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Potassium Accumulation in Muscle: A Test of the Binding Hypothesis

Abstract. *Living frog skeletal muscle can accumulate potassium in vitro to concentrations up to 580 millimolar. Both the amount of potassium accumulated and the relationship between intracellular and extracellular potassium concentrations indicate that potassium is "free" under all conditions, rather than bound to cellular macromolecules. The data also indicate that at most 20 percent of the cell water is "bound" in the sense that it excludes electrolytes.*

Several reports (*1*, *2*) have recently been published in support of the association-induction hypothesis of Ling (*3*). According to this theory, intracellular ion and nonelectrolyte contents are controlled not by membrane permeability and active transport, but by selective binding to cellular macromolecules and by exclusion by "ordered" or "bound" water. Thus, Na is kept at low concentrations within cells because of a low partition coefficient between cell water and extracellular water, whereas K is abundant within the cell in spite of this reduced ionic solubility because it is bound to intracellular charged sites with high selectivity over Na.

Sophisticated techniques have been used to probe the physical state of water and ions in cells to test this hypothesis. Intracellular ion activities measured with ion-selective electrodes are generally consistent with an activity coefficient for K close to that in extracellular fluids (*4*). However, in many cells, including the frog skeletal muscle, K is found to be at electrochemical equilibrium across the cell membrane. This is therefore also consistent with the binding hypothesis, which maintains that all solutes are at equilibrium between the cell and the extracellular fluid (*5*). Early nuclear magnetic resonance measurements of Na (*6*) and K (*7*) were interpreted as showing considerable immobilization of these ions. More recently, alternative explanations requiring only small amounts of bound ions have been presented (*8*). Conductivity studies (*9*) and diffusion coefficient measurements (*1*, *10*) have indicated that the mobilities of intracellular ions are between 10 and 50 percent of their mobilities in free solution. The binding hypothesis, however, does not necessarily predict drastically lowered mobilities, since site-to-site migration of ions could be rapid (*3*).

One crucial prediction of the association-induction hypothesis which can be tested relatively simply is that the K content of the cell should be a saturable function of external K. After the putative binding sites are filled, K, like Na, should be excluded from the ordered water system. Saturation at 90 to 100 μmole of K per gram of tissue (or about 140 mmole per liter of cell water) has been observed in frog skeletal muscle at external concentrations up to 10 mM (*11*, *12*). Boyle and Conway, in their classic 1941 paper (*13*), however, reported that as the external K was increased up to 300 mM

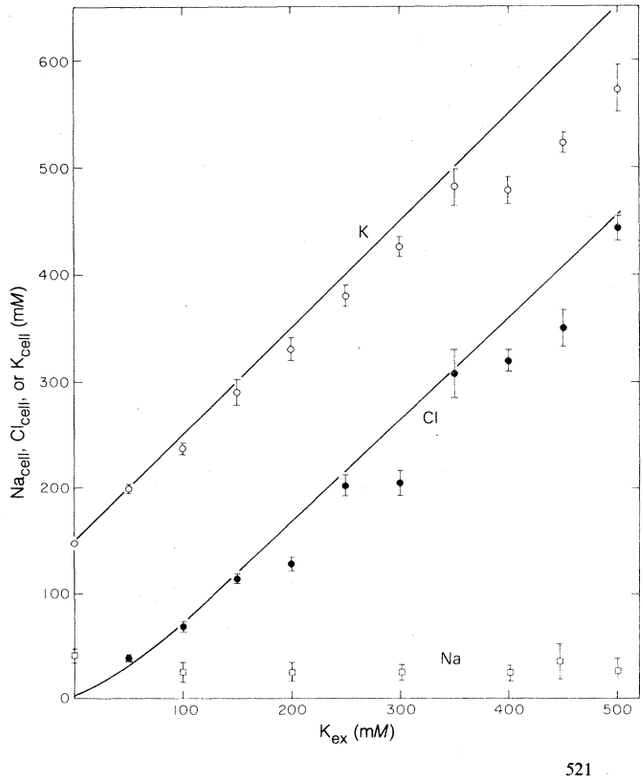

Fig. 1. Steady-state concentrations of K, Na, and Cl in the cells as a function of external K concentration. The cellular ion concentrations are given in millimoles per liter of cell water and are calculated from the equation $C_{cell} = (C_T - C_{ex}f_{ecs})/[1 - (W_d/W_w) - f_{ecs}]$ where C_T is the measured ion content of the tissue in micromoles per gram (wet weight), C_{ex} is the concentration of the ion in the external solution, W_d and W_w are the measured dry and wet weights of the tissues, and $f_{ecs} = 0.13$ is the extracellular space fraction of the tissue. The concentration of NaCl was held constant at 91 mM in each solution. The points shown for $K_{ex} \leq 400$ mM are from four to eight measurements (± standard error of the mean) made after 8-hour incubations. The points for $K_{ex} = 450$ mM and 500 mM were obtained after 1-hour incubations (*17*). The solid lines are theoretical fits drawn from Eqs. 1 and 2 (*14*). The parameter $(\epsilon - \eta)/2V$ was chosen to fit the measurement of K_{cell} at a physiological K_{ex}. The value obtained was 30 mM with $\epsilon/V = 145$ mM and $\eta/V = 85$ mM. These values were assumed to be constant in solutions of varying KCl.

the cell could accumulate K to levels far exceeding 100 μmole/g, as predicted for a simple Donnan equilibrium system (*14*). However, Boyle and Conway did not report routine measurements of the Na content of cells that had accumulated large amounts of K. They demonstrated steady-state levels of tissue Na only when muscles were incubated with external K in the range 30 to 150 m*M* at 0°C. Therefore, the possibility remained that the ordered cell water had broken down in solutions of higher external K concentrations. According to the ordered water theory, a rise in cell Na could imply a weakening of the cell water structure and the accumulation of cellular K could be explained simply by an increase of freely dissolved K in the cell water. We have repeated those experiments, using the rigorous standard that cell Na be no higher than that in fresh tissue as a condition for the viability of the cell.

Sartorius and semitendinosus muscles from *Rana pipiens pipiens* were excised and transferred to a Ringer solution (*15*) containing 2.5 m*M* K. A batch of four to six muscles was transferred to a solution made hypertonic by the addition of 50 m*M* KCl to the normal Ringer solution. These muscles were incubated for 30 minutes at room temperature (22° to 25°C) with constant stirring at 150 rev/min and then transferred to the next solution, containing additional KCl. This serial transfer was carried out in the order 50, 100, 200, 300, 400, and 500 m*M* KCl or 50, 150, 250, 350, and 450 m*M* KCl until the desired concentration was reached. Muscles transferred directly to very hypertonic solutions gained Na and H_2O, in violation of the criterion above. The serial transfer method extended the range of KCl concentrations in which the tissues would survive. The tissues were left in the final solution for 1 to 8 hours and then were analyzed for K, Na, Cl, H_2O, and trace [^{14}C]sucrose or inulin (*12, 16*).

Steady-state levels (*17*) reached for K, Na, and Cl are plotted as functions of external K concentration in Fig. 1. Two conclusions can be drawn from this figure. First, as much as 580 m*M* K, or 380 μmole/g, can be accumulated by the muscle while the Na content is maintained at low, physiological levels. An overestimate of the number of negative fixed charge sites in muscle can be made by assuming that the entire dry weight of the cell represents myosin, a highly charged protein which has 154 negative charges per 10^5 g (*18*). Using 0.2 g of protein per gram of tissue leads to an estimate of 310 μeq per gram of tissue, lower than the maximum amount of K actually accumulated. The discrepancy becomes much larger when it is considered that the K uptake shows only a slight trend toward saturation over this concentration range (*19*).

Second, at extracellular K concentrations from 2.5 to 350 m*M* the internal K increases nearly equal the external increases. This result is predicted from the simple Donnan formulation of Boyle and Conway (*14*) provided, as was approximately the case in our experiments (*20*), the cell volume remained constant. This suggests that osmotic equilibrium is established across the cell membrane by the accumulation of K and Cl ions which have osmotic coefficients in the intracellular water that are not much different from those in the extracellular water. Thus, in this important aspect the water and ions in the cell interact normally. Quantitatively, a linear regression analysis of the data in Fig. 1 yields a slope

$$d\mathrm{K_{cell}}/d\mathrm{K_{ex}} = 0.81$$

(correlation coefficient, $r^2 = 0.97$) which is close to the expected value (*14*). This slope could be explained if 19 percent of cell water strictly excluded water, or if the osmotic coefficient for K in the cell water was 20 percent higher than in the external solution. However, if only the data before the apparent discontinuity at $\mathrm{K_{ex}} = 350$ m*M* are used, the slope is:

$$d\mathrm{K_{cell}}/d\mathrm{K_{ex}} = 0.99$$

($r^2 = 0.997$). This simple analysis demonstrates that K is dissolved in cell water with a partition coefficient between cell water and extracellular water which is close to 1. This refutes the basic tenet of the association-induction hypothesis: that K is accumulated by the frog muscle by specific binding. It shows also that practically all the cell water may be accessible to KCl and that at least 80 percent of the water in the frog muscle cells is normal in its solvent properties.

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5. From the assumption of equilibrium

$$\frac{a_\mathrm{K}(\mathrm{cell})}{a_\mathrm{K}(\mathrm{ex})} = \frac{\gamma_\mathrm{K}(\mathrm{cell})C_\mathrm{K}(\mathrm{cell})}{\gamma_\mathrm{K}(\mathrm{ex})C_\mathrm{K}(\mathrm{ex})} =$$

$$\exp(-F\chi/RT) \cong 50 \text{ if } \chi = -100 \text{ mv}$$

Therefore

$$a_\mathrm{K}(\mathrm{cell}) = \gamma_\mathrm{K}(\mathrm{cell})C_\mathrm{K}(\mathrm{cell}) \cong 94 \text{ m}M$$

where the α_K's are the activities of K as measured by electrodes in the cell and the extracellular fluid, the γ_K's are the respective activity coefficients, the C_K's are the K concentrations, and χ is the electrical potential across the cell membrane. The usual interpretation is that γ_K(cell) is close to that in the Ringer solution and that C_K(cell) is 140 m*M* as measured chemically. Alternatively, if most of the cell **K** is bound, then C_K(cell) is small and $\gamma_\mathrm{K}(\mathrm{cell}) \gg 1$. This is consistent with the assumption of reduced solubility for K in the cell water.
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14. According to the formulation of Boyle and Conway (*13*),

$$\mathrm{K_{cell}} = \frac{M}{2} + \frac{\epsilon - \eta}{2V} \quad (1)$$

where η is the amount of osmotically active impermeant solute in the cell, ϵ is the amount of net negative impermeant charge in the cell, V is the cell volume (= 1 in the normal Ringer solution), and M is the total osmolarity of the external solution. Boyle and Conway found that $\epsilon \approx \eta$, so that if the external osmolarity was varied by the addition of KCl

$$\mathrm{K_{cell}} = \frac{M}{2} = \mathrm{K_{ex}} + \text{constant}$$

In our system $\epsilon \neq \eta$, but since the volume changed only slightly (*20*) the relations become

$$\mathrm{K_{cell}} = \frac{M}{2} + \text{constant}$$

and

$$\frac{d\mathrm{K_{cell}}}{d\mathrm{K_{ex}}} = 1$$

The internal chloride concentration in the formulation is given by

$$\mathrm{Cl_{cell}} = \mathrm{Cl_{ex}} \frac{\mathrm{K_{ex}}}{\mathrm{K_{cell}}} \quad (2)$$

15. The composition of the Ringer solution was (in millimoles per liter): NaCl, 91; $NaHCO_3$, 17.3; Na_2HPO_4, 1.2; NaH_2PO_4, 2.0; $CaCl_2$, 0.7; $MgCl_2$, 1.2; glucose, 5; and varying amounts of KCl. The solutions were bubbled with a mixture of 95 percent O_2 and 5 percent CO_2 to maintain *p*H 7.4.
16. The incubated muscles were gently blotted on moist filter paper and weighed. They were divided into two batches. Muscles in one batch were extracted overnight in 0.1*N* HNO_3; those in the other batch were first dried overnight at 98°C, weighed again, and then extracted in 0.1*N* HNO_3. The wet-weight extract was used for the determination of [^{14}C]sucrose, inulin, K, and Na. The dry-weight extract was used for the determination of H_2O, Cl, Na, and K. Inulin was measured colorimetrically; Cl was titrated with an Aminco-Cotlove automatic chloride titrator; Na and K were measured on a flame photometer with the use of proper counterions and standards; and the ^{14}C activity of the extracted sucrose was counted on a Packard β-scintillation counter. The sucrose space of muscles after a 1-hour incubation in sucrose was found to be 13

percent, and this value was used as the extra cellular space for correcting the data. This value was not significantly different at external K concentrations of 2.5, 100, and 400 m*M*, and was constant over an 8-hour period at the two higher concentrations. At 2.5 m*M* K the sucrose space rose gradually from 13 to 20 percent after 8 hours of incubation in sucrose. The inulin space of muscles in control solutions was 11 percent.

17. The time course of changes in the K, Na, Cl, and H_2O for up to 8 hours was determined in each of the solutions. It was found that steady levels were achieved in less than 1 hour after transferring the cells to the final solution. The steady levels were maintained for 8 hours in all solutions studied except for the two with the highest K_{ex}. In these solutions (K_{ex} = 450 and 500 m*M*), the cells gradually gained Na and H_2O above the physiological levels at 2 hours and beyond. Our results for Cl uptake differed somewhat from those of Adrian (*21*), who found a continuous rise in cell Cl from 1 to 5 hours in 100 m*M* KCl. The discrepancy may be due to a species difference or to slight differences in Ringer solutions.
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19. A double-reciprocal plot of the data ($1/K_{cell}$ against $1/K_{ex}$) is nonlinear. If the points at $K_{ex} \geq 150$ m*M* are used, the data fall approximately on a straight line, giving an extrapolated saturation value of 2500 μmole per gram of tissue. This is an order of magnitude larger than the number of fixed negative charges in the cell.
20. In solutions with high K_{ex} the muscles lost a small amount of water, the water content falling from 80 percent in control solution to 77 percent. As discussed by Adrian (*21*), this shrinking may reflect the inequality of the ϵ and η terms.
21. R. H. Adrian, *J. Physiol. (London)* **151**, 154 (1960).
22. The initial phases of this study were carried out at the Department of Molecular Biology, Pennsylvania Hospital, Philadelphia. Supported by PHS grant HL-07762.

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Carbon Monoxide: Effects on Oxygenation of the Fetus in utero

Abstract. *The partial pressure of oxygen in fetal blood decreases in proportion to the carboxyhemoglobin concentrations in fetal and maternal blood. Because fetal oxygen tensions normally equal 20 to 30 percent of the values for adults, this reduction can result in severe hypoxia of vital tissues. Decreases in oxygen tension may be a factor in the lower birth weights of infants born to women who smoke or are exposed to severe air pollution.*

Bernard (*1*) first showed that the physiologic effects of CO result from decreased capacity of the blood to transport O_2. Several workers (*2, 3*) have predicted that increased blood carboxyhemoglobin (HbCO) concentrations should result in decreased O_2 partial pressures in outflowing (venous) blood from a particular tissue bed. The question remains, however, to what extent these changes occur in vivo in the presence of possible compensatory changes in blood flow. An additional question concerns the effects of elevated blood HbCO concentrations on blood O_2 levels in the fetus, in which the O_2 partial pressure in arterial blood is normally only 20 to 30 torr, compared with about 100 torr in the adult. The work reported here was done to explore these questions.

I implanted small Tygon catheters in a maternal artery and a branch of the uterine vein of pregnant sheep. In the fetus, catheters were placed in a pedal artery and passed into the descending aorta, and in a pedal vein and passed into the inferior vena cava below the ductus venosus. Four or more days after recovery, I exposed the ewes to CO concentrations of 30, 50, or 100 parts per million (ppm) for 36 to 48 hours in an effort to achieve equilibrium between maternal and fetal partial pressures of carbon monoxide. Nine to 11 experiments were performed with six or seven different animals at each CO concentration. Experiments were not performed more frequently than at weekly intervals in animals that underwent repeated study. I measured oxygen partial pressures and HbCO concentrations repeatedly during CO uptake and equilibrium. Inspired CO was monitored with a CO analyzer (Ecolyzer, Energetics Science, Inc., Elmsford, N.Y.). Blood HbCO was determined spectrophotometrically by using an instrument (CO-Oximeter, model 182, Instrumentation Laboratory, Lexington, Mass.) that was recalibrated daily to maintain an accuracy of ± 1 percent in the HbCO range from 1 to 30 percent saturation. Blood partial pressures of O_2 and CO_2 and blood *p*H were determined by using appropriate microelectrodes (Radiometer model BMS 3, London Co., Westlake, Ohio).

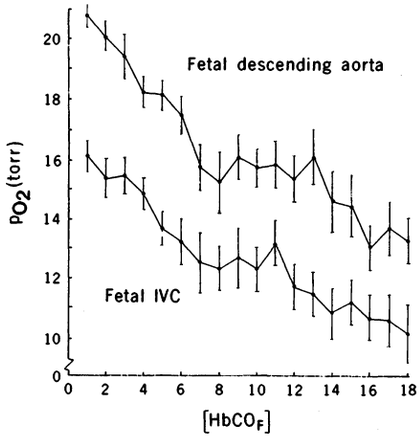

Fig. 1. Oxygen partial pressures in blood of the fetal descending aorta and the inferior vena cava (*IVC*) as a function of fetal HbCO concentrations during quasi-steady-state conditions of maternal-to-fetal CO exchange. Fetal inferior vena cava O_2 tension varies as a function of both maternal and fetal HbCO concentrations. The O_2 tension of fetal arterial blood is chiefly a function of maternal HbCO concentration. However, during steady-state conditions it is also related to the fetal HbCO concentration. Each point represents the mean ± standard error of the mean (vertical bars) of 6 to 20 determinations at each HbCO concentration. The indicated HbCO concentrations are actually ± 0.5.

Control values of maternal and fetal HbCO concentrations determined before exposure to CO, were, respectively, $[HbCO_M] = 1.1 \pm 0.2$ percent and $[HbCO_F] = 1.8 \pm 0.3$ percent (mean ± standard error of the mean). During maternal-to-fetal CO exchange after exposure, O_2 partial pressures decreased in the maternal uterine vein and in the fetal arterial and venous blood. The O_2 tension of uterine venous blood decreased from a control value of about 43 torr at a maternal HbCO concentration of 1 percent to about 39 torr at 10 percent. The least-squares regression equation for this relation was $pO_2 = 43.1 - 0.5[HbCO_M]$ (correlation coefficient $r = -0.96$), where pO_2 is the oxygen partial pressure.

Figure 1 shows that, under these quasi-steady-state conditions, the O_2 partial pressure in the fetal descending aorta decreased to 16 torr at a fetal HbCO concentration of 10 percent, from a control value of about 20.5 torr (*4*). The regression equation for this relation was $pO_2 = 20.1 - 0.4[HbCO_F]$ ($r = -0.94$). These O_2 tensions represent the mean values of 6 to 20 determinations at each HbCO concentration. An HbCO concentration of 10 percent actually represents the values that were greater than 9.5 percent but less than 10.5 percent, rather than exactly 10.0 percent. Figure 1 also shows the relation of O_2 tension in the fetal inferior vena cava, below the ductus venosus, to the fetal HbCO concentration. At a fetal HbCO concentration of 10 percent, the O_2 tension in the inferior vena cava decreased from a control value of 16 torr to about 13 torr. The regression equation for this relation was $pO_2 = 15.8 - 0.3[HbCO_F]$ ($r = -0.96$).

The O_2 tension in fetal arterial blood reflects the adequacy of placental exchange. Strictly speaking, it is a function of the O_2 tensions in the maternal and fetal placental end capillaries, which in turn are related to the maternal HbCO concentration. The O_2 partial pressure in fetal venous blood reflects the adequacy of tissue oxygenation. It decreases as a result of increased fetal and maternal HbCO concentrations (which result in