The key reaction in the β-oxidation of fatty acids is the acyl-CoA dehydrogenation, catalyzed by short chain, medium chain, and long chain acyl-CoA dehydrogenases. Acyl-CoA dehydrogenation reactions are also involved in the metabolism of the branched chain amino acids, where isovaleryl-CoA and 2-methylbutyryl-CoA dehydrogenases are involved and in the metabolism of lysine, 5-hydroxylysine and tryptophan, where glutaryl-CoA dehydrogenase functions. In all of these dehydrogenation systems reducing equivalents are transported to the main respiratory chain by electron transfer flavoprotein (ETF) and electron transfer flavoprotein dehydrogenase (ETFDH), which are common to all the dehydrogenation systems. The acyl-CoA dehydrogenation enzymes are dependent on flavin adenine dinucleotide (FAD) as coenzyme, for which riboflavin is the precursor. Patients with multiple acyl-CoA dehydrogenation deficiencies have been found in whom the defect has been located to ETF and/or ETFDH. A few patients with multiple acyl-CoA dehydrogenation deficiencies have been described, in whom no defects in acyl-CoA dehydrogenases, ETF or ETFDH have been found but who respond clinically and biochemically to pharmacological doses of riboflavin. This indicates a defect related to the metabolism of FAD. An uptake defect of riboflavin or a synthesis defect of FAD from riboflavin have been excluded by in vivo and in vitro studies. A mitochondrial transport defect of FAD or a defect in the binding FAD to ETF and/or ETFDH remains possible.

Multiple acyl-CoA dehydrogenation deficiency is the functional term for a group of human metabolic diseases characterised by defects in the oxidation of acyl-CoA esters in the fatty acid, branched chain amino acid, lysine, 5-hydroxylysine and tryptophan metabolism. The clinical picture can vary considerably from that reported in the first severe cases of the neonatal form (neonatal glutaric aciduria type II) (McKusick 23168), who exhibit serious metabolic disturbances (hypoglycaemia, metabolic acidosis, coma) leading to early death (Przyrembel et al., 1976; Gregersen et al., 1980; Goodman et al., 1980; Sweetman et al., 1980), to that in the later reported milder forms, from which the patients recover following symptomatic treatment (Mantagos et al., 1979; Dusheiko et al., 1979; Gregersen et al., 1982) after attacks of drowsiness (coma) accompanied by hypoglycaemia and acidosis.

Biochemically these diseases are characterised by the excretion of metabolites derived from accumulated acyl-CoA esters, i.e. C₆–C₁₀-dicarboxylic and hydroxymono- and dicarboxylic acids, ethylmalonic and methylsuccinic acids, free and glycine conjugated isobutyric, 2-methylbutyric, isovaleric, butyric, hexanoic and glutaric acids. Despite the qualitative similarity in the metabolic profiles, the quantitative pattern of metabolites is quite variable. In neonatal glutaric aciduria type II, the glutaric acid dominates the urinary metabolites, whereas the fatty acid derived metabolites dominate in some of the milder forms (Mantagos et al., 1979; Gregersen et al., 1982). Most certainly these differences in excretion pattern reflect the different molecular forms of the acyl-CoA dehydrogenation deficiencies, but it is also probable that they reflect an individual's ability to utilize alternative metabolic pathways for acyl-CoA esters (Gregersen et al., 1982; Gregersen, 1984).

THE ACYL-CoA DEHYDROGENATION COMPLEX

Acyl-CoA dehydrogenation is shown in Figure 1, where the dehydrogenation of hexanoyl-CoA is depicted as an example. The first enzyme involved is an acyl-CoA dehydrogenase, of which there are six. Two branched chain acyl-CoA dehydrogenases have been isolated.
dehydrogenase is unique because, in addition to length specificity (Furuta et al., 1981; Ikeda et al., 1983; Dommes and Kunau, 1984). Finally, glutaryl-CoA dehydrogenase is unique because, in addition to dehydrogenation, the enzyme also catalyzes the subsequent decarboxylation to crotonyl-CoA or the two enzymes' activities are closely integrated (Besrat et al., 1969; Noda et al., 1980).

The two other components of the dehydrogenation system, which transport the reducing equivalents to coenzyme Q in the respiratory chain (Figure 1), are electron transfer flavoprotein (ETF) and ETF dehydrogenase (ETFDH) (Hall and Kamin, 1975; Ruzicka and Beinert, 1977). ETF and ETFDH are common to all the dehydrogenation processes.

The acyl-CoA dehydrogenases, ETF and ETFDH, all require FAD as coenzyme. FAD is tightly bound in a non-covalent way to the apoenzymes. The $K_m$ values of rat liver acyl-CoA dehydrogenases for FAD are in the micromolar range (Ikeda et al., 1983) and the $K_m$ of human glutaryl-CoA dehydrogenase for FAD is approximately 5 $\mu$mol$^{-1}$ (Christensen and Brandt, 1978). The binding of FAD to ETF and ETFDH has not been measured experimentally, but it must be tight since FAD is not lost from ETF during purification procedures (Hall and Kamin, 1975; Ikeda and Tanaka, 1983), and the fact that ETF is not affected in riboflavin-deficient rats whereas the acyl-CoA dehydrogenase is decreased (Sakurai et al., 1982) indicates that FAD is more tightly bound to ETF than to the acyl-CoA dehydrogenases. As FAD remains bound to ETFDH to a considerable extent during purification procedures (Ruzicka and Beinert, 1977), it must also be tightly bound to this apoenzyme.

The binding of FAD to the apoenzyme determines the conformation of many holoflavoenzymes (Weimer and Neims, 1975) and may also stabilise the apoenzyme (Mapson and Isherwood, 1963; Massey, 1963). The presence of FAD in isolation buffers increases recoveries considerably (Dommes and Kunau, 1984); furthermore the activity of short chain acyl-CoA dehydrogenase in mitochondria isolated from riboflavin-deficient rats cannot be regained by the addition of FAD to the assay mixture (Sakurai et al., 1982) and indicates instability of the acyl-CoA dehydrogenase apoenzymes.

THE BIOSYNTHESIS OF FAD

The precursor of FAD is riboflavin (vitamin B$_2$) and normally supplied in the diet. The amounts required for maintenance of the body's metabolism and for compensating for urinary losses, is dependent on metabolic activity. The minimum requirement for adult man is approximately 1.7 mg day$^{-1}$ (Josko and Levy, 1975). A growing child aged 10–12 years should have a minimum intake of 1.75–2.00 mg day$^{-1}$, depending on the basis for the calculation (weight, calorie or protein intake). Pregnant women require approximately 0.3 mg more riboflavin per day than non-pregnant women (Josko and Levy, 1975). When given in pharmacological doses perorally the upper limit for absorption is about 25 mg per dose (Josko and Levy, 1975) and the unutilized vitamin is excreted during the following 8–24 h.

Following absorption riboflavin is converted first to flavin mononucleotide (FMN) by the cytosolic enzyme flavokinase (riboflavin kinase, EC 2.7.1.26) and then by the enzyme FAD pyrophosphorylase (flavin mononucleotide adenyltransferase, EC 2.7.7.2) to FAD (McCormick, 1975) which is then transported into the mitochondria by as yet unknown mechanisms.

RIBOFLAVIN-RESPONSIVE MULTIPLE ACYL-CoA DEHYDROGENATION DEFICIENCIES

The first patient with multiple acyl-CoA dehydrogenation deficiency which proved to be clinically and biochemically responsive to pharmacological doses of riboflavin, was a 3-year-old boy who was admitted to the hospital in a semicomatose condition. During the following days he developed a Reye-like syndrome with hypoglycaemia, metabolic acidosis and disturbances of consciousness (Gregersen et al., 1982). The liver was enlarged and liver aminotransferases and lactate dehydrogenase concentrations were elevated in the serum. He recovered rapidly after treatment with glucose and electrolytes. The urinary organic acids were characteristic of multiple acyl-CoA dehydrogenation deficiencies. Quantitatively, the metabolites derived from the acyl-CoA intermediates of fatty acid $\beta$-oxidation were the most abundant. After the acute attack the excretion rate of these metabolites fell to a lower, but constant, level. Controlled trials with oral riboflavin [100 mg three times per day, which is considerably above the absorption saturation level (see above)], resulted in dramatic improvement of the metabolic profile, and continuous riboflavin medication proved beneficial for the general development of the child (Gregersen et al., 1982). Rhead showed later that riboflavin-depleted cultured skin fibroblasts from the patient were more sensitive to the consequent FAD deficiency than control cells when fatty acid was oxidised, thus confirming the clinical riboflavin-response (Rhead and Fritchman, 1983).

A metabolic assessment after 3 years of riboflavin treatment showed that the excretion of acyl-CoA ester-derived metabolites has changed (Gregersen et al., unpublished results). Metabolites derived from the longer chain length acyl-CoA esters, i.e. the dicarboxylic acids and 5-hydroxycarboxylic acid, were excreted in lower amounts and excretion of metabolites derived from butyryl-CoA, i.e. ethylmalonic acid, had increased. This might indicate that the deficiency of $\beta$-oxidation has moved to a lower chain length, as is the case in the brother of the index case, where ethylmalonic acid is by far the most abundant metabolite (Gregersen et al., 1982). Another change during the 3 year period is that the excretion rate of free and esterified carnitine has decreased markedly, from 60 and 190 $\mu$mol (mmol creatinine)$^{-1}$ to 8 and 25 $\mu$mol (mmol creatinine)$^{-1}$, respectively. This is as low as control excretion (free carnitine: 3–22 $\mu$mol (mmol creatinine)$^{-1}$, esterified...