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INTERACTING SOLUTE FLOWS IN PERMEABILITY STUDIES ON THE SAND DOLLAR EGG

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SUMMARY

Equations are derived from the thermodynamics of irreversible processes which show that the flow of any permeable component across a semipermeable membrane may depend on the simultaneous flows of all other such components through solute–solute interactions. Experiments were performed on the sand dollar egg in an attempt to demonstrate such effects through their influence on classical swelling curves. Initial indications were obtained for the presence of such effects. Reflection coefficients determined individually for ethylene glycol, propylene glycol, and acetamide are compared with similar data for human erythrocytes.

INTRODUCTION

Several papers[1–5] have appeared in the past few years on the application of the thermodynamics of irreversible processes to problems involving membrane permeability. In general, these papers have emphasized the interactions which occur between solute and solvent flows. Such interactions have been shown to produce physiologically significant results: for example, a flow of water against the osmotic gradient under certain special conditions when the solute penetrates the membrane rapidly. Although more general equations have been presented for multicomponent systems, relatively little emphasis has been placed on the effects of solute–solute interactions. Such effects could be of real importance if the flow of one solute affected the flow of a second solute to a significant degree.

Our interest in this problem began when DUNLOP AND GOSTING at the University of Wisconsin[6,7] demonstrated that a concentration gradient of one solute caused a flow of a second solute in a freely diffusing system even when there was no initial concentration gradient of the second solute.

Although the effects observed in freely diffusing systems were relatively small, it was felt that such interactions might be considerably greater in flows across semipermeable membranes, and might be important in biological systems where many components are normally passing through the plasma membrane simultaneously. These effects would then have obvious significance in the interpretation of various transport phenomena. Accordingly, the flow equations necessary to test solute–solute

interactions among non-electrolytes were derived from the thermodynamics of irreversible processes, and initial experiments were performed on sand dollar eggs in an attempt to demonstrate such effects.

THEORY

The approach to problems of diffusion and permeation that utilizes the thermodynamics of irreversible processes has become familiar through the publications of KEDEM AND KATCHALSKY[1,2] and others. Accordingly, relatively little emphasis will be placed on the derivation of the necessary flow equations in this paper, and more emphasis will be placed on their significance.

The notation used throughout is generally in accordance with that used by KEDEM AND KATCHALSKY. Solute components are represented by subscripts; subscript 3 is reserved for an average of the impermeable solutes present. The positive flow of solute k per unit area of membrane in the direction from outside to inside is represented by $\dot{n}_k$. The subscript, v, refers to volume as in volume flow, J_v, and the subscript, w, to the solvent water. Superscripts, i and o, refer to the inside and outside of the sand dollar egg, respectively. Chemical potentials are represented by the convential symbol, μ, and $\Delta\pi_k$ for each solute is defined by the equation: $\Delta\pi_k = RT\Delta c_k$. Here, the quantity, Δc_k, is the concentration difference of component k between the outside and inside of the egg in moles/l, while R is the gas constant and T is the absolute temperature. Hydrostatic pressure difference between the outside and inside of the egg is represented by ΔP.

The dissipation function, $\Phi = T\mathrm{d}s/\mathrm{d}t$, where $\mathrm{d}s/\mathrm{d}t$ is the rate of internal entropy production per unit area of membrane, may be written for three solute components as:

$$\Phi = \dot{n}_w\Delta\mu_w + \sum_{k=1}^{3}\dot{n}_k\Delta\mu_k \tag{1}$$

In order to apply this equation several approximations must be made which are difficult to verify experimentally. Although the approximations make an exact quantitative interpretation of the equations difficult, they do not affect the general conclusion that the flow of one solute or the flow of the solvent may affect the flow of another solute.

When the concentration difference is small, the difference in chemical potential for the solute may be approximated by

$$\Delta\mu_k = \frac{RT}{c_k}\Delta c_k + v_k\Delta P = \frac{\Delta\pi_k}{c_k} + v_k\Delta P$$

where v_k is the partial molar volume for solute k. The chemical potential difference for the solvent is expressed with the help of the Gibbs–Duhem equation as

$$\Delta\mu_w = v_w\Delta P - \sum_{k=1}^{3}\frac{RT}{c_w}\Delta c_k = v_w\Delta P - \sum_{k=1}^{3}\frac{\Delta\pi_k}{c_w}$$

When these substitutions are made into the equation for Φ we get

$$\Phi = \dot{n}_w v_w + \sum_{k=1}^{3}\dot{n}_k v_k\Delta P - \sum_{k=1}^{3}\left[\frac{\dot{n}_k}{c_k} - \frac{\dot{n}_w}{c_w}\right]\Delta\pi_k \tag{2}$$

which is now in the usual form of a sum of products of forces, represented by ΔP and $\Delta\pi_k$, and fluxes defined as follows:

$$J_v \equiv \dot{n}_w \bar{v}_w - \sum_{k=1}^{3} \dot{n}_k \bar{v}_k \qquad J_{D_k} \equiv \frac{\dot{n}_k}{c_k} - \frac{\dot{n}_w}{c_w} \tag{3}$$

J_v is recognizable as the total volume flow into the cell per unit area of membrane, while J_{D_k} is the velocity of solute k relative to the solvent.

According to the basic principles of the thermodynamics of irreversible processes, these flows may be expressed as linear functions of the forces:

$$\begin{aligned} J_v &= L_P \Delta P + L_{PD_1} \Delta\pi_1 + L_{PD_2} \Delta\pi_2 + L_{PD_3} \Delta\pi_3 \\ J_{D_1} &= L_{D_1P} \Delta P + L_{D_1} \Delta\pi_1 + L_{D_1D_2} \Delta\pi_2 + L_{D_1D_3} \Delta\pi_3 \\ J_{D_2} &= L_{D_2P} \Delta P + L_{D_2D_1} \Delta\pi_1 + L_{D_2} \Delta\pi_2 + L_{D_2D_3} \Delta\pi_3 \\ J_{D_3} &= L_{D_3P} \Delta P + L_{D_3D_1} \Delta\pi_1 + L_{D_3D_2} \Delta\pi_2 + L_{D_3} \Delta\pi_3 \end{aligned} \tag{4}$$

When the reciprocal relationships

$$L_{PD_k} = L_{D_kP} \text{ and } L_{D_kD_j} = L_{D_jD_k}$$

are invoked; the substitutions,

$$L_P = -L_{PD_3},\ L_{D_1D_3} = -L_{PD_1}$$

and

$$L_{D_2D_3} = -L_{PD_2}$$

arising from the impermeability of solute 3 are made; the hydrostatic pressure difference is assumed to be zero; and the equations are rearranged, we get:

$$\begin{aligned} J_v &= L_{PD_1} \Delta\pi_1 - L_{PD_2} \Delta\pi_2 - L_P \Delta\pi_3 \\ \dot{n}_1 &= c_1(J_v + L_{D_1} \Delta\pi_1 + L_{D_1D_2} \Delta\pi_2 - L_{PD_1} \Delta\pi_3) \\ \dot{n}_2 &= c_2(J_v - L_{D_1D_2} \Delta\pi_1 - L_{D_2} \Delta\pi_2 - L_{PD_2} \Delta\pi_3) \end{aligned} \tag{5}$$

These equations are the results we are seeking from the thermodynamics of irreversible processes, and may be considered a starting point for the experimental investigation of the effects of one solute flow on the flow of another solute. They described the net volume flow and the flow of each permeable solute in the presence of any concentration gradient of the other solutes.

A comparison of these equations with equations which neglect solute–solvent and solute–solute interactions makes the significance of the new terms apparent. Classical equations which have no interaction terms are given below:

$$\begin{aligned} J_v &= -L'_P(\Delta\pi_1 + \Delta\pi_2 + \Delta\pi_3) \\ \dot{n}_1 &= c_1 L'_{D_1} \Delta\pi_1 \\ \dot{n}_2 &= c_2 L'_{D_2} \Delta\pi_2 \end{aligned}$$

Two points are immediately apparent. First, the volume flow in the classical equations depends in the same way on all concentration differences, either of permeable or impermeable solutes, while the new equations show a separate dependence of volume flow on each solute. Second, the rate of penetration of any permeable solute depends on the concentration difference of that solute alone when interactions are ignored, but depends on volume flow and concentration differences of all solutes independently when interactions are taken into account.

The varying contributions of different solutes to volume flow have been discussed fully by KEDEM AND KATCHALSKY who introduce STAVERMAN'S reflection coefficient, σ_k, defined by

$$\sigma_k = -L_{PD_k}/L_P,$$

to take these variations into account. The additional terms in the equations for solute flow are the ones we wish to focus attention on here.

It is clear from Eqns. 5 that the flow of a permeable solute depends not only on the concentration gradient of that solute, but also on the total volume flow and on the concentration gradients of the other solutes present. The exact significance of terms such as

$$L_{D_1D_2}\Delta\pi_2 \text{ and } -L_{PD_1}\Delta\pi_3 = L_{D_1D_3}\Delta\pi_3$$

in the flow of solute 1 can be seen in the defining Eqns. 4. Here, J_{D_1} may have a non-zero value in the absence of either a pressure gradient or gradient in concentration of solute 1 if

$$L_{D_1D_2}\Delta\pi_2 \text{ and } -L_{PD_1}\Delta\pi_3$$

differ from zero. Thus, a concentration difference of solute 2 or 3 can cause a flow of solute 1 relative to the solvent even when there is no pressure gradient or concentration gradient of solute 1 itself.

A system of differential equations describing volume and solute flows in a sand dollar egg may be obtained directly from Eqns. 5. If the right-hand side of each of these equations is multiplied by the area of the cell membrane, the resulting expressions give the rates of increase of volume and solute content of the cell. A difficulty arises in evaluating $\Delta\pi_k$ for the permeable solutes, however, because of the impossibility of measuring the internal solute concentration directly. In keeping with the assumptions of other authors, the internal concentration of solute k, c_k^i, has been set equal to $n_k^i/(V-b)$ where n_k^i is the number of moles of k within the cell, V is the cell volume, and b is the volume of osmotically inactive material within the cell.

No general solution of the resulting differential equations has been found. An appreciable simplification results if experimental conditions are chosen such that the initial rate of volume change is zero. The volume and concentration terms can then be expressed as power series expansions in time, carrying only the leading terms. Very satisfactory approximations to both experimentally observed volume changes and volume changes calculated numerically from the differential equations resulted from this simplified approach. Because of the complicated nature of the system and the limitations of the experimental method, however, no additional conclusions were drawn from this approach and it is not presented here in greater detail.

Initial rates of volume change, although somewhat difficult to evaluate experimentally, avoid some of these difficulties. Thus, by equilibrating the eggs in solutions

of known concentration before transferring them to experimental solutions, initial concentration differences can be determined for each component.

Further difficulties in interpretation might arise from solute–solute interactions at equilibrium which would alter the activity coefficients of each component. Thus far, there have been no determinations of activity coefficients for these solutes in solutions simulating the cell interior and such possible effects have, therefore, been neglected.

The use of Eqns. 5 and the experimental evaluation of the individual terms in the equations is considered in a later section.

EXPERIMENTAL METHODS

The investigation of solute–solute interactions involved direct measurements of total volume flow across the plasma membrane of the sand dollar egg (*Echinarachnius parma*). The general methods used were similar to those employed by JACOBS[8] and others in early studies on Arbacia eggs; the essential difference lay in the choice of initial conditions for the experiments.

The unfertilized sand dollar egg was chosen as a readily available experimental system with a low metabolic rate which would allow passive movement of the experimental solutes. Interaction between acetamide and propionamide was investigated first, but these substances seemed somewhat toxic to the eggs and ethylene glycol and propylene glycol were substituted. All of these solutes penetrate the eggs at a rate conveniently followed by visual methods.

The procedure for a typical experiment may be outlined as follows. Eggs were shed into sea water or experimental solution contained in Syracuse dishes by injection of 0.5 ml of 0.5 M KCl through the mouth of the sand dollar. Collection and all other manipulations were carried out at room temperature which was between 16° and 18° on experimental days. The entire unfertilized egg including jelly coat was employed for all experiments and comparisons between runs with and without the addition of a second solute were always performed on aliquots of the same batch of eggs. Each aliquot was drained and washed three times at 15-min intervals with the equilibrating solution, and in cases where a solute as well as water was required to equilibrate, 1 h elapsed before transfer of the eggs to a new experimental solution.

After equilibration, about 100 eggs were transferred to the bottom of another Syracuse dish containing the new solution mounted in the mechanical stage of an ordinary Bausch and Lomb light microscope. Time zero was taken from the moment when the eggs were released in the new solutions. Volume changes were observed initially through a 10 × objective and a 10 × measuring eyepiece, but all experiments reported here were recorded on 35-mm Plus-X film with a photomicroscopic attachment which allowed simultaneous visual observation of the swelling or shrinking eggs. This method of recording data was not only more precise, but allowed the volume changes in several different eggs to be followed at once. At some point during each experiment, the grid on a hemocytometer chamber was photographed for determination of the magnification factor. The grid itself had been calibrated previously on a toolmaker's microscope.

At the conclusion of the experiment, egg volumes were determined as a function of time by projecting the photographic image onto a glass-topped desk where the cross-sectional area of each egg could be determined with a Keuffel and Esser plani-

meter. Calibration of the planimeter and determination of the magnification factor involved tracing representative areas of the hemocytometer grid. At the sea water concentration employed, the eggs were nearly perfect spheres and it was possible to calculate total volume by the appropriate formula from the cross-sectional area.

A disadvantage of this technique is the time involved in tracing and plotting data on individual eggs. Such an approach is very useful, however, in establishing uniformity of response among different eggs and would serve as the basis for calibration of other optical methods which measure averages. Preliminary experiments indicated that useful swelling curves could be obtained with a light-scattering method.

Equilibrium volumes were determined as a function of sea water concentration on aliquots of eggs from several different sand dollars. The activity of the water component in sea water was determined by freezing in a Fiske osmometer, and the results were expressed both as osmolal and osmolar concentrations. In order to make this conversion, solid content of sea water was determined by evaporation to dryness at 135°.

Solutions were made by weighing quantities of solute, adding the required volume of sea water, and diluting to volume with distilled water. Eastman-grade highest purity chemicals were used throughout. Acetamide and propionamide were both dried *in vacuo* over silica gel, and the glycols were dried by passage through a silica-gel column. Freezing-point determinations were made on all experimental solutions.

A primary concern throughout the experiments was that the plasma membrane might be damaged either by manipulation or exposure to the solutes. On several occasions after the kinetic experiments had been completed, therefore, eggs were transferred from the experimental solutions to pure sea water, allowed to re-equilibrate, and fertilized. In experiments with the glycols, high rates of fertilization were obtained and initial cell divisions appeared normal. Similar experiments with the amides indicated damage to the eggs and led us to change solutes.

The first data obtained led to a relationship between equilibrium volume and sea water concentration. It was found that eggs transferred to sea water diluted by volume to 62.5 vol.% sea water were conveniently round and could either swell or shrink without losing their spherical configuration. Most experiments were performed, therefore, from this initial sea water concentration. The general plan of the experiments was to transfer aliquots of eggs from the 62.5 vol.% sea water to solutions more dilute in sea water but containing added glycol. Sea water and glycol concentration differences were carefully balanced to produce an initial volume flow close to zero. Parallel experiments were then performed with the second glycol present in equal concentrations both inside and outside the egg to note the effect of a second permeable solute.

RESULTS AND DISCUSSION

Dependence of equilibrium volume on sea water concentration

Before experiments with penetrating solutes were undertaken, certain general properties of the sand dollar egg were investigated. The eggs remained intact and changed volume reversibly over the concentration range from 40 vol.% to twice the normal strength of sea water. All kinetic experiments reported here, however, employed eggs initially equilibrated in 50–100 vol.% sea water.

The average volumes of 30 egg aliquots from one sand dollar are plotted against the reciprocal of the sea water concentration in Fig. 1. As shown in this figure, the equilibrium volume, V, follows the familiar expression, $c = a/(V - b)$, where c is the concentration of sea water, a is the effective impermeable solute content of the egg, and b is the osmotically inactive volume of the egg in the same units as V.

Variations in egg volume at any given sea water concentration for a single sand dollar were within ± 10 %. Although variation in size among eggs from different sand dollars was somewhat greater, the dependence on concentration was similar with both a and b increased or decreased by a factor related to the size of the eggs.

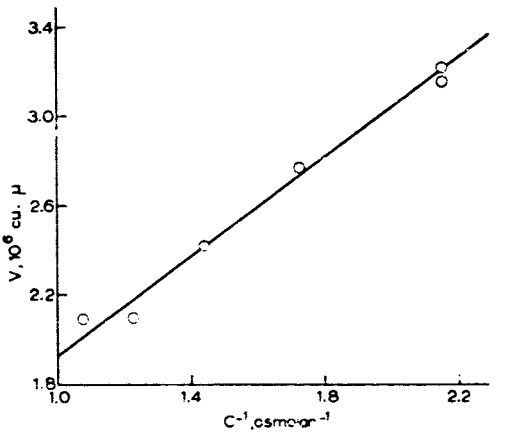

Fig. 1. Dependence of equilibrium volume of sand dollar eggs on sea water concentration.

Values for the constants in Fig. 1 are $a = 1.120 \cdot 10^6$ and $b = 0.802 \cdot 10^6$ when c is expressed in osmoles/l and V is in cubic micra. Comparison of b with the volume of an egg in pure sea water shows that nearly 40 % of the egg is osmotically inactive. This compares with only about 11 % in the Arbacia egg.

The osmotic concentration of sea water used in plotting Fig. 1, together with values for representative glycol solutions, are given below in Table I. The osmotic

TABLE I

OSMOTIC CONCENTRATIONS OF EXPERIMENTAL SOLUTIONS

Solution No.	*Vol. % sea water*	*Propylene glycol concn. (M)*	*Ethylene glycol concn. (M)*	*Osmolal concn.*	*Osmolar concn.*
1	37.5	0	0	0.350	0.346
2	62.5	0	0	0.578	0.568
3	87.5	0	0	0.814	0.797
4	100	0	0	0.948	0.933
5	37.5	0.25	0	0.637	0.626
6	37.5	0.50	0	0.944	0.902
7	37.5	1.00	0	1.610	1.477
8	62.5	0	0.50	1.152	1.103
9	37.5	0.50	0.50	1.502	1.390
10	33.0	0.50	0	0.878	0.838
11	33.0	0.50	0.50	1.479	1.370

concentration of the sea water solutions increased linearly with increased volume concentration of sea water, but mixtures with glycol showed some deviation from ideality.

Experiments with one permeable solute

As noted in the theoretical section, a detailed analysis of swelling curves is greatly simplified if conditions are chosen such that the initial volume flow is zero. Accordingly, a series of experiments was performed with each permeable solute in which eggs equilibrated in 62.5 vol. % sea water were transferred to solutions more dilute in sea water, but containing the permeable solute in varying concentrations.

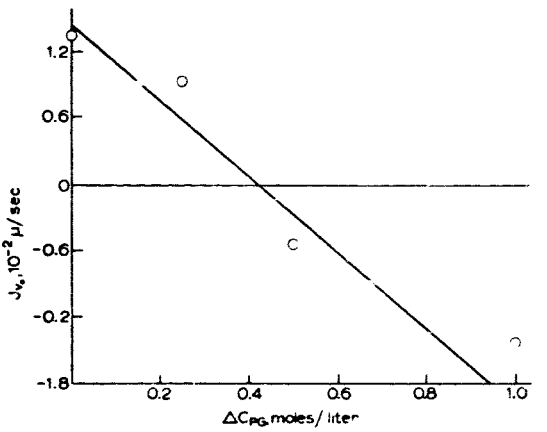

Fig. 2. Dependence of initial rate of swelling on external propylene glycol concentration. Eggs transferred from 62.5 vol.% sea water to 37.5 vol.% sea water containing varying concentrations of propylene glycol.

The results from one such experiment with propylene glycol are shown in Fig 2, where J_{v_o}, the initial volume flow into the egg, is plotted against the propylene glycol concentration difference.

In the experiments of Fig. 2, eggs were transferred from 62.5 vol.% sea water to solutions 1, 5, 6, and 7 of Table I. There was thus an initial concentration difference between the inside and outside of the egg of 0.22 osmole/l of sea water. To offset this concentration difference, and to produce an initial volume flow of zero, 0.42 M propylene glycol was required in the external solution. As may be seen from substitution in Eqns. 5 with $J_v = 0$, $\Delta\pi_1 = 0.42\,RT$, $\Delta\pi_2 = 0$, and $\Delta\pi_3 = -0.22\,RT$, the ratio $\sigma_{\text{propylene glycol}} = -L_{PD_1}/L_P$ then has the value 0.52.

In order to test Eqns. 5 for J_{v_o} over a wide range of initial conditions, RTL_P was determined from an average of swelling experiments involving a gradient of sea water concentration only. A value of $5.33 \cdot 10^{-2}$ was obtained when Δc_3 for sea water was expressed in osmoles/l and J_{v_o} was expressed in μ/sec. The constant, RTL_{PD_1}, was determined by multiplying the average value of RTL_P by the value of $-\sigma_{\text{propylene glycol}}$ from the experiment shown in Fig. 2, resulting in a value of $-3.05 \cdot 10^{-2}$. Experimentally determined intial rates were then plotted in Fig. 3 against those calculated from Eqns. 5 with $J_v = -3.05 \cdot 10^{-2}\,\Delta c_{\text{propylene glycol}} - 5.33 \cdot 10^{-2}\,\Delta c_3$. Each point in Fig. 3 represents the average rate for from two to

several different eggs under conditions varying from positive to negative concentration gradients in both sea water and propylene glycol. Agreement with the predicted value is fairly good and some of the residual scatter undoubtedly results from the inclusion of data on aliquots of eggs from several different sand dollars.

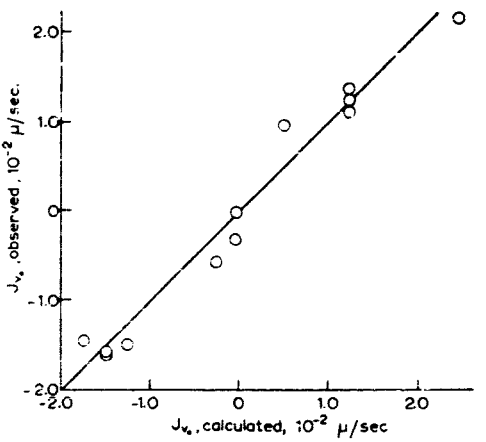

Fig. 3. Comparison between initial rates of swelling determined experimentally and calculated from Eqns. 5.

The expression for volume flow in Eqns. 5 thus appears to be adequate over a wide range of conditions. The experimental approach indicated above, with each permeable solute being studied separately, is also a practical method of evaluating 3 of the 6 constants in Eqns. 5.

Values for the reflection coefficient, σ, obtained in this manner on three different solutes are given below in Table II. Also included for comparison are data obtained by GOLDSTEIN AND SOLOMON[4] on the human erythrocyte. It is interesting to note that propylene glycol has a lower value of σ than ethylene glycol in the sand dollar, but a higher value in the erythrocyte. This difference may be related to the fact that these solutes are thought to penetrate through pores in the erythrocyte, but probably enter the sand dollar egg through a lipid phase.

Experiments with two permeable solutes

The remaining 3 constants in Eqns. 5 could be evaluated by comparing experimental swelling curves with those obtained from the numerically integrated form of the equations. The magnitude of $L_{D_1D_2}$ would then be a direct measure of the interaction between solutes 1 and 2. Such an approach was attempted here, but it was concluded that a direct measure of interaction utilizing tagged compounds would be a better experimental approach. The data presented here, therefore, simply indicate an effect of a second solute on the swelling curve obtained with the first solute.

Typical experiments were carried out in the manner described below. One aliquot of eggs was equilibrated in 62.5 vol.% sea water containing 0.5 M ethylene glycol. After equilibration, the first aliquot was transferred to a 33-vol.% sea water solution containing 0.5 M propylene glycol, and a swelling curve was obtained. A simil-

swelling curve was obtained when the second aliquot was transferred to a 33-vol. % sea water solution containing 0.5 M ethylene glycol and 0.5 M propylene glycol. Since there was no initial ethylene glycol concentration gradient in the second case, the ethylene glycol could alter the early part of the swelling curve only by interacting with the solvent or solute flow, or, possibly, by affecting the membrane.

TABLE II

REFLECTION COEFFICIENTS

Solute	Reflection coefficients	
	Sand dollar egg	Erythrocyte
Ethylene glycol	0.82	0.63
Acetamide	0.76	0.58
Propylene glycol	0.52	0.85

Some indication of interaction is shown in Fig. 4. In this figure, the comparison between swelling curves in the presence and absence of ethylene glycol is shown in 4 separate experiments. Each curve represents an average for all the eggs present in the photographic field. The only variation in the experimental design given above is that the dilute sea water in Run A was 37.5 vol.% in each case instead of 33 vol.%.

The curves in the presence of ethylene glycol seem different over their entire range, but it is difficult to evaluate how much of this difference is directly related to solute–solute interaction without a more elaborate kinetic treatment. Apparent differences in rate at time zero would not be predicted from Eqns. 5, and some of the observed difference may represent difficulty in extrapolating accurately back to time zero. Ethylene glycol could also affect the permeability of the membrane directly, altering all of the constants in Eqns. 5. We intend, therefore to obtain additional experimental data on individual solute flows with the aid of isotopically labelled

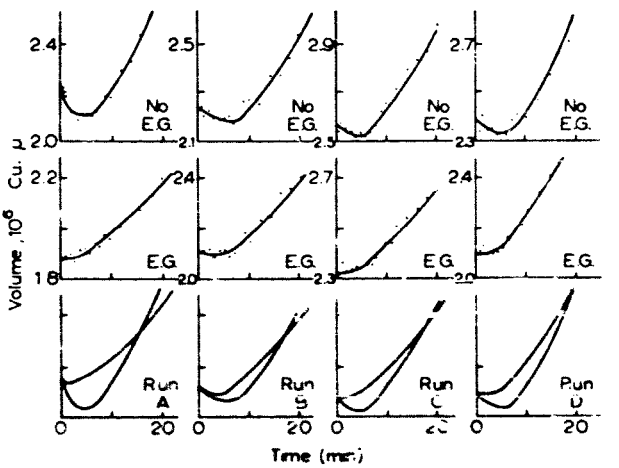

Fig. 4. Effect of ethylene glycol on propylene glycol swelling curves. Top row shows experimental data in the absence of ethylene glycol; the second row, in the presence of ethylene glycol, and the bottom row, a direct comparison.

compounds to evaluate the importance of each of these effects. We can, however, conclude from the present data that the addition of a second permeable solute markedly affects the nature of the swelling curves obtained with the first solute and that some of this effect may be related to solute–solute interactions.

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