(7) K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *Tetrahedron Lett.*, **1961**, 485.
(8) K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *J. Amer. Chem. Soc.*, **85**, 631(1963).
(9) K. Biemann and J. T. Watson, *Monatsh. Chem.*, **96**, 20 (1965).
(10) P. Tunmann and D. Wolf, *Z. Naturforsch. B*, **24**, 1665 (1969).
(11) S. Markey, K. Biemann, and B. Witkop, *Tetrahedron Lett.*, **1967**, 157.
(12) P. Tunmann and H. Guenther, *Pharmazie*, **25**, 361(1970).
(13) H. C. Wood, Jr., *Univ. Penn. Med. Bull.*, **23**, 1(1910).
(14) E. Bizet and Raymond-Hamet, *Compt. Rend.*, **204**, 1754 (1937).
(15) L. Floriani, *An. Farm. Bioquim.*, **2**, 215(1931).
(16) J. N. Banerjee and J. J. Lewis, *J. Pharm. Pharmacol.*, **7**, 46 (1955).
(17) B. Witkop, *J. Amer. Chem. Soc.*, **70**, 3712(1948).
(18) Raymond-Hamet, *Compt. Rend. Soc. Biol.*, **148**, 458(1954).
(19) Raymond-Hamet, *Compt. Rend.*, **253**, 1278(1961).
(20) *Ibid.*, **255**, 1482(1962).
(21) A. G. Kurmukov, *Farmakol. Toksikol.*, **31**, 47(1968).
(22) M. Gorman, A. L. Burlingame, and K. Biemann, *Tetrahedron Lett.*, **1963**, 39.
(23) M. Gorman, U. S. pat. 3,225,053 (Dec. 21, 1965); through *Chem. Abstr.*, **64**, 8256(1966).
(24) N. R. Farnsworth, R. N. Blomster, D. Damratoski, W. A. Meer, and L. V. Cammarato, *Lloydia*, **27**, 302(1964).
(25) D. J. Abraham, R. D. Rosenstein, R. L. Lyon, and H. H. S. Fong, *Tetrahedron Lett.*, **1972**, 909.
(26) A. Banerji, P. L. Majumder, and A. Chatterjee, *Phytochemistry*, **9**, 1491(1970).
(27) K. T. DeSilva, A. H. Ratcliffe, G. F. Smith, and G. N. Smith, *Tetrahedron Lett.*, **1972**, 913.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 5, 1972, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication August 21, 1972.

• Present address: Eli Lilly and Co., Indianapolis, IN 46206

▲ To whom inquiries should be directed.

Model Transport Studies Utilizing Lecithin Spherules II: Transport of 3-*O*-Methyl-^{14}C-D-glucose in D-Glucose Solution

ZAKA-UD-DIN T. CHOWHAN, TOSHIHISA YOTSUYANAGI, and WILLIAM I. HIGUCHI▲

Abstract □ Recently, quantitative methods were developed for determining the permeability coefficient of solutes in lecithin spherules. The technique involved following (*a*) the direct release of solutes from the dispersions, (*b*) the release after prior dilution, and (*c*) the release from dispersions partly equilibrated with the solutes for a predetermined period. A quantitative evaluation of several physical models indicated that the models that assume that the spherules are equally spaced, multiconcentric bilayers of lecithin were in satisfactory agreement with the experimental release data. In the present study, this technique was applied to the transport of 3-*O*-methyl-D-glucose in liposome dispersions prepared from lecithin–dicetyl phosphate (10:1) and lecithin–dicetyl phosphate–cholesterol (10:1:1). The transport results for 3-*O*-methyl-D-glucose yielded a permeability coefficient that was 50 times larger than that for D-glucose. The dispersions prepared from lecithin–dicetyl phosphate containing 10% cholesterol yielded a permeability coefficient that was 2.4 times smaller than the dispersions prepared without cholesterol. The analysis of the results indicated that, for relatively large permeability coefficients as obtained in these studies, the dilution-release experiments show greater sensitivity in the determination of this parameter compared to the direct-release experiments.

Keyphrases □ Permeability coefficients, 3-*O*-methyl-D-glucose, radiolabeled—liposome dispersions, comparison of three methods □ D-Glucose solution—transport of 3-*O*-methyl-^{14}C-D-glucose, lecithin spherule dispersions, permeability coefficients □ 3-*O*-Methyl-D-glucose, radiolabeled—transport in D-glucose solution, lecithin spherule dispersions, permeability coefficients □ Lecithin spherules—model transport studies, 3-*O*-methyl-^{14}C-D-glucose in D-glucose solution, comparison of permeability coefficients determined by three methods □ Transport studies, model using lecithin spherules—3-*O*-methyl-^{14}C-D-glucose in D-glucose solution, comparison of permeability coefficients determined by three methods

Several physical models were evaluated (1) in determining the permeability coefficients for solutes in complex aqueous liposome dispersions to quantitate the transport of drugs across phospholipid membranes. The solute transport experiments were conducted using three different initial boundary conditions. The first (direct-release experiment) was the solute release from the dispersions in which the solute was fully equilibrated between the spherule interior and the external aqueous phases. In the second situation (dilution-release experiment), the dispersion was diluted by about a factor of 10 just prior to beginning the release run. In the third case (uptake-release experiment), the dispersion was first prepared without the radioactive solute and then the spherules were allowed to absorb the radioactive solute for a predetermined period just prior to the release run.

A careful evaluation of several physical models in conjunction with these three experimental procedures helped significantly in both the selection of the best models and the determination of the best set of values for the parameters. Simple physical models that assume monosize or multisize single membrane-controlled solute transport failed to provide reasonable agreement between the experimental data and the theory. The models assuming multiconcentric layers of equal thickness were generally found to be in good agreement with the experimental transport data. The introduction of

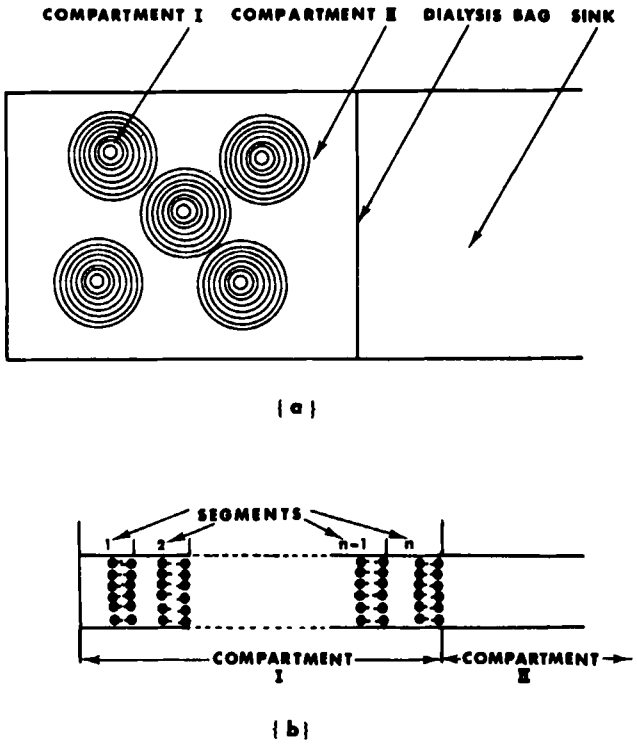

Figure 1—(a) *Schematic representation of the monosize, multiconcentric layer Model 2.* (b) *Schematic cross section of the multiconcentric layer Model 2 showing the division of Compartment I into* i = *1 to* i = n *number of segments of equal thickness.*

particle-size distribution into the theoretical analysis did not significantly alter the solute release curves. As a result of these findings, it was suggested that the monosize, multiconcentric models may be used in the determination of effective bulk permeability coefficients.

In the present studies the same procedures were used in determining the effective bulk permeability coefficients for 3-*O*-methyl-D-glucose.

EXPERIMENTAL

Materials—Egg yolk lecithin was purified using the procedures reported previously (1). Dicetyl phosphate[1] was used without further purification. Cholesterol[2] was recrystallized twice from ethanol. Anhydrous D-glucose[3] was analytical reagent grade. The dialysis bags[4] were used without pretreatment. The 3-*O*-methyl-^{14}C-D-glucose[5] was obtained in the crystalline solid form.

Procedures—The liposome dispersions were prepared from 5.0% egg yolk lecithin and 0.5% dicetyl phosphate, with or without 0.5% cholesterol. The procedures used in the preparation of dispersions and for the transport of solute were the same as reported earlier (1). The dispersions were prepared in 5.1% anhydrous D-glucose solution, which also served as a sink solution. Five milliliters of the dispersion was added to the dialysis bag, which was held by a square framework in a dialysis bag holder-stirring assembly. This assembly also served as a cover plate for the water-jacketed beaker containing 100 ml. sink solution. At the end of each sampling time, this assembly was transferred to another water-jacketed beaker containing fresh sink solution. The overall rate of the solute transport represented both the movement of solute out of the spherules and the transfer of solute across the dialysis bag. The procedures for the determination of radioactivity and for the particle-size distribution determinations by means of the Coulter counter were reported earlier (1).

[1] Sigma Chemical Co., St Louis, Mo.
[2] Fisher Scientific Co., Fair Lawn, N. J.
[3] J. T. Baker Chemical Co., Phillipsburg, N. J.
[4] Union Carbide Corp., Chicago, Ill.
[5] New England Nuclear, Boston, Mass.

THEORY

The monosize, multiconcentric layer models, Model 1 and Model 2, were previously investigated in the determination of the permeability coefficient for D-glucose (1). For molecules like D-glucose or 3-*O*-methyl-D-glucose which have relatively low partition coefficients, either model could be used in the determination of permeability coefficients. Theoretical analysis was, therefore, carried out in the present investigation using the monosize, multiconcentric layer Model 2.

A schematic representation of this model is presented in Fig. 1*a*. In Fig. 1*b*, a schematic cross section of this model, showing the division of Compartment I into $i = 1$ to $i = n$ segments, is shown. This model assumes that all of the spherules are of the same size and consist of multiconcentric layers (segments) of equal thickness. Each of these concentric layers consists of a lipid bilayer separating aqueous compartments. It is assumed that the solute instantaneously partitions between the inner aqueous–lipid interface and that the only barrier to solute transport is located at the external lipid–aqueous interface. It is also assumed that there is good mixing in both phases, so that there is no concentration gradient in each segment, neither in the lipid phase nor in the aqueous phase.

Equation 1 relates the average solution concentration to the aqueous and lipid contributions for the $i = 1$ to $i = n$th segment:

$$\begin{aligned} C_{1,t} &= (v_a + v_l \cdot k)C_{1,a} \\ &\vdots \\ C_{n,t} &= (v_a + v_l \cdot k)C_{n,a} \end{aligned} \qquad \text{(Eq. 1)}$$

Here, $C_{1,t}$ and $C_{1,a}$ are the average and aqueous solution concentrations in segment 1, respectively; v_a and v_l are the aqueous and lipid volume fractions, respectively; and k is the lipid–aqueous partition coefficient.

The rates at which solute leaves segments $i = 1$ to $i = n$ are given by a set of equations:

$$-V_1(v_a + v_l \cdot k)\frac{dC_{1,a}}{dt} = S_1 \cdot p(C_{1,a} - C_{2,a}) \qquad \text{(Eq. 2a)}$$

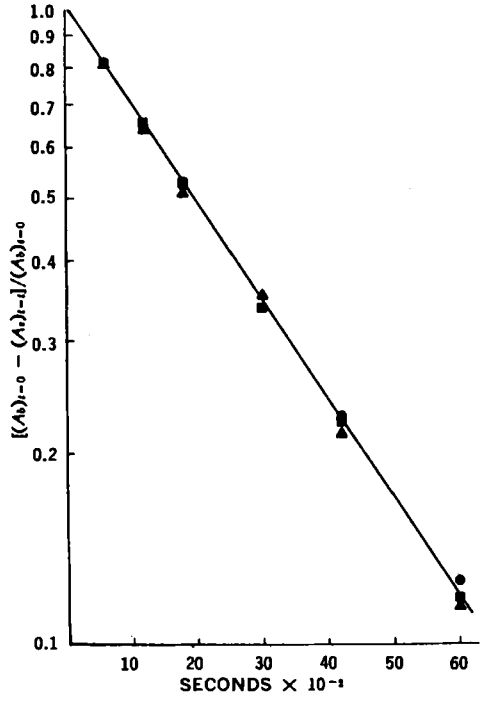

Figure 2—*First-order plots used for the determination of the bag constant for the transport of 3-O-methyl-D-glucose through the dialysis bag membrane. Different symbols represent data from the three different experiments.*

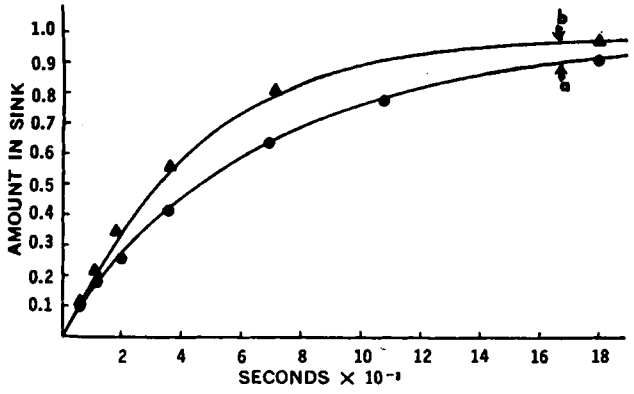

Figure 3—*Comparisons of the experimental data and results of computations using the monosize, multiconcentric layer Model 2 for the direct-release and dilution-release experiments, using a lecithin–dicetyl phosphate dispersion system. Key (symbols represent experimental data): ●, direct-release experiment; and ▲, dilution-release experiment. Curves represent results of computations. Curves a and b represent theory for the direct-release and dilution-release cases, respectively, and are based on the parameter values given in Table I. The ordinate represents A_s, the normalized amount of solute in the sink.*

$$V_2(v_a + v_1 \cdot k)\frac{dC_{2,a}}{dt} = S_1 \cdot p(C_{1,a} - C_{2,a}) - S_2 \cdot p(C_{2,a} - C_{3,a}) \quad \text{(Eq. 2b)}$$

$$V_n(v_a + v_1 \cdot k)\frac{dC_{n,a}}{dt} = S_{n-1} \cdot p(C_{n-1,a} - C_{n,a}) - S_n \cdot p(C_{n,a} - C_{II}) \quad \text{(Eq. 2c)}$$

Here, V_1 is the volume of segment 1, S_1 is the surface area of segment 1, p is the permeability coefficient per segment, and C_{II} is the concentration of solute in Compartment II, which is the external phase of the dispersion. The rate expression for solute in Compartment II is given by Eq. 3:

$$V_{II} \cdot \frac{dC_{II}}{dt} = S_n \cdot p(C_{n,a} - C_{II}) - S_b \cdot p_b \cdot C_b \quad \text{(Eq. 3)}$$

Here, V_{II} is the volume of Compartment II; S_b and p_b are the surface area and effective permeability coefficient of the bag, respectively; and C_b is the concentration of solute in the bag.

The rate of appearance of solute in the sink is given by Eq. 4:

$$\frac{dA_s}{dt} = p_b \cdot S_b \cdot C_b \quad \text{(Eq. 4)}$$

Here, A_s is the normalized amount of solute in the sink. These equations were solved by a digital computer[6] using the procedure described elsewhere (1).

RESULTS AND DISCUSSION

The results of the bag constant experiments for 3-*O*-methyl-D-glucose carried out by the procedure identical to that already reported (1) are presented in Fig. 2. The slope of the first-order plots represented the bag constant divided by the volume of the solution inside the bag. The bag constant was defined as the product of the surface area of the bag and the effective permeability coefficient of the bag.

The results of the direct-release and dilution-release experiments for 3-*O*-methyl-D-glucose in lecithin–dicetyl phosphate dispersions are given in Fig. 3. In Fig. 4 the results of the uptake release after 0 and 2 hr. prior to uptake through the same dispersion system are presented. In the monosize, multiconcentric Model 2, the partition coefficient is mainly a function of the initial slope of the direct-release experiment and was determined by the procedure outlined recently (1). Different values of p, the permeability coefficient per

[6] IBM 360.

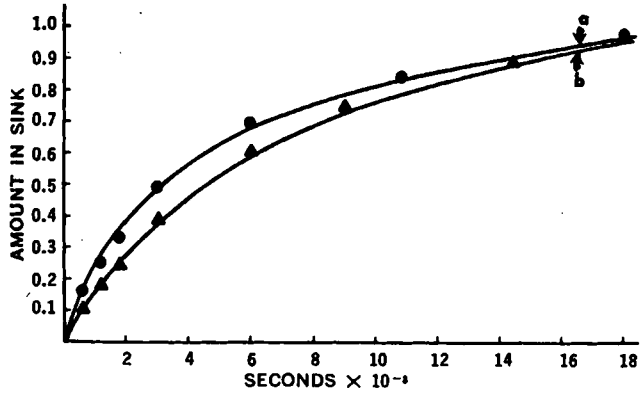

Figure 4—*Comparisons of the experimental data and results of computations using the monosize, multiconcentric layer Model 2 for the uptake-release experiments, using a lecithin–dicetyl phosphate dispersion system. Key (symbols represent experimental data): ●, 0-hr. uptake-release experiment; and ▲, 2-hr. uptake-release experiment. Curves represent results of computations. Curves a and b represent theory for the 0- and 2-hr. uptake-release cases, respectively, and are based on the parameter values given in Table I.*

segment, were then tried to obtain agreement between the experiment and the theory for the three types of experiments. The calculation results using a "best" set of parameter values, which are given in Table I, are compared with the experimental data in Figs. 3 and 4. The excellent agreement between the experimental data and the calculation results shows the usefulness of the technique in obtaining reliable values of the permeability coefficients. These results indicate that by substituting a hydroxyl group at the 3-position by a methoxy group in D-glucose the effective bulk permeability coefficient increased 50-fold. It is important to point out that this group substitution does not significantly change the partition coefficient of the solute in the liposome dispersions.

An important result of this study is revealed in Fig. 3. While significant differences between the direct-release and dilution-release experiments were not observed in the study of D-glucose transport (1), the shapes of the normalized direct-release and dilution-release curves for 3-*O*-methyl-D-glucose were significantly different. This resulted from the differences in the relative permeability coefficients of the two compounds (for D-glucose $p/n = 2.666 \times 10^{-10}$ cm. sec.$^{-1}$ and for 3-*O*-methyl-D-glucose $p/n = 1.33 \times 10^{-8}$ cm. sec.$^{-1}$). The dilution-release experiments showed greater sensitivity in the determination of permeability coefficients than the direct-release experiments when the permeability coefficient was relatively large. For example, it was found that in the direct-release

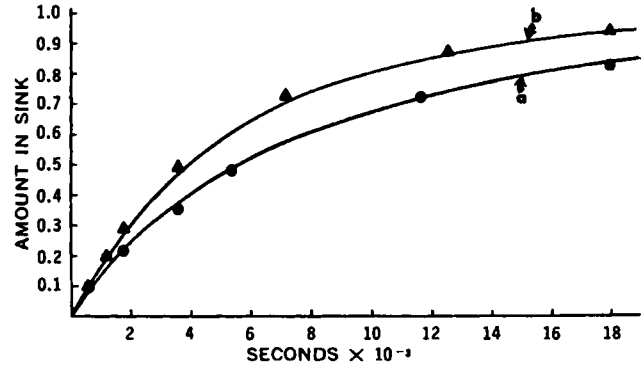

Figure 5—*Comparisons of the experimental data and results of computations using the monosize, multiconcentric layer Model 2 for the direct-release and dilution-release experiments, using a lecithin–dicetyl phosphate–cholesterol dispersion system. Key (symbols represent experimental data): ●, direct-release experiment; and ▲, dilution-release experiment. Curves represent results of computations. Curves a and b represent theory for the direct-release and dilution-release cases, respectively. The parameter values are given in Table I.*

Table I—Input Data Used in Calculations for a Lecithin–Dicetyl Phosphate and a Lecithin–Dicetyl Phosphate–Cholesterol Dispersion System Used in the 3-*O*-Methyl-D-glucose Transport Studies

Solute	Dispersion System	V_I, ml.	n	$p \times 10^7$, cm. sec.$^{-1}$	$p_b \cdot S_b \times 10^3$, cm.^{3} sec.$^{-1}$	Partition Coefficient, k	Mean Radius, μ
3-*O*-Methyl-D-glucose	Lecithin–dicetyl phosphate	1.05	45	6.0	1.764	20	0.685
3-*O*-Methyl-D-glucose	Lecithin–dicetyl phosphate–cholesterol	1.03	45	2.5	1.764	20	0.673
D-Glucose[a]	Lecithin–dicetyl phosphate	1.05	45	0.12	1.919	21	0.685

[a] Data taken from *Reference 1.*

curve a 20% change in the permeability coefficient was difficult to detect, while in the dilution-release case a 20% change in the permeability coefficient significantly changed the theoretical curve.

Several workers utilized liposome dispersions prepared from a lecithin–cholesterol–dicetyl phosphate mixture for solute transport studies (2–8). There is evidence in the literature (9) that dispersions prepared from lecithin–cholesterol are morphologically similar to those prepared without cholesterol. Papahadjopoulos and Miller (8) indicated that cholesterol in molar proportions of up to 50% could be incorporated without apparent change in the general morphology of the spherules. Although permeability coefficients have never been determined in such dispersion systems, a decrease in the permeability of glycerol (10) was observed when liposome dispersions were prepared from egg yolk lecithin and cholesterol compared to the dispersions prepared from lecithin alone. This decrease was proportional to the concentration of cholesterol. Similar observations were made (11) on chloride permeability. In the light of such studies, the results of the transport of 3-*O*-methyl-D-glucose in liposome dispersions prepared from lecithin–dicetyl phosphate containing cholesterol were found interesting. The experimental data of the direct-release and dilution-release cases are given in Fig. 5 and are compared with the computation results utilizing a best set of parameter values (Table I). In Fig. 6 the experimental results of 0- and 2-hr. uptake-release experiments are compared with the theory, utilizing the same set of parameter values. The effective bulk permeability coefficient obtained for 3-*O*-methyl-D-glucose in this dispersion system was smaller by a factor of 2.4 compared to the dispersion system without cholesterol.

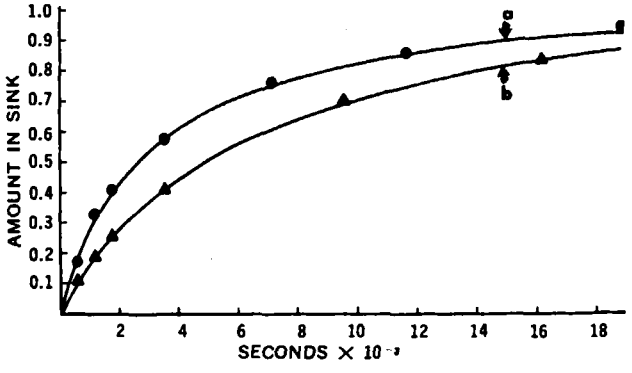

Figure 6—*Comparisons of experimental data and results of computations using the monosize, multiconcentric layer Model 2 for the uptake-release experiments, using a lecithin–dicetyl phosphate–cholesterol dispersion system. Key (symbols represent experimental data): ●, 0-hr. uptake-release experiment; and ▲, 2-hr. uptake-release experiment. Curves represent results of computations. Curves a and b represent theory for the 0- and 2-hr. uptake-release cases, respectively. The parameter values are given in Table I.*

CONCLUSION

The present study has demonstrated the effect of a group substitution on the permeability coefficient. A 50-fold increase in the permeability coefficient is obtained by the substitution of a hydroxyl group at the 3-position in D-glucose by a methoxy group.

This work has also shown that the dispersions prepared from a lecithin–cholesterol–dicetyl phosphate mixture yielded a smaller permeability coefficient than dispersions prepared without cholesterol.

In the development of the method (1), three different initial boundary conditions which led to three types of experimental procedures (direct release, dilution release, and uptake release) were selected. The usefulness of the choice of these procedures is supported by the present investigation. For solutes having relatively large permeability coefficients, the dilution-release experiments become more sensitive than the direct-release experiments in the determination of permeability coefficients.

REFERENCES

(1) Z. T. Chowhan, T. Yotsuyanagi, and W. I. Higuchi, *Biochim. Biophys. Acta*, **266**, 320(1972).

(2) A. D. Bangham, M. M. Standish, and G. Weissmann, *J. Mol. Biol.*, **13**, 253(1965).

(3) G. Weissmann and G. Sessa, *J. Biol. Chem.*, **242**, 616(1967).

(4) J. De Gier, J. G. Mandersloot, and L. L. M. Van Deenen, *Biochim. Biophys. Acta*, **137**, 143(1969).

(5) C. Sweet and J. E. Zull, *ibid.*, **173**, 94(1969).

(6) S. C. Kinsky, J. Haxby, C. B. Kinsky, R. A. Demel, and L. L. M. Van Deenen, *ibid.*, **152**, 174(1968).

(7) R. S. Smart and M. J. Wilson, *Nature*, **215**, 964(1967).

(8) D. Papahadjopoulos and N. Miller, *Biochim. Biophys. Acta*, **135**, 624(1969).

(9) A. D. Bangham and R. W. Horne, in "Metabolism and Physiological Significance of Lipids," R. M. C. Dawson and D. N. Rhodes, Eds., Wiley, New York, N. Y., 1964, p. 413.

(10) J. De Gier, J. G. Mandersloot, and L. L. M. Van Deenen, *Biochim. Biophys. Acta*, **150**, 616(1968).

(11) D. Papahagopoulos and J. C. Watkins, *ibid.*, **135**, 639(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 12, 1972, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication August 30, 1972.

▲ To whom inquiries should be directed.