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A MOLECULAR BASIS FOR AN IRREVERSIBLE THERMODYNAMIC DESCRIPTION ON NON-ELECTROLYTE PERMEATION THROUGH LIPID BILAYERS

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Summary

The non-electrolyte permeability of liposomal membranes has been investigated according to the concepts of irreversible thermodynamics. A strong interaction between the permeation of solute and water was observed. This solute-solvent interaction can be fully described by assuming that a number of water molecules will copermeate with each molecule of solute. This number of copermeating water molecules is independent of the nature of the permeant and of temperature, but depends on the osmotic concentration of impermeants inside the liposomes.

Introduction

Liposomes have been widely used for studying the permeability properties of lipid bilayers [1—3]. A method used frequently for determining the permeation rate of non-electrolytes through liposomal membranes is the measuring of the initial volume change of cells when suspended in an isotonic solution containing the permeant [2,3]. The isotonic swelling rate proved to be dependent on the lipid composition of the cell membrane and showed an exponential increase with rising temperature. From this temperature dependence values were obtained for the activation energy involved in these processes [2,3]. This method of measuring permeabilities was criticized by Hill and Cohen [4], based on the irreversible thermodynamic description of non-electrolyte permeation of Kedem and Katchalsky [5]. Using this approach as a starting point, the simul-

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taneous permeation of a non-electrolyte and water through a lipid bilayer should be described by a three parameter model. A method for the determination of these three parameters is given in ref. 6. It will be shown in this paper that measurement of the isotonic swelling rate is indeed insufficient for describing non-electrolyte permeation through lipid bilayers, since interaction between solute and solvent permeation is not taken into account. The results obtained with the method of isotonic swelling will be reinvestigated in the light of this irreversible thermodynamic approach. In addition, a molecular basis will be given for explaining this irreversible thermodynamic description of the system.

Materials and Methods

Materials. Egg phosphatidylcholine was purified from egg yolk by acetone precipitation and subsequent chromatography over alumina oxide and silica gel. Egg phosphatidic acid was prepared from egg phosphatidylcholine according to Davidson and Long [7]. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (14 : 0/14 : 0-phosphatidylcholine), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (18 : 1_c/18 : 1_c-phosphatidylcholine) and 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (22 : 1_c/22 : 1_c-phosphatidylcholine) were synthesized as described previously [8]. All other reagents were of Analytical Reagent Grade and used without further purification.

Permeability studies. Multilamellar liposomes, containing 4 mol% of egg phosphatidic acid were prepared [2] in 20 mM glucose (unless stated otherwise) up to a concentration of 2 μmol lipid/ml; 1 ml of this solution was injected in 9 ml of the desired solution in a thermostated cuvette equipped with a stirrer. Volume changes (dV/dt) were measured from relative changes in absorbance at 450 nm ($d A_{450}/dt$), using the experimental relationship $dV/dt = k\, d\, 1/A_{450}/dt$, in which k is a spectroscopic constant [9]. Changes in absorbance were measured on a Vitatron spectrophotometer (MPS type).

Results

Theory

In the irreversible thermodynamic considerations of Kedem and Katchalsky [5], the net flow of solute and water through the membrane of a selectively permeable cell are described by the following equations:

$$\frac{dV}{dt} = -AL_pRT(\Delta c_i + \Delta c_s) + AL_pRT(1-\sigma)\Delta c_s \tag{1}$$

$$\frac{dn_s}{dt} = A\omega RT\,\Delta c_s + \frac{dV}{dt}(1-\sigma)\frac{\Delta c_s}{\Delta \ln c_s} \tag{2}$$

In these equations V stands for volume and A for the outer area of the cells, while n_s is the amount of permeant trapped in the cells. Δc_i is the osmotic concentration difference over the membrane of all solutes which are impermeants and Δc_s the concentration difference of the single non-electrolyte which is a permeant.

The system parameters L_p (filtration coefficient), ω (permeation coefficient) and σ (reflection coefficient) are phenomenological coefficients describing the

conductivity of the membrane for water, the conductivity for the solute and the interaction between solute and solvent permeation, respectively. When a cell containing only impermeants is suspended in an isotonic solution containing a non-electrolyte which is a permeant with concentration c_s^0, the initial permeation rates of the solute and of water can be derived from the above general equations, giving:

$$\frac{dV}{dt} = AL_pRT(1 - \sigma)c_s^0 \tag{3}$$

$$\frac{dn_s}{dt} = A\omega RTc_s^0 \tag{4}$$

Each change in cell volume will be the result of a combination of the volume change produced by water transport and of that produced by solute transport. Since the latter can be found by multiplying the rate of solute permeation (Eqn. 4) by the molar volume of the solute ($\bar{V}_s$), the volume change produced by water permeation under these isotonic conditions is given by:

$$\frac{dV_w}{dt} = AL_pRTc_s^0\left((1 - \sigma) - \frac{\omega\bar{V}_s}{L_p}\right) \tag{5}$$

In case water and solute can permeate independently from each other $(1 - \sigma) = \omega\bar{V}_s/L_p$ (see ref. 5), and no initial water permeation will take place under these isotonic conditions. As will be shown, however, a high solute-solvent interaction takes place during non-electrolyte permeation through lipid bilayers, making $(1 - \sigma) >> \omega\bar{V}_s/L_p$.

It can be concluded that a net permeation of water takes place in the isotonic medium, as given by Eqn. 5, which can not be described by a process of osmosis. As will be shown in this paper, this net permeation of water can be fully described by assuming that for each molecule of solute passing the membrane a number (N) of water molecules will copermeate. The implication of this hypothesis is that each mole of solute passing the membrane will give rise to a volume change given by $\bar{V}_s + N\bar{V}_w$, in which $\bar{V}_w$ is the molar volume of water. The initial volume change taking place when a cell is suspended in an isotonic medium containing the permeant is then given by:

$$\frac{dV}{dt} = A\omega RTc_s^0(\bar{V}_s + N\bar{V}_w) \tag{6}$$

Since this process is also described by eqn. 3, it follows

$$(1 - \sigma) = \frac{\omega(\bar{V}_s + N\bar{V}_w)}{L_p} \tag{7}$$

and

$$N = \frac{\bar{V}_s}{\bar{V}_w}\left(\frac{(1 - \sigma) - \omega\bar{V}_s/L_p}{\omega\bar{V}_s/L_p}\right) \tag{8}$$

Since both σ and $\omega\bar{V}_s/L_p$ can be determined for liposomes, it is possible to calculate N. In this paper some of the properties of N will be analysed.

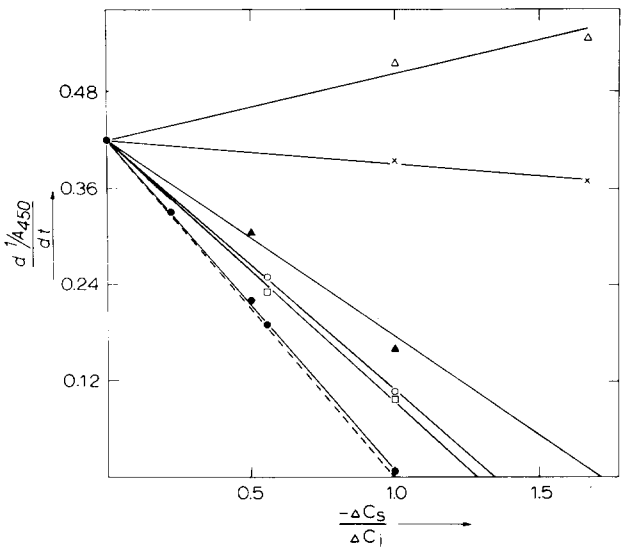

Fig. 1. Initial swelling rate as a function of permeant concentration in the outside medium for permeation of different solutes through bilayers of egg phosphatidylcholine/4 mol% egg phosphatidic acid at 15°C. Liposomes have been made in 20 mM glucose. - - - - -, glucose; ●———●, erythritol; □———□, glycerol; ○———○, urea; ▲———▲, thiourea; X———X, glycol; △———△, 1,3 propanediol.

Measurement of σ, $\omega\bar{V}_s/L_p$ *and* N

When a cell, containing only solutes which are impermeants, is suspended in a solution of a permeant, a linear relationship is found between the initial change in absorbance (450 nm) of the suspension and the concentration of the permeant in the outside medium (ref. 6, see also Fig. 1). From this linear relationship σ and kAL_pRT can be determined, in which k is a spectroscopic constant relating volume changes to changes in absorbance. When a cell is suspended in an isosmotic medium, defined by $\Delta c_i + \sigma\Delta c_s = 0$, the first phase with no change in volume is followed by a phase during which the cell volume increases linearly with time [6]. From this linear swelling rate $kA\omega RT$ can be determined (for more details see Appendix I). So, although L_p and ω can only be determined as relative parameters since k and A are normally unknown, the value of $\omega\bar{V}_s/L_p$ can be determined in an absolute way *.

Fig. 1 shows the initial change in absorbance as a function of permeant concentration for liposomes of egg phosphatidylcholine at 15°C for different solutes. Table I shows the values for the parameters σ, $\omega\bar{V}_s/L_p$ and N for the different solutes under these conditions. The values of σ can be determined fairly accurately with a standard error of about 0.03; since N and $\omega\bar{V}_s/L_p$ are measured in a rather indirect way, the relative errors in these parameters were found to be in the

* The parameter $\omega\bar{V}_s/L_p$ gives the ratio of the solute and water permeation coefficient for the free diffusion process corrected for differences in molar volume between solute and solvent. Since L_p can be visualized by the product of the permeation coefficient for water and the molar volume of water, the ratio of the permeation coefficients of water (ω_w) and solute (ω_s) is given by:

$$\omega_w/\omega_s = (\bar{V}_s/\bar{V}_w)\left(1\Big/\frac{\omega\bar{V}_s}{L_p}\right) \qquad (9)$$

TABLE I

IRREVERSIBLE THERMODYNAMIC PARAMETERS, DESCRIBING THE PERMEATION OF DIFFERENT NON-ELECTROLYTES THROUGH BILAYERS OF EGG PHOSPHATIDYLCHOLINE AT 15°C

Liposomes, containing 4 mol % egg phosphatidic acid, were prepared in 20 mM glucose. Molar volumes ($\bar{V}_s$) were calculated from molecular weight and density, and are expressed in l/mol. The molar volumes of water V_w = 0.018 l/mol (data taken from Handbook of Chemistry and Physics; The Chemical Rubber Co., 49th edition (1968)). Standard errors in σ are 0.01—0.03, and in $\omega\bar{V}_s/L_p$ and N between 10 and 20% (unless stated otherwise).

Solute	$\bar{V}_s$	σ	$\omega\bar{V}_s/L_p$	ω_w/ω_s	N
Glucose	0.115	1.00	—	—	—
Erythritol	0.084	0.98[5]	0.000017	300 000	3000—5000
Glycerol	0.073	0.78	0.000363	12 000	2450
Urea	0.045[5]	0.74	0.000226	11 000	2900
Thiourea	0.054	0.54	0.000501	6 500	2750
Glycol	0.056	0.08	n.d.	n.d.	n.d.
1,3-Propanediol	0.072	—0.4	?	?	?

order of 20%. Table I shows that for all solutes tested $(1-\sigma) >> \omega\bar{V}_s/L_p$, which is illustrative for a high solute-solvent interaction [5]. No significant variation in the value of N was observed for those solutes which could be tested accurately under the conditions used. It is striking that the value of N is very near to the ratio of water and solute molecules inside the liposomes (R). In order to test a possible correlation between these two parameters, the permeability properties of liposomes prepared in different glucose concentrations were investigated, thus varying the value of R. As is shown in Table II, the value of N indeed closely follows the value of R. The values of L_p and ω can not be compared directly for these different liposomes, since it is not known whether A changes or not. It is remarkable, however, that the value of $\omega\bar{V}_s/L_p$ changes with inside glucose concentration while the value of σ remains almost constant. Obviously the permeability properties of liposomes, prepared in different glucose concentrations, are difficult to compare.

Temperature dependence of non-electrolyte permeation

The initial swelling rate of liposomes in isotonic media of non-electrolytes was found to increase exponentially with rising temperature [3]. This made it

TABLE II

COMPARISON OF LIPOSOMES, PREPARED IN DIFFERENT GLUCOSE MEDIA, WITH RESPECT TO THEIR PERMEABILITY TO THIOUREA AT 15°C

Liposomes of egg phosphatidylcholine contained 4 mol % phosphatidic acid. R is the ratio of the number of water and solute molecules in the different glucose media.

Liposomes prepared in	L_p	σ	ω	$\omega\bar{V}_s/L_p$	N	R
10 mM glucose	34.5 *	0.52	0.174 *	0.000272	5300	5500
20 mM glucose	24.1 *	0.54	0.224 *	0.000501	2700	2750
40 mM glucose	20.0 *	0.59	0.349 *	0.000941	1300	1375

* Relative units.

possible to express the temperature dependence of such processes as an activation energy (normally given in kcal/mol). The value of the activation energy for isotonic non-electrolyte swelling was independent of the membrane lipid composition and depended only on the nature of the permeating non-electrolyte. From the correlation between the value of the activation energy and the polarity of the permeant, as was observed for certain poly-alcohols, it was concluded that the penetration of the non-electrolyte into the lipid bilayer in a dehydrated form is the rate limiting step for this permeation process [3,10]. Also the temperature dependence of water permeation through phospholipid bilayers in the liquid-crystalline state was independent of the lipid composition [11]. The values of the activation energies were reinvestigated by Blok et al. [12], showing that for certain cholesterol containing bilayers the activation energy for both water permeation and isotonic non-electrolyte swelling was enhanced, making them functions of both the nature of the permeant and of the membrane lipid composition. The permeability properties of these cholesterol-containing bilayers and also of bilayers in the gel state will be described in the accompanying paper [14]. Using the irreversible thermodynamic approach of Hill and Cohen [4], Cohen [13] measured the temperature dependence of the permeation coefficient for different solutes, finding values that differ significantly from those determined from isotonic swelling rates. However, their approach for measuring permeation coefficients turned out to be incorrect [6]. In this paper the temperature dependence of the irreversible thermodynamic parameters (σ, ω, L_p, $\omega\bar{V}_s/L_p$ and N) are investigated. Although ω and L_p can only be determined as relatively parameters, the temperature dependence of these parameters can be determined in an absolute way, assuming that the outer area and light-scattering parameters are independent of temperature.

Table III shows the temperature dependence of the irreversible thermodynamic parameters, all expressed in kcal/mol, for the permeation of glycerol through liposomal membranes as a function of the lipid composition. The activation energy for L_p, which is the activation energy for water permeation, and that for isotonic glycerol swelling were found to be constant and to be in

TABLE III

TEMPERATURE DEPENDENCE OF THE PERMEATION OF GLYCEROL AS A FUNCTION OF THE MEMBRANE LIPID COMPOSITION

Activation energies are given in kcal/mol, mean ± S.D.

Membrane lipid composition *	Isotonic swelling	L_p	ω	$1-\sigma$	$\omega\bar{V}_s/L_p$	N
Egg phosphatidylcholine	18.7 ± 1.5 (18) [3] **	11.3 ± 1.0 (10.6) [12]	20.0 ± 1.7 (11.0) [13]	7.0 ± 1.3	8.6 ± 3.2	−1.6 ± 3.3
18 : 1_c/18 : 1_c-Phosphatidylcholine	18.2 ± 1.6 (17) [3]	10.5 ± 1.3 (11.4) [12]	16.6 ± 0.8	7.1 ± 0.8	6.1 ± 1.3	+0.8 ± 1.1
22 : 1_c/11 : 1_c-Phosphatidylcholine	16.0 ± 2.0	8.5 ± 2.1 (11) [12]	16.4 ± 2.0	7.2 ± 2.4	7.8 ± 4.2	+0.7 ± 2.6

* Besides phosphatidylcholine the liposomes always contained 4 mol % egg phosphatidic acid.

** Values taken from literature. Reference no. is in square brackets.

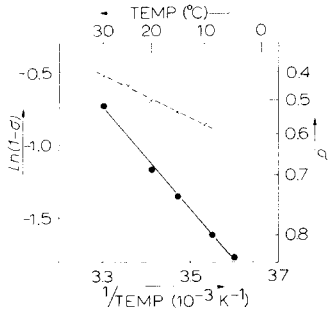

Fig. 2. Temperature dependence of the reflection coefficient (σ) for permeation of glycerol (●———●) and thiourea (X———X) through bilayers of egg phosphatidylcholine/4 mol % egg phosphatidic acid. Liposomes have been made in 20 mM glucose.

agreement with previous data [3,12]. According to Eqn. 3 the temperature dependence of isotonic swelling is in fact a combination of the temperature dependences of L_p and $(1-\sigma)$. It can be concluded therefore that $(1-\sigma)$ is strongly temperature dependent with an activation energy given by the difference in activation energies found for isotonic swelling and for water permeation. From this it follows that the temperature dependence of $(1-\sigma)$ is also independent of the membrane composition and is only a function of the nature of the permeating non-electrolyte. This is confirmed by the data presented in Table III and Fig. 2.

Measurements of ω as a function of temperature show that, within the error of determination, the activation energy of ω is not different from the value found from isotonic swelling rates, making N independent of temperature (see Eqn. 6). Table IV shows the temperature dependence of the permeation of different solutes through bilayers of egg phosphatidylcholine. In agreement with previous results, the temperature dependence of the isotonic swelling rate is a function of the nature of the permeating non-electrolyte, making the activation energy of $(1-\sigma)$ also a function of the permeant (see Fig. 2). Since

TABLE IV

TEMPERATURE DEPENDENCE OF THE PERMEATION OF NON-ELECTROLYTES THROUGH BILAYERS OF EGG PHOSPHATIDYLCHOLINE

Activation energies are given in kcal/mol, mean ± S.D.

Permeant	Isotonic swelling	L_p	ω	$1-\sigma$	$\omega\bar{V}_s/L_p$	N
Thiourea	14.3 ± 0.2	10.7 ± 1.1 (10.6) [12] *	13.5 ± 1.6 (8.4) [13]	3.0 ± 1.2	2.8 ± 1.2	+1.8 ± 2.0
Glycerol	18.7 ± 1.5 (18) [3]	11.3 ± 1.0 (10.6) [2]	20.0 ± 1.7 (11.0) [13]	7.0 ± 1.3	8.6 ± 3.2	−1.6 ± 3.3
Erythritol	21.3 ± 0.4 (21) [3]	10.6 ± 2.2 (10.6) [12]	22.9 ± 0.6 (16.1) [13]	10.3 ± 1.0	12.3 ± 1.8	−2.0 ± 1.4

* Values taken from literature. Ref. no. is in square brackets.

N is temperature independent for all solutes tested, it can be concluded that the temperature dependence of isotonic non-electrolyte swelling gives a good value for the temperature dependence of the permeation coefficient. In all cases significant differences were found between the values for the activation energies of ω presented here and those given by Cohen [13].

Discussion

The initial swelling rate of a cell suspended in an isotonic medium of a non-electrolyte has been used for measuring the permeation rate of small solutes which can cross the membrane in a way comparable with water. This is allowed if the permeation rate of water is so much faster than that of the permeant that each disturbance of isotonicity due to the permeation of the solute will be restored immediately. Since values are obtained in this paper for the ratio of water and solute permeation coefficients (ω_w/ω_s), it is possible to check the above hypothesis assuming that a mechanism of free diffusion and osmosis takes place. It will be shown in Appendix II that under the conditions used for the experiments in Table I, the ratio of solute and water permeation coefficients is such that a disturbance of isotonicity due to the permeation of the solute can not be fully restored by an osmotic flow of water. Since the actual number of water molecules that can cross the membrane by osmosis after the permeation of a solute molecule depends on the value of $\omega \bar{V}_s/L_p$ (see Appendix II), it will be strongly temperature dependent. It can, therefore, not be expected that under those conditions the temperature dependence of the isotonic swelling rate will coincide with that of the solute permeation coefficient.

From the following considerations, however, it can be seen that a mechanism of free diffusion and osmosis is not very realistic for the description of non-electrolyte permeation through lipid bilayers. When a cell is placed in an isosmotic medium of the permeant, the initial volume change of the cell will be zero because the volume change produced by the osmotic waterflow is exactly counterbalanced by the volume change produced by solute diffusion. The observation that for liposomes of egg phosphatidylcholine at 15° C the isosmotic concentration of thiourea is about twice the inside glucose concentration ($\sigma \approx 0.5$) can only be explained by a mechanism of free diffusion and osmosis ($(1 - \sigma) = \omega \bar{V}_s/L_p = 3\omega_s/\omega_w$) if the permeation coefficient of water is only 6 times that of thiourea. Besides the fact that such a ratio of water and solute permeation coefficient would not be very realistic, it is also not in agreement with the observed linear swelling rate of the cell in isosmotic media.

Using the irreversible thermodynamic description of non-electrolyte permeation of Kedem and Katchalsky [5], the system is not only described by a solute permeation coefficient (ω) and an osmotic coefficient for water permeation (L_p), but also by a third independent coefficient (σ), introducing the possibility of an interaction between the permeation of solute and solvent. Eqs. 1 and 2 can be interpreted in the following way. The first right-hand term of Egn. 1 describes the volume change of the cell produced by a net water flow due to the presence of a concentration difference of solutes over the membrane (osmosis). The second right-hand term of this equation describes the volume change produced by solute permeation and by permeation of water which is

driven by the solute gradient. This additional water flow is described in this paper by a copermeation of water molecules with the permeating solute molecules. The first right-hand term of Eqn. 2 describes the permeation of the solute driven by the solute gradient (diffusion). The second right-hand term of this equation describes the solute permeation driven by an osmotic gradient, and is only relevant when the permeant is present on both sides of the membrane. The amount of permeant both compartments have in common will be in constant exchange over the membrane including copermeating water. This fraction of the solute can be used for a net transport when a volume change of one of the compartments is required due to a difference in osmotic pressure over the membrane. Unless permeant is present inside the liposomes during the preparation, this term can be ignored for describing initial processes.

Since solute-solvent interaction ($(1-\sigma) >> \omega \bar{V}_s/L_p$) is important for the description of non-electrolyte permeation through lipid bilayers, a molecular model is required for explaining this interaction. The proposed model implying that during solute permeation water molecules are copermeating, not only explains the theoretically predicted initial water flow in the isotonic medium (see Eqn. 5), but also the linearity of the initial volume change of the cells as function of the permeant concentration in the outside medium (see Fig. 1). In addition, this model is able to explain why such a high concentration of solute is required for obtaining an isosmotic medium. This model also gives a clearer understanding of previous descriptions [10], stating that a solution of a permeant gives rise to a virtual osmotic pressure of $\sigma RT\, c_s$. Following this description solutions with the same value for RTc are called isotonic, and with the same value for σRTc isosmotic.

It is important to realize that copermeation of water is a process which is directly correlated with the solute permeation itself, and is therefore also relevant for the description of initial processes. An osmotic water permeation induced by solute permeation during the process of isotonic swelling, is not really an initial process and is, therefore, not described by the irreversible thermodynamic equations when applied to the conditions of zero time (see Eqns. 3 and 4). Whether induced osmotic water flows will interfere with the determination of the parameters for solute permeation will depend on the speed of the technique used for the measurements, also on the value of N (if N equals the water/solute ratio in the solution, no osmotic gradient is induced at all) and on the ratio of solute and water permeation coefficients, which determines to what extent an induced osmotic gradient can be levelled down. Computer simulation of the system has shown that interferences with non-initial processes could lead to deviations from the linearity observed in Fig. 1, resulting in an incorrect extrapolation towards the isosmotic medium. The real isosmotic medium with no initial volume change can always be found, however, by using a trial and error method for testing media around the extrapolated value. Large differences between the two methods were never observed; in case of discrepancies the experimentally measured isosmotic medium with an initial volume change of zero was used for determination of σ and ω (in this medium all initial volume changes will counterbalance, thus excluding the possibility that non-initial processes will interfere). The observed solute-solvent interaction during permeation of non-electrolytes through liposomal mem-

branes is not in agreement with the considerations of Lelievre and Rich [15] who assumed $(1 - \sigma) = \omega \bar{V}_s / L_p$ for these processes. However, when no interaction occurs ($N = 0$), measurements of isotonic swelling rates are sufficient for determining the permeation coefficient ω (see Eqn. 6), also without knowledge of σ (see ref. 15).

In this paper some of the properties of N are investigated. First of all, the linearity observed in Fig. 1 indicates that N is a parameter of the system. This means that independent of the outside concentration of the permeant always the same number of water molecules will copermeate per molecule of solute. Since N is very near to R, the number of copermating water molecules almost coincides with the number of water molecules that would have to cross the membrane by osmosis after the permeation of a solute molecule during isotonic swelling in order to restore isotonicity. This last equality does not hold, however, in media other than isotonic.

According to the principles of irreversible thermodynamics the driving force for permeation phenomena is a gain of entropy of the system. Therefore, interaction between the permeation of solute and solvent is only possible if it results in a higher gain of entropy per unit of time. It will be clear that during isotonic swelling the gain of entropy will be optimal when a number of water molecules will copermeate with each molecule of solute that equals the solute to water ratio in the solution. In this way it can be understood that the value of N is independent of the nature of the solute and of temperature, and in most cases also of the membrane lipid composition [14], but that it depends on the molarity of the solution in which the liposomes have been made.

In order to understand the molecular mechanism underlying this solute-solvent interaction, the following considerations can be taken into account. From the high values of the temperature dependence of the isotonic swelling rate it was concluded [10] that the rate limiting step during the permeation of this type of non-electrolytes is the penetration of the solute molecule into the lipid bilayer in a dehydrated form. Since the activation energy for ω closely correlates with the values obtained from isotonic swelling rates it can be assumed that this hypothesis still holds. No data can therefore be obtained in this way with respect to the rate of permeation of these dehydrated molecules inside the hydrophobic region of the bilayer. According to the theory of Dainty and Ginzburg [16], however, solute-solvent interaction results from a difference in the permeation rate of the non-electrolyte and of water inside the bilayer. It will be assumed therefore, that not only the rate of penetration, but also the rate of permeation through the bilayer is lower for the solute than for water.

Secondly, the amount of water that crosses the membrane per unit of time is very high. If, for example, an osmotic concentration difference of 20 mM is applied over the membrane, the observed net water transport will, in fact, be the result of an inward and outward flow which differ only 0.04%. The ratio of the permeation coefficients of water and thiourea during their permeation through bilayers of egg phosphatidylcholine at 15° C was found to be 6500 (see Table I). Since in an osmotic medium of 20 mM the concentration of water molecules is 2750 times that of the solute molecules, this implies that for each molecule of thiourea $1.8 \cdot 10^7$ molecules of water will enter the cell. In

addition an almost identical number of water molecules will leave the cell, because of the isotonicity of the system. From these considerations it can be seen that the "cavities" in the lipid bilayer through which these permeation processes take place, will constantly be used for water transport. The so-called kinks in the fatty acid chains of the lipid molecules are considered to be responsible for the formation of these "cavities" [17]. In this respect it should be realized that a water molecule has a diameter of 3.8 Å and a thiourea molecule of 5.5 Å (calculated from the molar volumes), while the average diameter of a lipid molecule is 8.7 Å (assuming an area per lipid molecule of 60 $Å^2$). This indicates that a "cavity" in the bilayer large enough for carrying a thiourea molecule can only be formed by a cooperative action of a number of lipid molecules. So, when a thiourea molecule penetrates the bilayer from outside, the lipid molecules in the outer monolayer will be compressed and the formation as other "cavities" in the direct environment of the solute molecule will be disfavoured. Therefore, the permeation of water molecules in the same direction of the solute molecule will be blocked in the micro-environment of the permeating solute until this molecule has reached the centre of the bilayer. At this state the outer monolayer will no longer be compressed and water molecules will now rapidly follow the permeating non-electrolyte. The permeation of water molecules in the direction opposite to the solute molecule will be blocked in this micro-environment during the whole period of time it takes the solute molecule to cross the bilayer. As soon as the solute molecule leaves the inner monolayer, water molecules can penetrate the bilayer again to move in the opposite direction, but the water molecules moving in the same direction as the solute molecule have then already reached the centre of the bilayer. In this way the water permeation opposite to the direction of the solute molecule will be reduced more than that in the same direction as the solute. The water molecules that will follow the permeating solute molecule and also the water molecules coming from the opposite direction can form complexes with the initially dehydrated non-electrolyte in the hydrophobic environment. The formation of these solute · water complexes will enlarge the size of the micro-environment and, thus, result in a reduction of water permeation in the direction opposite to the solute molecules over a larger area. The copermeation of water molecules during the permeation of a solute molecule is therefore partly due to a solute-water interaction inside the bilayer and partly due to a reduction of the water transport in the direction opposite to that of the permeating solute molecule.

From these considerations it can be imagined that each membrane will have a certain N value, which depends on the concentration of "cavities" of the size of the non-electrolyte and on the compressibility of the two monolayers. It will indeed be shown in the accompanying paper [14], that there is a maximum value of N for each membrane which can be correlated with membrane fluidity. The values for N observed in this study are less than this maximum value, which suggests that the system can compensate for the copermeation of water, for example by osmosis in the direction opposite to the solute molecule, until the thermodynamically favourable value of N will be reached.

Finally, it should be realized that the irreversible thermodynamic equations of Kedem and Katchalsky are based on an idealized description of membrane

permeability, since only concentration differences in the bulk of the solutions are considered. In a more detailed description the effects of unstirred layers at each side of the membrane on solute and water permeation should also be taken into account.

Appendix I

Measurement of the permeation coefficient ω

As has been shown by Hill and Cohen [4], the permeation coefficient ω can be determined from measurements of the changes in the cell volume if one is able to create a situation in which the cell volume changes linearly with time. From differentiation of Eqn. 1, they showed that a linear change of volume with time (v) is given by [1,4,6]:

$$v = \sigma \frac{dn_s}{dt}(c_i^i + \sigma c_s^i)^{-1} \tag{10}$$

in which c^i stands for inside concentration; the outside concentrations (c^o) are assumed to be constant with time. However, they were not able to calculate the inside concentrations of the permeants and impermeants during the linear part of the swelling under the conditions they were using [4].

When cells are suspended in an isosmotic medium, defined by $\Delta c_i + \sigma \Delta c_s = 0$, the absorbance of the suspension changes as a function of time, as shown in Fig. 3. The first phase with no volume change is followed by a second phase during which the volume changes linearly with time. The inside and outside concentrations of the permeant and impermeants present at the start of this linear phase can be calculated from the following considerations. When a cell is placed in an isosmotic medium no initial volume change will occur since the volume change produced by the inward solute flow and copermeation of water is exactly counterbalanced by the osmotic outward flow of water. This indicates that during this first phase permeant is entering the cell without changing the cell volume, so there is no alteration of the concentrations of the other solutes present.

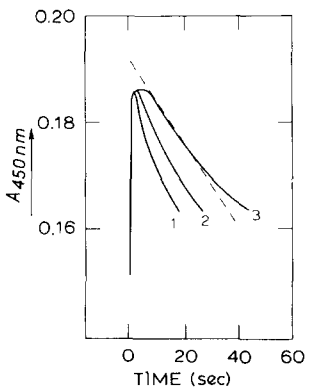

Fig. 3. Change in absorbance (450 nm) as a function of time for liposomes, prepared in 20 mM glucose, and suspended at 15°C in water (1), 20 mM thiourea (2) and 33.5 mM thiourea (3; isosmotic medium). 1 ml of the liposome solution (2 mM phospholipid) was injected in 9 ml of the desired solution. The dotted line represents the linear swelling rate of the liposomes in the isosmotic medium.

During the linear swelling phase the inside compartment will be constantly hyperosmotic when compared to the outside compartment, as can be seen from Eqn. 1:

$$c_i^i + \sigma c_s^i = \frac{v}{AL_pRT} + c_i^0 + \sigma c_s^0 \tag{11}$$

Since in the applied isosmotic medium and, therefore, also at the start of the linear swelling phase $c_t = c_i^i = c_i^o + \sigma c_s^o$, it follows that at the start of the linear swelling phase

$$c_s^i = \frac{v}{AL_pRT\sigma} \tag{12}$$

So, at the start of the linear swelling phase all inside and outside concentrations are known, including the rate of the linear swelling resulting from these conditions. Introduced in Eqn. 9 and using Eqn. 2, it follows

$$A\omega RT = v\left(\frac{c_t + v/AL_pRT}{\sigma(c_s^0 - c_s^i)} - \frac{(1-\sigma)}{\ln c_s^0/c_s^i}\right) \tag{13}$$

Under the conditions that during the linear swelling $c_s^i << c_s^o$, this equation is identical with the one given in ref. 6.

Appendix II

According to the concepts of a mechanism of free diffusion and osmosis, the initial volume change of a cell suspended in an isotonic solution of a non-electrolyte will depend on the permeation rate of the solute and on the number of water molecules that will follow each solute molecule by means of osmosis. If it is assumed that for each molecule of solute R water molecules can cross the membrane by osmosis which would result in a full restoration of isotonicity, Eqn. 6 can be used to describe the initial volume change of the cell using $N = R$. In this way a value for $\omega\bar{V}_s/L_p$ for the free diffusion process can be found from the ratio of the isotonic swelling rate and the initial swelling rate of the

TABLE V

CALCULATION OF THE NUMBER OF WATER MOLECULES THAT CAN PERMEATE PER SOLUTE MOLECULE BY MEANS OF OSMOSIS THROUGH BILAYERS OF EGG PHOSPHATIDYLCHOLINE AT 15°C

σ Values have been taken from Table I (R = 2750). For details see text.

Solute	σ	$\omega\bar{V}_s/L_p$	ω_w/ω_s	N_{diff}	N_{diff}/R
1,3-Propanediol	—0.4	0.002014	1 986	1557	0.561
Glycol	0.08	0.001030	3 022	1757	0.633
Thiourea	0.54	0.000496	6 048	2069	0.745
Urea	0.74	0.000237	10 666	2288	0.824
Glycerol	0.78	0.000321	12 634	2343	0.844
Erythritol	0.98[5]	0.000025	186 667	2734	0.984

cell when suspended in water $(1 - \sigma)$. The values for $\omega\bar{V}_s/L_p$ and ω_w/ω_s found in this way (see Table V) closely correlate with the values found according to the irreversible thermodynamic approach (see Table I), since the values of N found according to this method, although with a different interpretation, are also near to R. So assuming that no interaction between permeation of the solute and the solvent takes place, and assuming that a full osmotic water response is possible during the process of isotonic swelling, a value for the ratio of solute and water permeation coefficients can be calculated. It will be shown now, however, that these values of $\omega\bar{V}_s/L_p$ are such that a full osmotic response during isotonic swelling is not possible. Let us consider a liposome with volume V_o and outer area A. prepared in a solution of an impermeant with concentration c_t, which is suspended at zero time in an isotonic solution (c_t) of a permeant. It will be assumed that during the first part of the swelling c_s^i remains much smaller than c_t. Due to permeation of the solute and of water, the cell will start to swell and reach a volume V after time t, while then $A\omega RTc_t t$ mol of solute has entered the cell. So the volume change of the cell as a result of osmosis at this time t will be given by:

$$\frac{dV}{dt} = -AL_pRT\left(c_t - \frac{c_tV_0}{V} - \frac{A\omega RTc_t t}{V}\right)$$

This differential equation can readily be solved if it is assumed that during the initial part of the swelling the volume change of the cell per time will not be far from linear, making $V = V_o + (dV/dt) \cdot t = V_o + a_w \cdot t$, in which a_w is the volume change per time unit and $da_w/dt = 0$. From this if follows

$$a_w = AL_pRTc_t\left(\frac{A\omega RT \cdot t}{V} - \frac{a_w \cdot t}{V}\right)$$

or

$$V = \frac{AL_pRTc_t}{a_w}(A\omega RT - a_w)t,$$

and differentiation to time gives

$$\frac{dV}{dt} = a_w = \frac{AL_pRTc_t}{a_w}(A\omega RT - a_w)$$

from which a_w can be calculated. Since the volume change produced by the solute permeation is given by $(dV_s/dt) = a_s = A\omega RTc_t\bar{V}_s$, it can be calculated that

$$\frac{a_w}{a_s} = -\tfrac{1}{2}\left(\frac{1}{\omega\bar{V}_s/L_p}\right) + \frac{1}{2}\sqrt{\left(\frac{1}{\omega\bar{V}_s/L_p}\right)^2 + \frac{4}{(c_t\bar{V}_s)\cdot(\omega\bar{V}_s/L_p)}} \qquad (14)$$

Eqn. 14 shows that the ratio of the volume change produced by solute permeation and by the induced osmotic water flow can be determined from the value of $\omega\bar{V}_s/L_p$. We can now define the number of water molecules that can

follow each solute molecule by means of osmosis as

$$N_{\text{diff}} = \frac{a_w}{a_s} \cdot \frac{\bar{V}_s}{\bar{V}_w} \tag{15}$$

Table V shows that for the permeation process studies in Fig. 1 and Table I, the values of N_{diff} are less than R, indicating that only a partial restoration of isotonicity can be reached.

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