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# THE RELATIONSHIP BETWEEN CONCENTRATION AND UPTAKE BY RAT SMALL INTESTINE, *IN VITRO*, FOR TWO MICELLAR SOLUTES

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SUMMARY

The uptake by everted sacs of rat small intestine of labelled oleic acid and α-glycerol monooleyl ether solubilized in pure sodium taurocholate–sodium taurodeoxycholate (4:1, on a molar basis) was measured at varying lipid concentrations. The two lipids were tested either as the sole micellar solute or both solubilized in the same micellar solution. It was shown that uptake of both lipids is linearly dependent on concentration but that slope of the line relating uptake and concentration is different when the two lipids are compared. This suggests that the two lipids are taken up independently from micellar solutions although at least one interaction of the lipid fluxes was shown.

Free fatty acid and monoglyceride might be absorbed by the small intestine from micellar solutions either as single molecules or as part of intact micelles. If micelles were made containing two isotopically labelled micellar solutes and these two solutes were not taken up by everted sacs of rat intestine in the same ratio as in the micellar solution, this would be evidence against the uptake of intact micellar particles. Such an experiment has been done by several workers[1-3] with conflicting results. To try to resolve this conflict, everted sacs were incubated in micellar solutions containing two lipid solutes, fatty acid and α-glyceryl monooleyl ether at a variety of concentrations. It seemed possible that the relationship between concentration of micellar solute and rate of uptake might vary for different lipids. If the whole range of concentrations were considered it might be apparent that fortuitious agreement between incubation and mucosal ratios could occur at particular ratios.

Everted sacs of rat small intestine weighing 400–500 mg were prepared and incubated as previously described[2] for 15 min in an oxygenated phosphate buffer (pH 6.4). Sodium taurocholate and sodium taurodeoxycholate (purity >98%) were synthesised[4] and used in the molar ratio 4:1 to give a total concentration of 10 mM in all lipid media. The class purity of lipids was checked by thin-layer chromatography on 0.25 mm thickness silica gel G in the solvent system hexane–diethyl ether–glacial acetic acid (80:20:2, by vol). Labelled lipids were at least 98% pure and unlabelled 95% pure (200 μg spot). Micellar solutions of the lipids in the combinations to be described were prepared by sonication. After incubation, sacs were washed, the mucosa scraped off and the lipid extracted in chloroform–methanol (2:1, by vol.). An aliquot of this was dried and counted in a Nuclear Chicago liquid scintillation

counter using 10 ml of the scintillant 2,5-diphenyloxazole (PPO) (4g/l), 1,4-bis-(5-phenyloxazolyl-2)-benzene (POPOP) (0.05 g/l) in toluene. Quenching correction was by the channels ratio method[5]. In each experiment paired sacs were used to test each lipid mixture and at least three mixtures were tested in sacs from any single rat. From the known specific activity of the stock lipids, uptake of labels was expressed as $\mu$moles of lipid per g wet weight of tissue. The following statistical techniques were employed[6]: simple linear regression, comparison of regression coefficients and calculation of the correlation coefficient.

*Incubation with a single micellar solute.* Incubations were performed at 35° for 15 min with three concentrations of oleic acid. In Fig. 1 it can be seen that there is a linear relationship between uptake and concentration. The same is true for monoether as is also shown in Fig. 1. However, the slope of the line for oleic acid uptake against concentration is considerably (and significantly, $P < 0.01$) steeper than that for monoether. The intercepts for these lines are not significantly different from zero. These findings give no evidence for any carrier transport mechanism in the absorption of oleic acid or monoether, since the uptake is linearly related to concentration over the range tested. This suggests lipid uptake to be a simple diffusion process. The differing

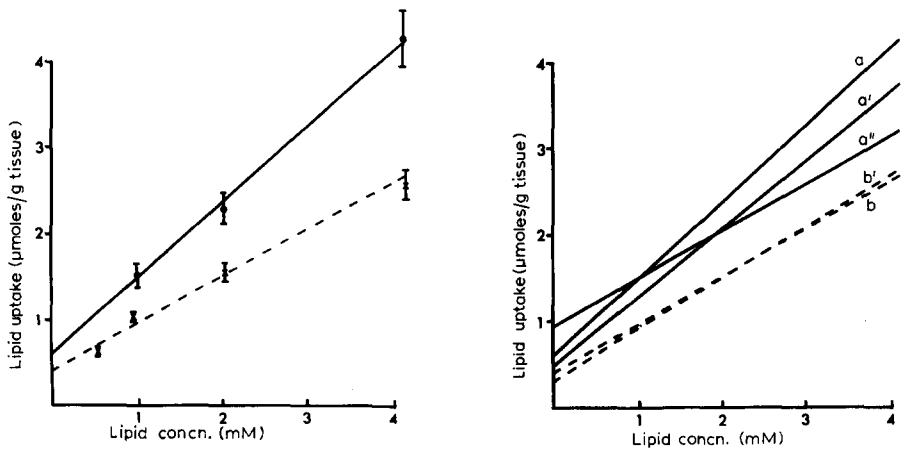


Fig. 1. Uptake *vs.* concentrations for a single micellar solute. Incubation was for 15 min at pH 6.4 in a bile salt mixture sodium taurocholate–sodium taurodeoxycholate in the molar ratio 4:1 to give a total concentration of 10 mM. The lipid composition was [1-$^{14}$C]oleic acid or $\alpha$-glyceryl mono[9,10-$^{3}H_2$]oleyl ether in the appropriate concentration. The points are ± S.E. (with six sacs per point). The equation for the oleic acid line is: $y = 0.5579 + 0.9083x$, $r = 0.93$. The equation for the monoether line is: $y = 0.3828 + 0.5592x$, $r = 0.95$. —, oleic acid uptake; – – –, monoether uptake.

Fig. 2. Uptake *vs.* concentration for two micellar solutes. Incubation was under the same conditions as for Fig. 1. For clarity the data points have been left off the lines. Each line is for at least three concentrations and at least six sacs per point. Two lines are as in Fig. 1. For incubations with mixed lipids, the lipids were equimolar being 0.5, 1, 2, 3, or 4 mM in each component. The mixtures were [1-$^{14}$C]oleic acid *plus* monoolein or [1-$^{14}$C]oleic acid *plus* $\alpha$-glyceryl mono[9,10-$^{3}H_2$]oleyl ether. The equation for oleic acid in the presence of monolein is: $y = 0.4540 + 0.8005x$, $r = 0.98$. The equation for oleic acid in the presence of monoether is: $y = 0.8963 + 0.5424x$, $r = 0.97$. The equation for monoether in the presence of oleic acid is: $y = 0.2684 + 0.6108x$, $r = 0.97$. The lines for oleic acid alone (a) and oleic acid in the presence of monoolein (a') are not significantly different from each other in slope ($p > 0.05$). These two lines are significantly different in slope from those for monoether alone (b), monoether in the presence of oleic acid (b') and oleic acid in the presence of monoether (a'') ($P < 0.01$). This latter three are not significantly different from each other.

slopes of the lines relating uptake and concentration for monoether and oleic acid suggest that each would be taken up independently from micellar solutions.

*Incubation with two equimolar micellar solutes.* To examine for any interaction between micellar solute fluxes across the mucosal cell membrane, incubations were performed at 35° for 15 min with media containing either equimolar labelled oleic acid and unlabelled monoolein or labelled oleic acid and labelled monoether. The concentration of each component was then 0.5, 1, 2, 3 or 4 mM in a particular medium. In Fig. 2 it can be seen the presence of monoolein does not significantly affect the slope of uptake with concentration for oleic acid. It can also be seen that the presence of oleic acid does not affect the slope of the line relating monoether uptake and concentration. But the presence of monoether does depress the slope of the line relating oleic acid uptake and concentration.

*Incubation with two micellar solutes in varying ratios.* The lines for oleic acid and monoether uptake when each was the sole micellar solute could be used to predict the mucosal lipid ratio following incubation with combinations of the two lipids in varying ratios. This would test further for any interaction of the two fluxes. Sacs were incubated under the usual conditions with mixtures oleic acid–monoether 1:1, 2:1, 4:1, 1:1, 1:2, 1:4 with the individual lipid concentrations 1, 2 or 4 mM. Both lipids were labelled.

In Table I are the predicted and observed mucosal ratios following the various incubations. It can be seen that there is appreciable deviation from the predicted values only at the higher monoether values. The effect of monoether was to depress the uptake of oleic acid.

The relationship between concentration and uptake for the equimolar oleic acid monoolein mixture as reported here was compared with that reported in a previous communication. In that report[2] the total concentration of the two lipids in the medium was held constant but the bile salt concentration was varied to vary the concentration of lipid in the micellar phase. To compare data from that report and this, uptake was expressed as μmoles of oleic acid/g wet tissue per min. Fig. 3 shows that the two sets of data fell on the same straight line. This suggests that oleic acid uptake is dependent only on its micellar concentration, *i.e.* the relationship shown in

TABLE I

INCUBATION WITH TWO MICELLAR SOLUTES IN VARYING RATIOS

Incubation was for 15 min at pH 6.4 in a bile salt mixture, sodium taurocholate–sodium taurodeoxycholate, in the molar ratio 4:1 at a total concentration of 10 mM. The lipid composition was [1-$^{14}$C]oleic acid and α-glyceryl mono[9,10-$^{3}H_2$]oleyl ether in the ratio indicated to give a concentration of 1, 2, or 4 mM in each component.

| *Incubation ratio oleic acid: monoether* | *Uptake (μmoles/g tissue)* | | *Mucosal ratio oleic acid: monoether* | |
|---|---|---|---|---|
| | *Oleic acid* | *Monoether* | *Observed* | *Predicted* |
| 1:1 | 1.304 ± 0.066 | 0.855 ± 0.042 | 1.52 | 1.56 |
| 2:1 | 2.006 ± 0.080 | 0.800 ± 0.026 | 2.51 | 2.50 |
| 4:1 | 3.389 ± 0.081 | 0.802 ± 0.025 | 4.25 | 4.45 |
| 1:1 | 1.506 ± 0.055 | 1.065 ± 0.044 | 1.41 | 1.56 |
| 1:2 | 1.361 ± 0.030 | 1.704 ± 0.040 | 0.80 | 0.97 |
| 1:4 | 1.002 ± 0.015 | 2.759 ± 0.064 | 0.36 | 0.56 |

the previous paper[2] was due to the varying micellar lipid concentration and not to the varying bile salt concentration.

The data presented suggested that the uptake of micellar lipids by everted sacs of rat intestine is as single molecules by a simple diffusion process. Although one interaction has been shown, the evidence is against uptake as intact micellar particles.

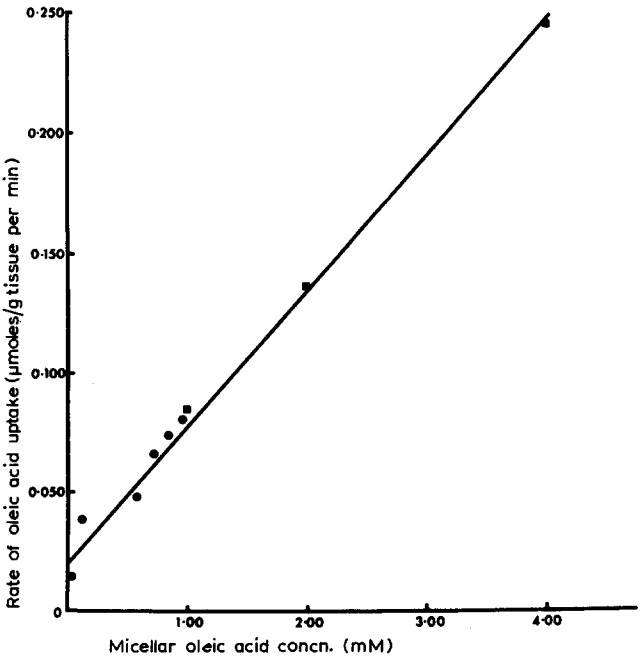


Fig. 3. Rate of uptake *vs.* concentration in the micellar phase For data set ■ incubation conditions were as in Fig. 1. The lipid composition was equimolar [1-$^{14}$C]oleic acid and monoolein. All solutions were optically clear and taken to be 100% solubilized. The incubation conditions for data set ● were as above: the same bile salt mixture was varied in total concentration from 0.5 to 10 mM. The lipid composition was constant at [1-$^{14}$C]oleic acid, 1 mM; monoolein, 1 mM. The lipid concentration in the micellar phase determined by ultracentrifugation. The regression coefficient for each set of data was not significantly different either from the other or from the regression coefficient for the total data set.

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