IONIC PARTITION BETWEEN SURFACE AND BULK WATER IN A SILICA GEL

A Biological Model

PHILIPPA M. WIGGINS

From the Department of Medicine, University of Auckland, School of Medicine, Auckland, New Zealand

ABSTRACT Distribution of the biologically important ions between two aqueous phases of different structure has been used as a model for ionic distribution in living tissue. When other sources of specificity had been eliminated or corrected for, surface-oriented water in a silica gel was found to have increased solvent power for water-structure-breaking ions and decreased solvent power for water-structure-making ions; and the relative solubility of an ion in the phase of enhanced structure increased regularly with the water-structure-breaking powers of the ion. The ionic selectivity was decreased in the presence of urea. The selectivity of the gel water for potassium relative to sodium increased to a maximum when the gel surface was partially ionized so that distribution of cations was not linked to distribution of anions, and then decreased as the surface changed from a hydrogen bonding to an ionic surface. It is pointed out that the distribution of ions across most living cell membranes is qualitatively the same as that found in this silica gel, and it is suggested that the membrane separates two aqueous phases of different structure, and that the enhanced structure of cell water contributes to the observed ionic distributions.

INTRODUCTION

If, as several workers have claimed (1–4), the structure of cell water is enhanced relative to that of bulk phase liquid water, then it may no longer be valid to assume that the solubility of a solute is the same in extracellular and intracellular water. This suggests the possibility of treating the two solutions separated by the cell membrane as effectively immiscible liquid phases in which ions and other solutes are dissolved. The condition for the equilibrium distribution of an ion then becomes

$$\mu_i^0 + RT \ln a_i = \mu_e^0 + RT \ln a_e - zFE_m , \qquad (1)$$

where μ^0 is the standard chemical potential and a the activity of the ion of charge z, E_m is the membrane potential and the subscripts i and e refer to the intracellular and extracellular phases, respectively. Solutes for which $\mu_i^0 > \mu_e^0$ would have lower free concentrations inside cells, and solutes for which $\mu_i^0 < \mu_e^0$ would have higher free con-

centrations inside cells than those expected for the simplest equilibrium distribution. It has been pointed out (5) that there is a striking correlation between the effect that an ion has upon the structure of water and its partition between most cell types and their surroundings. Nightingale (6) suggested that the best index of the effect that an ion has upon the structure of bulk water is its contribution to the energy of activation for viscous flow (ΔE^*). Ions with positive values of ΔE^* are net water structure makers; ions with negative values of ΔE^* are net water structure breakers. Of all the ions in the extracellular solution, only Na^+, Ca^{++}, Mg^{++}, and H^+ are water structure makers by this criterion, and these ions are commonly relatively excluded from living cells (5, 7). All the other ions in the extracellular solution (K^+, Cl^-, both NH_3^+ and COO^- ends of amino acids, HCO_3^-, $H_2PO_4^-$, HPO_4^{--}, and SO_4^{--}) are net water structure breakers, and they are in general accumulated by cells or normally distributed. This observation taken in conjunction with Eq. 1 suggests that $\mu_i^0 > \mu_e^0$ for structure-making ions, and $\mu_i^0 < \mu_e^0$ for structure-breaking ions, and that the enhanced structure of cell water might be to some extent determining the distributions of ions.

It is difficult to test this hypothesis directly in living tissue, because it is generally not possible to distinguish between steady-state ion distributions maintained by ion pumps, and equilibrium states. A model system in which equilibrium can unequivocally be established, has therefore been used. Electrolyte solutions have been equilibrated with a silica gel which contains inside its pores water which should have enhanced structure for two reasons. First the rigid surface of the gel should shield adjacent water molecules from thermal fluctuations, thus increasing the lifetime of intermolecular hydrogen bonds; secondly the silanol groups (Si-OH) which line the pores should stabilize existing water structures by participating in their hydrogen bonding, and perhaps promote new structures. Although there can be no sharp boundary between the two aqueous phases in the silica gel, one can as a first approximation treat the system as two immiscible aqueous phases of different structure and see whether the pattern of distribution of the biologically interesting ions is the same as that across the cell membrane, which it is suggested also separates aqueous phases of different structure.

MATERIALS AND METHODS

Preparation of the Gel

Davison silica gel code 950 was washed by upward elution with 0.5 N HNO_3 in a column connected to a Watson-Marlow peristaltic pump to remove ionic impurities, and then with deionized water until the pH of the eluent was not less than 5. The gel was then dried to constant weight at 110°C and stored in a vacuum desiccator until needed.

Determination of Residual Water in the Gel Dried at 110°C

A sample of gel dried to constant weight at 110°C was stirred in excess Karl-Fischer reagent (8) for 30 min, in which time it was found that all the water on the gel had reacted; it was then back titrated with a standardized water-methanol solution.

Determination of the Pore Volume of the Gel

Gel was equilibrated with saturated water vapor at 25°C until it reached constant weight. Samples were then removed, weighed, and dried to constant weight at 110°C.

Chemicals

All chemicals used were of analytical grade.

Equilibration of Gel with Electrolyte Solutions

Either 3 or 5 g gel were weighed into a Sorvall centrifuge tube (Ivan Sorvall, Inc., Newtown, Conn.), and 5 or 7 ml of electrolyte solution added. The tubes were capped and shaken vigorously at room temperature (25°C) overnight, in which time a constant distribution of ions, presumably equilibrium, was reached. The tubes were removed from the shaker, spun down at 12,000 rpm for 1 h in a Sorvall RCB-2 refrigerated centrifuge at 25°C, and approximately 1 ml of supernatant removed for analysis. Duplicate determinations of the distribution of 0.1 N KCl were included in each batch of tubes, so that any change in the properties of the gel as a result of repeated equilibration, washing, and drying would be detected. No such change was observed.

Analysis

Na^+ and K^+ were determined with an EEL flame photometer using external standards made up in 0.1 N HNO_3. Li^+ and Mg^{++} were determined with a Techtron AA5 atomic absorption spectrophotometer. Ca^{++} was determined by titration with sodium ethylenediaminetetraacetate using a 4% solution of Calcon in methanol as indicator (9). Cl^- was determined using an Aminco-Cotlove chloride titrator (America Instrument Co., Inc., Div. of Travenol Laboratories, Inc., Silver Spring, Md.).

Calculation of Results

The results were expressed in terms of an apparent partition coefficient [λ (apparent)] which was equal to the ratio of the apparent concentration of a salt in the pore water of the gel to its concentration in the bulk phase at equilibrium. This was given by

$$\lambda \text{ (apparent)} = (C_i V - C_f V')/V_p C_f W(1 - V_g) \qquad (2)$$

where C_i and C_f were the initial and final concentrations of salt in moles per liter; V, in milliliters, the volume of electrolyte solution added to the gel; V_p milliliters per gram of dry gel the pore volume of the gel; W_g the weight of gel dried to constant weight at 110°C; V_g grams per gram of wet gel the residual water content of the gel dried to constant weight at 110°C, and $V' = V + WV_g - V_pW(1 - V_g)$. The assumption is made in this calculation that the density of water inside the gel is unity.

RESULTS

Pore Volume and Residual Water in the Gel Dried at 110°C

It has been shown that when a silica gel is calcined at 450°C or higher temperature to remove all the water, both the pore volume and the surface area of the gel decrease

(10, 11). In the present experiments, therefore, the gel was dried only to constant weight at 110°C and the residual water on the gel found by Karl-Fischer titration to be 0.0306 ±0.0004 g/g wet gel (mean and standard error of 6 determinations). The pore volume of the gel corrected for this residual water content was found to be 0.40 ±0.01 g/g dry gel both for the gel as received from the manufacturer and for the gel washed in acid followed by deionized water, and dried to constant weight at 110°C; this value agreed very well with that supplied by the manufacturer (0.4 ml/g dry gel). Since the pore volume was unchanged by the preparative procedure, and since distribution of 0.1 N KCl did not change with time and was the same with gel as supplied by the manufacturer and as used in these experiments, it was considered valid to assume that the surface area of the gel was also unchanged. A value of 650 m^2/g was taken, as the mean of values given by the manufacturer.

Apparent Partition Coefficients of Salts

Equilibration of solutions of single salts with the gel showed that as Dalton et al. (12) had found the apparent partition coefficient decreased with decreasing concentration of salt. The values extrapolated to infinite dilution are therefore given in Table I, together with an estimate of the concentration dependence of the partition coefficient. A small correction was made for the hydrolysis of K_2SO_4. Measurement of both cation and anion concentration showed that ionization of the gel surface was negligible for concentrations of all the chlorides equal to or greater than 0.1 N. This was confirmed both for these salts and for other salts for which only one ion could be determined by the negligibly small change in pH of the supernatant that occurred during equilibration. The extrapolations to infinite dilution have therefore

TABLE I
APPARENT PARTITION COEFFICIENTS OF SALTS

Salt	λ_0(apparent)	$d\lambda/dc$	Number of experiments
		l/mol	
$MgCl_2$	0.34 ±0.004	0.20	24
$CaCl_2$	0.40 ±0.005	0.16	24
LiCl	0.43 ±0.003	0.15	12
NaCl	0.51 ±0.005	0.06	36
HCl	0.53 ±0.003	0.06	40
KCl	0.77 ±0.004	0.05	72
RbCl	0.82 ±0.009	0.05	12
CsCl	0.96 ±0.010	0.03	24
KNO_3	0.77 ±0.011	0.01	12
K_2SO_4	0.92 ±0.014	0.06	12

λ is the molar concentration of a salt in the pore water over its concentration in the bulk phase at equilibrium. The subscript 0 indicates the value extrapolated to infinite dilution.

been made from 0.1 N. Table I shows that all salts were excluded to some extent from the pores of the gel, the degree of exclusion increasing from CsCl to $MgCl_2$.

The Effect of Ionization of the Gel Surface

Samples of gel were equilibrated with solutions containing equal and constant concentrations of NaCl and KCl to which increasing and equal amounts of NaOH and KOH were added. Fig. 1 shows the change in the selectivity coefficient $K^{K^+}_{Na^+}$ of the gel and of the degree of ionization of the gel surface as the equilibrium pH of the bulk aqueous phase increased. $K^{K^+}_{Na^+} = \lambda_{K^+}/\lambda_{Na^+}$ and is a measure of the selectivity of the gel toward K^+ rather than Na^+. The concentration of ionized SiO^- on the gel surface cannot be calculated exactly because the partition of the OH^- ion between the pore water and the bulk phase water is unknown and cannot be measured. To a

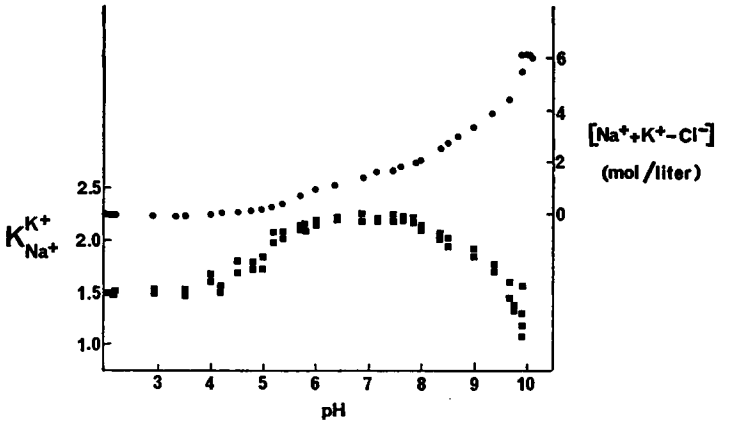

FIGURE 1 The change in the selectivity coefficient, $K^{K^+}_{Na^+}$ and of the concentration of fixed charge inside the pores of the gel with changing pH of the bulk phase at equilibrium. ●, $K^{K^+}_{Na^+}$; ■, $[Na^+ + K^+ - Cl^-]$.

good first approximation, however, the concentration of SiO^- can be taken as equal to the concentrations of Na^+ plus K^+ minus Cl^- in the pore water, because at low pH when the surface is lightly charged, the concentration of free OH^- ions is negligibly small, and at higher pH when the surface is highly charged, all anions are excluded to such a degree that their concentrations in the pore water are negligible compared with those of the cations which are strongly accumulated.

At low pH when the charge on the surface was effectively zero, $K^{K^+}_{Na^+} = 1.51$. As the surface began to ionize at about pH 5, $K^{K^+}_{Na^+}$ increased until it reached a value of 2.18, at which it remained constant over a pH range of about 7–9; it then decreased quite rapidly as the surface became more highly charged, dropping almost to unity, as the pH approached 11. The points at high pH were very scattered, and it seemed that ionization of the gel surface did not proceed much above 6 M.

The Effect of Urea upon Selectivity

Fig. 2 shows the results of three experiments at different pH's in which graded quantities of urea were added to solutions of constant concentrations of NaCl and KCl. At each pH as the concentration of urea increased $K^{K^+}_{Na^+}$ decreased steadily from its value in the absence of urea. The decrease in $K^{K^+}_{Na^+}$ was in each case predominantly due to an increase in λ_{Na^+}, λ_{K^+} decreasing only slightly. This point is illustrated in

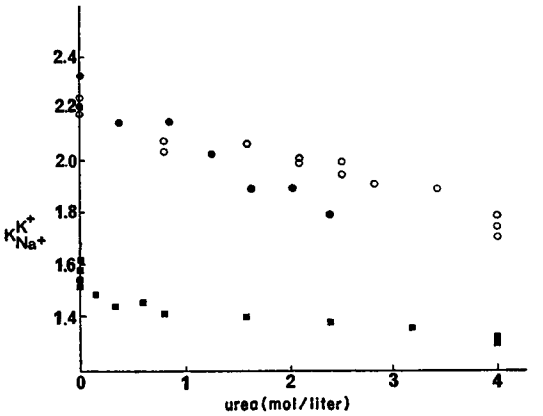

FIGURE 2 The change in the selectivity coefficient $K^{K^+}_{Na^+}$ when urea was added to solutions containing constant and equal concentrations of Na^+ and K^+ ions. Each experiment was at constant pH. ○, pH 7.2; ●, pH 7.8; ■, pH 3.5–4.

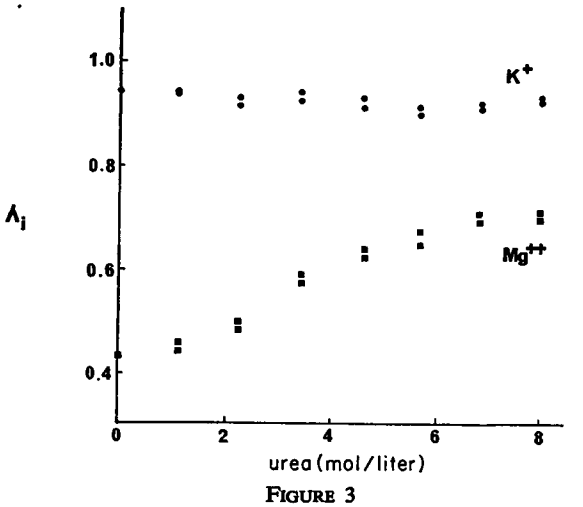

FIGURE 3

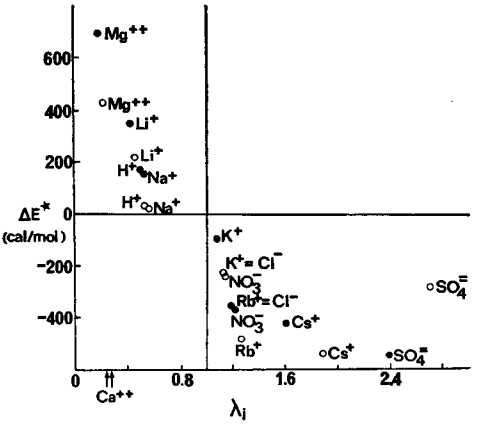

FIGURE 4

FIGURE 3 The change in the cationic partition coefficients λ_i when urea was added to constant and equal equivalent concentrations of KCl and $MgCl_2$.

FIGURE 4 Individual ionic partition coefficients (λ_i) plotted against the individual ionic contribution to the activation energy for viscous flow (ΔE^*). ○, assuming that $\lambda_{K^+} = \lambda_{Cl^-}$ and that $\Delta E^*_{K^+} = \Delta E^*_{Cl^-}$; ●, assuming that $\lambda_{Rb^+} = \lambda_{Cl^-}$ and that $\Delta E^*_{Rb^+} = \Delta E^*_{Cl^-}$.

Fig. 3 where the result of a similar experiment is shown. Here KCl and $MgCl_2$ were the equilibrating salts held at constant and equal equivalent concentrations. As the concentration of urea increased the partition coefficient of the K^+ ion decreased scarcely significantly, while the partition coefficient of the Mg^{++} ion increased from 0.43 to 0.71.

DISCUSSION

Correction for Inaccessible Pore Volume

McConnell et al. (10) attributed all the exclusion of salts from the pores of a similar silica gel to a geometric effect. They pointed out that adjacent to any surface there is a strip of inaccessible volume of thickness equal to the effective radius of the ion or molecule being considered, and they used their data to calculate effective radii of the various ions. The present results are corrected for this geometric effect in the following way. If V_p milliliters per gram is the absolute pore volume, then V_w, the pore volume accessible to water molecules, is given by

$$V_w = V_p - r_w A \times 10^{-4} \text{ ml/g}, \tag{3}$$

where r_w is the radius of a water molecule in angstroms, and A the surface area of the gel in square meters per gram. Similarly, V_i, the volume accessible to an ion, is given by

$$V_i = V_p - r_i A \times 10^{-4} \text{ ml/g}, \tag{4}$$

where r_i is the effective radius of the ion. Eliminating V_p from these equations, the volume accessible to an ion relative to that accessible to water molecules is given by

$$V_i/V_w = 1 - A(r_i - r_w) \times 10^{-4}/V_w . \tag{5}$$

Table II lists values for V_i/V_w that have been calculated for different ions putting $A = 650 \text{ m}^2/\text{g}$, $V_w = 0.40$ ml/g, and $r_w = 1.4$ Å, which is equal to half the nearest neighbor distance in ice and half the distance of the first peak in the radial distribution curve in liquid water (13). Values for r_i which should reflect the ion as a hydrated kinetic entity, have been obtained from the data of Nightingale (14) (derived from Stokes radii) and from the data of Conway et al. (15) (derived from mobility measurements). The value of V_i/V_w for any ion is sensitive to the value taken for the hydrated radius, so that the absolute values of V_i/V_w are not very reliable. The last column of Table II, however, gives values of V_i/V_{Cl^-}, the volume accessible to an ion relative to that accessible to the chloride ion, the two ionic radii being taken from the same set of data. It is clear that the relative volumes available to ions are independent of the particular set of data used in the calculation, so that correction of the data by either of these sets of V_i/V_w values removes any selectivity due to ion size.

In Table III the observed partition coefficients of salts extrapolated to infinite dilution are corrected for the geometric effect by dividing each value of λ_0 (apparent) by the factor V_i/V_w for the larger of the two ions of the salt. The corrected value of λ_0 should then represent the ratio of the concentration of a salt in all the pore water that was available to it to its concentration in the bulk aqueous phase with which it was in equilibrium, extrapolated to infinite dilution. It is immediately apparent that after correction for exclusion due to ion size, the pore water retains a consider-

TABLE II

THE RELATIVE PORE VOLUME ACCESSIBLE TO AN ION

Ion	Hydrated radius		V_i/V_w		V_i/V_{Cl^-}	
	N	C	N	C	N	C
	Å	Å				
H^+	2.82		0.769			
Li^+	3.82	3.28	0.607	0.695	0.882	0.889
Na^+	3.58	3.04	0.646	0.734	0.939	0.940
K^+	3.31	2.72	0.690	0.786	1.003	1.006
Rb^+	3.29	2.73	0.693	0.784	1.007	1.004
Cs^+	3.29	2.74	0.693	0.782	1.007	1.001
Ca^{++}	4.12		0.558			
Mg^{++}	4.28		0.532			
Cl^-	3.32	2.75	0.688	0.781	1.000	1.000
NO_3^-	3.35		0.683			
SO_4^{--}	3.79		0.612			

V_i/V_w is the pore volume accessible to an ion relative to that accessible to water molecules; V_i/V_{Cl^-} is the pore volume accessible to an ion relative to that accessible to a chloride ion, both radii being taken from the same set of data.
N, from the data of Nightingale (14); C, from the data of Conway et al. (15).

TABLE III

PARTITION COEFFICIENTS CORRECTED FOR EXCLUSION DUE TO ION SIZE

Salt	λ_0 (apparent)	V_i/V_w		λ_0 (corrected)	
		N	C	N	C
$MgCl_2$	0.34	0.532		0.64	
$CaCl_2$	0.40	0.558		0.71	
LiCl	0.43	0.607	0.695	0.71	0.62
HCl	0.53	0.688		0.77	
NaCl	0.51	0.646	0.734	0.79	0.71
KCl	0.77	0.688	0.781	1.12	0.99
RbCl	0.82	0.688	0.781	1.19	1.05
CsCl	0.96	0.688	0.781	1.49	1.23
KNO_3	0.77	0.683		1.13	
K_2SO_4	0.92	0.612		1.50	

The value of V_i/V_w used is that for the larger of the two ions.

able degree of selectivity, the partition coefficients of the salts ranging from 0.64 to 1.50 (using Nightingale's [14] hydrated radii). The change in the absolute values of the partition coefficients shows that exclusion due to ion size was a significant factor in determining the distribution of the salts; it was not, however, the predominant factor in determining specific effects. This is particularly well illustrated by the example of K_2SO_4. The hydrated radius of the SO_4^{--} ion is very close to that of the Li^+ ion (see Table II). Since the radii of K^+ and of Cl^- are nearly equal, one would expect that if the distribution of salts were determined primarily by ion size, that the values of λ_0(apparent) for LiCl and K_2SO_4 would be approximately equal. In fact they were 0.43 and 0.92, respectively (see Table I).

It seems probable that of the possible sources of specificity of ionic distribution in this system, only ion-water interactions can be important. Specificity due to ion size has been corrected for, specific ion-ion interactions have been abolished by extrapolation to infinite dilution, and specific interactions between cations and the negatively charged surface have been shown to be negligibly small under the conditions of the experiments. It is suggested, therefore, that the corrected values of λ_0 represent true partition coefficients of salts between two aqueous phases of different structure.

Individual Ionic Partition Coefficients

The distribution of a salt between these two aqueous phases would be determined partly by the anion and partly by the cation. In the Appendix the following relationship is derived between the partition coefficient of a salt and the partition coefficients of its constituent ions,

$$\lambda_{0B} = (\lambda_+^{\nu_+}\lambda_-^{\nu_-})^{1/(\nu_+ + \nu_-)}, \tag{6}$$

where λ_{0B} is the corrected partition coefficient at infinite dilution for a salt B which gives ν_+ positive ions and ν_- negative ions; λ_+ and λ_- are the individual ionic partition coefficients. In order to assign values to the individual ionic partition coefficients, it is necessary to make some nonthermodynamic assumption, the most common one being that K^+ and Cl^- ions make equal contributions to the properties of KCl. Nightingale (14), however, pointed out that a better assumption might be that Rb^+ and Cl^- make equal contributions to the properties of RbCl.

Two sets of values of the individual ionic partition coefficients λ_i have been calculated from the data of Table III using both these assumptions. Nightingale's hydrated radii (14) have been used, as values are available for all the ions considered. In Fig. 4 λ_i is plotted against the individual ionic contribution to the activation energy for viscous flow ΔE^* calculated from the data of Stokes and Mills (16) by the method of Nightingale and Benck (17) using the same two assumptions. The values of λ_i range from 0.19 for Mg^{++} to 2.38 for SO_4^{--} if $\lambda_{Rb^+} = \lambda_{Cl^-}$, and from 0.21 for Mg^{++} to 2.70 for SO_4^{--} if $\lambda_{K^+} = \lambda_{Cl^-}$. All the points lie on a fairly smooth curve of increasing λ_i with decreasing ΔE^*, with the exception of that for SO_4^{--} calculated assuming

that $\lambda_{K^+} = \lambda_{Cl^-}$, suggesting that for these data, at least, the assumption that $\lambda_{Rb^+} = \lambda_{Cl^-}$ is the better one. A value for ΔE^* for Ca^{++} could not be found, but since its B coefficient of viscosity (16), entropy and free energy of hydration, and partial molal volume (18–20) all lie between the corresponding values for Li^+ and Mg^{++}, it is probable that Ca^{++} also fits on the same smooth curve; the values for $\lambda_{Ca^{++}}$ are indicated on the abscissa. As the data are plotted all the points lie either in the quadrant for which $\Delta E^* > 0$ and $\lambda_i < 1$ or in the quadrant for which $\Delta E^* < 0$ and $\lambda_i > 1$. It was pointed out earlier that more reliance can be placed on the relative than on the absolute values of λ_i. If the absolute values shown here are in error, K^+ is the only ion which could cross the line into an unoccupied quadrant. Four values for λ_{K^+} have been calculated, (1.05, 1.12, 0.93, and 0.99) two using the hydrated radius of Nightingale (14) and two using that of Conway et al. (15) giving a mean and standard error of 1.02 ±0.04. If the gel contained micropores into which water molecules but no ions could penetrate, V_i/V_w would be overestimated and all the λ_i values underestimated. It is concluded, therefore, that λ_{K^+} is unlikely to be less than unity, and is probably slightly greater. The significance of the distribution of the points between only two quadrants in Fig. 4 is that compared with bulk phase water the pore water of enhanced structure has diminished solvent powers for structure-making ions, but increased solvent powers for structure-breaking ions.

Effect of Urea

Urea resembles the water molecule in its strong hydrogen-bonding potential and yet because of its plane triangular shape cannot fit into the existing water clusters; according to Franks (21) it therefore behaves by dilution as a statistical structure breaker. Its addition to the equilibrated gel-water-ion systems should therefore decrease the difference in structure between pore water and bulk phase water, and if the interpretation of the data in Fig. 4 is correct, all values of λ_i should approach unity. One would predict, for example, that λ_{K^+} should decrease, but only slightly, since it is already close to unity, that λ_{Na^+} should increase, and that $\lambda_{Mg^{++}}$ which is furthest from unity should increase still more. The results of Figs. 2 and 3 are consistent with this interpretation.

The Effect of Ionization of the Gel Surface

When NaCl and KCl were simultaneously distributed between pore water and bulk phase water, they would be expected to behave independently. The selectivity coefficient under these conditions should be equal to the ratio of the apparent partition coefficients of the two salts, i.e., $K^{K^+}_{Na^+} = 0.77/0.51 = 1.51$ (see Table I). In Fig. 1 this condition was satisfied for points at pH less than 5 where ionization of the gel surface was negligibly small; $K^{K^+}_{Na^+} = 1.51 \pm 0.04$ (mean and standard error of 12 determinations, only a few of which are shown in Fig. 1). As the surface of the gel became negatively charged, however, the distribution of the cations was no longer

rigidly linked to that of the chloride ion, which was largely excluded from the negatively charged pore, and increasingly the cations became free to distribute themselves in accordance with their individual ionic partition coefficients. Finally, with most of the neutralizing negative charge already present inside the pores one would expect the selectivity coefficient to reach a maximal value determined solely by the corrected individual cationic partition coefficients, which from Eq. 6 are given by

$$\lambda_{Na^+} = \lambda^2_{NaCl}/\lambda_{Cl^-} \quad \text{and} \quad \lambda_{K^+} = \lambda^2_{KCl}/\lambda_{Cl^-}.$$

From Table III $\lambda_{K^+}/\lambda_{Na^+} = 1.12^2/0.79^2 = 2.01$. The experimental value of $K^{K^+}_{Na^+}$, however, should be greater than this by the factor V_{K^+}/V_{Na^+} from Table III, so that the maximal experimental value of $K^{K^+}_{Na^+}$ should be 2.14. The only assumption used in deriving this figure is that the ratio of the pore volumes accessible to the Na^+ and K^+ ions is 0.94 which follows if the hydrated radii of the cations in the pore water are the same as those obtained by either Nightingale (14) or Conway et al. (15) for bulk phase water. The average of the values of $K^{K^+}_{Na^+}$ between pH 7 and 9 in Fig. 1 is 2.18 $\pm$0.03. This agreement is probably as good as could be expected and justifies the relative correction factors used for exclusion due to ion size and the interpretation of the data in terms of individual ionic partition coefficients.

The decrease in $K^{K^+}_{Na^+}$ as the pH rises above 9 was probably due to a breaking down of the enhanced structure of pore water as hydrogen-bonding OH groups were increasingly replaced by water-structure-breaking ionized Si-O^- groups. The decrease in selectivity was rapid when the pH was greater than the pK of the SiOH group (9.5–9.8) (22). If the increased selectivity of the gel at lower pH had been due to preferential association of K^+ rather than Na^+ with the surface charges, one would expect it to continue to increase as the surface became more highly charged.

Distributions of Ions in Living Tissue

The value of this model system has been to show that when ions are distributed between two adjacent aqueous phases of different structure, and when other sources of specificity have been eliminated or corrected for, that surface-oriented water of enhanced structure has increased solvent powers for water-structure-breaking ions, and decreased solvent powers for water-structure-making ions, and that the relative solubility of an ion in the phase of enhanced structure increases regularly as the water-structure-breaking powers of the ion increase.

Most water molecules inside cells are within a few molecular diameters of a protein or membrane surface (5) which should affect water structures, so that cell water might be predominantly a surface-oriented phase of enhanced structure. Partition of ions between such water and extracellular bulk phase water should then resemble partition of ions between the surface-oriented water of the silica gel and bulk phase water. Qualitatively such a distribution is frequently observed, most cell types having

lower free concentrations of Na^+, H^+, Ca^{++}, and Mg^{++} and higher concentrations of K^+ and the anions than expected for the simplest equilibrium distribution. Quantitatively, of course, the distributions are much more asymmetrical in living tissue than they are in the silica gel.

While any surface probably perturbs the structure of vicinal water to some degree, the kind and extent of ordering must depend upon the properties of the particular surface. Thus in Fig. 1 the silica gel surface appeared to lose its water-ordering powers as it changed from a strongly hydrogen-bonding surface to a structure-breaking ionic surface, maintaining, presumably, approximately the same total surface area.

Ionic selectivity and a low characteristic water content are properties of living cells which they lose when their metabolism is impaired, and which are therefore generally assumed to depend entirely upon energy-consuming membrane-bound pumps. An alternative or supplementary role for metabolic processes in the maintenance of ion distribution and water content might be that they change the properties of intracellular surfaces in such a way that they impose a high degree of order upon intracellular water.

For example the cell matrix could be held in a rather condensed metastable configuration such that the many hydrogen bond donor and acceptor groups on protein molecules were so oriented that existing water structures could be stabilized with little bending of hydrogen bonds; the stabilizing surfaces should have sufficient rigidity to insulate adjacent water molecules from thermal fluctuations; and each surface should be close enough to a similar surface so that two vicinal water structures could fuse without a region of mismatch. Such a metastable configuration stabilized by cooperative interactions between water and protein molecules could impose a high degree of order upon cell water, which should then accumulate structure-breaking ions and exclude structure-making ions. Upon impairment of metabolism the whole metastable structure would collapse, cells would swell, and ions redistribute themselves as the structure of cell water approached that of bulk phase water. That conformational changes do accompany metabolic processes was shown by Graham and Wallach (23) who found that erythrocyte membrane proteins underwent a transition to an antiparallel-structure when ATP was being hydrolyzed by the $Na^+ - K^+$-activated ATPase, which is generally considered to be the sodium pump.

It is particularly interesting that the conditions for maximal selectivity of cations probably exist in most cells, where proteins carry a net negative charge so that the distribution of cations should be fairly independent of the distribution of accompanying anions (cf. the maximum in $K^{K^+}_{Na^+}$ in Fig. 1). The fixed negative charge inside cells might also account for the much slighter degree of selectivity shown by cells for anions; their distributions would be tightly linked to that of cations, so that specific anion effects would be less apparent.

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APPENDIX

Consider a salt B distributed at equilibrium between the pore water of the gel and bulk phase water.

$$(\mu_B^0)_p + RT \ln (a_B)_p = (\mu_B^0)_b + RT \ln (a_B)_b , \qquad (A\ 1)$$

where the subscripts p and b denote the pore phase and the bulk phase, respectively, μ_B^0 is the standard chemical potential, and a_B the activity of the salt. If the salt yields ν_+ positive ions and ν_- negative ions then (24)

$$\mu_B^0 = \nu_+ \mu_+^0 + \nu_- \mu_-^0 , \qquad (A\ 2)$$

where μ_+^0 and μ_-^0 are the standard chemical potentials of the positive and negative ions, respectively, and

$$a_B = (\nu_+^{\nu_+}\nu_-^{\nu_-})c^{(\nu_+ + \nu_-)}\gamma_+^{\nu_+}\gamma_-^{\nu_-} , \qquad (A\ 3)$$

where c is the molar concentration of the salt and γ_+ and γ_- are the single ionic activity coefficients. Eq. A 1 can be written

$$(\mu_B^0)_b - (\mu_B^0)_p = (\nu_+ + \nu_-)RT \ln \frac{c_p}{c_b} + \nu_+ RT \ln \frac{(\gamma_+)_p}{(\gamma_+)_b} + \nu_- RT \ln \frac{(\gamma_-)_p}{(\gamma_-)_b}. \qquad (A\ 4)$$

At infinite dilution the activity coefficient terms disappear and $c_p/c_b = \lambda_B$, the partition coefficient of the salt at infinite dilution. Therefore

$$(\mu_B^0)_b - (\mu_B^0)_p = (\nu_+ + \nu_-)RT \ln \lambda_B . \qquad (A\ 5)$$

Similarly from Eqs. A 1 and A 2

$$(\mu_B^0)_b - (\mu_B^0)_p = \nu_+ RT \ln \lambda_+ + \nu_- RT \ln \lambda_- , \qquad (A\ 6)$$

where λ_+ and λ_- are the individual ionic partition coefficients at infinite dilution. Therefore

$$(\nu_+ + \nu_-)RT \ln \lambda_B = \nu_+ RT \ln \lambda_+ + \nu_- RT \ln \lambda_- , \qquad (A\ 7)$$

and

$$\lambda_B^{\nu_+ + \nu_-} = \lambda_+^{\nu_+}\lambda_-^{\nu_-} . \qquad (A\ 8)$$

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