observed in Fig. 9. The water content of the fiber is restored slowly in order to maintain osmotic equilibrium as the membrane potential returns to the resting value and K_i also returns towards its initial value.

There is still disagreement, however, on the laws governing the movement of water and ions or the establishment of intracellular concentrations. Boyle and Conway (1941) demonstrated that the behavior of whole frog sartorii muscles could be described by a Donnan partial equilibrium, and that the agreement with theory was independent of whether the external potassium was increased by making the bathing solution hyperosmotic or whether the external potassium was substituted progressively for sodium in equivalent relation (isosmotically). Adrian (1956) working on frog muscle and Sachar *et al.* (1964) working on crayfish muscle fibers also describe their preparation as conforming to the Donnan regime. Their conclusions were based solely on potential measurements. Both investigators, however, in order to explain discrepancies in their potential measurements invoke the permeability ratio of Na and K to allow for imperfect exclusion of sodium.

In this investigation the crayfish muscle fiber under some conditions appears to conform to the Donnan regime. In the hyperosmotic addition of KCl, the Donnan calculation predicts a large increase in internal potassium which agrees well with the measured values. For the hyperosmotic addition of K propionate, the internal potassium can be predicted based on the fiber behaving as an osmometer; yet, Reuben *et al.* (1964) show by their volume studies that the fiber, although main-

taining a volume displacement under this hyperosmotic condition, did not behave as a simple osmometer with a fixed dead space. The intercept on the volume ordinate of their pressure-volume curve is too large to be accounted for by the solid component of the single fiber and cannot be interpreted in terms of one or several fixed dead spaces. Similar findings have been reported by Blinks (1965) on frog fibers, by Mobley and Page (1971), on barnacle muscle, and by Freeman *et al.* (1966) on lobster and squid axon.

The fiber did not at all conform to the Donnan regime for isosmotic increases in KCl or K propionate (Fig. 8). Reuben *et al.* (1964) also found in crayfish fibers that the osmotic pressure-volume relationship differed depending upon whether the fiber was hyperosmotically or hypoosmotically challenged. In addition small-stepwise changes in K_0 resulted in different final volumes than did one large overall change in K_0. There is no apparent explanation for the discrepancy between the theoretical and actual curves. This difficulty highlights the limitation of the macroscopic technique of measuring the ionic concentration in terms of the contents of an entire fiber and formulating generalized conclusions concerning these ionic concentrations. The cell is not homogeneous and the ions are probably sequestered in various cellular compartments since muscle is a highly structured and complex system.

A scheme that would adequately describe the regulation of intracellular ionic concentrations must await the evaluation of ionic compartmentalization as well as metabolically controlled ionic distributions.

Appendix

Theoretical determination of intracellular potassium concentration. (1) The equations.

The derivation and nomenclature, with minor modifications, follow the Boyle and Conway (1941) analysis.

Let.

A, B, C, and G represent the total quantity (mM) of non-diffusible non-colloidal anions, non-colloidal cations, colloidal anions, and uncharged molecules, respectively, in 1 liter of cellular "fiber water" in normal bathing media.

k_i, d_i, the intracellular concentration (mM/l) of potassium and diffusible anions.

V, volume of "fiber water". In normal bathing media $V = 1$ liter.

a, b, the concentration (mM/l) of impermeable anions and cations in the extracellular solution.

k, d, the extracellular concentration (mM/l) of potassium and permeable anions.

c, the total extracellular concentration (mM/l).

If we assume that the membrane is inelastic and the activity coefficients of the univalent ions are identical so that we may deal with concentrations, we have for osmotic equilibrium under any condition

$$\frac{\Sigma A+\Sigma B+\Sigma C+\Sigma G}{V}+k_i+\Sigma d_i=\Sigma a+\Sigma b+\Sigma k+\Sigma d=c. \quad (10)$$

By letting

$$\eta\equiv\Sigma A+\Sigma B+\Sigma C+\Sigma G \quad (11)$$

where η is the total quantity of nondiffusible substance and substituting in Eq. (10) we get

$$k_i+\Sigma d_i=c-\eta/V. \quad (12)$$

For electrical neutrality

$$k_i+\frac{\Sigma mB}{V}=\Sigma d_i+\frac{\Sigma pA+\Sigma qC}{V} \quad (13)$$

where m, p, and q are valencies for the appropriate ions. Rearranging the preceding equation

$$k_i-\Sigma d_i=\frac{\Sigma pA+\Sigma qC-\Sigma mB}{V} \quad (14)$$

and if

$$\varepsilon\equiv\Sigma pA+\Sigma qC-\Sigma mB \quad (15)$$

where, ε is the total charge of internal nondiffusible anions and cations, Eq. (14) becomes

$$k_i-\Sigma d_i=\frac{\varepsilon}{V}. \quad (16)$$

The Donnan equilibrium relationship is $k_i d_i=kd$, the individual products can be summed to give

$$\Sigma k_i d_i=\Sigma kd. \quad (17)$$

(2) The determination of volume, V, and intracellular potassium k_i.

By subtracting Eq. (16) from Eq. (12) and rearranging we get

$$\Sigma d_i=\frac{c}{2}-\frac{\eta+\varepsilon}{2V} \quad (18)$$

by adding Eq. (16) to Eq. (12) and rearranging we get

$$k_i=\frac{c}{2}-\frac{(\eta-\varepsilon)}{2V}. \quad (19)$$

The product of Eqs. (18) and (19) yields

$$\Sigma k_i d_i=\Sigma kd=\left[\frac{c}{2}-\frac{(\eta-\varepsilon)}{2V}\right]\left[\frac{c}{2}-\frac{(\eta+\varepsilon)}{2V}\right]$$

and the volume, V, in quadratic form becomes

$$(c^2 - 4\Sigma kd)\, V^2 - 2\eta c\, V + (\eta^2 - \varepsilon^2) = 0. \tag{20}$$

(3) The evaluation of η and ε from the initial resting state.

With the fiber in the chloride control media (solution A) and a measured value of $k_i = 130$ mM/l, from Eq. (17)

$$\Sigma d_i = \frac{\Sigma kd}{k_i} = \frac{(5)(234.5)}{130} = 9 \text{ mM/l}$$

substituting in Eq. (12) for $V = 1$ liter

$$\eta = V[c - (k_i + \Sigma d_i)] = 455.5 - (130 + 9) \cong 316 \text{ mM}$$

and from Eq. (16)

$$\varepsilon = V(k_i - \Sigma d_i) = 130 - 9 = 121 \text{ meq}.$$

(4) Sample calculation.

The theoretical concentration for the hyperosmotic addition of KCl (Fig. 7) is carried out for the condition where external $k = 100$ mM/l

$$4\Sigma kd = (4)(100)(329.5) = 131,800$$

$$c = 645.5,\ \eta = 316,\ \varepsilon = 121.$$

Therefore from Eq. (20)

$$V = 1.19 \text{ liter}$$

from Eq. (19)

$$k_i = \frac{645.5}{2} - \frac{(316 - 121)}{(2)(1.19)} = 241 \text{ mM/l}.$$

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