TWO-STEP INTERFACIAL BARRIER MECHANISM FOR THE TRANSPORT OF MICELLE-SOLUBILIZED SOLUTE ACROSS AN OIL–WATER INTERFACE

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SUMMARY

Experiments on the transport of cholesterol, desmosterol and hydroxycholesterol from aqueous sodium taurocholate-lecithin and aqueous sodium cholate–lecithin micelle systems into hexadecane have been carried out as a function of lecithin to bile salt concentration ratios. These data strongly support the previously proposed hypothesis that the kinetics of transport of cholesterol, desmosterol and hydroxycholesterol is (1) interfacially controlled and (2) involves a two-step process in which there is first a collision of the solute–micelle complex with the oil–water interface and this is followed by the release of the solute from the micelle in a largely polar environment at the interface. The experimental evidence for this mechanism is that (1) effective interfacial barrier permeability coefficients for sterol transport has been found to be independent of bile salt–lecithin concentrations, when the ratio of lecithin to the bile salt was kept constant, and (2) when the lecithin–bile salt ratio was increased, keeping the bile salt concentration constant, it was found that the interfacial barrier permeability coefficients for the sterols decreased.

INTRODUCTION

Recent investigations[1–5] in these laboratories have shown that the transfer of sterols between an oil phase (hexadecane) and an aqueous micellar phase is governed by processes at the interface rather than by bulk diffusion and/or convection. Furthermore, for those situations in which sodium taurocholate–lecithin micelles and polysorbate 80 (polyoxyethylene sorbitan mono-oleate) micelles were involved, the interfacial transport rates were best described by a two-step process (see Fig. 1). In the proposed mechanism, there is first a collision of the solute–micelle complex with the interface and then this is followed by the release of the solute from the micelle in the interfacial environment, which appears to be relatively hydrophilic in nature.

The first line of reasoning which has supported this mechanism is that the effective permeability coefficient, P, for the interfacial transport has been found to be relatively independent of the surfactant concentration at constant solute concentration. Therefore, the process appears to be independent of the free sterol concentration in the bulk aqueous phase and consequently, these data agree with the idea that the solute–micelle complex rather than the free sterol is involved in the activated complex.

Secondly, the P values for the sterols were found to increase with increasing solute polarity in both the taurocholate–lecithin and the polysorbate 80 systems, *viz.* P(hydroxycholesterol) > P(desmosterol) > P(cholesterol) > P(β-sitosterol). This relationship suggested that the second step, *i.e.* the release of the solute from the micelle occurs not in the oil phase but at the interface where the environment is still predominantly aqueous or hydrophilic.
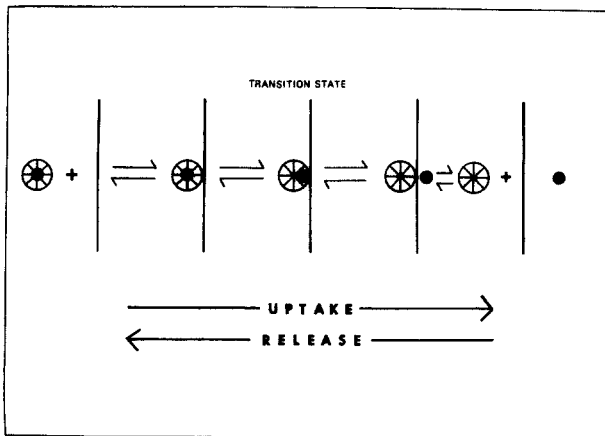

Fig. 1. Proposed model for the micelle delivery mechanism for the transport of solutes across the oil–water interface

The purpose of the present report is to describe the results of the experiments intended to generalize and to further explore the proposed mechanism. Experiments were conducted varying the taurocholate to lecithin ratios, and studies with sodium cholate–lecithin micelles were also carried out. As will be seen, the proposed two-step mechanism has been found to be consistent with the new experiments.

EXPERIMENTAL

The same general procedures utilized previously[5] were followed in the present studies. Basically the experiments involved the use of dispersions of micron size oil droplets as the oil phase into and out of which the solute was transferred as a function of time. Two different surfactant systems, mixtures of sodium taurocholate and lecithin and mixtures of sodium cholate and lecithin and three different sterols (obtained from New England Nuclear, Boston, Mass.), cholesterol, desmosterol and hydroxycholesterol were selected for these studies. (Sodium taurocholate, lecithin and sodium cholate were obtained from Mann Research Laboratories, New York, N.Y.)

A master emulsion was prepared for each system by mixing 8 ml of hexadecane (Matheson Coleman and Bell, Norwood, Ohio) and making up to 100 ml with the appropriate bile salt–lecithin solution prepared beforehand. The mixtures were homogenized for 75 s in the Waring blender (Sargent Welch Scientific Company, Chicago, Ill.).

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

the rate runs. Individual particle size distribution data for each emulsion were obtained by using the Coulter Counter (Model A, Coulter Electronics, Hialeah, Fla.) Fig. 2 shows the cumulative particle size distribution data obtained from the emulsion containing 1.0 % sodium taurocholate *plus* 0.1 % lecithin and 0.8 % hexadecane.
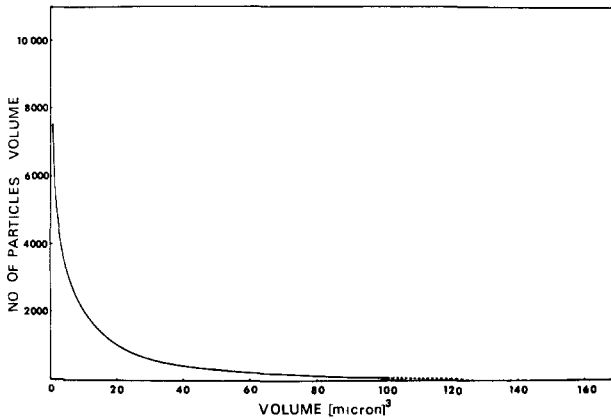

Fig 2 Cumulative particle size distribution data obtained from the emulsion of 1 0% sodium taurocholate + 0.1% lecithin + 0 8% hexadecane, using the Coulter Counter. Dotted portion of the curve represents the extrapolation of data.
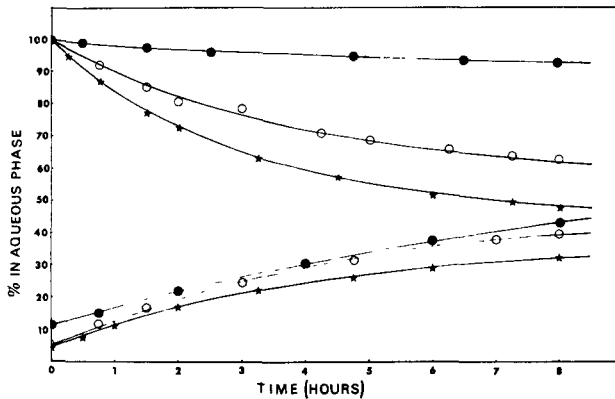

Fig 3. Comparison of experimental data with theory for the uptake and release of cholesterol in the sodium taurocholate–lecithin system with 0.8% hexadecane. ●—●, experimental, 1% sodium taurocholate + 0 5% lecithin; ○—○, 1% sodium taurocholate + 0 1% lecithin; ★—★ 1% sodium taurocholate + 0 05% lecithin Smooth curves represent theoretical predictions

These data were later used to obtain the differential size distribution employed in the treatment of the transport data. Similar data were obtained for each emulsion independently. It was also established that no significant size distribution changes occurred with any of the emulsions up to 8 h during a transport experiment.

The uptake and the release transport experiments were carried out essentially as before[5] employing radiolabeled sterols as the solutes.

RESULTS AND ANALYSIS OF DATA

The results of the transport experiments are presented in Figs 3–7. The plots give the percent of the radioactive compound in the aqueous phase as a function of time.

The following two equations derived earlier[5] were used to calculate the total bulk aqueous solute concentrations.

$$\frac{\mathrm{d}C_{0j}}{\mathrm{d}t} = \frac{3DP(C_b - C_{0j}/K)}{a_j(D + a_j P)} \tag{1}$$

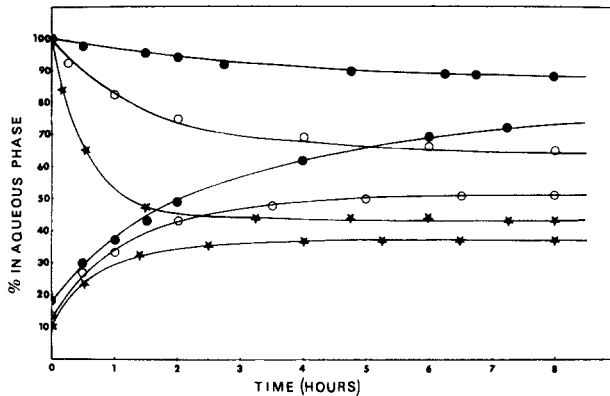
Fig 4. Comparison of experimental data with theory for the uptake and release of desmosterol in the sodium taurocholate–lecithin system with 0 8% hexadecane ●—●, experimental, 1% sodium taurocholate + 0 5% lecithin, ○—○, 1% sodium taurocholate + 0 1% lecithin, ★—★, 1% sodium taurocholate + 0 05% lecithin Smooth curves represent theoretical predictions
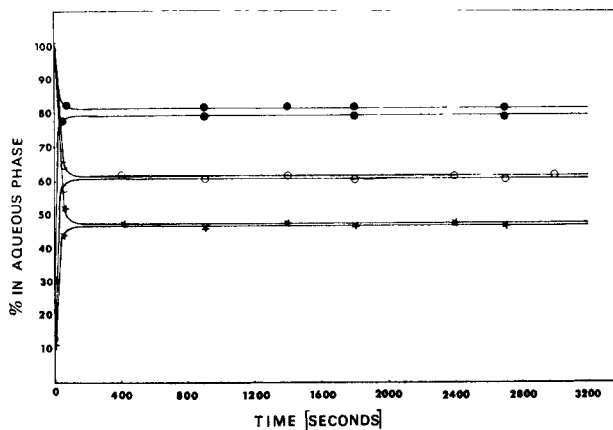

Fig 5 Comparison of experimental data with theory for the uptake and release of hydroxycholesterol in the sodium taurocholate–lecithin system with 0 8% hexadecane. ●—●, experimental, 2% sodium taurocholate + 0 2% lecithin, ○—○, 1% sodium taurocholate + 0 1% lecithin; ★—★, 0 5% sodium taurocholate + 0.05% lecithin. Smooth curves represent theoretical predictions

$$\frac{dC_b}{dt} = -\frac{4\pi}{3V_w} \sum_{j=1}^{L} a_j^3 N_J \frac{dC_{0J}}{dt} \qquad (2)$$

Where. a_J, radius of the oil droplet; P, the apparent permeability coefficient for the interfacial barrier, D, the relevant diffusion coefficient for cholesterol, desmosterol or hydroxycholesterol in the bile salt–lecithin solution; C_b, the total bulk aqueous solute concentration, C_{0J}, concentration of the solute in the oil droplet, K, the effective hexadecane/bile salt–lecithin partition coefficient for the solute; V_w, volume of the aqueous phase; N_J, number of droplets of sizes between a_J and a_{J+1}, L, the largest oil droplets in the system.

Eqns 1 and 2 may be solved for C_b with the help of IBM-360 digital computer when V_w, D, P, K and the particle size distribution data are known.
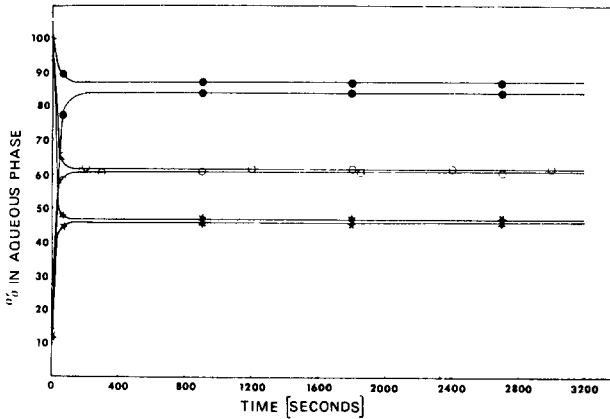

Fig 6 Comparison of experimental data with theory for the uptake and release of hydroxycholesterol in the sodium taurocholate–lecithin system with o 8% hexadecane ●—●, experimental, 1% sodium taurocholate + o 5% lecithin; ○—○, 1% sodium taurocholate + o 1% lecithin, ★—★, 1% sodium taurocholate + o o5% lecithin Smooth curves represent theoretical predictions
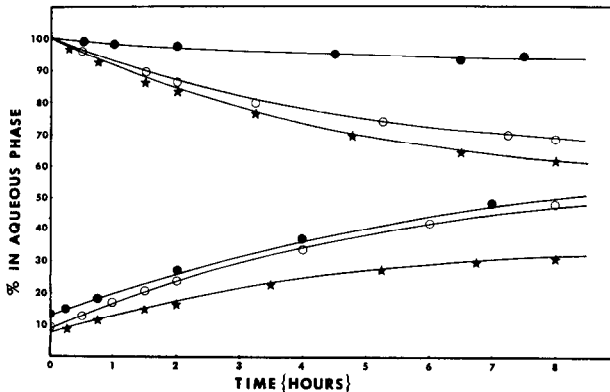

Fig 7 Comparison of experimental data with theory for the uptake and release of cholesterol in the sodium cholate–lecithin system with o 8% hexadecane ●—●, experimental, 1% sodium cholate + o 5% lecithin; ○—○, 1% sodium cholate + o 1% lecithin; ★—★, o 5% sodium cholate + o o5% lecithin. Smooth curves represent theoretical predictions.

Fitting of the experimental data by the theory was accomplished by using P as the only adjustable parameter. The K values were known from the plateau (equilibrium) portion of the uptake and release data. While the D values were not known accurately, their influence were estimated to be negligible in determining P for these systems.

The generally good fits obtained between experiments and theory are shown in Figs 3–7. This gives high credence to the P values as a quantitative measure of the interfacial transportability.

DISCUSSION

Influence of the lecithin–sodium taurocholate ratio upon P

Tables I–IV summarize the results of these studies. As was pointed out previously[5], varying the surfactant concentration while keeping the lecithin–sodium taurocholate ratios constant did not appear to change P It can be seen, however, that varying the lecithin–sodium taurocholate ratios markedly altered the P values for sterol transport. Increasing the lecithin–taurocholate ratio at constant taurocholate dramatically decreased the P values. This was found to be uniformly true for all three sterols.

The results appear to be significantly consistent with the proposed mechanism. The presence of lecithin in the micelle would be expected to greatly increase the affinity of the micelle for the sterols. Consequently, it is reasonable to expect that the release tendency for the sterol from the micelle should be reduced when lecithin is present in the micelle.

The decreases in P with increase in lecithin in the micelle parallels the decreases in K with lecithin. The increases observed in cholesterol solubility in taurocholate micellar solutions when increments of lecithin are added are also of the same order of magnitude[6]. This argues well in favour of the idea that the primary effect of lecithin upon P is to reduce the release tendency of the sterol from the micelle in the activated state.

It is of interest to note that K is decreased when either the taurocholate–lecithin

TABLE I

EFFECTIVE PERMEABILITY COEFFICIENTS (P) FOR CHOLESTEROL IN THE SODIUM TAUROCHOLATE–LECITHIN SYSTEM

Sodium taurocholate + lecithin (%)	K (Experimental)		K (Used)	$10^7 \times P$ (cm/s)	
	Uptake	Release		Uptake	Release
2 0 + 0.2	42	39	40	2 5	2 3
1 0 + 0 1	103	115	125	2 4	2 45
			110	2 5	2 1
0 5 + 0 05	205	200	220	2.2	2 05
			200	2.2	1 8
1 0 + 0 5	19 3	19 4	19.4	0 22	0 21
1.0 + 0 1	103 0	115 0	110	2 5	2 1
1.0 + 0.05	170.0	172 0	170	4 0	4 0

concentration or the lecithin–taurocholate ratio is increased. However, it is only in the latter situation that P is affected. This means that it is the individual micelle affinity for the sterol and not the collective micellar affinity for the sterol that is important in the interfacial mechanism. Therefore, these results strongly support the micelle involved interfacial step rather than the free solute involved interfacial transfer. Finally it appears (see Tables I and II) that, quantitatively, the influence

TABLE II

EFFECTIVE PERMEABILITY COEFFICIENTS (P) FOR DESMOSTEROL IN THE SODIUM TAUROCHOLATE-LECITHIN SYSTEM

Sodium taurocholate + lecithin (%)	K (Experimental)		K (Used)	$10^7 \times P$ (cm/s)	
	Uptake	Release		Uptake	Release
2 0 + 0 2	38	46	45	6 5	6.3
1 0 + 0 1	77	85	85	7 0	6 5
0 5 + 0 05	144	171	170	6.5	6.3
1.0 + 0 5	20 7	20 2	20 5	0 85	0.85
1 0 + 0 1	77 0	85 0	85	7 0	6.5
1 0 + 0 05	180.5	183 0	182	20 0	20 0

TABLE III

EFFECTIVE PERMEABILITY COEFFICIENTS (P) FOR HYDROXYCHOLESTEROL IN THE SODIUM TAUROCHOLATE–LECITHIN SYSTEM

Sodium taurocholate + lecithin (%)	K (Experimental)		K (Used)	$10^4 \times P$ (cm/s)	
	Uptake	Release		Uptake	Release
2 0 + 0 2	31.6	33 0	32.0	2 0	2.0
1.0 + 0 1	80 0	82 0	80.0	2 0	2.0
0 5 + 0 05	152 0	155 0	153 0	2 0	2 0
2 0 + 1 0	7.55	8 56	8 0	0 4	0.4
1 0 + 0 5	18.5	18 9	18 5	0 4	0 4
1 0 + 0 5	18 5	18 9	18 5	0 4	0 4
1 0 + 0 1	80 0	82 0	80.0	2 0	2.0
1 0 + 0 05	146 0	148 0	148 0	4 0	4.0

TABLE IV

EFFECTIVE PERMEABILITY COEFFICIENTS (P) FOR CHOLESTEROL IN THE SODIUM CHOLATE–LECITHIN SYSTEM

Sodium cholate + lecithin (%)	K (Experimental)		K (Used)	$10^7 \times P$ (cm/s)	
	Uptake	Release		Uptake	Release
1.0 + 0.5	15 5	18 5	16	0 3	0.3
1 0 + 0.1	83 4	87 0	85	2.0	2.0
0.5 + 0 05	163.0	162 0	162	2 1	2.0

of lecithin upon P is somewhat greater than the influence of lecithin upon K. There might be several possible explanations for this. One of these might be that the effective diffusivity of the micelle may decrease with increasing lecithin and this can reduce the micelle collision rate with the interface. Another explanation might be that lecithin may reduce the kinetic rate constant for the solute release and therefore, together with the reduced solute releasing tendency, permit P to decrease more than K.

Influence of the sterol polarity upon P

As was noted earlier, the polarity of the sterol appears to influence P. With increasing polarity, an increase in P has been found. This again argues well in favour of the sterol affinity idea and the greater tendency for the more polar sterols to be released in the activated state.

Comparison of the taurocholate-lecithin system with the cholate–lecithin and the polysorbate 80 systems

Table IV gives the results of the cholesterol transfer experiments with the sodium cholate–lecithin aqueous phase. Comparison of these results with the taurocholate–lecithin data in Table I shows remarkable similarities.

This dramatic insensitivity of the bile salt type suggests that the mechanism is probably of a rather general type and not one dependent strongly on details of the nature of the functional groups, charges *etc*. Recall also[3,4] that not only the rank order but the actual P values obtained for cholesterol and desmosterol transfer in the aqueous polysorbate 80–hexadecane system (Table V) are very close to those obtained in present studies with the bile salt–lecithin system.

TABLE V

EFFECTIVE PERMEABILITY COEFFICIENTS (P) FOR CHOLESTEROL, DESMOSTEROL, β-SITOSTEROL AND HYDROXYCHOLESTEROL IN THE POLYSORBATE-80 SYSTEM

Obtained by A Bıkhazı and W I Higuchi (taken from *Biochim Biophys Acta*, 233 (1971) 676)

Polysorbate 80 (%)	Cholesterol		Desmosterol		β-Sitosterol		Hydroxycholesterol	
	K	$10^7 \times P$ (cm/s)	K	$10^7 \times P$ (cm/s)	K	$10^8 \times P$ (cm/s)	K	$10^4 \times P$ (cm/s)
0 1	200	1 7–2 2	200	5 0–6 0	200	2 5	180	$\approx$3 0
0 3	100	1 2	—	—	—	—	—	—
0 5	—	—	70	5 0	72	2 5	46	$\approx$1 0
1 0	45	1 0	47	5 0	44	2 5	18 5	$\approx$5 0

The possible generalness of the proposed interfacial barrier transport mechanism for the sterols further underscores the probable importance of this mechanism in various biological situations such as sterol absorption in the gastrointestinal tract[3,4,7,8], transport of sterols into mammalian[9,10] and plant cells and in liver metabolism of cholesterol. The very recent work of Higuchi *et al.*[11-13] has shown that significant interfacial barriers are present during the dissolution of gallstones and that these barriers become increasingly important with increasing lecithin/bile

salt ratios in the dissolution media. Thus it is believed that the present findings should be very helpful in our eventual understanding of the many biological transport situations involving the sterols, especially cholesterol in the diseased and the normal states in man.

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