Mechanism of Cholesterol Gallstone Dissolution

I. The Determination of the Binding of Alkyl Amines to Bile Micelles Using Dynamic Membrane Transport Methods[1]

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Recent studies have shown that cholesterol monohydrate pellet dissolution rates in real and synthetic bile systems are enhanced dramatically in the presence of small concentrations of amines (primary, secondary, and tertiary), quaternary ammonium compounds, and cationic surfactants. These studies have shown that, in general, chain length, the degree of hydrophobicity, the ability to alter micellar charge, as well as the steric or structural features of these molecules seem to be important. In all of the past studies, however, the situations have been complicated by the fact that the accelerator molecules in question were bound to varying degrees to the micelles and other components of the synthetic bile. Meaningful structure activity relationships can only be considered when the concentrations of bound and unbound drug are known. A dynamic cellulose membrane dialysis technique was used; in evaluating the validity of the technique for the present purposes the following factors were considered: (a) the possible influence of membrane charge on dialysis, (b) the possible influence of bile acid-lecithin micelles on dialysis rate, (c) the possible influence of pore size and molecular structure on dialysis rate, and finally (d) the influence of Donnan membrane effects. Some of the results obtained using this technique were compared to the results obtained using the silicone rubber membrane system. There is very good agreement between the data obtained using the two methods. Thus, the good agreement underscores the validity of either method and rules out, for example, the possibility that an amine–chenodeoxycholate complex is being transported across either or both membranes.

INTRODUCTION

Recent studies in our laboratories (1–3) have shown that cholesterol monohydrate pellet dissolution rates in real and synthetic bile systems are enhanced dramatically in presence of small concentrations of amines (primary, secondary, and tertiary), quaternary ammonium compounds, and cationic surfactants. These studies have shown that, in general, chain length, the degree of hydrophobicity, the ability to alter the micellar charge, as well as the steric or structural features of these molecules seem to be important. Based on our observations, the fol-

lowing mechanisms may be proposed: (i) the adsorption of the charged form of the accelerator on the cholesterol crystal surface helps reduce the electrical repulsion between the negatively charged cholesterol surface and the negatively charged micelle; (ii) the long chain cations reduce the net negative charge on the micelle by formation of mixed micelles in bile thus facilitating the close approach of the micelle to the charged surface; (iii) alteration of the hydrophobicity of the micelle and/or the interface by the long chain cations; and (iv) some mechanism by which the unbound accelerator directly influences the rate-limiting step. In all of the past studies, however, the situations have been complicated by the fact that the accelerator molecules in ques-

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tion were bound to varying degrees to the micelles and other components of the synthetic bile. Meaningful structure–activity relationships can only be considered when the concentrations of bound and unbound drug are known. Thus appropriate methods need to be developed for determining free and bound accelerator molecules in bile and simulated bile solutions before further progress in the understanding of the mechanism of action of the cholesterol gallstone dissolution rate accelerators can be achieved.

The purpose of this study was to develop and to utilize dynamic membrane transport methods to determine the degree of bound and unbound accelerator in simulated bile. Mysels (4) has recently reviewed how micellar solubilization decreases the dialysis of a micellar solute. In the present work, a dynamic cellulose membrane dialysis technique was used and some of the results obtained were compared to the results obtained using the silicone rubber membrane system (5). Although each of these techniques are useful alone, there are situations where the simultaneous use of both methods is merited. Dimerization and self-association of free drug monomers can be determined separately in the absence of micelles; however, in the presence of micelles, e.g., in micellar cholate–amine systems, the possible transport of species such as the 1:1 complex between cholate and the protonated amine cannot be factored out easily using only one of the methods. However, if both methods are employed and the results are in quantitative agreement, the fraction free of these accelerators could be determined with great assurance.

CONSIDERATIONS IN THE DESIGN OF EXPERIMENTS

Dialysis rates of sodium benzoate, tetraethylammonium bromide, hexylamine, octylamine, nonyltrimethylammonium bromide, decyltrimethylammonium bromide, and dodecyltrimethylammonium bromide were to be studied with or without sodium

cholate and lecithin. The diffusion coefficients of these compounds were to be determined. By comparing the rates of benzoate ions (negatively charged) and those of tetraethylammonium bromide ions (positively charged) it was hoped that the possible influence of membrane charge on dialysis rate could be ascertained. Since the benzoate ions and tetraethylammonium bromide ions were not expected to bind to the bile acid-lecithin micelles, the possible direct influence of the presence of these micelles on dialysis rates could also be evaluated. By comparing the dialysis rates and the diffusion coefficients it was hoped that the possible influences of pore size and molecular structure could be determined. Donnan membrane equilibrium effects were to be studied by measuring dialysis rates of sodium benzoate with and without bile acid-lecithin micelles at high and low salt concentrations.

The cellulose membrane dialysis method for determining solute binding was to be compared to the silicone rubber membrane method using hexylamine and octylamine in chenodeoxycholic acid solutions. In the silicone rubber membrane system, the micellar solubilization of hexylamine and octylamine was to be studied between pH 9.0 and 9.75 for hexylamine and between pH 8.0 and 8.75 for octylamine where the aqueous diffusion layer effects are not important (5).

MATERIALS

Chemicals

Sodium benzoate,[2] tetraethylammonium bromide,[3] n-hexylamine,[3] n-octylamine,[4] nonyltrimethylammonium bromide,[3] decyltrimethylammonium bromide,[3] dodecyltrimethylammonium chloride,[5] sodium chloride,[6] picric acid,[7] methylene chloride,[6]

sodium phosphate[6] (monobasic and dibasic) were reagent grade and were used as received. Egg lecithin was prepared from fresh egg yolks and subsequently stored, according to the method of Singleton *et al.* (6). Chromatographically homogeneous lecithin was obtained. Cholic acid[8] was recrystallized from 95% ethanol. Sodium cholate solution was prepared by titrating equivalent amounts of cholic acid with sodium hydroxide. Chenodeoxycholic acid[9] was recrystallized from *n*-propanol-ethyl acetate mixture. Sodium chenodeoxycholate solution was prepared by titrating equivalent amounts of acid with sodium hydroxide.

Dialysis bags[10]. Suitable size bags were cut and boiled in double distilled water three times for 30 min each. The bags were then soaked overnight in buffer solution at pH and electrolyte levels to be used in the experiment.

Silver membranes[11]. Silver membranes were of 94 μm thickness with standard pore size of 0.45 μm.

Silicone rubber membranes. Silastic membranes[12] used in this study were previously described (5).

Apparatus

a. Dialysis. Figure 1 shows a schematic diagram of the apparatus used for the dialysis studies. The setup consisted of a water jacketed beaker and dialysis bag holder-stirring assembly. The temperature of the system was maintained at 37°C by circulating water. A 150 rpm synchronous motor and the dialysis bag holder were mounted on a polyethylene cover plate. The dialysis bag holder consisted of seven $1/16''$ gauge stainless-steel wires attached to a circular stainless-steel wire at the bottom, forming a cylindrical framework for the dialysis bag.

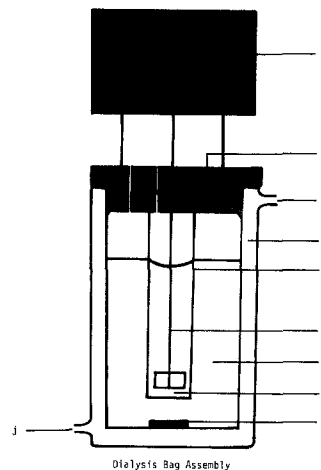[8] Schwarz/Mann, Orangeburg, N. Y.

[9] Canada Packers Ltd., Canada.

[10] Spectrum Medical Industries Inc., Los Angeles, Calif.

[11] Selas Flotronics, Huntington Valley, Pa.

[12] Dow Corning Corp., Midland, Mi.

Dialysis Bag Assembly

Key: a, synchronous motor
b, polyethylene cover
c, water outlet
d, jacketed glass beaker
e, cellophane dialysis bag and framework
f, Teflon[3] stirring paddle
g, receiver solution
h, donor solution
i, stirring bar
j, water inlet

FIG. 1. Diagramatic representation of the dialysis bag assembly.

The stirrer was made from Teflon[13]. Three holes were drilled in the polyethylene cover plate, one for the stirrer in the center of the dialysis bag holder and the other two for sampling from inside and outside the bag. The water jacketed beaker contained 100 ml of receiver solution which was stirred by a long Teflon magnetic stirring bar by means of another synchronous motor at 150 rpm placed at the bottom of the jacketed beaker.

b. Diffusion. The small-volume Diaphragm Cell Method was used (7, 8). The apparatus (Fig. 2) consisted of two well-stirred reservoirs separated by two silver filter membranes. The silver membranes were boiled in distilled water prior to assembly for about 30 min in order to remove all the entrapped air.

c. Silicone rubber membrane permeation cell. The apparatus used for determining the permeation rate was previously described (5). The diffusion cell consisted of two cylindrical half-cells with inside diameters of about 1.5 cm and a depth of about 5 cm.

[13] Registered trademark of E. I. dePont de Nemours and Company, Inc., Wilmington, De.

Synchronous motor

Cover

Stirring paddle
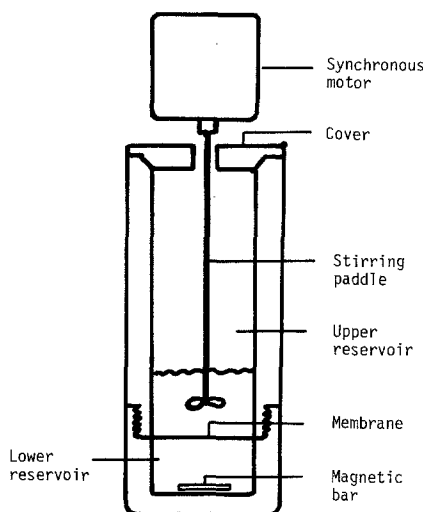
Upper reservoir

Membrane

Lower reservoir

Magnetic bar

FIG. 2. Diagramatic representation of the small-volume diaphragm diffusion cell.

This gave a half-cell volume of about 9 ml. The cell halves were assembled, with the silicone rubber membrane between half-cells using Teflon connection supports. The entire cell was immersed in a constant temperature bath. The cell contents were stirred by small stirrers on shafts driven at 150 rpm by a constant speed motor.

PROCEDURES

a. Dialysis Rate Determination

One hundred milliliters of the receiver solution was placed in the water jacketed beaker and 10 ml of the donor solution, previously equilibrated at 37°C, was placed inside the presoaked dialysis bag. Immediately, the bag with its contents was immersed in the receiver solution and stirring was started. At different time intervals, 5-ml samples were withdrawn from the outside compartment for the diffusate analysis. The receiver solution consisted of 0.05–0.5 M sodium chloride in 0.01 M phosphate buffer (pH 7.4). The donor solution consisted of 5 mM solute, 0.05–0.5 M sodium chloride, and 0.01–0.1 M phosphate buffer (pH 7.4) with or without bile acid and lecithin.

Sodium benzoate was assayed spectro-

photometrically at a wavelength of 225 nm. The amine assay procedure (9, 10) involved complexation of the amine with picric acid. The concentration of this picrate complex was determined spectrophotometrically. Five milliliters of receiver solution was shaken in a separatory funnel with two successive portions of 10 ml and 15 ml of a solution of 0.2% picric acid in methylene chloride. The absorbance of these solutions was determined spectrophotometrically at a wavelength of 420 nm.[14] Picric acid was used as the reagent for assaying the amines, because it has a strong spectrum and a single strong anionic functional group that has been shown to react quickly and quantitatively with the base. It is a very sensitive method, and allows the determination of small amounts of amines.

b. Diffusion Coefficient Determination

The lower reservoir, which has a volume of 3.4 ml, was filled in excess with the donor solution (i.e., the solution containing the diffusing species). The silver membranes were soaked for 2 hr in the donor solution and the membranes, held between two Teflon gaskets, were then inserted on top of the lower reservoir. The upper portion was screwed firmly in place and the apparatus was then mounted vertically and was allowed to equilibrate in a constant temperature bath at 37°C. Excess donor solution was then siphoned off and the upper reservoir was washed three times with the solvent medium. The upper reservoir was then filled with 3.4 ml of this solution previously equilibrated at 37°C. The solutions in both reservoirs were stirred at 150 rpm.

At a single time, t, where $t < t_{equilibrium}$ (about 60–90 min) both solutions were assayed for the diffusing species, using the assay procedure described in "Dialysis Rate Determination."

The apparent diffusion coefficient, D, was

then calculated using

$$D = \frac{1}{\beta t} \ln \frac{(C_0^L - C_0^U)}{(C_t^L - C_t^U)} \qquad [1]$$

where

β = cell constant (cm^{-2})
t = time (seconds)
C_0^L, C_0^U = concentration of the diffusing species at $t = 0$ in the lower and upper compartments, respectively
C_t^L, C_t^U = concentration of the diffusing species at time t in lower and upper compartments, respectively.

The cell constant, β, was determined by studying the diffusion of 0.1% benzoic acid, in 0.01 M HCl at 37°C, for which the diffusion coefficient was known to be 14 × 10^{-6} cm^2/sec (11). Uncertainties in diffusion coefficient values determined using this technique was less than 15%.

c. Silicone Rubber Membrane Permeation Procedure

Nine milliliters of donor solution was placed in one half-cell and 9 ml of receiver solution was placed in the other half-cell. The receiver solution consisted of 0.1 M sodium chloride and 0.1 M phosphate buffer. The donor solution consisted of hexylamine or octylamine, 0.1 M sodium chloride in 0.1 M phosphate buffer with or without chenodeoxycholate micellar solution. The contents of each half-cell was stirred at 150 rpm. At predetermined intervals, donor and receiver solutions were totally replaced for analysis. Total time involved in the exchange of the solutions was 1–2 min. The experiment was continued until the difference between two consecutive receiver compartment concentrations was less than 5%. The average of these concentrations was then used to calculate the flux, under conditions which were essentially steady-state. The amine was analysed as described in "Dialysis Rate Determination."

Since the amine flux is directly proportional to the free amine concentration, the fraction of unbound amine can be determined from the ratio of the slopes, in presence and absence of micelles.

d. Data Treatment—Dialysis

Under sink conditions, the rate at which the solute leaves the bag is given by Eq. [2]

$$\frac{-dD_t}{dt} = KD_f \qquad [2]$$

where

$-dD_t/dt$ = rate of loss of small molecule from the dialysis sac
K = first-order rate constant which characterizes the diffusion process and which incorporates the area and thickness of the membrane
D_f = the molar concentration of unbound small molecules.

Integration of Eq. [2] from initial time t_0, when initial solute concentration is C_0 to an assigned time t, when the solute concentration is C, leads to Eq. [3]

$$\ln \frac{C}{C_0} = -Kt. \qquad [3]$$

The relationship of concentrations in terms of amounts is given by Eq. [4]

$$C = \frac{(A_b)_{t=t}}{V_b}$$

$$C_0 = \frac{(A_b)_{t=0}}{V_b} \qquad [4]$$

where

A_b = the amount of solute in the dialysis bag
V_b = the volume of solution in the dialysis bag.

Substituting Eq. [4] into Eq. [3] gives Eq. [5]

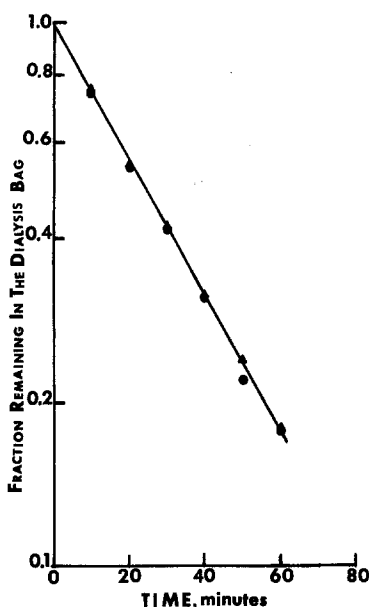$$\ln \frac{(A_b)_{t=t}}{(A_b)_{t=0}} = -Kt. \qquad [5]$$

FIG. 3. Dialysis rate of sodium benzoate. Key: ●, 6.94 mM sodium benzoate, 0.5 M sodium chloride, and 0.01 M phosphate buffer; ▲, 6.94 mM sodium benzoate, 0.05 M sodium chloride, 0.01 M phosphate buffer, 116 mM sodium cholate, and 32 mM lecithin. (The outside compartment contained 0.5 M sodium chloride in 0.01 M phosphate buffer.)

If the amount of solute in the pores of the bag is neglected, the total amount is given by Eq. [6]

$$(A_b)_{t=0} = (A_b)_{t=t} + (A_s)_{t=t} \qquad [6]$$

where

A_s = the amount in the sink.

Substituting for $(A_b)_{t=t}$ into Eq. [5] gives Eq. [7]

$$\ln \frac{(A_b)_{t=0} - (A_s)_{t=t}}{(A_b)_{t=0}} = -Kt. \qquad [7]$$

A plot of the log term in Eq. [7] vs time gives a straight line (Figs. 3–10). The slope of the straight line represents the rate constant K. In the presence of micelles Eq. [8] may be written

$$\ln \frac{(A_b)_{t=0} - (A_s)_{t=t}}{(A_b)_{t=0}} = -K_m t \qquad [8]$$

where

$K_m = K \times ff$

ff = fraction of unbound small molecule

K = rate constant in absence of micelles.

The fraction of unbound small molecule can be determined from the ratio of the slopes, in presence and absence of micelles.

$$ff = \frac{K_m}{K}. \qquad [9]$$

RESULTS AND DISCUSSION

Figures 3 and 4 show the dialysis rate data of sodium benzoate and tetraethylammonium bromide in the presence and absence of bile salt-lecithin micelles. Several points can be made with these data. As both the dialysis rates and the diffusivities are
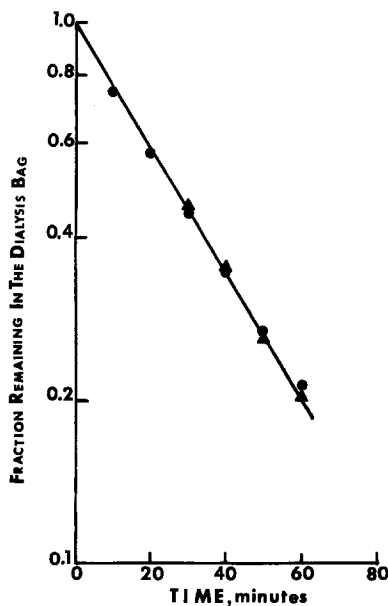

FIG. 4. Dialysis rate of tetraethylammonium bromide (TEAB). Key: ●, 5 mM TEAB, 0.5 M sodium chloride, and 0.01 M phosphate buffer; ▲, 5 mM TEAB, 0.5 M sodium chloride, 0.01 M phosphate buffer, 116 mM sodium cholate, and 32 mM lecithin. (The outside compartment contained 0.5 M sodium chloride in 0.01 M phosphate buffer.)

about the same for these two solutes (see Table II) electrical effects involving the membrane (e.g., pore charges or membrane potential gradients) are probably negligible. Since this is true both in the presence and absence of cholate-lecithin micelles, it suggests that cholate-lecithin has no significant effects upon the intrinsic permeability behavior of the dialysis membrane, either from the electrical standpoint or from the effective pore size standpoint (e.g., adsorption and partial blockage of pores). Thus, in general, fluxes obtained may be used directly in calculating the fraction free or the fraction bound of solutes unless there is significant interaction of a solute with the membrane.

Figure 5 shows the effect of electrolyte and bile salt-lecithin concentrations on the flux of sodium benzoate across the cellophane membrane. Significant Donnan membrane equilibrium effects on flux of sodium benzoate were observed in presence of high bile salt-lecithin concentrations when the electrolyte concentration was low. However, negligible Donnan membrane equilibrium effects were found at high electrolyte

FIG. 6. Dialysis rate of hexylamine. Key: ●, 5 mM hexylamine, 0.5 M sodium chloride, and 0.01 M phosphate buffer; ■, 5 mM hexylamine, 0.5 M sodium chloride, 0.01 M phosphate buffer, 11.6 mM sodium cholate, and 3.2 mM lecithin; ▲, 5 mM hexylamine, 0.5 M sodium chloride, 0.01 M phosphate buffer, 23.2 mM sodium cholate, and 6.4 mM lecithin; ●, 5 mM hexylamine, 0.5 M sodium chloride, 0.01 M phosphate buffer, 116 mM sodium cholate, and 32 mM lecithin. (The solution in the outside compartment contained 0.5 M sodium chloride in 0.01 M phosphate buffer.)

levels and/or at low bile salt-lecithin levels. These findings are consistent with expectations based upon the Donnan membrane theory (12) and define conditions under which fluxes can be a direct measure of the fraction free of the solute.

Figures 6–10 and Table I show the dialysis rate data of the amines and quaternary ammonium compounds at various concentrations of cholate-lecithin. It can be seen that in general, the dialysis rate decreased with an increase in bile salt-lecithin concentration. Since we have established (Figs. 3 and 4) that the presence of bile salt-lecithin micelles have little or no effect on the dialysis membrane, the data show that the amount of binding to the micelles increases with increasing micellar concentration. This result also shows that the amount of binding increases with increasing chain length of the amine and the quaternary.
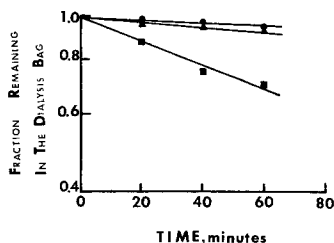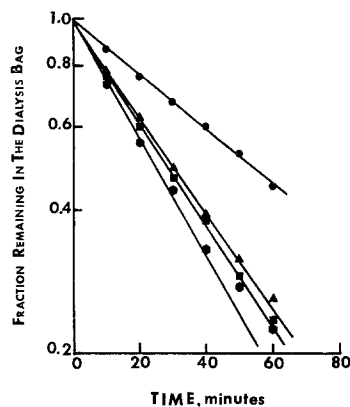
Table II shows a correlation between the

FIG. 5. Effect of sodium chloride concentration on sodium benzoate flux (Donnan equilibrium effect). Key: ●, 6.94 mM sodium benzoate, 0.05 M sodium chloride, and 0.01 M phosphate buffer; ▲, 6.94 mM sodium benzoate, 0.5 M sodium chloride, 0.01 M phosphate buffer, 116 mM sodium cholate, and 32 mM lecithin; ■, 6.94 mM sodium benzoate, 0.05 M sodium chloride, 0.01 M phosphate buffer, 116 mM sodium cholate, and 32 mM lecithin. (The outside compartment contained sodium chloride and phosphate buffer; same concentrations as inside the bag.)
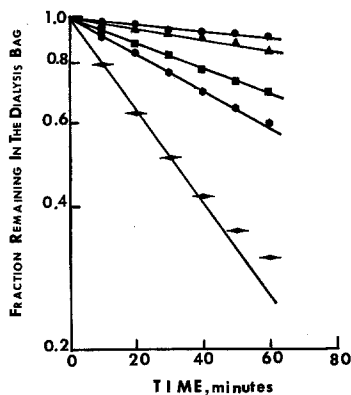
FIG. 7. Dialysis rate of octylamine. Key: ◆, 5 m*M* octylamine, 0.5 *M* sodium chloride, and 0.01 *M* phosphate buffer; ●, 5 m*M* octylamine, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 11.6 m*M* sodium cholate, and 3.2 m*M* lecithin; ■, 5 m*M* octylamine, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 23.2 m*M* sodium cholate, and 6.4 m*M* lecithin; ▲, 5 m*M* octylamine, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 58 m*M* sodium cholate, and 16 m*M* lecithin; ●, 5 m*M* octylamine, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 116 m*M* sodium cholate, and 32 m*M* lecithin. (The solution in the outside compartment contained 0.5 *M* sodium chloride in 0.01 *M* phosphate buffer.)

FIG. 9. Dialysis rate of decyltrimethylamminium bromide (DTAB). Key: ■, 5 m*M* DTAB, 0.5 *M* sodium chloride, and 0.01 *M* phosphate buffer; ▲, 5 m*M* DTAB, 0.05 *M* sodium chloride, and 3.2 m*M* lecithin; △, 5 m*M* DTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 23.2 m*M* sodium cholate, and 6.4 m*M* lecithin; ●, 5 m*M* DTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 58 m*M* sodium cholate, and 16 m*M* lecithin; ○, 5 m*M* DTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 116 m*M* sodium cholate, and 32 m*M* lecithin. (The solution in the outside compartment contained 0.5 *M* sodium chloride in 0.01 *M* phosphate buffer.)
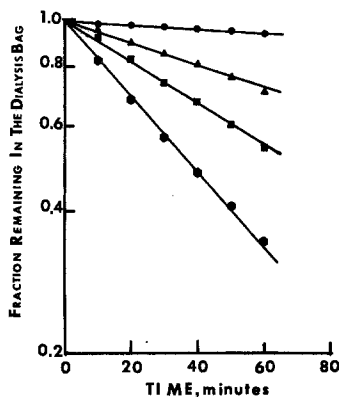

FIG. 8. Dialysis rate of nonyltrimethylammonium bromide (NTAB). Key: ●, 5 m*M* NTAB, 0.5 *M* sodium chloride, and 0.01 *M* phosphate buffer; ■ 5 m*M* NTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 11.6 m*M* sodium cholate, and 3.2 m*M* lecithin; ▲, 5 m*M* NTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 23.2 m*M* sodium cholate, and 6.4 m*M* lecithin; ●, 5 m*M* NTAB, 0.5 *M* sodium chloride, 0.01 *M* phosphate buffer, 116 m*M* sodium cholate, and 32 m*M* lecithin. (The solution in the outside compartment contained 0.5 *M* sodium chloride in 0.01 *M* phosphate buffer.)
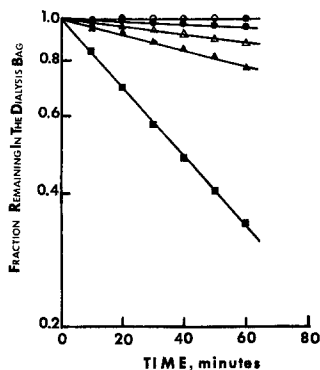
diffusivity and t_{50} (defined as the time required for 50% transport out of the dialysis bag). While the correlation is generally good, it can be seen, however, that for
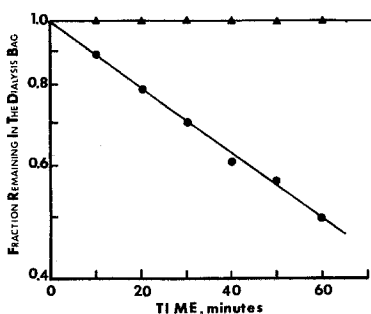
FIG. 10. Dialysis of dodecyltrimethylammonium bromide (DDTAB). Key: ●, 5 m*M* DDTAB, 0.5 *M* sodium chloride, and 0.01 *M* phosphate buffer; ▲, same as above in presence of: (11.6 m*M* sodium cholate and 3.2 m*M* lecithin) or (23.7 m*M* sodium cholate and 6.4 m*M* lecithin) or (116 m*M* sodium cholate and 32 m*M* lecithin). (The solution in the outside compartment contained 0.5 *M* sodium chloride in 0.01 *M* phosphate buffer.)

TABLE I

Influence of Micellar Concentration on Fraction
of Free Amines

Compound	Concentration (mM)		Slope (sec^{-1}) $K \times 10^3$	Fraction free
	Bile salt	Lecithin		
Octylamine	0.0	0.0	22.18	1.00
Octylamine	11.6	3.2	8.71	0.39
Octylamine	23.2	6.4	6.21	0.28
Octylamine	116.0	32.0	1.71	0.08
Hexylamine	0.0	0.0	27.75	1.00
Hexylamine	11.6	3.2	24.64	0.89
Hexylamine	23.2	6.4	23.11	0.83
Hexylamine	116.0	32.0	12.98	0.47
NTAB[a]	0.0	0.0	18.09	1.00
NTAB[a]	11.6	3.2	10.01	0.55
NTAB[a]	23.2	6.4	5.42	0.30
NTAB[a]	116.0	32.0	1.02	0.06
DTAB[b]	0.0	0.0	17.71	1.00
DTAB[b]	11.6	3.2	4.06	0.83
DTAB[b]	23.2	6.4	2.06	0.12
DTAB[b]	58.0	16.0	0.67	0.04

[a] Nonyltrimethylammonium bromide.
[b] Decyltrimethylammonium bromide.

TABLE II

Diffusivity and t_{50}[a] of Solutes

Compound	Diffusivity D (10^6 cm^2 sec^{-1})	t_{50} (min)
Hexylamine	12.49	24.44
Sodium benzoate	12.47	23.90
Tetraethylammonium bromide	11.31	25.30
Octylamine	9.88	30.08
Decyltrimethylammonium bromide	9.26	38.33
Dodecyltrimethylammonium bromide	7.39	60.00

[a] t_{50} is defined as the time required for 50% transport
out of the dialysis bag.

using the cellulose dialysis method and the
silicone rubber membrane method. There is
a very good agreement between the data ob-
tained using the two methods. These re-
sults are especially interesting in that the
predominant species being transported, in
the case of the cellulose dialysis membrane,
is the monomeric protonated amine. Thus,
the good agreement underscores the validity
of either method and rules out, for example,
the possibility that an amine-chenodeoxy-
cholate complex is being transported across
either or both membranes.

decyltrimethylammonium bromide and do-
decyltrimethylammonium bromide the t_{50} is
significantly greater than predicted by dif-
fusivity alone. This may be due to the pore
size–molecular size relationship or possi-
bly due to some effect upon the intrinsic
membrane permeability arising from an
interaction of the solutes with the mem-
brane. Such a question might be easily
answered by measuring the dialysis rate of
[^{14}C]tetraethylammonium bromide in the
presence and absence of dodecyltrimethyl-
ammonium bromide. In the case of dodecyl-
trimethylammonium bromide, it is also
possible that the critical micelle concentra-
tion (CMC) may have been exceeded (at
least at $t = 0$) as the reported CMC values
(13) for this compound under the conditions
of the present experiments are around
2.5 mM.

Table III compares the data obtained by

TABLE III

Comparison of Fraction Free of the Amine Ob-
tained by Using the Cellulose Membrane Dialysis
Method and Silicone Rubber Membrane Method

System	Fraction free	
	Silastic	Cellophane
5 mM Octylamine, 0.1 M PO$_4$, 0.1 M NaCl, 58 mM Cheno	0.126	0.104
5 mM Octylamine, 0.1 M PO$_4$, 0.1 M NaCl, 29 mM Cheno	0.189	0.181
5 mM Octylamine, 0.1 M PO$_4$, 0.1 M NaCl, 14.5 mM Cheno	0.328	0.338
5 mM Hexylamine, 0.1 M PO$_4$, 0.1 M NaCl, 58 mM Cheno	0.535	0.511
5 mM Hexylamine, 0.1 M PO$_4$, 0.1 M NaCl, 14.5 mM Cheno	0.845	0.887

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