STUDIES ON THE PHYSICAL STATE OF WATER IN LIVING CELLS AND MODEL SYSTEMS. III. THE HIGH OSMOTIC ACTIVITIES OF AQUEOUS SOLUTIONS OF GELATIN, POLYVINYLPYRROLIDONE AND POLY (ETHYLENE OXIDE) AND THEIR RELATION TO THE REDUCED SOLUBILITY FOR NA', SUGARS, AND FREE AMINO ACIDS.

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• *Very high osmotic activities of concentrated aqueous solutions of gelatin, polyvinylpyrrolidone, and poly(ethylene oxide) were recorded. These observed values are far above rhose predicrable from the molar concentrations of these polymers or those of aqueous solutions of native hemoglobin of equal concentrarions. It was shown rhar these high osmotic activities are closely associated with the ability of the gelatin- or polymer-dominated water to exclude Na^+ salts, sucrose, and glycine. Both phenomena are interpreted as reflecting the polarization of multilayers of water by the polymers enhancing the H_2O to H_2O interaction and also reducing rhe translational and rotational morional freedom of rhe water.*

INTRODUCTION

Gelatin, which is denatured collagen and a major component of glue, has long interested biologists and chemists. Thomas Graham used gelatin to represent a class of substances which he called colloids (κολλοσ, glue) (Graham[1]). Katz,[2] Kunitz,[3] Bungenberg de Jong and colleagues (Holleman et al[4]), Lloyd and Moran,[5] and others discovered many important and unusual attributes of the water in the gelatin-water system. Recently by making use of a modification of the dialysis technique, which was also introduced by Graham (i.e., the equilibrium dialysis method), Ling and coworkers[6,7] presented evidence that the unusual solvent properties of the gelatin-water system might arise from the extensive interaction of multilayers of water with the gelatin molecules. Their reasoning was as follows.

Due to the presence in the gelatin molecule of an abundance of glycine. proline, and hydroxyproline (Veis[8]), all well-known helix breakers (Chou and Fasman[9]), a major part of the gelatin polypeptide chain exists in an extended conformation and thus directly exposed to the bulk-phase water. According to the association-induction hypothesis, it is the NH and CO groups of the extended and exposed polypeptide chains that polarize and orient multilayers of water and cause the change in water solvency (Ling[10-12]).

In harmony with this view, 13 globular proteins, including hemoglobin, which show no or very little effect on water solvency acquired the ability to do so when these proteins were exposed to urea or guanidine HCI (Ling et al[6,7]). It was argued that native globular proteins affect water little or not at all because their NHCO groups are locked in α-helical or other macromolecular H-bonds. Urea and guanidine HCI unravel the secondary structure of proteins, thereby exposing the bulk-phase water to the polarizing influence of the NHCO groups; denaturants like SDS and n-propanol that unravel only the tertiary structures had little or no effect. Additional support for the role of exposed polypeptide groups in reducing water solvency came from the studies of certain macroscopically electrically neutral

polymers. Although these polymers lack the NH groups of the extended polypeptide chains, they too possess oxygen atoms at regular distances apart, and like the oxygen atoms of the peptide CO groups. the distances between the nearest neighboring oxygen atoms are roughly equal to twice the diameters of water molecules (Ling et al[6]). As anticipated, they cause solute exclusion from the surrounding water. The most outstanding among these polymers are polyvinylpyrrolidone (PVP). poly(ethylene oxide) (PEO), and polyvinylmethylether (PVME).

Our interest in gelatin and these synthetic polymers lies in the belief that they may serve as a model for certain important cellular proteins which endow water in living cells with some of its unique characteristics. From the viewpoint of the association-induction (AI) hypothesis (Ling[13-15]) the ability of water dominated by gelatin, PEO, PVP, etc., partially to exclude Na' and other solutes has great significance. It supports the view that the exclusion of these solutes in living cells may arise from a similar mechanism. That is, certain as yet unidentified matrix proteins (though actin, myosin, tubulin, and other cytoskeletal proteins are being considered as candidates) existing throughout the cell, may, like gelatin, ureadenatured proteins, PEO, PVP, etc., also exist in an extended conformation and in this state, polarize virtually all the intracellular water molecules. In the water thus polarized (in multilayers), the solubilities of small molecules and molecules that can fit into the multilayer dynamic structure remain normal (or even somewhat higher than normal as is known to be the case in living cells, Ling,[15] Ling et al[16]). For most molecules, due to enthalpic, entropic or both factors, the solubility decreases with increasing size and complexity of the solute involved (Size rule) (Ling[10,12]; Ling and Sobel[17]). Among the solutes excluded are hydrated Na', sugar, and glycine, which have also long been known to exist in much lower levels in living cells than in the surrounding media. A once popular theory, the membrane-pump theory, argued that these solutes are continually pumped out of the cells. However, extensive evidence now exists refuting this view (Ling[11,13,14,15,18]; Ling and Negendank[19]). Additional evidence against the membrane-pump theory is provided by the adsorbed state of cell K'.

Not all solutes exist in low concentration in the cell. Some solutes accumulate in living cells at a level substantially higher than that found in the surrounding medium, as it is in the case of K'. In the AI hypothesis, the preferential accumulation of K' involves selective adsorption of K' on β- and γ-carboxyl groups of intracellular proteins (Ling[13,20]; Ling and Ochsenfeld[21]). In voluntary muscles. these anionic groups are localized primarily in the A band and Z-line (Ling[22]). Therefore, most of the muscle cell K^+ is expected to be found in the A band and Z-line also. These predictions have been confirmed by investigators in West Germany, in Hungary, and in the USA. In this task, they used a total of four techniques: (i) autoradiography of air dried (Ling[22]) and frozen fresh muscle cells (Edelman[23]); (ii) direct EM visualization of electron dense Cs^+ and Tl^+ in frozen dried muscle cells after these ions had stoichiometrically and reversibly displaced the cell K^+ (Edelmann[24]); (iii) dispersive x-ray microprobe analysis (Edelmann,[25] Tigyi et al[26]); and (iv) laser mass-spectrometer microprobe analyais (LAMMA) (Edelmann[27]). Other experimental evidence showed that the K^+, Cs^+, and Tl^+ localization is the result of specific one-ion-on-one-site close-contact adsorption (Ling[10,22]). Since K^+ is the major cation of the cells, its adsorption and hence osmotic inactivity leaves unanswered the question, "What keeps the cell interior in osmotic equilibrium with an isotonic Ringer solution containing 0.1 M of free Na' and free Cl^-?"

Since osmotic activity is an expression of the decrease of the activity of the water present, the question posed above can be

restated as follows, "What component of the living cell causes the lowering of the activity of the bulk of cell water to match that of a Ringer solution, now that we know it cannot be free K'?" According to the association-induction hypothesis, this component is primarily the same "matrix proteins" mentioned above, which were postulated to lower the steady levels of Na', sugars, and free amino acids in the cell water (Ling[28]). If this idea is correct, we would expect that water dominated by PEO, PVP, and gelatin at a concentration high enough to lower the solvency of water for Na', sugars, and free amino acids should exhibit osmotic activity higher than that calculated on the basis of the molar concentration of the polymers. This report describes results from experiments designed to test this prediction.

MATERIALS AND METHODS

To measure the osmotic activity of polymer-water system, a Wescor Vapor Pressure Osmometer (Model 5100B, Wescor, Ind., Logan. Utah) was used. This small and versatile instrument measures the vapor pressure of the solution in a closed chamber by monitoring the dew-point temperature depression (which is a function of the vapor pressure) with a precision thermocouple hygrometer. While the instrument was designed originally for handling solutions of low viscosity. it was found suitable to measure osmotic activity of highly viscous solutions as most of the samples studied were. The main departure in the procedure used from the standard one was to deposit the sample in the sample holder first and to place the paper sample disc over the sample. Trials showed that this modification does not in any way adversely affect the results. Readings taken over a span of time yielded the same results. This verifying procedure was followed when new samples of different consistencies were measured.

Solutions (or gel) of three synthetic polymers and two proteins (gelatin and hemoglobin) were studied. The sources of these polymers were as follows: Polyvinylpyrrolidone (M.W. 360.000) (PVP-360). Lot 57c-0071 was from Sigma Chemical Co.; poly (ethylene oxide) (PEO) was a gift of Union Carbide. Gelatin. obtained from Eastman, was from pig skin (Lot A4-C, IEP 8.7, ash content 0.0340), and from calf skin (Lot B4B, IEP 4.7, ash content, 0.0290). Hemoglobin from bovine erythrocytes was also purchased from Sigma Chemical Co.; it existed as a mixture of methemoglobin and oxyhemoglobin.

We found that as a rule, samples of synthetic polymers as they were received from the suppliers contained very little ionic residues after ashing. On the other hand, gelatin, though of the highest quality obtainable commercially, did contain considerable ionic contaminants. To purify, dilute solutions (ca. 2%) of gelatin, hemoglobin as well as all the polymers were first prepared and then exhaustively dialyzed against ion-free distilled water until ashes prepared from the dried samples of the polymer solution (600° C, 24 hrs. in a muffle furnace) yielded no measurable osmotic activities in the Wescor osmometer when dissolved in 10 mM HCl. The water contents of the dialyzed polymer solutions. while still in the dialysis sacs. were reduced in steps by either being placed in front of a fan in a cold room or packed in a dry dust-free silica gel (Davidson, mesh size 6-16). Great care was taken not to let the polymer dry unevenly. These methods yield preparations of homogeneous samples of polymer-water systems of widely varying water contents. which were assayed by oven drying at appropriate temperature (100° C for PVP, PVME, hemoglobin, and gelatin; 60° in vacuo for PEO).

RESULTS

Figure 1 shows the osmotic activity of a

solution of exhaustively dialyzed gelatin. where the osmotic activity is expressed in units of OsMolal and the gelatin concentration in percentage (W/V). For comparison, data from hemoglobin solutions are also presented. Included also in this figure are six experimental points from the osmolarity measurements of hemoglobin of Adair (Adair,[29] Adair and Robinson[30]). Adair's data points in general agree with our own but they do not reach to as high a concentration as our own (up to 50%). Comparing the osmotic activity of gelatin with that of hemoglobin at equal protein concentrations one finds that the osmotic activity of gelatin is much higher especially at the higher concentration range. Neither the osmotic activity of gelatin nor that of hemoglobin is commensurate with the molar concentration of the proteins present. Thus a 50% hemoglobin solution is roughly $50016.7 \times 10^4 = 7.45$ mM, while the osmotic activity measured corresponds to a concentration of 450 mM. The molecular weight of gelatin (denatured collagen) is less clearly defined as it contains fractions with molecular weight as high as 10^6 (see Stainsky[31]). The molecular weight of the a-chain of collagen is close to 90,000 (Piez[22]). Using the lower value of 90.000, the molar concentration of a 50% gelatin solution is only $500/9 \times 10^4 = 5.5$ mM. compared to the measured osmotic activity which is equivalent to 2240 M! An equally remarkable trait of the gelatin curve is its pronounced sigmoid shape which indicates that the osmotic activity, while high at the lower concentration range already. abruptly increases to still higher levels when gelatin concentration reaches 45%.

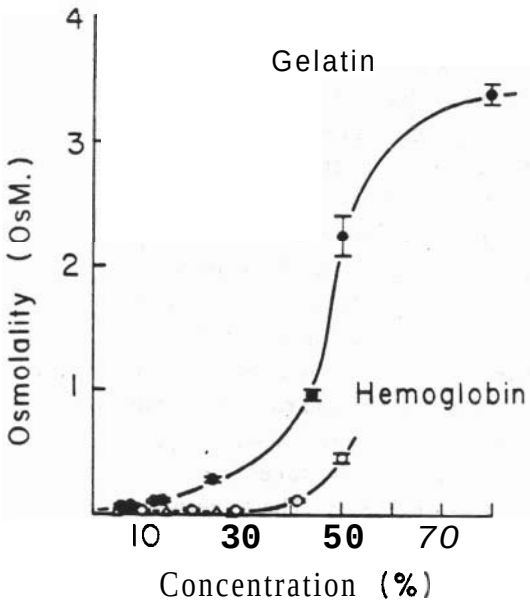

FIGURE 1. **Osmolality of gelatin and hemoglobin at varying concentrations. Osmolality is given in Osmolal concentration. Protein concentrations are in % (wt wt). Each point is the average of at least 4 independent determinations and the distance between horizontal bars are twice the standard errors. Six extra points on the hemoglobin curves shown as A's are taken from Adair's data (see text).**

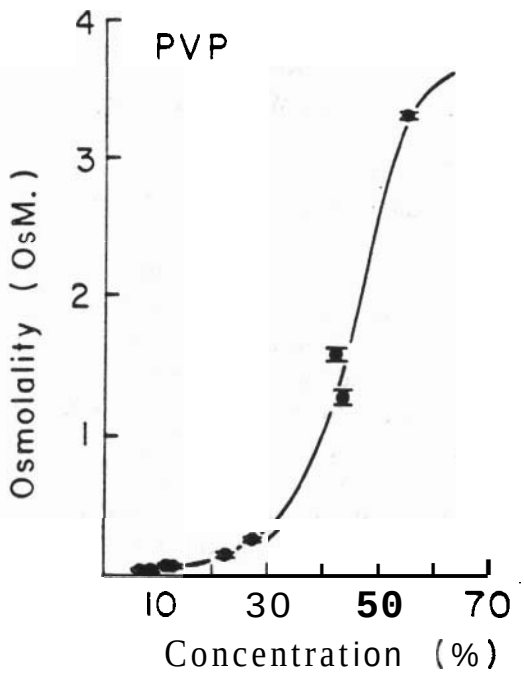

FIGURE 2. Osmolality of polyvinylpyrrolidone (PVP) solution at varying concentrations. Details are the same as in Figure 1.

Figures 2 and 3 show qualitatively similar curves for solutions of polyvinylpyrrolidone (PVP) and poly(ethylene oxide) (PEO). Quantitatively, PEO demonstrates the highest osmotic activity at the same molar concentrations even though the average molecular weight of PEO (600,000) is higher than that of PVP (360.000) or gelatin. A 50% PEO solution has a molar (or molal) concentration of only 400/600,000 = 0.67 mM; yet the measured osmolarity corresponds to that of a 2620 mM sucrose solution. This is nearly 4000 times higher than that calculated on the basis of its molar concentration. Similarly, at a 55% concentration, the molar concentration of PVP is only 1.53 mM, while the measured osmolarity is 3300 mM or 2160 times higher than that calculated on the basis of its molar concentration.

The PVP and PEO data shown in Figures 2 and 3 respectively are plotted in a different manner in Figures 4 and 5. Here the ordinate represents the measured osmotic pressure (π) in units of decimeters (dm) of H_2O divided by the polymer concentration C_2 in grams per liter of solution. The abscissa represents the polymer concentration. This plot is based on the theory of osmotic pressure given by Tombs and Peacocke.[33]

$$\frac{\pi}{C_2} = RT\frac{V_1^{\circ}}{V_1}[M_2^{-1} + BC_2 + CC_2^2 + DC_2^3 + \ldots.], \quad (1)$$

where R, T have the usual meanings. V_1° is the volume per mole of pure solvent used; V_1 is the partial molar volume of the solvent in the polymer solutions. M_2 is the molecular weight of the macromolecule in units of $gmol^{-1}$. B. C, and D are the second, third, and fourth virial coefficients in units of mole $l\ g^{-2}$, mol $l^2\ g^{-3}$, and mol $l^3\ g^{-4}$ respectively. However, following tradition, the virial coefficients tabulated from these data are given in units of mol ml g^{-2}, etc. (Table I).

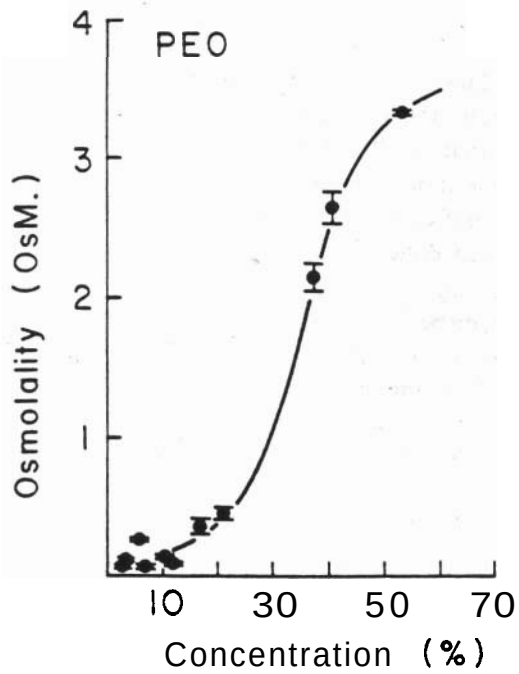

FIGURE 3, Osmolality of poly(ethylene oxide) (PEO) solution at varying concentrations. Details are the same as in Figure 1.

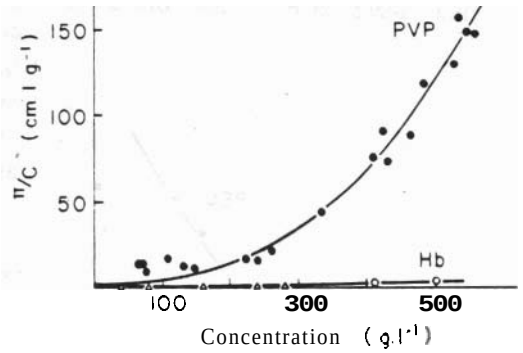

FIGURE 4. Plot of π C against C in PVP solutions. Osmotic pressure, π, is in units of cm of H_2O:C in prams of polymer per liter. Data are the same as in Figure 2, except that standard error bars are not represented. The solid line going through the experimental points are based on Equation I. Values of virial coefficients chosen to fit the data are given in Table 1. For comparison the hemoglobin data of our own and from Adair are also shown.

These plots show steep curvatures. Hence unusually large 3rd or even 4th virial coefficients are required to fit the data. Just how large these virial coefficients really are, is revealed by a comparison of the PVP and PEO curves with similar plots of hemoglobin in the same figures.

DISCUSSION

In answer to the main question raised in the Introduction, the observations presented in this communication show that the osmotic activities of the aqueous solutions of the two neutral polymers (PEO and PVP) and one charged protein (gelatin), are indeed much higher than that predicted by the molar concentration of the polymers present or the osmotic activity of the globular protein, hemoglobin, measured with the same instrument. These studies are in harmony with the theory that the osmotic pressure in living cells does not originate from free solutes like K^+ as was once widely believed but is primarily due to extended chains of some intracellular (matrix) proteins. However, before final acceptance of this conclusion, certain trivial causes for the observations must be considered.

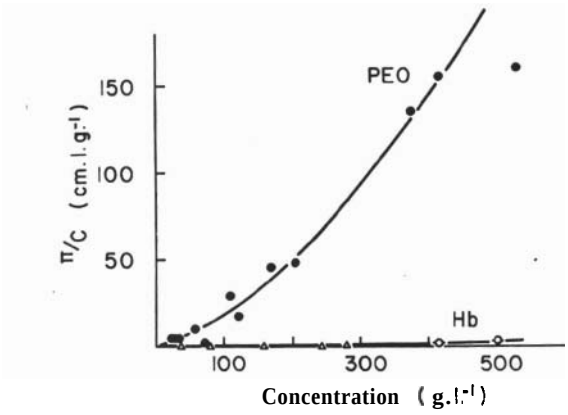

FIGURE 5. π/C vs. C plots of PEO solutions. Details are similar to those described in Figure 4. Value of virial coefficients art those given in Table I. For comparison hemoglobin data of our own and from Adair arc also shown.

Inadvertent oxidation reactions might give rise to, say, carboxyl groups and counterions which might increase the total osmotic activity beyond that of the originally neutral polymer. To test this possible trivial cause of the observed osmotic activity 1 analyzed the Na^+ contents of samples of PVP and PEO after first equilibrating them in 0.1 NaCl and then exhaustively dialyzing the solution in distilled water which was made slightly alkaline with the addition of NaOH. The data revealed a total Na^+ content amounting to a few micromolar in a 40% polymer solution. This level of contaminant is far too trivial in magnitude to make any significant difference to the data collected. A second possible source of error is the heterodisperse molecular weights of the polymers studied. However, the polymers had all been exhaustively dialyzed in dialysis tubings with a molecular cut-off point of abdut 12,000 daltons. Thus, even if the actual molecular weights of all the polymers studied were not as designated but were only 12,000, a 40% polymer solution would still be no more than $400/12{,}000 = 30$ mM. This osmotic activity is far from the recorded osmotic activity of more than 1000 milliosmolar. Thus heterodisperse molecular weights could not make any significant contribution to the activities observed either.

Having eliminated contaminants and heterodisperse molecular weights as the causes of the high osmotic activity observed. I conclude that gelatin, PVP and PEO, which have been shown to have the power to reduce the solubility of water for Na^+ salts, sugars, and free amino acids,.do indeed also have powerful effects in reducing the activity of water in general and especially when the polymer reaches a certain high concentration. This pattern of behavior of the two synthetic polymers, PEO and PVP, and gelatin is shared to a minor degree by hemoglobin. a native globular protein.

The Structural and Concentration Requirements of the Osmotic Effect of Polymers. The minimal structural requirements for the polymer to have the pronounced water-activity-reducing effect are the same as those found for producing the reduction of solubility for Na', sucrose, and glycine: the possession of oxygen atoms at regular intervals about two water diameters apart, with these oxygen atoms freely exposed to the bulk phase water and not locked in a-helical or other intra- or intermacromolecular H-bonds (Ling et al[7]).

The present findings thus provide new evidence that the unusual property of gelatin. and hence Graham's colloid, may reside in the powerful effect of its extended polymeric chains in reducing the activity of water in its surrounding medium.

Figure 1 clearly shows that at all concentration ranges, gelatin has much greater osmotic or water-activity-depressing effect than hemoglobin. A similar observation of the high osmotic effect was long ago noted by Kunitz[3] who studied the osmotic pressure of gelatin up to a concentration of only 18%. Yet we have shown in our new data that it is when the gelatin reaches the critical concentration at about 45% that the effect becomes truly pronounced. This type of behavior reminds one of the sigmoid-shaped oxygen uptake curve of hemoglobin which is generally acknowledged to be due to cooperative interaction among the heme-sites on which oxygen molecules are complexed; the binding of one oxygen molecule enhances the affinity of other sites for more oxygen. In our present case, what one sees is that as the polymer concentration increases, its effects on the water activity also demonstrate characteristically cooperative behavior. Let us examine what could be the basis of this phenomenon, focusing our attention first on PEO.

Being simply repeating units of $(-CH_2CH_2-O-)_n$, this polymer has no side chains and the only seats of direct interaction with water are the oxygen atoms in the chain. Therefore the effect of increasing PEO concentration on water activity could only be due to a synergism between the water-activity-reducing effect produced by one

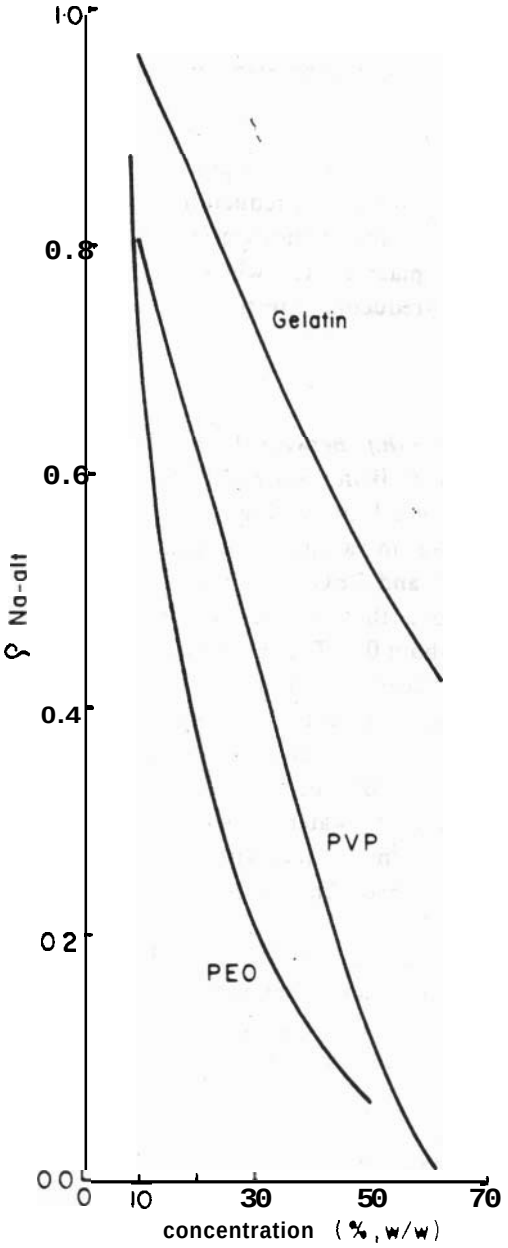

FIGURE 6. Plots of apparent equilibrium distribution coefficients of Na^+ salts (p-value) of polymer water system against polymer concentration given as % (w/w). (from Ling and Ochsenfeld[40]).

oxygen atom on one chain and similar effects exerted by oxygen atoms on other chains when the average chain-to-chain distance decreases to a close enough value.

Since gelatin. PVP and PEO all show similar sigmoid-shaped curves in their water-activity-reducing action and since the only H-bonding groups they share are the regularly and suitably separated oxygen atoms on the chain, the behavior of these models taken together supports the view that certain extended protein chains in living cells may also be responsible for the reduction of water activity to match that in the external medium of sea water, plasma, etc.. which owe their water-activity-reducing effects to free Na^+ and Cl^-.

The Relationship Between Water-Activity Reduction and Water-Solvency Reduction by Polymers and Proteins. Figure 6 plots the ρ-value for Na' in varying concentrations of gelatin, PVP, and PEO. Note that in a 40% PEO solution, the p-value for Na' has dropped to about 0.1. That is, at least 90% of the water has been so profoundly affected by the PEO that it has lost all its solubility for Na' citrate. In fact, this exclusion of Na' citrate could hardly be absolute for any specific volume of water. Thus it is more reasonable to suppose that virtually all the water has been profoundly affected by PEO in its solvency.

Strictly speaking, the PEO, PVP. and gelatin concentrations represented here in Figure 6 cannot be directly compared with those shown in Figures 1 and 3. While the osmotic activity measurements 'shown in Figures 1 and 3 were made on the basis of pure water-polymer systems containing no salts; the p-value data shown in Figure 6 were derived from polymer-water-systems containing Na' citrate. Indeed it was by varying the concentration of the Na' citrate that the polymer-water system enclosed in dialysis bags with different water contents were obtained. (The subject of swelling and shrinkage of polymer-water system in dialysis bags was briefly described by Ling[28]). However, the key comparison concerns 40% PEO. at which concentration the Na' citrate present in the polymer-water system is only 1/10 of that in the surrounding Na^+-citrate solution. Therefore, a comparison of the water-activity-reducing effect and the solvency reduction effects can be made legitimately. Closely parallel behaviors observed include sharp changes at certain polymer concentration and the relative effectiveness of both sets of effects among the three polymers studied in the rank order: gelatin $<$ PVP $<$ PEO.

One of the reasons previously given for the greater solvency-reducing effect of PEO than the two other polymers is that due to its extreme simplicity of structure and the lack of any side chains, it cannot form hydrophobic bonds or interaction H-bonds as is possible in the case of gelatin (Ling et al[6]).

The parallel behavior between the water-activity-reducing effect and the solvency-reducing effect of these oxygen-containing polymers and proteins is to be expected from the proposed mechanism for both phenomena. The water-activity-reducing effect is seen as the consequence of the polarization and immobilization by the propagated polarization emanating from the oxygen atoms and the consequent reduction of, in particular, the translational and rotational "partition functions" of the water molecules in statistical mechanical terms (Ling[10,11,12,34,35]; Ling et al[36]). Such a reduction of translational and rotational partition functions lowers the vapor pressure of the water, which was in fact what we actually observed with the Wescor vapor-pressure osmometer. At this point, it is most gratifying to learn that the recent quasi-elastic neutron scattering studies of Rorschach, et al[37], not only have demonstrated reduction of translational and rotational freedom of water in living cells of

brine shrimp cysts but also in PEO-dominated water (Rörschach[38]).

The solvency- educing effect has also been explained in terms of a translational but primarily rotational partition function reduction not of the water molecules themselves but of the large. complex solute molecules or hydrated ions (Ling[10,12]). However. reduction of motional freedom of these solutes is the consequence of the reduction of the motional freedom of the solvent water molecules. in the same sense that the loss of motional freedom of a butterfly when it is caught on a spider web is the consequence of the attachment to the immobilized elements of the spider web.

Quantitative Consideration of the Postulated Matrix Proteins in Living Cells. The concentration of polymers needed to produce a pronounced effect on water activity and on solvency is as a rule quite high (e.g., 40-50%). Can this system be compared with that of the living cells? The answer is yes for three reasons.

First. the required osmotic activity of most living cells falls in the range of 0.2 to 0.3 OsMolal. One does not require a 40-50% PEO solution to produce this level of osmotic activity: a 20% PEO or 25% PVP or gelatin can do quite well.

Second. according to the polarized multilayer hypothesis of cell water. the maximum effect on water polarization. solvency reduction and water-activity reduction occurs when the "matrix protein" chains are fully extended and are highly ordered as in the highly organized living cell interior. All the model systems studied are obviously far from this situation. being more like a randomly tangled mass. Testing the expected effect of ordering these disordered chains on lowering p-values. Ling et al[6,7] found that the ρ_{N_2} for the PVP-water system did indeed decrease with stirring, which tends to line up the linear chains. These results are in full accord with the earlier report 'of Woessner and Snowden[39] who produced NMR evidence for increased water structuring as a result of the stirring of another polymer-water system, a solution of Kelzan®, a bacterial polysaccharide.

The third point concerns the relation between protein contents and distances between protein chains where cell water is found. Even though some cells (e.g., human erythrocytes) contain as much as 40% protein, most living cells contain 20 to 30% protein. Furthermore. only a pan of this can be the matrix protein. The question is then. if the postulated matrix chains arc indeed fully extended. how would the content of these proteins affect the number of water molecules found between the nearest neighboring chains? Would there be enough matrix proteins effectively to control the properties of the bulk phase water? A simple calculation goes a long way toward answering these questions. Thus if one liter of cells contains n grams of matrix proteins. one can use an

TABLE I. Virial coefficients from the measured osmotic properties of aqueous systems of gelatin. PVP. and PEO.

	B ($mol.ml.g^{-2}$)	C ($mol.ml.^{-2}g^{-3}$)	D ($mol.ml.^{-3}g^{-4}$)
PEO	5.54×10^{-3}	217×10^{-2}	—
PVP	1.25×10^{-3}	—	3.28×10^{-2}
Gelatin	3.29×10^{-2}	5.15×10^{-2}	—
Hemoglobin			1.5×10^{-3}

average amino acid residue weight of 112 (see Ling[11], p. 48), an Avogadro's number of 6.06 $\times 10^{23}$, and a peptide linkage length of 3.5 X 10^{-8} cm, to find that the total length of the fully extended polypeptide chains of the matrix proteins equals $(n/112)$ X 6.06 X 10^{23} X 3.5 X 10^{-8} = 1.89 X 10^{14} X n cm. Cut into 10 cm long filaments, these filaments, uniformly distributed, in a 10× 10 X 10 cm cube, would be $\sqrt{1.89 \times 10^{13}}$ n or 4.34 X 10^{6} n filaments to each side. The distance between each nearest neighboring filament would be $10/(4.34 \times 10^{6}n)$ = 2.30 X $10^{-6}/n$ cm. Figure 7 shows a plot of the percentage of matrix proteins in a cell against the distance (right ordinate) and the number of water molecules between each pair of nearest neighboring chains (left ordinate), assuming a diameter of 3 Å for each water molecule.

What this figure demonstrates is that between the wide range of matrix protein contents from 5% to 80% an amazingly modest change in the number of water molecules between the protein filaments occurs. Indeed, even at a matrix protein concentration as low as 5%, there are no more than 10 water molecules between a pair of nearest neighboring chains. This is a concentration that one can reasonably expect of the postulated matrix proteins. especially if one recalls that other globular proteins not directly participating in polarizing the cell water must take up space and would thus help to reduce the average chain-to-chain distance between the matrix proteins to below 10 water molecules. On the other hand, if one or more of the other non-matrix proteins in the cell resembles hemoglobin. the data of Figure 1 suggests that it too will contribute. though to a much smaller extent, to the water-activity reduction essential for the maintenance of normal cell volume.

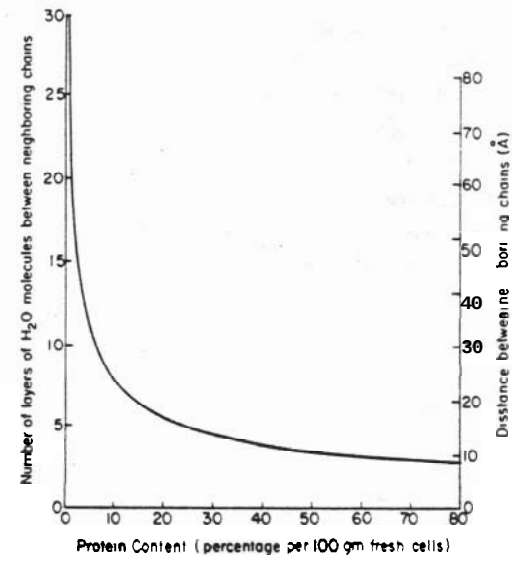

FIGURE 7. The theoretical distances both in Ångstrom units (right ordinate) and in number of water molecules between nearest neighboring (fully extended) protein chains in hypothetical case when the entire protein contents (abscissa. in % (w/w)) are in the fully extended state and occupy no space.

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