

SOME PECULIARITIES OF WATER TRANSPORT THROUGH PLASTICIZED NONPOROUS MEMBRANES

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ABSTRACT "Liquid" and "plasticized" solvent membranes are of interest as possible analogues of biological systems. Semipermeable homogeneous films are prepared by plasticizing polyvinylchloride with organic phosphates. Water permeability of such films is relatively high. For a material containing 70% of 1,4-dihydroxyphenyl-bis(dibutylphosphate), the diffusion coefficient of water at room temperature was estimated to be about 1×10^{-6} cm²/sec. Conditioning of a plasticized membrane, under the osmotic gradient of solution of sodium nitrate, leads to profound changes in its morphology and to a drastic increase of its water permeability. The induced changes are reversible to a large extent. Their reversibility in various solutions may be correlated with the respective differences in permselectivity. The structure of expanded membranes and the mechanism of changes taking place under the osmotic gradients are discussed.

INTRODUCTION

One of the model systems which have been used for comparison between porous and nonporous membranes is a polymer film plasticized with a phosphate ester. Such "solvent" plasticized membranes were developed for specific ion separation by dialysis (1, 2), but since the plasticizers dissolve water and do not dissolve sodium chloride and many other electrolytes, they also form highly selective osmotic membranes. Water flow in a membrane of this type was examined previously (3).

It is generally accepted that lipid-soluble substances pass across biomembranes by dissolution and diffusion. However, the mechanism of water transport across such membranes is still a matter of controversy: the question whether flow of water takes place through very small pores, or by diffusion through a continuous phase, is not yet settled. There is probably no sharp dividing line between the two mechanisms when pore radii of a few angstroms are considered.

The rate of permeation of different solutes through nonporous solvent membranes depends not only on their solubility in the membrane phase but also on the diffusion coefficient. It was recently pointed out by Lieb and Stein (4) that judging from relative rates of diffusion of various solutes, polymers rather than liquids may be good

models for the passage of nonelectrolytes through cell membranes. Moreover, in the plasticized dialysis membranes, the polyvinylchloride forms a rather loose network, and the phosphate ester is held in the membrane by interaction with the polymer. It may be interesting to compare this interaction between polymer and plasticizer to hydrophobic interactions in biological systems (5). The study reported here revealed a rather intriguing feature of the synthetic membranes, in that reversible changes were observed depending on the media in contact with membrane.

Such reversible changes in water and salt permeabilities under osmotic gradients were particularly striking in membranes prepared with 1,4-dihydroxyphenyl-bis-(dibutylphosphate) (BHQP) and results obtained with such membranes are the subject of this paper.

MATERIALS AND METHODS

Polyvinylchloride (PVC) powder: Rhodopas X.H.P.; grade C; Rhône-Poulenc, Paris.

Cyclohexanone; purum; Fluka AG, Switzerland.

Sodium nitrate, sodium chloride, Fisher reagent grade; Fisher Scientific Company, Pittsburgh, Pa.

Tritiated water (2×10^6 cpm/cm³).

Tetrabutylhydroquinone diphosphate is not commercially available and was synthesized in our laboratory.

A tetrachloride was prepared by refluxing hydroquinone with phosphoroxchloride and was butylated in ether in the presence of pyridine. The purified final product obtained after repeated high vacuum distillation has a refractive index $n_D^{25} = 1.4650$. Its purity was checked by thin-layer chromatography and its identity asserted by elemental analysis and by NMR.

About 80 μ thick membranes were cast from 9% PVC solution in cyclohexanone. The ratio PVC/BHQP was 1:2.5. Membranes formed on a glass plate could be easily peeled off the glass by using parafilm discs as supports. Such membranes were mounted in osmotic cells with thin nylon nets as a support. All osmotic experiments were conducted at 25°C. Proper stirring of the solutions in a cell was attained by its continuous mechanical shaking. Salt concentrations in the two compartments of the osmotic cell were monitored conductometrically by measuring the conductance of aliquot samples diluted by a factor of 100. The proper values of specific conductances were obtained from a plot of specific conductance vs. concentration by consecutive approximations. Radiometer's CDM2 conductivity meter (Radiometer Co., Copenhagen, Denmark) was used for these measurements. Total volume flow, AJ_V , (ml sec⁻¹) and total salt flow, AJ_s , (mole sec⁻¹), referred to compartment II, were calculated from changes in conductance. Since the total amount of salt in the two compartments must remain constant, volumes are related to concentrations by equations 1 and 2.

$$-AJ_v = V_0(C'_t + C''_t - C'_0)/(C'_t - C''_t)\Delta t \quad (1)$$

$$AJ_s = C''_t \cdot V''_t/\Delta t, \quad (2)$$

where C'_0 is the initial salt concentration in compartment I; C'_t and C''_t are the salt concentrations after time t in compartments I and II, respectively; V_0 is the initial volume in each of the two compartments ($V'_0 = V''_0 = 20$ ml). The initial salt concentration in compartment II was always zero.

In all measurements the nominal membrane area $A = 4.9 \text{ cm}^2$. During the osmotic experiments solutions were periodically removed from the cell and replaced by the original concentrated solutions on one side of the membrane and pure distilled water on the other side. Time intervals were adjusted in such a way that $C'_t \approx 0.9 C'_0$.

The diffusional permeability coefficient, ω_T (mole dyne $^{-1}$ sec $^{-1}$) was determined at steady-state conditions from the equation¹

$$RT\omega_T = \log \{R'_0/(R'_t - R''_t)\} / (1/V' + 1/V'')tA, \quad (3)$$

where R'_0 , R'_t , R''_t (counts min $^{-1}$ ml $^{-1}$) are radioactive counts at time zero and at time t in half-cells 1 and 2; R and T are gas constant and temperature; V' and V'' are the volumes of tritiated water in half-cells I and II (virtually constant during most of the experiments).

OSMOTIC EXPERIMENTS WITH SODIUM NITRATE AND SODIUM CHLORIDE SOLUTIONS

Data illustrating changes of water and salt fluxes with time in experiments conducted with 3 M NaNO₃ are collected in Table I.

TABLE I
DATA ILLUSTRATING CHANGES OF WATER AND SALT FLUXES WITH TIME
IN EXPERIMENTS CONDUCTED WITH 3 M NaNO₃

The over-all time of mem- brane exposure to osmotic gradients	$-AJ_v \times 10^4$	$AJ_s \times 10^9$	$-J_s/J_v \times 10^4$	$100 J_s/J_v C'_{av}$
<i>hr</i>	<i>ml/sec</i>	<i>mole/sec</i>	<i>mole/ml</i>	<i>%</i>
24	<0.01	0.2 ± 0.02	—	—
70	0.08 ± 0.02	1.0 ± 0.25	1.25	4.5
98	0.20 ± 0.02	3.3 ± 0.45	1.65	5.3
142	0.65 ± 0.02	5.4 ± 0.60	0.83	3.0
165	0.83 ± 0.05	6.8 ± 0.10	0.82	3.1
188	1.17 ± 0.06	6.0 ± 0.05	0.51	1.9
192	1.20 ± 0.09	16.3 ± 0.20	1.33	4.8
196	1.75 ± 0.25	17.2 ± 0.20	1.0	3.2
204	1.75 ± 0.30	17.2 ± 0.20	1.0	3.2

While water and salt fluxes increased as a result of membrane exposure to the conditions of an osmotic experiment with sodium nitrate, by more than a factor of 100, the ratio of salt and water permeabilities remained virtually constant. It seems, therefore, that the observed large increase of fluxes through a membrane may be due to changes in its morphology and not to any alteration of its basic properties. It should be emphasized that the asymmetric conditions prevailing during an osmotic ex-

¹ For the derivation of the integrated expression, see for example: Weissberger, A. 1965. *Physical Methods of Organic Chemistry*. Interscience Publishers, Inc., New York.

periment are essential for the development of high fluxes. Total immersion of a membrane in sodium nitrate solutions does not lead to any similar changes, the only detectable effect being some stiffness and shrinkage of the membrane by about 10%. The nature of the solute used in the osmotic experiment is, however, crucial. When sodium chloride or sucrose was used instead of sodium nitrate, no changes whatsoever were observed in membrane permeability during such osmotic experiments. Moreover, replacement of sodium nitrate by sodium chloride or by sucrose leads to a drastic reduction of water fluxes in a membrane in which high fluxes were already developed as a result of sodium nitrate treatment. This remarkable reversibility of membrane properties under the influence of various salts is illustrated by the plot of AJ_v vs. time given in Fig. 1. Segments *a* and *c* refer to osmotic water flow with 3 M sodium nitrate in one half-cell, and distilled water in the other, while segments *b* and *d* refer to water flows through the same membrane after 3 M sodium nitrate was replaced by 3 M sodium chloride. The reduction of water fluxes takes place in spite of the fact that the osmotic pressure gradient is even higher in sodium chloride than in sodium nitrate experiments since the osmotic coefficient, φ , for 3 M sodium chloride is higher than for 3 M sodium nitrate. The permeability of the investigated membrane to sodium chloride is much lower than to sodium nitrate, the respective $-J_s/J_v$ being 1×10^{-5} mole/ml for 3 M sodium chloride and 1×10^{-4} mole/ml for 3 M sodium nitrate. Hence, if the reflection coefficient, σ , is different from unity for either of the salts, we expect $\sigma_{\text{NaCl}} > \sigma_{\text{NaNO}_3}$ (6).

Profound changes in membrane morphology parallel to the changes in fluxes are revealed by inspection of membrane surfaces at different stages of the osmotic experi-

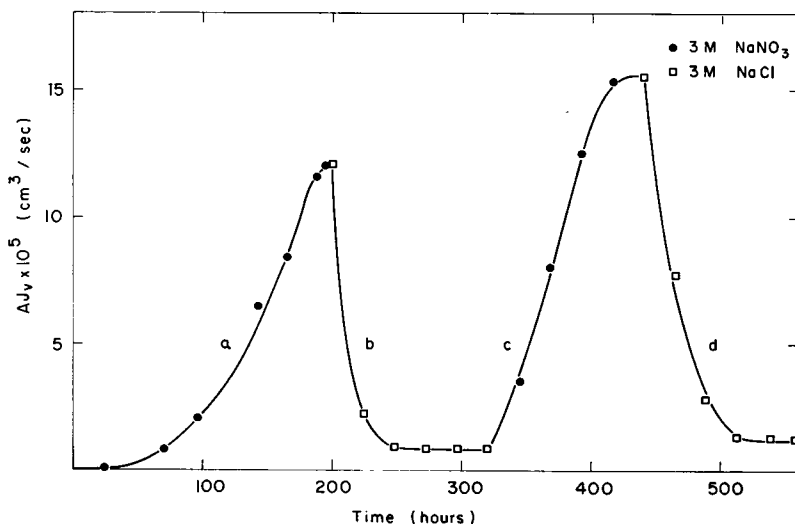


FIGURE 1 Water fluxes under the osmotic gradients of sodium nitrate or sodium chloride. *a*: 3 M NaNO₃ vs. H₂O. *b*: 3 M NaCl vs. H₂O. *c*: 3 M NaNO₃ vs. H₂O. *d*: 3 M NaCl vs. H₂O.

ment. A membrane, after 200 hr exposure to an osmotic gradient created by sodium nitrate, is shown in Fig. 2 *a*. It is obvious that the originally entirely homogeneous membrane was converted into an irregular expanded material. A remarkable decrease in membrane swelling is evident from Fig. 2 *b* for the same membrane 100 hr later after sodium nitrate was replaced by sodium chloride. Flux measurements on the same membrane showed that the rate of water flow was 15 times faster at the stage shown in Fig. 2 *a*. The observed reversible changes in the rates of flow through a membrane may be, therefore, correlated with changes of the effective area and thickness of a membrane exposed to conditions of an osmotic experiment.

An intriguing question arises as to the mechanism responsible for such changes. In order to answer this question one must first establish the exact nature of the expanded membrane. To examine whether one deals simply with a highly folded film or if a closed-pore structure was developed, the retention of tritiated water and of sucrose by such expanded membranes was measured. Membranes equilibrated with sucrose and with tritiated water were quickly rinsed with large amounts of water (twice for 30 sec) and then the tritiated water or sucrose, or both, retained by the membrane were extracted by prolonged shaking with a known quantity of distilled water. Since sucrose could be retained only in the open pores and folds of the membrane, while

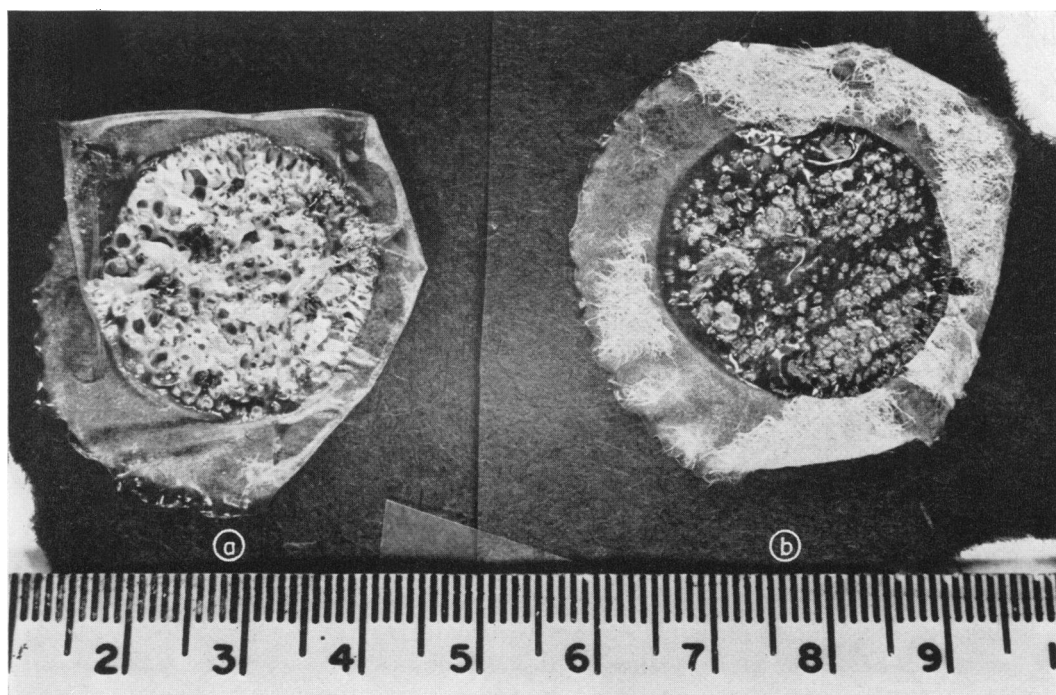


FIGURE 2 *a*: membrane after 200 hr with 3 M sodium nitrate/water. *b*: membrane *a* after additional 100 hr with 3 M sodium chloride/water.

tritiated water could penetrate into the closed pores (water-filled vacuoles) the difference between the amount of trapped tritiated water and trapped sucrose may be used as measure of the total volume of closed pores in the membrane. From the residual radioactivity one calculates that about 0.4 ml of aqueous solution equilibrated with tritiated water was retained by the membrane as compared to only 0.1 ml of "sucrose space". The volume of closed pores (vacuoles) in the expanded membrane amounts, therefore, to as much as 0.3 ml. The magnitude of membrane expansion becomes evident if one realizes that the volume occupied initially by the homogeneous unexpanded membrane used in our experiments was 0.04 ml only. Comparison of over-all fluxes through the expanded membrane with fluxes of tritiated water is also of interest. The so-called "g" ratio which relates the total volume flow through a membrane with tracer flow was suggested as criterion of membrane porosity (3, 7) and was used in the calculations of the apparent pore radius (8). g is defined by equation 4

$$g = L_p / \omega_T V_w, \quad (4)$$

where L_p (ml dyne⁻¹ sec⁻¹) is the hydraulic permeability, ω_T (mole dyne⁻¹ sec⁻¹) is the permeability of tritiated water $\omega_T = J_T / (RT\Delta C_T)$, and V_w (ml mole⁻¹) is the molar volume of water.

An expression relating the permeability of tritiated water to tracer flow in the presence of high total water flow was previously reported (9) namely

$$\omega_T = J_v / RT \ln (J_T / (J_T - C_T J_v)), \quad (5)$$

where J_v (ml cm⁻² sec⁻¹ \cong cm sec⁻¹) is the total volume flow, J_T (mole cm⁻² sec⁻¹) is the tracer flow, and C_T (mole ml⁻¹) is the molar concentration of tritiated water. Since by definition in an osmotic experiment, when hydraulic pressure gradient is equal to zero

$$L_p = J_v / 2\sigma\varphi RT\Delta C, \quad (6)$$

it follows that

$$g = \ln \{ J_T / (J_T - C_T J_v) \} / 2\sigma\varphi\Delta C \bar{V}_w. \quad (7)$$

Here σ is membrane reflection coefficient, $\sigma = (\Delta p / \Delta\pi)_{J_v=0}$; φ is salt osmotic coefficient; ΔC is salt concentration difference.

A typical plot showing changes of the radioactivity with time in an experiment, under gradient of 3 M sodium nitrate with tritiated water in the second half-cell, is shown in Fig. 3. From the initial slope one calculates $AJ_T / C_T = 10.0 \times 10^{-4}$ ml/sec and from changes in salt concentration (see equation 1), $AJ_v = 1.55 \times 10^{-4}$ ml/sec. The membrane reflection coefficient $\sigma \leq 1$, hence from equation 7 one calculates $g \geq 1.7$, similar to the value obtained with homogeneous PVC membranes plasticized with tributylphosphate (3). This clearly indicates absence of open pores in the ex-

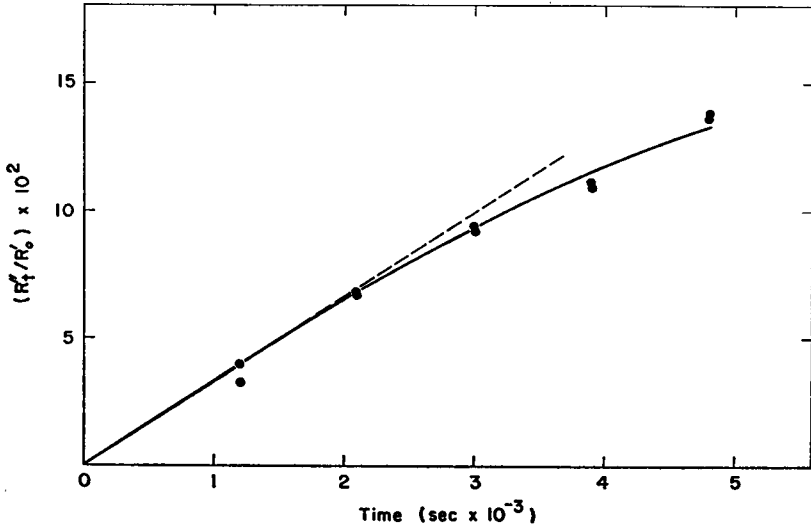


FIGURE 3 Osmotic experiment with 3 M sodium nitrate against tritiated water. Radioactivity of the sodium nitrate solution vs. time.

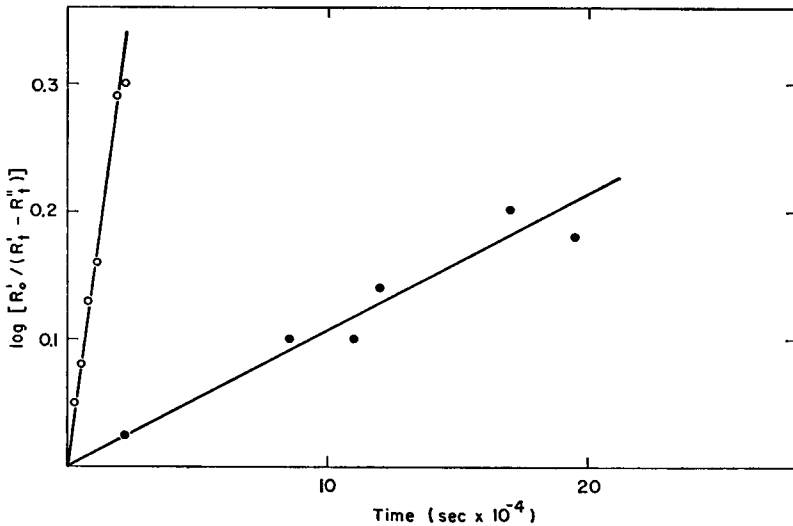


FIGURE 4 Diffusion of HTO. Half-cells: water and tritiated water. ●: water-conditioned membrane. ○: membrane exposed for 200 hr to osmotic gradient of sodium nitrate.

panded membrane. Since such a membrane was shown to have a composite structure, the diffusional flow through the dense continuous phase must be rate determining.

A large increase of the diffusional flow through an expanded membrane is also demonstrated by the results of diffusion experiments conducted with tritiated water on untreated and sodium nitrate pretreated membranes. From the slopes of the plots

of $\log R'_0/(R'_i - R''_i)$ vs. time shown in Fig. 4, one calculates that as result of membrane treatment the diffusional permeability increased by a factor of 16. It must be pointed out, that the actual increase may be even larger since water fluxes tend to decrease, if membranes are left for prolonged time periods with water after removal of sodium nitrate.

The increase of the diffusional water permeabilities of membranes exposed to the osmotic gradients of sodium nitrate may be again contrasted with a considerable decrease of membrane permeability after it is totally immersed in a sodium nitrate solution. A comparison between the diffusion of water through a water conditioned membrane and through the same membrane after it was conditioned with a 3 M sodium nitrate, and then tested with 3 M solution of sodium nitrate in tritiated water against 3 M solution of sodium nitrate in water, is very instructive. Typical plots of $\log \{R'_0/(R'_i - R''_i)\}/(1/V' + 1/V'')$ vs. time are shown in Fig. 5. The observed decrease of the diffusional water permeability in the presence of sodium nitrate by a factor of two is not surprising, if one remembers that the solubility of water in the plasticizer is considerably decreased by the addition of sodium nitrate and that the membrane contracts when immersed in sodium nitrate. From the results obtained for the untreated membranes, the value of the diffusion coefficient may also be calcu-

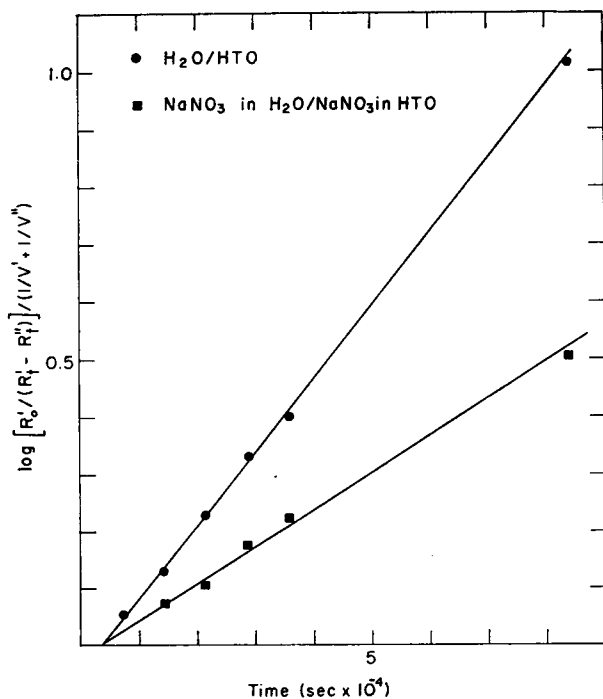


FIGURE 5 Diffusion of HTO. ●: water-conditioned membrane. Half-cells: water and tritiated water. ■: the same membrane after conditioning for 24 hr in sodium nitrate. Half-cells: 3 M sodium nitrate and 3 M sodium nitrate in tritiated water.

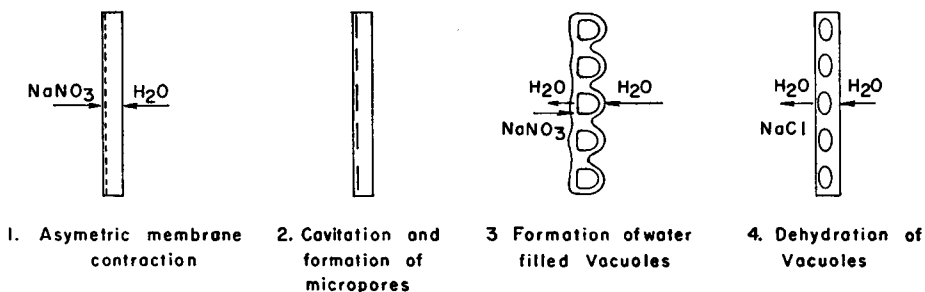


FIGURE 6 A schematic representation of changes taking place in a membrane exposed to osmotic gradients.

lated. Assuming that the water solubility in the untreated membrane is determined by the water solubility in the plasticizer (4.4%), one calculates $D = 1.0 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$, a value comparable to the diffusion coefficient of water in the plasticizer.

The following tentative explanation may be offered in order to account for the mechanism of the observed reversible changes of membrane structure.

As mentioned above, a membrane transferred from distilled water to a concentrated solution of NaNO_3 , contracts considerably. Thus, stresses must develop in a membrane when one surface is in contact with water and the other with $3 \text{ M } \text{NaNO}_3$. Cracks may be formed and these will contain water and some NaNO_3 . The osmotic pressure in this trapped solution must be lower than in compartment I and higher than in II. Whenever the permeability of the membrane layer between a crack and compartment II is relatively low, osmotic flow from compartment II will result in expansion into a water-filled vacuole.² The blown-up membrane has a much larger effective surface and thus permits higher water flow. Replacement of sodium nitrate in compartment I by the highly impermeable sodium chloride or by sucrose, will reverse osmotic flow with respect to the vacuoles. Due to the elasticity of the membrane material, the vacuoles will contract nearly to the dimensions of the primarily formed cracks. Consequently, the effective membrane area will be greatly reduced and its thickness increased. However, since the cracks remain, replacement of sodium nitrate should lead again to vacuole formation, at a faster rate than in a freshly prepared membrane. Comparison of sections *a* and *c* in Fig. 1 seems to indicate that this is indeed the case. While no changes could be detected in the rates of flow during the first 24 hr of an osmotic experiment conducted with sodium nitrate on fresh membranes, an immediate steep increase in the flow rate was observed when sodium nitrate replaced sodium chloride on previously pretreated membranes. A schematic representation of changes taking place in a membrane is given in Fig. 6 (see steps 1-4).

² The degree of swelling of a cell layer separating two solutions of different osmotic pressure was used by Hays and Leaf (10) to follow the changes in the relative water permeabilities of the membranes facing each solution.

The observations reported here demonstrate two phenomena which may be important in biological transport.

(a) In a plasticized polymer membrane, i.e. a polymer network mixed and interacting with a low molecular component, drastic changes in effective area are possible without disintegration or loss of selectivity. The macromolecules can readily alter shape and relative position and the plasticizer which fills the spaces between the polymer segments follows the displacements and no leak develops.

(b) Conformational change caused by interaction with a solute may lead to such reversible macroscopic changes of area in a membrane.

Hormone-induced changes in the permeability of toad bladder were extensively investigated (7, 8, 10). It was recently suggested (8) that this membrane is a composite structure and that the increase in the rate of diffusion of water across the dense part of such a membrane without change in porosity is responsible for the observed changes. Our investigation provides an example of the feasibility of such changes in a synthetic membrane.

Practical implications of the described phenomenon for the problems of mass transport through membranes are appealing. The treatment could also be of importance for processes requiring pressure gradients, if the expanded membrane structure could be "frozen" by a suitable chemical treatment, which at the same time would lead to improved physical properties of the membrane material.

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