THALLIUM AND CESIUM IN MUSCLE CELLS COMPETE FOR THE ADSORPTION SITES NORMALLY OCCUPIED BY K^+

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THALLIUM AND CESIUM IN MUSCLE CELLS COMPETE FOR THE ADSORPTION SITES NORMALLY OCCUPIED BY K^+

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• *It was shown that Tl^+ can stoichiometrically displace K^+ in frog muscle cells. This displacement is independent of the function of cell membrane and postulated pumps and of a macroscopic electric charge or Donnan effect. It follows that Cs^+ and Tl^+ compete for the adsorption sites normally occupied by K^+. On this basis, the association-induction hypothesis predicts that Cs^+ and Tl^+, like K^+, should be primarily localized in the A-band of the muscle myofibrils.*

INTRODUCTION

According to membrane-pump theory, the bulk of intracellular ions are in the free state. Ionic accumulation in living cells primarily follows the prediction of a Donnan equilibrium, modified by membrane pumps[1-4] and selective absolute membrane impermeability.[5] In the alternative association-induction hypothesis, the bulk of intracellular ions are in the adsorbed state.[6-9] Under the hypothesis, the theory of Donnan equilibrium is not applicable to ionic distribution, which is considered to be determined primarily by the solubility of the ion in cell water and the availability of adsorption sites.

To test these competing ideas, advantage has been taken of the proposed different effects of one ion upon the intra-extracellular distribution of a pair of ions. In the Donnan equilibrium case, the effect should be essentially the same as long as the pair of ions have the same valency. A different relation is predicted by the association-induction hypothesis; here the competing ion, as a rule, has different effects on the equilibrium distribution of a pair of ions even if the latter have the same valency. The divergent effects follow from their respective different adsorption energies on the same sites for which they all compete. Investigating along this line, Ling and Ochsenfeld showed that K^+ is three times as effective against Cs^+ accumulation as against K^+ accumulation, in agreement with the prediction of the association-induction hypothesis but contradicting the Donnan membrane equilibrium model.[10]

To further test the alternative theories I have attempted to find out, first, if in an effectively membrane-less and therefore pump-less cell preparation (EMOC) the

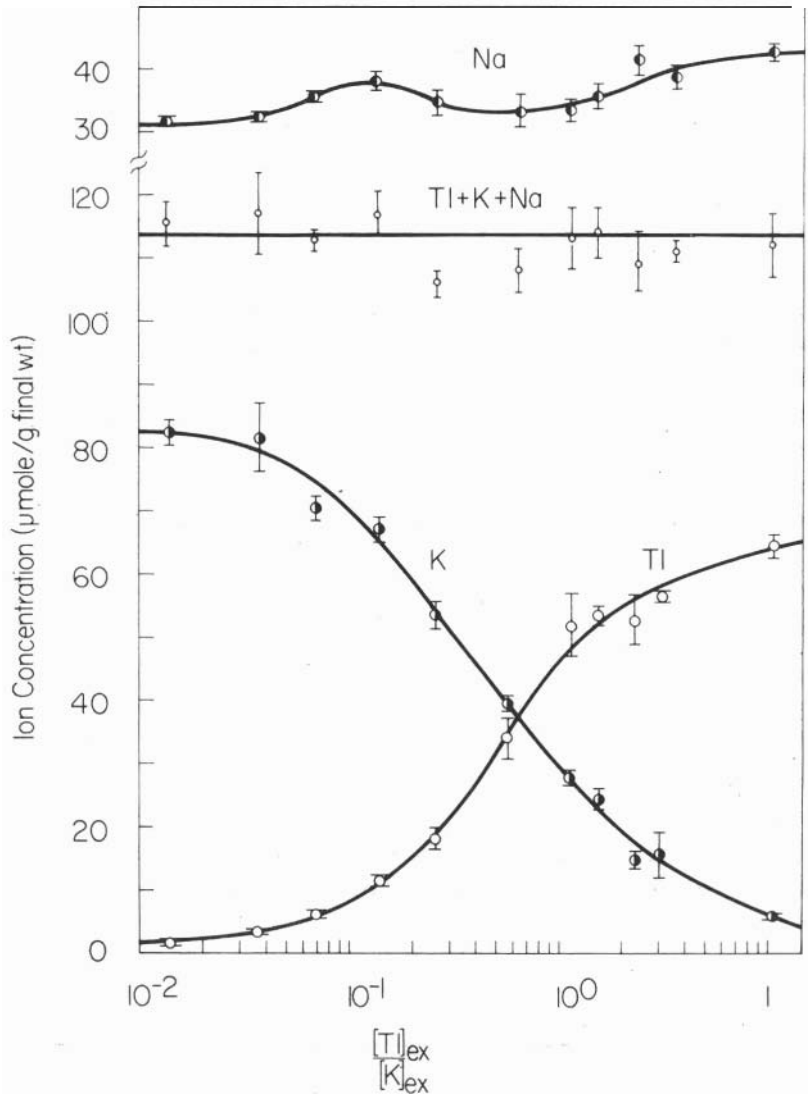

FIGURE **2.** ***Equilibrium distribution of Tl^+, K^+, and Na^+ in frog muscles.*** **To produce the wide range of $[Tl^+]_{ex}/[K^+]_{ex}$ represented on the abscissa, $[K^+]_{ex}$ was kept constant at 2.5 mM below a $[Tl^+]_{ex}/[K^+]_{ex}$ value of unity. Above that, $[Tl^+]_{ex}$ was kept constant at 4 mM. Each point represents the means of 4 or 5 determinations ± SE.**

and K^+, spanning 3 log cycles (1.4×10^{-2} to 1.1×10). To be noted is the observation that the sum of the concentrations of Tl^+, K^+, and Na^+ in the muscle cells remained constant at around 113 μmoles/g fresh tissues throughout the entire span of $[Tl^+]_{ex}/[K^+]_{ex}$ values. The primary event observed was a stoichiometric displacement of K^+ by Tl^+. It should be pointed out that muscles with their K^+ virtually completely replaced by Tl^+ remained quite normal in appearance and were

excitable. Figure 2 also shows that at the point of half exchange, $[Tl^+/K^+]_{ex} = 0.52$; this yields an intrinsic equilibrium constant $K^{\circ\circ}$ (K → Tl) for the Tl^+-K^+ exchange equal to 1.92 in favor of Tl^+. From a log-log plot of the ratio of Tl^+/K^+ in the cell against that in the medium, one obtains an n-value of 1.15 corresponding to a small nearest-neighbor interaction energy $(-\gamma/2)$ of 0.08 kcal/mole.

Accumulation of Cs^+ and Ti^+ in an effectively membraneless open-ended (EMOC) muscle cell preparation. In previous publications I have shown that in muscles suspended in air with only the cut end in contact with a Ringer solution containing labeled ions, the postulated membrane-pumps, though they would be intact, cannot function.[14] Yet in this cell preparation, K^+ accumulated to a level above that in the source medium while Na^+ was kept below that in the external medium much as in normal living cells.[15] Figure 3 shows the spatial ion distribution along the length of the sartorius muscle, from the exposed cut end toward the intact end suspended in air, after a 3-day exposure to a Ringer solution containing Cs^{134}-labeled Cs^+, Na^4-labeled Na^+, and Tl^{204}-labeled Tl^+. It is clear that the

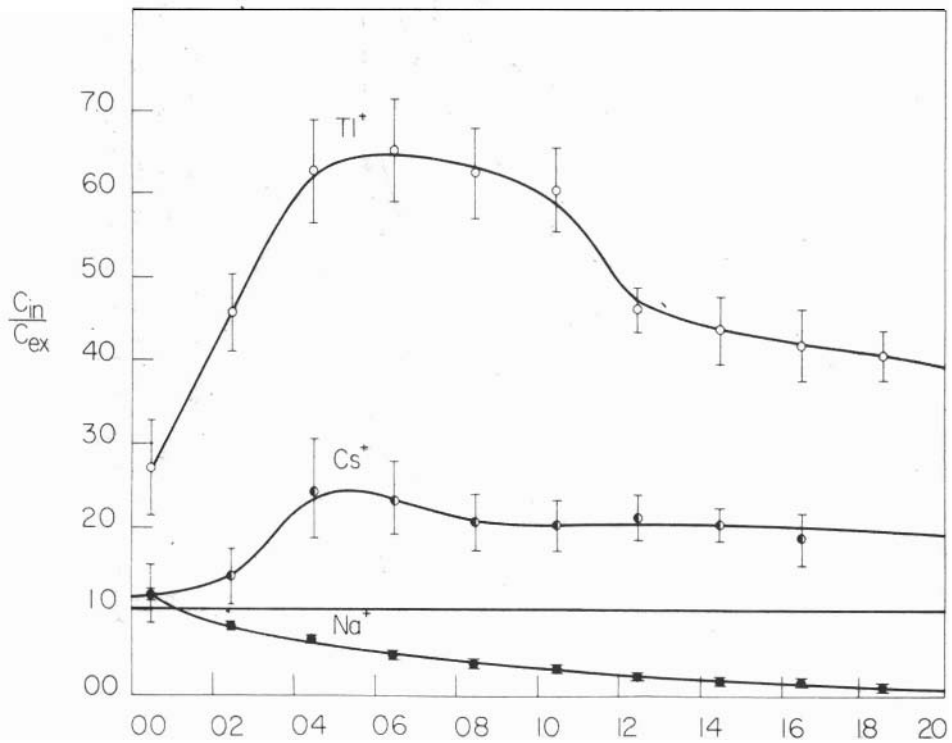

FIGURE 3. ***Accumulation of labeled Tl', Cs^+, and Na^+ in frog sartorius EMOC preparation.*** Source solution contained 1 mM Tl^{204}-labeled Tl, 1 mM Cs^{134}-labeled Cs^+, and 100 mM Na^{24}-labeled Na'. Ordinate represents ionic concentration in cell water divided by the final concentration of the same ion in the source solution bathing the cut-end at the conclusion of the experiment. Incubation was for 3 days at 25°C.

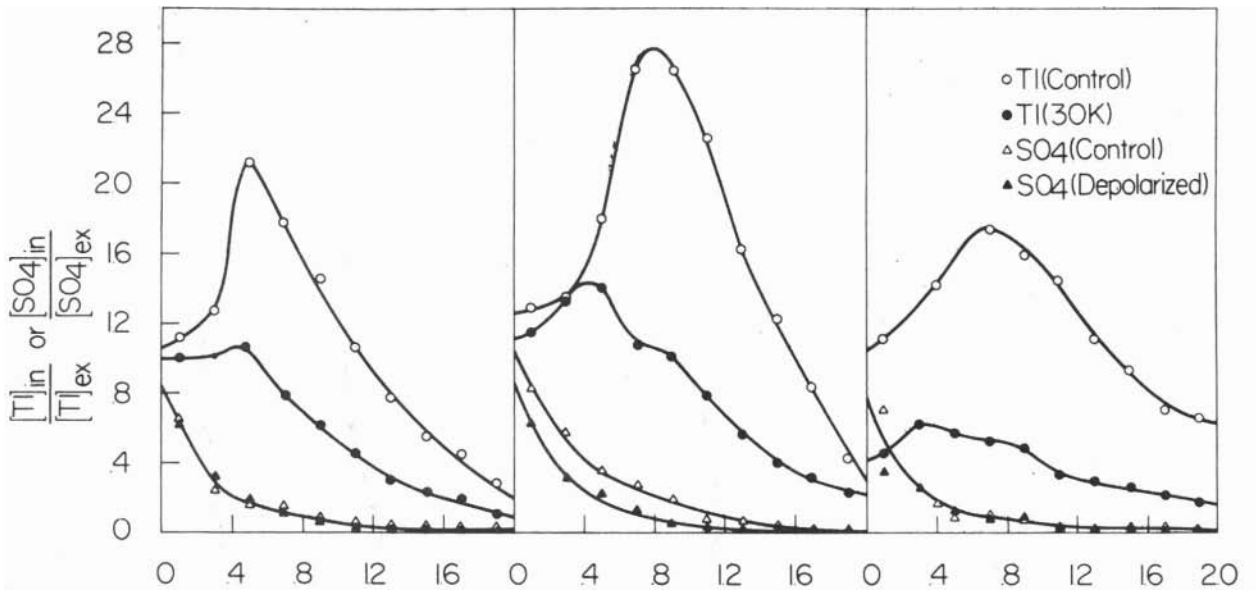

FIGURE 4. ***Influence of K⁺ and electrical depolarization upon the accumulation of cationic-labeled Tl⁺ and anionic-labeled sulfate.*** **Source solution contained either 0.5 mM KCl (wntrolj or 30 mM KC1 (experimental). Experimental muscles (but not the controls) were exposed with gentle shaking to K_2SO_4 Ringer for 1 h before being mounted in EMOC tubes. Incubation war at 25°C for 24 h.**

levels reached for these 3 monovalent ions follow the rank order $Tl^+ > Cs^+ > Na^+$, which is entirely reasonable since we have just seen that Tl^+ is preferred over K^+ by a factor of 1.92 and in earlier work Ling and Bohr have shown that K^+ is preferred over Cs^+ and Na^+ by factors of 1.81 and 260 respectively.[18]

Depolarization action of muscles exposed to K_2SO_4-Ringer containing 100 mM K^+. From 6 muscles and a total of 24 measurements, the resting potential of frog sartorius muscles after 48 h of incubation in a moist chamber following a' 1 h soaking in a 100 mM K_2SO_4 Ringer was 12.4 ± .4 mV in a comparison with the resting potentials measured in control muscles: 83.3 ± 0.42 mV.

Effect of including 30mM K^+ in the source solution bathing the cut end of the muscle and electrical depolarization of the whole muscle cells on the accumulation of Tl^+ and SO_4^{2-} in an EMOC preparation. Figure 4 compares the distribution of Tl^+ and SO_4^{2-} in muscles that had been exposed to a low K^+ Ringer containing 2.5 mM of labeled Tl^+, and the distribution in muscles that had first been depolarized by incubation in K_2SO_4 Ringer and subsequently exposed at the cut end to a Ringer solution containing 2.5 mM of labeled Tl^+ and 30 mM KCl.

The data show that exposure to 30 mM KCl in the source solution bathing the cut end following prior exposure to K_2SO_4-Ringer containing 122 mM K^+ strongly depresses the uptake of labeled Tl^+. Yet the same treatment did not change, or slightly decreased, the SO_4^{2-} uptake of the same muscles.

DISCUSSION

The maintained preferential accumulation of T1+ and Cs^+ over Na+ in the effectively membraneless open-ended muscle cell preparation, much as in a normal muscle, shows that the selective accumulation of Tl+ and Cs^+ and the exclusion of Na^+ have little to do with membrane functions, further confirming conclusions from earlier studies using the EMOC technique.[14,15] Indifference of the distribution of the anion SO_4^{2-} to the depolarization of the resting potential clearly shows that the distribution of ions in living muscle cells does not follow the prediction of the Donnan membrane theory. Were it otherwise, the suppression of Tl^+ uptake by high concentrations of K^+ applied should be accompanied by a corresponding gain of SO_4^{2-} as dictated by the fundamental relation of a Donnan membrane equilibrium:

$$r = ([K^+]_{in} / [K^+]_{ex}) = ([SO_4^{2-}]_{ex} / [SO_4^{2-}]_{in})^{1/2} \quad (1)$$

where the Donnan ratio r is equal to the cation K^+ concentration ratio and the square root of the reversed SO_4^{2-} concentration ratio. Furthermore, the membrane potential ψ should be related to r:

$$\psi = \frac{RT}{F} \ln r, \quad (2)$$

or $$r = \exp\left(\frac{\psi F}{RT}\right). \quad (3)$$

Under normal conditions, ψ is about 85 mV and the observed ratio of intracellular and extracellular K^+ and SO_4^{2-} are not too far from the prediction of Eqs. 1 and 3. However, by applying a Ringer solution containing a K^+ concentration exceeding 100 mM, $[K^+]_{in} / [K^+]_{ex} \rightarrow 1$ and ψ approaches zero. Hence the Donnan theory predicts an accompanying pronounced gain of intracellular SO_4^{2-}. In fact no such gain occurs. This observation offers further refutation of the applicability of Donnan membrane theory to the living cell as it has been shown that the displacement of cell cation is ion-specific and not' merely valency-specific, in contradiction to the prediction of the Donnan membrane equilibrium but in full agreement with the association-induction hypothesis. Thus Cs^+ and $T1^+$ compete with K^+ but in a way totally independent of membrane (pump) functions and a macroscopic electric charge or Donnan effects. These restrictions leave us with the only known alternative; suggested for example by the association-induction hypothesis: all three ions compete for the same adsorption sites in the muscle cells.

With this knowledge, the association-induction hypothesis can then further predict that Cs^+ and $T1^+$ should, like K^+ in normal cells, be adsorbed on myosin and thus be localized primarily in the A-band.

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