

C.1 Introduction

Organic acids comprise key metabolites of virtually all pathways of intermediary metabolism as well as exogenous compounds (Fig. C.1). Comprehensive quantitative analysis of organic acids in body fluids has therefore the potential of yielding information on the physiological and pathophysiological status of different metabolic pathways as well as their interrelationships. The very complexity and diversity of organic acids has, however, hampered the development of quantitative methods for organic acid analysis, and this methodology has not been and is still not widely available [1–3]. Organic acid analysis is generally performed by capillary gas-liquid chromatography/mass spectrometry of trimethylsilylated or methylated derivatives; identification and quantitation of metabolites by computer-based special user libraries containing both the mass spectra and retention indices. Less commonly used are liquid chromatography-chemical ionization mass spectrometric or automated capillary electrophoretic methods.

A critical step is the extraction of the acids from physiological fluids. This is usually accomplished by extraction with organic solvents or anion-exchange methods [1–4], and the results are at best semiquantitative. These methods are mainly suited to screen for gross elevations of organic acids in urine. More subtle abnormalities, such as in partial or vitamin responsive inborn errors of metabolism, e.g. biotinidase deficiency, or the methylmalonic acidemia of transcobalamin II deficiency [5], can remain undiagnosed. Similarly problematic can be the diagnosis of cerebral organic acid disorders

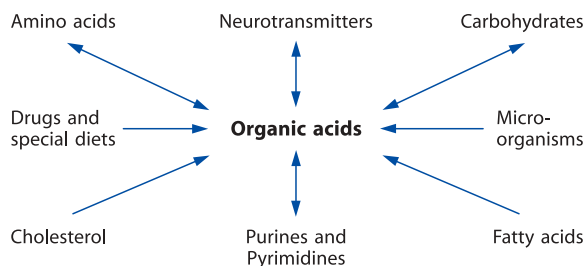


Fig. C.1. Organic acids in small molecule intermediary metabolism

such as 4-hydroxybutyric aciduria or glutaric aciduria type I. If semiquantitative methods are employed to analyze physiological fluids other than urines, e.g. plasma, cerebrospinal or vitreous fluids, even classical organic acid disorders may be missed [6]. Therefore, for specific diagnostic problems quantitative values by improved extraction procedures or even stable isotope dilution assays should be utilized (e.g. N-acetylaspartic acid, glutaric acid, 3-hydroxyglutaric acid, 3-hydroxyisovaleric acid and other hydroxy acids). However, misdiagnoses are also possible in laboratories utilizing sensitive quantitative methods as these detect hundreds of organic acids in biological fluids (Fig. C.1). Laboratories not familiar with a specific disorder may misinterpret characteristic metabolites [7]. Information obtained by organic acid analysis is ideally complemented by the results of analyses of acylcarnitines and acylglycines which was recently greatly facilitated by the development of appropriate electrospray tandem mass spectrometry techniques.

C.2 Preanalytical Conditions

■ Specimens

Organic acid analysis is generally performed on untimed random urine samples. As a guideline age-related control ranges are listed in Table C.1. There are as yet no strong recommendations with respect to time of sampling, although an early morning sample appears favourable. It is more concentrated and defects of amino acid catabolism and fatty acid oxidation are more distinct. Accurate and complete informations of the clinical status of the patient, any dietary manipulations and, most importantly, any medication are an indispensable prerequisite of optimal organic acid analysis.

Limited information is available on the diagnostic value of organic acid analyses in body fluids other than urine. Values of organic acids in plasma and CSF are given in Table C.2. One obvious indication for analyzing physiological fluids other than urine is the lack of an appropriate urine sample in a child who died acutely with symptoms compatible with an inborn error of metabolism. In these instances amino (see Chapter B), acylcarnitines and organic acids can be reliably analyzed in plasma, CSF and/or vitreous humour by quantitative analyses. Additionally, there are reports of the necessity of quantitative determinations of organic acids in CSF for the diagnosis of some patients with cerebral organic acid disorders, e.g. “cerebral” lactic acidemias [8], glutaryl-CoA dehydrogenase deficiency [9], and disorders of biotin metabolism [10]. In such patients urinary organic acid analyses has been reported to be normal on several occasions (see Table C.3).

■ Patient Status

The range of clinical and biochemical findings in organic acid disorders is extensive (Table C.4). Provisionally, it appears helpful to consider two main types of clinical presentations: Systemic intoxication or severe acute encephalopathy, which is the most common presentation (overview in [11]). The first episode usually occurs in (early) infancy, but it can also develop later in older children. Alternatively, older children can present with (sub-)acute neurological disease or variable fluctuating multisystemic manifestations. The former is the classical presentation of the cerebral organic acid disorders.

In acute severe systemic intoxication abnormalities of routine clinical chemistry are usually present such as metabolic acidosis, increased anion gap, cytopenia, hypoglycemia, hyperammonemia (which can mask acidosis), lactic acidemia, elevations of triglycerides and free fatty acids, and ketosis. Testing for ketonuria is an especially simple and useful first line investigation. In most newborns as well as in older children with organic acid disorders, including some patients with fatty acid oxidation defects, there is pronounced ketonuria. Ketonuria is only rarely observed even in very sick newborns without metabolic disease.

In older children the presentation of organic acid disorders is much more variable. Neurological manifestations are very common and often the leading and/or presenting feature. The initial presentation of these children may be nonspecific like developmental delay and/or epilepsy with abnormalities of routine clinical chemistry conspicuously absent. Also, elevations of diagnostic metabolites may be very small and be missed on semiquantitative “routine” organic acid analysis, e.g. in vitamin-responsive disorders [5] and glutaryl-CoA dehydrogenase deficiency [9]. On the other hand, early diagnosis of these patients is especially important, since treatment can be very successful with satisfactory longterm outcome. The untreated disease will lead to severe mental retardation, extrapyramidal movement disorders, pyramidal tract signs, cerebral atrophy, and often death [9, 12].

Early diagnosis of patients presenting (sub-)acutely with neurological disease relies on an increased awareness of these disorders. The clinical presentations include characteristic findings of ataxia, myoclonus, extrapyramidal symptoms, metabolic stroke and megalencephaly [13]. Routine clinical chemistry is often uncontributory. Sometimes, important diagnostic clues can be derived from neuroimaging studies. (Progressive) disturbances of myelination, cerebellar atrophy, frontotemporal atrophy, hypodensities and/or infarcts of the basal ganglia and any symmetrical (fluctuating) pathology, apparently independent of defined regions of vascular supply, are suggestive of an inherited metabolic disorder [13]. Chronic subdural effusions and/or hematomas following relatively mild traumas are characteristic findings in glutaryl-CoA dehydrogenase deficiency and may be mistaken as an indication of child abuse [12].

■ Specimen Collection

Due to the complexity of organic acids correct collection of specimens is most crucial (Table C.5). Samples for organic acid analysis should be frozen as soon as possible and kept and shipped frozen. At room temperature chemical instability causes substantial losses of some important groups of acids, such as oxo acids. For prolonged periods samples should be stored at -80°C instead of -20°C .

A number of different conditions can produce significant artifactual changes of organic acids in urine (Table C.6). The most common are bacterial contamination, which can be abolished by proper handling of specimens (Table C.5). Depending on their own metabolism bacteria can eliminate almost all known organic acids characteristic of inborn errors of metabolism. Furthermore, they can mimic a number of different organic acidurias. The most often observed abnormalities of bacterial origin include elevations and/or decreases of lactic acid and tricarboxylic acids, especially 2-oxoglutaric acid, succinic acid, etc., which can be mistaken as an indication of an underlying mitochondrial disorder. Preservatives, such as chloroform, are useful in preventing bacterial growth, if freezing is not possible.

C.3 Analysis

Optimal results of organic acid analysis require a close cooperation and, especially communication, between the referring physician and the biochemical genetic laboratory. Simply broadening the access to organic acid analyses does not automatically increase the diagnostic yield [14]. Detailed information on the patient must be provided to allow specific and sometimes extended analyses. All samples should be investigated by capillary gas chromatography/mass spectroscopy. It has been documented in our as well as other laboratories [15, 16] that a significant proportion of diagnoses is missed, if mass spectral identification is only performed on the grounds of suggestive abnormal gas chromatographic profiles. Quantitative results should be accurate within 20%. As methods of organic acid analysis vary with respect to isolation, identification and sensitivity, values between laboratories are often not comparable. This is even true for specified concentrations in the literature.

C.4 Interpretation and Normal Variation

The interpretation of organic acid analysis depends on several key diagnostic metabolites (Tables C.1, C.7, C.8) as well as characteristic patterns of abnormalities. Elevation of single compounds is often less informative than distortions of relative concentrations. In evaluating patients for organic acid disorders, it must be kept in mind that often a definitive diagnosis can only be reached after repeated quantitative urinary organic acid analyses, preferably during metabolic decompensations, and/or additional tests, e.g. evaluation of acylcarnitines, organic acids in CSF, loading studies, etc. In some patients with a proven enzyme defect urinary organic acid analysis has been documented to be normal on several occasions. On the other hand, metabolites may be found elevated several times above the upper normal limit during severe illnesses in children who do not suffer from an inborn error of metabolism. The final interpretation requires detailed information on the clinical status and course, routine clinical chemistry and, again, repeated quantitative organic acid analysis.

Hitherto little is known about age-specific changes or circadian rhythms of urinary organic acids reflecting metabolic changes and/or maturation of tubular function and the relevance of these changes for the diagnosis of organic acid disorders. Obviously, age-specific reference values should be used. Reference values may vary between laboratories, and the values listed in this chapter should only be used as a guideline.

C.5 Pathological Values/Differential Diagnosis

Cross links between relevant urinary organic acids and the disorders in which they are elevated are given in the last column of Table C.1. Key organic acids, requiring special attention already at borderline elevations, detailed information on the clinical status and course of the patient and an especially careful interpretation are specifically emphasized in Table C.7. In most disorders changes of metabolites in plasma and/or cerebrospinal fluid (Table C.2) are far less obvious than in urine except for some “cerebral” organic acid disorders, where a diagnostic elevation of organic acids may only be found in CSF (Table C.3).

Elevations of single organic acids may suggest different disorders. In most disorders a definitive diagnosis is approached by a careful evaluation of additional metabolites. Most importantly, it must always be kept in mind that only few organic acid disorders with constant high elevations of characteristic metabolites can be diagnosed unequivocally from a single urine specimen.

Table C.1. Reference values: organic acids in urine by age groups

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
eq/mol creatinine							
Total organic acids ^a	Mean	12.8	13.1	–	–	6.2	Any endogenous or exogenous organic acid disorder
	Min	6	3.8	–	–	4.5	
	Max	35	34.5	–	–	11	
mmol/mol creatinine							
Lactic acid ^b	Mean	49	51	86	76	25	Mitochondrial (27) & biotin (7) disorders; dihydrolipoyl (E ₃) dehydrogenase deficiency (6, 27); circulatory failure; bacteria MCAD (14)
	Min	1	0.5	33	35	13	
	Max	927	156	285	131	46	
Hexanoic acid	Mean	0.02	0.03	0.8	0.4	–	Hyperoxaluria I (26)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	0.5	0.4	2.3	2.2	–	
Glycolic acid	Mean	16.4	9.9	98	105	35	Hyperoxalurias (26)
	Min	6	1	0.2	43	18	
	Max	53	40	198	172	55	
Oxalic acid	Mean	n.d.	n.d.	0.6	0.5	0.3	Mitochondrial disorders (24); ketosis
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	n.d.	19	17	5	
2-Hydroxybutyric acid	Mean	1.8	0.2	2	2.5	n.d.	Propionic and methylmalonic acidemias (7); biotin disorders (7); 3-hydroxyisobutyric aciduria (7); bacteria Ketosis; defects of ketolysis (14)
	Min	n.d.	n.d.	0.2	n.d.	n.d.	
	Max	61	2	5.1	7.3	n.d.	
3-Hydroxypropionic acid	Mean	1.4	4	7.3	6.6	–	3-Hydroxyisobutyric aciduria (7); mitochondrial disorders (27); ketosis
	Min	n.d.	n.d.	1.0	n.d.	–	
	Max	8	19	36	20	–	
3-Hydroxybutyric acid	Mean	2.0	1.9	3.6	1.6	0.7	MSUD (6), dihydrolipoyl dehydrogenase (E ₃) deficiency (6, 27); ketosis
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	30	9	11.1	7.6	2	
3-Hydroxyisobutyric acid	Mean	10.9	5	54.8	45.1	11	3-Oxothiolase deficiency (7); 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency; propionic and methylmalonic acidemias (7); ketosis Malonic aciduria (8)
	Min	n.d.	n.d.	20.2	12.8	4.1	
	Max	82.5	38	118	137	19	
2-Hydroxyisovaleric acid	Mean	0.2	0.2	0.5	1.4	n.d.	
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	4	3	1.3	11.9	n.d.	
2-Methyl-3-hydroxybutyric acid	Mean	2.4	2	11.2	8.9	–	
	Min	n.d.	n.d.	3.2	1	–	
	Max	7.5	7.5	26.6	22.3	–	
Malonic acid		n.d.	n.d.	n.d.	n.d.	n.d.	

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
3-Hydroxyisovaleric acid	Mean	3.5	0.02	31.7	21.1	11	All leucine degradation defects (6); biotin disorders (7); ketosis
	Min	n.d.	n.d.	10.4	9.8	6.9	
	Max	17	18	67	50.2	25	
Methylmalonic acid	Mean	n.d.	0.2	n.d.	n.d.	n.d.	Methylmalonic acidemias (7); malonic aciduria (8)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	5	n.d.	n.d.	n.d.	
2-Ethyl-3-hydroxy-propionic acid	Mean	3	0.8	6.6	3.9	–	3-Hydroxy-isobutyric aciduria (7)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	18	12	19.9	19.8	–	
2-Hydroxyisocaproic acid	Mean	n.d.	0.02	n.d.	n.d.	n.d.	MSUD (6); dihydrolipoyl dehydrogenase (E ₃) deficiency (6, 27)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	5	n.d.	n.d.	n.d.	
3-Hydroxyvaleric acid		n.d.	n.d.	n.d.	n.d.	n.d.	Propionic and methylmalonic acidemias (7); ketosis
4-Hydroxybutyric acid	Mean	0.1	n.d.	n.d.	n.d.	0.6	4-Hydroxy-butyric aciduria (3)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	1.5	n.d.	n.d.	n.d.	2.8	
2-Hydroxy-3-methylvaleric acid	Mean	0.11	0.3	n.d.	n.d.	n.d.	MSUD (6); dihydrolipoyl dehydrogenase (E ₃) deficiency (6, 27)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	2	5	n.d.	n.d.	n.d.	
Benzoic acid	Mean	2.5	0.2	2.2	1.9	4.2	Benzoate treatment; bacteria
	Min	n.d.	n.d.	0.6	n.d.	1.9	
	Max	31	7	7.7	4.3	6.5	
Octanoic acid	Mean	1.1	0.3	0.8	0.4	–	MCAD (14)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	7	4	7.7	3	–	
4-Hydroxyisovaleric acid		n.d.	n.d.	n.d.	n.d.	n.d.	Isovaleric aciduria (6)
Glycerol		n.d.	n.d.	n.d.	n.d.	n.d.	Glyceroluria (17); ointment
Ethylmalonic acid	Mean	1.2	0.4	5.7	1.7	2.5	SCAD (14); glutaric aciduria II (14); mitochondrial disorders (27)
	Min	n.d.	n.d.	1.7	n.d.	0.4	
	Max	8.5	6.5	14.6	8.4	4.2	
Maleic acid	Mean	0.9	n.d.	n.d.	n.d.	n.d.	
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	34	n.d.	n.d.	n.d.	n.d.	
Succinic acid	Mean	53.4	39.8	44.1	21.6	7.5	Mitochondrial disorders (27)
	Min	5	13	17.6	4.9	0.5	
	Max	139	125	79.2	81.3	16	
Methylsuccinic acid	Mean	n.d.	n.d.	4.2	1.2	n.d.	SCAD (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	n.d.	8.8	4.4	n.d.	
Glyceric acid	Mean	17.4	5.3	12.1	12.5	1.7	Hyperoxaluria II (26); δ-glyceric aciduria (8)
	Min	2	0.2	4.2	2.6	0.2	
	Max	119	40.5	32.2	28.2	6	

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
Uracil	Mean	0.2	0.8	17	14.6	–	Dihydropyrimidine dehydrogenase deficiency (23); urea cycle disorders (14)
	Min	n.d.	n.d.	6.9	1.4	–	
	Max	2	5	56.4	64.5	–	
Fumaric acid	Mean	6.4	6.8	4.2	1.5	0.4	Fumaric aciduria (8); mitochondrial disorders (27)
	Min	n.d.	1	1.4	n.d.	0.2	
	Max	20.5	14	9.9	3.7	0.8	
2,3-Dihydroxybutyric acid	Mean	46.3	14	94.2	100	–	
	Min	13	2	32.1	34.2	–	
	Max	148	26.5	152	190	–	
Isobutyrylglycine	Mean	0.12	n.d.	n.d.	n.d.	n.d.	3-Hydroxy-isobutyric aciduria (7); glutaric aciduria II (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	4.5	n.d.	n.d.	n.d.	n.d.	
5-Hydroxyhexanoic acid	Mean	0.1	n.d.	1.5	1.6	n.d.	MCAD (14); MCT feeding
	Min	n.d.	n.d.	0.1	n.d.	n.d.	
	Max	5	n.d.	4.9	5.4	n.d.	
Glutaric acid	Mean	0.8	0.3	2.4	0.6	1.3	Glutaric aciduria I (12) & II (14)
	Min	n.d.	n.d.	n.d.	n.d.	0.6	
	Max	3.5	3	5.3	3.8	2.6	
2,4-Dihydroxybutyric acid	Mean	19.9	10.3	48.9	49.6	–	
	Min	5	2	23.6	11.9	–	
	Max	49	26	93.1	179	–	
3-Methylglutaric acid		n.d.	n.d.	n.d.	n.d.	n.d.	3-Methyl-glutaconic acidurias (6); 3-hydroxy-3-methylglutaric aciduria (6)
Propionylglycine		n.d.	n.d.	n.d.	n.d.	n.d.	Propionic and methylmalonic acidurias (7)
3,4-Dihydroxybutyric acid	Mean	112	45.1	207	151	–	4-Hydroxy-butyric aciduria (3)
	Min	16	14	109	58	–	
	Max	396	142	454	320	–	
3-Methylglutaconic acid	Mean	1.3	0.9	7.7	2.5	–	3-Methyl-glutaconic acidurias (6); 3-hydroxy-3-methylglutaric aciduria (7)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	5	9	19	11.4	–	
Glutaconic acid	Mean	0.6	0.9	n.d.	n.d.	n.d.	Glutaric aciduria I (12)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	9	11	n.d.	n.d.	n.d.	
Glyoxylic acid	Mean	7.4	1.7	3	2.4	–	Hyperoxaluria I (26)
	Min	n.d.	n.d.	0.29	0.2	–	
	Max	22.5	20	15.9	5.7	–	
Isovalerylglycine		n.d.	n.d.	n.d.	n.d.	n.d.	Isovaleric aciduria (6); biotin disorders (7); glutaric aciduria II (14)
3-Hydroxyadipic acid	Mean	0.1	n.d.	n.d.	n.d.	n.d.	Long-chain 3-hydroxy-CoA dehydrogenase deficiency (LCHAD) (14); MCAD (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	1	n.d.	n.d.	n.d.	n.d.	

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
Mandelic acid	Mean	23	2.1	–	–	–	Phenylketonuria (1)
	Min	n.d.	n.d.	–	–	–	
	Max	134	22.5	–	–	–	
Malic acid	Mean	18.5	18.2	5.6	2.3	2	Mitochondrial disorders; L-2-hydroxyglutaric acid- uria (8)
	Min	4	5	2.2	n.d.	0.7	
	Max	54	38	16.2	5.5	5.3	
Adipic acid	Mean	3.6	2.8	5.9	1.1	5.1	MCAD (14); glutaric acid- uria II (14); MCT feeding; ketosis
	Min	n.d.	n.d.	n.d.	n.d.	0.8	
	Max	15	32	34.3	5.3	35	
Pyruvic acid	Mean	9.6	27.8	10.1	9.6	5.4	Mitochondrial (27) & bio- tin (7) disorders; dihydro- lipoyl (E ₃) dehydrogenase deficiency (6, 27)
	Min	0.5	4.5	5.1	3.5	2.6	
	Max	187	130	22.6	17.3	7.9	
2-Oxobutyric acid	Mean	0.5	10.5	n.d.	n.d.	n.d.	
	Min	0	0	n.d.	n.d.	n.d.	
	Max	12	115	n.d.	n.d.	n.d.	
5-Oxoproline	Mean	11.6	15.4	58.1	34	26	5-Oxoprolinuria (9); haw- kinsinuria (4)
	Min	n.d.	n.d.	25.8	1.7	0.9	
	Max	40.5	42.5	92.2	80.9	63	
Thiodiglycolic acid	Mean	19.8	n.d.	n.d.	n.d.	n.d.	Prematurity
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	263	n.d.	n.d.	n.d.	n.d.	
3-Methyladipic acid	Mean	0.1	0.6	n.d.	n.d.	n.d.	
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	1.5	4	n.d.	n.d.	n.d.	
2-Oxoisovaleric acid	Mean	1.1	n.d.	n.d.	n.d.	n.d.	MSUD (6); dihydrolipoyl dehydrogenase (E ₃) defi- ciency (6, 27)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	36	n.d.	n.d.	n.d.	n.d.	
Mevalonic acid	Mean	0.4	0.4	0.2	0.2	0.1	Mevalonic aciduria (30)
	Min	0.3	0.3	0.1	0.1	0.1	
	Max	0.7	0.4	0.3	0.2	0.2	
3-Methylcrotonyl- glycine	Mean	n.d.	0.2	n.d.	n.d.	n.d.	3-Methylcrotonyl-CoA car- boxylase deficiency (6); 3- hydroxy-3-methylglutaric acidemia (6); biotin disorders (7)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	2.5	n.d.	n.d.	n.d.	
Tiglylglycine		n.d.	n.d.	n.d.	n.d.	n.d.	3-Oxothiolase deficiency (7); propionic aciduria (7)
2-Hydroxyphenylac- etic acid	Mean	0.03	n.d.	n.d.	n.d.	n.d.	Phenylketonuria (1)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	1	n.d.	n.d.	n.d.	n.d.	
2-Hydroxyglutaric acid	Mean	11.3	20	12.1	7.4	2.2	D- and L-2-hydroxygluta- ric aciduria (8); glutaric aciduria II (MAD) (14)
	Min	4	5	5	1.3	0.8	
	Max	30	69.5	26.8	13.9	52	

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
3-Hydroxyglutaric acid	Mean	0.03	0.7	2.2	1.6	–	Glutaric aciduria I (2)
	Min	n.d.	n.d.	1	n.d.	–	
	Max	1	3	4.2	4.6	–	
Acetoacetic acid	Mean	0.08	0.1	1.6	1.1	n.d.	Ketosis; defects of ketolysis (14)
	Min	n.d.	n.d.	0.2	n.d.	n.d.	
	Max	1.5	1.5	5.8	5	n.d.	
Phenyllactic acid	Mean	n.d.	n.d.	0.3	0.02	n.d.	Phenylketonuria (1)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	n.d.	1.3	0.2	n.d.	
2-Methylacetoacetic acid		n.d.	n.d.	n.d.	n.d.	n.d.	3-Oxothiolase deficiency (7)
3-Hydroxy-3-methylglutaric acid	Mean	28	22	22	10.3	3	3-Hydroxy-3-methylglutaric acidemia (6)
	Min	22	15	6.2	n.d.	n.d.	
	Max	40	43	49.7	28	10	
2-Oxoisocaproic acid	Mean	n.d.	1.2	n.d.	n.d.	n.d.	MSUD (6); dihydrolipoyl dehydrogenase (E ₃) deficiency (6, 27)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	7	n.d.	n.d.	n.d.	
2-Oxo-3-methylvaleric acid	Mean	n.d.	1	n.d.	n.d.	n.d.	MSUD (6); dihydrolipoyl dehydrogenase (E ₃) deficiency (6, 27)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	7	n.d.	n.d.	n.d.	
Hexanoylglycine		n.d.	n.d.	n.d.	n.d.	n.d.	MCAD (14)
4-Hydroxyphenylacetic acid	Mean	17.9	33.3	37	19.4	11	Tyrosinemias (all forms) (4); bacteria
	Min	3	3	12.3	7.4	3.5	
	Max	78	240	174	30.1	22	
<i>N</i> -acetylaspartic acid	Mean	13	15.4	20.2	11.2	–	<i>M.</i> Canavan (8)
	Min	8	5	7	6	–	
	Max	31	34	40.8	21.6	–	
2-Hydroxyadipic acid	Mean	0.3	0.1	0.9	0.4	–	2-Oxoadipic aciduria (12)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	4	1	2.8	1.5	–	
Octenedioic acid	Mean	0.4	0.1	1.9	1.4	–	MCAD (14); peroxisomal disorders (15)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	1	2	2.8	2.5	–	
3-Hydroxyadipic acid	Mean	0.6	2.1	7.9	3.6	–	Long-chain 3-hydroxy-CoA dehydrogenase deficiency (LCHAD) (14)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	6	7.5	15.7	13.3	–	
Suberic acid	Mean	2.8	0.3	2.2	1.4	0.5	MCAD (14); LCAD (14); glutaric aciduria II (14); MCT feeding; ketosis
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	16	20	10.1	8.8	2.9	
Aconitic acid	Mean	19.1	23.8	106	79	13	
	Min	8	10	26.87	20.5	2.7	
	Max	38.5	54	189	135	44	
Orotic acid	Mean	0.03	n.d.	1.6	0.7	n.d.	Urea cycle disorders (11); orotic aciduria (11)
	Min	n.d.	n.d.	0.02	n.d.	n.d.	
	Max	1	n.d.	3.6	1.9	n.d.	

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
Homovanillic acid	Mean	6.8	7	7.4	4.8	2.3	Neuroblastoma
	Min	4	2.5	4.2	0.7	0.9	
	Max	15	18.5	13.2	10.3	5.5	
Azelaic acid	Mean	0.4	0.3	6.4	9.1	4.8	Ketosis; peroxisomal disorders (25)
	Min	n.d.	n.d.	n.d.	n.d.	1.3	
	Max	4	4	15.4	46.7	15	
Homogentisic acid		n.d.	n.d.	n.d.	n.d.	n.d.	Alcaptonuria
Hippuric acid	Mean	62.2	48	495	275	290	
	Min	n.d.	2	119	58	170	
	Max	162	122	1390	746	390	
Isocitric acid	Mean	39.5	40.3	52.8	68.2	58	
	Min	25	19.5	11.3	34	36	
	Max	65	120	81.4	141	84	
Citric acid	Mean	401	480	385	386	155	
	Min	93	117	75	120	70	
	Max	1022	1422	667	582	226	
Methylcitric acid	Mean	n.d.	n.d.	2.5	2.2	0.1	Propionic and methylma- lonic acidurias (7); biotin disorders (7)
	Min	n.d.	n.d.	0.5	0.2	n.d.	
	Max	n.d.	n.d.	5.3	5.8	2	
Decenedioic acid	Mean	1.3	0.5	0.4	0.2	–	MCAD (14); peroxisomal disorders (25)
	Min	n.d.	n.d.	n.d.	n.d.	–	
	Max	13.5	7.5	3.1	1.6	–	
Vanillinmandelic acid	Mean	1.78	2.92	8	9	–	Neuroblastoma
	Min	n.d.	n.d.	0.7	1	–	
	Max	8	14	17	15	–	
Sebacic acid	Mean	3.3	0.04	0.4	0.1	n.d.	MCAD (14); LCAD (14); glutaric aciduria II (14); MCT feeding; ketosis
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	40	57	1.4	1.5	n.d.	
Decadienedioic acid	Mean	2	n.d.	0.4	0.05	n.d.	Fatty acid oxidation disorders (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	53	n.d.	2.6	0.5	n.d.	
4-Hydroxyphenyll- actic acid	Mean	16.5	3.7	1.3	1	1.1	Tyrosinemias (all forms) (4); hawkinsinuria (4); peroxisomal (25) and mi- tochondrial disorders (27)
	Min	n.d.	n.d.	0.03	n.d.	0.2	
	Max	74.5	48	3.1	3.6	2.6	
2-Oxoglutaric acid	Mean	56	115	66	36	24	2-Oxo-glutaric aciduria (8); dihydrolipoyl (E ₃) dehydrogenase deficiency (6, 27); mitochondrial disorders (27)
	Min	n.d.	4	29.8	2.4	4	
	Max	233	524	117	94.8	74	
Phenylpyruvic acid	Mean	5	6.1	n.d.	n.d.	n.d.	Phenylketonuria (1)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	19.5	15.5	n.d.	n.d.	n.d.	
Phenylpropionylgly- cine		n.d.	n.d.	n.d.	n.d.	n.d.	MCAD (14)

Table C.1 (continued)

Compound		Premature infants ≤36 weeks	Term newborns >36 weeks	Children ≤5 years	Children >5 years	Adults	Disorder to be considered (Chapter number)
Vanillinlactic acid	Mean	5	trace	n.d.	n.d.	n.d.	Neurotransmitter defect (2)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	40	20	10	5	n.d.	
2-Hydroxysebacic acid		trace	trace	trace	trace	trace	Peroxisomal disorders (25)
2-Oxoadipic acid	Mean	1.3	n.d.	n.d.	n.d.	n.d.	2-Oxoadipic aciduria (12)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	29	n.d.	n.d.	n.d.	n.d.	
3-Oxoadipic acid	Mean	n.d.	0.04	n.d.	n.d.	n.d.	
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	1.5	n.d.	n.d.	n.d.	
3-Hydroxysebacic acid	Mean	2.7	4	2.3	0.2	n.d.	Long-chain 3-hydroxy-CoA dehydrogenase deficiency (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	19	65.5	9.1	2.0	n.d.	
<i>N</i> -acetyltyrosine	Mean	75	1.6	n.d.	n.d.	n.d.	Tyrosinemias (all forms) (4)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	781	6.4	n.d.	n.d.	n.d.	
Indolelactic acid	Mean	n.d.	0.5	0.46	0.1	n.d.	
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	n.d.	8	3.4	0.6	n.d.	
5-Hydroxyindole- acetic acid	Mean	6.9	4.4	4.7	3	1.5	
	Min	n.d.	n.d.	0.2	n.d.	n.d.	
	Max	57	11.5	11.5	8.7	7.2	
Suberylglycine	Mean	0.8	n.d.	n.d.	n.d.	n.d.	MCAD (14)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	15	n.d.	n.d.	n.d.	n.d.	
4-Hydroxyphenyl- pyruvic acid	Mean	18.2	8	0.03	0.02	n.d.	Tyrosinemias (all forms) (4); hawkinsinuria (4)
	Min	n.d.	n.d.	n.d.	n.d.	n.d.	
	Max	276	74	0.4	0.3	n.d.	
Succinylacetone		n.d.	n.d.	n.d.	n.d.	n.d.	Tyrosinemia (hepatorenal form only) (4)

Reference values were derived from 30 premature infants (≤36 weeks of gestation), 34 term newborns (≥36 weeks of gestation), 13 children younger than 5 years, 20 children older than 5 years, and 9 control adults. All control subjects were healthy with no evidence of severe systemic or especially metabolic disease. The compounds are listed in the order of their chromatographic appearance, i.e. in the order of their MUs (DB5 30 m×0.25 mm I.D. fused-silica capillary column, film thickness 1 µm, J&W, Rancho Cordova, CA, USA). For a detailed description of the methodology see ref. (18). Abbreviations employed were Min.=minimal, and Max.=maximal values, n.d.=not detectable (<1 mmol/mol of creatinine), – = undetermined.

Disorders listed in the last column are indicated by elevations of the respective organic acid.

^a Total organic acids, units equ/mol of creatinine.

^b Individual organic acids, units = mmol/mol of creatinine.

Table C.2. Reference values: concentrations of organic acids in control CSF and plasma samples

Compound	Cerebrospinal fluid			Plasma		
	Mean (% Occ.)	Min.	Max.	Mean (% Occ.)	Min.	Max.
	meq/l			meq/l		
Total organic acids	10.4 (100%)	6 μ mol/l	15 μ mol/l	12.2 (100%)	5 μ mol/l	19 μ mol/l
Lactate	850 (100%)	450	2100	1700 (100%)	700	3300
2-Hydroxyisobutyrate	n.d. (0%)	n.d.	n.d.	7 (13%)	n.d.	9
Hexanoate	trace (32%)	n.d.	1.5	17 (75%)	n.d.	105
Glycolate	54 (100%)	5	250	27 (100%)	9	42
2-Hydroxybutyrate	35 (100%)	11	86	54 (100%)	8	80
3-Hydroxypropionate	4.4 (50%)	n.d.	9.5	2.3 (40%)	n.d.	4
3-Hydroxybutyrate	48 (100%)	trace	280	180 (100%)	22	700
3-Hydroxyisobutyrate	18 (93%)	trace	38	20 (100%)	4	48
2-Hydroxyisovalerate	6.8 (75%)	n.d.	18	7.7 (80%)	n.d.	19
Octanoate	2.5 (3%)	n.d.	2.5	8 (32%)	5	19
Succinate	3 (25%)	n.d.	5	9 (73%)	n.d.	32
Glycerate	34 (100%)	trace	95	10 (93%)	n.d.	24
Fumarate	trace (5%)	n.d.	trace	1.5 (78%)	n.d.	4
Glutarate	trace (5%)	n.d.	trace	0.8 (17%)	n.d.	1.8
2,4-Dihydroxybutyrate	84 (44%)	n.d.	260	2 (40%)	n.d.	7
3,4-Dihydroxybutyrate	15 (28%)	n.d.	73	18 (50%)	n.d.	54
Decanoate	n.d. (0%)	n.d.	n.d.	11 (10%)	5	17
Malate	3 (13%)	n.d.	4.5	12 (78%)	n.d.	21
Pyruvate	71 (95%)	trace	102	92 (100%)	27	160
Pyroglutamate	41 (100%)	10	96	51 (100%)	13	161
2-Oxoisovalerate	8.2 (63%)	n.d.	15	14 (72%)	n.d.	28
Erythronate	5 (30%)	n.d.	21	2 (13%)	n.d.	5
2-Hydroxyglutarate	1 (15%)	n.d.	3	1.5 (6%)	n.d.	1.5
Acetoacetate	6 (84%)	n.d.	32	21 (95%)	n.d.	86
2-Oxo-3-methyl- <i>n</i> -valerate	2 (77%)	n.d.	8	18 (100%)	8	31
2-Oxoisocaproate	5 (93%)	n.d.	9.4	28 (93%)	n.d.	58
Laurate	2.8 (64%)	n.d.	6.3	12 (100%)	2	37
Suberate	1.7 (3%)	n.d.	1.7	3.6 (8%)	n.d.	10
Acetonitrate	2 (5%)	n.d.	4	n.d. (0%)	n.d.	n.d.
Azelate	17 (17%)	n.d.	35	27 (44%)	n.d.	58
Isocitrate	10 (100%)	1	22	6 (90%)	n.d.	10
Citrate	350 (100%)	90	590	190 (100%)	30	400
Hippurate	n.d. (0%)	n.d.	n.d.	3 (13%)	n.d.	5
Myristate	5 (18%)	n.d.	14	25 (100%)	8	70
2-Oxoglutarate	2 (80%)	n.d.	9	7 (84%)	n.d.	23
Palmitoleate	2 (8%)	n.d.	6	31 (100%)	5	85
Palmitate	18 (93%)	n.d.	30	250 (100%)	75	780
Linoleate	11 (8%)	n.d.	14	110 (100%)	42	370
Oleate	36 (40%)	n.d.	120	460 (100%)	120	1830
Stearate	10 (87%)	n.d.	37	85 (100%)	31	470

Reference values from 35 paired control specimens of CSF and plasma from children aged 0.2 to 16 years (from 17). The compounds are listed in the order of their chromatographic appearance, i.e. in the order of their MUs (DB5 Megabore 30 m \times 0.53 mm I.D. fused-silica capillary column with a 1.5- μ m bonded film; J&W, Rancho Cordova, CA, USA). The values given in parentheses behind the means were the percentages of samples, in which the compound could be detected and/or quantified (% Occ.). Abbreviations employed were Min., minimal, and Max., maximal values. "Trace" reflects values approximating 1 μ mol/l, around the limit of detection. n.d. indicates not detectable, i.e. <1 μ mol/l. For interpretation of results and differential diagnosis of pathological values see last column of Table C.1.

Table C.3. Indications for organic acid analysis

Indications for quantitative determinations of organic acids in CSF

Screening for organic acid disorders (no urine available)

High clinical suspicion for organic acid disorders with possibly isolated elevations of pathological metabolites in CSF ("cerebral" lactic acidemias; glutaryl-CoA dehydrogenase deficiency; disorders of biotin metabolism)

Patients with organic acid disorders manifesting neurological disease

Table C.4. Organic acid analysis

Indications

Routine clinical chemical indices

(Unexplained) metabolic acidosis
Increased anion gap
(Hypoketotic) hypoglycemia
Massive ketosis
Ketonuria (especially suggestive in newborns)
Lactic acidosis
Hyperammonemia
Hyperuricemia
Hypertriglyceridemia
Granulocytopenia and thrombocytopenia

Clinical signs and symptoms

Consanguinity
Uneventful pre-/perinatal history
Systemic intoxication
Tachypnoea/acidotic breathing
Refusal of feeding
Adverse reaction to feeding
Protracted episodic vomiting
Pyloric stenosis (with acidosis)
Reye or "Reye-like" syndromes
Hepatosplenomegaly
Myeloproliferative syndrome
Failure to thrive
Peculiar smell
(Near missed) sudden infant death syndrome
Symptomatic sibling of sudden infant death syndrome victim
Acute metabolic encephalopathy
Hypotonia/lethargy/coma
Myoclonus
(Myoclonic, intractable) seizures
Acute profound dyskinesia
Pseudotumor cerebri
Cerebral/intraventricular hemorrhage in full term babies
Neurological presentation
Progressive psychomotor retardation
Fluctuating symptomatology/crises/coma
Macrocephaly

Table C.4 (continued)

(Metabolic) stroke
(Progressive) ataxia
Hypotonia
Dystonia, athetosis
Myoclonus
(Myoclonic, intractable) seizures
(Progressive) peripheral neuropathy
Pyramidal signs/paraspasm/cerebral palsy
Speech retardation
Myopathy and/or cardiomyopathy
Congenital cerebral malformations
Fluctuating multisystemic disease
Failure to thrive
(Chronic, recurrent) pancreatitis
Hepatosplenomegaly
Reye or "Reye-like" syndromes
Recurrent severe infections
(Non-immune hemolytic) anemia
Myeloproliferative syndrome
(Cave: Routine chemical abnormalities such as hypoglycemia, metabolic acidosis, lactic acidosis, hyperammonemia or ketonuria, the usual concomitants of disorders of organic acid metabolism, can be conspicuously absent in the last two categories)
Neuroradiological and neurophysiological signs
(suggestive are (fluctuating, progressive) symmetrical pathological changes, apparently independent of defined regions of vascular supply)
White matter disease
Leukodystrophy (centrum semiovale)
Spongiform encephalopathy (arcuate fibers)
Cerebellar atrophy
Prominent basal ganglia involvement
Calcifications
"Holes"
Atrophy
Transient germinaloid cysts at the caudothalamic pit
Fronto-temporal "atrophy"
Chronic subdural effusions and/or hematomas
(Cave: child abuse)
Burst-suppression EEG pattern

Table C.5. Specimen collection and handling

Specimen	
Urine	Collect random sample without preservatives and keep it frozen until shipment on dry ice. Volume should be 5 to 10 ml. Timed urine collections are unnecessary. If shipped unfrozen, a few drops of chloroform should be added to the urine as a preservative
Plasma	Centrifuge heparinized blood immediately and keep supernatant plasma (at least 1 ml) frozen until shipment on dry ice
CSF	Collect two 1 ml aliquots without preservatives. Freeze on dry ice at the bedside and keep frozen at -80°C until shipment on dry ice.
Vitreous humour	Aspirate at least 1 ml from the lateral angle of the eye and keep frozen until shipment on dry ice

Table C.6. Artefacts in urinary organic acid analysis

Condition	Organic acid(s) involved	Value (\uparrow/\downarrow)
Dietary origin	Adipic acid	\uparrow
	Furoic acid	\uparrow
	Tartaric acid	\uparrow
	N-Acetyltyrosine	\uparrow
	N-Acetyltryptophane	\uparrow
MCT feeding	Sebacic \rightarrow suberic \rightarrow adipic acid	\uparrow
	7-Hydroxyoctanoic acid;	\uparrow
	octanoylglucuronide	\uparrow
Nutramigen feeding	5-Oxoproline	\uparrow
Pregestimil feeding	di-(2-ethylhexane)phthalate	\uparrow
Medications	Cyclohexanediol	\uparrow
i. v. Solutions	Propanediol	\uparrow
L-DOPA	Vanillinlactic acid	\uparrow
Paracetamol (acetaminophen)	5-Oxoproline	\uparrow
Valproic acid	Numerous metabolites (including dicarboxylic acids)	\uparrow
Acetylsalicylic acid	2-Hydroxyhippuric acid	\uparrow
Ethosuximide	Numerous metabolites	\uparrow
Phenytoin	Numerous metabolites	\uparrow
Primidone	Numerous metabolites	\uparrow
Ethylene glycol poisoning	Ethylene glycol	\uparrow
	Glycolic acid, oxalic acid	\uparrow
	Benzoic acid	\uparrow
	Hippuric acid	\downarrow
	Glutaric acid	\uparrow
Bacterial contamination	3-Hydroxypropionic acid	\uparrow
	4-Hydroxyphenylacetic acid	\uparrow
	D-Lactic acid	\uparrow
	2-Oxoglutaric acid	\uparrow
	Phenylpropionylglycine	\uparrow
	Succinic acid	\uparrow
	Uracil	\uparrow

Table C.6 (continued)

Condition	Organic acid(s) involved	Value (↑/↓)
Blind loop syndrome	2-Hydroxyglutaric acid	↑
	Benzoic acid	↑
	3-Hydroxybenzoic acid	↑
	4-Hydroxybenzoic acid	↑
	D-Lactic acid	↑
	Lactylactate	↑
Contaminations		
with soap	Palmitic and stearic acid	↑
with ointment	Glycerol	↑

Table C.7. Key and “problem” organic acids requiring special attention, repeated and additional investigations already at borderline elevated levels

Elevated compound	Disorder to be considered (chapter number)
<i>N</i> -Acetylaspatic acid	M. Canavan [2]
Ethylmalonic acid	Mitochondrial disorders [24]
	Short-chain acyl Co-A dehydrogenase deficiency [15]
Fumaric acid	Fumaric acidemia [8]
Glutaric acid	Glutaryl-CoA dehydrogenase deficiency [13]
3-OH-Glutaric acid	
Glutaconic acid	
4-Hydroxybutyric acid	4-Hydroxybutyric acidemia [3]
4-Hydroxycyclohexylacetic acid	Hawkinsinuria [4]
3-Hydroxydicarboxylic acids	Long-chain 3-hydroxyacyl-CoA Dehydrogenase deficiency [15]
3-Hydroxyisovaleric acid	Disorders of biotin metabolism [7]
Malonic acid	Malonyl-CoA decarboxylase deficiency [8]
Methylcitric acid	Disorders of propionate metabolism
3-Methylglutaconic acid	3-Methylglutaconic acidemias [6]
2-Methyl-3-hydroxybutyric acid	3-Oxothiolase deficiency [7]
Tiglylglycine	
2-Methylacetoacetic acid	
Methylmalonic acid	Disorders of B ₁₂ metabolism [7]
Suberic acid	Medium-chain acyl-CoA
Sebacic acid	Dehydrogenase deficiency [15]
Hexanoylglycine	
Phenylpropionylglycine	
Decenedioic acid	Very-long-chain acyl-CoA
Decadienedioic acid	Dehydrogenase deficiency [15]
Succinylacetone	Tyrosinemia type I [4]

Table C.8. “Non-organic acidurias” detectable by organic acid analysis

Metabolic pathways	Disorder	Compound	Value
Purines and pyrimidines	Dihydropyrimidine dehydrogenase deficiency	Uracil	↑↑
		Thymine	↑↑
	Dihydropyrimidase deficiency	Uracil	↑
		Thymine	↑↑
		DHU+DHT	↑
		Uracil	↑
	Ureidopropionase deficiency (β -alanine synthase deficiency)	Thymine	↑
		DHU+DHT	↑↑
		β -ALA+ β -AIB	↑↑
		Xanthine	↑
Urea cycle	Xanthine oxidase deficiency	Orotic acid	↑↑
	Ornithine transcarbamylase deficiency	Uracil	(↑)–↑↑
	Citrullinemia		
	Argininemia		
Carbohydrate	D-Glycerate kinase deficiency	D-Glyceric acid	↑↑
Glycerol	Glycerokinase deficiency	Glycerol	↑↑
Neurotransmitter	Aromatic L-amino acid decarboxylase deficiency	Vanillin lactic acid	↑
Nongenetic disorder	Neuroblastoma	HVA	↑
		VMA	↑

DHU, Dihydrouracil; DHT, dihydrothymine; β -ALA, β -alanine; β -AIB, β -aminoisobutyric acid; HVA, homovanillic acid; VMA, vanillinmandelic acid.

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