Organic Acidurias

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Organic acidurias as a group of inherited metabolic disorders have been recognized for more than 20 years since the first description of a patient with methylmalonic acidemia. Until that moment the screening for inborn errors of metabolism was limited to groups of substances that could unambiguously be detected by simple staining reagents, such as ninhydrin for amino acids. Specific reagents for the detection of organic acids do not exist. Therefore, the introduction of gas chromatography combined with mass spectrometry (GC-MS) in the pediatric biochemical laboratory was necessary to reveal the various and exciting organic acidemias.

Many of the organic acids that accumulate in this type of disorder are relatively strong acids and will accordingly give rise to changes of the acid-base status of the blood plasma. The extent of the acid-base changes is highly dependent on the concentration of the organic acid, and it will be shown below that many patients with organic acidemia do not have a metabolic acidosis.

This chapter deals with organic acidurias that result from defects of the catabolism of amino acids and carbohydrates. Disorders of fatty acid β-oxidation leading to hypoketotic hypoglycemia are dealt with in the chapter by Bartlett (*this volume*), while defects in the metabolism of branched-chain amino acids are covered in Saulubray’s chapter. Some of the disorders of organic acid metabolism are accompanied by a specific hyperaminoacidemia, which could theoretically lead to their diagnosis by amino acid analysis. However, in several cases the analysis of organic acids gives direct and indispensable information, enabling their unequivocal diagnosis. A good example is tyrosinemia due to fumarylacetoacetase deficiency. For this reason tyrosinemia is included in this chapter. A complete list of the organic acidurias discussed here is shown in Table 1. Emphasis is placed on the “newer” defects and on those defects for which the diagnostic approach contains potential difficulties.

**ANALYTICAL CHEMICAL ASPECTS**

Urinary and plasma organic acids have to be analyzed by gas chromatography-mass spectrometry. This technique is highly selective and sensitive and will detect
TABLE 1. Organic acidurias not including the defects of branched-chain amino acid catabolism

1. 3-Methylglutaconic aciduria (two types)
2. Mevalonic aciduria
3. (Methyl)malonic semialdehyde dehydrogenase deficiency
4. Succinic aciduria
5. Fumaric aciduria
6. Malonic aciduria
7. Lactic acidemia due to fructose-1,6-diphosphatase deficiency
8. Disorders associated with ketosis
9. Glutaric aciduria type I
10. 4-Hydroxybutyric aciduria
11. β-Glyceric acidemia
12. Defect in 2-oxobutyric acid metabolism
13. N-Acetylaspartic aciduria
14. Fumarylacetoacetase deficiency

virtually all organic acidurias. Organic acids may display a wide range of physical properties; in particular, their hydrophilic character varies considerably. Knowledge of these properties is important for the choice of a method for isolation of these substances from biological material. A good overview is given by Chalmers and Lawson (1).

Isolation by extraction with an organic solvent such as ethyl acetate or diethyl ether is the most simple and direct approach. It has been stated many times that solvent extraction does not given 100% recovery for all acids, but it is our experience that reliable calibration curves can be made for each acid, provided that an authentic standard is available. Advantages over ion-exchange methods, such as those using DEAE-Sephadex, lie in the speed of the procedure and the less prominent interference from urinary substances such as inorganic salts. Solid-phase extraction techniques have not yet found wide applications; they may turn out to be promising, however.

With the exception of the volatile fatty acids, all organic acids have to be derivatized prior to gas chromatography. The method of choice nowadays includes protection of the ketoacids by (eth)oximation followed by trimethylsilylation. Despite the difficulties in interpreting the electron impact mass spectra of these derivatives, the ease of the overall procedure has convinced the majority of investigators to follow it. Glycine conjugates and related substances are better analyzed as their methyl esters. If one is really interested in detecting conjugated organic acids, one may apply alkaline hydrolysis of the sample followed by the standard analysis. Ion-exchange separations prior to the hydrolysis step allows the detection of pure hydrophilic—and thus nonextractable—compounds such as acylcarnitines. Phosphate esters such as glycerol-3-phosphate can be analyzed by GC-MS as their trimethylsilyl esters following isolation via anion-exchange procedures (e.g., Dowex-2, elution with formic acid).

Organic acidurias have the reputation of being associated with the urinary excretion of huge amounts of metabolites. Certainly this holds true for long-established
defects such as isovaleric acidemia and methylmalonic acidemia. On the other hand, it cannot be neglected that some organic acidurias are characterized by moderate (or even low) excretions of abnormal organic acids. In this respect we should like to point to 4-hydroxybutyric aciduria, tyrosinemia type I, and 3-methylglutaconic aciduria type II. Furthermore, it has been shown recently that patients with glutaric aciduria type I may also show extremely low glutarate excretions. These considerations require the pediatric laboratory not only to check for major abnormalities of organic acid excretion, but also to evaluate carefully the minor anomalies of the excretion profile.

Mass spectrometric identification of urinary organic acids on a routine basis can be achieved with a relatively simple quadrupole instrument (e.g., the bench-top version equipped for electron impact MS). For more detailed studies it is necessary to have more options disposable, such as chemical ionization and fast atom bombardment. Proton NMR-spectroscopy has been used successfully for the identification of urinary organic acids, with the additional advantage that extensive purification of the sample can be omitted. The complexity of the instrumentation will probably preclude its wide application in pediatric laboratories.

Interesting new observations can be made using whole body in vivo NMR-spectroscopy. This technique will reveal abnormal metabolite concentrations in various organs such as the brain. We have shown that patients with so-called cerebral lactic acidosis may vary in the actual site of lactate overproduction. Some of them show a generalized accumulation in the brain; other patients accumulate lactate in the basal ganglia region only (2). It can easily be foreseen that many more applications of this technique will be found in the next few years.

DESCRIPTION OF ORGANIC ACIDURIAS

3-Methylglutaconic Aciduria (Types I and II)

Only two brothers with a deficiency of 3-methylglutaconyl-CoA hydratase (type I) have been reported. Both had mild clinical symptoms, including speech retardation. The organic acid excretion profile was characteristic, including 3-methylglutaconic acid (521–934 mmol/mol creatinine), 3-methylglutaric acid (5–9), and 3-hydroxyisovaleric acid (144–207).

Type II seems to be more common: at least 15 patients have been reported so far (3). Clinically, patients with the type II defect are severely affected; almost invariably they suffer from major neurological abnormalities with retarded psychomotor development, hypotonia and/or spasticity, convulsions or EEG abnormalities, and sensorineural changes of the eye and ear.

Biochemically, the type II patients differ from type I by a slightly lower excretion of 3-methylglutaconic acid (usually less than 400 mmol/mol creatinine), a somewhat higher excretion of 3-methylglutaric acid (see Fig. 1), and a normal excretion of 3-hydroxyisovalerate. Oral loading with l-leucine did not result in an increased urinary
FIG. 1. 3-Methylglutaconic aciduria. Urinary excretion of 3-methylglutaconic acid and 3-methylglutaric acid in patients with 3-methylglutaconyl-CoA hydratase deficiency (▲), patients with 3-methylglutaconic aciduria type II (■), and controls (○). 3-Methylglutaconic acid is present as two peaks (cis- and trans-?), the ratio of which was not consistent in either of the groups.
metabolite excretion. The activity of 3-methylglutaconyl-CoA hydratase was normal in all tissues tested (Dr. H. Ibel, Munich, FRG, personal communication). It can safely be concluded that in these cases 3-methylglutaconate is not derived from leucine catabolism in the liver. More investigations are inevitably needed for the elucidation of the underlying defect. An exact stereochemical characterization of urinary 3-methylglutaconate could be of help in this respect. We have recently come across several adult female 3-methylglutaconate excretors who—being healthy themselves—gave birth to neurologically handicapped children. This raises the question of a possible teratogenic effect of 3-methylglutaconate or any of its hitherto unknown precursors.

Mevalonic Aciduria

A deficiency of mevalonate kinase represents the first documented inherited disorder of cholesterol biosynthesis. As such it is a very intriguing model system for the study of regulatory mechanisms in this biosynthetic pathway. Surprisingly, none of the three patients reported so far did have a decreased plasma cholesterol level.

In vitro studies on cholesterol metabolism carried out with cultured fibroblasts also gave normal results. One of the possible explanations lies in the very high production of mevalonate—probably by an increased HMG-CoA reductase—which overcomes the decreased affinity of the enzyme and permits the mevalonate pathway to function. An argument to support this hypothesis is the level of urinary mevalonate excretion, which is exceptionally high: 167–56.200 mmol/mol creatinine. The variability of the clinical presentation—ranging from mild cerebellar ataxia with hypertonia to severe failure to thrive and early death—seems to correlate with the urinary mevalonate excretion but not with the residual enzyme activity in fibroblasts or lymphocytes (4). Organic acid analysis of these patients’ urine displays a simple profile: an impressive peak of mevalonolactone is mainly observed, with a minor peak of mevalonate itself. Plasma mevalonate did not exceed values over 0.5 mmol/liter; hence metabolic acidosis due to accumulation of organic acid did not occur.

(Methyl)malonic Semialdehyde Dehydrogenase Deficiency

The catabolism of uracil, thymine, and valine proceeds via malonic and methylmalonic semialdehydes. As an example, the formation of propionyl-CoA from valine involves the action of methylmalonic semialdehyde dehydrogenase. To date a single patient with a deficiency of this enzyme has been described. A characteristic excretion profile can be observed comprising the presence of 3-hydroxypropionate, 3-hydroxyisobutyrate, 2-ethylhydracrlylate, and the amino acids β-alanine and β-aminoisobutyric acid. Clinically, the patient was essentially free of symptoms (5).
Succinic Aciduria

One report has appeared in the literature. It dealt with a neonate who died at the age of 5 weeks with a complex I deficiency (6). Plasma succinate was measured on one occasion: the value was found to be 2.23 mM with a concomitant lactate of 27 mM. Urinary succinate was normal on two consecutive days, but was strongly increased on the next day. A subsequent pregnancy in this family was terminated at 22 weeks; fetal serum contained 6.3 mmol/liter succinate.

We think one should be very careful interpreting these data because (a) succinate is easily formed nonenzymatically from 2-oxoglutarate, and (b) extremely high succinate levels are usually found in serum samples that have been collected perimortally.

Fumaric Aciduria

Fumarase, an enzyme of the citric acid cycle, has been shown to be present in at least six fractions. Both cytosolic and mitochondrial forms can be distinguished. Fumaric aciduria has been described in only a few isolated cases where the enzyme defect was (a) nonexistent, (b) confined to the cytoplasm, or (c) generalized (7). It is to be expected that more variants will be found. Screening for organic acids revealed increased urinary levels of fumarate (151–772 mmol/mol creatinine) and succinate (153–194 mmol/mol creatinine). Plasma fumarate did not exceed 5.5 μmol/liter.

The clinical spectrum of fumaric aciduria ranges from speech retardation with mental regression to severe failure to thrive with cerebral atrophy and early death. In general, the clinical histories appear to be nonspecific so far.

Malonic Aciduria

Malonic aciduria is another of the "moderate" organic acidurias. Malonic acid is the main urinary metabolite (150–3,900 mmol/mol creatinine); one of the two reported patients also excreted methylmalonic acid (210 mmol/mol creatinine), probably as a result of the inhibition of methylmalonyl-CoA mutase by malonyl-CoA. A deficiency of mitochondrial malonyl-CoA decarboxylase—which plays only a minor role in the metabolism of malonyl-CoA—could be established in cultured fibroblasts. Clinically important were the following signs: (recurrent) vomiting, metabolic acidosis, and seizures (8).

Lactic Aciduria Due to Fructose-1,6-diphosphatase Deficiency

Gluconeogenesis is regulated by four key enzymes: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose-6-phos-
phatase. Defects of all enzymes are generally accompanied by severe lactic acidosis, a symptom without significance for the differential diagnosis. The deficiency of glucose-6-phosphatase, or glycogen storage disease type I, can be recognized by its clinical presentation and the secondary abnormalities of triglyceride and urate metabolism.

Fructose-1,6-diphosphatase acts in the upper half of the gluconeogenic pathway, which involves not only the metabolism of pyruvate but also that of glycerol. This opens new possibilities for the differential diagnosis, as has recently been shown. Dremsek and co-workers (9) described an increased urinary excretion of glycerol during episodes of metabolic decompensation, a finding that became more important since the description of glycerol-3-phosphate in the urine of a similar patient (10). We have studied another four patients with fructose-1,6-diphosphatase deficiency and observed glycerol-3-phosphate in all cases (for the method of detection, see Analytical Chemical Aspects).

Urinary glycerol reached values up to 90 mmol/liter, a figure comparable to those seen in glycerol kinase deficiency. A missing link in the gluconeogenic pathway from glycerol (i.e., dihydroxyacetone phosphate) could not be traced in any of the patients’ urine samples. As a rule we always measure glycerol-3-phosphate in urine samples containing abnormal amounts of glycerol and lactate.

The specificity of glycerol-3-phosphate has some limitations, as exemplified by a patient with hereditary tyrosinemia type I. During episodes of hypoglycemia—probably as a result of impaired liver function—she excreted both glycerol and glycerol-3-phosphate in excess. The activity of liver fructose-1,6-diphosphatase could not be assessed, but should be subject of further studies associated with tyrosinemia.

Disorders Associated with Ketosis

Ketone bodies, which are produced by the liver, are important sources of energy to the brain, especially during starvation. Defects of ketone body utilization will thus result in a decreased energy supply to the brain, with severe clinical consequences. Two defects have been unraveled so far: (A) deficiency of succinyl-CoA:3-ketoacid CoA-transferase (11) and (B) deficiency of acetoacetyl-CoA thiolase (12).

Defect A can be suspected in children who have a permanent ketonemia in the nonfasting as well as in the fasting state. Postprandial plasma 3-hydroxybutyrate levels of 0.7–0.9 mmol/liter have been reported. Affected patients suffer from attacks of ketoacidosis without hypoglycemia. Defect B is somewhat more difficult to understand. Both acetoacetyl-CoA and 2-methylacetoacetyl-CoA are substrates for the thiolase. It has been proposed that there are two forms of the enzyme: a hepatic form and an extrahepatic form. Only the extrahepatic form is necessary for ketone body utilization. Hence a deficiency of the extrahepatic isoenzyme alone would leave the catabolism of 2-methylacetoacetyl-CoA in the liver unaffected, and consequently, no abnormal organic aciduria is observed. Clinically, a severe hypoglycemic ketoacidosis following gastroenteritis in a 10-month-old girl was observed. Inter-
mittent ketoacidosis is not an extremely rare condition in childhood, but a sound biochemical explanation is found in only a few cases. Therefore, additional methods of investigation have to be devised for the investigation of children who suffer from recurring attacks of ketoacidosis. It will not be sufficient to relate the degree of ketosis to the glucose levels, but accurate measurements of brain ketone body consumption will be needed.

**Glutaric Acidemia Type I**

One of the organic acidurias accompanied by the worst clinical symptomatology is the deficiency of glutaryl-CoA dehydrogenase. The usual clinical course starts with a sudden onset of dystonia following an infection between the age of 3 months and 3 years. Patients do not recover completely and may even deteriorate further after subsequent attacks. Dystonia and choreoathetosis are among the most striking features of the disease.

Now that almost 20 cases of glutaric aciduria have been described, the large variation of the clinical presentation becomes manifest. Within one family both an acute and a chronic clinical course may occur. Even healthy affected siblings may be encountered, which stresses the need for careful family investigations (13).

Urinary glutarate in the patients reported thus far ranged from 587 to 11,800 mmol/mol creatinine with two exceptions. These were patients in whom no free glutarate could be detected on several occasions, but who permanently excreted abnormal amounts of glutaryl carnitine. Although it seems attractive to correlate these variations of metabolite excretion with those of residual enzyme activity, this did not appear to be true.

We have diagnosed three siblings with glutaryl-CoA dehydrogenase deficiency, of whom only one showed the classical clinical picture. Even this affected girl produced abnormal amounts of free glutarate exclusively during infections. During quiet periods all three siblings excreted abnormal quantities of conjugated glutarate, which was tentatively identified as glutaryl carnitine. Therefore it is advisable to analyze all patients suspected of having glutaric aciduria type I for conjugated glutarate. Another characteristic metabolite of this condition could be 3-hydroxyglutarate, a compound that is difficult to separate from the rather common 2-hydroxyglutarate, however.

Glutaric acidemia type I is theoretically accessible to dietary treatment by decreasing the intake of lysine and tryptophan. Thus far no real success has been achieved by this approach, however. Carnitine treatment is needed at least by a number of patients.

**4-Hydroxybutyric Aciduria**

There are data which suggest that 4-OH-butyric acid acts as a neurotransmitter. Accordingly, patients who accumulate this acid are likely to have neurological im-
pairment. Several of the 10 or so patients diagnosed so far had a marked ataxia, hypotonia, and mental retardation. Milder variants (e.g., with speech retardation as the only clinical symptom) are now coming to light. Biochemically, all patients are diagnosed by the finding of large amounts of 4-OH-butyrate and its lactone in urine (e.g., 350 mmol/mol creatinine) and moderately increased levels of this compounds in plasma and CSF (levels in CSF always predominating over those in plasma). The activity of succinic semialdehyde dehydrogenase was deficient in lymphocyte extracts (14). Succinic semialdehyde itself was detected in trace amounts in the urine of several patients. Experimental treatment with a GABA analog has recently been proposed.

\[\text{D-Glyceric Acidemia}\]

This very rare organic acidemia has been described in only five patients. A delayed psychomotor development and neurological abnormalities were common clinical characteristics of the patients. The urinary excretion of glyceric acid was fairly high, but there was a large variation from 0.5 to 116 mmol/liter. It is important to realize that glycerate has a very bad extraction recovery with organic solvents; hence quantitative measurements have to be performed with care.

Oral loading tests with fructose (1 g/kg), dihydroxyacetone (1 g/kg), or \(\alpha\)-serine (200 mg/kg) have been useful in provoking exaggerated glycerate excretions. \(\text{D}\) and \(\text{L}\)-glycerate are easily separated by capillary gas-liquid chromatography of their O-acetylated (\(-\))methyl esters (15). It is not certain whether all patients had the same enzyme defect: only very recently a deficiency of \(\text{D}\)-glycerate kinase could be established in one of the patients (16). The human liver enzyme appeared to be very unstable and could only be assayed when the tissue had been homogenized in a special medium containing inorganic phosphate, EGTA, and glycerate. Theoretically, a deficiency of triokinase is also possible, especially in patients who do not respond to serine loading. Apparently, more studies in this area are needed.

\[\text{Defect of 2-Oxobutyrate metabolism}\]

A brother and a sister with symptoms of cyclic vomiting and ketoacidosis without hypoglycemia were found to have elevated levels of 2-OH-butyratate and 2-amino-butyratate in plasma and urine. The concentrations of both substances increased upon methionine loading, with a concomitant rise of urinary sulfur excretion. A defect of 2-oxobutyrate metabolism (dehydrogenation?) was postulated (17).

\[\text{N-Acetylaspartic Aciduria}\]

Canavan disease is a form of autosomal recessively inherited leukodystrophy with associated megalencephaly, blindness, and spasticity. The neurological findings usu-
ally appear in the first few months of life. Recently, it was shown that some cases of Canavan disease can be attributed to aspartoacylase deficiency.

This enzyme cleaves the acetyl group, yielding free aspartic acid. Affected patients excrete 800–3600 mmol/mol creatinine of N-acetylaspartate in their urine (controls <20). Cultured fibroblasts are well suited as starting material for the enzyme assay (18). N-Acetylaspartate is present only in the brain; however, its function is still unknown 30 years after its discovery. The pathogenesis of the clinical symptoms of aspartoacylase deficiency remains to be elucidated. One would expect both an increased brain N-acetylaspartate level and a decreased aspartate level. None of these phenomena have been studied up to now.

**Fumarylacetoacetase Deficiency**

Hereditary tyrosinemia type I due to fumarylacetoacetase deficiency is a severe disorder leading to liver disease, eventually with hepatocellular carcinoma, and renal tubular abnormalities. Both an acute and a chronic form occur. Treatment with a low-tyrosine diet does not give successful long-term results, and hence liver transplantation appears to be the ultimate form of treatment.

To make a correct diagnosis, a very careful analytical approach has to be chosen. Most patients are identified by finding an increased urinary excretion of succinylacetone, the key metabolite that is formed by reduction and decarboxylation of the primary accumulating product fumarylacetoacetate. As succinylacetone has been reported to be somewhat unstable, it is preferable to take a fresh urine sample. Furthermore, the usual ethoxime formation does not function very well for this substance: treatment with hydroxylamine will result in a stable derivative with a ring structure. Urinary excretion levels of succinylacetone may vary considerably: values from less than 1 µmol/liter to more than 500 µmol/liter for untreated patients were observed in our laboratory. In this respect it is helpful to analyze the urinary excretion of δ-aminolevulinic acid. This amino acid cannot react to form porphobilinogen due to the inhibition by succinylacetone of the corresponding enzyme. As shown in Fig. 2, the excretion of δ-aminolevulinic acid could be a more sensitive, albeit possibly less specific, parameter of tyrosinemia type I. Finally, one should be fairly liberal in assaying fumarylacetoacetase in cells of suspected patients.

One of the major problems of follow-up studies of tyrosinemia is the prediction of the moment at which hepatocellular carcinoma will develop and the timing of the liver transplantation. To date no reliable biochemical parameter has been found. Even the practical value of serial α-fetoprotein determinations has to be discussed. For the time being imaging techniques remain the sole possibility to check the condition of the patient’s liver.

**CONJUGATION OF ORGANIC ACIDS**

Conjugation is an effective means of detoxification of potentially hazardous substances, which may be of exogenous or endogenous origin. In view of their possible
neurotoxic action, short chain and medium chain organic acids are therefore readily conjugated. It was originally thought that only glycine conjugates were formed, as exemplified by isovalerylglucose in isovaleric acidemia, but more recent investigations have brought to light other conjugating substrates, such as carnitine and glucuronic acid. These observations have taught us many novel insights on secondary metabolic pathways (Table 2). All three conjugation reactions take place in different regions of the cell: glycine conjugation is an essentially mitochondrial process, the formation of carnitine esters is associated with the mitochondrial membrane, and glucuronidation takes place in the microsomal fraction. As virtually all catabolic

### TABLE 2. Conjugation mechanisms for short chain and medium chain organic acids in humans

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Type of conjugate</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Glycine ester</td>
<td>Glucuronide</td>
<td>Carnitine ester</td>
<td></td>
</tr>
<tr>
<td>Propionyl-CoA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Isovaleryl-CoA</td>
<td>++</td>
<td>(+)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3-CH₃-crotonyl-CoA</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tiglyl-CoA</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hexanoyl-CoA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Octanoyl-CoA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Valproyl-CoA</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Benzoyl-CoA</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Salicyloyl-CoA</td>
<td>++</td>
<td>+</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>7-OH-octanoyl-CoA</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glutaryl-CoA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Suberyl-CoA</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Urinary carnitine excretion in various types of organic acidemia*

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Free carnitine</th>
<th>Acylcarnitine</th>
<th>Acyl/free</th>
</tr>
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<tbody>
<tr>
<td>Methylmalonic acidemia</td>
<td>32</td>
<td>415</td>
<td>13.0</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>27</td>
<td>267</td>
<td>9.9</td>
</tr>
<tr>
<td>3-Ketothiolase ceficiency</td>
<td>12</td>
<td>285</td>
<td>23.7</td>
</tr>
<tr>
<td>3-Methylcrotonylglycinuria</td>
<td>10</td>
<td>254</td>
<td>25.4</td>
</tr>
<tr>
<td>Isovaleric acidemia</td>
<td>14</td>
<td>240</td>
<td>17.1</td>
</tr>
<tr>
<td>3-Methylglutaconic aciduria I</td>
<td>67</td>
<td>219</td>
<td>3.3</td>
</tr>
<tr>
<td>3-OH-3-Methylglutaryl aciduria</td>
<td>28</td>
<td>261</td>
<td>9.3</td>
</tr>
<tr>
<td>Glutaric aciduria I</td>
<td>19</td>
<td>346</td>
<td>18.2</td>
</tr>
<tr>
<td>Mevalonic aciduria</td>
<td>242</td>
<td>505</td>
<td>2.1</td>
</tr>
<tr>
<td>4-OH-butyric aciduria</td>
<td>251</td>
<td>229</td>
<td>0.9</td>
</tr>
<tr>
<td>Controls (20)</td>
<td>7–128</td>
<td>24–127</td>
<td>0.7–3.4</td>
</tr>
</tbody>
</table>

* Concentrations expressed in μmol/g creatinine.

Pathways of short chain acyl-CoAs are located inside the mitochondrion, it is logical to assume that accumulating acyl-CoAs primarily react with glycine to restore the intramitochondrial CoA homeostasis. A beautiful example is given by patients with isovaleric acidemia, who start to excrete isovalerylglucuronide during attacks of metabolic decompensation only when their glycine N-acylating capacity is apparently surpassed (19).

Based on the theory of mass action, one should be able to change the pattern of conjugation by adding large amounts of a different substrate. We have achieved this by giving oral carnitine (100 mg/kg-day) to a clinically well patient with isovaleric acidemia. Subsequently, the ratio of glycine to carnitine ester in her urine changed from 96:4 to 80:20. Carnitine treatment is considered worthwhile to try only in those cases where glycine conjugation is not very effective, and secondary carnitine deficiency is thus a real threat. It can be seen from Table 3 that especially patients with propionic acidemia and glutaric acidemia type I are candidates for carnitine supplementation.

Virtually all short chain and medium chain acyl-CoAs are able to form carnitine esters by reactions catalyzed by carnitine acetyltransferase or carnitine octanoyltransferase. Apparently, there are no large differences in affinity for the various acyl-CoAs as were observed toward glycine N-acylase (20).

Formation of glucuronides has been described for a few substrates only so far. In our opinion this extramitochondrial process becomes operative only when large amounts of short chain fatty acids escape from the mitochondria. The finding of glucuronides must therefore mean that the patient is in a particularly bad condition.

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**DISCUSSION**

**Dr. Van den Berghe:** I wonder if the excretion of glycerol-3-phosphate that you observed could derive from an accumulation of this compound in the liver, combined with liver lesions. Although it is biochemical dogma that phosphorylated compounds do not as a rule cross cell membranes, isolated hepatocytes have been shown to release such metabolites, including glycerol-3-phosphate (1). I should therefore like to ask you if you looked for the accumulation of glycerol-3-phosphate in the liver of your patients, and also in other conditions in which glycerol-3-phosphate may accumulate, such as hereditary fructose intolerance and following alcohol
intake. Is there also an increase in the excretion of glycerol-3-phosphate during a fructose tolerance test?

**Dr. Duran:** We have not checked the presence of glycerol phosphate in the liver cell. I think one of the things to do would be to look at animal or human liver using in vivo NMR spectroscopy. We have not investigated the production of glycerol phosphate after fructose loading because we don’t like to give a lot of fructose to fructose-1,6-diphosphatase-deficient patients. Finally, we have not looked at alcoholic cirrhosis.

**Dr. Van den Berghe:** Did you also look for excretion of glycerol-3-phosphate in children with chronic liver disease such as biliary atresia?

**Dr. Duran:** The only case is a patient with tyrosinemia type 1, who developed severe hypoglycemia and then started to excrete glycerol phosphate. We do not know if this was secondary to liver dysfunction or if it was due to inhibition of fructose-1,6-diphosphatase.

**Dr. Krywawycz:** I assume that glycerol-1-phosphate does not cross the cell wall. In the patient with fructose-1,6-diphosphatase deficiency in whom we found a glycerol-1-phosphate in the urine we suspected that this compound was coming from the renal tissue as a result of renal damage. In fructose-1,6-diphosphatase deficiency there is renal involvement, and also glycerol-1-phosphate can accumulate in the kidney in this disease as it is a gluconeogenic organ. We did not perform fructose loading tests but made several measurements in this patient both during hypoglycemia and normoglycemia. The largest excretion of glycerol-1-phosphate occurred during periods of hypoglycemia.

**Dr. Saudubray:** I remember a very old paper from Senior, trying to explain why in glucose-6-phosphatase deficiency there is hypoketonemia. I remember he gave a hypothesis connected with glycerol phosphate accumulation in glucose-6-phosphatase deficiency. I don’t agree when you state that in fructose-diphosphatase deficiency there is a ketoacidotic state. Acidosis occurs, it is true, but ketosis—not really. In many patients affected with fructose-diphosphatase deficiency there is a problem of differential diagnosis with fatty acid oxidation defects. Two patients affected with fructose-diphosphatase deficiency were referred to our unit with a possible diagnosis of fatty acid oxidation defect, because of low plasma ketone levels.

**Dr. Duran:** One of our patients with fructose-1,6-diphosphatase deficiency had a plasma 3-hydroxybutyrate concentration of 6 mmol/liter, together with a blood glucose of 1.5 mmol/liter. This certainly is not hypoketotic.

**Dr. Saudubray:** I agree, but it depends largely on the conditions of measurement. When we measured blood ketones in six patients affected with fructose-diphosphatase deficiency, using a fasting test performed when the patients were in good nutritional condition, we found that blood ketones were not very raised even when blood glucose levels were lowered. Of course, there was some accumulation but not very high compared to what we find in glycogenosis type 3, for example.

**Dr. Duran:** I think it is a good hypothesis to test if there is any effect of glycerol phosphate on ketogenesis.

**Dr. Hobbs:** You have described two serious clinical conditions that might justifiably be treated by bone marrow transplant. In glutaryl-CoA dehydrogenase deficiency you mention that the defect occurs also in the leukocytes. You could therefore devise an in vitro test to see if normal leukocytes would correct defective fibroblasts or another tissue grown from the patient. The other is the type 2 methylglutaconic acidemia, where the exact defect is not known. Nevertheless, it would again be possible to test in vitro the addition of normal leukocytes, to see if they could correct defective cells from a patient. If in either of these situations, normal leukocytes could compensate, then following a bone marrow transplantation you would have the provision of a detoxification system for that patient.
Dr. Wanders: In the first disease you treated, 3-methylglutaconic aciduria, in those patients in which the hydratase is normal, you mentioned the possibility that methylglutaconic acid comes from the other side, and you suggested some of the intermediates involved in cholesterol biosynthesis. Has cholesterol synthesis in these patients been tested? And is it normal or abnormal?

Dr. Duran: As far as I know they have normal cholesterol levels in their body fluids. Synthesis starting from precursors such as 3-hydroxy-3-methylglutaric acid has not been tested.

Dr. Saudubray: We have observed a patient with 3-methylglutaconic aciduria presenting with a very severe neurological dysfunction with myopathy. In addition to 3-methylglutaconic aciduria he had severe lactic acidosis. On muscle biopsy there was a generalized defect of the respiratory chain complex. Since you mentioned this very interesting finding of 3-methylglutaconic accumulation in mothers of microcephalic children, maybe it would be interesting to check systematically for respiratory chain disorders in these children. Have you done it?

Dr. Duran: It was checked as far as I know in only one patient, the patient from München. Dr. Endres could perhaps give the details.

Dr. Endres: The enzymes of the respiratory chain were normal. But this patient had a severe cardiomyopathy and died at the age of 5 months, after having already had a life-threatening event in the neonatal period. I think patients with type 2 3-methylglutaconic aciduria are so severely ill that leukocyte infusions would fail to be of any help.

Dr. Saudubray: I am not sure that your explanation of the possible teratogenic role of 3-methylglutaconic is true, because it is evident that in your two forms of the disease, one was due to hydratase deficiency and the other was not. In your first patients, the ones with hydratase deficiency, there was a very high level of 3-methylglutaconic, and these patients were not very sick. They presented with ketoacidosis but not with severe mental retardation, whereas the others were very severely retarded.

Dr. Van Hoof: Coming back to the family you report with one child excreting glutaric acid in urine while his two other sibs had low glutaryl-CoA dehydrogenase in blood cells. I presume that the activity of control enzymes has been measured in leukocytes and I suggest that the enzyme should also be assayed on cultured fibroblasts in Dr. Christensen’s laboratory.

Dr. Duran: That has been done. Dr. Christensen has confirmed the diagnosis glutaryl-CoA dehydrogenase deficiency in the patient’s fibroblasts.

Dr. Van Hoof: Have they performed a loading test of the fibroblasts with glutaric acid?

Dr. Duran: The dehydrogenase has been assayed using the labeled substrate, and it is deficient. This was done in fibroblast homogenates; we did not perform whole cell oxidation studies, as this is not usual in glutaric aciduria type I.

Dr. Van Hoof: It is always wise to look for substrate accumulation in living cells when an enzyme deficiency has been demonstrated in vivo.

Dr. Duran: Maybe I did not make myself clear enough, but all three members of this family excreted abnormal amounts of conjugated glutarate at one time or another. So the free glutarate was often very low, but the conjugated glutarate was increased in all three patients.

Dr. Van Hoof: And none of them was excreting large amounts of glutaconic acid?

Dr. Duran: We have not been able to find large amounts of glutaconic acid in other patients with glutaric acidemia type I. Also if you screen the literature on glutaric aciduria carefully, you will not find many reports dealing with increased glutaconic acid excretion.

Dr. Mowat: Could I come to fumarylacetacetae deficiency? I understand that some Scandinavian workers found deficiency of this enzyme in normal individuals. I wonder if you could comment on that.

Dr. Duran: That is a pseudodeficiency that represents a genetic variant occurring in appar-
ently healthy individuals. This genetic variant has important implications for the use of fumarylacetocacetase analysis in the diagnosis of tyrosinemia.

Dr. Saudubray: Do you have any idea why in some patients with tyrosinemia type 1, succinylacetone excretion is so low, contrasting with a complete absence of fumarylacetocacetase?

Dr. Duran: There has been a hypothesis for a few years that the accumulating toxic metabolites inhibit the 4-hydroxyphenylacetate dioxygenase to such an extent that no metabolite gets through to fumarylacetocacetase anymore. I don’t know if this is a true hypothesis.

Dr. Jaeken: Regarding the detection of hepatoma in tyrosinemia, magnetic resonance imaging (MRI) seems to be a sensitive test. Do you have experience with this?

Dr. Duran: We have had one MR image in one of our tyrosinemia patients, but it is really difficult to follow these cases by MRI because all our patients are below the age of 3 years. You have to give them anesthesia and it is not very nice to give that every 2 or 3 months. We try to do it by ultrasound.

Dr. Sokal: As a pediatrician involved in liver transplantation, I don’t see why you should delay liver transplantation in a child once you have diagnosed tyrosinemia. There is no point in waiting for transplantation and this is true for all life-threatening metabolic disorders.

Dr. Duran: No, there is no point in waiting. But, at least in our country, donor organs are so scarce that it is almost impossible to get the patients on the list for transplantation.

Dr. Hobbs: I would like to support Dr. Sokal. I think it is a mistake to wait. What we would like from you experts in genetic disease and involved with the DNA probes are tests to identify which patients are going to get the serious disease. They could then be transplanted before they suffer irreversible damage. I think that in one of the families you described there was one child of 3 years who had not yet been affected. These are the children we should be transplanting, not the ones who have progressed to end-stage disease.

Dr. Leroy: I wonder whether anyone can tell if the hepatoma in tyrosinemia patients is different from hepatoma in other conditions. Is it in any way specific? It looks as if we may be confronted by a condition here where either the liver cell is intoxicated, with the suppression of a tumor suppressor gene as a consequence, or the inborn error serves as a selecting mechanism favoring those cells in which some proto-oncogene or oncogene has become amplified. If this type of hepatoma is pathologically very similar to any other hepatoma, one can surmise that a less specific mechanism must have caused it. Maybe we missed the chance here to see some specific regulatory gene disturbances due to specific metabolic disorders of tyrosine and its derivatives.

Dr. Duran: I have no information on this.

REFERENCE