

# Electrically Silent Anion Transport through Lipid Bilayer Membranes Containing a Long-Chain Secondary Amine

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**ABSTRACT** The permeability properties of planar lipid bilayers made from egg lecithin, *n*-decane and a long-chain secondary amine (*n*-lauryl [trialkylmethyl]amine) are described. Membranes containing the secondary amine show halide selectivity and high conductance at pH <6, as estimated by measurements of zero-current potentials generated by NaBr activity gradients. In the absence of halide ions, the membranes show H<sup>+</sup> selectivity, although the total membrane conductance is relatively low. In 0.1 M NaBr both the membrane conductance ( $G_m$ ) and the Br<sup>-</sup> self-exchange flux ( $J_{Br}$ ) are proportional to H<sup>+</sup> concentration over the pH range of 7 to 4, and both  $J_{Br}$  and  $G_m$  saturate at pH <4. However,  $J_{Br}$  is always more than 100 times the flux predicted from  $G_m$  and the transference number for Br<sup>-</sup>. Thus, >99% of the observed (tracer) flux is electrically silent and is not a Br<sub>2</sub> or HBrO flux because the reducing agent, S<sub>2</sub>O<sub>3</sub><sup>=</sup>, has no effect on  $J_{Br}$ . At pH 7,  $J_{Br}$  is proportional to Br<sup>-</sup> concentration over the range of 1–340 mM, with no sign of saturation kinetics. Both urea and sulfate tracer permeabilities are low and are unaffected by pH. The results can be explained by a model in which the secondary amine behaves as a monovalent, titratable carrier which exists in three chemical forms (C, CH<sup>+</sup>, and CHBr). Br<sup>-</sup> crosses the membrane primarily as the neutral complex (CHBr). The positively charged carrier (CH<sup>+</sup>) crosses the membrane slowly compared to CHBr, but CH<sup>+</sup> is the principal charge carrier in the membrane. At neutral pH >99% of the amine is in the nonfunctional form (C), which can be converted to CH<sup>+</sup> or CHBr by increasing the H<sup>+</sup> or Br<sup>-</sup> concentrations. The permeability properties of these lipid bilayers resemble in many respects the permeability properties of red cell membranes.

## INTRODUCTION

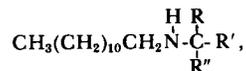
Electrically silent anion transport processes are important in many plant and animal cell membranes. The transport process may be either anion exchange (e.g., Cl<sup>-</sup>-Cl<sup>-</sup> or Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup>) or cotransport of a cation and anion (e.g., NaCl or HCl). The best characterized anion transport occurs in erythrocytes, where a divalent "titratable carrier" mediates anion exchange diffusion and, under some conditions, a net transport of anions and protons (Gunn, 1972; Jennings, 1976).

Recent studies indicate that the molecule responsible for anion exchange in red cells is a protein (molecular weight ca.  $10^5$ ) which spans the membrane (Steck, 1974; Rothstein et al., 1976).

The possible role of membrane lipids in anion exchange was first suggested by Bangham et al. (1965) and Papahadjopoulos and Watkins (1967), who found that liposomes of both phosphatidylcholine and phosphatidylserine display rapid anion (but not cation) exchange fluxes (see also Nicholls and Miller, 1974). This work was extended by Pagano and Thompson (1968) and Toyoshima and Thompson (1975 *a, b*), whose results suggest that  $\text{Cl}^-$  exchange diffusion in lecithin bilayers is a carrier mediated process involving a lecithin-HCl complex.

In seeking information about the molecules or functional groups responsible for anion exchange through biological membranes, we are testing the effects of various lipids and proteins on anion transport through planar lipid bilayer membranes. The planar (Mueller-Rudin) type membrane is ideal for studies of this type, because both electrically silent and "electrogenic" diffusion processes can be studied simultaneously. This paper describes  $\text{Br}^-$  transport through membranes made from egg lecithin and a long-chain secondary amine (Amberlite LA-2) in *n*-decane.

Amberlite LA-2 (Rohm and Haas Co., Philadelphia) is a mixture of long-chain secondary amines with molecular weights ranging from 353 to 395. The structural formula is



and the three side chains (R, R', and R'') contain a total of 11–14 carbons. The amine is insoluble in water but readily soluble in organic solvents. Thus, it can be incorporated into the membrane simply by adding it to the membrane forming solution. An early study by Shean and Sollner (1966) showed that this compound induces high anionic permselectivity in thick liquid membranes, and we obtained similar results in preliminary experiments with planar lipid bilayers (Gutknecht and Tosteson, 1970). In this paper we show that in lipid bilayers Amberlite LA-2 promotes electrically silent anion exchange which resembles in many respects anion exchange through the red cell membrane.

#### METHODS

Lipid bilayer (optically black) membranes were made by the brush technique of Mueller and Rudin (1969). Unless otherwise specified, the membranes were formed from a mixture of egg lecithin (50 mg/ml) and Amberlite LA-2 (lauryl[trialkylmethyl]amine) (50 mg/ml) in *n*-decane, which gives lecithin:Amberlite:decane mole ratios of 1:2:82. Membranes were formed on a 1.5-mm diameter hole in a polyethylene partition which separated two magnetically stirred solutions of 1.2 ml each. The temperature was 22–24°C.

Halide fluxes in most experiments were measured with  $^{82}\text{Br}^-$  rather than  $^{36}\text{Cl}^-$ , because  $^{82}\text{Br}^-$  is available in higher specific activities. The bathing solution in most experiments was NaBr (0.1 M) plus various pH buffers as specified with each experiment. In a few experiments a reducing agent,  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mM) was added to the aqueous solutions, but  $\text{S}_2\text{O}_3^{2-}$  had no effect on the  $\text{Br}^-$  fluxes through lecithin-Amberlite membranes (see Fig. 3). In a few experiments we measured  $^{24}\text{Na}^+$ ,  $^{35}\text{SO}_4^{2-}$ ,  $^{36}\text{Cl}^-$ , and  $^{14}\text{C}$ -urea fluxes, using techniques similar to those described for  $^{82}\text{Br}^-$ .

After a stable membrane was formed,  $^{82}\text{Br}^-$  (as  $\text{NaBr}$  or  $\text{NH}_4\text{Br}$ ) was injected into the rear compartment. The rate of appearance of radioactivity in the front compartment was then measured by continuous perfusion (1–2 ml/min) and collection of samples at 3–10-min intervals. The perfusate was aspirated from the front compartment and collected in a vacuum trap. The rear compartment was sampled periodically with a microsyringe. The samples were dried in 2-inch diameter planchets and counted in a low-background counter. The one-way flux of  $\text{Br}^-$  was then calculated by the equation:

$$J_{\text{Br}} = \frac{{}^{82}\text{Br}^F}{t A \text{SA}^R}, \quad (1)$$

where  $J_{\text{Br}}$  is the flux ( $\text{mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ),  ${}^{82}\text{Br}^F$  is the total amount of tracer (counts per minute) entering the front compartment during the sampling interval,  $t$  (seconds),  $A$  is the surface area of the membrane ( $\text{cm}^2$ ), and  $\text{SA}^R$  is the specific activity of the tracer in the rear compartment (counts per minute/mole). Each flux was computed from the mean of at least three consecutive samples.

We calculated the steady-state membrane conductance ( $G_m$ ), using Ohm's Law, from the membrane potential produced by applying a known voltage pulse across the membrane in series with a known resistance. The membrane potential ( $V_m$ ) was recorded as the potential difference between two calomel-KCl electrodes which made contact with the front and rear solutions. Membrane conductance was measured at 5–10-min intervals throughout each experiment.

Ion transference numbers were estimated from the zero-current potentials produced by imposing 5–10-fold ionic activity gradients across the membrane (see Andreoli et al., 1967). A variety of buffer ions (e.g., Tris, histidine, glutamate) were used in these experiments, and we assumed that the buffer ion conductance was negligible. In a few experiments, the buffer concentrations were varied by 10-fold (e.g., 1–10 mM) with no effect on the membrane conductance.

From the transference number ( $t_{\text{Br}}$ ) and the total membrane conductance ( $G_m$ ), we estimated the conductive  $\text{Br}^-$  flux by the equation:

$$J_{\text{Br}^-} = \frac{R T t_{\text{Br}} G_m}{z_{\text{Br}}^2 F^2}, \quad (2)$$

where  $R$  is the gas constant,  $T$  is the absolute temperature,  $z_{\text{Br}}$  is the ionic valence, and  $F$  is the Faraday (Hodgkin, 1951). This equation assumes that the ions move independently through the membrane. This calculated (conductive) flux was subtracted from the observed (tracer) flux in order to estimate the electrically silent component of the observed flux.

Egg lecithin, phosphatidylserine (bovine), monogalactosyldiglyceride (plant), and *n*-decane were obtained from either Supelco, Inc. (Bellefonte, Pa.) or from Lipid Products (Surrey, England). Amberlite LA-2 was a gift from Rohm and Haas Co. (Philadelphia, Pa.).  $^{82}\text{Br}^-$  (as  $\text{NaBr}$  or  $\text{NH}_4\text{Br}$ ),  $^{24}\text{Na}^+$  (as  $\text{Na}_2\text{CO}_3$ ),  $^{35}\text{SO}_4^-$  (as  $\text{Na}_2\text{SO}_4$ ),  $^{36}\text{Cl}$  (as  $\text{NaCl}$ ), and  $^{14}\text{C}$ -urea were obtained from International Chemical and Nuclear Corp., ICN Pharmaceuticals, Inc. (Irvine, Ca.).

## RESULTS

### *Cl<sup>-</sup> and Br<sup>-</sup> Fluxes through Lecithin, Phosphatidylserine, and Monogalactosyldiglyceride Membranes*

Table I shows the  $\text{Br}^-$  and  $\text{Cl}^-$  fluxes through membranes made from egg lecithin or bovine phosphatidylserine or plant monogalactosyldiglyceride in *n*-decane. We tested the egg lecithin and phosphatidylserine to provide control

data and also for comparison with the results of Pagano and Thompson (1968) and Papahadjopoulos and Watkins (1967). We tested the monogalactosyldiglyceride because Kuiper (1968) found that this particular lipid was associated with  $\text{Cl}^-$  accumulation in plant roots and also increased the rate of  $\text{Cl}^-$  diffusion through liquid "membranes" made of *n*-pentanol. As shown in Table I,  $\text{Cl}^-$  and  $\text{Br}^-$  fluxes through all these "control" membranes are low (ca.  $10^{-12}$   $\text{mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ), although still more than 50 times the maximum flux predicted from the membrane conductance. Thus, almost all of the halide flux through these control membranes occurs by an undefined electrically silent mechanism.

Our results agree qualitatively with those of Papahadjopoulos and Watkins (1967) and Hauser et al. (1972), who observed relatively high  $\text{Cl}^-$  exchange

TABLE I  
ONE-WAY FLUXES OF  $\text{Cl}^-$  OR  $\text{Br}^-$  THROUGH LIPID BILAYER MEMBRANES  
COMPOSED OF EGG LECITHIN OR PHOSPHATIDYLSERINE (BOVINE) OR  
MONOGALACTOSYLDIGLYCERIDE (PLANT) IN *n*-DECANE

Ion	Lipid	Bathing solution	Flux	Flux	Membrane conductance
			observed	predicted	
			$10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	$10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	$10^{-8} \text{ mho} \cdot \text{cm}^{-2}$
$\text{Cl}^-$	Egg lecithin	NaCl (0.1 M) pH 5.6	$1.5 \pm 0.5$ (2)	<0.02	$8 \pm 0$ (2)
$\text{Br}^-$	Egg lecithin	NaBr (0.1 M) pH 5.0	$4 \pm 2$ (2)	<0.02	$8 \pm 1$ (2)
$\text{Br}^-$	Egg lecithin	NaBr (0.1 M) pH 7.3	$1.6 \pm 0.1$ (2)	<0.005	$2 \pm 2$ (2)
$\text{Br}^-$	Phosphatidylserine	NaBr (0.1 M) pH 7.3	$3.2 \pm 1.4$ (3)	<0.002	$0.5 \pm 0.3$ (3)
$\text{Br}^-$	Monogalactosyldiglyceride (plant)	NaBr (0.1 M) pH 5.0	0.1 (1)	<0.002	0.8 (1)

The front and rear bathing solutions are identical except for the addition of tracer to the rear compartment. The pH 5.0 solutions are buffered with sodium acetate (1 mM), the pH 7.3 solutions are buffered with sodium phosphate (2 mM), and the pH 5.6 solutions are unbuffered. The NaBr solutions also contain  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mM). The predicted fluxes, calculated from Eq. 2, are upper limits because they are computed from total membrane conductance rather than the specific conductance of  $\text{Cl}^-$  or  $\text{Br}^-$ . The lipid concentrations in decane are 20–25 mg/ml.

Results are quoted in the form: mean  $\pm$  SE (number of membranes).

fluxes in both egg lecithin and phosphatidylserine vesicles. However, our halide exchange fluxes are only about 5% of those observed in large spherical egg lecithin bilayers by Pagano and Thompson (1968). Toyoshima and Thompson (1975 *a, b*) postulated that a slow flip-flop of a lecithin-HCl complex could account for most of the electrically silent exchange flux through lecithin bilayers, but subsequent demonstrations of the slowness of phospholipid flip-flop in bilayers necessitated a modification of this hypothesis. A more recent model proposes that molecular HCl may be released into the bilayer interior as a result of the "bobbing up and down" of lecithin-HCl complexes in the membrane surface (Robertson and Thompson, 1977). Our data provide no insights into the mechanism of electrically silent anion exchange through pure phospholipid bilayers. However, as we show below, the anion exchange fluxes through all these control membranes are insignificant compared to the anion

exchange fluxes through bilayers containing the secondary amine, Amberlite LA-2.

*Electrical Properties of Lecithin-Amberlite Bilayers*

Fig. 1 shows that Amberlite LA-2 increases the  $\text{Br}^-$  transference number in a pH-dependent manner. Membranes containing Amberlite LA-2 are slightly anion-selective at  $\text{pH} > 7$  and highly anion-selective at  $\text{pH} < 6$ . In contrast, pure egg lecithin-decane bilayers show no ionic selectivity in NaBr solutions. Fig. 2 shows that the steady-state conductance of lecithin-Amberlite-decane membranes increases from about  $10^{-7}$  to  $10^{-4}$   $\text{mho} \cdot \text{cm}^{-2}$  as pH decreases from about 7 to 3. In contrast, the conductance and ionic selectivity of lecithin-decane bilayers is far less sensitive to pH (Table I, Fig. 1, and Redwood et al., 1971),

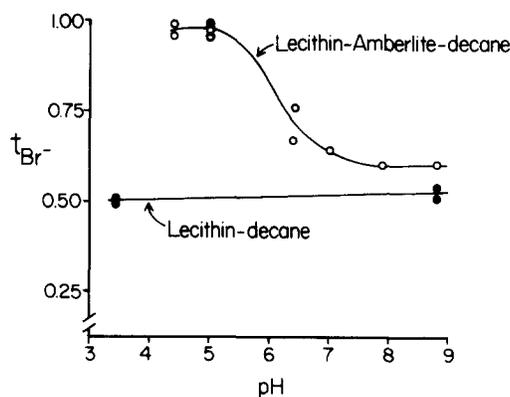


FIGURE 1. Transference numbers for  $\text{Br}^-$  in lecithin-Amberlite-decane bilayers (○) and lecithin-decane bilayers (●) as a function of pH. Solutions contain NaBr and buffers as described in the legend of Fig. 2. Transference numbers are calculated as described in Methods. Each point represents a single membrane.

which is expected because the net charge on egg lecithin is constant over the pH range of 3.5 to 11 (Papahadjopoulos, 1968).

The data shown in Figs. 1 and 2 indicate that the high conductance of the lecithin-Amberlite membranes at low pH is mainly a  $\text{Br}^-$  conductance. In addition to the data shown in Figs. 1 and 2, we find that 5-fold  $\text{H}^+$  activity gradients over the pH range of 4.0–4.7 produce diffusion potentials of  $< 2$  mV in the presence of 0.1 M NaBr and  $\text{Na}^+$  glutamate buffer (0.05 M). A similar lack of  $\text{H}^+$  selectivity was found earlier for the pH range 5–7 (Gutknecht and Tosteson, 1970). However, in  $\text{Br}^-$ -free solutions ( $\text{Na}^+$  glutamate buffer, 0.05 M), the membrane shows  $\text{H}^+$  selectivity, giving  $\text{H}^+$  diffusion potentials of 45–50 mV/decade over the pH range of 4.0–4.6. In  $\text{Br}^-$ -free solutions, however, the membrane conductance is much lower than in 0.1 M NaBr. For example, at pH 4.0  $G_m$  is  $6(\pm 4) \times 10^{-7}$   $\text{mho} \cdot \text{cm}^{-2}$ , which is  $< 5\%$  of  $G_m$  in 0.1 M NaBr (cf. Fig. 2). Thus, in  $\text{Br}^-$ -free solutions lecithin-Amberlite bilayers show  $\text{H}^+$  selectivity but relatively low conductance, whereas in 0.1 M NaBr the membranes show high  $\text{Br}^-$  selectivity and high conductance.

*Br<sup>-</sup> and Na<sup>+</sup> Fluxes through Lecithin-Amberlite Bilayers*

Fig. 3 shows that the addition of Amberlite to lecithin-decane bilayers increases the Br<sup>-</sup> exchange flux in a pH-dependent manner. The results from two different lecithin-Amberlite-decane mixtures are shown, i.e., 50 mg each per milliliter decane (mole ratio = 0.5) (curve A) and 30 mg lecithin and 3 mg Amberlite per milliliter decane (mole ratio = 5) (curve B). The dashed line is calculated from Eq. 8, which is derived in the Appendix. From the measured values of  $G_m$  and  $t_{Br}$  (Figs. 1 and 2), we estimate that the conductive Br<sup>-</sup> flux (calculated by Eq. 2) ranges from 0.001 to 1% of the observed flux for both curve A and curve B. Thus, >99% of the one-way flux occurs by an electrically silent mechanism.

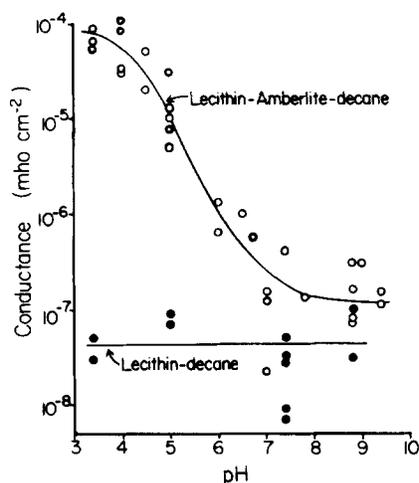


FIGURE 2. Conductance of lecithin-Amberlite-decane bilayers (O) and lecithin-decane bilayers (●) as a function of pH. Both front and rear solutions contain NaBr (100 mM) and the following buffers: pH 3.4, no buffer; pH 4.0–5.0, acetate (1–2 mM); pH 6.0–7.4, phosphate or histidine (2 mM); pH 7.6–8.8, Tris (5 mM); pH 9.4, borate or histidine (5 mM). Each point represents a single membrane.

The reducing agent,  $S_2O_3^{2-}$ , has no effect on  $J_{Br}$  (open squares in Fig. 3). Thus, an electrically silent Br transport via  $Br_2$  or  $HBrO$  is unlikely (see Gutknecht et al., 1972). A more likely transport mechanism, which is consistent with the known chemical properties of Amberlite LA-2, involves a reversible reaction between  $Br^-$  and the protonated amine, i.e.,  $Br^- + CH^+ \rightleftharpoons CHBr$ , followed by diffusion of the neutral complex ( $CHBr$ ) through the membrane. Such a mechanism would account qualitatively for both the pH dependence and the electrically silent nature of the Br<sup>-</sup> exchange flux.

At pH < 4 the flux saturates at either  $8 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  (curve A) or about  $3 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  (curve B). The higher value is close to the maximum rate at which Br<sup>-</sup> can diffuse through the aqueous unstirred layer, which in our system has a combined thickness of about 130  $\mu\text{m}$  (Gutknecht and Tosteson, 1973). Thus, the maximum flux in curve A is probably unstirred layer-limited, whereas the maximum flux in curve B is membrane-limited.

At  $\text{pH} > 8$  the  $\text{Br}^-$  flux attains a minimum value of about  $10^{-10} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  (Fig. 3, curve A), which is about 50 times higher than the lecithin-decane controls (cf. Table I). The reason for this high residual  $\text{Br}^-$  flux at  $\text{pH} > 8$  is not clear, inasmuch as the expected titration of the secondary amine should ultimately produce a  $\text{Br}^-$  flux similar to the controls, i.e., ca.  $10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ . One possible mechanism for the electrically silent  $\text{Br}^-$  flux at high  $\text{pH}$  is an Amberlite-mediated cotransport of  $\text{Na}^+$  and  $\text{Br}^-$ . However, Table II (lines 1 and 2) shows that the  $\text{Na}^+$  exchange flux is  $< 0.5 \times 10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ , which is  $< 1\%$  of the  $\text{Br}^-$  flux. Thus, the secondary amine does not facilitate  $\text{NaBr}$

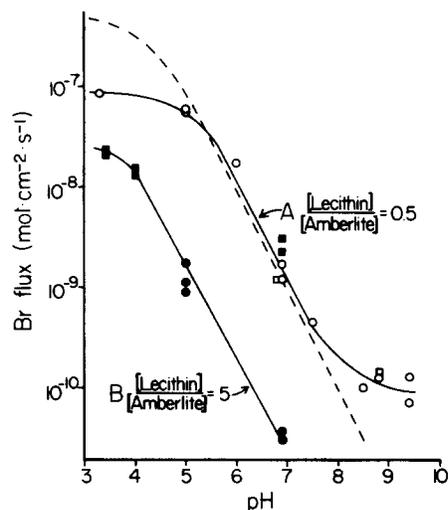


FIGURE 3. One-way (self-exchange) flux of  $\text{Br}^-$  through lecithin-Amberlite bilayers as a function of  $\text{pH}$ . Solutions contain  $\text{NaBr}$  (100 mM) and buffers as described in the legend of Fig. 2. In curve A the membrane forming solution is the standard mixture of egg lecithin and Amberlite LA-2, i.e., 50 mg each per ml decane (mole ratios = 1:2:82). In curve B the membrane forming solution contains 30 mg lecithin and 3 mg Amberlite per ml decane (mole ratios = 0.61:0.13:82). The dashed line is calculated from Eq. 8, which is described in the Appendix. The open squares indicate that the aqueous solutions contain  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mM), and the closed squares indicate that the solutions contain sodium trinitroresolate (1 mM). Each point represents a single membrane.

transport, even when  $[\text{H}^+]$  is very low. A remaining possibility, which we have not been able to assess, is the presence of trace amounts (ca. 0.1%) of a contaminating amine which is more basic than Amberlite LA-2. This possibility is also suggested by the titration curves in Figs. 1 and 2.

Fig. 4 shows that the  $\text{Br}^-$  flux is proportional to  $\text{Br}^-$  concentration over a range of 1–340 mM  $\text{Br}^-$ . As in previous experiments, virtually all of the observed flux is electrically silent. The slope of 1.0 indicates a 1:1 stoichiometry between  $\text{Br}^-$  and the transport mechanism, and the linearity of the relationship indicates that the apparent  $K_m$  or  $K_{1/2}$  for the transport process is high, i.e.,  $> 300 \text{ mM}$ . The dashed line is calculated by Eq. 8, which is discussed in the Appendix.

*SO<sub>4</sub><sup>=</sup> and Urea Fluxes through Lecithin-Amberlite Bilayers*

To obtain additional information about the selectivity mechanism in lecithin-Amberlite bilayers, we measured sulfate and urea fluxes at pH 7.4 and 5.0. The SO<sub>4</sub><sup>=</sup> fluxes are shown in Table II (lines 3 and 4), and the sulfate and urea permeability coefficients are shown in Table III. Strictly speaking, all these

TABLE I I  
Na<sup>+</sup>, Br<sup>-</sup>, AND SO<sub>4</sub><sup>=</sup> FLUXES THROUGH LECITHIN-AMBERLITE BILAYERS

Ion	Bathing solution and pH	Flux observed	Flux predicted	Membrane conductance
		$10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	$10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$	$10^{-6} \text{ mho} \cdot \text{cm}^{-2}$
Na <sup>+</sup>	NaBr, 0.1 M, pH 8.8	<0.5 (3)	0.03	0.3±0.2 (3)
Br <sup>-</sup>	NaBr, 0.1 M, pH 8.8	131±10 (2)	0.02	0.12±0.1 (2)
SO <sub>4</sub> <sup>=</sup>	Na <sub>2</sub> SO <sub>4</sub> , 0.05 M, pH 7.4	1.5±0.2 (2)	0.02	0.24±0.16 (2)
SO <sub>4</sub> <sup>=</sup>	Na <sub>2</sub> SO <sub>4</sub> , 0.05 M, pH 5.0	1.7±1.0 (2)	<0.34	4.1±3.3 (4)

Front and rear solutions are identical except for the addition of tracer to the rear compartment. The pH 8.8 solutions are buffered with tris (5 mM), the pH 7.4 solutions are buffered with HEPES (1 mM), and the pH 5.0 solutions are buffered with acetate or citrate (1 mM). The predicted fluxes are calculated from  $G_m$  and  $l_i$  by Eq. 2.

Results are quoted in the form: mean ± SE (number of membranes).

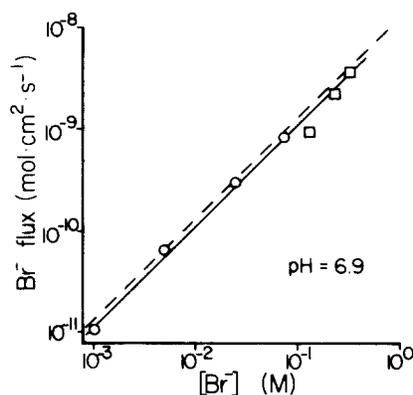


FIGURE 4. Br<sup>-</sup> flux through lecithin-Amberlite-decane bilayers as a function of Br<sup>-</sup> concentration at pH 6.9 (histidine buffer, 2 mM). The dashed line is calculated from Eq. 8, which is described in the Appendix. The data points indicated by circles are from one membrane, and the data points indicated by squares are from one membrane.

values should be regarded as upper limits in that we did not assess the radiochemical purity of tracer coming through the membrane. Nevertheless, the SO<sub>4</sub><sup>=</sup> exchange flux is consistently low (ca.  $10^{-12} \text{ mol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ ), although still apparently larger than that predicted from the membrane conductance. Furthermore, neither the SO<sub>4</sub><sup>=</sup> tracer permeability ( $3 \times 10^{-8} \text{ cm} \cdot \text{s}^{-1}$ ) nor the urea permeability ( $2 \times 10^{-6} \text{ cm} \cdot \text{s}^{-1}$ ) are affected by pH over the range of 7.4 to 5.0 (Table III). In contrast, the Br<sup>-</sup> permeability increases by more than 100-fold, i.e., from about  $5 \times 10^{-6}$  to  $5 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$  over the same range of pH (cf. Fig. 3), and a parallel increase occurs in the Br<sup>-</sup> conductance (cf. Figs. 1 and 2).

Thus, the selectivity mechanism is highly specific for  $\text{Br}^-$  as compared to  $\text{SO}_4^{2-}$  and urea. These results argue against the possibility of a hydrophilic pathway or channel mechanism for the conductive flux of  $\text{Br}^-$ . Further evidence against a channel mechanism is the fact that thick (i.e., colored) membranes of lecithin, Amberlite and decane show pH dependent  $\text{Br}^-$  conductances (and fluxes) similar (within a factor of 5) to those observed in optically black films.

## DISCUSSION

*The Mechanism of Anion Exchange and Conductance through Lecithin-Amberlite Bilayers*

As first pointed out by Shean and Sollner (1966), ion transport through liquid ion exchange membranes resembles carrier-mediated ion transport through biological membranes. Our results suggest that in lipid bilayer membranes Amberlite LA-2 functions as a mobile, titratable, monovalent anion carrier

TABLE III  
 $\text{SO}_4^{2-}$ , UREA, AND  $\text{Br}^-$  PERMEABILITIES OF LECITHIN-AMBERLITE  
BILAYERS AT HIGH AND LOW pH

Solute	Bathing solution	Permeability coefficient	
		pH 7.4	pH 5.0
		$10^{-6} \text{ cm} \cdot \text{s}^{-1}$	$10^{-6} \text{ cm} \cdot \text{s}^{-1}$
$\text{SO}_4^{2-}$	$\text{Na}_2\text{SO}_4$ , 0.05 M	$0.031 \pm 0.005$ (2)	$0.032 \pm 0.017$ (2)
Urea	$\text{NaBr}$ , 0.1 M; Urea, 0.01 M	$1.7 \pm 0.5$ (2)	$2.1 \pm 0.8$ (3)
$\text{Br}^-$	$\text{NaBr}$ , 0.1 M	5.5 (1)	$550 \pm 30$ (2)

The pH 7.4 solutions are buffered with HEPES (1 mM), and the pH 5.0 solutions are buffered with acetate or citrate (1.0 mM). The permeability coefficient is calculated as flux/concentration, since  $V_m = 0$ . The  $\text{Br}^-$  flux data are taken from Fig. 3.

Results are quoted in the form: mean  $\pm$  SE (number of membranes).

which exists in three chemical forms, C,  $\text{CH}^+$ , and  $\text{CHBr}$ . In our experiments the predominant ion transport process is an electrically silent exchange of  $\text{Br}^-$ , presumably mediated by the neutral complex ( $\text{CHBr}$ ). From electrical data we infer that the rate of proton transport via the charged complex ( $\text{CH}^+$ ) is small compared to the transmembrane movement of  $\text{CHBr}$  over the pH range of 8.0 to 4.0. In the Appendix we derive an expression for the  $\text{Br}^-$  exchange flux as a function of the Amberlite,  $\text{H}^+$ , and  $\text{Br}^-$  concentrations. Then, from the data in Figs. 1 and 3, we derive estimates of the dissociation constants,  $[\text{C}][\text{H}^+]/[\text{CH}^+]$  and  $[\text{CH}^+][\text{Br}^-]/[\text{CHBr}]$ , which can be used to predict some of the permeability properties of these anion selective bilayers.

Although the permeability properties of lecithin-Amberlite bilayers are dominated by the enormous anion exchange fluxes, the conductance increase caused by Amberlite is also of interest. As pointed out by Sandblom and Orme (1972), in a liquid ion exchange membrane the mechanisms of ionic exchange and ionic conductance are basically different and may show very different selectivities. A model which describes qualitatively both the ionic exchange and ionic conductance properties of lecithin-Amberlite bilayers is shown schematically in Fig. 5.

According to this model, anion exchange is mediated by the neutral complex, CHBr, whereas the transmembrane current is carried by CH<sup>+</sup>.

The model shown in Fig. 5 can explain qualitatively both the electrically neutral Br<sup>-</sup> exchange and the observed H<sup>+</sup> selectivity in Br<sup>-</sup>-free solutions. According to this model, the H<sup>+</sup> conductance in halide-free solutions is analogous to the K<sup>+</sup> conductance induced by neutral carriers such as valinomycin (Lauger, 1972). However, to explain the Br<sup>-</sup>-dependent conductance and Br<sup>-</sup> selectivity seen in Figs. 1 and 2, we must assume that (a) CHBr crosses the membrane faster than CH<sup>+</sup>, and (b) the formation of CHBr is faster than the formation of CH<sup>+</sup> (see also Appendix). If these assumptions are correct, then the membrane conductance is Br<sup>-</sup>-dependent because Br<sup>-</sup> provides a parallel pathway for the transmembrane movement of CH<sup>+</sup>. For example, when a constant transmembrane voltage is imposed under symmetrical conditions, net charge transport through the membrane is carried by CH<sup>+</sup>. However, the net steady-state ion flux through the membrane is mainly Br<sup>-</sup>, because most of the CH<sup>+</sup> recycles via the lower pathway in Fig. 5. Stated in another way, if the

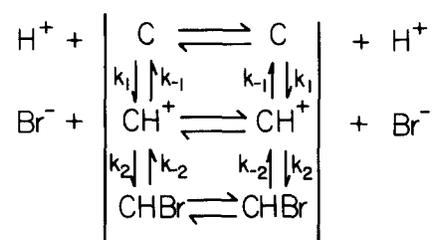


FIGURE 5. Model for ionic exchange and conductance through lecithin-Amberlite bilayers. See text for details.

carrier cycled preferentially via the upper pathway in Fig. 5, then the conductance would depend on [H<sup>+</sup>] but not on [Br<sup>-</sup>]. According to this model, under open-circuit conditions a transmembrane Br<sup>-</sup> gradient causes a net flow of CHBr through the membrane with the consequent creation of an oppositely directed gradient of CH<sup>+</sup>, some of which moves back across the membrane and generates a Br<sup>-</sup> diffusion potential. Finally, the absence of H<sup>+</sup> selectivity in high Br<sup>-</sup> solutions can be explained by a rapid transmembrane movement of CHBr, which tends to equalize the CH<sup>+</sup> concentrations on either side of the membrane in spite of the transmembrane pH gradient.

#### *Comparison between Lecithin-Amberlite Bilayers and Red Cell Membranes*

The similarities between anion transport in erythrocyte membranes and liquid ion exchange membranes were first pointed out by Wieth (1972). Gunn (1972) extended this comparison and developed a "titratable carrier" model for anion exchange through the red cell membrane. Table IV compares by orders of magnitudes some permeability properties of lecithin-Amberlite bilayers and red cell membranes. In both types of membranes the anion exchange fluxes are enormous and >99% electrically silent. In both membranes the halide/Na<sup>+</sup> and halide/SO<sub>4</sub><sup>=</sup> tracer permeability ratios are very high. In both membranes the

conductance is very low, and the permselectivity of the conductance pathway ( $G_{\text{halide}}/G_{\text{Na}}$ ) is lower than the halide/ $\text{Na}^+$  tracer permeability ratio. Furthermore, the apparent turnover number for Amberlite LA-2 in bilayers may be of the same order of magnitude as that for the red cell anion carrier (see Appendix). Finally, the urea permeability of red cell membranes is about 100-fold higher than that of lecithin-Amberlite bilayers, which probably reflects the

TABLE IV  
COMPARISON BETWEEN THE PASSIVE PERMEABILITY PROPERTIES OF  
LECITHIN-AMBERLITE BILAYERS AND MAMMALIAN RED CELL  
MEMBRANES

Transport property	Lecithin-Amberlite bi-layer (pH ca. 6)	Mammalian red cell membrane (pH ca. 7)	Reference for red cell data
Halide permeability of the exchange pathway ( $\text{cm} \cdot \text{s}^{-1}$ )	$10^{-4}$	$10^{-4}$	1
Halide permeability of the conductance pathway ( $\text{cm} \cdot \text{s}^{-1}$ )	$10^{-8}$	$10^{-8}$	2-4
Halide/sodium tracer permeability ratio ( $P_{\text{halide}}/P_{\text{Na}}$ )	$>10^4$	$10^6$	2,6
Halide/sulfate tracer permeability ratio ( $P_{\text{halide}}/P_{\text{SO}_4}$ )	$10^4$	$10^4$	5
Total membrane conductance (mho $\text{cm}^{-2}$ )	$10^{-6}$	$10^{-6}$	2
Permselectivity of conductance pathway ( $G_{\text{halide}}/G_{\text{Na}}$ )	$>10^1$	$10^2$	2-4,6
Turnover number for anion carrier ( $\text{s}^{-1}$ )	$>10^3$	$10^4$	7
Halide concentration at one-half maximal flux (M)	$>10^{-1}$	$10^{-1}$	8
Urea permeability ( $\text{cm} \cdot \text{s}^{-1}$ )	$10^{-6}$	$10^{-4}$	9

References and experimental temperatures for the red cell data are as follows:

<sup>1</sup> Tosteson (1959), 23°C.

<sup>2</sup> Tosteson et al. (1973), 37°C.

<sup>3</sup> Hunter (1976), 32-37°C.

<sup>4</sup> Knauf et al. (1977), 37°C.

<sup>5</sup> Schnell et al. (1973), 0°C.

<sup>6</sup> Wieth (1972), 10-18°C.

<sup>7</sup> Brahm (1977), 25-38°C.

<sup>8</sup> Brazy and Gunn (1976), 0°C.

<sup>9</sup> Sha'afi et al. (1971), 24°C.

Because most of the listed parameters are highly pH and temperature dependent, our comparison is based on orders of magnitude only.

presence of a polar pathway through the red cell membrane. If the titratable carrier model shown in Fig. 5 is correct, it should also be possible to demonstrate cotransport of protons and anions as was shown recently in red cells by Jennings (1976).

Of course, the red-cell anion "carrier" and Amberlite LA-2 differ in many important respects. First, anion exchange in red cells is mediated by a protein, not a lipid (see Rothstein et al., 1976). Although this protein may contain a mobile component which facilitates anion exchange through a hydrophobic

barrier, the rotational movement of the entire protein molecule is much too slow to account for the rate of anion transport (Cherry et al., 1976). Second, Amberlite LA-2 behaves as a monovalent titratable carrier, whereas the red cell anion carrier appears to change from monovalent to divalent as pH decreases (Gunn, 1972; Jennings, 1976). A third difference is the apparent lack of saturation kinetics in Amberlite-mediated  $\text{Br}^-$  exchange (Fig. 4). In red cells,  $\text{Cl}^-$  exchange has a  $K_{1/2}$  of about 65 mM (Brazy and Gunn, 1976). As we discuss in the Appendix, the absence of saturation kinetics for  $\text{Br}^-$  transport through lecithin-Amberlite bilayers at neutral pH can be explained mainly by a large reservoir (>99%) of nonfunctional carriers (C) which can be converted by mass action to functional carriers ( $\text{CH}^+$ ) as the  $\text{Br}^-$  concentration increases.

A fourth difference between lecithin-Amberlite bilayers and red cell membranes is the effect of the aromatic anion, trinitrocresolate ( $\text{TNC}^-$ ). At neutral pH,  $\text{TNC}^-$  is a potent, reversible inhibitor of anion exchange in red cells (Gunn and Tosteson, 1971) but not in lecithin-Amberlite bilayers (Fig. 3). Presumably,  $\text{TNC}^-$  inhibits anion exchange in red cells by binding to at least one of the positively charged anion transport sites, which have apparent pKs of >11 and ca. 6.2 (Funder and Wieth, 1976). However,  $\text{TNC}^-$  and other aromatic anions are also known to induce negative surface potentials in lipid bilayers (Ginsburg and Stark, 1976). In erythrocytes the three main effects of  $\text{TNC}^-$  would all be inhibitory to halide exchange, i.e., binding directly to the transport site as well as decreasing the pH and decreasing the halide concentration near the transport site. In lecithin-Amberlite bilayers, however, any decrease in surface potential caused by  $\text{TNC}^-$  would have two opposing effects on  $\text{Br}^-$  transport at neutral pH, because the  $\text{Br}^-$  flux is proportional to both  $[\text{Br}^-]$  and  $[\text{H}^+]$  (Figs. 3 and 4). Furthermore, the binding of  $\text{TNC}^-$  to the functional carrier ( $\text{CH}^+$ ) will be largely compensated by the formation of additional  $\text{CH}^+$ , since the membrane contains a reservoir of uncomplexed carrier (C) at neutral pH (see also Appendix).

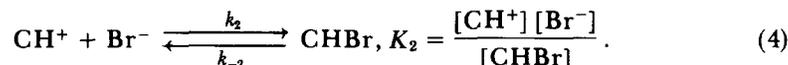
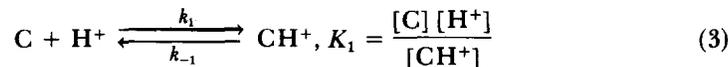
#### *Conclusion*

In conclusion, we have shown that in lipid bilayers the long-chain secondary amine, Amberlite LA-2, induces a rapid anion exchange which resembles in some respects the anion exchange through erythrocyte membranes. Although this hydrophobic amine is obviously not the anion carrier in any biological membrane, comparative studies of this sort may provide insights into the nature of the molecules or functional groups responsible for ion transport through biological membranes. A variety of other hydrophobic amines, both synthetic and natural, offer interesting prospects for future studies. For example, our preliminary experiments with spermine and tridodecylamine show that both are capable of facilitating rapid anion exchanges through lipid bilayer membranes (Graves, unpublished). Finally, liquid ion exchangers such as Amberlite LA-2 might be incorporated into biological membranes by means of fusion with lipid vesicles. If so, the resulting altered cell membranes might show some interesting transport properties.

## APPENDIX

*Br<sup>-</sup> Exchange Flux as a Function of Amberlite Concentration, Br<sup>-</sup> Concentration, and pH*

Amberlite LA-2, a secondary amine, exists in three chemical forms (C, CH<sup>+</sup>, and CHBr) which are related by the following reversible reactions and dissociation constants:



If we assume that Amberlite molecules behave as monomers, then the total Amberlite concentration in the membrane is given by

$$[C_{tot}] = [C] + [CH^+] + [CHBr]. \quad (5)$$

Since the observed Br<sup>-</sup> exchange flux ( $J_{Br}$ ) is >99% electrically silent, we assume that all of the flux is carrier mediated, i.e., that Br<sup>-</sup> crosses the membrane only as the neutral complex, CHBr. We also assume symmetrical conditions and that the rate of Br<sup>-</sup> exchange is limited by the mobility of the neutral complex in the membrane. Thus,

$$J_{Br} = P_{CHBr} [CHBr], \quad (6)$$

where  $P_{CHBr}$  is the membrane permeability coefficient for CHBr.

Solving Eq. 3 for [C], solving Eq. 4 for [CH<sup>+</sup>], and substituting into Eq. 5 gives

$$[C_{tot}] = [CHBr] \left( \frac{K_1 K_2}{[H^+][Br^-]} + \frac{K_2}{[Br^-]} + 1 \right). \quad (7)$$

Solving Eq. 6 for [CHBr], substituting into Eq. 7, and rearranging gives

$$\frac{1}{J_{Br}} = \frac{1}{P_{CHBr}[C_{tot}]} \left( \frac{K_1 K_2}{[H^+][Br^-]} + \frac{K_2}{[Br^-]} + 1 \right), \quad (8)$$

which contains four unknowns, i.e.,  $P_{CHBr}$ ,  $[C_{tot}]$ ,  $K_1$  and  $K_2$ . Although none of these is directly measurable in our system, we can make some order-of-magnitude estimates from the data shown in Figs. 1-3.

Fig. 2 shows that when pH = 6,  $G_m$  is about  $10^{-2}$  times its maximum value. If we then assume that the increase in  $G_m$  reflects a proportional increase in [CH<sup>+</sup>], we can estimate that  $[CH^+]/[C_{tot}] \leq 10^{-2}$  at pH 6. This is probably an upper limit since the increasing membrane surface charge at low pH will tend to reduce [CH<sup>+</sup>]. Fig. 3 (curve B) shows that when pH = 6,  $J_{Br}$  is about  $10^{-2}$  times its maximum value. Therefore, we estimate that when pH = 6,  $[CHBr]/[C_{tot}] \leq 10^{-2}$ . We regard this as an upper limit because increasing the [Br<sup>-</sup>] at low pH would probably increase the apparent  $J_{max}$  which we obtain from curve B, and this would decrease our estimate of  $[CHBr]/[C_{tot}]$  at pH 6. Nevertheless, at pH

6,  $[\text{CH}^+]$  and  $[\text{CHBr}]$  are both small and thus  $[\text{C}_{\text{tot}}] \approx [\text{C}]$ . Therefore,  $[\text{CH}^+]/[\text{C}] \leq 10^{-2}$  and  $[\text{CHBr}]/[\text{CH}^+] \leq 10^0$ . Inserting these values into Eqs. 3 and 4 yields  $K_1 \geq 10^{-7} \text{ mol} \cdot \text{cm}^{-3}$  and  $K_2 \geq 10^{-4} \text{ mol} \cdot \text{cm}^{-3}$ .

To estimate  $[\text{C}_{\text{tot}}]$  we assume that the bilayer has the same mole ratio of lecithin:Amberlite as the membrane-forming solution, i.e., 0.5, and we assume also that the volume occupied by decane in the bilayer is small compared to that of lecithin and Amberlite. These assumptions lead to an estimate for  $[\text{C}_{\text{tot}}]$  of about  $10^{-3} \text{ mol} \cdot \text{cm}^{-3}$ .

An estimate of  $P_{\text{CHBr}}$  can be obtained by assuming, as before, that at pH 6,  $[\text{CHBr}]/[\text{C}_{\text{tot}}] \leq 10^{-2}$ . Thus, at pH 6,  $[\text{CHBr}] \leq 10^{-5} \text{ mol} \cdot \text{cm}^{-3}$ . Inserting this value and the observed  $J_{\text{Br}}$  at pH 6 into Eq. 6 yields  $P_{\text{CHBr}} \geq 10^{-3} \text{ cm} \cdot \text{s}^{-1}$ . An apparent turnover number for the  $\text{Br}^-$  carrier is obtained by dividing  $P_{\text{CHBr}}$  by the membrane thickness, ca.  $5 \times 10^{-7} \text{ cm}$  (White, 1975) and multiplying by two. This yields a value  $\geq 4 \times 10^3 \text{ s}^{-1}$ , which is comparable to the turnover numbers for valinomycin in lipid bilayers and the anion carrier in red cells, both about  $10^4 \text{ s}^{-1}$  at  $25^\circ\text{C}$  (Lauger, 1972; Brahm, 1977).

An estimate of  $P_{\text{CH}^+}$  can be obtained by assuming that the membrane conductance (ca.  $10^{-6} \text{ mho} \cdot \text{cm}^{-2}$  at pH 6) is limited by the mobility of  $\text{CH}^+$  in the membrane. Using Eq. 2 and the lower limit of  $[\text{CH}^+]$  obtained above, we get  $P_{\text{CH}^+} \geq 10^{-8} \text{ cm} \cdot \text{s}^{-1}$ , five orders of magnitude smaller than  $P_{\text{CHBr}}$ .

Using the above limiting values of  $K_1$ ,  $K_2$ ,  $[\text{C}_{\text{tot}}]$  and  $P_{\text{CHBr}}$ , we have calculated  $J_{\text{Br}}$  as a function of pH and  $[\text{Br}^-]$ , using Eq. 8. The results are shown in Figs. 3 and 4 (dashed lines). Our model does not take into account the diffusional resistance of the unstirred layers, and thus the calculated flux in Fig. 3 (curve A) saturates at a value above the experimental flux. Although the agreement with the data is otherwise satisfactory, the properties which are successfully predicted by the model (i.e., an exchange flux which is proportional to  $[\text{H}^+]$  but saturates at high  $[\text{H}^+]$ , and the lack of saturation of the exchange flux with increasing  $\text{Br}^-$  at neutral pH) could probably be explained by models other than the one presented. Furthermore, as pointed out previously, our model does not explain the residual exchange flux at high pH (Fig. 3). Finally, our model does not explain the greater than ten-fold difference between curves A and B in Fig. 3, which could be caused by the formation of complexes containing more than one Amberlite molecule. Flux measurements with varying mol ratios of Amberlite, lecithin and decane are needed to resolve this question.

The lack of saturation kinetics which is observed and predicted by the model (Fig. 4) is due mainly to the large value of  $K_1$ , i.e.,  $\geq 10^{-7} \text{ mol} \cdot \text{cm}^{-3}$  or  $\geq 10^{-4} \text{ M}$ . This is equivalent to a surprisingly low  $\text{pK}_a$  of  $\leq 4.0$  for the dissociation of  $\text{CH}^+$  (Eq. 3). However, the true  $\text{pK}_a$  of Amberlite is probably much higher than 4.0 for two reasons. First, the  $\text{H}^+$  concentration at the membrane surface is lower than that in the bulk solution due to the net positive surface charge. Second, due to the hydrophobic nature of Amberlite, probably only a small fraction of the total uncomplexed molecules (C) are exposed to the aqueous phase and are thus available to react with  $\text{H}^+$ . If so, this could have two consequences. First, the value of  $[\text{C}]$  which we use to calculate  $K_1$  would be too high and we would thus underestimate the true  $\text{pK}_a$  of the amine. Second, a low concentration of C at the membrane-water interface could limit the rate of formation of  $\text{CH}^+$ , and

this could explain why the carrier seems to recycle preferentially via the lower pathway in Fig. 5.

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#### REFERENCES

- 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**:1729-1745.
- 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**:238-252.
- BRAHM, J. 1977. Temperature-dependent changes of chloride transport kinetics in human red cells. *J. Gen. Physiol.* **70**:283-306.
- BRAZY, P. C., and R. B. GUNN. 1976. Furosemide inhibition of chloride transport in human red blood cells. *J. Gen. Physiol.* **68**:583-599.
- CHERRY, R. J., A. BURKLI, M. BUSSLINGER, G. SCHNEIDER, and G. R. PARISH. 1976. Rotational diffusion of band 3 proteins in the human erythrocyte membrane. *Nature (Lond.)*. **263**:389-393.
- FUNDER, J., and J. O. WIETH. 1976. Chloride transport in human erythrocytes and ghosts: a quantitative comparison. *J. Physiol. (Lond.)*. **262**:679-698.
- GINSBURG, H., and G. STARK. 1976. Facilitated transport of di- and trinitrophenolate ions across lipid membranes by valinomycin and nonactin. *Biochim. Biophys. Acta.* **355**:685-700.
- GUNN, R. B. 1972. A titratable carrier model for both mono- and divalent anion transport in human red blood cells. In *Oxygen Affinity of Hemoglobin and Red Cell Acid-Base Status*. M. Rørth and P. Astrup, editors. Munksgaard, Copenhagen. 823-827.
- GUNN, R. B., and D. C. TOSTESON. 1971. The effect of 2,4,6-trinitro-m-cresol on cation and anion transport in sheep red blood cells. *J. Gen. Physiol.* **57**:593-609.
- 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**:359-374.
- GUTKNECHT, J., and D. C. TOSTESON. 1973. Diffusion of weak acids across lipid bilayer membranes: effects of chemical reactions in the unstirred layers. *Science (Wash. D. C.)*. **182**:1258-1261.
- GUTKNECHT, J., L. J. BRUNER, and D. C. TOSTESON. 1972. The permeability of thin lipid membranes to bromide and bromine. *J. Gen. Physiol.* **59**:486-502.
- HAUSER, H., M. C. PHILIPS, and M. STUBBS. 1972. Ion permeability of phospholipid bilayers. *Nature (Lond.)*. **239**:342-344.
- HODGKIN, A. L. 1951. The ionic basis of electrical activity in nerve and muscle. *Biol. Rev. Camb. Philos. Soc.* **26**:339-365.
- HUNTER, M. J. 1976. Human erythrocyte anion permeabilities measured under conditions of net charge transfer. *J. Physiol. (Lond.)*. **268**:35-49.
- JENNINGS, M. L. 1976. Proton fluxes associated with erythrocyte membrane anion exchange. *J. Membr. Biol.* **28**:187-205.
- KNAUF, P. A., G. F. FUHRMANN, S. ROTHSTEIN, and A. ROTHSTEIN. 1977. The

- relationship between anion exchange and net anion flow across the human red blood cell membrane. *J. Gen. Physiol.* **69**:363-386.
- KUIPER, P. J. C. 1968. Ion transport characteristics of grape root lipids in relation to chloride transport. *Plant Physiol. (Bethesda)*. **43**:1372-1374.
- LAUGER, P. 1972. Carrier-mediated ion transport. *Science (Wash. D. C.)*. **178**:24-30.
- MUELLER, P., and D. O. RUDIN. 1969. Translocators in bimolecular lipid membranes: their role in dissipative and conservative bioenergy transductions. *Curr. Top. Bioenerg.* **3**:157-249.
- NICHOLLS, P., and N. MILLER. 1974. Chloride diffusion from liposomes. *Biochim. Biophys. Acta.* **356**:184-198.
- PAGANO, R., and T. E. THOMPSON. 1968. Spherical lipid bilayer membranes: electrical and isotopic studies of ion permeability. *J. Mol. Biol.* **38**:41-57.
- PAPAHADJOPOULOS, D. 1968. Surface properties of acidic phospholipids: interaction of monolayers and hydrated liquid crystals with uni- and bivalent ions. *Biochim. Biophys. Acta.* **163**:240-254.
- PAPAHADJOPOULOS, D., and J. C. WATKINS. 1967. Phospholipid model membranes. II. Permeability properties of hydrated liquid crystals. *Biochim. Biophys. Acta.* **135**:639-652.
- REDWOOD, W. R., F. R. PFEIFFER, J. A. WEISBACH, and T. E. THOMPSON. 1971. Physical properties of bilayer membranes formed from a synthetic saturated phospholipid in n-decane. *Biochim. Biophys. Acta.* **233**:1-6.
- ROBERTSON, R. N., and T. E. THOMPSON. 1977. The function of phospholipid polar groups in membranes. *FEBS Fed. Eur. Biochem. Soc. Lett.* **76**:16-19.
- ROTHSTEIN, A., Z. I. CABANTCHIK, and P. KNAUF. 1976. Mechanism of anion transport in red blood cells: role of membrane proteins. *Fed. Proc.* **35**:3-10.
- SANDBLOM, J., and F. ORME. 1972. Liquid membranes as electrodes and biological models. In: *Membranes: a Series of Advances*. G. Eisenman, editor. Marcel Dekker, Inc., New York. **1**:125-177.
- SCHNELL, K. F., S. GERHARDT, and H. PASSOW. 1973. Asymmetric inhibition by phlorizin of halide movements across the red blood cell membrane. *Biochim. Biophys. Acta.* **318**:474-477.
- SHA'AFI, R. I., C. M. GARY-BOBO, and A. K. SOLOMON. 1971. Permeability of red cell membranes to small hydrophilic and lipophilic solutes. *J. Gen. Physiol.* **58**:238-258.
- SHEAN, G. M., and K. SOLLNER. 1966. Carrier mechanisms in the movement of ions across porous and liquid ion exchanger membranes. *Ann. N. Y. Acad. Sci.* **137**:759-776.
- STECK, T. L. 1974. The organization of proteins in the human red blood cell membrane. *J. Cell Biol.* **62**:1-19.
- TOSTESON, D. C. 1959. Halide transport in red blood cells. *Acta Physiol. Scand.* **46**:19-41.
- TOSTESON, D. C., R. B. GUNN, and J. O. WIETH. 1973. Chloride and hydroxyl ion conductance of sheep red cell membrane. In *Erythrocytes, Thrombocytes, Leukocytes*. E. Gerlach, K. Moser, and E. Deutch, editors. Georg Thieme Verlag KG., Stuttgart. 62-66.
- TOYOSHIMA, Y., and T. E. THOMPSON. 1975*a*. Chloride flux in bilayer membranes: the electrically silent chloride flux in semispherical bilayers. *Biochemistry.* **14**:1518-1524.
- TOYOSHIMA, Y., and T. E. THOMPSON. 1975*b*. Chloride flux in bilayer membranes: chloride permeability in aqueous dispersions of single-walled bilayer vesicles. *Biochemistry.* **14**:1525-1531.
- WHITE, S. H. 1975. Phase transitions in planar bilayer membranes. *Biophys. J.* **15**:95-117.
- WIETH, J. O. 1972. The selective ionic permeability of the red cell membrane. In: *Oxygen Affinity of Hemoglobin and Red Cell Acid-Base Status*. M. Rørth and P. Astrup, editors. Munksgaard, Copenhagen. 265-278.