FIG. 2. Schematic representation of fatty acid oxidation. This metabolic pathway is divided into the carnitine cycle (A), the inner mitochondrial membrane system (B), and the mitochondrial matrix system (C). The carnitine cycle includes the plasma membrane transporter, carnitine palmitoyltransferase I (CPT I). The reactions shown in B occur in the inner mitochondrial membrane. The carnitine-acylcarnitine translocase system and carnitine palmitoyltransferase II (CPT II). The inner mitochondrial membrane system includes the very-long-chain acyl-CoA dehydrogenase (VLCAD) and the trifunctional protein with three catalytically active sites. Long-chain acylcarnitines enter the mitochondrial matrix by the action of CPT II to yield long-chain acyl-CoAs. These thioesters undergo one or more cycles of chain shortening catalyzed by the membrane-bound system. Chain-shortened acyl-CoAs are degraded further by the matrix beta-oxidation system. Medium-chain fatty acids enter the matrix beta-oxidation system directly and are activated to the medium-chain acyl-CoAs before degradation by the matrix beta-oxidation system. Tp, carnitine transporter; TL, carnitine-acylcarnitine translocase; LC, long chain; EH, 2-enoyl-CoA hydratase; CoA, coenzyme A; VLC, very long chain; AD, acyl-CoA dehydrogenase; AS, arginosuccinate; HD, 3-hydroxyacyl-CoA dehydrogenase; CPT, carnitine palmitoyltransferase; KT, 3-ketoacyl-CoA thiolase; MC, medium chain; SC, short chain. (From Ref. 8, with permission.)

Carnitine Palmitoyltransferase Deficiency

Mitochondria contain two CPT enzymes, CPT I and II, that are vital in the transport of LCFA into mitochondria. CPT I is located in the inner aspect of the outer mitochondrial membrane, whereas CPT II is bound to the inner aspects of the inner mitochondrial membrane (Fig. 2). CPT I deficiency presents in infancy as attacks of potentially fatal, fasting-induced, hypoketotic hypoglycemia. The hypoglycemic episodes manifest as lethargy, coma, and seizures. They can lead to psy-
chomotor developmental delay, hemiplegia, and generalized epilepsy. Myopathy is not a typical manifestation, and serum CK has been elevated in only two siblings (36); another patient had a lipid-storage myopathy (37). The diagnosis is confirmed by demonstrating decreased CPT I activity in cultured fibroblasts, leukocytes, or hepatocytes. CPT I activity is normal in skeletal muscle, accounting for the absence of clinical myopathy is probably due to tissue-specific isoforms. The first mutations in the CPT I gene have been identified in a patient with the hepatic form (37a).

In contrast, CPT II deficiency has variable clinical manifestations. Three forms of CPT II deficiency have been described: an infantile, late-infantile, and adult form. The early infantile phenotype is rare and presents at birth with severe hypoketotic hypoglycemia and generalized steatosis and can cause death in a few days. Multiple organ malformations are often present, including renal cystic dysplasia, nephromegaly, microgria, neuronal heterotopia in the brain, and facial dysmorphism. The late-infantile hepatomuscular form is clinically similar to CPT I deficiency, with acute episodic fasting hypoglycemia and hypoketosis, lethargy, coma, and death. Seizures, hepatomegaly, cardiomegaly, arrhythmias, and pancreatitis have also been described (5). In both infantile forms, CPT II activity is less than 10% of normal.

The adult form of CPT II deficiency was first described in 1973 and is a common cause of exercise-induced myoglobinuria (9,38). It typically presents in young adulthood with complaints of muscle pain and panniculitis after prolonged exercise. Severe bouts of rhabdomyolysis with myoglobinuria can cause acute renal failure. Some infants have presented with acute muscle breakdown induced by fever. Adult patients may also have rhabdomyolysis precipitated by fever or other stress. CPT activity is less than 30% of normal. CPT II is a homotetrameric enzyme and the gene (CPTI) encoding the subunit has been characterized. Missense, frameshift, and deletion mutations CPTI segregate with the clinical phenotypes. In the adult muscular form, the most common mutation is a C-to-T transversion at nucleotide (nt) 439, which changes a highly conserved serine to leucine (S113L) (39).

**Carnitine-Acylcarnitine Translocase Deficiency**

Rare patients with carnitine-acylcarnitine translocase deficiency (5) were first described clinically by Stanley et al. (41) in a young boy with stunted growth, recurrent vomiting, and coma from birth. At age 2.5 years, he had muscle weakness, cardiomyopathy, and fasting hypoglycemia. Hypoketosis, increased serum long-chain acylcarnitines, and normal CPT activity were later found. Carnitine-acylcarnitine translocase activity was less than 5% of normal control subjects.

**BETA-OXIDATION DEFECTS**

The breakdown of fatty acid in mitochondria requires two related systems: the inner mitochondrial membrane portion that metabolizes long-chain acyl-CoA and the mitochondrial matrix beta-oxidation spiral that acts on medium- and short-chain acyl-CoA (Fig. 2).

**Beta-Oxidation Defects of the Inner Mitochondrial Membrane System**

Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency presents in infancy with hypoketotic hypoglycemia, hepatic steatosis, cardiomyopathy, and elevated plasma levels of long-chain acylcarnitines (42). Metabolic acidosis, dicarboxylic aciduria, and increased serum CK with myoglobinuria have also been noted. Patients with recurrent myoglobinuria have the same clinical phenotype as those with CPT II deficiency. Immunoblot analyses for VLCAD have shown absence of the protein in fibroblasts from patients (5). The cDNA for the human VLCAD has been cloned, and a 105-base pair (bp) deletion in the gene has been identified in two unrelated infants (5).

Patients with defects of trifunctional protein have isolated long-chain-L-3-hydroxyacyl-coA dehydrogenase (LCHAD) deficiency, whereas a small number of individuals have a combined defect of its three enzyme components. The clinical feature of LCHAD deficiency include onset in infancy, Reyes-like episodes, hypoketotic hypoglycemia with hepatic dysfunction, progressive myopathy, recurrent myoglobinuria, cardiomyopathy, and sudden infant death syndrome (4,5). Jackson et al. (43) first reported the combined defect of the three functional enzyme activities in an infant with recurrent limb weakness, hypotonia, and anorexia precipitated by intermittent illnesses, who died at age 4.5 years during a severe metabolic crisis with an elevated serum CK, hyperammonemia, and lactic acidosis.

The treatment of patients with inner mitochondrial membrane defects of fatty acid metabolism is mainly dietary. Affected patients should avoid prolonged fasts and long-chain fatty acid ingestion. Intravenous glucose should be given during acute intermittent illnesses (4).

**Beta-Oxidation Defects of the Mitochondrial Matrix System**

The mitochondrial beta-oxidation matrix system shortens the fatty acid backbone of acyl-CoA by two carbon fragments during each turn through the beta-oxidation spiral. In this process, acetyl-CoA is produced and is oxidized in the Krebs cycle. In addition, electron transfer flavoprotein (ETF) is reduced; the reduced ETF provides reducing equivalents to the oxidative-phosphorylation pathway through the action of ETF CoQ oxidoreductase. Human diseases are caused by defects in several steps of this matrix system (Fig. 2).
Defects of long-chain, medium-chain, and short-chain acyl-CoA dehydrogenases generally occur in infancy. Medium-chain acyl-CoA dehydrogenase (MCAD) is, along with CPT II deficiency, the most frequent defect of beta-oxidation, with more than 200 identified patients and a disproportionately high incidence in Anglo-Saxon whites (44). MCAD typically begins in the first 2 years of life with fasting intolerance, nausea, vomiting, hypoketotic hypoglycemia, lethargy, and coma; however, clinical expression is variable and some patients are asymptomatic. MCAD activity in most tissues, including fibroblasts, lymphocytes, and liver, is low, generally 2% to 20% of normal. Early diagnosis and treatment can lead to a favorable outcome. Dietary therapy is aimed at avoidance of fasting and provision of adequate caloric intake. Most MCAD-deficient patients have an A-to-G transition mutation at nt 985 of the cDNA, causing a lysine-to-glutamate substitution at amino acid 304 of the mature protein, which leads to impaired homotrimeric assembly and instability of the protein (45,46). Long-chain acyl-CoA dehydrogenase deficiency is less common, and many reported patients instead have VLCAD deficiency. Short-chain acyl-CoA dehydrogenase deficiency has been documented in only a few patients with varying phenotypes, including one adult with progressive myopathy and massive lipid storage in type I muscle fibers and in several infants with failure-to-thrive and nonketotic hypoglycemia who died in early childhood (47,48).

Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency was identified in a 9-month-old child with episodes of hypoglycemia and Reyes-like encephalopathy (49). At age 16 years, she had an attack of hypoketotic hypoglycemia, acute myoglobinuria, and encephalopathy. She developed arrhythmia and died of a dilated cardiomyopathy. Deficiency of 2,4-dienoyl-CoA reductase was identified in muscle and liver of a dysmorphic infant with hypotonia who died at 4 months of age (50).

Multiple acyl-CoA dehydrogenase deficiency (MAD) or glutaric aciduria type II is a clinical syndrome characterized by metabolic acidosis, hypoketotic hypoglycemia, strong sweaty-feet odor, and early death. Three distinct clinical presentations of MAD deficiency exist: a severe neonatal form with congenital abnormalities, a severe neonatal form without congenital abnormalities, and a mild later-onset form (4,5). Pathology reveals fatty degeneration of kidney, liver, heart, and skeletal muscle. The biochemical abnormality is characterized by decreased activities of various acyl-CoA dehydrogenases with urinary excretion of large amounts of numerous organic acids. Three defects lead to MAD deficiency, namely, ETF deficiency, ETF CoQ oxidoreductase deficiency, and riboflavin (B2)-responsive MAD.

DEFECTS OF MYOADENYLATE DEAMINASE

In 1978, myoadenylate deaminase (mAMPD) deficiency was first described in association with exercise-related myalgia and cramps (51). At present, it is also detected in 1% to 3% of skeletal muscle biopsies in asymptomatic patients so studied (52). Fishbein (53) proposed two forms of mAMPD deficiency: primary or hereditary mAMPD deficiency characterized by myopathy, exercise intolerance, myalgias, and cramps with negligible or less than 1% of normal residual activity and lack of cross-reactive material in muscle, and secondary or acquired mAMPD deficiency, associated with other well-defined neuromuscular disorders, higher residual enzyme activity, and detectable cross-reactive material. In the forearm ischemic exercise test, affected patients show a normal elevation of venous lactate, without a rise in ammonia and inosine monophosphate, the products of the mAMPD reaction.

DEFECTS OF OXIDATIVE-PHOSPHORYLATION

Since the initial discovery of mitochondrial DNA mutations in 1988, our understanding of the mitochondrial encephalomyopathies has advanced at an astonishingly rapid pace. This topic is reviewed extensively elsewhere. In this section, we illustrate some fundamental clinical and scientific themes.

Mitochondrial respiratory chain disorders often do not conform to single enzyme defects. The measured enzyme activities may be normal or multiple enzymes can be affected; therefore, a biochemical classification system can have limitations. Molecular genetics provides an alternative perspective of mitochondrial diseases. One can gain a better understanding of mitochondrial disorders by considering the several unusual genetic characteristics of mitochondria. They are unique organelles because they possess their own genetic material, mitochondrial DNA (mtDNA), which is a small circular molecule of 16.5 kilobases (kb) (58). Each mtDNA encodes 22 transfer RNAs, 13 polypeptides, and two ribosomal RNAs. The mtDNA-encoded polypeptides are functionally important because they are subunits of the respiratory chain. Most mitochondrial proteins are encoded in the nuclear DNA (nDNA); thus, mitochondria are the products of two genomes. Defects in either genome can cause mitochondrial dysfunction. To date, most respiratory chain defects characterized at the molecular genetic level are due to mtDNA mutations (59).

Four important characteristics of mtDNA contribute to the expression of a given mt gene defect. The first is heteroplasmacy. Each mitochondrion contains 2 to 10 copies of mtDNA, and in turn, each cell contains multiple mitochondria. Therefore, there are numerous copies of mtDNA in each cell. Mutations of mtDNA may be present in some mtDNA molecules (heteroplasmacy) or in all molecules (homoplasmacy). As a consequence of heteroplasmacy, the proportion of a deleterious mtDNA mutation can vary widely. An individual that harbors a large proportion of mutant mtDNA will be more severely affected.