The Coupling of Solute Fluxes in Membranes

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ABSTRACT Our previous description of solute drag on a synthetic membrane has been extended to include the solutes mannitol, sucrose, raffinose, inulin, and dextran. Labeled and nonlabeled forms of these solutes were used in pairs to quantitate solute flux interaction. Three membranes with pore sizes of 350, 80, and 20 Å, respectively, have been utilized. It is shown that solute flux interaction occurs with all the solutes and that the extent of interaction is related directly to solute permeability, concentration, and molecular size. The magnitude of solute interaction is reciprocally related to the radii of the membrane pores, greater interaction occurring with small pored membranes. Solute drag is seen as an increased flux of tracer solute in the direction of the diffusion gradient of a second solute as well as a decreased tracer flux into the diffusion gradient. Values are given for self-diffusion and interaction coefficients as well as for a new coefficient, the “effectiveness coefficient.”

INTRODUCTION

We have previously shown that a net solute flux may be created across a synthetic membrane even when the solute has no apparent chemical potential difference to cross the membrane (1). This net flux is observed when a chemical gradient is present for a second solute and is directed down the concentration gradient of the second solute. Our earlier papers (2, 3) suggest that the asymmetric solute flux is created by interaction between the fluxes of the two solutes. We have labeled the phenomenon “solute drag.” Our previous study (1) on a nonbiological membrane appears to explain the findings of flux asymmetry described by Ussing (4) and Franz and Van Bruggen (2, 3) in frog skin, making it unnecessary to postulate the participation of biological mechanisms as done by Ussing (4). Biber and Curran (5) have recently described an analogous asymmetric solute flux in toad skin which concurs with our hypothesis that the net flux across the skin is related to the coupling of solute fluxes.

In the initial publication, details were given for a single synthetic membrane and a single pair of solutes. This paper extends the studies to other membranes and other solute pairs and reveals new quantitative as well as qualitative as-
pects of the solute drag process. In addition, it is shown that the membrane is involved in the expression of the process, distinguishing the mechanism of solute drag from the coupling of solute flows known to take place in free solution (6-8).

**Materials and Methods**

**Membranes**

The membranes involved in the present study are the Diaflo UM-3 ultrafiltration membrane, the S & S Bact-T-Flex B20 membrane, and a General Atomic Type B desalinization membrane. Characteristics of these membranes are given in Table I.

| TABLE I | CHARACTERISTICS OF SYNTHETIC MEMBRANES |
|---------|----------------------------------------|
|         | Diaflo UM-3 | S & S B20 | GA Type B |
| 1. Composition | Poly Electrolyte | Cellulose acetate | Cellulose acetate |
| 2. Thickness, μ | ~200 | ~100 | ~80 |
| 3. Effective pore radius, A | ~300-350* | ~70 | ~20 |
| 4. Lp , cmdyne^{-1}sec^{-1} | 2.0 × 10^{-10} | 0.66 × 10^{-10} | 1.3 × 10^{-10} |
| 5. Reflection coefficients | Procedure | Procedure | Procedure |
| Urea | Not determined | 0.008 | 0.005 | 0.070 |
| Mannitol | Not determined | 0.033 | 0.058 | 0.282 |
| Sucrose | 0.02 | 0.092 | 0.088 | 0.524 |
| Raffinose | 0.03 | 0.032 | 0.103 | 0.096 |

* By the method of Goldstein and Solomon (12).
† By the method of Paganelli and Solomon (13).
§ = ΔP/ΔCRT.
\[ \sigma = \frac{-LpD - LpD}{Lp} \]

The Diaflo UM-3 membrane (Amicon Corporation, Cambridge, Mass.) is an anionic, hydrated polymer ultrafilter which is thermally and dimensionally stable, being cast from mixtures of poly(vinyl benzyl trimethylammonium chloride) with poly(sodium styrene sulfonate) (9). It is a "skinned" anisotropic membrane (analogous to cellulose acetate membranes) and consists of a "skin" about 1 μ thick made up of consolidated layers of the polyelectrolyte complex. Beneath this skin are thick, spongy, opaque layers of the same polymers which give structural support to the barrier skin layer but offer little resistance to flow (9).

The S & S B20 cellulose acetate membrane filter is available from the Carl Schleicher and Schuell Company (Keene, N. H.). As stated by the manufacturer, these filters "have an extremely uniform micropore structure and are approximately 100 μ thick" (10). There is evidence (11) that cellulose acetate membranes are similar in structure to that of the polyelectrolyte membranes such as the Diaflo membrane, for it has been shown by electron microscopy (11) that cellulose acetate mem-
branes have a thin and dense surface skin overlying a more porous and thick backing. It is felt that the dense skin layer is responsible for the filtration properties of the membrane.

The third membrane is a desalinization membrane material produced by General Atomic Division of General Dynamics (San Diego, Calif.). This material, designated Type B by the manufacturer, is also a cellulose acetate membrane.

**Solute**

Reagent grade urea and sucrose were obtained from Merck and Co. (Rahway, N. J.). Mannitol and raffinose were obtained from Matheson Coleman and Bell (East Rutherford, N. J.). The melting points of the latter two solutes agreed with those of the pure substances. 0.35 molal solutions of these solutes showed 354, 354, 350, and 357 milliosmoles per liter, respectively. Osmolalities were determined by freezing point depression on an osmometer (model 66-31KS, Advanced Instruments, Inc., Newton Highlands, Mass.). Solutes were checked for purity by descending paper chromatography in butanol, pyridine, benzene, and H₂O (50:30:4.5:30) and it was determined that each migrated as a single spot and showed characteristic Rᵣ values. Dextran (Type 15), Sigma Chemical Company (St. Louis, Mo.), was stated by the supplier to have an average molecular weight of 19,900. In the present studies, ~0.008 molal (m) solutions of this dextran were used.

**Tracer Solutes**

d-Mannitol-1-¹⁴C was obtained from Nuclear Research Chemicals, Inc. (Orlando, Fla.) and from New England Nuclear Corporation (Boston, Mass.). Sucrose-U-¹⁴C, inulin-carboxyl-¹⁴C, and inulin-methoxy-²H were obtained from New England Nuclear. In each case, the stock solution of tracer was purged with ¹²CO₂ to remove volatile impurities. Raffinose-²H was prepared by the Wilzbach reaction. 200 mg of raffinose was reacted for 2 wk under an atmosphere of 3 Ci of tritium gas by the New England Nuclear Corporation. The product, approximately one-eighth of which was labeled raffinose, was purified by Dr. Harriet Frush of the National Bureau of Standards using descending paper chromatography in a N-butanol-pyridine-benzene-H₂O (50:30:4.5:30) solvent system on Whatman No. 17 paper. The labeled material thus purified migrated at the same Rᵣ as nonlabeled raffinose in two other solvent systems with descending paper chromatography.

Dextran-¹⁴C of two molecular sizes were obtained from the New England Nuclear Corporation. These dextran hydrolysates were stated by the supplier to have molecular weight of 16,000-19,000 and 60,000-90,000. Both fractions were supplied as carboxyl-¹⁴C dextran. Solutions of these solutes were purged with ¹²CO₂ before use to eliminate volatile impurities.

Samples of the dextrans-¹⁴C and the inulin-¹⁴C were chromatographed on Sephadex G50 and G75. The preparations eluted in single peaks and showed no evidence of low molecular weight contaminants. Radioassays were done by liquid scintillation spectrometry using 4% Cab-o-sil (Cabot Corporation, Boston Mass.) in Bray’s solution (14, 15). This system permits
the dependable radioassay of up to 0.25 ml of hyperosmotic solutions in 10 ml of counting fluid.

Apparatus

Experiments were carried out in the apparatus shown in Figs. 1 and 2. Each membrane was mounted as a barrier between two plastic chambers shown in Fig. 1. The membrane was supported between two flattened 20 mesh stainless steel screens held in parallel planes by metal rings. The cassette so formed is shown suspended between the open chamber halves in Fig. 1. The system is closed by wing nut-applied pressure, the seal between the stainless steel rings of the cassette and the chambers being made by neoprene O-rings embedded in the faces of the chambers. The solutions bathing the membrane are stirred by Teflon-coated magnetic bars driven by external magnets mounted on 600 rpm synchronous clock motors. The chambers are filled and sampled through ports in the top. These ports are sealed with disposable rubber closures, Critocaps J (Clay-Adams, Inc., N. Y.), and the closures are held in place by hollow threaded plastic plugs. The drain ports in the bottom of the chambers are sealed by metal stopcocks (Becton, Dickinson & Company, Rutherford, N. J.). The other two ports toward the front of the chamber may also be sealed by metal stopcocks and are...
used for the measurement of volume flow or the application of hydrostatic pressure. The metal stopcocks are joined to the chambers with the use of a cement (Dolphan CN-1065 Epoxy Adhesive, John C. Dolph Co., Monmouth Junction, N. J.).

The stopcock on the left chamber may be attached to a pressure device as seen to the left of the chamber assembly in Fig. 2. This mercury manometer device, developed for these studies, is used to apply hydrostatic pressure to the solution on the left side of the membrane. The pressure is attained by pumping air into the mercury reservoir by a hand-operated bulb or by an air line and is measured by the height of the mercury column. Fine adjustment of the pressure is made by the displacement of a screw-driven plunger in the barrel of a 10 ml syringe. An airtight seal between the barrel and plunger is made by a mercury reservoir seal fashioned at the top of the syringe barrel. In cases in which pressures greater than 150 cm of mercury are used, cylinder gas and

![Figure 2. View of assembled apparatus showing the essential components. In the center is the assembled chamber, on the right the microburet used for measuring volume changes. The left side of the chamber is attached to a mercury pressure device for the imposition of pressures up to 2 atm. When greater pressures are needed the mercury device is replaced by a compressed gas cylinder and gauge arrangement.](image-url)
an Ashcroft 100 psi test gauge (Industrial Air Products Co., Portland, Ore.) are used to measure the hydrostatic pressures.

To the stopcock on the right chamber is attached a glass capillary standpipe with a reservoir bulb and a reference marker corresponding to the top of the fluid level in the chamber. A ground-glass ball-joint makes the connection between the standpipe and a modified micropipet-buret with automatic zeroing and digital readout (Manostat Digi-Pet 2404-U10, Greiner Scientific Corp., N.Y.). The total capacity of the microburet is 1 ml and each division on the digital readout is equal to 0.2 µl.

This apparatus is used to measure volume flows across the membrane. To measure such flows, the plunger of the microburet is either advanced or retracted to bring the water meniscus in the standpipe to the reference point marked on the standpipe. The amount of fluid added or removed from the system in order to maintain the level of the meniscus in the standpipe is the net volume flow across the membrane and is read directly from the microburet. In this way, volume flows across the membrane may be measured whether they are caused by hydrostatic or osmotic pressure.

**Experimental Procedures**

All experiments were done with zero net volume flow ($J_v = 0$). The hydrostatic pressure required to maintain $J_v = 0$ was chosen for each membrane and each hyperosmotic agent by the use of a pressure vs. volume flow plot, previously obtained experimentally. During the experiments in which tracer fluxes were measured, observation of the constancy of the height of the fluid in the capillary standpipe allowed minor adjustments of the hydrostatic pressure to be made. All tracer fluxes were made in a steady-state condition after an initial equilibration period. Values reported are generally the means of eight samples.

**Determination of Reflection Coefficients**

The reflection coefficients of the solutes (Table I) were found by the use of two procedures. The first was by determining the hydrostatic pressure necessary to block osmotic flow, in effect by measuring the osmotic pressure created by a known concentration of the test solute. This observed osmotic pressure was used to calculate $\sigma$, the reflection coefficient.

\[
\sigma = \frac{\pi_0}{\Delta CRT}
\]

Where $\pi_0$ is the observed osmotic pressure; $\Delta CRT$ is the theoretical van't Hoff osmotic pressure; $\Delta C$ is the concentration difference of the solute across the membrane, and $R$ and $T$ are the standard constants.

The second method used to determine $\sigma$ is based upon the ability of the solute tested to create osmotic flow (16-18). In this method use is made of the fact that the reflection coefficient is the ratio of the osmotic coefficient, $L_{Dn}$, to the pressure filtration coefficient, $L_p$, as in the following:

\[
\sigma = -\frac{L_{Dn}}{L_p}
\]
RESULTS

In the previous paper (1) it was shown that with osmotic water flow blocked, the flux of tracer inulin out of the hyperosmotic solution was increased and that its flux into the hyperosmotic solution was decreased. The flux ratio (out of hyper/into hyper) in this case was greater than 1.0. It was suggested that the tracer solute fluxes are changed from their normal rate by interaction with the hyperosmotic agent, sucrose, as it diffused down its concentration gradient. If this is so, the degree of interaction of the hyperosmotic agent with the two tracer flows should be similar, and the magnitude of interaction should be related to the amount of hyperosmotic agent present in the system.

In order to determine the effect of sucrose concentration on inulin fluxes a series of experiments were done with the S & S B20 membrane. Fig. 3 records these results.

The outflux values are seen to increase linearly with the sucrose concentration while the influxes decrease, also linearly. Notice that the slopes of the fluxes are nearly the same but of opposite sign. This indicates that not only is the outflux increased over some initial value by the action of the hyperosmotic agent, but also that the influx of solute into the hyperosmotic solution is decreased by approximately the same amount. Hence, the effect of hyperosmolarity appears to be symmetrical with respect to the unidirectional transmembrane fluxes. The lines of best fit (by regression analysis) for the experimental points intersect the ordinate at essentially the same point, indicating that there will be zero net flux in the absence of the hyperosmotic agent. This

![Figure 3](image-url)
intercept at about $8.0 \times 10^{-3}$ is the predicted $P$ value for a zero concentration of sucrose. This $P$ of $8.0 \times 10^{-3}$ is less than the experimentally determined value of $10.9 \times 10^{-3}$ found when $H_2O$ bathes both sides of the membrane. Presumably, the difference between the values lies in the fact that viscosity effects of the hypertonic solutions on $P$ values are not corrected for in extrapolating to zero sucrose concentration. As shown in Figs. 6 and 7, $P$ values in 0.35 molal sucrose solutions are 20–30% less than those determined with $H_2O$ bathing both sides of the membrane.

The flux ratio is the ratio of the unidirectional flux values (outflux/influx). Although the unidirectional fluxes are linearly related to the concentration of the hyperosmotic solute, a plot of the flux ratios vs. sucrose concentration (Fig. 4) is not linear. For this reason it is important that unless the effect of the hyperosmotic solute concentration on the flux ratio is known, it is not advisable to extrapolate flux ratio data to concentrations other than those determined experimentally. After having established that the concentration of the hyperosmotic agent determines the magnitude of the flux asymmetry, it was desirable to examine the relationship between the net flux of hyperosmotic agent and the flux of the tracer solute.

Fig. 5 presents the results of parallel experiments carried out to determine the relationship between the fluxes of the two solutes. Both sucrose-$^{14}$C and

![Figure 4](image_url)

**Figure 4.** The relationship between the inulin flux ratio and the hyperosmotic sucrose concentration. The flux ratios represented by each point are calculated from the mean values of at least eight experimental determinations of unidirectional inulin fluxes across the S & S B20 membrane.
inulin-14C outfluxes were determined at four different concentrations of sucrose, the hyperosmotic agent. Clearly the inulin outflux is a linear function of the concomitant sucrose flux. This finding strongly suggests a coupling between the fluxes of the two solutes. Similar observations have been presented by Biber and Curran (5) for toad skin with the solute pair mannitol and urea.

When it had been shown that coupling of solute fluxes may occur in membrane systems, studies were undertaken to determine the mechanism of this coupling. First to be studied was the role of the molecular size of solutes with respect to their performance as hyperosmotic and tracer solutes.

In Figs. 6 and 7 the P values for THO, mannitol, sucrose, raffinose, inulin, and dextran are presented as bar graphs showing influx, outflux, and flux ratio values. Fig. 6 presents the P values of the tracer solutes with only 1 mm solutions of the tracer on both sides of the membrane. In Fig. 7 the P values obtained with 0.35 m sucrose bathing both sides of the membrane are presented. It is seen that the permeabilities of the solutes decrease with increasing molecular size, as do diffusion coefficients (19). The relative order of P values in the H2O system is retained in the sucrose system; i.e., \( P_{\text{man}} > P_{\text{aug}} > P_{\text{rat}} > P_{\text{inul}} > P_{\text{dex}} \). It should be noted that the absolute magnitude of the P values, except those for THO, has decreased some 20–30% in the sucrose system. This decrease is probably related to the decrease in diffusion coefficients in the more viscous sucrose solution (19).

It is to be noted that with water or sucrose on both sides of the membrane the flux ratios do not differ from 1.0, as determined by the t test at a 0.95 confidence level. These ratios of 1.0 indicate that neither membrane nor chamber asymmetry is responsible for experimentally found flux ratios that differ from 1.0.
Figs. 8 through 11 summarize experiments in which solutions of the indicated hyperosmotic agent were placed on one side of the membrane and tracer solute added to an appropriate side. Both bathing solutions were made 1 mM with the nonlabeled form of the tracer solute. In this system then, a finite concentration gradient existed only for the hyperosmotic agent. Hydrostatic pressure was applied to the chamber containing the hyperosmotic agent until the net volume flow across the membrane was less than ± 2 μL cm⁻² hr⁻¹. Tracer fluxes across the membrane were measured under steady-state conditions.

Attention is directed to several findings as revealed in Figs. 8–11:

1. It is easily seen that the flux ratios of all the tracers are greater than 1.0. This phenomenon on the S & S B20 membrane is similar to that shown earlier by us for the Diaflo membrane (1). It can be concluded that flux asymmetry of a solute created by the chemical gradient of another solute is a general phenomenon of all six solutes tested and thus is not due to a peculiar interaction between only two solutes nor is it characteristic of only a single membrane.

2. Another and most interesting finding is that the flux ratios created by a given hyperosmotic solute increase with the size of the tracer species; i.e., the flux ratios increase progressively in the series tracer mannitol, sucrose, raffinose, and inulin. Table II summarizes data from Figs. 8–11 and illus-
trates this progressive increase, from left to right, for each of the hyperosmotic agents. Notice, that with any one hyperosmotic agent, moderate increases in flux ratios are seen from tracer mannitol to sucrose and to raffinose. The increases in flux ratios correspond to increases in the molecular radii of the tracer solutes from 4.4 \( \rightarrow \) 5.3 \( \rightarrow \) 6.1 \( \text{ Å} \), respectively. The flux ratio increase from tracer raffinose to tracer inulin for hyperosmotic sucrose corresponds to a threefold increase in flux asymmetry.\(^1\) This large increase coincides with an approximate threefold increase in molecular radii (6.1 \( \rightarrow \) \~15 \( \text{ Å} \)) for the pair raffinose and inulin. The apparent close relationship of solute size and flux ratio increase does not extend to the case of the dextrans, which because of their molecular shape cannot be considered in the series of the four smaller, more spherical solutes. Inulin is considered to have an axis ratio of about 5 to 1.

3. A similar progressive increase in flux ratios for any one tracer with increasing large hyperosmotic agent does not appear to be present as a

\[ \frac{3.54 - 1}{1.72 - 1} = \frac{2.54}{0.72} = 3.5. \]

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3. A similar progressive increase in flux ratios for any one tracer with increasing large hyperosmotic agent does not appear to be present as a
perusal of the vertical columns of Table II will reveal. Although the larger
hyperosmotic agent might be expected to have a greater interaction with
tracer solutes, the decreased permeability of the larger agents limits the
effect of the interaction.

![Graph showing flux ratios for tracer solutes with 0.35 molal mannitol on one
side of the membrane and with osmotic flow blocked by hydrostatic pressure; i.e., $J_o = 0$. Flux ratios are calculated from the $P$ values for outfluxes and influxes. The $P$ values (as cm hr$^{-1}$) for the unidirectional fluxes reported are the means of at least eight experimental periods. The standard errors of the means are seen below.]

| Tracer Solute | $P$ (Outflux) | $P$ (Influx) |
|---------------|---------------|--------------|
| $M$ = mannitol| ±6.8          | ±9.5         |
| $S$ = sucrose | ±4.9          | ±4.9         |
| $R$ = raffinose| ±2.2          | ±1.1         |
| $I$ = inulin  | ±0.4          | ±0.4         |
| $D_I$ = 16,000-19,000 mol wt dextran | ±0.4 | ±0.4 |
| $D_{II}$ = 60,000-90,000 mol wt dextran | ±0.6 | ±0.4 |

Expressions derived to express the transport of solutes across membranes
based on the formulation of nonequilibrium thermodynamics offer a convenient
means of quantitating the magnitude of coupling of solute transport pro-
cesses. The equation of Kedem and Katchalsky (20) which describes the
passive flux of solute across a membrane may be expanded to include the
possibility of interaction between solute fluxes. This modified equation may be written as:

\[ J_1 = \dot{C}_1 (1 - \sigma) J_e + P_{11} \Delta C_1 + P_{12} \Delta C_2 \]

(3)

![Graph showing P values and flux ratios](image)

**Figure 9.** *P* values and flux ratios for tracer solutes with 0.35 molal sucrose on one side of the membrane and with osmotic flow blocked by hydrostatic pressure; i.e., \( J_e = 0 \). Flux ratios are calculated from the \( P \) values for outfluxes and influxes. The \( P \) values (as cm hr \(^{-1} \)) for the unidirectional fluxes reported are the means of at least eight experimental periods. The standard errors of the means are seen below.

| Solute                  | Outflux (\( \pm \)) | Influx (\( \pm \)) |
|-------------------------|---------------------|--------------------|
| Mannitol                | \( \pm 8.9 \)       | \( \pm 5.0 \)       |
| Sucrose                 | \( \pm 5.9 \)       | \( \pm 1.6 \)       |
| Raffinose               | \( \pm 2.9 \)       | \( \pm 1.8 \)       |
| Inulin                  | \( \pm 0.5 \)       | \( \pm 0.13 \)      |
| D\(_t\) = 16,000-19,000 mol wt dextran | \( \pm 0.2 \) | \( \pm 0.4 \) |
| D\(_{II}\) = 60,000-90,000 mol wt dextran | \( \pm 0.9 \) | \( \pm 0.2 \) |

Here \( J_{1u} \), the flux of solute 1, is equal to: (a) The contribution of solvent drag expressed by the first term of the equation where \( \dot{C}_1 \) is the mean concentration of the solute, \( \sigma \) is the reflection coefficient of the solute, and \( J_e \) is the volume

\(^1\text{A similar equation has been presented in terms of } \omega \text{ by Biber and Curran (5).}\)
flow across the membrane; (b) the self-diffusion of the solute when $P_{11}$ is the self-permeability coefficient and $\Delta C_1$ is the concentration difference of solute 1 across the membrane; (c) the effect of solute 2 on the diffusion of solute 1 described as $P_{12}\Delta C_2$ when $P_{12}$ is the cross-coefficient and $\Delta C_2$ is the concentration difference of solute 2 across the membrane.

For each additional permeable solute in a system with a concentration gradient across the membrane one must add similar terms $P_{13} C_3$, $P_{14} C_4$, ..., 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure10.png}
\caption{P values and flux ratios for tracer solutes with 0.35 molal raffinose on one side of the membrane and with osmotic flow blocked by hydrostatic pressure; i.e., $J_o = 0$. Flux ratios are calculated from the P values for outfluxes and influxes. The P values (as cm hr$^{-1}$) for the unidirectional fluxes reported are the means of at least eight experimental periods. The standard errors of the means are seen below.}
\end{figure}

| Tracer Solute | Outflux $\pm$ S.E. | Influx $\pm$ S.E. |
|---------------|--------------------|-------------------|
| $M =$ mannitol | $\pm 5.7$           | $\pm 4.3$         |
| $S =$ sucrose  | $\pm 3.2$           | $\pm 1.5$         |
| $R =$ raffinose| $\pm 4.2$           | $\pm 1.3$         |
| $I =$ inulin   | $\pm 0.6$           | $\pm 0.3$         |
| $D_1 =$ 16,000-19,000 mol wt dextran | $\pm 1.3$ | $\pm 0.2$ |
| $D_{11} =$ 60,000-90,000 mol wt dextran | $\pm 0.6$ | $\pm 0.4$ |
Figure 11. $P$ values and flux ratios for tracer solutes with $\sim 0.008$ molal $\sim 16,500$ mol wt dextran on one side of the membrane and with osmotic flow blocked by hydrostatic pressure; i.e., $J_s = 0$. Flux ratios are calculated from the $P$ values for outfluxes and influxes. The $P$ values (as cm hr$^{-1}$) for the unidirectional fluxes reported are the means of at least eight experimental periods. The standard errors of the means are seen below.

| Tracer Solute | Outflux | Influx |
|---------------|---------|--------|
| $M = \text{mannitol}$ | ±5.5 | ±5.1 |
| $S = \text{sucrose}$ | ±4.7 | ±3.5 |
| $R = \text{raffinose}$ | ±2.3 | ±1.4 |
| $I = \text{inulin}$ | ±0.7 | ±0.6 |
| $D_1 = 16,000\text{--}19,000$ mol wt dextran | ±1.2 | ±1.1 |
| $D_{II} = 60,000\text{--}90,000$ mol wt dextran | ±0.3 | ±0.7 |

**Table II**

COMPENDIUM OF FLUX RATIOS—S & S 220

| Tracer solute | Mannitol | Sucrose | Raffinose | Inulin |
|---------------|----------|---------|-----------|--------|
| Hyperosmotic agent |        |         |           |        |
| None ($H_2O$)  | 1.05     | 0.95    | 1.10      | 1.08   |
| Mannitol       | 1.32     | 1.57    | 1.61      | 2.98   |
| Sucrose        | 1.32     | 1.54    | 1.72      | 3.54   |
| Raffinose      | 1.20     | 1.48    | 1.80      | 3.29   |
etc. Thus for the general case equation 3 becomes

\[ J_1 = \tilde{C}_1(1 - \sigma)J_* + P_{11}\Delta C_2 + \sum_{j=2}^{n} P_{ij}\Delta C_j. \]

(4)

In the experiments reported in this paper, the volume flow across the membranes was held at zero, no concentration gradient existed for solute 1, and only solute 2 had a concentration gradient across the membrane. Consequently, the flux of solute 1 across the membrane is described by

\[ J_1 = P_{12}\Delta C_2 \quad J_* = 0, \quad \Delta C_1 = 0 \]

(5)

The effect of solute 2 on the flux of solute 1 may then be shown as

\[ P_{12} = \frac{J_1}{\Delta C_2} \quad J_* = 0 \quad \Delta C_1 = 0 \]

(6)

Since the flux of solute 2 in our system is given by \( J_2 = P_{22}\Delta C_2 \), substituting \( J_2/P_{22} \) for \( \Delta C_2 \) in equation 5 we find

\[ J_1 = \left( \frac{P_{12}}{P_{22}} \right) J_2 \quad J_* = 0 \quad \text{and} \quad \Delta C_1 = 0 \]

(7)

This new expression unitizes the effect of solute 2 on the flux of solute 1 in terms of the unit flux of the second species rather than unit concentration difference across the membrane as expressed by equation 6.

Since larger hyperosmotic solutes less readily penetrate the membrane the "interaction ratio" \( P_{12}/P_{22} \) expression is particularly useful in that it makes possible a unitized comparison of various size hyperosmotic agents on their ability to interact with tracer solutes. Thus it is possible to determine whether large solutes interact with the tracer solutes to a greater or lesser degree. In practice, \( J_1 \), the net flux of solute 1, is obtained by subtracting its flux into from the flux out of the hyperosmotic solution. The \( P_{22} \) value used in the present paper is the permeability coefficient of solute 2 out of a 350 milliosmol solution of itself into water in which hydrostatic and hyperosmotic pressures are made equal. The concentration difference across the membrane for the hyperosmotic solutes is 350 milliosmols except for dextran which is ~8.

Table III presents \( P_{12} \) and \( P_{12}/P_{22} \) values calculated from the data shown in Figs. 8–11. It can be seen that the values for the cross-coefficients \( P_{12} \) are all greater than 0 indicating that the effect of solute 2 on solute 1 is to increase the flux of solute 1. It is of considerable interest that for each of the six tracer solutes, the interaction coefficients \( (P_{12}/P_{22}) \) increase with increasing size of the hyperosmotic agents. This shows that the force created by the addition of a diffusable hyperosmotic agent is proportional to the size of the agent as well as
to the size of the tracer solute, as shown above. Notice that the largest increase in \( P_{12}/P_{22} \) comes between raffinose and dextran at which point there is the largest increase in molecular size between any two of the hyperosmotic agents investigated.

**Table III**

| Solutes  | Tracer Hyperosmotic | Cross-coefficient | Interaction ratio |
|----------|----------------------|-------------------|------------------|
|          | 1 \( P_{12} \) 2 \( P_{22} \) \( P_{12}/P_{22} \) \( P_{12}/P_{22} \times 10^4 \) |
| Mannitol | M 0.127 6.8 9.2 10.1 | S 0.151 | R 0.101 |
|          | D* 2.59 749.7         |                  |                  |
| Sucrose  | M 0.174 9.4           | S 0.170 10.3     | R 0.124 12.5     |
|          | D* 4.66 135.1         |                  |                  |
| Raffinose| M 0.123 6.6           | S 0.137 8.3      | R 0.124 12.5     |
|          | D* 4.85 140.6         |                  |                  |
| Inulin   | M 0.029 1.5           | S 0.036 2.1      | R 0.042 4.2      |
|          | D* 4.64 134.5         |                  |                  |
| Dextran I| M 0.007 0.3           | S 0.026 1.5      | R 0.038 3.8      |
|          | D* 1.44 417.3         |                  |                  |
| Dextran II| M 0.021 1.1          | S 0.045 2.7      | R 0.032 3.2      |
|          | D* 0.613 177.6         |                  |                  |

* \( D = \sim 16,500 \) mol wt dextran at 0.008 molal.

In order to determine the effect of the pore size of the membrane on the process producing flux asymmetry, two additional membranes were studied. The characteristics of these membranes are cited in Table I. The Diaflo and General Atomic membranes are shown to have effective pore radii of 350 and 25 A respectively, whereas the S & S B20 membrane used in the studies above has an effective pore radius of 75 A.

Table IV presents some \( P \) values and flux ratios for the Diaflo and General
Atomic membranes. It can be seen that: (a) The $P$ values for mannitol, sucrose and raffinose do not differ as greatly from each other on the wide pore Diaflo membrane as they do for the narrower pore S & S membrane shown in Figs. 8–11. (b) Flux ratios greater than 1.0 are obtained on both of the membranes and these flux ratios increase in size with increasing size of the tracer solute as shown before with the S & S B20 membrane.

### Table IV

| Membrane     | Hyperosmotic solute | Tracer solute | Outflux $P \times 10^4 \pm 1$ s⁻¹ | Influx $P \times 10^4 \pm 1$ s⁻¹ | Flux ratio Outflux/influx |
|--------------|---------------------|---------------|--------------------------------|--------------------------------|------------------------|
| UM-3 No. 2   | Mannitol            | M             | 30.6 ± 1.7                     | 29.5 ± 1.6                     | 1.04                   |
|              |                     | S             | 22.1 ± 0.8                     | 17.4 ± 0.5                     | 1.27                   |
|              |                     | R             | 20.6 ± 1.2                     | 15.3 ± 1.0                     | 1.34                   |
| Sucrose      | M                   | —             | —                              | —                              | —                      |
|              | S                   |               | 21.3 ± 1.1                     | 18.4 ± 0.6                     | 1.15                   |
|              | R                   |               | 19.6 ± 0.8                     | 16.0 ± 1.2                     | 1.22                   |
| Raffinose    | M                   |               | 27.8 ± 1.6                     | 24.2 ± 1.4                     | 1.15                   |
|              | S                   |               | 20.1 ± 1.1                     | 17.3 ± 0.8                     | 1.16                   |
|              | R                   |               | 21.6 ± 1.3                     | 13.9 ± 0.8                     | 1.56                   |
| G.A. B       | Mannitol            | M             | 222.9 ± 5.5                    | 139.7 ± 5.8                    | 1.59                   |
|              |                     | S             | 83.9 ± 3.6                     | 29.0 ± 1.2                     | 2.89                   |
|              |                     | R             | 70.4 ± 1.8                     | 27.2 ± 0.6                     | 2.60                   |
| Sucrose      | M                   |               | 176.5 ± 5.0                    | 121.4 ± 5.8                    | 1.45                   |
|              | S                   |               | 89.6 ± 3.1                     | 25.8 ± 1.3                     | 3.48                   |
|              | R                   |               | 80.9 ± 2.2                     | 22.4 ± 0.9                     | 3.60                   |
| Raffinose    | M                   |               | 171.8 ± 7.7                    | 132.0 ± 5.2                    | 1.30                   |
|              | S                   |               | 96.9 ± 2.2                     | 34.0 ± 1.4                     | 2.85                   |
|              | R                   |               | 94.1 ± 3.1                     | 29.2 ± 1.0                     | 3.22                   |

A comparison of flux ratios for the three membranes is presented in Table V. It is to be seen that as one reads from left to right, the effective pore radius decreases, while in contrast, the flux ratios increase.

The effectiveness of a hyperosmotic agent in creating asymmetric solute flow, as measured by the $P_{12}/P_{22}$ term, also increases with smaller pore radii. Table VI lists $P_{12}/P_{22}$ values. With only one exception, the "effectiveness coefficient," $(P_{12}/P_{22})$, increases, from left to right, as the pore size decreases. The effect of increasing the size of the hyperosmotic agent generally is to increase flux ratios.
TABLE V
COMPARISON OF FLUX RATIOS FOR THREE MEMBRANES DIFFERING IN EFFECTIVE PORE RADIUS

| Solutes | Flux ratios |
|---------|-------------|
|         | UM-3 (<350 Å) | S & S B20* (~75 Å) | GA B (~25 Å) |
| Hyperosmotic | Tracer | |
| Mannitol | M | 1.04 | 1.32 | 1.59 |
|           | S | 1.27 | 1.57 | 2.89 |
|           | R | 1.34 | 1.61 | 2.60 |
| Sucrose | M | — | 1.32 | 1.45 |
|          | S | 1.15 | 1.54 | 3.48 |
|          | R | 1.22 | 1.72 | 3.60 |
| Raffinose | M | 1.15 | 1.20 | 1.30 |
|           | S | 1.16 | 1.48 | 2.85 |
|           | R | 1.56 | 1.80 | 3.22 |

*From Table II.
† Effective pore radius. See Table I for details.

TABLE VI
COMPARISON OF \( P_{12}/P_{22} \) VALUES FOR MEMBRANES WITH DIFFERING EFFECTIVE PORE RADIi

| Solutes | \( P_{12}/P_{22} \times 10^4 \) |
|---------|------------------|
|         | UM-3* | S & S B20† | GA B§ |
| Tracer | Hyperosmotic | |
| Mannitol | M | 1.0 | 6.8 | 14.9 |
|          | S | — | 9.2 | 16.5 |
|          | R | 4.9 | 10.1 | 12.0 |
| Sucrose | M | 4.3 | 9.4 | 7.0 |
|          | S | 4.0 | 10.3 | 20.3 |
|          | R | 3.7 | 12.5 | 17.8 |
| Raffinose | M | 4.9 | 6.6 | 5.5 |
|           | S | 4.7 | 8.3 | 18.6 |
|           | R | 9.3 | 12.5 | 19.6 |

* Approximate effective pore radius = 350 Å.
† Approximate effective pore radius = 75 Å.
§ Approximate effective pore radius = 25 Å.

DISCUSSION

Our previous description (1) of a net solute flux across a nonbiological membrane, down the concentration gradient of a second, diffusible solute, is in accord with the solute flux asymmetry reported for biological systems by ourselves, Ussing, and Biber and Curran (2, 4, 5). We suggested that the net movement of a solute may result from its interaction with a second diffusible
solute moving down a concentration gradient. Such interaction is known to occur in free solution (6-8). Membrane systems differ from free solution in that the interface between solutions of unequal concentration is a permanent, rigid structure with a limited diffusion area, bathed by well-stirred solutions. In the case of the nonbiological or synthetic membranes, the movement of solute through the membrane must take place through "pores." Since all solutes crossing the membrane must share these diffusion spaces, interaction between solutes may well differ from the more random interaction that may occur in free solution.

A possible explanation of the flux asymmetry created by the presence of an osmotic gradient may be visualized in the following way. As a result of random diffusion, a molecule of a tracer solute having no chemical gradient enters a "pore" of the membrane. It may then move back out of the pore or it may move on through the pore. The direction of movement of the tracer, in a system without bulk flow, will depend upon the direction from which comes a "force," in this case the bombardment by other solute molecules. More specifically, as the hyperosmotic solute diffuses down its concentration gradient it will collide (interact) with the tracer molecule, tending to drive it in the direction of its own movement. As a consequence, movement into the hyperosmotic solution will be less and movement out of the hyperosmotic solution will be greater, thus creating an asymmetric movement of tracer. If molecules are to collide or interact with other molecules, it seems reasonable to expect that the cross-sectional area of both the tracer (the driven species) and the hyperosmotic agent (the driver) will influence the magnitude of the interaction. It is clear that the concentration gradient, or chemical potential, of the driver solute will determine the amount of this solute diffusing and thus the magnitude of flux asymmetry. An additional factor to be considered is the influence of the pore itself. Reduction of the pore size should restrict the randomness of solute interaction and increase the vectorial effect of the solute gradient, thus producing more effective solute interaction.

With regard to the model system described above, let us consider the data obtained from the experiments we have conducted. In the experiments on sucrose and inulin fluxes in which the concentration of hyperosmotic agent was varied, the unidirectional fluxes and the flux ratios showed a change proportionate to the imposed gradient, as predicted by the model.

The significant role played by solute size is well-documented by the experiments in which tracer and hyperosmotic sizes were varied. The effect of increasing tracer size, as measured by flux ratios for a given driver solute, is seen in Tables II and IV. Here it is shown that, in keeping with the prediction of the model, the flux ratios increase progressively with increasing cross-sectional area of the tracer. The hyperosmotic agent or driver solute also shows that with increasing size there is an increase in the effectiveness of inter-
action (see $P_{12}/P_{21}$ data in Tables III and VI). The effects then of tracer and driver solute sizes are in accord with the proposed model.

The role of pore size in solute interaction is confirmed by the data showing increasing flux ratios and interaction ratios with decreasing effective pore size (Tables V and VI). With the particular solute pairs used in these studies, the flux ratios in the widest pore membrane were generally not too different from 1.0. The same solutes, with the narrow pore (25 A) membrane, showed flux ratios two to three times larger. Consequently, one must conclude that there is a critical relationship between the effective size of the pore, the size of the solutes and the degree of solute interaction.

We do not presume that the size relationship just cited describes all parameters of solute-pore interaction. To be considered in the ultimate understanding of solute drag are factors such as pore length, tortuosity, surface charge, bound water, molecular shape of the solutes, and hydrogen bonding.

The anomalous behavior of the dextrans requires comment. The smaller dextrans generally yielded flux ratios greater than did the large dextrans. Also, the flux ratios with the dextrans are usually smaller than those obtained with inulin although the dextrans are several times larger than inulin. The dextrans used in our studies are believed by some (21) to be long, rod, or thread-like structures. It may be that the anomalous behavior is attributable to the orientation of the molecules in the pores, an orientation created by the diffusing solutes in a manner analogous to that suggested by Soll (22) for systems with bulk solvent flow. A more complete treatment of this subject must await further investigations.

Consideration must be given to alternative explanations for the observed flux asymmetry. First to be mentioned is the matter of the activity of the tracer solute. Although activity coefficients for all our solute pairs are not readily available, data for the sucrose-mannitol system are given by Robinson and Stokes (23). They show an increase of less than 3% in the concentration range relevant to this paper. Thus, it is unlikely that changes in activity produced by the presence of the hyperosmotic agent would be sufficient to produce the observed findings. This opinion is shared by Biber and Curran (5) for their mannitol-urea system.

A second potential explanation of the observed flux asymmetry is related to a circulatory flow of water through the membrane. Such a flow might create flux asymmetry but the presence of this flow is difficult to prove experimentally. We have measured THO flows under conditions yielding solute flux asymmetry but these were not different from those obtained in "open" experiments where pure diffusion conditions were present and there was no reason to suspect the presence of a circulatory flow. Unequivocal experimental evidence on this point is not yet available.

1 Patlak, C. S., and Rapoport, S. I. Personal communication.
That coupling of processes may occur has been discussed by a number of authors (24–26). However, it does not appear that the description given by these papers anticipate fully the solute interaction, and the role of solute and pore size that is presented in this paper for a nonmetabolizing system in which physical interaction alone is occurring.

The discovery that solute drag may be demonstrated with biological systems such as frog and toad skin suggests that the process may be a functional component in the transmembrane movement of solutes at the cellular level. One can envision that the concentration gradients of a wide variety of bio-solutes may act to influence cellular and extracellular compositions. For example, the concentration gradient for a solute such as glucose after feeding may cause the delivery into the cell of not only glucose but also other solutes previously in thermodynamic equilibrium across the cell membrane. Consequently one must consider the role of solute drag in evaluating the results of what may have been presumed to be the presence of a gradient for a single solute. The potential participation of solute drag also in biological transport mechanisms cannot be discounted.

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