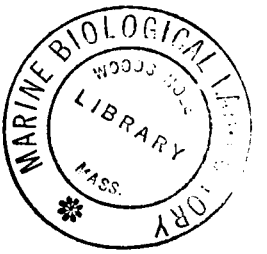

SOME OBSERVATIONS ON THE PARTITION OF Na^+ AND K^+ INTO A LIPID PHASE

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ABSTRACT It is likely that sodium and potassium must traverse a lipid membrane surrounding cells and that this membrane has to do with intracellular cation selection. Electrolyte theory is inadequate to predict the partition of salts of these cations between water and a lipid phase. The data obtained here demonstrate the partition and the cation selection to be a function of the anion species (or ionized lipid), the solvent and the presence of the unionized form of the lipid. Specificity in lipid partition is not synonymous with the cation specificity in precipitation of ionic crystals.

INTRODUCTION

One of the most intriguing problems in biology is the accumulation of K^+ in excess of sodium inside animal and plant cells which are bathed in extracellular fluid or sea water containing much more sodium than potassium. The concentration ratio ($S_{K/Na}$), or K/Na inside divided by K/Na outside, is commonly over 300. There is much evidence that this ion selection is a function of the cell membrane. This evidence includes the ability of erythrocyte "ghosts" to reaccumulate K in excess of Na, and the fact that microelectrodes placed inside cells relate sodium and potassium activity in nearly the same ratio as their intracellular concentration. (Hinkle, 1959). This degree of ion selectivity is not obtainable in a water solution. Osterhout (1933) postulated that cation selectivity was a function of one or both lipid layers of the cell membrane of the algae which he studied. He constructed a model using guaiacol saturated with water as a lipid phase, or "membrane," separating 2 aqueous phases. Sodium and potassium guaiacolates placed in "extracellular water" would partition into the lipid phase with $S_{K/Na} \doteq 2.5$. If CO_2 (or an acid) were added to the "intracellular water" Na and K guaiacolates would be decomposed to $NaHCO_3$ and $KHCO_3$, which were sparsely soluble in the lipid phase and would partition into the intracellular phase with $S_{K/Na} \doteq 1$. If water transfer through the lipid layer was then limited by an osmotic agent, a steady state could be reached with CO_2 continuously transferring out of the cell and with K exceeding Na within

the cell. In this model the relative intracellular cation concentrations are an inate function of $S_{K/Na}$ of the lipid and of ΔpH in the water phases.

Many observations could be cited to support this model. Intracellular pH is usually lower than extracellular. A lipid layer is present in many if not all cells and cations must traverse this layer. It contains phosphatides and perhaps other ionizable lipids. Recently ln $S_{K/Na}$ for the erythrocyte has been found to be a linear function of intracellular lactate production. (Thurber and Thompson, 1965). However, information is lacking on the partition of cations into a lipid phase.

Theory. Following the observations of Osterhout (1933), Shedlovsky and Uhlig (1933) synthesized the partition coefficient of sodium and potassium guaiacolates between mutually saturated guaiacol and water over a range of concentration. The extrapolation of the experimentally obtained lipid/water partition coefficient (S) to infinite dilution (S_0) was approximated by the Born equation. This equation, based on long-range coulombic forces, may be written for these purposes.

$$\ln S_0 = \frac{e^2}{4kT}\left(\frac{1}{D} - \frac{1}{D_1}\right)\left(\frac{1}{r^+} + \frac{1}{r^-}\right)$$

where D and D_1 are the dielectric constants of the lipid and water phases, r^+ and r^- are the radii of the "hydrated" ionic species, and the other terms have the usual meaning. Appropriate values of r^+ and r^- were obtained by adding the thickness of one layer of water molecules to the crystal radius of the cations and to the Stokes radius of guaiacolate. In this approximation S_0 will be greater for the K^+ than for Na^+ salt of a given anion.

Since the activity to a common reference will be constant in all phases at equilibrium, S differs from S_0 by the inverse of the mean activity coefficient of the salt at a given concentration referred to infinite dilution in the same solvent. Ion association is prominent in media of low dielectric constant. In order that the term γ may refer to the mean activity coefficient of the dissociated species in the lipid medium it must be multiplied by a term for the dissociation fraction, θ. The equation becomes,

$$S = S_0\left(\frac{\gamma_1}{\gamma\theta}\right)$$

Values of γ_1 and γ were estimated from the Debye-Huckel theory with appropriate estimates of necessary constants. Values of θ were then derived and found to agree with values derived from conductivity measurements in wet guaiacol. The resultant dissociation constants (K) were about 3.5×10^{-5} for the Na^+ salt and 5.5×10^{-5} for the K^+ salt. Since S was found to be a function of S_0^2 as well as of $1/K$, it was predicted that the salt of the larger cation would have a higher S as well as S_0 in spite of being more dissociated in the lipid phase. Should these approximations

be adequate then the difference between cations should be amplified as the dielectric constant of the lipid phase decreases and as the temperature rises.

Owen (1932) refers to the logarithm of the ratio of activity coefficient of an electrolyte in a less aqueous solvent to that in water at the same concentration ($-\log S$) as the total medium effect. His primary medium effect is the limit to which the total medium effect converges as the electrolyte concentration approaches zero ($-\log S_0$) and his secondary medium effect is the difference between the primary and the total effects (corresponding to $-\log \gamma \theta$). He demonstrated that the Born equation was inadequate to describe the primary medium effects of organic solvents on silver bromate (Owen, 1933). Instead, the effects were specific for the solvents used. Similarly, the dielectric constant of the medium has been found inadequate to describe the dissociation constant in organic media (Harned and Owen, 1958). A closer fit is obtained with the dipole moment. In addition to ion pairing, triple and quadruple ion formation will affect the secondary medium effect. The appropriate K and K_3 has been shown to be related to the geometry of the ions and short range forces.

It may be concluded that present theory is inadequate to predict the partition coefficient for potassium and sodium salts and in turn inadequate to predict the ion selectivity. In the survey of lipid partitions to follow, this is realized.

Experimental. Since $S_{K/Na}$ is probably a monotonically increasing function of the value at infinite dilution, most values were determined at only one concentration. The lipid phase was either an organic solvent or the ionizable lipid whose salt was partitioned. These ionizable lipids are carboxylates, phenols, primary phosphates (including lecithin and phosphatidylserine), an aliphatic alcohol, and a sulfonate. One milliequivalent of KCl and one of Na^+ were added to 5 ml of water and 5 ml of organic phase, except as mentioned. Sodium was added either as the hydroxide to react with a large excess of lipid acid, or as a salt in the absence of excess acid. In one instance, the partition into caprylic acid, a repeat with 0.6 meq Na^+ and 0.1 meq K^+ gave results similar to the higher concentration (compare No. 30 with No. 14 in Table I). Phosphatidylserine, 50 μmole in 1 ml chloroform, was equilibrated with 10 ml of 0.05 M Na^+ and 0.05 M K^+ as the phosphates at 3 pH's, 8.4, 7.4, and 6.4. All mixtures were shaken for 30 min at room temperature (23 $\pm$ 1°C) and then separated by centrifuging. The cations were usually recovered from the organic phase by partition into HCl. In those instances in which cation recovery was incomplete, e.g. phosphatidylserine, an aliquot of the lipid phase was wet ashed with H_2SO_4 and $HClO_4$ and appropriate blanks subtracted. Sodium and potassium were determined by flame photometry to a precision of about 1% except in the instances noted. In these instances (marked *) they were determined using Na^{22} and K^{42} to a precision of about 0.2%. Reproducibility was found to be limited to about 2% largely by temperature change; e.g., warming in the centrifuge. In the instance of a solid acid in an organic solvent (e.g. α-methoxy-

TABLE I

LIPID-WATER PARTITION COEFFICIENTS OF NA^+ AND K^+

Salts added	Lipid phase	S_{Na}	S_K	$S_{K/Na}$
1. $Na(C_8O)_2PO_4$ + KCl	diethyl ether	0.403	0.351	0.871
2. $Na(C_8O)_2PO_4$ + KCl	diethyl ether + cholesterol 250 mg%	0.438	0.364	0.831
3. $Na(C_8O)_2PO_4$ + KCl	isooctanol	0.781	0.706	0.905
4. $Na(C_8O)_2PO_4$ + KCl	isooctanol + 1 meq $H(C_8O)_2PO_4$	0.651	0.638	0.981
5. $Na(C_8O)_2PO_4$ + KCl	isooctanol + 2 meq $H(C_8O)_2PO_4$	0.623	0.647	1.04
6. $Na(C_8O)_2PO_4$ + KCl	isooctanol + 9 meq $H(C_8O)_2PO_4$	0.571	0.742	1.30
7. $Na(C_8O)_2PO_4$ + KCl	$H(C_8O)_2PO_4$	0.74	1.0	1.35
8. Na + K phosphates, pH 8.4	phosphatidyl serine, 50 mM in chloroform	0.440	0.407	0.925
9. Na + K phosphates, pH 7.4	phosphatidyl serine, 50 mM in chloroform	0.392	0.363	0.926
10. Na + K phosphates, pH 6.4	phosphatidyl serine, 50 mM in chloroform	0.390	0.351	0.90
11. NaOH + KCl, pH 11.9	10% lecithin in isooctanol	0.359	0.354	0.986
12. NaOH + KCl	Butyric acid	0.421	0.681	1.62
13. NaOH + KCl	caproic acid	0.446	1.13	2.53
14. NaOH + KCl	caprylic acid	0.439	1.34	3.06
15. NaOH + KCl	80% caprylic acid in isooctane	0.551	1.21	2.19
16. NaOH + KCl	60% caprylic acid in isooctane	0.534	1.09	2.05
17. NaOH + KCl	40% caprylic acid in isooctane	0.416	0.801	1.93
18. NaOH + KCl	80% caprylic acid in cottonseed oil	0.481	1.29	2.68
19. NaOH + KCl	60% caprylic acid in cottonseed oil	0.401	0.968	2.41
20. NaOH + KCl	40% caprylic acid in cottonseed oil	0.337	0.705	2.09
21. NaOH + KCl	oleic acid	0.485	1.01	2.08
22. NaOH + KCl	linoleic acid	0.516	1.06	2.05
23. NaOH + KCl pH 7.46	stearic acid in $CHCl_3$	0.285	0.492	1.73
24. NaOH + KCl pH 7.8	stearic acid in $CHCl_3$ + lecithin, 250 mg	0.703	0.645	0.92
25. Na caprylate + KCl	isooctanol	0.042	0.042	1.00
26. Na caprylate + KCl	isooctanol + 1 meq caprylic acid	0.064	0.065	1.02
27. Na caprylate + KCl	isooctanol + 9 meq caprylic acid	0.264	0.359	1.36
28. Na caproate + KCl	isooctanol	0.0026	0.0025	0.98
29. Na caproate + KCl	isooctanol + 1 meq caproic acid	0.0036	0.007	2.0
30. NaOH 0.6 meq + KCl 0.1 meq	caprylic acid	0.50	1.51	3.03
31. NaOH + KCl	sebacic acid in isooctanol	0	trace	
32. Na dodecyl benzene sulfonate + KCl	isooctanol	0.355	0.441	1.25
33. NaOH + KCl	α-methoxyphenylacetic acid in chloroform	0.151	0.381	2.52
*34. Na guaiacolate + KCl	isooctanol	0.0024	0.00149	0.63
*35. Na guaiacolate + KCl	isooctanol + 1 mM guaiacol	0.0020	0.00168	0.84
*36. Na guaiacolate + KCl	isooctanol + 9 mM guaiacol	0.0054	0.00652	1.17
37. NaOH + KCl	guaiacol	0.184	0.413	2.25
38. NaOH + KCl	phenol	0.350	0.471	1.35
39. NaOH + KCl	o-cresol	0.052	0.109	2.10
40. NaOH + KCl	m-cresol	0.246	0.481	1.96
41. NaOH + KCl	p-cresol	0.321	0.667	2.08
42. NaOH + KCl	p-vanillin in $CHCl_3$	0.0035	0.0132	3.8
43. NaOH + KCl	o-vanillin in $CHCl_3$	0.0062	0.024	3.8

phenylacetic acid in chloroform) the values were found to vary with the quantity of acid added. The values given represent a near saturated solution in the organic phase before equilibration.

RESULTS

The results are given in Table I. They are expressed as the lipid/water concentration ratios, S_K and S_{Na}, and as S_K/S_{Na} or $S_{K/Na}$.

DISCUSSION AND CONCLUSION

Several observations can be made from the data: (*a*) Sodium and potassium salts are quite soluble in organic solvents with low dielectric constants if the anion is a lipid moiety of sufficient size to pull the charged site and counterion into the lipid phase. A lesser lipid moiety is necessary with a more polar lipid phase. Greater cation selectivity is possible in a lipid-water partition than has been observed in ion association in an aqueous medium, including the extreme instance of ion exchange resins. (Kitchener, 1957). Selectivity as high as that of the cell membrane has not been found in simple lipid partition.

(*b*) In four instances without an excess of ionizable lipid $S_{K/Na}$ was less than 1. Upon adding the unionized form of the lipid to the system, $S_{K/Na}$ exceeded 1; i.e., the lipid then selects a potassium over sodium (a primary phosphate, No. 1, 4, 5, 6, 7; two carboxylates, No. 28, 29, 13, and 25, 26, 27, 14; and a phenolate, No. 34, 35, 36, 37).

(*c*) In a sequence of fatty acids $S_{K/Na}$ increases from butyric to caprylic acids, but is lower in oleic and linoleic acids. Dilution of caprylic acid with cottonseed oil or with isooctane, a very nonpolar solvent, resulted in a lower ratio.

(*d*) Phosphatidylserine in chloroform appears nonselective at 3 pH's. In each instance sodium plus potassium equaled about ¾ of the phosphorus. This differs from $S_{K/Na}$ of 14.2 reported for the solid lipid alone. (Solomon et al., 1956). Perhaps the difference is related to dilution by the solvent used, or to the lower cation content in this work. Lecithin in chloroform behaves similarly, but with a higher aqueous pH.

(*e*) Stearic acid in excess in chloroform selects K over Na. Addition of lecithin solubilizes more stearates in the chloroform, but results in a reversal of cation selection. This may be due to the choline residue of lecithin.

(*f*) Ion selection in lipid-water partition is not synonymous with ion selection in a crystal lattice. In the same equilibrium (No. 33) in which sodium crystallizes out as the hydrogen double salt of α-methoxyphenylacetic acid, $S_{K/Na} = 2.5$.

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