Partition of lipids between emulsified oil and micellar phases of glyceride–bile salt dispersions

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ABSTRACT The composition of the emulsified oil and of the micellar phases obtained when a glyceride–fatty acid mixture is dispersed in bile salt solution has been defined. The micellar phase in equilibrium with the emulsified oil phase was obtained by filtration through Millipore filters.

The behavior of different lipids in such systems was defined as the partition ratio, micellar/emulsified oil phase (m/o).

Partition of fatty acids was found to be strongly dependent on the chain length of the fatty acid and the pH of the dispersion. The curve for partition against pH for oleic acid was interpreted to show a pK_a for oleic acid in bile salt solution of approximately 7.

The partition between micellar and oil phases is given for a series of lipids of different polarity. No significant difference in behavior was found for cholesterol and sitosterol.

A relationship was found between the partition m/o and filtration rates through a Millipore filter in micellar solution. The lower the partition coefficient the lower was the rate of filtration. The results obtained are discussed in relation to the mechanism of absorption of fat from the small intestine.

KEY WORDS bile salts · detergent · emulsion · sonication · micelles · glycerides · fatty acids · pK_a · cholesterol · cholesteryl esters · sitosterol · acylsarcosyl taurates · partition · fat absorption

DURING INTESTINAL DIGESTION of dietary fat, the lumen of the small intestine has been shown to contain an oil phase dispersed in a micellar bile salt solution (1). The emulsified oil phase is mainly made up of tri- and diglycerides, whereas the micellar phase contains bile salt and the polar end products of pancreatic lipolysis, namely monoglycerides and free fatty acids. Other lipid-soluble substances are distributed between these two phases. Evidence at hand indicates that absorption of lipids takes place mainly from the micellar phase (2) and that this micellar phase is continuously generated from the emulsified oil phase by reactions catalyzed by pancreatic lipase (3). The redistribution of lipid-soluble substances originally present in the oil phase to the micellar phase might therefore be important in the rate and extent of their absorption.

We have determined the partition ratios of various lipid-soluble substances between the emulsified oil phase and the micellar phase formed when glycerides are dispersed in a bile salt solution in vitro. The partition figures that are the basis of this communication were determined in equilibrium experiments carried out with Millipore filters.

MATERIALS

Triolein, trilaurin, and trioctanoin were obtained from Fluka AG (Buchs, Switzerland) and purified by elution from a column of aluminum oxide Grade III with ethyl ether–petroleum ether 2:98. Trilinolein was synthesized from the acid chloride by standard procedures and purified as above. The 1,2-diglycerides of oleic, lauric, and octanoic acids were synthesized according to Krabisch and Borgström (4). Monoolein was a product of Distillation Products Industries (Rochester, N. Y.). Mono- and dilinolein were gifts from Dr. L. W. Beck of the Procter & Gamble Co., Cincinnati, Ohio. The 1-monoglycerides of lauric and octanoic acid were synthesized according to standard procedures (5). The fatty acids used were commercial products. The acid chlorides were made by the use of oxalyl chloride and distilled. Cholesterol was obtained from Eastman Kodak Co. (Rochester, N. Y.) and recrystallized. Sitosterol was prepared from stigmasterol (Nutritional Biochemicals Corporation, Cleveland,

Abbreviations: TO, triolein; DO, diolein; MO, monoolein; OA, oleic acid; NaTDC, sodium taurodeoxycholate; CMC, critical micellar concentration; TLC, thin-layer chromatography.

Ohio). The stigmasterol was dissolved in light petroleum–benzene 8:1, washed with alkaline ethanol–water 1:1, and crystallized from light petroleum and then from acetone. Stigmasterol was then converted into sitosterol as described by Fernholz and Ruigh (6) via the isostigmasterol methyl ether by hydrogenation. Cholesteryl ethers were prepared as described by McKennis (7). Octadecane was obtained from Fluka AG (Buchs, Switzerland) (>99% pure).

Sodium taurodeoxycholate was prepared according to Hofmann (8) and was at least 97% pure on TLC. Sodium dodecyl sulfate was synthesized in this laboratory. Oleoyl taurine and decanoyl taurine were synthesized by the method used for preparation of taurine-conjugated bile salts (8). Sodium decanoyl-sarcosyl taurate and sodium oleoyl-sarcosyl taurate were synthesized similarly starting from sarcosyl taurine, which was generously provided by Dr. A. H. A. van den Oord, Utrecht, The Netherlands. The acylsarcosyl taurates were made in this laboratory by Dr. R. G. H. Morgan. The yields for the decanoyl and oleoyl derivatives were 80 and 34% respectively.

The purity of the synthesized detergents was checked by TLC by means of the systems described by Hofmann for conjugated bile salts (9). The purification was carried to the point when 100 μg gave only one distinct spot. Pluronic F 68, a polyoxyethylene–polyoxypropylene surfactant, was obtained from the Wyandotte Chemicals Corp., Wyandotte, Mich. Its molecular weight has been reported to be 8000 and its CMC 6.9 μM (10).

The ^{14}C-labeled fatty acids linoleic, oleic, myristic, lauric, decanoic, octanoic, and hexanoic and oleic acid-9, 10-^{3}H were obtained from the Radiochemical Centre, Amersham, Bucks, England. Some preparations of ^{3}H-labeled oleic acid were found to contain polar components and were purified by TLC. Cholesterol-4-^{14}C and cholesterol-^{3}H were also obtained from the Radiochemical Centre. Their radiopurity, checked by TLC, was in general found to be better than 98%.

Sitosterol-^{3}H was prepared as for the nonlabeled compound except that the hydrogenation was performed by the Radiochemical Centre with tritium gas. Analysis by gas–liquid chromatography showed the sitosterol to be more than 95% pure by mass, the remainder being campesterol that did not contain any radioactivity. Octadecane-^{14}C was obtained from the Radiochemical Centre. The product was found by TLC to contain some 5% of polar impurities and was therefore purified on a silicic acid column, the light petroleum eluate being more than 99% pure.

Fatty acid-labeled triolein and monoolein were prepared according to standard procedures and purified to better than 99% radiopurity by combinations of column and thin-layer chromatography.

METHODS

The micellar phases were separated from the bile salt dispersions in filtration chambers designed according to Ogston and Phelps (11) with slight modification. Two compartments, each of 5 ml volume, were separated by a Millipore filter (Millipore Corp., Bedford, Mass.) 25 mm in diameter. Both compartments could be filled and sampled separately. Three different types of experiments were performed. In the first two, the filter was vertical.

Method 1. The emulsion of the glycerides in bile salt solution was placed in one compartment and allowed to filter until the volume was the same on both sides of the Millipore filter.

Method 2. The emulsion in bile salt solution was placed on one side of the filter and a micellar solution on the other and the two solutions were allowed to equilibrate.

The first method was found to be more convenient and was generally used. With the 100 A filter, filtration was allowed for 3–4 days; with the 500 A filter, for 1–2 days.

The chambers were at 37°C and were shaken to and fro at about 50 strokes/min with an amplitude of 3–4 cm. To allow filtration but prevent evaporation we connected the compartments by plastic tubing.

Method 3. The chambers used for filtration were held with the filter horizontal. 4 ml of the emulsion in bile salt solution was put into the upper chamber, which was connected by plastic tubing to a reservoir of solution that was used to replace the volume filtered. Filtered micellar solution was collected in test tubes by means of a fraction collector.

The emulsions were prepared either by sonication for 1 min in a Branson sonicator (Branson Instruments, Inc., Stamford, Conn.) at maximum intensity in a volume of 5–10 ml, or by vigorously shaking by hand the glyceride mixture with the bile salt solution in a stoppered test tube, or with the aid of a Super-Mixer (Lab-Line Instruments, Inc., Melrose Park, Ill.) for about 30 sec. The effect of these different procedures will be discussed later.

To prevent oxidation during filtration and equilibration we flushed the solutions and the chambers with nitrogen before filling them. When linoleic acid or its derivatives were used, 1% hydroquinone was added to the oil phase prior to dispersion.

Determinations of lipid partition in most experiments were based on radioactivity determinations of labeled compounds, which were in general used in pairs (one ^{14}C-, the other ^{3}H-labeled). In some experiments the glycerides were extracted and separated by TLC (3) and the different species were determined chemically after elution from the adsorbent (12).

Radioactivity was determined by liquid scintillation counting in a Packard Model 4000 spectrometer. For

counting, 1 ml of each sample was added to 10 ml of scintillator solution (3).

Calculation of Lipid Partition between Micellar and Oil Phases

In the experiments to be discussed an oil phase was dispersed in a micellar solution. Lipids present in such a system partition between the micelles and the oil (for definition of micellar and oil phases see footnote 1). With the assumption that (*a*) the filter allowed the passage of only the micellar solution, (*b*) the micelles filtered were in equilibrium with the micelles in the compartment containing the retained emulsion, and (*c*) the micelles in the emulsion were in equilibrium with the oil phase of the emulsion, the partition of lipids between the micellar and the oil phase can be calculated.

Suppose the final volume on each side of the filter is V, then the original volume—in which the concentration of lipid was T— was 2 V. Total lipid in the system is therefore 2 TV. At the end of the experiment, the amount of lipid on the filtered side is MV, M being the concentration of lipid in the micellar solution. On the retained side the amount of lipid is $MV + O$, where O is the amount present dissolved in the oil phase. Hence $2\ TV = MV + MV + O$ and $O = 2\ V(T - M)$. The partition of lipids between the micellar solution and the oil phase on the retained side of the filter is expressed by MV/O or $M/2(T - M)$. As all the lipid in the micellar solution can be assumed to be in the micelles and the volume in which both the micelles and the oil are dispersed is the same the expressions above can be used to express the partition of lipid between the micellar and oil phases, m/o, in the retained emulsion.

In the initial experiments the concentration of lipid was measured directly in the content of the two compartments to give MV and $MV + O$. When it was observed that the emulsion tended to cream during filtration, calculations were based on the determination of M and T.

RESULTS

Effects of Filter Pore Size on the Separation of Micellar Solution from an Emulsion

In the initial experiments cholesterol-^{14}C dissolved in triolein-^{3}H was sonicated in 5 ml of bile salt solution. The emulsion obtained was diluted tenfold with bile salt solution, and 5 ml was transferred to one side of filtration chambers that contained Millipore filters of different pore sizes. When the volumes on both sides of the filter seemed to be equal (equilibrium time, a few hours to 3 days according to pore size), samples were withdrawn and their radioactivity was counted. The amount of cholesterol and triolein in the filtered solution was calculated as a percentage of that present in the original solution before filtering (see Fig. 1). The smallest filter pore diameter used was a nominal 0.01 μ (100 A), the largest 1.2 μ (12,000 A). The solutions filtered by the 100 and 500 A filters were optically clear; with larger pore sizes increasingly more turbid filtrates were obtained. Samples of the optically clear solutions obtained with the 100 and 500 A filters were subjected to gel filtration and shown to be micellar (13). As can be seen from Fig. 1 (100 and 500 A filters only), the percentage of micellar cholesterol present increased with increase in bile salt concentration. Only a small fraction of the triolein was in micellar solution even at high bile salt concentration.

Effect of Method of Dispersion on the Particle Size Distribution

The preliminary experiments discussed above were done with triolein dispersed in bile salt solution. For this mixture fairly stable emulsions could be produced only by sonication. In the following work I generally used a lipid mixture that included tri-, di-, and monoglycerides and free fatty acids in ratios similar to those found in intestinal content of human during digestion of a fat-containing meal (1). This lipid mixture emulsified almost spontaneously when a bile salt solution was added. Shaking by hand or with a Super-Mixer for 30–60 sec produced an

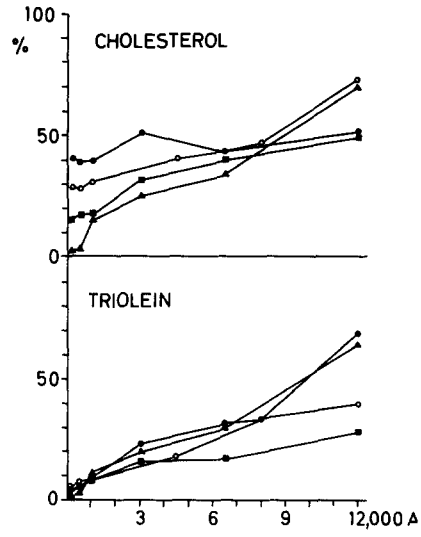

FIG. 1. Effect of filter pore size on the composition of the filtrate obtained using *method I* (see Methods) at different bile salt concentrations. 10 μmoles of TO-^{3}H and 1 μmole of cholesterol-^{14}C were dispersed per ml of bile salt solution with concentration 1.2 (▲), 2.4 (■), 6 (○), and 12 (●) mM, respectively, at pH 6.3. Dispersions were made by sonication. TO and cholesterol in the filtrates were determined by radioactivity and the figure was converted to a percentage of that in the original dispersion.

[1] The term "micellar phase" is used to denote the micelles of a micellar solution, including any dissolved lipid. "Oil phase" or "emulsified oil phase" is used to denote only the oil dispersed in the emulsion. Neither term includes the water that constitutes the bulk of the dispersion.

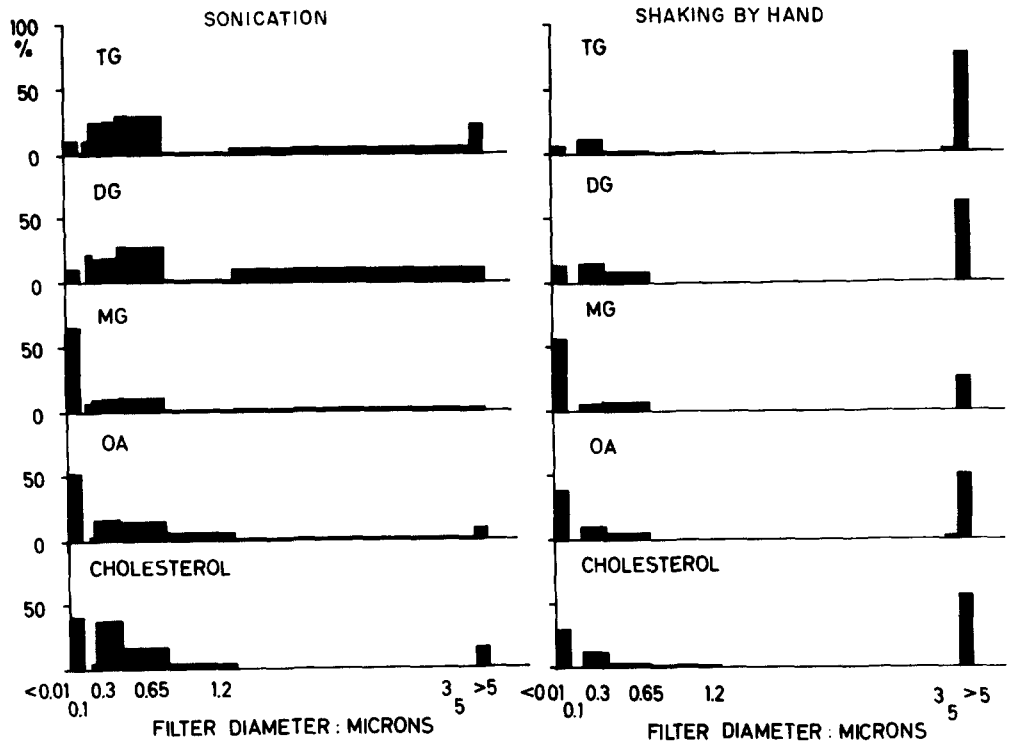

FIG. 2. Particle size profile of a dispersion of glycerides, fatty acid, and cholesterol in bile salt solution made either by sonication (left) or hand-mixing (right). The dispersion was 2.5 mM each in TO, DO, and MO, 7.5 mM in OA (i.e., free fatty acid equivalent to the free hydroxyl groups of the partial glycerides), 1 mM in cholesterol, and 6 mM in NaTDC.

The filters had a pore diameter specified as 0.01 and 0.05 μ, 0.1, 0.3, 0.65, 1.2, 3, and 5 μ. The filtrates obtained were analyzed for radioactivity (OA and cholesterol) and for glycerides (chemical determination after TLC). The bars represent the additional material filtered by the larger pore sizes above that passing through the 0.01 μ filter. The first bar to the left thus represents the filtrate obtained by the 0.01 μ filter, the 0.05 μ filter did not filter more material than the 0.01 μ; the 0.1 μ filter gave only little material over that of the 0.05 μ filter, etc.

emulsion that was stable for hours. Sonication of this dispersion produced even more stable emulsions. A particle size distribution profile of these dispersions was made with the aid of Millipore filters of different pore size. The results are given in Fig. 2. Sonication produced at least three different families of particles. The first one had a particle size that passed the 0.01 and 0.05 μ filters and was an optically clear micellar solution. The second family contained particles that passed filters in the range of 0.1–1.2 μ pore size. These dispersions were turbid and behaved on gel filtration as an emulsion (in a micellar solution). The third family represented particles not passing the 1.2 μ filter, ranging up to particles that were retained also by the 5 μ filter. These particles also clearly had the characteristics of an emulsion.

The particle size distribution in the dispersion produced by simple shaking by hand or by the Super-Mixer for short times was different. Even though the same families could be seen, their relative sizes were different. The size of the micellar fraction was almost the same, but the 0.1–1.2 μ fraction was small when hand mixing had been used and a much larger fraction consisted of particles that did not pass even the 5 μ filter. Continued mechanical agitation by simple shaking or by means of a magnetic stirrer resulted in an increase, with time, of the 0.1–1.2 μ fraction with a corresponding decrease of larger particles. The particle size distribution produced by such means became nearly the same as that obtained by sonication.

The relative lipid composition of the different fractions was quite different. The micellar fraction was relatively high in monoolein, oleic acid, and cholesterol and low in tri- and diglycerides.

The values in Table 1 established that the partition of the different lipid species between the micellar and emulsified oil phases when the 0.01 μ filtrate was used was not affected to any great degree by the method of dispersion.

Importance of the Method of Filtration

The method of equilibration from one side of the filter only (A, Fig. 3) gave the same distribution of labeled cholesterol between the compartments as filtration (B),

TABLE 1 EFFECT OF METHOD OF DISPERSION ON PARTITION RATIOS (M/O) BETWEEN MICELLAR AND OIL PHASES

Lipid Species	Sonicated	Super-Mixer
TO	0.029	0.019
DO	0.069	0.054
MO	0.50	0.48
OA	0.33	0.30
Cholesterol	0.27	0.25

TO, DO, and MO (2.5 μmoles each), OA (7.5 μmoles), and cholesterol (0.1 μmole) were dispersed per ml in a solution of 6 mM NaTDC at pH 6.3 (phosphate buffer, Na^+ 0.15 M). A 0.5 ml dispersion was made either by sonication with a Branson sonifier at maximum intensity for 1 min or by agitation in a Super-Mixer at maximum speed for 1 min. Partition ratios (m/o) were calculated[1] after filtration (0.01 μ filter) as described in Methods. The data for cholesterol and oleic acid were calculated from radioactivities in five experiments in which oleic acid-^{3}H and cholesterol-^{14}C were used. The glyceride figures were obtained from one experiment in which the glycerides were extracted, separated by TLC, and chemically determined. The differences obtained for oleic acid and cholesterol were not statistically significant.

which indicates that the same equilibrium was reached in each case. Filtration (*method 1*) was preferred in general as it involved the preparation of only one dispersion for each experiment. The equilibrium times for the 0.01, 0.05, and 0.3 μ filters were found to be 3 days, 1 day, and 8 hr., respectively. Complete filtering of the dispersion through a horizontal filter gave results indicating that the dispersion medium, water, filtered more rapidly than the micellar phase. As a continuous change in concentration of the emulsion occurs during filtration, the partition coefficient in this case does not refer to the conditions present in the original solution but to those at half filtration. Filtration by pressure almost always led to a decreasing rate of filtration and sometimes to complete clogging of the filter and was therefore generally not used for obtaining a micellar solution.

Partition of Different Lipids between the Micellar and Emulsified Oil Phases

Partition figures (micellar to oil phase, as defined) were determined for the triolein–bile salt system and the more complex system containing tri-, di-, and monoolein and oleic acid in bile salt dispersion.

The amount of triolein in the micellar phase was found to be small. In this determination it was important to exclude the effect of labeled polar impurities, such as free fatty acids and monoglycerides, present in the triolein from the beginning or formed by sonication. In one experiment in which the triolein partition was determined by recovering triolein in the micellar phase by TLC and chemical determination, the partition m/o for triolein was found to be 0.013 (TO 10 mM, NaTDC 12 mM).

The effect of bile salt concentration on the partition ratios at pH 6.3 for the different components of the mixed glycerides–free fatty acid system is seen in Fig. 4. In these

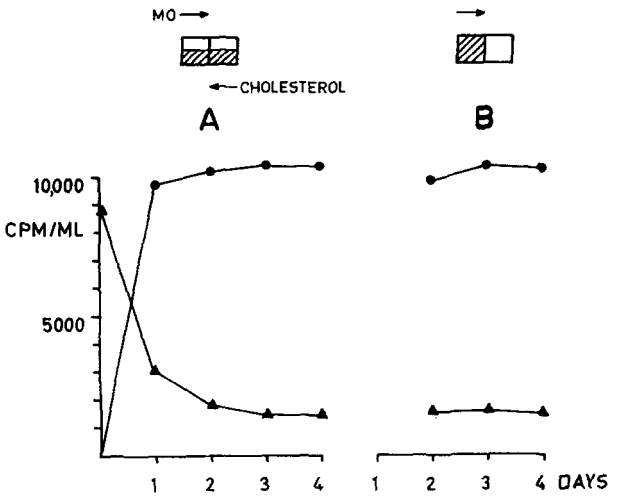

FIG. 3. Equilibration (A) versus filtration (B) for the determination of distribution of cholesterol and monoolein between the two compartments of the filtration chamber used. A and B refer to *methods 2* and *1*, respectively, as given in Methods.

In A, 2 ml of a dispersion 10 mM in TO and 5 mM in MO-^{3}H in a solution of 6 mM NaTDC was placed in the left compartment. The right compartment contained 2 ml of a micellar solution 5 mM in MO and 0.2 mM in cholesterol-^{14}C in 6 mM NaTDC. The right-hand compartment was analyzed for radioactivity after 1–4 days.

In B equal volumes of the two dispersions that were placed on each side of the filter in A were mixed, and 4 ml of this mixture was placed in the left compartment of three filtration chambers. The filtrate that collected in the right compartment was analyzed for radioactivity after 2–4 days.

Monoolein ▲, cholesterol, ▲.

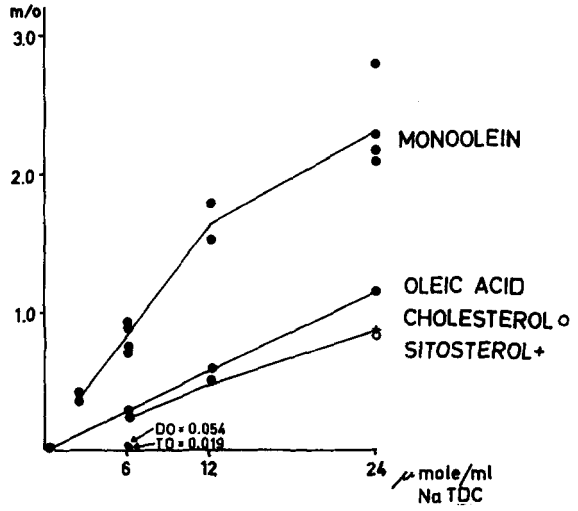

FIG. 4. Partition ratios (m/o) versus bile salt concentration for different glycerides, oleic acid, and sterols in dispersions of 2.5 μmoles each TO, DO, and MO, 7.5 μmoles of oleic acid, and 1.0 μmole of sterol per ml in 6 mM NaTDC, pH 6.3. The values for cholesterol and sitosterol at 6 and 12 μmoles/ml NaTDC were identical and the symbols therefore blend.

experiments the relative proportions of tri- and diglycerides were determined chemically after their separation by TLC. The oleic acid and monoolein distributions were calculated from isotope data. The partition ratios for the

polar oleic acid and monoolein were higher than those for the nonpolar di- and triglyceride and increased with bile salt concentration. The partition ratio for monoolein in the glyceride–oleic acid–bile salt dispersion at higher bile salt concentration shows rather large variability in different determinations. It should, however, be clear that it is a natural feature of the partition coefficient as defined here that at higher values, a small change in distribution has a large effect on the coefficient.

Effect of pH on the Partition of Oleic Acid

When the partition m/o was determined for oleic acid at different pH values, the results shown in Fig. 5 were obtained. At low pH values the partition ratio was constant, whereas a steep increase was seen as the pH was made to increase from 6 to 8.

Partition of Fatty Acids of Different Chain Length

At pH 6.3 the partition values m/o of linoleic and oleic acid are identical (Fig. 6). Decreasing the chain length of the fatty acid resulted in an increase in the partition ratio in favor of the micellar phase, decanoic acid being approximately equally distributed between the micellar and oil phases at this pH. Studies using a dispersion of the lipids in buffer instead of bile salt clearly showed that the short-chain acids were also distributed preferentially into the aqueous phase under these conditions. Because acids of short and medium chain length have a definite solubility in water the presentation of results in terms of a single coefficient, as in Fig. 6, is an oversimplification.[2]

Partition of Lipids Other than Glycerides and Fatty Acids

The results previously discussed refer to the lipids which make up the chief components of the oil phase during digestion under normal dietary conditions. In the following, I will discuss the partition behavior of substances added to the oil phase as minor components. Most of the substances studied are usually considered nonpolar. Their partition (m/o) ratios are given in Table 2 and have been determined under identical conditions in the mixed glyceride–oleic acid–bile salt system described above. The highest values, those for cholesterol and sitosterol, are of the same order of magnitude as those of long-chain fatty acids in slightly acid solution (see Fig. 4). The highly nonpolar cholesteryl oleate has the lowest partition m/o. The cholesteryl ethers show a partition m/o that decreases with the chain length of the aliphatic alcohol residue. Octadecane has a partition m/o approximately equal to that of cholesteryl methyl ether. Increasing the bile salt

[2] Figures identically calculated for the partition of fatty acids of different chain length in dispersions in buffer pH 6.3 in the absence of bile salt were as follows: C_6, C_8, C_{10}, C_{12} acids >100, >100, 0.29, and 0.02. The amount of oleic acid and linoleic acid in the aqueous phase was not measurable.

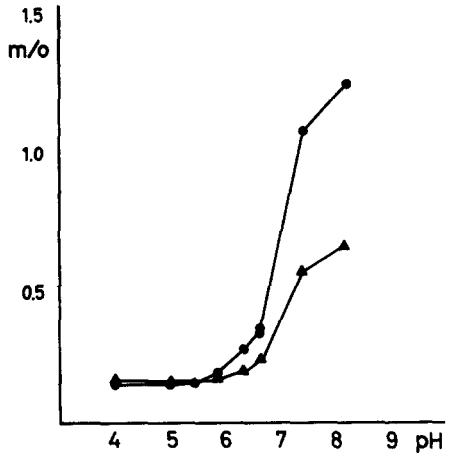

FIG. 5. Effect of pH on the partition ratio (m/o) of oleic acid (●) and cholesterol (▲). Dispersion made up of 2.5 μmoles each of TO, DO, and MO, 7.5 μmoles of oleic-^{3}H, and 0.1 μmole of cholesterol-^{14}C per ml in 6 mM NaTDC at the pH indicated. Buffers (citrate, phosphate, and borate) were all 0.15 M with respect to Na^+.

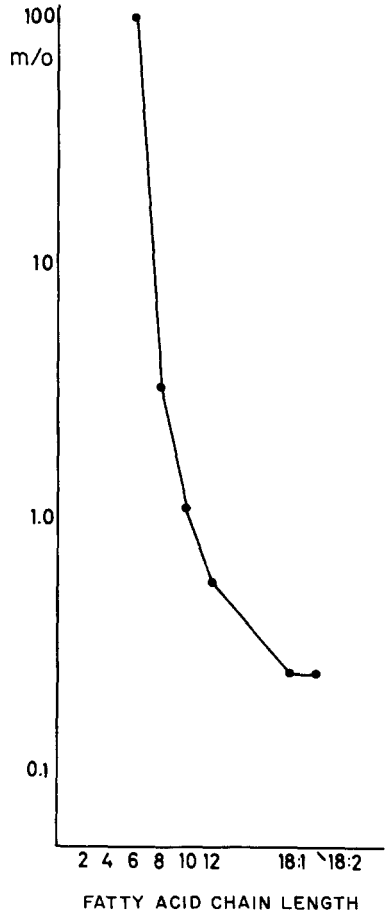

FIG. 6. Partition ratio (m/o) for fatty acids of different chain length. 2.5 μmoles each of TO, DO, and MO and 7.5 μmoles of the fatty acid were dispersed per ml of 6 mM NaTDC, pH 6.3.

TABLE 2 PARTITION RATIOS (M/O) FOR SEVERAL LIPIDS

Lipid	Ratio m/o
Cholesterol-^{14}C	0.25
Sitosterol-^{3}H	0.23
Cholesteryl oleate-^{3}H	0.033
Cholesteryl-^{14}C methyl ether	0.068
" propyl "	0.047
" amyl "	0.035
" decyl "	0.021
Octadecane-^{14}C	0.050

The original dispersion contained one of the labeled lipids dissolved in the oil dispersed in bile salt solution. The original dispersion contained 2.5 μmoles each of TO, DO, and MO and 7.5 μmoles of OA per ml in a solution of 6 mM NaTDC, pH 6.3. The concentration of the labeled solutes studied was 0.1 μmole/ml in the original dispersion.

TABLE 3 PARTITION BETWEEN MICELLAR AND OIL PHASES FOR OCTADECANE AND CHOLESTEROL IN GLYCERIDE–OLEIC ACID–BILE SALT DISPERSIONS

Amount of Octadecane	Ratio m/o	
	Octadecane	Cholesterol
μmoles/ml		
0	—	0.25
0.1	0.07	0.25
0.2	0.064	0.25
1.0	0.063	0.21
2.0	0.061	0.19
5.0	0.047	0.18

TO, DO, and MO (2.5 μmoles each), OA (7.5 μmoles), cholesterol (1.0 μmole), and the indicated amounts of octadecane were dispersed per ml of a solution of 6 mM NaTDC, pH 6.3. The micellar phase was separated as described in the text.

TABLE 4 EFFECT OF AMOUNTS OF CHOLESTEROL AND SITOSTEROL ON THE PARTITION OF CHOLESTEROL IN A GLYCERIDE–FATTY ACID–BILE SALT DISPERSION

Amounts in Original Emulsion		Ratio m/o
Cholesterol	Sitosterol	
μmoles/ml		
0.2	—	0.23
1.1	—	0.25
10.1	—	0.08
0.1	0.1	0.23
0.1	1.0	0.26
0.1	10.0	0.10

TO, DO, and MO (2.5 μmoles each) and OA (7.5 μmoles) with the amounts of sterol indicated above were dispersed per ml of a solution of 6 mM NaTDC.

concentration results in an almost linear increase in m/o for cholesterol and sitosterol (Fig. 4) starting approximately from the CMC of the bile salt used.

An increase in total concentration of the nonpolar component within certain limits does not affect the partition to any great extent. This is demonstrated both for octadecane (Table 3, first two columns) and for cholesterol (Table 4). The fall in partition ratio with higher concentration of cholesterol (Table 4) is due to the fact that the solubility of cholesterol in the micelles is exceeded. Whether the mass of sterol is cholesterol or sitosterol is of no importance for the sterol partition (Table 4).

At high concentrations of octadecane in the system, the partition of cholesterol is decreased (Table 3). The pH of the dispersion also affects the partition of cholesterol (see Fig. 5), which indicates that the inclusion of soaps into the micelles favors the distribution of cholesterol to the micellar phase.

Effect of Fatty Acid Composition on the Partition of Cholesterol

Glyceride–fatty acid–bile salt dispersions with different fatty acid species present in the glyceride ester bonds and as free fatty acid were compared. The different fatty acids used were oleic, linoleic, and the saturated C_{12} and C_8 acids. The results given in Fig. 7 show that the fatty acid structure is important for cholesterol distribution. Cholesterol was distributed more in favor of the oil phase when the component acid was oleic acid than when it was linoleic acid, the difference observed being statistically significant.

The highest figures were obtained when lauric acid was the component acid. Also the octanoic acid compounds gave a partition m/o higher than that for the oleic acid system.

Effect of Some Other Detergents

Table 5 includes the relative partition ratios obtained for monoolein and cholesterol between micellar and oil phases with the glyceride–oleic acid–cholesterol system dispersed in the micellar solution of a few anionic detergents and one nonionic detergent. The anionic detergents were used in a concentration of 6 mM. They all have a CMC below 1 mM and therefore for all of them 5 or more μmoles/ml were in micellar form. The nonionic detergent Pluronic 68 has a much higher molecular weight and was therefore used at lower concentrations to keep the micellar volume about the same as that for the anionic detergents. In the table, the partition m/o for monoolein and cholesterol is given as a percentage of that obtained with NaTDC. NaTDC is the only detergent that gives a high partition ratio for monoolein; although it also gives the highest figure for cholesterol, some of the other detergents give quite similar values.

Transport Studies

In the experiments so far described the partition of lipids between micellar and emulsified oil phase at equilibrium was studied. As the filter allows the separation of a micellar solution, it was also of interest to study the trans-

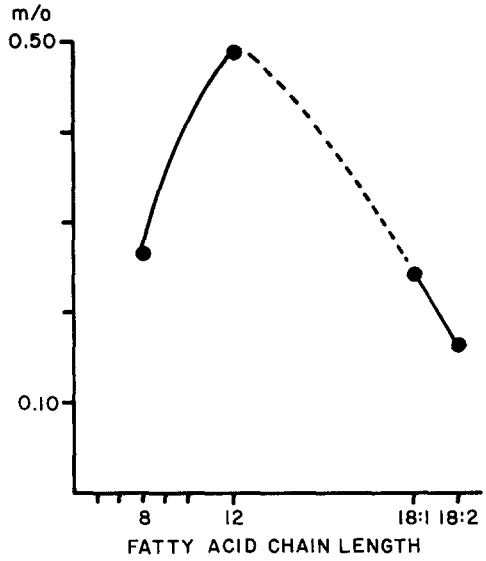

FIG. 7. Effect of chain length of the component fatty acids on the partition of cholesterol between micellar and oil phases. Tri-, di-, and monoglyceride with fatty acids of the chain length given in the figure, each 2.5 mM, the corresponding fatty acid (7.5 mM), and cholesterol (1 mM) were dispersed in 6 mM NaTDC, pH 6.3.

port of lipids through the membrane in micellar form and the importance of the partition ratio for this transport. In these experiments the filtration chamber was placed so that the filter was horizontal and the lipid dispersion, usually a total volume of 4 ml, was introduced in the upper chamber (*method 3*, see Methods). A head of bile salt solution was applied to maintain a constant volume in the upper chamber and fractions were collected from the lower chamber. Mixing in the upper chamber was accomplished by the use of a to and fro shaker.

When a dispersion of the amphiphilic monoolein and oleic acid in bile salt solution at a concentration well above their micellar solubility was placed in the upper

TABLE 5 EFFECT OF SUBSTITUTION OF VARIOUS DETERGENTS FOR BILE SALT ON THE M/O FOR MONOOLEIN AND CHOLESTEROL IN DISPERSION WITH GLYCERIDE–OLEIC ACID

Detergent	Monoolein	Cholesterol
	relative m/o	
None	—	—
NaTDC	100	100
Decanoylsarcosyl taurate	5	9
Oleoylsarcosyl taurate	29	81
Decanoyl taurate	6	16
Oleoyl taurate	24	81
Dodecyl sulfate	11	28
Pluronic F 68 I	14	51
" II	29	88

TO, DO, and MO (2.5 μmoles each), OA (7.5 μmole), and cholesterol (0.1 μmole) were dispersed per ml of detergent solution in buffer pH 6.3. All detergents except Pluronic were used in a concentration of 6 mM. The two Pluronic F 68 Samples (I and II) contained 0.2 and 0.6 μmole/ml of this detergent respectively.

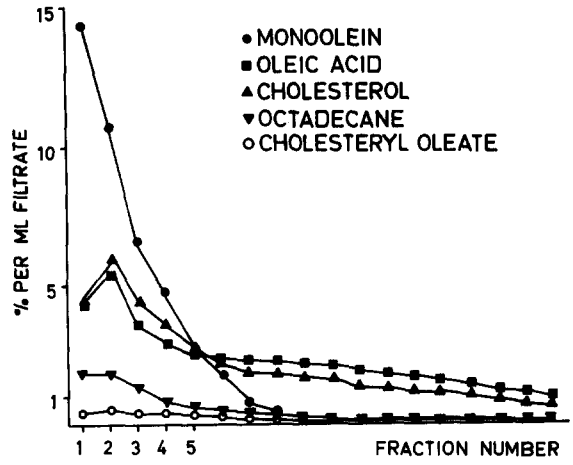

FIG. 8. Filtration of different solutes through Millipore filter in micellar form. Dispersions were made of TO, DO, MO (each 2.5 mM), OA (7.5 mM), and different labeled compounds in pairs in the concentrations given below in a solution 6 mM in NaTDC, pH 6.3. 4 ml of the respective dispersions were placed in the upper compartment of a filtration chamber oriented to hold the filter in horizontal position. The micellar phase was filtered using 0.3 μ Millipore filter; as filtration proceeded 6 mM NaTDC was continuously added to maintain a constant volume above the filter. Fractions of filtrate were collected by means of a drop counter; weight of the fractions varied between 1.32 and 2.60 g depending on the surface tension of the filtrate. The curves for the different compound listed on the figure are taken from three different experiments each using pairs of labeled compounds: (*a*) Monoolein-^{14}C and cholesteryl oleate-^{3}H (0.1 mM); (*b*) Oleic acid-^{3}H and cholesterol-^{14}C (1.0 mM); (*c*) Octadecane-^{14}C (1 mM) and cholesterol-^{3}H (0.4 mM). The values for cholesterol are from the second experiment only. Those obtained in the other two experiments were similar. The amounts of labeled monoolein and oleic acid added were small compared to the mass of these substances in the dispersions.

chamber, the fresh bile salt solution entering subsequently solubilized and passed the monoolein and oleic acid through the filter in micellar form. No oil phase was left behind and the small amount of cholesterol present in the dispersion was cosolubilized and filtered almost completely.

In other experiments dispersions of tri-, di-, and monoolein and oleic acid, containing labeled compounds in different combinations and dispersed in bile salt solution, were filtered in the same way. The results are given in Fig. 8.

Almost 100% of the monoolein was recovered in the first eight fractions (total volume approximately 18 ml). In the same volume the other compounds were found in amounts related to their partition between micellar and oil phase in the equilibrium experiments. The amounts of tri- and diglyceride filtered were in all cases lower than those of monoolein and oleic acid. These compounds therefore remained the main components of the oil phase. It is obvious from the results of Fig. 8 that the filtration in micellar solution of compounds with a relatively low partition ratio (m/o) is favored as long as micelles containing

monoolein and to a lesser extent oleic acid are filtered. No difference in extent of filtration of cholesterol and sitosterol was found under similar condition.

When triolein containing labeled cholesterol was dispersed in bile salt solution in the upper compartment, the concentration of cholesterol in the filtrate was low compared to when the dispersion contained more polar lipids such as monoolein and oleic acid, and was directly proportional to the cholesterol content of the triolein. Addition of pancreatic lipase to the bile salt solution entering the upper compartment resulted in a 6- to 8-fold increase in the concentration of cholesterol in the micellar filtrate. This type of experiment, again demonstrating the importance of the polar products of pancreatic lipolysis for the transport of cholesterol in micellar solution, usually was difficult to carry to completion because the filter became clogged soon after lipase was added to the bile salt solution.

DISCUSSION

Bile salts are biological detergents and at a concentration above their CMC can dissolve lipids by polar or nonpolar solubilization (14). When the solubilizing ability of the bile salt is exceeded, the excess of the lipid substance will be dispersed, depending on its molecular structure and composition, either as an emulsified oil phase or as a mesophase. If an oil is dispersed in excess of its micellar solubility in a detergent solution, an emulsified oil phase and a micellar phase will coexist and interact. Such systems occur in the lumen of the small intestine during the digestion of fat, the oil phase initially being made up mainly of long-chain triglycerides, substrates for pancreatic lipase. That the interaction between emulsified oil phase and micellar phase could be described as a partition was suggested by Hofmann and Borgström (1) based on the concepts of McBain and Hutchinson (17) and Shinoda and Hutchinson (18). Hofmann and Borgström (1) measured the partition coefficient of monoolein between triolein and micellar bile salt solution and defined it as (μmoles of monoolein/μmoles of triolein)/(μmoles of monoolein/μmoles of micellar bile salt).

The present paper describes more detailed studies of the properties of such systems, especially the distribution of different lipid classes between an emulsified oil phase and bile salt micelles. Such a description will give information necessary for an understanding of the complex processes that take place during lipid digestion and absorption from the intestine, and the mechanism(s) of specificity behind the marked selectivity in absorption of lipids of different chemical structure. The interaction between the micellar phase and emulsified oil in this study has been expressed as a partition, m/o, of different lipids between these two phases.

As the phase volumes cannot be exactly calculated, the relationship m/o does not strictly correspond to a partition coefficient as usually defined. Under the general experimental conditions of this investigation—when a glyceride–fatty acid mixture was dispersed in 6 mM bile salt solution—the volumes of the micellar and oil phases were calculated[3] to make up approximately 1% of the volume of the emulsion and to be of the same order of magnitude. The partition between micellar and oil phases in most of these experiments therefore corresponds to a partition coefficient $K'_{m/o}$ that is uncorrected for phase volumes.

The partition values between micellar and oil phases have been measured using Millipore filters of a pore size that allows the separation of the micellar solution from a dispersion containing the same micellar solution in equilibrium with the emulsified oil phase.

In most of the experiments, I used a lipid mixture simulating that present in intestinal content during the digestion of dietary fat. The lipid mixture contained equimolar quantities of tri-, di-, and monoolein and an amount of oleic acid equivalent to the number of free hydroxy groups of the mono- and diglycerides. The partition of these different components between an emulsified oil phase and a micellar phase was determined at different bile salt concentrations. Increase in bile salt concentration above the CMC results in an increase in m/o. This effect is presumably related only to an increase in the total micellar volume and not to a change in the concentration of lipid in the micellar phase. Very little tri- or diolein was present in the micellar phase, while the amphiphilic monoolein was, at intermediate bile salt concentration, about equally distributed between the two phases.

The distribution of fatty acids was found to be highly dependent on the pH of the solution and also on the chain length of the fatty acid. At low pH values the partition for long-chain fatty acids is in favor of the emulsified oil phase, but between pH 6 and 8 a marked increase occurs in favor of the micellar phase. The most probable ex-

[3] An approximate volume of the micelles can be obtained from data for the equivalent radius of the bile salt micelles and their anhydrous particle weight as determined by ultracentrifugation (15) and gel filtration (16). Assuming an anhydrous particle weight of 11,900, a bile salt micelle contains approximately 23 molecules with a hydrated volume of $4/3\pi \times 20$ A^3. Now a 6 mM solution of NaTDC contains approximately 5 μmoles of micellar bile salt per ml (CMC < 1 μmole/ml). The number of micelles per ml of solution will then be $(5 \times 6.02 \times 10^{17})/23$ and their volume $(5 \times 6.02 \times 10^{17})/23 \times 4/3\pi \times 20^3$ A^3 = 4.4×10^{21} A^3 or 4.4×10^{-3} ml per ml of solution.

The lipid mixture per ml of emulsion usually contained 2.5 μmoles of each tri-, di-, and monoolein and 7.5 μmoles of fatty acid. This corresponds to 6.8 mg of lipid per ml, or a volume of approximately 5×10^{-3} ml/ml.

The calculated volumes will be changed by the distribution of a fraction of the lipids between the phases, and by the concentration of the emulsified oil phase due to filtration of the micellar solution.

planation is that the curve for partition versus pH represents the dissociation curve for oleic acid under these conditions. The low partition value thus represents that of the undissociated fatty acid. The pK_a for oleic acid under the conditions of these experiments then can be estimated as being approximately 7.0. This figure is in good agreement with the results obtained from titration (19).

The results of the present experiments clearly show the importance of fatty acid chain length in the distribution of fatty acids in an emulsified oil–micelles–water system. The long-chain fatty acids, at the slightly acid pH which occurs in intestinal content, are distributed in favor of the oil phase and their concentration in molecular solution in the water phase is extremely low. With decrease in chain length the distribution to the micellar phase is increased, decanoic acid being approximately equally distributed between oil and micellar phase. In the absence of bile salt the concentration of acid in the water phase is still very low for dodecanoic acid. The fatty acids with shorter chains are shifted more and more from the emulsified oil phase to both the micellar phase and to the molecular dispersed form, octanoic being almost completely in the latter form at pH 6.3.

The pK_a of fatty acids in bile salt solution will be affected by their tendency to aggregate. As soon as aggregation occurs the ionization will be depressed because of the effect of ionized neighbors (the bile salt) and the change in dielectric constant as the hydrocarbon chains dissolve (or are solubilized) in the interior of the aggregates. The shorter the chain length of the fatty acid, the less they tend to aggregate. The pK_a for medium-chain fatty acids in bile salt solution is not known but it seems reasonable to conclude, from their behavior in the partition experiments, that the C_8 and C_6 acids, in the weakly acid solutions used here, are present mainly in the form of soaps.

The fact that the shorter-chain fatty acids are present mainly as soaps in molecular dispersion at slightly acid pH values can be an important determinant for their mechanism of transport and absorption and might well explain the old finding that decanoic acid is the dividing point for the route of transport of fatty acids via the portal or lymphatic pathway during absorption. The results also explain the unimportance of bile salt in absorption of the so-called medium-chain triglycerides.

The present results are interesting in connection with the mechanism of lipase action and the influence of such factors as bile salt, pH, etc. In a closed system at a slightly acid reaction, the long-chain fatty acids that have been split off the primary glyceride ester bonds will stay mainly in the oil phase as the undissociated acid and the monoglyceride will also be present in considerable quantities in the emulsified oil phase. The bile salt may have a more direct effect on pancreatic lipolysis, but its most important functions seem to be those in an open system such as that presented in the transport experiments in this paper and those prevailing in the intestinal lumen during digestion and absorption. In such an open system, one important function of the bile salt is the transport of the amphiphilic split products of pancreatic lipolysis from the oil phase to the brush border of the mucosa cells. This results in continuously changing equilibrium conditions at the interphases which are governed also by the presence of the enzyme and the physicochemical behavior of the hydrolysis products.

Nonglyceride lipids dissolved in the emulsified oil phase also distribute between the oil phase and the micellar phase. Their distribution seems to be related to their polarity; the more nonpolar they are, the more they distribute in favor of the oil phase. The free sterols partition as the undissociated long-chain fatty acids. Cholesteryl oleate, on the other hand, has a partition m/o that is approximately 10 times lower than that of the free sterol. Since the highly nonpolar octadecane partitions more in favor of the micellar phase than cholesteryl oleate and many of the cholesteryl ethers, not only the polarity but also the chemical structure must be important in the partition. It seems quite feasible that the partition is related to the stereochemical "fit" of the substance to the micellar structure. The fatty acid structure of the phase components is also important for the behavior of nonpolar lipids in these systems, distribution to the micellar phase being favored by lower unsaturation (oleic compared to linoleic) and shorter chain length (lauric compared to oleic). The possible importance of these differences in the absorption of sterols from different triglyceride carriers in vivo is under investigation.

Cholesterol and sitosterol have approximately the same partition behavior, as would be expected from their closely similar chemical structure. This is in contrast to the great difference in their extent of absorption from the intestinal tract.

The partition of a lipid between the micellar and emulsified oil phases is largely independent of its concentration, as long as its solubility in the micellar phase is not exceeded. In this respect the system functions as would be expected for a partition system in general.

In the transport type experiments three factors were found important for the mass of nonpolar lipid filtered in micellar form. These factors are (*a*) the presence of an oil phase, (*b*) the presence of amphiphilic substances such as monoolein and to some extent fatty acids, and (*c*) the partition of the nonpolar compound transported between the micellar and emulsion phase. In the absence of an oil phase the substance was transported in relation to its micellar solubility. The presence of an oil phase "retained" the nonpolar compound. The main transport took place in the mixed micelles containing bile salt and

the more polar products of the system, the monoglycerides and fatty acids. When these had passed the filter or were absent from the start, the transport of nonpolar compounds became much smaller, indicating the importance of the polar products of pancreatic lipolysis and especially the monoglycerides for this function. If the transport of lipids from the oil to the micellar phase is important in vivo, it will constitute one level of specificity in absorption which will be based on physicochemical factors related to molecular structure.

In the models used in these experiments a system has been devised in which an oil phase was present to the end of the experiment. Under in vivo conditions, the oil phase is continuously used up with the production of polar products. It seems reasonable to assume that under such conditions a fraction of nonpolar substances with a partition m/o that is low in relation to monoglycerides and fatty acids will remain dispersed but not solubilized in the bile salt solution when the oil phase has been "used up." The fraction of a substance remaining, that is, not transported to the micellar phase, should be related to its phase "partition coefficient."

The mass of nonpolar compound transported was found to be directly proportional to the total concentration of the compound in question in the oil phase.

The two-phase emulsion system thus functions as a generator of a micellar solution in which the concentration of nonpolar lipids present, dissolved in the oil phase from the beginning, is proportional to the partition ratio and to the concentration in the emulsified oil phase. If the micellar phase is the substrate for absorption by the intestinal cells, the percentage absorption of nonpolar lipids should be largely independent of their concentration in the triglyceride oil in which they are fed.

The unusual properties of bile salts as detergents for the interaction with the polar product of pancreatic lipolysis—the monoglycerides—were again demonstrated (14). A series of anionic and one nonionic detergent gave partition ratios (m/o) for monoolein in the glyceride–oleic acid detergent dispersion that did not exceed 30% of that obtained with a typical bile salt. The partition of cholesterol in this system was less dependent on detergent structure. It is of some interest that the acylsarcosyl taurates, present in the digestive tract of the crab (20), did not compare to bile salt in the interaction with monoolein. From evidence at hand it seems that the effectiveness of bile salt in its interaction with the products of pancreatic lipolysis is related to its hydroxy functions.

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