Oleic Acid Absorption from Micellar Solutions and Emulsions in the Rat[1] (37141)

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In animals without luminal bile salts, long-chain fatty acid absorption is impaired but not absent, while absorption of sterols and fat-soluble vitamins is virtually abolished. The reason for this was suggested by Hofmann and Borgström (1) who pointed out that lipid is solubilized in bile salt micelles which could shuttle between emulsion droplets or debris in the bowel lumen and the micro villi of the mucosal cell. In the absence of such a micellar phase, the water insoluble sterols would not be absorbed but the long-chain fatty acids, having a finite if low solubility in water at the pH of the intestine, might be expected to be absorbed to a small extent. Surprisingly, experiments designed to show a difference in absorption of oleic acid from solutions with and without a micellar phase failed to do so (2). Recent work by Hoffman (3, 4) has provided an explanation for some of these results. He found that the supposed micelle-free solutions used previously (2.5 m*M* bile salts) did, in fact, contain micelles; when the experiments were repeated, using truly micelle-free solutions (1 m*M* bile salts), the effect of micellar solubilization of fatty acid on its absorption could be demonstrated, whether bile salts or the nonionic detergent pluronic acid were used. However Hamilton (5) using a different technique failed to show the advantage of having a micellar phase present either *in vitro* or *in vivo*. In these experiments we have studied further the effect of micellar solubilization on the absorption of oleic acid using closed loops of rat jejunum; both taurocholate and Pluronic acid were used to produce micellar solutions.

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Materials and Methods. Chemicals. Uniformly ^{14}C-labeled oleic acid was purchased from the Radio Chemical Centre, Amersham, oleic acid (99% pure) from the Fatty Acid Project, the Hormel Institute, Minnesota and sodium taurocholate from Maybridge Chemicals, Tintagel, Cornwall. The nonionic detergent Pluronic F68, a polyoxyethylene–polyoxypropylene copolymer, stated to have an average molecular weight of 8000, was a gift from Jacobson, Van Den Berg and Co., Ltd.

Solutions. Uniformly ^{14}C-labeled oleic acid was dissolved in a solution containing NaCl, KCl, sodium phosphate buffer pH 6.4 and either sodium taurocholate, 1 m*M* or 15 m*M*, or Pluronic acid F68 70 mg/ml to give a final concentration of 135 m*M* Na^+ and 15 m*M* K^+. When the concentration of oleic acid was above its micellar solubility the solution was sonicated for 3–5 min in a M.S.E. 60 Watt Ultrasonic Disintegrator.

Method. Male rats of Wistar strain weighing 230–300 g were used. The bile duct was cannulated with polythene tubing which was led out to drain into a glass panier stitched to the back, after the technique of Van Zyl (6). The rats were kept, unrestrained, in individual cages, on a grid to prevent coprophagy, and allowed free access to a solution of 5% glucose, 0.9% NaCl and 0.04% KCl; 20–24 hr later, under ether anesthesia, two adjacent loops of jejunum, approximately 10 cm long, were tied off and a short length of fine polythene tubing tied into each. Using a 1-ml tuberculin syringe, 0.2 ml of the solution to be tested was taken for a standard, 0.2 ml put into one loop, the tubing tied off from the loop and removed, and a second 0.2 ml standard taken. Two test solutions were used for each rat and put alternatively into proximal and

distal loops. The loops were replaced in the abdomen which was closed and the animal left anesthetized for 1 hr. At the end of this time the loops were removed for extraction.

Extraction. The loops were hydrolysed overnight at 55° in 25 ml 30% KOH and 25 ml methanol. After acidification with concentrated HCl, the lipid was twice extracted with 50 ml hexane, the extract pooled and adjusted to 100 ml. Five drops of concentrated HCl and 1 ml methanol were added to each of the 0.2-ml solutions taken as standards and they were then extracted with 10 ml hexane; 2-ml aliquots of the hexane extracts from loops and standards were taken for determination of radio activity by liquid scintillation. Absorption was calculated as the difference between the mean of the two standards and the radioactivity remaining in the loop and expressed as m*M* of oleic acid or as % standard.

Results. Absorption from 1 mM and 15 mM sodium taurocholate (Fig. 1). There was greater absorption of oleic acid from a 15 m*M* NaTc solution containing micelles compared with a 1 m*M* taurocholate solution which Hoffman showed to be without a micellar phase (4). This difference and the % absorption were constant over a wide range of oleic acid concentration 0.2–7.0 m*M*; at high concentrations of oleic acid in 15 m*M* sodium taurocholate there was an emulsion phase and a micellar phase present, however, the amount of oleic acid absorbed was proportional to the total amount of oleic acid present rather than to its concentration in the micellar phase. No saturation of absorptive capacity was noted under these conditions.

Comparison of absorption from sodium taurocholate and pluronic acid F68 solutions (Table I). When the concentration of oleic acid was high, 10 m*M*, pluronic acid was as effective as 15 m*M* taurocholate in promoting absorption, 15 ± 1.2% and 17 ± 1.3%, respectively. At very low concentration of oleic acid, 0.1 m*M*, absorption from a Pluronic acid micellar solution (13 ± 1.7%) was the same as from a nonmicellar emulsion in 1 m*M* taurocholate (12 ± 1.3%) and this anomalous result was confirmed in a second group of rats done a few months later 9 ± 1% and 9 ± 1.4%.

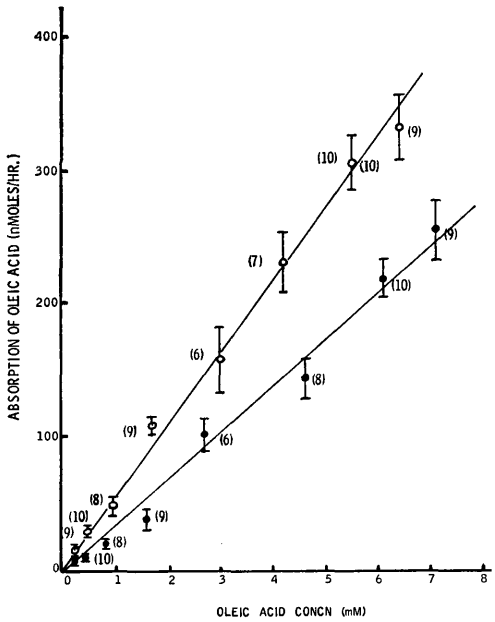

FIG. 1. 0.2 ml of solution containing uniformly ^{14}C-labelled oleic acid in 1 m*M* or 15 m*M* sodium taurocholate solution at pH 6.4, into adjacent jejunal loops in a rat under ether anesthesia for 1 hr. Loops removed and hydrolyzed overnight, then acidified and extracted with hexane. Absorption given as nanomoles oleic acid ± SE/hr removed from loop. Numbers in parenthesis are numbers of observations at each point. (○) 15 m*M* taurocholate solution. (●) 1 m*M* taurocholate solution.

Discussion. These results confirm the observation of Hoffman (3, 4) that a micellar phase is important in the absorption of oleic acid and that the function of bile is not solely as an emulsifying agent. The contradictory findings of Hamilton (5) may be due to technique, for he showed an apparently high absorption (20%) only 10 sec after the test solution had been placed in a jejunal loop; this has not been our experience.

The appreciable absorption of oleic acid from nonmicellar 1 m*M* taurocholate solution suggests that at pH 6.4 sufficient oleic acid is in solution to act as a shuttle between the emulsion and the mucosal cells, although to a less extent than when the aqueous solubility of the oleic acid is increased by the presence of micelles.

TABLE I. Comparison of Absorption of Oleic Acid from Solutions Containing Pluronic Acid F68 or Sodium Taurocholate by Rat Jejunal Loops *in Vivo*.[a]

Oleic Acid mM		Oleic Acid absorbed (%) in 1 hr) Pluronic Acid F68 70 mg/ml	Sodium Taurocholate 1 mM	Sodium Taurocholate 15 mM	p[b]
0.1	(10)	13 ± 1.7	12 ± 1.3		
	(12)	13 ± 2.2		25 ± 2.8	<0.01
	(5)	9 ± 1	9 ± 1		
10.0	(25)	15 ± 1.2		17 ± 1.3	
	(11)	17 ± 1.6	9 ± 1		<0.002

[a] Mean ± standard error. Two solutions being used in each rat. Values in parenthesis are number of paired observations.

[b] Calculated using Wilcoxon's sum of ranks test.

The fact that no saturation of absorptive capacity was seen over a wide range of oleic acid concentration, with and without a micellar phase present, would be compatible with the hypothesis that the rate limiting step is one of diffusion, this is supported by the *in vitro* experiments of Hoffman and Simmonds (7), Willix (8) and Wilson *et al.* (9). However, the present experiment, showing absorption to be proportional to the total concentration of oleic acid rather than to its concentration in the micellar phase, although in agreement with those of Simmonds *et al.* (2) and Hoffman *et al.* (4) using *in vivo* perfusions are at variance with Hoffman's *in vitro* observations (3). It has already been shown in relation to fat absorption (10) and to selectivity in steroid absorption (11, 12) that it is inadvisable to extrapolate from *in vitro* to *in vivo* situations.

Our anomalous results with Pluronic F 68 are difficult to explain; at the higher concentration of oleic acid, 10 m*M*, they are in agreement with those of Simmonds *et al.* (2), who, perfusing a solution with a mixed lipid concentration of 7 m*M* *in vivo*, also found no difference in absorption from a Pluronic F 68 and a bile salt solution. A slow uptake of fatty acid from Pluronic micelles compared with bile salt micelles was demonstrated *in vitro* by Hoffman (10) and was explained on the basis of larger micelles and slower diffusion of Pluronic F 68 (11); but it is a problem to account for our finding with 0.1 m*M* oleic acid that a Pluronic micellar solution had no advantage for absorption over a nonmicellar solution.

Summary. Closed loops of rat jejunum *in vivo* have been used to study the absorption of oleic acid from nonmicellar and micellar taurocholate solutions and compared with that from micellar solutions of a nonionic detergent Pluronic Acid F 68. (a) Absorption from 15 m*M* taurocholate was greater than from 1 m*M* taurocholate over a range of oleic acid concentrations. (b) Absorption of oleic acid was proportional to its total and not its micellar concentration. (c) Pluronic acid micelles were as efficient as taurocholate micelles in promoting absorption from a 10 m*M* oleic acid solution but had no advantage over a nonmicellar solution at low oleic acid concentration 0.1 m*M*.

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