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THE RELATIONSHIP BETWEEN UPTAKE *IN VITRO* OF OLEIC ACID AND MICELLAR SOLUBILIZATION

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SUMMARY

1. Uptake of labelled oleic acid by everted sacs of rat jejunum was compared above and below the critical micellar concentrations for two detergents, (a) pure sodium taurocholate–sodium taurodeoxycholate (4:1, on molar basis); (b) a high molecular weight non-ionic detergent, Pluronic F68.

2. Uptake was greater from micellar media of both detergents indicating an effect of a micellar phase *per se* in promoting uptake.

3. Uptake has been shown to correlate with the percentage free fatty acid solubilized in a micellar phase.

4. Uptake was measured from media which contained both labelled free fatty acid and a labelled monoglyceride analogue, 1-monoether. The relative rates of uptake of the two labels suggested that uptake is as single molecules rather than as intact micelles.

5. The efficiency of esterification has been shown to correlate with the rate of uptake of fatty acid.

6. Preliminary experiments were done to examine the nature of labelled fatty acid efflux into unlabelled media. It is suggested that fatty acid in efflux comes from two compartments, one rapidly and one slowly exchanging.

INTRODUCTION

Solubilization of lipid in bile salt micelles accelerates uptake from the lumen of the small intestine and promotes absorption[1,2] but the exact physicochemical mechanism is not yet clear. There are two main possibilities: (i) uptake of whole micelles or of their whole lipid content following collision with the microvillus membrane; (ii) uptake as single molecules following aqueous diffusion, micelles acting as a reservoir to maintain the concentration gradient. There is the further possibility that bile salt might have an intracellular metabolic effect[3].

To obtain further information on the role of micelles the following experiments have been done on everted sacs of rat small intestine: (i) Uptake of labelled fatty acid was measured from a lipid mixture of constant composition and concentration either completely in micellar solution or in fine emulsion with no micellar phase. In the latter case, in contrast to previous work, no additional emulsifier was introduced. The same detergent was used but below the critical micellar concentration. (ii)

Uptake in the presence and absence of micelles was compared with a high molecular weight, non-ionic detergent as well as with bile salts. With the non-ionic detergent, an effect of micellar solubilization *per se* should be seen. Comparisons between the two detergents might indicate differences attributable to bile salts *per se*. (iii) With constant lipid the concentration of detergent was varied over a range to show how uptake was affected by the proportion of lipid solubilized in micelles as distinct from the mere presence or absence of a micellar phase. (iv) Uptake of two lipid molecules of different physical properties, fatty acid and glyceryl-1-monopalmityl ether was compared when both were in the same micelles or emulsion particles. Uptake of lipid in the same proportions as the mixture solubilized in micelles would indicate penetration of intact micelles. Previous evidence has been conflicting[4,5].

It has been shown that uptake of labelled free fatty acid is accompanied by efflux of free fatty acids into the medium[6]. The characteristics of this efflux have not been studied so far. Since quantitative features of this efflux could influence interpretation of data on net uptake, preliminary experiments have been done with various combinations of media for preloading and subsequent efflux.

MATERIALS AND METHODS

Tissue

Male rats, 200–220 g, of a Wistar strain locally interbred for 12 years were fasted overnight. The small intestine was removed under ether anaesthesia and rinsed with saline at room temperature. The bowel was everted on a stainless steel rod and sacs made from upper small intestine below the ligament of Treitz to give a wet weight of 200–300 mg in uptake experiments and 500–600 mg in efflux experiments. The serosal space was filled with oxygenated phosphate buffer. In each experiment sets of paired sacs from the same animal were used to compare variables.

Materials

The non-ionic detergent Pluronic F68 was the gift of Wyandotte Chemical Corporation and was stated to have an average molecular weight of 8000. It was used as supplied. Sodium taurocholate was Puriss grade as purchased from Koch–Light Laboratories Ltd. and was used as supplied. Sodium taurodeoxycholate was prepared by the HOFMANN[7] modification of the method of Norman. A 2-mg sample of each bile salt ran as one spot on thin-layer chromatography when developed in the system ethyl acetate–methanol–glacial acetic acid (70:20:10, by vol.). [1-^{14}C]Oleic acid and [9,10-$^{3}H_2$]oleic acid were purchased from Radio Chemical Centre, Amersham. Both were in excess of 95% pure on thin-layer chromatography in the system hexane–diethyl ether–glacial acetic acid (80:20:2, by vol.). They were used as supplied. Both labelled and unlabelled glyceryl-1-monopalmityl ether were the gift of Dr. A. F. Hofmann. The glyceryl-1-mono[1-^{14}C]palmityl ether was purified to better than 98% by thin-layer chromatography in the hexane–diethyl ether–glacial acetic acid system. The unlabelled chemical was better than 98% pure as supplied.

Unlabelled oleic acid was purchased from Koch–Light Laboratories Ltd.; unlabelled glycerol monooleate was purchased from Calbiochem. The monoolein was contaminated with a small amount of diglyceride and free fatty acid, the oleic acid contained a small amount of diglyceride. Both were used as supplied. All other

chemicals and solvents were of analytical grade and were used as supplied except for ethanol which was re-distilled.

Solutions

Solutions were made up in a phosphate buffer (pH 6.4) of the following composition: HPO_4^{2-}, 7.5 mM; $H_2PO_4^-$, 15 mM; Cl^-, 137 mM; Ca^{2+}, 1 mM; K^+, 7.5 mM; Na^+, 157 mM; glucose, 1 mM. This buffer was oxygenated with O_2–CO_2 (95:5, by vol.) for 15 min prior to use. A mixture of purified bile salts was used, sodium taurocholate–sodium taurodeoxycholate (4:1, on a molar basis). Concentrations used in the text refer to the total molar concentration.

From solubilization curves the critical micellar concentration of our bile salt mixture was shown to be approx. 2 mM and the critical micellar concentration for Pluronic F68 was 0.75 mg/ml, about 0.1 mM. Bile salt solutions were either 1 mM or 10 mM and for Pluronic F68 0.66 mg/ml or 30 mg/ml.

A stock solution of 40 mM monoolein and [1-^{14}C]oleic acid *plus* unlabelled carrier to make oleic acid 40 mM was made up in chloroform. The specific activity of the oleic acid was measured. An appropriate volume of the stock was evaporated to dryness and buffer *plus* detergent added to give a solution 1 mM in monoolein and 1 mM in oleic acid. This was warmed and insonated briefly in a Branson Sonifier. Similarly a solution was made of [9,10-^{3}H$_2$]oleic acid and glyceryl-1-mono[1-^{14}C]-palmityl ether, 1 mM in each constituent.

Uptake

5 ml of the labelled lipid were taken in a 10-ml round bottomed stoppered flask and shaken in a water bath at 35°. After allowing time for temperature equilibration, freshly prepared sacs were added and incubated for times varying from 2 min to 2 h.

Efflux

Sacs were incubated for 15 min as above. At the conclusion of this time they were washed in buffer and then transferred to 5 ml of micellar (or emulsified) media of detergent *plus* unlabelled 1 mM oleic acid and 1 mM monoolein. At the end of 2 min the sac was touched lightly on the flask neck and then transferred to a similar medium and the procedure repeated at 4, 6, 10, 15, 30, 60 and 120 min.

Extraction and counting

At the conclusion of an uptake or efflux experiment the sac was washed with 0.15 M NaCl, the serosal fluid drained and the wet weight of tissue measured. The mucosa was scraped off and homogenized by insonation. The homogenate was made up to volume and aliquots were then extracted by solvent partition in ethanol–diethyl ether–light petroleum (1:1:1, by vol.) and the lower phase washed twice with pre-equilibrated upper phase[8]. Duplicate aliquots were taken to dryness under N_2 scintillant added and they were counted in a Nuclear Chicago liquid scintillation counter. For efflux experiments 2 ml of the initially unlabelled medium in each vessel were extracted in the same way.

Thin-layer chromatography (silica gel G, 0.25 mm thickness) was performed on an aliquot of extract from each sac in the system hexane–diethyl ether–glacial acetic acid (80:20:2, by vol.). The lipids were divided into 3 fractions, (i) triglyceride *plus*

cholesterol esters, (ii) free fatty acid, (iii) diglyceride, cholesterol, monoglyceride and phospholipids. Fraction (iii) was sometimes subdivided further to (iiia) diglycerides and cholesterol, (iiib) monoglyceride and phospholipid. Silica gel was removed from the glass plates[9], lipid eluted with chloroform–methanol into scintillation vials and evaporated to dryness.

The scintillant used was 10 ml of the mixture 2 g 2,5-diphenyloxazole and 0.025 g 1,4-bis-(5-phenyloxazoyl-2)benzene in 500 ml toluene. Quench correction was by the channels ratio method[10].

Calculations

From the known specific activity of the isotopes, uptake was expressed as μmoles of free fatty acid (or glyceryl monoether) per g wet weight of tissue. From the total uptake and the distribution of labelled lipid on thin-layer chromatography the partition of labelled lipid was calculated as μmoles of labelled fatty acid per g wet weight of tissue in each fraction.

In efflux experiments the uptake of labelled fatty acid during preloading was calculated from the amount of isotope in the sac at the end of efflux *plus* the total isotope collected in the media during efflux. In all reported experiments this calculated value was within one standard deviation of the mean value determined in separate experiments after 15-min uptake from the appropriate solutions. It was shown by thin-layer chromatography that all label passing into the efflux media was in free fatty acid. It was also found that the amount of labelled esterified fatty acid in the mucosa at the end of the efflux was within one standard deviation of the mean value for experiments in which the mucosa was extracted at the end of 15-min isotopic uptake. These two facts suggested that efflux of label was from a pool of free fatty acid and that no label came from esterified fatty acid by lipolysis. Thus the initial size of the labelled free fatty acid pool could be calculated and also the percentage of the pool remaining at a given time.

RESULTS

Uptake from 1 mM [1-^{14}C]oleic acid and 1 mM monoolein

Uptake from micellar media was significantly ($P<0.01$) greater than from emulsion media at all times for both detergents. For micellar media, uptake from bile salts was 2–3-fold greater than from the non-ionic detergent (significant at all times). For emulsions the mean uptake was also greater with bile salts but this difference was small and significant only at 60 and 120 min at the $P<0.05$ level (Fig. 1). The rate of isotopic accumulation decreased with time following a quadratic function. This implies that uptake is determined by at least two time-dependent processes.

The total incorporation of isotopic fatty acid into esters (Fig. 1) in general followed the same trend as the total uptake in that it was greater from micelles than from emulsions and greater from bile salts than from Pluronic F68 micelles. When esterification was expressed as a percentage of uptake the same effects were apparent. It was notable, however, that variations in percentage esterification were due to altered incorporation into triglyceride. The percentage of isotope incorporated into other esters was unaffected by varying conditions (Table I).

The higher percentage incorporation into ester from bile salt micelles might

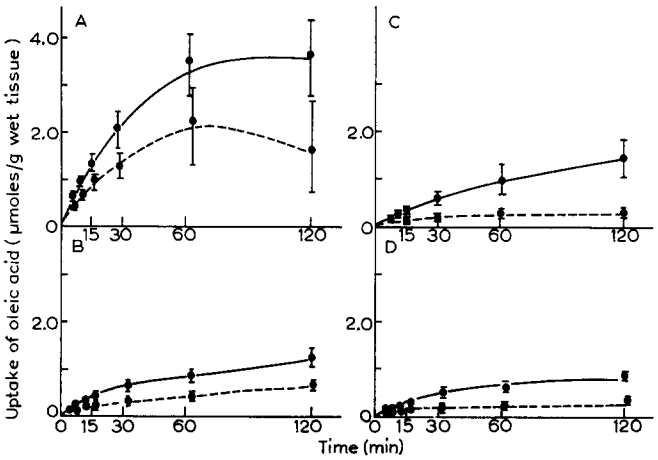

Fig. 1. The uptake and esterification of labelled oleic acid from micellar and non micellar solutions of two detergents. The lipid mixture was 1 mM [1-^{14}C]oleic acid and 1 mM monoolein. ———, uptake as μmoles/g wet tissue as calculated from the specific activity of oleic acid; – – – –, total esterification as μmoles of oleic acid/g wet tissue. Error bars are ±S.E. A and B are for micellar media, the detergent in A being sodium taurocholate–sodium taurodeoxycholate (4:1, on molar basis) to give a total bile salt concentration of 10 mM, the detergent in B, Pluronic F68, 30 mg/ml. C and D are non micellar media, the detergent in C being the above bile salt mixture to give a total concentration of 1 mM; the detergent in D being Pluronic F68, 0.66 mg/ml. *n* for A and C was 6 or 8, *n* for B and D was 22 at 15 min, 14 at 30 min and 4 at other times.

TABLE I

PERCENTAGE OF FREE FATTY ACID ESTERIFIED WITH VARYING MEDIA AND TIME

Lipid composition: 1 mM [1-^{14}C]oleic acid and 1 mM monoolein. All figures are mean ±S.E. TG = percentage of label in the thin-layer chromatography fraction which included triglyceride and cholesterol esters. PG = percentage label in the thin-layer chromatography fraction which included phospholipid, monoglyceride and diglyceride. The detergents used were: (a) emulsion media: (i) bile salts sodium taurocholate–sodium taurodeoxycholate (4:1, on a molar basis), to give total concentration of 1 mM, (ii) Pluronic F68, 0.66 mg/ml; (b) micellar media: (i) bile salts in the mixture as above to give a total concentration of 10 mM, (ii) Pluronic F68, 30 mg/ml.

Time (min):		*2*	*5*	*10*	*15*	*30*	*60*	*120*
Bile salts								
Micellar medium	TG		54.8 ± 4.7	62.7 ± 1.3	62.5 ± 2.4	53.3 ± 2.7	47.4 ± 3.2	27.7 ± 3.7
	PG		11.7 ± 1.9	11.2 ± 1.0	9.5 ± 0.2	10.2 ± 0.4	11.3 ± 1.2	11.7 ± 1.1
Emulsion medium	TG		12.1 ± 4.3	22.3 ± 2.7	30.6 ± 4.7	19.0 ± 5.5	10.2 ± 2.0	6.4 ± 1.5
	PG		10.1 ± 1.0	12.2 ± 0.9	13.6 ± 1.5	10.2 ± 2.0	9.9 ± 1.7	8.3 ± 1.1
n			4	4	6	6	6	6
Pluronic F68								
Micellar medium	TG	21.9 ± 5.9	26.5 ± 9.4	27.7 ± 9.0	27.6 ± 3.3	25.0 ± 5.3	32.5 ± 4.6	33.0
	PG	13.3 ± 1.6	13.4 ± 1.2	13.2 ± 0.9	12.0 ± 1.0	11.4 ± 1.9	12.2 ± 1.9	15.0
Emulsion medium	TG	22.6 ± 3.0	22.5 ± 2.6	19.9 ± 2.0	13.0 ± 1.3	15.7 ± 4.2	13.5 ± 3.3	9.4
	PG	14.4 ± 1.3	16.3 ± 1.7	17.3 ± 1.2	11.9 ± 1.3	10.3 ± 1.2	13.5 ± 1.9	12.2
n		4	4	4	14	6	6	2

suggest a specific effect of bile salts on esterification. However, as will be discussed later, this effect may be mediated by the increased rate of entry of free fatty acids into the mucosa.

The falling percentage incorporation into triglyceride (Table I) suggested that efficiency of esterification declined after the first 15 min particularly in bile salt media. However, the total isotope as ester did not fall significantly which indicated that lipolysis was not a major factor.

The effect of bile salts serosally

In an attempt to dissociate possible intracellular effects of bile salts from effects on uptake 11 everted sacs were filled with dilute phosphate buffer (pH 6.4) containing the usual bile salt mixture, 5 mM, while in 9 controls bile salts were omitted as in all the previous experiments. Both groups of sacs were pre-incubated for 30 min in buffer solution and then incubated for 30 min in micellar solution of the usual lipid mixture in non-ionic detergent. Bile salts serosally had not effect, compared with controls, on uptake (0.661 $\pm$ 0.031 and 0.584 $\pm$ 0.039 μmole/g wet wt., $\pm$ S.E.), on total radioactivity incorporated into ester (0.168 $\pm$ 0.010 and 0.162 $\pm$ 0.052 μmole labelled acid/g wet wt.) or on percentage incorporation into triglyceride (18.9 $\pm$ 1.5 and 17.3 $\pm$ 3.8). In both groups uptake and percentage incorporation were somewhat lower than in previous experiments with non-ionic detergent in which sacs were incubated in radioactive lipid without 30 min pre-incubation at 37° in buffer. The differences were statistically significant and possibly reflected some deterioration in performance. In another group of experiments, sacs with bile salts, 5 mM, serosally were incubated in buffer for 60 min (corresponding to 30 min pre-incubation, followed by 30 min post-incubation with lipid). Following this the serosal fluid was drained and the sac carefully washed. The mucosa was scraped off, extracted in 95% (v/v) ethanol and bile salts estimated[11]. The bile salt concentration in mucosa was 1.0 μmole/g wet wt.

The effect of varying percentage solubilization

Bile salt concentration was varied over a range from 0.5 to 10 mM with constant lipid composition: 1 mM [1-^{14}C]oleic acid 1 mM monoolein. Uptake did not alter with increasing bile salt concentration below the critical micellar concentration (Fig. 2) but rose sharply with increasing concentration of bile salts above the critical micellar concentration.

Particularly striking is the way in which the increase in uptake parallels the percentage solubilization of free fatty acids with increasing bile salt concentration (Fig. 2). The solubilization curve was established for the same lipid mixture as used for incubation, centrifugation at $2 \cdot 10^7 \times g \cdot$min being used to separate the micellar and non micellar phases.

Comparison of oleic acid and 1-monoether uptake

Everted sacs were incubated in a lipid mixture consisting of 1 mM [9,10-$^3H_2$]-oleic acid and 1 mM glyceryl-1-mono[1-^{14}C]palmityl ether. This was either micellar or non micellar in buffer and bile salts. Uptake of oleic acid was faster, relative to its micellar concentration, than uptake of 1-monoether (Table II). This did not support penetration of absorptive cells by micelles as intact lipid aggregates. In the absence

of micelles the uptake of both 1-monoether and oleic acid was decreased to about the same slow rate.

Esterified monoether was incorporated mainly into fraction (iiia) (Table II) which included diglycerides and monoalkoxymonoglyceride, and it was labelling in this fraction which was promoted by micellar media. It can also be seen that there was more oleic acid label in fraction (iiia) than was seen for fraction (iii) in Table I when 1-monoolein was used in the incubate instead of 1-monoether. Label derived from oleic acid and 1-monoether were almost equivalent in fraction (iiia) as would be expected if labelled oleic acid were present as monoalkoxymonoglyceride.

These results are compatible with others[12] which suggest that 1-monoether is esterified mainly to monoalkoxymonoglyceride. They also imply that labelled oleic acid is not diluted appreciably by unlabelled free fatty acids, *i.e.* the endogenous intracellular pool of free fatty acids is very small.

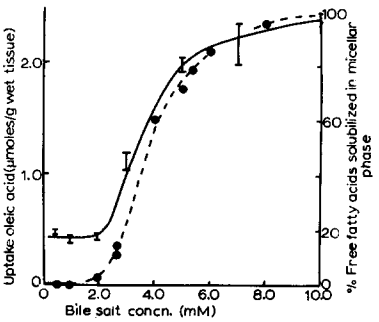

Fig. 2. The correlation between micellar solubilization and uptake of oleic acid. The lipid mixture was 1 mM [1-^{14}C]oleic acid and 1 mM monoolein. The incubation time was 30 min. ———, uptake of free fatty acids by sacs in μmoles/g wet tissue ± S.E., there being 8 sacs at 10 mM bile salts, 6 sacs at 1 mM bile salts and 4 sacs at each other point; – – – –, the percentage of oleic acid solubilized in a micellar phase which was separated by centrifugation at $2 \cdot 10^7 \times g \cdot$min.

TABLE III

EFFLUX OF LABELLED OLEIC ACID INTO UNLABELLED MEDIA

Preload consisted of a 15-min incubation at 35° in a lipid mixture of 1 mM [1-^{14}C]oleic acid, 1 mM monoolein and detergent concentrations as indicated. Efflux was into unlabelled media of the same lipid composition at 35° with detergent concentration as indicated. Detergent concentrations were as indicated in Table I. The figures for $t_{\frac{1}{2}}$ were calculated from the lines C_1 and C_2 for curves drawn as in Fig. 3. Each curve was drawn from mean points from 4 experiments. Pool sizes were calculated by dividing the total labelled free fatty acids pool (see *Calculations*) in the proportions indicated by extrapolating C_2 to zero time. Pool size is expressed as μmoles/g wet tissue.

Efflux medium	*Preload media*	*Bile salts*				*Pluronic F68*			
		$t_{\frac{1}{2}}$ *(min)*		*Pool size*		$t_{\frac{1}{2}}$ *(min)*		*Pool size*	
		C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
Micellar	Micellar	5	66	0.129	0.584	4	90	0.149	0.197
	Emulsion	6	60	0.320	0.107	5	95	0.173	0.097
Emulsion	Micellar	9	300	0.075	0.368	16	95	0.166	0.130
	Emulsion	5	220	0.118	0.171	14	75	0.132	0.133

TABLE II

UPTAKE FROM DOUBLE LABELLED LIPID MIXTURES

The lipid mixture was 1 mM glyceryl-1-mono[1-^{14}C]palmityl ether and 1 mM [9,10-$^{3}H_2$]oleic acid. The detergent was sodium taurocholate–sodium taurodeoxycholate (4:1, on a molar basis) 10 mM for micellar media and 1 mM for emulsion media. Incubation was for 30 min. Uptake = uptake of label as μmoles/g wet tissue. Total = total percentage of label esterified. TG = percentage esterified as triglyceride, monoalkoxydiglyceride or cholesterol ester. PG = percentage esterified as diglyceride or monoalkoxymonoglyceride. Amount = amount of label in PG expressed as μmoles/g wet tissue. All figures are the mean of 6 experiments ± S.E.

Medium	*Glyceryl-1-mono[1-^{14}C]palmityl ether esterification*					*[9, 10-$^{3}H_2$]oleic acid esterification*				
	Uptake	*Total*	*TG*	*PG*	*Amount*	*Uptake*	*Total*	*TG*	*PG*	*Amount*
Micellar	1.891 ± 0.085	44.4 ± 3.0	9.1 ± 0.6	35.3 ± 2.1	0.675 ± 0.062	2.731 ± 0.175	68.3 ± 3.6	39.8 ± 2.5	26.3 ± 1.1	0.720 ± 0.070
Emulsion	0.382 ± 0.033	20.7 ± 2.1	7.4 ± 1.4	13.3 ± 1.3	0.048 ± 0.004	0.416 ± 0.033	41.0 ± 3.2	19.5 ± 1.8	12.8 ± 1.3	0.050 ± 0.005

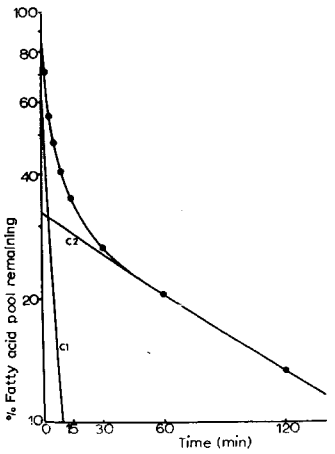

Fig. 3. A representative efflux curve. This curve is the mean of 4 experiments. The lipid medium for the 15-min preloading incubation was 1 mM [1-^{14}C]oleic acid and 1 mM monoolein in buffered Pluronic F68 solution (0.66 mg/ml). The efflux medium was 1 mM oleic acid and 1 mM monoolein in buffered Pluronic F68 (30 mg/ml). C_1 represents a pool of rapidly exchanging labelled free fatty acids and C_2 a slowly exchanging pool of labelled free fatty acids. The slope of C_1 was determined by the method of subtraction.

Efflux

The logarithm of the percentage of isotopic free fatty acid remaining in the tissue was plotted as a function of time (see *Calculations*) and a representative curve is shown (Fig. 3). In all experiments this curve was readily resolved, by the method of subtraction, into two straight lines which represented two functional compartments of grossly differing half time of exchange (Table III). In most groups of experiments the half time for the fast compartment was 4–9 minutes and for the slow compartment 60–95 min.

The amount of isotopic free fatty acids in each compartment at the commencement of efflux was estimated by extrapolation. While this involves assumptions which have yet to be verified it will be noted (Table III) that the amount of rapidly exchanging free fatty acids was little affected by the medium used for pre-incubation. The amount of slowly exchanging free fatty acids was considerably increased by pre-incubation in micellar bile salt media and was possibly increased for non-ionic micelles when compared with non-ionic emulsions.

DISCUSSION

Other workers have compared uptake of free fatty acid from bile salt micellar solutions with uptake from suspensions stabilized with albumin[2,6]; in the present experiments a more clear cut comparison was possible. The effect of bile salts was compared above and below the critical micellar concentration and the same was done for a second detergent, the non-ionic Pluronic F68. Since uptake was greater from micellar media than from emulsion for both detergents an effect of solubilization *per se* was clearly indicated. Any intracellular effect of Pluronic would be most unlikely since, with a molecular weight of 8000, it would not be absorbed. The greater uptake for bile salt micellar media than from Pluronic micelles might suggest an additional intracellular effect of bile salt micelles. However, in Fig. 2 it can be seen that uptake and solubilization vary together, there being a sharp inflection at the critical micellar concentration. This strongly suggests that uptake is primarily dependent on the physico-chemical nature of the incubation medium rather than any enzymic effect of bile salts. The absence of such an effect is further supported by the experiments in which bile salts, when introduced into the serosal space reached the mucosal layer but failed to influence uptake.

If the greater uptake from bile salts than from Pluronic F68 is not attributable to an intracellular effect, then an explanation must be sought in terms of the mechanism by which micellar solubilization promotes uptake. Penetration of the cell membrane by intact bile salt micelles but not by the larger non-ionic micelles[14] is a possible explanation. Micellar penetration has been supported by some workers[4,13]. The differing rates of uptake of two molecular species from the same bile salt micelles in this work (Table II) and that of some others[5] is, however, difficult to reconcile with penetration of intact micelles.

The alternative is that micelles have a transport function carrying lipid up to the membrane. It has been shown that micelles promote passage of single lipid-soluble molecules across membranes which exclude intact micelles[15], by increasing the driving force for diffusion in the bulk aqueous phase[16]. Information is needed on how this effect differs for different detergents.

It has been claimed that bile salts may contribute to intestinal absorption by specifically stimulating intracellular re-esterification[17,3]. Others have claimed the effect to be associated with a stimulation of uptake[6,2]. Evidence has been presented for the stimulation of uptake of free fatty acid by a micellar phase of a non-ionic detergent or bile salts. However, it was also found that esterification was more efficient, *i.e.* was greater relative to uptake, from micellar media and that this effect was greater for bile salt micelles. While this might suggest a specific effect of bile salts, Fig. 4 shows that the efficiency of esterification increases with increasing rate of uptake for data including micellar and non-micellar media of both Pluronic F68 and bile salts. This linear correlation was highly significant. Efficiency of esterification might therefore be related to the rate of uptake rather than to any enzymic effect of bile salts. The preliminary efflux experiments suggest a possible explanation of the phenomenon in terms of a three compartment model, two compartments containing freely exchanging free fatty acids and the third being a non-exchanging compartment which accumulated esterified fatty acid. Whilst this data cannot be finally interpreted until some evidence is available on the specific activity and rate of exchange of mass of these compartments, it can be seen (Tables I and III) that percentage of label esterified correlates with percentage of free fatty acids label in the slowly exchanging compartment.

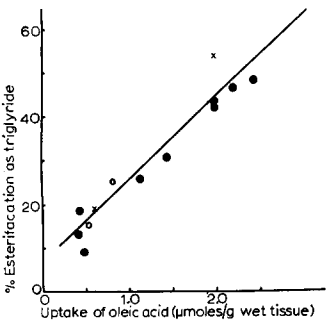

Fig. 4. The correlation of percentage esterification as triglyceride with the rate of uptake of labelled oleic acid. Incubation media 1 mM [1-^{14}C]oleic acid and 1 mM monoolein. ●, data from experiments (Fig. 2) in which uptake was measured at varying bile salt concentrations; ×, from experiments (Fig. 1, Table I) in which uptake at 30 min was measured for bile salts above and below the critical micellar concentration; ○, from experiments (Fig. 1, Table I) in which uptake at 30 min was measured for Pluronic F68 above and below the critical micellar concentration. The line is the regression line ($y = 6.5 + 18.5x$, $P < 0.001$).

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