The Permeability of Thin Lipid Membranes to Bromide and Bromine

JOHN GUTKNECHT, L. J. BRUNER, and D. C. TOSTESON

From the Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710. Dr. Bruner's present address is the Department of Physics, University of California, Riverside, California 92502.

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 ^{82}Br. The unidirectional flux of Br (in 50–100 mM NaBr) was $1–3 \times 10^{-12}$ mole/cm²sec. This flux is more than 1000 times the flux predicted from the membrane electrical resistance ($> 10^8$ 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 $S_2O_3^=$ suggests that the Br flux is due chiefly to traces of Br_2 in NaBr solutions. As expected, the addition of Br_2 to the NaBr solutions greatly stimulated the Br flux. However, at constant Br_2 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 Br_2 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 Br_2 but that rapid equilibration of Br between Br_2 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 10^3–10^7 times less than the ionic permeability of most biological membranes. Estimates of the conductances for K^+ and Na^+ (g_K and g_{Na}), 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°–24°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 $Na_2S_2O_3$.

Radioactive samples were dried in 2-inch planchets containing (when necessary) a small amount of $Na_2S_2O_3$. 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

$$M_{Br} = \frac{^{82}Br^F[Br^-]^R}{tA[^{82}Br]^R}$$

where M_{Br} (in moles per square centimeter seconds) is the Br flux, $^{82}Br^F$ (in counts per minute) is the total radioactivity collected from the front compartment, $[Br^-]^R$ (in moles per cubic centimeter) is the Br^- concentration in the rear solution, $[^{82}Br]^R$ (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 $\pm 20\%$ (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_5^- 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.

$$Br_2 + H_2O \rightleftharpoons 2Br^- + 2H^+ + \tfrac{1}{2}O_2 \qquad K = 1.5 \times 10^{-5} \qquad (1)$$

$$Br_2 + H_2O \rightleftharpoons H^+ + HBrO + Br^- \qquad K = 5 \times 10^{-9} \qquad (2)$$

$$Br_2 + Br^- \rightleftharpoons Br_3^- \qquad K = 17 \qquad (3)$$

$$2Br_2 + Br^- \rightleftharpoons Br_5^- \qquad K = 20 \qquad (4)$$

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_5^- concentration, however, is always small (10^{-9}–10^{-11} 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

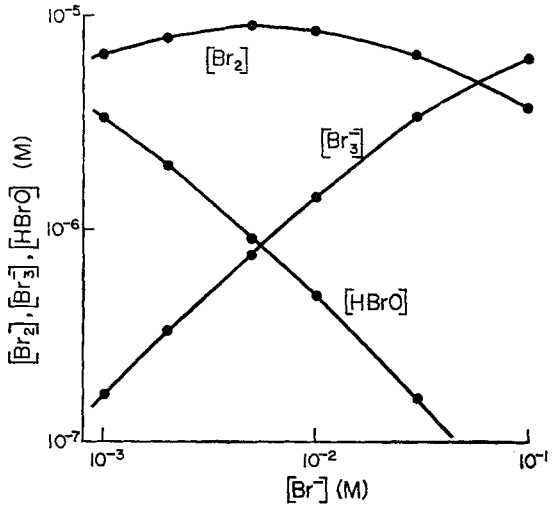

FIGURE 1. Computed equilibrium concentrations of Br_2, Br_3^-, and HBrO as a function of Br^- concentration at pH 5.0. Initial $[Br_2] = 1 \times 10^{-5}$ M. The values on the ordinate, when expressed as fractions of the initial $[Br_2]$, can be used to compute the equilibrium concentrations of Br_2, Br_3^-, and HBrO for any initial $[Br_2]$, provided that $[Br_2] \ll [Br^-]$ and that pH = 5.0.

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 × 10^{-12} mole/cm² sec. This value is more than 1000 times the Br flux predicted from the membrane resistance (R_m) and transference num-

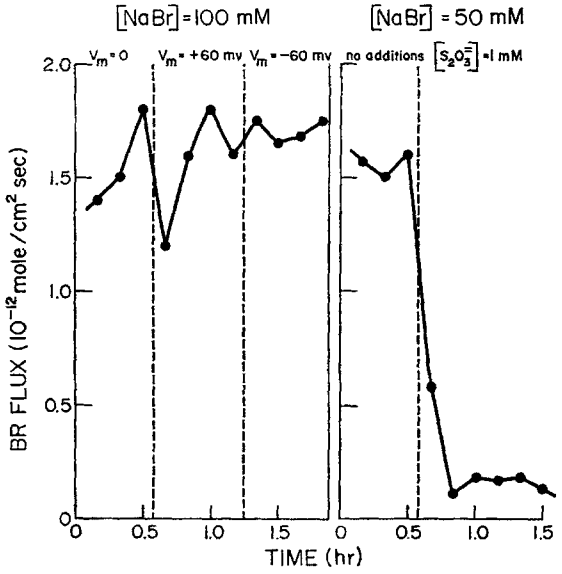

FIGURE 2. Effects of membrane voltage and thiosulfate on the Br flux across thin lipid membranes. In the first experiment (left-hand side) the NaBr solutions were buffered at pH 7.0 with sodium phosphate (1 mM). In the second experiment (right-hand side) the NaBr solutions were unbuffered (pH = 5.9). Membrane resistance was 1–3 × 10^3 ohm-cm² throughout both experiments.

ber for Br^- (T_{Br^-}), i.e., $(RT/z^2F^2)(T_{Br^-}/R_m)$ (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 $S_2O_3^=$ suggests that Br crosses the mem-

brane chiefly as Br_2, which may be present in trace amounts in NaBr solutions. From equation 1 we estimate that the Br_2 concentration in 100 mM NaBr at pH 5.0 could be as high as 3×10^{-8} M. Thiosulfate (10^{-3} M) should reduce the Br_2 concentration to less than 10^{-20} 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}Br rather than a radiochemical impurity. To check this possibility we

TABLE I

EFFECTS OF BROMINE ON THE ELECTRICAL PROPERTIES OF BIMOLECULAR LIPID MEMBRANES

Br_2 added	Solute concentrations at equilibrium		Membrane resistance	Membrane voltage	Equilibrium potential
	Front	Rear			
M	*M*	*M*	*ohm-cm²*	*mv*	*mv*
None	Br^-, 10×10^{-3}	100×10^{-3}	$2\text{–}6\times10^8$	−25 to −40	+55.3
	Na^+, 11×10^{-3}	101×10^{-3}			−53.0
	Ac^-, 1×10^{-3}	1×10^{-3}			0
2×10^{-5}	Br^-, 10×10^{-3}	100×10^{-3}	$1\text{–}2\times10^6$	+22 to +24	+55.3
	Na^+, 11×10^{-3}	101×10^{-3}			−53.0
	Ac^-, 1×10^{-3}	1×10^{-3}			0
	Br_2, 1.72×10^{-5}	0.74×10^{-5}			—
	Br_3^-, 0.28×10^{-5}	1.26×10^{-5}			+34.5
	Br_5^-, 0.6×10^{-10}	1.1×10^{-10}			+14
	HBrO, 1.0×10^{-6}	0.1×10^{-6}			—

The NaBr solutions were buffered at pH 5.0 with NaAc (1 mM). Concentrations of Br_2, Br_3^-, Br_5^-, 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 $\frac{59}{z_j} \log \frac{a^R}{a^F}$, where a^F and a^R 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 Br_3^- and Br_5^- 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 $S_2O_3^{=}$. The half-life of ^{82}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 $1\text{–}6 \times 10^8$ ohm-cm². Cationic diffusion potentials of 25–40 mv were generated by a 10-fold concentration gradient of NaBr (Table I). The addition of Br_2 (2×10^{-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, $S_2O_3^=$ (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 (3×10^{-5} M). In a second experiment 30 mM NaCl was added to 2 mM NaBr plus Br_2 (1×10^{-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 1×10^{-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 1×10^{-5} M shifted the curve upward without greatly altering the slope.

The main effects of Br_2 and Br^- on the Br flux can be summarized as follows:

(*a*) The Br flux is extremely sensitive to the Br_2 concentration. The slopes of the log Br flux vs. log $[Br_2]$ curves are 2–4 (Fig. 3). The largest fluxes shown in Fig. 3 approach 10^{-7} mole/cm² sec, which is nearly 10 times the chloride-for-chloride exchange flux in human red blood cells (18).

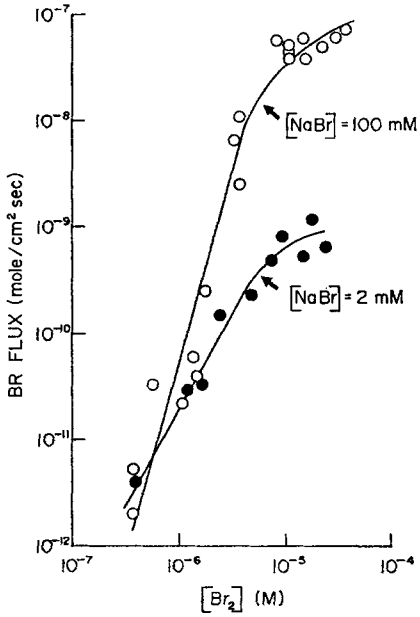

FIGURE 3

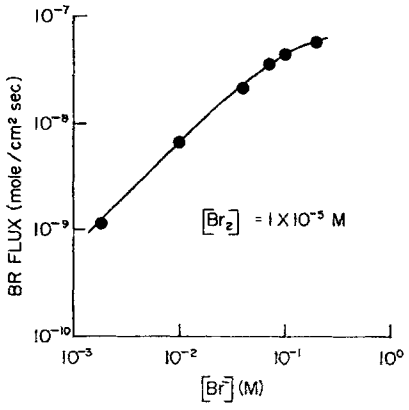

FIGURE 4

FIGURE 3. The Br flux as a function of the equilibrium concentration of Br_2 at constant $[Br^-]$. The equilibrium concentration of Br_2 was computed from equations 2 and 3, as shown in Fig. 1. Solutions were buffered at pH 5.0 with sodium acetate (1 mM).

FIGURE 4. The Br flux as a function of the Br^- concentration at constant $[Br_2]$. The equilibrium concentration of Br_2 (1×10^{-5} M) was computed from equations 2 and 3, as shown in Fig. 1. Solutions were buffered at pH 5.0 with sodium acetate (1 mM).

(*b*) Virtually all of the Br flux is electrically silent. As indicated in Table I and Fig. 3, the effect of Br_2 on the Br flux is much more pronounced than the effect of Br_2 on the membrane resistance.

(*c*) The Br flux apparently saturates at high Br_2 concentrations, and the saturation level is roughly proportional to the Br^- concentration (Fig. 3).

(*d*) The Br flux is roughly proportional to the Br^- concentration over the range 2–200 mM, with apparent saturation of the flux at high $[Br^-]$ (Fig. 4).

DISCUSSION

The flux of Br across membranes bathed with NaBr is probably chiefly due to Br_2 rather than to Br^-, Br_3^-, or Br_5^-. The presence of trace amounts of Br_2 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^{-13} mole/

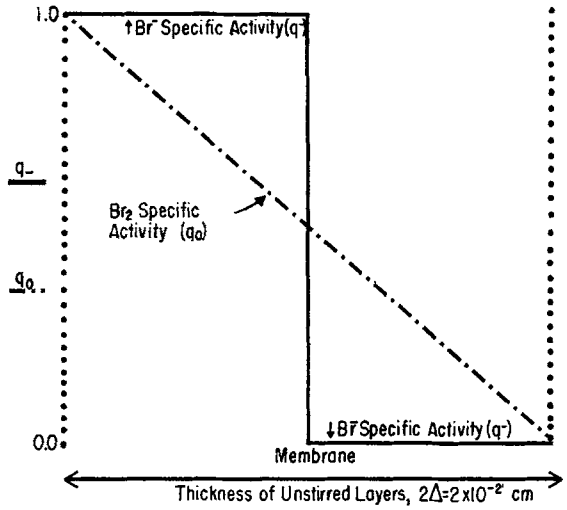

FIGURE 5. Model for Br transport across bilayers with no isotopic exchange between Br_2 and Br^-. The specific activities of Br^- (q_-) and Br_2 (q_0) are plotted as a function of distance through the unstirred layers from the labeled cis (left), to the nonlabeled, trans (right), side of the membrane. The curves were computed from the assumptions that Br traverses the membrane only as Br_2 and that isotopic exchange between Br_2 and Br^- occurs in the bulk bathing solutions but not in the unstirred layers. The specific activity of both Br_2 and Br^- in the cis solution is assumed for simplicity to be 1.0.

cm^2 sec) is still much larger than the flux predicted from electrical measurements (about 10^{-15} mole/cm^2 sec). We have no information yet about the nature of this residual Br flux.

The stimulating effect of Br_2 on the Br flux (Fig. 3) might be explained by the equilibration of added Br_2 with $^{82}Br^-$ in the well-stirred bathing solution, followed by the diffusion of labeled Br_2 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_0) decreases linearly from a value equal to q_- in cis bulk solution to zero in the trans bulk solution. In the unstirred layers, q_0 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 B_1 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/cm³ and the thickness (Δx) 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} cm²/sec, then the Br flux should be given by

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

This calculated Br flux is more than 1000 times less than the observed Br flux at $[Br_2] = 10^{-8}$ mole/cm³ (Fig. 3). The same argument applies to the results of Läuger et al. (21). They measured an I flux of 5×10^{-9} mole/cm² sec when the I_2 concentration was 6×10^{-9} mole/cm³ and the I^- concentration was 10^{-5} mole/cm³.[1] The computed rate of diffusion of I across the unstirred layer as I_2 (assuming no exchange with I^-) is 6×10^{-12} mole/cm² 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_0) 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,

$$K = \frac{[I_2][I^-]}{[I_3^-]} \simeq 1.3 \times 10^{-3} \text{ M (21).}$$

isotopic exchange of Br_2 and Br^-. The characteristic relaxation length (γ^{-1}) may be thought of as the mean distance over which a labeled molecule of $^{82}Br_2$ can diffuse before it is destroyed by isotopic exchange and is very short ($<10^{-4}$ cm) compared to the effective thickness of the unstirred layers (about 10^{-2} cm). An important property of this model is that a concentration gradient for $^{82}Br^-$ is set up in the nustirred layers despite the fact that no Br^- traverses the membrane. Indeed, since q_- and q_0 are equal

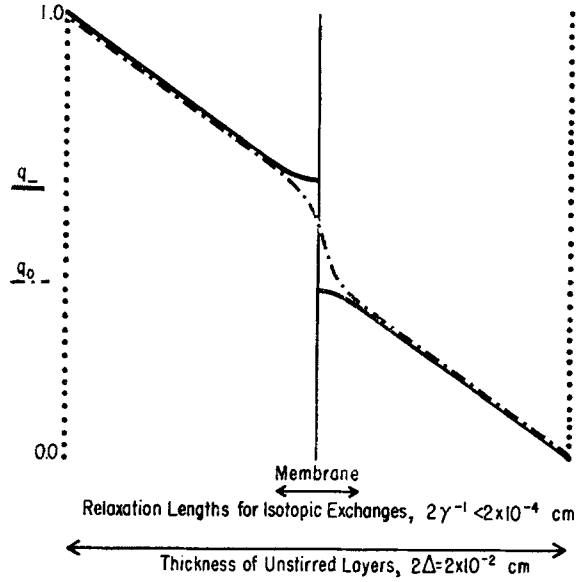

FIGURE 6. Model for Br transport across bilayers with isotopic exchange between Br_2 and Br^-. Similar to Fig. 5 except that isotopic exchange between Br_2 and Br^- is assumed to occur *both* in the bulk bathing solutions *and* in the unstirred layers. γ^{-1} is the mean distance which a $^{82}Br_2$ atom can diffuse before it is destroyed by an isotopic exchange reaction with Br^-. For details see text and Appendix.

throughout most of the unstirred layer, and since $[Br^-] \gg [Br_2]$, 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 Br_2 concentrations. When the concentration of Br_2 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 Br_2 is higher (but not so high as to alter appreciably the sum of $[Br_2]$ and $[Br^-]$), the flux of Br becomes limited by the

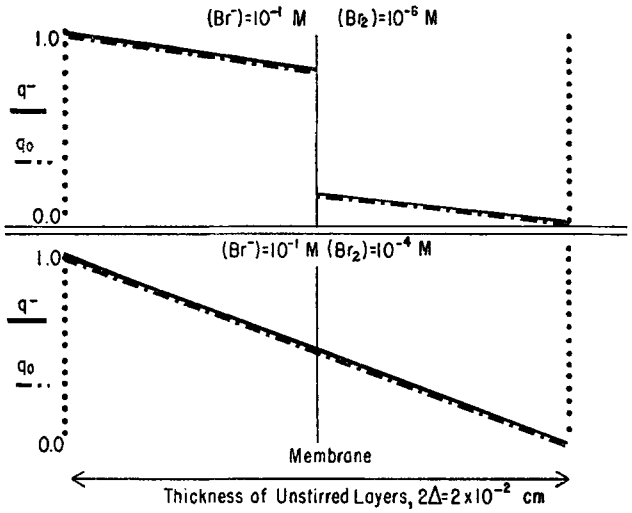

FIGURE 7. Model for Br transport across bilayers; effect of Br_2 concentration. Similar to Fig. 6 except that the relaxation zones are eliminated for clarity of presentation. At low Br_2 concentration (top), Br flux is limited by diffusion of Br_2 through the membrane. At high Br_2 concentration (bottom) Br flux is limited by diffusion of Br^- through the unstirred layer. For details see text and Appendix.

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

$$\begin{aligned} M_{Br} &\simeq D_{Br^-}([Br^-]/\Delta x) \\ &\simeq 10^{-5}\ \text{cm}^2/\text{sec}\ (10^{-4}\ \text{mole/cm}^3\ \text{per}\ 10^{-2}\ \text{cm}) \\ &\simeq 10^{-7}\ \text{mole/cm}^2\ \text{sec}. \end{aligned}$$

A similar calculation for 2 mM Br^- gives a saturation flux of 2×10^{-9} mole/cm^2 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. Br_2 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 $[Br_2]$ 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

$$Br_2 + {}^{82}Br^- \underset{k}{\overset{k}{\rightleftharpoons}} {}^{82}Br_2 + Br^- \tag{5}$$

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

$$q_- = \frac{[{}^{82}Br^-]}{[{}^{82}Br^-] + [Br^-]} \tag{6}$$

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

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

and

$$\frac{d^2}{dx^2}(q_- - q_0) - \gamma^2(q_- - q_0) = 0. \tag{8}$$

where

$$\beta_-{}^2 = \frac{k[Br^-][Br_2]}{D_{Br^-}[Br]}, \tag{9}$$

$$\beta_0{}^2 = \frac{k[Br^-][Br_2]}{D_{Br_2}[Br_2]}, \tag{10}$$

and

$$\gamma^2 = \beta_-{}^2 + \beta_0{}^2. \tag{11}$$

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, occuring 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 Br^- concentration is always much greater than the Br_2 concentration. Thus $(\gamma)^{-1}$ is the average distance that a $^{82}Br_2$ can diffuse before being destroyed by isotopic exchange with a 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 $^{82}Br_2$ to be $\simeq 10^{-9}$ sec. Therefore $(\gamma)^{-1} \simeq 10^{-7}$ cm is obtained as a lower limit for the relaxation length. Even if only one encounter in 10^6 actually resulted in exchange we would still have $(\gamma)^{-1} \simeq 10^{-4}$ cm. For an unstirred layer of thickness $\Delta \simeq 10^{-2}$ cm, the condition

$$\gamma\Delta \gg 1 \tag{12}$$

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 $-\Delta \leq x < 0$, solution of equations 7 and 8 yields

$$q_-(x) = q^R - A\left[\left(\frac{\beta_-}{\gamma}\right)^2 \gamma\{\Delta + x\} - \left(\frac{\beta_-}{\gamma}\right)^2 e^{\gamma x}\right] \tag{13}$$

and

$$q_0(x) = q^R - A\left[\left(\frac{\beta_-}{\gamma}\right)^2 \gamma\{\Delta + x\} + \left(\frac{\beta_0}{\gamma}\right)^2 e^{\gamma x}\right] \tag{14}$$

where

$$A = \frac{\beta_0^2}{\gamma} \frac{M_{^{82}Br}}{k[Br^-][Br_2]} \tag{15}$$

and $M_{^{82}Br}$ 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 Br^- is presumed not to penetrate the membrane. Corresponding relations for the unstirred layer occupying the region $0 < x \leq \Delta$, 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

$$M_{s^2Br} = P^m_{Br_2} \{(q_0)_{x=0-} - (q_0)_{x=0+}\} [Br_2] \quad (16)$$

where $P^m_{Br_2}$ is the coefficient of membrane permeability to Br_2. This suffices to permit derivation of an expression for the total tracer flux involving q^R, $[Br^-]$, $[Br_2]$, $P^m_{Br_2}$, and the unstirred layer permeability coefficients, defined by

$$P^u_{Br_2} = D_{Br_2}/2\Delta \quad (17)$$

and

$$P^u_{Br^-} = D_{Br^-}/2\Delta. \quad (18)$$

The specific activity, q^R, 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, $P^u_{Br^-} \simeq P^u_{Br_2}$ while $[Br^-] \gg [Br_2]$, we present, instead of the exact result of the model, the simpler approximate expression

$$\frac{1}{M_{Br}} = \frac{1}{P^u_{Br^-}[Br^-]} + \frac{1}{P^m_{Br_2}[Br_2]} + \frac{1}{\gamma\Delta P^u_{Br_2}[Br_2]} \quad (19)$$

which introduces negligible error.

Equation 19 is most effectively considered in the context of Fig. 3, where the variation of M_{Br} with $[Br_2]$ is illustrated for fixed values of $[Br^-]$. 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 $[Br_2]$, being determined solely by the permeability of the unstirred layers to Br^-. At low $[Br_2]$ either the second or the third term will fix the flux. If $P^m_{Br_2} < \gamma\Delta P^u_{Br_2}$, 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^- 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 $[Br_2]$ at low $[Br_2]$, 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^-.

The authors acknowledge valuable discussions with D. F. Hastings, M. Tieffenberg 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, $^{82}Br_3^-$, 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.

REFERENCES

1. MUELLER, P., and D. O. RUDIN. 1969. Translocators in bimolecular lipid membranes: their role in dissipative and conservative bioenergy transductions. *Curr. Top. Bioenerg.* **3**:157.
2. HENN, F. A., and T. E. THOMPSON. 1969. Synthetic lipid bilayer membranes. *Annu. Rev. Biochem.* **38**:241.
3. ROTHFIELD, L., and A. FINKELSTEIN. 1968. Membrane biochemistry. *Annu. Rev. Biochem.* **37**:463.
4. VREEMAN, H. J. 1966. Permeability of thin phospholipid films. *Proc. Kon. Ned. Akad. Wetensch. Ser. B.* **69**:564.
5. PAGANO, R., and T. E. THOMPSON. 1968. Spherical lipid bilayer membranes: electrical and isotopic studies of ion permeability. *J. Mol. Biol.* **38**:41.
6. BANGHAM, A. D., M. M. STANDISH, and J. C. WATKINS. 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* **13**:238.
7. PAPAHADJOPOULOS, D., and J. C. WATKINS. 1967. Phospholipid model membranes. II. Permeability properties of hydrated liquid crystals. *Biochem. Biophys. Acta.* **135**:639.
8. ANDREOLI, T. E., J. A. BANGHAM, and D. C. TOSTESON. 1967. The formation and properties of thin lipid membranes from HK and LK sheep red cell lipids. *J. Gen. Physiol.* **50**:1729.
9. TOSTESON, D. C. 1967. Electrolyte composition and transport in red blood cells. *Fed. Proc.* **26**:1805.
10. HUNTER, F. R., 1967. Facilitated diffusion in the chloride shift in human erythrocytes. *Biochim. Biophys. Acta.* **135**:784.
11. SCARPA, A., A. CECCHETTO, and G. F. AZZONE. 1970. The mechanism of anion translocation and pH equilibration in erythrocytes. *Biochim. Biophys. Acta.* **219**:179.
12. DALMARK, M., and J. O. WIETH. 1970. Chloride and sodium permeabilities of human red cells. *Biochim. Biophys. Acta.* **219**:525.
13. GUTKNECHT, J., and D. C. TOSTESON. 1970. Ionic permeability of thin lipid membranes: effects of *n*-alkyl alcohols, polyvalent cations, and a secondary amine. *J. Gen. Physiol.* **55**:359.
14. LIEBHAFSKY, H. A. 1934. The equilibrium constant of the bromine hydrolysis and its variation with temperature. *J. Amer. Chem. Soc.* **56**:1500.
15. GRIFFITH, R. O., A. MCKEOWN, and A. G. WINN. 1932. The bromine-bromide-tribomide equilibrium. *Trans. Faraday Soc.* **28**:101.
16. HODGKIN, A. L. 1951. The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26**:339.
17. LANGE, N. A. (editor). 1961. Handbook of Chemistry. McGraw-Hill Book Company, New York. 10th edition. 1212.
18. TOSTESON, D. C. 1959. Halide transport in red blood cells. *Acta Physiol. Scand.* **46**:19.
19. ANDREOLI, T. E., V. W. DENNIS, and A. M. WEIGL. 1969. The effect of amphotericin B on the water and nonelectrolyte permeability of thin lipid membranes. *J. Gen. Physiol.* **53**:133.
20. HOLZ, R., and A. FINKELSTEIN. 1970. The water and nonelectrolyte permeability induced in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *J. Gen. Physiol.* **56**:125.
21. LÄUGER, P., J. RICHTER, and W. LESSLAUER. 1967. Electrochemistry of bimolecular phospholipid membranes. *Ber. Bunsenges. Phys. Chem.* **71**:906.

22. HARRIS, E. J., and B. C. PRESSMAN. 1967. Obligate cation exchanges in red cells. *Nature (London)*. **216**:918.
23. HOFFMAN, J. F., and U. V. LASSEN. 1971. Plasma membrane potentials in amphiuma red cells. *Int. Congr. Physiol. Sci. Lect. Symp. 25th.* **9**:746A.
24. GUNN, R. B., M. DALMARK, J. O. WIETH, and D. C. TOSTESON. 1971. A comparison of normal and inhibited chloride permeability in human red blood cells. *Int. Congr. Physiol. Sci. Lect. Symp. 25th.* **9**:657A.
25. LEBLANC, O. H., JR. 1971. The effect of uncouplers of oxidative phosphorylation on lipid bilayer membranes: carbonylcyanide m-chlorophenyl-hydrazone. *J. Membrane Biol.* **4**:227.
26. CLARK, J. D., and R. P. WAYNE. 1969. The theory of elementary reactions in solution. *In* Comprehensive Chemical Kinetics. C. H. Bamford and C. F. H. Tipper, editors. Elsevier Publishing Company, Amsterdam. **2**:302.