Intestinal absorption in vivo of micellar and nonmicellar lipid

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Knoebel, Leon K. Intestinal absorption in vivo of micellar and nonmicellar lipid. Am. J. Physiol. 223(2):255-261. 1972.—Lymphatic transport of absorbed fatty acid and rate and site of uptake from intestinal lumen were measured in bile-fistula rats which received the lipid mixture, 10 mM oleic acid and 10 mM monoolein, by steady intraduodenal infusion. The concentration of fatty acid solubilized in the four infusates used varied from 40% (10 mM bile salts) to monomolecular solubility (2.5 mM and 1 mM bile salts or 1% gum acacia). A model of micellar and nonmicellar absorption, based on the balance between absorption rate per unit area of intestine and utilization of available surface area, is proposed. Fatty acid was completely absorbed from the proximal intestine for the infusate with a large micellar phase. When the diffusion gradient was reduced with the nonmicellar infusates, the ileum acted as a reserve absorptive area. Differences in lymphatic output of fatty acid between groups given nonmicellar infusates are explained on the basis of intraluminal fluid absorption which resulted in intraluminal concentration of bile salts to varying extents, and thus different degrees of solubilization of fatty acid. Studies on metabolism of absorbed fatty acid in mucosa and lymph support the idea that intracellular triglyceride formation is promoted by increased rate of delivery of fatty acid into the mucosa. The data do not support a specific role of the bile salts in intracellular esterification.

Methods

Male albino rats of the Wistar strain weighing 200-250 g were used. Operations were performed under ether anesthesia after fasting overnight. The abdominal thoracic lymph duct was cannulated according to the method of Bollman, Cain, and Grindlay (3) with a polyethylene cannula (id 0.5 mm). A bile fistula was prepared by cannulating the common bile duct above the junction of the bile and pancreatic ducts with a silicone cannula tipped with polyethylene tubing (id 0.28 mm). A silicone tube was passed through a small incision in the fundus of the stomach into the duodenum and its tip secured near the entrance of the bile duct. Postoperatively the rats were transferred to restraining cages without access to food and water. Saline solution (8.5 g/liter NaCl, 0.3 g/liter KCl per liter) was continuously infused through the duodenal cannula, except during those times when replaced by an infusate containing lipid, the administration of which was never earlier than 48 hr after operation. Rate of infusion was always 3 ml/hr.

Four infusates containing lipid were used. Each of these contained oleic acid and 1-monoolein in a molar ratio of 1:1 with a total lipid concentration of 20 mM. In three of the infusates, the concentration of bile salts was either 10, 2.5, or 1 mM. A mixture of sodium taurocholate and sodium taurodeoxycholate in a molar ratio of 4:1 was used. In the fourth infusate which contained no bile salts, 1% gum acacia was used to stabilize the emulsion. Stock solutions of oleic acid and monoolein were mixed in the desired proportions on the day of the experiment, a tracer amount of oleic acid-14C was added, and the solvent was completely evaporated under a stream of nitrogen. The required amount of detergent in a small volume of buffer solution was added. The buffer solution was composed of 0.3 mM Na2HPO4 and 0.15 mM NaH2PO4, 5.5:4.5 v/v, diluted 1 to 10 with saline solution and adjusted to pH 6.4 using a pH meter. Buffer solution was then added in 5-ml increments and the mixture was ionized after each addition with a Branson insonator (40 w at 20,000 Hz) for 2 min until the final volume was 1 ml (11), which is well below the critical micellar concentration. This report deals with experiments in which the steadily infused load of fatty acid was 30 μmoles/hr. Lymphatic transport of absorbed fat and rate and site of uptake from lumen were measured in rats receiving infusates in which the concentration of fatty acid solubilized was varied 70-fold from 4 X 10^-3 M (40% solubilized) to 6 X 10^-3 M (monomolecular solubility).

Uptake, in vitro, of labeled fatty acids by everted sacs of small intestine decreases with decreasing concentration of solubilized fatty acid in the incubate (10, 15). However, this simple relationship does not appear to hold in experiments in vivo. Bile-fistula rats absorbed fatty acid infused at rates of 6 μmoles/hr (11) or 13 μmoles/hr (18) equally well under steady-state conditions either when all fatty acid was in bile salt micellar solution (10 mM bile salts) or when at most a trace of micellar fatty acid was present (2.5 mM bile salts). These results might be explained by the reserve area, in vivo, which has been proposed to exist for the absorption of fat (4). Under steady-state conditions, fatty acid infused as micellar solution was absorbed before reaching the ileum (11). It is conceivable that when the proportion of fatty acid solubilized in the infusate is small (2.5 mM bile salts), fatty acid which escapes absorption in the proximal intestine is absorbed distally. However, under less favorable conditions, a stage could be reached at which all of the absorbing area was exposed to infusate and absorption would fail to keep pace with the steady infused load. This seemed to occur when the concentration of bile salts in the infusate was 1 mM (11), which is well below the critical micellar concentration.
attained. The emulsions produced were stable at room temperature for several days.

In order to determine the extent to which oleic acid was solubilized in each of the four infusates, 9 ml of emulsion were spun in a Beckmann L2-65 ultracentrifuge for 22 hr at 25,000 rpm ($7 \times 10^7$ g-min) at 30 C. At the end of a run, about 5 ml of the lower, clear, aqueous (isotropic) phase was withdrawn, an aliquot taken, and lipid extracted according to the method of Bligh and Ahrens (1). The solvent was evaporated and radioactivity was measured as described below. A sample of the original emulsion before centrifugation was similarly treated. Percentage solubilization of fatty acid in the isotropic phase was calculated by dividing the concentration of labeled fatty acid in the isotropic phase by that in the unspun emulsion (Table 1). The fact that isotopic fatty acid was about 40% solubilized in the infusate, 10 mM bile salts, shows that this emulsion contained a relatively large micellar phase. On the other hand, the amount of oleic acid solubilized in the other three infusates was quite low and equivalent to a concentration of about $6 \times 10^{-5}$ M, a value which is consistent with monomolecular dispersion; i.e., a micellar phase was absent. It should be recognized, however, that the concentration of bile salts in the infusate, 2.5 mM bile salts, was probably close to the critical micellar concentration for this bile salt mixture (4, 18).

Optical densities of the four infusates were measured with a Beckmann DB spectrophotometer at 650 nm, light path 1 mm (Table 1). Although this determination provides no information regarding absolute particle size, the results suggest that relative mean particle size increased with decreasing bile salt concentration.

Rats prepared in the manner described above were used in two types of experiments. In one instance, hourly lymph samples were collected into heparinized, graduated centrifuge tubes for the hour before and the 8 hr during the infusion of lipid. A 4-hr lymph collection, followed by an 11-hr collection, made up the overnight lymph recovery. Aliquots of all lymph collections were extracted with ethyl acetate-diethyl ether, 3:1 v/v, and aliquots of the extracts were counted for radioactivity. The 8th hr lymph collection was made over ice. Lipid was extracted from this lymph sample by the method of Blankenhorn and Ahrens (1), and an aliquot of the extract was used to determine by thin-layer chromatography (TLC) the distribution of radioactivity between various lipid classes. In many animals, a second test was performed the following day, but with an infusate different from that administered the 1st day.

Lymph-fistula, bile-fistula rats were also used in experiments which, in addition to monitoring labeled fatty acid in the lymph, measured the recoveries of isotopic oleic acid in gastrointestinal contents and wall. Hourly lymph samples were collected for 6 hr, after which the animals were killed and the gastrointestinal tract was ligated to isolate the stomach, upper and lower halves of the small intestine, and the colon. The contents of the stomach and both segments of small intestine were collected for radioactivity. The wall of the upper and lower small intestine and that of the colon plus its contents were homogenized separately by grinding in chloroform-methanol, 2:1 v/v, in a glass homogenizer. Each homogenate was allowed to stand overnight in a stoppered test tube, filtered, and the residue washed a number of times with solvent. An aliquot of the filtrate was analyzed for radioactivity, and in the case of the proximal and distal segments of small intestine, another aliquot was used to determine by TLC the distribution of label between various lipid types.

In another group of experiments, the concentration of bile salts in the contents of the proximal three-fourths of the small intestine was estimated in rats prepared only with bile fistulas and duodenal cannulas. These animals were infused for 6 hr with the lipid mixture containing bile salts, 2.5 and 1 mM, killed, and the contents collected as completely as possible by milking the intestinal segment. The concentration of the taurocholate in the contents recovered was determined by the method of Lee and Herrnau (13). Since this method estimates only trihydroxy bile salts (taurocholate) and since the bile salt mixture infused was taurocholate-taurodeoxycholate molar ratio 4:1, all values determined were corrected by multiplying by 1.25 to give total bile salt concentration.

Radioactivity in the various samples described above was measured in a Nuclear Chicago liquid scintillation counter. Solvents were evaporated and radioactivity was determined in the scintillant mixture, 2.5 diphenyloxazole, 4 g/liter, and 1,4-bis[2-(5-phenyloxazoyl)] benzene, 0.05 g/liter, in toluene. Quenching correction was made by the channels-ratio method (8). Chloroform was used as the quenching agent to establish the correction curves.

Thin-layer chromatography was carried out on 0.25-mm layers of silica gel G (E. Merck, Darmstadt, Germany) on plates activated at 110 C for 30 min prior to use. Up to 0.5 mg of lipid was applied and developed for 15 cm. The solvent system used to separate free fatty acids and glycerides was hexane-diethyl ether-glacial acetic acid, 80:20:2 v/v/v. The plate was stained with iodine and lipid classes were identified by referring to standards run on the same plate. The lipids were scraped and eluted with chloroform-methanol, 2:1 v/v, by the method of Goldrick and Hirsch (7). Monooolein was checked for purity using a hexane-diethyl ether-glacial acetic acid, 30:70:2 v/v/v, solvent system. Bile salts were checked for purity in the solvent system, ethyl acetate-methanol-water, 70:20:10 v/v/v.

### Table 1. Percentage solubilization of oleic acid in infusates and optical density of infusates

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Oleic acid Solubilized, %</th>
<th>Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile salts, 10 mM</td>
<td>40.1 ± 0.7 (4)</td>
<td>0.000 ± 0.001 (5)</td>
</tr>
<tr>
<td>Bile salts, 2.5 mM</td>
<td>0.63 ± 0.02 (4)</td>
<td>0.169 ± 0.016 (5)</td>
</tr>
<tr>
<td>Bile salts, 1 mM</td>
<td>0.69 ± 0.03 (4)</td>
<td>0.201 ± 0.076 (5)</td>
</tr>
<tr>
<td>Gum acacia, 1%</td>
<td>0.39 ± 0.01 (3)</td>
<td>1.29 ± 0.03 (7)</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of determinations are shown in parentheses. The percentage of oleic acid solubilized in an infusate was calculated by dividing the concentration of labeled fatty acid in the isotropic phase by that in the unspun emulsion.
The plate was stained with 10% phosphomolybdic acid in ethanol.

Materials. Oleic acid (May and Baker, Ltd., Dragenham, England) was 99% pure and used as supplied. Glycerol-1-monoooleate (Calbiochem, Los Angeles), 90% pure, was shown by TLC to contain small amounts of diglycerides and free fatty acids and was purified by solvent partition. The resultant product ran as one spot on TLC. Radiochemical purity of oleic acid-1-\(^{14}\)C (Radiochemical Center, Amersham, England) was certified as 98% pure and was used as supplied. Sodium taurocholate and sodium taurodeoxycholate were prepared according to the method of Norman, as modified by Hofmann (9). A 2-mg sample of each bile salt ran as one spot on TLC. All other reagents used were of analytical grade, except ethanol, which was redistilled.

RESULTS

Lymphatic absorption. Lymphatic absorption of labeled oleic acid was studied in the rat during an 8-hr intraduodenal infusion of the lipid mixture, oleic acid-monoolein (molar ratio 1:1), in a concentration of 20 mM. The concentration of bile salts in this infusate was either 10, 2.5, 1, or 0 mM (1% gum acacia). In the case of the infusate, 10 mM bile salts, a relatively large proportion of the oleic acid infused was in micellar solution, whereas for the infusates, 2.5 mM bile salts, 1 mM bile salts, and 1% gum acacia, the concentration of fatty acid in the clear aqueous phase was very low and compatible with nonmicellar monomolecular solution.

The results are summarized in Fig. 1. A steady transfer of labeled oleic acid into the lymph was attained by the 6th hr in the groups receiving the infusates, 10 mM bile salts (Fig. 1A), 2.5 mM bile salts (Fig. 1B), and 1% gum acacia (Fig. 1D). Despite the fact that oleic acid was about 40% solubilized in the infusate, 10 mM bile salts, as compared to 0.6% in the infusate, 2.5 mM bile salts, the pattern of lymphatic recovery of isotope was the same for both groups. In these instances, about 80% of the hourly radioactivity infused was recovered in the lymph during the steady-state absorption. On the other hand, lymphatic recovery of labeled oleic acid during the steady state in the group receiving the infusate, 1% gum acacia, was only about 40% of the hourly radioactivity infused, even though the amount of oleic acid solubilized was the same as in the infusate, 2.5 mM bile salts.

Individual rats within those groups which attained a steady rate of lymphatic absorption rather closely followed the corresponding average patterns of recovery of isotope in the lymph. On the other hand, the rats in the group infused with the lipid mixture, 1 mM bile salts, did not behave uniformly as shown by the greater variance in Fig. 1C and by the individual experiments depicted in Fig. 2. However, five rats in this group did attain a steady state of lymphatic absorption by the 6th hr, and the average pattern of appearance of label in lymph for group as a whole is shown in Fig. 1C.

Regional recoveries of isotope. The same four infusates were given in another four groups of rats. These were killed after 6 hr of infusion for measurement of recoveries of labeled fatty acid in intestinal lumen and wall. Figures 1 and 2 show that most animals reached a steady state by this time.

![Figure 1](image1.png)

![Figure 2](image2.png)
and 2.5 mM, as shown by the very small recoveries of label with the result that mean lymphatic output was lower than in the first series and about the same as the gum acacia emulsion. The lymphatic recoveries of labeled oleic acid as it was administered in infusates containing bile salts, 10 mM, were very similar to those in the first 6 hr of the 8-hr infusion of the contents and wall of the small intestine and colon of the rats could be classified as “slow absorbers” (see Fig. 2), four groups of rats. Oleic acid was absorbed almost as fast as it was administered in infusates containing bile salts, 10 mM, 2.5 mM, 1 mM, and 1 % gum acacia, were 53.4 % ± 3.2, 26.7 % ± 4.2, 26.3 % ± 3.6, 53.4 % ± 3.2, 26.7 % ± 4.2, and 38.1 % ± 1.8, respectively.

Figure 3 shows the recoveries of labeled oleic acid from the contents and wall of the small intestine and colon of the four groups of rats. Oleic acid was absorbed almost as fast as it was administered in infusates containing bile salts, 10 mM, 2.5 mM, and 1 % gum acacia, were 53.4 % ± 3.2, 26.7 % ± 4.2, and 38.1 % ± 1.8, respectively.

The low recoveries of label in the lymph of animals receiving the infusates, 1 mM bile salts or 1 % gum acacia, indicated that absorption of fatty acid was incomplete in these two groups. Compared with the first two groups, much larger amounts of unabsorbed fatty acid were recovered from the contents of the distal small intestine and an appreciable proportion of the infused dose passed into the colon (Fig. 3, C and D). Recoveries of label from the wall of the proximal and distal small intestine of these two groups were similar to those for rats infused with 2.5 mM bile salts and showed that some of each of the infusates was absorbed from both regions of the small intestine. However, it appeared that utilization of the distal absorptive area, although complete, was not sufficient to compensate for a limited proximal absorption.

**Mucosal metabolism of fatty acid.** Table 2 shows the distribution of labeled fatty acid between various lipid classes in the wall of the small intestine. When the groups receiving the infusates, 2.5 mM bile salts, 1 mM bile salts, and 1 % gum acacia, are compared to those that infused with 10 mM bile salts, it can be seen that in the absence of a micellar phase in the infusates a greater proportion of the label remained as free fatty acid in the proximal wall of the small intestine. Furthermore, a greater proportion of the label was incorporated into phospholipid and partial glycerides, but less into triglycerides. The same comparison cannot be made for the distal wall of the small intestine, because very little labeled fatty acid escaped absorption in the proximal intestine of the

**TABLE 2. Total radioactivity and lipid class distribution of radioactivity in wall of small intestine**

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Proximal Wall</th>
<th>Distal Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL*</td>
<td>MG, PL†</td>
</tr>
<tr>
<td>Bile salts, 10 mM (4)</td>
<td>24.6±2.1</td>
<td>8.2±3.2</td>
</tr>
<tr>
<td>Bile salts, 2.5 mM (3)</td>
<td>9.1±0.8</td>
<td>23.3±1.6</td>
</tr>
<tr>
<td>Bile salts, 1 mM (5)</td>
<td>5.7±1.2</td>
<td>23.7±2.7</td>
</tr>
<tr>
<td>Gum acacia, 1% (4)</td>
<td>6.3±2.1</td>
<td>24.4±4.5</td>
</tr>
</tbody>
</table>

Values are means ± se. Number of experiments shown in parentheses. The following abbreviations are used: TL, total lipid; MG, monoglycerides; PL, phospholipids; DG, diglycerides; C, cholesterol; FFA, free fatty acids; TG, triglycerides; CE, cholesterol ester. *Values are expressed as a percentage of the total dose infused. †Values are expressed as a percentage of the total radioactivity recovered from the wall.
rats infused with 10 mM bile salts and, thus, the data obtained from the distal wall are to be viewed with caution.

It should be noted for both proximal and distal wall that there were no substantial differences in distribution of label when the groups receiving the 2.5 or 1 mM bile salts are compared with that in which the infusate contained no bile salts (Table 2). Also, the pattern in the proximal mucosa of these three groups was similar to that in the distal mucosa.

Table 3 shows that there were no differences between groups in the distribution of absorbed label among lipid classes in the lymph which was collected during the final hour of the 8-hr infusion experiments. It will be particularly noted that there was no increase in the proportion of free fatty acid in the lymph of the three groups which received the infusates with no detectable micellar phase, compared to the group in which 40% of the fatty acid in the infusate was micellar.

**Concentration of bile salts in intestinal contents.** In the groups infused with bile salts, 2.5 or 1 mM, the concentration of bile salts in the infusates entering the duodenum was either on the borderline of the critical micellar zone or well below it. There was no detectable micellar solubilization of fatty acid in either infusate as determined by ultracentrifugation. However, most of the fluid was absorbed in the small intestine, as shown by the recovery at the end of a 6-hr infusion of less than 0.5 ml of contents from the proximal three-fourths of small intestine. If fluid absorption in this region of small intestine proceeded more rapidly than absorption of conjugated bile salts, a process which takes place mainly in the terminal ileum, bile salts might be concentrated above the critical micellar concentration, and some solubilization of fatty acid could occur in the intestinal contents. The volume of contents was too small to verify this directly. Instead, the bile salt concentration was measured in 0.1-0.3 g of contents, which was all that could be expelled without saline washing. The rats in these experiments received the infusates, 2.5 or 1 mM bile salts, but were not used for lipid recovery experiments.

Table 4 shows that, compared with the infusates, there was about a sixfold concentration of bile salts in the proximal three-fourths of the small intestine of both groups of rats. Therefore, some micellar solubilization of fatty acid in the intestinal contents was possible. It must be borne in mind, however, that the total amount of micellar fatty acid must have been quite small, because the total quantity of bile salts was small. On the other hand, it is possible that a small number of micelles can facilitate a considerable transfer of fatty acid from intestinal lumen to intestinal wall.

**DISCUSSION**

Evidence has accumulated in recent years in support of the concept that penetration of fatty acid into the mucosal cell is a nonenergy-requiring process, presumably diffusion (12). If diffusion is the mechanism by which fatty acid moves from lumen into cell, it might be expected that rate of lymphatic absorption would decrease with a decreased diffusion gradient; i.e., decreased solubilization of solubilized fatty acid in emulsions presented to the absorptive surface. However, this expectation is not always realized (Fig. 1). On the other hand, these results might be explained by considering possible variations in the intestinal absorptive reserve area. Under normal circumstances, the jejunum is the major site of fat absorption in the rat (5). However, under conditions in which the proximal absorptive area is saturated, the absorption of fat can be completed in the ileum. When lymph-fistula, bile-fistula rats are given a steady intraduodenal infusion of micellar or nonmicellar lipid, as in the present experiments, a model of absorption, as based on the balance between rate of absorption per unit area of intestine and utilization of available surface area, can be proposed.

When the infusate contains a relatively large micellar phase, in which case the gradient for diffusion of fatty acid is large, fatty acid would be absorbed from the proximal small intestine almost as fast as it is infused. There would be a high recovery of fatty acid in the lymph and the proximal wall of the small intestine, but virtually no lipid would be found in the contents of any region of the small intestine or in the wall of the distal intestine. The findings for the rats infused with 10 mM bile salts fit this case.

The diffusion gradient for uptake would be reduced with a decreased aqueous concentration of fatty acid. In this circumstance, it would be anticipated that rate of uptake of fatty acid per unit area of intestine would be reduced and, thus, recovery of lipid from the proximal wall would be decreased. Some or all of the fatty acid which escaped absorption in the proximal intestine would be absorbed in the distal intestine and lipid would be recovered from the distal wall. If combined proximal and distal absorption is adequate to cope with the amount of lipid infused, lymphatic output of lipid would reach a value similar to that described above, and the amount of lipid recovered from the contents of the small intestine would remain small. All rats infused with 2.5 mM bile salts and some of those which received 1 mM bile salts gave these results. There is also the case in

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**TABLE 3. Lipid class distribution of radioactivity in lymph**

<table>
<thead>
<tr>
<th>Infusate</th>
<th>MG, PL, DG, C</th>
<th>FFA</th>
<th>TG, CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile salts, 10 mM (4)</td>
<td>5.7 ± 1.4</td>
<td>3.5 ± 0.8</td>
<td>90.8 ± 2.2</td>
</tr>
<tr>
<td>Bile salts, 2.5 mM (4)</td>
<td>4.9 ± 1.4</td>
<td>1.5 ± 0.3</td>
<td>93.6 ± 1.6</td>
</tr>
<tr>
<td>Bile salts, 1 mM (7)</td>
<td>2.8 ± 0.6</td>
<td>1.6 ± 0.2</td>
<td>95.6 ± 0.7</td>
</tr>
<tr>
<td>Gum acacia, 1% (4)</td>
<td>4.9 ± 1.2</td>
<td>4.3 ± 0.8</td>
<td>90.8 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± se and are expressed as a percentage of the radioactivity recovered from the lymph. Number of experiments shown in parentheses. Abbreviations used are the same as in Table 2.

**TABLE 4. Concentration of bile salts in infusates and in contents recovered from proximal three-fourths of small intestine**

<table>
<thead>
<tr>
<th>Infusate</th>
<th>Infusate, µmoles/ml (4)</th>
<th>Contents, µmoles/g</th>
<th>C/I*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile salts, 2.5 mM</td>
<td>2.56 ± 0.02</td>
<td>16.23 ± 3.34</td>
<td>6.33</td>
</tr>
<tr>
<td>Bile salts, 1 mM</td>
<td>1.00 ± 0.02</td>
<td>6.78 ± 1.00</td>
<td>6.78</td>
</tr>
</tbody>
</table>

Values are expressed as means ± se. Number of experiments shown in parentheses. *Contents/infusate was calculated by dividing the mean concentration of bile salts in the contents by the mean concentration of bile salts in the infusate and is an estimate of the extent to which infused bile salts are concentrated in the intestinal lumen by the absorption of fluid.
which combined proximal and distal absorption would not keep pace with the infusion when the isotropic concentration of fatty acid is low. In this instance, lymphatic recoveries would decrease and unabsorbed lipid would accumulate in the lumen. The accumulation would be mainly distal because transit through the proximal small intestine is rapid (4). The extent to which fatty acid is passed on to the colon would depend on the emptying rate of the distal small intestine. The remainder of the animals which received 1 mM bile salts and all of those infused with 1% gum acacia followed this pattern.

In order to distinguish the group infused with 2.5 mM bile salts from that given 1% gum acacia, both of which received infusates with the same low concentration of solubilized fatty acid, it would have to be assumed that the absorption of fluid from the infusate, 2.5 mM bile salts, would concentrate the bile salts which were already on the borderline of the critical micellar zone. Formation of micelles would result and fatty acid would be solubilized during transit through the small intestine. It has previously been shown that the concentration of bile salts in the contents of successive quarters of the small intestine of rats fed a stock diet progressively increased through the first three-quarters of small intestine (6). Although it has been suggested that gum acacia may form micelles (16), it seems unlikely that the intraluminal concentration of gum acacia would have produced appreciable solubilization of fatty acids, because in additional ultraacentrifugation experiments in the present study, a threefold increase in gum acacia concentration increased isotropic fatty acid concentration to only 1.47 X 10^{-4} M. Even this increase is open to doubt due to the tendency of gum acacia to form gels in the ultracentrifuge tubes at the higher concentration, thereby making it difficult to prevent contamination of the aqueous phase with unsolubilized fatty acid.

A similar intraluminal concentration of bile salts by the absorption of fluid, but starting with a bile salt concentration considerably below the critical micellar zone, would explain the variability in the group infused with 1% bile salts. Some rats in this group would achieve solubilization whereas others would not and would behave much like rats infused with 1% gum acacia. This group would be marginal, because the full reserve of absorptive area was utilized and the concentration gradient was critical.

A possibility that cannot be completely excluded in the interpretation of the results is that, in the absence of a micellar phase, the rate-limiting step was transfer of fatty acid from emulsion particles to aqueous phase. The emulsion particles were probably much coarser with gum acacia and smaller for infusates with 2.5 mM bile salts than for those with 1 mM bile salts (Table 1). No direct evidence is available on size of emulsion particles as a rate-limiting factor in vivo. No evidence for such an effect was found in vitro (14).

There is conflicting evidence on a possible intracellular rule of bile salts in promoting esterification of absorbed fatty acid in the mucosa (17). When solubilization of fatty acid was varied independently of bile salt concentration in vitro, it was found that both uptake of fatty acid and incorporation into triglyceride varied with the concentration of solubilized fatty acid in the incubation medium and not with the concentration of bile salts per se (10). In the present series of experiments in vivo, the proportion of labeled fatty acid incorporated into mucosal triglyceride was less when the infusates contained very little solubilized fatty acid, as compared to when there was a large micellar phase (Table 2). The lower percentage esterification was associated with smaller mucosal pools of lipid (Fig. 3). Rate of uptake from lumen per unit area or per absorptive cell must have been lower in these groups than in that infused with 10 mM bile salts. Even in the group, 2.5 mM bile salts, with an overall rate of lymphatic absorption similar to the group, 10 mM bile salts, a larger absorptive area was utilized and, thus, the rate of uptake per unit area must have been lower. Consequently, it seems that the lower incorporation of fatty acid may be related to slow uptake in the absence of solubilized fatty acid. Furthermore, since incorporation into triglyceride in the group in which the infusates contained no bile salts was the same as in the groups, 2.5 and 1 mM bile salts, a specific effect of bile salts on mucosal esterification is minimized. Finally, it should be noted that differences in mucosal metabolism were not reflected in the lymph, in which nearly all of the absorbed, labeled fatty acid was esterified irrespective of the rate of absorption or the presence or absence of bile salts in the lumen.

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