The Permeability of Thin Lipid Membranes to Bromide and Bromine

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ABSTRACT Thin lipid (optically black) membranes were made from sheep red cell lipids dissolved in n-decane. The flux of Br across these membranes was measured by the use of tracer 82Br. The unidirectional flux of Br (in 50-100 mM NaBr) was 1-3 X 10^-2 mole/cm²sec. This flux is more than 1000 times the flux predicted from the membrane electrical resistance (>10⁶ ohm·cm²) and the transference number for Br⁻ (0.2-0.3), which was estimated from measurements of the zero current potential difference. The Br flux was not affected by changes in the potential difference imposed across the membrane (±60 mv) or by the ionic strength of the bathing solutions. However, the addition of a reducing agent, sodium thiosulfate (10⁻³ M), to the NaBr solution bathing the membrane caused a 90% reduction in the Br flux. The inhibiting effect of S₂O₃⁻ suggests that the Br flux is due chiefly to traces of Br₂ in NaBr solutions. As expected, the addition of Br₂ to the NaBr solutions greatly stimulated the Br flux. However, at constant Br₂ concentration, the Br flux was also stimulated by increasing the Br⁻ concentration, in spite of the fact that the membrane was virtually impermeable to Br⁻. Finally, the Br flux appeared to saturate at high Br₂ concentrations, and the saturation value was roughly proportional to the Br⁻ concentration. These results can be explained by a model which assumes that Br crosses the membrane only as Br₂ but that rapid equilibration of Br between Br₂ and Br⁻ occurs in the unstirred layers of aqueous solution bathing the two sides of the membrane. A consequence of the model is that Br⁻ "facilitates" the diffusion of Br across the unstirred layers.

INTRODUCTION

Studies of the properties of bimolecular (black) lipid membranes are contributing to our understanding of the molecular structure and function of biological membranes. Although there are many similarities between synthetic lipid bilayers and biological membranes, the ionic permeability properties of these two types of membrane are radically different (1–3, 8). The ionic permeability of synthetic bilayers, measured electrically, is 10⁴–10⁷ times less than the ionic permeability of most biological membranes. Estimates of the conductances for K⁺ and Na⁺ (gK and gNa), based on measurements of the
zero current membrane potential across bilayers made from sheep red cell lipids, yield values of less than $10^{-8}$ ohm$^{-1}$ cm$^{-2}$ (8). These cation permeabilities of bilayers are of the same order of magnitude as those observed in mammalian red blood cell membranes (9) but are considerably less than those characteristic of the membranes of nerve and muscle cells (16).

Isotopic flux measurements have confirmed that the permeability of lipid bilayers to alkali metal ions is extremely low (4-6). On the other hand, a rapid, but electrically silent, movement of Cl across large spherical bilayers made from egg lecithin was recently reported (5). This report extends earlier observations that small anions like Cl$^-$ traverse the surface of both lecithin and phosphatidylserine liposomes much more rapidly than do monovalent cations (6, 7). However, estimates of $g_{Cl}$ based on electrical measurements of bilayers made from sheep red cell lipids yield values less than those for $g_K$ and $g_{Na}$ ($10^{-8}$ ohm$^{-1}$ cm$^{-2}$ [8]). These observations are of interest in the search for an explanation of the extraordinarily high permeability of mammalian red cell membranes (9, 18) to halides such as Cl$^-$, permeabilities which are several orders of magnitude higher than those observed in bilayers (5). Recent evidence suggests that most of the Cl$^-$ transport across the red cell membrane may occur by a process which cannot carry electrical current (9-12, 22-24).

The experiments reported in this paper were undertaken for two reasons. First, we sought to find whether a rapid, electrically silent exchange of halides like that observed by Pagano and Thompson (5) across egg lecithin bilayers also occurs across membranes formed from sheep red cell lipids. Secondly, we hoped to compare the anion permeabilities of intact red cell membranes and of bilayers made from lipids extracted from these membranes in an attempt to discover the molecular basis of the high permeability of red cells to physiologically important anions such as Cl$^-$ and HCO$_3^-$.

**METHODS**

**Lipid Source and Membrane Formation**

Lipids were extracted from sheep red cell ghosts either with n-butanol (13) or with chloroform-methanol (8) as described previously. The lipid extract contained phospholipids in the following approximate percentages: sphingomyelin 48%, phosphatidylethanolamine 29%, phosphatidylserine 14%, and phosphatidylinositol 4%. The cholesterol:phospholipid molar ratio was about 1:1.

Membranes were formed at room temperature ($22^\circ$-$24^\circ$C) from a solution of red cell lipids in n-decane (20-25 mg/ml). The lipid solution was brushed across a circular hole, 3 mm$^2$ in area, in a polyethylene partition about 0.2 mm thick. The partition separated two open compartments which each contained 1.2 ml. Perfusion of both front and rear compartments (usually not simultaneously) was by gravity flow with withdrawal by aspiration through a vacuum system. Both compartments were
stirred continuously with magnetic stirrers. The average life of a membrane under these conditions was about 2 hr.

Electrical Measurements

Membrane resistance, membrane voltage, and ionic transference numbers were measured as described previously (8). Briefly, the membrane resistance was calculated, using Ohm's law, from the membrane potential produced by applying a calibrated voltage pulse across the membrane plus a known resistance in series with the membrane. The membrane voltage was recorded as the potential difference between two calomel-KCl electrodes which made direct contact with the front and rear solutions. Ionic transference numbers were estimated from the steady potential difference which was generated by ionic activity gradients across the membrane.

Flux Measurements

The unidirectional flux of Br was measured by means of $^{82}$Br, obtained as NaBr from Cambridge Nuclear Corp., Cambridge, Mass. First, a membrane was formed in a nonradioactive NaBr solution and allowed 5–20 min to become optically black. Then the membrane resistance was checked, and only membranes with an initial $R_m > 10^8$ ohm-cm$^2$ were used for flux measurements. Then the rear compartment was perfused with 5–10 ml of Na$^{82}$Br solution, and a 5 µl sample was withdrawn and measured for radioactivity. The rear vacuum system was then disconnected, and the rear compartment was covered with plastic tape. Perfusion of the front chamber was then started and maintained at a rate of 0.6–0.8 ml/min. Samples of 6–8 ml were collected at 10-min intervals in a vacuum trap. To protect against possible loss of $^{82}$Br$^2$ to the vacuum system, the samples were collected into a reducing solution containing Na$_2$S$_2$O$_4$. Radioactive samples were dried in 2-inch planchets containing (when necessary) a small amount of Na$_2$S$_2$O$_4$. The samples were counted in a gas flow, low background detector (Beckman Wide Beta II, Beckman Instruments, Inc., Fullerton, Calif.). Self absorption corrections were made when necessary.

The unidirectional Br flux was calculated by the expression

\[
M_{Br} = \frac{\text{NaBr}[\text{Br}^-] R}{t A[\text{Br}^2]} 
\]

where $M_{Br}$ (in moles per square centimeter seconds) is the Br flux, $^{82}$Br$^-$ (in counts per minute) is the total radioactivity collected from the front compartment, [Br$^-$]$^z$ (in moles per cubic centimeter) is the Br$^-$ concentration in the rear solution, [$^{82}$Br]$^z$ (in counts per minute per cubic centimeter) is the concentration of $^{82}$Br in the rear solution, $t$ (in seconds) is the sampling time, and $A$ (in square centimeters) is the surface area of the membrane. Backflow of tracer from front to rear was neglected because the specific activity of $^{82}$Br in the front solution was always less than 1% of the specific activity in the rear. The flux values quoted are always the averages of two to four consecutive samples. The variation among these several samples was usually less than ±20% (see, for example, Fig. 2).
Composition of Solutions

Stock bromine solutions (10^-2 M) were prepared fresh daily by dissolving liquid bromine in distilled water. Small amounts of this solution were then added to the experimental NaBr solutions to give the desired Br_2 concentration.

Several equilibria involving Br^-, Br_2, HBrO, Br_3^-, and Br_4^- play a possible role in the experiments to be discussed below. The relevant reactions and their approximate equilibrium constants at 24°C are listed below.

\[
\begin{align*}
\text{Br}_2 + \text{H}_2\text{O} & \rightleftharpoons 2\text{Br}^- + 2\text{H}^+ + \frac{1}{2}\text{O}_2 & K = 1.5 \times 10^{-5} \\
\text{Br}_2 + \text{H}_2\text{O} & \rightleftharpoons \text{H}^+ + \text{HBrO} + \text{Br}^- & K = 5 \times 10^{-9} \\
\text{Br}_2 + \text{Br}^- & \rightleftharpoons \text{Br}_3^- & K = 17 \\
2\text{Br}_2 + \text{Br}^- & \rightleftharpoons \text{Br}_5^- & K = 20
\end{align*}
\]

Reaction 1 goes very slowly (14), whereas reactions 2, 3, and 4 go rapidly to equilibrium (14, 15). Fig. 1 shows computed concentrations of Br_2, Br_3^-, and HBrO as a function of the Br^- concentration at pH 5.0 over the range used in this study. From Fig. 1 it is clear that the addition of small amounts of Br_2 to NaBr solutions can produce substantial proportions of Br_3^- and HBrO which must be taken into account. The Br_- concentration, however, is always small (10^-9-10^-1 M) and probably plays an unimportant role in the experiments to be described.

Unless otherwise indicated, the experimental solutions were buffered at pH 5.0

![Figure 1](image-url)
with sodium acetate (1 mM). This rather low pH was used to minimize the formation of HBrO as shown in reaction 2.

RESULTS

The Br Flux across Thin Lipid Membranes

The unidirectional flux of Br across membranes bathed with 50–150 mM NaBr was $1-3 \times 10^{-12}$ mole/cm$^2$ sec. This value is more than 1000 times the Br flux predicted from the membrane resistance ($R_m$) and transference number for Br$^-$ ($T_{Br^-}$), i.e., $(RT/z^2F)(T_{Br^-}/R_m)$ (16). Measurements of $R_m$ and $T_{Br^-}$ are described in the next section. The disparity between the observed and predicted Br fluxes indicates that more than 99% of the Br is crossing the membrane in an electrically neutral form. This interpretation is confirmed by the fact that clamping the membrane voltage at ±60 mv had no effect on the Br flux (Fig. 2).

The addition of a reducing agent, sodium thiosulfate ($10^{-3}$ M), to the NaBr solutions on both sides of the membrane reduced the Br flux to about 10% of the control level (Fig. 2), whereas the addition of sodium sulfate (not shown) had no effect. The inhibiting effect of $S_2O_3^{2-}$ suggests that Br crosses the mem-
brane chiefly as \( \text{Br}_2 \), which may be present in trace amounts in NaBr solutions. From equation 1 we estimate that the \( \text{Br}_2 \) concentration in 100 mM NaBr at pH 5.0 could be as high as \( 3 \times 10^{-8} \) M. Thiourea (10^{-3} M) should reduce the \( \text{Br}_2 \) concentration to less than \( 10^{-2} \) M (17).

Before proceeding further, it was important to confirm that the radioactivity appearing on the nonlabeled (trans) side of the membrane was, in fact, \( ^{82}\text{Br} \) rather than a radiochemical impurity. To check this possibility we

**Table 1**

Effects of Bromine on the Electrical Properties of Bimolecular Lipid Membranes

| \( \text{Br}_2 \) added | Solute concentrations at equilibrium | Membrane resistance | Membrane voltage | Equilibrium potential |
|--------------------------|-------------------------------------|---------------------|-----------------|---------------------|
|                          | Front \( M \) | Rear \( M \) | cm\(^{-2}\) M | mv | mv |
| None | \( \text{Br}^- \), \( 10 \times 10^{-3} \) | \( 10 \times 10^{-3} \) | \( 2.6 \times 10^6 \) | -25 to -40 | +55.3 |
| Na\(^+\), \( 11 \times 10^{-3} \) | 101 \times 10^{-3} | 1 \times 10^{-2} | 0 | -53.0 |
| Ac\(^-\), \( 1 \times 10^{-3} \) | 1 \times 10^{-3} | 0 | 0 | 0 |
| \( \text{Br}_2 \), \( 10 \times 10^{-3} \) | \( 10 \times 10^{-3} \) | \( 1 \times 10^{-3} \) | \( 1.2 \times 10^6 \) | +22 to +24 | +55.3 |
| Na\(^+\), \( 11 \times 10^{-3} \) | 101 \times 10^{-3} | 1 \times 10^{-2} | 0 | -53.0 |
| Ac\(^-\), \( 1 \times 10^{-3} \) | 1 \times 10^{-3} | 0 | 0 | 0 |
| \( \text{Br}_2 \), \( 1.72 \times 10^{-5} \) | \( 1 \times 10^{-3} \) | \( 0.74 \times 10^{-5} \) | \( 1.26 \times 10^{-5} \) | +34.5 |
| \( \text{Br}_2 \), \( 0.8 \times 10^{-6} \) | \( 1 \times 10^{-5} \) | \( 1.6 \times 10^{-5} \) | \( 1 \times 10^{-4} \) | +14 |
| HBrO, \( 1.0 \times 10^{-4} \) | \( 0.1 \times 10^{-4} \) | \( 0 \) | \( 0 \) | \( 0 \) |

The NaBr solutions were buffered at pH 5.0 with NaAc (1 mM). Concentrations of \( \text{Br}_2 \), \( \text{Br}_2^- \), \( \text{Br}_2^+ \), and HBrO were calculated from equations 2, 3, and 4 and the initial composition of the bulk solutions. The sign of the membrane voltage is that of the rear solution with respect to the front. The equilibrium potential is \( \frac{59}{z_j} \log \frac{a_j^*}{a_j^*} \), where \( a_j^* \) and \( a_j^* \) are the ionic activities in the front and rear solutions, and \( z_j \) is the valence of the ion \( j \). The ratio of the activity coefficients for \( \text{Br}_2^- \) and \( \text{Br}_2^+ \) on the two sides of the membrane were assumed to be similar to the ratio of the activity coefficients for 100 mM to 10 mM NaBr (i.e., 0.87).

measured the half-life of several samples obtained from the trans side of the membrane during flux measurements. The \( t_{1/2} \) values were 35 ± 1 hr for the control fluxes and 40 ± 5 hr for two samples of very low activity obtained during exposure of the membrane to \( \text{SO}_4^- \). The half-life of \( ^{82}\text{Br} \) is 35.3 hr.

**Effects of Bromide and Bromine on the Electrical Properties of Thin Lipid Membranes**

Membranes bathed with NaBr (1–200 mM), pH 5.0, had resistances of 1–6 \( \times \) \( 10^6 \) ohm-cm\(^2\). Cationic diffusion potentials of 25–40 mv were generated by a 10-fold concentration gradient of NaBr (Table I). The addition of \( \text{Br}_2 \) (2 \( \times \) \( 10^{-5} \) M) to both front and rear bathing solutions lowered the membrane resistance by about two orders of magnitude, and caused the membrane
permselectivity to change from cationic to anionic. In Table I, the diffusion potential observed under these conditions is compared with the equilibrium potentials for Br\(^{-}\), Br\(_3\)\(^{-}\), or Br\(_5\)\(^{-}\), computed from the assumption that the concentrations of these ions in the fluid bathing the membrane surfaces are those defined by the composition of the bulk solutions and by equations 2, 3, and 4. This assumption is not entirely justified because the Br\(_2\) concentration in the unstirred layers is not the same as in the bulk solutions for reasons described below. Therefore, the situation is complicated and the data do not permit assignment of transference numbers to Br\(^{-}\), Br\(_3\)\(^{-}\), and Br\(_5\)\(^{-}\). The effect of Br\(_2\) on membrane resistance was reversible. Washing the membrane with Br\(_2\)-free solutions for 30 min restored \(R_m\) to within 10% of the control level. We have not studied in further detail the effects of Br\(_2\) on the electrical properties of thin lipid membranes because, as shown below, the Br fluxes at all Br\(_2\) concentrations were more than 99.9% electrically silent.

**Effects of Bromine and Bromide on the Br Flux**

The addition of Br\(_2\) to the NaBr solutions bathing the membrane greatly stimulated the Br flux (Fig. 3) and, as expected, S\(_2\)O\(_3\)\(^{-}\) (1 mm) completely blocked this stimulating effect of Br\(_2\) (not shown). The addition of Br\(^{-}\) to solutions containing a constant [Br\(_2\)] also stimulated the Br flux (Fig. 4). This result was surprising because, as shown in Fig. 2, Br\(^{-}\) itself cannot cross the membrane in appreciable amounts. The possibility that the stimulating effect of Br\(^{-}\) on the Br flux was due to an increase in the ionic strength of the bathing solutions was checked and rejected. In one experiment the ionic strength was increased by adding 50 mm NaNO\(_3\) to 2 mm NaBr plus Br\(_2\) (3 \(\times\) 10\(^{-6}\) M). In a second experiment 30 mm NaCl was added to 2 mm NaBr plus Br\(_2\) (1 \(\times\) 10\(^{-5}\) M). In neither case did the increase in ionic strength alter the Br flux, which was measured both before and after the addition of salt. Concentrations of Br\(_2\) higher than those shown in Fig. 3 usually caused the membrane to break.

In describing the effects of Br\(_2\) and Br\(^{-}\) on the Br flux, we had to take into account the formation of HBrO and Br\(_3\)\(^{-}\), as indicated in equations 2 and 3 and in Fig. 1. For example, at a Br\(^{-}\) concentration of 2 mm the equilibrium concentration of Br\(_2\) was 80% of the initial Br\(_2\) concentration and, at a Br\(^{-}\) concentration of 100 mm, the equilibrium concentration of Br\(_2\) was 37% of the initial Br\(_2\) concentration. These two correction factors (0.80 and 0.37) were applied in calculating the curves shown in Fig. 3, which resulted in a leftward shift of the original curves without changing the slopes. In Fig. 4 the amount of Br\(_2\) added to give an equilibrium concentration of 1 \(\times\) 10\(^{-5}\) M was somewhat different for each Br\(^{-}\) concentration. In this case, converting the [Br\(_2\)] from an initial value to an equilibrium value of 1 \(\times\) 10\(^{-5}\) M shifted the curve upward without greatly altering the slope.
The main effects of Br\textsubscript{2} and Br\textsuperscript{-} on the Br flux can be summarized as follows:

(a) The Br flux is extremely sensitive to the Br\textsubscript{2} concentration. The slopes of the log Br flux vs. log [Br\textsubscript{2}] curves are 2-4 (Fig. 3). The largest fluxes shown in Fig. 3 approach 10\textsuperscript{-7} mole/cm\textsuperscript{2} sec, which is nearly 10 times the chloride-for-chloride exchange flux in human red blood cells (18).

(b) Virtually all of the Br flux is electrically silent. As indicated in Table I and Fig. 3, the effect of Br\textsubscript{2} on the Br flux is much more pronounced than the effect of Br\textsuperscript{-} on the membrane resistance.

(c) The Br flux apparently saturates at high Br\textsubscript{2} concentrations, and the saturation level is roughly proportional to the Br\textsuperscript{-} concentration (Fig. 3).

(d) The Br flux is roughly proportional to the Br\textsuperscript{-} concentration over the range 2-200 mM, with apparent saturation of the flux at high [Br\textsuperscript{-}] (Fig. 4).
DISCUSSION

The flux of Br across membranes bathed with NaBr is probably chiefly due to Br₂ rather than to Br⁻, Br₃⁻, or Br₅⁻. The presence of trace amounts of Br₂ would account for the insensitivity of the Br flux to the membrane voltage, as well as for the inhibition of the Br flux by the reducing agent, thiosulfate (Fig. 2). There is no indication so far for a large exchange diffusion of Br⁻, such as that reported for Cl⁻ in lecithin bilayers (5). Note, however, that the flux remaining after thiosulfate inhibition (about $10^{-16}$ mole/cm² sec) is still much larger than the flux predicted from electrical measurements (about $10^{-15}$ mole/cm² sec). We have no information yet about the nature of this residual Br flux.

The stimulating effect of Br₂ on the Br flux (Fig. 3) might be explained by the equilibration of added Br₂ with ³⁵Br⁻ in the well-stirred bathing solution, followed by the diffusion of labeled Br₂ across the membrane and adjacent unstirred layers. The consequences of this model are depicted in Fig. 5. The specific activity of Br⁻ ($q_-$) in the unstirred layer on the cis side of the membrane (left) bathed by labeled solution is constant and equal to the value established in the well-stirred bulk solution. The specific activity of the Br⁻ on the trans side of the membrane (right) is zero throughout both bulk solu-
tions and unstirred layers. The specific activity of the Br$_2$ ($q_o$) decreases linearly from a value equal to $q_-$ in cis bulk solution to zero in the trans bulk solution. In the unstirred layers, $q_o$ is not equal to $q_-$ because isotopic exchange is assumed to be slow compared to diffusion of Br$_2$. In this treatment, the membrane itself is assumed to offer resistance to Br$_2$ diffusion which is negligible compared to the unstirred layers. Läuger et al. (21) suggested a similar model to account for their observations of I movement through bilayers in the presence of I$^-$ and I$_2$.

However, the observed fluxes of Br$_2$ are much too large to be due solely to simple diffusion of Br$_2$ through the membrane and unstirred layers. To illustrate this point, we computed, from Fick’s law and the approximate thickness of the unstirred layer, the Br flux that would occur if the membrane offered no resistance to Br$_2$ diffusion. For example, if [Br$_2$] = 10$^{-8}$ mole/cm$^3$ and the thickness ($\Delta x$) of the unstirred layer is about 10$^{-2}$ cm (19, 20), and the diffusion coefficient for Br$_2$ ($D_{Br_2}$) is about 10$^{-5}$ cm$^2$/sec, then the Br flux should be given by

$$M_{Br} = D_{Br_2}([Br_2]/\Delta x) \approx 10^{-11} \text{ mole/cm}^2\text{sec.}$$

This calculated Br flux is more than 1000 times less than the observed Br flux at [Br$_2$] = 10$^{-8}$ mole/cm$^3$ (Fig. 3). The same argument applies to the results of Läuger et al. (21). They measured an I flux of $5 \times 10^{-8}$ mole/cm$^3$ sec when the I$_2$ concentration was $6 \times 10^{-8}$ mole/cm$^3$ and the I$^-$ concentration was $10^{-8}$ mole/cm$^3$.$^1$ The computed rate of diffusion of I across the unstirred layer as I$_2$ (assuming no exchange with I$^-$) is $6 \times 10^{-12}$ mole/cm$^2$ sec, again 1000 times less than the observed value. Furthermore, both the saturation of the Br flux at high Br$_2$ concentrations and the dependence of the flux upon the Br$^-$ concentration are incompatible with a mechanism of simple diffusion of Br$_2$ across the membrane and unstirred layers. In the light of these considerations, the model depicted in Fig. 5 must be rejected as an explanation for both our data and the data of Läuger et al.

A model which can explain most of our results involves, in addition to the diffusion of Br$_2$ across the membrane, a rapid equilibration of Br between Br$_2$ and Br$^-$ within the aqueous unstirred layers. The model is shown schematically in Fig. 6 and the equations describing the model are given in the Appendix. In this case the specific activities of Br$^-$ and Br$_2$ ($q_-$ and $q_o$) are assumed to be equal throughout most of the unstirred layers except for a relaxation zone immediately adjacent to the two surfaces of the membrane. The thickness of this zone is determined by the relative rates of diffusion and

$^1$ This I$_2$ concentration is estimated from the equilibrium constant,

$$K = \frac{[I^-][I_2]}{[I_2^2]} \approx 1.3 \times 10^{-9} \text{ M} \ (21).$$
isotopic exchange of Br₂ and Br⁻. The characteristic relaxation length \((\gamma^{-1})\) may be thought of as the mean distance over which a labeled molecule of \(^8\text{Br}_2\) can diffuse before it is destroyed by isotopic exchange and is very short \(< 10^{-4} \text{ cm}\) compared to the effective thickness of the unstirred layers (about \(10^{-2} \text{ cm}\)). An important property of this model is that a concentration gradient for \(^8\text{Br}^-\) is set up in the unstirred layers despite the fact that no Br⁻ traverses the membrane. Indeed, since \(q_-\) and \(q_0\) are equal throughout most of the unstirred layer, and since \([\text{Br}^-] \gg [\text{Br}_2]\), almost all of the transported Br moves across this region in the form of Br⁻. It is this property which accounts for the apparently anomalously high Br flux across the unstirred layers. Br⁻ can be considered to “facilitate” the diffusion of tracer Br across the unstirred layer in the same sense that hemoglobin “facilitates” the diffusion of oxygen.

Fig. 7 shows how the model can also account for the apparent saturation of Br flux at high Br₂ concentrations. When the concentration of Br₂ is low, the flux across the membrane is low and the gradient in \(q_-\) required to produce the same flux across the unstirred layer is small (top half of Fig. 7). When the concentration of Br₂ is higher (but not so high as to alter appreciably the sum of \([\text{Br}_2]\) and \([\text{Br}^-]\)), the flux of Br becomes limited by the
rate of diffusion of Br⁻ across the unstirred layer, which is maximal when $q_-$ at the membrane is one-half of its value in the cis solution (bottom half of Fig. 7). The magnitude of this maximal flux depends on the concentration of Br⁻. For example, if the unstirred layer is about $10^{-2}$ cm thick (19, 20) the maximum Br flux in 100 mM NaBr should be approximately

$$M_{Br} \approx D_{Br^-} [(Br^-)/\Delta x]$$

$$\approx 10^{-4} \text{ cm}^2/\text{sec} \quad (10^{-4} \text{ mole/cm}^2 \text{ per } 10^{-2} \text{ cm})$$

$$\approx 10^{-7} \text{ mole/cm}^2 \text{ sec.}$$

A similar calculation for 2 mM Br⁻ gives a saturation flux of $2 \times 10^{-9}$ mole/cm² sec. Both values agree roughly with the respective extrapolated values in Fig. 3.

Although this model explains most of our data, it does not account for the steepness of the slopes of the Br flux vs. Br₂ concentration curves shown in Fig. 3. According to our model the slope of these curves should be between 1.0 and 1.5 depending on the effect of [Br₂] on the relaxation length (see Appendix). Further work will be necessary to resolve this discrepancy. However, we present the model now partly because it accounts for most of our observations and partly because it may have more general application. The same considerations apply to any case in which a transported substance exists in at least two chemical forms to which a membrane offers widely different resistances to transport, e.g., the undissociated and dissociated forms of a weak
acid or base. If the concentration of the slowly penetrating form is much greater than that of the rapidly penetrating form, the former may "facilitate" the transport of the latter across the unstirred layers. A treatment of a case like this which is formally similar to that proposed in this paper has recently been published by LeBlanc (25).

APPENDIX

The Permeability of Thin Lipid Membranes to Bromide and Bromine

Isotopic exchange occurs by the reversible reaction

$$\text{Br}_2 + ^{82}\text{Br}^- \xrightarrow{k} ^{82}\text{Br}_2 + \text{Br}^-$$  \hspace{1cm} (5)

where the forward and reverse rate constants ($k$) are equal since the reacting species on each side are identical, apart from location of the tracer atom. Introduce

$$q_- = \frac{[^{82}\text{Br}^-]}{[^{82}\text{Br}^- + \text{Br}^-]}$$  \hspace{1cm} (6)

as the specific activity of tracer Br$^-$, and also $q_0$, similarly defined, as the specific activity of tracer Br$_2$.

If, for each tracer species, we combine Fick's Law and the equation of continuity, then a pair of differential equations for $q_-$ and $q_0$ is obtained. For the steady-state case these are most conveniently expressed as

$$\frac{d^2}{dx^2} \left( \frac{q_- + q_0}{\beta_- \beta_0} \right) = 0$$  \hspace{1cm} (7)

and

$$\frac{d^2}{dx^2} \left( q_- - q_0 \right) - \gamma^2 (q_- - q_0) = 0$$  \hspace{1cm} (8)

where

$$\beta_- = \frac{k[\text{Br}^-][\text{Br}_2]}{D_{\text{Br}^-}[\text{Br}^-]}$$  \hspace{1cm} (9)

$$\beta_0 = \frac{k[\text{Br}^-][\text{Br}_2]}{D_{\text{Br}_2}[\text{Br}_2]}$$  \hspace{1cm} (10)

and

$$\gamma^2 = \beta_- + \beta_0$$  \hspace{1cm} (11)

These results are obtained subject to the assumption that the tracer specific activities are much smaller than unity.
Equation 7, integrated once, simply states that the total tracer flux is everywhere constant in the steady state.

Application of the mass action law to reaction 5 shows that \(q_- = q_0\) in isotopic exchange equilibrium. Thus equation 8 describes how a steady-state deviation from equilibrium, occurring at the membrane surface, is relaxed in an adjacent unstirred bulk phase. The relaxation is exponential, with a characteristic length of \((\gamma)^{-1}\). The condition \(\beta_0^2 \gg \beta_-^2\) is appropriate to the experiments described here since the \(\text{Br}^-\) concentration is always much greater than the \(\text{Br}_2\) concentration. Thus \((\gamma)^{-1}\) is the average distance that a \(^8\text{Br}_2\) can diffuse before being destroyed by isotopic exchange with a \(\text{Br}^-\). If reaction 5 is assumed to be diffusion controlled (26), with every encounter between appropriate species resulting in exchange, then in 100 mM NaBr we estimate the lifetime of a \(^8\text{Br}_2\) to be \(\approx 10^{-6}\) sec. Therefore \((\gamma)^{-1} \approx 10^{-3}\) cm is obtained as a lower limit for the relaxation length. Even if only one encounter in \(10^4\) actually resulted in exchange we would still have \((\gamma)^{-1} \approx 10^{-4}\) cm. For an unstirred layer of thickness \(\Delta \approx 10^{-2}\) cm, the condition

\[
\gamma \Delta \gg 1
\]

is clearly appropriate.

We adopt a coordinate system in which the membrane, assumed to be of negligible thickness, lies in the plane \(x = 0\). Then the boundaries between the unstirred layers and the bulk solutions lie in the planes \(x = \pm \Delta\). Let the negative \(x\) axis extend into the rear compartment, where the specific activity of each tracer species is \(q_r\). Then, for the unstirred layer lying in the interval \(-\Delta < x < 0\), solution of equations 7 and 8 yields

\[
q_-(x) = q^0 - A \left[ \frac{(\beta_-)}{\gamma} \gamma \Delta + x \right] - \left[ \frac{(\beta_-)}{\gamma} \gamma \Delta + x \right] \left[ \frac{(\beta_0)}{\gamma} \gamma \Delta + x \right]
\]

and

\[
q_0(x) = q^0 - A \left[ \frac{(\beta_-)}{\gamma} \gamma \Delta + x \right] + \left[ \frac{(\beta_0)}{\gamma} \gamma \Delta + x \right]
\]

where

\[
A = \frac{\beta_0^2}{\gamma} \frac{M_{\text{stBr}}}{k[\text{Br}^-][\text{Br}_2]}
\]

and \(M_{\text{stBr}}\) is the total tracer flux. These results establish the linearity of the tracer profiles throughout most of the unstirred layer. Deviations are confined to the relaxation region, having a thickness of the order of \((\gamma)^{-1}\), immediately adjacent to the membrane. Similarly, isotopic exchange equilibrium, for which \(q_- = q_0\), is maintained everywhere except in the relaxation region. Note that the gradient of \(q_-(x)\) vanishes at \(x = 0\) because \(\text{Br}^-\) is presumed not to penetrate the membrane. Corresponding relations for the unstirred layer occupying the region \(0 < x < \Delta\), in the front compartment, are readily obtained subject to the condition that \(q_- = q_0 = 0\) for all \(x \geq \Delta\) in the stirred bulk solution.
Finally we introduce the relation

\[ M_{Br} = P_{Br2}^{m} \left\{ (q_{0})_{r=0}^{+} - (q_{0})_{r=0}^{-} \right\} [Br_{2}] \]  

(16)

where \( P_{Br2}^{m} \) is the coefficient of membrane permeability to \( Br_{2} \). This suffices to permit derivation of an expression for the total tracer flux involving \( q^{u} \), [Br\textsuperscript{−}], [Br\textsubscript{2}], \( P_{Br2}^{m} \), and the unstirred layer permeability coefficients, defined by

\[ P_{Br2}^{u} = D_{Br2}/2\Delta \]  

(17)

and

\[ P_{Br-}^{u} = D_{Br-}/2\Delta. \]  

(18)

The specific activity, \( q^{u} \), enters as a multiplicative factor on the entire expression. Therefore, since we are interested in the total unidirectional Br flux, we simply replace it by unity in the final result. Since, under the experimental conditions which are pertinent here, \( P_{Br-}^{u} \sim P_{Br2}^{u} \) while \([Br\textsuperscript{−}] \gg [Br\textsubscript{2}]\), we present, instead of the exact result of the model, the simpler approximate expression

\[ \frac{1}{M_{Br}} = \frac{1}{P_{Br2}^{u}[Br\textsuperscript{−}]} + \frac{1}{P_{Br2}^{m}[Br\textsubscript{2}]} + \frac{1}{\gamma\Delta P_{Br2}^{u}[Br\textsubscript{2}]} \]  

(19)

which introduces negligible error.

Equation 19 is most effectively considered in the context of Fig. 3, where the variation of \( M_{Br} \) with \([Br\textsubscript{2}]\) is illustrated for fixed values of [Br\textsuperscript{−}]. At sufficiently high \( Br_{2} \) concentration the first term on the right will determine the flux. This will typify the saturation region in which the flux is independent of [Br\textsubscript{2}], being determined solely by the permeability of the unstirred layers to Br\textsuperscript{−}. At low \([Br\textsubscript{2}]\) either the second or the third term will fix the flux. If \( P_{Br2}^{m} < \gamma\Delta P_{Br2}^{u} \), the second term will predominate, and transport of \( Br_{2} \) through the membrane will be rate limiting. If the sense of the inequality is reversed the third term will be most important, and the Br flux will be limited by the transport of \( Br_{2} \) through the relaxation zones adjacent to the membrane. It is clear that, although the tracer flux may be carried primarily by Br\textsuperscript{−} through most of the unstirred layers, the tracer must nevertheless be transferred to \( Br_{2} \) within the aqueous phase and then diffuse to the membrane boundary before it can penetrate the membrane. This transfer step can be rate limiting under the conditions set forth above. Equation 19, as a relation between reciprocal terms, clearly reflects the series nature of the three conductances which determine the flux.

As previously noted in the text, the steepness of the curves of log \( M_{Br} \) versus log \([Br\textsubscript{2}]\) at low \([Br\textsubscript{2}]\), shown on Fig. 3, is not adequately explained by this model. It may be that the membrane permeability coefficient is itself dependent upon the \( Br_{2} \) concentration, a point upon which further independent experimental evidence is necessary. In any case the location of the “knees” on the curves of Fig. 3 implies that the membrane permeability to \( Br_{2} \) is at least \( 10^4 \) times that of the unstirred layers to Br\textsuperscript{−}.

The authors acknowledge valuable discussions with D. F. Hastings, M. Tiefenblog Tosteson, and H. Ginzburg.

This work was supported by Grant HE 12157 from the National Institutes of Health.
Note Added in Proof  The model presented here does not take into account the existence of the tribromide ion, $\text{Br}_3^-$, as an intermediate complex in the isotopic exchange reaction. However, we have recently formulated a model which takes this complication into account and have found that it yields results which do not differ significantly from those given above.

Received for publication 25 August 1971.

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