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Propionic acidaemia: clinical, biochemical and therapeutic aspects

Experience in 30 patients

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Abstract Comprehensive data on 30 patients with propionic acidaemia, diagnosed by selective screening for inborn errors of metabolism, are presented. The most valuable diagnostic metabolites found were methylcitric-, 3-hydroxypropionic-, and 2-methyl-3-oxovaleric acids. Hyperlysinaemia and hyperlysinuria are also characteristic findings in this disease. The metabolic pattern found in propionic acidaemia is discussed extensively as are enzymatic findings. Residual activity of propionyl-CoA carboxylase is neither a predictive marker for severity nor for outcome of the disease. Propionate fixation assays were less reliable for confirmation of propionic acidaemia and of no prognostic value. Clinical presentation of the disease is discussed in detail. Besides the well-known unspecific findings (poor appetite, feeding difficulties, vomiting, dehydration, weight loss, muscular hypotonia, dyspnoea, somnolence, apathy, convulsion, coma, severe metabolic acidosis, hyperammonaemia) various skin abnormalities have been detected in about 50% of all patients. In 27% "dermatitis acidemica" was found.

Key words Propionic acidaemia
Propionyl-CoA carboxylase deficiency · Skin lesions
Inborn errors of metabolism

Abbreviations *GC-MS* gas chromatography-mass spectrometry *MCC* 3-methylcrotonyl-CoA carboxylase · *OLCFA* odd-numbered long-chain fatty acids · *PA* propionic acidaemia · *PC* pyruvate carboxylase *PCC* propionyl-CoA carboxylase *P-CoA* propionyl-CoA *SSS-syndrome* staphylococcal scalded skin syndrome · *MSUD* maple syrup urine disease

Introduction

Propionic acidemia (PA) is a rare autosomal recessively inherited inborn error of propionate metabolism presenting in most cases as a life-threatening disease during the neonatal period with severe metabolic acidosis, hyperammonaemia, hyperglycinaemia, and hyperglycinuria.

In 1968, Hommes et al. [30] demonstrated elevated concentrations of propionate in serum and odd-numbered long-chain fatty acids (OLCFA) in the liver of an infant with severe metabolic acidosis who died on the 5th day of life. Deficiency of propionyl-CoA carboxylase (PCC), (E.C. 6.4.1.3) was suspected. In 1969, Hsia et al. [32] demonstrated PCC deficiency in leucocytes from a 6-year-old girl with ketotic hyperglycinaemia and later in fibroblasts [33] from an older sibling of this patient with a similar clinical course [12]. Gompertz et al. [21] finally confirmed PCC deficiency in the liver of a patient with PA.

Subsequently many patients with PA have been described, several of whom were included in a clinical update published by Wolf et al. in 1981 [98]. During the last 15 years (1975–1990) we diagnosed 30 patients with PCC deficiency by selective screening for inborn errors of metabolism using gas chromatography (GC) and gas chro-

matography-mass spectrometry (GC-MS). Diagnosis was verified by enzyme analysis in most cases. In this paper we present our accumulated experience.

Patients

Most patients were from Germany (22), some from Austria (5) and 3 from Switzerland, 15 of them were of Turkish and 1 of Lebanese origin. Patients were selected for investigation for inborn metabolic disorders by the local clinical paediatricians according to their individual experience with this group of diseases.

Biochemical methods

Analysis of organic acids

Random or morning urine samples (1–10 ml), treated with 1–2 drops of chloroform for preservation, were transported by mail at ambient temperature.

Preparation of samples and analysis by GC and GC-MS respectively as methyl ester derivatives was performed as described [44].

Enzymatic investigations

Cultured skin fibroblasts from 22 out of 30 patients, lymphocytes from 1, and liver from another patient obtained after death, were available for enzyme studies. There was no material from 6 patients. One of these was a sibling of an enzymatically proven case. Fibroblasts were routinely cultured in a basal medium (MEM) containing 10% fetal calf serum which provides a final biotin concentration of 10^{-8} mol/l. Fibroblasts from most patients were also cultured in basal medium supplemented with biotin to give a final concentration of 10^{-5} mol/l. PCC and a further biotin-dependent enzyme, 3-methylcrotonyl-CoA carboxylase (MCC)(EC 6.4.1.4) were assayed directly in the same cell or tissue homogenates in most of the patients by measuring the incorporation of ^{14}C-bicarbonate into acid-stable products as described earlier [5, 84], ^{14}C-Propionate fixation in intact fibroblasts was measured by the method of Willard et al. [93], except that the propionate concentration was 0.65×10^{-4} mol/l.

Results

Metabolic pattern and other laboratory findings

Tables 1 and 2 summarize urinary organic acid excretion and other laboratory parameters. With respect to reliability of diagnosis, the organic acids most consistently found in our patients were 2-methyl-3-oxovaleric, methylcitric and to some extent 3-hydroxypropionic acids. Serum carnitine was measured only in a few of our most recently detected patients and in all of them a deficiency was apparent. Of the children, 96% had clear hyperglycinaemia and 57% hyperglycinuria. In 90% of all patients severe metabolic acidosis was present. Two, however, although severely ill, had no disturbances of acid/base balance. Hyperlysinaemia and hyperlysinuria were often found. A further sign characteristic of PA, especially valuable as a pointer to an inborn error of metabolism, is hyperammonaemia, found in 88% of all patients. In addition haematological abnormalities were frequently observed.

Table 1 Urinary organic acids in PA (initial specimens)

	Number of patients with a finding / investigated	(%)
2-Methyl-3-oxovaleric acid	27 / 27	100
Methylcitric acid	25 / 27	93
Lactic acid	17 / 26	65
3-Hydroxypropionic acid	17 / 27	63
3-Hydroxy-2-methylbutyric acid	13 / 27	48
N-Propionylglycine	12 / 27	44
Other glycine conjugates with short-chain fatty acids	10 / 27	37
Ketonuria	9 / 26	35
3-Oxovaleric acid	6 / 27	22
3-Hydroxy-n-valeric acid	6 / 27	22
2-Methyl-3-oxobutyric acid	6 / 27	22

Table 2 Other laboratory findings in PA (at any time)

	Number of patients with a finding / investigated	Patients with a finding (%)
Serum carnithine deficiency	6 / 6	100
Hyperglycinaemia	23 / 24	96
Metabolic acidosis	27 / 30	90
Hyperammonaemia	22 / 25	88
Leucopenia	24 / 29	83
Hyperlysinuria	19 / 23	83
Anaemia	21 / 29	72
Thrombopenia	18 / 29	62
Hyperlactic acidaemia	10 / 17	59
Hyperlysinaemia	13 / 23	57
Hyperglycinuria	13 / 23	57
Pancytopenia	12 / 29	41
Hypoproteinaemia	10 / 30	33
Hypoglycaemia	5 / 29	17
Hyperglycaemia	5 / 29	17

Enzyme studies

PCC deficiency was confirmed by direct assay of PCC activity in 24 patients (Table 3). In 12 of 14 patients studied with the sensitive assay, activities varied from 0.4% to 1.4% of mean normal values in fibroblasts grown in basal medium. In one patient (patient 1) with a typical severe course of the disease, clearly higher residual activities of approximately 3,8% were reproducibly found. In contrast, PCC activity was 0.9% of normal in the only patient (patient 2) with a mild course of the disease (first symptoms at 18 months of age).

Table 3 Activities of the biotin-dependent enzymes PCC and MCC in patients and controls

Subject	Material	Enzyme activities, pmol/min/mg protein		
		Expressed as	PCC	MCC
12 patients	Fibroblasts[a]	Mean ± S.D. (range)	9.5 ± 5.0 (4.1–19.2)	460 ± 180 (291–755)
Patient 1[b]	Fibroblasts	Single assay	41.8	461
Patient 2[c]	Fibroblasts	Single assay	9.5	293
21 controls	Fibroblasts	Mean ± S.D. (range)	1087 ± 356 (564–2005)	549 ± 158 (237–1798)
8 patients[d]	Fibroblasts	Range	<0–13.4	Not done
1 patient	Lymphocytes	Single assay	9.9	525
19 controls	Lymphocytes	Mean ± S.D. (range)	694 ± 86 (492–811)	468 ± 102 (288–681)
1 patient	Liver	Single assay	202	1307
6 control children	Liver	Mean ± S.D. (range)	18550 ± 4078 (14000–23800)	1580 ± 1300[e] (20–3233)

[a] Fibroblasts were grown in basal medium
[b] The only patient with >2% residual activity
[c] The only patient with a mild course of the disease
[d] PCC assay performed before 1981 (less sensitive assay)
[e] PCC activity is stable but MCC activity is unstable in post mortem liver tissues [5]

In 13 patients the effect of biotin supplementation on PCC activities was measured in fibroblasts. In 9 of them PCC activity in cells grown in a medium supplemented with biotin was lower or equal (range: 30%–100%, mean 60%) to that in cells grown simultaneously in the basal medium. In the other 4 cases 1.05–1.5 fold higher activities were found. Such variations of the very low levels of specific PCC activities (see Table 3) are probably within the overall variation of the assay. These results provide no evidence for an in vitro response to biotin in our patients. PCC activities were determined also in parents from three families (five subjects). In two families, both with two children having a severe clinical course, intermediate activities were found in the parents, consistent with the pccA mutation. In the third family with a child showing a relatively benign course, normal activities were found in both parents, consistent with pccBC group (see: Enzymatic and genetic aspects).

The incorporation of ^{14}C-propionate into macromolecules in intact fibroblasts was measured in 21 patients. Although propionate fixation was reduced in all these patients, the range of activity varied widely between 2.3% and 46% (mean: 21%) of simultaneous control values. Similar variations were obtained between different experiments with the same cell lines, e.g. 8.8%–33% of simultaneous control values in one cell line. The variations were in contrast to the clear differences between PCC activities in patient and control cell lines. Furthermore, as was the case with PCC activity, no significant increases in propionate fixation were observed in cells grown in biotin supplemented medium.

Table 4 Family history and the most frequent initial findings in our patients with PA

Family history	Number of patients with a finding / investigated	(%)
Consanguinity of parents	10 / 30	33
Unexplained deaths	14 / 30	47
Affected siblings	5 / 30	17
Pregnancy, delivery, and age of clinical onset		
Uneventful pregnancy	28 / 28	100
Born at term	25 / 28	89
Time of clinical onset:		
Within the 1st week	20 / 30	67
Within the first 2 weeks	23 / 30	77
Earliest manifestation (few hours)	1 / 30	3
Latest manifestation (1.5 years)	1 / 30	3
Unknown	5 / 30	17
Most frequent initial symptoms		
Poor feeding, weight loss	25 / 30	83
Muscular hypotonia	22 / 30	73
Somnolence, apathy	22 / 30	73
Vomiting	21 / 30	70
Respiratory problems	17 / 30	57
Convulsions	13 / 30	43
Hepatomegaly	11 / 30	37
Exsiccation, diarrhoea	11 / 30	37
Hypothermia	11 / 30	37
Obstipation	2 / 30	7

Clinical findings

The most important initial and later clinical findings in our patients are summarized in Tables 4 and 5 respectively. Striking is the frequency of skin lesions (53%). In two patients hair loss as seen in biotinidase deficiency occurred. Recently alopecia in PA has been described to result from selenium deficiency [99]. Our patients were not investigated in this respect. In several patients osteoporosis developed.

Table 5 Clinical findings in PA (observed at any time) and clinical course

	Number of patients with a finding / investigated	(%)
Feeding difficulties		
Poor feeding, weight losss	26 / 30	87
Vomiting	22 / 30	73
Neurological compromise		
Muscular hypotonia	24 / 30	80
Somnolence, apathy, coma	23 / 30	77
Convulsions	16 / 30	53
Respiratory problems		
Tachypnoea, apnoea, respiratory failure	19 / 30	63
Other symptoms		
Recurrent infections	18 / 30	60
Exsiccation, diarrhoea	16 / 30	53
Skin involvement	16 / 30	53
Candida infection	12 / 30	40
Dermatitis acidemica	8 / 30	27
Hair loss	2 / 30	7
SSS-syndrome	2 / 30	7
Hepatomegaly	11 / 30	37
Hypothermia	11 / 30	37
Obstipation	2 / 30	7
Clinical course		
Initial death	9 / 30	30
Death during crisis lateron	12 / 30	40
Frequent crisis, moderate outome	4 / 30	13
Mild course	8 / 30	27

Discussion

Biochemical, laboratory, and pathophysiological considerations

Propionyl-CoA (P-CoA), formed by degradation of several essential amino acids (valine, isoleucine, threonine, methionine), cholesterol side-chains, odd-numbered [30] or alpha-methylbranched-chain [62] fatty acids, is normally converted to methylmalonyl-CoA by the biotin-dependent enzyme PCC. In PA, deficiency of PCC causes accumulation of the metabolically highly active P-CoA. As a consequence a large variety of secondary metabolites is formed and excreted in the urine (Fig. 1).

Propionic acid is derived from hydrolysis of P-CoA and is detected in all body fluids in concentrations up to several mmol/l during metabolic crises [21, 30, 48]. Considerable amounts are also formed by gut bacteria and may aggravate metabolic crisis in disturbances of propionate metabolism [4, 77, 88]. A further source of propionic acid to be taken into account is pristanic – and its immediate precursor phytanic acid. Both are significant components of ruminant milk fat [62]. As shown by Gregersen [24], short-chain fatty acids and especially propionic acid exert a strong inhibitory effect on mitochondrial energy production and may thus contribute generally to the pathophysiology of acute episodes in PA. Furthermore propionate inhibits the growth of bone marrow stem cells, leading to leucocytopenia, thrombocytopenia, and/or pancytopenia [83], a condition which has been described repeatedly in PA [49, 86].

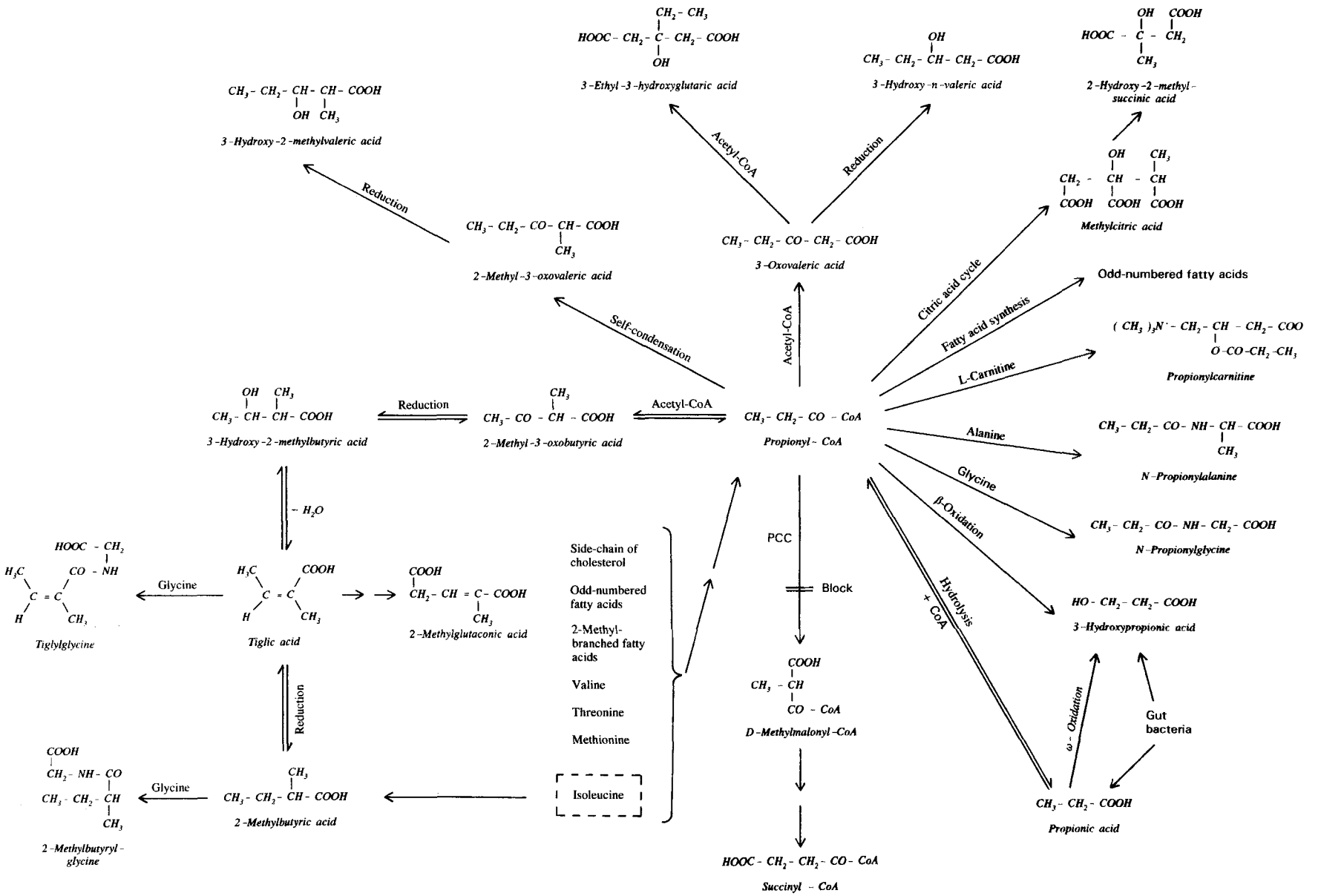
Beta- and/or omega-oxidation of P-CoA or propionic acid results in formation of 3-hydroxypropionic acid [1], a metabolite also found in short bowel disease [51] and other gastro-intestinal abnormalities [61]. Only 17 out of 27 of our patients investigated (63%) excreted clearly detectable amounts of 3-hydroxpropionic acid (Table 1).

Conjugation with glycine, alanine or L-carnitine produces propionylglycine [65], propionylalanine [19], and propionylcarnitine [18, 53] respectively. In 44% of our patients we found excretion of significant quantities of propionylglycine (Table 1). Propionylalanine was detected only inconsistently. Measurements of propionylcarnitine were not performed in this study.

Utilization of P-CoA as a primer in fatty acid synthesis instead of acetyl-CoA leads to OLCFA in PA [30]. Subsequently these lipids may contribute to increased urinary excretion of pathognomonic metabolites due to accelerated degradation during fasting [87] and catabolic conditions in disorders of propionate metabolism. Prolonged fasting should therefore be avoided in PA. Wendel and coworkers [90] suggested the use of the content of OLCFA in membrane lipids of erythrocytes for quality control of long-term dietary therapy. Further studies revealed that OLCFA production is active already prenatally [92]. By this mechanism part of the highly active P-CoA is incorporated into different lipids of all organs including the brain already during fetal life. Surprisingly these alterations seem not to influence normal pregnancy and birth. As a consequence enhanced postnatal lipolysis may lead to increased production of toxic P-CoA and secondary metabolites not only from proteins but also from OLCFA.

Incorporation of P-CoA instead of acetyl-CoA into the citric acid cycle leads to two diastereomeric methylcitric acids [2] with (1S,2S)- and (1S,2R)-configurations [9]. The diagnostic value of these metabolites was somewhat limited in our former analytical system using packed columns [48] because of serious overlapping with p-hydroxyphenylacetic acid, a compound found in every urine in large amounts. With capillary columns, however, methylcitric acid is one of the most important metabolites for PA detection. Considerable amounts are found also in methylmalonic aciduria, biotinidase deficiency and holocarboxylase synthetase defect.

2-Hydroxy-2-methylsuccinic (citramalic) acid is thought to be formed by further action of the citric acid cycle en-


3-Ethyl-3-hydroxyglutaric acid
3-Hydroxy-n-valeric acid
2-Hydroxy-2-methyl-succinic acid
3-Hydroxy-2-methylvaleric acid
Acetyl-CoA
Reduction
Reduction
Methylcitric acid
2-Methyl-3-oxovaleric acid
3-Oxovaleric acid
Self-condensation
Acetyl-CoA
Citric acid cycle
Fatty acid synthesis
L-Carnitine
Odd-numbered fatty acids
Propionylcarnitine
3-Hydroxy-2-methylbutyric acid
Reduction
2-Methyl-3-oxobutyric acid
Acetyl-CoA
Propionyl-CoA
Alanine
N-Propionylalanine
Glycine
β-Oxidation
N-Propionylglycine
- H2O
PCC
Block
Hydrolysis + CoA
3-Hydroxypropionic acid
Tiglylglycine
Glycine
Tiglic acid
2-Methylglutaconic acid
Side-chain of cholesterol
Odd-numbered fatty acids
2-Methyl-branched fatty acids
Valine
Threonine
Methionine
Isoleucine
D-Methylmalonyl-CoA
ω-Oxidation
Gut bacteria
Reduction
Glycine
2-Methylbutyric acid
Propionic acid
2-Methylbutyryl-glycine
Succinyl-CoA

zymes on methylcitrate [25]. It was not observed in considerable amounts in our patients.

Condensation of P-CoA with acetyl-CoA leads to 3-oxovaleryl-CoA [16], a homologue of acetoacetyl-CoA. Hydrolysis produces 3-oxovaleric acid. Reduction yields 3-hydroxy-n-valerate, the excretion of which was first described by Stokke et al. [82] in a patient who died from possible methylmalonic aciduria and later in PA by Sweetman et al. [85] and others [16, 45].

Reaction of 3-oxovaleryl-CoA with acetyl-CoA leads to 3-hydroxy-3-methylglutaryl-CoA synthase-catalysed formation of 3-hydroxy-3-ethylglutaric acid, a metabolite described previously in PA [40]. Although this compound was included in our library of mass spectra, we did not detect it in any considerable amount.

The occurence of 2-methyl-3-oxovaleric acid in patients with PA, described by Lehnert et al. [45, 48], is explained by self-condensation of two molecules of P-CoA. In our hands it turned out to be the most characteristic metabolite in diagnosing PA. Reduction produces 3-hydroxy-2-methylvaleric acid [89], which was also detected in some of our patients.

Several metabolites are formed from the break-down of isoleucine in PCC deficiency. Excess of 2-methylbutyryl-CoA is trapped by glycine and excreted as 2-methylbutyrylglycine [45, 85]. Excessive tiglyl-CoA is eliminated in a similar manner as tiglylglycine [66].

Small amounts of tiglyl-CoA are carboxylated by biotin-dependent 3-methylcrotonyl-CoA carboxylase yielding (E)-2-methylglutaconic acid. This has also been found in patient with methylmalonic aciduria and β-ketothiolase deficiency [17].

Minor quantities of tiglic acid detected by several authors [57] may be the result of hydrolysis of tiglylglycine during storage of the urine samples [16] or may of course be formed by hydrolysis of tiglyl-CoA within the cell.

Hydration of tiglyl-CoA results in formation of 3-hydroxy-2-methylbutyryl-CoA and dehydrogenation of the latter produces 2-methyl-3-oxobutyryl-CoA which likewise is formed by condensation of acetyl-CoA with propionyl-CoA. Hydrolysis produces the corresponding acids (3-hydroxy-2-methylbutyric- and 2-methyl-3-oxobutyric acid respectively). Both metabolites are foremost characteristic of β-ketothiolase deficiency. However, the former is detectable in small amounts in almost any urine.

Unusual ketones are likely to be formed by non-enzymatic degradation of the corresponding thermolabile 3-oxoacids [21, 48, 52]. Decarboxylation of both, 2-methylacetoacetic and 3-oxovaleric acids produces butanone and decomposition of 2-methyl-3-oxovaleric acid yields 3-pentanone. In contrast to the report of Menkes [52], 2-pentanone and 2-hexanone were not detected by the gas chromatographic method of Lehnert et al. [48].

Hyperglycinaemia and hyperglycinuria is a well documented finding in PA, already observed before the disease had been established as a distinct entity. Because of this finding, the disorder was called "ketotic hyperglycinaemia" [12, 70]. In our patients {Table 2) glycine excretion was highest after having received larger quantities of protein for a prolonged time. After adequate treatment, hyperglycinaemia and hyperglycinuria diminishes, but only slowly. As shown earlier, metabolites of isoleucine seem to have an inhibitory effect on the glycine-cleavage system in cultured fibroblasts [28, 29]. This may in part explain the accumulation of glycine. So far there is insufficient experimental evidence, however, to finally elucidate the mechanism whereby hyperglycinaemia and hyperglycinuria occur in PA. It is noteworthy that considerable amounts of glycine are also found in methylmalonic aciduria and β-ketothiolase deficiency.

Little attention has been paid to hyperlysinaemia and hyperlysinuria in PA. Gompertz et al. [21] described an amino acid pattern in serum, characteristic of nonketotic hyperglycinaemia together with elevated levels of lysine. Furthermore hyperlysinaemia was mentioned in four out of eight patients described by Duran et al. [16], three of them showing combined cystine-lysinuria. Parvy et al. [60] found hyperlysinaemia in three patients investigated and elevated cystine-lysinuria in four out of eight patients. They attributed their findings to a direct effect of propionate or it's metabolites on renal reabsorption of dibasic amino acids. Of our patients, 57% had hyperlysinaemia and 83% hyperlysinuria (Table 2). This was most noticeable during acute metabolic derangement. Combined cystine-lysinuria was detected in 14% only. There was no correlation between hyperglycin- and -lysinuria. The accumulation of lysine might be due to an interaction of P-CoA and/or it's metabolites on lysine-forming saccharopine dehydrogenase, the first step in lysine degradation.

Hyperammonaemia is a frequent finding in PA [43] (Table 2). It is thought to be caused by inhibitory effects of propionic acid [20], P-CoA, and perhaps secondary metabolites of P-CoA on the urea cycle. As shown by Coude et al. [13] and Stewart and Walser [81] the principal mechanism is deficiency of N-acetylglutamate (an allosteric effector of carbamylphosphate synthetase I) by depletion of acetyl-CoA and P-CoA-mediated competitive inhibition of N-acetylglutamate synthetase.

Blood ammonia concentrations correlate well with blood propionate levels [96] and may be helpful in monitoring therapy of an acute crisis. Out of 25 of our patients, 22 had hyperammonaemia during a metabolic crisis (Table 2). Because of it's availability even in peripheral hospitals, blood ammonia measurement is one of the most important parameters in the diagnosis of inborn errors of metabolism.

Kuhara and colleagues [39] observed elevated excretion of precursors of acetyl-CoA (glutarate, 3-hydroxyiso-

◀ **Fig. 1** Formation of P-CoA and secondary metabolites in PA. Explanations are given in more detail discussing biochemical, laboratory, and pathophysiological considerations. Metabolites are dealt with counterclockwise

valerate, 3-methylglutaconate, and lactate) during acute episodes in PA as compared with levels under favourable clinical conditions. This can be explained by inhibition of the corresponding metabolising enzymes by P-CoA and its metabolites. Lactic acidosis is common in PA. Of our patients, 65% excreted elevated amounts of lactate. One of the reasons for lactic acidosis is competitive inhibition of the pyruvate dehydrogenase complex by P-CoA [24]. The increased ketogenesis observed in PA and methylmalonic aciduria seems to be due to inhibition of the citric acid cycle enzymes by propionate and/or it's metabolites [69].

The wide range of severity of clinical symptoms and time of onset in PA is not easy to explain. Of our patients, 80% experienced their first metabolic decompensation within the first 2 weeks of life. One child was well until 18 months of age. In the literature at least two cases with extremely late onset of the disease have been reported [75, 97]. Time and severity of metabolic derangement depend on several factors, (1) residual activity of PCC; (2) quantity of protein ingested; (3) conditions precipitating catabolism (infections, fasting, other stress situations); and (4) activity of alternative pathways, mediating consumption of toxic P-CoA. However, there was no correlation between residual activity of PCC and the clinical course in our patients (see below).

Theory of chaos tells us, that future, also causally determined, is unpredictable in complex biological systems even with a small set of parameters because minimal deviations of initial variables may effect serious changes in final state. This also must be accounted for, when searching for correlations at the level of inborn metabolic disorders.

Biotin responsive PA has been considered as a possibility for many years but no well documented case of biotin-responsive PCC deficiency has been discovered so far.

Enzyme and genetic aspects

PCC is one of the 3 mitochondrial biotin-dependent carboxylases in man, the other two being 3-methylcrotonyl-CoA carboxylase (MCC; EC 6.4.1.4) and pyruvate carboxylase (PC; EC 6.4.1.1). Isolated PCC deficiency, i.e. PA, is inherited as an autosomal recessive trait. The frequency of PA, although not yet determined by experimental newborn screening, has been estimated to be approximately 1 in 50000 [44] from the number of patients with PA and methylmalonic aciduria respectively, detected by selective screening and the known incidence of methylmalonic aciduria published earlier [14]. Native PCC has been reported to be a dodecamer [22, 26] composed of 6 alpha- and 6 beta-subunits. The alpha-subunits contains the covalently bound biotin ligand. Complementation analysis of cultured skin fibroblasts from patients with PA has revealed two main complementation groups, i.e. pccA and pccBC, which are intergenic [23]. The pccBC group is complex, consisting of three intragenic subgroups (B, C, BC) [23, 74]. PccA mutants have a primary defect in the PCCA gene which codes for the alpha-chain of PCC and is located on the human chromosome 13 [41, 42]. In pccBC mutants the defect is in the PCCB gene which codes for the beta-subunit [41, 42] and is located on the human chromosome 3 [38, 42]. Investigations of skin fibroblasts at the molecular level have revealed considerable heterogeneity within the main complementation groups [59].

Parents of pccBC group patients have a normal level of PCC activity, while parents of pccA group patients have activity of about 50% of normal [95]. Measurement of enzyme activities in parents (performed only in three families in this study), might be valuable in defining the main complementation group, particularly in relation to direct analysis of mutations.

Variation of the clinical features of affected individuals does not seem to correlate with complementation group assignment [23]. Indeed, extreme variations in clinical presentation of PA have been found within the same complementation group [36] and even within the same family [97].

Although analysis of organic acids in urine is usually sufficient to make a correct diagnosis, it is valuable to determine PCC activity, especially if prenatal diagnosis, systematic studies of clinical outcome or genetic studies are considered. A rapid confirmation of diagnosis can be performed by measuring the three mitochondrial carboxylases in blood lymphocytes with the advantage to simultaneous exclusion of multiple carboxylase deficiencies due to defects in either holocarboxylase synthetase or biotinidase [84]. Cultured fibroblasts are valuable for more detailed studies, e.g. the effect of biotin supplementation on PCC activities and on propionate fixation. Several other tissue samples, e.g. liver, can also be used for the diagnosis. In contrast to MCC and PC activities, PCC activity is stable in post mortem tissues. It is resistant to storage at –70° C for several months [5].

Prenatal diagnosis is possible by measuring PCC activity in chorionic villi biopsies, cultured chorionic villi, cultured amniotic fluid cells or by measuring methylcitrate concentration in amniotic fluid [34].

The finding of clearly detectable residual activities (0.4%–1.4% of mean normal values) in fibroblasts from the 15 patients studied with our "sensitive" assay is in agreement with published data of Wolf [94] who reported the presence of residual PCC activity in all PA patients. Clearly higher residual activity (3.8% of normal values) was found in one patient (patient 1) who presented, however, with a typical severe clinical course whereas the only patient with a mild course of the disease (patient 2) had a low residual PCC activity of only 0.9%. These observations support previous results [23] suggesting a lack of correlation between clinical outcome and the level of residual activity.

There was no significant increase of PCC activity in fibroblasts of any of our patients in response to supplementation of the culture medium with high concentrations of biotin. This is in contrast to the observation of Wolf who reported a slight increase of PCC activities either in vivo (leucocytes) or in vitro (fibroblasts) by biotin supplementation although the patients did not seem to respond clinically [94].

^{14}C-Propionate fixation in intact fibroblasts has been widely used as a relatively simple method for confirmation of the diagnosis in patients with PA and methylmalonic aciduria. This indirect assay, in contrast to the determination of PCC activity, gives relatively high and variable activities even in cell lines with a profound enzyme deficiency. We therefore conclude that propionate fixation is a less suitable method for confirmation of the diagnosis and that conclusive and reproducible results can be obtained by the direct determination of PCC activity.

Studies using cDNA clones coding for the alpha- and beta-subunits as probes have revealed natural polymorphism associated with PCCA and PCCB genes [42]. As specific mutations become recognized it is likely that DNA analysis will play an increasing role in the diagnosis of PA.

Clinical presentation of PA

A frequent finding in our families with PA is consanguinity, which was found in 33% of all pedigrees (Table 4). In almost half of our families with PA at least one unexplained death, most probably from PA, was reported and in 5 families affected siblings existed.

In all cases pregnancy was uneventful and most children were born at term with normal body weight and length. This shows that P-CoA and its metabolites do not interfere with normal somatic development, although OL-CFA production and accumulation starts already during fetal life [92].

Usually a symptom-free interval lasting several days was observed in most of our patients. In more than 66%, clinical manifestation occured within the first week and in nearly 80% within the first 2 weeks of life (mean: 6 days). There were no significant differences with respect to clinical onset of PA as compared to other organic acidurias [73]. One patient experienced his first severe metabolic crisis at the age of 18 months. Real late-onset type of PA, as described recently in an adult with chorea and dementia [75] was not found. We also did not detect asymptomatic PA, as observed by Wolf et al. [97] in a 13-year-old girl following diagnosis in her younger brother. Of our patients, 80% were breast fed and no precipitating factor for metabolic decompensation was found. The remaining 20% had signs of intercurrent infections or septicaemia (data not shown). While PA with neonatal course follows a typical clinical pattern with unspecific symptoms such as poor appetite, feeding difficulties, vomiting, dehydration, weight loss, muscular hypotonia, dyspnoea, somnolence, apathy, convulsions, coma, and severe metabolic acidosis, diagnosis of the late onset form is still a challenge. Atypical clinical patterns such as acute infantile hemiplegia [76] or chorea and dementia [75] have to be considered.

Feeding problems (Tables 4 and 5) are one of the most common initial symptons (83%) in PA, often combined with heavy vomiting, in part appearing as the projectile type, resembling pyloric stenosis. According to the literature, several patients with an organic aciduria have been subjected to pyloromyotomy [55] before the correct diagnosis had been established. In one of our cases post mortem investigation revealed hypertrophic pyloric stenosis in addition to the verified PA [49]. Therefore unexplained vomiting, especially during the neonatal period and in infancy should always arouse the suspicion of an inborn metabolic disorder. Furthermore feeding remains a problem in later life. Several of our patients had to be at least partially fed by naso-gastric tube until the age of 5 years and even longer to assure intake of the minimum amount of calories necessary for maintenance and growth.

Neurological symptoms are common in PA (Table 5). The accumulation of toxic metabolites such as organic acids and ammonia leads to dysfunction of the central nervous system. First somnolence, apathy and seizures and then severe metabolic coma may result. Seizures as the only symptom, however, should not be overestimated as a characteristic sign for an inborn error of metabolism. Although 53% of our patients with PA had convulsions, we did not diagnose a single metabolic disorder in hundreds of patients who only had seizures. As a rule, patients with PA with convulsions, can also be expected to exhibit several of the typical symptoms summarized in Tables 4 and 5. It is noteworthy, that the initial gastro-intestinal symptoms and neurological deterioration may improve dramatically for several days after stopping protein intake and administrating intraveneously adequate amounts of glucose. In such cases diagnosis can be delayed for weeks, especially when cow milk intolerance is suspected.

As shown in Tables 4 and 5, respiratory problems in PA are common during initial crises (57%) and also later on (63%). These include tachypnoea (respiratory compensation for metabolic acidosis) as well as apnoea and respiratory failure requiring assisted ventilation.

Other more common symptoms in our patients (Tables 4 and 5) were recurrent infections (60%), diarrhoea with signs of exsiccation (53%), hepatomegaly (37%), and hypothermia (37%).

The frequency of skin lesions is striking. In about 50% of patients skin alterations varying from nappy rash with *Candida* infection to staphylococcal scalded skin syndrome (SSS-syndrome) have been observed (Table 5). Koopman and Happle [37] described acrodermatitis enteropathica-like skin lesions in two patients with methyl-

malonic aciduria showing sharply demarcated erythematous eruptions with a flaky or lammelar desquamation around the mouth, in the nappy area, the palms and the plantar surface of the feet. He called this type of skin inflammation "dermatitis acidemica". This was found to be associated not only with, methylmalonic aciduria, but also with PA [63, 78, 100]. We observed "dermatitis acidemica" in 8 out of 30 patients (Table 5). The cause may be lack of trace elements or special amino acids. In several of our patients zinc deficiency was ruled out. In one boy, a sibling of an affected patient, skin involvement occured as early as at the 7th day of life, rendering a nutritional deficiency unlikely. Association with SSS-syndrome was found in two of our patients (Table 5) and has been already described in others [58]. Although alterations in T- and B-cell function [54] and hypogammaglobulinaemia, more likely reflecting malnutrition (see hypoproteinaemia in Table 2) than a specific combined immunodeficiency [98], may partly explain the frequency of recurrent infections in PA (Table 5) as well as the described skin lesions, there is so far no convincing explanation of the striking skin involvement.

Neutropenia, thrombopenia, anaemia, and pancytopenia, often observed during acute attacks in PA (Table 2) are thought to result from propionate-mediated supression of proliferation and maturation of stem cells [31, 83]. Recently we investigated the influence of propionic acid on haematopoietic progenitor cells and T-lymphocytic colony formation and found a clear dose-dependent suppressive effect [64].

Myelination disturbances or demyelination of the CNS in PA have first been described by Behbehani et al (case included in this study) [6]. Clinical and morphological evidence suggest, that basal ganglia are especially vulnerable in PA [27]. Recent MRI studies, however, of brain in three patients and one heterozygote parent revealed no major abnormalities (own observation).

In several patients we detected osteoporosis with "spontaneous" fractures of bones. This phenomenon is also known in other organic acidurias such as methylmalonic aciduria but so far has not yet been satisfactorily explained.

Diagnosis

The clinical presentation of many metabolic disorders in the neonate and infant is very similar and routine laboratory measurements do not adequately reflect the underlaying disturbance. Therefore selective screening for metabolic disorders is essential, including amino acid evaluation and organic acid analysis by GC-MS as well as thin-layer chromatography of sugars to detect as wide a range of inborn metabolic diseases as possible.

In our patients commonly considered differential diagnoses were septicaemia, meningitis, gastro-intestinal obstructions, congenital infections and intracranial haemorrhage. Some children were suspected to have other metabolic diseases such as urea cycle disorders or nonketotic hyperglycinaemia.

In laboratories, where methyl esters are analysed [7, 15, 48] 2-methyl-3-oxovaleric together with methylcitric acid have proven to be the most reliable specific markers for PA (Table 1). Other metabolites (Fig. 1, Table 1) further support the diagnosis. In centres, where trimethylsilyl derivatives are being analysed, methylcitric- and 3-hydroxypropionic acids have been used most frequently as the diagnostic markers [16, 85]. Detection and determination of propionic acid is not necessary for diagnostic purposes [10, 16], (own experience) but could be valuable in therapeutic monitoring if readily available.

Irrespective of the method of derivatization employed, PA can be reliably diagnosed using modern capillary GC-MS. The lower levels of metabolites found after treatment by protein restriction and/or peritoneal dialysis may cause severe diagnostic difficulties, however, if GC is used without MS (own experience). It is therefore advisable to screen all samples by GC-MS.

Preselection of patients for selective metabolic screening, aided only by unspecific clinical symptoms and routine laboratory findings, is an important and difficult task. This great responsibility lies with the neonatologists and padiatricians. To facilitate their work, a list of indications for selective screening has been devised, distributed to children's hospitals [46, 47, 78], and used successfully for many years. Diagnosis of PA has to be confirmed by biochemical investigations. Prenatal diagnosis is available (see: Enzyme and genetic aspects).

Treatment

Usually therapy of PA has to begin during an acute metabolic crisis, when the nature of the disease is still unknown. At that time the whole spectrum of organic acidurias, urea cycle disorders, and defects in degradation of sugars and lipids has to be considered. As summarized in Table 6 a special therapeutic strategy is necessary at this early stage, until information from selective screening has become available. After establishing the diagnosis, a more specific therapy is introduced. Finally, when the acute crisis has been overcome, management of the chronic disease is required, including careful continuous metabolite monitoring. Three main principles are stressed in treatment of PA:

1. Stop and prevention of accumulation of toxic intermediary products (restriction of protein intake [50, 56]; inhibiton of endogeneous protein catabolism [35, 91]; reduction of propionate production by gut bacteria [88].

2. Effective elimination of toxic metabolites (blood exchange transfusions; peritoneal dialysis [72]; haemodialysis [71]; continuous arteriovenous haemofiltration [67, 79, 101]).

Table 6 Therapeutic regimen in PA

A. During acute metabolic crisis before diagnosis

1. Buffering, assisted ventilation (if necessary)
2. Restriction of protein intake [35]
3. High caloric parenteral nutrition [glucose 25–30 g/kg body weight and day)/insulin (0.05–0.2 IU/kg body weight and hour), and lipids (2–4 g/kg body weight and day) only, if β-oxidation defects had been ruled out] via a central venous line and careful monitoring
4. High fluid intake, forced diuresis (150–180 ml/kg body weight and day), with or without diuretics
5. L-Carnithine (100 mg/kg body weight per day) together with L-arginine hydrochloride (2 mmol/kg body weight within 2 h, then 2 mmol/kg body weight and day) i.v. [3]
6. Vitamins as a trial: 10–20 mg biotin/day, 100–200 mg riboflavin/day, and 300 mg thiamine/day, 1 mg vitamin B_{12}, i.v.
7. Detoxification, if necessary (peritoneal dialysis, haemodialysis, continuous arteriovenous haemofiltration)

B. During acute metabolic crisis after diagnosis (GC-MS analysis)

1. Termination of unspecific measures (L-arginine, vitamins)
2. Continuation of intensive therapy to induce anabolism; introduction of lipids
3. Early (re)administration of natural protein via nasogastric or nasoduodenal tube (0.5–0.7 g/kg body weight and day) and careful introduction of a nonpropiogenic amino acid mixture (free of Val, Ile, Thr, Met) [56] to achieve positive nitrogen balance
4. Prophylactic antibiotic treatment [4, 77, 88]
5. Substitution of erythrocytes, albumin and trace elements, if necessary
6. Careful clinical and metabolic monitoring of the patient

C. Long-term specific therapy

1. Carefully balanced intake of natural protein according to the individual point of tolerance (0.7–1.5 g/kg body weight and day)
2. Supplementation with nonpropiogenic amino acids up to the age-appropriate requirement of total protein
3. High-caloric intake
4. L-Carnithine (100 mg/kg body weight), orally
5. Training and education of the parents (to apply nasogastric tubes, to reduce protein intake during infections etc.)
6. Availability of a specialised and engaged metabolic centre within a few hours

3. Supportive measures (assisted ventilation, if necessary; correction of pH and fluid imbalances; administration of L-carnitine [8, 11, 68]; therapeutic trial with biotin; prevention of infections).

Long-term mangement of PA is a difficult task. Minimal disturbances provoked either by too high protein or too low caloric intake or catabolic situations (intercurrent infections, immunization, trauma, surgery) may cause life-threatening imbalances and decompensation. Thus metabolic equilibrium has to be monitored carefully. The following parameters may be helpful:

1. Clinical condition of the patient,
2. Somatic development of the child,
3. OLCFA in total lipid fraction of erythrocyte membranes [80, 90]. Patients continuously in good clinical condition show levels below 2%.
4. Blood gases, serum ammonia, lactate, and propionate levels (these become abnormal secondary to metabolic decompensation and indicate, that derangement has already taken place). For details see Table 6 and [58].

Prognosis

In this study patients with PA were diagnosed during a period of about 15 years. From the point of view of prognosis they can be divided into two groups, i.e., patients detected before 1986 and patients diagnosed thereafter. Before 1986 only 30% of all cases were treated adequately between onset of the disease and diagnosis, including 15%, where the nature of disease was already known because of prenatal diagnosis. In the other patients, however, protein was administered continuously (50%) or intermittently (20%) until diagnosis was obvious. After 1986, when selective screening for organic acidurias became established in central Europe, prognosis improved markedly. Selective screening programmes apparently increased the awareness amongst paediatricians of the correct handling of patients with acute metabolic crises. After 1986 75% of all new patients with PA were treated adequately between onset and diagnosis of the disease as well as during recurrent metabolic deterioration applying protein restriction, high caloric intake, specific measures for detoxification and L-carnitine supplementation. The time between onset and diagnosis, however, did not change markedly over the whole 15-year period. Nevertheless survival from the first metabolic crisis improved from 14/22 (64%) before 1986 to 8/8 (100%) thereafter. We therefore conclude that early diagnosis of PA is important but not as crucial as adequate therapy before diagnosis and during recurrent metabolic crises.

Of our patients with PA nine have so far survived. At the end of 1992 they were 2, 3, 3, 5, 5, 6, 7, 12 and 27 years old. Of the survivors three had normal psychomotor development, five are moderately and one is severely retarded.

With increasing age protein tolerance increases and metabolic decompensation occurs less frequently. In some patients demyelinisation of the brain has been observed by computed tomography. It is not clear, however, whether this finding is a consequence of endogenic intoxication during acute metabolic deterioration or a long-term effect of the disease. Two of our patients developed impaired renal function.

We conclude that normal psychomotor development is possible with PA, so long as early diagnosis, adequate treatment and careful therapeutic monitoring are consequently achieved. However, even short periods of poor control may result in a disastrous outcome or sudden death.

Conclusions

Selective screening procedures for inborn errors of metabolism have contributed considerably to the early diagnosis of PA and other organic acidurias. Adequate management of acute metabolic crises between onset and diagnosis as well as improved treatment of recurrent metabolic derangements has markedly improved the outcome of patients with PA. Although reliable and rapid diagnosis, using GC-MS and/or enzymatic investigations is possible within a single day, we are still unaware of a predictive marker for prognosis of the disease at the time of diagnosis. Influence of metabolites on haematopoietic and central nervous systems as well as reasons for peculiar feeding problems are further challenging fields of future research. The therapy of PA should be guided by more strict protocols elaborated by centres with adequate experience. The long-term firm guidance of parents and patients by established diagnostic and therapeutic centres is indispensable for a benefical outcome.

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