

Water Structure as a Determinant of Ion Distribution in Living Tissue

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All ions perturb the structure of water. The biologically important ions can be divided into two classes which have opposite effects. Na^+ , Ca^{2+} and Mg^{2+} are net structure-orderers; K^+ and anions are net structure-disorderers.

Many living cells apparently discriminate between these two groups, largely excluding the “water-structure-ordering ions” and freely taking up “water-structure-disordering ions”. It has been elsewhere suggested that cell water is considerably more ordered than is ordinary liquid water; it is proposed here that this difference in the structure of intracellular and extracellular water could result in the observed ion distributions in the following way. Water inside a cell, being already ordered by the cell matrix, would be less readily available to hydrate ions than would water in the extracellular solution. All ions might therefore be expected to have diminished solubility in cell water, but the effect should be greatest for those ions which are stable only when they can impose a highly ordered structure of their own upon water. Thus ions should be increasingly excluded from a cell as their water-structure-ordering powers increase.

Examples of non-biological systems in which such a mechanism of selective ion distribution operates are discussed, and it is shown that it is thermodynamically possible that the structural nature of cell water could be solely responsible for the observed distribution of ions in living tissue.

1. Introduction

Recent evidence (Odeblade, Bhar & Lindstrom, 1956; Bratton, Hopkins & Weinberg, 1965; Pocsik, 1966; Ling, 1967; Fritz & Swift, 1967; Cerbon, 1969; Cope, 1969; and Hazelwood, Nichols & Chamberlain, 1969) suggests that cell water is significantly more ordered than is water in a simple electrolyte solution. It is probable, therefore, that intracellular and extracellular water interact differently with the biologically important ions, Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , HCO_3^- and phosphate, so that the activity coefficient of an ion might have a different value on either side of the membrane. Szent-Gyorgyi and co-workers had in fact suggested, in 1957, that the commonly observed

asymmetrical distribution of Na^+ and K^+ between a living cell and its extra-cellular solution might be a consequence of the "ice-likeness" of cell water (Baird, Karreman, Mueller & Szent-Gyorgyi, 1957).

The interactions that ions make with ordinary liquid water have been extensively investigated. (Noyes, 1963; Samoilov, 1965; Stokes & Mills, 1965; Nightingale, 1966.) The properties of water, both thermodynamic (Shereshfsky & Carter, 1950; Folman & Shereshfsky, 1955) and kinetic (Adlfinger & Peschel, 1970) are perturbed for some distance out from an interface between the liquid and a polar solid (Drost-Hansen, 1969). This has been interpreted to mean that a degree of organization has been imposed upon the liquid. There are reports in which water ordered in this way has specific effects upon the activity coefficients of ions (Dalton, McClanahan & Maatman, 1962; Jones, Mysels & Scholten, 1965; Horne, 1966; Horne, Day, Young & Yu, 1968; Allen & Matijevic, 1969). An attempt will be made, therefore, to show that if water inside a cell is surface-oriented and relatively immobile, then (1) partition of ions between such water and bulk phase water should not be simple, and (2) that the commonly observed distributions of ions is in fact predictable.

2. Interactions of Ions with Water

The interactions of water with some biologically important ions are summarized in Fig. 1, where one kinetic property (the viscosity B -coefficient) is plotted against one thermodynamic quantity (the free energy of hydration of a single ion). This plot has been chosen to illustrate the general observation that kinetic and equilibrium properties of ion-water systems show the same trends.

The viscosity B -coefficient is a measure of the specific effect that an ion has upon the mobility of water molecules in its immediate neighbourhood. As constriction of adjacent water molecules increases, B becomes more positive. The free energy of hydration which represents the affinity between an ion and water becomes more negative as dehydration of an ion becomes more difficult.

According to Samoilov's analysis (1965), an ion which attracts its neighbouring water molecules more strongly than did the water molecule it has replaced has a positive B -coefficient because it decreases the mobility of neighbouring water molecules. Such an ion shows positive hydration, or is a water-structure-ordering ion (Nightingale, 1966) in the sense that it has imposed upon the water a different structure which is more highly ordered than was the intrinsic structure. In order of increasing effectiveness, Na^+ , Li^+ , Ca^{2+} and Mg^{2+} are water-structure-ordering ions. Moreover their dehydration requires large and increasing amounts of energy. An ion which

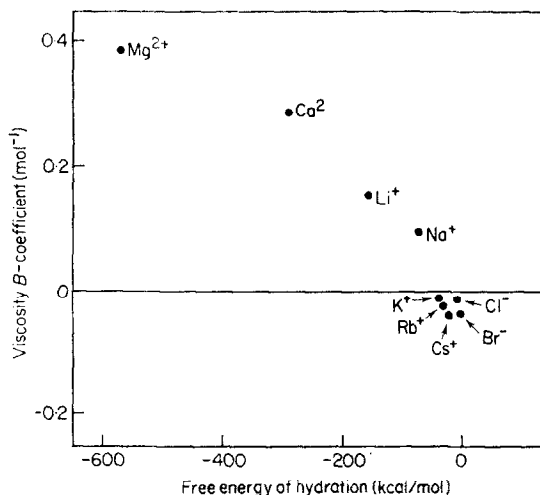


FIG. 1. Interactions of biologically important ions with water. The viscosity B -coefficients are taken from the data of Stokes & Mills (1965) and the free energies of hydration from Noyes (1963). The temperature is 25°C. The structure-ordering ions (Mg^{2+} , Ca^{2+} , Li^+ and Na^+) have positive hydrations; the structure-disordering ions (K^+ , Rb^+ , Cs^+ , Br^- and Cl^-) all have negative hydrations.

attracts its neighbouring water molecules less strongly than did the water molecule it has replaced, has a negative B -coefficient because it increases the mobility of adjacent water molecules. Such an ion shows negative hydration (Samoilov, 1965) or is a water-structure-disordering ion (Nightingale, 1966). Included in this class are most anions (only Cl^- and Br^- are shown in Fig. 1 as precise data are not available for HCO_3^- , phosphate and lactate, the other biologically important anions) and the inorganic cations K^+ , Rb^+ and Cs^+ which in order of increasing effectiveness loosen the structure of water and require relatively small and decreasing amounts of energy for their dehydration.

Thus the ions in Fig. 1 are separated by the line $B = 0$ into two distinct classes which have opposite effects upon water. It is of particular interest that this division separates Na^+ and K^+ which in other respects are so similar chemically. Other properties of aqueous solutions confirm that these two classes are different and that the division occurs between Na^+ and K^+ . Fig. 2 illustrates three kinetic properties of aqueous electrolyte solutions which show a change of sign between Na^+ and K^+ ; they are the self-diffusion coefficient of water ($D_{\text{H}_2\text{O}}$), the reciprocal of the proton spin-lattice relaxation time ($1/T_1$) and the ultrasonic absorption (α/ν^2), all of which are related to the mobility of water molecules. Wang (1954), showed that $D_{\text{H}_2\text{O}}$ is increased by the addition of KCl (up to 2M) but is decreased by the addition of NaCl ; i.e.

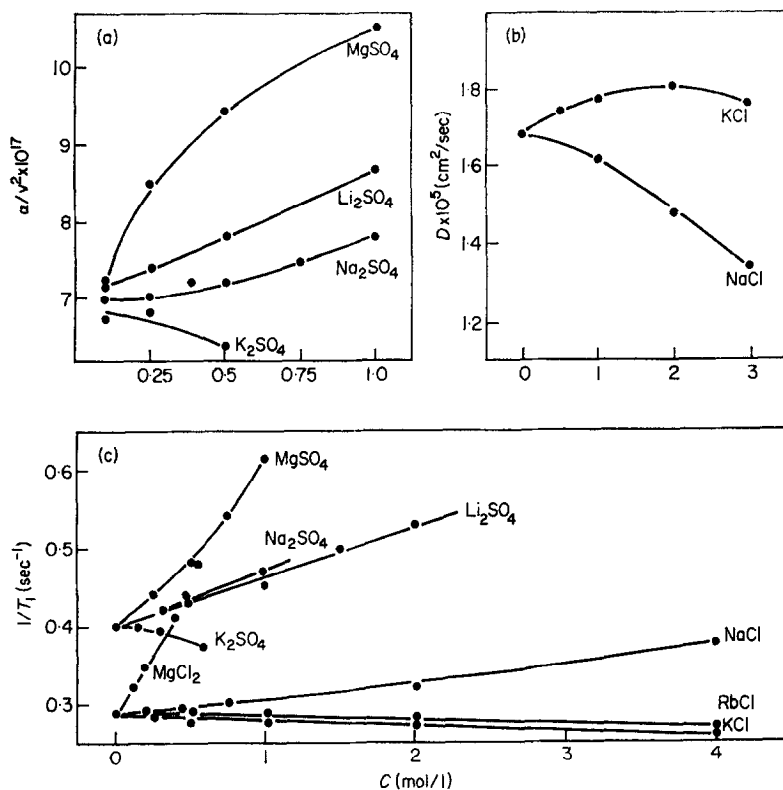


FIG. 2. Kinetic properties of aqueous electrolyte solutions with a change in sign between Na^+ and K^+ . In each case the abscissa shows the molar concentration of added salt. The ordinate shows: (a) ultrasonic absorption at 18°C (data of Fittipaldi *et al.*, 1967); (b) self-diffusion coefficient of water at 10°C (data of Wang, 1954); (c) reciprocal of the spin-lattice relaxation time (sulphates at 18°C from Fittipaldi *et al.*, 1967; chlorides at 25°C from Jones & Powles 1964).

K^+ ions loosen the structure of water and facilitate its diffusion, Na^+ ions tighten the structure of water and hinder its diffusion. $1/T_1$ is decreased by the addition of RbCl and KCl (Jones & Powles, 1964) and K_2SO_4 (Fittipaldi, Ragazzino & Viggiano, 1967) but is increased by the addition of NaCl and MgCl_2 (Jones & Powles, 1964) and Na_2SO_4 , Li_2SO_4 and MgSO_4 (Fittipaldi *et al.*, 1967). Fittipaldi *et al.* (1967) also showed that α/v^2 , which is inversely related to the mobility of water molecules, was increased by the addition of Na_2SO_4 , Li_2SO_4 and MgSO_4 , but was decreased by the addition of K_2SO_4 .

Figure 3 shows two thermodynamic properties which illustrate the difference between Na^+ -type and K^+ -type ions. Millero (1969) showed that \bar{V}° ,

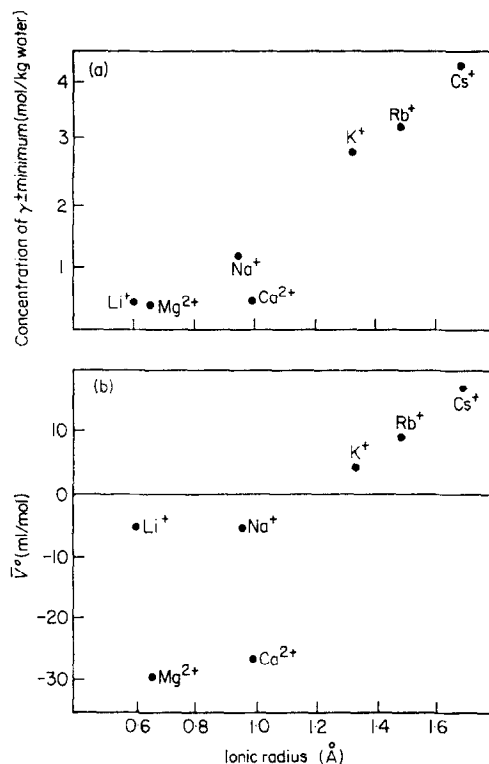


FIG. 3. Equilibrium properties of aqueous electrolyte solutions illustrating the difference between Na^+ -type and K^+ -type cations. Along the abscissa is plotted Pauling's crystal radii (Robinson & Stokes, 1959). The ordinate shows: (a) the molal concentration at which the mean activity coefficient of the chlorides of the various cations is a minimum (data from Robinson & Stokes, 1959); and (b) the partial molar volume of the ions at infinite dilution (alkali metal ions from Millero, 1969; Ca^{2+} and Mg^{2+} from Glueckauf, 1965).

the partial molal volume of ions in aqueous solution at 25°C is negative for Li^+ and Na^+ , but positive for K^+ , Rb^+ , Cs^+ and anions; i.e. Na^+ -type ions constrict water; K^+ -type ions expand water. Values of γ_{\pm} the mean activity coefficient for chlorides in aqueous solution (Robinson & Stokes, 1959) show minima at concentrations where ion-water interactions begin to predominate over ion-ion interactions. For the cations of interest these minima fall into two groups separated by a relatively wide concentration gap [Fig. 3(b)]. Mg^{2+} , Ca^{2+} , Li^+ and Na^+ chlorides all show minima over the narrow concentration range 0.4 to 1.2 M; K^+ , Rb^+ , and Cs^+ chlorides have minima between 3 and 4 M.

The biological interest in the classification of ions shown in Fig. 1 lies in

the observation that living cells apparently discriminate between the water-structure-ordering, Na^+ -type class, and the water-structure-disordering K^+ -type class. Table 1 shows the distribution of ions between frog muscle cells and their surroundings (Ling, 1962). Similar distributions are commonly observed in other tissues. For example mammalian kidney, liver, nerve and muscle cells have K^+ as their principal cation and relatively low concentrations of Na^+ , Mg^{2+} and Ca^{2+} .

The intracellular concentration of an ion which is passively distributed and for which the activity coefficient is the same inside and outside the cell can be calculated from the Nernst equation

$$E_m = -(59/z) \log m_i/m_o \quad \text{mv.} \quad (1)$$

at 25°C , where E_m is the membrane potential, z the valence of the ion, and m_i and m_o its molal concentration in the intracellular and extracellular water respectively. The intracellular molalities calculated from the experimental values of m_o and E_m are shown in column 4, and the ratio of observed to calculated m_i in column 5.

The first three ions, Mg^{2+} , Ca^{2+} , and Na^+ , which are all of the water-structure-ordering class, are clearly largely excluded from the cell; the ratios

TABLE 1
Ions classified according to their distribution in frog muscle

| Ion | m_o (mmol/kg water) | m_i (obs) (mmol/kg water) | m_i (calc) (mmol/kg water) | m_i (obs)/ m_i (calc) |
|------------------|-----------------------------|-----------------------------------|------------------------------------|---------------------------|
| Mg^{2+} | 1.23 | 15.8 | 1968 | 0.008 |
| Ca^{2+} | 2.02 | 5.65 | 3232 | 0.002 |
| Na^+ | 105 | 16.9 | 4200 | 0.004 |
| K^+ | 2.53 | 128 | 101.2 | 1.26 |
| Cl^- | 76.8 | 1.04 | 1.92 | 0.54 |
| HCO_3^- | 26.4 | 9.2 | 0.66 | 13.9 |
| Phosphate | 1.07 | 5.4 | 0.01 | 540 |
| Lactate | 3.42 | 3.5 | 0.09 | 38.9 |

The values of the second and third columns were taken from the data of Ling (1962). m_i (obs) is obtained from the difference between total ions and extracellular ions. For Na^+ and Cl^- which have high m_o , m_i (obs) is strongly dependent upon the value taken for the extracellular space. There is therefore some uncertainty about their absolute values but they are of the right order. The values in the fourth column were calculated from the Nernst equation

$$E_m = -(59/z) \log m_i/m_o \quad \text{mv}$$

at 25°C where E_m is the membrane potential (Ling, 1962, found an average value of -94.6 mv), z the valence of the ion and m_i and m_o the molal concentrations in the intracellular and extracellular water respectively.

of column 5 are all much less than one. The calculated values of m_i appear unrealistic, as a cell containing such high accumulations of cations would require a net negative charge on its macromolecules of about 9.5 M, whereas from column 3 the real net non-diffusible charge, the difference between diffusible cations and anions, amounts to only 170 mM. If, however, the restriction that the net negative non-diffusible charge was to remain constant at 170 mM were used, the membrane potential could not be -94.6 mV. These conditions are incompatible. The calculated molality of column 4 and the ratios of column 5 are therefore valid indications of the degree of exclusion of the ions from the cell, given that the membrane potential has its experimental value of -94.6 mV.

The remaining ions, which are all of the water-structure-disordering class, appear to be passively distributed, or accumulated by the cell; the ratios of column 5 are of the order of one or greater.

This striking correlation between ions classed according to their partition between cell and surroundings, and ions classed according to their interactions with water, has prompted this investigation of the possibility that the kind of interaction that an ion makes with water might be a factor in determining whether that ion is taken up or excluded by a cell. Accordingly, model systems which appear to show effects of specific ion-water interactions will be considered. The first and simplest of these is an aqueous electrolyte solution to which a non-electrolyte has been added.

3. Interactions in Three-component Systems

The activity coefficients of most non-electrolytes are increased (i.e. the compounds are salted out) by the addition of simple electrolytes. The approximate Setschenow equation (see Long & McDevit, 1952) relates the activity coefficient of the non-electrolyte (γ_3) to the molal concentration of added salt (m_2)

$$\log \gamma_3 = k_{32} m_2. \quad (2)$$

Here k_{32} the empirical Setschenow coefficient is positive for salting out and negative for salting in. Representative values of k_{32} are shown in Table 2 where for all the compounds listed except two (ammonia and trimethylamine) k_{32} decreases in the order $\text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$; i.e. of this series of cations Na^+ increases the activity coefficient of the non-electrolyte most, and Cs^+ least. In the majority of cases for which k_{32} has been measured for the entire series of alkali metal ions, the greatest difference between adjacent ions falls between Na^+ and K^+ .

TABLE 2

Setschenow interaction coefficients for representative non-electrolytes

| Non-electrolyte | NaCl | KCl | RbCl | CsCl |
|-------------------------------|-------|-------|--------|--------|
| (a) <i>Non-polar</i> | | | | |
| Benzene | 0.195 | 0.166 | 0.141 | 0.088 |
| γ -Butyrolactone | 0.067 | 0.035 | | |
| Hydrogen | 0.114 | 0.102 | | |
| Oxygen | 0.141 | 0.132 | | |
| Nitrous oxide | 0.123 | 0.099 | 0.076 | 0.024 |
| (b) <i>Basic</i> | | | | |
| Ammonia | 0.033 | 0.057 | | |
| Trimethylamine | 0.16 | 0.20 | | |
| Aniline | 0.16 | 0.13 | 0.09 | 0.02 |
| (c) <i>Acidic</i> | | | | |
| Phthalic acid | 0.178 | 0.093 | 0.053 | 0.001 |
| Benzoic acid | 0.182 | 0.144 | | |
| Succinic acid | 0.088 | 0.018 | —0.005 | —0.026 |
| Phenol | 0.172 | 0.133 | | |
| (d) <i>Intermediate polar</i> | | | | |
| Carbon dioxide | 0.101 | 0.073 | 0.063 | |
| Sulphur dioxide | 0.01 | —0.04 | | |
| Cobaltic trinitrotri-amine | —0.16 | —0.24 | | |
| Acetone | 0.110 | 0.101 | | |
| Diacetone alcohol | 0.159 | 0.142 | | |

Values of the Setschenow interaction coefficient k_{32} when chlorides of Na^+ , K^+ , Rb^+ and Cs^+ are added to saturated solutions of some representative non-electrolytes. k_{32} is the gradient of the line obtained when $\log \gamma_3$ (the activity coefficient of the non-electrolyte) is plotted against m_2 the concentration of added salt. All these values have been taken from Long & McDevit (1952).

While salting in and out of non-electrolytes has been extensively investigated few measurements have been made of the reverse effect which is more pertinent to this discussion; viz. the effects of non-electrolytes upon the activity coefficients of ions. Haugen & Friedman (1956) however, measured both the effect of added electrolyte upon the solubility of nitromethane, and the effect of added nitromethane upon the solubility of electrolytes. Their results fitted equations of the form

$$\log \gamma_3 = k_{32} m_2 \quad (3)$$

and

$$\log \gamma_2 = k_{23} m_3, \quad (4)$$

where γ_3 and m_3 are the activity coefficient and molality of nitromethane, and γ_2 and m_2 the mean activity coefficient and molality of the electrolyte; k_{32} and k_{23} , the mutual interaction coefficients were constant over the ranges of concentrations measured. Friedman (1955) showed that in the limit of infinite dilution

$$k_{32}^0 = k_{23}^0. \quad (5)$$

Haugen & Friedman (1956) confirmed this for systems containing KClO_4 or CsClO_4 and nitromethane. They then calculated the free energy of transfer (at infinite dilution) of ions from pure water to water saturated with nitromethane and showed that it should decrease in the order $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+ > \text{ClO}_4^- > \text{ReO}_4^- > \text{Cl}^-$, the greatest difference between adjacent ions being 120 cal/mol between Na^+ and K^+ .

The relevance of this work to the problem of partition of ions between a cell and its surroundings is clarified by considering partition of ions when water saturated with nitromethane is separated from pure water by a hypothetical membrane impermeable only to nitromethane. At equilibrium an ion for which the activity coefficient is increased by the presence of nitromethane would have a higher concentration in the water phase than in the water-nitromethane phase. This relative exclusion from the water-nitromethane phase should decrease in the order $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$ becoming a relative accumulation when k_{23} became negative. Quantitatively, using Haugen & Friedman's (1956) value for the difference between the free energies of transfer of Na^+ and K^+ at infinite dilution, if the molalities of Na^+ and K^+ were very low and were equal in the aqueous phase, the ratio of their molalities in the water-nitromethane phase would be given by

$$m_{\text{K}^+}/m_{\text{Na}^+} = e^{-120/RT} = 1.23 \quad (6)$$

i.e. in this system Na^+ would be excluded relative to K^+ by a factor of 1.23.

Although the precise equality of equation (5) holds only at infinite dilution, the results of Haugen & Friedman (1956) showed an important correspondence between k_{32} and k_{23} in solutions up to 2 M in nitromethane and 0.1 M in electrolyte. Unlike most chlorides, perchlorates decreased the activity coefficient of nitromethane; similarly nitromethane decreased the mean activity coefficients of perchlorates; i.e. both k_{32} and k_{23} were negative. Moreover they found that k_{32} increased in the order $\text{Cs}^+ < \text{K}^+ < \text{Na}^+$ when the anion was Cl^- , and that k_{23} increased in the same order $\text{Cs}^+ < \text{K}^+ < \text{Na}^+$ when the anion was ClO_4^- and $\text{Cs}^+ < \text{Rb}^+ < \text{K}^+$ when the anion was ReO_4^- . Thus for nitromethane at least the effect of a non-electrolyte upon the activity coefficients of ions is of the same sign and shows the same trend with changing cation as the effect of ions upon the activity coefficient of the non-electrolyte in solutions of finite concentration. In the absence of

more experimental data one must assume that for other ions with other non-electrolytes there should be a direct qualitative relationship between k_{32} and k_{23} as Haugen & Friedman found for the system nitromethane-water-ions.

Since the order of the effect of alkali metal ions upon the activity coefficients of non-electrolytes is almost always the same, exclusion of Na^+ relative to K^+ should be a general phenomenon in any system

non-electrolyte-water/membrane/water

where the membrane is impermeable to the non-electrolyte. Or to generalize any ion in the series Na^+ , K^+ , Rb^+ , Cs^+ should be excluded relative to any later ion in the series. If this conclusion is to be applied usefully to partition of ions between a cell and its surroundings a mechanism must be postulated.

4. Mechanism of Specific Ion Effects

Frank & Evans (1945) suggested that solution in water of a non-polar molecule or of a molecule with a significant non-polar residue increased the proportion of open-structured, hydrogen bonded ice-like regions in water. Frank & Wen (1957) and Frank (1958) developed this concept in terms of "flickering clusters" of hydrogen-bonded water molecules, which they suggested would be stabilized by the presence of non-polar solutes which do not transmit disruptive fluctuations to the clusters adjacent to them. The result would be a longer life-time of the clusters and a greater degree of ice-likeness of the water. Ions added to an aqueous solution of a non-electrolyte might therefore be expected to have rather specific effects dependent upon the type of the interactions they make with water. A water-structure-ordering ion (Li^+ , Na^+ , Ca^{2+} , Mg^{2+}) should compete strongly for water molecules, disrupting the open-structured regions in which the non-electrolytes are stable, and force them out of solution (salting out, positive Setschenow constant, increase of activity coefficient). A water-structure-disordering ion (most anions and K^+ , Rb^+ and Cs^+) should free molecules from the bulk of liquid water, and make them available to form more stabilizing hydrogen-bonded structures round non-polar regions of solute molecules. This should result in a relative salting in (negative Setschenow constant, and decrease in activity coefficient).

Salting-out effectiveness might then be expected to decrease with the water-structure-ordering powers of the ions. This in fact is usually the observed order (Long & McDevit, 1952) $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+ > \text{Cs}^+$.

Departure from this strict order would be expected when specific ion-non-electrolyte interactions became important. Thus Li^+ and H_3O^+ are often

out of order as is Na^+ for a few basic non-electrolytes (see Table 2). Long & McDevit (1952) suggested that this might be due to a localized hydrolysis.

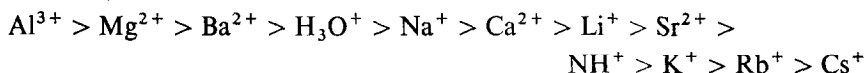
According to this mechanism, in the system

water-non-electrolyte/membrane/water

a structure-ordering ion in the non-electrolyte-containing phase would have to compete with the non-electrolyte for water molecules which it needs for stability; in the simple aqueous phase there would be no such competition. A structure-disordering ion, on the other hand, would not have to do work to perturb existing water structures round the non-electrolytes and therefore could fit readily into the non-electrolyte solution. Thus the structure-ordering ion would be excluded relative to the structure-disordering ion.

The degree of selectivity towards Na^+ and K^+ shown by these simple systems is orders of magnitude lower than that of living cells. It is, however, in the right direction. Moreover, it is increased in other non-biological systems in which the same mechanism probably operates.

Dalton *et al.* (1962) found that nitrates of various cations were partially excluded from the water-filled pores of a silica gel. The nitrate ion was not excluded, and cations were excluded in the order



In this series the percentage availability of the water, ranged from 24% for Al^{3+} to 100% for Cs^+ . Again the ions are increasingly excluded as their water-structure-ordering powers increase, and again the greatest difference between adjacent alkali metal ions is between Na^+ and K^+ . In this case Na^+ was excluded relative to K^+ by a factor of 1.35; i.e. selectivity is greater than in the non-electrolyte systems. Silica gel presents to water a hydrophilic surface containing both hydrogen-bonded and freely rotating hydroxyl groups, from which it is probable that many layers of water molecules are oriented and have reduced mobility. The capillary condensed state in the pores of the gel is probably a three-dimensional network of hydrogen-bonded molecules (Nair & Thorp, 1965) which presumably could accommodate water-structure-disordering ions more readily than water-structure-ordering ions.

The degree of selectivity resulting from this mechanism might be expected to increase as the proportion of surface-oriented water to bulk phase water increased. Jones *et al.* (1966) showed that formation of a second black film of about 44 Å thickness from the first black film of sodium dodecyl sulphate was very sensitive to the nature of the cation present. All the water within the bimolecular leaflet was probably surface-oriented and electrostricted, because its thickness was estimated to be only 27 Å. The second black film

formed spontaneously in the presence of 0.22 M-NaCl. If, however, low concentrations of KCl were added, less NaCl was needed. For example 0.01 M-KCl would replace 0.09 M of the NaCl. When LiCl was added the film would not form at all unless more than the 0.22 M-NaCl was present. Thus relative to Na^+ , Li^+ inhibited the formation of the second black film, and K^+ strongly promoted it. This phenomenon is also probably due to the diminished capacity of ordered water to accommodate water-structure-ordering ions.

Allen & Matejevic (1969) found strikingly different effects of Li^+ and Na^+ chlorides on the one hand, and of K^+ and Cs^+ chlorides on the other, upon coagulation of colloidal silica. With increasing pH the critical coagulation concentrations of LiCl and NaCl decreased exponentially, but of KCl and CsCl showed minima at pH 9. Absolute critical coagulation concentrations also varied widely. At pH 7, for example, they were LiCl, 3.2; NaCl, 2.5; KCl, 0.58; CsCl, 0.25 M. This high degree of specificity could again be attributed to relative exclusion of the water-structure-ordering cations Li^+ and Na^+ from the surface-oriented water. Coagulation proceeded as metal ions replaced hydrogen ions from the silanol hydroxyl groups: higher concentrations of the excluded ions would be needed for exchange to take place.

5. The State of Water in Living Cells and Near Surfaces

Nuclear magnetic resonance (n.m.r.) studies of muscle (Cope, 1969; Hazelwood *et al.*, 1969; Bratton *et al.*, 1965; and Sussman & Chin, 1966) of red blood cells (Odeblade *et al.*, 1956) of nerve (Chapman & McLauchlan, 1967; Fritz & Swift, 1967; and Swift & Fritz, 1969) and of brain (Cope, 1969) have shown that most of the intracellular water of these tissues has sufficient mobility to be seen by high resolution n.m.r., but is significantly more ordered than is water in a simple electrolyte solution. Such relative crystallinity of cell water is also consistent with rates of diffusion of water into frog eggs (Ling, 1967), the failure of animal cells and non-vacuolar plant cells to behave as perfect osmometers (Troschin, 1966) and changing density of muscle water at different phases of drying (Pocsik, 1966).

The organization of proteins, nucleic acids and lipids into intracellular organelles and membranes is such that most cell water is probably close enough to a hydrophilic surface to be influenced by it. There have been reports for a long time that the structure of water adjacent to an interface is perturbed for some distance into the bulk liquid phase (Bangham & Saweris, 1938; Bangham, 1946; Bond, Griffith & Maggs, 1948). Experimentation with boundary phases is difficult, and some claims of surface-oriented water have

subsequently been shown to be the result of artifacts (e.g. see Gittens, 1969). Nevertheless there is a large body of evidence, much of it recently reviewed by Drost-Hansen (1969), which suggests that surfaces impose a degree of organization upon vicinal water extending several molecular diameters into the bulk phase. Most recently Adlfinger & Peschel (1970) measured the viscosity of interfacial liquid zones in the neighbourhood of polished silica surfaces. They found that the viscosity was five times its bulk value at 400 Å from the surface, and twice its bulk value as far out as 1000 Å.

In a muscle cell an ordered parallel array of myofilaments presents to water molecules hydrophilic surfaces separated by about 200 Å (Buchthal & Rosenfalck, 1957). Few tissues contain more than 80% water (Altman & Dittmer, 1964), a variable amount of which is extracellular. Muscle, with 76% water is near this upper limit so that it can be assumed that most water molecules inside cells of most tissues are within about 100 Å of a surface and could possibly constitute a surface-oriented boundary phase rather than a bulk phase. Moreover the possibility of organization of water is enhanced by the amphoteric nature of proteins. The presence of both cationic and anionic groups increases the probability that structures imposed upon water by neighbouring regions of the cell might be compatible, so that the intervening regions of mismatch would be small (see Drost-Hansen, 1969).

6. Intracellular Ions

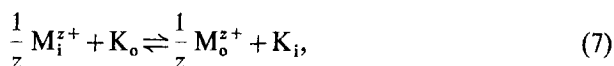
Like silica gel, cells should show selective exclusion of ions, increasing as the water-structure-ordering powers of the ions increase. The degree of selectivity could be high if all the water close enough to surfaces to constitute a boundary phase were in fact surface-oriented and ordered, or low if the conformation of intracellular surfaces was such that co-operative ordering of water molecules did not take place. The n.m.r. evidence suggests that in some cells, at least, much of the water is relatively immobile and might therefore be expected to contribute appreciably to the selective ion distribution illustrated in Fig. 1, where water-structure-ordering ions (Na^+ , Mg^{2+} , and Ca^{2+}) are relatively excluded, and water-structure-disordering ions (K^+ and anions) are passively distributed or relatively accumulated.

At physiological pH there is a net negative charge on cell proteins, lipids and nucleic acids. Counter-cations are therefore necessary to preserve electro-neutrality and total exclusion of cations is not possible, however low the solvent power of cell water for ions might be. This requirement should further increase the degree of selectivity in a cell with long-range ordered water.

7. Energy Requirement of this Mechanism

The mechanism proposed then, is that regions of the solution inside the cell where structure is imposed upon the water by, for example, the presence of a charged interface, should exclude structure-ordering ions (e.g. Na^+ , Ca^{2+} and Mg^{2+}) relative to structure-disordering ions (e.g. K^+ and anions).

If this mechanism were solely responsible for the observed distribution of ions (cf. Table 1) all the ions would be in equilibrium. Therefore in the exchange reaction



where M^{z+} can be Na^+ , Ca^{2+} or Mg^{2+} and z is its valence, the total free energy change would be zero.

$$\Delta G_{(\text{total})} = RT \ln (a_{\text{K}^+})_i (a_{\text{M}^{z+}})_o^{1/z} / (a_{\text{K}^+})_o (a_{\text{M}^{z+}})_i^{1/z} = 0. \quad (8)$$

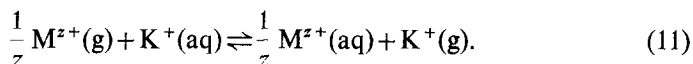
$\Delta G_{(\text{total})}$ can be divided into an ideal and non-ideal part

$$\Delta G_{(\text{total})} = \Delta G_{(\text{ideal})} + \Delta G_{(\text{non-ideal})} \quad (9)$$

$$= RT \ln (m_{\text{K}^+})_i (m_{\text{M}^{z+}})_o^{1/z} / (m_{\text{K}^+})_o (m_{\text{M}^{z+}})_i^{1/z} + \\ RT \ln (\gamma_{\text{K}^+})_i (\gamma_{\text{M}^{z+}})_o^{1/z} / (\gamma_{\text{K}^+})_o (\gamma_{\text{M}^{z+}})_i^{1/z}. \quad (10)$$

$\Delta G_{(\text{ideal})}$ is equal to the work that would have to be done against concentration gradients to exchange K^+ from its low concentration outside the cell for Na^+ , $\frac{1}{2} \text{Ca}^{2+}$ or $\frac{1}{2} \text{Mg}^{2+}$ from inside the cell. If the proposed mechanism were the only one operating, then $\Delta G_{(\text{non-ideal})}$ should describe quantitatively the specific effects of water structure upon the activity coefficients of the cations inside and outside a cell. The problem, then, is to decide whether $-(\Delta G_{(\text{non-ideal})})$ could be great enough to be equal to $\Delta G_{(\text{ideal})}$ so that $\Delta G_{(\text{total})}$ could be zero and the ions could in fact be in equilibrium.

Suppose that water inside the cell were totally crystalline and unavailable as solvent for ions, so that an intra-cellular ion had to be dehydrated, then a model for the non-ideal part of the exchange reaction (7) would be



The standard free energy change of this reaction ($\Delta G_{(\text{hydration})}$) would be equal to the difference between the free energies of hydration of $1/z \text{M}^{z+}$ and of K^+ ; $-(\Delta G_{(\text{hydration})})$ would be the upper limit of values attainable by $-(\Delta G_{(\text{non-ideal})})$ and would apply only to this extreme case in which intra-cellular ions were totally dehydrated.

Table 3 shows values of $\Delta G_{(\text{ideal})}$ [calculated from the values of m_i (observed) and m_o given in Table 1] and of $\Delta G_{(\text{hydration})}$ (taken from the data of Noyes, 1963) for the three cations Na^+ , Ca^{2+} and Mg^{2+} .

TABLE 3

Values of $\Delta G_{(ideal)}$, the ideal part of the total free energy change of exchange reaction (7), and of $\Delta G_{(hydration)}$, the standard free energy change of the exchange reaction (11)

| Ion | $\Delta G_{(ideal)}$ (kcal/mol) | $\Delta G_{(hydration)}$ (kcal/mol) |
|------------------|------------------------------------|--|
| Mg ²⁺ | 2.32 | -242 |
| Ca ²⁺ | 2.02 | -103 |
| Na ⁺ | 3.41 | -33 |

For each cation

$$\Delta G_{(ideal)} \ll -(\Delta G_{(hydration)}).$$

It follows that $-(\Delta G_{(non-ideal)})$ could have a value much lower than its upper limit $-(\Delta G_{(hydration)})$ and be equal to $\Delta G_{(ideal)}$.

The proposed mechanism is therefore quite reasonable if cell water were on an average in a state intermediate between ice and ordinary liquid water such that the difference in solvation energy would be just sufficient to balance the work that would have to be done against concentration gradients to accumulate K⁺ and relatively exclude Na⁺, Ca²⁺ and Mg²⁺.

Hechter (1965) proposed a pluralistic solution to the problem of ion distribution in living tissue, saying, in effect, that proponents of membrane pumps and proponents of selective binding were both partially right and partially wrong. This paper offers a third mechanism to fit into that pluralistic scheme.

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