BBA 75684

THE INTESTINAL UPTAKE AND ESTERIFICATION, *IN VITRO*, OF FATTY ACID AS A DIFFUSION LIMITED PROCESS

N. E. HOFFMAN AND W. J. SIMMONDS

Department of Physiology, The University of Western Australia, Nedlands, W.A. 6009 (Australia)

(Received March 24th, 1971)

SUMMARY

The rate of uptake and esterification of fatty acid by everted intestinal sacs is reduced when micellar media of a non-ionic detergent are compared with bile salt micellar media[1] (HOFFMAN, *Biochim. Biophys. Acta*, 196 (1970) 193). In this paper it has been shown that:

(1) The reduced rate of uptake correlates with a reduced diffussion coefficient of fatty acid in the non-ionic detergent micellar solution.

(2) The sac preparation has a maximal esterifying capacity.

(3) This is the same for non-ionic detergent and bile salt micellar media.

INTRODUCTION

When a non-ionic detergent, Pluronic F68, was used as a solubilizer, the uptake of fatty acid from micellar solutions by everted sacs was slower than from bile salt micelles. The absolute and percentage esterification of labelled fatty acid was less[1].

The reduced rate of uptake of fatty acid in vitro may be explained by the reduced diffusion coefficient of fatty acid in Pluronic F68 micellar media as compared with the bile salt media[2]. If this were so, the regression coefficient of the relationship between uptake and fatty acid concentration should also be reduced for Pluronic F68 media.

The reduced esterification might in turn be due to slower uptake from Pluronic F68 micelles, since a relationship between rate of uptake and percentage esterification has been demonstrated[1]. If bile salts have no specific intercellular effect, the relationship between rate of uptake and esterification of labelled fatty acid should be the same for Pluronic F68 and bile salt micellar media although for Pluronic micelles a higher fatty acid concentration would be needed for a given rate of uptake. This paper describes experiments to test the above two suggestions.

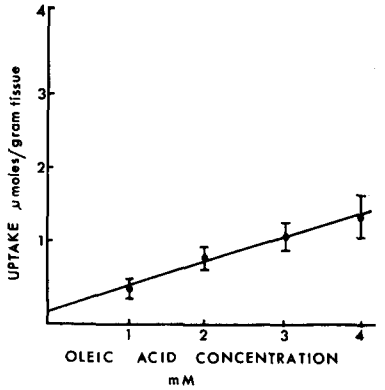
RESULTS AND DISCUSSION

Everted sacs of rat small intestine weighing 400–500 mg were prepared and incubated for 15 min as previously described[1]. Micellar media were made up in an oxygenated phosphate buffer (pH 6.4). Pluronic F68 was a gift of the Wyandotte Chem. Corp. and was used as supplied. Sodium taurocholate and sodium taurodeoxy-

cholate were synthesized and purified[3] to greater than 98 % pure and used in the molar ratio 4:1 to give a total concentration of 10 mM. Isotopic lipids were > 98 % class pure by thin-layer chromatography on silica gel G in the solvent system hexane–diethyl ether–glacial acetic acid (80:20:2, by vol.). Unlabelled lipids were greater than 95 % on thin-layer chromatography in the same solvent system (1 mg spot). Lipids were extracted from intestinal mucosa with chloroform–methanol (2:1, v/v). Liquid scintillation counting was performed in a Nuclear Chicago Mark I counter using the 10 ml of the scintillant mixture 2,5-phenyloxazole (4 g/l)–1,4-bis-(5-phenyloxazolyl -2)-benzene (0.05 g/l) in toluene. In each experiment paired sacs were used to test each lipid mixture. At least three lipid mixtures were tested with sacs from any single rat. From the known specific activity of the stock oleic acid solution the uptake or esterification of oleic acid could be expressed as μmoles of oleic acid per g wet tissue.

Uptake vs. fatty acid concentration

The uptake of fatty acid was shown to be linearly related to its concentration in Pluronic F68 micellar media (see Fig. 1) with a regression coefficient of 0.30. The Pluronic F68 concentration and the lipid mixture (equimolar oleic acid and monoolein) were chosen to conform with that used by WILLIX[2]. The regression coefficient would be a function of the diffusion coefficient of fatty acid in the media, the surface area of intestine per g tissue and the thickness of the unstirred layer of fluid adjacent to the mucosa. These latter two terms may, as an approximation, be taken as constants since we used rats of an inbred colony and constant incubation conditions. Thus the only



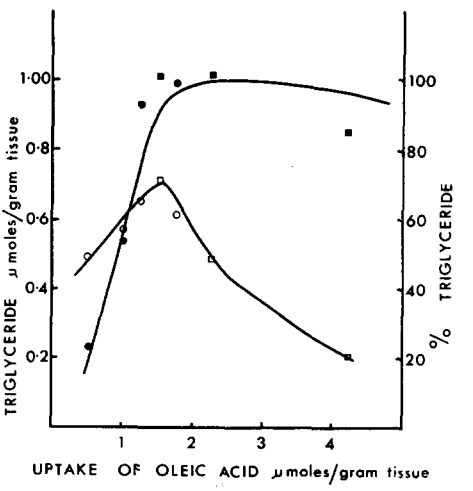


Fig. 1. Uptake *vs.* fatty concentration. Incubation was for 15 min at pH 6.4, 35°. The Pluronic F68 concentration was 16 mg/ml and the lipid mixture equimolar [1-^{14}C]oleic acid, monoolein to give the oleic acid concentration indicated. The regression line is $y = 0.153 + 0.304\,x$ where y is uptake and x is oleic acid concentration. Each point is the mean ± S.E. for 6 sacs.

Fig. 2. The amount of oleic acid esterified to triglyceride with varying rates of uptake. Sacs were incubated for 15 min at 35°, pH 6.4. The lipid was [1-^{14}C]oleic acid at the concentration indicated. The bile salt mixture was sodium taurocholate–sodium taurodeoxycholate (4:1 on a molar basis) at a total concentration of 10 mM. The Pluronic F68 concentration was 30 mg/ml. The amount esterified is indicated ■ for bile salts and ● for Pluronic F68 solution. The percentage esterified is indicated □ for bile salts and ○ for Pluronic F68 solution. Each point is the mean for 6 sacs.

variable between Pluronic F68 and bile salt micellar solutions would be the diffusion coefficient of fatty acid. WILLIX[2] found the ratio of the diffusion coefficient of fatty acid in bile salt media to that in Pluronic F68 media was 2.16. In a previous communication[4] the regression coefficient for fatty acid uptake against its concentration in bile salt micellar media and this lipid mixture was 0.80. Therefore the ratio of regression coefficient would be 0.80/0.30, that is 2.67. This level of agreement suggests that the reduced uptake of fatty acid by sacs from Pluronic F68 micellar media is due to the diffusional properties of the fatty acid rather than a specific intracellular effect of bile salts.

Esterification of fatty acid vs. rate of uptake

Sacs were incubated for 15 min in micellar solutions of fatty acid in bile salts or Pluronic F68. In Fig. 2 it can be seen that for bile salt solutions, with an increasing rate of uptake, the percentage esterification fell, whereas the absolute amount of isotopic lipid esterified remained virtually constant. With Pluronic F68 micellar solution it can be seen that the percentage and absolute amount of labelled fatty acid rises with the rate of uptake. The absolute amount esterified has a maximum value which is equivalent to that seen for bile salt micellar incubation. This suggests that the everted sac has a limited capacity to esterify fatty acid. CLARK *et al.*[5] have also demonstrated the limited capacity of *in vitro* preparation to esterify fatty acid. But up to that capacity, the proportion of fatty acid esterified is related to the rate of uptake, and bile salts have no specific effect on esterification, beyond that due to enhanced diffusion as a consequence of solubilization in the medium.

ACKNOWLEDGEMENTS

Mrs. V. J. Yeoh provided very capable technical assistance. This work was supported by grants from the National Health and Medical Research Council of Australia and the Medical School Research Grants of the University of Western Australia. N.E.H. is a National Health and Medical Research Council Medical Post Graduate Scholar.

REFERENCES

1 N. E. HOFFMAN, *Biochim. Biophys. Acta*, 196 (1970) 193.
2 R. L. S. WILLIX, *J. Pharm. Sci.*, 59 (1970) 1439.
3 A. F. HOFMANN, *Acta Chem. Scand.*, 17 (1963) 173.
4 N. E. HOFFMAN AND V. J. YEOH, *Biochim. Biophys. Acta*, 223 (1971) 53.
5 M. L. CLARK, H. R. LANZ AND J. R. SENIOR, *J. Clin. Invest.*, 48 (1969) 1587.