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Electrolytes Control Flows of Water and Sucrose Through Collagen Membranes

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Summary. The physical state of a collagen membrane is determined, among other factors, by the concentration of electrolytes in the bathing solutions, going from a crystalline to an amorphous phase as the concentration increases. Thus, the permeation of uncharged solutes and water is strongly dependent upon the salts in the bathing solutions, which through the induced phase transition control not only the thickness and the solvent content of the membrane but also affect the magnitudes of the frictional coefficients of transport. These changes in physical parameters are reflected in variations of several hundred per cent in the values of the phenomenological coefficients ω_s, L_p and σ. Experiments were performed to determine the physical state and the permeability properties of the membrane as functions of the controlling electrolyte, in this instance $CaCl_2$, in the bathing solutions. In particular the filtration coefficient L_p, the permeability coefficient for sucrose ω_s, and the reflection coefficient for sucrose σ were determined via flow measurements at different salt concentrations. Complementary measurements of swelling and length variations were made. Data were reduced to membrane thickness, solvent volume-fraction, and the phenomenological coefficients. These in turn were reduced to the frictions f_{sm}, f_{sw} and f_{wm}; there was a direct correlation between the behavior of these frictions and the physical state of the collagen membrane as indicated by the length and volume variations.

The magnitudes of the flows of uncharged solutes and water through a simple collagen membrane are controlled by the concentration of electrolytes in the bathing solutions. Significant variations in the permeabilities of the order of several hundred per cent are caused primarily by phase changes in the membrane matrix, the collagen molecules going from a crystalline helix conformation to an amorphous random-coil conformation as the elec-

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trolyte concentration increases to the transition point. This cooperative phenomenon of melting in the membrane results in large changes in its water content, its physical structure and dimension, and in the frictional interactions between solute, solvent and membrane. In addition to those effects caused by melting, further changes in water content and physical dimension are created by *salting in* or *salting out* (depending upon the type of electrolyte used: *see* Ciferri, Rajagh & Puett, 1965; Veis, 1967) and, at higher electrolyte concentrations, by mechanical stresses developed in the membrane which is clamped at a prescribed area (Gliozzi, Penco, Battezzati & Ciferri, *in press*). These induced changes in the physical state of the membrane are reflected in more or less abrupt changes in the phenomenological coefficients: the solute permeability ω_s, the reflection coefficient σ, and the filtration coefficient L_p.

It is the phenomenological coefficients, given as ratios of flows-to-forces, that are measured, and they, being gross parameters functionally dependent in turn upon a variety of more fundamental parameters, are not well-suited to separate and characterize the various physical changes induced in the membrane by variations in the electrolyte concentration in the bathing solutions. The phenomenological coefficients, with the aid of experimentally determined values of the solvent volume-fraction φ_w and the membrane thickness Δx are resolved into three parameters: (1) the solvent-membrane frictional coefficient f_{wm}; (2) the solute-membrane frictional coefficient f_{sm}; and (3) the solute-solvent frictional coefficient f_{sw}. Thus, the control of nonelectrolyte permeability by electrolytes is characterized by five parameters: φ_w, $\Delta x, f_{wm}, f_{sm}, f_{sw}$. If it is assumed that there is a viscous flow of solvent through transmembrane pores, then hydraulic flow is characterized by an effective pore radius R rather than by f_{wm}.

This study of the changes in permeability experienced by a given solute as structural changes are induced in the membrane matrix is, to a degree, the converse of the classic experiments of Collander and Barlund (1933) wherein the permeability as a function of the structure of the permeating species was studied.

Materials and Methods

Collagen Membrane

Doubly-oriented collagen films, 25 μ thick, obtained by casting a dispersion of fibrils from steer tendons, were kindly supplied to us by the Ethicon Company. The characteristics of these membranes have been previously described in Gliozzi, Morchio and Ciferri (1969) and Gliozzi, Vittoria and Ciferri (1972). It was not necessary to perform a cross-linking reaction since the membranes proved to be completely insoluble in the employed solutions. In the investigations of the salt-induced phase transition, the membrane was

equilibrated in 0.2 M sucrose solutions having values of $CaCl_2$ concentration in the range of 1 to 5 M. The occurrence of the phase transition was determined by following the isothermal variations in length and elastic modulus with concentration, as well as the isothermal swelling behavior. The crystal-amorphus transition occurred at about 2.5 M $CaCl_2$ in the first transition, while in the subsequent melting processes it occurred in a much broader range at lower concentration values (*cf.* Fig. 1). After the first transition the subsequent melting processes proved to be almost completely reversible. Unless explicitely indicated, all the experiments reported in the following, were performed by decreasing the salt concentration, when the membrane had undergone a contraction-expansion cycle.

The degree of swelling, given as the ratio of the volume of the swollen membrane with respect to the dry membrane V/V_0 was determined from measurements of the weight and density of the membrane in each state. The membrane, equilibrated in the salt solution for 12 hr, was gently blotted to remove surface moisture and then weighed. The blotting procedure was repeated 5 to 6 times, and the mean value of the weights was used. Two series of measurements were made on each of the two membranes considered: first by increasing the salt concentration from 1 to 4.5 M and then by subsequently reducing it. The thickness of the membrane when clamped at constent area in the flow cell was derived from these measurements.

Volume Flow Determination

The volume flow through the membrane per unit time and unit area J_V was measured under a pressure difference of about 20 cm of H_2O. The membrane was clamped between two half-cells which contained aqueous solutions of sucrose and $CaCl_2$ of identical composition. The apparatus and details of the method are described in Gliozzi *et al.* (1969) and Gliozzi *et al.* (1972). The temperature was kept constant at 26 ± 0.1 °C. The membrane was equilibrated in the cell with the employed solutions for about 12 hr; during this period, no pressure was applied. After application of pressure, the volume flow reached a constant, steady-state value in about 30 min. All measurements were performed with the same membrane at different values of $CaCl_2$ concentrations, decreasing from 4.5 to 1 M. The estimated precision of values of J_V is of the order of $\pm$ 5%.

Determination of the Reflection Coefficient σ

The reflection coefficient, defined as $\sigma = (\Delta P/\Delta \pi_s)_{J_V=0}$, was determined using the same cell employed for the volume flow measurements. The two half-cells were initially filled with identical $CaCl_2$ solutions with no sucrose since the equilibration time is rather long and the osmotic pressure difference would change over such a long period if the permeant species were present. After 12 hr of equilibration, one of the salt solutions was substituted by another solution with the same $CaCl_2$ concentration but containing in addition 0.2 M sucrose.

Pressure was then applied to the side containing sucrose, thus reducing the water flow due to osmotic forces. The applied pressure required was small enough to assure that the operations occurred within the linear pressure-volume flow range. The steady-state volume flow was then measured. The procedure was repeated at different values of the pressure difference, and a linear plot of J_V versus ΔP was thus obtained. An extrapolation of J_V to zero gave the value of the hydrostatic pressure difference required to counterbalance the osmotic pressure difference. The reflection coefficient is given as the ratio of this pressure difference to $\Delta \pi_s$, calculated for an ideal solution of sucrose. The estimated precision in the value of σ is 5%.

Even though there exists a sucrose gradient across the interior of the membrane which arises from having sucrose in only one of the half-cells, the membrane remains a homo-

geneous phase. Since the swelling of the membrane is an indirect measure of the interaction of the membrane with the other components of the system, we performed swelling measurements in 2 M $CaCl_2$ with sucrose concentrations ranging from 0 to 0.2 M. No variation in swelling was found, thus providing indirect proof that under the conditions of the flow experiments sucrose does not change the structure of the collagen membrane. Further support is given by measurements of swelling as a function of $CaCl_2$ concentration in the presence or absence of 0.2 M sucrose; no substantial change was found.

Determination of Sucrose Permeability ω_s

Permeability to sucrose was determined using sucrose labeled with ^{14}C. The same cell employed for the volume flow determination was used for these tracer measurements. The membrane was equilibrated in the cell with identical solutions of $CaCl_2$ and 0.2 M sucrose on both sides. When equilibrium was attained, 0.5 cc of labeled sucrose, with an activity of 0.025 mC, was added to one side of the cell. Samples were collected every 5 min for 45 min. These samples were analyzed with a Packard scintillation spectrometer.

The value of the permeability ω_s was deduced from the slope of the straight-line plot of activity α as a function of time using the relationship

$$\alpha_1 = \frac{RTA\,\omega_s(\alpha_2 - \alpha_1)}{V_1}\,t \tag{1}$$

where V_1 is the volume of the cold half-cell, α_1 is the ^{14}C activity of the cold half-cell at time t, A is the area of the membrane, and $\alpha_2 - \alpha_1$ is the difference in ^{14}C activity between the two half-cells. The preceding equation is linear because $\alpha_2 - \alpha_1 \simeq \alpha_2$ during the course of the measurements. The error in the determination of ω_s is of the order of 6%.

Theory and Data Reduction

Membrane Thickness Δx

The membrane is mechanically clamped so that it has a constant prescribed area; hence, for a flow determination at a given salt concentration we have

$$\frac{\Delta x}{V} = \frac{\Delta x_0}{V_0}, \tag{2}$$

where V_0 is the volume of the dried membrane and Δx_0 is its thickness. In place of a direct determination of the volume V of the membrane swollen by the bathing salt solution, the mass of the wet membrane can be used in the calculation of Δx. If the mass of the swollen membrane is M and the density of the bathing solution which fills the pores in the membrane is ρ_s, we have

$$\Delta x = \frac{\rho_s V_0 + M - M_0}{\rho_s V_0}\,\Delta x_0. \tag{3}$$

The values of Δx calculated through Eq. (3) are in agreement with those obtained by use of a direct optical technique (Gliozzi *et al.*, 1969). Ginzburg

and Katchalsky (1963) demonstrated that the thickness of the unstirred layers, which varies from 10^{-5} to 10^{-3} cm, need not be considered in those membrane systems with ω_s of the order of 10^{-15} or less. It can safely be ignored here. It should be noted that we have measured the swelling effect on the free membrane, while during the experiment the membrane, being clamped at constant area, is subjected to stresses at high values of salt concentrations. However, it has been shown (Gliozzi *et al.*, *in press*) that the applied stress has only secondary effects upon the flows as compared to the effects of changing concentration.

Solvent Volume-Fraction φ_w

Since the volume of excess solute in the membrane is negligible compared to that of the matrix V_0 and the solution V_w (Katchalsky & Oplatka, 1965) we have:

$$V_w + V_0 \cong V$$

or

$$\varphi_w = 1 - \frac{V_0}{V}. \tag{4}$$

By Eqs. (2) and (4) we see that for a membrane with a fixed area, four, not five, parameters suffice to characterize the flows since both Δx and φ_w are simple functions of V_0/V.

Frictional Coefficients $\mathrm{f_{sm}}$ *and* $\mathrm{f_{sw}}$

The equations used here to determine f_{sm} and f_{sw} (Richardson, 1970) are similar to those of Ginzburg and Katchalsky (1963), the latter however requiring the value of L_p to make the J_w to J_V correction. For a highly swollen membrane, where the solute partition coefficient can be set to φ_w, (*see*, e.g., Katchalsky & Curran, 1965), we have

$$f_{sw} = \varphi_w(1-\sigma)/\omega_s \Delta x \tag{5}$$

and

$$f_{sm} = \varphi_w[1-(1-\sigma)(1+\bar{C}_s \bar{V}_s)]/\omega_s \Delta x, \tag{6}$$

where $\bar{V}_s$ is the partial molar volume of the solute and $\bar{C}_s$ is average concentration of the solute in the two bathing solutions. It is assumed that for these highly swollen membranes where φ_w is of the order of 50%, we can set the tortuosity equal to one.

Characterization of Solvent Flow f_{wm} *and* R

The characterization of solvent flow through membranes remains an open problem (*see*, e.g., Mikulecky, 1967, 1972), and it is instructive here to consider two possible models: one based upon frictional coefficients and a second upon an effective pore radius. On one hand, if the membrane is tight, the primary resistance to the flow of solvent is the frictional interaction between the solvent and the membrane molecules f_{wm}; in that case (Ginzburg & Katchalsky, 1963),

$$f_{wm}=\frac{\varphi_w \bar{V}_w}{\Delta x}\left[\frac{1}{L_p}-\frac{(1-\sigma)(\sigma+\omega_s \bar{V}_s/L_p)\,\bar{C}_s}{\omega_s}\right]. \tag{7}$$

On the other hand, if the membrane is highly swollen with water, the majority of water molecules have little or no interaction with the membrane matrix, the primary resistance to water flow thus being viscous forces. If in this case one assumes that the solvent flows through right cylindrical pores penetrating the membrane, then (*see*, e.g., Katchalsky & Curran, 1965):

$$R=\left(\frac{8\eta L_p \Delta x}{\varphi_w}\right)^{\frac{1}{2}} \tag{8}$$

where η is the viscosity of the solvent. Since the pore radii calculated via Eq. (8) are 5 to 10 times greater than the radius of a water molecule[1], it appears that the primary mode of solvent transport through these collagen membranes is viscous flow.

Having measured values of φ_w and Δx, we need not resort to the observation of Pappenheimer, Renkin and Borrero (1951), who saw that the ratio of L_p to ω_s gives an expression for R free from Δx, which fortunately for those working with biological membranes cancels out. In addition, their calculation assumes that one knows the value of D_s in the membrane phase. We calculate R directly from Eq. (8). Because the membrane is actually not an array of cylindrical pores, but rather a loose mesh of fibrils, the calculated values of R are, of course, only effective values. Nevertheless, they do provide an indication of the physical changes induced in the structure of the membrane by variations in the salt concentration.

Evaluation of f_{wm} from the data shows that this coefficient bahaves in a manner similar to f_{sw} and f_{sm}, its behavior being, as expected, opposite to that of R. The solvent-membrane frictional coefficient and the effective pore radius both appear therefore to be reliable indicators of the physical-chemical processes occurring in the membrane as its structure is changed by the variation of the electrolyte $CaCl_2$.

1 The radius of a water molecule is about 1.5 Å as compared to 4.7 Å for sucrose.

Results and Discussion

Swelling and Length Variation

Fig. 1*a* depicts the variation of the length of a strip of collagen membrane with changes of the concentration of salt in the bathing solution. The length relative to the dry length L/L_0 is plotted as a function of the electrolyte, i.e. the salt, concentration C_e. The subscript e notation is used so that subscript s can be used to denote quantities related to the solute. The arrows on the graph indicate that the length measurements were performed with an increasing salt concentration through the first transition and with a decreasing C_e in the subsequent one. The abrupt change in length at $C_e \cong 2.5$ M corresponds to the crystal-amorphous transition.

It is evident that the first transition is a highly cooperative phenomenon which can be compared to the melting of oriented polymers (Flory & Garrett, 1958). The data reported in Fig. 1*a* indicate that, following the first transition, the structural change brought about by recrystallization occurs in a much broader C_e range. The continuous increase in length with decreasing concentration might be attributable to the formation of crystalline domains which differ in size and degree of cross-linking; it is expected that different crystallites are formed at different salt concentrations (Harrington & Von Hippel, 1961).

Measurements of the ratio of the swollen volume V to the dry volume V_0 as a function of salt concentration are plotted in Fig. 1*b*. These measurements were performed in the presence as well as the absence of 0.2 M sucrose at various $CaCl_2$ concentrations. No significant difference in the swelling behavior was found, thus indicating that within the given $CaCl_2$ concentration range the collagen interacts primarily with the salt. Fig. 1*b* shows an inflection point at $C_e \cong 2.5$ M in the swelling of crystalline collagen with increasing concentration corresponding to the initiation of the melting process. This melting is almost completed in the 2.5 to 3 M concentration interval. The de-swelling occurring at higher values of C_e indicates the salting-out power of the $CaCl_2$. At these higher values of C_e the membrane can be considered to be an amorphous network.

The swelling behavior in the re-crystallization process differs from that observed in the first transition in two respects: there is a higher degree of swelling at low concentrations and a shift downwards to about 1.5 M in the value of C_e giving the maximum swelling. The first difference in behavior can be ascribed to residual amorphous regions, which can be highly swollen at low concentrations. The second difference has already been observed in collagen tendons (Ciferri *et al.*, 1965). A possible explanation might lie in the cross-link-

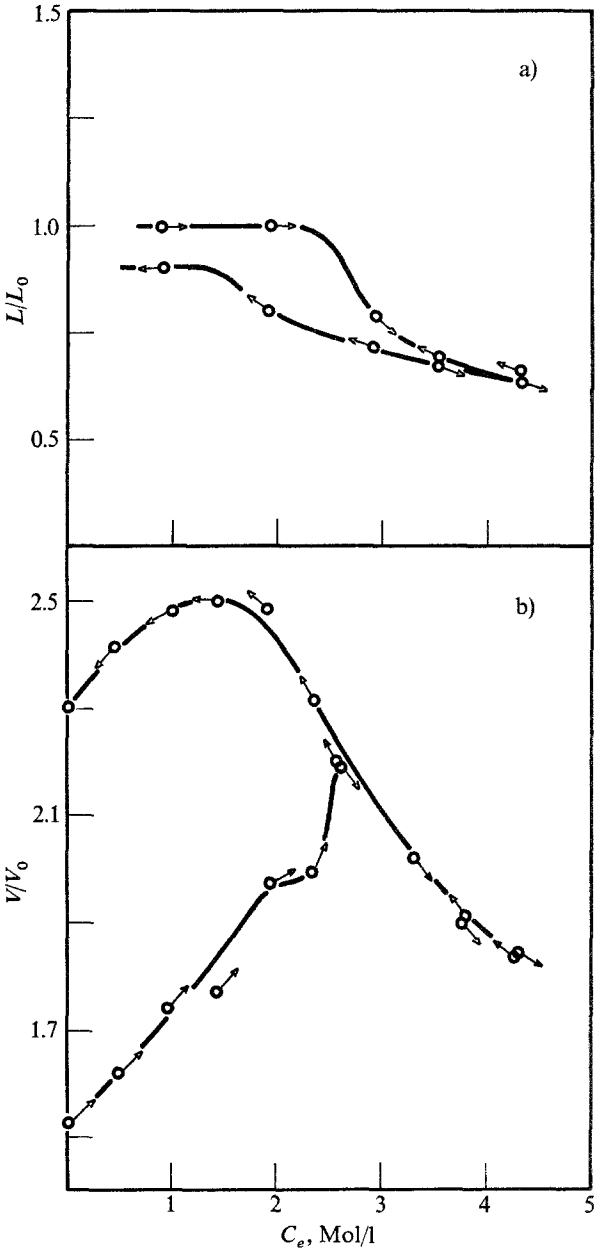

Fig. 1. Dependence of physical structure of collagen upon electrolyte (i.e. salt) concentration C_e. (*a*) Length relative to dry length L_0. (*b*) Volume relative to dry volume V_0. Path of increasing salt concentration through first transition ⇢. Path of decreasing salt concentration ⇠. T = 26 °C

ing power of Ca^{++} ions in the amorphous state, stabilizing the amorphous state and thus hindering the re-crystallizing process. In any case, these two

phenomena together with the length-concentration curve indicate that the degree of crystallinity found during the initial transition is not regained in subsequent transitions.

Phenomenological Coefficients

In this system higher order correction terms may be neglected in the calculation of the filtration coefficient L_p: *see* discussions in Gliozzi *et al.* (1969) and Gliozzi *et al.* (1972). Fig. 2 gives L_p, derived directly from the $J_V/\Delta P$ measurements, as a function of the salt concentration. One observes that the trend of L_p is similar to that of $V/V_0 \cdot L_p$ has a maximum at the maximum value of swelling, and decreases at $C_e \gtrsim 1.5$ M where swelling decreases.

This behavior differs with a previous finding (Gliozzi *et al.*, 1969) in which the first transition was studied. In those measurements L_p and V/V_0 followed the same trend during the phase transition and in the amorphous phase but had opposite trends in the crystalline region. This behavior is discussed in a forthcoming paper analyzing the effect of stress upon permeability properties of collagen membranes (Gliozzi *et al.*, *in press*) where it is

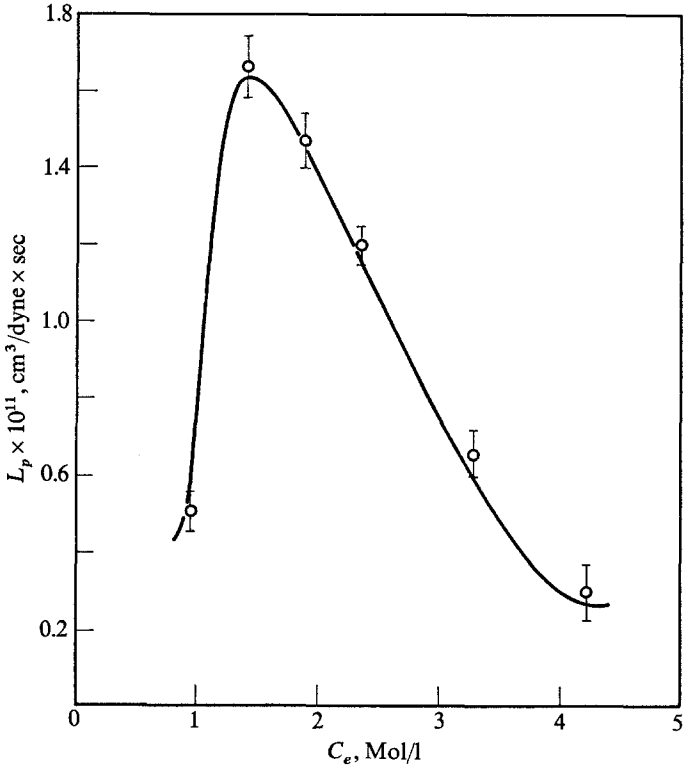

Fig. 2. Filtration coefficient L_p as a function of electrolyte (i.e. salt) concentration C_e. T = 26 °C

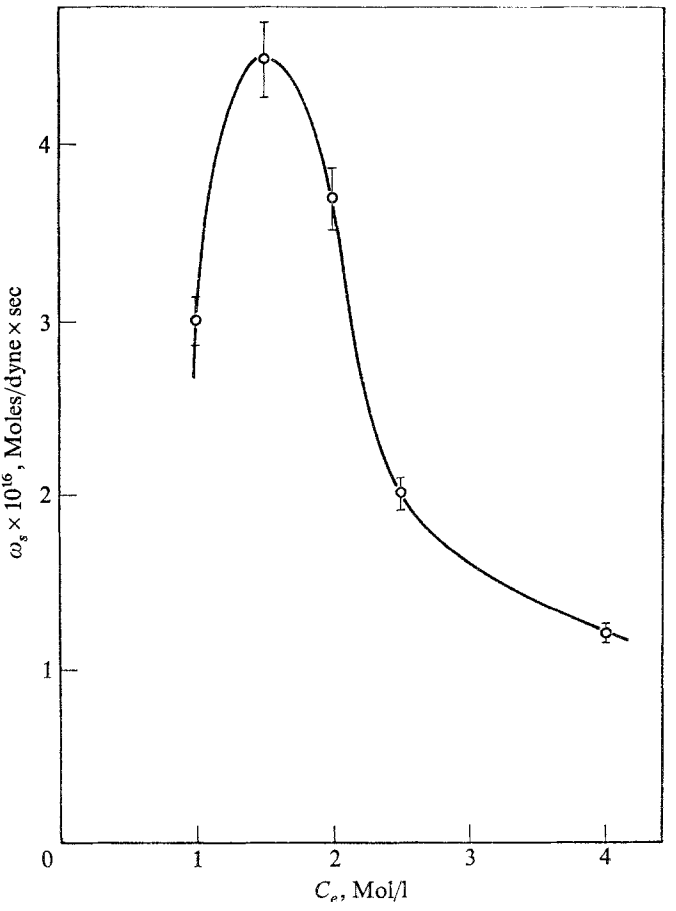

Fig. 3. Permeability coefficient of sucrose ω_s as a function of electrolyte (i.e. salt) concentration. T = 26 °C

concluded that opposite trends of L_p and V/V_0 can be attributed to the limited degree of crystallinity attained after the first transition. In the concentration range of 1 M $< C_e <$ 1.5 M, the membrane in the present system is still undergoing the amorphous-crystal transition, and a large percentage of disordered regions are present. In this range of concentration the membrane should therefore be regarded as a granular substance wherein the crystallites are more permeable than the accompanying amorphous material.

The permeability of the membrane to sucrose ω_s calculated by Eq. (1) is plotted as a function of C_e, the controlling salt concentration, in Fig. 3.

The reflection coefficient σ deduced from the data as $\sigma = (\Delta P/\Delta \pi_s)_{J_V=0}$, is presented as a function of C_e in Fig. 4. The upper curve is for the first transition, obtained by increasing the external salt concentration, and the lower curve is for the subsequent transition, obtained by decreasing C_e in the

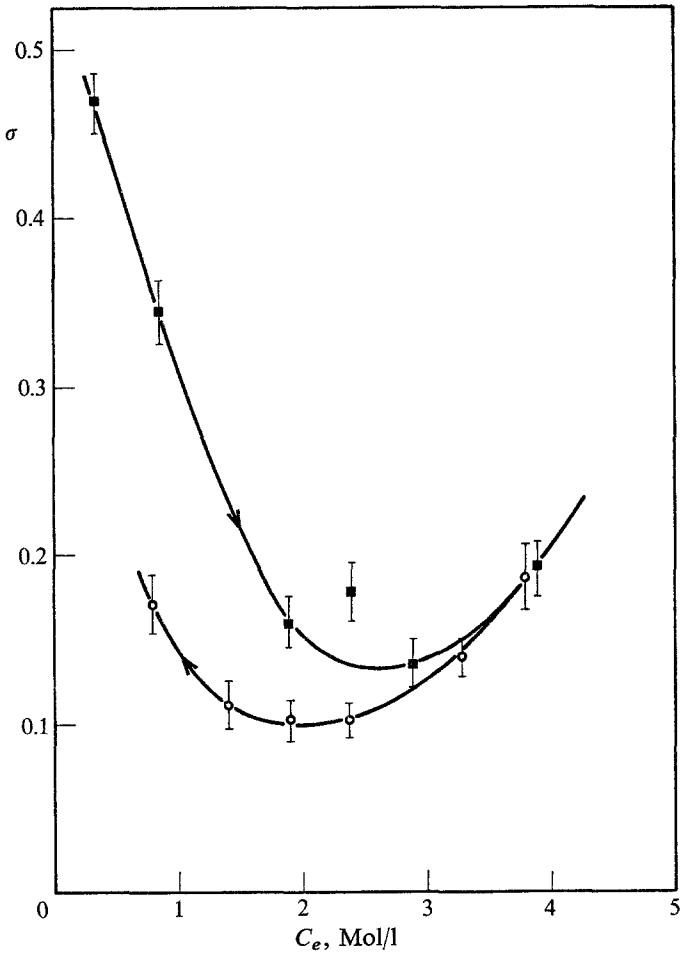

Fig. 4. Sucrose reflection coefficient σ as a function of electrolyte (i.e. salt) concentration. Path of increasing salt concentration through first transition ▪. Path of decreasing salt concentration ∘. T = 26 °C

course of the experiment. As expected, the curves are similar but do not coincide, showing that the initial state was not regained upon lowering the concentration. Notice that σ is much lower at $C_e \cong 1$ M in the second transition, providing indirect support for our contention that a much lower degree of crystallinity is attained in transitions following the first one.

It should be noted that the inversion point in the trends of all these practical parameters of transport, L_p, ω_s and σ, correspond to changes in membrane structure revealed by the various measures of solvent-membrane interactions, e.g. V/V_0. The sucrose and water flows are controlled directly by structural changes in the collagen membrane induced by varying the salt concentration.

Frictional Coefficients and Effective Pore Radius

From the behavior of the phenomenological coefficients it is difficult to deduce directly the causal relationship between particular changes in membrane structure and the resulting variation in transport properties. For example, the measured variations in L_p induced by varying C_e reflect significant variations in f_{wm} and in Δx and φ_w. Thus, the set of coefficients f_{sw}, f_{sm}, f_{wm}, Δx and/or R constitute a more fundamental and independent set of elements with which to investigate the basis of the control of nonelectrolyte transport by salt-induced membrane phase transitions.

The frictional coefficients, calculated from the data via Eqs. (5), (6) and (7), are presented in Fig. 5. The estimated precision of these values is 8%. The similar behavior exhibited by the curves is immediately apparent. In all cases there is a minimum in the curve at the amorphous-crystalline transition at $C_e \cong 1.5$ M, where maximum swelling occurs. The reduced interactions within the membrane matrix can be attributed to the expansion of the network with swelling and to the greater mobility of the polymeric chains in the region of phase transition. The effective pore radius, calculated by Eq. (8) and shown in Fig. 6, provides direct insight into the structural variations induced by changes in the electrolyte concentration.

The trend of the values of the frictional coefficients is always opposite to the trend of the swelling curve, thus indicating that the changes in interactions arise from the expansion of the network. However, considering the actual magnitudes of the friction coefficients, it is seen that, relative to their values in the crystalline state ($C_e \cong 1$ M), f_{sm} and f_{sw} increase two- or threefold at higher concentrations in contrast to f_{wm} which, even at high concentrations, always has a value smaller than that in the crystalline state. This behavior can be attributed to the *salting-out* property of the amorphous protein in the salt solution (Ciferri *et al.*, 1965; Orofino, Ciferri & Hermans, 1967). Therefore, in spite of the greater stiffness of the membrane matrix at high C_e (indicated by the higher value of f_{sm} and by the trend of the effective pore radius) the poor quality of the solvent in the amorphous state [poor in that the liquid admitted or expelled by the membrane is in a relatively looser state (Orofino *et al.*, 1967) than in the crystalline state] makes f_{wm} in the amorphous state smaller than in the crystalline state.

At this point it should be pointed out that the frictional interaction between the membrane and the salt solution as considered here must not be confused with the selective binding (Mandelkern, 1964; Ciferri, Garmon & Puett, 1967) of Ca^{++} ions accompanying the phase transition. In fact, we have treated the salt solution as a one-component diluent and thus have

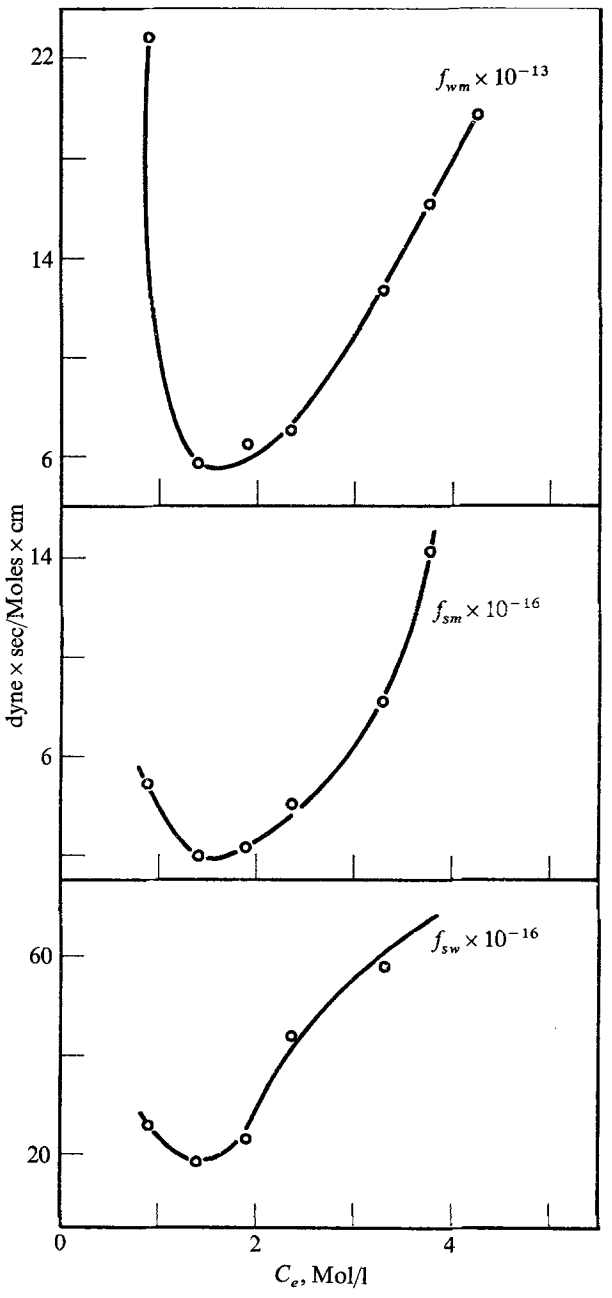

Fig. 5. The frictional coefficients f_{wm}, f_{sm} and f_{sw} as functions of the electrolyte (i.e salt) concentration

disregarded all *enrichment* effects, or disproportion between water and salt binding within the membrane (Katchalsky & Oplatka, 1965; Rubin, Piez & Katchalsky, 1969). Here these effects are implicitly taken into account by

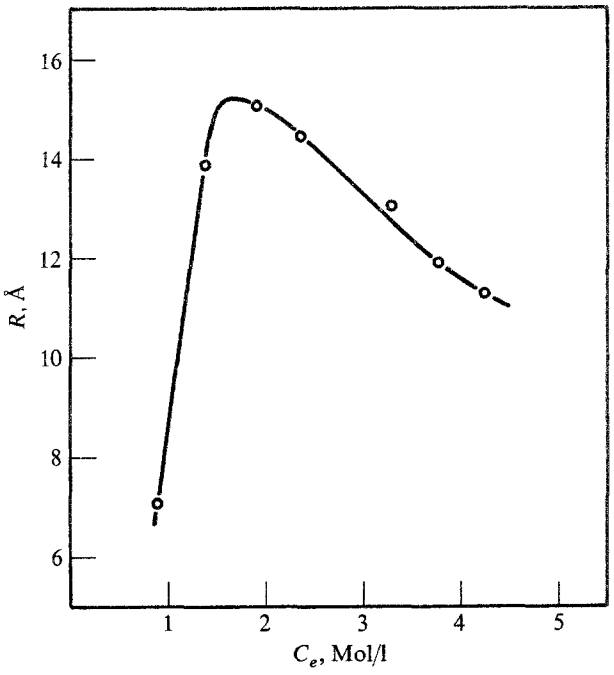

Fig. 6. The effective pore radius R as a function of electrolyte (i.e. salt) concentration

considering their influence on the structure of the membrane, which, in turn, can be considered (Orofino *et al.*, 1967) as a polymer-water-salt complex swollen by a solution behaving as a conventional diluent. The parameter denoted here by f_{wm} is therefore characteristic of the frictional interaction between the modified membrane and the bulk salt solution flowing through it.

Conclusion

Although this report is limited to the effect that Ca^{++} has upon the flow of solvent and sucrose through a simple collagen membrane, the phenomenon is not limited to the passive diffusion of nonelectrolytes since the ultimate mechanism by which the permeability is controlled lies within changes in the physical state of the membrane matrix. As to the agent of control, phase transitions in collagen, in particular, and proteins, in general, can be induced by variations in pH at low ionic strength, in salt concentration, and even in temperature.

The transition in collagen from the crystalline to the amorphous phase is a cooperative phenomenon and hence, near transition, is quite sensitive to small changes in electrolyte concentration. It is possible that for those

flows in biological membranes posited to occur through proteinous pores, small changes in the local pH or salt concentration might induce phase changes in the proteins, thus producing significant changes in permeability. These local variations in electrolyte concentration need not be large if the system is poised close to the transition point. Tasaki (1959) proposed that conformational transitions might be involved in the initiation and propagation of nerve impulses. Evidence of these transitions is being sought by the use of optical techniques such as light scattering and birefringence changes (Cohen, Keynes & Hille, 1968), by the detection of changes in extrinsic fluorescence in voltage-clamped squid axons (Conti & Tasaki, 1970), and by the measurement of changes in axon impedance induced by pH changes in bathing solutions (Clark & Strickholm, 1971). In the latter experiments, the pH dependence of the impedance strongly resembles a protein dissociation curve; the authors propose a pH-dependent cooperative transition in membrane surface proteins. Of naturally occurring nonelectrolytes, sugar appears to be one of the most extensively studied, but the complexity of its mode of transport makes difficult the interpretation of such observations of Holloszy and Narahara (1967). They propose that the enhanced permeability of sugar associated with muscle contraction is indirectly related to the influx of Ca^{++}. There is a local increase in salt concentration, but any effect upon the membrane matrix is masked by the effects of Ca^{++} in a chemical role. It is possible that an enzymatic process occurring in a membrane or at its surface and having hydrogen among its substrates or products could result in a change in local pH, thus inducing permeability changes in the membranes. Goldman, Kedem and Katchalski (1968) show that membrane-embedded enzymes may give rise to concentration gradients of 2 to 3 pH units across a 200- to 300-Å thick membrane; the rate of the enzymatic reaction controls the intramembrane pH and might in turn control permeability.

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