lysine metabolism has been measured in rats fed a diet low in trimethyl-lysine and carnitine (Davis & Hoppel, 1983, 1986). Under these steady-state conditions, the total body trimethyl-lysine content is similar to the total body carnitine content. The tissue distribution of peptide-linked and free trimethyl-lysine shows that approximately 68% of the total trimethyl-lysine is present in skeletal muscle. In the steady-state, the urinary excretion of trimethyl-lysine and its metabolites will measure the amount of trimethyl-lysine produced and its conversion into carnitine. We have measured both urinary free trimethyl-lysine and \( N^2 \)-acetyl-trimethyl-lysine and determined that the plasma clearance of trimethyl-lysine is less than 5% of the glomerular filtration rate. Acetyl-trimethyl-lysine accounts for about 70% of total trimethyl-lysine excretion. Other metabolites, such as 2-oxo-trimethyl-ammonio-hexanoate, trimethylammonio-pentanoate and trimethylammonio-butanooate, account for less than 10% of trimethyl-lysine metabolism and are considered minor metabolites. In the steady state, carnitine excretion represents between 60 and 80% of the combined excretion of trimethyl-lysine and carnitine. Therefore, the conversion of endogenous trimethyl-lysine into carnitine appears to occur with a much higher efficiency than that observed with exogenous trimethyl-lysine. These data support the notion that trimethyl-lysine is converted into an intermediate, trimethylammonio-butanooate, in the tissue of origin and it is the intermediate that is the transported precursor for hepatic carnitine synthesis. Skeletal muscle contains the majority of trimethyl-lysine in the body and protein turnover rates in skeletal muscle are sufficiently high to suggest that it contributes substantially to the availability of trimethyl-lysine. The proposed sequence for formation of carnitine from extrahepatic sources of trimethyl-lysine would then be:

1. skeletal muscle free trimethyl-lysine → skeletal muscle trimethylammonio-butanooate,
2. transport into plasma,
3. uptake of trimethylammonio-butanooate into liver, and
4. conversion to carnitine.

Thus, experiments employing injections of labelled trimethyl-lysine into the rats are affected by the tissue differences in cellular transport and measure the contribution of the kidney to the initial metabolism, whereas in situ the initial metabolism occurs in the tissue of origin. Because skeletal muscle does not readily extract trimethyl-lysine during perfusion (Zaspel et al., 1980) it seems reasonable to assume that its export would be similarly restricted.

**Characterization and properties of carnitine acyltransferases**

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The most extensively studied carnitine acyltransferase is mitochondrial (CPT), which is required for the \( \beta \)-oxidation of long-chain fatty acids (Bremer, 1983). Early studies showed that peroxisomes, microsomes and mitochondria from liver contain both short-chain (carnitine acyltransferase; CAT) and medium-chain (carnitine octanoyltransferase; COT) carnitine acyltransferase activity (Markwell et al., 1973; Markwell & Bieber, 1976). The microsomal enzymes are membrane-bound, very labile and remain poorly characterized (Hahn & Secombe, 1980; Bieber & Markwell, 1981; Valkner & Bieber, 1982). Some of our studies of the peroxisomal and mitochondrial carnitine acyltransferases purified from mouse liver and beef heart are summarized herein.

**Materials and methods**

The assays and materials were identical to those described elsewhere (Fiol & Bieber, 1984; Bieber & Fiol, 1984). 2-Tetradecyglycyl-CoA was a generous gift from Drs. Paul and Linda Brady, Washington State University, Pullman, Washington.

**Results and discussion**

The peroxisomal carnitine acyltransferases are soluble,
very stable, and have a broad acyl-CoA substrate specificity. They have been purified from mouse liver by us (Farrell & Bieber, 1983; Farrell et al., 1984) and from rat liver by others (Miyazawa et al., 1983a, b; Ozasa et al., 1983). Their kinetic properties greatly favour acylcarnitine formation, primarily due to a favourable $K_a$ for both acyl-CoAs and carnitine (see Table 1 and Farrell & Bieber, 1983; Farrell et al., 1984). The reverse reaction for both CAT and COT is kinetically unfavourable due to the high $K_a$ for acyl-carnitines. Formation of short-chain and medium-chain acylcarnitines in liver peroxisomes is consistent with the roles proposed for these enzymes in transferring chain-shortened acyl groups of acyl-CoAs, produced by the β-oxidation system of peroxisomes, to l-carnitine (Bieber et al., 1981, 1982; Bieber & Farrell, 1983).

In contrast to the broad substrate specificity of the peroxisomal and mitochondrial COT of mammalian systems, CAT from unicellular systems has a very narrow acyl specificity (Gilbert & Klein, 1982; Bieber et al., 1982; Emaus & Bieber, 1983). It apparently functions in the shuffling of acetyl units into the mitochondria matrix. The properties and substrate specificities of various CAT preparations are summarized in Table 1 of Bieber (1983). The COT activity of both rat liver mitochondria (Miyazawa et al., 1983a, b) and beef heart mitochondria (Clarke & Bieber, 1981a, b) is due primarily to the medium-chain carnitine acyltransferase activity of CPT with some contribution by CAT. Neither of the above-mentioned groups of investigators was able to isolate a separate COT from mitochondria. Antibodies against peroxisomal COT do not cross-react with mitochondrial CPT (Farrell & Bieber, 1983; Miyazawa et al., 1983b). CPT from rat liver has a molecular mass of about 300 000 daltons with a monomeric molecular mass of 69 200 daltons (Miyazawa et al., 1983; Ozasa et al., 1983), while the enzyme from beef heart has an aggregated molecular mass of about 510 000 daltons with a monomeric molecular mass of 67 000 daltons (Fiol & Bieber, 1984). The latter enzyme contains several moles of tightly bound phospholipid (Fiol & Bieber, 1984). Both of the mitochondrial enzymes of rat liver (CPT and CAT) are synthesized as proenzymes, which have molecular masses about 2500 daltons larger than the monomeric molecular mass (Miyazawa et al., 1983a, b; Ozasa et al., 1983).

Peroxosomal COT has low but significant activity with palmitoyl-CoA (Farrell et al., 1984). This activity could be mistaken for easily soluble CPT or non-mitochondrial CPT. It could also be present as a contaminant in mitochondria isolated by conventional techniques. It is interesting that COT is inhibited by 2-bromopalmitoyl-CoA but soluble CPT is not inhibited (Miyazawa et al., 1983a). Therefore the bromopalmitoyl-CoA inhibition of the 'loosely associated' CPT activity of liver mitochondria (West & Tubbs, 1971) could be due to the inhibition of contaminating peroxisomal COT.

Purified and membrane-bound mitochondrial CPT show complex kinetic behaviour (Bieber et al., 1981, 1982; Saggerson, 1982). Its kinetic properties are affected by pH (Fiol & Bieber, 1984), assay conditions (Clarke et al., 1983a, b), physiological state of the animal (Zammit, 1984) and possibly by phosphorylation/dephosphorylation (Harano et al., 1985). The membrane-bound, but not the purified, enzyme is potently inhibited by malonyl-CoA. Our data, with intact mitochondria, show that malonyl-CoA acts like a classical negative allosteric effector in that it shifts the $V$ versus [$S$] curve to the right, i.e. 20 μM-malonyl-CoA raises the $K_m$ for decanoyl-CoA from <5 μM to about 160 μM without changing the Hill coefficient (L. L. Bieber & C. J. Fiol, unpublished work). Both membrane-bound and purified CPT of beef heart mitochondria show co-operativity for l-carnitine and the acyl-CoA (Fiol & Bieber, 1984; Bieber & Fiol, 1984; L. L. Bieber & C. J. Fiol, unpublished work). The $K_m$ for l-carnitine of the purified enzyme shows a dependence on the chain-length of the acyl-CoA. With the longer-chain acyl-CoA co-substrates, a much greater affinity for carnitine is found (Fiol & Bieber, 1984); we have not done similar studies with CPT of intact mitochondria.

CPT activity of mitochondria and intact organs can be altered by some substrate analogues. One such compound is 2-tetradecylglycidic acid (Tutwiler & Brentzel, 1982; Kioreps et al., 1984; Declereq et al., 1985). Its CoA ester is a potent inhibitor of CPT-1. In vivo, it inhibits fatty acid-promoted gluconeogenesis (Tutwiler & Brentzel, 1982). Evidence has been presented showing it forms a covalent complex with a 90 000 dalton protein of rat liver mitochondria (Kioreps et al., 1984). Although it was concluded that the 90 000 dalton protein is native CPT, this seems highly unlikely, because the proenzyme in rat liver for the CPT monomer has a molecular mass of 71 600 daltons (Miyazawa et al., 1983b). We recently have determined the effect of 2-tetradecylglycidyl-CoA on the kinetic parameters of purified CPT. Fig. 1 shows that 2-tetradecylglycidyl-CoA alters the shape of the $V$ versus [$S$] curve for palmitoyl-CoA.

<table>
<thead>
<tr>
<th>Acyl group</th>
<th>COT</th>
<th>CAT</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Acyl-CoA (μM)</td>
<td>Carnitine (μM)</td>
</tr>
<tr>
<td>C-2</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>C-4</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>C-18</td>
<td>64</td>
<td>3</td>
</tr>
</tbody>
</table>

*From Table I of Farrell et al. (1984).
†From Table IV of Farrell & Bieber (1983).
Among the carnitine acyltransferases present in the liver, the

Table 2. Effect of 2-tetradecylylglycidyl-CoA on the kinetic parameters of purified CPT for palmitoyl-CoA at pH 6.0

<table>
<thead>
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<th>2-Tetradecylylglycidyl-CoA conc.</th>
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<tr>
<td>K_m (μM)</td>
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<tr>
<td>V_max (μmol/min)</td>
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<tr>
<td>Hill n</td>
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<td>1.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>


Carnitine acyltransferases in the physiological setting: the liver

Among the carnitine acyltransferases present in the liver, the

Fig. 1. Effect of 2-tetradecylylglycidyl-CoA on the kinetics of purified CPT

See legend to Table 2 for experimental details. Abbreviation: TDGACoA, 2-tetradecylylglycidyl-CoA.