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Organic Acidurias and Related Abnormalities

Denis C. Lehotay[1] and Joe T.R. Clarke[2]*

[1]Department of Clinical Biochemistry and [2]Division of Clinical Genetics, Departments of Pediatrics and Genetics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada M5G 1X8

Referee: P. Renaldo, Yale University School of Medicine.

* To whom correspondence should be addressed. Dr. Denis Lehotay, Department of Clinical Biochemistry, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

ABSTRACT: Organic acid analysis is a powerful technique in the diagnosis of inborn errors of metabolism. Since the development of the technique over twenty-five years ago, it has evolved into a sophisticated and powerful method and is an essential tool in the diagnosis of the organic acidurias. The chemistry and biochemistry of organic acids, as well as sample preparation, instrumentation, and many aspects of the more commonly used methods for the analysis of these compounds, are reviewed. The biochemical and clinical characteristics of each of the primary organic acidurias are described. In addition, the various noninherited causes of secondary organic acidurias that lead to the excretion of abnormal organic acids are also described, and ways of differentiating primary from secondary causes are discussed.

KEY WORDS: mass spectrometry, organic acid, organic aciduria, inborn errors of metabolism, gas chromatography, diagnosis.

I. INTRODUCTION

The application of sophisticated mass spectrometric methods to analysis of constituents of urine has resulted in the identification of a large and growing number of inborn errors of metabolism characterized by organic aciduria: the excretion of excess amounts of normal urinary organic acids or the appearance in the urine of organic acids not normally present. Individually these diseases are rare; however, as a group, they constitute a significant clinical load.[1] Early detection and appropriate treatment are often lifesaving. However, the interpretation of organic aciduria is complicated by the observation that considerable overlap occurs between the abnormalities encountered in different primary disorders of organic acid metabolism, and the type and quantities of organic acids in urine may be profoundly affected by the patient's diet and medication. Moreover, in some disorders, typical diagnostic abnormalities may be present only intermittently.

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In this article, we review the chemistry and biochemistry of organic acids commonly found in urine, current analytic methodologies, and a number of primary organic acidurias and causes of secondary organic aciduria. Much of the information is based on our own experience at the Hospital for Sick Children in Toronto. Because the field is advancing continuously and rapidly, any review of this nature will be, by the time of publication, necessarily incomplete.

II. CHEMISTRY AND BIOCHEMISTRY

Organic acids, as understood in this article, are compounds that contain one or more carboxylic acid or acidic phenolic functional groups, but do not contain any primary amino groups that might react with ninhydrin. Accordingly, amino acids and amino aciduria are generally excluded from this review. Similarly, inorganic acids and disorders of inorganic acid metabolism are not discussed. In addition to carboxylic acid or acidic phenolic groups, organic acids may contain other structural characteristics, such as hydroxyl, oxo, amides, esters, or thiol groups, as well as olefinic groups.[2] The organic acids are named systematically on the basis of the alkane from which they are derived. However, because many of the compounds are better known by their trivial names, these are also used in this review.

In the solid or liquid phase, organic acid molecules exist predominantly as dimers.[3] The physiologically important organic acids exist as liquids at body temperature; saturated, unbranched aliphatic acids with fewer than five carbons are miscible with water. As chain length and molecular weight increase, water solubility decreases. The sodium or potassium salts are generally more soluble in water than the undissociated acids. The organic acids are weakly acidic with dissociation constants (K_a) between 3.0 and 6.0; at physiological pH, they are generally almost completely dissociated. Long-chain fatty acids (>16 carbons) are lipophilic, and some of their biological activity is related to their ability to become integrated within cell membranes in which they may act as anionic surfactants with membrane-disrupting effects.[4]

In principle, any inherited disease occurring as a result of deficiency of an enzyme involved in organic acid metabolism results in accumulation of the substrate of the reaction affected. Identification of the accumulating compound is an important clue to localization of the defect. However, insolubility or localization of the compound to some inaccessible subcellular compartment, such as mitochondria, often makes detection of the immediate substrate of the defective reaction difficult or impossible. For example, inborn errors of fatty acid oxidation, and many of the other primary disorders of organic acid metabolism, commonly result in localized accumulation of acyl-coenzyme A (acyl-CoA) molecules. Demonstration of the accumulation of abnormal acyl-CoA compounds is rarely feasible because these molecules are not transported across cellular membranes to any extent, the quantitation in tissue is technically cumbersome, and metabolic ex-

change of the CoA for carnitine occurs rapidly as one of the mechanisms that has evolved to conserve free coenzyme A (CoASH). On the other hand, identification of secondary metabolic consequences of the defect is often technically easy and diagnostically important. In the example already described, deesterification of the acyl-CoA substrate of the defective enzyme commonly results in the accumulation of the free organic acid and of corresponding carnitine esters in urine, both of which are technically relatively easy to demonstrate with the aid of modern mass spectrometry (MS). Other organic acids, particularly alpha-substituted aliphatic acids that are less readily metabolized, and aromatic acids are eliminated in the urine conjugated with glycine or with glucuronic acid.[5] In isovaleric acidemia, an inborn error of leucine metabolism, most of the accumulated isovalerate in urine occurs as isovalerylglycine; many drug-derived organic acids are excreted as glucuronides.

Unbranched long-chain fatty acids (aliphatic monocarboxylic acids with >16 carbons) are metabolized predominantly in mitochondria by beta-oxidation to acetyl-CoA, which is further oxidized to CO_2 and water via the tricarboxylic acid (TCA) cycle.[6] In some physiological circumstances, and under many pathological conditions, they are also oxidized by another beta-oxidation system localized in peroxisomes. Peroxisomal beta-oxidation differs from mitochondrial beta-oxidation in four important respects: the electron acceptor for the fatty acyl-CoA dehydrogenases is a flavoprotein that transfers the electrons to water without the generation of ATP, oxidation results in the production of H_2O_2, the enoyl-CoA hydration and 3-hydroxyacyl-CoA dehydrogenation occur as a concerted reaction catalyzed by a single enzyme, and oxidation of the hydrocarbon does not proceed beyond an 8-carbon chain length.[7] Medium-chain fatty acids (8 to 12 carbons), derived from peroxisomal beta-oxidation or from the diet, are oxidized in mitochondria, by beta-oxidation, or by microsomal omega-oxidation. Branched-chain fatty acids, such as phytanic acid derived from the metabolism of chlorophyll, are oxidized by alpha-oxidation by a microsomal enzyme system. When the capacity for mitochondrial beta-oxidation is exceeded, either because of increased pressure on fatty acid oxidation for energy production, such as occurs in diabetes mellitus or during long-term starvation, or owing to deficiency of one of the enzymes involved, peroxisomal and microsomal oxidation is increased, resulting in accumulation of medium-chain dicarboxylic acids in the urine.

In certain organic acidopathies, accumulation and excretion of acylcarnitines cause carnitine depletion with secondary effects on fatty acid metabolism. One of the important metabolic functions of carnitine is to facilitate the transport of long-chain fatty acids into mitochondria, a process involving transesterification of fatty acyl-CoA with carnitine catalyzed by carnitine palmitoyltransferase I (CPT I), translocation across the inner mitochondrial membrane, and regeneration of fatty acyl-CoA in a reaction catalyzed by CPT II.[8] The metabolic consequences of secondary carnitine depletion, including organic aciduria, are difficult to differentiate from primary carnitine deficiency and from primary disorders of fatty acid oxidation.

Most inborn errors of amino acid metabolism involve reactions beyond transamination, the first step in the catabolism of most amino acids, resulting in the accumulation of some derivative organic acid. Accordingly, most of the amino acidopathies are associated with organic aciduria. However, those conditions, such as maple syrup urine disease in which massive accumulation of the relevant amino acids occurs, are generally classified as amino acidopathies rather than as organic acidopathies.

Metabolic acidosis is a prominent feature of some of the inborn errors of organic acid metabolism, such as methylmalonic acidemia, and the clinical manifestations of disease are dominated by the tachypnea attributable to accumulation of low-molecular-weight anions. Many aliphatic acyl compounds with three or more carbons also have encephalopathic properties that are apparently independent of their metabolic effect as low-molecular-weight anions.[9] Octanoate, which accumulates in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, one of the most common hereditary fatty acid oxidation defects, has been shown to inhibit CoA metabolism and to produce anesthesia, apparently as a result of its ability to dissolve in cellular membranes.[10]

Secondary metabolic effects are a prominent cause of symptoms in patients with primary disorders of organic acid metabolism. A comprehensive discussion of these effects is beyond the scope of this review. A brief discussion of one example will suffice to indicate the potential complexity of the issue. As already mentioned, accumulation of abnormal acyl-CoAs is a feature of many organic acidopathies, and may have far-reaching consequences. These include direct inhibition of several enzymes of mitochondrial oxidative metabolism, insufficient production of ATP, NADH, and other high-energy compounds, and it is also associated with decreased intracellular free CoASH. The ratio of free-to-esterified CoA in mitochondria is important in the regulation of pyruvate dehydrogenase (PDH) and 2-oxoglutarate dehydrogenase activities. Decreased availability of CoASH and resultant depletion of acetyl-CoA have widespread effects, including decreased gluconeogenesis, owing to the lack of allosteric stimulation of pyruvate carboxylase, and impaired detoxification of ammonia resulting from decreased synthesis of *N*-acetylglutamate, an essential allosteric activator of carbamylphosphate synthase I (CPS I). Hypoglycemia and life-threatening hyperammonemia are prominent clinical problems in many patients with primary or secondary defects of organic acid metabolism. Direct effects on mitochondrial energy metabolism have also been demonstrated in animals administered octanoate,[10,11] one of the principal metabolites that accumulate in patients with MCAD deficiency.[12]

III. METHODOLOGY

The identification, by Tanaka *et al.* in 1966, of isovaleric acidemia using gas chromatography (GC) and MS paved the way for the application of these tech-

niques to the study of organic acidurias.[13] Investigators recognized from the beginning that the ideal method for organic acid analysis should be comprehensive enough to allow all the organic acids of interest to be extracted, analyzed, and identified in a single run.[14] Because of the large number of organic acids found in urine, and the complexity of the mixtures, linked GC-MS was subsequently proposed as the method that came closest to meeting the need for a general analytical method with sufficient sensitivity and resolution, wide dynamic range, and ability to detect any biologically relevant organic compound that might occur in urine.[15,16] Although a large number of GC-MS methodologies have been described, they are all applications of a physical union of the high resolving power of gas-liquid chromatography with the sensitivity and structural analytical power of MS.

A. Analysis of Free Organic Acids

1. Extraction

Analysis of organic acids in body fluids by GC-MS requires that the acids first be extracted from aqueous solution as a group. Plasma, urine, cerebrospinal fluid (CSF), and vitreous humor have all been used by various laboratories as samples for organic acid analysis. Urine is the most easily obtained and it is the physiological fluid in which organic acid levels are generally highest. A variety of approaches to extraction of organic acids from physiological fluids have been described over the years. A commonly used method in our laboratory involves partitioning of underivatized organic acids from acidified urine, plasma, or CSF by exhaustive extraction with volatile organic solvents such as diethyl ether and/or ethyl acetate. Some procedures call for preliminary removal of amino acids by alkaline extraction prior to acid extraction. However, interference by contaminating amino acids is rarely a problem, and alkaline extraction may cause hydrolysis of organic acid esters that may be diagnostically significant. Most contemporary methodologies omit the preliminary alkaline extraction. Because the efficiency of extraction of different organic acids varies from one another, regardless of the extraction method employed, an internal standard is added to each sample prior to extraction. The ideal internal standard has not yet been discovered; many laboratories employ more than one internal standard per sample, one for low-molecular-weight compounds, another for higher-molecular-weight organic acids, and still another for ketoacids. An external standard is also added immediately prior to injection of the sample to monitor the volume of sample injected, and possible variations in detector sensitivity. These standards allow the calculation of relative response factors that may be used in combination with standard curves to estimate the amount of each organic acid that has been identified.

2. Derivatization

The biologically important organic acids occurring in physiological fluids all require conversion to stable, volatile derivatives that will permit gas-phase resolution without prohibitive loss through pyrolysis in the inlet of the GC or on the column. The minimum requirement is esterification of the carboxylic acid group. Methylation has given way over the years to trimethylsilylation, which has the advantage of simultaneously derivatizing other functional groups such as hydroxyls. Reliable recoveries of 2-ketoacids usually require that they be converted to oximes prior to trimethylsilylation.

3. GC-MS

Much literature has accumulated on the results of organic acid analysis achieved using various supports, liquid phases, and open tubular or capillary columns.

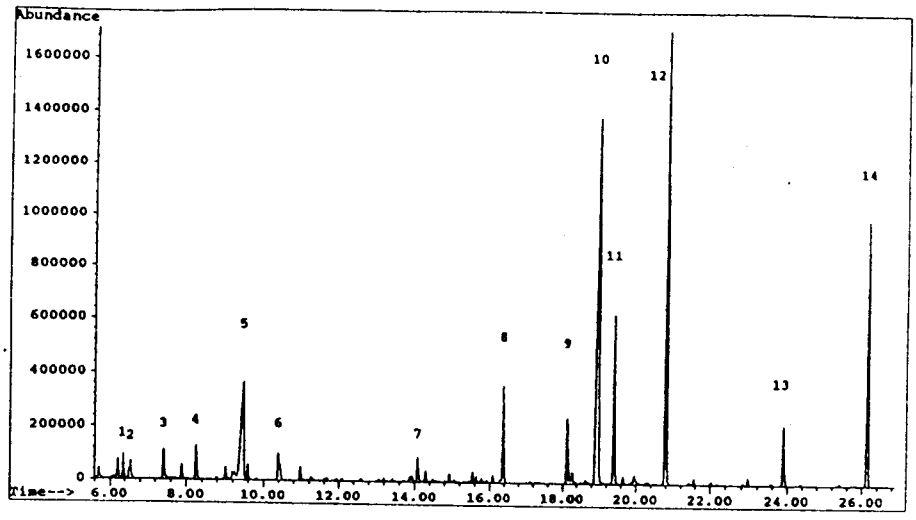
MS may be by magnetic sector or by quadrupole instruments. For routine organic acid analysis quadrupole instruments are preferred because of the reduced cost, increased reliability, and simpler operation. The various approaches used in the analysis of organic acids have been reviewed in detail.[17–33]

In our laboratory fresh random urine specimens are collected and volumes containing 2.5 µmol of creatine are diluted with distilled water to 1.0 ml. An internal standard is added (2-oxo-caproic acid or pentadecanoic acid), and if chemical spot tests indicate the presence of ketones, or if the clinical condition of the patient warrants it, the sample is oximated by treatment with 0.2 ml of hydroxylamine-HCl at 60°C for 30 min. The sample is then acidified with 6.0 *N* HCl to ~pH 1.0, saturated with NaCl, and extracted once with ethyl acetate, and once with diethyl ether. The organic phases are combined, a C-24 hydrocarbon is added as an external standard, the sample is dried with anhydrous sodium sulfate, and the solvent is removed by evaporation under a stream of nitrogen. A 0.1-ml aliquot of BSTFA with 1% TMCS is added to the dried residue and heated at 60°C for 10 min. The sample is diluted with 0.9 ml of hexane and analyzed by GC-MS on an HP-5890 GC equipped with an autosampler, connected to an HP-5971 Mass Selective Detector. The sample is injected in splitless mode onto an open tubular glass capillary column (SPB-1, 30 m, 0.25 mm ID, 0.25 µm coating, made by Supelco), and the injector is kept at 250°C. The carrier gas is helium, with a flow rate of 1 ml/min. The GC oven is held at 90°C for 4 min, then raised at 8°C/min to 300°C, and maintained at that temperature for 4 min. The peaks are identified by reference to a mass spectral library (see Figure 1).

B. Analysis of Acrylcarnitines

Analysis of carnitine esters has become an integral part of the investigation of organic aciduria and conditions associated with secondary carnitine insufficiency

Panel A



Panel B

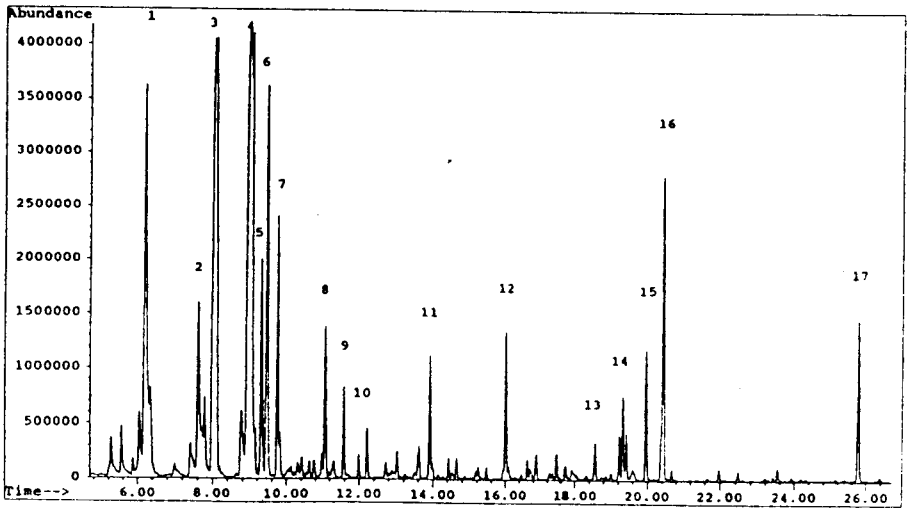


FIGURE 1. (A) Normal organic acid pattern. Numbered peaks were identified by MS as follows: 1, lactate; 2, glycolate; 3, oxalate; 4, 3-hydroxyisobutyrate; 5, urea; 6, phosphoric acid; 7, 2-hydroxy-2-methylsuccinate; 8, 4-hydroxyphenylacetate; 9, aconitate; 10, hippurate; 11, citrate; 12, int. std.; 13, 4-hydroxyhippurate; 14, ext. std. (B) Pattern observed in methylmalonic acidemia. Numbered peaks are as follows: 1, lactate; 2, 3-hydroxypropionate; 3, 3-hydroxybutyrate; 4, methylmalonate; 5, acetoacetate; 6, 3-hydroxyvalerate; 7, succinate; 8, int. std-1; 9, 5-hydroxyoctanoate; 10, glutarate; 11, adipic acid; 12, 2-oxoglutarate; 13, hippurate; 14, methylcitrate; 15, 4-hydroxyphenylacetate; 16, int. std-2; 17, ext. std.

by virtue of the extent of acylcarnitine formation and excretion in urine under circumstances in which free organic acids accumulate. A number of technically difficult and time-consuming methods, based on resolution of complex mixtures of organic acids by high-performance liquid chromatography (HPLC), have been developed for the separation and quantitation of acylcarnitines.[34–36] For the routine analysis of samples of urine, fast atom bombardment (FAB) MS is rapid and relatively simple, and it has become the most commonly used technique.[37] Our laboratory employs this method for the analysis of urinary acylcarnitines using a Trio-2 quadrupole mass spectrometer from Fisons Instruments.

More recently, Millington and co-workers[38,39] have been advocating the use of FAB combined with tandem mass spectrometry (FAB-MS-MS) as a more sensitive method for the analysis of plasma acylcarnitines for the diagnosis of disorders of organic acid metabolism. Combined liquid chromatography-mass spectrometry (LC-MS) has also been used for acylcarnitine analysis.[40] This technique provides more structural information about the acyl moieties of the acylcarnitines than FAB-MS-MS, but the LC part of the method has lower resolution than GC on capillary columns and further development is required. Both LC-MS and FAB-MS-MS are relatively expensive, and most laboratories cannot afford the cost of the instrumentation or the level of expertise that is required. However, recent advances in LC-MS-MS[41] and the gradual reduction in the cost of mass spectrometers have raised the possibility that this powerful technique will provide new opportunities for the analysis of both organic acids, and other, less volatile metabolites that were difficult or impossible to analyze in the past.

Recent technical developments have promised to place acylcarnitine analysis within the financial reach of a larger number of diagnostic laboratories. One important advance has been the development of a novel derivatization technique[42–45] that might make GC-MS analysis of acylcarnitines possible with sensitivity comparable to that of FAB-MS-MS. The method relies on derivatization of the acylcarnitines to form volatile lactones that are analyzed by GC-MS using either electron impact or chemical ionization. Sample preparation is relatively easy and rapid, and the analysis may be possible on simple, affordable quadrupole bench-top GC-MS systems.

C. Analysis of Acylglycines

The development of rapid and technically easy acylcarnitine analysis by GC-MS has greatly facilitated the diagnosis of fatty acid oxidation defects in which diagnostic abnormalities in free organic acids in the urine may be frustratingly intermittent. However, the sensitivity of the method appears to depend critically on adequate availability of carnitine *in vivo*. Because carnitine depletion is a commonly secondary metabolic problem in the organic acidopathies, analysis of acylcarnitines is likely to be least sensitive in that group of patients in whom it is most important. To ensure adequate carnitine stores prior to analysis of urinary

acylcarnitines, many investigators have stressed the importance of preliminary loading with L-carnitine immediately before collecting the urine for acylcarnitine analysis.[38] Other groups have focused on the detection and identification of other organic acid derivatives excreted in the urine of patients with various organic acidopathies.

In 1988, Rinaldo *et al.*[46] reported the abnormal excretion of acylglycines in the urine of patients with MCAD deficiency, the most common of the primary disorders of fatty acid oxidation. The acylglycines in urine appeared to be derived in part from accumulation of endogenous substrates of the defective enzyme (ω-hexanoylglycine and suberylglycine) and from intestinal bacterial metabolism (3-phenylpropionylglycine). The sensitivity of the approach appears to be greater than that of urinary acylcarnitine analysis,[47] but may not be more sensitive than FAB-MS-MS analysis of acylcarnitines in blood.[39] Acylglycine analysis is technically demanding. It is carried out by stable isotope dilution analysis with the use of appropriate deuterated standards of the glycine derivatives to be quantitated. False-negative results have been encountered in patients in whom the bacterial production of 3-phenylpropionic acid is decreased by age- or treatment-related alterations in gut flora.[48,49] For this reason, some investigators have advocated coupling analysis of urinary acylglycines with preparatory oral loading with the 3-phenylpropionic acid.[50] In our laboratory urinary acylglycines are analyzed by the method of Rinaldo *et al.*[47] without prior loading with 3-phenylpropionic acid.

D. Computer-Assisted Interpretation of Organic Acid Analyses

Organic acid analysis has been facilitated by the development of powerful, inexpensive, and user-friendly computers and software coupled with the availability, from most mass spectrometer manufacturers, of organic acid libraries and tools for the automated quantitation of hundreds of components. As a result of these developments, the technical component of organic acid analysis has become relatively easy. However, interpretation of the data requires consideration of the entire organic acid profile, the identity of all the specific organic acids present, as well as the relative amounts of each. This potentially complex task is facilitated by the application of algorithms to take into account the presence of normal organic acids, compounds derived from diet or drugs, and consideration of the age of the patient. This is an aspect of organic acid analysis that will no doubt be advanced considerably by computer software developments. The final interpretation of the results of organic acid analyses, in terms of disease, drug exposure, diet, and other variables, requires attention to the clinical condition of the patient, the time and conditions under which the urine was collected for analysis, and any history of environmental exposure.

Our experience, as well as that of others, has been that automation of the technical and data processing aspects of organic acid analysis speeds up the work significantly. However, manual checking of the data is still mandatory to eliminate

the possibility of errors arising as a result of the complexity of the organic acid pattern. For example, most data systems currently in use are not capable of reliably distinguishing overlapping chromatographic peaks; in this situation, the judgement of an experienced operator is more reliable than the output of an automated data processing system.

A number of quality control programs currently exist to check on the reliability and diagnostic efficiency of laboratories engaged in organic acid analysis.[51,52] Samples from healthy individuals, as well as from patients with various inborn errors of metabolism, are sent several times during the year to participating laboratories. The laboratories report the results of their analyses and their diagnostic interpretation of the data that are then checked against information provided by the originating reference laboratory.

E. Artifacts

A number of chemical and biological artifacts have been identified in the analysis of urinary organic acids. Inappropriate sample handling and storage may result in confusion caused by leeching of chemicals from containers in which urine is collected. Azelaic and pimelic acids are odd-chain-length dicarboxylic acids that may be elevated in the urine of patients with defects either in peroxisomal or mitochondrial fatty acid oxidation. In these situations, the organic acids are primarily of endogenous origin, and they are present in much lower concentration than the even-chain-length dicarboxylic acids of the same chain length. However, both may arise artifactually as a result of storage of urine in plastic containers containing plasticizers.[53]

Age-related differences in organic acid excretion are well known and have been attributed to delayed maturation of the kidney and to immaturity of liver enzymes responsible for the metabolism and clearance of a variety of compounds. In premature infants, artifactual organic aciduria has been reported as a result of fermentation of carbohydrates by gut microorganisms that do not occur to a significant extent in older children.[54] The role of gut bacteria in organic aciduria is discussed in more detail elsewhere.

Two recent articles highlight the potentially damaging confusion that may be caused by the similarity between the analytical and clinical features of chemical intoxication and of some inborn errors of organic acid metabolism.[55,56] The authors of the first paper report a case of misidentification of propionic acid as ethylene glycol in the blood of a patient with methylmalonic acidemia. In the second, an infant presenting with a history of recurrent metabolic acidosis, initially attributed to an unidentified inborn error of metabolism, was found to be a victim of intentional ethylene glycol poisoning. These cases underscore the importance of considering the possibility of either intoxication or inherited metabolic disease in patients presenting with severe metabolic acidosis and organic aciduria. The presence of unusual organic acids in urine may also arise as a result of benign

genetic heterogeneity in the metabolism of drugs or dietary constituents. The extent of this contribution to normal variations in the pattern of organic aciduria is still unknown.

IV. PRIMARY DISORDERS OF ORGANIC ACID METABOLISM

Recognition of the possibility that disease may be due to an inborn error of organic acid metabolism is the most critical step in the ultimate diagnosis and treatment of the disorder. This may occur as a result of finding unusual compounds in the urine in the course of screening for organic acids with little or no specific clinical grounds for suspecting an organic acidopathy. More often, the clinician is alerted to the possibility by the clinical presentation of the patient. Not surprisingly, the clinical manifestations of the various organic acidopathies vary markedly, depending on the metabolic pathway involved, the nature of accumulating metabolites, the secondary metabolic effects of the defect, and whether the defect is partial or complete. However, certain clinical situations are particularly common among the organic acidopathies and should alert the clinician to the possibility.[57,58] They include acute metabolic acidosis, acute or intermittent encephalopathy (including hypoketotic hypoglycemia or Reye-like disease), chronic encephalopathy, myopathy (including cardiomyopathy), and failure to thrive. This classification of clinical presentation is an overlapping one: in many cases, patients may present with combinations of symptoms, such as acute metabolic acidosis on a background of chronic encephalopathy, or chronic encephalopathy and failure to thrive, or persistent metabolic acidosis and chronic encephalopathy, along with failure to thrive. Moreover, the same metabolic defect may present in different ways in different patients, even among different affected members of the same family, depending on the severity of the defect, the age of the patient, and various poorly understood gene-gene and gene-environment interactions that contribute to the individuality of every patient. Each of the primary disorders of organic acid metabolism is associated with urinary organic acid abnormalities. However, in some cases, particularly the fatty acid oxidation defects, the abnormalities may be subtle or intermittent, resulting in delays in diagnosis. In general, analysis of urinary organic acids is most reliable when the urine is collected during acute metabolic decompensation. Alternatively, the presence of an abnormality that is only intermittently associated with organic aciduria may be exposed by carefully controlled provocative testing — either by controlled starvation or by loading with metabolic intermediates.

A. Branched-Chain Organic Aciduria

Branched-chain organic aciduria is a prominent feature of a number of primary genetic defects in the metabolism of the branched-chain amino acids leucine, isoleucine, and valine (Figure 2).

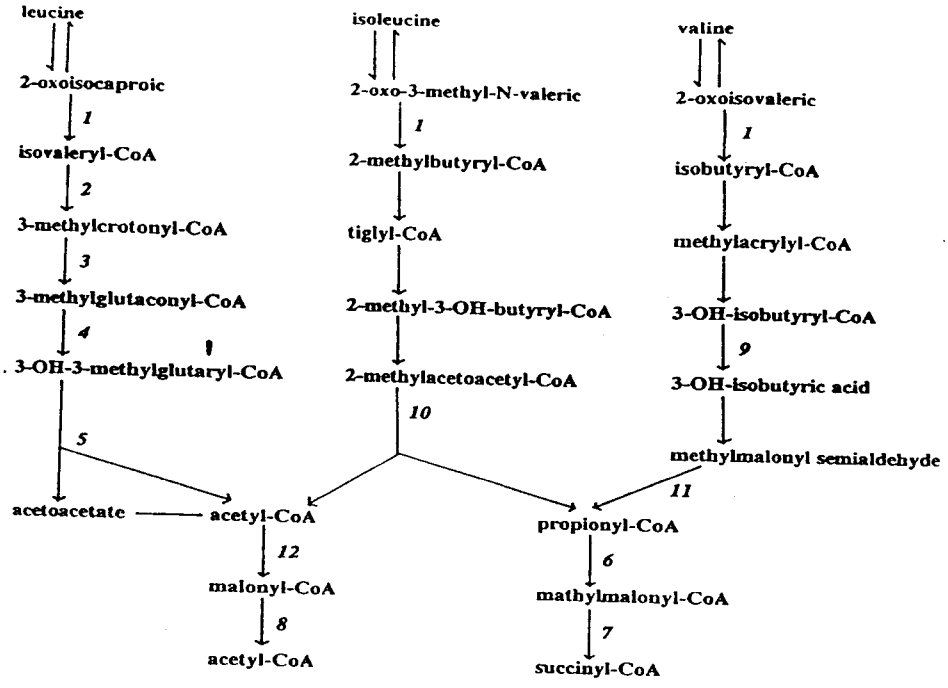


FIGURE 2. Known enzyme deficiencies in Figure 1 are indicated by the corresponding number in the Figure. Deficiencies numbered 1 to 8 are discussed in the text. 1, Branched-chain keto acid decarboxylase; 2, Isovaleryl-CoA dehydrogenase; 3, 3-Methylcrotonyl-CoA carboxylase; 4, 3-Methylglutaconyl-CoA hydratase; 5, HMG-CoA lyase; 6, PCC; 7, Methylmalonyl-CoA racemase; 8, Malonyl-CoA decarboxylase; 9, 3-Hydroxyisobutyryl-CoA deacylase; 10, 2-Methylacetoacetyl-CoA thiolase; 11, Methylmalonyl semialdehyde dehydrogenase; 12, Acetyl-CoA carboxylase. (From Ogier et al., *Inborn metabolic diseases: diagnosis and treatment.* Pp. 271–99, 1990.)

1. Branched-Chain 2-Ketoaciduria

Excretion of large amounts of 2-oxoisocaproic, 2-oxo-3-methylvaleric, and 2-oxoisovaleric acids is characteristic of maple syrup urine disease due to deficiency of branched-chain 2-ketoacyl-CoA dehydrogenase, a multisubunit enzyme complex with features in common with PDH and 2-ketoglutaryl-CoA dehydrogenase (KGDH) with which it shares a common E_3 subunit.[59] Clinically, the disease presents in the newborn period as an acute encephalopathy (poor feeding, vomiting, seizures, and drowsiness progressing to stupor and coma) associated with severe ketoacidosis. However, variants occur that are characterized by intermittent episodes of ketoacidosis triggered by intercurrent illness in otherwise apparently healthy children, or by moderate to severe mental retardation in the absence of ketoacidosis.[60] Analysis of plasma amino acids shows marked elevation of the

three normal branched-chain amino acids as well as accumulation of alloisoleucine, the product of keto-enol tautomerization and transamination of 2-oxo-3-methylvalerate. Reliable identification of the 2-ketoacids in the urine of affected patients may require derivatization with hydroxylamine and analysis of the organic acids as the oximes. However, in addition to the 2-ketoacids, the urine also generally contains large amounts of the corresponding branched-chain 2-hydroxyacids, derived from enzymic reduction of the 2-keto groups *in vivo;* the branched-chain 2-hydroxyacids are detectable by GC-MS and are sufficiently characteristic of the disease to make easy diagnosis possible.

2. Isovaleric Aciduria

Isovaleric acid is derived from the oxidative decarboxylation of 2-oxoisocaproic acid in the metabolism of the amino acid, leucine. Large amounts of isovaleric acid are excreted in the urine of patients with primary isovaleryl-CoA dehydrogenase deficiency, a disease commonly presenting in the newborn period as acute encephalopathy associated with ketoacidosis and hyperammonemia.[61] As in the case of other primary organic acidopathies, late-onset variants occur in which periods of apparently good health are interrupted by intermittent episodes of metabolic decompensation with metabolic acidosis, hyperammonemia, and encephalopathy. The urine of affected patients contains very little free isovalerate, even during metabolic decompensation. Instead, accumulated isovalerate appears in the urine as isovalerylglycine derived from the condensation *in vivo* of isovalerate and glycine. During episodes of acute metabolic decompensation, the urine of affected patients also contains 3-hydroxyisovalerate, in addition to high concentrations of 3-hydroxybutyrate, acetoacetate, and lactate. Isovaleric aciduria also occurs in glutaric aciduria, type II, due to deficiency of mitochondrial flavoprotein-dependent acyl-CoA dehydrogenases (see below). However, the presence of high concentrations of glutaric acid, and other organic acids, makes differentiation from primary isovaleric aciduria obvious (see below).

3. 3-Methylcrotonic Aciduria

3-Methylcrotonic acid is an intermediate in leucine metabolism, derived from the enzymic dehydrogenation of isovaleryl-CoA. Accumulation occurs as a result of deficiency of the enzyme, 3-methylcrotonyl-CoA carboxylase, one of the four biotin-dependent carboxylases. Isolated specific deficiency of 3-methylcrotonyl-CoA carboxylase is a rare disorder characterized clinically by episodic hypoglycemia associated with severe ketoacidosis.[62] Analysis of the urinary organic acids shows the presence of 3-hydroxyisovalerate and 3-methylcrotonylglycine in addition to 3-methylcrotonic aciduria.

3-Methylcrotonic aciduria is one of several organic acids found in the urine of patients with hereditary defects of biotin metabolism characterized metabolically by deficiencies of all four biotin-dependent carboxylases. Multiple carboxylase deficiency occurs in two forms as a result of deficiency either of holocarboxylase synthetase or biotinidase. The former enzyme catalyzes the biotinylation of the four carboxylase apoenzymes; the latter catalyzes the reverse reaction, the hydrolysis of biotin from the apoenzyme in the course of the normal turnover of the enzyme protein. Disease due to holocarboxylase synthetase deficiency commonly presents early in infancy with severe metabolic ketoacidosis and hyperammonemia.[63] In contrast, biotinidase deficiency is characterized by neurological symptoms (seizures, hypotonia, developmental delay, sensorineural hearing loss, and optic atrophy), skin rash and hair loss, metabolic acidosis, and hyperammonemia, developing somewhat later in infancy or childhood.[64] Treatment of either form of multiple carboxylase deficiency with very large doses of biotin begun prenatally or very early in the course of the disease is associated with a good clinical and biochemical response; failure to treat early is often lethal.

The pattern of organic aciduria in multiple carboxylase deficiency is the same as the patterns encountered in each of the specific carboxylase deficiencies taken together: 3-methylcrotonate, 3-methylcrotonylglycine, and 3-hydroxyisovalerate (due to deficiency of 3-methylcrotonyl-CoA carboxylase); lactate, acetoacetate, and 3-hydroxybutyrate (due to deficiency of pyruvate carboxylase); and propionate, 3-hydroxypropionate, methylcitrate, and tiglylglycine (due to deficiency of propionyl-CoA carboxylase).

4. 3-Methylglutaconic Aciduria

3-Methylglutaconic aciduria, generally accompanied by excretion of smaller amounts of 3-methylglutaric acid, has been encountered in at least four distinct and apparently unrelated inherited metabolic diseases.[65]

a. Type I

3-Methylglutaconic acid is an intermediate in leucine metabolism, derived from the enzymic carboxylation of 3-methylcrotonyl-CoA. The majority of patients in whom 3-methylglutaconic aciduria has been found have normal hydratase activity. However, in a small number of patients, designated type I 3-methylglutaconic aciduria, accumulation and excretion of large amounts of the compound have been shown to occur as a result of hereditary deficiency of 3-methylglutaconyl-CoA hydratase.[66] Clinically, the patients exhibited mild developmental delay and fasting hypoglycemia associated with ketoacidosis. In addition to 3-methylglutaconic acid, the urine contained smaller amounts of 3-hydroxyisovalerate and 3-methylglutarate. The concentrations of all three metabolites were increased by leucine loading in patients with this condition.

b. Type II

Small amounts of 3-methylglutaconic acid are found in the urine of some patients with Barth syndrome, an X-linked recessive disorder characterized by cardiomyopathy and persistent neutropenia. In addition to 3-methylglutaconic and 3-methylglutaric acids, the urine contains 2-ethylhydracrylic acid. The mechanism of the organic aciduria is unknown. 3-Methylglutaconyl-CoA hydratase activity in these patients, classified as type II 3-methylglutaconic aciduria, is normal. 3-Methylglutaconic acid excretion is not increased by leucine loading, indicating that the defect lies in some other metabolic pathway.

c. Type III

3-Methylglutaconic aciduria is also a feature of Costeff optic atrophy syndrome characterized by the onset in early infancy of optic atrophy and severe, progressive mental retardation, choreoathetosis, spasticity, and seizures.[65,67,68] 3-Methylglutaconyl-CoA hydratase activity in these patients has also been shown to be normal, and the mechanism of the organic aciduria is unknown.

d. Type IV

This variant of 3-methylglutaconic aciduria is characterized clinically by severe multiple organ involvement, including hypertrophic cardiomyopathy, hepatocellular dysfunction, mental retardation, failure to thrive, optic atrophy, seizures, and multiple congenital malformations, including inguinal hernias, undescended testes, subaortic stenosis, and cerebellar hypoplasia.[69] A number of similar, previously unclassified, clinical variants of 3-methyl-glutaconic aciduria have been reported in recent years, all with normal 3-methylglutaconyl-CoA hydratase activity.[65] In many, the urine contains increased concentrations of lactic acid and TCA cycle intermediates, in addition to 3-methylglutaconic and 3-methylglutaric acids. The pathophysiology of the organic aciduria is not yet known; a defect in cholesterol biosynthesis has been postulated, but the results of direct analysis of cholesterol metabolism have been normal.[70]

Some patients with similar clinical features and 3-methylglutaconic aciduria have been reported in whom various defects in mitochondrial energy metabolism have been demonstrated. Holme and associates[71] described mitochondrial ATP-synthase (complex V) deficiency in a child with 3-methylglutaconic aciduria. Ibel *et al.*[72] and Muller-Hocker *et al.*[73] showed combined deficiencies of mitochondrial electron transport complexes in their patients. Lichter-Konecki *et al.*[74] reported the same urinary organic acid abnormalities in a patient with Pearson syndrome, a mitochondrial depletion syndrome characterized by pancreatic insufficiency, sideroblastic anemia, hepatocellular dysfunction, lactic acidosis, cardiomyopathy, and mental retardation.

What has been classified as type IV 3-methylglutaconic aciduria is almost certainly a heterogeneous group of diseases in which the organic aciduria is likely a secondary, and relatively nonspecific, phenomenon. In all but type I, measurements of 3-methylglutaconyl-CoA hydratase activity *in vitro* have been reported consistently normal. 3-Methylglutaconic aciduria has also been found, apparently as a nonspecific secondary metabolic phenomenon, in patients with a variety of other, well-defined metabolic disorders, such as carbamyl phosphate synthetase deficiency, long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, renal Fanconi syndrome,[70] and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase deficiency,[75] further complicating the issue. What is clear is that the accurate interpretation of 3-methylglutaconic aciduria is difficult and cannot be done without considerable detailed clinical information.

5. HMG Aciduria

HMG-CoA is an intermediate in leucine metabolism (Figure 2) as well as an important intermediate in normal ketogenesis and in cholesterol biosynthesis. HMG-CoA formed in the course of ketogenesis is produced by condensation of acetoacetyl-CoA with acetyl-CoA, catalyzed by the liver enzyme, HMG-CoA synthase. HMG-CoA derived from leucine metabolism is formed by hydration of 3-methylglutaconyl-CoA. HMG-CoA derived either from leucine degradation or ketogenesis is hydrolyzed to acetoacetate and acetyl-CoA by a cytosolic reaction catalyzed by HMG-CoA lyase. Primary HMG-CoA lyase deficiency is characterized clinically by intermittent episodes of vomiting, hypotonia, hepatomegaly, and stupor progressing to coma, associated with hypoglycemia, metabolic acidosis, elevated transaminases, and sometimes hyperammonemia.[76,77] Large amounts of HMG are excreted in the urine along with 3-methylglutaconate, 3-hydroxyisovalerate, and 3-methylglutarate.[78,79] The presence of the latter three metabolites suggests that leucine is the principal source of the accumulating HMG. Analysis of urinary carnitine esters shows the presence of 3-methylglutarylcarnitine, which may account for the carnitine depletion that is another characteristic of the disease.[80]

B. Short-Chain Organic Aciduria

1. Propionic Aciduria

Propionic acid is derived from the metabolism of cholesterol, odd-chain fatty acids, and the amino acids valine, isoleucine, methionine, and threonine. Propionic acid and propionylcarnitine accumulate in the urine as a result of deficiency of the biotin-dependent enzyme, propionyl-CoA carboxylase (PCC). Clinically, isolated PCC deficiency (propionic acidemia) classically presents in the newborn period with severe metabolic acidosis, hyperammonemia, neutropenia, and thrombo-

cytopenia. Late-onset variants have been described in which ketoacidosis and encephalopathy occur in the absence of hyperammonemia or hematological abnormalities. The presentation in a small number of cases is subacute with recurrent episodes of moderate-to-severe ketoacidosis on a background of chronic developmental delay or failure to thrive. Recently, Massoud and Leonard[81] reported a series of patients with organic acidopathies, including patients with propionic acidemia, in whom acute cardiac failure occurred as a result of metabolic cardiomyopathy. The pathogenesis of the myocardial involvement in these patients is unclear.

PCC deficiency, occurring as a result of mutations in the apoenzyme, is associated with excretion of large amounts of 3-hydroxybutyrate and acetoacetate and smaller amounts of methylcitrate, propionylglycine, and 3-hydroxypropionate in addition to propionic acid and propionylcarnitine. In the course of the analysis of urinary organic acids by conventional GC-MS, the large excess of propionic acid may be missed due to losses during sample preparation, and also because it often elutes in the solvent peak. In this situation, the presence of methylcitrate, 3-hydroxypropionate, and/or propionylglycine in the urine should be regarded as highly suggestive of the underlying disorder of propionate metabolism and appropriate additional studies undertaken. Analysis of urinary or plasma acylcarnitines invariably shows the presence of propionylcarnitine, which is virtually diagnostic of the PCC deficiency. Propionic aciduria also occurs in patients with multiple carboxylase deficiency; however, the presence of other organic acids, such as 3-methylcrotonate and 3-hydroxyisovalerate, generally makes discrimination between the diseases relatively straightforward (see above).

2. Methylmalonic Aciduria

Methylmalonic acid is derived from the enzymic carboxylation of propionyl-CoA. Methylmalonic aciduria occurs as a result of deficiency of the cobalamin-dependent enzyme, methylmalonyl-CoA mutase, due either to mutations of the mutase itself or secondary to genetic defects in cobalamin metabolism.[82] It is also a prominent biochemical feature of nutritional vitamin B_{12} deficiency (see below). Apart from the response to treatment with large doses of vitamin B_{12}, primary methylmalonyl-CoA mutase deficiency (the *mut*^{0} and mut$^-$ variants) is clinically indistinguishable from the cobalamin variants, cblA and cblB, characterized by defects in the adenosylation of cobalamin. Affected patients often present in the newborn period with severe ketoacidosis, hyperammonemia, and acute encephalopathy indistinguishable from propionic acidemia due to PCC deficiency or isovaleric acidemia due to isovaleryl-CoA dehydrogenase deficiency (see above). However, the clinical spectrum of methylmalonic acidemia due to mutase mutations is vast, including benign, asymptomatic variants that may be detected coincidentally by organic acid analysis[83,84] (also see Figure 1).

Analysis of urinary organic acids shows the presence of large amounts of methylmalonic acid, 3-hydroxybutyrate, and acetoacetate, and smaller concentrations of propionate and 3-hydroxypropionate, in addition to methylcitrate derived from intramitochondrial condensation of propionate with oxaloacetate.[85] Analysis of plasma amino acid shows accumulation of glycine. Methylmalonic aciduria due to other cobalamin defects (cblC, cblD, cblE, cblF, and cblG) is associated with hematological abnormalities and homocystinuria.[86] However, the urinary organic acid abnormalities are otherwise the same as those seen in patients with primary mutase deficiency. Similarly, the urinary organic acid abnormalities in nutritional vitamin B_{12} deficiency, whether due to inadequate intake or impaired absorption, although milder, are also the same.

3. Glutaric Aciduria

The unbranched, five-carbon dicarboxylic acid, glutaric acid, is a product of leucine, lysine, and tryptophan metabolism. The excretion of large amounts of glutaric acid in the absence of any other significant urinary organic acid abnormalities is characteristic of glutaric aciduria, type I (GA I). This is a rare disorder due to isolated deficiency of mitochondrial glutaryl-CoA dehydrogenase. Clinically, the disease is characterized by slowly progressive developmental regression, opisthotonus, dystonia, and athetoid posturing with onset in the first few months of life.[87,88] The otherwise slowly progressive clinical course is often punctuated by episodes of intractable vomiting, ketosis, hepatocellular dysfunction, and acute encephalopathy.

Glutaric acid is also prominent in the urine of infants and children with glutaric aciduria type II (GA II),[89] also called multiple acyl-CoA dehydrogenase (MAD) deficiency. This condition is due to deficiency of mitochondrial electron transport flavoprotein (ETF) or ETF dehydrogenase (ETF-DH).[90,91] ETF and ETF-DH are central to the mitochondrial oxidation of fatty acids and several amino acids. Clinically, the disease varies markedly from one patient to another. In its most severe form, affected infants present in the newborn period with characteristic congenital malformations (facial dysmorphism, rocker-bottom feet, muscular defects of the abdominal wall, abnormal genitalia, and cystic degeneration of the kidneys and brain), severe neurological impairment, hepatomegaly, hypoglycemia, and metabolic acidosis. Other patients present early in infancy with the metabolic abnormalities but none of the congenital malformations associated with the most severe variant of the disease. In its mildest form, the disease presents later in infancy or childhood with episodes of intractable vomiting, stupor, hepatomegaly, myopathy, hepatocellular dysfunction, and hypoglycemia.

Deficiency of ETF or ETF-DH results in the excretion of large amounts of a number of metabolites in the urine (Table 1), such as glutarate, ethylmalonate, 3-hydroxyisovalerate, 2-hydroxyglutarate, 5-hydroxyhexanoate, and the dicarboxylic

TABLE 1
Urinary Organic Acids in Various Inherited Disorders of Fatty Acid Oxidation

MCAD deficiency	SCAD deficiency	LCAD deficiency	LCHAD deficiency	ETF/ETF-DH deficiency
5-Hydroxyhexanoate	**Ethylmalonate**	Adipate	Adipate	3-Hydroxybutyrate
Adipate	**Methylsuccinate**	Suberate	3-Hydroxyadipate	**Glutarate**
Suberate		Octenedioic	Suberate	**Ethylmalonate**
Octenedioic	**Butyrylglycine**	Decenedioate	Octendioate	**Methylsuccinate**
7-Hydroxyoctanoate		**Dodecandioate**	**3-Hydroxysuberate**	
Sebacate		**Tetradecandioate**	Sebacate	*n*-Butyrylglycine
Decenedioate			Decendioate	Isobutyrylglycine
3-Hydroxysebacate			**3-Hydroxysebacate**	2-Methylbutyrylglycine
			Dodecandioate	**Isovalerylglycine**
Hexanoylglycine			**3-Hydroxydodecandioate**	**Hexanoylglycine**
Suberylglycine			**3-Hydroxydodecendioate**	
Phenylpropionylglycine			**3-Hydroxytetradecandioate**	
			3-Hydroxytetradecendioate	
Octanoylcarnitine				

Note: MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; ETF/ETF-DH, electron transfer flavoprotein/electron transfer flavoprotein dehydrogenase.

acids, adipate, suberate, sebacate, and dodecanedioate, as well as isovalerylglycine, isobutyrylglycine, and 2-methylbutyrylglycine. Lactate is usually also present, but 3-hydroxybutyrate and acetoacetate are generally absent or present in only trace amounts.

4. 2-Hydroxyglutaric Aciduria

2-Hydroxyglutaric acid occurs in normal urine in both D and L configurations. Excess L-2-hydroxyglutaric aciduria has been reported in a number of patients with neurological disease, including extrapyramidal movement disorders and seizures.[92] The primary metabolic defect is unknown. An infant with a severe seizure disorder, hypsarrhythmia, and developmental delay has been described with D-2-hydroxyglutaric aciduria.[93]

5. 4-Hydroxybutyric Aciduria

4-Hydroxybutyric acid accumulates in the urine of patients with succinic semialdehyde dehydrogenase deficiency, a rare autosomal recessive disorder of 4-aminobutyric acid (GABA) metabolism, characterized clinically by mental retardation, hypotonia, and severe ataxia and dysarthria.[94–96]

6. Malonic Aciduria

Excess malonic acid excretion has been described in a small number of developmentally delayed infants with hereditary malonyl-CoA decarboxylase deficiency. In one child, the defect was also associated with cardiomyopathy.[97]

7. Ethylmalonic Aciduria

Ethylmalonate and the C-6 dicarboxylic acid, adipate, are particularly prominent in the urine of patients with the mild form of MAD deficiency;[90] the condition has been called ethylmalonic-adipic aciduria. Ethylmalonate, along with methylsuccinate, also occurs in the urine of patients with short-chain acyl-CoA dehydrogenase deficiency in which it may be the only demonstrable organic acid abnormality[98] (see below).

Burlina *et al.*[99] recently described four unusual Italian patients with persistent ethylmalonic and 2-methylsuccinic aciduria, lactic acidosis, orthostatic acrocyanosis, chronic diarrhea, and progressive neurological abnormalities culminating in death at about 2 years of age in three of them. Intensive investigations of fatty acid

oxidation were all normal; however, one of the four patients had partial cytochrome c oxidase deficiency. Garavaglia *et al.*[100] described an additional two Italian patients, with virtually the same clinical features, who also exhibited isolated ethylmalonic aciduria, persistent lactic acidosis, and deficiency of muscle cytochrome c oxidase. Ethylmalonic aciduria without methylsuccinic aciduria in other patients with progressive neurodegenerative disease due to muscle cytochrome c oxidase deficiency have been reported by Christensen *et al.*[101] and Lehnert and Ruitenbeek.[102]

The interpretation of ethylmalonic aciduria is often difficult. Low concentrations of ethylmalonate have been reported as an apparently nonspecific finding in the urine of some infants with various inherited metabolic diseases, such as hereditary fructose intolerance and 3-methylcrotonyl-CoA carboxylase deficiency, as well as in some apparently healthy children.[103] Transient ethylmalonic aciduria has also been reported, along with lactic, methylmalonic, and 3-hydroxypropionic aciduria, in infants with disturbances of gastrointestinal flora.[54,104] We have also observed this phenomenon, especially in premature infants with acute gastroenteritis in whom the abnormality has always resolved with elimination of the infection and was not associated with any other biochemical or clinical evidence of any of the inherited metabolic diseases generally associated with ethylmalonic aciduria.

8. Fumaric Aciduria

Massive, isolated, excretion of fumarate in the urine was reported in two young mentally retarded adults.[105] The observation that the excretion of very large amounts of fumarate was not accompanied by any other urinary organic acid abnormality, and the determination that the plasma fumarate concentrations in the patients were not elevated suggested to the investigators that the primary defect in the patients involved failure of renal tubular reabsorption of the organic acid. The mechanism of the mental handicap in the patients is not clear.

9. Succinylacetonuria

Succinylacetone is derived from succinylacetoacetate (SAA), which accumulates in the urine of patients with hereditary tyrosinemia type I, caused by deficiency of fumarylacetoacetate (FAA) hydrolase.[106] Accumulated FAA is reduced enzymatically to SAA, which then undergoes nonenzymatic conversion to chemically stable succinylacetone. Therefore, although the urine of patients with hereditary tyrosinemia type I generally contains small amounts of SAA and FAA, succinylacetone is the most characteristic urinary organic acid abnormality. The clinical picture in hereditary tyrosinemia type I is generally dominated by evidence of severe hepatocellular dysfunction, including anasarca, ascites, hyperbilirubinemia,

hyperammonemia, and coagulopathy, in addition to hypoglycemia.[107] Analysis of plasma amino acids characteristically shows abnormalities, such as increased concentrations of tyrosine, phenylalanine, and methionine, commonly associated with severe hepatocellular disease. Marked elevation of plasma alpha-fetoprotein is a characteristic feature of the disease. Rickets, occurring as a result of phosphate losses owing to renal tubular dysfunction, is also common. In addition to succinylacetone, the urine contains large concentrations of 4-hydroxyphenylpyruvate and other tyrosine metabolites, as a result of nonspecific deficiency of 4-hydroxyphenylpyruvic acid dioxygenase (see below). The diagnosis is confirmed by measurement of FAA hydrolase in liver.

The management of hereditary tyrosinemia has been dramatically altered by the recent discovery that pharmacological inhibition of *p*-hydroxyphenylpyruvic acid dioxygenase by administration of the drug, NTBC, appears to arrest the otherwise rapidly progressive hepatocellular damage of the disease, apparently by preventing accumulation of FAA and maleylacetoacetate.[108]

10. Oxalic Aciduria

Hyperoxalic aciduria (or hyperoxaluria) occurs in a wide variety of circumstances as a result of the ingestion of certain drugs and toxins (e.g., ascorbic acid, ethylene glycol, methoxyflurane), increased intestinal absorption in patients with inflammatory bowel disease, or increased dietary intake. Two primary metabolic defects causing excessive oxalate biosynthesis have been described.[109,110] Primary hyperoxaluria type I is caused by deficiency of peroxisomal alanine:glyoxylate aminotransferase[111] and is associated with excretion of large amounts of glycolate and glyoxylate. Affected patients present with renal colic or asymptomatic hematuria, sometimes in early infancy. Primary hyperoxaluria type II, also called L-glyceric aciduria, is caused by a very rare defect in hydroxypyruvate metabolism and is distinguishable from type I disease by the presence of L-glyceric acid in the urine.[109] Secondary hyperoxaluria due to ethylene glycol intoxication or to increased absorption of dietary oxalate in patients with intestinal malabsorption is much more common than either of the primary disorders of oxalate metabolism.[110]

11. N-Acetylaspartic Aciduria

Variably increased concentrations of *N*-acetylaspartate are found in the urine of infants with Canavan disease, a severe early-onset neurodegenerative disease caused by hereditary deficiency of aspartoacylase.[112]

C. Medium-Chain Dicarboxylic Aciduria

Medium-chain dicarboxylic aciduria is a general feature of inherited defects in mitochondrial fatty acid oxidation.[113–116] Mitochondrial fatty acid oxidation occurs

by the sequential action of four different groups of enzymes, each group consisting of genetically distinct subgroups with differing substrate specificities based on the chain length of the fatty acid intermediates. Fatty acid intermediates accumulating as a result of defects in mitochondrial beta-oxidation are oxidized by omega-oxidation in the endoplasmic reticulum, and by peroxisomal beta-oxidation, to shorter-chain dicarboxylic acids. The enzymes involved in peroxisomal beta-oxidation are not capable of catalyzing the oxidation of fatty acids or dicarboxylic acids with chain lengths less than six to ten carbons, and the corresponding C-6, C-8, and C-10 dicarboxylic acids (adipic, suberic, and sebacic acids, respectively) accumulate and are excreted in high concentration in the urine. Clinically, this group of disorders may present as hypoketotic hypoglycemia with evidence of acute hepatocellular dysfunction, acute encephalopathy, or myopathy (including cardiomyopathy), or as combinations of these.

Medium-chain dicarboxylic aciduria occurs in children on valproate, in infants fed formulas containing medium-chain triglycerides (MCT), and in patients with marked ketoacidosis, irrespective of the cause. The presence of large amounts of 3-hydroxybutyrate and acetoacetate in these situations distinguishes this group of patients from those with hereditary fatty acid oxidation defects.

1. MCAD Deficiency

The most common of the inherited fatty acid oxidation defects associated with dicarboxylic aciduria is MCAD deficiency.[117–122] This condition is characterized clinically by acute-onset anorexia and vomiting, lethargy progressing to stupor and coma, hepatomegaly with evidence of hepatocellular dysfunction, hypotonia, hypoglycemia, and hyperammonemia. In a number of cases, death occurred so rapidly and unexpectedly that sudden infant death syndrome (SIDS) was suspected.[123,124] The condition is often clinically indistinguishable from Reye's syndrome,[120,122,125] and many affected children are asymptomatic.[126] Onset in the first 2 years of life, a positive family history, and recurrence of acute metabolic decompensation in the face of otherwise trivial intercurrent illness or fasting should alert the clinician to the likelihood that the patient has MCAD deficiency.

Analysis of urinary organic acids during acute metabolic decompensation characteristically shows the presence of large amounts of the adipate, suberate, and sebacate, and the (omega-1)-hydroxy derivatives of hexanoate and octanoate: 5-hydroxyhexanoate and 7-hydroxyoctanoate, respectively, with little or no 3-hydroxybutyrate and acetoacetate.[114,118,127,128] Routine organic acid analysis of urine obtained when the child is well may be completely normal. However, analysis of urinary acylcarnitines and acylglycines from affected patients, irrespective of their clinical condition, generally shows the presence of octanoylcarnitine and hexanoylglycine and phenylpropionylglycine.[46,47] The presence of these organic acid esters in urine is virtually pathognomonic of the disease.

Analysis of acylcarnitines and acylglycines has been found to be particularly helpful in the identification of inherited fatty acid oxidation defects.[47,128,129] Analysis of urinary acylcarnitines is diagnostically reliable only if the patient has adequate stores of carnitine. Because carnitine depletion is a common feature of the fatty acyl-CoA dehydrogenase deficiencies, some have recommended that acylcarnitine analysis be done on urine collected 4 to 8 h after a loading dose of L-carnitine (100 mg/kg body weight).[37] Analysis of urinary acylglycines is technically more cumbersome, but may be more sensitive in the detection of MCAD deficiency and other fatty acid oxidation defects. Phenylpropionate and derivatives are probably products of intestinal bacterial metabolism that are normally absorbed and further metabolized by mitochondrial beta-oxidation. Some have recommended preliminary phenylpropionate loading to increase the sensitivity of the detection of MCAD deficiency by analysis of urinary acylglycines.[50]

Analysis of organic acids in plasma or dried blood spots is also helpful in the investigation of asymptomatic patients, or if the patient has died and no urine is available for analysis. Accumulation of the medium-chain, unsaturated, ten-carbon, fatty acid, *cis*-4-decenoic acid in plasma has been considered to be particularly characteristic of MCAD deficiency.[130–134] In many populations, a single point mutation, an A to G transition at nucleotide position 985 of the cDNA producing a substitution of glutamate for lysine at codon 329,[135] accounts for over 90% of the MCAD mutations.[136–139] Molecular testing for the presence of this common mutant allele is helpful confirmatory evidence for MCAD deficiency, and it is useful in those cases as a method for accurate carrier detection.

2. Long-Chain Acyl-CoA Dehydrogenase (LCAD) Deficiency

In patients with LCAD deficiency, dicarboxylic aciduria includes excretion of C-12 and C-14 dioic acids in addition to adipate, suberate, and sebacate.[140,141] Plasma carnitine levels are decreased and the proportion of carnitine that is esterified is increased; analysis of urine or plasma during acute metabolic decompensation shows the presence of long-chain acylcarnitines. The clinical course in many patients with LCAD deficiency may be indistinguishable from that associated with MCAD deficiency or Reye's syndrome. Acute encephalopathy (vomiting, lethargy, stupor, coma), hypoketotic hypoglycemia, hepatomegaly, and hypotonia are common. On the other hand, the onset of disease is generally earlier in LCAD deficiency than in MCAD deficiency, the myopathy is more severe, and cardiomyopathy, which is virtually never seen in MCAD deficiency, is prominent.[122,142]

3. Short-Chain Acyl-CoA Dehydrogenase (SCAD) Deficiency

SCAD deficiency is much less common than MCAD or LCAD deficiency. Among the small number of patients who have been reported, chronic skeletal

muscle weakness, cardiomyopathy, neutropenia, metabolic acidosis, failure to thrive, and developmental delay have been described.[143–145] In an adult with lipid myopathy, ethylmalonic aciduria, and secondary carnitine deficiency, SCAD deficiency was restricted to muscle; activity in cultured skin fibroblasts was normal.[146]

Hypoglycemia has been reported in some infantile patients with SCAD deficiency, but ketogenesis is apparently normal. The urinary organic acid patterns in SCAD deficiency is characterized by the presence of variable amounts of ethylmalonate, methylsuccinate, and adipate;[98,143,145] butyrylglycine and butyrylcarnitine are also present in the urine in some. Dicarboxylic aciduria is not a prominent feature of the disease. The urinary organic acid pattern seen in patients with demonstrated SCAD deficiency has also been seen in patients with normal enzyme activity; definitive diagnosis requires analysis of enzyme activity in muscle, as well as in fibroblasts, with precautions taken to ensure that the SCAD deficiency is not obscured by hydrolysis of substrate by MCAD.[98]

4. 3-Hydroxydicarboxylic Aciduria

3-Hydroxydicarboxylic acids are intermediates in the mitochondrial beta-oxidation of fatty acids. Intramitochondrial fatty acyl-CoA esters are dehydrogenated to Δ^2 unsaturated derivatives in reactions catalyzed by three fatty acyl-CoA dehydrogenases, with substrate specificities based on the fatty acid chain length, short (e.g., butyric acid), medium (e.g., octanoic acid), and long (e.g., hexadecanoic acid). Further oxidation of the Δ^2 intermediates is catalyzed by two trifunctional enzymes, short- and long-chain enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase/3-oxoacyl-CoA thiolase.[147,148] Accumulation of long-chain (C-12, C-14) 3-hydroxydicarboxylic and monocarboxylic acids in the urine occurs in patients with specific deficiency of LCHAD activity. The clinical course of this disease is variable. Patients most commonly present in early infancy with episodes of hypoketotic hypoglycemia, hepatocellular dysfunction, skeletal myopathy, and cardiomyopathy.[149–154] However, it may also present somewhat later, at 12 to 24 months of age, in a form in which the skeletal and cardiac myopathy dominate,[155] in some cases in association with peripheral neuropathy.[156,157] This group of disorders is particularly difficult to diagnose because the organic aciduria is characteristically intermittent; the urinary organic acid pattern is generally normal between episodes of acute, metabolic decompensation, and even during symptomatic episodes the long-chain 3-hydroxydicarboxylic aciduria is rarely as obvious as the abnormalities seen in infants with MCAD deficiency. Patients affected with combined deficiency of all three activities of the trifunctional protein, who were clinically indistinguishable from many with specific deficiency of LCHAD activity, have been described:[158] the urinary organic acid profile in these cases was quite

benign, apparently showing only small amounts of ethylmalonic, adipic, and suberic acids.

Recently, deficiency of LCHAD activity has been implicated in the pathogenesis of acute fatty liver of pregnancy.[159,160] Women who are heterozygous for LCHAD deficiency would appear to be at high risk for the development of severe, acute fatty liver while pregnant with a fetus homozygous for the disease. The clinical differences between patients with LCHAD deficiency and those with MCAD deficiency, together with the association of partial deficiency of LCHAD activity with acute fatty liver of pregnancy, indicates that disease in patients with 3-hydroxyacyl-CoA dehydrogenase deficiency is not simply due to deficiency of fatty acid oxidation; the tissue distribution of the pathology and the clinical severity of the condition suggest that accumulation of some, as yet unidentified, metabolic intermediate results in severe, tissue-specific cytotoxicity.

Deficiency of short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) would appear to be extremely rare. The only reported case was a teenaged girl with a history of muscle weakness, intermittent rhabdomyolysis, encephalopathy, and dilated cardiomyopathy.[161] Organic aciduria was only intermittent and not clearly related to the clinical severity of her disease. The pattern resembled the organic aciduria seen in patients with LCHAD deficiency.

We and others have encountered a significant number of sick infants, including many with hepatocellular disease, in whom increased levels of 3-hydroxydodecanedioic acid were found in the urine during the acute phase of their disease, but subsequently disappeared with resolution of the underlying hepatopathy, and analysis of LCHAD activities was normal. Long-chain 3-hydroxydicarboxylic and monocarboxylic aciduria have also been reported as a secondary phenomenon in some patients with type III glycogen storage disease[162] and in some with mitochondrial respiratory chain defects.[163]

The interpretation of long-chain 3-hydroxydicarboxylic and monocarboxylic aciduria is difficult. Although the clinical presentation and course may suggest strongly the presence of a hereditary defect in mitochondrial fatty acid beta-oxidation, confirmation requires specific analysis of the relevant enzymes. Cultured skin fibroblasts are generally suitable for these analyses.

D. Aromatic Organic Aciduria

1. Phenylpyruvic Aciduria (and Phenyllactic Aciduria)

Phenylpyruvic and phenyllactic acids are derived from the transamination of phenylalanine; high concentrations of the compounds in urine were first identified in one of the most widely recognized chemical abnormalities occurring in phenylketonuria (PKU) due to hereditary phenylalanine hydroxylase deficiency. The diagnosis of the disease is made by demonstrating increased levels of phenyl-

alanine in plasma and ruling out primary defects of the metabolism of the pteridine cofactor, tetrahydrobiopterin.

2. Phenylacetic Aciduria

The bulk of the phenylacetate in urine is derived from bacterial metabolism and absorption from the gut. Phenylacetate accumulates as a minor component in the urine of patients with PKU. Excess excretion also occurs in patients with severe hepatocellular disease.

3. 4-Hydroxyphenylpyruvic Aciduria (and 4-Hydroxyphenyllactic Aciduria)

4-Hydroxyphenylpyruvic acid (4-HPPA) is derived from the transamination of tyrosine. Accumulation occurs as a result of a deficiency of 4-hydroxyphenylpyruvic acid dioxygenase that catalyzes the conversion of 4-HPPA to homogentisic acid. The reaction requires the presence of ascorbic acid and involves the concerted hydroxylation of the aromatic ring, migration of the side chain, and oxidation and decarboxylation of the side-chain pyruvate to acetate.[164] Partial deficiency of the enzyme is common in premature infants, apparently as a result of developmental immaturity. This results in transient hypertyrosinemia that is associated with excretion of large amounts of 4-hydroxypyruvate in the urine and elevated levels of tyrosine and phenylalanine in the plasma. Apart from other possible complications of prematurity, infants with transient tyrosinemia of the newborn are generally well. Treatment with large doses of ascorbic acid for several days usually results in resolution of the organic aciduria.

Hepatocellular dysfunction, irrespective of etiology, is another common cause of 4-hydroxyphenylpyruvic aciduria. In infants, differentiation of type I hereditary tyrosinemia from other causes of severe hepatocellular disease may be difficult. In severe liver disease, plasma tyrosine and phenylalanine concentrations are generally increased, and excretion of 4-hydroxyphenylpyruvate and other tyrosine metabolites may be massive. However, in type I hereditary tyrosinemia, the plasma alpha-fetoprotein level is markedly elevated and the urinary organic acid analysis shows the presence of succinylacetone, a compound that does not occur in the urine under any other circumstances (see above).

4-Hydroxyphenylpyruvic aciduria also occurs in primary hereditary deficiency of 4-HPPA dioxygenase, classified as hereditary tyrosinemia type III, an extremely rare disease. It is characterized clinically by mild mental retardation and intermittent ataxia.[165,166] Hereditary deficiency of cytosolic tyrosine transaminase, classi-

fied as type II hereditary tyrosinemia (also called Richner-Hanhart syndrome or occulocutaneous tyrosinemia), is characterized by corneal erosions, opacification, and scarring, along with painful blistering and hyperkeratosis of the palms and soles. Mental retardation is an inconstant finding.[167]

4. Homogentisic Aciduria

Homogentisic acid, derived from the metabolism of phenylalanine and tyrosine, accumulates and is excreted in the urine of patients with alkaptonuria caused by a primary deficiency of homogentisic acid oxidase.[168] The urine of patients with the condition characteristically turns dark on exposure to air and light. The condition is relatively benign, although it is associated with late-developing degenerative joint disease as a result of the effects of homogentisic acid on collagen cross-linking.

5. Xanthurenic Aciduria

Xanthurenic acid is derived metabolically from the amino acid, 3-hydroxykynurenine, an intermediate in tryptophan metabolism. Kynureninase is a pyridoxine-dependent enzyme that catalyzes the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid; pyridoxine deficiency is associated with increased excretion of kynurenine, 3-hydroxykynurenine, and xanthurenic acid in response to tryptophan loading. Xanthurenic aciduria due to hereditary pyridoxine-responsive kynureninase deficiency has been reported.[169] A pyridoxine-resistant defect in the same enzyme appears to be the cause of hydroxykynureninuria in which xanthurenic aciduria also occurs.[170] Although some of the original patients with xanthurenic aciduria were mentally retarded, the metabolic abnormality may be clinically benign.

E. Lactic Aciduria

The occurrence of increased quantities of lactic acid in the urine is one of the most common abnormalities encountered in the analysis of urinary organic acids.

1. Acquired Disorders of Lactate Metabolism

Systemic lactic acidosis is a feature of a number of acquired disorders, and in most of these situations, the presence and nature of the underlying condition are obvious (Table 2). Overall, lactic acidosis resulting from tissue hypoxia, termed type A lactic acidosis by Cohen and Woods,[171] is probably the most

TABLE 2
Clinical Classification of Lactic Acidosis

Acquired	Inborn errors of metabolism
Hypoxemia	**Primary**
Circulatory collapse	**Defects of pyruvate metabolism**
Shock	PDH deficiency
Congestive heart failure	Pyruvate carboxylase deficiency
Severe systemic disease	PDH phosphatase deficiency
Liver failure	**Defects of NADH oxidation**
Kidney failure	Mitochondrial electron transport defects
Diabetic ketoacidosis	**Secondary**
Acute pancreatitis	**Disorders of gluconeogenesis**
Acute leukemia	Glycogen storage disease, type 1
Intoxication	Hereditary fructose intolerance
Ethanol	PEP-carboxykinase deficiency
Methanol	Fructose-1,6-diphosphatase deficiency
Ethylene glycol	**Fatty acid oxidation defects**
Oral hypoglycemic drugs	**Defects of biotin metabolism**
Acetylsalicylic acid	Biotinidase deficiency
Nutritional deficiency	Holocarboxylase synthetase deficiency
Thiamine deficiency	**Defects of organic acid metabolism**
	HMG-CoA lyase deficiency
	Propionic acidemia
	Methylmalonic acidemia
	Other organic acidopathies

common cause of lactic aciduria, although it is also a feature of many severe systemic diseases in which tissue perfusion appears to be adequate. Lactic acidosis due to hypoxemia is generally reversed rapidly by correction of the hypoxic state; lactic acidosis attributable to inborn errors of metabolism is generally more resistant to treatment.

When the lactic acidosis is associated with systemic disease, diagnostic difficulty may occur when the underlying condition, such as severe parenchymal liver disease, is itself due to an inherited metabolic disease, such as a fatty acid oxidation defect, hereditary tyrosinemia, or some primary disorders of lactate metabolism. Lactic acidosis is a specific feature of some intoxications, such as salicylism, acute ethanol or methanol intoxication, or ingestion of ethylene glycol. It is also encountered in malnourished infants treated with high carbohydrate infusions[172] and in patients on total parenteral nutrition (TPN).[173]

2. Inborn Errors of Lactate Metabolism

Inborn errors of metabolism account for a relatively small fraction of all patients with lactic acidosis, but a high proportion of those in whom the acidosis is persistent.[59] Lactate is derived from the NADH-dependent reduction of pyruvate, catalyzed by the enzyme lactate dehydrogenase (LDH).[174] The reaction is freely

reversible, and in the presence of adequate concentrations of NAD^+/NADH rapidly reaches thermodynamic equilibrium. Lactate is also a "dead-end" metabolite, meaning that metabolic removal occurs only through its precursor, pyruvate. The steady-state concentration of lactate in any given metabolic compartment is, therefore, directly proportional to the concentrations of pyruvate, NAD^+, and NADH. Inborn errors of metabolism associated with lactic acidosis are generally classifiable into those due to accumulation of pyruvate, those attributable to accumulation of NADH, or those in which the defect results in accumulation of both. Accumulation in either case may be the result of a defect in removal, of accelerated production of one or other of the compounds, or of both.

Pyruvate accumulation as a result of a defect in pyruvate removal is the direct cause of the lactic acidosis occurring in patients with primary disorders of pyruvate oxidation, pyruvate carboxylase deficiency, PDH deficiency, and PDH phosphatase deficiency. The mechanism of the pyruvate accumulation in inborn errors of metabolism associated with secondary lactic acidosis, such as the disorders of gluconeogenesis, is less clear and probably involves both increased pyruvate production and decreased removal. Irrespective of the cause, lactic acidosis associated with pyruvate accumulation is accompanied by increased concentrations of the amino acid, alanine, in plasma, owing to reversible transamination of pyruvate.

Lactic acidosis due to NADH accumulation is a feature of the inherited disorders of mitochondrial electron transport.[59] In patients with electron transport defects, pyruvate production is also increased, but the increase is modest compared with the accumulation of NADH. As a result, the lactate-to-pyruvate ratio in the blood of patients with these disorders is characteristically increased. In patients with mitochondrial electron transport defects, the degree of the lactic acidosis is variable but rarely as severe as is encountered in patients with primary disorders of pyruvate metabolism.

Irrespective of the cause, severe lactic acidosis is often accompanied by excretion of small to moderate amounts of 2-hydroxybutyrate[175] possibly derived from methionine, homocysteine, homoserine, and threonine. The occurrence of the compound in the urine under these circumstances is not due to a primary defect of 2-hydroxybutyrate metabolism.

3. D-Lactic Aciduria

The detection of increased concentrations of lactic acid by physicochemical analytical techniques, such as GC, in patients with metabolic acidosis but normal blood lactate levels, as measured by commonly employed enzymatic techniques, suggests that the accumulated acid is D-lactate, a bacterial metabolite accumulating in patients with bowel stasis.[176,177] Accumulation of the compound has been sufficient in some cases to cause encephalopathy.[178,179]

F. Ketonuria

Ketonuria (excretion of increased concentrations of acetoacetate and 3-hydroxybutyrate) is a reflection of increased fatty acid oxidation, whether as part of the normal physiological response to fasting or due to primary or secondary defects in glucose oxidation. To the extent that the metabolic abnormalities in many inherited disorders of organic acid metabolism include secondary defects in glucose utilization, increased excretion of acetoacetate and 3-hydroxybutyrate is a common characteristic of conditions like maple syrup urine disease and methylmalonic and propionic acidemia. Ketosis, irrespective of cause, is also associated with increased excretion of the dicarboxylic acids, adipate, suberate, and sebacate.

Massive ketonuria as a result of a primary defect in ketone utilization occurs in mitochondrial acetoacetyl-CoA thiolase (β-ketothiolase) deficiency. Affected patients are often completely well between intermittent episodes of metabolic decompensation, generally precipitated by intercurrent infection or fasting. Metabolic decompensation is signaled by the onset of intractable vomiting, lethargy, somnolence, and tachypnea associated with hypoglycemia, metabolic acidosis, and massive ketosis. Urinary organic acid analysis shows the presence of 2-methylacetoacetate and 2-methyl-3-hydroxybutyrate in addition to very large amounts of 3-hydroxybutyrate and acetoacetate.

G. 5-Oxoprolinuria

5-Oxoprolinuria (pyroglutamic aciduria), due to glutathione synthetase deficiency, is a rare inherited metabolic condition that may present in early infancy as persistent or acute metabolic acidosis associated with chronic hemolytic anemia.[180] In older patients, the disease may present as acute metabolic decompensation on a background of mental handicap, spinocerebellar degeneration, peripheral neuropathy, and myopathy. In some individuals, the condition is completely asymptomatic. Oxoprolinuria has also been associated with acetaminophen ingestion,[181] and with treatment with the new anticonvulsant drug, vigabatrin.[182]

H. Mevalonic Aciduria

Mevalonic acid is a central intermediate in the biosynthesis of the isoprenoid precursors of cholesterol, ubiquinone, and the dolichols. Massive mevalonic aciduria occurs in patients with mevalonate kinase deficiency,[183] a rare autosomal recessive disease characterized in severely affected infants by developmental delay, dysmorphic features, cataracts, hepatosplenomegaly, anemia, and intestinal malabsorption with failure to thrive. Patients with less severe forms of the disease show

mental retardation, myopathy, ataxia, and cerebellar atrophy; all patients experience recurrent episodes of fever, lymphadenopathy, arthralgia, edema, and skin rash associated with marked elevation of the erythrocyte sedimentation rate. Urinary organic acid analysis reveals marked mevalonic aciduria. Plasma ubiquinone-10 levels are generally decreased, but cholesterol levels are normal. Plasma leukotriene E_4 levels are uniformly increased.

I. Hawkinsinuria

Hawkinsin [(2-L-cystein-*S*-yl-1,4-dihydroxycyclohex-5en-1-yl)-acetic acid] is derived from 1,4-dihydroxycyclohexylacetic acid, an apparently transient intermediate in the enzymic oxidation of 4-HPPA to homogentisic acid.[184] Hawkinsinuria is a rare organic acidopathy that appears to be transmitted as an autosomal dominant condition.[185] It presents in infancy with persistent metabolic acidosis associated with marked failure to thrive. The urine of affected patients contains 4-hydroxycyclohexylacetic acid in addition to hawkinsin. Treatment with a reduced dietary protein intake supplemented with ascorbic acid results in a dramatic reversal of symptoms.

V. SECONDARY ORGANIC ACIDURIAS AND CARNITINE DEFICIENCIES

A number of genetic and acquired conditions, including drugs and diet, are associated with excretion of abnormal organic acids. Some are also associated with symptomatic secondary carnitine depletion.

A. Drugs

1. Valproic Acid

Valproate (dipropylacetate [VPA]) is an eight-carbon branched-chain fatty acid that is a commonly used antiepileptic drug in children and adults. It is metabolized mainly in the liver and undergoes biotransformation by several metabolic routes. Beta-oxidation and glucuronidation are predominant, whereas omega and omega-1 oxidation are minor pathways.[186] Valproate is activated to its CoA ester and is metabolized extensively in mitochondria and peroxisomes.[187] In humans, 75% of the VPA administered orally is excreted as valproyl glucuronide within 24 h; the remainder is eliminated in urine in the form of valproylcarnitine and other metabolites.[188,189]

Valproate interferes with both branched-chain amino acid and fatty acid metabolism. In patients receiving VPA, the excretion of the deaminated metabolites of leucine, isoleucine, and valine was significantly increased.[190] The authors suggested that this may have been due to inhibition of the branched-chain acyl-CoA dehydrogenase. Total ketone body production and 3-hydroxybutyrate levels are also decreased following VPA, there is often dicarboxylic aciduria, and secondary carnitine deficiency is common.[191-193] Urinary excretion of both free and acylcarnitine is increased, accompanied by decreased reabsorption of free carnitine. This results in low carnitine levels in the blood, muscle, and liver. Some of the minor metabolites of VPA, particularly 4-en-VPA, have been implicated in the hepatotoxic effects of the drug.[194] It has been suggested that 4-en-VPA may be capable of generating free radicals and cause lipid peroxidation injury in hepatocytes, which may be one of the possible mechanisms of irreversible hepatic injury associated with VPA treatment.[195]

The widespread use of valproate revealed a small population of patients who are sensitive to VPA-induced hepatic injury that manifests as hepatocellular microvesicular steatosis, and may result in irreversible liver damage and death. The incidence of patients suffering irreversible hepatic injury and death was highest (1 in 500) in children under 2 years of age who were receiving the drug as part of a multidrug regimen.[196] The incidence of severe adverse reactions to VPA declines with age, and may be as low as 1 in 35,000 in otherwise healthy well-nourished adults who are being treated with VPA alone.[193]

There are also dose-related reversible abnormalities associated with valproate administration that normalize after reduction or discontinuation of the drug. A complex organic acid pattern has been observed in patients being treated with this drug. Organic acids include valproyl-glucuronide, unmetabolized VPA, 2-*n*-propylglutarate, 2-*n*-propylsuccinate, 3-, 4-, and 5-hydroxy-VPA, as well as 3-oxo-VPA and 4-oxo-VPA. Mono-unsaturated (4-en-VPA) as well as doubly unsaturated (2,4-dien-VPA) have also been detected.[197] VPA and its metabolites induce both hyperglycinemia and hyperglycinuria.[198,199] In VPA-treated patients the excretion of both acylcarnitines and acylglycines is elevated and abnormal.[200,201] VPA causes metabolic abnormalities that resemble those found in inborn errors of mitochondrial beta-oxidation, which are not corrected by L-carnitine.

The drug also inhibits urea synthesis and causes hyperammonemia both in humans and experimental animals.[202,203] In the absence of hepatic dysfunction, these metabolic changes are reversible side effects of VPA therapy.

2. Pivalate

Pivampicillin and pivmecillinam are commonly prescribed antibiotic precursors that undergo rapid enzymatic hydrolysis to the respective antibiotics, ampicillin and methecillin, and the xenobiotic free drug, pivalate. Pivalate undergoes esteri-

fication and is excreted as the acylcarnitine.[204] We have also detected pivaloyl glycine and pivaloyl glucuronide in the urine of patients on pivampicillin. The increased excretion of acylcarnitine results in secondary carnitine deficiency with subnormal serum carnitine levels. Tissue levels may be decreased to 10% of normal in patients on long-term treatment with the drug.[205] Patients with carnitine deficiencies have a reduced ability to metabolize fatty acids, and dicarboxylic aciduria is commonly observed. The pivaloylcarnitine in urine from patients on pivampicillin cannot be differentiated either by FAB-MS or FAB-MS-MS from isovalerylcarnitine or other five-carbon acylcarnitines.

3. Etomoxir

Etomoxir, 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate, is a candidate antidiabetic drug.[206] Substituted 2-oxiranecarboxylic acids such as etomoxir have several metabolic effects as a consequence of inhibition of long-chain fatty acid oxidation in mitochondria. Etomoxir is hypoglycemic in fasted animals and its CoA ester strongly inhibits mitochondrial beta-oxidation at the level of CPT I.[207] In experimental animals etomoxir has also been shown to cause significant dicarboxylic aciduria.[208] This dicarboxylic aciduria resembles that caused by the administration of hypoglycin or methylenecyclopropylglycine, inhibitors of mitochondrial beta-oxidation.[209,210] Peroxisomal beta-oxidation was unaffected.[205]

B. Diet

1. Food Constituents and Toxins

Food additives and naturally occurring constituents of some food items may give rise to organic aciduria as a result of the appearance of the material itself in the urine, or owing to the secondary metabolic effects of the compound.

Adipic acid is a common additive in Jello. This gives rise to an isolated adipic aciduria that is asymptomatic and is not associated with any inborn error of metabolism. The organic aciduria disappears rapidly when the responsible food item is removed from the diet.

Ingestion of hypoglycin, a plant toxin present in the unripe fruit and seeds of ackee (*Blighia sapida*), an edible plant, is the cause of Jamaican vomiting sickness, a disease characterized by vomiting, severe metabolic acidosis and hypoglycemia, accompanied by depletion of glycogen. Hypoglycin is converted to 3,4-methylene-pent-4-enoyl-Coenzyme A, which is a specific inhibitor of isovaleryl-CoA dehydrogenase,[211] and intoxication is associated with organic aciduria similar to that seen in isovaleric acidemia caused by isovaleryl-CoA dehydrogenase deficiency. However, in addition, the organic acid pattern in hypoglycin-poisoned patients

suggests that the toxin inhibits a number of dehydrogenases involved in glutarate, fatty acid, lysine, isoleucine, and valine metabolism.[212,213]

2. Octenylsuccinate

Octenylsuccinic acid (1,2-dec-4-ene-dicarboxylic acid [OSA]) is a component of one form of modified cornstarch. OSA-modified cornstarch has been used for over 20 years in puddings and sauces, but since 1987 it has been incorporated into several elemental infant formulas. An unusual pattern of organic acids in children whose diet included OSA-modified starch was subsequently shown to be due to the presence of OSA and its metabolites.[214–216] OSA is a branched-chain organic acid that is metabolized by a combination of microsomal omega-1 and omega-oxidation and mitochondrial and peroxisomal beta-oxidation. Like VPA, OSA has secondary effects on oxidative metabolism associated with increased concentrations of glutaric acid and 2-ketoglutarate in the urine.[215] In several instances, this has led to confusion with primary glutaric aciduria. While VPA is known to form carnitine esters and lead to secondary carnitine deficiency (see above), no evidence has been published to date to indicate that OSA has similar effects on carnitine metabolism.

3. MCT

MCT containing fatty acids of 6 to 12 carbons are metabolized differently from long-chain triglycerides. MCT enter the portal circulation directly and once hydrolyzed, medium-chain fatty acids enter mitochondria where they undergo beta-oxidation, independent of the highly regulated carnitine acyltransferase transport system.[217] MCT is widely used as a nutritional supplement in infants and children with intestinal malabsorption or failure to thrive. Ingestion of MCT-enriched formulas is associated with the excretion of increased concentrations of 5-hydroxyhexanoate, 7-hydroxyoctanoate, adipate, suberate, and sebacate.[218,219] In adult diabetic subjects treated with MCT, suberic and 7-hydroxyoctanic acid excretion was increased 55- and 30-fold, respectively.[220] The dicarboxylic acids excreted during ketosis, or as a result of a fatty acid oxidation defect, usually include more adipic, less suberic, and even less sebacic and 3-hydroxysebacic acids. In the urine of patients on MCT-enriched diets, the levels of sebacic acid are particularly high and the levels of adipic and suberic acids are low. Some commercial formulas contain different ratios of medium-chain fatty acids, resulting in a dicarboxylic aciduria with prominent suberate excretion, and thus different from that described above.[221] The excretion of 5-hydroxyhexanoate and 7-hydroxyoctanoate is also increased in infants and children on MCT formulas. Kuhara *et al.*[222] also reported the presence of octanoyl glucuronide in the urine of children on MCT-enriched formulas. MCT-enriched diets also might interfere in the diagnosis

of fatty acid oxidation defects. Patients receiving MCT excreted greater amounts of octanoylcarnitine and hexanoylcarnitine in their urine than normals.[200] To correctly interpret the results of acylcarnitine analysis, a history of recent MCT administration must be considered before a diagnosis can be made. The organic aciduria in patients being treated with MCT consists mostly of saturated C-6 to C-10 dicarboxylic acids, 5-OH-hexanoate, and 7-OH-octanoate. In dicarboxylic acidurias caused by fatty acid oxidation defects or secondary carnitine deficiencies, unsaturated dicarboxylic acids are also found in addition to the saturated and hydroxydicarboxylic acids. This may also be useful in differentiating the various causes of dicarboxylic aciduria.

4. Vitamin Deficiency

Dietary deficiency of cobalamin (vitamin B_{12}), an obligatory cofactor in the metabolism of methylmalonyl-CoA to succinyl-CoA and in the conversion of homocysteine to methionine, is associated with methylmalonic aciduria, although the levels are rarely as high as are seen in patients with inherited defects of cobalamin metabolism or methylmalonic acidemia due to mutase mutations[223–225] (see above). The principal dietary sources of vitamin B_{12} are meat, eggs, dairy products, and some fermented foods. These are the food items avoided by strict vegetarians, who are at high risk for deficiency and associated methylmalonic aciduria. Breast-fed infants of vegetarian mothers are at particularly high risk;[226–228] in the course of a single year, we encountered two such breast-fed infants presenting with clinically significant metabolic acidosis due to methylmalonic acid accumulation owing to subclinical maternal vitamin B_{12} deficiency.

Riboflavin is the electron transfer prosthetic group of flavin adenine dinucleotide (FAD)-dependent acyl-CoA dehydrogenases involved in the mitochondrial beta-oxidation of fatty acids.[6] In experimental animals, nutritional riboflavin deficiency is associated with a characteristic dicarboxylic aciduria.[229,230] Breast-fed neonates receiving phototherapy for the treatment of hyperbilirubinemia have been shown to be at high risk for riboflavin deficiency;[231,232] however, studies of urinary organic acid excretion in full-term neonates with phototherapy-induced riboflavin deficiency yielded normal patterns.[233] Premature neonates may be at high risk for metabolically significant riboflavin deficiency owing to reduced dietary intake, increased severity and duration of jaundice, making prolonged phototherapy more likely, and greater penetration of the skin by the ultraviolet radiation responsible for the degradation of riboflavin. More studies of this problem are required to determine its clinical significance.

Pyridoxine deficiency is reported to cause hyperoxaluria.[109,110] However, we have never encountered this in several thousand organic acid analyses carried out over several years. Thiamine deficiency is a documented cause of severe, often

life-threatening lactic acidosis, particularly in infants receiving TPN containing high proportions of carbohydrate.[172,234]

5. TPN

Carnitine stores in muscle in term neonates have been shown to be significantly lower than those in muscle from adults, and the stores in premature infants are drastically lower.[235] TPN with nutrient solutions containing no supplemental carnitine, for as short as 5 d, has been shown to result in a 50% decrease in plasma carnitine levels.[236] Not until age 12 years are children who receive carnitine-free TPN able to maintain normal plasma carnitine concentrations.[237] Carnitine excretion in both children and adults on long-term TPN appears to be reduced to conserve body stores.[238,239] However, during stress-induced catabolism such as during the immediate postoperative period,[240] following multiple injuries,[241] during sepsis, and after burns,[242] plasma carnitine levels may be below normal. Plasma concentrations of carnitine do not reflect tissue stores, which may be more depleted than the plasma levels would suggest. The metabolic effects of TPN-induced carnitine deficiency are not well understood. Whether TPN-associated carnitine deficiency is of sufficient magnitude to induce organic acid disturbances and dicarboxylic aciduria is not known and has not been explored in sufficient detail to date.

C. Contributions of Bacterial Metabolism in the Gut

In healthy individuals, bacterial fermentation of dietary polysaccharides and protein reaching the large gut gives rise to potentially toxic short-chain fatty acids and other low-molecular-weight organic acids that are rapidly absorbed in the colon and metabolized in the liver.[243–246] Administration of short-chain fatty acids induces coma in experimental animals and it may be important in the pathogenesis of hepatic coma in humans.[247,248] Intestinal bacteria are the source of a significant proportion of the urinary propionic acid and its metabolites in patients with propionic acidemia due to PCC deficiency,[249,250] and of urinary methylmalonic acid in primary methylmalonic acidemia.[251] Treatment of affected patients with metronidazole results in significant decreases in the excretion of these organic acids.

Unabsorbed phenylalanine is degraded by gut bacteria to 3-phenylpropionic acid (PPA), which is normally esterified with CoA in the liver and oxidized to benzoyl-CoA in a reaction catalyzed by mitochondrial MCAD.[252] Benzoyl-CoA is rapidly converted to hippuric acid by condensation with glycine and is excreted in the urine. The central role of MCAD in the metabolism of PPA has been exploited in the diagnosis of MCAD deficiency by analysis of urinary acylglycines[46] (see

above). As pointed out under Methodology, screening for MCAD deficiency by urinary 3-phenylpropionylglycine analysis may not be reliable in early infancy or following treatment with certain antibiotics, which are both associated with major alterations in gut flora.

Severe metabolic acidosis with excretion of large amounts of lactic acid in the urine has been encountered in patients with conditions associated with bowel stasis;[176,177,253–256] in some cases, it has been associated with severe metabolic encephalopathy mimicking an inborn error of metabolism.[178,179] Patients with this condition have plasma lactate levels, as measured enzymatically, that are normal or well below what might be expected on the basis of the high concentrations of lactate in the urine. The excess lactate in urine is due to the accumulation of D-lactate, a product of bacterial carbohydrate metabolism that is not distinguishable from endogenous L-lactate by GC-MS. The discrepancy between L-lactate, determined enzymatically, and total lactate, determined by GC-MS, provides the clue to the bacterial origin of the compound in these patients. Treatment by diet or antibiotics results in resolution of the acidosis and elimination of the excretion of excess lactate in the urine.

Acute gastroenteritis is a common medical problem in infants and children. Abnormal urinary excretion of methylmalonic, ethylmalonic, and 3-hydroxypropionic acids has been reported recently in two neonates with viral gastroenteritis.[257] After treatment and resolution of the diarrhea, repeat organic acid analyses were normal at 12 and 14 weeks of age, and the infants were developing normally. The authors suggested that the abnormal metabolites may be due to bacterial metabolism in patients with underlying enteric disease.

D. Prematurity

The urinary organic acid pattern of infants born before 33 weeks gestation is different from the pattern in term babies.[258] The differences are accountable in part by the relative immaturity of tyrosine metabolism in the liver and solute reabsorption in the kidney. Metabolites of tyrosine and phenylalanine are commonly found in the urine of preterm infants as the combined result of immaturity of 4-hydroxyphenylpyruvic acid dioxygenase, and from the products of gut bacterial metabolism. The urine of healthy preterm infants also contains increased concentrations of lactate, a variety of TCA cycle intermediates, such as fumarate, aconitate, succinate, 2-hydroxyglutarate, and citrate. In addition, many excrete increased amounts of the ketone precursor, 3-hydroxy-3-methylglutaric acid, which is not normally found in the urine of older children or adults; 3-hydroxybutyrate and acetoacetate levels are generally low or absent. Many samples of urine from premature infants contain adipic and suberic acids, but only trace amounts of sebacic acid. This pattern of dicarboxylic aciduria was attributed to the increased utilization of fat for energy production in newborn infants. We, as well as others,[103]

have observed the presence of trace to small amounts of ethylmalonic acid in the urine of a number of healthy neonates. A few infants also excrete small amounts of methylmalonic acid, along with ethylmalonic acid, 2,3-butanediol, and acetoin, presumed to be derived from bacterial carbohydrate fermentation in the gut.[54]

E. Renal Failure

The kidney is a major site of carnitine biosynthesis from trimethyllysine.[259] The kidney also plays a key role in the conservation of body carnitine stores, over 90% of filtered carnitine being reabsorbed in a threshold-dependent manner. The renal clearance of carnitine esters is four to eight times greater than that of free carnitine; metabolic circumstances characterized by increased acylcarnitine formation are also associated with increased carnitine losses in the urine. In patients undergoing chronic hemodialysis, low plasma carnitine levels are commonly observed, and the ratio of acylcarnitine to unesterified carnitine in plasma is increased,[260] possibly as a result of a disproportionate loss of free carnitine. Patients with secondary carnitine deficiency due to excessive renal loss of carnitine may have reduced ability to metabolize fatty acids, and dicarboxylic aciduria may be present.

F. Birth Asphyxia

Abnormal organic acid patterns have been documented in infants who have suffered varying degrees of birth asphyxia immediately following the event and presumably due directly or indirectly to tissue hypoxia.[261] The changes include marked increases in urinary lactic and pyruvic acid levels, as well as increased excretion of 3-hydroxybutyrate, tyrosine metabolites (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, 4-hydroxyphenylacetate), and oxo- and hydroxy-acids derived from the oxidative degradation of the branched-chain amino acids.

VI. FUTURE PROSPECTS

One aspect of organic acid analysis that will probably become automated in the near future is sample preparation. Currently, most laboratories spend a significant amount of time extracting, purifying, and derivatizing organic acids from urine before a patient sample is ready to be injected into the GC-MS. Because many of the preanalytical preparative steps are performed manually, this area is ripe for automation. Recently, a number of automated liquid handling and robotic devices have become available that will make automated sample preparation a common practice. Some of these devices are capable of a variety of operations, including

solvent extraction, application of sample to solid phase extraction (SPE) columns, elution of SPE columns, heating, vortexing, evaporation under nitrogen, derivatization, etc. These are the steps that are required for most samples being prepared for GC-MS. In addition to the obvious labor-saving features, the advantages of automated sample preparation include more uniform and reproducible analytical results and less breakdown of the sample while it is waiting to be analyzed.

The development of either rule-based expert systems or neural network-type artificial intelligence programs using either laboratory data alone or preferably in combination with clinical information capable of providing a diagnosis is not available yet. This area is one where powerful and inexpensive desktop computers and the availability of software that can be used to develop such applications will likely result in the appearance of diagnostic programs that will be helpful for both laboratory workers and pediatricians interested in the diagnosis of inborn errors.

Another area where new developments may occur is in the use of stable isotopes in developing new and better tests for the diagnosis of inborn errors. Another area in which new technology might assist in the diagnostic process is in the application of noninvasive testing such as percutaneous analysis of abnormal blood constituents, or the analysis of saliva or breath for the detection of inborn errors.

VII. SUMMARY AND CONCLUSIONS

Organic acid analysis as a tool in the diagnosis of inborn errors of metabolism and also as an analytical technique for the study of organic acidurias secondary to drugs, diet, diseases, etc. has been in use for over 25 years. During this period, the instrumentation, the methods, the interpretation, and our understanding of the significance of changes in organic acid patterns have evolved significantly.

The study of organic acids in inborn errors of metabolism has made it possible to gain greater insights into some of the biochemical pathways by which amino acids and fatty acids are metabolized. In addition to inherited metabolic diseases, a large number of noninherited diseases, vitamin deficiencies, diet, bacterial metabolism in the gut, medications, food additives, etc. can cause abnormal organic acids to be excreted in the urine. These complicate the interpretation of organic acid patterns and require additional information regarding medications, diet, etc. before a correct diagnosis can be made. The increasing understanding of the role of carnitine and carnitine esters in organic acid metabolism has also contributed to our understanding of the production and removal of abnormal or toxic organic acids from the body.

New techniques in MS, the commercial availability of simple, relatively easy to operate, user-friendly mass spectrometers, as well as the development of new modes of ionization (LC-MS, MS-MS, FAB-MS-MS) that allow the study of molecules that could not be analyzed before, have been significant factors in

contributing to advances in this field. The advances in computer technology have also allowed a major part of the data analysis, storage, retrieval, and transformation of data to be automated.

Our present understanding of the abnormalities of organic acid metabolism and the significance of the excretion of specific organic acids has increased greatly and will continue to grow in the future as well.

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