

## THE BEHAVIOR AND SOLUBILITY OF MONOGLYCERIDES IN DILUTE, MICELLAR BILE-SALT SOLUTION

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(Received December 7th, 1962)

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### SUMMARY

1. The behavior and solubility of a number of pure monoglycerides in dilute, micellar bile-salt solution has been studied *in vitro*, using experimental conditions simulating those present in human small-intestinal content during fat digestion with respect to  $\text{Na}^+$  (0.15 M), pH (6.3) and temperature ( $37^\circ$ ).

2. Monoglycerides with a chain length of 10 carbon atoms or more have a low solubility in buffer alone, although certain unsaturated monoglycerides (*e.g.* 1-monoolein, 1-monolinolein, 2-monoolein) may interact with buffer to form liquid crystalline states; these, however, disperse poorly.

3. In bile-salt solution, the solubility of monoglycerides is greater and their behavior and solubility is chiefly related to melting point. At  $37^\circ$ , monoglycerides with melting point under about  $65^\circ$  have a striking micellar solubility, being solubilized as amphiphiles. Higher-melting-point monoglycerides (*e.g.* 1-monopalmitin or 1-monomyristin) are solubilized as non-polar solutes and have much lower solubilities.

4. 1-Monoolein and 2-monoolein are solubilized identically by bile-salt solutions. 2-Monopalmitin has a higher solubility than 1-monopalmitin, but the difference may only reflect the former's lower melting point. 2-Monoolein and 2-monopalmitin isomerize slowly in dilute bile-salt solution (5 % in 1 h; 55 % in 24 h) with the experimental conditions cited.

5. If two amphiphilic monoglycerides are equilibrated with bile-salt solution, they are solubilized competitively; however, the solubilization of an amphiphilic monoglyceride generally enhances the solubility of a higher-melting-point (non-polar) monoglyceride.

6. Glycerol 1-monoethers and 1-monoglycerides containing the same alkyl radical are solubilized identically by bile-salt solutions, with the appropriate experimental conditions.

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### INTRODUCTION

Any understanding of the complicated process of fat absorption requires clarification of the physico-chemical state of lipids in intestinal content during digestion and absorption. Ingested triglyceride is hydrolyzed by pancreatic lipase chiefly to fatty

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Abbreviations: TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

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acids and monoglycerides, and these polar lipids are solubilized in bile-salt micelles to form an isotropic micellar solution<sup>1,2</sup>. A previous report<sup>3</sup> has discussed the solvent properties of solutions of conjugated bile salts in general. Also described was the behavior of 1-monoolein *in vitro* in solutions of different conjugated bile acids and the effect of experimental variables (pH, temp., Na<sup>+</sup> concentration and bile-salt concentration) on this.

All lipids dissolve to some extent in micellar solutions and this phenomenon is termed solubilization<sup>4</sup>. Two types of micellar solubilization are distinguished, amphiphilic or polar, and non-polar<sup>3-5</sup>. Amphiphilic or polar solutes have a higher micellar solubility, are believed to be oriented with their polar groups at the micelle's surface, and when present in concentrations higher than that which can exist in isotropic micellar solution, interact with the micellar solution generally forming liquid crystalline states<sup>6</sup>. Their solubilization may increase the micellar solubility of non polar solutes. Non polar solutes have a much lower micellar solubility, are believed to be incorporated into the center of the micelle, and when present in excess do not interact with the saturated micellar solution. This classification of solute behavior in micellar solutions is quite useful, and may be applied to the behavior of lipids in micellar bile-salt solution, although the molecular arrangement of the bile-salt micelle containing solubilized amphiphile may be quite different from that of the typical ionic detergent micelle containing amphiphile.

This paper compares the behavior and solubility *in vitro* of a number of monoglycerides in dilute bile-salt solution (<20 mM) with their behavior and solubility in phosphate buffer. The experimental conditions were constant and such as to simulate conditions in the human small-intestinal lumen during fat digestion and absorption with respect to Na<sup>+</sup> (0.15 M), pH (6.3) and temperature (37°) (see ref. 7). In a few experiments, structural analogues (glyceryl 1-monoethers and glycol monoesters) were studied.

An accompanying paper<sup>8</sup> presents observations on the action of pancreatic lipase on monoglycerides, the substrate being in the form of a mixed bile-salt monoglyceride micelle.

#### MATERIALS AND METHODS

As previous experiments and additional preliminary experiments had shown that the behavior of 1-monoolein and 1-monolaurin was qualitatively similar in solutions of a number of conjugated bile salts, all experiments were performed with sodium taurodeoxycholate. The preparation had been synthesized by the method of NORMAN<sup>9</sup> as described<sup>10</sup>, and was at least 98 % pure by TLC<sup>11-13</sup>. Phosphate buffer with pH 6.3 and 0.15 M in Na<sup>+</sup> was prepared as described<sup>3</sup>.

#### Monoglycerides

The 1- and 2-monoglycerides used were of high purity with respect to class and homologue composition. 1-Monomyristin, 1-monolaurin, 1-monoolein and 1-monolaidin were generously supplied by Dr. L. BECK, Proctor and Gamble Laboratories, Cincinnati, Ohio (U.S.A.). 1-Monopalmitin, 1-mono[1-<sup>14</sup>C]palmitin, 1-monodecanoin, 1-monooctanoin, 2-monopalmitin, and 2-monoolein were synthesized in this laboratory by Dr. L. KRABISCH or Dr. B. BORGSTRÖM. Class purity was assayed by TLC as described<sup>13,14</sup> and by periodate titration<sup>15</sup>. 1-Monoglycerides were at least 97 % pure

by class; 2-monoolein contained about 5% 1-isomer, and 2-monopalmitin 7% 1-isomer. The 2-monoolein and 2-monopalmitin gave a periodate titration value within 3% of the theoretical value, after  $\text{HClO}_4$ -induced isomerization, using the correction factor of 1.15 (ref. 16). Homologue purity, examined by GLC, was greater than 95% except for 1- and 2-monoolein which contained about 10% palmitoleic acid, as monopalmitolein.

### *Monoglyceride structural analogues*

Glyceryl 1-monododecyl ether, glyceryl 1-monooleyl ether (selachyl alcohol), and glycol monooleate were synthesized by Dr. L. KRABISCH, and purified to class purity (TLC) by column chromatography on silicic acid. Homologue purity was as described for 1- and 2-monoolein.

Bile-salt solutions, 0.04 M in bile salt and 0.15 M in  $\text{Na}^+$  were prepared as described<sup>3</sup>. Ampoules containing solute, bile salt and buffer were prepared as described<sup>3</sup> and incubated at 37° until equilibrium was reached. All experiments were performed in duplicate.

### *Solubility determinations*

These were performed according to the behavior of the solute in buffer or bile salt.

1-Mono[1- $^{14}\text{C}$ ]palmitin solubility was determined as follows: the solution was filtered through a 100- $\mu$  Millipore R filter. The filtrate was allowed to stand several days. During this time, a micro-crystalline precipitate would appear and was allowed to sediment spontaneously; occasionally centrifugation (at 37°) was necessary. The water-clear supernatant was carefully aspirated, and transferred to a graduated glass-stoppered tube; the volume was recorded. Three volumes of diethyl ether – heptane – ethanol (1:1:1, v/v) was added, and most of the upper phase removed. The lower phase was then extracted two additional times with the upper phase of pre-equilibrated diethyl ether–heptane–ethanol–water (1:1:1:1, v/v)<sup>17</sup>. The pooled extracts were evaporated, and the residue counted in a liquid scintillation counter.

1-Monomyristin solubility was determined similarly, except that the monoglyceride in the final pooled extracts was determined by weighing or by titration after saponification.

For these two monoglycerides, the values plotted cannot be considered to be very accurate, as no satisfactory method could be found for complete separation of the micellar solution from the finely divided crystalline excess. The results are approximately correct, for ampoules prepared with 10% less than the reported solubilities showed complete solution and ampoules prepared with 10% more showed a crystalline excess. Results were the same if reached from over-saturation or under-saturation and duplicates agreed closely.

The remaining monoglycerides and the structural analogues behaved as amphiphiles in bile-salt solution. They possessed a high micellar solubility and when an excess was present, turbidity appeared whose extinction generally was roughly proportional to the excess present, over a small range of excess. The solubility was determined turbidometrically as described<sup>3</sup>.

No satisfactory method was developed for the exact determination of the solubility of most of the monoglycerides in buffer alone. When the excess was crystalline, the solubility was determined as outlined above for 1-monopalmitin in bile-salt

solution. When the excess was liquid crystalline or unemulsified droplets, the solubility was determined by inspection. A series of ampoules containing increasing amounts of solute was equilibrated with buffer, then examined with a powerful beam of light in a darkened room. Extremely accurate values are not considered essential for the interpretation of the results presented.

In some experiments, a mixture of two monoglycerides was incubated with a bile-salt solution. A single bile-salt concentration was used. A number of groups of ampoules were prepared. Each group had a constant concentration of one monoglyceride, while the concentration of the other monoglyceride was varied in individual ampoules. The concentration of the first monoglyceride was increased progressively in each group of ampoules. Thus a complete range of concentrations of both monoglycerides was present in the experiment. The ampoules were examined with respect to rapidity of equilibration, whether or not complete solution occurred, and the form and amount of excess present after equilibration was obtained.

1-Monooctanoin and 1-monolaurin had an appreciable buffer-solubility. To determine whether or not any micellar aggregation occurred prior to the appearance of turbidity, azobenzene solubilization studies were performed as described<sup>2</sup>.

#### *Rate of isomerization of 2-monoglycerides in dilute bile-salt solutions*

The 2-monoglycerides were added in heptane to ampoules, and the heptane was removed by suction; both operations were performed at 8°. Bile salt and buffer were added and the ampoules rapidly sealed by fusion. They were then shaken vigorously for 5 min in a 37° water bath (this was sufficient time for equilibration of the 1-monolein, but not the 1-monopalmitin). The ampoules were then carried to a 37° constant-temperature room and placed on a to-and-fro shaker. The zero-time sample was removed, the contents transferred to a graduated glass-stoppered test tube, and extracted immediately as described above for 1-monopalmitin-solubility determinations. At appropriate time intervals, subsequent samples were removed from the shaker and treated similarly. If extraction could not be performed immediately, the sample was frozen instantaneously on removal from the shaker, in a dry ice-alcohol bath, and kept frozen until extraction could be done. The pooled extracts were evaporated at room temperature with suction, then dried in a desiccator over P<sub>2</sub>O<sub>5</sub>. The 1- and 2-isomer content was then determined<sup>15,16</sup>.

Control experiments had shown that the extraction procedure resulted in no isomerization of the unstable 2-isomer, and this point was confirmed by the experimental results. The completeness of the extraction procedure was also clear from the analytical results. As a further check, samples were taken at 12 and 24 h and analyzed for total monoglyceride content by periodate titration after HClO<sub>4</sub>-induced isomerization; recovery was 95–100 %.

### RESULTS

The solubility of the monoglycerides in sodium taurodeoxycholate is shown in Fig. 1. Such curves are termed solubilization curves<sup>2</sup>; their slope is termed the saturation ratio, *i.e.* micellar monoglyceride/micellar bile-salt.

#### *Behavior of monoglycerides and structural analogues in buffer alone*

1-Monopalmitin and 1-monomyristin have a very low solubility in buffer; their

polar groups have little influence. The excess is crystalline and usually adherent to the glass surface.

1-Monolaurin behaves quite differently. It has a higher solubility in buffer and when excess is present, it is liquid. Equilibrium is very low if crystalline 1-monolaurin is incubated with buffer, but reached rapidly if the monolaurin is melted prior to incubation by brief warming. The excess rises, but is dispersed easily. It is not birefringent.

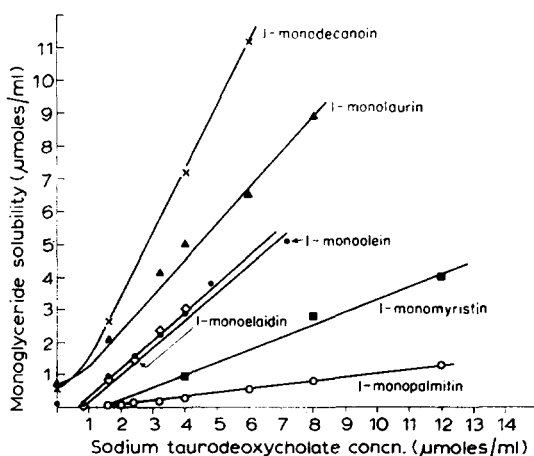


Fig. 1. Solubility of 1-monoglycerides in sodium taurodeoxycholate, in sodium phosphate buffer (pH 6.3,  $\text{Na}^+$  0.15 M,  $37^\circ$ ). The slope of the curve is the ratio of micellar monoglyceride to micellar bile salt. The intercept of the curve with the ordinate is the solubility of the monoglyceride in buffer alone, which, except for 1-monolaurin and 1-monodecanoin, is very low. The solubility of 1-monooctanoin in sodium taurodeoxycholate (4  $\mu\text{moles/ml}$ ) is about 34  $\mu\text{moles/ml}$ , which lies off the graph.

1-Monodecanoin remains crystalline when incubated with buffer at  $37^\circ$ . Its solubility is lower than that of 1-monolaurin. It disperses more readily than 1-monomyristin or 1-monopalmitin. There was no azobenzene solubilization at  $37^\circ$  or  $55^\circ$  by solutions of 1-monodecanoin. At  $55^\circ$  excess 1-monodecanoin was present as a crude emulsion.

1-Monooctanoin has an appreciable buffer solubility (about 3.8  $\mu\text{mol/ml}$ ). In excess, it is present as poorly emulsified droplets. No azobenzene solubilization was observed.

1- and 2-monoolein are almost insoluble in buffer alone, but hydrate, forming myelin figures. Despite the formation of the liquid crystalline state, the compounds do not disperse, but remain adherent to the glass surface. 1-Monoelaidin behaves similarly at  $37^\circ$  but does not form myelin figures at  $23^\circ$ , as do both 1- and 2-monoolein.

1-Mono- $\alpha,\alpha$ -dimethyldecanoin has a low solubility in buffer and in excess floats as unemulsified droplets on the surface. It does not form myelin figures in buffer at  $37^\circ$  when examined with the polarizing microscope.

Glyceryl 1-monododecyl ether has a low solubility in buffer alone; at  $37^\circ$ , it does not form myelin figures and in excess it remains crystalline. At higher temperatures, it does form myelin figures. Glyceryl 1-monooleyl ether (selachyl alcohol) behaves as 1- and 2-monoolein; it has a low solubility and forms myelin figures. Glycol monooleate behaves as 1- and 2-monoolein and selachyl alcohol.

*Behavior of monoglycerides and structural analogues in dilute bile-salt solution at 37°*

1-Monopalmitin has a very low saturation ratio, and the excess is crystalline; its behavior can be compared to that of a non-polar solute such as azobenzene<sup>3</sup>.

1-Monomyristin behaves similarly. Its saturation ratio is higher in accordance with its smaller molar volume, although this may not be the only factor.

1-Monolaurin behaves as an amphiphile. Its saturation ratio is considerably higher than that of 1-monomyristin. The excess forms a viscous, slightly turbid phase which slowly settles; the phase is not birefringent.

1-Monodecanoin and 1-monooctanoin behave similarly to 1-monolaurin, but their micellar solubilities or saturation ratios are much higher. 1-Monooctanoin (not plotted) is considerably more soluble than 1-monodecanoin. In neither case is the excess birefringent.

1- and 2-monoolein behave as typical amphiphiles, the excess forming a uniformly turbid system, which shows little tendency to separate. In some bile salts, *e.g.* sodium glycochenodeoxycholate, a turbid phase slowly settles. No birefringence has been noted in the turbid system. In one case the turbid phase was concentrated by ultracentrifugation<sup>3</sup>; when examined microscopically, it was not birefringent. The saturation ratios of 1- and 2-monoolein are identical, based on determinations at two bile-salt concentrations. The experiments with 2-monoolein were read after 1 h, at which time not more than 5 % isomerization to the 1-isomer had occurred.

1-Monoelaidin behaves as 1- and 2-monoolein at 37°, although it does not behave as an amphiphile at 23° when it behaves as 1-monomyristin or 1-monopalmitin (at 37°). 1-Monolinolein behaves as 1- and 2-monoolein and has about the same saturation ratio. The experimental values are not plotted, because the sample available was not of high purity.

2-Monopalmitin has an appreciably higher solubility than 1-monopalmitin in dilute bile-salt solution. This could not be measured accurately because equilibration of the crystal form was so slow that the monoglyceride in solution had already isomerized. If the solution was pre-warmed at 60° to obtain rapid equilibration, the higher solubility of the 2-isomer at 37° was apparent, but some isomerization always occurred. The excess at 37° is crystalline.

1-Mono- $\alpha,\alpha$ -dimethyldecanoin has a far lower saturation ratio than its straight-chain homologues. The excess forms a viscous, opalescent, non-anisotropic phase which settles out.

Glyceryl 1-monododecyl ether and glyceryl 1-monooleyl ether (selachyl alcohol) behave as 1-monoolein, although their saturation ratios are slightly higher. The excess forms a uniformly turbid system, which is not birefringent.

Glycol monooleate is less soluble than 1-monoolein in bile-salt solution. When present in excess it shows uniform, opalescent turbidity, whose extinction rises extremely little for the amount of excess present, compared to 1-monoolein. Solubility values could not be determined satisfactorily and are not plotted.

*Behavior of monoglyceride mixtures in dilute bile salt solution*

*Two amphiphilic monoglycerides.* Fig. 2 is a phase diagram of the 1-monoolein-1-monolaurin-sodium taurodeoxycholate system. The curve plotted is the upper limit of the isotropic micellar solution. Any mixture whose composition falls under the curve will be micellar solution; any mixture corresponding to a point above the

line is turbid. The curve slopes upward toward the 1-monolaurin ordinate because of the 1-monolaurin's higher buffer-solubility and saturation ratio. The deviations from linearity are probably within experimental error.

*An amphiphilic monoglyceride and a non-polar monoglyceride.* The addition of an amphiphilic monoglyceride such as 1-monoolein or 1-monolaurin to bile-salt solution considerably increases the solubility of a non-polar monoglyceride such as 1-monopalmitin or 1-monomyristin. The enhancing effect of 1-monoolein on 1-monopalmitin solubility has been reported<sup>2</sup>. When the micelle is nearly saturated with amphiphile, the solubilization of a monoglyceride such as 1-monopalmitin becomes competitive.

#### *Isomerization of 2-monoolein and 2-monopalmitin*

The rate of isomerization of 2-monoolein and 2-monopalmitin to their respective 1-isomers in 4 mM sodium taurodeoxycholate (pH 6.3, 37°) is shown in Fig. 3.

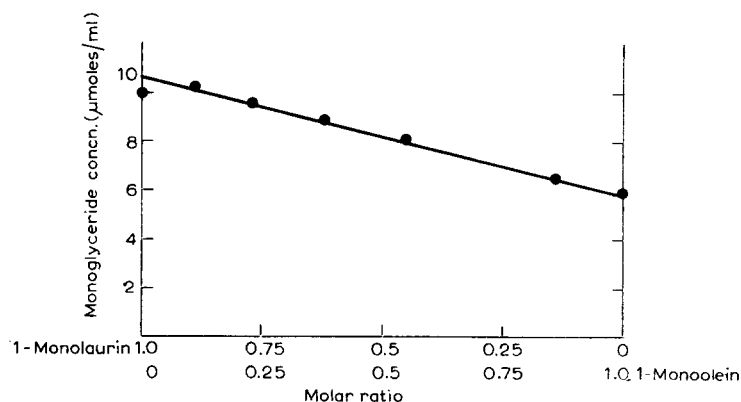


Fig. 2. Phase diagram, illustrating competitive solubilization of 1-monolaurin and 1-monoolein by sodium taurodeoxycholate (4 μmoles/ml) in sodium phosphate buffer (pH 6.3, Na<sup>+</sup> 0.15 M, 37°). Any mixture of a composition falling below the line exists in isotropic, micellar solution; mixtures of composition lying above the line are turbid.

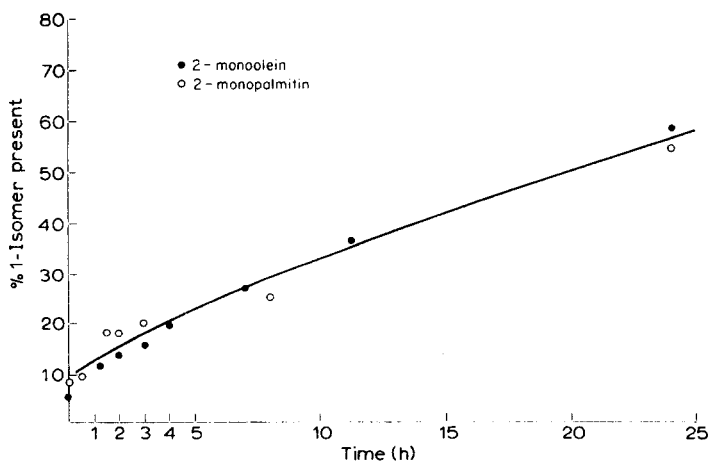


Fig. 3. Rate of isomerization of 2-monoglycerides in sodium taurodeoxycholate (4 μmoles/ml) in sodium phosphate buffer (pH 6.3, Na<sup>+</sup> 0.15 M, 37°). The monoglycerides were present in isotropic, micellar solution at the beginning of the experiment.

## DISCUSSION

The solubility of saturated 1-monoglycerides in buffer is similar to that of a series of aliphatic homologues containing no or weak polar groups. The low solubility of 1-monopalmitin can be explained in terms of the crystal lattice or by surface energy considerations<sup>18,19</sup>. 1-Monomyristin has a lower melting point or heat of fusion, and accordingly a higher solubility than 1-monopalmitin. A similar explanation applies to 1-monodecanoin. 1-Monooctanoin is liquid at 37° and, as anticipated<sup>19</sup> has a considerably higher solubility in buffer than the monoglycerides which are solid at 37°.

The anomalous behavior of 1-monolaurin is noteworthy. MCBAIN AND MARSDEN<sup>20</sup> presented X-ray diffraction evidence for 1-monolaurin's being in a liquid crystalline state in water, but in my experiments, no birefringence was observed. The excess 1-monolaurin can be considered to be present in a phase which contains some water and which has a melting point under 37°. Such behavior is commonly observed with phenol in water and other examples are known<sup>18</sup>.

The behavior of 1-monoolein in buffer has been discussed previously<sup>3</sup>. The molecular structure of liquid crystalline states has been well established by electron microscopy<sup>21</sup>.

The curves in Fig. 1, which show the solubility of monoglycerides in dilute bile-salt solution are the limits of the isotropic, micellar area in the ternary phase-diagram of bile-salt-monoglyceride-water<sup>3,5</sup>. However, bile salts do not form liquid crystalline states in the binary system of bile-salt-water<sup>3,22</sup>, and no microscopic evidence for liquid-crystal formation has been observed in the ternary systems reported here. The ternary phase diagram for the bile-salt-amphiphile-water system is apparently quite different from those reported for typical anionic detergent-amphiphile-water systems.

As elucidated by LAWRENCE<sup>5</sup>, the temperature-composition phase diagram for a ternary soap-amphiphile-water system shows an eutectic temperature for the liquid crystalline phase, *i.e.* the liquid crystalline phase, which is composed of amphiphile-soap and water, can exist well below the Krafft point of the soap solution or the freezing point of the amphiphile. Both the bile-salt-amphiphile micelle and the turbid phase, composed of bile salt, amphiphile and water possess such an eutectic point. This is evidenced by the high micellar solubilities at 37° of 1-monolaurin (m.p. 63°), 1-monodecanoin (m.p. 56.5°) and 1-monoelaidin (m.p. 58.5°)\*, as well as the liquid state of the system when these monoglycerides are present in a turbid excess.

Although the behavior of the higher-melting-point monoglycerides fulfills the usual criteria for non-polar solubilization, these monoglycerides behave as amphiphilic or polar solutes at higher experimental temperatures. Conversely, amphiphilic monoglycerides with lower melting points, such as 1-monolaurin, behave as non-polar solutes at lower experimental temperatures. The bile-salt micelle, in all probability, is liquid, as evidenced by the solubility of azobenzene in micellar bile-salt solutions<sup>3</sup>. The data suggest that the amount of incorporation of any monoglyceride into the bile-salt micelle is related to the effect of the solubilized monoglyceride on the apparent freezing point of the resultant mixed micelle, the restriction being that the micelle

\* The melting points listed are from DEUEL<sup>23</sup> and refer to the  $\beta$ -polymorphic form, as this form was used in all experiments. The melting point of the  $\alpha$ -polymorphic form for 1-monolaurin is 44°, and for 1-monoelaidin, 42°, both above 37°, also.



always remains liquid. Therefore, relative to the experimental temperature, little high-melting-point monoglyceride, or a great deal of low-melting-point monoglyceride is incorporated into the micelle.

When the experimental temperature is below the eutectic point for the bile-salt-amphiphile micelle, the monoglyceride is partitioned between the liquid micelle and the solid crystalline, excess monoglyceride; the amount solubilized by the micelle is less than that which lowers the micelle's freezing point below the experimental temperature. When the experimental temperature is above the eutectic point for the bile-salt-amphiphile micelle, the monoglyceride is solubilized to an infinite extent in the micelle. As the amount of low-melting-point monoglyceride in the system increases, the maximum micellar volume is reached, and the system becomes heterogeneous.

Based on this hypothesis, which was derived from LAWRENCE<sup>5</sup>, the experiments in which an amphiphilic monoglyceride and a non-polar monoglyceride were equilibrated simultaneously with bile-salt solution become intelligible. Initially, only the amphiphilic monoglyceride can be solubilized to any extent. As its concentration in the micelle is increased, the resulting micelle can dissolve progressively more of the high-melting point monoglyceride and remain liquid. When the micelle contains a great deal of amphiphilic monoglyceride, it can dissolve a relatively large amount of the higher-melting-point monoglyceride without freezing; but now the limitation of micellar volume is superimposed, and the system becomes heterogeneous. Solubilization at this point is described as competitive, because the addition of either low-melting-point or high-melting-point monoglyceride to the system induces turbidity.

The higher solubility of 2-monopalmitin (m.p. 69°) which possesses a lower melting point than 1-monopalmitin (m.p. 77°) is explained similarly. To prove the validity of this explanation, namely that the arrangement of hydroxyl groups has no significant influence on the solubility in bile-salt solution, one could compare the solubility of glyceryl 1-monopalmityl ether and glyceryl 2-monopalmityl ether at a temperature where both compounds would behave as amphiphiles.

A detailed description of the molecular interaction in amphiphilic solubilization cannot be given, as no information is available on molecular packing in the bile-salt micelle or the bile-salt-amphiphile micelle. However, the high saturation ratios of amphiphilic monoglycerides suggest that the two hydroxyl groups are important for the behavior of monoglycerides in bile-salt solution. The significantly lower solubility of glycol monooleate and the negligible solubility of oleyl alcohol are consistent with this view.

As amphiphile is added to an aqueous micellar soap solution, there is usually a change from an isotropic micellar solution to a liquid crystalline state<sup>6</sup>. When excess amphiphile is added to bile-salt solution, a turbid phase appears which contains bile salt, monoglyceride and water<sup>3</sup>, and yet possesses no birefringence. It may therefore be termed a coacervate<sup>24</sup>, although the name adds little in understanding.

It is clear therefore that any monoglyceride may behave as an amphiphilic or non-polar solute in bile-salt solution, depending on the experimental temperature. But in man, body temperature is constant at 37°. The classification of monoglycerides with melting point under about 65° ( $\beta$ -polymorphic form) as amphiphilic probably has little application to normal fat digestion. The normal products of pancreatic lipolysis are chiefly 2-monoglycerides and fatty acids<sup>1</sup>. The 2-monoglycerides will

generally be unsaturated<sup>25,26</sup> and therefore amphiphilic. Since it is well known that binary mixtures of fatty acids have a melting point below the melting point of either pure fatty acid<sup>5</sup> and that amphiphilic monoglycerides and fatty acids are competitively solubilized by bile-salt solutions<sup>2</sup>, it is most probable that the mixture of fatty acids and 2-monoglycerides occurring in intestinal content after the ingestion of a normal meal will be amphiphilic, if considered as a single species. They will be partitioned between the bile-salt micelles and the emulsified oil phase, the latter containing most of the di- and triglyceride present in intestinal content<sup>2</sup>.

Application of these data to dietary experiments where high-melting-point triglycerides, such as tristearin, are fed is quite hazardous, for several of the steps in fat digestion, *e.g.* emulsification, lipolysis, or partition may be altered.

The slow rate of isomerization of the 2-monoglycerides in bile-salt solution agrees with recent data on the isomerization rate of 2-monoglycerides incubated in buffer alone<sup>16</sup>. However, the results of these experiments cannot be applied to the situation in intestinal content, for other substances present may influence the isomerization rate. Nevertheless, fresh intestinal content, extracted and chromatographed immediately, may contain only the 2-isomer<sup>14</sup>.

The behavior of the structural analogues was not unexpected as the replacement of an ester linkage by an ether linkage should have little influence on their amphiphilic properties. The much lower saturation ratio of the branched-chain monoglyceride, 1-mono- $\alpha$ , $\alpha$ -dimethyldecanoin, emphasizes the importance of packing in micellar solubilization. Changes in the configuration of the paraffin chain seem of less importance in view of the virtually identical saturation ratios of 1-monoolein, 1-monoelaidin, and 1-monolinolein.

#### ACKNOWLEDGEMENTS

Valuable suggestions were received from Professor B. BORGSTRÖM. Miss G. ÖSTBERG, Miss B. ÅKESSON, and Miss U. HANSSON gave competent technical assistance.

This work was begun while a fellow of the National Foundation (U.S.A.) and completed while a postdoctoral fellow of the National Heart Institute. Additional support from the National Heart Institute (Grant H-5302-Metabolism) is acknowledged.

#### REFERENCES

- <sup>1</sup> B. BORGSTRÖM, in K. BLOCH, *Lipide Metabolism*, Wiley, New York, 1960, p. 128.
- <sup>2</sup> A. F. HOFMANN AND B. BORGSTRÖM, *Federation Proc.*, 21 (1962) 43.
- <sup>3</sup> A. F. HOFMANN, *Biochem. J.*, in the press.
- <sup>4</sup> M. E. L. MCBAIN AND E. HUTCHINSON, *Solubilization and Related Phenomena*, Academic Press, New York, 1955.
- <sup>5</sup> A. S. C. LAWRENCE, in K. DURHAM, *Surface Activity and Detergency*, MacMillan, London, 1961, p. 158.
- <sup>6</sup> A. S. C. LAWRENCE, *Nature*, 183 (1959) 1491.
- <sup>7</sup> B. BORGSTRÖM, A. DAHLQVIST, G. LUNDH AND J. SJÖVALL, *J. Clin. Invest.*, 36 (1957) 1521.
- <sup>8</sup> A. F. HOFMANN AND B. BORGSTRÖM, *Biochim. Biophys. Acta*, 70 (1963) 316.
- <sup>9</sup> A. NORMAN, *Arkiv Kemi*, 8 (1955) 331.
- <sup>10</sup> A. F. HOFMANN, *Acta Chem. Scand.*, 17 (1963) 173.
- <sup>11</sup> E. STAHL, *Angew. Chem.*, 73 (1961) 646.
- <sup>12</sup> A. F. HOFMANN, *J. Lipid Res.*, 3 (1962) 127.
- <sup>13</sup> A. F. HOFMANN, in A. C. FRAZER, *Proc. 7th Intern. Congr. Biochem. Probl. of Lipids*, Birmingham, Elsevier, Amsterdam, 1963, in the press.
- <sup>14</sup> A. F. HOFMANN, *J. Lipid Res.*, 3 (1962) 391.

- <sup>15</sup> P. DESNUELLE AND M. J. CONSTANTIN, *Biochim. Biophys. Acta*, 9 (1952) 531.
- <sup>16</sup> F. H. MATTSON AND R. A. VOLPENHEIN, *J. Lipid Res.*, 3 (1962) 281.
- <sup>17</sup> D. H. BLANKENHORN AND E. H. AHRENS JR., *J. Biol. Chem.*, 212 (1955) 69.
- <sup>18</sup> S. GLASSTONE, *Textbook of Physical Chemistry*, Van Nostrand, New York, 1946, p. 755.
- <sup>19</sup> G. S. HARTLEY, in R. T. HOLMAN, W. O. LUNDBERG AND T. MALKIN, *Progress in the Chemistry of Fats and Other Lipids*, Vol. 3, Pergamon Press, London, 1955, p. 19.
- <sup>20</sup> J. W. MCBAIN AND S. S. MARSDEN JR., *J. Chem. Phys.*, 15 (1947) 211.
- <sup>21</sup> W. STOECKENIUS, J. H. SCHULMAN AND L. M. PRINCE, *Kolloid-Z.*, 169 (1960) 170.
- <sup>22</sup> R. D. VOLD AND J. W. MCBAIN, *J. Am. Chem. Soc.*, 63 (1941) 1296.
- <sup>23</sup> H. J. DEUEL JR., *The Lipids*, Vol. 1 (Chemistry), Interscience, New York, 1951, p. 268.
- <sup>24</sup> H. G. BUNGENBERG DE JONG, in H. R. KRUYT, *Colloid Science*, Elsevier, Amsterdam, 1949, p. 243.
- <sup>25</sup> F. H. MATTSON AND R. A. VOLPENHEIN, *J. Biol. Chem.*, 236 (1961) 1891.
- <sup>26</sup> P. SAVARY, J. FLANZY AND P. DESNUELLE, *Biochim. Biophys. Acta*, 24 (1957) 414.

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