metabolism in a reconstituted drug metabolizing enzyme system and suggests a functional role for cytochrome P-450 in the metabolism and utilization of medium-chain non-esterified fatty acids in the membrane of the hepatic endoplasmic reticulum.

We thank the M.R.C. and University of Surrey Research Committee for financial support and P. P. T. is grateful to the S.R.C. for a research studentship. We are grateful to Dr. John B. Schenkman for his original stimulus in this study.


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In mammals, the bulk of the fatty acids is transported as triacylglycerol in the plasma chylomicrons and very-low-density lipoproteins. Uptake of the fatty acids by the extra-hepatic system that closely resembles that of mammals, although the existence of an alternative mechanism in which lipid is transported as non-esterified fatty acid in the plasma has been reported (Robinson & Mead, 1973; Kayama & Eijima, 1976). The nature of the fatty-acid carrier has not been determined. In this communication we report on the characterization of the chylomicron-like particles in the plasma of trout that have been maintained on a high-lipid diet, and also provide evidence that suggests that the high-density-lipoprotein fraction of trout plasma binds non-esterified fatty acids.

Trout, fed on a high-lipid diet over a period of 2 weeks, were anesthetized in fresh water containing 0.03% of ethyl m-amino benzoate methanesulphonate (Sigmob) and blood was withdrawn from the caudal vein by means of a syringe containing EDTA and trisodium citrate to give a final concentration in the blood of 0.01% EDTA and 15mg of citrate/ml. Chylomicrons were prepared by centrifuging trout plasma for 70 min at 10°C and 80000g, in the type 5OTi rotor of the Beckman model L2 ultracentrifuge. The largest proportion of particles appeared in fraction 4 with a diameter of approx. 100nm.

For the determination of the fatty-acid-binding properties of trout plasma, [3H]oleic acid (5μCi) was added to trout serum (3ml). Lipoproteins were prepared and electrophoresis procedures were carried out as previously described (Skinner & Rogie, 1978).

The trout very-low-density-lipoprotein fraction contained particles of 35-60nm diameter, which were electron-lucent, and, in addition, some particles of 300-600nm diameter were detected. It was found that a 50-fold increase in the number of particles of diameter greater than 300nm was detected by electron microscopy in the chylomicron fraction when the diet of the fish was changed from standard fish pellets (10% lipids) to a diet containing 20% lipid. These particles were spherical and electron-lucent, resembling mammalian chylomicrons. The distribution of chylomicron particle sizes between the different fractions was as follows: fraction 1 (top fraction), >400nm; fraction 2, 150-400nm; fraction 3, 100-150nm; fraction 4 (bottom fraction), 60-100nm. The nature of the fatty-acid carrier has not been determined. In this communication we report on the characterization of the chylomicron-like particles in the plasma of trout that have been maintained on a high-lipid diet, and also provide evidence that suggests that the high-density-lipoprotein fraction of trout plasma binds non-esterified fatty acids.

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Trout, fed on a high-lipid diet over a period of 2 weeks, were anesthetized in fresh water containing 0.03% of ethyl m-amino benzoate methanesulphonate (Sigma) and blood was withdrawn from the caudal vein by means of a syringe containing EDTA and trisodium citrate to give a final concentration in the blood of 0.01% EDTA and 15mg of citrate/ml. Chylomicrons were prepared by centrifuging trout plasma for 70 min at 10°C and 80000g, in the type 5OTi rotor of the Beckman model L2 ultracentrifuge and collecting the upper turbid layer by tube-slicing. This fraction was mixed with NaBr solution of density 1.020g/ml and re-centrifuged under the same conditions. A portion of the chylomicron fraction (2ml) were collected from each tube by means of the rotor of the Beckman model L2 ultracentrifuge. The chylomicrons were further fractionated on a continuous densiflow and the fraction washed by repeating this procedure three times.

The chylomicrons were further fractionated on a continuous sucrose gradient (28-35%; v/v) by a modification of the method of Pinter & Zilversmit (1962). The chylomicron fraction (2ml) adjusted to contain 60% (v/v) sucrose, was placed in a 5ml centrifuge tube, overlayed with the sucrose gradient (3.2ml) and centrifuged for 30 min at 10°C and 7280g, in the SW65 Ti rotor. Three fractions (1ml) and a final bottom fraction (2ml) were collected from each tube by means of the Buchler Autodensiflow and dialysed against 0.15 M NaCl before analysis.

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The differential inhibition by cerulenin of fatty acid synthesis and elongation in the psychrophilic bacterium \textit{Micrococcus cryophilus}

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The psychrophilic bacterium \textit{Micrococcus cryophilus} regulates its phospholipid acyl chain length by a mechanism that may involve an elongation enzyme system that is distinct from fatty acid synthetase (Sandercock & Russell, 1980). The present paper compares the effects of the antibiotic cerulenin on fatty acid synthesis and elongation. Cerulenin inhibits condensation reactions, such as the \( \beta \)-oxacyl-acyl-carrier-protein synthetase of fatty acid synthetase (Omura, 1976). In view of the similarity of fatty acid elongation and synthesis cerulenin would be expected to inhibit both processes; this has been demonstrated for plants (Jordan & Harwood, 1980) and \textit{Acholeplasma laidlawii} B (Saito \textit{et al.}, 1978). However, the sensitivity of the condensing enzymes of synthesis and elongation may well differ, so that cerulenin could be used to differentiate between the two processes (e.g. see Jordan & Harwood, 1980).

Cultures of \textit{M. cryophilus} (A.T.C.C. 15174) were grown to mid-exponential growth phase at 20°C in a defined casamino acids (Difco)-salts medium (Russell, 1974). Fatty acid synthesis was assayed by adding 0.93kBq of carrier-free sodium \([\mathrm{U}^{}-^{14}\mathrm{C}]\)acetate (sp. radioactivity 2.2 GBq/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) to 1 ml portions of bacterial culture, which were incubated for 30 min at 20°C. In those assays where cerulenin was added, bacteria were pre-incubated for 5 min in the presence of the inhibitor before starting the assay with isotope. Incorporation was stopped, and the lipids were extracted by adding 3.75 ml of chloroform/methanol (1:2, v/v); after 1 h the lipid extract was washed by the procedure of Garbus \textit{et al.} (1963). The lower chloroform phase (total lipid extract, comprising 85% phospholipid) was transferred to a scintillation vial, the solvent evaporated and radioactivity was measured by liquid-scintillation counting.

Fatty acid elongation was assayed by using radio-g.l.c. analysis of fatty acid methyl esters (for details see Sandercock & Russell, 1980) prepared from total lipids extracted as described above, except that the assay was scaled up 10-fold.

In some experiments the incorporation and elongation of \([1-^{14}\mathrm{C}]\)palmitic acid (sp. radioactivity 2.2 GBq/mmol; The Radiochemical Centre) was determined by using the same methods.

Cerulenin (the generous gift of Professor S. \Ömura, Kitasato Institute, Tokyo, Japan) was dissolved either directly in absolute ethanol or in water by sonication for 30 min in a Mettler Electronics ME 4.6 cleaning bath (Mettler Electronics Corp., Anaheim, California, U.S.A.) to give a final concentration of 1 mg/ml.

Fatty acid elongation was found to be more sensitive than was synthesis to inhibition by cerulenin dissolved in water (Table 1); 10 \( \mu \)M-cerulenin inhibited elongation, whereas 50–100 \( \mu \)M-cerulenin was required to inhibit synthesis. However, we were concerned about the poor solubility of cerulenin in water, even though an apparently clear solution was obtained after sonication (see above). Therefore, the experiments were repeated with cerulenin dissolved in ethanol. Similar results were obtained, but their interpretation was complicated by the inhibition of both fatty acid synthesis and elongation by ethanol alone, even in small amounts (0.12–0.23%, v/v), in which the cells were viable. Elongation was particularly sensitive to ethanol, which may reflect the membrane location of the elongation enzymes (Sandercock & Russell, 1980).

Cerulenin also inhibited the elongation of palmitate to \( \mathrm{C}_{16} \) fatty acids, the amount of inhibition being comparable with that obtained using acetate as precursor.

In conclusion, the present results show that, although both fatty acid synthesis and elongation are inhibited by cerulenin, elongation is more sensitive. This fact is interpreted as reflecting the different affinities for cerulenin of the respective condensing enzymes involved in synthesis and elongation. These data are

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\begin{array}{cccccccc}
| \text{Cerulenin} (\mu M) | \text{Cerulenin in water} & \text{Cerulenin in ethanol}^* & \text{Cerulenin in water} & \text{Cerulenin in ethanol} & \text{C}^{14}/\text{C}^{14} \text{ fatty acid ratio of radioactivity} \\
0 & 4343 (100) & 5663 (100) & 6.53 & 8.46 \\
1 & 4721 (109) & 5013 (92) & --- & --- \\
2 & 4409 (102) & 5250 (100) & --- & --- \\
5 & 4706 (108) & 4933 (108) & --- & --- \\
10 & 4391 (101) & 3674 (102) & 5.02 & 8.71 \\
20 & 4541 (105) & 3723 (130) & --- & --- \\
50 & 4245 (98) & 3456 (82) & 5.21 & 2.52* \\
100 & 3406 (78) & 1919 (71) & 2.33 & 0.90* \\
200 & 1894 (44) & --- & 0.68 & --- \\
400 & 1519 (35) & --- & --- & --- \\
\end{array}
\]

\* For details see text.

\* The percentages have been calculated taking into account the inhibition by an equivalent volume of ethanol alone.

\* The corresponding ratios for controls in which an equivalent volume of ethanol was added in the absence of cerulenin were 4.91 and 2.00 respectively.