Effects of Solvent and Solute Drag on Transmembrane Diffusion

J. T. VAN BRUGGEN, BRENT CHALMERS, and MERTIE MULLER

From the Department of Biochemistry, School of Medicine, Oregon Health Sciences University, Portland, Oregon 97201

ABSTRACT The present study compares and quantitates both solvent drag and solute drag forces in a system with both heteropore and homopore membranes. It is shown that tracer solute permeability can be increased if solution flow or driver solute flux is in the direction of tracer diffusion. Either force can decrease tracer permeability if the force is opposite to the direction of tracer diffusion. The two forces can be additive or one force may reduce the effect of the other force. In the particular system quantitated, solute drag is shown to be some 300 times more effective than solvent drag on a mole-to-mole basis. The use of a number of solute pairs on other homopore and heteropore membranes confirms the finding that the two drag forces can be analyzed or manipulated in a variety of systems.

INTRODUCTION

Solute drag and solvent drag are two physical forces, each of which can produce an asymmetry of tracer solute flux across biological or synthetic membranes. Solute drag results from solute-solute interaction as a hyperosmotic permeable solute (the driver) diffuses through the membrane pores, down its concentration gradient. As it does so, it physically interacts with another permeable solute (the tracer), which is diffusing without a concentration gradient. This solute-solute interaction results in an increase of tracer flow in the direction of the downhill gradient flux of the driver and a decrease in the flux of tracer diffusing against the driver flux (Franz et al., 1968; Galey and Van Bruggen, 1970; Van Bruggen et al., 1974). Solvent drag, on the other hand, results from a permeable solute being carried in the bulk flow of solution, which results from osmotic or hydrostatic gradients. Solvent drag may also increase or decrease the flow of tracer solute as it moves with the bulk flow or against it (Anderson and Ussing, 1957).

Both of these two drag forces may be operative when hyperosmotic gradients exist and one or the other force may be increased or decreased by the imposition of a hydrostatic force. The net effect of the expression of the multiple drag forces has not previously been quantitatively predictable. The
direction and rate of solute fluxes will depend upon the size of the osmotic
gradient, the magnitude of the hydrostatic force, and the permeability char-
acteristics of the driver and tracer solutes. The basic transmembrane perme-
abilities depend upon solute size and shape and upon the structure and
porosity of the membrane.

To understand the mechanisms of biological transmembrane transport, it
is necessary to consider the potential role of both solute drag and solvent drag
forces. Previous studies have not included both forces nor have the two forces
been compared by the same parameters. In the current studies we have used
synthetic heteroporous and homoporous membranes in separate and simul-
taneous studies of the two drag forces. Additional variables that have been
considered include solute concentration, molecular weight and size, solution
viscosity, and membrane pore size.

The present studies demonstrate that the transmembrane flow of a tracer
solute can be manipulated at will by the imposition of either solvent or solute
drag forces. These two forces can be coupled or opposed to yield a selected
direction and rate of tracer solute diffusion.

A model is proposed for the interplay of the solute and solvent drag forces
and the model is substantiated with experimental data permitting quantitative
comparisons of the two forces by the same parameters.

MATERIALS AND METHODS

Membranes

The experiments reported here were carried out on synthetic membranes of two types,
homoporous polycarbonate and heteroporous cellulose acetate.

The homoporous membranes were experimental ones prepared by the Nucleopore
Corp., Pleasanton, Calif. These polycarbonate membranes had been programmed
to have $6 \times 10^8$ pores cm$^{-2}$ and pore diameters between 100 and 300 Å. Pieces
of membrane were cut from the sheet provided and mounted in the same holders as
before (Galey and Van Bruggen, 1970). The small pore size prevented the electron
micrographic determination of pore count and size such as we did previously (Van
Bruggen et al., 1974). Each piece, however, was characterized by bulk water flow and
tracer diffusion measurements as described in the Appendix. In experiments where
a number of pieces of membrane were required to complete the study, data from several
pieces of the membrane having similar pore diameters were used regardless of the
number of pores calculated for the pieces. The results of these pooled data are given
as the average value of the data that were first normalized to be a function of the
total open area per square centimeter of the membrane so that the area parameter of
permeability is per square centimeter with an average number of pores.

The heteroporous cellulose acetate membranes (Carl Schleicher & Schuell Co.,
Keene, N. H.) were selected for their range of pore sizes. These membranes have an
extremely thin, dense surface skin with an overlying porous, thick backing. The
"brush pile" structure of the "skin" with its variable and/or tortuous pore character-
istics is contrasted with the regular, uniform pore size of the polycarbonate membrane.
Despite the asymmetrical structure of the cellulose acetate membranes, we found only
symmetrical diffusive permeabilities for tracer water under the conditions of our
studies. Table I lists characteristics of the membranes.
Solute Drag on Transmembrane Diffusion

Table II lists the solutes used as hyperosmotic agents and compares their physical properties.

Reagent grade urea, sucrose, and raffinose were obtained from J. T. Baker Chemical Co., Phillipsburg, N. J., as were the polyethylene glycols (PEG) with average molecular weights of 400 or 600. Mannitol was obtained from Mallinckrodt Inc., St. Louis, Mo.

**Table I**

**Characteristics of the Synthetic Membranes**

| Name            | Nucleopore | A     | B     | S and S3C2 | S and SAC62 |
|-----------------|------------|-------|-------|------------|-------------|
| Composition     | Polycarbonate | Cellulose acetate | Cellulose acetate |
| Thickness (µm)  | 6          | 6     | 100 (see text) | 90 (see text) |
| Effective pore  | 265*       | 110   | 132   | 181        |
| (Å Diam)        | 360‡       | 110   | 100   | 120        |
|                 | 260§       | 105   | 70    | 90         |
| Lp (cm$^2$ dyn$^{-1}$ s$^{-1}$ × 10$^{25}$) | 100-300 | 100-300 | 50-100 | 50-100 |
| P H$_2$O (cm$^{-3}$ s$^{-1}$ × 10$^{6}$) | 100 | 27.7 | 652 | 451 |
| Reflection coefficient | 0.02* | 0.05 | 0.043 | 0.031 |

* Calculated (see Appendix).
‡ By the method of Goldstein and Soloman (1960).
§ By the method of Renkin (1954).
∥ Manufacture’s designation.
¶ Method of Durbin (1960).

**Table II**

**Molecular Dimensions of Solutes**

| Solute | Molecular weight | Molecular radius$^*$ |
|--------|------------------|---------------------|
|        |                  | By diffusion        | By viscosity      |
| DHO    | 19               | 1.9                 | —                 |
| Urea   | 60               | 2.7                 | —                 |
| Mannitol | 182           | 4.4                 | 3.8               |
| Sucrose | 342            | 5.3                 | 5.0               |
| Raffinose | 504           | 6.1                 | 5.7               |
| PEG 600 | 570-630       | 7.7                 | —                 |

* By diffusion: Durbin (1960); by viscosity: Pappenheimer et al. (1951).

Radioactively labeled sucrose [$^{14}$C(U)] and raffinose [$^{3}$H(G)] were obtained from New England Nuclear, Boston, Mass. Tracer mannitol[$^{13}$C] was obtained from Amersham Corp., Arlington Heights, Ill. These compounds were dissolved in distilled and microfiltered water before being used in diffusion experiments. Tritiated water for determining the diffusive permeability of solvent was obtained from New England.
Nuclear. Radioassays were done by liquid scintillation spectrometry using Aquasol (New England Nuclear).

Solvent water after double-glass distillation was filtered through 0.05-µm Nucleopore membranes. Care was taken in the preparation of solutions to avoid contamination by dust or other foreign substances because the clogging of pores by foreign particles in these tight membranes can substantially alter permeability results. Membranes were periodically checked with tritiated water and other solutes to insure their basic permeability.

**Apparatus**

The experiments were carried out in Lexan chambers slightly modified from those previously described by us (Galey and Van Bruggen, 1970). Volume flow was measured with a dial gauge micro-buret (Beckman Instruments, Inc., Fullerton, Calif.) having a total capacity of 1 ml; each complete revolution of the dial is equal to 1 µl. Volume flows occurring from either hydrostatic or osmotic pressure were measured with a precision of ± 0.2 µl min⁻¹. However, the measurement of volume flows, particularly the low flows of certain experiments, was found to be unnecessarily tedious and time consuming. In these cases, in place of an actual volume flow reading, the flow was read from a previously prepared graph relating imposed hydrostatic pressures (0–60 cm Hg) to volume flows. The figures document this flow-pressure relationship.

**The Model System**

In our previous studies with two permeable solutes (Galey and Van Bruggen, 1970), the flux of solute 1 was described as being due to three principle forces:

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

where \( \bar{C} \) is the mean concentration of the solute, \( \sigma \) is the reflection coefficient of the solute, \( J_v \) is the volume flow across the membrane, \( P_{11} \) is the self permeability of the tracer, and \( \Delta C_1 \) is the concentration difference of solute 1 across the membrane. The effect of solute 2 on the diffusion of solute 1 is described in the third term of the equation, where \( P_{12} \) is the cross coefficient of diffusion and \( \Delta C_2 \) is the concentration difference of solute 2 across the membrane. In our previous studies with \( J_v = 0 \) and \( \Delta C_1 = 0 \), only the third term or solute-solute interaction was quantitated. In the present study we report on the manipulation of volume flows (first term) and use of different tracer solutes (second term), as well as use of different driver solutes (third term).

The following model (Fig. 1) for tracer diffusion illustrates the interaction of diffusion, solvent drag and solute drag forces upon tracer permeability \( P_T \).

As before (Van Bruggen et al., 1974), the driver solute was placed in chamber 2, and hydrostatic pressure could also be applied to chamber 2. Tracer solute was added to either chamber 2 or chamber 1. The unidirectional movement of tracer \( P_T \) is shown as \( P_T^{2 \rightarrow 1} \) or \( P_T^{1 \rightarrow 2} \), the net flux of driver \( J_d \) as \( J_d^{2 \rightarrow 1} \) and the bulk flow as \( J_v \) being either \( J_v^{2 \rightarrow 1} \) or \( J_v^{1 \rightarrow 2} \). With no net flow \( J_v = 0 \). The role of the forces is summarized below.

The abscissa indicates the direction and size of the volume flow. Note that the volume flow is centered around \( J_v = 0 \), the direction of flow \( J_d^{2 \rightarrow 1} \) is into the hyperosmotic driver solution. At \( J_v = 0 \) the imposed hydrostatic pressure has now blocked the osmotically induced volume flow. In the area \( J_v^{2 \rightarrow 1} \), the hydrostatic pressure is greater than the osmotic pressure so that bulk flow is reversed. At some
point the hydrostatic pressure will approach 0 and the bulk flow will then be the maximum that the osmotic gradient can induce given the nature of the driver solute and its reflection coefficient. Additional \( J^2 - 1 \) beyond this point would require hydrostatic pressure on side 1.

The ordinate is shown as \( \log P_T/P_T \) to indicate increases or decreases in the measured permeability of the tracer \( P_T \) as deviations from the basic permeability of the tracer \( P_T \) at 1 mM with \( J = 0 \) and \( J_D = 0 \).

Figure 1. Model showing the effects of solvent drag and solute drag forces upon the permeability of a tracer solute. Solution flow, \( J_v \), is shown centered around \( J_v = 0 \) with the appropriate flow direction indicated adjacent to the \( J_v = 0 \) axis. Effects on tracer permeability are shown as increases or decreases from a basic permeability \( P_T \) determined with the tracer at 1 mM without the presence of the second solute (driver). The separate or combined effects of solvent and solute drag upon the diffusion of tracer are described in detail in the text.

The effects of various factors studied can be illustrated by reference to the numbered points on Fig. 1.

1 Reflects the basic \( P_T \) of the tracer at 1 mM on both sides without the presence of driver or bulk flow.

1–8 Increase in \( P_T \) proportional to volume flow (solvent drag), \( T \) diffusing with the bulk flow.

1–9 Decrease in \( P_T \) proportional to volume flow (solvent drag), \( T \) diffusing against bulk flow.
1-5 and 10-4 Decrease in $P_t$ due to presence of driver in the pore environment (viscosity?).

1-3 Decrease in $P_t$ due to solute drag with tracer moving against driver flux $J_D^{2-1}$ while $J_o = 0$.

1-2 Increase in $P_t$ due to solute drag with tracer moving with driver flux $J_D^{2+1}$ at $J_o = 0$.

3-7 Decrease of $P_t$ proportional to volume flow $J_o^{2+1}$ with tracer moving against driver flux $J_D^{2-1}$ and against bulk flow $J_o^{2-1}$.

3-4 Increase of $P_t$ proportional to volume flow $J_o^{2-1}$ with tracer against driver flux $J_D^{2-1}$ but with volume flow.

2-6 Increase in $P_t$ proportional to volume flow $J_o^{2+1}$ with tracer moving with volume flow and driver flux $J_D^{2+1}$.

2-4 Decrease in $P_t$ proportional to volume flow $J_o^{2-1}$ (osmotic flow) with tracer moving against driver flux $J_D^{2-1}$.

3-4 Increase of $P_t$ proportional to volume flow $J_o^{2-1}$ with tracer against driver flux $J_D^{2+1}$.

4-12 and 4-11 Rectification of direction of tracer movement when solvent drag is greater than solute drag.

**Statistical Treatment of Data**

In Figs. 2-5, the many individual data points have not been shown on the figures; rather, the data were treated to linear regression analysis to obtain the lines, slopes, and intercepts reported. The notation SEE, the standard error of estimate, is essentially the standard deviation of actual $Y$ values from the $Y'$ values predicted from the line on the figure (at a particular value of $X$). Some authors use the notation $E y/x$ or $s_y/x$ to describe the standard error of estimate of $Y$ (at indicated values of $X$).

In Figs. 6 and 7 the lines reported were fitted to the data points by the method of least squares.

**RESULTS**

Experiments were conducted to test the validity of the proposed model.

**Solvent and Solution Flow: Homopore Membrane**

The first study, shown in Fig. 2, is that of pressure/flow relationships on a homopore membrane (A) with solute pairs of differing composition. The upper line represents the $J_o^{2-1}$ flow obtained from the indicated applied pressure when only water bathed both sides of the membrane. The response was linear and has a slope of 0.57 $\mu l$ min$^{-1}$ cm$^{-2}$. When 0.25 M PEG 600 replaced the solute-free bathing solution, the flow response was decreased (slope 0.24) but the response was linear over these pressure and flow ranges. The lesser flow of the solution, over that of the solvent itself, is an indication of the changed pore environment (viscosity?) and the greater pressure that is required to cause a particular flow.

The bottom line of Fig. 2 represents an experiment in which both osmotic pressure and hydrostatic pressures were operative. 0.5 M PEG 600 was placed in chamber 2 and H$_2$O in chamber 1, this being the case in most of our studies of solute drag. When no hydrostatic pressure was applied, the flow $J_o^{2-1}$ was caused by the osmotic force. As pressure was applied, the flow was decreased in a linear manner until it reached $P = 30$ cm Hg, $J_o = 0$, and hydrostatic pressure = osmotic pressure. Above 30 cm Hg, the flow became $J_o^{2+1}$. It is to be noted that the slope of the lower line (0.26) is essentially the same as the
slopewith one-half the concentration of PEG on both sides of the membrane. This similarity of slopes suggests a similarity of the pore environments in the cases of the two PEG systems (0.25 PEG/0.25 PEG and 0.5 PEG/H₂O). This is in part the basis of our use of the mean concentration of solute $\bar{C}$ as the “effective solute concentration” of the pore environment. In the following, knowledge of $P\bar{C}$ is of value in quantitation of the solute and solvent drag effects.

The study above was done with the homopore polycarbonate membrane,
which has right angle, cylindrical pores of known number, diameter, and length. In some respects the surface of the homopore membrane may resemble a biological surface having extracellular transmembrane pores over a fraction of its surface. However, a heteroporous cellulose acetate membrane may also bear a similarity to some biological surfaces in that the “compressed brush pile” structure leads to a greater number of pores. These pores range widely in size, the number is not easily determined, and the pore channel may be tortuous and of unknown length.

It is of interest then to compare the solvent and solute drag characteristics of the pore systems of these two membranes that differ so grossly in structure.

**Solvent and Solution Flow: Heteropore Membrane**

Fig. 3 shows the pressure/flow relationships of one of the heteropore membranes cited in Table I. This S and SRC52 membrane has a calculated effective pore diameter of 70–100 Å and permeability characteristics not too dissimilar from the homopore used above (see Table I for details).

The upper line of Fig. 3 indicates that with 0.175 M sucrose bathing both sides, the solution flow with applied pressure was linear over the ranges studied. The slope of the upper line is 0.67. When the solutions were replaced with 0.35 M sucrose/H₂O (lower line), an osmotic flow of 19.9 μl min⁻¹·cm⁻² occurred. This flow was reduced as hydrostatic pressure was imposed and brought to Jₑ = 0 at 30 cm Hg. The slope of this response (lower line) is 0.66.

It appears that the heteropore membrane, although having a more complex “pore” system, does allow the solute to obtain a C concentration similar to the homopore system above.

**Tracer Mannitol Diffusion with Solvent and Solute Drag: Homopore Membrane**

With knowledge of the volume flow-pressure relationship for several driver solutes on both homopore and heteropore membranes, it was possible next to follow the effect of volume flow upon the diffusion of tracer solutes. Fig. 4 shows a solvent drag and solute drag study such as was illustrated in the model (Fig. 1). The solutes of interest here are tracer mannitol and driver solute PEG 400. The tracer mannitol-C₁₄ was added to one side at a time with both bathing solutions being 1 mM mannitol.

The figure shows the effect of solution flow upon the apparent permeability value for tracer mannitol as it diffused along with or against the flow Jₑ of cause by hydrostatic pressure on chamber 2. The actual Pₑ value for the tracer at Jₑ = 0 is 4.5 × 10⁻⁶ cm·s⁻¹. These experimental points are shown as point IA and 1B close to the center intersection of the two base lines and is similar to the theoretical point 1 on the model system diagram of Fig. 1. The unidirectional Pₑ value is shown to increase (IA–8) when solution flow occurred in the same direction as the tracer diffusion and to decrease proportionally (1B–9) when diffusion of tracer was in the opposite direction to solution flow. Solvent drag is seen to exert either a positive or negative effect upon the diffusion of tracer. These Pₑ effects are found to be symmetrical in that the slopes are equal but of opposite sign. The described changes in Pₑ values may not hold much beyond the actual pressure-volume relationship reported in the figures. At higher pressures, mechanical problems and changes
Figure 3. Effect of imposed hydrostatic pressure on solution flows through heteropore membrane S and SRC52 during the two conditions: 0.175 M sucrose/0.175 M sucrose and 0.35 M sucrose/H₂O. Details of the linear regression analysis of the experimental data are shown below. SEE is the standard error of estimate.

| Condition          | Number of samples | Slope  | Intercept | SEE  |
|--------------------|--------------------|--------|-----------|------|
| 0.175 M sucrose/0.175 M sucrose | 5                  | 0.67   | -0.03     | 0.03 |
| 0.35 M sucrose/H₂O     | 4                  | 0.66   | -19.9     | 0.01 |

The flow shown by the upper line is hydrostatically induced. The flow shown in the lower line is osmotically induced and hydrostatically repressed.

In fluid dynamics introduce deviations from the linear plot of log values. The present studies have, however, used pressures up to 60 cm Hg.

When solute drag effects, in addition to solvent drag, are imposed upon tracer diffusion, larger changes in the apparent permeability $P_T$ are elicited.
FIGURE 4. Effects of solvent and solute drag upon tracer mannitol permeability using PEG 400 as the driver solute. Homopore membrane B with pores of ~110 Å Diam was used between the chambers. The figure represents an experimental test of the model proposed in Fig. 1. Details of the linear regression analysis of the data are presented below. SEE is the standard error of estimate.

| Line segment | 4–6 | 4–7 | 1A–8 | 1B–9 |
|--------------|-----|-----|------|------|
| Number of samples | 94 | 90 | 32 | 29 |
| Slope | 1.35 | -1.21 | 1.12 | -0.26 |
| Intercept | 0.19 | 0.012 | -0.024 | -0.024 |
| SEE | 0.09 | 0.08 | 0.05 | 0.07 |

The upward-sloping lines show the increase in apparent P values as either solvent or solute drag exert their effect in the same direction as the tracer is diffusing. Similarly, the downward-sloping lines show that either solvent or solute drag can decrease the permeability of a diffusing tracer solute. The text more fully describes the specific singular effects of the two drag forces.

Solute drag and solvent drag were followed with driver solute PEG 400 placed in chamber 2. The decreased permeability shown at point 5 is to be expected from the solute-solute interaction that occurs in free solution (Dunlop, 1957; Ellerton and Dunlop, 1967a and b). A further decrease in $P_f$ is shown at point 3, where the diffusion of tracer $P_f^{2-1}$ is against the flux of the
driver. In contrast, point 2 represents the increase in $P_T^{2-1}$ afforded by the flux of driver $J_D^{2-1}$, adding to the concomitant diffusion of tracer. These two large changes (1-2 and 1-3) in $P_M/P_{1\text{mM}}$ indicate a significant solute drag effect.

The increase in $P_T$ values shown on the upper line, 2–6, is the result of the additive positive effects of solvent drag $J_o^{2-1}$ and solute drag $J_D^{2-1}$. From point 2 toward 4, the positive solute drag effect at point 2 is reduced by the osmotically induced bulk flow $J_o^{2-1}$, which is also directionally against the $P_T$ of the tracer. The osmotic flow was allowed by reduction of the hydrostatic pressure on side 2 from 46 cm Hg at $J_o = 0$ down to $0$ at $J_o^{2-1} = 0.12 \mu l \text{ min}^{-1}$, as is indicated by the lower end of the upper line. Thus far, it is clear from the figure that the solute drag and solvent drag effects can be roughly compared on a quantitative basis, although with different parameters. The increase in $P_T$ from the base to point 2 is $\approx 1.5$-fold. This increase is due to solute drag. With this solute drag effect continuing to operate, there was required a volume flow of $0.18 \mu l \text{ min}^{-1} \cdot \text{cm}^{-2}$ to reduce the $P_T$ value to the base level.

The lower line in Fig. 4 describes the interplay of the forces detailed above in terms of the movement of tracer $P_T$ against the flux of driver $J_D^{2-1}$. The effect of these forces will be briefly described.

Point 3 indicates the decrease of $P_T$ caused by tracer diffusion, $P_T^{2-1}$, against the flux of driver $J_D^{2-1}$ at the balance point of hydrostatic and osmotic forces, i.e., $J_o = 0$. $P_T$ is further decreased when bulk flow $J_o^{2-1}$ also opposes $P_T$. When hydrostatic pressure is lowered and osmotic flow in the direction of $P_T^{2-1}$, the latter measurement increases up to the maximum afforded by the osmotic flow (at $\approx 0.18 \mu l \text{ min}^{-1} \cdot \text{cm}^{-2}$). The solute drag effect of reducing $P_T^{2-1}$ movement (lower line) is overcome by the $J_o^{2-1}$ or solvent drag assistance to $P_T^{2-1}$.

To illustrate the broad or general nature of the solvent and solute interactions detailed above, portions of three other studies are given below. In these, four variables have been investigated: pore size, heteropore vs. homopore membranes, tracer size, driver size, and driver concentration. These are being presented to contrast the two drag forces and to illustrate the principles previously described by this laboratory (Van Bruggen et al., 1974). The following are not meant to be exhaustive studies of each of the variables. Graphic information on tracer flow in H$_2$O/H$_2$O systems is not included.

**Tracer Sucrose Diffusion with Solvent and Solute Drag: Homopore Membrane**

Fig. 5 can be contrasted with portions of Fig. 4 and shows that the larger tracer sucrose and PEG 600, a larger driver, show good solute drag effects, although the larger pore size, 260 vs. 110 Å, would decrease solute/solute interaction. The effect of the larger molecular size of the driver 600 vs. 400 PEG is shown by the large decrease in $\bar{C} \log P/P_{1\text{mM}}$ to 0.25, in contrast to the 0.07 decrease shown in Fig. 4 on the $J_o = 0$ axis. It is this more restricted pore environment that leads to the lower position of Fig. 5 on the ordinate as compared with Fig. 4. In this larger, pored homopore system, the osmotic
pressure using the larger driver PEG 600, with its larger reflection coefficient, was sufficient to produce a flow $J_{c_{-1}}$ of $\approx 1.0 \mu l \ min^{-1} \ cm^{-2}$.

**Tracer Raffinose and Sucrose Diffusion with Solvent and Solute Drag: Heteropore Membranes**

The interplay of solvent and solute drag forces across heteroporous membranes is illustrated in Figs. 6 and 7. These two membranes were selected because of similar “pore” diameters to the homopore membrane shown in Fig. 4. These

**Figure 5.** Effect of solvent and solute drag on tracer sucrose permeability using PEG 600 at 0.5 M as driver solute. Homopore membrane A with pore diameters of $\sim 260 \ \AA$ was used. Details of the linear regression analysis of the data are summarized below. SEE is the standard error of estimate.

| Lines | Number of samples | Slope       | Intercept  | SEE   |
|-------|-------------------|-------------|------------|-------|
| 4-3   | 20                | -0.022      | -0.39      | 0.04  |
| 4-2   | 19                | +0.013      | -0.10      | 0.02  |

The figure shows the effects of a larger tracer (sucrose) and a larger driver (PEG 600) used with a larger pored (260 \AA) homopore membrane. The position of the figure on the coordinates and the slope of the lines confirms the roles of the dual drag forces.

heteropore membranes were, however, an order of magnitude more permeable to water flow than the homopore membranes, due to their larger open area. In general, the heteropore membranes had similar responses to the homopore, as shown by the comparable shapes of Figs. 4 and 5 with Figs. 6 and 7. It is clear that there is an interplay between solute and solvent drag forces across either type of membrane.

These membranes can be used to compare other aspects of the solute drag concept presented previously (Van Bruggen et al., 1974). The log $P/P_1$ \ mM
value identified by the intercept of each line with the $J_v = 0$ axis is a measure of the positive or negative effect of the driver upon the tracer. In Fig. 7, with sucrose as both the driver and tracer of intermediate size, driver sucrose at 0.35 M shows its effect upon tracer sucrose but the interaction is not as large as that shown for driver PEG 600 and tracer sucrose (Fig. 5), although the larger pore size of Fig. 5 would tend to lower the solute interaction. With this degree of interaction, less bulk flow, $J_v^{2-1}$, was required for the solvent drag effect to equal the solute drag effect. When the tracer size was increased to that of the trisaccharide raffinose and the sucrose driver concentration was increased to 0.5 M, a greater degree of interaction (Fig. 6) took place, as shown by the intercepts with $J_v = 0$ and the location of the cross-over of the lines at a higher $J_v^{2-1}$.

DISCUSSION

In an earlier preliminary study (Franz et al., 1968), we contrasted the effects of solvent drag and solute drag upon inulin fluxes crossing a synthetic membrane. With 0.3 M sucrose bathing both sides of the membrane, fluxes of 4.0 $\mu$mol cm$^{-2}$·h$^{-1} \times 10^3$ were found in both directions. With 0.3 M sucrose on one side only, the flux with the osmotic flow was 8.9 and against it was 1.6 (ratio = 0.2). When the osmotic flow was blocked by a hydrostatic head, the flux of 1.6 increased to 23.3 and the 8.9 decreased to 1.7 (ratio = 13.7).

It was clear that either solvent drag or solute drag were forces capable of causing tracer solute asymmetric fluxes and that an adequate regard for these effects is required for an understanding of biological transmembrane transport. This problem of tracer flux asymmetry became increasingly apparent to us as we sought to study mechanisms of gut absorption in an attempt to identify solute drag as a potential contributing force in absorption (T. Mullen, unpublished data).

When luminal perfusate solutions were made hyperosmolar to initiate solute drag, fluid was drawn into the lumen while solutes (and fluid) were being absorbed into the vascular system. At least for certain routes of absorption, the movement of a tracer molecule from the lumen into the blood is subjected to both solute drag effects (lumen → blood) and solvent drag effects (lumen ← blood). As in many other biological studies, it is not possible to control the osmotic volume flow hydrostatically and it is necessary to carry out complex, multiple-label experiments that are usually difficult to interpret precisely. We became aware that to understand these multiple physical forces influencing diffusion, a more precise description of the interactions of the solvent and solute drag forces was required.

The present studies on a simple nonbiological system are an attempt to analyze the two forces for quantitative aspects that may subsequently be applied to biological systems.

To study the drag effects, membranes must be chosen that are traversed by pores so that solvent may flow in bulk in the required amount, and the pores must be of a size to accommodate the flux of solute caused by the force involved. For solvent drag, the pores must be large enough to allow passage
of the solutes carried by the solvent flow. For solute drag also, the pores must be large enough to allow passage of the solute pairs (driver and tracer), but in contrast to solvent drag, the pores must also be small enough to permit a finite interaction between solutes and their fluxes. This dependence of solute drag on pore size (Galey and Van Bruggen, 1970) is in contrast to the solute interaction that takes place in free solution (Dunlop, 1957; Ellerton and Dunlop, 1967a and b).

The model system proposed in Fig. 1 has been tested with a number of solutes and two basically different membranes. It is acknowledged that Fig. 1 represents a particular case for a tracer solute and its driver, each present at a certain concentration, when the donor and receiver compartments are separated by a particular membrane. The slopes of the plotted lines, the location of the intercepts, and the degrees of interaction shown between the forces will be peculiar to a particular experiment. This is illustrated by the different shapes of the various figures shown in Results.

The experiments were conducted to reveal the various effects caused by solution flow, solution composition, and membrane pore effects.

When solution is made to flow by imposition of a hydrostatic head, the flow rate was shown to be linear, within the pressure-flow limits stated in this text. The volume flow is, however, also determined by solution composition. When both bathing solutions contained 0.25 M PEG 600, the flow rate was reduced to less than one-half of the H₂O/H₂O solution flow. With the donor side containing 0.5 M PEG 600 (against H₂O), the flow rate was the same as the experiment with 0.25 M PEG/0.25 M PEG. Reduction of the flow rate by the presence of solute in the solvent is generally considered as an effect of viscosity. In fact, the effect of solution composition upon solution flow reflects
the summation of the interaction of all components of the system, i.e., solvent, solutes, pore structure, etc. In some systems, these can be separately described in terms of specific frictional coefficients, but it is the summation of these frictional interactions that will determine the degree of solute movement and it is this summation of effects we have followed.

It is clear that solvent flow and solution composition can modify the rate of solute diffusion through membranes and that each factor must be under control of the observer of the diffusion events. The effects of the imposition of multiple solvent and solute forces are illustrated by the experiments shown in Figs. 4–7.

The study reported in Fig. 4 has included in it the two chief forces under discussion in this paper. The system contained a homopore membrane with small pores of 100 Å Diam, and two solutes, tracer-mannitol and driver PEG 400. The effect of solution flow and/or solute drag is presented as increases or decreases in the rate of diffusion of tracer mannitol compared with its basic diffusion in an H₂O/H₂O system without driver solute or net solvent flow. As predicted by the model, the rate of tracer diffusion is shown to increase or decrease severalfold when bulk solution flow is produced by hydrostatic or osmotic pressure. Tracer diffusing “upstream” is slowed and tracer diffusing “downstream” is accelerated. These effects of solvent flow upon tracer diffusion are shown in both the H₂O/H₂O system and in the presence of a gradient of driver solute PEG 400. Because solvent flow initiated by either osmotic or hydrostatic forces does cause solvent drag and tracer solute asymmetry, the
effect of this force must either be given its quantitative role or eliminated from consideration.

As illustrated in Fig. 4, when volume flow is reduced to a negligible value represented as \( J_v = 0 \), the effect of other factors can be studied. In the case of Fig. 4, the \( P \) value for tracer mannitol in an \( H_2O/H_2O \) system is \( 5.36 \times 10^{-6} \) cm·s\(^{-1} \). When the system is made to contain 0.5 M PEG 400/H\(_2\)O, and \( J_v = 0 \) is maintained with hydrostatic pressure, a number of effects upon tracer diffusion become apparent. It is expected that the \( P \) value will decrease because of solute-solute interactions that occur in free solution (Dunlop, 1957; Ellerton and Dunlop, 1967a and b). This is represented by point 5 determined for \( C \) of 0.25 M PEG.

The effect of the solute drag force can now be seen by further inspection of point 2 on the \( J_v = 0 \) axis. When the tracer is diffusing in the same direction as the driver flux, there is an approximate twofold increase in the rate of permeation for the tracer over the base rate (point 5 to point 2). This increase in \( P_T \) illustrates the potential large role the solute drag force may exert. The degree of solute-solute interaction seen here is, of course, peculiar to this particular system in that the driver PEG 400 is of adequate molecular size and is used at a high enough concentration (\( C = 0.25 \) M). The tracer mannitol is also of such a molecular size to afford good interaction with the driver. The fourth factor to be considered on equal terms with the others is the matter of pore size. The \( \sim 110 \) Å Diam. pores provide the spaces for a high degree of solute-solute interaction, allowing a net and directional effect of one solute flow upon the other solute. As we have shown previously (Galey and Van Bruggen, 1970), larger pores would lessen the net solute drag effect and smaller pores would increase the effect.

When solvent drag and solute drag forces are coupled, an additional increase in \( P_T \) is seen (point 2 to point 6). The tracer molecule in the pores is swept along by the solvent flow and simultaneously interacts with the net flux of driver solute moving within (and with) the solvent flow.

When the tracer diffusion is in a direction opposite to the driver flux, the \( P_T \) value is decreased by the interaction of the tracer with the driver flux (see point 3). This decrease is similar in magnitude to the increase discussed above. Additional reduction of \( P_T \) occurs when bulk flow is introduced in the same direction as the driver solute flux so that both solvent and solute drag oppose the tracer diffusion (see point 7).

The above considerations relate to events described by the points and lines to the right of the \( J_v = 0 \) axis in Fig. 4. To the left of this line are shown the effects of imposing a hydrostatic pressure of \(< 46 \) cm Hg, which permitted rectification of bulk flow so that the solvent drag force would operate against the solute drag force. As the hydrostatic pressure was lowered, increased osmotic flow into chamber 2 occurred, which contained the 0.5 M PEG 400.

The maximum positive solute drag effect shown at point 2 is reduced and the maximum negative effect shown at point 3 is also reduced. These responses are shown by the solid lines 2-4 and 3-4. At the intercept of the line at \( J_v = 0 \) of 0.59 µl min\(^{-1}\)·cm\(^{-2}\), the maximum osmotic flow had been reached and no
net hydrostatic flow was present. Point 4 represents a "stand-off" effect of solvent drag upon solute drag—an effect in which the diffusion of tracer ($P_T$) is not dissimilar from its diffusion in a PEG 400, $\bar{C}$ solution (see point 5).

That solute and solvent drag effects are common to other membranes and other solute pairs is illustrated in Figs. 5–7. The increase in pore size to ~260 Å diam has the effect of lessening solute coupling. However, in the study shown in Fig. 5, the molecular size of both the driver and the tracer were increased over that reported in Fig. 4. As a result, good solute drag effects were seen on this membrane. This interaction is seen in the presence of increased osmotic flow permitted by the larger pores and the larger osmotic solute PEG 600.

Solute and solvent drag effects similar to those above were demonstrated on heteropore membranes (see Fig. 6 and 7). The figures also illustrate the effects of driver size and concentration and the greater effect on tracer raffinose over that of sucrose.

To this point we have reported on a comparison of solute and solvent drag forces in terms of changes in $P_T$ values above a base rate. For a quantitative comparison between the magnitudes of these two drag forces, it is necessary to describe and compare them by the same parameters.

As a basis for this comparison, consider the nature of the two forces. Solute drag can be described as driver solute collisions with the tracer solute as the driver solute diffuses down its concentration gradient. Effects on the tracer will be to increase or decrease the tracer diffusion rate, depending upon the direction of tracer diffusion.

In a similar fashion, solvent drag results from the interaction within the membrane pores of a dissolved solute with the solvent that is moving through the membrane because of an osmotic or hydrostatic force. The interaction is that of collisions between solvent molecules and dissolved solute molecules. With the solvent moving in a particular direction, collisions will yield increases or decreases in the net diffusion movement of the solute. In this regard, solvent molecules may be considered similar to solute molecules as the driving force.

Solvent and solute drag forces may be roughly quantitated and compared in a particular system in terms of the number of moles of solvent and/or moles of driver solute required to cause a stated amount of permeability disturbance.

For the system described in Fig. 4, the following calculations can be made.

**Solute Drag**

At point 2 Fig. 4, the flux of driver PEG 400, is calculated as:

\[
P_{\text{PEG 400}} = 2 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}
\]

\[
[\bar{C}] = 0.25 \times 10^{-3} \text{ mol cm}^{-3}
\]

\[
J_{\text{PEG 400}} = P \times \bar{C}
\]

\[
= 2 \times 10^{-6} \times 0.25 \times 10^{-3} \times 60 \text{ (min)}
\]

\[
= 3 \times 10^{-8} \text{ mol min}^{-1} \cdot \text{cm}^{-2}.
\]
Solvent Drag

From line 1–8, Fig. 4, it is seen that at a water flow of 0.165 μl/min⁻¹.cm⁻² the P value for tracer is increased to the same degree as in point 2:

\[
0.175 \text{ μl min}^{-1} = 0.175 \times 10^{-3} \text{ ml min}^{-1} \\
0.175 \times 10^{-3} \text{ ml min}^{-1} = 0.175 \times 10^{-5} \text{ g min}^{-1} \\
\frac{0.175 \times 10^{-3}}{18} \text{ g min}^{-1} = 9.7 \times 10^{-6} \text{ mol min}^{-1}/8 \text{ cm}^{2} \\
\frac{9.7 \times 10^{-6}}{8 \text{ cm}} \text{ mol min}^{-1} = 1.2 \times 10^{-6} \text{ mol min}^{-1} \cdot \text{cm}^{-2}.
\]

It is seen that the solvent drag effect requires \( \sim 1 \times 10^{-6} \) mol of water compared with the \( 3 \times 10^{-8} \) mol of PEG 400. Solute drag in this case is seen to be some 300 times more effective than is the solvent drag effect, although the PEG 400 molecule is only some 20 times larger than the water molecule.

It is not profitable to attempt a truly molecular explanation of the nature of the interacting solute species. The molecular structure of water involved in this system is unknown, and it may be an aggregate of many molecules of water. The PEG 400 in water solution may have much water in molecular association so that its effective size is considerably larger. Even less is known about how the solution species of these substances interact in collision. This paper does not attempt to resolve these problems.

The present findings are in agreement with our earlier studies (Galey and Van Bruggen, 1970), in which it was shown that in a comparison of the size of solute drivers that a positive correlation was seen with increasing driver size and the effects upon the tracer. A precise, arithmetic effect of molecular sizes was not found in the earlier studies. In the present study, solvent and solute drag forces have been compared by assuming a common collision mechanism of solvent and solute flows.

SUMMARY

Two physical forces, solvent drag and solute drag, have been imposed upon transmembrane diffusion of selected tracer solvents. It has been shown that each force is capable of causing tracer diffusion asymmetry. The two forces can be coupled to accent each other, or opposed to lessen or negate each other. The forces have similar effects across both heteropore and homopore membranes, but the solute drag force is membrane pore size dependent.

Considering a common mechanism of action of the two forces, i.e., collision of components of the solutions, the forces can be compared on a quantitative basis. Solute drag of selected solute pairs in a selected membrane system can be demonstrated to be of greater magnitude than solvent drag.

The application of these findings to biological transport systems may have relevance in the correct description of biological transport mechanisms.
APPENDIX

Characterization of Membranes

The permeability of a membrane to a solute due to diffusion of the solute through the membrane is:

\[ P_{\text{theo}} = \frac{n\pi d^2 D_m}{4l} \]  

(1)

where \( P_{\text{theo}} \) is the permeability in centimeters per second; \( n \) is the number of pores per centimeters; \( d \) is the diameter of pores in the membrane in centimeters; \( D_m \) is the diffusion coefficient; and \( l \) is the length of diffusion path.

The permeability of a solute due to diffusion is determined experimentally by measuring the flux of tracer across the membrane as:

\[ P_{\exp} = \frac{\Delta \text{cts}}{t \cdot A_m \cdot \text{ctsD}} \]  

(2)

where \( \Delta \text{cts} \) is the change in radioactivity in receiver chamber; \( t \) is the time interval for \( \Delta \text{cts} \) in seconds; \( A_m \) is the area of membrane in square centimeters; \( \text{ctsD} \) is the activity in the donor chamber.

The hydraulic flow of water through a membrane is:

\[ L_{p_{\text{theo}}} = \frac{n\pi d^4}{128\eta} \]  

(3)

where \( L_p \) is the coefficient of hydraulic conductivity, in cubic centimeters per dyne per second; \( \eta \) is the viscosity of water in poise as dynes per second per centimeters\(^2\); \( \pi, d, l, \) and \( n \) have the same meaning as above.

The hydraulic flow of water is determined experimentally as:

\[ L_{p_{\exp}} = \frac{F}{t \cdot A_m \cdot Pr} \]  

(4)

where \( F \) is the bulk water flow in cubic centimeters and \( Pr \) is the pressure applied in dynes per centimeters\(^2\).

I. Calculation of Pore Diameter from Diffusion and Flow Information

It is not possible to calculate pore diameters from either \( P \) or \( L_p \) experimental values alone when \( n \), the number of pores, is not known. When the two experimental values \( P \) and \( L_p \) are known, the pore diameter can be calculated \((d_{\text{cal}})\) as follows.

Solve Eq. 1 and 3 for \( d \).

\[ d^2 = \frac{41P}{n\pi D_m} \text{ (from permeability experiments)} \]  

(5)

\[ d^4 = \frac{128nl L_p}{\pi \eta} \text{ (from bulk flow experiments).} \]  

(6)

If the solute used as tracer in Eq. 5 is small enough so that its diffusion through the pore is not significantly hindered (see Beck and Schultz, 1972; Van Bruggen et al., 1974), then the ratio of solute diffusion in the membrane to that in free solution will approach 1.0 or \( D_m = D_o \).

In this case, substitute \( D_o \) for \( D_m \) and divide Eq. 6 by Eq. 5:

\[ d^2_{\text{cal}} = \frac{d^4_{Lp}}{d^2} = \frac{128nl L_p \pi D_o}{41P \pi n} \]
\[ d^2_{\text{cal}} = 128 D_0 \eta L_p / 4 P. \]

Let \( K_1 = 128 D_0 \eta / 4 \) (all constants for the particular system). Then \( d^2_{\text{cal}} = K_1 L_p / P \) and \( d_{\text{cal}} = (K_1 L_p / P)^{1/2} \). \hfill (7)

II. Approximation of Relative Pore Diameters from Diffusion Using Pairs of Tracers

In the case where \( D_{ox} \) is the free solution diffusion coefficient for tracer \( x \) and \( D_{oy} \) is the coefficient for tracer \( y \), and the membrane shows less hindrance for \( x \) than for \( y \), then \( D_{mx} > D_{my} \).

The hindrance factor \( Z_H \) is defined as:

\[ Z_H = \frac{D_{my}}{D_{mx}} \times \frac{D_{ox}}{D_{oy}}. \] \hfill (8)

Membrane hindrance to the transmembrane diffusion of \( y \) will cause \( D_{my} \) to be less than \( D_{oy} \) and thus \( Z_H \) will be <1.0.

Experimentally determined \( P \) values can be substituted for corresponding values of \( P_m \) because

\[ \frac{P_y}{P_x} = \frac{n \pi d^2 D_{my} / 4l}{n \pi d^2 D_{mx} / 4l} = \frac{D_{my}}{D_{mx}}; \] \hfill (9)

then

\[ Z_H = \frac{P_y}{P_x} \times \frac{D_{ox}}{D_{oy}}. \] \hfill (10)

Since \( D_{ox} \) and \( D_{oy} \) are constants,

\[ Z_H = K_2 P_y / P_x. \] \hfill (10)

In practice, one compares \( Z_H \) values on a particular membrane with other values obtained on membranes that have known pore sizes and offer more or less hindrance. This allows an approximation of pore size within the limits of the test membranes used.

III. Graphic Method Relating Solute Size and Permeability to Pore Diameters

Approximations of membrane pore size can also be made by comparing solute permeabilities on several membranes. An illustration of this application is shown in the following plot (Fig. 8). The molecular weights of a number of tracers are shown on the abissasa. On the ordinate is a plot of the ratio of the relative permeabilities \( (P_m) \) or diffusion \( (D_o) \) of the tracer over the corresponding value for tracer water. The log of the diffusion ratio is linearly related to the log of the molecular weight of the solutes as is indicated by the upper solid line in Fig. 8. When a membrane is put into the system, the log of the permeability ratio will also be linear as long as there is no membrane hindrance to diffusion. This is seen by the comparison of membranes 86 and 678. These membranes have calculated pore diameters of 120 and 308 Å, respectively. It can be seen that the 678 membrane with the largest (300 Å)
pore diameter shows little hindrance until the tracer solute approaches a molecular weight of 1,000. After this, the $P_T/P_{H_2O}$ ratio for PEG 4000 and insulin (5,000 mol wt) are far removed from the solid line, which indicates considerable hindrance. Membrane 86, on the other hand, consistently showed hindrance throughout the range of molecular weights of 90 to 1,000. Even glycerol (90 mol wt) showed considerable hindrance.

For another membrane with unknown pore size, the location of the $P_T/P_{H_2O}$ ratio of suitable tracers would allow approximation of the pore diameters.

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