Interfacially Controlled Transport of Micelle-Solubilized Sterols across an Oil/Water Interface in Two Ionic Surfactant Systems

VIJAY SURPURIYA and W. I. HIGUCHI▲

Abstract □ The kinetics of cholesterol and desmosterol transfer from aqueous sodium lauryl sulfate solutions and from sodium taurocholate–lecithin solutions into hexadecane and vice versa were 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. It was shown in this study that in the case of sodium taurocholate–lecithin systems, the resistances to the transport of cholesterol and desmosterol across the oil droplet interface were of the order of 10^5 times greater than that for the diffusion-controlled mechanisms. These values were comparable to, and had the same rank order as, those found in the polysorbate 80 surfactant systems. Changing the concentrations of sodium taurocholate–lecithin but keeping their ratio constant had very little effect on the permeability coefficient of cholesterol and desmosterol. These results suggested that for this case an interface-controlled mechanism is operative, which involves the solute–micelle complex in the rate-determining step. In the sodium lauryl sulfate systems, increasing the surfactant concentration caused a decrease in the effective permeability coefficients for cholesterol and desmosterol. This decrease was approximately inversely related to the surfactant concentration. These results support a mechanism in which the free solute is mainly involved in the interfacial rate-determining step for the sodium lauryl sulfate case.

Keyphrases □ Cholesterol transfer—oil/water interface, ionic surfactant system, interfacially controlled □ Desmosterol transfer—oil/water interface, ionic surfactant system, interfacially controlled □ Transport kinetics—interface controlled, sterols across oil/water interface in ionic surfactant systems □ Multiparticulate dispersion technique—sterols across oil/water interface, ionic surfactant systems

Recent investigations (1–4) in these laboratories led to the development of techniques that are well suited to the study of interfacial factors influencing interphase transport of drugs and other pharmaceutically and physiologically important compounds. These studies demonstrated the existence of significant resistances to solute transfer at oil/water interfaces under a wide variety of conditions. One interesting recent finding involving the use of these techniques was that the transport of some sterols across the polysorbate 80–aqueous hexadecane interface is best explained by an interface-controlled mechanism in which the drug-containing micelle participates in the rate-determining step.

The purpose of this report is to describe the results of interfacial barrier-controlled transfer of sterols with two ionic surfactants: sodium lauryl sulfate and the sodium taurocholate–lecithin combinations. As will be seen, the micelle delivery mechanism was found to be consistent with the results of the sodium taurocholate–lecithin experiments. However, the data obtained with the sodium lauryl sulfate system support the mechanism in which the free sterol is mainly involved in the rate-determining step.

EXPERIMENTAL

General Considerations in Experimental Design—The multiparticulate dispersion technique (1, 2) provides both good reproducibility and sensitivity for interfacial barrier determinations. It was decided to carry out both water-to-oil (solute uptake) and oil-to-water (solute release) transport experiments to assure the reliability of the deduced interfacial permeability coefficients. Two different surfactant systems, sodium lauryl sulfate and mixtures of sodium taurocholate and lecithin, were selected for these studies.

Preparation of Stock Emulsions—Because emulsions with satisfactory particle-size distributions could not be prepared using sodium lauryl sulfate as the emulsifying agent, the stock emulsion for the sodium lauryl sulfate system studies was made using polysorbate 80 as the emulsifying agent. This was then diluted into sodium lauryl sulfate[1] solutions for the transport experiments[2]. This stock emulsion was prepared by mixing 8 ml. of hexadecane[3] and 10 ml. of a 1.0% aqueous polysorbate 80[4] solution, making up to 100 ml. with distilled water, and then homogenizing this mixture for 75 sec. using a blender[5].

For the sodium taurocholate[6]–lecithin[6] studies, emulsions were prepared by mixing 8 ml. of hexadecane and making up to 100 ml. with solutions of different concentrations of sodium taurocholate–lecithin prepared beforehand. The mixtures were homogenized for 75 sec. in the blender.

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

Figure 1 shows the cumulative particle-size distribution data obtained from a hexadecane–sodium taurocholate–lecithin emulsion using the Coulter counter[7]. These data were used to obtain the

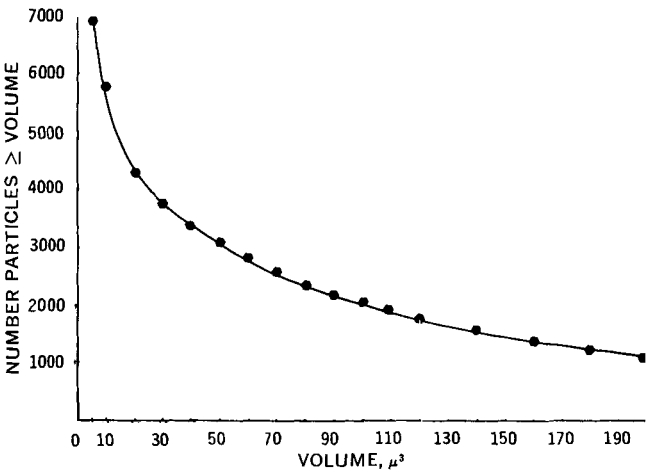

Figure 1—*Cumulative particle-size distribution data obtained from a hexadecane–sodium taurocholate–lecithin emulsion, using the Coulter counter.*

[1] Eastman Kodak Co., Rochester, N. Y.
[2] The sodium lauryl sulfate experiments always contained 0.01% polysorbate 80 in addition to the much larger amounts of sodium lauryl sulfate.
[3] Matheson, Coleman & Bell, Norwood, Ohio.
[4] Emulsion Engineering Inc., Elk Grove Village, Ill.
[5] Waring, Sargent Welch Scientific Co., Chicago, Ill.
[6] Mann Research Labs, New York, N. Y.
[7] Model A, Coulter Electronics, Hialeah, Fla.

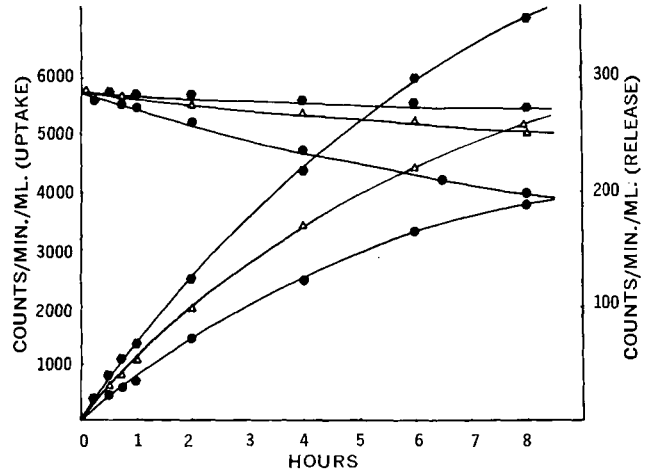

Figure 2—*Comparison of experimental data with theory for the uptake and release of cholesterol in sodium lauryl sulfate system with 0.8% hexadecane. Key: ⬢, experimental, 1% sodium lauryl sulfate; Δ, experimental, 0.5% sodium lauryl sulfate; and ●, experimental, 0.3% sodium lauryl sulfate. Smooth curves represent theoretical predictions.*

differential size distribution employed in the treatment of the transport data. Similar results were obtained from the emulsion used in the sodium lauryl sulfate studies. Mass balance calculated from the size distribution data was generally found to be 85–95%. No significant size distribution changes were found with any of the emulsions for up to 8 hr. during a transport experiment.

Uptake Experiment—Predetermined dilutions of the emulsions were made in sodium lauryl sulfate or sodium taurocholate–lecithin solutions. Then 1.5×10^{-7} g. of cholesterol-4-^{14}C or desmosterol-26-^{14}C (specific activity: cholesterol-4-^{14}C = 30.6 mc./mmole, and desmosterol-26-^{14}C = 48.5 mc./mmole) in 3 ml. of a sodium lauryl sulfate or a sodium taurocholate–lecithin solution was added to 100 ml. of the diluted emulsion containing the same surfactants, and the mixture was shaken gently at 30° in a shaker[8]. Five-milliliter samples were pipeted out at different time intervals, and the aqueous supernatant phases were separated from the oil by high speed centrifugation[9] for 1–1.5 min. The total time of sampling and centrifugation was very short compared to the experimental times and, therefore, was neglected in the data analysis. Out of the clear aqueous solution collected, 1 ml. was pipeted out into a liquid scintillation vial. Then 10 ml. of a liquid scintillation cocktail[10] was added to the vial, and the samples were quantitatively analyzed with the liquid scintillation system[11].

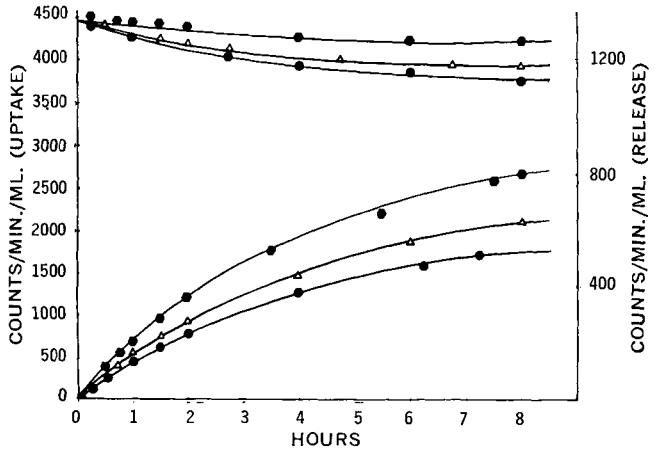

Figure 3—*Comparison of experimental data with theory for the uptake and release of desmosterol in sodium lauryl sulfate system with 0.8% hexadecane. Key: ⬢, experimental, 1% sodium lauryl sulfate; Δ, experimental, 0.5% sodium lauryl sulfate; and ●, experimental, 0.3% sodium lauryl sulfate. Smooth curves represent theoretical predictions.*

[8] Burrell Wrist-Action, Burrell Corp., Pittsburgh, Pa.
[9] Lourdes Instrument Corp., Brooklyn, N. Y.

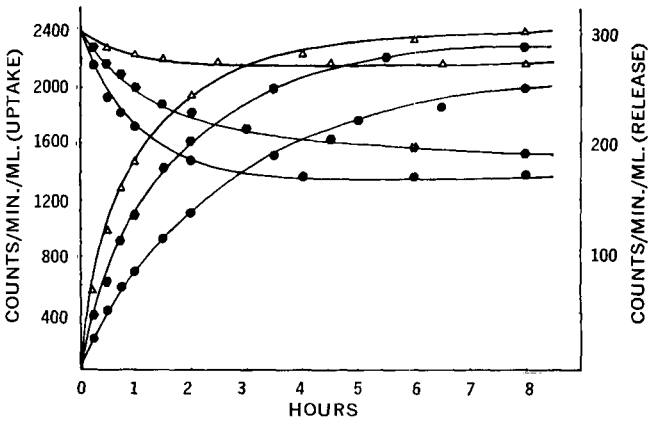

Figure 4—*Comparison of experimental data with theory for the uptake and release of cholesterol in the sodium taurocholate–lecithin system with 0.8% hexadecane. Key: Δ, experimental, 2% sodium taurocholate + 0.2% lecithin; ⬢, experimental, 1% sodium taurocholate + 0.1% lecithin; and ●, experimental, 0.5% sodium taurocholate + 0.05% lecithin. Smooth curves represent theoretical predictions.*

Release Experiments—For the release experiments, the same procedure was used for the preparation of the stock emulsions. However, the radioactive compound was added to the oil (6.68×10^{-7} g. of cholesterol or desmosterol/ml. of hexadecane) prior to the emulsification step. Aliquots of the stock emulsion containing the radioactive compound were then added at zero time to predetermined volumes of sodium lauryl sulfate or sodium taurocholate–lecithin solutions. Sampling and analysis of the aqueous phases were carried out in the same manner as in the uptake experiments.

RESULTS

In all of the uptake and release experiments, significant changes in the aqueous radioactive compound concentrations were found up to 8 hr. The results of the transport experiments are presented in Figs. 2–5. The uptake plots give the actual aqueous concentrations of the radioactive compound as a function of time. In the release plots, the initial (zero-time) expected aqueous concentrations were subtracted from all of the determinations.

Figure 6 shows the model used 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.

The rate of transport of the radioactive compound into or out of the oil droplet is given by:

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

where a_j = radius of the oil droplet, P = apparent permeability coefficient for the interfacial barrier, D = relevant diffusion coefficient for cholesterol or desmosterol in sodium lauryl sulfate or sodium taurocholate–lecithin solution, C_b = total bulk aqueous radioactive compound concentration, and C_{bj}' is defined by:

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

where K is the effective hexadecane–aqueous sodium lauryl sulfate or hexadecane–sodium taurocholate–lecithin partition coefficient

[10] The cocktail consisted of: 2,5-diphenyloxazole (7 g.), 1,4-bis[2-(5-phenyloxazolyl)]benzene (50 mg.), naphthalene (50 g.) and *p*-dioxane (*q.s.* 1000 ml.).
[11] Beckman Instruments, Inc., Fullerton, Calif.

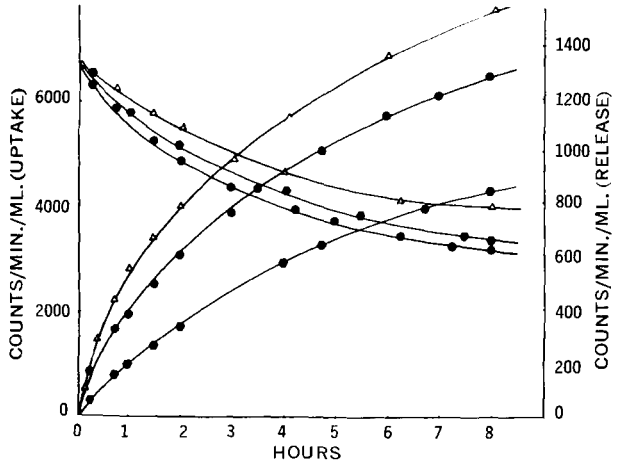

Figure 5—*Comparison of experimental data with theory for the uptake and release of desmosterol in the sodium taurocholate–lecithin system with 0.8% hexadecane. Key: Δ, experimental, 2% sodium taurocholate + 0.2% lecithin; ●, experimental, 1% sodium taurocholate + 0.1% lecithin; and ⬢, experimental, 0.5% sodium taurocholate + 0.05% lecithin. Smooth curves represent theoretical predictions.*

for cholesterol or desmosterol, and C_{oj} is the concentration of the radioactive compound in the oil droplet.

When G_j is positive, the situation is for uptake; when G_j is negative, the situation is for release.

When $a_jP \ll D$, then a_jP can be neglected and Eq. 1 reduces to:

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

which is the appropriate limiting expression for the interfacial barrier-controlled transfer of the radioactive compound.

For the rate of transport of the radioactive compound into or out of the droplet, one can also write:

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

where $V_{oj} = {}^4/_3\pi a_j^3$, the volume of the oil droplet, and t is the time.

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

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

From the material balance considerations in the system, one can also write:

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

Table I—Effective Permeability Coefficients (P) for Cholesterol and Desmosterol in the Sodium Taurocholate–Lecithin System[a]

Sodium Taurocholate + Lecithin, %	K (Experimental) Uptake	K (Experimental) Release	K (Used)	P, cm./sec. Uptake	P, cm./sec. Release
Cholesterol					
2 + 0.2	42	39	40	2.5×10^{-7}	2.3×10^{-7}
1 + 0.1	103	115	125	2.4×10^{-7}	2.45×10^{-7}
			110	2.5×10^{-7}	2.1×10^{-7}
0.5 + 0.05	205	200	220	2.2×10^{-7}	2.05×10^{-7}
			200	2.2×10^{-7}	1.8×10^{-7}
Desmosterol					
2 + 0.2	38	46	45	6.5×10^{-7}	6.3×10^{-7}
1 + 0.1	77	85	85	7.0×10^{-7}	6.5×10^{-7}
0.5 + 0.05	144	171	170	6.5×10^{-7}	6.3×10^{-7}

[a] The calculations also show the sensitivity of the P values to the K used.

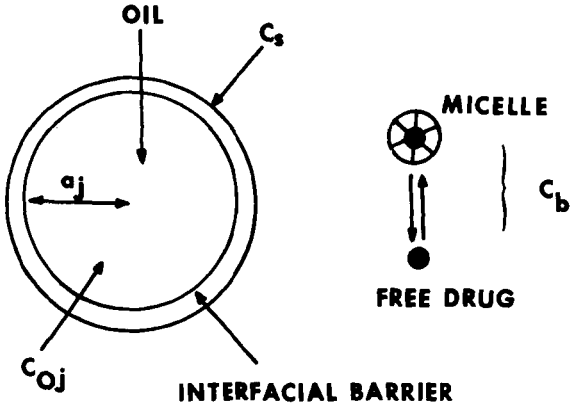

Figure 6—*Physical model describing the uptake and/or release of solutes by the oil droplet. Key:* a_j = *radius of the oil droplet;* C_{oj} = *solute concentration in the oil phase;* C_s = *aqueous solute concentration just outside the adsorbed film; and* C_b = *solute concentration in the aqueous phase.*

where Vw is the volume of the aqueous phase, N_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.

Equations 5 and 6 are used to solve for C_b when Vw, D, P, K, and the particle-size distribution data are known. The IBM-360 digital computer was used for calculation. The flow diagram based on the FORTRAN IV language for solving Eqs. 5 and 6 is given in Fig. 7.

The computer calculations were carried out for both uptake and release experiments. These are presented as smooth curves in Figs. 2–5.

In previous investigations (3, 4) with polysorbate 80 as the surfactant, it was shown that the resistance to the transport of cholesterol and desmosterol across the oil droplet interface was of the order of 10^6–10^7 times greater than that for diffusion-controlled mechanisms. In the present studies with the two surfactant systems, all of the results (Tables I and II) were also found to be consistent with the interfacial barrier-controlled interpretations; that is, the rates of transport were found to be much too small to be explained on the basis of bulk diffusion-controlled processes. In all calculations, choices for D from 10^{-6} to 10^{-10} made no significant differences in the results.

In studies of this type, one factor which might limit the accuracy of the permeability coefficient determinations is the particle-size distribution data obtainable with the Coulter counter. However, the situation in the present study was quite favorable because it yielded a 85–95% mass balance with all emulsions prepared for sodium lauryl sulfate and sodium taurocholate–lecithin experiments.

DISCUSSION

The results of the experiments, the accompanying treatment of the data, and the consideration of the basic assumptions in Eqs. 5 and 6 show that the method is highly quantitative mechanistically; for example, fits of the experimental data to the theory with

Table II—Effective Permeability Coefficients (P) for Cholesterol and Desmosterol in the Sodium Lauryl Sulfate System

Sodium Lauryl Sulfate, %	K (Experimental) Uptake	K (Experimental) Release	K (Used)	P, cm./sec. Uptake	P, cm./sec. Release
Cholesterol					
1.0	20	30	30	2.0×10^{-8}	2.2×10^{-8}
0.5	65	95	80	5.0×10^{-8}	6.2×10^{-8}
0.3	290	340	315	1.2×10^{-7}	2.2×10^{-7}
Desmosterol					
1.0	10	10.5	13	4.3×10^{-8}	4.2×10^{-8}
0.5	25	23	30	7.8×10^{-8}	7.6×10^{-8}
0.3	51	65	60	1.0×10^{-7}	1.3×10^{-7}

the single adjustable parameters (P) were found to be extremely good in most instances (Figs. 2–5). Consequently, the meaning of the permeability coefficient (P) is not physically ambiguous to the extent of distinguishing between an interfacial barrier-controlled and bulk diffusion-controlled situation.

The model expressed by Eqs. 5 and 6 has not considered whether only free solute (*i.e.*, nonmicellar solute) is involved in the rate-determining step. It is possible then that either or both free solute and solute species interacting with sodium lauryl sulfate or sodium taurocholate–lecithin (*e.g.*, micellized solute) in the aqueous phase may be crossing the interfacial barrier. In such cases, the P values obtained (Tables I and II) would be effective permeability coefficients representing free solute and/or micelle-solubilized transport.

If only the free solute is involved in the transition state, *i.e.*, the rate-determining transport step, one should expect P to be a function of the ratio, R, of the free solute to the total solute in the aqueous phase so that:

$$P_f = \frac{P}{R} \qquad \text{(Eq. 7)}$$

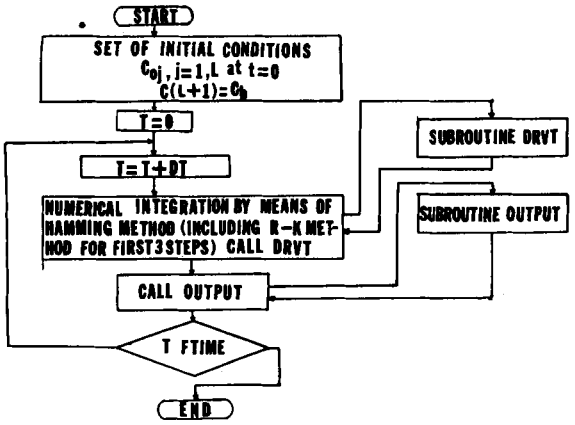

Figure 7—*Computer flow diagram showing the procedure for computation of* C_b *and* C_{oj}.

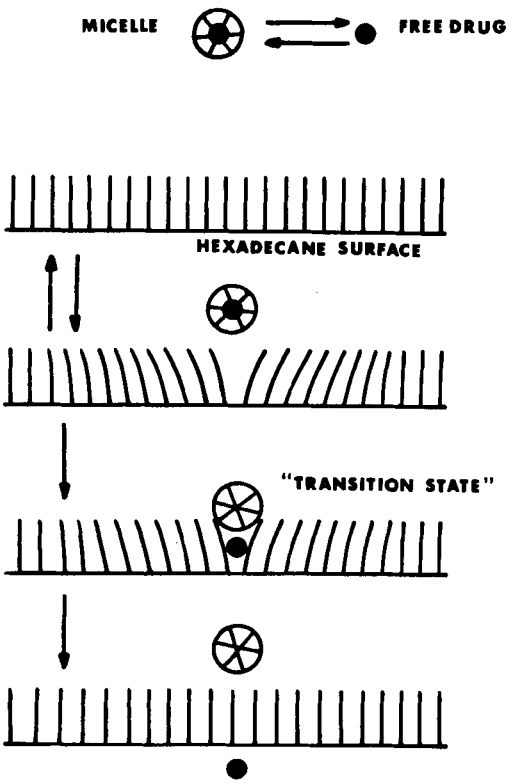

Figure 8—*Proposed model for the micelle delivery mechanism for the transport of solutes across the oil/water interface.*

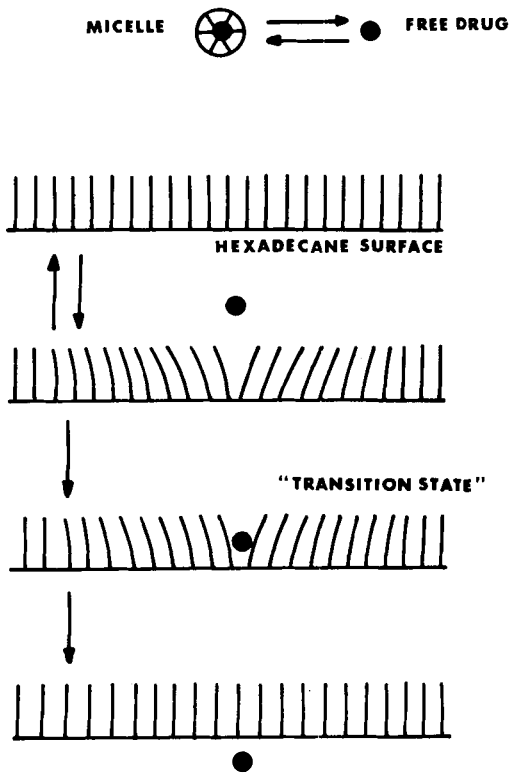

Figure 9—*Proposed model for the free drug delivery mechanism for the transport of solutes across the oil/water interface.*

where P_f is the intrinsic interfacial permeability coefficient for the free solute and is constant for constant barrier conditions and independent of the total solute in the aqueous phase.

On the other hand, if micellized solute were to be primarily involved in the transition state, then:

$$P_m = P(1 - R) \qquad \text{(Eq. 8)}$$

would give the intrinsic permeability coefficient, P_m, for the interfacial transfer of the micelle-solubilized solute.

In the sodium taurocholate–lecithin system, it was found that changing the concentrations but keeping their ratio constant had very little effect on the permeability coefficient for cholesterol and desmosterol (Table I). Thus, the P values are not dependent on the concentration of sodium taurocholate and lecithin. This strongly suggests that a mechanism involving solute-containing micelles is operative. These results are consistent with the interpretations proposed by Bikhazi and Higuchi (3, 4) for the polysorbate 80 system.

Figure 8 shows the proposed model for the micelle delivery mechanism. Note that even though free drug as well as micelle-containing drug is present in the solution, only the micelle takes part in the transfer of drug across the hexadecane–water interface. It interacts with the hexadecane–water interface and unloads the solute, which passes into the oil phase, and the micelle returns to the aqueous phase.

The difference in the P values between cholesterol and desmosterol may be rationalized on the basis of the proposed model if it is assumed that because of the somewhat greater hydrophobic nature of cholesterol, the free cholesterol concentration in the "transition state" is less than that for desmosterol. This is the same interpretation proposed to describe the P value differences among the sterols investigated in the polysorbate 80 system.

In strong contrast to the findings with the sodium taurocholate–lecithin and the polysorbate 80 systems, it was found that the permeability coefficients in the sodium lauryl sulfate studies were dependent upon the surfactant concentration (Table II). As can be seen, there is almost a direct proportionality between the K and P values. Thus, in these experiments, the data closely approximate the relationship given by Eq. 7 and support the model in which the free solute is involved in the rate-determining step.

Figure 9 shows the proposed model for free drug delivery. Even though micelle-containing drug as well as free drug is present in the solution, according to this model, the micelle does not take part in the transfer of solute across the hexadecane–water interface and only the free drug is involved in the rate-determining step.

It should be stressed, however, that the limited data presented in Table II cannot exclude the possibility that some micelle transport may have contributed to the interphase transfer of the sterols. More extensive studies are desirable with regard to this question.

Since the present findings with the sodium taurocholate–lecithin system are very similar to those obtained with the polysorbate 80 system, the likelihood that an interfacial barrier-controlled process governs sterol absorption in rats (5) has been strengthened.

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Absorption Kinetics of Aspirin in Man following Oral Administration of an Aqueous Solution

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Abstract □ The absorption of aspirin was studied in four male subjects following an oral solution of 650 mg. The absorption process appeared to follow first-order kinetics, with a half-life ranging from 4.5 to 16.0 min. between subjects. Comparison of the area under the aspirin plasma concentration–time curve following intravenous and oral routes indicated that only 68% of the dose reached the peripheral circulation intact. A two-compartment model for both aspirin and its metabolite, salicylic acid, involving a constant fractional hydrolysis of aspirin during absorption, satisfactorily described both the aspirin and resultant salicylic acid plasma data. Methods used to calculate aspirin absorption kinetics are discussed.

Keyphrases □ Aspirin, oral—absorption kinetics, aqueous solution, man □ Absorption kinetics—aspirin, oral aqueous solution, man □ Pharmacokinetics, aspirin, oral aqueous solution—absorption kinetics, calculations, half-life, plasma concentration–time curves, two-compartment model □ Half-life—orally administered aspirin, first-order kinetics, man

The clinical importance of aspirin as a weak analgesic has led to numerous studies concerning the absorption of this drug in man (1–8). Several of these studies confirmed that, although aspirin is known to be rapidly hydrolyzed to salicylic acid *in vivo*, intact drug is absorbed and blood levels of aspirin can be demonstrated up to 1 hr. following an oral dose of the drug (1–8). Using a specific assay, Rowland and Riegelman (9) examined the kinetics of distribution and metabolism of aspirin in man following intravenous administration as its *N*-methylglucamine salt (10). The decay was biexponential with half-lives of 4 and 15 min. for the initial and final phases, respectively. A two-compartment model was the minimum that could be proposed which fitted both the observed aspirin and resulting salicylic acid plasma level data.

The present study was undertaken to examine both the absorption kinetics of aspirin and the availability or amount of unchanged drug reaching the peripheral circulation since, together with elimination, they influence the amount of aspirin in the body at any time. This drug was given in solution to obviate the effects of varying dissolution rates from tablets (11). A preliminary communication of these findings was published previously (12).

EXPERIMENTAL

Human Studies—Four male subjects were used in whom the pharmacokinetics of aspirin and salicylic acid following intravenous administration had been determined previously. Each subject ingested (within 15 sec.) 650 mg. aspirin dissolved in 250 ml. water, prepared just prior to administration. They remained seated for at least 2 hr. following the dose. Blood samples (5 ml.) were drawn from the antecubital vein usually at 0, 3, 6, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 240, 360, and 480 min. following aspirin administration. In all but one, a similar study was conducted following oral administration of 500 mg. salicylic acid in 250 ml. water. On another occasion, two subjects received intravenous logarithmic infusions of 650 mg. aspirin using an infusion pump (Harvard). The logarithmic infusion was achieved by lowering the rate of infusion at constant intervals. The duration of this interval determined the half-life. Plasma samples were assayed for aspirin using GLC, and salicylic acid was determined fluorometrically (9).

Everted Gut Studies—New Zealand white rabbits, weighing 3 kg., were used. Animals were killed with intraperitoneal thiopental, and various parts of the GI tract were removed immediately. Approximately an equal weight of each tissue (4 g.) was everted, filled with 8 ml. Krebs-Ringer solution, and placed into a 125-ml.