The catabolism of medium- and long-chain dicarboxylic acids

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The ω-oxidation of fatty acids results in the formation of medium- and long-chain dicarboxylic acids. By this accessory metabolic pathway monocarboxylic acids are first converted into ω-hydroxymonocarboxylic acids, a reaction catalysed by the microsomal mixed-oxidase function system (Pettersen, 1972). These intermediates are subsequently converted into ω-oxomonocarboxylic acids and dicarboxylic acids by the action of cytosolic alcohol- and aldehyde-dehydrogenases (Mitz & Heinrikson, 1961). Medium-chain dicarboxylic acids can be formed from long-chain dicarboxylic acids and be excreted in urine. This organic aciduria is increased in a variety of conditions with excessive fatty acid degradation such as ketosis and diabetes mellitus or secondarily to defective mitochondrial fatty acid oxidation, including inherited metabolic defects, hypoglycin intoxication and riboflavin deficiency, as well as in subjects receiving medium-chain triglycerols. Dicarboxylic acids can be activated to their acyl-CoA esters by a rat liver microsomal synthetase (Vamecq et al., 1987). In clofibrate-treated rats, esters of long- and medium-chain dicarboxylic acids can be β-oxidized by both liver mitochondrial and peroxisomal fractions (Kolvraa & Gregersen, 1986), whereas in control animals this catabolism is mainly peroxisomal (Vamecq, 1987). Our studies were thus mainly dedicated to the catabolism of dicarboxylic acids in control, riboflavin-deficient and clofibrate-treated rats in vivo. The shortening of dicarboxylic acids by isolated mitochondria from the livers of control rats was also investigated.

The rate of oxidation of dodecanedioic acid was studied in the three groups of animals by the following procedure. Anaesthetized rats were cannulated to give direct access to blood, and urine was continuously collected from the bladder. Dodecanedioic acid was infused in the form of its Na salt in doses of 11, 22 and 44 μmol per 100 g body weight during a 30 min period. Urine was collected over 30 min periods from 1 h before to 3 h after infusion. Organic acids were extracted, and trimethylsilylated derivatives were separated and detected by gas chromatography using an Intersmat IGC-121 DC gas chromatograph (Suresnes, France) coupled to an Intersmat ICR-1B integrator and equipped with a 50 m capillary column (0.32 mm in diameter, Chrompack CP Sil 5 fused silica) as previously described (Draye et al., 1987).

In control and riboflavin-deficient rats, dodecanedioic acid (22 μmol/100 g body weight) was rapidly transformed into adipic, suberic and sebacic acids, which appeared in urine (Fig. 1). Excretion of succinic acid remained low and was not modified by the infusion. In control animals, this process lasted less than 2 h, and urinary dicarboxylic acids amounted to 28.6 ± 1.0% (mean ± S.E.M.; n = 4) of the dose administered. Over 60% of this excretion occurred within 30 min after the infusion period. In riboflavin-deficient rats, the process was slower and not complete 3 h after infusion; dicarboxylic acids in urine amounted to 75.6 ± 15.5% (n = 3) of the infused dose. In clofibrate-treated rats, only 4.4 ± 1.2% (n = 3) of dodecanedioic acid was recovered as shorter dicarboxylic acids in urine.

The maximal apparent velocity of dicarboxylate oxidation as calculated from the initial rate of appearance of medium-chain dicarboxylic acids in urine is similar in control and riboflavin-deficient animals. This rate of β-oxidation corresponds to the formation of 10.0 μmol of acetyl-CoA/30 min per 100 g body weight in controls, and 14.8 μmol/30 min per 100 g body weight in riboflavin-deficient rodents. The livers of the latter animals were 1.4 times larger than those of controls, when expressed as a percentage of body weight. In control rats the experiment was repeated with 11 and 44 μmol of dodecanedioic acid and, surprisingly, the same proportions of shorter dicarboxylic acids were recovered in urine (22.1 and 24.9%, respectively compared to 28.6%). Over 70% of the infused dodecanedioic acid was never recovered in urine, irrespective of dose, the rate of which is presently unknown.

Using the ferricyanide method of Osmundsen & Bremer (1977) we showed in preliminary experiments that there was no detectable oxidation by intact mitochondria from control rat liver of sebacyl-CoA in the presence of L-carnitine. The same acyl-CoA was a convenient substrate for the peroxi-

![Fig. 1. Urinary excretion of dicarboxylic acids in control, riboflavin-deficient and clofibrate-treated rats](image-url)

Dodecanedioic acid (DC12) was infused intravenously (22 μmol/100 g body weight) at a concentration of 5 mg/ml in 5% (w/v) bovine serum albumin in 0.15 M-NaCl during the period 0, as indicated by hatched areas. Urinary adipic, suberic and sebacic acids were quantified by gas chromatography.

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Inherited defects of mitochondrial fatty acid oxidation

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Introduction

During the last decade, several new inborn errors of mito-
chondrial and peroxisomal fatty acid oxidation have been
described. These inborn errors of metabolism are likely to be
much more common than has been recognized until now,
and they may present in a variety of different ways. This
review will be confined to the inborn errors of mitochon-
drial β-oxidation, and it will report the recent progress that
has been made in the study of these disorders. Progress in
understanding disorders of peroxisomal β-oxidation has
been described elsewhere (Fahimi & Sies, 1987).

Investigations of defects of fatty acid oxidation

The most important initial investigations to be performed
in patients with suspected defects of fatty acid oxidation are
the determination of the circulating blood concentrations of
intermediary metabolites and of urinary organic acids.
Hypoglycaemia, when associated with low blood ketone
body concentrations and normal or high non-esterified fatty
acid concentrations, strongly suggests that a defect of fatty
acid oxidation is likely to be present. A defect of mitochon-
drial β-oxidation is often associated with the excretion of
abnormal metabolites in the urine, and these metabolites can
be detected by analysing the urine by gas chromatography,
gas chromatography–mass spectrometry and high perform-
ance liquid chromatography. Thus, in the presence of a
defect of mitochondrial fatty acid oxidation, long-chain fatty
acids may be directed to α-oxidation which is followed by
peroxisomal β-oxidation to form dicarboxylic acids that are
subsequently excreted (Van Hoof et al., 1988). Moreover, the
accumulation of acyl-CoA esters in the mitochondrial
matrix is associated with the increased urinary excretion of
acylcarnitines and glycine conjugates owing to the action of
carnitine acyltransferases and glycine-N-acylase, respect-
ively. These metabolites can be of substantial diagnostic
value, thus for example, the presence of medium-chain di-
carboxylic acids, suberylglycine and octanoylcarnitine, are
very suggestive of medium-chain acyl-CoA dehydrogenase
deficiency (Bhuiyan et al., 1987). However, the urinary
excretion of abnormal metabolites can be extremely variable,
and some patients under non-stressed conditions may not
excrete any abnormal metabolites. It is imperative, therefore,
to obtain appropriate blood and urine samples when the
patient has a spontaneous episode of illness. If this is not pos-
sible, then samples should be collected during metabolic
stress induced by carefully controlled fasting in hospital. The
starvation test is potentially very dangerous, particularly in
children, and it should only be performed by individuals
experienced in metabolic investigations. The next step in the
investigation is to measure the flux through fatty acid oxida-
tion either radiochemically or spectrophotometrically using
cultured fibroblasts or tissues from the patient. If these in-
vestigations are abnormal, direct assay of the individual
enzymes of β-oxidation is required.

Enzyme defects

There are 15 enzymes involved in the β-oxidation of satu-
rated fatty acids and defects of seven of these have been
described to date (see below). No defects have yet been
reported in patients of carnitine/acylcarnitine translocase,
the enoyl-CoA hydratases, the hydroxyacyl-CoA dehydro-
genases, the long-chain 3-oxoacyl-CoA thiolase or of the
auxiliary enzymes required for the oxidation of polyunsatu-
rated fatty acids. Carnitine deficiency has been discussed
recently (Turnbull & Sherratt, 1985). The syndromes in
which enzyme deficiencies have been found will now be
discussed.