Inborn Errors of the Metabolism of Branched-Chain Amino Acids

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METABOLIC PATHWAYS OF BRANCHED-CHAIN AMINO ACIDS

The three essential neutral branched-chain amino acids, L-leucine, L-valine, and L-isoleucine, are unique among the amino acids in that they undergo oxidation to a greater extent in the peripheral tissues than in the liver. Transamination is the initial metabolic step, resulting in branched-chain 2-oxo acids. Hypervalinemia and hyperleucine-isoleucinemia have been identified as disorders of branched-chain amino acid transamination in extremely rare cases. The next step is an irreversible oxidative decarboxylation to the corresponding acyl-CoA derivatives. All three branched-chain 2-oxo acids are decarboxylated by a single mitochondrial multienzyme complex, called branched-chain 2-oxo-acid dehydrogenase.

This multienzyme complex resembles pyruvate and 2-oxoglutarate dehydrogenase complexes in composition (three catalytic components: E₁ = branched-chain 2-oxo acid decarboxylase, E₂ = branched-chain acyltransferase, and E₃ = dihydrolipoyl dehydrogenase), cofactor requirement, and regulation. The branched-chain 2-oxo acid dehydrogenase complex activity is deficient in patients with maple syrup urine disease (branched-chain ketoaciduria, leucinosis). Immunologic and recombinant DNA techniques have shown that the E₁ and eventually the E₂ component of the complex are affected (1,2). A deficiency of E₃, which is a common catalytic subunit for all three 2-oxo-acid dehydrogenase complexes, produces a syndrome with features of congenital lactic acidosis, branched-chain ketoaciduria, and 2-oxoglutaric aciduria (3).

Leucine Metabolism

Leucine is further metabolized to acetoacetate and acetyl-CoA and hence into the Krebs cycle. Specific enzymes deficiencies at every stage of this metabolic pathway
FIG. 1. Metabolism of branched-chain amino acids showing the position of the known inherited metabolic disorders. 1, BCAA decarboxylase; 2, isovaleryl-CoA dehydrogenase (FAD-ETF) (isovaleric aciduria); 3, 3-methylcrotonyl-CoA carboxylase; 4, 3-methylglutaconyl-CoA hydratase; 5, 3-hydroxy-3-methylglutaryl-CoA lyase; 6, 2-methylacetacetyl-CoA thiolase; 7, 3-hydroxisobutyryl-CoA deacylase; 8, methylmalonyl-semialdehyde dehydrogenase; 9, propionyl-CoA carboxylase (biotin, propionic aciduria); 10, methylmalonyl-CoA racemase and mutase (B12) (methylmalonic aciduria); 11, malonyl-CoA decarboxylase. From Ogier H, et al. (13).

are known. Isovaleryl-CoA produced by the oxidative decarboxylation step of leucine is metabolized by a specific mitochondrial FAD-ETF-dependent dehydrogenase to 3-methylcrotonyl-CoA (Fig. 1, step 2). A defect in this dehydrogenase activity may occur secondary to apoenzyme mutation (isovaleric acidemia) or to FAD-ETF system dysfunction (glutaric aciduria type II).

3-Methylcrotonyl-CoA is carboxylated by a specific biotin-dependent acyl-CoA carboxylase (BMCC) to form 3-methylglutaconyl-CoA (step 3). Deficient activity of this enzyme leads to 3-methylcrotonylglycinuria. Variants are known, including defects in biotin metabolism that are responsible for multiple carboxylase deficiency (MCD). Isolated and biotin-resistant BMCC deficiency is much less common than that of MCD.

3-Hydroxy 3-methylglutaconyl-CoA is metabolized to 3-hydroxy-3-methylglutaral-CoA by 3-methylglutaconyl-CoA hydratase (step 4). Defective activity leads to 3-methylglutaconic aciduria. However, there are at least two distinct disorders among patients affected with 3-CH₃-glutaconic aciduria, as most of them display a
normal hydratase activity in fibroblasts. These diseases responsible for major developmental delay are not amenable to treatment (see Duran et al., this volume).

3-Methylglutaryl-CoA is converted to acetoacetate and acetyl-CoA by a specific lyase (HMG-CoA lyase) (step 5). In addition to its role in leucine degradation, HMG CoA lyase is involved in the cycle of ketogenesis, which explains most of the clinical manifestations of this enzyme deficiency (see Bartlett et al., this volume).

**Isoleucine/Valine Metabolism: The Propionyl-CoA Pathway**

The metabolism of valine and isoleucine is of particular interest since both are major precursors of propionyl-CoA and methylmalonyl-CoA. A deficiency of L-valine catabolism at the step of 3-hydroxyisobutyryl-CoA deacylase (Fig. 1, step 7) and methylmalonic semialdehyde dehydrogenase (Fig. 1, step 8), respectively, have been observed in only one and two patients (see Duran et al., this volume). In addition, two patients with malonyl-CoA decarboxylase deficiency (step 11) have been described. The latter enzyme serves an important function in the mitochondria by preventing accumulation of malonyl-CoA from acetyl-CoA through propionyl-CoA carboxylase (4).

The final unique step in the metabolism of L-isoleucine involves the ketolysis process at the cleavage of 2-methylacetocetyl-CoA to acetyl-CoA and propionyl-CoA (step 6). L-Valine is also metabolized ultimately to propionyl-CoA and thus these two BCAA form the major precursors of propionyl-CoA. Other amino acid precursors, including threonine and methionine, are also metabolized to this intermediate via 2-oxobutyryl-CoA (Fig. 2). The β-oxidation of fatty acids containing odd numbers of carbon, which are minor components of dietary fats and body lipids, yield propionyl-CoA, too. Through the peroxisomal β-oxidation, the side chain of cholesterol is also a minor precursor of propionate. Finally, the potential importance of propionate synthesis by gut bacteria is of interest in disorders of propionate metabolism (5).

Propionyl-CoA is carboxylated to α-methylmalonyl-CoA by a mitochondrial biotin-dependent carboxylase enzyme (step 9). This carboxylation is readily reversible. It is also of interest that either α-butyryl-CoA or acetyl-CoA can substitute for propionyl-CoA to yield ethylmalonyl-CoA or malonyl-CoA, respectively. A defect in propionyl-CoA carboxylase activity results in propionyl-CoA accumulation and hence propionic acidemia. Propionyl-CoA accumulation also occurs in MCD due to defective activities of all three biotin-dependent carboxylases.

α-Methylmalonyl-CoA is converted by methylmalonyl-CoA racemase into the L-isomer, which in turn is converted into succinyl-CoA by the vitamin B12-dependent methylmalonyl-CoA mutase (step 10). The succinyl-CoA subsequently enters the Krebs cycle. Deficient activity of the apomutase enzyme leads to methylmalonic aciduria, and because of the requirement by the apomutase for adenosylcobalamin, abnormal B12 metabolism leads to variant forms of methylmalonic aciduria.
FIG. 2. Metabolism of propionyl-CoA: major alternative pathways in case of propionyl-CoA accumulation. 1, acetyl-CoA carboxylase; 2, β- or ω-oxidation; 3, citrate synthase; 4, acetyl-CoA and propionyl-CoA condensation and reduction; 5–6, glycine-N acylase and carnitine-N acylase. (From Ogier H, et al. [13]).

In this chapter we consider only the four main inborn errors of BCAA: MSUD, IVA, PA, and MMA.

METABOLIC CONSEQUENCES OF INBORN ERRORS OF BRANCHED-CHAIN AMINO ACIDS

Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is due to an inherited deficiency of the branched-chain 2-oxo acid dehydrogenase activity, affecting the metabolism of all three branched-chain 2-oxo amino acids. This defect produces marked increases in plasma, urinary, and CSF branched-chain amino acids (3). Smaller amounts of the respective 2-hydroxy acids, especially of 2-hydroxyisovaleric acid, are formed by reduction of the oxo acids. L-Alloisoleucine is constantly found in blood from MSUD patients. This compound is endogenously formed from (3S)2-oxo-3-methyl-n-valeric acid through nonenzymic racemization to (3R)2-oxo-3-methyl-n-valeric acid and further transamination to L-alloisoleucine (3).

The leucine/2-oxoisocaproic acid pair is the most toxic of the branched-chain metabolites. Probably 2-oxoisocaproic acid is the main neurotoxic substance. In maple
syrup urine disease, leucine and 2-oxoisocaproate are always present in plasma in about equimolar concentrations (6,7). Valine and isoleucine are of less clinical significance.

**Isovaleric Aciduria**

Isovaleric aciduria is the first organic aciduria described using GC-MS. It is caused by a defect in isovaleryl-CoA dehydrogenase (IVCoA-DH), leading to an accumulation of isovaleryl-CoA (IVCoA) and its by-products. Biochemically, the disease is characterized by a greatly increased excretion in the urine of N-isovalerylglucine (IVG) and 3-hydroxyisovaleric acid (3OHIVA), which are diagnostic. The concentration of free isovaleric acid (IVA) is usually increased in both blood and urine, but normal levels have been reported. In this disorder, the majority of the accumulating IVCoA is conjugated with glycine to IVG by the action of glycine N-acylase, which shows a high affinity for the IVCoA as a substrate (8). IVG thus formed is rapidly excreted in the urine (9). IVCoA accumulation also favors isovaleryl carnitine (IVC) synthesis by carnitine N-acylase, which leads to high IVC urinary excretion (10). Considering the treatment of this condition, these two alternative pathways are of interest as they allow the transformation of the highly neurotoxic IVA into nontoxic by-products with a high renal clearance.

**Propionic Acidemia**

Isolated propionic acidemia (PA) is secondary to a defect in the synthesis or structure of the apoenzyme propionyl-CoA carboxylase (PCC). The enzyme has the structure $\alpha_4\beta_4$, with the alpha chain containing a biotin prosthetic group. Irrespective of the clinical phenotype, severe reduction but not complete absence of PCC activity (1–5%) has been found in cultured skin fibroblasts of all patients. Two complementation groups have been demonstrated, pcc A and pcc BC, the latter further divided into two subgroups pccB and pccC, which showed intragenic complementation. At the molecular level, pcc A and pcc BC correspond to the genes named PCCA and PCCB coding for the alpha and beta subunits of PCC, respectively. The $\beta$ chain is unstable in the absence of the alpha chain (11,12). These data account for the heterozygote expression in PCC deficiency. Mean PCC activity in fibroblast extracts or peripheral blood leukocytes from pcc A heterozygotes is 50% of that in controls. This contrasts with PCC activity in pcc C heterozygotes, which is indistinguishable from that of controls, the latter data being consistent with compensatory balancing rates of synthesis and degradation for the two subunits in pcc C heterozygotes. Theoretically, beside the common biotin-nonresponsive PA, and independently of combined carboxylase deficiency, a biotin-responsive form of PA may exist. However, the first reports are confusing and its existence remains uncertain.

Biochemically, PA is characterized by greatly increased concentrations of free propionate in blood and urine. However, this major sign may fail and the diagnosis
is based on multiple organic acid by-products present in the urine. Methylcitrate and 3-hydroxypropionate are major diagnosis metabolites. The latter product is formed either by β- or ω-oxidation of propionyl-CoA. Methylcitrate arises via condensation of propionyl-CoA with oxaloacetate by the action of citrate synthase. Otherwise, organic aciduria could include low levels of tiglylglycine, tiglic acid, and propionylglycine. During ketotic episodes, 3-hydroxyvaleric acid is formed via propionyl-CoA and acetyl-CoA condensation and reduction. Some lactate, 3-hydroxybutyrate, methylmalonate, 2-methyl-3-hydroxybutyrate, and several other organic acids may be present (13).

**Methylmalonic Acidemia**

Deficient activity of methylmalonyl-CoA apomutase and, because of the requirement by the apomutase for adenosyl cobalamin (AdoCbl), defects at any step of AdoCbl metabolism, can lead to methylmalonic acidemia (MMA). Nine classes of MMA are defined on the basis of complementation studies. Approximately one half of patients have a mutase apoenzyme defect further divided into mut+ and mut− groups. Mut+ group is B12 nonresponsive both in vivo and in vitro and CRM − . Mut− group is usually B12 nonresponsive in vivo, partially responsive in vitro and CRM + . It corresponds to a defective coenzyme-apoenzyme affinity. Recently, the human mutase gene has been cloned and assigned to chromosome 6 and the first molecular characterization of normal and mutant mutase are henceforth available (14). The remaining patients are cobalamin variants. Among them, Cbl A and Cbl B types implicate AdoCbl synthesis. CblA is due to a defect in mitochondrial cobalamin reductase and CblB to defective AdoCbl transferase. All Cbl A patients and 40% of Cbl B are B12 responsive. Cbl C, D, and F are characterized by reduced function of both methylmalonyl-CoA mutase and methionine synthase, resulting in combined methylmalonic aciduria and homocystinemia. Cbl C and D are due to a defect in cytoplasmic reduction of Cob(III)alamin. Most but not all patients are B12 responsive in vivo. The Cbl F is due to abnormal lysosomal release of cobalamin (15). Only one patient has been described with this defect.

Biochemically, impairment of mutase activity leads to accumulation of methylmalonyl-CoA and secondary propionyl-CoA, which is reflected by the presence in blood and urine of greatly increased amounts of MMA and PA. Propionyl-CoA metabolites such as methylcitrate, 3-hydroxypropionate, and 3-hydroxyisovalerate are usually found in the urine. Vitamin B12 deficiency must be excluded when excessive amounts of urinary MMA are found, even more so in infants who are breast-fed by a mother who is either a strict vegetarian (16) or is suffering from subclinical pernicious anemia. Some patients without B12 deficiency have also been found (especially in the neonatal period) with methylmalonic aciduria 10–50 times the normal level. However, they are “well babies,” and no confusion with the true congenital MMA should occur if the proper analytical procedures of organic aciduria are employed.
Secondary Metabolic Disorders Common to PA and MMA

Propionyl-CoA and its metabolites are known to produce a variety of metabolic disturbances which have major effects on intermediary metabolism (inhibition of citrate synthase, pyruvate dehydrogenase complex, N-acetylglutamate synthetase, the glycine cleavage system, and pyruvate carboxylase). These inhibitions may explain some clinical features common to both disorders, such as hypoglycemia, mild hyperlactacidemia, hyperammonemia, and hyperglycinemia (13).

Patients affected with PA or MMA have increased acylcarnitines in blood and urine in which propionylcarnitine is the major metabolite. Thus a relative insufficiency of L-carnitine may occur in a state of continual propionyl-CoA accumulation.

Odd-carbon number fatty acids are precursors of propionyl-CoA, but propionyl-CoA can replace acetyl-CoA or n-butyryl-CoA as “primer” for de novo long chain fatty acid synthesis leading to the formation of odd-numbered fatty acids via malonyl-CoA and ethylmalonyl-CoA. By competition with malonyl-CoA, methylmalonyl-CoA is responsible for the accumulation of methyl-branched long chain fatty acids. This secondary effect has recently been studied in erythrocytes and reported as a potentially useful means for long-term assessment of these disorders (17).

CLINICAL PRESENTATION

Children with MSUD, IVA, PA, and MMA have in common many clinical and biochemical symptoms, which can be divided into three schematic presentations: a severe neonatal onset form, with metabolic distress; an intermittent late onset form; and a chronic progressive form presenting as hypotonia, failure to thrive, and developmental delay. In addition to these three presentations, the prospective data gathered by the Massachusetts metabolic disorders newborn screening program and the systematic screening of siblings have demonstrated the relative frequency of asymptomatic forms mainly for MMA (18). Most of the severe neonatal MMA forms belong to mut8 class and are B12 unresponsive in vivo. Many late onset forms belong to CbLA or CbLB classes. There is an extremely rare thiamin-responsive MSUD which is always present with a late onset form (19).

Severe Neonatal Onset Form

The general presentation of this form can be summarized as a neurological distress “intoxication type” with ketoacidosis and belongs to type II of the classification of the neonatal inborn errors of metabolism (20) (Tables 1 and 2). An extremely evocative clinical setting is that of a full-term baby born after a normal pregnancy and delivery who, after an initial symptom-free period, undergoes a relentless deterioration that has no apparent cause and is unresponsive to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of metabolic block, and is not necessarily correlated to the
protein content of the feeding. Typically, the first reported sign is poor sucking and diminished feeding, after which the child sinks into an unexplained coma despite supportive measures. At a more advanced stage, neurovegetative problems with respiratory distress, hiccup, apneas, bradycardia, and hypothermia may appear. In the comatose state, most patients have characteristic changes in muscle tone and involuntary movements. Generalized hypertonic episodes with opisthotonus are frequent, and boxing or pedaling movements as well as slow limb elevations, spontaneously or upon stimulation, are observed. Another suggestive neurological pattern

### TABLE 1. Five neonatal types of inherited metabolic distress

<table>
<thead>
<tr>
<th>Type</th>
<th>Clinical symptoms</th>
<th>Acidosis</th>
<th>Ketosis</th>
<th>Hyperlact-acidemia</th>
<th>Hyperamonemia</th>
<th>Most frequent diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Neurological distress</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>Maple syrup urine disease</td>
</tr>
<tr>
<td>II</td>
<td>Neurological distress</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>Organic acidurias</td>
</tr>
<tr>
<td>III</td>
<td>Neurological distress</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>Congenital lactic acidemias</td>
</tr>
<tr>
<td>IVA</td>
<td>Neurological distress</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Urea cycle defects</td>
</tr>
<tr>
<td>IVB</td>
<td>Neurological distress</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Nonketotic hyperglycemia, sulfite oxidase deficiency, peroxisomal disorders, respiratory chain defects</td>
</tr>
<tr>
<td>V</td>
<td>Hepatomegaly, liver dysfunction, seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>Gluconeogenesis defects, galactosemia, tyrosinemia type I, α1-antitrypsin deficiency</td>
</tr>
</tbody>
</table>

### TABLE 2. Clinical presentation before treatment in 69 neonates*

<table>
<thead>
<tr>
<th></th>
<th>MSUD (n = 26)</th>
<th>MMA (n = 18)</th>
<th>PA (n = 15)</th>
<th>IVA (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom-free period (days)</td>
<td>5</td>
<td>2.9</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Feeding refusal</td>
<td>100</td>
<td>61</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Coma</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hypertonia and abnormal movements</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>90</td>
</tr>
<tr>
<td>Ketosis</td>
<td>100</td>
<td>83</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>Dehydration</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Acidosis</td>
<td>0</td>
<td>94</td>
<td>87</td>
<td>90</td>
</tr>
<tr>
<td>Hyperammonemomia</td>
<td>19</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>0</td>
<td>72</td>
<td>73</td>
<td>70</td>
</tr>
</tbody>
</table>

* Data are expressed as the percentage of n, the total number of patients.
is axial hypotonia and limb hypertonia with large amplitude tremors and myoclonic jerks which are often mistaken for convulsions. In contrast, true convulsions occur late and inconsistently. The EEG often shows a periodic pattern in which bursts of intense activity alternate with nearly flat segments.

Dehydration is a frequent finding in propionic and methylmalonic acidemia. A moderate hepatomegaly may be observed in IVA, PA, and MMA. Sometimes, the importance of vomiting associated with abdominal distension and constipation may suggest gastrointestinal abnormalities such as pyloric stenosis or intestinal obstruction. In isovaleric acidemia, a strong “sweaty feet” odor of urine and skin is constantly present in sick neonates as well as in late onset acute episodes. In MSUD concomitantly with the onset of neurologic symptoms the infants start emitting an intensive (sweet, caramel-like) maple syrup–like odor.

In IVA, PA, and MMA, laboratory abnormalities include metabolic acidosis (pH < 7.30) with increased anion gap, associated with ketonuria (Acetest 2 to 3+ when it is checked before, or early at the beginning of intravenous glucose infusion). However, ketoacidosis can be moderate and transient and is often responsive to symptomatic therapy. Hyperammonemia is a constant finding. When it is very high (>800 μmol/liter) it can induce a respiratory alkalosis and lead to the erroneous diagnosis of a urea cycle defect. Moderate hypocalcemia (<1.7 mmol/liter) and hyperlactacidemia (3–6 mmol/liter) are frequent symptoms. The physician should be wary of attributing marked neurological dysfunction merely to these findings. Blood glucose can be reduced or elevated. In some patients it may reach 20 mmol/liter or more before glucose administration. If associated with glycosuria, ketoacidosis, and dehydration, it may mimic insulin-dependent diabetes. Neutropenia, thrombocytopenia, nonregenerative macrocytic anemia, and pancytopenia are other frequent findings and often responsible for confusion with sepsis.

The final diagnosis is made by identifying specific abnormal metabolites by GLC-MS of blood and urine. Free carnitine plasma levels are constantly low, with abnormal excretion of specific acylcarnitine. By contrast, plasma and urine amino acid chromatography are often normal or may show a non specific profile such as a slight increase in glycine.

In MSUD, the dinitrophenylhydrazine test is strongly positive, whereas urine tests for acetone may be negative. None of our patients with maple syrup urine disease had an initial blood pH less than 7.30 (20). The diagnosis is confirmed by serum amino acid chromatography, which displays an elevation of the branched chain amino acids leucine (usually higher than 2 mmol/liter), valine, isoleucine, and the presence of alloisoleucine (3).

**Intermittent Late Onset Form**

In approximately one third of the patients, the disease presents with a late onset after a symptom-free period commonly longer than 1 year, or even in adolescence or adulthood. Recurrent attacks are frequent and in between the child may seem
entirely normal. This makes the diagnosis difficult if adequate investigations have not been performed during the acute attack itself. Onset of acute disease may be precipitated by an infection or even severe constipation. Excessive protein intake, and all conditions that enhance protein catabolism, may exacerbate such decompensations. However, sometimes no overt cause is found. Recurrent attacks of coma and lethargy with ataxia are the main presentations of these late onset acute forms. The most frequent variety of coma is that presenting with ketoacidosis accompanied by low, normal, or high blood glucose levels, the latter condition mimicking insulin-dependent diabetes. Exceptionally, ketosis may be absent. Hyperammonemia is rarely encountered in attacks of ketoacidosis that occur later in infancy or childhood. Unlike patients with PA and MMA, patients with IVA are mostly normoglycinemic. Although most recurrent comas are not accompanied by neurological signs, we have observed PA, MMA, and MSUD patients who presented with acute hemiplegia and hemianopsia mimicking a cerebrovascular accident or cerebral tumor. A few patients with MMA developed acute extrapyramidal disease and corticospinal tract involvement after metabolic decompensation. The neurologic findings resulted from bilateral destruction of the globus pallidus with variable involvement of the internal capsule. This complication is not related to a specific gene defect, as it was observed irrespective of mutant class type and in vivo cobalamin responsivity (21). Cerebellar hemorrhage has also been observed in IVA, PA and MMA patients. These disorders must therefore be considered in the diagnostic list of metabolic strokes after respiratory chain disorders and urea cycle defects.

These apparent initial manifestations have frequently been preceded by other premonitory symptoms which had been missed or misdiagnosed. These symptoms include acute ataxia, unexplained episodes of dehydration, persistent anorexia, chronic vomiting associated with failure to thrive, hypotonia, and progressive developmental delay. Severe hematological manifestations are frequent in IVA, PA, and MMA, mostly concomitant with ketoacidosis and coma, sometimes as the presenting problem. Neutropenia is regularly observed in both neonatal and late onset forms of IVA, PA, and MMA. Thrombocytopenia occurs only in infancy and anemia only in the neonatal period. Recurrent infections are common and there appears to be a special relationship with Candida. Chronic mucocutaneous candidiasis is frequent and reflects the clinical status of the patient. Toxins and metabolites might suppress the cellular immune system (22). Some children with frequent infections sustain a concurrent mild generalized hypogammaglobulinemia which improves when metabolic control is achieved (23).

### Chronic Progressive Forms

Persistent anorexia, chronic vomiting, failure to thrive, and osteoporosis are frequent revealing signs. This “digestive” presentation is easily mistaken for cow’s milk protein intolerance, celiac disease, late onset chronic pyloric stenosis, or fructose intolerance, particularly as these symptoms start after weaning and diversifi-
cation of food. The anorexia is a prominent feature of these disorders. Some patients present with severe hypotonia, muscular weakness, and poor muscle mass and can simulate congenital or metabolic myopathies. Cardiomyopathy has been observed in PA and MMA. As plasma carnitine levels are severely lowered, as in IVA (24), the diagnosis of idiopathic systemic or muscular carnitine deficiency must be questioned in such patients. Nonspecific developmental delay and progressive psychomotor retardation as well as seizures can also be observed during the course of the disease. However, these rather unspecific symptoms are rarely observed as the only presenting symptom.

Complications

Skin Disorders

We have observed patients (with PA and with MMA) who presented a generalized staphyloccal cutaneous epidermolysis concomitant with an ultimate metabolic decompensation leading to death despite supportive measures. Rajhore described severe skin lesions with vesicobullous and eczematous eruption in a PA patient. Complete healing of the skin was accomplished within a week by daily infusions of fresh frozen plasma (25).

Renal Complications

With early diagnosis and appropriate treatment, an increasing number of patients with the most severe forms of MMA are surviving longer. As a result, long-term complications are becoming apparent. Chronic renal impairment in MMA is becoming increasingly recognized, although only a few cases have been documented (26). However, in some patients affected with the subacute form, renal complications can be the presenting symptom. A transient tubular dysfunction presenting as a pseudocystinuriasinuria is also frequently observed in the severe neonatal form of PA, whereas it is not present in IVA and MMA (27). Acute pancreatitis have been recently described in MMA, PA, IVA, and MSUD (34).

TREATMENT AND PROGNOSIS

Over the past three decades, several hundred patients have been observed. Evidence is accumulating that the central nervous system dysfunction often associated with such organic acidurias can be prevented by early diagnosis, emergency treatment, and then by compliance to the restricted diet. This aspect is important in view of a more radical therapeutic development such as liver transplantation, which represents a real hope for the most severe cases of PA and MMA, who have to deal with recurrent life-threatening episodes of metabolic acidosis.

As discussed earlier in this chapter, patients affected with BCAA disorders are
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divided into neonatal onset forms and late onset forms. This heterogeneity of manifestation is reflected in the variety of therapeutic management strategies. Neonatal onset forms require early toxin removal. Thereafter, the restricted food pattern, essential to limit formation of organic acid by-products, is applied to survivors of the newborn period as well as to the patients affected with the late onset form. In both, the prevention and early treatment of recurrent episodes of metabolic imbalance is crucial. Each of these recurrent episodes is life-threatening and parents must be taught to recognize early warning signs and have an immediate plan for intervention.

The emergency management of BCAA disorders in the neonate has two main goals: toxin removal and promotion of anabolism. Toxin removal is achieved with blood exchange transfusions and peritoneal dialysis in IVA, PA, and MSUD; and with hydration and exchange transfusions in MMA (28). Additionally, thiamine 50 mg/day in MSUD, glycine 500 mg/kg-day in IVA, biotin in PA, and vitamin B12 in MMA should be tried in all cases, although the neonatal forms of these defects are rarely vitamin responsive. L-Carnitine (200 mg/kg) is systematically given in IVA, PA, and MMA. Additional treatment such as insulin or growth hormone may be considered. Anabolism is met by early effective continuous enteral nutrition with a protein-free diet. A special amino acid mixture free of precursors is added to the formula immediately in MSUD and as soon as ammonia levels are below 80 μmol/liter in IVA, PA, and MMA (3,13,20,28).

Long-term dietary treatment is aimed at reduction of toxic metabolites accumulated, in parallel with maintenance of normal development, nutrition status, and prevention of catabolism. In IVA, leucine intake can be increased up to 800 mg/day during the first year and then most of children can tolerate 20–30 g/day of vegetable protein if associated with oral l-glycine and l-carnitine therapy. In most PA and MMA early onset forms, the intake of valine must be severely restricted to 250–500 mg/day for the first 3 years of life, subsequently slowly increased to 600–800 mg/day by the age of 6–8 years. Supplementation with a synthetic amino acid mixture containing none of the amino acid precursors is generally recommended, although still controversial. In general, these infants are severely anorexic and the diet must be totally delivered through a nocturnal gastric drip feeding using a peristaltic pump. Long-term carnitine treatment may be considered. Metronidazole has recently been found to be effective in reducing excretion of propionate metabolites because of its activity against gut anaerobic bacteria (29). In MSUD, the BCAA intake has to be adjusted according to their plasma levels, which have to be monitored several times a week during the first weeks of life; later in life the intervals can be prolonged to 2–4 weeks. Leucine blood levels ranging from 0.1 to 0.5 mmol/liter are sufficient for normal growth and appear to prevent neurologic damage. In our experience, these levels can be achieved in most of the severe neonatal forms with a daily leucine intake ranging 400–600 mg/day during the first 5 years of life. There is, however, a considerable interindividual variation of the BCAA requirements during the first 3 months of life.

The long-term outcome of classic maple syrup urine disease under dietary therapy
is still uncertain as both mental retardation and cerebral palsy are common. The outcome depends on early diagnosis and management. However, the most recent reviews (1,12,22,30–32) suggest that normal somatic growth and normal psychomotor development can be achieved if early diagnosis and treatment, subsequent long-term management, and meticulous attention to catabolic states accompanying even minor illnesses are guaranteed. A reasonable number of children with classic maple syrup urine disease are now developing normally and are performing well in the early elementary school grades (30).

Most of the late onset forms are easier to manage, patients tolerate up to 1.5–2 g/kg-day of protein, and amino acid mixtures are no longer necessary. In all CblA and 40% of CblB patients, hydroxocobalamin at a dose of 1 mg IM/day is very efficient. Some patients have gradually interrupted chronic B12 therapy without apparent discomfort. In MSUD, since some variants may respond to thiamin (19), a prolonged trial with supraphysiologic amounts of thiamin (20–200 mg/day) may be given, although additional treatment, such as a reduction of dietary branched-chain amino acids, must not be omitted (3). These late onset forms, as well as the vitamin-responsive forms, have an excellent long-term prognosis, although they may decompensate at any age and in unpredictable situations (33).

GENETIC COUNSELLING

All forms of MSUD, IVA, PA, and MMA are autosomal recessive disorders. The true incidences are estimated about 1/50,000 for MMA, 1/100,000 for PA and MSUD, and 1/200,000 for IVA. All forms of MSUD, IVA, PA, and MMA can be diagnosed early in pregnancy through the measurement of the defective enzyme activity in uncultured chorionic villi, and for IVA, PA, and MMA, through the direct measurement of abnormal metabolites accumulated in amniotic fluid as early as week 12 of gestation (3,13).

REFERENCES

DISCUSSION

**Dr. Wendel:** You told us that there is no hyperlactacidemia in neurological distress "intoxication type II" (organic acidurias). In some cases there is lactic acidosis.

**Dr. Saudubray:** Yes, you are right. I tried in this presentation to emphasize the leading symptoms. The problem is to find the leading symptom and to choose from among the constellation of signs the best hallmark of the disorder. Lactic acidosis is not a leading symptom when you have this clinical presentation with ketosis, metabolic acidosis, and hyperammonemia.

**Dr. Casaei:** I should like to ask whether focal clinical signs, secondary to brain edema during metabolic deterioration, are seen more frequently in younger infants than in older infants and children. The reason I ask this is that there is some clinical evidence that brain edema can remain more localized in young infants, while in older infants and children it has the tendency very quickly to become a diffuse edema of the whole brain.

**Dr. Saudubray:** I have never observed localizing neurological signs in neonates. I guess this is because the patient is deteriorating very rapidly, so he is hospitalized immediately in the intensive care unit and has no time to develop neurological symptoms. I don't know the exact pathogenesis of this very strange case of hemianopsia and hemiplegia. In some patients the diagnosis of cerebral tumor was very strongly considered until a CT scan and even ventriculography and encephalography were performed.

**Dr. Wendel:** You told us that there are no congenital malformations in patients with branched-chain organic acidurias. That is true. But I would like to stress that fetuses affected with propionic acidemia accumulate large amounts of odd-numbered fatty acids in body lipids, including brain. At 22 weeks of gestation various tissue lipids contain 6–7% of C15 and C17 fatty acids compared to about 1% in normal controls. We do not know the relevance of the odd-chain fatty acids accumulated in the fetal brain for the later psychomotor development. Most probably, during severe tissue catabolism such as after birth, the breakdown of adipose tissue during lipolysis might contribute to excessive accumulation of propionyl-CoA leading to symptoms of severe intoxication.

**Dr. Saudubray:** Odd-numbered fatty acids represent a very important source of propionyl CoA. Through stable isotope studies performed by Thompson and us in methylmalonic and propionic acidemia we were able to show that approximately one third of the propionyl CoA turnover comes from protein catabolism, one third comes from gut bacteria (anaerobic flora), and the final third probably comes from lipolysis. We are now doing experiments with the C13 propionate turnover to demonstrate this important source of propionate production.

**Dr. Wang:** I am interested by your methylmalonic acidemia group. During the past 3 years we have had four cases of MMA, and only one of them was responsive to vitamin B12; the other three belong to the mut group. One of these persisted with minimal enzyme activity, while the other two had total deficiency of mutase activity. The outcome is that two of them died eventually, and one has psychomotor retardation. What is the outcome of your 31 MMA patients?

**Dr. Saudubray:** Our 31 MMA patients were collected over 20 years, so you cannot compare the patients we found in 1967 and the patients we found last year or yesterday. So collectively about two thirds of our patients (19 or 20) are mutase deficient, B12 unresponsive, with a complete absence of methylmalonyl-CoA mutase activity. Among this first group we need to divide the patients we observed in the first 10 years of our survey (i.e., in the late 1960s and the 1970s) from the patients we have observed in the 1980s. We have now changed our management completely, and we systematically insert a nasogastric tube immediately after the
diagnosis, whatever the infant’s appetite. We teach the parents to feed the child for a minimum of 20 h out of 24. Since we started with this method the patients are doing very well. Of course, it is a major constraint for the parents, but they don’t have the terrible problem of feeding their child. These children are not anorectic at all, because they are not fed orally. They grow well because they are permanently in an insulin-secreting state. So since the 1980s these patients have been doing well. In the second group, composed of patients with the variant forms of methylmalonic aciduria (I mean either some B12 variants, CBLA, CBLB, or two patients with a minus mutation) the prognosis is good. It is very easy to manage these patients because they need only a slightly protein restricted diet and B12 therapy.

Dr. Brodehl: I also was interested in the long-term outcome of these patients. How are the patients with MSUD on long-term treatment?

Dr. Saudubray: I have had very good experience with the long-term outcome of MSUD patients. I feel it is really easy to manage these patients. It is as easy as PKU. You have to make a distinction between the 1960s and 1970s and the 1980s, of course, because our management has greatly improved. My general impression of the outcome in patients who have been treated since the beginning of the 1980s is good. I have five patients now attending regular school, in the regular level, and two of them are very bright, first in the class. So I am pretty sure that the results are even better than in PKU, because we are managing MSUD patients more carefully than the PKU ones. In PKU it is not acutely dangerous in the day-to-day management if plasma levels over 12 mg/dl occur, because there are no immediate symptoms. In MSUD, on the other hand, you get immediate consequences of daily variations in control.

Dr. Wendel: Clinicians need tools for monitoring patients on long-term treatment, not only methods for making diagnoses. In four out of six of our patients with propionic acidemia and methylmalonic acidemia there was a relative abundance of odd-chain fatty acids in erythrocyte lipids, while two extremely well treated patients had normal odd-chain fatty acid levels, which are about 0.7% of the total fatty acids. In one patient with methylmalonic aciduria and a very high odd-chain fatty acid level of over 5%, efforts were made to improve metabolic control. When oral treatment with metronidazole was started in order to reduce the propionate production by the gut bacteria, the level of odd-chain fatty acids came down very much to below 2%. To me the odd-chain fatty acid content in various lipids seems to be a valuable long-term measure of control in patients with disorders of propionate metabolism, and could be a useful indicator of disease severity in individual patients and hence a possible indicator of prognosis, like glycosylated hemoglobin (HbA1) in diabetes.

Dr. Saudubray: I guess this must be one of the most convenient ways of monitoring long-term therapy of methylmalonic and propionic acidemia. In our prospective protocol in which we are starting to study the value of metronidazole therapy for the long-term management of methylmalonic and propionic acidemia we shall check the concentration of odd-numbered fatty acids in erythrocytes as an indicator of metabolic equilibrium.

Dr. Roe: I would like to ask about the late onset of propionic aciduria. I recently encountered a family in which a previously asymptomatic child presented for the first time at 2 years of age with ketoacidosis. His elder sibling of 4 years had propionic acidemia and this 2-year-old turned out to have the condition as well. His mother was pregnant and the fetus was also affected. I found it very unusual that the child was growing reasonably well. It is clearly a very mild form. I wonder if you have experience with similar types of families and if there is any explanation for the difference.

Dr. Saudubray: I have no explanation for this. I don’t know of any similar situations. In my experience patients with late onset forms of propionic acidemia are in very bad health because paradoxically they presented with very severe hypotonia and may have destroyed their brain.
at the first attack. But I have no fortuitous association, within the same family, of one severe case and one mild case. Maybe you have to look at the possibility of composite heterozygosity with a combination of two different mutations. We observed in an MSUD family the coexistence of a mild form and severe neonatal form. The father was a compound heterozygote, both for the classical mutation and for another mild variant form as well. The mother was a classical heterozygote for the classical form. So they produced two kinds of children: one with the classical neonatal form due to true homozygosity with the classical mutation, and the other a heterozygote compound like the father.

**Dr. Otten:** Do you use tube feeding only for neonates? How long do you do it for, and do you expect that these children will ever eat by themselves?

**Dr. Saudubray:** When I teach the family what is in effect a management contract, I explain that it will last for a minimum of 5 years. After 1 year I reevaluate the tolerance of the nasogastric tube. If it is badly tolerated, we perform a gastrostomy for 5 years. Why 5 years? Because it is the most important period for the rapid growth. During this period you have very important variations in anabolism, depending on intercurrent infectious diseases. Feeding by gastric tube minimizes these variations.

**Dr. Dufour:** What is the cause of dehydration in MMA and PA?

**Dr. Saudubray:** The cause of dehydration is methylmalonic acidemia is very clear. Methylmalonic acid, secondary to accumulation of methylmalonic CoA, is a “cul-de-sac” of metabolism. The organism is unable to use this methylmalonic acid. The only way for detoxication is excretion through the urinary flow. The renal clearance of methylmalonic acid is very high. Dehydration is due to this very high renal clearance inducing enhanced diuresis. When you look carefully at these patients, both neonates and older children during an acute attack in the late onset forms, very frequently dehydration contrasts with enhanced diuresis. This association suggests that it is an osmotic mechanism. In propionic acidemia the situation is not so clear. Maybe the same explanation can be given for methyl citrate.