Model Transport Studies Utilizing Lecithin Spherules IV: Transport of D-Glucose in Spherules Prepared from Lecithin, Dicetyl Phosphate, and Cholesterol

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Abstract □ The transport of D-glucose in liposome dispersions prepared from lecithin–dicetyl phosphate–cholesterol was studied. The transport experiments were carried out using previously developed techniques in which the release of the solute is studied from dispersions contained in dialysis bags. The experimental results of the direct-release and uptake-release experiments were analyzed by the monosize, multiconcentric layer Model 2, which was developed and used recently for the 3-*O*-methyl-D-glucose transport studies. Uniformly good fits of the experimental data with the model were observed, and the permeability coefficients and the aqueous–lipid partition coefficients were calculated for liposome dispersions containing different percentages of cholesterol. A direct relationship between the percent cholesterol in liposome dispersions and the effective bulk permeability coefficient was obtained. The incorporation of cholesterol into liposome dispersions does not seem to alter the effective aqueous–lipid partition coefficient.

Keyphrases □ Lecithin spherules—transport of D-glucose in lecithin–dicetyl phosphate–cholesterol spherules, permeability and partition coefficients □ D-Glucose—transport in lecithin–dicetyl phosphate–cholesterol spherules, permeability and partition coefficients □ Cholesterol—transport of D-glucose in lecithin–dicetyl phosphate–cholesterol spherules, permeability and partition coefficients □ Permeability—lecithin–dicetyl phosphate–cholesterol liposome dispersions, model transport studies □ Partition coefficients—lecithin–dicetyl phosphate–cholesterol liposome dispersions, model transport studies

In previous reports (1–3), quantitative methods were developed and used in the determination of effective bulk permeability coefficients for D-glucose, 3-*O*-methyl-D-glucose, and taurocholic acid-[cholic-^{3}H (G)] in complex aqueous liposome dispersions. The release of the solutes from dispersions contained in a dialysis bag was studied as a function of time using the direct-release, the dilution-release, and the uptake-release experimental procedures. The data were analyzed using the monosize, multiconcentric layer models, which assume that the spherules consist of multiconcentric bilayers of equal thickness separating aqueous compartments.

Since cholesterol is abundant in many biological membranes, several experimental model membrane systems have been used to understand its unique properties in the membranes. Several investigators used cholesterol in combination with lecithin for the preparation of liposome dispersions (4–14). The evidence derived from electron microscope studies (15) indicated that equimolar mixtures of lecithin and cholesterol arrange themselves in the form of concentric skins of bimolecular leaflet in aqueous solutions which are basically the same as lecithin alone. Reported observations (10–14, 16) indicated a reduction in permeability caused by the presence of cholesterol in liposome dispersions. The effective bulk permeability coefficient of 3-*O*-methyl-D-glucose was 2.4 times smaller for dispersions prepared from lecithin–dicetyl phosphate–cholesterol (10:1:1) as compared to dispersions prepared without cholesterol (2).

The purpose of this investigation was to use the liposome dispersion system prepared from lecithin–dicetyl phosphate–cholesterol in determining the effective bulk permeability coefficient of D-glucose. The effect of different percentages of cholesterol used in the preparation of liposome dispersions was expected to elucidate the role of cholesterol in biological membranes.

EXPERIMENTAL

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

Procedure—The liposome dispersions were prepared from 5.0% egg yolk lecithin, 0.5% dicetyl phosphate, and varying percentages of 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, and at zero time the dialysis bag holder–stirring assembly was lowered into 100 ml of sink solution maintained at 25°. The dispersion and the sink solution were stirred at 150 rpm by different stirring arrangements. At the end of each sampling time, the dialysis bag holder–stirring assembly was transferred to another water-jacketed beaker containing 100 ml of 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 particle-size distribution[6] were reported earlier (1).

RESULTS AND DISCUSSION

The results of the direct-release experiment for the release of D-glucose in liposome dispersions containing different percentages of cholesterol are given in Fig. 1. The data are compared with the results of calculations utilizing the monosize, multiconcentric Model 2 (see *Appendix*). Since the results of the dilution-release experiments were very close to those of the direct-release experiments, and since the former were found to give the same good agreement between theory and experimental values, they are not shown here. The results of the uptake-release experiments are given in Fig. 2 and are compared to the results of computations using the same model and the same set of parameter values as were used for the direct-release case (Table I).

The generally good agreement obtained between the model-predicted results, the experimental results, and the results from different types of experimental procedures indicates the usefulness of

[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.
[6] Using a Coulter counter.

Table I—Input Data Used in Calculations for D-Glucose Transport Studies in a Liposome Dispersion System Containing Cholesterol

Cholesterol, %	V_I, ml	Mean Surface Radius, μm	n	p_b ($S_b \times 10^3$), cm^3 sec^{-1}	p, cm sec^{-1}	k
0.2	0.97	0.655	45	1.919	1×10^{-8}	21
0.5	1.03	0.673	45	1.919	6×10^{-9}	21
0.8	0.94	0.653	45	1.919	2.5×10^{-9}	21

the method in determining the effective bulk permeability coefficients. The relatively low partition coefficient value for this solute and the range of permeability coefficients found in this study make the determination of this latter parameter very sensitive. Significantly different release curves are obtained (Fig. 1) only because of these differences in permeability coefficients. Furthermore, the incorporation of cholesterol into the liposome dispersions does not seem to alter the effective aqueous–lipid partition coefficients. An earlier report (14) on the decrease in permeability of glucose due to the presence of cholesterol may, therefore, be related to the reduction in the permeability coefficient. Although the liposome dispersion system prepared from lecithin–cholesterol in equal molar proportions has been described as basically the same as lecithin alone (15), the molecular arrangement in the two dispersion systems may not be exactly the same. While making comparisons between the two systems, one must recognize this limitation. Therefore, the results given in Fig. 3 showing the relationship between the percent cholesterol in liposome dispersions and the effective bulk permeability coefficient as defined in the *Appendix* (Eq. A5) were indeed interesting.

The linear relationship obtained in Fig. 3 indicates that the decrease in the permeability coefficient is directly related to the concentration of cholesterol used in the preparation of liposome dispersions. The investigations on mixed monolayers of cholesterol and phospholipids (17) demonstrated reductions in the average area per molecule compared with those in films of phospholipids alone. The direct relationship between the percent cholesterol in the liposomes and the permeability coefficient seen in this study could be explained on the basis of reductions in the average volume per molecule in the presence of cholesterol.

APPENDIX

Monosize, multiconcentric layer models assume that all spherules are of the same size and consist of $i = 1$ to $i = n$ number of segments. In Model 2, the outermost lipid layer is considered as a part of the spherule so that each segment consists of an aqueous phase and a lipid phase. The basic assumptions and equations were reported earlier (1, 2). A summary of the equations used in Model 2 is presented here.

Equation A1 relates the average solution concentration to the aqueous and lipid contributions for the $i = 1$ to $i = n$th segment:

$$\begin{aligned} C_{1,t} &= [v_a + (v_l)(k)]C_{1,a} \\ &\vdots \\ C_{n,t} &= [v_a + (v_l)(k)]C_{n,a} \end{aligned} \qquad \text{(Eq. A1)}$$

where $C_{1,t}$ 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 effective lipid–aqueous partition coefficient.

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

$$-V_1[v_a + (v_l)(k)]\frac{dC_{1,a}}{dt} = S_1[p(C_{1,a} - C_{2,a})] \qquad \text{(Eq. A2}a\text{)}$$

$$V_2[v_a + (v_l)(k)]\frac{dC_{2,a}}{dt} = S_1[p(C_{1,a} - C_{2,a})] - S_2[p(C_{2,a} - C_{3,a})] \qquad \text{(Eq. A2}b\text{)}$$

$$\vdots$$

$$V_n[v_a + (v_l)(k)]\frac{dC_{n,a}}{dt} = S_{n-1}[p(C_{n-1,a} - C_{n,a})] - S_n[p(C_{n,a} - C_{II})] \qquad \text{(Eq. A2}c\text{)}$$

where 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 of solute in Compartment II is given by Eq. A3:

$$V_{II}\left(\frac{dC_{II}}{dt}\right) = S_n[p(C_{n,a} - C_{II})] - (S_b)(p_b)(C_b) \qquad \text{(Eq. A3)}$$

where 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.

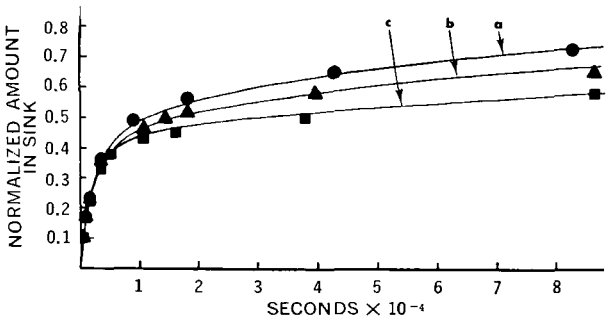

Figure 1—*Comparisons of the experimental data and results of computation using the monosize, multiconcentric layer Model 2 for the direct-release experiments using lecithin–dicetyl phosphate dispersion systems containing different percentages of cholesterol. Key (symbols represent experimental data): ●, 0.2% cholesterol; ▲, 0.5% cholesterol; and ■, 0.8% cholesterol. Curves a, b, and c represent calculations using parameter values taken from Table I for the 0.2% cholesterol, 0.5% cholesterol, and 0.8% cholesterol, respectively.*

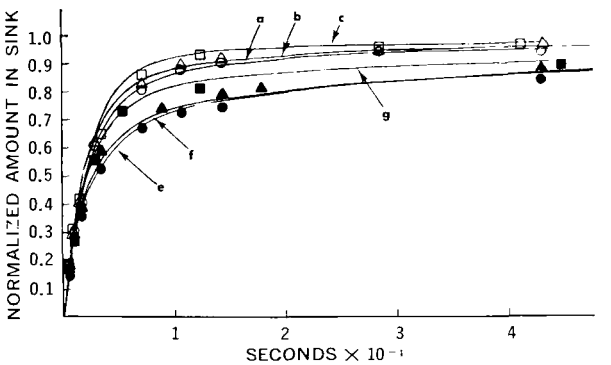

Figure 2—*Comparisons of the experimental data and results of computations using the monosize, multiconcentric layer Model 2 for the uptake-release experiments using lecithin–dicetyl phosphate dispersion systems containing different percentages of cholesterol. Key (symbols represent experimental data): ○, 0.2% cholesterol after 2 hr uptake; △, 0.5% cholesterol after 2 hr uptake; □, 0.8% cholesterol after 2 hr uptake; ●, 0.2% cholesterol after 10 hr uptake; ▲, 0.5% cholesterol after 10 hr uptake; and ■, 0.8% cholesterol after 10 hr uptake. Curve a (0.2% cholesterol), curve b (0.5% cholesterol), and curve c (0.8% cholesterol) represent 2-hr uptake calculations. Curve e (0.2% cholesterol), curve f (0.5% cholesterol), and curve g (0.8% cholesterol) represent 10-hr uptake calculations. The parameter values were taken from Table I.*

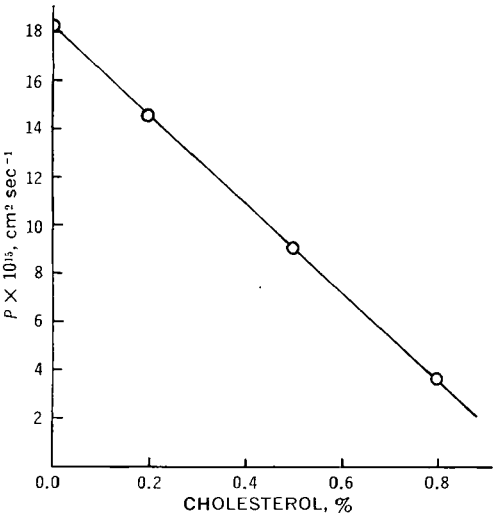

Figure 3—*Relationship between the percent cholesterol in liposome dispersions and the effective bulk permeability coefficient. The effective bulk permeability coefficient,* P, *was calculated using Eq. A5. The permeability coefficient per segment,* p, *value for the 0% cholesterol data point was taken from Ref. 1.*

The rate of appearance of solute in the sink is given by Eq. A4:

$$\frac{dA_s}{dt} = (p_h)(S_h)(C_h) \qquad \text{(Eq. A4)}$$

where A_s is the normalized amount of solute in the sink.

For multiconcentric layer models, it was shown earlier (1) that the choice for the permeability coefficient, p, depends upon both the radius, r, of the spherules and the number, n, of segments used in the calculations according to the relationship:

$$p = \frac{Pn}{r} \qquad \text{(Eq. A5)}$$

where P is regarded as the effective bulk permeability coefficient when the spherule is treated as a homogeneous sphere. At constant P, the convergence behavior of the theoretical curves was shown earlier (1) as n was increased. For $n = 45$, the release curve was approximately 2% higher than that for the $n \rightarrow \infty$ case. This was the basis for selecting the value of n used in the calculations.

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