Interfacial Barrier Limited Interphase Transport of Cholesterol in the Aqueous Polysorbate 80–Hexadecane System

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Abstract □ The kinetics of transfer of cholesterol from an aqueous polysorbate 80 solution into hexadecane and vice versa was studied by means of the multiparticulate dispersion technique. The experimental data were quantitatively analyzed by the physical model which accounts for the effects of bulk diffusion, interfacial resistance, interfacial area, and the lipid–water partition coefficient. For the 0.1% polysorbate 80, a P value around 1.7 to 2.2 × 10^{-7} cm. $sec.^{-1}$ was found that was consistent with all of the data on water-to-oil as well as oil-to-water transfer experiments. These findings suggest such large nonspecific interfacial barriers to be important in many biological and biopharmaceutical situations.

Keyphrases □ Interfacial barrier limited transport—interphase □ Cholesterol interphase transport—aqueous polysorbate 80–hexadecane system □ Emulsions—hexadecane–water–polysorbate 80 □ Particle-size distribution—emulsions □ Partition coefficient, cholesterol—hexadecane–polysorbate 80 system

Recently (1–5), there has been increased interest in the possibility of utilizing physical models to gain an understanding of the transport of drugs and other biologically important substances from body fluids into tissues and across membranes. The main characteristic of the physical model approach is the intimate interaction of realistic physical concepts with well-designed experiments. Thus the previous studies (1–5) have shown that it should be possible to interrelate such factors as the pKa, the partition coefficient and the diffusional characteristics of the drug, the buffer characteristics and the pH of the aqueous phases involved, and the heterogeneous nature of the membrane, and then to test such relationships experimentally.

One of the factors in interphase transport of drugs which has escaped serious consideration until recently (1, 2) is the possible existence of significant interfacial resistances at the oil–water interface. The present studies along with those of Ghanem *et al.* (1) appear to represent for the first time the likelihood of the frequent domination of interfacial barriers in interphase transport.

The purpose of the present communication is to describe the interfacial barrier-controlled transfer of cholesterol across an oil–water interface. As will be shown, very large interfacial resistances were found. Because of the probable nonspecific nature of the interfacial barrier, these findings may be very pertinent to a number of biological and pharmaceutical situations, for example, the deposition of gallstones, the initiation of atherosclerosis, and the transport and the absorption of cholesterol and other steroids (6) from the gastrointestinal tract.

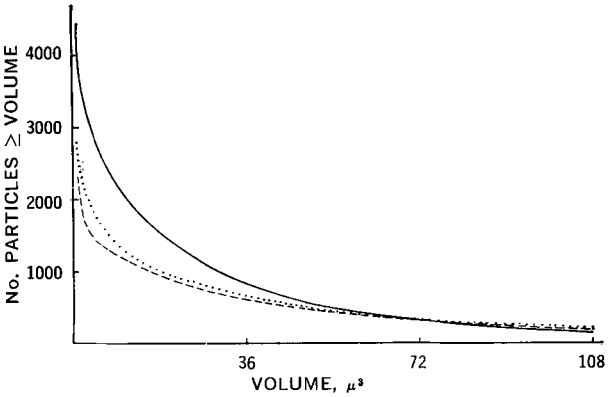

Figure 1—*Cumulative particle-size distribution data from Emulsions I and II obtained using the Coulter counter model A. A plot of number of particles* ≥ *volume* versus *volume gave a 95% mass balance for Emulsion I, a 66% mass balance for Emulsion II as shown in Table I, and a 73% mass balance for Emulsion III. Key: —, Emulsion I; - - -, Emulsion II; and · · ·, Emulsion III.*

EXPERIMENTAL

General Considerations in the Design of Experiments—It was decided to employ the multiparticulate dispersion technique (1, 2) which provides both good reproducibility and sensitivity for interfacial barrier determination. Both water-to-oil ("solute uptake") as well as oil-to-water ("solute release") transport experiments were carried out in order to assure the reliability of the interfacial permeability coefficient. Different oil particle-size distributions were utilized to demonstrate further that an interfacial barrier was rate controlling.

Preparation of Stock Emulsions—Two 8% hexadecane–water emulsion stocks using 0.1% polysorbate 80[1] were prepared in such a manner that the particle-size distributions differed significantly. Emulsion I was prepared by mixing 8 ml. of hexadecane[2] with 1 ml. of 10% aqueous polysorbate 80 solution and then making up to 100 ml. with distilled water. The mixture was then homogenized for 75 sec. in a Waring blender. Emulsion II was prepared by mixing 8 ml. of hexadecane with 0.5 ml. of the 10% polysorbate 80 solution and then making up to 100 ml. with distilled water. This mixture was then homogenized for 45 sec., after which 0.5 ml. of 10% surfactant was added to make the final surfactant concentration of

[1] Obtained from Atlas Chemical Industries, Inc., Wilmington, Del.
[2] Hexadecane Spectroquality Reagent, Matheson Coleman & Bell, Norwood, Ohio.

Table I—Oil Droplet Size Distribution and Data Treatment of Emulsion Systems I and II Given in Fig. 1 with Their Respective Mass Balance

j	Mean Radius (Micron)	Δ(Volume) (Micron)[3]	No. of Particles	Total Volume (Micron)[3]
		System I		
1	0.688	0.90	625.00	850.00
2	0.815	0.91	475.00	1073.50
3	0.912	0.90	300.00	954.00
4	0.990	0.91	200.00	814.00
5	1.060	0.90	162.50	809.25
6	1.120	0.91	137.50	807.13
7	1.175	0.90	125.00	847.50
8	1.225	0.91	100.00	770.00
9	1.275	0.90	100.00	870.00
10	1.335	1.81	162.50	1616.88
11	1.415	1.82	150.00	1777.50
12	1.485	1.81	137.50	1883.75
13	1.545	1.80	112.50	1743.75
14	1.605	1.82	100.00	1690.00
15	1.695	4.53	225.00	4590.00
16	1.815	4.52	187.50	4706.25
17	1.915	4.53	162.50	4761.25
18	2.010	4.53	137.50	4661.25
19	2.095	4.52	112.50	4331.25
20	2.175	4.53	75.00	3240.00
21	2.250	4.53	75.00	3577.50
22	2.325	4.52	50.00	2640.00
23	2.385	4.53	50.00	2870.00
24	2.495	4.55	62.50	4075.00
25	2.505	4.51	62.50	4112.50
26	2.560	4.52	50.00	3520.00
27	2.640	9.06	50.00	3850.00
28	2.740	9.05	37.50	3232.50
29	2.830	9.05	37.50	3562.50
30	2.920	9.06	37.50	3907.50
31	3.085	42.28	137.50	16981.25
			Sum	95126.00
			Mass Balance	95%
		System II		
1	0.688	0.90	525	714.00
2	0.815	0.91	187.50	423.75
3	0.912	0.90	112.50	357.75
4	0.990	0.91	87.50	356.13
5	1.060	0.90	50.00	249.00
6	1.120	0.91	50.00	293.00
7	1.175	0.90	37.00	253.13
8	1.225	0.91	25.00	192.50
9	1.275	0.90	25.00	217.50
10	1.335	1.81	75.00	746.25
11	1.415	1.82	62.50	740.63
12	1.485	1.81	62.50	856.25
13	1.545	1.80	50.00	775.00
14	1.605	1.82	50.00	845.00
15	1.695	4.53	112.50	2295.00
16	1.815	4.52	75.00	1882.50
17	1.915	4.53	75.00	2197.50
18	2.010	4.53	62.50	2118.75
19	2.095	4.52	62.50	2406.25
20	2.175	4.53	62.50	2700.00
21	2.250	4.53	37.50	1788.75
22	2.325	4.52	37.50	1980.00
23	2.385	4.53	25.00	1435.00
24	2.495	4.55	50.00	3260.00
25	2.505	4.51	31.25	2056.25
26	2.560	4.52	37.50	2640.00
27	2.640	9.06	37.50	2887.50
28	2.740	9.05	37.50	3232.50
29	2.830	9.05	37.50	3562.50
30	2.920	9.06	37.50	3907.50
31	3.140	28.97	150.00	19425.00
			Sum	66795.37
			Mass Balance	66%

0.1%. These emulsions were gently shaken for about 15 min. prior to their use in the rate runs.

In addition to these two emulsions, a third emulsion (Emulsion III) was prepared in a similar manner but utilizing a polysorbate 80 ester (Polyol-Free).[1]

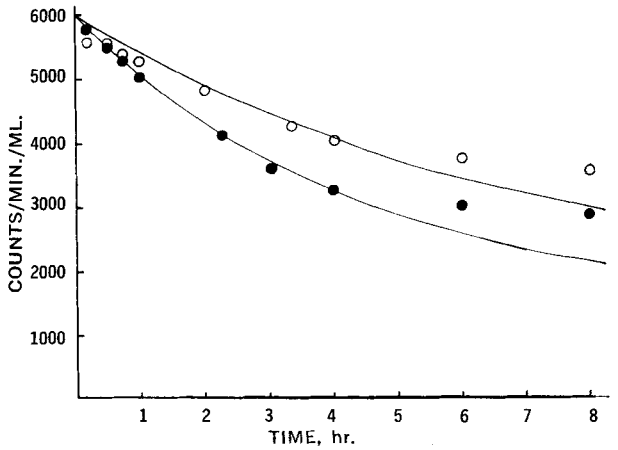

Figure 2—*Comparison of experimental data with theory for the uptake of cholesterol from Emulsion Systems I and II; counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 2% oil of System I, ●; and experimental points from 2% oil of System II, ○. Curves are theoretically computed values.* V_W *= 49 ml. of aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$*;* K *value is 200.*

Figure 1 shows the cumulative particle-size distribution data from the three emulsions obtained using the Coulter counter.[3] The data given in Fig. 1 were used to obtain the differential size distribution in Table I. No significant particle-size distribution changes were found with these emulsions up to 8 hr.

Uptake Experiments—Predetermined dilutions of these stocks were made in 0.1% polysorbate 80 solution. Then 1.5×10^{-7} g. of 4-^{14}C-cholesterol contained in 3 ml. of a 0.1% polysorbate 80 solution was added into 47 ml. of the diluted emulsions and the mixture shaken gently at 30° in the Burrell Wrist-Action shaker.[4] Three- or five-milliliter samples were pipeted out at different time intervals and the aqueous phases were analyzed by either filtration (7) of the sample employing 0.20-μ pore size Gelman Metricel filters[5] (GA-8) or by high-speed centrifugation[6] at 21,600×g for 1.5 min. Out of the clear aqueous solution collected, 1 ml. was pipeted into a liquid scintillation vial. To the latter, 10 ml. of a liquid scintillation cocktail was added, and the samples quantitatively analyzed in the Beckman liquid scintillation system.[7] In the filtration procedure it was found that a small amount (≃ 10%) of the cholesterol was lost to the filter during filtration. Therefore, a correction for the adsorption loss was obtained by this technique.

Release Experiments—For the release experiments the same procedure was used for the preparation of the stock emulsions. However, radioactive cholesterol was added to the oil (6.68×10^{-7} g. of 4-^{14}C-cholesterol/ml. of hexadecane) prior to the emulsification step. Aliquots of the stock emulsion containing the 4-^{14}C-cholesterol were then added at zero time to predetermined volumes of 0.1% polysorbate 80 solutions. Sampling and analysis of the aqueous phases were carried out in the same manner as in the uptake experiments.

Partition Coefficient Determinations—The hexadecane–0.1% polysorbate 80 partition coefficient for the 4-^{14}C-cholesterol was determined in emulsion systems containing 0.24 to 8% hexadecane and equilibrating for 48 and 72 hr. A value of 200 ± 10% was found which was used in the analysis of the data.

RESULTS AND ANALYSIS OF THE DATA

In all of the uptake and the release experiments, significant changes in the aqueous cholesterol concentrations were found up to 8 hr. The results of the experiments for uptake and release are

[3] Model A, Coulter Electronics, Hialeah, Fla.
[4] Burrell Corp., Pittsburgh, Pa.
[5] Gelman Instrument Co., Ann Arbor, Mich.
[6] Lourdes Instrument Corp., Brooklyn, N. Y.
[7] Beckman Instruments, Inc., Fullerton, Calif.

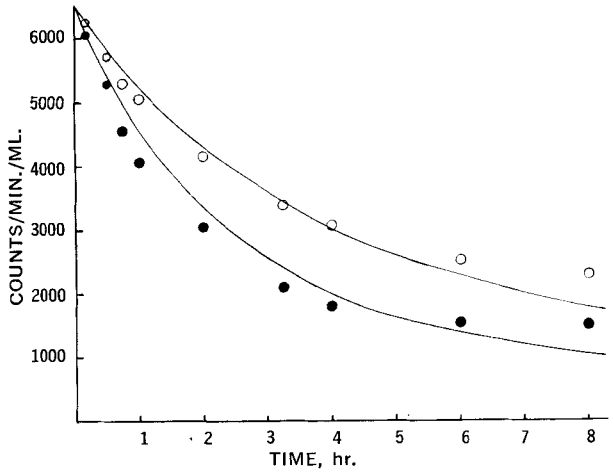

Figure 3—*Comparison of experimental data with theory for the uptake of cholesterol from Emulsion Systems I and II. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 4% oil of System I, ●; and experimental points from 4% oil of System II, ○. Curves are theoretically computed values.* V_W = *48 ml. aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

presented in Figs. 2–9. In the uptake plots (Figs. 2–5) the ordinate gives the actual aqueous concentrations of 4-^{14}C-cholesterol as a function of time. In the release plots (Figs. 6–9) the initial (zero time) expected aqueous concentrations were subtracted from all of the determinations of the aqueous cholesterol.

The following procedure was developed for the analysis of the experimental results. The general relationships apply to both uptake and release, the two situations differing only by the difference in sign of the concentration gradient.

It is helpful to refer to the model in Fig. 10. For an oil droplet of radius a_j, the rate of cholesterol transport into (or out of) the droplet is given by Eq. 1:

$$G_j = \frac{4\pi a_j{}^2 PD(C_b - C_{bj}')}{D + a_j P} \qquad \text{(Eq. 1)}$$

where P is the apparent permeability coefficient for the interfacial barrier, D is the relevant diffusion coefficient for cholesterol in the 0.1% polysorbate 80 solution, C_b is the total bulk aqueous cholesterol concentration, and C_{bj}' is defined by Eq. 2:

$$K = \frac{C_{oj}}{C_{bj}'} \qquad \text{(Eq. 2)}$$

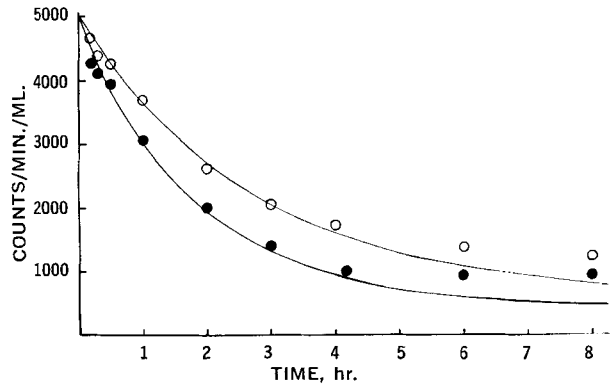

Figure 4—*Comparison of experimental data with theory for the uptake of cholesterol from Emulsion Systems I and II. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 6% oil of System I, ●; and experimental points from 6% oil of System II, ○. Curves are theoretically computed values.* V_W = *47 ml. aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

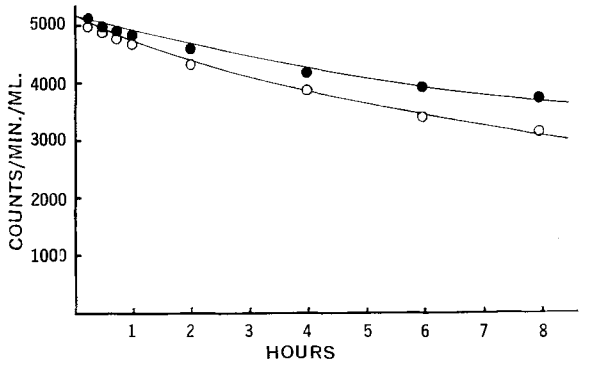

Figure 5—*Comparison of experimental data with theory for the uptake of cholesterol from Emulsion III. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 ester phase* versus *time in hours. Key: experimental points from 0.8% oil, ●; and experimental points from 1.2% oil, ○. Curves are theoretically computed values.* P *value for both dilutions is* 2.2×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

where K is the effective hexadecane–0.1% polysorbate 80 partition coefficient for cholesterol and C_{oj} is the cholesterol concentration in the oil droplet. When G_j is positive the situation is for uptake; when G_j is negative, one has cholesterol release from the droplet.

It is noteworthy that when $a_jP \ll D$, then a_jP may be neglected in the denominator of Eq. 1. In this case, one may write

$$G_j = 4\pi a_j{}^2 P(C_b - C_{bj}') \qquad \text{(Eq. 3)}$$

which is the appropriate limiting expression for the interfacial barrier-controlled transfer of cholesterol. One may also write:

$$G_j = V_{oj}\frac{dC_{oj}}{dt} \qquad \text{(Eq. 4)}$$

where $V_{oj} = \frac{4}{3}\pi a_j{}^3$ is the volume of the oil droplet and t is the time.

Equations 1, 2, and 4 may be combined to give

$$\frac{dC_{oj}}{dt} = \frac{3DP(C_b - C_{oj}/K)}{a_j(D + a_jP)} \qquad \text{(Eq. 5)}$$

Now, from material balance considerations in the system, one may write

$$\frac{dC_b}{dt} = -\frac{4\pi}{3V_W}\sum_{j=1}^{L} a_j{}^3 \Delta N_j \frac{dC_{oj}}{dt} \qquad \text{(Eq. 6)}$$

where V_W is the volume of the aqueous phase, ΔN_j is the number of droplets of sizes between a_j and a_{j+1}, and L represents the largest oil droplets in the system.

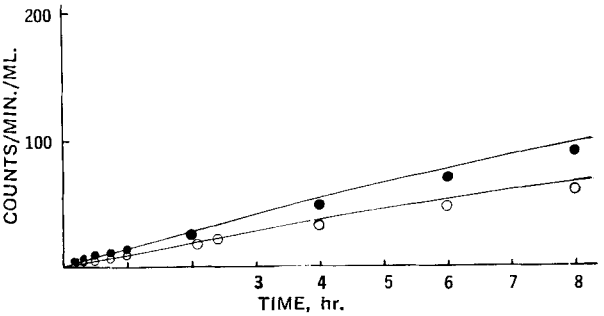

Figure 6—*Comparison of experimental data with theory for the release of cholesterol from Emulsion Systems I and II. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 0.24% oil of System I, ●; and experimental points from 0.24% oil of System II, ○. Curves are theoretically computed values.* V_W = *49.88 ml. aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

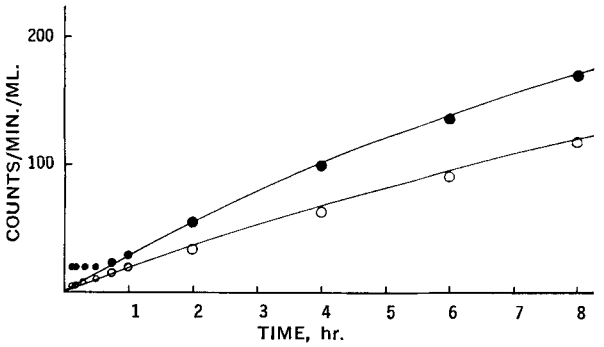

Figure 7—*Comparison of experimental data with theory for the release of cholesterol from Emulsion Systems I and II. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 0.48% oil of System I, ●; and experimental points from 0.48% oil of System II, ○. Curves are theoretically computed values.* V_W = *49.76 ml. aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

As is easily seen, the two equations, Eqs. 5 and 6, may be used to solve for C_b as a function of time when V_W, D, P, K, and the particle-size distribution (*e.g.*, Table I) are known. However, this cannot be done analytically and one must resort to numerical methods. The flow diagram based on the FORTRAN IV language (IBM 360 digital computer) for solving Eqs. 5 and 6 is given in Fig. 11.

The computer calculations for the various experimental conditions were carried out for both cholesterol uptake and release. These are presented as the smooth curves in Figs. 2–9. As can be seen, a P value of around 1.7 to 2.2×10^{-7} cm. sec.$^{-1}$ was found to give good agreement of all experiments with the theoretical relations of Eqs. 5 and 6. The experiments with the polysorbate 80 ester sample appeared to correspond to slightly higher rates ($P \simeq 2.2 \times 10^{-7}$ cm. sec.$^{-1}$) than those with the unpurified surfactant ($P \simeq 1.7 \times 10^{-7}$ cm. sec.$^{-1}$).

In all of these calculations, a choice of D from 10^{-6} to 10^{-10} cm.^{2} sec.$^{-1}$ made no significant difference in the results. The lower limit (10^{-10} cm.^{2} sec.$^{-1}$) would be an unexpectedly low value even when the principal species in 0.1% polysorbate 80 is micellar in nature.

An idea of the sensitivity of the fit of the experimental data to the theoretical predictions can be obtained by referring to Fig. 12. It can be seen that the precision in the determination of P is in the neighborhood of ± 10% for the release experiments. For the

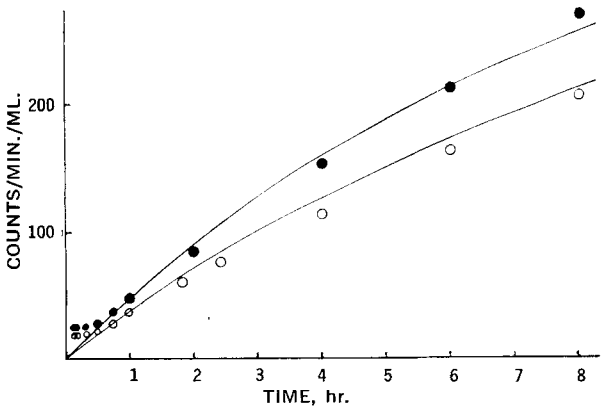

Figure 8—*Comparison of experimental data with theory for the release of cholesterol from Emulsion Systems I and II. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 phase* versus *time in hours. Key: experimental points from 0.96% oil of System I, ●; and experimental points from 0.96% oil of System II, ○. Curves are theoretically computed values.* V_W = *49.52 ml. aqueous polysorbate 80 phase;* P *value for both systems is* 1.7×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

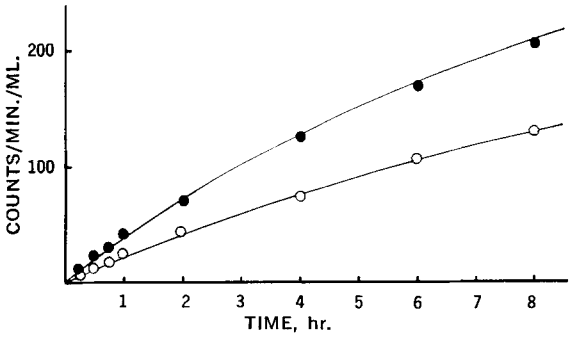

Figure 9—*Comparison of experimental data with theory for the release of cholesterol from Emulsion III. Counts/min./ml. of drug in the aqueous 0.1% polysorbate 80 ester phase* versus *time in hours. Key: experimental points from 0.4% oil, ○; and experimental points from 0.8% oil, ●. Curves are theoretically computed values.* P *value for both dilutions is* 2.2×10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

uptake experiments, while the general agreement of the experimental data with theory is good, somewhat larger discrepancies were found at larger times.

One of the factors which may limit the accuracy of the treatment of the transport by Eqs. 5 and 6 is the particle-size distribution data obtainable with the Coulter counter. The situation in the case of Emulsion I of this study is quite satisfactory as it has yielded a 95% mass balance. In other experiments, comparably good mass balances have been found (1). However, one should question the accuracy of the analysis procedure when, for example, only 66% (Emulsion II) of the oil phase can be accounted for by the Coulter counter data.

Whenever the mass balance is poor, the cause may be attributed to one of five possibilities: (*a*) volumetric errors in transferring the oil in the stock emulsion to the reaction flask; (*b*) significant number of droplets below the Coulter counter sensitivity; (*c*) significant number of droplets too large to be accurately sized; (*d*) accuracy of the Coulter counter itself; and (*e*) solubilization of the oil in the aqueous media (intrinsic solubility of solubilization by, for example, surfactant).

In the experiments with Emulsion II, it is most likely that the discrepancy is not likely to be due to the following: (*a*) because of good precision of the partition coefficient data obtained with many solutes; (*b*) because this would require an unusual, essentially bimodal, distribution of droplet sizes; (*d*) because many other emulsions have yielded better mass balances than 66%; and (*e*) because no time effects on size distribution were observed. It is therefore proposed that the absence of good mass balance in the case of Emulsion II is the result of (*c*), *i.e.*, the presence of a significant number of large droplets that was not measured by the instrument.

In order to assess the effect of neglecting the large droplets in the calculations with Eqs. 5 and 6, a computation was carried out with a particle-size distribution for Emulsion II (see Table I, Column 9), but for which a 93% mass balance was obtained by including more

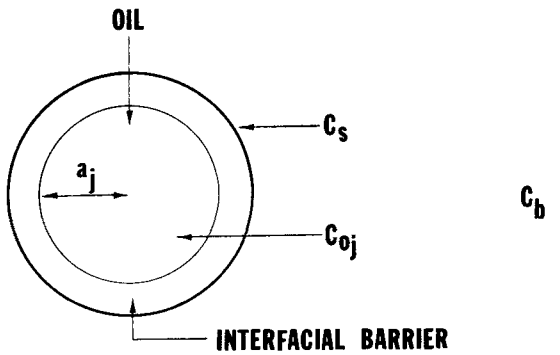

Figure 10—*The physical model that describes the uptake and/or release of the solute (cholesterol) across the oil droplet. Key:* a_j = *droplet radius;* C_{oj} = *solute concentration in the oil phase;* C_s = *aqueous solute concentration just outside the adsorbed film;* C_b = *solute concentration in the aqueous 0.1% polysorbate 80 phase.*

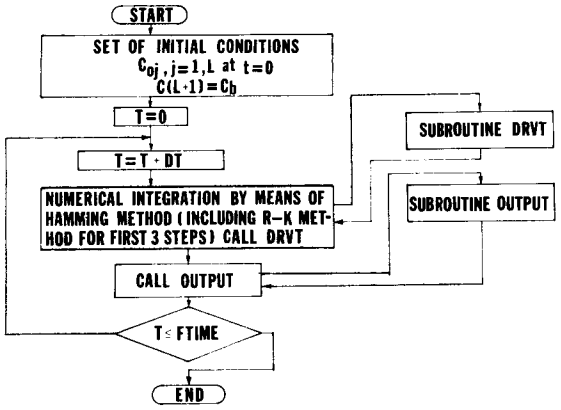

Figure 11—*Computer flow diagram showing the procedure for computation of* C_b *and* C_{oj}.

large droplets in the extrapolation. In Fig. 13, the broken curve shows the computed release-time behavior for this situation. As can be seen, for initial release the difference between the 66% mass balance and the 93% mass balance is relatively small and comparable to the scatter of the experimental data.

DISCUSSION

The results of the analysis of data clearly show that an interfacial barrier was operative in these experiments. The effective permeability coefficient value of 1.7 to 2.2 × 10^{-7} cm. sec.$^{-1}$ corresponds to a rather large interfacial resistance to transport which might appear to be rather surprising for the simple oil–water system in these studies.

It is of interest to compare this value with those reported in the literature for transport of organic compounds across biological and "synthetic" biological membranes. Bean *et al.* (8) reported on the bilayer lipid membrane permeability coefficient of a number of

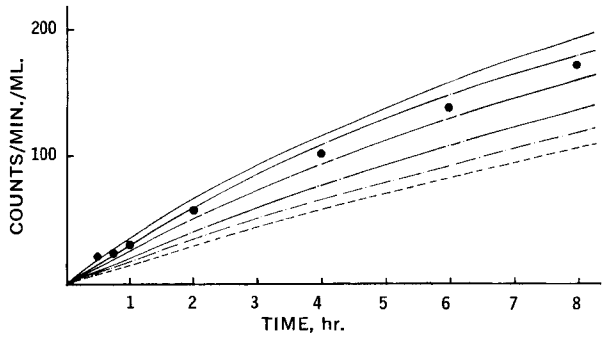

Figure 12—*Sensitivity of the fit of the experimental data to the theoretical predictions. The points are experimental results for 0.48% release experiments whose data gave these theoretical curves with a change in the* P *values. Key:* P *value 9* × 10^{-8} *cm. sec.*$^{-1}$, - - -; P *value 1* × 10^{-7} *cm. sec.*$^{-1}$, —·—; P *value 1.2* × 10^{-7} *cm. sec.*$^{-1}$, ·-·-·; P *value 1.5* × 10^{-7} *cm. sec.*$^{-1}$, — - - —; P *value 1.8* × 10^{-7} *cm. sec.*$^{-1}$, — ·; P *value 2* × 10^{-7} *cm. sec.*$^{-1}$, —; K *value is 200.*

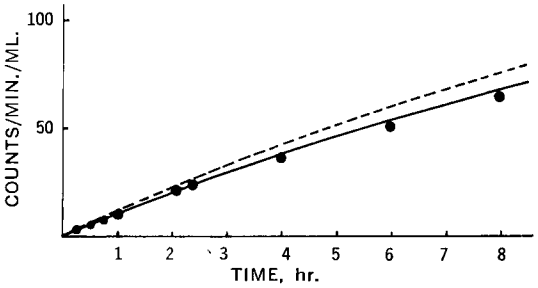

Figure 13—*Effect of neglecting the large droplets in the calculations with Eqs. 5 and 6 as computed for Emulsion II. Key: the broken curve, - - -, shows the computed release-time behavior for a 93% mass balance; and the full curve, —, shows the computed release-time behavior for a 66% mass balance.* P *value for both systems is 1.7* × 10^{-7} *cm. sec.*$^{-1}$; K *value is 200.*

organic compounds. The authors found that the permeability coefficients for most simple organic molecules are in the range of 10^{-4} to 10^{-6} cm. sec.$^{-1}$. Holder and Hayes (9) found red blood cell permeability coefficients of a number of sulfonamides to be in the range of 10^{-4} to 10^{-7} cm. sec.$^{-1}$. Rothblat *et al.* recently (10) reported data on the uptake of cholesterol by $L_{5178}Y$ tissue culture cells. A rough calculation with their data yields a permeability coefficient in the neighborhood of 10^{-6} cm. sec.$^{-1}$. Thus the interfacial barrier for cholesterol at the hexadecane–0.1% polysorbate 80 system is of the same order of magnitude if not somewhat greater than those observed in a number of biological situations.

It is hoped that this investigation and the continuing studies will provide a basis for understanding the detailed molecular factors in the various biological and biopharmaceutical situations.

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