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SIMULTANEOUS NET ACCUMULATION OF BOTH K^+ AND Na^+ BY LYMPHOCYTES AT 0°C

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Summary

Human lymphocytes at 0°C in low Na^+ medium accumulate both K^+ and Na^+ to levels higher than in the external medium. This is not due to an impermeable compartment or a Donnan equilibrium, and is incompatible with the membrane Na^+-pump concept. In contrast, it supports prior evidence that ion exchange in lymphocytes is mediated by adsorption onto and desorption from fixed anionic sites within the cell. Additional aspects of ion and water contents of cells in low Na^+ medium are described and are explained by this concept.

The conventional interpretation of the asymmetric distribution of K^+ and Na^+ between cells and their environment assumes that the physical state of cell water and ions is essentially that of a dilute aqueous solution and that ions that are dissolved in cell water have thermodynamic activity coefficients that are similar to those of ions in the external medium. Therefore, the difference in the macroscopic concentrations of cell and medium ions is assumed to reflect true chemical (or electrochemical) gradients. The tendencies of K^+ and Na^+ to leak down these gradients are felt to be balanced by a coupled K^+ and Na^+ pump, the $(Na^+ + K^+)$-ATPase. In the membrane theory, the maintenance of normal cell ion levels is ultimately dependent upon the pump. If it were inhibited, as at low temperature, then there should be a gain of cell Na^+ and a loss of cell K^+, and the levels of cell ions should either approach those in the external medium, or reach an electrochemical equilibrium of the Donnan type.

We have, however, found considerable evidence that the amount and exchange of the major part of lymphocyte K^+, and of Na^+ that may replace it,

are determined not by the plasma membrane, but by adsorption onto and desorption from fixed anionic sites throughout the cell [1—5]. In this view, the maintenance of cell ion levels is not dependent upon the function of an enzymatic pump. Rather, the levels of both K^+ and Na^+ that are dissolved in cell water are less than in the external medium, reflecting their decreased solubility in water that exists in a more ordered (polarized) physical state; while the high level of total cell K^+ is due to selective adsorption onto intracellular macromolecules.

To test further these opposing concepts, we determined the ion contents of human lymphocytes at 0 and 37°C in both normal (145 mM) and low (19 mM) Na^+-containing media.

Lymphocytes were prepared from normal fresh blood. Cell separation, the composition of the incubation medium, centrifugation of cells from the medium (without washing), the measurement of trapped space in the cell pellet with poly(ethylene glycol) (10.1% of weight), the determination of K^+ and Na^+ by atomic spectroscopy, and the determination of water content were as described previously [1,4]. The incubation medium was similar to Hank's medium and contained 10% autologous serum, 5 mM K^+, and either 145 or 19 mM Na^+. The 19 mM Na^+ medium contained 7.3 g sucrose/100 ml to maintain approximate isotonicity.

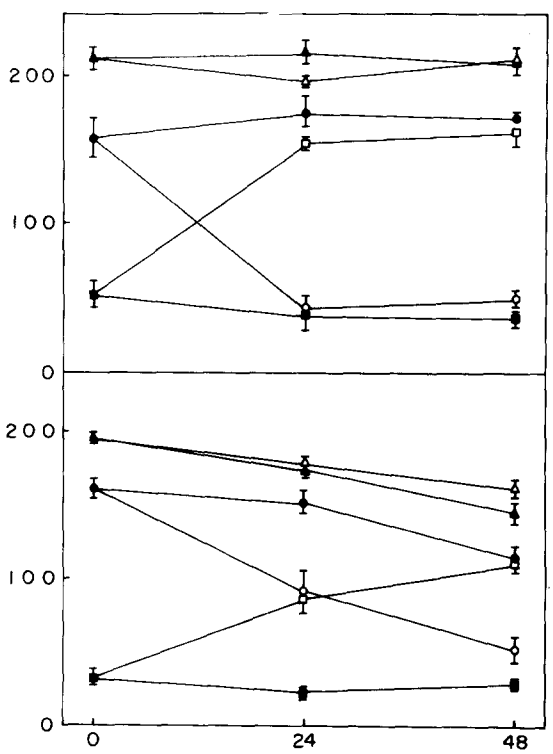

Fig. 1. Ion contents over 48 h. (top) Cells are in 145 mM Na^+; (bottom) cells are in 19 mM Na^+. ●, K^+; ■, Na^+; and ▲, $K^+ + Na^+$ at 37°C. ○, K^+; □, Na^+; and △, $K^+ + Na^+$ at 0°C. Means ± S.E., n = 3—5. Data at '0' time at the bottom are within 10 min after transfer to 19 mM Na^+. Ordinate: cell ion contents (mmol/l cell water). Abscissa: time (h).

TABLE I

ION CONTENTS AT 48 h

All media contained 5.4 mM K^+. Results are given as means ± S.E. of three to four separate experiments. $[K^+]$ and $[Na^+]$ are expressed as mmol/l cell water. Gradients are expressed as mM (internal)/mM (external).

$[Na]_o$ (mM)	Temperature (°C)	H_2O (% wet wt.)	$[K^+]$	$[Na^+]$	Gradients	
					K^+	Na^+
145	37	78 ± 2.5	171 ± 5	37 ± 7	32	0.3
145	0	75 ± 3.0	49 ± 10	162 ± 10	9.1	1.1
19	37	68 ± 2.0	114 ± 7	30 ± 1	21	1.6
19	0	69 ± 4.1	53 ± 9	110 ± 5	9.8	5.8

Cells incubated in 5 mM K^+, 145 mM Na^+ at 37°C maintain normal K^+ and Na^+ levels for at least 48 h (Fig. 1, top). Cells in the same medium at 0°C lose K^+ and replace it mole-for-mole with Na^+ (Fig. 1, top). The same levels of cell ions at 24 and 48 h are also reached in cells that are pre-depleted of K^+ and pre-loaded with Na^+, at both 37 and 0°C [4].

Cells incubated in 5 mM K^+, 19 mM Na^+ at 37°C maintain a near normal level of K^+ for 24 h and then show a moderate decrease in K^+ at 48 h. Their Na^+ level is less than normal within 10 min after transfer to 19 mM Na^+ ('0' time) and remains low through 48 h (Fig. 1, bottom). Cells in 19 mM Na^+ at 0°C, however, slowly gain Na^+ to reach a level of 110 mM at 48 h (Fig. 1, bottom).

Ion and water contents at 48 h are summarized in Table I, and cell-to-medium ion gradients are calculated. Cells in 145 mM Na^+ at 0°C have an Na^+ gradient that is near to unity. This would seem to fit with the absence of Na^+ pump activity at 0°C. However, the K^+ gradient is maintained at 9.1. Furthermore, cells in 19 mM Na^+ at 0°C not only still have a K^+ gradient of 9.8, but gain Na^+ to reach a gradient of 5.8.

We consider four mechanisms that might possibly contribute to ion accumulation at 0°C.

Impermeable membranes: relatively impermeable surface or compartment membranes cannot account for any of the data at 0°C since (a) the cell Na^+ levels are increasing, and at 19 mM external Na^+, cell Na^+ is increasing against the apparent chemical gradient; (b) the cell K^+ levels at 48 h are reached not only by loss of K^+ but also by gain of K^+ in K^+-depleted cells at both 0 and 37°C [4]; and (c) the rates of isotopic ion exchange at all temperatures, and at 19 mM Na^+, are much faster than the rates of net changes in ion contents [3–5].

Donnan equilibrium: in an electrochemical equilibrium of the Donnan type, the presence of fixed anionic charges within the cell requires cationic counterions in order to maintain electrical neutrality. This creates a chemical gradient of the balancing cations, but the net result is a true, electrochemical, equilibrium. The concept of counterions existing in a chemical gradient, and the Donnan equilibrium itself, are based upon the assumption that the cations and water exist as in a dilute aqueous solution. As is well known, a Donnan equilibrium is incompatible with osmotic equilibrium unless there is a counterbalanc-

ing hydrostatic pressure provided by a rigid membrane or cell wall or by a rigid macromolecular matrix within the cell. This, however, was ruled out many years ago when it was realized that animal cells swell and shrink to a considerable extent. If there is no source of hydrostatic pressure, then a Donnan equilibrium could only occur if there is a 'double' Donnan system, in which there is the equivalent of a fixed charge on the outside of the membrane as well. This was realized by Boyle and Conway [6] in their classical study of muscle in 1940, and they thought that external Na^+ played this role by virtue of its inability to penetrate the cell membrane, while K^+, which was penetrable, served as the internal counterion. The finding, in the same year, that Na^+ does in fact readily penetrate cells led to the postulation of the outwardly directed Na^+ pump. This leads to two consequences: (1) a Donnan equilibrium of K^+ can only exist if there is a pump working to keep out Na^+, and (2) the maintenance of cell volume and prevention of swelling due to the forces at work in the Donnan equilibrium are dependent upon the Na^+ pump [7].

The question as to whether a Donnan equilibrium can explain our data at 0°C must be considered in two ways, i.e., with and without a functioning outward Na^+ pump.

(1) If the Na^+ pump is not working at 0°C, then one must postulate some other restraining force that keeps the cell from swelling. The ability of lymphocytes to swell rapidly and reversibly [8] rules out the presence of a rigid membrane or matrix. Sucrose could play this role in the experiments at 19 mM external Na^+ (if sucrose were not able to penetrate the cell), but not at 145 mM external Na^+, since sucrose was not present. Even if we accept the ad hoc postulation that there appears some restraining force to keep the cells from swelling, the failure of K^+ and Na^+ to reach the same distribution ratio between cell water and medium rules out a Donnan equilibrium, since in a Donnan equilibrium the counterions remain free in dilute solution and there is no way to achieve selective ion accumulation.

(2) If the Na^+ pump is working at 0°C, then a Donnan equilibrium of K^+ may exist. However, there remains the requirement for a net outward Na^+ pumping. This does not occur, for at 145 mM external Na^+ the cell-to-medium Na^+ ratio is near to unity, and at 19 mM external Na^+ it is greater than unity with a net inward accumulation of Na^+.

There is, therefore, no way to explain consistently our data using an electrochemical equilibrium of the Donnan type. Additional evidence against a Donnan equilibrium in lymphocytes has been given [1,3,4].

Pump: cells in 145 mM external Na^+ at 0°C allow Na^+ to replace K^+ (Fig. 1, top) and have an Na^+ gradient that is near to unity. This would suggest the absence of Na^+ pump activity at 0°C. However, cells in 19 mM external Na^+ at 0°C actually accumulate Na^+ (Fig. 1, bottom). Within the context of the membrane pump-leak theory, this observation forces one to postulate not only that the pump is able to work at 0°C after all, but that it is working in a mode that allows it to pump both K^+ and Na^+ inwardly at the same time. This observation has also been made on muscle [9]. It contradicts the fundamental concept of the mechanism of the $(Na^+ + K^+)$-ATPase membrane pump [10] and cannot be accounted for by any of its five postulated modes [11]. The normal forward reaction of the postulated 'transport ATPase' system [11] pumps Na^+ out, not

in; the reverse reaction requires and is driven by very steep ion gradients and cannot pump Na^+ in; the Na^+-Na^+ exchange is one-for-one Na^+ exchange and hence performs no pumping work; the ouabain-sensitive K^+ efflux cannot account for the Na^+ distribution or for the high gradients of K^+ in-to-out; and the ouabain-sensitive Na^+ efflux requires media free of Na^+ and K^+ and also performs no pumping work. It should also be noted, in regard to the normal forward reaction of the postulated pump, that the Na^+ levels reached in our cells at 0°C are well above the K_m value for activation of lymphocyte ($Na^+ + K^+$)-ATPase [12], and hence the enzyme should be maximally stimulated to pump Na^+ out if it is in fact functioning. Thus, even if there were residual activity of the ATPase at 0°C, the simultaneous, net accumulation of both K^+ and Na^+ at 0°C cannot be explained by the ($Na^+ + K^+$)-ATPase pump concept.

Adsorption: selective association with macromolecular anionic sites remains the most plausible explanation for simultaneous net K^+ and Na^+ accumulation at 0°C. These data complement recent extensive studies of lymphocyte ions [1—5] which conclude (1) that the level and exchange of the major part of K^+, and of Na^+ that may replace it, are determined by adsorption onto and desorption from fixed anionic sites, within the cell, that interact with one another in a critical cooperative fashion, and (2) that the relative exclusion of Na^+ from the cell is due to its reduced solubility in cell water that exists in a physical state approximating that of polarized multilayers.

These two processes are mirrored in both the ion distributions and the kinetics of ion exchange of both K^+ and Na^+. Thus, there are two fractions of cell K^+ [1,3]. The larger accounts for about 97% of normal cell K^+, is saturable, and exchanges slowly ($t_{1/2}$ = 400 min) with external K^+. The smaller is nonsaturable, is dissolved in cell water to the extent that it bears a constant ratio to external K^+ of 0.6, and exchanges rapidly ($t_{1/2}$ = 2 min). Na^+ also exists in two (or more) fractions [1,5]. Normally, about half of the cell Na^+ is adsorbed, and the adsorbed fraction increases mole-for-mole in exchange for K^+ whenever K^+ is lost from the cell (as at 0°C, Fig. 1, top). The other half of normal cell Na^+ is dissolved in cell water in a ratio to external Na^+ of 0.15, and exchanges rapidly ($t_{1/2}$ = 2 min). It is the rapidly exchanging fractions of K^+ and Na^+ that reflect the surface membrane barrier [3,5].

Two additional features of ion accumulation at 19 mM Na^+ (Fig. 1, bottom) are explained by these concepts.

First, cell Na^+ at 19 mM external Na^+ at 37°C is at all times less than at 145 mM Na^+ at 37°C. If this reflects the Na^+ dissolved in cell water that exchanges with a $t_{1/2}$ value of 2 min, and that bears a ratio to external Na^+ of 0.15, then one may predict that the cells should rapidly lose ($0.15 \times 145 - 0.15 \times 19$ or 19 mM) Na^+. In fact, cell Na^+ at 19 mM external Na^+ is 15—20 mM less (Fig. 1, bottom). At 37°C, the Na^+ level remains low since the adsorption sites still prefer K^+. There is a critical transition in the sites at 3°C (see Ref. 4), so that at 0°C they now prefer Na^+. When present at 145 mM in the medium, Na^+ competes for and displaces K^+ well within 24 h at 0°C (Fig. 1, top). When present at 19 mM, however, Na^+ is, as expected, less able to displace adsorbed K^+ and is still rising at 48 h (Fig. 1, bottom).

Second, total cell $K^+ + Na^+$ dropped from 210 mM normally to either 144 or 165 mM at 48 h in media containing 19 mM Na^+. This, and the slight water loss

(Table I), are not due to the sucrose in the medium, since the drop in $K^+ + Na^+$ (Fig. 1) and the drop in water are gradual in time (water, as % wet weight ± S.E. of four experiments, was 79.0 ± 2.0% before transfer to 19 mM Na^+ at 0°C, and 77.2 ± 2.1, 73.5 ± 1.9, and 69.2 ± 4.1% at 10 min, 24 h and 48 h, respectively). Part of the decrease in $K^+ + Na^+$ (15—20 mM) is due to the fraction of Na^+ described above. We suggest that the remainder reflects salt-linkage formation among macromolecules, in which fixed anionic sites that normally adsorb K^+ or Na^+ associate instead with fixed cationic sites. Macromolecules with a higher degree of salt-linkage would be less able to orient and hold water by the polarizing mechanism outlined in Ref. 13, and this would explain the shrinkage and loss of cell water that occur slowly in cells incubated in 19 mM Na^+.

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