# STUDIES ON THE PHYSICAL STATE OF WATER IN LIVING CELLS AND MODEL SYSTEMS. VI. CONCENTRATION-DEPENDENT SUSTAINED VOLUME CHANGES OF DIALYSIS SACS CONTAINING .AQUEOUS SOLUTION OF NATIVE AND DENATURED PROTEIN, GELATIN, AND OXYGEN-CONTAINING POLYMERS IMMERSED IN SOLUTIONS OF Na SALT AND OF SUGAR AND SUGAR ALCOHOL

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It is common knowledge that the basic unit of all life are the living cells. It is also well known that although there are variations from one type of living cell to another, each type of living cell, as a rule, has essentially a constant chemical composition. To explain the unchanging chemical composition of a living cell the conventional theory has postulated continued activities of specific membrane pumps. Decisive experiments against this concept were presented 25 years ago when it was demonstrated that after suppression of both respiration and glycolysis, frog muscle maintained their ionic concentration essentially unchanged for as long as 7 hours $(0°C)$. Under this condition the minimal energy need of one pump alone (the Na pump) exceeds the maximal energy available — almost all in the form of utilizable free energy stored in the so-called "high energy phosphate bonds" of ATP and creatine phosphate — by a factor of 1500% to 3000% (Ling, 1962). In fact, these figures were gross underestimates because it is now well established that these phosphate bonds do not carry a large package of utilizable energy (George and Rutman, 1960); by taking this fact into account the energy discrepancy becomes vastly larger than even the 1500% to 3000% figures cited.

With so much effort spent on the mechanism underlying the constancy of the level of one ion, $Na^+$ accumulating at very low concentration in the living cell, it is rather remarkable that the constancy of the most abundant molecules in the cell has rarely been discussed, i.e., water. For each cell in a defined medium the ratio of cell water to cell proteins are also constant. Since in the membrane-pump theory there is no connection between the bulk of cell water and cell proteins, there is no reason that the water content should be a direct function of the protein contents.

It is true that a water pump has been also postulated (Robinson, 1950) but this idea was challenged (Conway and McCormack, 1953; Leaf, 1956) and apparently abandoned (Robinson, 1956). The fact that muscle cell segments without an intact membrane can not only maintain normal volume but also swell in hypotonic as well as isotonic KCl solutions in a way not distinguishable from normal intact cells and also the lack of energy for pumping mentioned above make the water pump concept even less tenable.

What does the constancy of water content of each type of cells really signify? We believe that the simplest answer is that the presence of water in the cell is due to the presence of cell proteins and that from the amount of the protein there is a predictable amount of cell water, i.e., $H_2O$/protein ratio is constant. It then can be readily understood why the water

content by and large does not change with either gain of protein during cell growth or loss of cell protein due to cell atrophy. This concept was first introduced in 1965 and called the polarized multilayer theory of cell water as part of the association-induction hypothesis (Ling, 1965).

In this theory the bulk of cell water exists in an adsorbed state in multilayers on the exposed NHCO groups of fully extended polypeptide chains of certain intracellular proteins referred to generically as "matrix proteins."

According to the polarized multilayer theory of cell water, only proteins existing in the fully extended state with the backbone NHCO groups directly exposed to the bulk phase water can polarize it in multilayers. A prediction of this theory is that native proteins polarized little water since most of its backbone NHCO groups are locked in $\alpha$-helical and other intra- and intermacro-molecular H-bonds. Gelatin, on the other hand, due to the preponderance of non-helix forming amino acids (i.e., lysine, proline, and hydroxyproline) in its amino acid composition exists partly in the fully extended state. In agreement with the theory, at vapor pressure corresponding to that of Ringer solution (0.9969), gelatin takes up water quantitatively comparable to that of the living cells while native proteins take up far less (Ling, 1984, p. 286; Ling and Hu, to be published).

Not only does gelatin sorb much more water from a vapor phase in equilibrium with a Ringer solution, the water taken up by gelatin has reduced solvency for $Na^+$ salts, sugars, and amino acids, solutes usually found in low concentration in living cells. In agreement with theory, urea, guanidine HCl denatured proteins — which unravel secondary structures — but not sodium dodecyl sulfate (SDS) — which only unravels tertiary structure — reduce the solvency for $Na^+$ salts, sugars, and free amino acid.

While the constancy of the water content of living cells agrees with the results of these model studies, a more stringent test of the polarized multilayer theory can be made if one can also demonstrate on these protein and polymer models of the living cells the striking feature of the living cell which has intrigued biologists since the earliest days: volume changes in solutions of salts and sugars. Indeed the membrane theory was founded by Pfeffer (1877) largely as a result of studies of the relation between cell volume and varying concentrations of salt and non-electrolyte solutions. When $Na^+$ (Cl), sugars were later shown to be permeant to the cell membrane, the original concept of cells behaving like osmometers due to the presence of a cell membrane permeable to water but not to these solutes, became no longer tenable. The energy problem and sustained swelling of cells without intact membranes in hypotonic solution have refuted later remedial hypothesis where "effective membrane impermeability" was postulated to be a consequence of the Na pump.

In contrast, the AI hypothesis has offered a theory of volume control that is in harmony with all findings supporting as well as refuting the membrane-pump theory of cell volume regulation. This AI hypothesis of cell volume control will be described in the next section under "Theory."

THEORY

1. *A Qualitative Description of the Theory of Cell Volume Maintenance in Solutions of Different Concentrations.* The relative vapor pressure of an aqueous solution measures the *activity* of water in the solution. Increasing the concentration of the solute in the solution decreases the relative vapor pressure and the water activity. This decrease of relative vapor pressure or water activity is also referred to as the osmotic activity of the system. Multi-

layer polarization of water on gelatin and various oxygen polymers like polyvinylpyrrolidone and polyethylene oxide generate high osmotic activity much as the osmotic activity of water is increased by the dissolving of sucrose in the water. Thus, if a closed dialysis sac containing gelatin or polyethylene oxide polymer is exposed to water, the sac will expand, taking in more and more water.

If now a non-electrolyte is suddenly introduced into the solution bathing the dialysis sac, the osmotic activity will be suddenly increased, and, depending on the final concentration of the solution, the inward movement of water into the sac will be arrested or even reversed.

However, the dialysis sac membrane is also quite permeable to the solute and as a result the solution will also move into the sac. Full equilibrium will be reached when the osmotic activity due to the protein or polymer (present only inside the sac) equals that due to the difference of the concentration of the solute in the external solution and in the water in the sac, which is determined by the equilibrium distribution coefficient, or q-value of the solute between the polarized water within the sac and outside the sac.

Thus a qualitative prediction of this model of volumes of cells and cell models is that in those cases where the protein or polymer in the dialysis sac is capable of polarizing water in multilayers, and the sac is placed in a solution containing primarily a solute with q-value below unity, the volume of the sac will reach different levels and maintain that volume depending on the concentration of solute in the external medium. On the other hand, in solutions of proteins that do not provide fully extended polypeptide chains, the q-value may be close to unity and the volume change, if observed, will not be as pronounced or maintained.

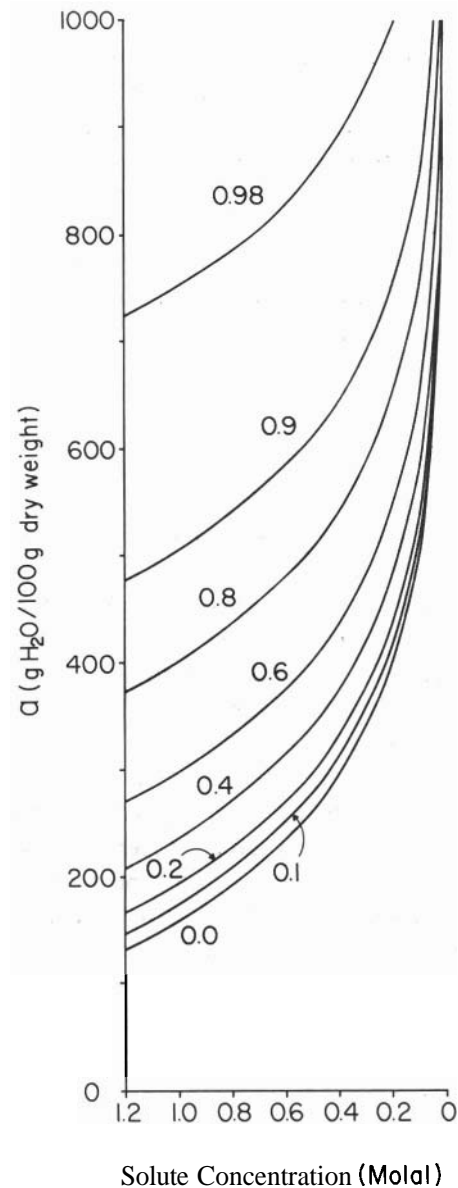A quantitative formulation of this theory is given in the section immediately following.



FIGURE 1. Theoretical curves of the relation between solute concentration and the equilibrium distribution coefficient (q-value) and the water uptake calculated according to Equation 1. Numbers varying from 0.0 to 0.98 are q-values of the solute between cell water and external aqueous medium. q-value of 0.0 means the solute is completely excluded from the cell or model system.

2. A *Quantitative Formulation of the Theory of Cell Volume Maintenance in Solutions of Different Concentrations.* In a preceding paper, Ling (1987) has introduced an equation relating the water content of living cells (and model system) to the molal concentration of a solute in the external medium which is permeant to the cell and distributes in the cell water at an equilibrium distribution coefficient equal to q:

$$\log \left[ 1 + \frac{n_2(1-q)}{n_1} \right] = K_1^a \, K_3 + K_4 , \quad (1)$$

when a is the water content of the cell or model system in units of grams of water taken up by 100 grams of dry proteins (or other polymers in model system). $n_2$ and $n_1$ are the number of solute and solvent $(H_2O)$ molecules in a unit volume of the external medium at equilibrium. Under specified conditions $K_1$, $K_3$, and $K_4$ are all constants.

Figure 1, reproduced from the above-mentioned paper, presents plots of Equation 1 with solutes that have different q-values. Note that at the same molal concentrations, much more water is expected to be taken up at equilibrium by a solute of high q-value than another solute with a lower q-value. The bottom curve was calculated for a solute that has q-value of zero and is thus effectively the same as a solute that cannot pass through the cell membrane.

## MATERIALS AND METHODS

Solutions of two synthetic polymers and three proteins were studied. The polymers used were poly(ethylene oxide) (Polyox WSR-205) with an approximate molecular weight of 600,000 from Union Carbide and poly(ethylene glycol) (Carbowax, PEG 20,000) with a molecular weight of 15-20,000 from Fisher Scientific, Lot 714714. All proteins were obtained from Sigma Chemical Co.: bovine serum albumin, Fraction V powder (lots 121F-0134, 92F-0721), hemoglobin from bovine erythrocytes, Type 1 (Lot 100F-9365), and gelatin from swine skin, Type 1 (Lot 29C-0532).

Aqueous polymer solutions (30 to 40%) and protein solutions (26 to 40%) were projected into pre-weighed 1/4 inch dialysis tubing (Spectra Por 2 or Spectra Por 4, MW cutoff 12-14000). The ends of the tubing were then tied and the initial weight of the sac determined.

Individual sacs were incubated with gentle shaking in solutions of various concentrations of Na citrate, D-glucose or sorbitol. Sodium penicillin (0.1 mg/ml), streptomycin sulfate (0.1 mg/ml) and thymol crystals (0.15 mg/ml) were included in the solutions. Incubation was at $23°C \pm 1°C$ in a constant temperature room for the Na citrate studies, $4°C$ for D-glucose and sorbitol.

After differing periods of time the sacs were removed from the incubation solution, carefully blotted dry and weighed. After the weighing the sacs were returned to the solution for further incubation.

The contribution of the dialysis tubing to the weight change was determined by treating a section of cut-open tubing in the same manner as the filled sacs.

The final water contents of the polymers were determined by two methods: (a) drying 48 hours under vacuum in the presence of $P_2O_5$, and (b) in an oven set at $80°C$ also under vacuum.

## RESULTS

1. *In Solutions of Na Citrate*
    1.1. *Oxygen-containing Polymers*
        1.1.1. *Polyethylene Oxide*

A 40% (w/w solution of polyethylene oxide (PEO) (WSR 205)) was placed inside lengths of ¼ inch dialysis tubing and knots were tied with surgical threads to form chains of four fully filled "sausages" more or less like those shown under 0.5 in Figure 2. Each individual

sausage, initially containing about 0.5 ml of the 40% PEO solution, soon changed its weight after immersion in solutions of Na citrate of different concentration until a new steady weight was achieved. A glance at Figure 2 shows the sacs, at more or less its initial weight in 0.5 M Na citrate (the middle links of sacs under 0.5), swelled in more dilute solution of Na citrate but shrank in more concentrated solutions. The behaviors seen here bear a resemblance to swelling and shrinkage of living cells. The dialysis tubing here used was Spectra Por 2 with a molecular weight cut-off of 12,000 to 14,000 daltons and hence perfectly permeable to Na and citrate ions; the sustained swelling and shrinkage has nothing to do with sac membrane permeability. Therefore the phenomenon is most relevant to the observed volume changes in living cells with the intactness of the cell membrane already destroyed (Ling and Walton, 1976).

Figure 3 shows a more detailed set of data on the weight changes of 40% PEO-filled sacs like those shown in Figure 2. A new steady weight was reached rapidly in Na citrate solution ranging in concentration from 0.1 M (A) to 1.5 M (F) and maintained a more or less constant level for 14 to 17 days (25°C). The gradual fall in weight in the most dilute solutions and the gain in the most concentrated solutions were within 5%. The most likely cause was leakage of some components of the hetero-disperse mixtures of low molecular weight (PEO) even though the "approximate molecular weight" given by the manufacturer for the Polyox WSR-205 used (600,000 daltons) was much larger than the molecular cutoffs of the dialysis tubing (12,000 to 14,000 daltons).

Figure 4 shows a more detailed study of the gain and loss of weights of the 40% PEO-filled sacs in solutions of Na citrate when they were transferred first into 0.25 M solution, then to 1.5 M solution and once more returned to a 0.25 M solution. Here a 5.3% drop in the final level of weight in the 0.25 M solution was also seen.
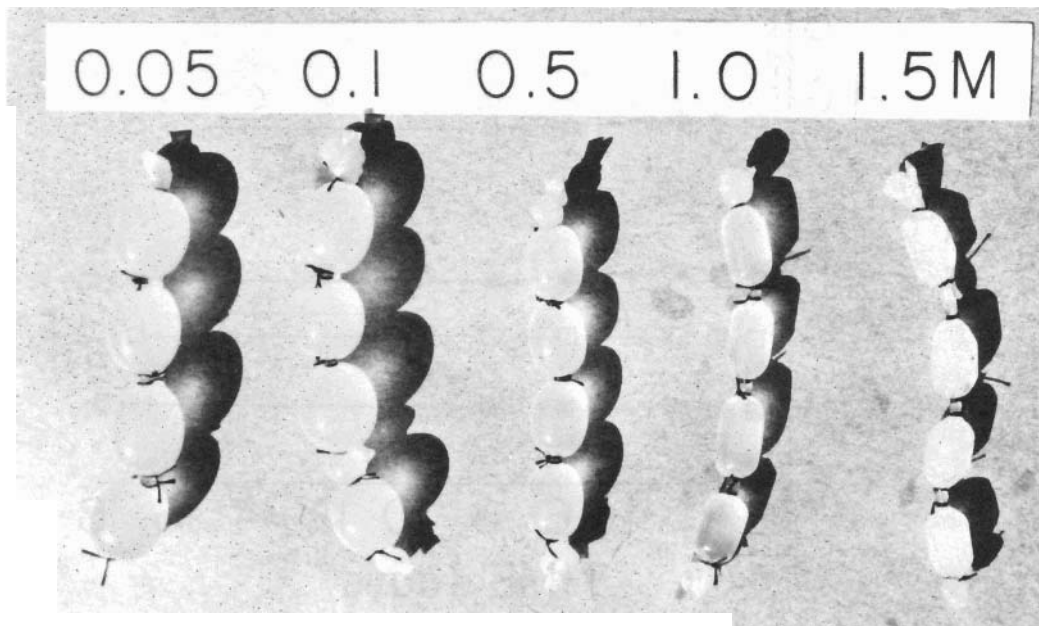


FIGURE 2.    Dialysis sacs filled with 30% aqueous solution of polyethylene oxide (PEO) immersed in Na citrate solution of concentration ranging from 0.05 M to 1.5 M (25°C).

### 1.1.2. *Polyethylene Glycol*

Figure 5 shows the time course of weight changes of sacs filled with a solution containing initially 30% (w/w) of polyethylene glycol (PEG 20,000) when introduced into solution of Na citrate of different concentrations. In more dilute solutions, the weight peaked at first weighing followed by a slow decline, the gradual loss of weight ranging between 13 to 37%, while in concentrated solutions (D, E, F) little further change of weight occurred after the first weighing at from 2 to 3 days following immersion. The more pronounced loss of weight here than with PEO in the dilute Na citrate solution, are to be expected because the molecular weights given by the manufacturer was 15 to 20,000 daltons. The dialysis tubing cut-off was 12,000 to 14,000 daltons, which on further stretching in the more dilute solutions could easily permit loss of a considerable amount of the fraction of the PEG molecules with smaller molecular weights. Despite all these changes, it is obvious that swelling and shrinkage of PEO solutions behave similarly to PEO solutions and both resemble living cells when exposed to solutions of Na salts of varying strength.

The loss of polymer explains the lesser swelling of 30% PEG solution in 0.25 M Na citrate than 40% PEO. One may surmise that equal swelling might have been observed if dialysis tubing with smaller pore sizes are used. A more significant comparison between PEO and PEG solution concerns shrinkage.
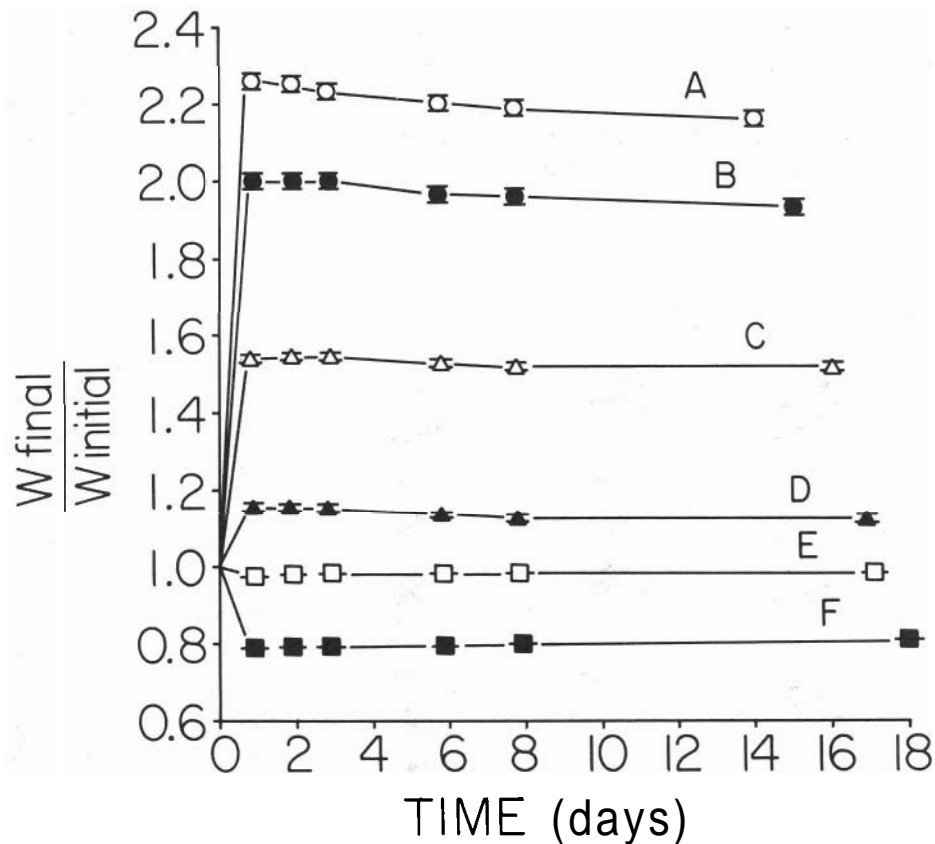


FIGURE 3. Time course of volume (or weight) change of dialysis sacs filled with 30% PEO immersed in different concentration of Na citrate (25° C). Ordinate represents ratio of final weight over initial weight of the sacs. A. 0.1 M Na Citrate, B. 0.25 M, C. 0.5 M, D. 0.75 M, E. 1.0 M, F. 1.5 M.

Note that in 1.5 M Na citrate, the ratio of final weight over initial weight, was only 0.8 for PEO, while it reached 0.65 for PEG solution. This is in agreement with a greater effectiveness of PEG in polarizing water and reducing p-value for citrate (see Figure 15 below).

## 1.2. *Native Proteins*
### 1.2.1. *Bovine Serum Albumin*

Figure 6 shows that bovine serum albumin solution initially at a concentration of 26.6% (w/w), in solutions of Na citrate from 0.1 M to 1.5 M, does not swell nor, except for an initial transient one, shrink more than 0.4%. Even this minor degree of shrinkage did not follow increasing concentration of the Na citrate and was within the random error of each set. The overall volume change of the

bovine serum albumin solution thus stood in sharp contrast to similarly treated solutions of PEO, PEG, as well as gelatin (see below), and is in accord with the prediction that native bovine serum albumin with its backbone NHCO groups locked in a-helical and other intra-macromolecular H-bonds, do not polarize the bulk phase water to any significant degree.

### 1.2.2. *Bovine Hemoglobin*

When sacs containing 36% bovine hemoglobin were exposed to Na citrate solutions of different concentrations, there was some moderate irregular swelling at the lower concentrations (Figure 7). In 1.0 M Na citrate, the initial shrinkage was followed by gradual swelling, while in 1.5 M, the shrunken sacs did not rise up again.
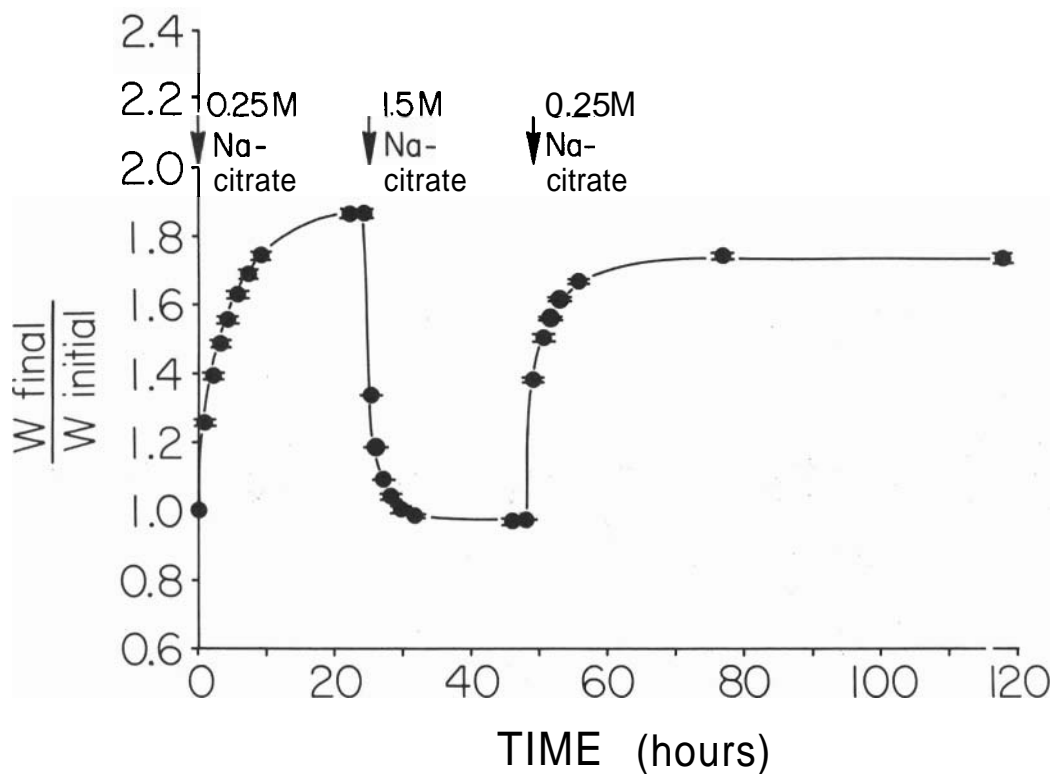


FIGURE 4. Volume changes of PEO-filled dialysis sacs when moved from a Na citrate solution of one concentration to one of another concentration.
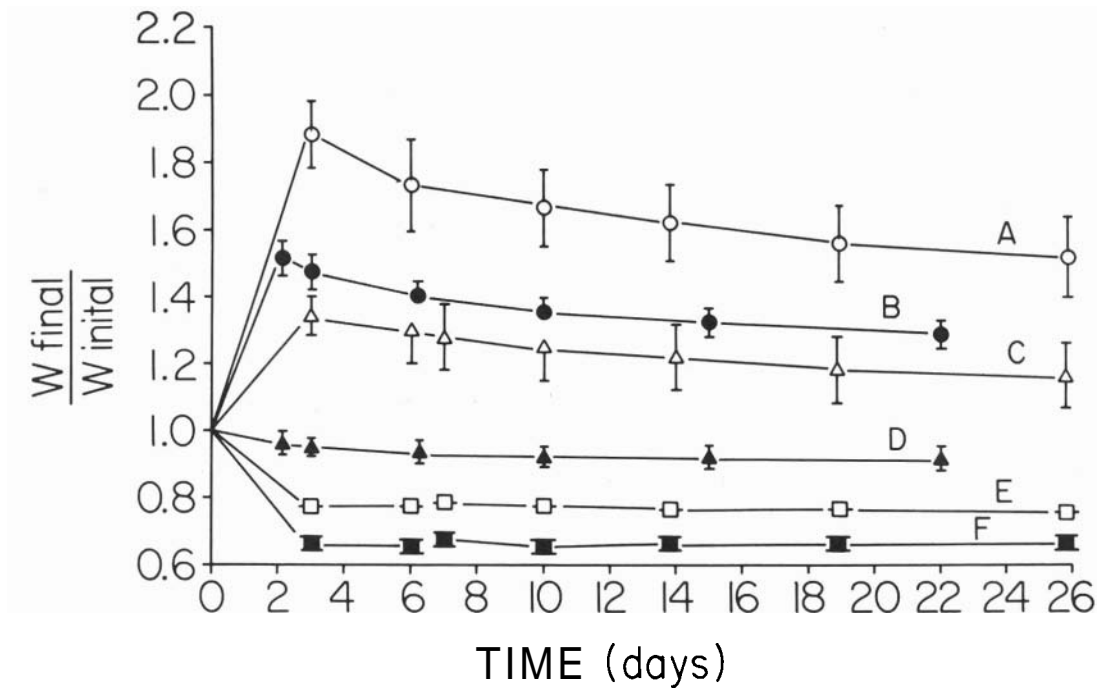
**FIGURE 5.** Time course of volume (or weight) changes of 30% solution of polyethylene glycol in different concentrations of Na citrate. A. 0.1 M, B. 0.25 M, C. 0.5 M, D. 0.75 M, E. 1.0 M, F. 1.5 M.
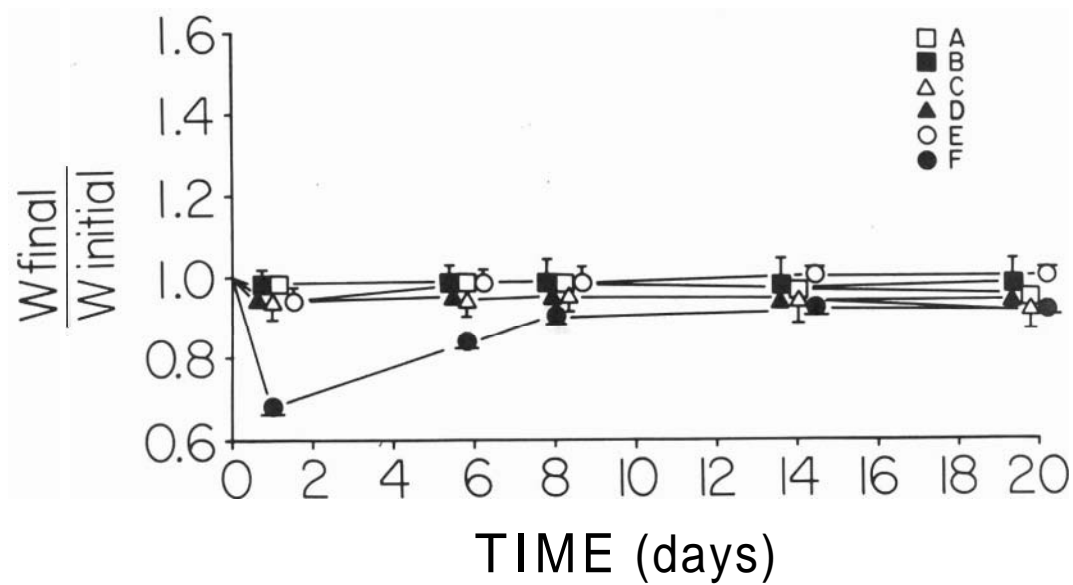


**FIGURE 6.** Time course of volume (or weight) changes of 26.6% solution of bovine serum albumin in different concentrations of Na citrate. A. 0.1 M, B. 0.25 M, C. 0.5 M, D. 0.75 M, E. 1.0 M, F. 1.5 M.
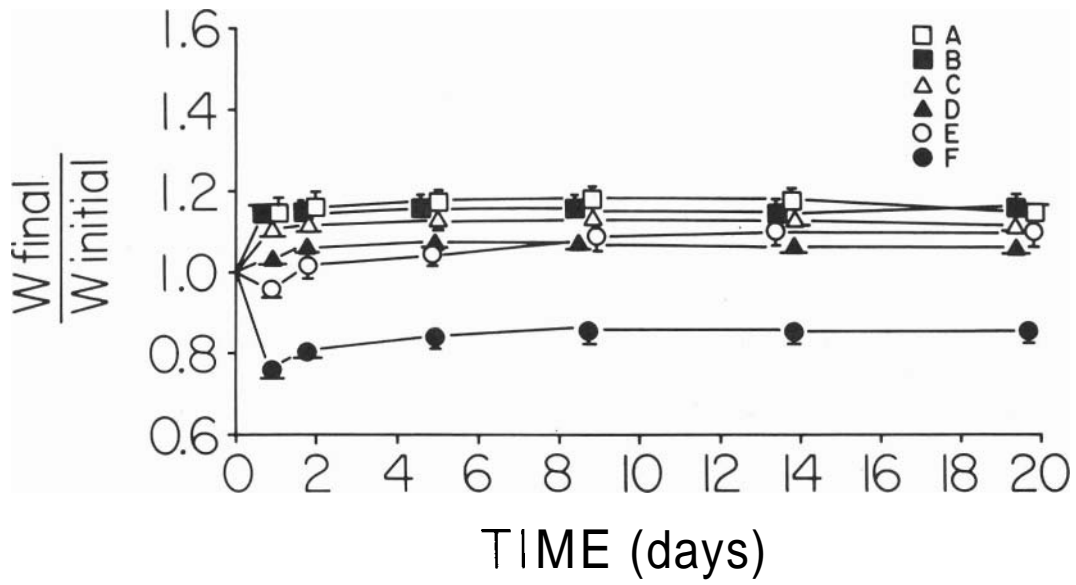
**FIGURE 7.** Time course of volume (or weight) changes of **36.1%** solution of bovine hemoglobin in different concentrations of Na citrate. A. **0.1** M, B. **0.25** M, C. **0.5** M, D. **1.0** M, E. **0.75** M, F. **1.5** M.
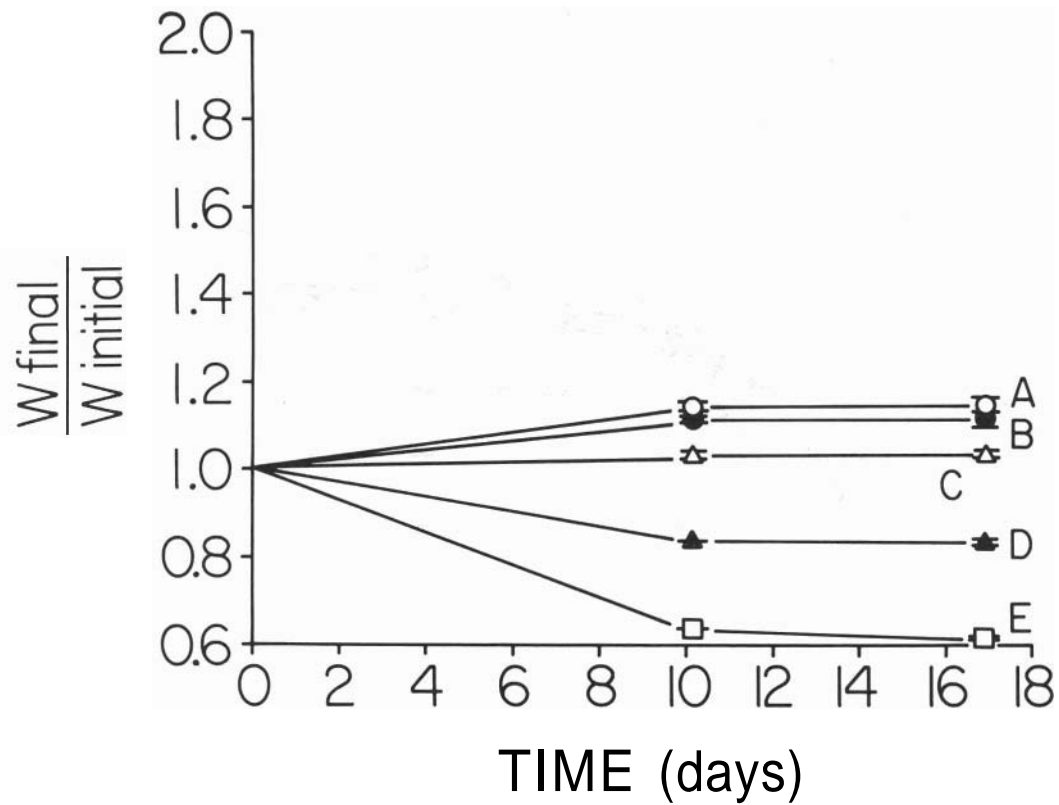


**FIGURE 8.** Time course of volume (or weight) changes of 29% solution of gelatin in different concentrations of Na citrate. A. **0.1** M, B. **0.25** M, C. **0.5** M, D. 0.75M, E. 1.0 M.

### 1.3. *Denatured Proteins*
#### 1.3.1. *Gelatin*

Figure 8 shows the time course of change of a gelatin solution from an initial concentration of 28.5% in solutions of Na citrate ranging from 0.1 M (A) to 1.0 M (E). Swelling was much more limited even in a solution of Na citrate as the most dilute ones used in the PEG and PEO experiments. Restricted swelling of gelatin could have been due to cross-links due to interaction of the side chain functional groups formed in the gel state. Neither PEO nor PEG possess side chains, nor do they gel. While gelatin had only slight swelling, it did shrink slowly. Thus even in a 1.0 M Na citrate its weight had dropped to close to 0.6, while PEO solution in a Na citrate solution of equal strength did not shrink at all

and PEG shrank to about 0.77 of its initial weight. However, the p-value actually measured was not very low (0.4 or higher) (Ling and Ochsenfeld, 1983). This apparent discrepancy suggests the presence of adsorbed $Na^+$ on the anionic side chains of gelatin. If so, the true equilibrium distribution or q-value for Na citrate would have been much lower and perhaps comparable to those of PEG and PEO (ca. 0.I).

#### 1.3.2. *Urea-denatured Proteins*

In the experiments illustrated in Figure 9, a 37.6% solution of bovine serum albumin (bsa) was first dialyzed against 8 M urea solution for three days (23°C), before exposure to different concentrations of Na citrate. The urea-denatured bsa solution dialysed in dis-
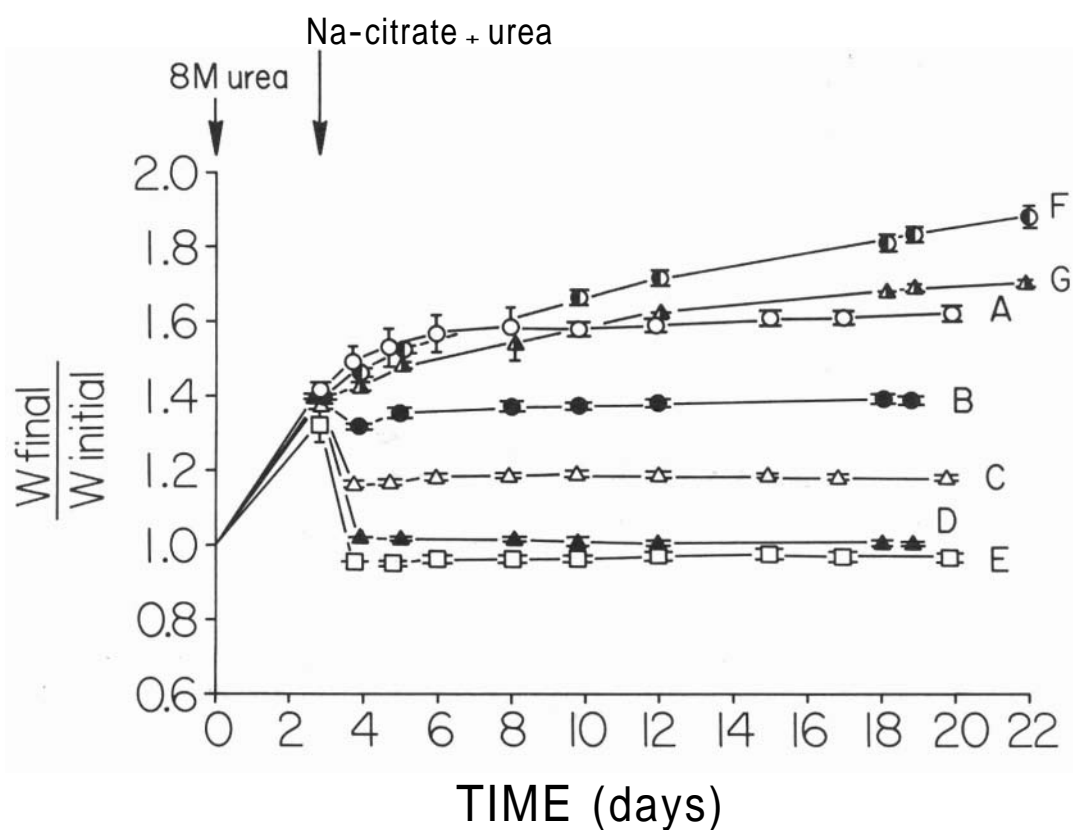


FIGURE 9. Time course of changes of urea-treated 37.6% bovine serum albumin in the presence of different concentrations of Na citrate. A. 0.1 M, B. 0.25 M, C. 0.5 M, D. 0.75 M, E. 0.9 M, F. 0.0 M, G. 0.05 M.

tilled water continued to swell even after 19 more days of incubation, so did other sacs in 0.05 M and even 0.1 M Na citrate solution, though to a lesser degree.

At first glance, the data of Figure **7** may suggest a lack of shrinkage of the urea-denatured bag in all concentrations of Na citrate. In truth, exposure to 8 M urea caused considerable swelling. Referring the initial weight to that at the moment of transfeer to the different solution containing both 8 M urea and varied concentrations of Na citrate, what one sees includes both swelling and shrinkage with shrinkage occurring at lower concentrations of Na citrate than in the solutions of PEO and PEG. This offers evidence that the fully extended polypeptide chains are highly effective in polarizing multilayers of water once their a-helical and other "inward" directed H-bonds are broken.

### 1.3.3. *SDS-denatured Proteins*

Our attempts to study the effect of sodium dodecyl sulfate (SDS) on swelling and shrinkage of bsa solution met with difficulty. Solution containing 0.1 M SDS and either $Na_2SO_4$ or Na citrate above 0.25 M formed a strong gel. For this reason, only two concentrations of Na citrate in the presence of 0.1 M SDS were studied more in the way of a preliminary examination. The data shown in Figure 10 reveal some swelling in solution containing 0.1 M(A) and 0.25 M (B) solution of Na citrate. Comparing with urea-denatured bas in similar concentrations of Na citrate (A and B), the difference is much less pronounced. Thus taking into account the very limited data on hand, one can only tentatively say that SDS had less effect when compared to 8 M urea in concentrating solutions of native bsa. This general trend is thus in accord with other comparative studies of SDS vs. urea-denatured bsa when other physico-chemical traits were measured.
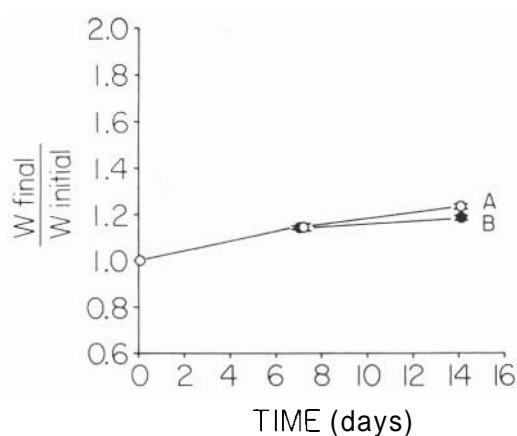


**FIGURE 10.** Time course of volume (or weight) change of bovine serum albumin treated with sodium dodecyl sulfate (SDS) in different concentrations of Na citrate. A. 0.1 M, B. 0.25 M.

### 1.3.4. *Alkali-denatured Proteins*

Exposure of a solution of bovine serum albumin to 0.2 M NaOH caused marked swelling. Varying degree of shrinkage promptly followed the introduction of Na citrate of different concentrations (Figure 11). Figure 12 shows that NaOH-denatured proteins responded to different concentrations of Na citrate in a manner quite similar to PEO and PEG solutions shown in Figures 2 and 3 respectively.

### 2. *In Solutions of Sugars and Sugar Alcohol*

Figure 13 shows the volume change of PEO solution (40.8%) after immersion in solution of D-glucose of different concentrations. Even though the glucose concentrations varied between 0.5 M to 3 M, all cause swelling, however the final volume reached roughly decreased with increase of D-glucose concentration. In Figure 14 a comparable set of data were obtained for volume changes of PEO solution in different concentration of sorbitol solution. In this case, at the highest concentration of sorbitol concentration, the PEO solution-filled dialysis sacs shrank.
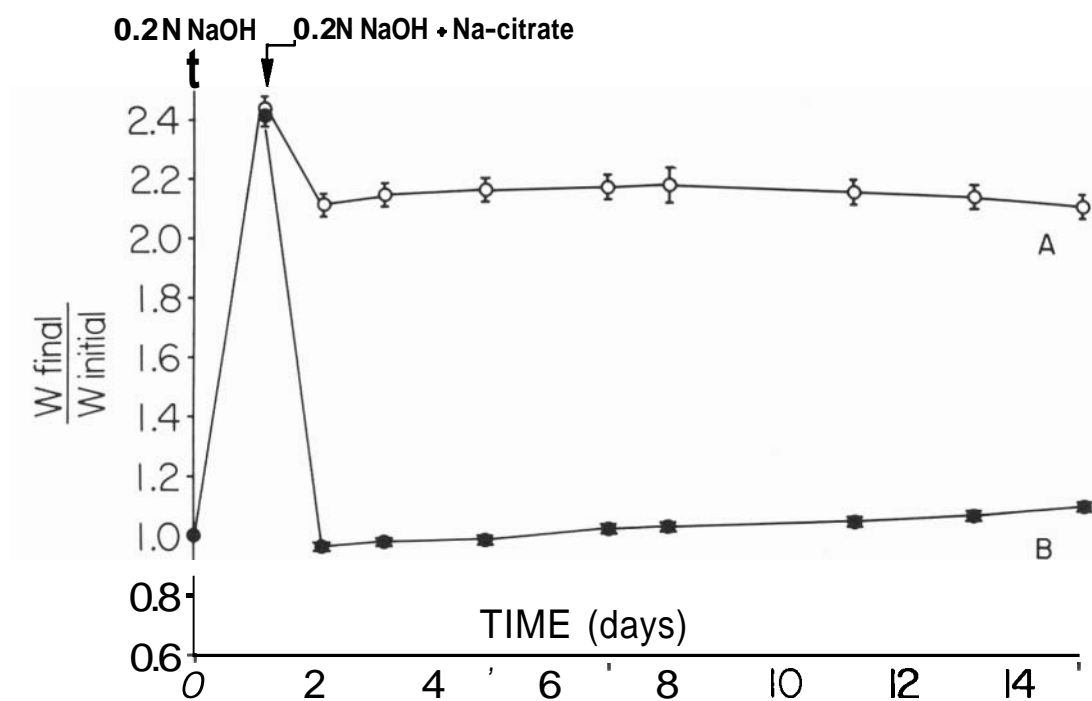
FIGURE 11.   Time course of volume (or weight) changes of NaOH-treated bovine serum albumin in the presence of two different concentrations of Na citrate. A. 0.25 M, B. 1.5 M.
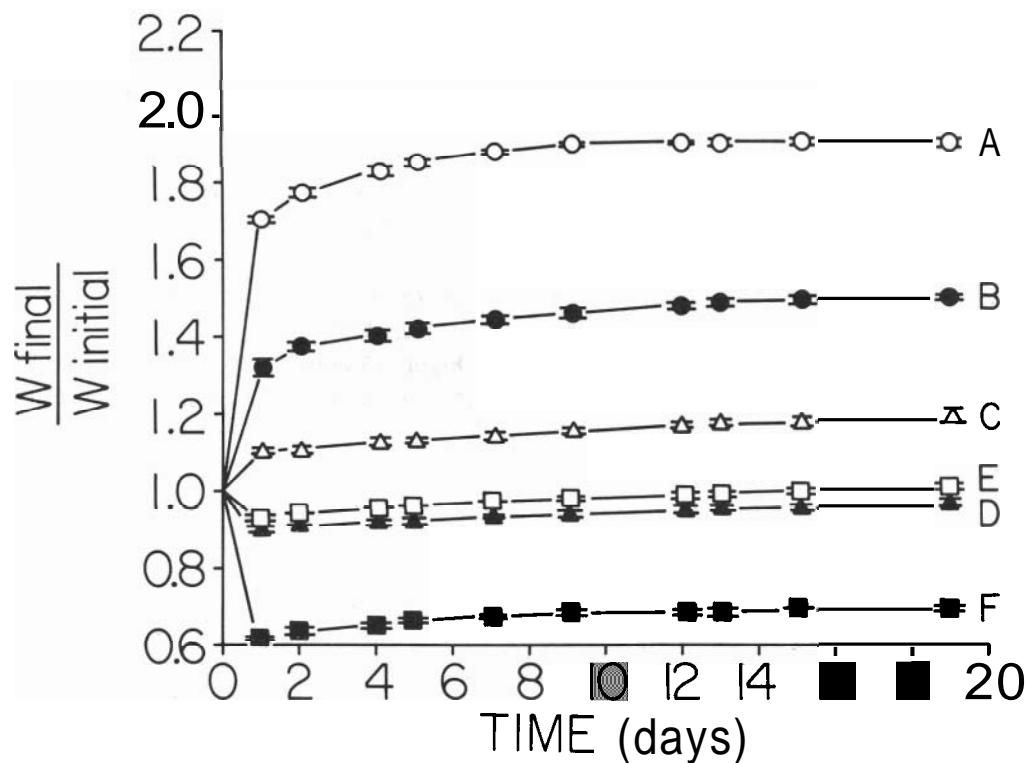


FIGURE 12.   Time course of volume (or weight) changes of NaOH-treated bovine serum albumin (35.4%) in the presence of different concentrations of Na citrate. A. 0.1 M, B. 0.25 M, C. 0.5 M, D. 1.0 M, E. 0.75 M, F. 1.5 M.
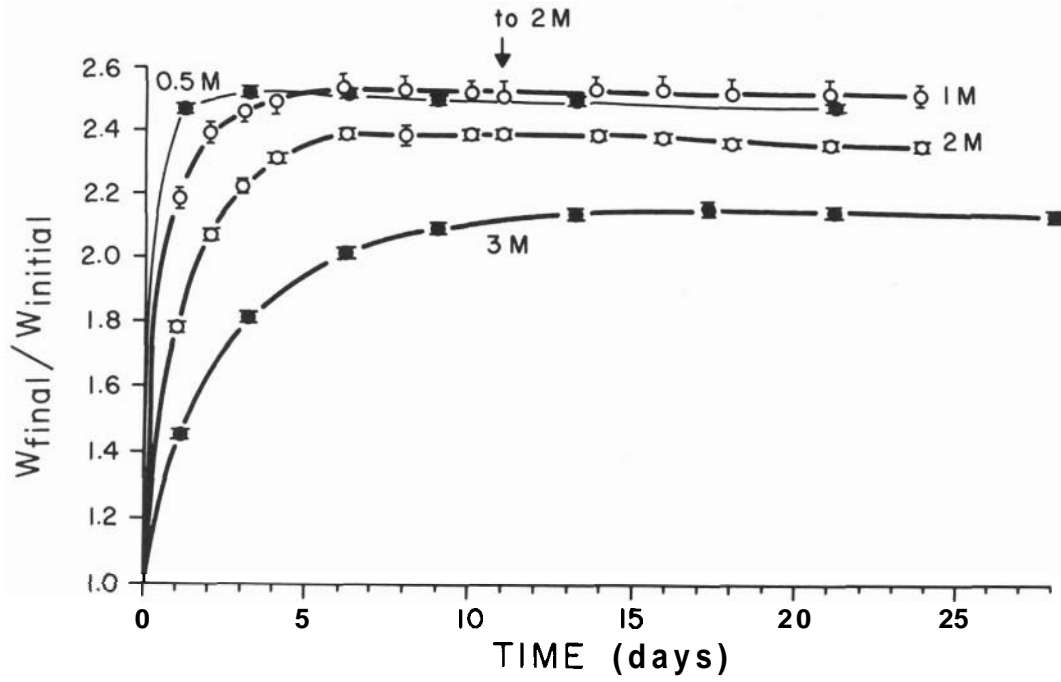
**FIGURE 13.** Time course of volume (or weight) changes of dialysis sacs filled with 40.9% PEO solution in different concentrations of D-glucose.
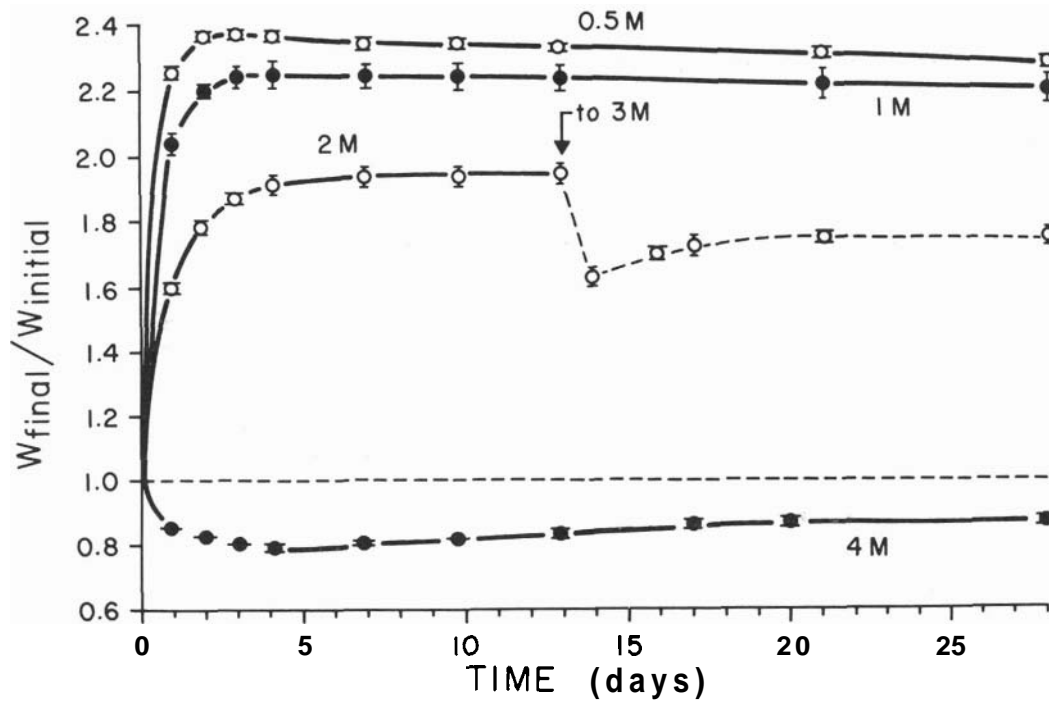


**FIGURE 14.** Time course of volume (or weight) changes of dialysis sacs filled with 41.0% PEO solutions in different concentrations of sorbitol.

### 3. *Quantitative Relationship Between the Concentration of Na Citrate and the Volume of Polymer Solution*

Figure 15 plots the amount of water in the PEO and PEG solutions in the sac after prolonged incubation in Na citrate solution of different strength. The general contour of the curves agree with theory shown in Figure 1. Note that at the same concentration of Na citrate, PEG takes up more water than PEO even though at first glance PEG seemed to take up less water (Figure 4) than PEO in the same environment (Figure **3**). As pointed out earlier, much PEG had leaked out of the dialysis sac. a, given in grams of water per 100 grams of dry polymer, was calculated on the basis of final dry weight from which the weight of Na citrate was subtracted. The weight of Na citrate in the sac was in turn calculated from the p-value of Na citrate determined and shown in Table I.

### DISCUSSION

First, it must be made very clear that we are not dealing with an electrostatic or Donnan effect because polymers like PEO and PEG are neutral polymers and they respond to neutral solutes like D-glucose or sorbitol in a way basically not different from the way they responded to electrolytes like Na citrate.

In the ability of the right type of models to respond to dilute solutions with sustained swelling and to concentrated solutions with sustained shrinkage, one can add yet another set of physiological manifestations that can cogently simulate the behavior of living cells. The obediance to Equation 1 in the volume change of dialysis sacs filled with PEO and PEG shown in Figure 15 and of frog muscle cells described in the companion paper (Ling, 1987) provides greater measures of the comparable complex behavior between living cells and a confined body of water existing in the

state of polarized multilayers.

Since the dialysis sac has a molecular weight cut-off at 12,000 daltons and is thus fully permeable to all the solutes studies, i.e., Na citrate, D-glucose, and sorbitol, the sac
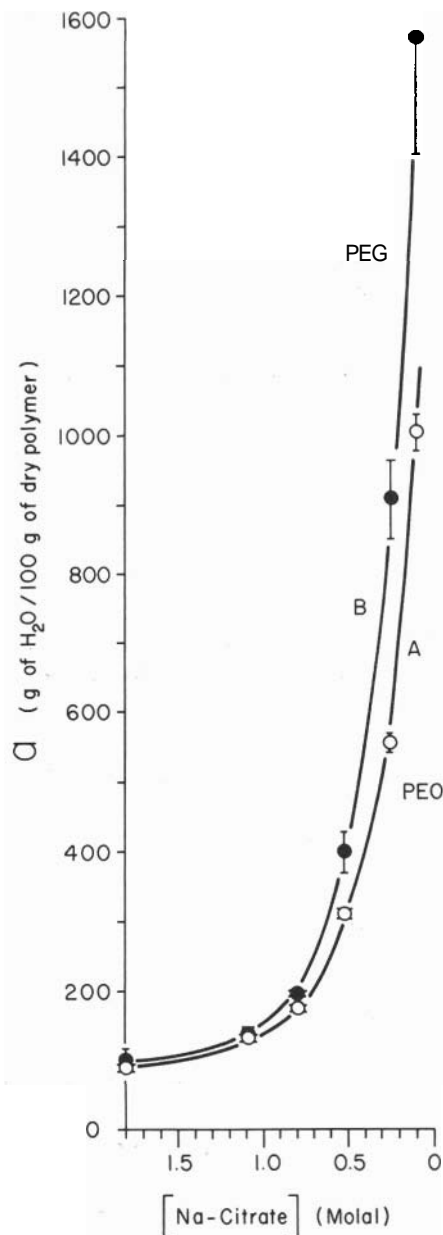


**FIGURE 15.** A plot of the water content of PEO and PEG solution equilibrated in different concentrations of Na citrate.

membrane is not "semipermeable" in the physiological sense but acts only to restrain and confine the proteins and polymers from dispersion. Therefore, the sustained volume change in response to varying solute concentration is not a membrane phenomenon but in essence a manifestation of the protein (or polymer) to polarize water in multilayers and that water so polarized has reduced solvency, or q-value, for large and complex solutes like Na citrate, D-glucose, and sorbitol.

Equally fascinating is that in volume regulation, one sees yet another set of **physico**chemical manifestations that find close parallelism between living cells and aqueous solutions of gelatin, PEO, PEG, and **urea**denatured proteins. In contrast, solutions of native proteins and SDS-denatured proteins do not simulate the behaviors of the living cells.

This volume regulation is yet another attribute to be seen in the gelatin-PEO-PEG-ureadenatured proteins conglomerate but not in the native protein-SDS-denatured proteins in addition to the previously reported attributes of size-dependent solvency reduction (Ling et al., 1980; Ling and Ochsenfeld, 1983) of extremely high osmotic activity (Ling, **1983),** of extensive water sorption at near saturation vapor pressure (Ling and Hu, to be pub-

lished). Together they offer powerful confirmation of the **AI** hypothesis according to which the living cells owe these physiological traits to the existence of cell proteins in the fully-extended conformation which then polarize the bulk of cell water to assume the dynamic structure of polarized multilayers.

This is the first time in which we have introduced alkali-denatured proteins as another example of proteins converted from their non-water polarizing native state to the state in which the bulk of cell water has reduced solvency for Na salts and sugars just like ureadenatured proteins. Why **NaOH** should produce such a far-reaching effect on water will be the subject of discussion in a forthcoming paper.

## SUMMARY

In this paper we studied the volume changes of dialysis sacs containing concentrated solutions of native and denatured proteins and of oxygen-containing polymers after immersion in aqueous solution of Na-citrate, D-glucose, and sorbitol of varying concentration. The results confirm the theory of cell volume regulation: volume changes of living cells in different solutions represent a balance between the tendency of intracellular proteins

TABLE I. **Water contents of the apparent equilibrium distribution coefficient or p-value of** $Na^+$ **(citrate) in the water of PEO solution in the presence of different concentrations of Na citrate.**

| Na citrate Concentration | | PEO | | PEG | |
|---|---|---|---|---|---|
| | | $H_2O$ Content | $\rho_{Na}$ | Hz0 Content | $\rho_{Na}$ |
| (M) | (molal) | (gm $H_2O$/ 100 gm polymer) | | (gm $H_2O$/ 100 gm polymer) | |
| 0.1 | 0.1 | $603.5 \pm 6.30$ | $3.01 \pm 0.027$ | $1602 \pm 256$ | $0.860 \pm 0.030$ |
| 0.25 | 0.252 | $604 \pm 6.30$ | $0.617 \pm 0.005$ | $910 \pm 77.0$ | $0.746 \pm 0.025$ |
| 0.5 | 0.52 | $264 \pm 1.50$ | $0.430 \pm 0.003$ | $401 \pm 27.5$ | $0.416 \pm 0.036$ |
| 0.75 | 0.80 | $174 \pm 2.67$ | $0.244 \pm 0.004$ | $194 \pm 0.96$ | $0.200 \pm 0.004$ |
| 1.0 | 1.09 | $137.8 \pm 1.06$ | $0.215 \pm 0.002$ | $142 \pm 1.74$ | $0.121 \pm 0.013$ |
| 1.5 | 1.71 | $91.4 \pm 0.54$ | $0.169 \pm 0.003$ | $104 \pm 9.4$ | $0.115 \pm 0.015$ |

— which exist in the fully extended conformation — to polarize, sorb, and draw into the sac or cell more water and the opposite tendency to lose water from the sac or cell created by the lower level of the solutes in the cell or sac water than in the external medium. The lower level of the solutes is the consequence of the reduced solvency of the polarized water in the sac or cell water for large and complex solutes like sugar and free amino acids. This study adds another important physico-chemical attribute of the living cell that can be duplicated by aqueous solutions of gelatin, oxygen-containing polymers like **PEO** and **PEG as** well as urea-denatured proteins but not, or only weakly so, by aqueous solutions of native proteins or SDS-denatured proteins.

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