Solute Fluxes in Fat Absorption

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Abstract □ Diffusion rates of fatty acid (oleic and palmitic acids) in bile salt solution and in polyoxyethylene–polyoxypropylene copolymer solution depend on the amount of fatty acid solubilized and also on any physical restrictions to flow. Micelles of the two types of surfactant are significantly different in size. Assuming a passive process for the uptake of fatty acid, diffusion rates correlate with the observed uptake of fatty acid into everted intestinal sacs of the rat.

Keyphrases □ Fat absorption—solute fluxes, *in vitro* □ Diffusion cells—fat transfer □ Radioactive solute—fat transfer, diffusion cells □ Refractometry, differential—diffusion rates □ Scintillometry—analysis

The surface-active property of the natural surfactants, bile salts, in mediating lipolysis (1) and absorption by the small intestine of hydrophobic material, otherwise poorly soluble in an aqueous medium (2–4), is well accepted.

Synthetic nonionic surfactants, such as polyoxyethylene–polyoxypropylene copolymer,[1] also promote absorption of free fatty acids by everted intestinal sacs of the rat but less effectively than bile salts (5). They exert an effect *in vivo* from which conclusions are obscured due to the absence of a clear distinction between uptake from micellar solutions compared with uptake from emulsions (6).

Phase separation by ultracentrifugation has established that, for any one concentration of either surfactant and lipid composition, a fixed amount of fatty acid partitions between the micellar and emulsion phases (6); and for absorption *in vitro*, there is a clear relationship between the amount of fatty acid (oleic acid) solubilized and uptake into the everted intestinal sac (5).

If it is generally agreed that lipid is absorbed almost exclusively from the isotropic phase of intestinal content, micellar solubilization could promote uptake by increasing the chemical potential or effective concentration of lipid in the systems considered. An understanding of the mechanism, however, requires a knowledge of the dynamic nature of the physicochemical events in the absorptive process. Diffusion coefficients in the aqueous phase and the interfacial resistances to fatty acid movement between emulsion globules, micelles, and the surrounding aqueous medium are the kinetic quantities that provide the link between solubilization and uptake in this luminal stage of fat absorption.

The method involves the measurement of the fatty acid flux across a confined volume of solution, in practice a solution that is limited to the interior of a diaphragm and made less sensitive to gradients that lead to bulk flow in free solution. In addition, the use of diaphragms of different porosities provides data on solute fluxes when the movement of fatty acid in one or more of its states of aggregation, monomers, micelles, or emulsion globules is restricted.

The method has been applied to lipids in a qualitative manner (7) and follows from studies (8, 9) on the effect of solubilizing agents in increasing the driving force for diffusion of water-insoluble dyes. The capacity of Millipore filters, under conditions of free filtration, in separating emulsion and micellar phases of lipid dispersed by bile salts has been previously demonstrated (10). Using Millipore and sintered-glass diaphragms, the present study provides quantitative data on the diffusive movement of fatty acid, specifically oleic acid, in lipid systems of bile salts and of polyoxyethylene–polyoxypropylene copolymer.

The validity of a diffusion-limited model for the luminal stage of fatty acid absorption implies the presence in the gut of a still layer near the absorptive cell. While this is not proven, the glycocalyx coat could well produce an unstirred layer of fluid of greater thickness than at a clean cell surface; the presence of microvilli with some of the characteristics of physical pores (11) improves the analogy with the physicochemical model, making the data on fatty acid movement in its different states of aggregation perhaps directly applicable to the biological situation.

An understanding of the luminal stage of the absorptive process through the comparison of different surfactants may then provide insight into the much more complicated *in vivo* problem where, as yet, few clearcut physicochemical parameters have emerged.

EXPERIMENTAL

Materials—Sodium taurocholate (NaTC) moved as a single spot on activated silica gel at room temperature with ethyl acetate–methanol–glacial acetic acid (70:20:10). It was used as supplied.[2]

Sodium taurodeoxycholate (NaTDC) was synthesized from taurine and deoxycholic acid by the method of Norman (12) as modified by Hofmann (13). Bile salts were used in a 4:1 ratio with NaTC–NaTDC in all experiments.

Polyoxyethylene–polyoxypropylene copolymer (nominal molecular weight 8000) had a specific refractive index increment of 0.139 ml. $g.^{-1}$ and was used as supplied.

Glycerol 1-monooleate (monoolein) was shown by TLC to contain small amounts (~5%) of diglyceride and free fatty acid. By GLC the fatty acid composition was $C_{12:0}$ 1, $C_{16:1}$ 5, $C_{18:1}$ 85, $C_{18:2}$ 5.

Oleic acid and palmitic acid were ~98% pure by GLC. Radiochemical purity of oleic acid-1-^{14}C was certified[3] as 95–98% pure $C_{18:1}$ of which 7% was elaidic acid; 97% of the material was free fatty acid by TLC. Palmitic acid-9,10-^{3}H was certified 96% pure; 94% of the activity was in the fatty acid band.

Sodium dihydrogen phosphate and monohydrogen disodium phosphate were used as supplied.[4]

Methods—Two types of diaphragm cells are described. One uses a sintered-glass disk of ~30-μ average pore diameter, 4.9 cm.[2]

[1] Pluronic F68, Wyandotte Chemicals Corp., Wyandotte, MI 48193

[2] Koch-Light Laboratories, Colnbrook, England.
[3] Radiochemical Centre, Amersham, England.
[4] Ajax Chemicals, Sydney, Australia.

Table I—Cell Constants

Diaphragm	Cell Constant β (cm.$^{-2}$)
Sintered glass (SG), 30 μ	0.25 ± 0.02
Millipore (MP), 1.2 μ	9.54 ± 1.1
Millipore, 0.010 μ	4.37 ± 0.06

in area and 2 mm. thick, in the Stokes modification (14) of the Northrop–Anson (15) diffusion cell, in which stirring is effected by two polyethylene-encased[5] metal bars mounted one on each side close to, but not in contact with, the diaphragm. The cell was filled by suction and the lower chamber was closed with a tightly fitting ground-glass stopper. A film of silicone grease was placed on the external joint, and the entire lower chamber port was enclosed in a small polyethylene bag. The cell was immersed vertically in the thermostat and held in a rigid clamp to minimize vibration.

When steady-state conditions prevailed in the diaphragm, the solution in the upper chamber was removed and replaced with the solution of interest, either a nonradioactive solution of lower concentration if a chemical concentration gradient was to be maintained across the diaphragm or a nonradioactive solution of the same lipid concentration if the system was to be at chemical (but not isotopic) equilibrium. The upper chamber was closed with a ground-glass stopper with a perforation to the atmosphere, and the run was commenced by activating the stirrer with rotating magnets. At the end of the diffusion period, the entire solution (24.2 ml.) from each chamber was removed for analysis. Radioactivity measurements were made on triplicate samples (refractive index measurements also were made if surfactant diffusion in concentration gradient experiments were being followed).

The other diaphragm cell utilized Millipore cellulose acetate filters (25 mm. in diameter, 0.15 mm. thick) of different porosities, mounted vertically to expose 2.90 cm.^{2} to the solutions in each chamber (initially always 20 ml. in volume) and stirred by centrally located metal bars encased in Perspex and driven by rotating magnets mounted on the cell support platform. Solutions in both chambers were always of the same composition and concentration in lipid, *i.e.*, at chemical but not isotopic equilibrium.

At timed intervals over the period of one experiment, 0.5-ml. samples (usually six in all) were removed from each chamber for radioactivity analysis, but the liquid level at the end of the diffusion period never fell below the top of the diaphragm. Before use the cell was tested for leaks with an impermeable barrier.

Cells were calibrated using KCl as the standard diffusing solute. Chloride ion in stock solutions and in each chamber at the experiment end was determined by AgCl turbidity in 50% ethanol–water (after dilution to reduce Cl^- concentration to a convenient level) according to the method of Luce *et al.* (16). The calibration curve at 350 mμ was linear up to 14 mcg./ml. Cl.

Where surfactant diffusion rates were monitored, differential refractometry (in 10-cm. cells) with a Hilger–Rayleigh interferometer was used. The instrument was calibrated with sucrose solutions of known refractive index to give drum readings with respect to the refractive index: $\Delta l/\Delta n = 4.81 \times 10^{-6}$. However, since the solute concentration term in the diffusion equation (discussed later) is independent of the unit of measurement, drum readings were later taken directly as measures of concentration. When such diffusion rates were followed in the presence of lipid, lipid concentrations were such that the refractive index measurement of $\Delta n/\Delta c = 0.140$ ml. g.$^{-1}$ polyoxyethylene–polyoxypropylene copolymer compared with 0.139 ml. g.$^{-1}$ polyoxyethylene–polyoxypropylene copolymer in the absence of lipid. From this it was concluded that, at these lipid concentrations, lipid contributed little to the refractive index and that refractive index changes were due mainly to the movement of surfactant.

For radiochemical assay, fatty acids were extracted with a 3:1 v/v quantity of a 1:1:1 v/v/v mixture of ethanol–heptane–diethyl ether. Two further extractions with the upper phase of ethanol–water–heptane–diethyl ether ensured that > 99% of the fatty acid was in the organic extract, the solvent was driven off under dry N_2, and the residue was counted in a liquid scintillation counter[6] after dissolution in toluene containing 2,5-diphenyloxazole (4 g./l.) and 1,4-bis-2-(5-phenyloxazolyl)benzene (0.05 g./l.) as scintillation solutes. Count times were such as to achieve 1% standard deviation. Corrections for quenching were made by the channels ratio method.

Solutions taken to be nonradioactive at the start of a diffusion period were tested for adventitious amounts of radioactivity.

Emulsions and micellar solutions of lipid were prepared by 5-min. ultrasonic irradiation (40 w. at 20 kc.) at constant anode current on a Branson sonifier.

Solubilization was determined by separation of emulsion and micellar phases by ultracentrifugation at 10^7 *g* min. (~36°), at the end of which the lower aqueous micellar phase was sampled and analyzed for radioactivity.

All solutions were 0.15 *M* NaH_2PO_4/0.07 *M* Na_2HPO_4 in phosphate buffer at pH 6.4 ± 0.1, and all experiments were performed at 37 ± 1°.

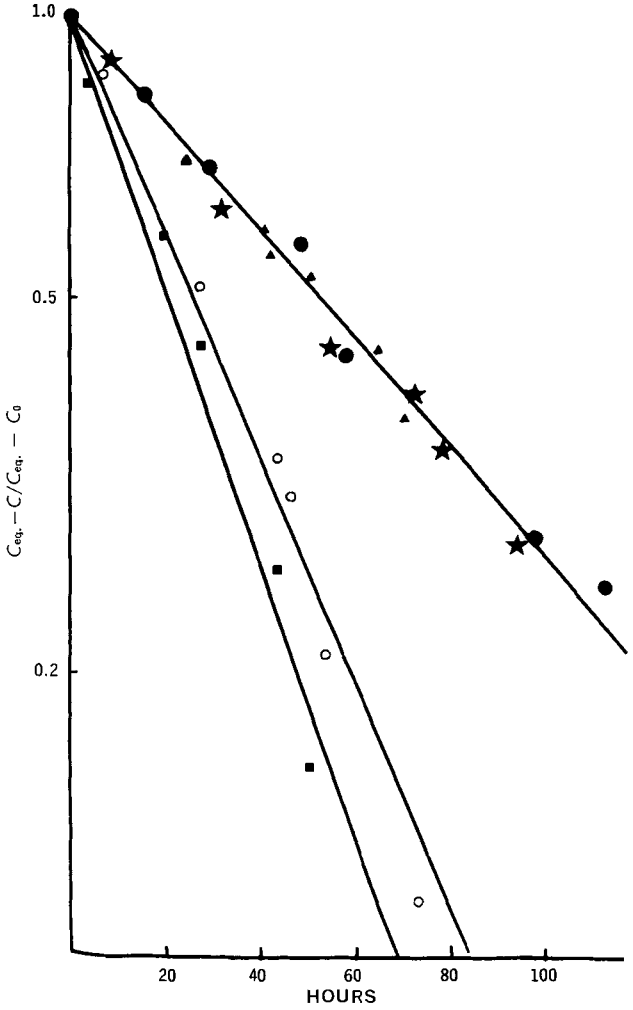

Figure 1—*Semilogarithmic plots of* ^{14}C *and* ^3H *isotopic concentration function* $(C_{eq.} - C)/(C_{eq.} - C_0)$ versus *time for 4:4:2 mM monoolein–oleic acid-*^{14}C*–palmitic acid-*^3H *equilibrating through 10-mμ MP. Key: mM 4:1 sodium taurocholate–sodium taurodeoxycholate: ●, 2.5; ▲, 5.0; ★, 7.5; ○, 10.0; and ■, 15.0.*

RESULTS AND DISCUSSION

Diffusion Equation—The usual application of Fick's law of diffusion as applied to diaphragms (17) is followed:

$$\log \frac{C_{eq.} - C}{C_{eq.} - C_0} = -\bar{D}_T \left[\frac{A}{2.3l} \frac{V_P + V_Q}{V_P V_Q}\right] t \qquad \text{(Eq. 1)}$$

where A is the total area of the diaphragm pores exposed at right angles to the direction of flow, l the thickness of the diaphragm (and associated unstirred layers of solution), V_P and V_Q the volumes of

[5] Polythene.
[6] Nuclear Chicago.

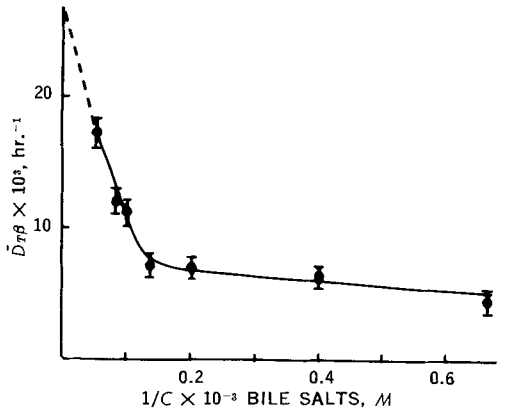

Figure 2—*Fatty acid equilibration rate* $\overline{D}_T\beta$ *as a function of the reciprocal of the bile salt concentration, 4:4:2 m*M *monoolein–oleic acid–palmitic acid, through 10-m*μ *MP.*

each compartment P and Q, $C_{eq.}$ the concentration or specific activity of the diffusing solute at equilibrium, C_0 initially, and C at time t. $\bar{D}$ is the average value of the diffusion coefficient for the concentration range of the experiment and is expressed as $\bar{D}_T$ to allow for all states of aggregation of fatty acid.

The expression in square brackets is the cell constant β and is determined with KCl, given that $D_{KCl} = 2.45 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ in 0.5 M solution at 37° (Table I). Three measurements with separate Millipore (MP) filters of each stated pore size were made for β, whereas β for the sintered-glass (SG) disk was obtained from nine separate determinations over the period of use (several months) to detect significant erosion. The 8% variability in β for the SG disk is higher than can be achieved with such diaphragms, but was considered satisfactory for the experiments described here. With SG diaphragms, diffusion coefficients were calculated from one experimental point. Since kinetic curves were not obtained, the results were more liable to error than in the MP procedure. This was partly offset by the duplication of experiments with the SG diaphragms.

Tortuosity and blocked capillaries in MP filters are serious limitations if such structural characteristics are a feature of many pores in the one filter and are not even approximately reproduced from filter to filter. However, the range in β for any one porosity suggested no gross irregularities.

Preliminary experiments established a rate of stirring above which the results were insensitive to stirring rate, *i.e.*, the "still" layers associated with the diaphragm were at their minimum thickness. In this respect, one limitation of the method is whether the dimensions of the unstirred layer are comparable for KCl and lipid solutes. When considered against the overall thickness of the diaphragm, the effect is likely to be small.

With MP filters, semilogarithmic plots of the concentration term–time give acceptable straight lines (Fig. 1). Correlation coefficients were never less than −0.95. The gradients $\bar{D}_T\beta$, obtained by regression analysis, were within 8% for both oleic acid and palmitic acid loss from one compartment and gain in the other compartment in any one experiment. Such parallelism in the rates of loss and gain suggest steady-state conditions in the diaphragm and the reasonable balance of activity-limited retention by the diaphragm.

Bile Salts—*Effect of Solubilization*—Figure 1 shows fatty acid, equilibration rates across a 0.010-μ MP where, in each experiment both chambers P and Q have the same lipid composition (4 mM oleic acid, 2 mM palmitic acid, and 4 mM monoolein), with one chamber containing ^{14}C- and ^{3}H-labeled fatty acids; bile salt concentrations alter from experiment to experiment. The effect of surfactant concentration on equilibration rate ($\bar{D}_T\beta$) is shown in Fig. 2.

Fatty acid solubilization in this system (Fig. 3*a*) suggests a CMC in the region 2.0–2.5 mM bile salts with total solubilization above 10.0 mM. When the gradient $\bar{D}_T\beta$ is expressed as a function of the reciprocal of the bile salt concentration (Fig. 2), no change in $\bar{D}_T\beta$ is apparent until the bile salt concentration reaches ~7.0 mM. This may be a result of pore blockage by fine globules, so appreciable transport rates are achieved only when there is near total solubilization. On the other hand, the solubilization curve reveals that only 30% of the fatty acid is solubilized at this bile salt concentration and that an increase in the solubilizing power, apparent from the change in the gradient of the solubilization curve, occurs in this region. A significant change in the flux is then to be expected near this surfactant concentration.

Furthermore, although all the lipid was solubilized near 10 mM bile salts, the diffusion function continued to increase with increasing surfactant concentration. At 10 mM bile salts and the stated lipid concentration, about 40 molecules of lipid are associated with each micelle on the basis of an even distribution of solubilizates and an aggregation number of ~40 for the bile salt micelle (discussed later). Given the tendency of lipid to swell micelles (18, 19), increasing the concentration of surfactant with the same overall lipid concentration would suggest the formation of more, and smaller, micelles. An increase in the rate of equilibration $\bar{D}_T\beta$ with increasing surfactant concentration is then understandable on the basis of micelle size. The reciprocal concentration plot is made to allow extrapolation to such high bile salt concentrations ($1/C = 0$), *i.e.*, so large a number of micelles that on the average the amount of lipid per micelle (at this fixed total lipid concentration) scarcely alters the dimension of the micelle. The extrapolated $\bar{D}_T$ so determined is 1.7×10^{-6} cm.^{2} sec.$^{-1}$.

Effect of Pore Size—The diffusion coefficient of oleic acid solubilized in bile salts and equilibrating through the SG diaphragm is 3.9×10^{-6} cm.^{2} sec.$^{-1}$ (Table II). The difference between this figure and 1.7×10^{-6} cm.^{2} sec.$^{-1}$ is probably due to the factor ~3 between micellar diameter (~30 Å) and average pore size not being adequate for the process to approach that of diffusion in free solution, *i.e.*, with no geometrical limitations on movement. This fits with the observation that for the same lipid mixture in 10 mM bile salts equilibrating through a 1.2-μ MP, $\bar{D}_T$ is 4.4×10^{-6} cm.^{2} sec.$^{-1}$ (Table II).

The 20% lower $\bar{D}_T$ when there is a chemical concentration dif-

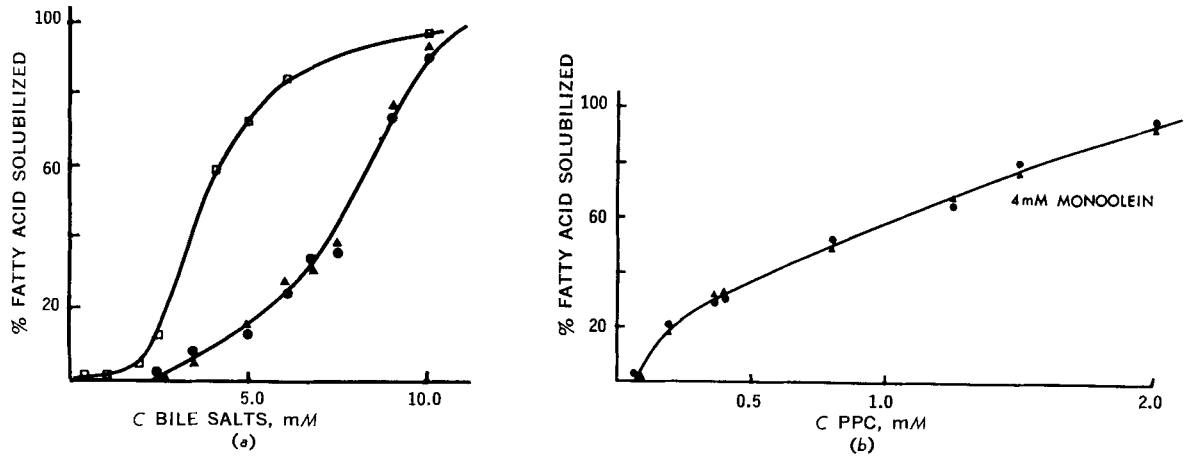

Figure 3—(*a*) *Fatty acid solubilization in bile salt solution. Total lipid concentrations: oleic acid* mM: □, *1.0, and* ●, *4.0; palmitic acid* mM: ▲, *2.0; and monoolein* mM: *upper curve, 1.0, and lower curve, 4.0.* (*b*) *Fatty acid solubilization in polyoxyethylene–polyoxypropylene copolymer (PPC) solution. Total lipid concentrations: oleic acid* mM, ●, *4.0; palmitic acid* mM, ▲, *2.0; and monoolein* mM, *4.0.*

Table II—Diffusion Coefficients $\bar{D}_T$ in Bile Salt Solutions

Chamber P		Chamber Q			$\bar{D}_T \times 10^6$ (cm.^{2} sec.$^{-1}$)	
Bile Salt, mM	Lipid, mM[a]	Bile Salt, mM	Lipid, mM	Diaphragm	Bile Salts	Oleic Acid
10.0	—	5.0	—	30-μ SG	5.28	
10.0	1.0 OA, 1.0 MO	5.0	—	30-μ SG	5.43	3.25
					4.75	3.40
					4.63	3.10
					5.0 ± 0.4	3.2 ± 0.2
10.0	1.0 OA, 1.0 MO	10.0	1.0 OA, 1.0 MO	30-μ SG		3.47
						4.47
						3.9 ± 0.5
10.0	4.0 OA, 4.0 MO	10.0	4.0 OA, 4.0 MO	1.2-μ MP		4.4 ± 0.5
2.0	1.0 OA, 1.0 MO	2.0	—	30-μ SG		0.59
2.0	1.0 OA, 1.0 MO	2.0	1.0 OA, 1.0 MO	30-μ SG		0.41
						0.33
						0.37 ± 0.04
2.0	4.0 OA, 4.0 MO (2.0 PA)	2.0	4.0 OA, 4.0 MO (2.0 PA)	0.010-μ MP		0.25 ± 0.04

[a] MO = monoolein, OA = oleic acid, and PA = palmitic acid.

ference (rather than just an isotopic concentration gradient) is outside the error limits for the experiments described.

For bile salt concentrations below the CMC, that is, lipid exists mainly in the emulsion phase, the kinetic constant is effectively the same when a 0.010-μ MP rather than a 30-μ SG diaphragm is interposed between the equilibrating systems; this figure is an order of magnitude lower than when the lipid is totally solubilized.

Polyoxyethylene–Polyoxypropylene Copolymer—*Effect of Pore Size*—When each chamber contains lipid of composition 4:4:2 mM in monoolein–oleic acid–palmitic acid and 2 mM polyoxyethylene–polyoxypropylene copolymer, equilibration rates over the MP porosity range from 0.010 to 1.2 μ are as shown in Fig. 4. The CMC for this nonionic surfactant is near 0.10 mM, based on solubilization data in Fig. 3*b*, and 2.0 mM polyoxyethylene–polyoxypropylene copolymer ensures better than 95% solubilization of fatty acid in the lipid system used. Hence, the interpretation is not complicated by the presence of an emulsion phase. Nevertheless, the equilibration rate is roughly constant over the MP porosity range from 0.010 to 0.200 μ and increases with larger pore sizes, even when variation in the cell constant β is allowed for (lower curve of Fig. 4).

In the lower porosity range, $\bar{D}_T$ is 3.2 × 10^{-7} cm.^{2} sec.$^{-1}$ and increases to 9.7 × 10^{-7} cm.^{2} sec.$^{-1}$ for the 1.20-μ MP; from this rate/reciprocal pore dimension display, ~3 μ is the MP dimension at which the diffusion coefficient of fatty acid in the 2.0 mM polyoxyethylene–polyoxypropylene copolymer system approaches that obtained with the SG diaphragm corresponding to free diffusion (Table III). Due to the steep nature of the plot in this region, this can only be an estimate; but the point emphasized is that although the fatty acid is solubilized, the flux is much reduced below 0.200 μ.

Effect of Solubilization—A detailed study of the effect of solubilization on equilibration rate was not carried out in the polyoxyethylene–polyoxypropylene copolymer system, since preliminary experiments suggested that similar effects to the bile salt system were being observed; that is, when micelles are freely transported and emulsion globules restricted in their movement, increased solubilization due to an increasing concentration of surfactant, at a fixed lipid composition, results in an increased flux. The difference is that these effects are observable in the 1-μ pore range compared with 100 times lower for the bile salt micelle.

This behavior is expected from the data in Fig. 5, which summarizes the throughput of fatty acid in ultrafiltration experiments, under a differential pressure of 60 cm. Hg. While only approximate at best, ultrafiltration does show that with increasing polyoxyethylene–polyoxypropylene copolymer concentration the proportion

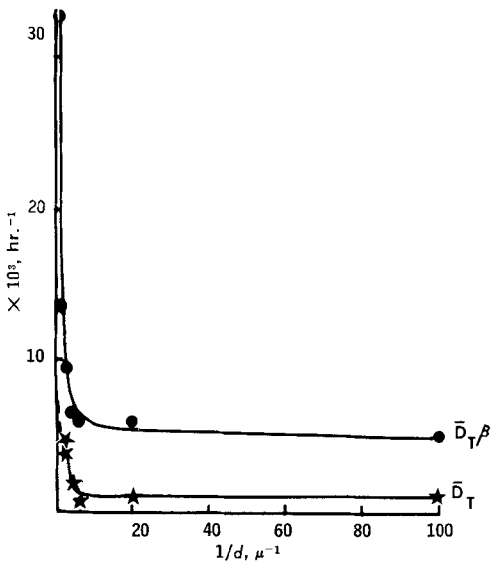

Figure 4—*Fatty acid equilibration rate* $\bar{D}_T\beta$, *upper curve,* $\bar{D}_T$, *lower curve,* versus *reciprocal of average pore dimension (MP): 4:4:2* m*M monoolein–oleic acid–palmitic acid in 2.0* m*M polyoxyethylene–polyoxypropylene copolymer.*

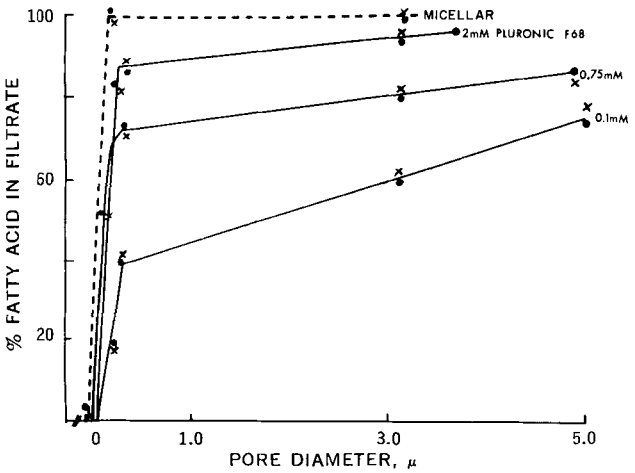

Figure 5—*Ratio of fatty acid, filtrate/unfiltered dispersion as a function of average pore dimension (MP): 4:4:2* m*M monoolein–oleic acid–palmitic acid in polyoxyethylene–polyoxypropylene copolymer. Key: oleic acid* m*M,* ●, *4.0; palmitic acid* m*M,* ×, *2.0;* ——, *filtration of emulsion–micellar dispersion; and* - - -, *filtration of micellar solution obtained by ultracentrifugation of 2* m*M polyoxyethylene–polyoxypropylene copolymer–lipid dispersion.*

Table III—Diffusion Coefficients $\bar{D}_T$ in Polyoxyethylene–Polyoxypropylene Copolymer (PPC) Solutions

Chamber P		Chamber Q			$\bar{D}_T \times 10^6$ (cm.^{2} sec.$^{-1}$)	
PPC, mM	Lipid, mM	PPC, mM	Lipid, mM	Diaphragm	PPC	Oleic Acid
2.0 (16 mg./ml.)	—	0.1	—	30-μ SG	2.68	
2.0	1.0 OA, 1.0 MO	1.0	—	30-μ SG	1.83	2.20
					1.89	2.05
					2.63	2.01
					2.49	1.13
					2.2 ± 0.4	1.8 ± 0.4
2.0	1.00 OA, 1.0 MO	2.0	1.0 OA, 1.0 MO	30-μ SG		2.0
2.0	4.0 OA, 4.0 MO	2.0	4.0 OA, 4.0 MO	0.010-μ MP		0.32 ± 0.04
2.0	4.0 OA, 4.0 MO	2.0	4.0 OA, 4.0 MO	1.20-μ MP		0.97 ± 0.11

of fatty acid in the filtrate increases appreciably only with filters above 0.100-μ pore size. Moreover, while the bulk of fatty acid in polyoxyethylene–polyoxypropylene copolymer micellar solution is transmissible through 0.300-μ pores (Fig. 5), pore sizes in excess of 5.0 μ are necessary to recover all the fatty acid from a filtered polyoxyethylene–polyoxypropylene copolymer stabilized emulsion.

By contrast, a 0.010-μ MP under the same hydrostatic pressure retains no fatty acid from an emulsion stabilized with 2.0 mM bile salts.

The diffusion data in the polyoxyethylene–polyoxypropylene copolymer system are summarized in Table III.

Comparison of Micelles of the Two Surfactants—At 37°, pH 6.4, $Na^+ = 0.15$ M, and in the absence of lipid, the micellar molecular weights (MMWs) of 4:1 NaTC–NaTDC is 2.0×10^4 (*i.e.*, aggregation number 40) and 5×10^5 for polyoxyethylene–polyoxypropylene copolymer from a light-scattering study made in conjunction with the present investigation. The former value is in fair agreement with 1.2–2.3×10^4 (depending on the sodium-ion concentration) for the taurodeoxycholate micelle at 25° (20). MMWs of the magnitude found for polyoxyethylene–polyoxypropylene copolymer are not unusual for nonionic surfactants (21).

From the Stokes relation, the diffusion coefficient D is inversely proportional to the radius. If the diffusing entities are roughly spherical and their densities about the same, D is inversely proportional to $\sqrt[3]{\text{MMW}}$. Also, if the two media have about the same viscosity,

$$\frac{D^{BS}}{D^{PPC}} \sim \frac{\sqrt[3]{5 \times 10^5}}{\sqrt[3]{2 \times 10^4}} = 2.9 \qquad \text{(Eq. 2)}$$

Above the CMC the apparent diffusion coefficient $\bar{D}_T$ is the weighted mean of the coefficient for monomolecular and micellar species (22, 23):

$$\bar{D}_T C_T = D_{\text{mono.}} C_{\text{mono.}} + D_{\text{mic.}} C_{\text{mic.}} = L_1 X_1 + L_2 X_2 \qquad \text{(Eq. 3)}$$

where C's are the concentrations (see *Appendix*).

If solubilization does not greatly alter the CMC, *i.e.*, the CMC of each surfactant in the absence of lipid solubilizate is close to that in the presence of lipid, then from the observed values of the CMC by the solubilization method and the measured diffusion coefficients of monomeric and micellar surfactant:

$$\frac{\bar{D}^{BS}}{\bar{D}^{PPC}} = \frac{4.9 \times 10^{-6}}{1.6 \times 10^{-6}} \sim 3.0 \qquad \text{(Eq. 4)}$$

in fair agreement with the expected ratio.

Because of the necessarily lower concentrations of surfactants and the interferometric method of estimation, $D_{\text{mono.}}$ has low accuracy. However, the monomeric contribution to the total flux is relatively small, and relatively large errors in the detemination of the diffusion coefficient of monomer result in relatively small changes in $D_{\text{mic.}}$

Correspondingly, using 6×10^{-6} cm.^{2} sec.$^{-1}$ for the diffusion coefficient of monomeric oleic acid (24) and 4×10^{-5} M for its solubility (6), the diffusion coefficients of the lipid-containing (1.0 mM solutions) bile salt and polyoxyethylene–polyoxypropylene copolymer micelles are 3.9×10^{-6} cm.^{2} sec.$^{-1}$ and 1.8×10^{-6} cm.^{2} sec.$^{-1}$, respectively, based on the movement of radioactively labelled fatty acid.

CONCLUSIONS

An increase in the driving force for diffusion of lipid with an increase in solubilization is clearly apparent and, provided that events in the luminal stage determine the kinetics of absorption of fatty acid, this would account for the improved fatty acid uptake observed in everted sacs from micellar systems compared with emulsions (5).

Another factor that emerges from this study is the necessity for unrestricted movement of micelles if their maximum contribution to the diffusive flux is to be achieved. With bile salt micelles, the transport function approaches the diffusion coefficient in free solution for pore sizes somewhat in excess of 10 mμ; whereas with polyoxyethylene–polyoxypropylene copolymer micelles, dimensions greater than 200 mμ are necessary for unhampered micellar movement.

The significance of these findings to fat absorption *in vivo* is not clearcut, but a reasonable expectation is that incomplete mixing in the gut preserves a layer of fluid near the absorptive cell in which diffusion-controlled processes dominate. Physical restrictions on the movement of micelles due to the microvilli and the associated glycocalyx coat are possible, but further experiments are required to assess their importance.

However, even neglecting the possible existence of a greater macroscopic barrier to the movement of the polyoxyethylene–polyoxypropylene copolymer micelle, the factor of 2 between the diffusion coefficients of the lipid-containing bile salt micelle compared with the lipid-containing polyoxyethylene–polyoxypropylene copolymer micelle would largely account for the difference of absorption rates in everted intestinal sacs observed for the two types of micellar systems.

APPENDIX

The usual application of irreversible thermodynamics is followed.

The flux of fatty acid (*e.g.*, oleic acid) in the systems described may be written

$$J_T = J_1 + J_2 + J_3 \qquad \text{(Eq. A1)}$$

where the subscripts 1, 2, and 3 refer to the contributions of monomers, micelles, and emulsion globules ("monomer" including all associated forms of fatty acid that exist outside the micelles and emulsions).

Flows and forces are related by

$$J_i = \sum_k L_{ik} X_k \qquad \text{where } i = 1, 2, 3 \ldots n \qquad \text{(Eq. A2)}$$

and L_{ik} is the coefficient that expresses the effect of the force X_k on the flow J_i. This set of n equations includes all possible interactions between flows and forces in the system.

In the absence of driving forces such as temperature and pressure gradients, the driving forces are equatable to chemical potential gradients or concentration gradients if the usual equivalence between thermodynamic activity and concentration, at low solute concentrations, is assumed.

Since the flux of surfactant can also be described as the sum of monomer J_I and micellar J_{II} fluxes, the fatty acid flux can be wholly described by

$$J_1 = L_1 X_1 + L_{12} X_2 + L_{13} X_3 + L_{1I} X_1 + L_{1II} X_{II} \qquad \text{(Eq. A3)}$$

$$J_2 = L_2X_2 + L_{21}X_1 + L_{23}X_3 + L_{2I}X_I + L_{2II}X_{II} \quad \text{(Eq. A4)}$$

$$J_3 = L_3X_3 + L_{31}X_1 + L_{32}X_2 + L_{3I}X_I + L_{3II}X_{II} \quad \text{(Eq. A5)}$$

The first term in each equation is, as expected, an expression of Fick's diffusion law, $J_i = L_iX_i = -D(dc_i)/(dx)$. For example, in Eq. A3 the flux of monomer J_1 is related to the concentration of monomer X_1 through the diffusion coefficient L_1, the second term takes into account "coupling" between monomer fatty acid and micellar fatty acid, and the third term takes into account coupling between monomer oleic acid and emulsified oleic acid. The last two terms in Eq. A3 allow for association between monomer oleic acid and monomer and micellar surfactant, respectively, and so on for J_2 and J_3. These equations attempt to account for all associations between fatty acid and surfactant in their different aggregated forms (with the proviso that the presence of monoolein alters the description little).

From the reciprocal relation postulate of Onsager, $L_{12} = L_{21}$, $L_{13} = L_{31}$, *etc.* The near similarity of $\bar{D}_T$ in an emulsified system when the movement of emulsion globules is, or is not, restricted by the diaphragm, *e.g.*, 0.010-μ MP compared with SG, suggests that the flux of emulsion is small, *i.e.*, $J_3 \sim 0$. Correspondingly and considering the relatively small interface of the emulsion, that is, molecules aggregated in the emulsion must interact less with fatty acid in free solution compared with the extent of interaction if the same amount of material was all dispersed in solution, coupling between emulsion and monomeric and emulsion and micellar fatty acid may be neglected. Then

$$J_1 = L_1X_1 + L_{12}X_2 + L_{1I}X_I + L_{1II}X_{II} \quad \text{(Eq. A6)}$$

and

$$J_2 = L_2X_2 + L_{21}X_1 + L_{2I}X_I + L_{2II}X_{II} \quad \text{(Eq. A7)}$$

are the only quantities of importance.

Coupling between surfactant and fatty acid movement should be apparent in the comparison of two systems, one where there is a net concentration gradient of surfactant and the other where chemical concentrations are the same in both chambers, but a difference exists only in the concentration of isotopic label. The identity (within the limits stated) of $\bar{D}_T$ for oleic acid in polyoxyethylene–polyoxypropylene copolymer diffusing in sintered glass under the two conditions described suggests that

$$L_{1I} \sim L_{1II} \sim L_{2I} \sim L_{2II} = 0 \quad \text{(Eq. A8)}$$

Considering the weights of monomers and micelles of bile salts compared with polyoxyethylene–polyoxypropylene copolymer, one may be led to expect that if interaction between the flows of fatty acid and surfactant were significant, a lesser effect would be observed with bile salts than with the nonionic surfactant. The reverse is the case, which is the justification for the application of Eq. A8 to the bile salt system.

The argument is somewhat complicated in a system with a solute concentration gradient by the movement of solvent (*i.e.*, water) in the opposite direction to the flow of solute, which may reduce the observed (*i.e.*, mutual) solute diffusion coefficient from the isotopic (~differential or thermodynamically ideal) value. However, this solvent flow effect is likely to be small when the chemical potential differences are as small as in the systems described here. The magnitude of solute concentrations in the present work also justifies comparisons between 1 mM and 4 mM lipid systems. Then,

$$J_T = L_1X_1 + L_2X_2 + L_{12}(X_1 + X_2) \quad \text{(Eq. A9)}$$

from the mentioned equivalence of L_{12} and L_{21}. L_{12} is likely to be small. But since the extent of this coupling between monomer and micellar fatty acid is uncertain, Eq. A9 is best written

$$J_T = (L_1 + L_{12})X_1 + (L_2 + L_{12})X_2 = L_1'X_1 + L_2'X_2 \quad \text{(Eq. A10)}$$

The measured diffusion coefficients L_1' and L_2' equal L_1 and L_2, respectively, if L_{12} is negligibly small.

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