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Inborn Metabolic Diseases

Diagnosis and Treatment

Second Edition

With 52 Figures and 50 Tables

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Preface

Five years after its predecessor, the second edition of *Inborn Metabolic Diseases: Diagnosis and Treatment* is appearing in an almost completely revised form. Its main feature is an even stronger emphasis on the clinical presentation of inborn errors of metabolism. For that reason, clinical approach has not only remained the central theme of the first chapter, but also become the starting point for all chapters dealing with either a single specific disorder or a group of diseases. Particular attention has been paid to clinical presentation under acute, subacute or chronic forms, to the appearance of initially aspecific symptoms evolving into a more characteristic syndrome at a later age, or vice versa, to clinical heterogeneity and its relation to genetic and biochemical heterogeneity. Brief reference to other diagnostic possibilities is also given in all disease-related chapters. Description of the metabolic derangements is restricted to the main pathophysiological features which provide the rationale for diagnosis and treatment. Methods to ascertain the diagnosis and diagnostic tests are listed comprehensively. As in the first edition, treatment is discussed extensively. Details are given for dietary treatment and drug administrations in acute situations, during infections and in maintenance treatment. The impressive progress of knowledge with respect to genetic lesions in inborn errors of metabolism remains condensed to the essentials. For more detailed information, particularly with respect to pathophysiology and genetics, we highly recommend the seventh edition of *The Metabolic Basis of Inherited Disease*, by Charles R. Scriver et al. (McGraw-Hill, 1995).

Most of the second edition of this book has thus been rewritten or extensively revised. A few chapters from the first edition have been deleted, because their contents are discussed in other chapters (for instance "Prenatal Diagnosis"). A couple of new chapters have been introduced, including the chapters on "Diagnostic Procedures", "Emergency Treatments", "Disorders of Small Peptides", "Carbohydrate-Deficient Glycoprotein Syndromes", "Inborn Errors of Bile Acid Synthesis" and "Bilirubin". Moreover, the chapter on "Peroxisomal Disorders" has been expanded to form Part XIV on "Organelle Disorders", also including "Lysosomes" and the "Golgi and Pre-Golgi systems".

The editors welcome new authors of old and new chapters and pay tribute to the authors who, though not participating this time, laid the framework for this book.

K. Tada was succeeded by G. van den Berghe as a new editor, and we are grateful for the continued participation of Dr. Tada as author and consulting editor.

Hattem, The Netherlands
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December 1994

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Part I
Diagnosis and Treatment: General Principles

Clinical Approach to Inherited Metabolic Diseases

J.-M. Saudubray, H. Ogier de Baulny, and C. Charpentier

CONTENTS

Inborn errors of metabolism are individually rare, but collectively numerous. As a whole, they cannot be recognized through systematic neonatal screening tests, which are too slow, too expensive or unreliable. This makes it an absolute necessity to use a simple method of clinical screening before deciding to initiate sophisticated biochemical investigations. Clinical diagnosis of inborn errors of metabolism may at times be difficult. A number of generally accepted ideas contribute to this difficulty:

- Many physicians think that because individual inborn errors are rare, they should be considered only after more common conditions (such as sepsis) have been excluded.
- In view of the large number of inborn errors, it might appear that their diagnosis requires precise knowledge of a large number of biochemical pathways and their interrelationships. As a matter of fact, adequate diagnostic approach can be based on the proper use of only a few screening tests.
- The neonate has an apparently limited repertoire of responses to severe overwhelming illness, and the predominant clinical signs and symptoms are nonspecific: poor feeding, lethargy, failure to thrive etc. It is certain that many patients with such defects succumb in the newborn period without having received a specific diagnosis, death often having been attributed to sepsis or other common causes.
- Classical autopsy findings in such cases are often unspecific. Infection is often suspected as the cause of the death, since sepsis is a common accompaniment of metabolic disorders.
- Many general practitioners and pediatricians only think of inborn errors of metabolism in very unspecific clinical circumstances such as psychomotor retardation or seizures. Conversely, they ignore most of the highly specific symptoms which are excellent keys to the diagnosis. Another common mistake is to confuse "syndrome" (such as Leigh syndrome or Reye syndrome), which is a set of symptoms possibly due to different causes, with the etiology itself.
- Although most genetic metabolic errors are hereditary and transmitted as recessive disorders, the majority of cases appear sporadic, because of the small size of sibships in developed countries.
- Finally, "hereditary" does not mean "congenital," and many patients can present a late onset form in childhood, adolescence, or even in adulthood.

Based mostly upon personal experience over 25 years, this chapter gives an overview of clinical keys to the diagnosis of inborn errors of metabolism.

Clinical Classification and Presentation

From a pathophysiologic perspective, the metabolic disorders can be divided into the following three diagnostically useful groups.

Group 1. This group includes diseases that disturb the synthesis or the catabolism of complex molecules. Symptoms are permanent, progressive, independent of intercurrent events and not related to food intake. All lysosomal disorders, peroxisomal disorders, disorders of intracellular trafficking and processing such as alpha-1-antitrypsin and carbohydrate deficiency glycoprotein (CDG) syndrome belong to this group.

Group 2. This group includes inborn errors of intermediary metabolism that lead to an acute or progressive intoxication from accumulation of toxic compounds proximal to the metabolic block. In this group are the aminoacidopathies (phenylketonuria, maple syrup urine disease, homocystinuria, tyrosinemia etc.), most of the organic acidurias (methylmalonic, propionic, isovaleric etc.), congenital urea cycle defects, and sugar intolerances (galactosemia, hereditary fructose intolerance). All the conditions in this group present clinical similarities, including a symptom-free interval and clinical signs of "intoxication", which may be acute (vomiting, lethargy, coma, liver failure, thromboembolic complications etc.) or chronic (progressive developmental delay, ectopia lentis, cardiomyopathy etc.). There are frequently humoral disturbances (acidosis, ketosis, hyperammonemia, hypoglycemia etc.). Clinical expression is often late in onset and intermittent. Biologic diagnosis is easy and relies mostly on plasma and urine amino acid or organic acid chromatography. Treatment of these disorders requires removal of the toxin by special diets, exchange transfusion and peritoneal dialysis or hemodialysis.

Group 3. This group consists of inborn errors of intermediary metabolism with symptoms due at least partly to a deficiency in energy production or utilization resulting from a defect in liver, myocardium, muscle, or brain. Included in this group are glycogenosis, gluconeogenesis defects, congenital lactic acidemias (deficiencies of pyruvate carboxylase and pyruvate dehydrogenase), fatty acid oxidation defects, and mitochondrial respiratory chain disorders. Symptoms common to this group include hypoglycemia, hyperlactacidemia, severe generalized hypotonia, myopathy, cardiomyopathy, failure to thrive, cardiac failure, circulatory collapse, sudden infant death syndrome (SIDS), and malformations [1].

Presentation. A great diversity of signs and symptoms can lead to the diagnosis of inborn errors of metabolism. Besides systematic screening in the general population (as for phenylketonuria) or in at-risk families (in which the diagnosis depends on specific biologic tests), there are four groups of clinical circumstances in which physicians are faced with metabolic disorders:

- Acute symptoms in the neonatal period
- Late-onset acute and recurrent symptoms (such as coma, ataxia, vomiting, acidosis)
- Chronic and progressive general symptoms (mainly "digestive" and "neurological")
- Specific and permanent symptoms indicative of an inborn error of metabolism (cardiomyopathy, hepatomegaly, lens dislocation etc.).

These four categories of clinical circumstances are presented below. Only those which present as emergencies or with chronic general symptoms are detailed with a tentative diagnostic approach. Other permanent and progressive symptoms are classified according to the organ involvement (cardiac, ocular, neurologic, liver, renal etc.). For each symptom a diagnostic list encompasses not only inborn errors of metabolism presenting with this unequivocal symptom, but also diverse inherited autosomal recessive syndromes which mimic and are possibly related to inborn errors, and some non-inherited disorders which represent classical differential diagnosis in pediatrics [2].

Acute Symptoms in the Neonatal Period [3–6]

Clinical Presentation

The neonate has a limited repertoire of responses to severe illness and at first glance presents with unspecific symptoms such as respiratory distress, hypotonia, poor sucking reflex, vomiting, diarrhea, dehydration, lethargy, seizures, all symptoms which could be attributed easily to infection or some other common cause. If present, death of affected siblings may have been falsely attributed to sepsis, heart failure, or intraventricular hemorrhage, and it is important to review clinical records and autopsy reports critically when they are available.

In the *intoxication type of metabolic distress*, an extremely evocative clinical setting is the course of

a full-term baby born after a normal pregnancy and delivery who, after an initial symptom-free period during which the baby is completely normal, deteriorates relentlessly for no apparent reason and does not respond to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the metabolic block and the environment.

Investigations routinely performed in all sick neonates, including chest X-ray, cerebrospinal fluid (CSF) examination, bacteriologic studies, and cerebral ultrasound yield normal results. This unexpected and "mysterious" deterioration of a child after a normal initial period is the most important signal of the presence of an inherited disease of the intoxication type. If present, careful reevaluation of the child's condition is warranted. Signs previously interpreted as nonspecific manifestations of neonatal hypoxia, infection, or other common diagnoses take on a new significance in this context. In *energy deficiencies*, clinical presentation is often less evocative and displays variable severity. A careful reappraisal of the child is warranted for the following aspects:

Neurologic Deterioration. Most inborn errors of both the intoxication or energy deficiency type are brought to a doctor's attention because of neurologic deterioration. In the intoxication type, the initial symptom-free interval varies in duration depending on the condition (see chapter by H. Ogier et al. on "Branched-Chain Organic Acidurias"). Typically, the first reported sign is poor sucking and feeding, after which the child sinks into an unexplained coma despite supportive measures. At a more advanced state, neurovegetative problems with respiratory abnormalities, hiccups, apneas, bradycardia, and hypothermia can appear. In the comatose state, characteristic changes in muscle tone and involuntary movements appear. Generalized hypertonic episodes with opisthotonus are frequent, and boxing or pedaling movements as well as slow limb elevations, spontaneously or upon stimulation, are observed. Conversely, most nonmetabolic causes of coma are associated with hypotonia, so that the presence of "normal" peripheral muscle tone in a comatose child reflects a relative hypertonia. Another neurologic pattern suggesting metabolic disease is axial hypotonia and limb hypertonia with large amplitude tremors and myoclonic jerks, which are often mistaken for convulsions. An abnormal urine and body odor is present in some diseases in which volatile metabolites accumulate; the most important examples are the maple syrup odor of maple syrup urine disease (MSUD) and the sweaty feet odor of isovaleric acidemia (IVA) and type II glutaric acidemia. If one of the preceding symptoms is present, metabolic disorders should be given a high diagnostic priority.

In energy deficiencies, the clinical presentation is less evocative and displays a more variable severity. In many conditions, there is no symptom-free interval. The most frequent symptoms are a severe generalized hypotonia, hypertrophic cardiomyopathy, rapidly progressive neurologic deterioration, possible dysmorphia, or malformations. However, in contrast to the intoxication group, lethargy and coma are rarely inaugural signs. Hyperlactacidemia with or without metabolic acidosis is a very frequent symptom.

Only few lysosomal disorders with storage symptoms are expressed in the neonatal period. By contrast, most peroxisomal disorders present immediately after birth with dysmorphia and severe neurological dysfunction.

Seizures. True convulsions occur late and inconsistently in inborn errors of intermediary metabolism, with the exception of pyridoxine-dependent seizures and some cases of non-ketotic hyperglycinemia (NKH), sulfite oxidase deficiency (5), and peroxisomal disorders, where they may be important inaugural elements in the clinical presentation. Convulsions are the unique symptom in pyridoxine-dependent convulsions. This rare disorder should be considered with all refractory seizures in children under 1 year of age. In contrast, newborns with MSUD, organic acidurias, and urea cycle defects rarely experience seizures in the absence of preexisting stupor or coma, or hypoglycemia. The electroencephalogram (EEG) often shows a periodic pattern in which bursts of intense activity alternate with nearly flat segments.

Hypotonia. Hypotonia is a very common symptom in sick neonates. Whereas many nonmetabolic inherited diseases can give rise to severe generalized neonatal hypotonia (mainly all severe fetal neuromuscular disorders), only a few inborn errors of metabolism present with predominant hypotonia in the neonatal period. Discounting dis-

orders in which hypotonia is included in a very evocative clinical context of major bone changes, dysmorphia, malformations, or visceral symptoms, the most severe metabolic hypotonias are observed in hereditary hyperlactacidemias, respiratory chain disorders, urea cycle defects, NKH, SO, peroxisomal disorders, and trifunctional enzyme deficiency. In all these circumstances, the diagnosis is mostly based upon the association with the central hypotonia of lethargy, coma, seizures, and neurologic symptoms in NKH, SO, and peroxisomal disorders and with characteristic metabolic changes in congenital lactic acidosis and urea cycle disorders (hyperammonemia). Severe forms of Pompe disease (alpha-glucosidase deficiency) can mimic at first respiratory chain disorders or trifunctional enzyme deficiency when generalized hypotonia is associated with cardiomyopathy. However, Pompe disease does not strictly start in the neonatal period. Finally, one of the most frequent diagnoses is Willi Prader syndrome, where hypotonia is central and apparently an isolated symptom at birth.

Hepatic Presentation. Three main clinical groups of hepatic symptoms can be identified:

- Hepatomegaly with hypoglycemia and seizures suggest glycogenosis type I and III, gluconeogenesis defects, or severe hyperinsulinism.
- Liver failure syndrome (jaundice, hemorragic syndrome, hepatocellular necrosis with elevated transaminases, and hypoglycemia with ascitis and edema) suggests fructosemia (in the case of a fructose-containing diet), galactosemia, tyrosinosis type I (after 3 weeks), neonatal hemochromatosis, and respiratory chain disorders.
- Predominantly cholestatic jaundice with failure to thrive is observed in alpha-1-antitrypsin deficiency, Byler disease, inborn errors of bile acid metabolism, peroxisomal disorders, and Niemann-Pick type C disease.

Hepatic presentations of inherited fatty acid oxidation disorders and urea cycle defects consist of acute steatosis or Reye syndrome with normal bilirubin, slightly prolonged prothrombin time, and moderate elevation of transaminases rather than true liver failure. One must emphasize frequent difficulties in investigating patients with severe hepatic failure. At an advanced state, many unspecific symptoms secondary to liver damage can be present. Mellituria (galactosuria, glycosuria, fructosuria), hyperammonemia, hyperlactacidemia, short fast hypoglycemia, hypertyrosinemia (>200 μmol/l), and hypermethioninemia (sometimes higher than 500 μmol/l) are encountered in advanced hepatocellular insufficiency.

Cardiac Presentation. Sometimes metabolic distress can strike with predominant cardiac symptoms. Cardiac failure revealing or accompanying a cardiomyopathy (dilated hypertrophic) and most often associated with hypotonia, muscle weakness, and failure to thrive suggests respiratory chain disorders, Pompe disease, or fatty acid oxidation disorders. Recent observations suggest that some respiratory chain disorders are tissue specific and are only expressed in the myocardium. The new multisystemic CDG syndrome can sometimes present in infancy with cardiac failure due to pericardial effusions and cardiac tamponnade. Many defects of long-chain fatty acid oxidation can be revealed by cardiomyopathy and/or heart beat disorders (auriculoventricular block, bundle branch blocks, ventricular tachycardia) responsible for cardiac arrest.

Metabolic Derangement

Once clinical suspicion of an inborn metabolic error is aroused, general supportive measures and laboratory investigations must be undertaken simultaneously (Table 1). Abnormal urine odors can be detected on a drying filter paper or by opening a container of urine which has been closed at room temperature for a few minutes. Although serum ketone bodies reach 0.5–1 mmol/l in early neonatal life, acetonuria, if observed in a newborn, is always abnormal and an important sign of a metabolic disease. The dinitrophenylhydrasine (DNPH) test screens for the presence of alpha-keto acids such as seen in MSUD. The test can be considered significant only in the absence of glucosuria and acetonuria, which also react with DNPH. Hypocalcemia and elevated or reduced blood glucose are frequently present in metabolic diseases. The physician should be wary of attributing marked neurologic dysfunction merely to these findings.

The metabolic acidosis of organic acidurias is usually accompanied by an elevated anion gap. Urine pH should be below 5; otherwise, renal aci-

Table 1. Emergency protocol of investigations

	Immediate investigations	Storage of samples
Urines	Smell (special odor) Look (special color) Acetone (Acetest, Ames) Reducing substances (Clinitest, Ames) Keto acids (DNPH) pH (pHstix Merck) Sulfitest (Merck) Brand reaction Electrolytes (Na, K) Uric acid	Urine collection Collect separately each fresh micturition and put it in the fridge Freezing Freeze at −20°C samples collected before treatment and afterward an aliquot of 24-h collection on treatment Do not use them without having taken expert metabolic advice
Blood	Blood cell count Electrolytes (search for anion gap) Glucose, calcium Blood gases (pH, PCO_2, HCO_3H, PO_2) Uric acid Prothrombin time Transaminases (and other "liver tests") Ammonia Lactic, pyruvic acids 3-Hydroxybutyrate, acetoacetate Free fatty acids	Plasma heparinized 5 ml at −20°C Blood on filter paper (as "Guthrie" test) Whole blood 10–15 ml collected on EDTA and frozen (for molecular biology studies)
Miscellaneous	Lumbar puncture Chest X-ray Cardiac echography, ECG Cerebral ultrasound, EEG	Skin biopsy (fibroblasts culture) CSF 1 ml frozen Postmortem Liver, muscle biopsies (see postmortem protocol) Autopsy

DNPH, dinitrophenylhydrazine; CSF, cerebrospinal fluid; ECG, electrocardiogram; EEG, electroencephalogram; EDTA, ethylenediaminetetra-acetic acid.

dosis is a consideration. A normal serum pH does not exclude hyperlactacidemia, as neutrality is usually maintained until serum levels of 5 mmol/l are present. Ammonia and lactic acid should be determined systematically in newborns at risk. An elevated ammonia level in itself can induce respiratory alkalosis; hyperammonemia with ketoacidosis suggests an underlying organic acidemia. Elevated lactic acid levels in the absence of infection or tissue hypoxia are a significant finding. Moderate elevations (3–6 mmol/l) are often observed in organic acidemias and in the hyperammonemias; levels higher than 10 mmol/l are frequent in hypoxia. It is important to measure lactate (L), pyruvate (P), 3-hydroxybutyrate (3OHB), and acetoacetate (AA) on a plasma sample immediately deproteinized at the bedside in order to appreciate cytoplasmic and mitochondrial redox states through the measurement of L/P and 3OHB/AA ratios, respectively. Some organic acidurias induce granulocytopenia and thrombocytopenia, which may be mistaken for sepsis.

The storage of adequate amounts of plasma, urine, and CSF is an important element in diagnosis. The utilization of these precious samples should be carefully planned after taking advice from specialists in inborn errors of metabolism.

Once the above clinical and laboratory data have been assembled, specific therapeutic recommendations can be made. This process is completed within 2–4 h and often precludes long waiting periods for sophisticated diagnostic results. On the basis of this evaluation, most patients can be classified into one of five groups (Table 2).

Diagnostic Tests

According to the major clinical presentations and to the proper use of the laboratory data described above, most patients can be assigned to one of five schematical syndromes. In our experience, type I (MSUD), type II (organic acidurias), type IVa (urea cycle defects), and nonketotic hyper-

Table 2. Five neonatal types of inherited metabolic distress

Types	Clinical type	Acidosis/ketosis	Other signs	Most usual diagnosis	Elective methods of investigation
I	Neurological distress of intoxication type Abnormal movements Hypertonia	Acidosis 0 DNPH +++ Acetest 0/±	NH_3 N or ↗ ± Lactate N Blood count N Glucose N Calcium N	MSUD (special odor)	Aminoacid chromatography (plasma, urine)
II	Neurological distress of intoxication type Dehydration	Acidosis ++ Acetest ++ DNPH 0/±	NH_3 ↗ +/++ Lactate N or ↗ ± Blood count: leucopenia thrombopenia Glucose N or ↗ + Calcium N or ↘ +	Organic acidurias (MMA, PA, IVA, MCD) Ketolytic defects	Organic acid chromatography by GLCMS (urine, plasma) Carnitine(plasma) Carnitine esters (urine, plasma)
	Neurological distress of energy deficiency type with liver or cardiac symptoms	Acidosis ++/± Acetest 0 DNPH 0	NH_3 ↗ ±/++ Lactate ↗ ±/++ Blood count N Glucose ↘ +/++ Calcium N or ↘ +	Fatty acid oxidation and ketogenesis defects	Idem above Loading test Fasting test Fatty acid oxidation studies on lymphocytes or fibroblasts
III	Neurological distress of energy deficiency type Polypnea Hypotonia	Acidosis +++/+ Acetest ++/0 Lactate +++/+	NH3 N or ↗ ± Blood count: anemia or N Glucose N or ↘ ± Calcium N	Congenital lactic acidosis (PC, PDH, Krebs cycle, respiratory chain) MCD	Plasma redox potential states (L/P, OHB/AA ratios) Organic acid chromatography (urines) Polarographic studies Enzyme assays (muscle, lymphocytes or fibroblasts)
IVa	Neurological distress of intoxication type Moderate hepatocellular disturbances Hypotonia Seizures, coma	Acidosis 0 (alcalosis) Acetest 0 DNPH 0	NH_3 ↗ +/+++ Lactate N or ↗ + Blood count N Glucose N Calcium N	Urea cycle Triple H Fatty acid oxidation defects (GAII, CPTII, LCAD, 3LCHAD)	AAC (plasma, urines) Orotic acid (urines) Liver or intestine enzyme studies (CPS, OTC)
IVb	Neurological distress Seizures Myoclonic jerks Severe hypotonia	Acidosis 0 Acetest 0 DNPH 0	NH_3 N Lactate N Blood count N Glucose N	NKH SO ± XO Pyridoxine dependency Peroxisomal disorders Trifunctional enzyme	AAC (NKH, SO) VLCFA, phytanic acid in plasma (PSO)

IVc	Storage disorders Coarse facies Hepatosplenomegaly Ascitis, hydrops fetalis Macroglossia Bone changes Cherry red spot Vacuolated lymphocytes	Acidosis 0 Acetest 0 DNPH 0	NH_3 N Lactate N Blood count N Glucose N Hepatic signs	GM1, gangliosidosis ISSD (sialidosis type II) I-cell disease Niemann-Pick type A MPS VII Galactosialidosis	Enzyme studies (lymphocytes, fibroblasts)
V	Hepatomegaly Hypoglycemia	Acidosis ++/+ Acetest +	NH_3 N Lactate ↗ +/++ Blood count N Glucose ↘ ++	Glycogenosis type I (acetest −) Glycogenosis type III (acetest ++) Fructose diphosphatase	Fasting test Loading test Enzyme studies (liver, lymphocytes, fibroblasts)
	Hepatomegaly Jaundice Liver failure Hepatocellular necrosis	Acidosis +/0 Acetest +/0	NH_3 N or ↘ + Lactate ↗ +/++ Glucose N or ↘ ++	Fructosemia, galactosemias Tyrosinosis type I Neonatal hemochromatosis Respiratory chain disorders	Enzyme studies (fructosemia, galactosemia) Organic acids and enzyme studies (tyrosinemia type I)
	Hepatomegaly Cholestatic jaundice ± Failure to thrive ± Chronic diarrhea	Acidosis 0 Ketosis 0	NH3 N Lactate N Glucose N	Alpha-1-antitrypsin Inborn errors of bile acid metabolism Peroxisomal disorders	Protein electrophoresis Organic acid chromatography (plasma, urine, duodenal juice) VLCFA, phytanic acid, pipecolic acid
	Hepatosplenomegaly "Storage" signs ± Failure to thrive ± Chronic diarrhea	Acidosis 0 Ketosis 0	NH3 Lactate N or ↗ Glucose N	Storage disorders	Oligosaccharides, sialic acid Mucopolysaccharides Enzyme studies

N, normal (normal values = NH3 < 80 μ*M*; lactate < 1.5 m*M*; glucose 3.5–5.5 m*M*); ±, slight; +, moderate; ++, marked; +++, significant/massive; ↗, elevated; ↘, decreased; 0, absent (acidosis) or negative (Acetest; dinitrophenylhydrazine, DNPH); AAC, amino acid chromatography; CPT II, carnitine palmitoyltransferase II; GA II, glutaric aciduria type II; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ISSD, infantile sialic acid storage disease; IVA, isovaleric acidemia; LCAD, long-chain acylCoA dehydrogenase; LCHAD, 3-hydroxy long-chain acylCoA dehydrogenase; MCD, multiple carboxylase defiency; MMA, methylmalonic acidemia; MPS VII, mucopolysaccharidosis type VII; MSUD, maple syrup urine disease; NKH, nonketotic hyperglycinemia; PA, propionic acidemia; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; SO, sulfite oxidase; TL, translocase; XO, xanthine oxidase; L, lactate; P, pyruvate; OHB, hydroxybutyrate; AA, acetoacetate; GLCMS, gas liquid chromatography mass spectrometry; CPS, carbamylphosphate synthetase; OTC, ornithine transcarbamylase; PSO, peroxisome; VLCFA, very long chain fatty acids.

glycinemia (the most common disease in type IVb) encompass more than 65% of the newborn infants with inborn errors of intermediary metabolism, but fatty acid oxidation defects and respiratory chain disorders are increasing rapidly. The experienced clinician will, of course, have to carefully interpret the metabolic data, especially in relation to time of collection and ongoing treatment. It is important to insist on the need to collect at the same time all the biochemical data listed in Table 1. Some very significant symptoms (such as metabolic acidosis and especially ketosis) can be moderate and transient, largely depending on the symptomatic therapy. Conversely, at an advanced state, many nonspecific abnormalities (such as respiratory acidosis, severe hyperlactacidemia, secondary hyperammonemia) can disturb the original metabolic profile. This applies particularly to disorders with a rapid fatal course such as urea cycle disorders, in which the initial characteristic presentation of hyperammonemia with respiratory alkalosis and without ketosis shifts rapidly to a rather nonspecific picture of acidosis and hyperlactacidemia.

Type I. This syndrome involves neurologic distress of the intoxication type with ketosis and is represented by MSUD. It is one of the commonest aminoacidopathies.

Type II. This syndrome involves neurologic distress of the intoxication type with ketoacidosis and hyperammonemia and encompasses many of the organic acidurias. In addition to methylmalonic (MMA) and propionic (PA) acidemia and IVA, a large number of rare organic acidurias, usually presenting with neurologic distress and metabolic acidosis, have been described in recent years as organic acid analysis techniques have become more available and reliable. Among them, glutaric aciduria type II or multiple acyl coenzyme A (acyl-CoA) dehydrogenase deficiency and 3-hydroxy-3-methylglutaryl (HMG)-CoA lyase deficiency have many similarities with MMA, PA, and IVA, except that ketosis is absent and hypoglycemia is frequent. Very rare conditions in this group are succinyl-CoA transferase deficiency, biotin-dependent multiple carboxylase deficiency (MCD) due to holoenzyme synthetase deficiency, short-chain acyl-CoA dehydrogenase (SCAD) deficiency, 3-methylglutaconicuria, and glycerol kinase deficiency, which all display ketoacidosis. Pyroglutamic aciduria is a rare condition which can start in the first few days of life with a severe metabolic acidosis, but without ketosis nor abnormalities of blood glucose, lactate, and ammonia. The final diagnosis of all these organic acidurias is made by identifying specific abnormal metabolites by gas chromatography-mass spectrometry (GCMS) of blood and urine.

Type III. This syndrome involves lactic acidosis with neurological distress of the energy deficiency type. The clinical presentation of these children varies. Unlike the previous disease category, in which moderate acidosis is noted during the evaluation of an acutely ill comatose child, the main medical preoccupation in group III patients is the acidosis itself, which clinically may be surprisingly well tolerated. However, the acidosis can at times be mild. An elevated anion gap exists, which can be explained in part by the presence of equimolar amounts of lactic acid in the blood. Often the acidosis recurs soon after bicarbonate therapy, in the absence of adequate treatment.

If a high lactic acid concentration is found, it is urgent to rule out readily treatable causes, especially hypoxia. Ketosis is present in most of the primary lactic acidemias, but is absent in acidosis secondary to tissue hypoxia. Biotin-responsive MCD may present as lactic acidosis, and biotin therapy is indicated in all patients with lactic acidosis of unknown cause after baseline blood and urine samples have been taken. Primary lactic acidoses form a complex group. A definite diagnosis is often elusive and is attempted with specific enzyme assays and by considering metabolite levels, redox potential states, and fluxes under fasting and fed conditions. Many cases remain still unexplained.

Type IV. This can be divided into three groups, IVa, IVb, and IVb.

Type IVa. This involves neurologic distress of the intoxication type with hyperammonemia and without ketoacidosis i.e., urea cycle defects. As mentioned above, this group of patients is one of the most important among those with neonatal inborn errors of metabolism. An important diagnostic clue to separate urea cycle defects from organic acidurias with hyperammonemia is the universal absence of ketonuria. As already stated, at an advanced state, neurovegetative

disorders rapidly give rise to unspecific findings including acidosis and hyperlactacidemia. An especially important diagnostic consideration is transient hyperammonemia of the neonate. Long-chain fatty acid oxidation disorders (mainly carnitine palmitoyltransferase II, (CPT II), and translocase deficiency) can also, though rarely, present in the neonatal period with hyperammonemia and mimick urea cycle disorders. They are mostly associated with hypoglycemia, hepatic dysfunction, muscular and cardiac symptoms, or sudden infant death.

Type IVb. This involves neurologic deterioration of the energy deficiency type without ketoacidosis and without hyperammonemia. The most frequent diseases of type IVb are NKH, SO, and inborn errors of peroxisomal metabolism. SO is probably underdiagnosed, as its clinical pattern shares many similarities with common acute fetal distress. In addition, some patients with respiratory chain disorders can present in the neonatal period without evidence of lactic acidosis. Beside these four disorders, an increasing number of other rare conditions has been described in recent years, and we can assume that the list of disorders of this group will expand substantially in the near future.

Fatty acid oxidation disorders can also be observed in the neonatal period without acidosis. They present with hypoglycemia, hepatic dysfunction possibly associated with muscular and cardiac symptoms, or sudden infant death. Trifunctional enzyme deficiency can reveal by severe hypotonia and neurologic distress without obvious metabolic disturbances.

Type IVc. This group is comprised of patients with storage disorders. Only a few lysosomal disorders are expressed clinically in the neonatal period. They can be associated with hydrops fetalis, neonatal ascitis, and edema.

Type V. This involves hepatomegaly and liver dysfunction. In this type, four main clinical groups of hepatic symptoms lead to diagnosis of more than 20 inborn errors of metabolism.

Recurrent Acute Presentations

In approximately one third of the patients with inborn errors of intermediary metabolism, disease onset is late. The symptom-free period is often longer than 1 year and may extend into late childhood, adolescence, or even adulthood. Each attack can present a rapid course toward either spontaneous improvement or unexplained death despite supportive measures in the intensive care unit. Between attacks the child may appear normal. Onset of acute disease may be precipitated by a minor viral infection, fever, or severe constipation or may occur without overt cause. Excessive protein intake and all conditions that enhance protein catabolism may exacerbate such decompensations.

The initial approach to the late-onset acute forms of inherited metabolic disorders, like the approach to acute neonatal distress, is based on the proper use of a few screening tests. As with neonates, the laboratory data listed in Table 1 must be collected during the acute attack, all at the same time, and both before and after treatment.

Coma and Attacks of Vomiting with Lethargy

All types of comas in pediatrics can signal inborn errors of metabolism, including those presenting with focal neurologic signs (Table 3). Neither the age at onset, nor the accompanying clinical-signs (hepatic, digestive, neurologic, psychiatric etc.), nor the mode of evolution (improvement, sequelae, death), nor the routine laboratory data allow an inborn error of metabolism to be ruled out a priori. Three categories can be distinguished:

Metabolic Coma Without Neurologic Signs. The main varieties of metabolic comas may all be observed in these late-onset, acute diseases: coma with predominant metabolic acidosis, coma with predominant hyperammonemia, coma with predominant hypoglycemia, and combinations of these three major abnormalities. A rather confusing finding in some organic acidurias and ketolytic defects is ketoacidosis with hyperglycemia and glycosuria that mimic diabetic coma. The diagnostic approach to the metabolic derangements is developed below (see "Metabolic Acidosis," "Ketosis," "Hyperlactacidemias," and "Hypoglycemia," Tables 5–8).

Neurologic Coma with Focal Signs, Seizures, or Severe Intracranial Hypertension. Although most recurrent metabolic comas are not accompanied by

Table 3. Diagnostic approach to recurrent attacks of coma and vomiting with lethargy

Clinical presentation	Metabolic derangements or other important signs		Most frequent diagnosis	Differential diagnosis
Metabolic coma (without focal neurological signs)	Acidosis (metabolic) pH < 7.20 CO_3 H < 10 mmol PCO_2 < 25 mmHg	Ketosis + (acetest ++)	Respiratory chain disorders MCD, PC Organic acidurias (MMA, PA, IVA, GA I, MSUD) Ketolysis defects Neoglucogenesis defects	Diabetes Intoxication Encephalitis
		Ketosis−	PDH deficiency Ketogenesis defects Fatty acid oxidation defects FDP deficiency	
	Hyperammonemia NH_3 > 100 μmol/l Gazeous alcalosis pH > 7.45 PCO_2 < 25 mmHg	Normal glucose	Urea cycle disorders Triple H, LPI	Reye syndrome Encephalitis Intoxication
		Hypoglycemia	Fatty acid oxidation defects HMGCoA lyase deficiency	
	Hypoglycemia <2 mmol/l	Acidosis +	Gluconeogenesis defects MSUD HMG-CoA lyase deficiency	Drugs and toxin Ketotic hypoglycemia Adrenal insufficiency (and other endocrine etiologies)
		Acidosis−	Fatty acid oxidation defects	Hypopituitary coma
	Hyperlactacidemia >4 mmol/l	Normal glucose	PC, MCD, respiratory chain disorders Krebs cycle defects (with ketosis) PDH (without ketosis)	
		Hypoglycemia	Gluconeogenesis defects (ketosis variable) Fatty acid oxidation defects (moderate hyperlactacidemia, no ketosis)	

Neurologic coma (with focal signs, seizures, or intracranial hypertension)	Biochemical signs are very variable, can be absent or moderate, see metabolic coma	Cerebral edema	MSUD, OTC	
		Hemiplegia (hemianopsia)	MSUD, OTC, MMA, PA, PGK	Cerebral tumor Migraine Encephalitis
		Extrapyramidal signs	MMA, GA I, Wilson disease Homocystinuria classic	
		Stroke-like	Urea cycle, MMA, PA, IVA, Respiratory chain (MELAS), Homocystinurias CDG syndrome, Thiamine responsive Megaloblastic anemia Fabry disease (rarely revealing)	Moya moya Vascular hemiplegia Cerebral thrombophlebitis Cerebral tumor
	Abnormal coagulation Hemolytic anemia	Thromboembolic accidents	Antithrombin III, Protein C or S deficiency, Homocystinurias, Sickle cell anemia	
Hepatic coma (hepatomegaly, cytolysis or liver failure)	Normal bilirubin Slight elevation of transaminases	Steatosis	Fatty acid oxidation defects Urea cycle disorders	
	Hyperlactacidemia	Liver failure	Respiratory chain disorders	Reye syndrome Hepatitis Intoxication
	Hemolytic jaundice	Cirrhosis Chronic hepatic dysfunction	Wilson disease	
	Hypoglycemia Exsudative enteropathy		Hepatic fibrosis with enteropathy	

MCD, multiple carboxylase deficiency; PC, pyruvate carboxylase; MMA, methylmalonic acidemia; PA, propionic acidemia; IVA, isovaleric acidemia; GA, glutaric aciduria; MSUD, maple syrup urine disease; PDH, pyruvate dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; FDP, fructose 1–6 diphosphatase; LPI, lysinuric protein intolerance; OTC, ornithine transcarbamylase; PGK, phosphoglycerate kinase; MELAS, mitochondrial encephalopathy lactic acidosis stroke-like episodes.

neurologic signs other than encephalopathy, we have observed several patients with organic acidemias who presented with focal neurologic signs or cerebral edema. Several of these patients were mistakenly diagnosed as having cerebrovascular accident or cerebral tumor. In two patients with classic homocystinuria who were subsequently demonstrated to be vitamin B_6 responsive, the associated disease struck in late childhood with an acute cerebrovascular accident. A few patients with MMA have had acute extrapyramidal disease and corticospinal tract involvement after metabolic decompensation. The neurologic findings resulted from bilateral destruction of the globus pallidus with variable involvement of the internal capsule. Cerebellar hemorrhage has also been observed in IVA, PA, and MMA.

GA type I can also be revealed by encephalopathic episodes with acute metabolic derangements that occur in connection with gastrointestinal and viral infections.

Mitochondrial encephalopathy lactic acidosis stroke-like episodes (MELAS) syndrome is another important diagnostic consideration in such late-onset and recurrent comas.

Early episodic central nervous system problems possibly associated with liver insufficiency or cardiac failure have been inaugural symptoms in some cases of CDG syndrome.

In summary, all these disorders should be considered in the expanding differential diagnosis list of strokes or stroke-like episodes. Certain vaguely defined and/or undocumented diagnoses such as encephalitis, basilar migraine, intoxication, or cerebral thrombophlebitis should therefore be questioned, especially when even moderate ketoacidosis, hyperlactacidemia, or hyperammonemia is present. In fact, these apparent initial acute manifestations are frequently preceded by other premonitory symptoms, which may be unrecognized or misinterpreted. Such symptoms include acute ataxia, persistent anorexia, chronic vomiting, failure to thrive, hypotonia, and progressive developmental delay, all symptoms that are often observed in urea cycle disorders, respiratory chain defects, and organic acidurias.

Certain features or symptoms are characteristic of particular disorders. For example, macrocephaly is a frequent finding in glutaric aciduria type I; unexplained episodes of dehydration may occur in organic acidurias; and hepatomegaly at the time of coma is an important, although inconsistent finding in fructose diphosphatase deficiency. Severe hematologic manifestations and recurrent infections are common in IVA, PA, and MMA.

Hepatic Coma with Liver Dysfunction or Hepatomegaly. When coma is associated with hepatic dysfunction, Reye syndrome secondary to disorders of fatty acid oxidation and of the urea cycle should be considered. Hepatic coma with liver failure and hyperlactacidemia can be a revealing sign of respiratory chain disorders. Finally, hepatic coma with cirrhosis, chronic hepatic dysfunction, hemolytic jaundice, and various neurologic signs (psychiatric, extrapyramidal) is a classical, but underdiagnosed manifestation of Wilson disease.

Recurrent Attacks of Ataxia

Intermittent acute ataxia (Table 4) and abnormal behavior can be the presenting signs of late-onset MSUD and organic acidurias, in which they are associated with ketoacidosis and sometimes with hyperglycemia, which can mimic ketoacidotic diabetes. Late-onset forms of congenital hyperammonemia, mainly partial ornithinetranscarbamylase (OTC) deficiency, can strike late in childhood or in adolescence with psychiatric symptoms. Because hyperammonemia and liver dysfunction can be mild even at the time of acute attacks, these intermittent late-onset forms of urea cycle disorders can be easily misdiagnosed as hysteria, schizophrenia, or intoxication. Acute ataxia associated with peripheral neuropathy is a frequent presenting sign of pyruvate dehydrogenase deficiency (PHD); moderate hyperlactacidemia with a normal L/P ratio supports this diagnosis. Finally, patients affected with homocysteine remethylation defects may present with schizophrenia-like, folate-responsive episodes.

Metabolic Acidosis

Metabolic acidosis (Fig. 1) is a very common symptom in pediatrics. It can be observed in a large variety of acquired circumstances, including infections, severe catabolic states, tissue anoxia, severe dehydration, and intoxication, all of which

Table 4. Diagnostic approach to recurrent attacks of ataxia

Clinical presentation	Metabolic derangements or other important signs		Most frequent diagnosis	Differential diagnosis
Acute ataxia	Ketoacidosis	Special odor Neutropenia Thrombopenia Hyperglycemia	Late-onset MSUD MMA, PA, IVA	 Diabetes
	Hyperammonemia	Respiratory alkalosis, hepatomegaly	Urea cycle defects (OTC, ASS)	Intoxication
	Hyperlactacidemia	Normal L/P ratio, no ketosis Peripheral neuropathy	PDH	 Encephalitis Migraine Cerebellitis (varicella)
		High L/P ratio, ketosis Cutaneous signs	Multiple carboxylase deficiency Respiratory chain disorders	Polymyoclonia Acetazolamide responsive ataxia
	No metabolic disturbance	Skin rashes, pellagra, sun intolerance	Hartnup disease	Acute exacerbation in chronic ataxias
Psychiatric symptoms (hallucinations, delirium,	Hyperammonemia	Slight liver dysfunction, vomiting, failure to thrive	Urea cycle disorders (OTC, ASS, arginase deficiency, LPI)	
dizziness, aggressivity,	Ketoacidosis	Ataxia, neutropenia	Organic acid disorders, MSUD	
anxiety, agitation, agony, schizophrenic-like	Portowine urines	Abdominal pain, all kinds of neuropathy, vomiting	Acute intermittent pophyria, Hereditary coproporphyria	Hysteria
behaviour)	Positive brand reaction	Stroke, seizures, myelopathy	Methylene tetrahydrofolate reductase	Schizophrenia

MSUD, maple syrup urine disease; MMA, methylmalonic acidemia; PA, propionic acidemia; IVA, isovaleric acidemia; PDH, pyruvate dehydrogenase; L, lactate; P, pyruvate; AAS, arginino succinate aciduria; LPI, lysinuric protein intolerance.

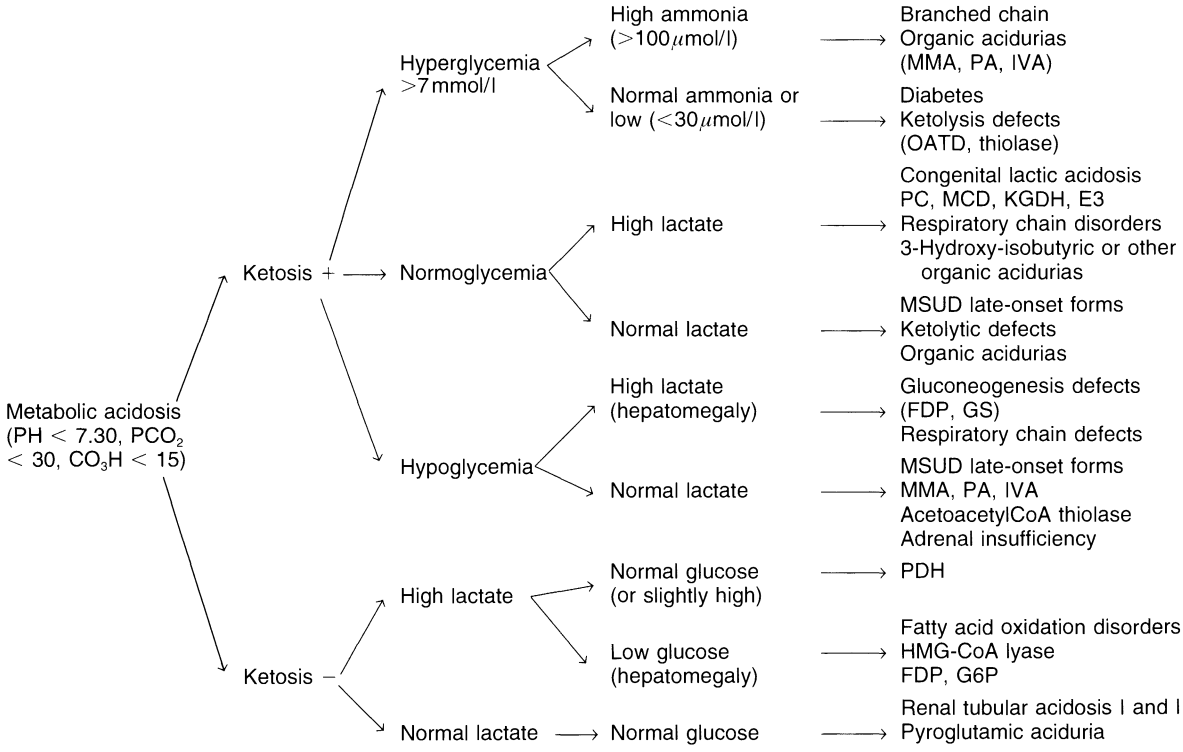

Fig. 1. Metabolic acidosis. *MMA*, Methylmalonic aciduria; *PA*, propionic aciduria; *IVA*, isovaleric aciduria; *PC*, pyruvate carboxylase; *MCD*, multiple carboxylase deficiency; *MSUD*, maple syrup urine disease; *GS*, glycogen synthetase; *PDH*, pyruvate dehydrogenase; *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *G6P*, glucose-6-phosphatase, *OATD*, oxoacid CoA transferase; *KGDH*, αketoglutarate dehydrogenase; *E3*, lipoamidooxido reductase; *FDP*, fructose diphosphatase

should be ruled out. However, these circumstances can also trigger an acute decompensation of an unrecognized inborn error of metabolism. The presence or absence of ketonuria associated with metabolic acidosis is the major clinical key to the diagnosis.

When metabolic acidosis is not associated with ketosis, PDH, fatty acid oxidation disorders, and some disorders of gluconeogenesis should be considered, particularly when there is moderate to severe hyperlactacidemia. All these disorders except PDH deficiency have concomitant fasting hypoglycemia. Although fructose diphosphatase deficiency is classically considered to give rise to ketoacidosis, some patients were referred with the tentative diagnosis of fatty acid oxidation disorders because of low concentrations of ketone bodies during hypoglycemia.

When metabolic acidosis occurs with a normal anion gap and without hyperlactacidemia or hypoglycemia, the most frequent diagnosis is renal tubular acidosis (RTA) type I and II. Pyroglutamic aciduria also can present early in life with permanent, isolated metabolic acidosis, which can be mistaken for RTA type II.

Ketoacidotic states compose the second large group of inherited metabolic disorders. The range of serum ketone body concentration varies with age and nutritional state (see chapter by Fernandes and Saudubray on "Diagnostic Procedures")

Many metabolic disorders of childhood may lead to ketoacidosis, including insulin-dependent diabetes, inborn errors of branched-chain amino acid metabolism, congenital lactic acidoses such as MCD and PC deficiencies, inherited defects in enzymes of gluconeogenesis and of glycogen synthesis (glycogen synthase, GS), and ketolytic defects.

When metabolic acidosis is associated with ketosis, the first parameter to be considered is the glucose level, which can be elevated, normal, or low. The distinction between the different disorders is also based on ammonia and lactate levels, which are generally increased in organic acidemias and normal or low in ketolytic defects. When ketoacidosis is associated with hypoglycemia, the

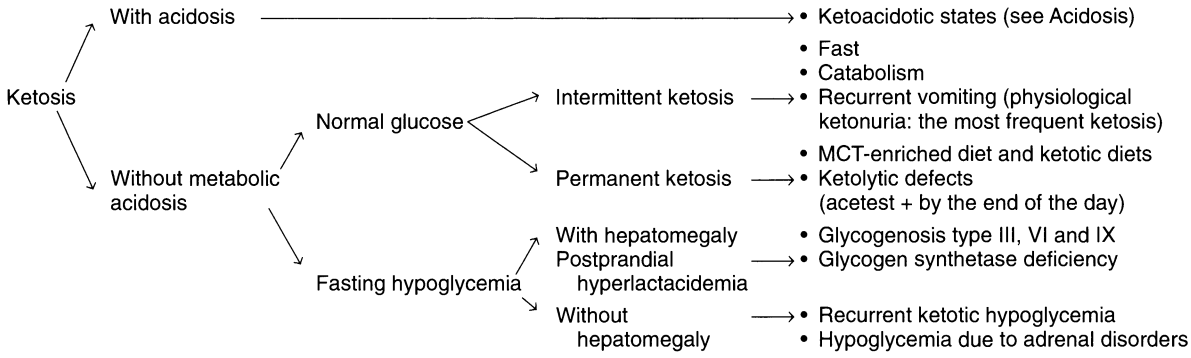

Fig. 2. Ketosis (see also Fig. 1.) MCT, medium chain triglycerides

first classical group of diseases to be considered is the gluconeogenesis and glycogenosis defects. The main symptoms are hepatomegaly and hyperlactacidemia, though they are not constant findings. When there is no significant hepatomegaly, late-onset forms of MSUD and of organic acidurias must be considered as well. A classic differential diagnosis is adrenal insufficiency, which can strike as a ketoacidotic attack with hypoglycemia.

When blood glucose levels are normal, congenital lactic acidosis must be considered in addition to the disorders discussed above. According to this schematic approach to inherited ketoacidotic states, a simplistic diagnosis of fasting ketoacidosis or ketotic hypoglycemia should be questioned when there is a concomitant severe metabolic acidosis.

Ketosis

While ketonuria should be always considered abnormal in neonates, it is a physiological finding in many circumstances of late infancy, childhood, and even adolescence. However, as a general rule, one can assume that hyperketosis at a level that produces metabolic acidosis is not physiologic. Ketosis (Fig. 2) which is not associated with acidosis, hyperlactacidemia, or hypoglycemia is likely to be a normal physiological reflection of the nutritional state (fasting, catabolism, vomiting, medium-chain triglyceride-enriched or other ketogenic diets). Of interest are ketolytic defects (succinyl-CoA transferase and 3-ketothiolase deficiencies) that can present as permanent moderate ketonuria occurring mainly in the fed state at the end of the day.

Significant fasting ketonuria without acidosis is often observed in glycogenosis type III in childhood and in the very rare GS defect in infancy. In both disorders, there is hepatomegaly (inconstant in GS), fasting hypoglycemia, and postprandial hyperlactacidemia.

Finally, ketosis without acidosis is observed in ketotic hypoglycemias of childhood and in association with hypoglycemias due to adrenal insufficiency. Absence of ketonuria in hypoglycemic states, as well as in fasting and catabolic circumstances induced by vomiting, anorexia, or intercurrent infections, is an important observation, suggesting an inherited disorder of fatty acid oxidation or ketogenesis disorder. It can also be observed in hyperinsulinemic states and in growth hormone deficiency.

Hyperlactacidemia

Lactate and pyruvate are normal metabolites. Their plasma levels reflect the equilibrium between their cytoplasmic production from glycolysis and their consumption by different tissues. The blood levels of lactate and pyruvate and the L/P ratio reflect the redox state of the cells.

Blood lactate accumulates in circulatory collapse, in hypoxic insult, and in other conditions involving failure of cellular respiration. The conditions must be ruled out before an inborn error of lactate–pyruvate oxidation is sought. Persistent hyperlactacidemias (Table 5) can result from many acquired conditions, such as diarrhea, persistent infections (mainly of the urinary tract), hyperventilation, and hepatic failure. Ketosis is absent in most hyperlactacidemias secondary to tissue hypoxia, while it is a nearly constant finding

Table 5. Diagnostic approach to hyperlactacidemias

Time of occurrence	Main clinical signs	Redox potential states	Diagnosis
Only after feeding (or exacerbated after feeding)	Hepatomegaly Fasting ketotic hypoglycemia	Not diagnostic	Glycogenosis type III Glycogen synthetase
		Normal L/P ratio No ketosis	PDH
	Neurologic signs Encephalomyopathy	L/P high 3OHB/AA low Postprandial hyperketosis	PC (citrullinemia), MCD Alpha-KDH (isolated or multi-ketodecarboxylase)
		L/P high 3OHB/AA high Postprandial ketosis	Respiratory chain (3CH3 glutaconic aciduria Krebs cycle intermediates)
		L/P high No ketosis	Respiratory chain
Only after fasting (or exacerbated after fasting)	Prominent hepatomegaly Hypoglycemia	Not diagnostic (normal L/P ratio)	Glycogenosis type I Fructodiphosphatase (ketosis inconstant)
	Moderate hepatomegaly Hypoketotic hypoglycemia	Not diagnostic	Fatty acid oxidation disorders (cardiac, muscle symptoms) Fructose diphosphatase
Permanent	Moderate hyperlactacidemia Recurrent attacks of ketoacidosis	Not diagnostic	Organic acidurias (MMA, PA, IVA)
	Predominant hyperammonemia	Not diagnostic	Urea cycle defects (in neonates)
	Predominant hypoglycemia Hepatomegaly	Not diagnostic	Glycogenosis type I Fructose diphosphatase
	Neurological signs Encephalomyopathy Important hyperlactacidemia (>10m*M*)	Highly diagnostic (see above "after feeding")	Congenital lactic acidemias (see above "after feeding")

L, lactate; P, pyruvate; 3OHB, 3-hydroxybutyrate; AA, acetoacetate; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; MCD, multiple carboxylase deficiency; KDH, ketoglutarate dehydrogenase; MMA, methylmalonic acidemia; PA, propionic acidemia; IVA, isovaleric acidemia.

in most inborn errors of metabolism (except in PDH deficiency and glycogenosis type I). On the other hand, the level of lactic acidemia is not discriminating; some acquired disorders are associated with very high levels, whereas some inborn errors of lactate–pyruvate metabolism cause only moderate hyperlactactacidemia. Nutritional state also influences the levels of lactate and pyruvate.

Once the organic acidurias, urea cycle defects (mainly citrullinemia), and fatty acid oxidation defects that cause secondary hyperlacidemia have been excluded as possible diagnoses, four types of inherited disorders remain to be considered:

- Disorders of liver glycogen metabolism
- Disorders of liver gluconeogenesis
- Abnormalities of lactate–pyruvate oxidation, the PDH complex, or Krebs cycle defects
- Deficient activity in one of the components of the respiratory chain

The diagnosis of hyperlactacidemias is largely based upon two metabolic criteria: time of occurrence of lactic acidosis relative to feeding and determinations of L/P and ketone bodies ratios.

In disorders of gluconeogenesis (fructose diphosphatase and glucose-6-phosphatase deficiencies), hyperlactacidemia reaches its maximum

level when the patient is fasting and hypoglycemic (up to 15 m*M*). By contrast, in glycogenosis types III and VI and in GS deficiency, hyperlactacidemia is observed only in the postprandial period in patients on a carbohydrate-rich diet. Here, hyperlactacidemia never exceeds 7 m*M*. In pyruvate carboxylase deficiency, hyperlactacidemia is present in both the fed and the fasted state, but tends to decrease with a short fast. In disorders of PDH, alpha-ketoglutarate dehydrogenase, and respiratory chain function, maximum lactate levels are observed in the fed state (although all hyperlactacidemias exceeding 7 m*M* appear more or less permanent). In these disorders, there is a real risk of missing a moderate (although significant) hyperlactacidemia when the level is checked only before breakfast after an overnight fast (as is usual for laboratory determinations).

The second clue to diagnosis is the simultaneous determination of plasma L/P and 30HB/AA ratios before and after meals. These ratios indirectly reflect cytoplasmic (L/P) and mitochodnrial (30HB/AA) redox potential states. They must be measured in carefully collected blood samples (see chapter by Fernandes and Saudubray on "Diagnostic Procedures").

Three metabolic profiles are nearly pathognomonic of an inborn error of lactate–pyruvate metabolism:

- When the L/P ratio is normal or low (<10) without hyperketonemia, PDH deficiency is highly probable, regardless of the lactate level.
- When the L/P ratio is very high (>30) and is associated with postprandial hyperketonemia and with a normal or low 30HB/AA ratio (<1.5), a diagnosis of pyruvate carboxylase deficiency (isolated or secondary to biotinidase or holocarboxylase synthetase deficiency) or alpha-ketoglutarate dehydrogenase deficiency is virtually mandatory.
- When both L/P and 30HB/AA ratios are elevated and associated with a significant postprandial hyperketonemia, respiratory chain disorders should be suspected.

All other situations, especially when the L/P ratio is high without hyperketonemia, are compatible with respiratory chain disorders, but all acquired anoxic conditions should also be ruled out (see above).

Hypoglycemia

Our approach to hypoglycemia (Table 6) is based on the following clinical criteria:

- Liver size
- Characteristic schedule of hypoglycemia (unpredictable, postprandial, or after fasting)
- Association with lactic acidosis
- Association with hyperketosis or hypoketosis

Other clinical signs of interest are hepatic failure, vascular hypotension, dehydration, short stature, neonatal body size (head circumference, weight, height), and evidence of encephalopathy, myopathy, or cardiomyopathy.

Liver size can be used to separate the hypoglycemias into two large groups as discussed below.

Hypoglycemia with Permanent Hepatomegaly. Most of the hypoglycemias associated with permanent hepatomegaly are due to inborn errors of metabolism. All conditions, acquired or inherited, that are associated with severe liver failure can give rise to severe hypoglycemia, which appears after 2–3 h of fasting and involves moderate lactic acidosis and no ketosis. When hepatomegaly is the most prominent feature without liver insufficiency, gluconeogenesis defects (involving glucose-6-phosphatase or fructose diphosphatase), glycogenosis type III, and GS deficiency are the most probable diagnoses. Disorders presenting with hepatic fibrosis and cirrhosis, such as hereditary tyrosinemia type I, can give rise to hypoglycemia. The late-onset form of fructose intolerance is rarely, if ever, revealed by isolated postprandial hypoglycemic attacks. *S*-adenosyl homocysteine hydrolase deficiency presents with fasting hypoglycemia and hepatocellular insufficiency, often triggered by high protein or methionine ingestion, and is associated with hepatic fibrosis, mental retardation, and marked hypermethioninemia. Respiratory chain disorders can present with hepatic failure and hypoglycemia. The amazing familial association of hepatic fibrosis and exsudative enteropathy can strike by hypoglycemia early in infancy [7].

Hypoglycemia Without Permanent Hepatomegaly. It is important to discover the timing of hypoglycemia and to search for metabolic acidosis and ketosis when the patient is hypoglycemic. As a general rule, most if not all hypoglycemias that are

Table 6. Hypoglycemia: general approach

With permanent hepatomegaly	Severe liver failure Hepatic necrosis	Permanent short fast hypoglycemia	Neonatal to early in infancy	Galactosemia Fructosemia Tyrosinosis type I Neonatal hemochromatosis Respiratory chain Other severe hepatic failure
		Postprandial hypoglycemia (triggered by fructose) Vomiting		Fructose intolerance Glycerol intolerance
	Fibrosis Cirrhosis	Mental retardation Hypermethioninemia Hepatic failure induced by methionine	Early in infancy	Glycogenosis type IV SAH hydrolase deficiency Respiratory chain
		Exsudative enteropathy Cholangitis attacks Short fast hypoglycemia	Early in infancy	Familial hepatic fibrosis with exsudative enteropathy
	Isolated hepatomegaly	Fasting hypoglycemia and lactic acidosis Ketosis	Infancy	G6P FDP PEPCK
		Protuberant abdomen Fasting hypoglycemia and ketosis Postprandial hyperlactacidemia		Glycogenosis type III Glycogen synthetase
Without permanent hepatomegaly	With ketoacidosis	Recurrent attacks Hyperlactacidemia	Infancy to childhood	Organic acidurias Late-onset MSUD Ketolysis defects Glycerol kinase FDP
		Dehydration, collapsus Hyponatremia		Adrenal insufficiency (central or peripheral)
	Acidosis without ketosis	Moderate hyperlactacidemia Reye syndrome (± muscle/cardiac symptoms)	Neonatal to infancy	HMGCoA lyase (frequent) FAO disorders (rare) Idiopathic Reye syndrome
	Ketosis without acidosis	Fasting hypoglycemia Low lactate Small for age Macrocephaly	1–6 years	Recurrent hyperketotic hyperglycemia Adrenal insufficiency (central or peripheral)
		Unpredictable and post-prandial hypoglycemia reactive to glucagon	Neonatal to childhood	Hyperinsulinism Cortisol deficiency
	Without acidosis	Short stature Short, fast hypoglycemia	Infancy	Growth hormone deficiency and related disorders
	Without ketosis	Long, fast hypoglycemia Reye syndrome Moderate hepatomegaly Transient cytolysis	Neonatal to infancy	FAO disorders (frequent) HMGCoA lyase (rare) FDP (rare)

G6P, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; MSUD, maple syrup urine disease; HMG-CoA, 3-hydroxy-3-Methylglutaryl coenzyme A; SAH, S. adenosyl homocysteine hydrolase; FDP, fructose diphosphatase; FAO, fatty acid oxidation defects.

not accompanied by hepatomegaly and are caused by inborn errors of metabolism appear after at least 8h of fasting. This is especially true of hypoglycemias due to inherited fatty acid oxidation disorders except in the neonatal period. Conversely, unpredictable postprandial or very short fasting hypoglycemias (2–6h) are mostly due to hyperinsulinism and growth hormone deficiency or related disorders. When ketoacidosis is present at the time of hypoglycemia, organic acidurias, ketolytic defects, late-onset MSUD, and glycerol kinase deficiencies should be considered. Here, hypoglycemia is very rarely the initial metabolic abnormality. Adrenal insufficiencies should be systematically considered in the differential diagnosis, especially when vascular hypotension, dehydration, and hyponatremia are present. Severe hypoglycemia with metabolic acidosis and absence of ketosis, in the context of Reye syndrome, suggests HMG-CoA lyase deficiency or fatty acid oxidation disorders. Fasting hypoglycemia with ketosis occurring mostly in the morning and in the absence of metabolic acidosis suggests recurrent functional ketotic hypoglycemia, which presents mostly in late infancy or childhood in children who were small for their gestational age or in children with macrocephaly. Conversely, in our experience, this pattern is rarely associated with inborn errors of metabolism.

Hypoketotic hypoglycemias encompass several groups of disorders including hyperinsulinemic states, growth hormone deficiency, inborn errors of fatty acid oxidation, and ketogenesis defects (see "Ketosis" above).

Chronic and Progressive General Symptoms

As already stated, many apparently delayed-onset acute presentations of inherited disorders are preceded by insidious premonitory symptoms which have been misinterpreted. These symptoms fall schematically into three categories: digestive, neurologic, and muscular symptoms.

Digestive Symptoms

Digestive symptoms (anorexia, chronic vomiting, failure to thrive) occur in a wide variety of inborn errors of metabolism. Unfortunately, their cause often remains unrecognized, thus delaying the correct diagnosis. Persistent anorexia, feeding difficulties, chronic vomiting, failure to thrive, frequent infections, osteopenia, and generalized hypotonia in a context of chronic diarrhea are the presenting symptoms in a number of constitutional and acquired diseases in pediatrics. They are easily misdiagnosed as cow's milk protein intolerance, celiac disease, chronic ear, nose, or throat infections, late-onset chronic pyloric stenosis etc. Congenital immunodeficiencies are also frequently considered, although only a few present early in infancy with such a clinical picture.

From a pathophysiological viewpoint, it is possible to define two groups of inborn errors of metabolism presenting with chronic diarrhea and failure to thrive:

- Disorders of the intestinal mucosa or the exocrine function of the pancreas, for example congenital chloride diarrhea, glucose–galactose malabsorption, lactase and sucrase–isomaltase deficiencies, abetalipoproteinemia type II (Anderson disease), enterokinase deficiency, acrodermatitis enteropathica, and selective intestinal malabsorption of folate and vitamin B_{12}
- Systemic disorders which also give rise to digestive abnormalities

In clinical practice, these groups are sometimes very difficult to distinguish, because a number of specific intestinal disorders can give rise to various systemic clinical abnormalities and the reverse. This is summarized in Table 7.

Neurologic Symptoms

Neurologic symptoms encompass progressive psychomotor retardation, seizures, and a number of neurologic abnormalities in both the central and peripheral system as well as sensorineural defects. The main keys to the diagnosis of such nonspecific symptoms are age at onset and their being progressive, not explained by an obvious cause, associated with (an)other extraneurologic symptom(s), preceded or accompanied by an acute episode which is triggered by a catabolic stress, and associated with a metabolic disturbance (such as slightly low plasma bicarbonate, hyperlactacidemia, ketonuria).

Tables 8–10 present a general approach to inborn errors of metabolism involving neuro-

Table 7. Chronic diarrhea, poor feeding, vomiting, failure to thrive

Severe watery diarrhea Attacks of dehydration	No meconium, nonacidic diarrhea, metabolic alkalosis, hypochloremia and hypochloruria	Congenital to infancy	Congenital chloride diarrhea
	Acidic diarrhea, reducing substances in stool, glucosuria	Neonatal	Glucose galactose malabsorption Lactase deficiency
	Acidic diarrhea, reducing substances in stool After weaning or after starch dextrins are added to the diet	Neonatal to infancy	Sucrase isomaltase deficiency
	Skin lesions, alopecia (late onset), failure to thrive	Neonatal or after weaning	Acrodermatitis enteropathica
Fat-soluble vitamins malabsorption	Unexplained cholestatic jaundice	Neonatal to infancy	Bile acid synthesis defects Infantile Refsum disease
Severe hypocholesterolemia Osteopenia, steatorrhea	Hepatomegaly, hypotonia, slight mental retardation, Retinitis pigmentosa, deafness (after at least 1 year)	Infancy	Infantile Refsum disease CDG syndrome
	Abdominal distension, failure to thrive, anorexia Acanthocytosis, peripheral neuropathy, ataxia Retinitis pigmentosa[a]	Infancy	Abetalipoproteinemia type I Abetalipoproteinemia type II (no acanthocytes, no neurological signs)
	Pancreatic insufficiency, neutropenia, pancytopenia	Early in infancy	Pearson syndrome Schwachman syndrome
Severe failure to thrive Anorexia, poor feeding, with predominant hepatosplenomegaly	Severe hypoglycemia, inflammatory bowel disease, neutropenia, recurrent infections	Neonatal to early infancy	Glycogenosis type Ib (glucose-6-phosphate carrier deficiency)
	Hypotonia, vacuolated lymphocytes, adrenal gland calcifications	Neonatal	Wolman disease
	Recurrent infections, inflammatory bowel disease, dermatitis, stomatitis	Infancy	Chronic granulomatosis (X-linked)
	Megaloblastic anemia, stomatitis, muscle weakness, peripheral neuropathy, homocystinuria, methylmalonic aciduria	1–5 years	Intrinsic factor deficiency
	Leukoneutropenia, osteopenia, recurrent attacks of hyperammonemia, interstitial pneumonia, orotic aciduria	Infancy	Lysinuric protein intolerance

Table 7. (*Contd.*)

Leading symptoms	Other signs	Age of onset	Diagnosis
Severe failure to thrive Anorexia, poor feeding, with megaloblastic anemia	Oral lesion, stomatitis, pancytopenia, peripheral neuropathy, infections, homocystinuria, methylmalonic aciduria	1–2 years	Transcobalamin II deficiency Intrinsic factor deficiency
	Stomatitis, infections, peripheral neuropathy, intracranial calcifications	Infancy	Congenital folate malabsorption
	Severe pancytopenia, vacuolization of marrow precursors, exocrine pancreas insufficiency, lactic acidosis	Neonatal	Pearson syndrome
Severe failure to thrive Anorexia, poor feeding	Severe hypoprotidemia, putrefaction diarrhea	Infancy	Enterokinase deficiency
Hypotonia No significant hepatosplenomegaly No megaloblastic anemia	Diarrhea after weaning, cutaneous lesion (periorificial) alopecia, modest diarrhea, low alkaline phosphatase, low plasma zinc	Infancy	Acrodermatitis enteropathica
	Ketoacidosis, metabolic attacks, frequent infections, vomiting	Infancy	Organic acidurias (MMA, PA) Mitochondrial DNA deletions (lactic acidosis)
	Vomiting, lethargy, hypotonia, metabolic attacks, hyperammonemia	Infancy	Urea cycle defects (mainly OTC)
	Frequent infections, lymphopenia, bone changes, severe combined immune deficiency	Infancy	Adenosine deaminase deficiency

MMA, methylmalonic acidemia; PA, propionic acidemia; CDG, carbohydrate-deficient glycoprotein; OTC, ornithine transcarbamylase.

[a] While abdominal distension, failure to thrive, anorexia, and acanthocytosis start in infancy, peripheral neuropathy, ataxia and retinitis pigmentosa only appear after 5 years of evolution.

logic and/or mental deterioration. Diseases are classified according to their age of onset, the presence or absence of associated extraneurologic signs, and the neurologic presentation itself; the latter is based largely on the clinical classification of Adams and Lyon [8]. Inborn errors of metabolism with neurologic signs presenting in the neonate (birth to 1 month; Table 2) and those presenting intermittently as acute attacks of coma, lethargy, ataxia, or acute psychiatric symptoms are presented earlier (Tables 3, 4).

It must be stressed that a large number of inborn errors of intermediary metabolism present with nonspecific, early, progressive developmental delay, poor feeding, hypotonia, some degree of ataxia, and frequent autistic features. The list has lengthened rapidly as new laboratory techniques have been applied. The relationship between clinical symptoms and biochemical abnormalities is not always firmly established. Many aminoacidopathies that were first described in the late 1950s and 1960s, when plasma and urine amino acid chromatography was systematically used in studying mentally retarded children, must now be questioned as definite causes of neurologic disturbance. This is the case for histidinemia, hyperlysinemia, hyperprolinemia, alpha-aminoadipic aciduria, and Hartnup "disease."

Table 8. Progressive neurologic and mental deterioration (1–12 months) (see also Table 2)

Leading symptoms	Other signs	Diagnosis
With obvious extraneurologic symptoms		
Visceral signs	Hepatosplenomegaly Coarse facies Storage signs	Landing I-cell disease Sialidosis type II Niemann-Pick A
	Hepatosplenomegaly Opisthothonos Vegetative state	Gaucher type II
Hair and cutaneous symptoms	Steely hair	Menkes disease (X-linked; hypothermia, osteopenia)
	Ichthyosis	Sjögren-Larsson syndrome (spastic paraplegia, cataract)
	Alopecia Cutaneous rashes	Biotinidase deficiency (ketoacidosis, hyperlactacidemia)
	Peculiar fat pads on buttocks	Carbohydrate-deficient glycoprotein syndrome (episodic failure of multiorgan, liver heart tamponade)
Megaloblastic anemia (see this symptom)		Inborn errors of folate metabolism Inborn errors of cobalamin metabolism Hereditary orotic aciduria
With specific or suggestive neurologic signs		
Extrapyramidal signs	Major parkinsonism	Inborn errors of biopterin metabolism Aromatic amino acid decarboxylase deficiency
	Choreoathetoid movements Self-mutilation Cyanosis Methemoglobinuria	Lesch-Nyhan syndrome (X-linked; hyperuricemia) Cytochrome b-5-reductase deficiency
	Dystonia	Pelizaeus-Merzbacher (X-linked; laryngeal stridor, nystagmus)
	Acute-onset pseudoencephalitis	Glutaric aciduria type I (choreoathetosis, dystonia)
Macrocephaly, startle response to sound, ocular symptoms	Myoclonic jerks	Tay-Sachs, Sandhoff (cherry red spot)
	Megalencephaly	Canavan, Van Bogaert, Bertrand (aspartoacylase deficiency)
	Optic atrophy	Alexander disease
	Incessant crying Irritability	Krabbe (infantile) (peripheral neuropathy, hyperproteinorachia)
	Dystonia Choreoathetosis	Glutaric aciduria type I
	Dystonia Hemiballistic movements	Creatine deficiency in the brain
Recurrent attacks of neurologic deterioration	Mental regression Hyperventilation attacks Various neurologic signs	Leigh syndrome (pyruvate carboxylase and dehydrogenase, respiratory chain disorders)
Without suggestive symptoms: nonspecific mental retardation		
With evidence of developmental arrest	Infantile spasms Hypsarrhythmia Autistic features	Classic untreated phenylketonuria Inborn errors of biopterin metabolism
Without specific symptoms	Frequent autistic feature Poor feeding Failure to thrive Hypotonia	Hyperammonemia (late-onset subacute form) 4-Hydroxybutyric aciduria Mevalonic aciduria (recurrent attacks of fever, rash, arthralgia) Adenylosuccinase deficiency Dihydropyrimidine dehydrogenase Other organic acidurias Homocystinurias (iridodonesis) Salla disease (ocular nystagmus) NKH late onset forms SO deficiency (lens dislocation)

NKH, nonketotic hyperglycinemia; SO, sulfite oxidase.

Table 9. Progressive neurologic and mental deterioration (1–5 years)

Symptoms		Diagnosis
With visceral, craniovertebral, or other somatic abnormalities		
Coarse facies, skeletal changes, hirsutism, corneal opacities		Hurler (MPS I), Hunter (X-linked) (MPS II), San Filipo (MPS III) Pseudo-Hurler polydystrophy (MLP III)
Coarse facies, subtle bone changes, hepatosplenomegaly, vacuolated lymphocytes, ± lens dislocation		Mannosidosis (gingival hyperplasia) Fucosidosis (angiokeratoma) Aspartylglucosaminuria (macroglossia, leukopenia, joint laxity)
Hepatosplenomegaly, progressive dementia, myoclonic jerks		Niemann-Pick type C and related disorders (late infantile form) (vertical supranuclear ophtalmoplegia)
Splenomeagly + hepatomegaly, osseous lesions, various neurologic signs (ataxia, myoclonus)		Gaucher type III (subacute neuronopathy) (supranuclear ophtalmoplegia)
Major visual impairment, blindness		Mucolipidosis type IV (corneal clouding)
With progressive paraplegia, weakness, hypotonia, or spasticity of the lower limbs due to corticospinal tract involvement or to peripheral neuropathy		
Flaccid paraparesis ± pyramidal signs, high protein content in CSF		Metachromatic leukodystrophy (abnormal nerve conduction velocity)
Flaccid paraparesis, no change in CSF, optic atrophy, early mental regression		Neuroaxonal dystrophy (Seitelberger disease) (normal nerve conduction velocity)
Progressive spastic diplegia, scissoring or "tiptoe" gait		Arginase deficiency (hyperargininemia, high orotic acid excretion)
With unsteady gait, uncoordinated movements when standing, walking, sitting, reaching for objects, speaking, and swallowing due to cerebellar syndrome, sensory defects or involuntary movements (Athetosis choreiform movements)		
Without disturbances of organic acid excretion	Ataxia with choreoathetosis Oculocephalic asynergia	Ataxia telangiectasia (conjunctival telangiectasias, sinopulmonary infections, low IgA level)
	Ataxia, difficulty in walking, mental deterioration (speech)	GM1 gangliosidosis (Landing) (late infantile form) (spastic quadriparesis, pseudobulbar signs)
	Ataxia, spinocerebellar degeneration, psychotic behavior	GM2 gangliosidosis (Tay-Sachs, Sandhoff) (late infantile form)
	Ataxia, pyramidal signs (hemiplegia, paraplegia), vision loss	Krabbe disease (late infantile form) (peripheral neuropathy)
	Ataxia, muscular atrophy in lower extremities	Carbohydrate-deficienc glycoprotein syndrome (peripheral neuropathy)
	Seizures and myoclonic jerks, postictal coma, transient hemiplegia	Alpers syndrome (respiratory chain disorders) (hepatic syndrome, hyperlactatemia)
With disturbances of organic acid excretion	Progressive ataxia, intentional tremor, cerebellar atrophy	L-2-hydroxyglutaric aciduria (spongiform encephalopathy)
	Combined degeneration of the spinal cord	Cobalamin deficiencies (homocystinuria, low methionine)
	Ataxia, peripheral neuropathy	PDH deficiency (hyperlactatemia)
	Ataxia, muscular weakness, retinitis pigmentosa myoclonic epilepsy	Respiratory chain disorders, MERF syndrome (hyperlactatemia, Krebs cycle intermediate excretion)

Table 9. (*Contd.*)

Symptoms	Diagnosis
Ataxia, peripheral neuropathy, retinitis pigmentosa	3-hydroxy-acylCoA dehydrogenase deficiency (hypoketotic hypoglycemia, 3-hydroxy-dicarboxylic aciduria)
Acute attacks resembling encephalitis, choreoathetosis, macrocephaly	Glutaric aciduria type I (glutarylCoA dehydrogenase deficiency) (glutaric aciduria)
With convulsions, seizures and myoclonus, ataxia, frequent falling due to intention myoclonus or to cerebellar ataxia	
Rapidly advancing psychomotor degeneration, myoclonic jerks, blindness	Santavuori-Hagberg (infantile ceroid lipofuscinosis) (early-flattening EEG)
Akinetic myoclonic petit mal, retinitis pigmentosa, typical EEG on slow rate, photic stimulation	Jansky Bielschowski (late infantile ceroid lipofuscinosis) (do not misdiagnose with Lennox-Gastaut syndrome) (vacuolated lymphocytes)
Rapid regression, myoclonic seizures, spasticity	Schindler disease (alpha-*N*-acetyl galactosidase deficiency) (optic atrophy, severe osteoporosis)
Myoclonic epilepsy, volitional and intentional myoclonias, muscular weakness	MERF syndrome (respiratory chain disorders) (hyperlactacidemia)
With splenomegaly and hepatomegaly, myoclonic epilepsy can be revealing	Niemann-Pick type C, Gaucher type III (supranuclear ophtalmoplegia)
Seizures and myoclonic jerks, uncoordinated movements	Alpers syndrome (respiratory chain disorders) (hepatic symptoms, hyperlactatemia)
Disorders with arrest or regression of psychic and perceptual functions as the preponderant or unique revealing symptom	
Autistic behavior, regression of high-level achievements, stereotyped movements of fingers	Rett syndrome (only girls), sporadic cases of unknown etiology (acquired microcephaly, secondary epilepsy)
Regression of high-level achievements, loss of speech, agitation	San Filippo (hirsutism)

Ig, immunoglobulin; PDH, pyruvate dehydrogenase; CoA, coenzyme A; EEG, electroencephalogram; CSF, cerebrospinal fluid; MPS, mucopolysaccharidosis; MLP, mucolipidosis; MERRF, myoclonic epilepsy ragged red fibers.

The same story may now be developing with organic acidurias, so that it is more and more important to try to define pathophysiological links between clinical symptoms and metabolic disturbances. Conversely, it is more and more difficult to screen patients on clinical grounds when the clinical symptoms consist only of developmental delay, hypotonia, and convulsions. Among the new categories of inborn errors of intermediary metabolism that present with uninformative clinical manifestations are, for example, adenylosuccinase deficiency, dihydropyrimidine dehydrogenase deficiency, 4-hydroxybutyric aciduria, and some other organic acidurias, such as Salla disease (sialic acid excretion). These disorders rarely, if ever, cause true development arrest; rather, they cause progressive subacute developmental delay. Conversely, there is still an important gap between neurologic descriptions and biochemical investigations. Many well-known heritable neurologic or polymalformative syndromes have not been considered from a pathophysiological perspective and should be submitted to a comprehensive biochemical evaluation. This is illustrated for example by the story of Canavan disease, in which *N*-acetylaspartic aciduria was not found until 1988, even though the clinical phenotype had been identified in 1949 and the procedure for identifying *N*-acetylaspartate in urine was available in 1972.

Table 10. Progressive neurologic and mental deterioration (5–15 years)

Symptoms		Diagnosis
With predominant extrayramidal signs, parkinson syndrome, dystonia, choreoathetosis (see also symptom "extrapyramidal signs")		
Torsion, dystonia, no mental retardation		Dystonia musculorum deformans
Dystonia on lower extremities, gait difficulties, normal intellect		Segawa disease (diurnal fluctuation of dystonia, dopa responsive)
Lens dislocation, marfanoid morphology		Classic homocystinuria
Progressive disorders of locomotion, dystonic posture, severe mental regression		Hallervorden Spatz (retinitis pigmentosa, acanthocytosis)
Generalized parkinsonian rigidity, scholastic failure		Wilson disease (hepatic signs, Kayser-Fleischer ring)
Rigidity, fine tremor abolished by movements, dementia, seizures		Huntington chorea (dominant inheritance)
Parkinsonism, difficulties in reading and writing, alacrima, dysphagia due to achalasia		Familial glucocorticoid deficiency (hypoglycemia due to selective cortisol deficiency)
With severe neurologic and mental deterioration, diffuse central nervous system disorders, bipyramidal paralysis, incoordination, seizures, visual failure, dementia		
With visceral signs (hepatosplenomegaly)		Niemann-Pick type C, Gaucher type III
Without visceral signs		Metachromatic leucodystrophy (juvenile form) Adrenal leukodystrophy (X-linked) (many variants) Leigh syndrome Krabbe disease (infantile form), GM1 and GM2 (juvenile form) Respiratory chain disorders
With polymyoclonia		
Generalized epilepsy, dementia		Lafora disease
Intellectual deterioration, loss of sight, retinitis		Spielmeyer-Vogt (juvenile neuronal ceroid lipofuscinosis) (vacuolated lymphocytes)
Proeminent seizures, myoclonic epilepsy		Gaucher type III
Cerebellar ataxia, cherry red spot		Late GM2 gangliosidosis (Sandoff, Tay-Sachs)
Hepatomegaly, splenomegaly		Niemann-Pick type C
Myoclonic epilepsy, lactic acidosis		Respiratory chain disorders (MERFF etc.)
With predominant cerebellar ataxia		
Without significant mental deterioration	Dysarthria	Friedreich ataxia (pes cavus, cardiomyopathy)
	Spinocerebellar degeneration	Other hereditary ataxias
	Chronic diarrhea, retinitis pigmentosa, peripheral neuropathy	Abetalipoproteinemia (low cholesterol acanthocytosis)
	Oculocephalic asynergia, conjunctival telangiectasias	Ataxia telangiectasia (see age of onset 1–5 years)
	Peripheral neuropathy, retinitis pigmentosa	Refsum disease (ichthyosis)
With deterioration and dementia		Lafora disease Cererotendinous xanthomatosis GM1, GM2, Gaucher, Niemann-Pick type C, Krabbe juvenile form Metachromatic leukodystrophy Respiratory chain disorders

Table 10. (*Contd.*)

Symptoms	Diagnosis
With predominant polyneuropathy	
Acute attacks	Porphyrias Tyrosinemia type I
Progressive	Metachromatic leukodystrophy Krabbe disease Refsum disease Abetalipoproteinemia Leigh syndrome Respiratory chain disorders Pyruvate dehydrogenase deficiency 3-Hydroxydicarboxylic aciduria Carbohydrate-deficient glycoprotein syndrome
With psychiatric symptoms as the only presenting sign	
Behavior disturbances, personality and character changes, mental regression, dementia, schizophrenia before any significant neurologic or extraneurologic sign	San Filippo, metachromatic leukodystrophy, krabbe disease, Niemann-Pick type C X-linked adrenoleukodystrophy Leigh syndrome Spielmeyer-Vogt disease (lipofuscinosis) Hallervorden-Spatz disease Wilson disease Cerebrotendinous xanthomatosis Huntington chorea (juvenile form) OTC deficiency Methylene tetrahydrofolate reductase deficiency

OTC, ornithine transcarbamylase

Muscular Symptoms

Many inborn errors of metabolism can present with severe hypotonia, muscular weakness, and poor muscle mass. These include most of the late-onset forms of urea cycle defects and many organic acidurias. Severe neonatal generalized hypotonia and progressive myopathy associated or not with a nonobstructive idiopathic cardiomyopathy can be specific revealing symptoms of a number of inherited energy deficiencies. The most frequent conditions actually observed are mitochondrial respiratory chain disorders and other congenital hyperlactacidemias, fatty acid oxidation defects, peroxisomal disorders, muscular glycogenolysis defects, alpha-glucosidase deficiency, and some other lysosomal disorders.

Specific Symptoms Suggestive of an Inborn Error of Metabolism

A number of clinical or biologic symptoms can reveal or accompany inherited inborn errors of metabolism. Some of these phenotypes are rare and very distinctive (e.g., lens dislocation and thromboembolic accidents in homocystinuria), whereas others are common and rather nonspecific (e.g., hepatomegaly, seizures, mental retardation). The most important ones are listed in the Appendix. The diagnostic checklist presented in this Appendix is mostly based upon the author's personal experience and, of course, is not exhaustive. It should be progressively extended by the personal experience of all readers.

In searching for the diagnosis, we must reemphasize the importance of not confusing a syndrome due to different causes with the etiology itself. Hence, Leigh syndrome and Reye syndrome have been incorporated in the list of symptoms and must also be considered as the actual diagnosis. Some other well-known recessive syndromes (such as Joubert, Usher, Cockayne etc.) have been listed under inborn errors of metabolism in order to highlight the necessity of performing extensive metabolic and genetic investigations before attributing a label of false security to a patient. The recent demonstration of a cholesterol synthesis

defect in Smith-Lemli-Opitz syndrome is an illustration of this statement [9].

Appendix

In the following list, symptoms are arbitrarily classified according to the main organ involved (heart, skin, brain etc.).

Cardiology

Cardiac Failure, Heart Beat Disorders

With cardiomyopathy (see this symptom)
With tamponade, multiorgan failure:

- Carbohydrate deficient glycoprotein (CDG) syndrome

With apparently primitive heart beat disorders:

- Adrenal dysfunction (hyperkalemia)
- Hypoparathyroidism (hypocalcemia)
- Thiamine deficiency-dependent states
- Triose phosphate isomerase deficiency
- Fatty acid oxidation disorders – carnitine palmitoyltransferase II (CPT II), translocase, long-chain acyl coenzyme A dehydrogenase (LCAD), 3-hydroxy LCAD (LCHAD), very long chain acyl-CoA dehydrogenase (VLCAD)
- Kearn-Sayre syndrome

Cardiomyopathy

- Respiratory chain disorders (revealing sign)
- Fatty acid oxidation disorders (revealing sign)
- Pompe disease (revealing sign)
- Glycogenosis type III and IV
- Phosphorylase β kinase (revealing sign)
- 3-Methylglutaconic aciduria
- Propionic acidemia
- Methylmalonic aciduria (Cb1 C)
- Mevalonic aciduria
- Carbohydrate-deficient glycoprotein syndrome
- Mucopolysaccharidosis
- Friedreich ataxia
- Steinert disease – myotonic dystrophy
- Congenital muscle dystrophies

Sudden Infant Death Syndrome

See "Reye Syndrome"

Dermatology

Alopecia

Age at onset, neonatal to infancy:

- Menkes disease (X-linked)
- Biotin-responsive multiple carboxylase defects
- Methylmalonic and propionic acidurias
- Acrodermatitis enteropathica
- Essential fatty acid deficiency
- Zinc deficiency
- Hepatoerythropoietic porphyria
- Congenital erythropoietic porphyria
- Calciferol metabolism defects (vitamin D-dependent rickets)
- Ehlers-Danlos type IV
- Netherton syndrome
- Conradi-Hunermann syndrome

Age at onset, adulthood:

- Steinert
- Porphyria cutanea tarda

Angiokeratosis

- Fabry disease
- Fucosidosis
- Galactosialidosis
- Aspartylglucosaminuria
- β-Mannosidosis
- Schindler disease (adult form)

Ichthyosis (with Congenital Erythrodermia)

- Conradi-Hunermann syndrome (X-linked chondrodysplasia punctata)
- Multisystemic triglyceride storage disease
- Sjögren-Larsson syndrome
- Austin disease
- Steroid sulfatase deficiency (X-linked)
- Netherton syndrome
- Refsum disease (adult form)

Hyperkeratosis

- Ichthyosis (see above)
- Tyrosinemia type II (keratosis on palms and soles)

Laxity (Dysmorphic Scarring, Easy Bruising)

Inborn errors of collagen:

- Ehlers-Danlos syndrome (nine types)
- Occipital horn syndrome
- Cutis laxa

Nodules

- Farber lipogranulomatosis
- Carbohydrate-deficient glycoprotein syndrome

Photosensitivity and Skin Rashes

Age at onset, neonatal to childhood:

- Congenital erythropoietic porphyria
- Erythrohepatic porphyria
- Erythropoietic protoporphyria
- Hartnup disease
- Respiratory chain disorders
- Xeroderma pigmentosum(nine varieties)
- Mevalonic aciduria (with fever and arthralgia)

Age at onset, adulthood:

- Porphyria variegata
- Hereditary coproporphyria
- Porphyria cutanea tarda

Pili Torti

- Menkes disease
- Netherton syndrome

Telangiectasias – Purpuras

- Prolidase deficiency

Trichorrhexis Nodosa

- Argininosuccinic aciduria
- Argininemia
- Lysinuric protein intolerance
- Menkes disease
- Netherton syndrome

Ulceration (Skin Ulcers)

- Prolidase deficiency

Vesiculo bullous Skin Lesions

- Acrodermatitis enteropathica
- Zinc deficiency
- Holocarboxylase synthetase deficiency (biotin responsive)
- Biotinidase deficiency (biotin responsive)
- Methylmalonic, propionic acidemias

Dehydration (Attacks)

With Severe Diarrhea (Digestive Causes)

- Glucose-galactose malabsorption
- Congenital lactase deficiency
- Congenital chloride diarrhea
- Sucrase isomaltase deficiency
- Acrodermatitis enteropathica

With Ketoacidosis (Organic Acidurias)

- Diabetic coma
- Methylmalonic, propionic, isovaleric acidurias
- 3-Kethiolase deficiency
- Hydroxyisobutyric aciduria

With Renal Tubular Dysfunction (*see "Tubulopathies"*)

- Cystinosis
- Nephrogenesis diabetes insipidus
- Renal tubule acidosis (RTA) type I, II, and IV

With Salty Sweat

- Cystic fibrosis

With Salt-Losing Syndrome (*see this symptom*)

- Adrenal dysfunctions

Dysmorphology

Coarse Facies

Age at onset, present at birth:

- Landing
- Sialidosis type II

- Galactosialidosis (early infancy)
- Sly (mucopolysaccharidosis, MPS type VII) (rare)
- I-cell disease

Age at onset, early infancy:

- Fucosidosis type I
- Sialidosis type II
- Salla disease
- Hurler (MPS type Is)
- Sly (MPS type VII)
- Mannosidosis
- Austin
- Maroteaux-Lamy (MPS type V)

Age at onset, childhood:

- Hunter (MPS type II)
- Aspartylglucosaminuria
- Pseudo-Hurler polydystrophy
- San Filippo (MPS type III)

Congenital Malformations and Dysmorphic Syndromes

Inborn errors affecting the fetus

- 3-Hydroxy isobutyrylCoA deacylase deficiency
- Mevalonic aciduria (mevalonate kinase deficiency)
- Glutaric aciduria type II (maltiple acyl CoA dehydrogenase deficiency, MADD)
- Carnitine palmityl transferase II deficiency
- Peroxisomal disorders (Zellweger and variants, CDP)
- Pyruvate dehydrogenase deficiency
- Respiratory chain defects
- Inborn errors of collagen
- Hypoparathyroidism
- Hypophosphatasia
- Leprechaunism
- Lysosomal storage disorders
- Smith-Lemli-Opitz syndrome (inborn error of cholesterol synthesis)

Metabolic disturbances of the mother

- Phenylketonuria
- Alcohol
- Diabetes
- Drugs
- Vitamin deficiencies (riboflavin)

Endocrinology

Diabetes (and Pseudodiabetes)

- Respiratory chain disorders – Wolfram syndrome
- Diabetes, deafness, and thiamine-responsive megaloblastic anemia
- Organic acidurias (methylmaloric aciduria, MMA; propionic aciduria PA, isovaleric aciduria IVA; ketolytic defects)

Hypogonadism – Sterility

- Galactosemia

Hypoparathyroidism

- Respiratory chain disorder

Salt-Losing Syndrome

- Disorders of adrenal steroid metabolism

Sexual Ambiguity

- Disorders of adrenal steroid metabolism
- Congenital adrenal hyper- and hypoplasias

Short Stature – Growth Hormone Deficiency

- Respiratory chain disorder

Gastroenterology

Abdominal Pain (Recurrent)

With meteorism, diarrhea, loose stool:

- Lactose malabsorbers
- Congenital sucrase isomaltase deficiency

With vomiting, lethargy, ketoacidosis:

- Urea cycle defects (ornithine transcarbamylase, OTC, arginosuccinic aciduria, AS)
- Organic acidurias (MMA, PA, IVA)

- Ketolysis defects
- Diabetes

With neuropathy, psychiatric symptoms:

- Porphyrias
- Tyrosinemia type I
- OTC deficiency (late onset)

With hepatomegaly (± splenomegaly):

- Cholesteryl ester storage disease
- Lipoprotein lipase deficiency
- Lysinuric protein intolerance
- Hemochromatosis

With pain in extremities:

- Fabry disease
- Aladehydratase deficiency
- Sickle cell anemia

With hemolytic anemia:

- Coproporphyria
- Hereditary spherocytosis
- Sickle cell anemia

Acute Pancreatitis

- Organic acidurias (MMA; PA; IVA; maple syrup urine disease, MSUD)
- Hyperlipoproteinemia type I and IV

Chronic Diarrhea
See Table 9

Hypocholesterolemia

- Peroxisomal disorders
- Infantile Refsum disease
- CDG syndrome
- Mevalonic aciduria
- Smith-Lemli-Opitz syndrome
- Abetalipoproteinemia type I and II
- Tangier disease

Hematology

Acanthocytosis

- Wolman disease
- Abetalipoproteinemia
- Inborn errors of cobalamine (Cb1 C)
- Hallervorden-Spatz syndrome

Nonmacrocytic anemia (Hemolytic or Due to Combined Mechanisms)

- Red blood cells glycolysis defects
- Pyroglutamic aciduria
- Galactosemia
- Wolman disease
- Wilson disease
- Abetalipoproteinemia
- Hemochromatosis
- Severe liver failure
- Erythropoietic protoporphyria
- Congenital erythropoietic porphyria
- Erythropoietic porphyria
- Lecithin cholesterol acyltransferase deficiency
- Carnitine transport defect

Megaloblastic Anemia

- Inborn errors of folate metabolism:
  Dihydrofolate reductase deficiency
  Glutamate formimino transferase deficiency
  Congenital folate malabsorption
- Inborn errors of cobalamin metabolism:
  Imerslund disease
  Intrinsic factor deficiency
  TC II deficiency
  Cbl C, Cbl E, Cbl G
  Methionine synthase deficiency
- Thiamine responsive megaloblastic anemia
- Respiratory chain disorders
- Pearson syndrome (mitochondrial DNA deletion)
- Hereditary orotic aciduria
- Mevalonic aciduria
- Dyserythropoiesis type II (respiratory chain disorders)

Bleeding Tendency

- Glycogenosis type Ia and Ib
- Gaucher disease
- Inborn errors with severe liver failure
- Primitive disorders of homeostasis
- Severe thrombopenias

Pancytopenia – Neutropenia – Thrombopenia

- Inborn errors of cobalamin metabolism
- Inborn errors of folate metabolism

- Organic acidurias (MMA, PA, IVA)
- Respiratory chain disorders
- Pearson syndrome
- Johansson-Blizzard syndrome
- Schwachman syndrome
- Lysinuric protein intolerance
- Glycogenosis type Ib (neutropenia)
- Gaucher type I and III
- Other conditions with large splenomegaly
- Aspartylglucosaminuria

Vacuolated Lymphocytes

- Aspartylglucosaminuria
- I-cell disease (mucolipidosis type II)
- Landing disease (GM1)
- Niemann-Pick type A
- Wolman disease
- Ceroid lipofuscinosis
- Mucopolysaccharidosis
- Austin disease
- Sialidosis
- Pompe disease

Hepatology

Cholestatic Jaundice

- Alpha-1-antitrypsin deficiency
- Byler disease
- Inborn errors of bile acid metabolism
- Peroxisomal disorders
- Niemann-Pick type C

Cirrhosis

- Fructose intolerance
- Galactosemia
- Glycogenosis type IV
- Homocysteine hydrolase deficiency
- Phosphoenol pyruvate carboxykinase deficiency
- Tyrosinemia type I
- Alpha-1-antitrypsin deficiency
- Alpers progressive infantile poliodystrophy
- Cystic fibrosis
- Familial hepatic fibrosis with exsudative enteropathy
- Gaucher disease
- Wolman disease
- Cholesterylester storage disease
- Hemochromatosis
- Niemann-Pick disease
- Wilson disease
- CDG syndrome

Hepatocellular Deficiencies

Age at onset, congenital (hydrops fetalis):

- GM1 gangliosidosis (Landing)
- Niemann-Pick A and C
- Galactosialidosis
- Sialidosis type II
- Mucopolysaccharidosis type VII
- Barth hemoglobin

Age at onset, neonatal (<1 month):

- Galactosemia
- Fructosemia
- Fructose diphosphatase deficiency
- Neonatal hemochromatosis
- Respiratory chain disorders
- Tyrosinemia type I (after 3 weeks)
- Fatty acid oxidation disorders

Age at onset, infancy:

- Same defects as in neonatal period
- Ketogenesis defects
- Phosphoemolpyruvate carboxykinase (PEPCK) deficiency
- Pyruvate carboxylase (PC) deficiency
- Alpha-1-antitrypsin deficiency
- Urea cycle defects
- Wolman disease
- Cholesteryl ester storage disease
- *S*-Adenosyl homocystine hydrolase deficiency
- Familial hepatic fibrosis with exsudative enteropathy
- Cystic fibrosis
- CDG syndrome

Age at onset, childhood to adolescence:

- Wilson disease

Reye Syndrome

- Fatty acid oxidation disorders
- Ketogenesis defects
- Urea cycle defects
- Gluconeogenesis defects
- Respiratory chain disorders
- Organic acidurias
- Fructose intolerance

Myology

Exercise Intolerance and Recurrent Myoglobinuria

Glycolytic defects (muscle "glycogenosis"):

- Phosphorylase deficiency (MacArdle)
- Phosphofructokinase deficiency
- Phosphoglycerate kinase deficiency
- Phosphoglycerate mutase deficiency
- Lactate dehydrogenase deficiency
- Glucose-6-phosphate dehydrogenase deficiency
- Phosphorylase β kinase deficiency

Fatty acid oxidation defects:

- Carnitine palmitoyl transferase II
- LCAD, LCHAD
- SCHAD (restricted to muscles)
- Others undescribed (translocase, TL; CPT I; short-chain acyl-CoA dehydrogenase, SCAD?)

Miscellaneous:

- Myoadenylate deaminase deficiency
- Respiratory chain disorders
- Duchenne and Becker muscular dystrophies
- Idiopathic familial recurrent myoglobinuria

Myopathy (progressive)

- Adenylate deaminase deficiency
- Glycogenosis type II (acid maltase deficiency)
- Glycogenosis type III
- Fatty acid oxidation disorders
- Respiratory chain disorders (Kearns-Sayre and others)
- Multisystemic triglyceride storage disease
- Steinert disease

Nephrology

Hemolytic Uremic Syndrome

- Inborn errors of cobalamin metabolism (Cbl C)

Nephrolithiasis/Nephrocalcinosis

- Cystinuria (cystine)
- Hyperoxaluria type I and II (oxalic)
- Xanthine oxidase deficiency (xanthine)
- Molybdenum cofactor deficiency (xanthine)
- Lesh-Nyhan (uric acid)
- Phosphoribosyl pyrophosphate (PRPP) synthase superactivity (uric acid)
- Hereditary renal hypouricemia (uric acid)
- Adenine phosphoribosyltransferase (APRT) deficiency (2–8 dihydroxy adenine)
- Hereditary hyperparathyroidism (calcium)
- Renal tubular acidosis type I

Nephrotic Syndrome

- Respiratory chain disorders

Nephropathy (Tubulointerstitial)

- Glycogenosis type I
- Methylmalonic aciduria
- Respiratory chain disorders (pseudo Senior-Loken syndrome)

Renal Polycystosis

- Zellweger syndrome
- Glutaric aciduria type II
- CPT II deficiency
- CDG syndrome

Tubulopathy

Fanconi syndrome:

- Fructose intolerance – galactosemia
- Respiratory chain disorders (complex IV or with DNA deletion)
- Tyrosinemia type I
- Glycogenosis with tubulopathy (Bickel Fanconi syndrome)
- Lowe syndrome (X-linked)
- Cystinosis

Renal tubular acidosis:

- Renal tubular acidosis type I (distal)
- Renal tubular acidosis type II (proximal)
- Pyruvate carboxylase deficiency
- Methylmalonic aciduria
- Glycogenosis type I
- CPT I deficiency

Urine (Abnormal Odor)

- 3-CH3-crotonylglycinuria (cat)
- Glutaric aciduria type Ii (sweaty feet)
- Isovaleric acidemia (sweaty feet)
- Trimethylaminuria (fish)
- MSUD (maple syrup)
- Tyrosinemia type I (boiled cabbage)
- Phenylketonuria (musty odor)

Urine (Colored)

- Alkaptonuria (black)
- Myoglobinuria (red)
- Porphyria (red)
- Indicanuria (blue)

Neurology

Cerebellar Hypoplasia (and Olivopontocerebellar Atrophy)

- Carbohydrate-deficient glycoprotein syndrome
- 3-Hydroxy-isobutyric aciduria
- Peroxisomal disorders
- L-2-Hydroxyglutaric aciduria
- 3-Methylglutaconic aciduria
- Mevalonic aciduria
- Joubert syndrome (vermis atrophy)

Chronic Ataxia
See Tables 8–10

Corpus Callosum agenesis

- Pyruvate dehydrogenase deficiency
- Peroxisomal disorders
- Respiratory chain disorders
- 3-Hydroxyisobutyric aciduria
- Nonketotic hyperglycinemia
- Adrenocorticotrophic hormone (ACTH) deficiency
- Aicardi syndrome

Dementia
See "Psychiatric Symptoms"

Extrapyramidal Signs (Dyskinesia, Dystonia, Choreoathetosis, Parkinsonism)
See Tables 8–10

Hyperventilation Attacks

- Hyperammonemias
- Metabolic acidosis
- Joubert syndrome
- Leigh syndrome (idiopathic or due to various inborn errors)
- Rett syndrome (only girls)

Hypotonia in the Neonatal Period

Evocative clinical context, visceral symptoms, malformations (dysmorphia, bone changes):

- Hypophosphatasia
- Calciferol metabolism defects
- Osteogenesis imperfecta
- Peroxisomal disorders
- Lowe syndrome (X-linked)
- Chromosomal abnormalities
- Other multiple-malformation syndromes (such as Walker-Warburg, Fukuyama, muscular dystrophy)
- Lysosomal disorders (see Table 2)
- Smith Lemei Ofitz syndrome

Neurological neonatal distresses (see Table 2)
Apparently isolated at birth:

- Severe fetal neuromuscular disorders
- Steinert
- Myasthenia
- Congenital myopathy
- Hereditary sensorimotor neuropathy
- Familial dysautonomy
- Congenital dystrophy
- Werdnig-Hoffmann (spinal muscular atrophy, SMA type I)
- Prader-Willi syndrome
- Pelizaeus-Merzbacher
- CDG syndrome

Intracranial Calcifications

- Inborn errors of folic acid metabolism
- Inborn errors of biopterin metabolism
- Congenital lactic acidemias
- 3-Hydroxyisobutyric aciduria
- Leigh syndrome
- Cockayne syndrome

Leigh Syndrome

- Respiratory chain disorders
- Pyruvate carboxylase deficiency
- Pyruvate dehydrogenase deficiency
- Biotinidase deficiency
- Fumarase deficiency
- Sulfite oxidase deficiency
- 3-Methylglutaconic aciduria

Mental Regression – Neurological Deterioration
See Tables 8–10

Myoclonic Epilepsy (Polymyoclonia)
See Table 2, 9, 10

Neuropathy (Peripheral)
See Tables 8–10

Self-Mutilation

- Lesch-Nyhan syndrome
- Tyrosinemia type I
- Phenylketonuria (untreated)
- 3-Methylglutaconic aciduria

Sensorineural Deafness

Detectable in neonatal to early infancy:

- Zellweger and variants
- Rhizomelic chondrodysplasia punctata
- AcylCoA oxidase deficiency
- Cockayne syndrome
- Alport syndrome

Detectable in late infancy to childhood:

- Infantile Refsum disease (pseudo Usher)
- PRPP synthetase overactivity
- Mucopolysaccharidosis type I, II, and IV
- Mannosidosis (alpha)
- Mucolipidosis type II (I cell disease)
- Biotinidase deficiency (biotin responsive)
- Megaloblastic anemia and diabetes (thiamine responsive)
- Wolframm syndrome
- Neutral lipid storage disorder
- Mitochondrial encephalomyopathy
- Mitochondrial encephalopathy lactic acidosis stroke-like episodes (MELAS), myoclonic epilepsy ragged red fibers (MERRF), Kearn. Sayre syndrome
- Flyn aird syndrome
- Hallgren syndrome

Detectable in late childhood to adolescence:

- β-Mannosidosis
- Refsum disease (adult form)
- Usher syndrome type II
- MERRF, Kearn-Sayre syndromes

Spastic Paraparesia (*see also Tables 9, 10*)

- Hyperargininemia
- Triple H syndrome
- Metachromatic leucodystrophy
- Pyroglutamic aciduria
- Sjögren-Larsson syndrome
- L-2-Hydroxyglutaric aciduria

Ophthalmology

Cataracts

Detectable at birth (congenital):

- Lowe syndrome (X-linked)
- Peroxisomal biogenesis defects (Zellweger and variants)
- Rhizomelic chondrodysplasia punctata
- Cockayne syndrome
- Sorbitoldehydrogenase deficiency

Detectable in the newborn period:

- Galactosemias
- Peripheral epimerase deficiency
- Marginal maternal galactokinase deficiency

Detectable in infancy:

- Galactokinase deficiency
- Galactitol or sorbitol accumulation of unknown origin
- Sialidosis
- α-Mannosidosis
- Hypoglycemia (various origins)
- Respiratory chain disorders

Detectable in childhood (1–15 years):

- Hypoparathyroidism
- Pseudohypoparathyroidism
- Diabetes mellitus
- Wilson disease
- Sjögren-Larsson syndrome
- Lysinuric protein intolerance

- Neutral lipid storage disorders
- Mevalonic aciduria

Detectable in adulthood (>15 years):

- Heterozygotes for galactoseuridyltransferase (GUT) and galactokinase
- Carriers for Lowe syndrome
- Lactose malabsorbers
- Ornithine aminotransferase deficiency
- Cerebrotendinous xanthomatosis
- Glucose-6-phosphate dehydrogenase deficiency
- Steinert dystrophy (cataract can be revealing sign)

Cherry Red Spot

- Gangliosidosis GM1 (Landing)
- Galactosialidosis (neuraminidase deficiency)
- Cytochrome C oxidase deficiency
- Sialidosis type I
- Niemann-Pick type A, C, and D
- Nephrosialidosis
- Sandhoff disease
- Tay-Sachs disease

Corneal Opacities (Clouding)

Visible in early infancy:

- Tyrosinosis type II (tyrosine amino transferase)
- Cystinosis
- I-cell disease (mucolipidosis type II)
- Hurler, Sheie (MPS I)
- Maroteaux-Lamy (MPS VI)
- Steroid sulfatase deficiency

Visible in late infancy to early childhood:

- Morquio (MPS IV)
- Mucolipidosis type IV
- α-Mannosidosis (late-onset form)
- Tangier disease
- Lecithin cholesterol acyltransferase deficiency
- Pyroglutamic aciduria

Visible in late childhood, adolescence to adulthood:

- Fabry disease (X-linked)
- Galactosialidosis (juvenile form)
- Wilson disease (green Kaiser Fleischer ring)

Ectopia Lentis (Dislocation of the Lens)

- Classical homocystinuria
- Sulfite oxidase deficiency
- Marfan syndrome
- Marchesani syndrome

Keratitis (*see "Corneal Opacities"*)

- Tyrosinemia type II
- Fabry disease (X-linked)

Microcornea

- Ehlers-Danlos type IV

Ptosis, External Ophtalmoplegia, Abnormal Eye Movements

- Respiratory chain disorders (Kearns-Sayre)
- Niemann-Pick types C and D
- Gaucher type III
- Ataxia telangiectasia (ocular contraversion)
- Cogan syndrome (ocular contraversion)
- Steinert disease
- Azomatic amino acid decarboxylase deficiency

Retinitis Pigmentosa

Inborn errors of lipid metabolism:

- Abetalipoproteinemia
- Vitamin E malabsorption
- LCHAD deficiency
- Sjögren-larsson syndrome

Ceroid lipofuscinosis:

- Santavuori-Hagberg (infantile)
- Jansky-Bielchovsky (juvenile)
- Spielmeyer-Vogt (juvenile)

Peroxisomal disorders:

- Zellweger and variant forms
- Neonatal adrenoleucodystrophy
- Infantile Refsum disease
- Isolated fatty oxidation defects
- Classical Refsum disease (adult form)

Carbohydrate-deficient glycoprotein syndrome
Respiratory chain disorders:

- Kearn-Sayre syndrome
- Others (mitochondrial DNA deletions)

Cobalamin metabolism dfects (Cbl C)
Recessive autosomal syndromes without known etiology:

- Cockayne
- Hallervorden-Spatz (severe neurologic regression, dystonia, acanthocytosis)
- Laurence-Moon-Biedl (obesity, polydactyly, mental retardation)
- Usher type II (deafness, severe mental retardation)
- Joubert (mental retardation, vermis atrophy, hyperventilation attacks)
- Others (Senior-Loken, Alsthom etc.).

Isolated retinitis pigmentosa:

- Gyrate atrophy with ornithine amino transferase (OAT) deficiency
- "Primary retinitis pigmentosa" X-linked, autosomal recessive or dominant

Osteology

Osteoporosis

- Lysinuric protein intolerance
- Infantile Refsum disease
- Homocystinuria
- I-cell disease (mucolipidosis type II)
- Cerebrotendinous xanthomatosis

Punctate Epiphyseal Calcifications

- Peroxisomal disorders (Zelwegger and variants)
- Chondrodysplasia punctata rhizomelic type
- Conradi Hunermann syndrome
- Familial resistance to thyroid hormone
- Warfarin embryopathy
- β-Glucuronidase deficiency

Pneumology

Pneumonitis (Interstitial)

- Dibasic aminoaciduria
- Niemann-Pick type B

Psychiatry

Acute Attacks of Delirium, Hallucinations, Mental Confusion, Hysteria, Psychosis
See Tables 4, 9, 10

Progressive Disorders with Intellectual Disintegration, Mental Regression, Psychosis
See Tables 8–10

Rheumatology

Arthritis – Joint Contractures – Bone Necrosis

- Alkaptonuria
- Gaucher type I
- Lesch Nyhan syndrome
- Farber disease
- Familial gout
- I-cell disease
- Mucolipidosis type III
- Homocystinuria
- Mucopolysacchridosis type I S
- Mevalonic aciduria (recurrent crisis of arthralgia)

Bone Crisis

With bone changes (rickets):

- Calciferol metabolism deficiency
- Hereditary hypophosphatemic rickets

With hemolytic crises (± abdominal pain):

- Porphyrias
- Tyrosinemia type I
- Sickle cell anemia

With progressive neurologic signs:

- Krabbe disease
- Metachromatic leucodystrophy
- Gaucher type III

Apparently isolated (revealing symptom):

- Fabry disease
- Gaucher type I

Vascular Symptoms

Raynaud Syndrome

- Fabry disease

Thromboembolic Accidents

Stroke-like Episodes
See Table 3

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Diagnostic Procedures: Function Tests and Postmortem Protocol

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CONTENTS

The best function test is performed by nature itself during an acute metabolic stress, such as caused by an acute infection, inadvertent fasting, or consumption of a nutrient for which a metabolic intolerance exists. If symptoms arise as discussed in Chap. 1, blood and urine should be collected and stored in the correct way to perform the endocrinologic and metabolic screening assays given in Table 1 in the chapter by Saudubray et al. on the "Clinical Approach to Inherited Metabolic Diseases." If no material is available or if the results are incomplete or ambiguous, a function test which challenges a metabolic route may provide a tentative diagnosis.

When performing a function test, it is very important to adhere to a strictly defined protocol to attain a maximum of diagnostic information and to minimize the risk of metabolic complications. Protocols applicable to a variety of inborn errors are discussed here. Other function tests which are solely used for the elucidation or confirmation of one particular disorder, are discussed in the relevant chapters.

Function Tests

Exploration Around the Clock

Indications. These include any clinical situation in which a metabolic derangement is suspected or a previous hormonal or metabolic incident of unknown etiology.

Procedure. Blood samples from an indwelling venous catheter (kept open with a saline infusion) are taken before and and after meals and twice during the night, as outlined in Table 1. Immediate deproteinization (with perchloric acid) at the bedside is the only guarantee of obtaining interpretable results for calculating redox potential ratios. Samples should be forwarded on ice to the laboratory.

Interpretation. As hyperinsulinemia is sometimes erratic and difficult to (dis)prove, repeat insulin assays are required. A plasma insulin concentration greater than 10 mU and a concomitant plasma glucose concentration of less than 2.8 mmol/l (50 mg/dl) at any time suggests the existence of hyperinsulinemia. The interpretation of metabolic derangements, which may characteristically differ in the fasting and in the fed state in some disorders, is given in the chapter by Saudubray et al. on the "Clinical Approach to Inherited Metabolic Diseases" (Figs. 1, 2; Tables 2–6).

Glucose Test

Indications. These include hypoglycemia and/or hyperlactacidemia of unknown etiology.

Preparation. Previous fasting for 3–8 h is necessary, depending on the meals interval. An indwelling venous catheter is inserted and flushed with a saline drip. In the case of hypoglycemia,

Table 1. Exploration of intermediary metabolism in the fed and the fasting state

Parameters in blood or plasma	Breakfast		Lunch		Dinner		Night	
	0	1 h	0	1 h	0	1 h	0	4 h
Glucose	x	x	x	x	x	x	x	x
Insulin	x	x	x	x	x	x	x	x
Ionogram	x	x						
Acid-base	x	x						
Amino acids	x	x						
Carnitine (total and free)	x							
Profile[a]	x	x	x	x	x	x		

Urine collected overnight and during the daytime is assayed for amino acids, organic acids, ketone bodies, and carnitine.
[a]Lactate pyruvate, 3-hydroxy butyrate acetoacetate, free fatty acids (FFA), ammonia.

the test is started at a plasma glucose concentration between 3.3 and 2.6 mmol/l, the approximate threshold levels for the secretion of counter-regulatory hormones and the development of neuroglycopenic symptoms, respectively [1, 2].

Procedure. Glucose (2 g/kg body weight; maximum, 50 g) as a 10% solution in water should be ingested orally or by tube in 5–10 min. Two separate blood samples are taken before the glucose administration. Blood is then sampled every 30 min for 3 or 4 h after the completion of glucose intake. All blood samples are assayed for glucose, lactate, pyruvate, 3-hydroxybutyrate (30H-butyrate), and acetoacetate (AA).

Interpretation. Normal fasting values are as follows:

- Glucose $\geq$2.6 mmol/l
- Lactate 0.5–2.0 mmol/l
- Lactate to pyruvate ratio between 10 and 20
- Total ketone bodies (KB) $\leq$2.0 mmol/l
- 30H-Butyrate to AA ratio 1.2–3.5

Glucose. A short-lived increase in plasma glucose, followed by a precipitous decrease, is observed in hyperinsulinemia.

Lactate. A marked decrease from an elevated level at zero time is observed in disorders of gluconeogenesis and in glucose-6-phosphatase deficiency; an exaggerated increase occurs in other glycogen storage diseases, glycogen synthase deficiency, and pyruvate dehydrogenase deficiency; the lactate to pyruvate ratio is increased in pyruvate carboxylase deficiency and in mitochondrial disorders. KB decrease in defects of gluconeogenesis, glycogen synthase deficiency, and glycogenoses types III and VI. They can paradoxically increase in pyruvate carboxylase deficiency (with a low 30H-butyrate to AA ratio) and in respiratory chain disorders (with a high 30H-butyrate to AA ratio). KB at fasting are very low in hyperinsulinemia and defects of fatty acid oxidation.

Note of Caution. The test should be interrupted at a plasma glucose level of 2.6 mmol/l or less and the complete metabolic profile taken at that time.

Glucagon Test

Indications. These include glycogen storage diseases except glucose-6-phosphatase deficiency, glycogen synthase deficiency, and hyperinsulinemia.

Preparation. This is similar to that for the glucose test (GT) or at the time of spontaneous hypoglycemia.

Procedure. Glucagon (30 μg/kg body weight; maximum, 1 mg) is administered intramuscularly. Blood samples are taken at 0 ($\times$2), 5, 10, 15, 30, 45, and 60 min for glucose and lactate.

Interpretation. Glucose normally rises by 1.4–2.8 mmol/l within 15–45 min [3]. In the case of hyperinsulinemia, a rise in plasma glucose of more than 30 mg/dl (1.7 mmol/l) might separate this condition from hypoglycemia not due to excessive insulin [4].

Galactose Test

Indications. These include glycogen storage diseases except glucose-6-phosphatase deficiency and glycogen synthase deficiency.

Preparation. See comments for GT.

Procedure. Galactose (2 g/kg body weight; maximum, 50 g) is administered as a 10% solution in water taken orally or by tube in 5–10 min; dura-

tion of the test is 4 h. Blood samples are taken at 0 min (×2), then every 30 min for glucose and lactate.

Interpretation. Lactate rises markedly in the above-mentioned diseases.

Note of Caution. Do not perform a galactose test if a defect of galactose metabolism is suspected.

Fructose Test

Indications. These include disorders of fructose metabolism and fructose-1,6-bisphosphatase deficiency.

Preparation. A diet devoid of fructose and sucrose should be prescribed for at least 2 weeks before the test; an intravenous saline drip is inserted before the test.

Procedure. Fructose (0.2 g/kg body weight) is injected as a 10% solution intravenously in 2 min; duration of the test is 90 min. Blood samples are taken at 0 (×2), 5, 10, 15, 30, 45, 60, and 90 min for glucose, fructose, phosphate, magnesium, and urate after finishing the injection of the fructose solution.

Interpretation. Glucose and phosphate decrease within 10–20 min, and magnesium and urate increase in parallel in hereditary fructose intolerance. In fructose-1,6-bisphosphatase deficiency, these changes tend to be less pronounced. A marked fructose rise is the only abnormality in fructokinase deficiency; for a review see [5].

Note of Caution. The test should be started at a slightly elevated plasma glucose concentration between 4.0 and 5.0 mmol/l in view of a potential hypoglycemic effect of the fructose administration. The test should not be performed if liver function tests are abnormal.

Fasting Test [6, 7]

Indications. These include disorders of fatty acid oxidation, disorders of ketolysis, disorders of gluconeogenesis, organic acidemias, and disorders of hormonal glucose homeostasis.

Preparation. Fasting tolerance differs considerably, depending on age and disorder. Therefore, fasting should start at the time at which the child is accustomed to take his last meal before the night or at most 2 h earlier by giving that meal earlier. An indwelling venous catheter with a saline drip should be inserted at zero time.

Procedure. Fasting is continued during 20 h for young children up to 2 years and 24 h for older children. It is terminated earlier at a plasma glucose level of 2.6 mmol/l or bicarbonate level of less than 15 mmol/l or if neurologic symptoms develop.

Substrates and Hormones in Plasma. Glucose and acid-base status is assessed after 12 h, then hourly until glucose is 3.3 mmol/l or less, then every 30 min until glucose is 2.6 mmol/l or bicarbonate less than 15 mmol/l. FFA, 30H-butyrate, AA, lactate, and pyruvate are determined as soon as glucose is 3.3 mmol/l or less; this metabolic profile should then be continued simultaneously with the glucose assays. At the end of the test, carnitine (total and free), ammonia, amino acids, insulin, cortisol, growth hormone, adrenocorticotrophic hormone (ACTH), (glucagon, adrenaline) are also measured. The complete profile should be taken earlier if the test has to be terminated earlier. Two milliliters of plasma should be stored at −70°C.

Substrates in Urine. Substrates assessed in 4-h urine starting after 12 h fasting (12–16, 16–20, and eventually 20–24 h) include lactate, KB, amino acids, organic acids, and carnitine.

Interpretation. The results of the fasting metabolic profile are compared with the normal values for the particular age (Table 2).

Note of Caution. A fasting test should not be performed if metabolic abnormalities in blood or urine are already present or before 2 weeks have passed after a metabolic incident.

Exercise Test

The exercise test is a means to disclose patients suspected of having a metabolic myopathy. Several methods exist:

- Semi-ischemic forearm exercise test
- Bicycle ergometer test [8]

Table 2. Metabolic profile during fasting tests in children of different ages (from [6])

	<12 months		1–7 yrs		7–15 yrs	
	20 h	24 h	20 h	24 h	20 h	24 h
Glucose (m*M*)	3.5–4.6	2.7–4.5	2.8–4.3	2.8–3.8	3.8–4.9	3.0–4.3
Lactate (m*M*)	0.9–1.8	0.8–2.0	0.5–1.7	0.7–1.6	0.6–0.9	0.4–0.9
FFA (m*M*)	0.6–1.3	1.1–1.6	0.9–2.6	1.1–2.8	0.6–1.3	1.0–1.8
KB (m*M*)	0.6–3.2	1.5–3.9	1.2–3.7	2.2–5.8	0.1–1.3	0.7–3.7
β-OH-butyrate(m*M*)	0.5–2.3	1.1–2.8	0.8–2.6	1.7–3.2	<0.1–0.8	0.5–1.3
Carnitine (free; μ*M*)	15–26	13–23	16–27	11.5–18	24–46	18–30
Glucose × KB	3–11	7.6–11.5	4.8–11.5	8.3–13	0.4–4.6	2.4–7.3
FFA/KB	0.3–1.4	0.3–0.7	0.4–1.5	0.4–0.9	0.7–4.6	0.5–2.0

Normal values at the end of the fast or when the patient is hypoglycemic, irrespective of the age of the patient, are: insulin, less than 10 mU at a glucose level of less than 2.8 mmol/l, cortisol, greater than 120 ng/ml, adrenocorticotrophic hormone (ACTH) less than 80 pg/ml; growth hormone, greater than 10 ng/ml; glucagon and adrenaline, no normal values.
FFA, free fatty acids; KB, ketone bodies.

- Treadmill test
- Sophisticated P-31 magnetic resonance [9]

The forearm test and the bicycle test are only applicable in adults and older children who can squeeze the sphygmometer balloon or ride a bicycle. The treadmill test offers the advantage that it can be used from the age at which the child is able to walk. Furthermore, the treadmill can be manipulated by changing belt speed and angle of inclination. A walking velocity of 3–5 km/h and a pulse rate of 150–180 beats/min are compatible with a *sub*maximal workload. This is a safeguard to prevent severe complications such as rhabdomyolysis, myoglobinuric anuria, and metabolic acidosis. Experience with the treadmill test for the screening of metabolic myopathies is very limited so far. The interpretation of the results can only be extrapolated from those of the classical exercise tests in which the rises of venous lactate, ammonia, urate, and the O_2 consumption are measured [8, 10, 11]. Peak levels for venous lactate and ammonia are produced 2 min after exercise, and for urate 1 h after exercise. Normal increments for lactate are 2.51 ± 0.35 mmol/l, for ammonia 25.7 ± 5.7 μmol/l, and for urate 0.1 mg/dl [8]. Any exercise test should be immediately interrupted if muscle cramps develop.

Postmortem Protocol

Since the first description of a postmortem protocol for suspected genetic disease by Kronick [12], some refinements have become available to enhance the diagnostic value of the original recommendations. In the protocol given below, the time schedule for proper conservation of specimens determines the sequence of the diagnostic procedures. It comprises the following elements of investigation.

- Cells and tissues for enzyme assays
- Cells and tissues for chromosome and DNA investigations
- Body fluids for chemical investigation
- Photography, radiography, ultrasound
- Complete autopsy

Enzyme Assays

Liver (minimum 10–20 mg wet weight) and muscle (minimum 20–50 mg wet weight) biopsies are taken by needle puncture or better via open incision.

The advantage of obtaining multiple samples of skeletal muscle and heart muscle is that some enzyme defects are expressed in a tissue-specific pattern. If possible, a brain biopsy should be taken within 2–4 h postmortem. The tissues are immediately frozen in small plastic cups in liquid nitrogen, followed by storage at −70°C. If the muscle biopsy is taken from muscle above the fascia lata, it can be combined with a fascia lata biopsy (diameter, 3 mm) for a fibroblast culture. As most, but not all enzymes are stable at that temperature, the biochemist should be consulted whether the processing of fresh material is also indicated for additional biochemical assays. Part of the liver biopsy should be fixed for histological and electronmicroscopic investigation prior to freezing (see "Autopsy" below).

Blood cells must be conserved for enzyme assays and for chromosome and DNA investigation. A total of 20 ml blood is collected by peripheral or intracardiac puncture in a heparin-coated syringe; 10 ml is transferred to the laboratory for isolation of erythrocytes or white blood cells and the biochemist is notified, and at least 10 ml is conserved for chromosome analysis and DNA extraction (see below).

Chromosome and DNA Investigation

A total of 10–20 ml blood is collected in ethylenediaminetetra-acetic acid (EDTA) and frozen at −70°C for chromosome and DNA investigations. Alternatively, blood spots dried on filter paper (as in the Guthrie test) are useful for many investigations and should always be collected. These samples can also be used for DNA analysis after polymerase chain reaction (PCR) amplification.

Skin Fibroblasts. At least two biopsies (size, 10 × 5 mm) are taken under sterile conditions as early as possible, one from the forearm and the other from the upper leg (fascia lata, see above), and conserved in culture medium or alternatively in sterile saline for one night at room temperature. Cultured fibroblasts may also serve to investigate enzymes.

Chemical Investigation

Plasma from the centrifuged blood sample, urine (≈10 ml), and cerebrospinal fluid (≈4 ml) are immediately frozen at −20°C. If no urine can be obtained by suprapubic puncture or catheterization, the bladder may be filled with 20 ml saline solution and diluted urine harvested. Alternatively, vitreous liquor can also be collected by intraocular puncture (1–3 ml) and frozen. This liquid is comparable with urine with respect to its solubility for organic acids.

It must be realized that many biochemical parameters are impossible to interpret postmortem, due to rapid tissue lysis. Among them are carnitine (total and free), ammonia, lactate, and amino acids, which rapidly increase without any specific significance. In contrast, the acylcarnitine ester profile (determined from dried blood spots) is highly diagnostic for many disorders of fatty acid oxidation and organic acidemias.

Imaging

Photographs are made of the whole body and specific dysmorphic anomalies, if present. Total body radiographs in anteroposterior and lateral views are performed as well as ultrasound of the skull, thorax, and abdomen.

Autopsy

The autopsy should be complete, including the cranium, provided that parents give their permission. The pathologist conserves important tissues for histology and electronmicroscopy in buffered formaldehyde 4% and Karnofski fixative, respectively.

Mentioning the autopsy as the last item does not mean that it is the least important. On the contrary, the first three items are complementary to it, and only the rapid decay of enzymes and vital cells gives their conservation a higher priority.

Notes

An explanation of the importance of a complete workup is generally well received by modern parents. However, even if a complete autopsy is refused, permission to take photographs and X-rays, to take blood, urine, and cerebrospinal fluid samples and to do needle biopsies of liver and muscle is usually given.

An assembly kit containing all the material for collecting and conserving the specimens is a highly recommended means of enhancing the speed and completeness of the postmortem protocol.

The results of the postmortem protocol provide an essential source of information for parents and for genetic counseling, which would otherwise be impossible.

Cells cultured for chromosome and DNA studies should be transferred to a repository for future reference.

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Emergency Treatments

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CONTENTS

The emergency treatment of children suspected of having an inborn error of metabolism implies three simultaneous actions: collecting all samples necessary for the diagnosis, deciding on adequate treatment, and organizing clinical and biochemical supervision. As already stated in the first chapter (Saudubray et al.), management depends on the physiopathology involved. This chapter focuses on the emergency treatment of those disorders which lead to an acute intoxication secondary to accumulation of toxic compounds proximal to the metabolic block. Mainly, they comprise branched-chain organic acidurias (BCOA), urea cycle defects (UCD), and some β-oxidation defects. Age of onset (neonatal versus late-onset) and intercurrent decompensations are other aspects which influence management.

Management of Neonatal-Onset Metabolic Derangement

After the diagnosis of an inborn error of metabolism of the intoxication type has been established, the treatment is directed towards the suppression of the production of toxic metabolites from catabolism of endogenous protein and the stimulation of their elimination by extrarenal procedures and specific alternate pathways, if available.

Supportive Care

Most of these very ill newborns require ventilatory and circulatory supports as well as correction of electrolyte, calcium, and phosphate imbalance. The following points need special attention.

Hydration

Poor feeding, increased renal fluid losses, and consequent hypovolemia and prerenal failure are frequently observed during metabolic decompensation. Thus, rehydration and maintenance of good hydration is often necessary. In addition, efficient diuresis is an important means of detoxication in situations where the renal clearance of metabolites and byproducts such as methylmalonate, acyl-carnitines, or hippurate is high.

Acid-Base Equilibrium

Acidosis, if severe (pH < 7.15), can be partially corrected with i.v. bicarbonate, especially if it does not improve with the first measures of toxin removal. However, it should be stressed that overcorrection sometimes carries more risk than persistent mild acidosis. Some patients affected with UCD may present with mild acidemia, a situation which should not be corrected, as acidosis protects against NH_4 dissociation and toxicity.

Infections

It has been demonstrated that newborns affected with a metabolic crisis frequently suffer from con-

comitant septicemia. This in turn results in persistent catabolism and thus therapeutic failure. Therefore, infections must be thoroughly searched for and prevented. Due to acidosis, patients with BCOA easily develop oral and digestive moniliasis, requiring appropriate therapy.

Venous Catheterization

Insertion of a central venous catheter should be considered at once on rapidly meet a high energy requirement (see below).

Specific Therapeutic Means

Nutrition

Whatever the disease, suppression of toxic metabolite production from breakdown of endogenous protein is essential. However, we must be aware that hypercaloric nutrition by itself is seldom sufficient to correct a metabolic imbalance rapidly and that its use as a sole therapeutic means may compromise the neurologic outcome [1, 2]. Conversely, any toxic removal procedure will fail without concomitant anabolism.

► When the clinical status allows continuous enteral nutrition, it must be considered as a first choice. However, in most cases, digestive intolerance or application of invasive removal techniques for toxin removal will preclude effective enteral feeding. In that case, total parenteral nutrition (TPN) is the method of choice to provide hypercaloric nutrition all along the emergency treatment. Once the signs of metabolic decompensation abate, enteral nutrition is reintroduced in progressive amounts. As an example, parenteral solutions providing 100 kcal/kg per day to a 3.5-kg baby are shown in Table 1. If a suitable amino acid solution is not immediately available, a mixture of glucose (15%–20%) and lipid (2–3 g/kg per day) solutions is started with. In the case of prolonged digestive intolerance despite normalization of the metabolic status, commercially available amino acid solutions can be introduced in the TPN. Initially, the amino acids are introduced in an amount sufficient to meet the minimal daily requirement and then titrated against biochemical controls. The method is safe if the amino acid solution is evenly distributed over the whole day and checked biochemically [3].

The composition of the enteral formulas is based on a glucose–lipid mixture. Depending on the defect involved, an appropriate amino acid mixture is added to cover the protein requirement. In order to titrate gastric tolerance, nutrition is given at a low rate, for instance 10 ml/3 h for the first 3 h and increased every 3–6 h until the full fluid requirement is met. Simultaneously, the TPN infusion rate is decreased. Finally, the diet should provide 110–130 cal/kg per d. Nutrients, osmolarity, and renal solute load must be checked in order to provide the recommended dietary allowance (RDA) for the newborn and prevent diarrhea and dehydration.

Once the toxic metabolites have "normalized," natural proteins are introduced using quantitated amounts of infant formula. A specific amino acid mixture is added to increase the total amount of protein to the RDA level. At this step, attention must be paid to both the protein and the essential amino acid requirements. For patients with an inborn error blocking a catabolic pathway, intakes of natural protein and essential amino acids must cover the minimal requirements (protein accretion + nonurinary losses) which are 50%–60% below the normal requirements (protein accretion + nonurinary losses + urinary losses) and should not follow the RDA [4]. These minimal requirements represent the basis for initiation of a protein-controlled diet. Then natural protein and amino acid intakes are adjusted to growth with frequent monitoring of biochemical status.

Exchange Transfusion

Theoretically, exchange transfusion (ET) is an in- ◄ adequate removal procedure for metabolites distributed throughout total body water. However, ET with large volumes of fresh blood has long been recognized as an effective means in numerous inborn errors of metabolism: maple syrup urine disease (MSUD), methylmalonic, propionic, and isovaleric acidurias (MMA, PA, IVA), and even in UCD [5]. But its transient effect limits its use, and ET should only be applied in association with other methods such as peritoneal dialysis [6] or in view of long-standing patterns such as multiple or continuous exchanges. Multiple exchanges use 1.5- to four-volume exchanges repeated four to six times within 24 h. Continuous exchange using 600 ml/kg body weight within 15 h has been successfully performed in patients affected with

Table 1. Examples of the composition of a parenteral nutrition for a 3.5-kg newbom (minerals, vitamins and nutrients should be added at the recommended allowances for age)

	Without adequate amino acid i.v. solution					With amino acid i.v. solution, free of BCAA				
	Vol (ml)	N (g)	Lip (g)	Glu (g)	Energy (kCal)	Vol (ml)	N (g)	Lip (g)	Glu (g)	Energy (kCal)
Amino acids (9%)[a]	–	–	–	–	–	20	0.27	–	4	23
Intralipid (20%)	50	–	10	–	90	50	–	10	–	90
Glucose (15%)	400	–	–	60	240	375	–	–	56	225
Total	450	–	10	60	330	445	2.6	10	60	338
Water (ml/kg per day)	130	–	–	–	–	127	–	–	–	–
Total energy provision (%)		–	27	73	–	–	2	27	71	–
Amino acids (6.53%)[a]	28	0.26	–	–	7					
Intralipid (20%)	50	–	10	–	90					
Glucose (15%)	400	–	–	60	240					
Total	475	2.6	10	60	337					
Water (ml/kg per day)	135	–	–	–	–					
Total energy provision (%)	–	2	27	71	–					

Lip, lipid; Glu, glucose.
[a] Calculation based on the branched-chain amino acid (BCAA)-free amino acid i.v. solution used by Berry et al. [1] in patients affected with maple syrup urine disease (MSUD). It contains 9 g/dl amino acids mixed with 20%–25% glucose.
[b] Calculation based on commercially available i.v. amino acid solution (Vaminolact; Pharmacia) containing 9.3 g/l total nitrogen and 65.3 g/l amino acids; 28 ml provides 200 mg leucine, 103 mg valine, 89 mg isoleucine, 37 mg methionine, and 103 mg threonine.

MSUD [7]. Fresh blood is highly recommended to avoid ammonia loading. Each cycle is slowly performed to maximize the detoxification.

Peritoneal Dialysis

► Efficacy of peritoneal dialysis (PD) for the emergency treatment of newborns with various metabolic disorders has long been demonstrated [8]. Manual PD requires minimal technical expertise and can be rapidly initiated in any pediatric intensive care unit (PICU). A catheter inserted through the lateral abdominal wall, with its tip placed into the contralateral iliac fossa or pelvis, is connected to a dialysis line and a drainage system. Warmed dialysate solutions buffered with bicarbonate are delivered by gravity with volumes of 40–50 ml/kg body weight. One-hour cycles (15 min filling up, 30 min dwell time, 15 min drainage) are repeated over 24–36 h, during which most of the toxin removal occurs. Prolonged PD is usually not necessary, except for UCD. Continuous flow peritoneal dialysis with inflow and outflow catheters could be a way to circumvent severe technical problems such as poor drainage and leakage of dialysate [9]. Careful records of the inflow and outflow must be kept, and net water exchange frequently checked by weighing the patient. Dehydration must be prevented by i.v. infusion (TPN when available). When overhydration exists, the dialysis fluid should be made hypertonic by addition of glucose (3 g/100 ml = 100 mOsm/l). Due to glucose absorption from the dialysis fluid (200–300 mg/kg per h), hyperglycemia may develop and require insulin therapy.

In terms of clearance, PD is far less efficient than hemodialysis and hemofiltration. It has, however, the avantage of simplicity. The main cause of failure, is poor splanchnic flow secondary to shock and septicemia. Clearances average 6–12 ml/min per m^2 for ammonia [5, 10, 11], leucine [6, 12, 13], PA, and MMA [6]. Only methylmalonate has a spontaneous renal clearance twice higher than that of PD: MMA patients therefore do not require PD.

Continuous Hemofiltration

Hemofiltration (HF) has recently appeared as an ◄ effective means of treatment in newborns and in-

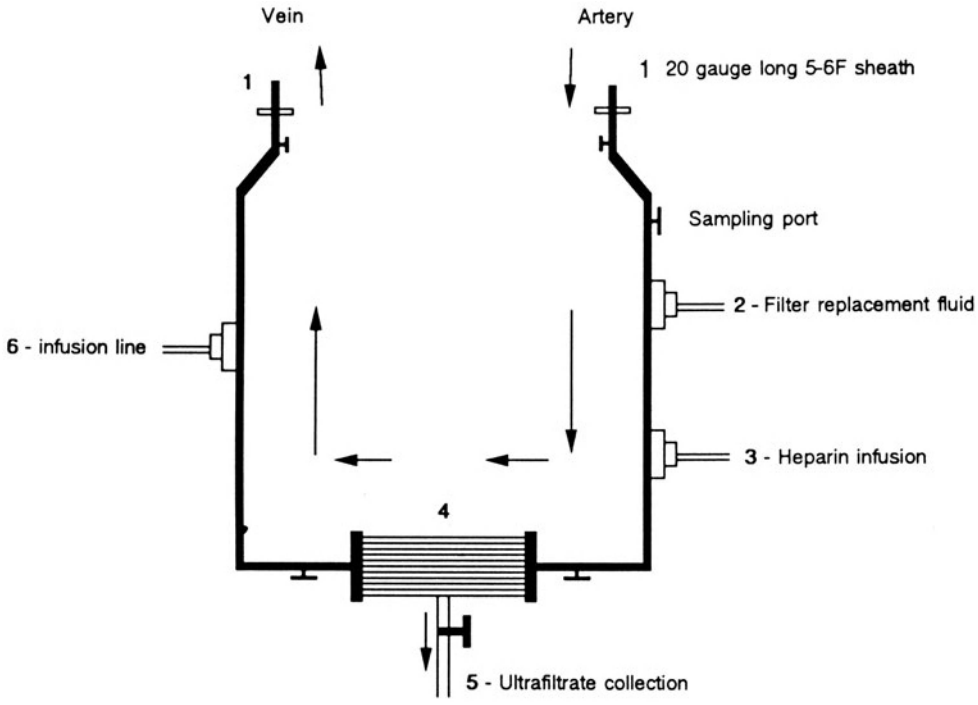

Fig. 1. Continuous arteriovenous hemofiltration (CAVH) circuit in the predilution configuration. *1*, Artery and vein access. CAVH, percutaneous canulation of a major artery (*right*) and vein (*left*). Continuous venovenous hemofiltration (CVVH), canulation of a main vein with a dual-luminal catheter. Up to 5 days of age, umbilical vessels can be used. Spontaneous or pumped blood flow ±20–30 ml/min. Air detector placed on venous line is essential in any pumped circuit. *2*, Filter replacement fluid composition: Na, 140 mEq/l; K, 3.5 mEq/l; Cl, 120 mEq/l; bicarbonate, 25 mEq/l; Ca, 2.6 mEq/l; Mg, 1.6 mEq/l; P, 1.4 mEq/l; dextrose, 1.25 g/l. Flow rate = ultrafiltrate rate = total parenteral nutrition (TPN) rate. *3*, Heparinization: loading dose (20–50 IU/kg) 3–5 min before the circuit connection + continuous infusion ±5 IU/kg. Postfilter activated clotting time, 200 s. *4*, Fiber hemofilter, type FH 22 Gambro. Total volume of the extracorporeal cicuit ≤10% of the patient's blood volume. *5*, Ultrafiltrate flow rate ±30% of the blood flow rate. *6*, Infusion line for TPN and/or drug infusion

fants suffering acute decompensation of various metabolic disorders. The basic components of HF are shown in Fig. 1. The procedure consists of a low-resistance extracorporeal circuit connected to a small-fiber hemofilter that is permeable to water and non-protein-bound small solutes [14]. In continuous arteriovenous hemofiltration, blood is driven through the circuit by the cardiac arteriovenous pressure. However, in order to maintain adequate blood flow (20–30 ml/min), external pump assistance is usually necessary. This disadvantage makes continuous venovenous hemofiltratrion, with a venous dual-luminal catheter, more suitable. The ultrafiltrate of plasma formed by convection is concurrently replaced by electrolyte and TPN solutions. The advantages are simplicity of logistics, high tolerance in neonates or infants who present with hemodynamic instability, multiorgan failure, and hypercatabolic state, and the possibility to use a large volume of TPN without the risk of overhydration. Nevertheless, application of such procedures requires a PICU trained in techniques of extracorporeal circulation. Hemodiafiltration (HDF) increases solute removal by the addition of diffusive transport exerted by a dialysis solution flowing upstream through the ultrafiltrate compartment of the hemofilter [15]. However, most of the basic simplicity of the HF is then lost.

The ultrafiltrate formed during HF has essentially the same small-solute composition as plasma water. Therefore, the clearance of these solutes approximates the ultrafiltration rate. Clearances of leucine and ammonia have been reported to vary from 8 to 50 and from 8 to 21 ml/min per m^2, respectively [16–19]. Even though the procedure has been pursued during 18–48 h, the toxin removal has been achieved much earlier (8–10 h) without any rebound in the circulation of toxic metabolites. This allows resumption of effective

anabolism through TPN, a major prerequisite for final success [20].

Hemodialysis

► Hemodialysis (HD) is the most effective and rapid method of removing small solutes [5, 11]. However, the logistics are such that it is difficult to mobilize this procedure for acute management of a newborn infant, and it cannot be performed without the assistance of a dialysis staff. With this method, clearances of PA, branched-chain amino acids, and ketoacids reach around 60 ml/min per m^2 [21, 22] and that of ammonia 80–100 ml/min per m^2, results which are undoubtedly better than those obtained by any other procedure [11, 22]. Two- to four-hour hemodialysis cycles appeared to be sufficient to sustained improvement in MSUD and PA patients. By contrast, the procedure has not allowed hyperammonemic patients to survive, despite large ammonia removal [5, 22, 23]. These poor results could be due to the difficulty in obtaining prompt anabolism in severely hyperammonemic neonates, who often develop hemodynamic instability and multiorgan failure.

Assessment of Biochemical Progress

In order to evaluate the efficiency of toxin removal procedures, the general rule is to sample blood, urine, and dialysate or ultrafiltrate within timed periods. Specific and nonspecific biochemical values must be checked regularly. Attention must be paid to blood glucose, plasma electrolytes, and calcium, which should be appropriately corrected. Regular blood counts are also important, as in organic aciduria neutropenia and thrombocytopenia may be present or develop after the initiation of the treatment and may require specific transfusions. Repeated searches for septicemia must be systematically done and treatment initiated as soon as suspision arises.

Additional Therapies

Enhancing Anabolism

Owing to its well-known anabolic effect, insulin is used to treat severe catabolism. However, to attain this goal, high infusion doses (0.2–0.3 IU/kg per h) have to be used, combined with high glucose infusion and frequent control of glycemia [24, 25]. Human growth hormone should not be used, as its beneficial role to sustain protein anabolism in a short-time situation is highly improbable.

Alternative Pathways

The stimulation of alternative pathways depends on the catabolic pathway involved. It is detailled in Chaps. 11, 14, and 20, which deal with those emergency situations.

Vitamin Therapy

Megadoses of specific vitamins must be systematically tested in all cases of potentially vitamin-dependent disorders (Table 2). As the response to the vitamin may be masked by simultaneous use of other therapies, this trial should be repeated in a later stable metabolic period and compared with in vitro studies.

Sometimes, therapy must be initiated with a general plan of management that is adapted as soon as the exact metabolic disorder is recognized. Figure 2 summarizes the nonspecific emergency treatment and investigations to be used in the neonatal period.

Table 2. Cofactors used in various metabolic disorders

Cofactors (doses mg/d)	Disorders
Thiamin, B_1 (10–50)	MSUD Hyperlactatemia (PDH)
Biotin (10–20)	Propionic aciduria MCD Hyperlactatemia (PC)
Cobalamin, B_{12} (1–2)	Methylmalonic aciduria
Riboflavin, B_2 (20–40)	Glutaric aciduria β-Oxidation defects
Carnitine (50–100 p.o., 400 i.v.)	Branched-chain organic acidemia Dicarboxylic Acidemia Primary hyperammonemia Hyperlactatemia

MSUD, maple syrup urine disease; PDH, pyruvate dehydrogenase; MCD, multiple carboxylase deficiency; PC, pyruvate carboxylase.

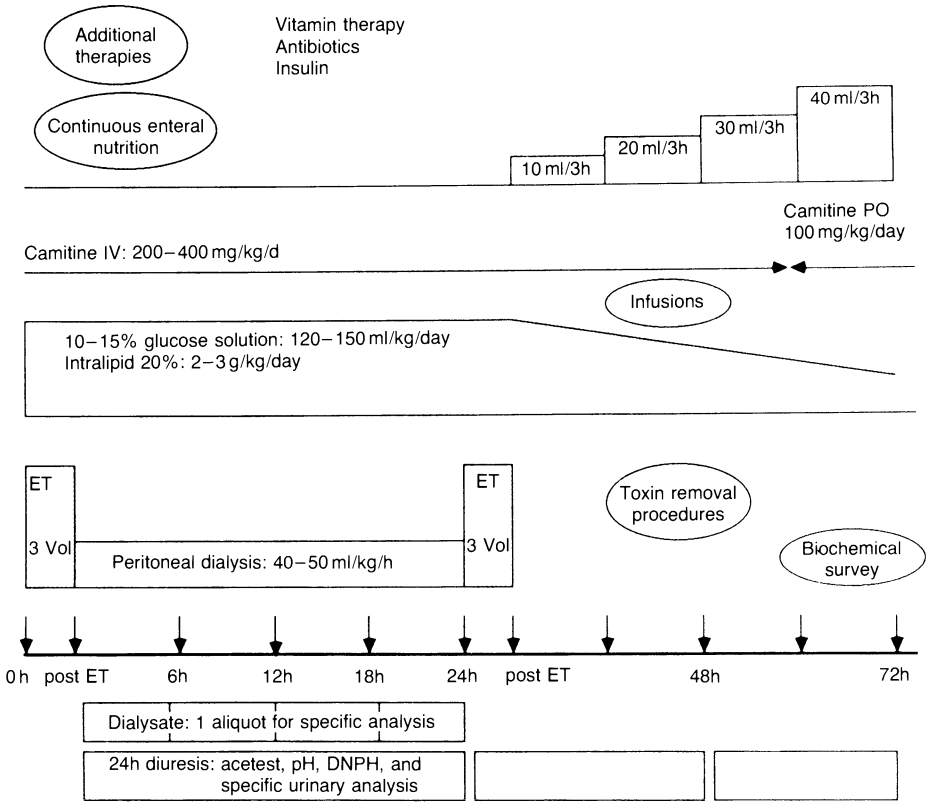

Fig. 2. General guidelines for the nonspecific treatment of inborn errors of metabolism. *ET*, exchange transfusion; *DNPH*, dinitrophenylhydrasine

Management of Late-Onset Metabolic Derangement

Compared with the approach for neonates, the management of late, acute forms is more supportive, exogenous removal procedures being less often necessary. Specific therapies are essentially similar.

Supportive Care

Cerebral Edema

Patients with metabolic disorders are at particular risk of cerebral edema, and all along emergency treatment care should be taken to reduce this risk [26]. Cerebral edema due to hypotonic fluid overload is probably an underestimated cause of death in these late decompensations [27]. In the early phase, fluid restriction to maintenance, intubation, and hyperventilation may be sufficient. If insufficient, mannitol (0.25–0.50 g/kg), furosemide (1 mg/kg), and even phenobarbital must be considered. For any seriously ill patient, monitoring of intracranial pressure or of cerebral perfusion should be considered.

Hydration

Many patients, especially those with metabolic ketoacidosis, present with intracellular dehydration, the extent of which is often underestimated. In this situation, agressive rehydration with hypotonic fluids and alkalinization may cause or exacerbate preexisting cerebral edema. Therefore, an individualized rehydration schedule should be planned over 48 h, with fluid infusion of less than 3 l/m^2 per day. If shock is present, the first priority is to replenish the intravascular space using colloids (10–20 ml/kg within 30 min). The repair fluid should contain an average concentration of 70–85 mmol/l Na^+ (4–5 g/l), 30–40 mmol/l K^+ (2–3 g/l), and 5% dextrose. To correct severe acidosis (pH $<$ 7.15, HCO^{3-} $<$ 10 mEq/l), sodium bicarbonate may be substituted for one quarter to one half of the Na^+ requirements during the first 6–12 h of rehydration. In order to prevent precipita-

tion with calcium, the bicarbonate solution should be connected with a Y-connector to the infusion line.

Specific Therapeutic Means

Nutrition

► Early high-energy nutrition is essential to prevent further protein and fat catabolism. Continuous enteral feeding must be considered first, as it might be tolerated even if recurrent vomiting has previously occurred. There are important advantages: it allows the provision of more energy than by peripheal venous infusion, and it is often sufficient to obtain rapid clinical and biologic recovery. Initially, in parallel with intravenous infusion, energy and water requirements can be provided with 80056 powder (Mead-Johnson) in 16%–20% dilution. 80056 Powder is a glucose – fat formula which, per 100 g of powder, contains: 72 g glucose, 22 g fat, 540 mg calcium, 300 mg phosphorus, some other nutrients, and vitamins. Depending on the disorder, specific amino acid mixtures with additional water are added. Once toxic metabolites have normalized, natural proteins are introduced, and appropriate long-term dietary treatment initiated. When clinical status prevents effective oral nutrition, TPN must be considered following a general pattern similar to that already described for neonates [1].

Exogenous Toxic Removal Procedures

In some cases, the situation deteriorates so rapidly that toxic removal procedures become necessary. Emergency blood ET using a single main vein canulated with a dual-luminal catheter could be rapidly effective in BCOA, but fails in primary hyperammonemia. Due to lower peritoneum area relative to body weight, PD appears to be much less effective in children than in newborns. HD and HF, if available, are probably better choices. However, the selection of the procedure is also influenced by local resources and experience, keeping in mind that intervention started too late is likely to fail.

Additional Therapies

Each therapy that has already been discussed for neonates should be considered. Vitamin responsiveness is more likely in late onset-forms than in neonatal diseases.

Prevention of Acute Intercurrent Decompensation

Most patients are stabilized on a diet appropriate for their metabolic disease. However, stress such as infectious disease, immunization, trauma, and surgery may precipitate decompensation and cause complications. To oppose these catabolic effects, the main adaptations comprise reinforcement of energy supply and a further reduction of the protein intake for patients treated with a low-protein diet. The latter step is the most difficult. On the one hand, further protein reduction, if not necessary, may lead to protein malnutrition and chronic imbalance; on the other hand, a patient's stress tolerance is so unpredictable that the same child could tolerate an acute febrile infection well and develop a life-threatening coma secondary to an apparently trivial illness. Therefore, parents need guidelines in order to recognize early situations of impending protein catabolism and metabolic imbalance.

General Emergency Diet

For all disorders the major source of energy will be provided by glucose. A glucose polymer diluted in water, in an oral rehydration solution, or in fruit juice (for some older children) is the simplest [28]. Otherwise, a 16%–20% solution of 80056 powder is useful for those patients already used to this formula. For children treated with specific amino acid mixtures, the usual supplements can be added, being aware that they increase osmolarity and that their taste renders nasogastric tube feeding quite unavoidable.

At home, the solution is given orally in small frequent drinks, at intervals of 2–4 h day and night. Minimum volume intake should approximate 85% of prescribed intake during every 12-h period. If the child occasionally vomits, it may still be possible to feed orally by giving frequent sips. In these situations, children receiving a nocturnal feeding can benefit from total nasogastric feeding [28, 29]. However, if the child is vomiting frequently or is obviously unwell, hospitalization is essential for clinical, biochemical, and therapeutic evaluation. The glucose – fat basic diet should not be continued for more than 48 h, because it does

not provide adequate nutrition and carries the risk of inducing sustained metabolic imbalance. Once the child has normalized or stabilized, the usual diet is progressively reintroduced along with frequent high-energy drinks until the full diet is reestablished. For patients with a low-protein diet, the protein intake is usually increased daily, resuming the usual diet within 3–4 days. Regular daily clinical assessment and measurement of specific metabolites restrict home treatment to situations of mild decompensation. Attention must be paid to the fact that large discrepancies may occur between the clinical and biochemical status. Parents who feel that their child is not well are most often right even if after the first investigations there is "no cause for worry," and therapeutic measures should be anticipated. An individualized energy regimen must be taught to the parents to cope with metabolic derangements in the future.

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Psychosocial Care of the Child and Family

J.C. Harris

CONTENTS

Since Garrod's initial description of inborn errors of metabolism in 1923 [1], a large number and a great variety of inherited metabolic disorders have been identified. Increasingly physicians have come to recognize the complexities of psychosocial care for the child with an inborn error of metabolism. Although the special features of each condition dictate an individualized treatment approach, there are general issues of adaptation to the illness, styles of coping, and mechanisms of defense against anxiety that are pertinent to all families with affected children.

This chapter will review the psychosocial issues that commonly arise when dealing with affected children and their families. Among the situations that families must cope with that will be discussed are: (1) lifelong treatment with potentially favorable outcome; (2) gradual deterioration and unfavorable outcome; (3) onset in infancy and chronic course; and (4) deterioration and death.

The types of problems that occur in representative disorders illustrate the complexity and range of problems in behavior that may result from inborn errors. In each of these conditions, acknowledging the diagnosis, finding school programs, and participating in treatment of associated physical and behavioral disorders are all tasks the family must confront. The physician must be available in times of crises and, when there is the need, support the family through the child's chronic deterioration or terminal illness.

Psychosocial Issues with Families

Orientation to the Family Interview. When the parents and child come to the physician for treatment, they are concerned. It is advisable to consider that the interview begins with a statement of concern rather than the traditional chief complaint. An emphasis on the family's concerns rather than on complaints subtly reframes the interview to one that may facilitate the doctor-patient relationship in the treatment of illnesses that are long lasting and require the establishment of considerable trust from the time of first contact with the patient and family. The family's disquietude must be appreciated as symptoms are elicited and signs understood so that confidence in the physician and in the treatment program can be established. The physician has an important role to help family members to become confident as they cope with the genetic [2–4], medical, and psychosocial [5, 6] aspects of these conditions. Instilling confidence through a confiding relationship helps the family member and the child develop the capacity to confront, actively struggle and persevere despite emotional frustration and confusion. The physician interview is an opportunity to establish understanding, develop confidence, and encourage interpersonal rapport so that recommendations can be carried out effectively and appropriately. This approach to the patient is biopsychosocial [7] and not exclusively biomedical in nature. It addresses the biological presentation of the disease, its social and interpersonal antecedents, the consequences of the illness, and facilitates the child's and parents' capacity for psychological adjustment.

Ongoing Counseling. Following the initial interviews, supportive counseling is a continuous process for the family and for the child who must deal with major psychosocial issues in care, such as confusion and delay in diagnosis, uncertainties in clinical course, questions about the screening of siblings, and day-to-day stresses inherent in man-

agement. Considerable emphasis has been placed on the generic family's psychological adjustment. This has led to families often being considered as homogeneous in their responses. However, each family is unique and must be given individual consideration. It is a particular family's response to the stress of the illness that is at issue. It remains essential to identify characteristic vulnerabilities to stress in parents and family members, to provide sensitive support, and to appreciate the psychological phases involved in the adaptation to illness. Still it is also imperative to appreciate the reality of the stresses of unmet service needs and not necessarily attribute repeated questions as a failure in emotional adaptation. Respite or day care during holidays and weekends, baby sitting, and help with transportation are critical to facilitate family adaptation [8].

Genetic Counseling. Genetic counseling is individualized according to the type of case. Specific issues must be addressed according to the particular disorder in regard to counseling adults about recurrence risk for future pregnancies and about sibling screening. The majority of these conditions are associated with mental retardation or problems in learning and behavior which require support for parents.

Crisis Intervention. Crisis intervention [9] includes both anticipatory guidance and preventive intervention. Anticipatory guidance is the approach taken when a crisis can be predicted. Preventive intervention is a method of guidance for parents and children during the crisis itself. These approaches recognize the family's increasing dependency and need for continuing support during the crisis. Help is provided by facilitating communication among family members and by discussion of their concerns and their plans for coping. During these discussions, the physician should point out that negative feelings are normal, empathize with the family's frustration, and encourage the sharing of tasks among family members in recognition of the fatigue that may develop with ongoing family care of chronic conditions. Interview sessions should focus on present problems and not emphasize discussion of past failures. Identification of psychological needs and the development of confiding relationships [9] among family members is essential in preventive intervention. If this natural support does not occur spontaneously, it is appropriate for the professionals involved to arrange active support.

Coping at Times of Predictable Crisis

Times when family crises may develop include:

- Establishment of a diagnosis and discussion of its implications;
- Living with the child at home and participating in the specific management program;
- Time of school entry;
- Entry into adolescence;
- Dealing with loss of function and deterioration; and
- Future family planning.

The way a family copes depends on the support that family members can offer one another and the ability of each member to adapt to loss, both potential and actual, as well as the availability of community care. It is the physician's first responsibility to convene a support group at the time of diagnosis. The first step in this is to counsel both parents together, rather than one or the other parent alone, and then to assist the family in finding local resources through the extended family, recognized parent support groups for the given condition, community agencies, and religious organizations. Meeting with other parents who have faced similar crises can be particularly helpful. Kazak [10] studied three samples of families with disabled children in regard to stress and social networks. Mothers and fathers of 125 handicapped/chronically ill children were compared with parents of 127 matched nondisabled children from three separate samples with respect to personal stress, marital satisfaction, and social network size and density. Only mothers of disabled children experienced higher levels of stress than comparison parents. No differences were found in marital satisfaction. Although few group differences were found for social network variables, mothers of handicapped children had higher-density networks than comparison mothers, illustrating the importance of extensive psychosocial support.

Interviewing. Successful interviews with the parents should result in an acknowledgment of the nature of the child's illness and an awareness of what they can do for their child. During the inter-

view, the parent is reassured not only by what is said, but also by how it is said. Self awareness by the physician is critical to empathetic listening to the parents' perceptions of the child's difficulties. It is basic to his understanding of the parents' adaptation to illness that he understand the psychological mechanisms that are normally present in a time of stress.

When experiencing stress, individuals may use a variety of defense mechanisms to minimize experienced anxiety. If the individual is not able to cope with the disorder, the use of defenses against anxiety may be heightened and may interfere with his ability to understand and utilize appropriate recommendations for care. The most commonly used defenses are denial, guilt or self-blame, projection or blaming others, and excessive dependency by the parent on the physician, family, or community members [11]. Unexpected and unwanted information is normally responded to with denial and disbelief, followed by mixed feelings of sadness and anger, before eventual acknowledgment occurs. To determine the degree of parents' adaptation, the following questions are suggested in the interview:

- Whom do you talk to when you are concerned about your child?

This question is needed to establish the degree to which the parent has become isolated and whether there is a confiding relationship with another person. It also helps to clarify if the parent is denying the seriousness of the child's illness, thereby putting the child at risk.

- Do you blame yourself for your child's illness?

This question clarifies guilt and should be pursued since the self-blaming parent may be demonstrating symptoms of depression.

- Do you have doubts about the staff's ability to provide care for your child?

This question deals with projection and excessive suspiciousness. Not uncommonly, parents criticize caregivers as an expression of their own projected fears and anxiety.

- Do you feel adequate to take care of the child yourself, or do you frequently seek directions from others and feel dependent on them?

This question deals with dependency and passivity, which may present in the overwhelmed parent. When this occurs, the physician often sees signs of helplessness in the parent, receives frequent telephone calls, and is asked to make more and more decisions for family members.

Coping. All these processes, i.e., denial, guilt, projection, and dependency occur normally [11] and are problematic only if one of these means of coping becomes predominant and persists. Throughout the coping process, parental anticipatory adaptation and efforts at maintaining confidence are taking place as the child's condition changes. Several phases in adaptation are identified: acknowledgment, the progressive realization of the seriousness of the condition; grieving, the experience and expression of impending loss; and reconciliation, the process of developing the perspective that restores the parents' confidence in the worth of the child's life [12].

In order to cope with day-to-day events, the parents must gain and maintain confidence. This is accomplished by mastery operations and an affirmation of life. Mastery operations include efforts to obtain information about everything involved in the child's care, searching out the best care available, and coming to terms with feeling responsible for their child's illness. Participation in care is of vital importance for many families. They need to help with procedures and "do everything possible" for their child. These efforts should, of course, be balanced with care of other family members and continuation of daily tasks in order to maintain psychological equilibrium. Mastery operations are most prominent in the phases of acknowledgment and grieving and lead to reconciliation. Affirmation of life is a response to the fact that the child's illness often threatens the parents' optimism about the value of life. The majority of families gradually come to terms with their feelings of resentment and hopelessness through devotion to the child and make full use of treatment facilities.

Crises in Psychosocial Management

There are a variety of inborn errors which illustrate the complexity and range of psychosocial issues that may result in the care of children with intracellular enzymatic deficiencies. The following examples represent types of problems that may be encountered:

Long-term Treatment with Potentially Favorable Outcome. Phenylketonuria (PKU) is the most common of the amino acid disorders, which, if untreated, leads to mental subnormality. Early dietary control, an environmental intervention, requires that the parent act as a co-therapist to prevent mental retardation. Moreover, current practice suggests that the diet must be carefully monitored during the school years since early dietary discontinuation may result in learning and behavioral problems [13, 14]. Behavioral disturbance and neuropsychological disturbance [15] are present in many patients with early-treated PKU and are more prevalent in those with higher phenylalanine concentrations. With early discontinuation of diet, the patients or their families may complain of lack of concentration and emotional instability. However, after returning to a "relaxed" phenylalanine-restricted, tyrosine-enriched diet, the impaired neuropsychological and behavioral functions appear to be reversible. Behavioral problems may compound the intellectual problems that are often present; emotional stress and neurologic dysfunction are their likely causal factors. An increased frequency of deviant behavior, then, may be a result of an interaction of psychological stress and neurologic impairment. Behavioral disorders, including attention deficit disorder and pervasive developmental disorder, may be associated with PKU [16]. Careful monitoring and family counseling is critical to prevent these complications. When they occur, appropriate referral is needed for treatment of the disorder and to counsel parents in their role in treatment.

Parents of phenylketonuric children face difficulties which may disrupt family life. In an early study, McBean [17] in Glasgow, Scotland, evaluated and followed up 59 families with a total of 204 children, 79 of them with PKU. Most of the parents had low incomes, poor housing, and the fathers were often unemployed. The study was not controlled; however, it indicates common problems that are seen in families with an affected child. Twenty-five of 59 families reported problems of behavior in the home. These included marital separations in 15 families and one divorce. The fathers were reported to have more difficulty than the mothers, and alcoholism, family violence, and infidelity were reported. The authors interviewed two family groups. In the first, the diagnosis was made late and an already retarded child was in the family, and in the second, the index case was ascertained by screening in the newborn period. Those families in the second group were reported since it is with this group that preventive intervention to prevent mental retardation may occur.

The families were interviewed to gather pilot information. Parents were asked the following specific questions:

- Their reaction to the PKU screening test
- Their reaction to diagnosis of PKU in the infant and their appreciation of what the diagnosis meant
- Their understanding of the diet and the problems involved in following through with the diet
- Their thoughts about the future for the infant
- The effect on the family and on their marriage of the phenylketonuric child
- Their attitude toward having additional children.

The initial Guthrie test was wanted; parents were anxious that it be done. The request for a second confirmatory test was stressful, particularly if it was accompanied by ill-advised comments from the medical staff. The admission to the hospital for assessment of an apparently normal infant was remembered as a particularly unhappy time.

All the parents when told of the diagnosis of PKU acknowledged feelings of anxiety and disbelief and found it difficult to assimilate the facts about the disorder given at the first interview. The mode of inheritance of the disease, which was discussed at the time when the diagnosis was given, was a difficult one for many parents to understand. Most appreciated that both parents are involved in the transmission of the disorder, but grandparents tended to blame one or the other side of the family. The parents frequently had not understood what was thought by the professional staff to have been an adequate and full explanation.

In regard to understanding the administration of the diet, all parents expressed considerable difficulty. When they could not understand or accept the consequences of dietary neglect, their belief that the diet was essential to the well-being of the child was often modified. When there was no previous experience of a retarded or phenylketonuric child, it was difficult for the parents to appreciate the form the mental retardation might take or how quickly it would occur. A threat of mental deterioration led to concern about the diet and sometimes an increased involvement of the mother

with the phenylketonuric child, at times to the exclusion of the father from the child's care. When some mothers found that dietary indiscretion does not lead to immediate mental retardation, they were inclined to question the diagnosis.

When asked about the future of the phenylketonuric child, few parents looked beyond their child starting school and feared that special schooling might be necessary. Others were more concerned with the supervision of the diet while the child was away from home. Very few parents mentioned secondary school or future work plans. Fewer still looked ahead to the possibility of marriage for the child or child-bearing.

Based on this pilot study, McBean [17] suggests that the parents be given information and simple and repeated explanations of the cause of PKU, the course of the disease, and the reasons underlying treatment. Although pamphlets may be helpful, alone they are inadequate. For all of the families, the moment of initial diagnosis was traumatic. Genetic counseling can be very difficult and particularly hard if they already have two or more affected children or know of other families where this situation has occurred. There is a need for constant emotional and practical support, which must be available for as long as the diet continues and may be necessary for even longer to help in dealing with the problems of adolescence in those who began treatment for PKU in childhood.

In the 20 years since McBean's study was carried out, there has been considerable experience in working with families. Pueschel et al. [18] used questionnaires, informal group meetings, and individual interviews with parents and their children with PKU to understand their attitudes and experiences surrounding discontinuance of the phenylalanine-restricted diet. These authors stressed the importance of understanding changing social interactions as termination of the restricted diet progresses. Preparatory discussions with parents and children prior to the change in diet should be held to avoid undue stress and conflict in such families.

Reber et al. [19] have provided a recent systematic study of family factors. They studied a population of 41 young children with early-treated PKU, and included family investigation to determine relationships between dietary phenylalanine control and patient functioning and family functioning. Children received neuropsychological tests, and parents completed behavior checklists on their child. They also completed four self-report measures aimed at evaluating family adjustment, stress, and social interaction. Significant correlations were found between concurrent phenylalanine control and patients' intelligence test scores, and lifetime phenylalanine control and patients' social competence. Children with PKU had lower social competence scores than a comparison control group. Parent-report measures of family psychological adjustment, stress, interaction, and socioeconomic status showed no significant association with children's dietary phenylalanine control. Family cohesion and adaptability correlated positively with the patients' cognitive performance. Mothers of PKU children perceived their families to be significantly less cohesive (more separated) and less adaptable (more rigid) than matched mothers of non-PKU children. Fathers of PKU children perceived their families to be less adaptable. The reported reduced cohesion and rigidity may have negative implications on test performance by PKU children. These findings suggest that both metabolic and family factors be considered in evaluating outcome of early-treated PKU. Longitudinal study of families whose children have PKU is needed to better understand the effects of the illness on family cohesion and adaptability.

Gradual Deterioration with Unfavorable Outcome. The lysosomal storage diseases lead to particular problems in psychosocial management. The conditions are normally identifiable by the characteristic facial appearance along with skeletal deformities and physical features associated with involvement of other organ systems. The descriptive term, gargoylism, graphically illustrates the altered physical appearance which can be of particular psychosocial importance. Mental deterioration with mental retardation is characteristic of the severe forms, but intelligence may be normal in the milder variants. A period of apparent early normality with a later decline in function is important to address with families. The severe form is a particular challenge to family adaptation. Behavioral problems related to central nervous system involvement may be present in the severe forms and complicated by interpersonal management problems. Those severely affected may die in childhood. In the milder variants, survival into adulthood requires ongoing specific support for the young person who is affected. A specific metabolic diagnosis is important in regard to prognosis and genetic counseling [20].

Psychosocial problems were investigated in a national study in the United Kingdom [21] of mucopolysaccharidosis (MPS) II (Hunter's syndrome), a sex-linked recessive condition. The sample consisted of families who volunteered for interview and hospital records; there was no control group and specific rating instruments were not used. Visits were made to 33 sets of parents with a total of 44 affected sons, 27 with the severe form, and 17 with the mild form of the disease. Information about the behavioral pattern in a further 22 boys was obtained from hospital records. Serious behavioral disturbance was reported in 36 of the 38 severely affected boys. The mildly affected boys generally adapted to the condition but often suffered from stigma related to their physical appearance. Adaptation to adult life after leaving special schooling is problematic for the mildly involved, highlighting the need for long-term support for the families and the boys themselves.

In the early-onset severe group, the initial behavioral complaint was overactivity (29 of 38 boys), commonly beginning in the second year and continuing to age 8 or 9 years when the disease process progressed to the point that the boys were more inactive and lethargic. Aggression towards others was reported in 16 out of 38 and, in some instances, related to rapid growth in the early years. However, ten were described as particularly affectionate and playful. The prevalence of aggression and oppositional behavior is comparable to the prevalence of behavior disorder in other reports of severe mental retardation. The rate of hyperactivity is quite high and, according to the authors, not responsive to pharmacotherapy. In the mild form of MPS II, overactivity, sleep disturbance and violence were not reported. In regard to family adjustment, most families indicated that they had received considerable psychosocial support from professionals, though particular concern was expressed in regard to adult support services.

Crocker and Cullinane [22] addressed clinical and educational issues for families and personality development in the child with both MPS IH and II. They emphasized specific problems faced by families including orthopedic, cardiac, and ear, nose and throat management and describe the work of an interdisciplinary team involved in the management of three cases. They studied: (1) response to the diagnosis; (2) the family's view of long-term needs; (3) reaction to genetic consequences; (4) continuing parental adjustment; (5) household emotional tone; and (6) response to guidance. The first family denied the disorder, did not plan for long-term care, avoided the mental retardation initially, then showed painful acknowledgment, and avoided the genetic issues which led to three interrupted pregnancies. The mother became clinically depressed and the father left home; the home atmosphere was one of mourning, and the diagnosis was not accepted. The second family showed prolonged bereavement regarding the diagnosis, was cautious about the future, showed partial acceptance of the mental retardation, showed disappointment and avoided thinking about the hereditary aspect, maintained a stressful and precarious marriage, and showed partial understanding regarding efforts at support. The third family accepted the diagnosis, made plans for the future, accommodated to the diagnosis but had some difficulty regarding siblings' anxieties, coped together with problems, oriented their attention to the child, and effectively used medical and psychosocial guidance. The first family remained disorganized, the second was in a process of continuous reintegration, and the third was maturely adapted.

To facilitate mature adaptation, Crocker and Cullinane [22] suggest:

- Establishment of a relationship with the family early on, preferably before the diagnosis is reached
- Identification of parental attitudes relevant to positive adaptation and initiating contact with parent organization and community support groups
- formulation of specifics of the patient care program and a clear outline of how they can be accomplished
- Orientation of the program toward the family's rights and needs
- attention to the needs of siblings
- Continuous regular support for parents, and
- Provision of genetic counseling and ongoing provision of information about new research related to the disorder.

Onset in Infancy and Chronic Course. The Lesch-Nyhan disease is a disorder of purine metabolism associated with gross uric acid overproduction, dystonias, problems in speech articulation, mental retardation and chronic self-injury [23]. The self-biting has been described by Nyhan as a "behav-

ioral phenotype," suggesting that a behavioral pattern may be a characteristic feature of a disorder. It is of psychosocial and psychiatric importance because of the association with mental retardation and self-injury. In type, the behavior is different from that seen in other mental retardation syndromes of self-injury where self-hitting and head banging are the most common presentations. The self-injury occurs although all sensory modalities, including the pain sense, are intact. Because of their self-injurious behavior, the patient may be restrained. Despite their dystonias, when restraints are removed the patient may appear terrified and quickly and accurately place a hand in the mouth. The child may ask for restraints to prevent elbow movement. When restraints are placed, the child may appear relaxed and more good-humored. The dysarthric speech may result in interpersonal communication problems; however, the higher functioning children can express themselves and participate in their treatment. Hemiballismic arm movements can also create difficulty, since the raised arm is sometimes interpreted as a threatening gesture by others rather than a neurological symptom. Psychosocially parents must cope throughout the child's life with multiple hospitalizations and continuous surveillance of their child.

The self-mutilation is conceptualized as an obsessive behavior which the child tries to control but generally is unable to resist. As the patient becomes older, he becomes more adept at finding ways to control it, including enlisting the help of others to protect himself against these impulses. Some older children show aggression towards others by pinching, grabbing, or using verbal forms of aggression.

In regard to treatment, the motivation for self-injury [24] must be considered as well as the biological basis of self-injury [25, 26].

Behavioral techniques, using operant conditioning approaches alone, may have limited effectiveness in Lesch-Nyhan disease. Pharmacological approaches to reduce anxiety and spasticity with medication also have met with mixed results. However, combined psychological, behavioral, and pharmacological approaches have been more successful. Parents report that attending to physical comfort, adjusting restraints, discussing concerns, and other stress management procedures are most helpful. Of the psychopharmacological agents used, the drug reported by parents to be most effective is diazepam. An emphasis on parent training is of particular importance for drug compliance and generalization of treatment effects. Continuous education and family support is essential. ◄

Period of Normal Development with Deterioration and Death. Adrenoleukodystrophy (ALD) is a disorder of peroxisomal fatty acid metabolism, with infantile, juvenile, and adult onset. Early juvenile symptoms include learning and attentional problems with progression to deterioration in motor and cognitive abilities and, finally, dementia and death.

The leukodystrophies are inherited progressive, nonselective disorders of the central and peripheral nervous system [27]. The child comes to medical attention during infancy or early childhood. Subtle changes in affect, behavior and attention are among the early symptoms of the leukodystrophies. Frequently, nonmedical management is attempted prior to the discovery of the specific diagnosis. In some instances, psychiatric disorders are diagnosed and children are placed in school programs for the emotionally disturbed, or in other instances, in programs for learning disabilities. A common behavioral disturbance is an alteration of the sleep-wake cycle. The child may have difficulty getting to sleep and staying asleep. Muscle spasms may wake the child at night and be part of the disease.

As the leukodystrophic process develops, a major therapeutic focus is to maintain muscle tone and support bulbar muscle functions. Bulbar muscle control is needed for normal respiratory toilet, eating and normal gastrointestinal activity. With the deterioration in muscle control, handling oral secretions and nutritional support is frequently impaired. This requires changes in feeding patterns to the use of pureed and soft foods. In some instances, as the ability to handle oral liquids is lost, invasive measures, including nasogastric tubes, nasoesophageal tubes, or a gastrostomy, may be required to administer medication, food and water.

Since children with leukodystrophies have problems in learning and attention and later may have cognitive disorders, the coordination of school services becomes particularly important. The purpose of the education program is to enhance the quality of life and provide as much normalization as possible. When the child can no

longer attend school, home and hospital teaching services focus on positioning, handling and transporting techniques, and relaxation activities. These latter services may involve the use of physical and occupational therapy along with a teacher certified to work with the multiply-handicapped.

Medication may be needed for a variety of problems that present, including the sleep disorder, the attention deficits, and the treatment of the muscle spasms. The management of these symptoms is often problematic for parents since the child frequently requires one-to-one supervision. As difficulties in understanding and processing auditory experience and interpreting what is seen occur, the child requires increasing support. The family must fill in the missing information or compensate for the child's losses.

From a psychosocial perspective, grief, frustration and anger about the lack of a specific therapy and the experience of dealing with progressive deterioration affects both the patient and family as well as the physician and other professionals who are involved in care. There are experimental dietary treatments that are currently being tested, but in conducting them, one must work carefully with the family to minimize discomfort, inconvenience, and additional time involved in care.

The severity of medical problems is compounded by the necessity of coping with a fatal illness which threatens family functioning. When parent organizations are available, families should be encouraged to contact them. Family organizations provide newsletters and monthly mailings regarding current research, provide opportunities to talk to other parents about patient management and offer personal experiences in coping with the disorder. Through helping one another, the family can enhance their own ability to cope with the situation. As the family begins to understand the severity of the condition, they may begin to withdraw investment in the child. The physician, then, has a major role in helping to maintain confidence and hope. To the degree that the final phase of the illness is adequately supported, the parents may develop a realistic and meaningful perspective on the individual child's life as part of the family.

Issues Regarding New Forms of Treatment

Bone Marrow Transplantation. New forms of treatment have risks as well as benefits; for example, bone marrow [28–31] transplantation is an approach which has been applied to about 50 inborn errors of metabolism, and gene therapy will become available in the future. Bone marrow transplantation represents a major medical advance and provides hope to children and families. Increasingly, it is being made available to children with a variety of disorders. Pfefferbaum, Lindamood and Wiley [29] have reported that although psychosocial factors do not influence survival for bone marrow transplantation, they may be critical in the management of many cases.

The first psychosocial issues emerge at the time of referral for treatment. Children are commonly referred for bone marrow transplantation to facilities that are distant from their homes because of limited bed availability and the location of bone marrow transplantation programs in tertiary care facilities. Such social disruption may place a considerable burden on family members. When proposing bone marrow transplantation, in addition to providing knowledge of the procedure itself, an important psychosocial issue is the child's motivation and willingness to have this procedure done. Moreover, feelings of guilt, and misgivings about the procedure by the parents should be discussed prior to the transplantation. Considerable psychosocial support may be needed to help the child and parent work through their feelings about an impending transplantation.

In preparing parents and children for transplantation, it is important to elicit a history of the child's previous emotional responses to stress and hospitalization. Assessment procedures may include pre-treatment psychological evaluation regarding the child's level of intelligence, history of past emotional problems, and typical coping strategies used by the child. Common problems include anxiety related to the procedure itself, the feeling of being a burden to the family, low self-esteem, and a sense of vulnerability. During the hospital stay, depression, anxiety, excessive dependence, aggressive behavior, and anger may be noted. This may be demonstrated by less tolerance for repeated procedures along with periodic refusal to cooperate with treatment. Ultimately, the emotional events linked to bone marrow transplantation for a family relate to the recipient's medical course, the type and extent of the family members' psychological strengths and weaknesses, and the disruptions in family organization that are required for the transplantation.

Psychological stress may occur when adapting to disruptions brought about by the move. More-

over, during bone marrow transplantation procedures, stress can be severe and prolonged. Patients may be isolated in sterile environments and subjected to high doses of chemotherapy. The child and family must then cope with the secondary effects of a treatment. The possibility of infection, rejection of the graft, relapse, and graft-versus-host responses may lead to chronic high anxiety. Commonly, patients stay for several months in the hospital and require close follow-up at discharge, requiring that family members remain with them, away from the rest of the family, for considerable lengths of time. Following discharge, the readjustment phase can also be stressful.

During the hospitalization, the child's response to other patients must also be considered. It is not uncommon for children to be placed on adult units where bone marrow transplantations are carried out for both children and adults with potentially terminal disorders, such as aplastic anemia and leukemia. Although the child with an inborn error does not have these conditions, the possible negative effect of this exposure to other patient groups must be anticipated and measures taken to provide appropriate support. For example, the parents of other hospitalized children or relatives of other patients may become emotionally close to a child on a bone marrow transplantation unit. Should another patient on the unit die, then the child not only loses that person but also their visitors and family with whom he may have become familiar.

Stresses within the marriage and problems with siblings must also be considered. The experience of participating in a life-saving or life-enhancing procedure may produce a strong and intimate bond between parent and child. In a bone marrow transplantation, the donor may be a parent or sibling. If it is a sibling, competition among siblings may occur for the role of donor. In the case of successful transplantations, a special relationship may develop between the child and the donor. Yet, in some instances, the donors may feel that they have not received adequate recognition for their contribution. Moreover, if medical complications develop, the donor may worry about the part played by his marrow in the illness. If death occurs, the donor may experience a sense of guilt about letting a sibling or child down. Siblings may experience a sense of loss if a parent is the donor and goes to a distant medical center for the transplantation. Parent expectations about the transplantation may also be excessive and can lead to unrealistic promises about how things might turn out after the transplantation.

Differences may also be noted in coping with transplantation if one parent is primarily involved with the child and the other parent is not. There is always a dilemma when parents are differentially involved in the day-to-day events concerning the illness and its treatment. Due to differences in life experience with the child's illness, there may be misunderstandings about what is happening, the meaning that the parents assign to the event, and the timing of their emotional responses.

The medical professional should try to ensure that the patient and family enter discharge planning with realistic expectations. This involves not only helping them to develop a positive attitude toward recovery but also the recognition of possible complications. Following the successful completion of a bone marrow transplantation, patients and their families may experience ambivalence about leaving the protected environment of the bone marrow transplantation unit, and there may be unrealistic expectations about outcome. Children may become quite dependent during the time of the procedure and, when isolation has been necessary, there may be concerns about the home being a sufficiently safe environment [31]. Parents sometimes may establish excessive precautions at home in planning for discharge. Therefore a discharge meeting where there is encouragement for the parents and siblings to articulate expectations and to review the course of the hospital stay can be very helpful. Issues such as the time to return to school and participation in peer group activities should be included. Sensitization of parents to the possible presence of unresolved feelings of siblings about the transplantation procedure may act as a form of psychological immunization if difficulties do emerge.

After discharge, parents require reassurance and recognition that a new equilibrium in their relationship may have to be established following prolonged separation and the stresses that may be involved with the procedure. Follow-up visits are particularly important and should be scheduled immediately following discharge when parents may be apprehensive about the expected course, and then subsequently, when lingering unresolved feelings may re-emerge.

In summary, factors which affect family coping with bone marrow transplantation include the length of the patient's previous illness, the degree and duration of family geographical dislocation

from home, and preexisting and intercurrent stresses within the family, such as marital conflict, separation/divorce, and changes in family role relationships. High levels of physical and emotional stress, intense relationships which develop between the bone marrow transplantation team members and recipients during the procedure, and psychosocial phases in the transplantation procedure must also be considered. Other issues include the psychological impact of bone marrow transplantation on donors, the long-term family consequences of bone marrow transplantation, and long-term cognitive, neuroendocrine, sexual, reproductive and psychosocial status of bone marrow transplantation survivors.

Organ Transplantation. In regard to pediatric organ transplantation, Gold et al. [32] have reviewed the parents' perspectives. They report that there are three specific stages that the family must cope with, which they divide into preoperative, perioperative, and long-term postoperative. Preoperative psychosocial issues include the initial hospital experience, which involves preparation for hospital stay and an initial building of trust in staff members. It also involves making plans for family members who will be waiting at home as well as financial issues. During the perioperative period, the first 24 h are associated with anxiety about outcome, often followed by exhilaration during the first 2 weeks if the transplantation has been successful. They describe a "roller coaster phase" following this, where there may be fear of rejection or infectious disease, continued guilt and fear of loss, and ongoing isolation and marital stress. There is need for preparation for hospital discharge. In doing so, there is the need to build confidence and work through the dependency that has developed during the hospital stay. Finally, in the postoperative period, issues regarding returning home involve readaptation to parental roles and readjustments in family structure. Uncertainty must be endured until the final results of the procedure become apparent.

Gene Therapy. Finally, gene therapy is a new approach to treatment for inborn errors that is currently under development. Clear data on psychosocial adjustment are not yet available; however, one would expect that the psychological adjustment may be similar to other potentially corrective procedures, such as bone marrow transplantation.

Conclusion

The challenge for society in dealing with these families is to provide care through offering comprehensive services, including genetic counseling, modern treatment, and follow-up supportive counseling. These approaches must ensure confidentiality and freedom of choice to avoid misunderstanding and stigmatization. The primary objective of screening programs should be to maximize the options available to families at risk. Whenever possible, the ongoing treatment should be coordinated with and include the involvement of the primary health care physician working with consultation from the metabolic specialist.

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Part II
Carbohydrate Metabolism

Glycogen Storage Diseases

J. Fernandes and Y.-T. Chen

CONTENTS

Glycogen storage diseases (GSD) are caused by enzyme defects of glycogen degradation. Some enzyme defects cause glycogen storage in the liver due to the fact that the deficient enzyme is mainly localized in the liver. Some enzyme defects are localized in muscles, and some are more generalized. The classification is presented in Table 1, which shows that GSD are not only defined by the deficient enzyme, but also by a type number, which reflects the historical sequence of elucidation. In this chapter the GSD are not described according to that chronological order, but rather according to the clinical presentation, mainly hepatic, generalized, or mainly myogenic.

The degradation of glycogen to pyruvate and the reverse pathway are shown in Fig. 1. The conversion of glycogen into pyruvate is divided into two parts: glycogenolysis from glycogen to glucose-6-phosphate, and glycolysis from glucose-6-phosphate to pyruvate. The synthesis of glycogen from pyruvate is also divided into two parts: gluconeogenesis from pyruvate to glucose-6-phosphate (see the chapter by Buist on "Disorders of Gluconeogenesis") and glycogen synthesis from glucose-6-phosphate.

The enzyme defects currently known are indicated in Fig. 1.

Hepatic Glycogen Storage Diseases

Glucose-6-Phosphatase Deficiency (GSD Ia, Von Gierke's Disease)

Clinical Presentation

"Hepatonephromegalia glykogenica" was the title of the first description of glucose-6-phosphatase deficiency by von Gierke [1]. A protruded abdomen because of marked hepatomegaly, truncal obesity, a rounded "doll face," hypotrophic muscles, and short stature are conspicuous clinical findings. The liver may already be enlarged at birth. Its size increases gradually, and the lower border may extend to well below the umbilicus. Initially, the liver has a normal consistency and a smooth surface, but beyond the age of 10–20 years the surface may become uneven and the consistency much firmer because of the development of adenomas. Cirrhosis does not develop. The kidneys are moderately enlarged, whereas the spleen remains normal sized. Usually, the patient's growth lags behind, unless intensive dietary treatment has started early. The patient bruises easily, and nose bleeds may be troublesome due to impaired platelet function [2]. Episodes of diarrhea or loose stool may occur, presumably due to impaired active absorption of glucose [3]. Profound hypoglycemia occurs frequently and can be elicited by trivial events such as a short delay of a meal or a lower food intake induced by an intercurrent illness. Hypoglycemic symptoms are usually accompanied by hyperventilation, a symptom of lactic acidosis. Exceptionally,

Table 1. Classification of glycogen storage diseases

Type	Deficient enzyme	Tissue involved	Main clinical findings
Ia	Glucose-6-phosphatase	Liver, kidney	Hypoglycemia, hepatomegaly, lactic acidosis, hyperlipidemia
Ib Ic Id	Glucose-6-phosphatase-related transport	Liver	In addition: neutropenia and infections in Ib
II	Acid α-glucosi-dase (lyso-somal)	Generalized	Infant form: cardio-respiratory failure Later forms: myopathy
III	Debranching enzyme	Liver/muscle heart	Hypoglycemia, hepatomegaly, myopathy
IV	Branching system	Liver	Hepatosplenomegaly, cirrhosis
V	Phosphorylase	Muscle	Exercise intolerance
VI	Phosphorylase	Liver	Hepatomegaly
VII	Phosphofructokinase Phosphoglycerate kinase Phosphoglycerate mutase	Muscle	Exercise intolerance
IX	Phosphorylase b kinase	Liver	Hepatomegaly
O	Glycogen synthase	Liver	Hypoglycemia

hypoglycemic symptoms do not occur even during prolonged fasting, though other clinical features and metabolic abnormalities persist. Long-term cerebral function is normal as long as hypoglycemic damage is prevented.

Metabolic Derangement

Hypoglycemia, *hyperlactacidemia*, *hyperlipidemia* and *hyperuricemia* are the most characteristic metabolic derangements.

Hypoglycemia occurs as soon as exogenous sources of glucose are exhausted, because the enzyme defect between glucose-6-phosphate and glucose blocks glucose release from both glycogenolysis and gluconeogenesis. However, the degradation of glycogen to pyruvate is intact and is intensified under hormonal stimulation as soon as the provision of glucose fails. The resulting increased pyruvate and lactate production is a useful mechanism as long as pathological lactic acidosis does not develop, because lactate may serve as a fuel to the brain [4]. This alternate brain fuel may protect some patients against cerebral symptoms, even when the blood glucose concentration is close to zero.

More important is the increased synthesis of fatty acids and cholesterol from the conversion of excess lactate/pyruvate into acetylcoenzyme A (acetyl-CoA), the end product of glycogen degradation. This, together with decreased lipoprotein lipase activity, results in hyperlipidemia, which is observed in most patients. Serum triglycerides predominate, and cholesterol (esters) and phospholipids are less elevated. The hyperprebetalipoproteinemia is characterized by high levels of apolipoproteins (Apo) B, C (particularly C III), and E and low levels of Apo A and D [5]. Ketosis does not occur, in contrast to in the other types of GSD [6]. This is due to the conversion of excess acetyl-CoA into malonyl-CoA, which inhibits fatty acid oxidation.

Hyperuricemia is another metabolic derangement. It is caused by both a decreased renal clearance of urate by inhibition from lactic acid, and increased production of uric acid. The overproduction of uric acid is elicited by a mechanism similar to that elicited by fructose administration to a patient with hereditary fructose intolerance (see the chapter by Van den Berghe on "Disorders of Fructose Metabolism").

Diagnostic Tests

For the diagnosis, preliminary screening is helpful, as the enzyme defect can only be detected in a liver biopsy.

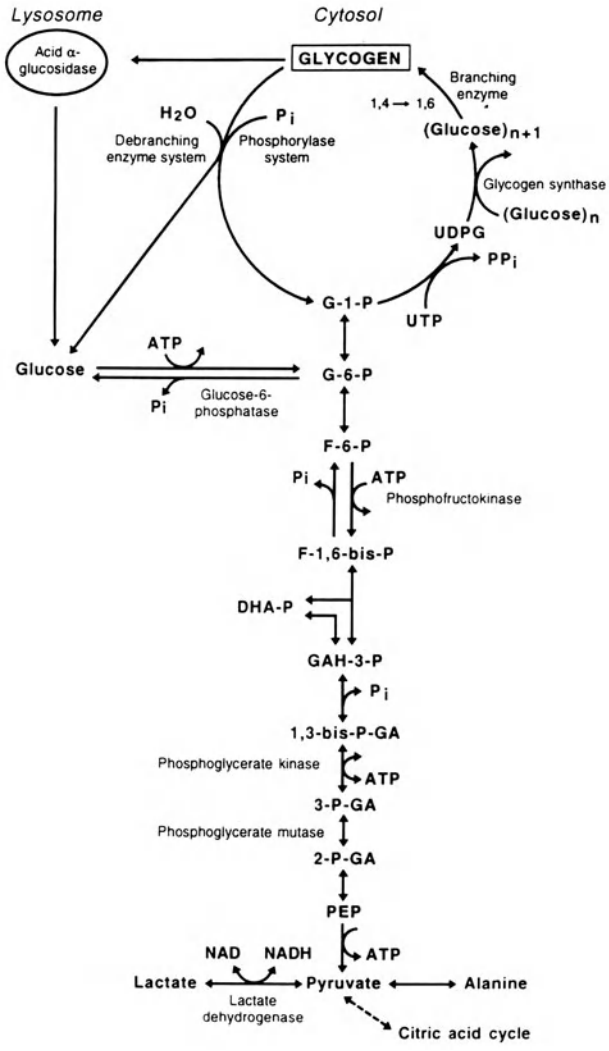

Fig. 1. The glycogenolytic–glycolytic pathway. *UDPG*, uridine diphosphoglucose; *UTP*, uridine triphosphate; *G-1-P*, glucose-1-phosphate; *G-6-P*, glucose-6-phosphate; *ATP*, adenosine triphosphate; *Pi*, anorganic phosphate; *PPi*, pyrophosphate; *F-6-P*, fructose-6-phosphate; *F-1,6-bis-P*, fructose-1,6-bis-phosphate; *DHA-P*, dihydroxyacetone-phosphate; *GAH-3-P*, glyceraldehyde-3-phosphate; *1,3-bis-P-GA*, 1,3-bis-phosphoglycerate; *3-P-GA*, 3-phospho-glycerate; *2-P-GA*, 2-phosphoglycerate; *PEP*, phosphoenolpyruvate; *NAD*, nicotinamide-adenine dinucleotide; *NADH*, reduced NAD

The safest approach is to perform an oral glucose tolerance test and to determine the blood glucose and lactate concentrations. It is not the glucose, but the lactate curve that provides the clue for the diagnosis. The initially increased lactate concentration, which reflects fasting, decreases when glucose increases in a reciprocal way. This is opposite to the normal situation, in which blood lactate is low and increases slightly. For details of the test see Chap. 2. Some authors prefer a glucagon test, which shows a flat or descending blood glucose curve and a markedly rising lactate curve. However, in view of the risk of hypoglycemia due to the continuation of fasting this test is not recommended as a *first* test. It might be used as a second test to differentiate between other GSD.

A biochemical assay of glucose-6-phosphatase activity in a liver biopsy is indicated to confirm the tentative diagnosis.

Differentiation between deficiency of glucose-6-phosphatase itself (GSD Ia) and that of other proteins of the multienzyme system is very important (see under GSD Ib). At histological examination, fat accumulation often predominates over that of glycogen.

Complications

Numerous complications have been observed, such as liver adenoma, kidney stones, proteinuria and progressive renal failure, gout, xanthomas, pancreatitis, anemia, osteoporosis, and ovarian cysts.

Liver Adenoma. This develops, singly or multiply, in the majority of patients in their second decade. Its presence can be suspected by palpation and confirmed by ultrasound. The ultrasound appearance varies between hyper-, iso-, and hypoechogenic [7]. Liver adenomas are mostly benign, but malignant transformation (carcinoma) can occur [8]. Acute hemorrhage in a liver adenoma has also been reported. It should be treated with continuous intravenous glucose administration for a few days in order to restore the presumed abnormal thrombocyte aggregation.

In view of the risk of malignancy, ultrasound of the liver should be performed regularly, for instance once a year. A nodule which increases in size or changes from being circumscribed to having poorly defined margins or the appearance of a "nodule in a nodule" should be checked by an angiogram or scintigram [8]. Also, serum α-fetoprotein should be determined twice a year, although some doubt has arisen about its sensitivity as a tumor marker. Therefore, we have added the determination of des-γ-carboxyprothrombin to the monitoring of liver adenoma [9], but there is no proof of its value yet. Enucleation of a carcinoma or orthotopic liver transplantation are the final options. The cause of adenoma development is not known. It is speculated that hyperglucagonemia or long-term toxicity of excess substrates (lactate, lipid) are contributing factors, and this speculation is supported by regression of adenomas with intensive dietary treatment. This is exceptional, however, as adenomas usually remain constant during many years of optimal treatment.

Kidney Stones. These have been reported incidentally, as has nephrocalcinosis with renal colic and hematuria [10]. Acid urine (hyperlactaciduria), hypercalciuria, and hyperuricemia may be contributing factors.

Proteinuria and Progressive Renal Failure. Much more serious are proteinuria, deteriorating kidney function, and hypertension due to focal segmental glomerulosclerosis and interstitial fibrosis, described by Chen [10] and others. It starts with a "silent" glomerular hyperfiltration and hyperperfusion, followed by microalbuminuria. This slowly evolves to overt proteinuria and eventually a decrease in the glomerular filtration rate. In the final stage, hemodialysis and renal transplantation are the ultimate options. Glomerulosclerosis with its long latency is observed in almost all older patients whose disease was not adequately treated. Other renal abnormalities include amyloidosis, a Fanconi-like syndrome [11], and a distal renal tubular acidification defect.

Gout. Hyperuricemia occurs in almost all patientls and may lead to gout and urate kidney stones.

Xanthomas. These may be observed on the buttocks, elbows, and knees, similar to those observed in some primary lipid disorders (see Chap. 25). Their occurrence has become rare since the introduction of intensive dietary treatment.

Pancreatitis. Reflected by bouts of abdominal pain and diarrhea, this can be elicited by severe hyperlipidemia. Its presence can be screened for by looking for elevated levels of serum amylase, lipase, and trypsin and can be proved by computed tomography (CT) and endoscopic retrograde cholangiopancreatography [12].

Anemia. Older patients are prone to develop normochromic anemia [13]. Erythropoietin deficiency has been observed.

Osteoporosis. This has received relatively little attention up to now. However, its occurrence becomes important as it might precede osteomalacia due to renal insufficiency in adulthood. Chronic lactic acidosis might contribute to the decalcification of bones by increased calcium desorption from bone and increased urinary calcium excretion [14]. Bone densitometry should be determined at least once in every older patient.

Atherosclerosis. This is remarkably rare despite the chronic hyperlipidemia and the atherogenic profile of serum lipoproteins, one report from 40 years ago excepted [15].

Polycystic Ovaries. These have been observed in adolescent patients. Ultrasound might be performed in that age group in search of this complication.

Treatment and Prognosis

The aim of dietary treatment is to prevent hypoglycemia and suppress secondary metabolic derangements as much as possible. The methods

to achieve this have changed considerably during the last 20 years. The first breakthrough was the introduction of nocturnal drip feeding via a nasogastric tube [16]. It allowed the patient and the parents to sleep during the night instead of waking up every 2–3 h for obligatory feeding. The second improvement was the introduction of uncooked starch, from which glucose is much more protractedly released than from cooked starch [17].

Both methods have been compared during follow-up studies and the results are equally favorable [18]. Their application is shown in Table 2. The glucose requirement in the formula, in gastric drip feeding, in meals containing precooked starch (cereals etc.), or in the form of uncooked starch is calculated from the theoretical glucose production rate, which decreases with age. Three elements of the diet need special consideration: formula feeding, gastric drip feeding, and uncooked starch.

Formula Feeding. A milk-based, low-lactose, sucrose-free formula is enriched with maltodextrin until the calculated requirement, if breast milk is not available. This sugar is gradually replaced by precooked starch (rice, corn), maximum 6%, in order to prolong gastric emptying time. This might allow a wider spacing of the feeding frequency from 2- to 3-h intervals to 3- to 4-h intervals.

Gastric Drip Feeding. Gastric drip feeding at night may be introduced in young infants at the time of the diagnosis. The feeding may be identical to the above-mentioned formula or it may contain only glucose or a glucose polymer in water. Theoretically, the former complete formula is preferable.

Gastric drip feeding should not be started later than 1 h after the last meal, and breakfast should be within 15 min of removal of the drip tube. The technical outfit of the pump and the connection of the tube and its fixation to the patient should be meticulously explained to the parents, as a fatal outcome due to disconnection of the system has been described. Some parents cannot cope with the technical and emotional implications of infusion pumps and tube feeding and prefer to switch to uncooked corn starch.

Uncooked Starch. Initially, uncooked corn starch was used to replace nocturnal drip feeding, in order to allow adolescents more freedom from the tight dietary schedule [17]. Its effect is based on the fact that glucose is slowly released and absorbed from uncooked corn starch, so that normoglycemia may be maintained for 6–8 h instead of 3 h after an equivalent intake of glucose in water.

Corn starch can be introduced directly at the age of 1 year, the approximate age at which pancreatic amylase activity has sufficiently matured. Side effects of bowel distension, flatulence, and loose stool are usually transient and can be mitigated by slowly increasing the dose. For infants under 2 years of age, corn starch can be introduced at a dose of 1.6 g/kg every 4 h. The response is variable. As the child grows older, the regimen can be changed to every 6 h and the dose increased to 1.75–2.5 g/kg. The starch is mixed with water in a starch to water ratio of 1:2. Adding glucose is contraindicated, as its insulin stimulation offsets the advantage of the starch. However, mixing the uncooked starch with yoghurt, diet drink, or milk increases its palatability for some children without significantly affecting the period of euglycemia. The efficacy of corn starch to keep the blood glucose concentration at or above 3.9 m*M* (70 mg/dl) is investigated by means of a starch tolerance test. Its procedure is similar to that of a glucose tolerance test (see Chap. 2). Depending on the results of the test, the 4- or 6-h dose for the diet is adapted (Table 2). With a large amount of corn starch consumed, the diet plan to provide other essential nutrients for growth and development needs to be carefully designed and followed. Corn starch can be varied with other slow carbohydrates such as rice, wheat, or tapioca with similar slow-releasing properties. Another approach is to prescribe slow carbohydrates twice per night and semislow carbohydrates twice during the daytime as a cover between the meals. Semislow carbohydrates which release glucose during 4 h are: cooked rice, macaroni, rolled oats, barley groats, millet, couscous, legumes, and lentils.

The variation between slow and semislow carbohydrates may prevent corn starch monotony if only that starch is administered four times per day. This is important in view of the observation of amyluria in GSD I patients consuming corn starch [19]. This means "persorption" and incorporation of undissolved starch particles in various tissues. The long-term effects of this phenomenon are not known.

With respect to rapid carbohydrates, the consumption of lactose is limited to the amount present in 0.5 l milk per day, as milk and dairy products are important sources for protein and

Table 2. Feeding schedule of patients with glycogen storage disease (GSD) I

Age	Schedule	Gastric drip[a]	Starch processed in meals	Uncooked starch (g/kg)	Glucose[b] (mg/kg/min)
0–12 months	Formula 2- to 3-h intervals	Possibly	Rice, corn 0%–6% in low-lactose and sucrose-free formula	–	7–9
1–3 years	3 meals 2 snacks	35% energy in 12 h	Cereals, bread, rice, macaroni, legumes	Cornstarch 6 × 1.6	7
3–6 years	3 meals 2 snacks	See above	See above	Slow starch 4 × 1.75–2.5 Semislow[c] 2 × 1.0–1.5	6–7
6–14 years	3 meals 1–2 snacks	30% energy in 10 h	See above	See above	5–6
Adolescents	3 meals 1–2 snacks	Possibly	See above	Slow 2 × 1.5 Semislow 2 × 1.0	5
Adults	See above	–	See above	See above	4

[a] Gastric drip feeding and uncooked starch are mutually exchangeable or may be complementary.
[b] The total requirement of glucose expressed in this way is used for the calculation of the amount of glucose in gastric drip feeding, starch in meals, or uncooked starch.
[c] One or two doses of slow starch can be interchanged for semislow starches.

calcium. Sucrose and fructose are prohibited, except in a limited amount of fruit, as these sugars enhance the production of lactate. The total amount of carbohydrates should be 60%–65% of total energy, protein 10%–15%, and fat the remainder (20%–30%).

Dietary Adequacy. The laboratory parameters used to control the adequacy of the dietary treatment are summarized in Table 3. In this approach, slightly elevated blood lactate levels between 2.0 and 5.0 m*M* (normal, <2.0 m*M*) are acceptable. Higher levels may reflect insufficient carbohydrate intake or too wide spacing of the meals. Lower levels, though to be preferred theoretically, may reflect overtreatment with too much carbohydrate, which increases the proneness to preprandial hypoglycemia. The lactate concentrations in urine, collected at home in 12-h portions or as freshly voided samples and delivered to the laboratory in the frozen state provide valuable information about the adequacy of the diet in the home situation. The lactate concentration should not exceed 0.6 m*M*.

Hyporesponders. The dietary refinements of gastric drip feeding and uncooked corn starch are very effective to induce "catch-up" growth or to maintain growth close to the expected target curve in most patients. However, a small group of hyporesponders exists with growth lagging behind despite all efforts to attain optimal metabolic adjustment [20]. In these patients urinary lactate is higher than in responders, although even in the latter group with a more favorable course, serum lactate, lipids, and urate usually do not normalize.

Focal Glomerulosclerosis. Up till now, no preventive treatment exists, as the etiology of this late complication is not yet known. Even with early dietary treatment, glomerular hyperfiltration is still seen [21]. In analogy with diabetic nephropathy, patients with proteinuria are treated with a moderate protein restriction and Captopril, an inhibitor of angiotensin-converting enzyme.

Hyperuricemia. Hyperuricemia, defined as serum urate concentration greater than 0.36 m*M* (6 mg/dl), should be treated with Allopurinol, a xanthine–oxidase inhibitor, at a dosage between 10–15 mg/kg per day, orally. Alkalization of the urine with $NaHCO_3$, 1–2 mmol (85–170 mg) per kg per day is optional.

Table 3. Laboratory parameters for dietary adjustment of glycogen storage disease (GSD) I patients

Parameter	Value
Blood	
Glucose profile during the daytime before meals and during gastric drip feeding	≥3.9 m*M* (70 mg/dl)
Lactate before each meal or 2 h after the start of drip feeding	2.0–5.0 m*M* (18–45 mg/dl)
Urine	
Lactate concentration (sample or 12 h)	≤0.6 m*M*
Lactate to creatinine ratio	≤0.12

Hyperlipidemia. Premature atherosclerosis appears not to be a feature of GSD I. Nevertheless, a low-fat, low-cholesterol diet and the use of vegetable oils with a high linoleic acid content is emphasized, although this regime in itself does not improve hyperlipidemia. In short-term experiments, the administration of 10 g fish oil per day lowered serum triglycerides and cholesterol markedly and improved the lipoprotein profile, presumably by enhancing fat catabolism [22].

Osteoporosis. The risk for osteoporosis is probably increased, because chronic lactacidemia and the gradual development of renal insufficiency are expected to promote calcium desorption and to limit peak bone mass at adult age. Therefore, preventive measures are indicated: 10–20 μg vitamin D_3 (400–800 IU) per day for countries with a higher latitude and 0.5–1.0 g calcium per day in the case of limited milk intake.

Intercurrent Infections. During infections dietary treatment is endangered because of anorexia and vomiting. This may lead to hypoglycemia and lactic acidosis, which both increase nausea. The parents should administer a small amount of a polycose syrup (low osmolarity) while bringing their child to the hospital. For further treatment of hypoglycemia, see Chap. 3.

Preparation for Elective Surgery. Bleeding time and platelet adhesiveness must be investigated before operation. If abnormal, continuous gastric drip feeding for 1 week or intensive intravenous glucose infusion for 24–48 h should be instituted to normalize the bleeding tendency prior to surgery.

Liver Transplantation. Orthotopic liver transplantation, followed by complete resolution of the metabolic derangements, is a final option for patients with malignancy of a liver adenoma, and for nonresponders to dietary treatment (Chap. 41). It is not yet known whether the kidney abnormalities regress.

Dietary treatment and a few drugs have considerably improved the prognosis of this severe disorder of carbohydrate metabolism, and a few patients have passed the age of 50 years. However, late kidney abnormalities are of great concern.

Genetics

Inheritance is autosomal recessive. The gene has been cloned and localized to chromosome 17 [23]. This might allow antenatal diagnosis.

Other Defects of the Multicomponent Glucose-6-Phosphatase Protein: GSD Ib, Ic, and Id

Patients with GSD Ib are clinically and metabolically not discernible from those with GSD Ia, except for an additional propensity to infections and *immunologic abnormalities*, based on neutropenia and *defective leukocyte function*. Patients with GSD Ic and Id are too rare to allow conclusions about specific symptoms. For the sake of conciseness, only abnormalities that are different from those in GSD Ia are described.

Clinical Presentation

Bacterial infections, recurrent or chronic, occur in most patients. Even brain abscesses have been reported [24]. A decreased number of neutrophils and defective neutrophil and monocyte functions underlie the infections. The number of neutrophils is usually below 1500/μl, but a normal count does not exclude proneness to infections. Bone marrow examination shows hypercellularity instead of the expected hypocellularity (for a review see [25]). Chronic inflammatory bowel disease similar to Crohn's disease is presumably related to the neutrophil dysfunction [26], as is acute myelogenous leukemia [27].

Metabolic Derangement

As the metabolic derangements do not permit us to differentiate GSD Ia from the other defects of the glucose-6-phosphatase enzyme system, biochemical assay of a liver biopsy is the only means to obtain the diagnosis. The condition of the liver tissue is very important, as the reliability of the biochemical assays depends on the intactness of the microsomes. Therefore, consultation with the biochemist before performing the biopsy is necessary. A working model of the enzyme system is shown in Fig. 2 [28].

Glucose-6-phosphatase itself is located at the inner luminal wall of the endoplasmic reticulum. Three translocases exist which import or export substrates for the enzyme:

- T1 allows the entry of glucose-6-phosphate.
- T2 exchanges phosphate for pyrophosphate.
- T3 exports glucose.

Deficiencies are known of all three translocases: T1 deficiency for GSD Ib, T2 deficiency for GSD Ic, and T3 deficiency for GSD Id [29].

Treatment and Prognosis.

Treatment with recombinant human granulocyte colony-stimulating factor (GCSF) has reversed the gradually deteriorating clinical course of the disorder into rapid abatement of infections and healing of abscesses, ulcers, and inflammatory bowel disease [26]. Impressive weight gain and improved vitality occur simultaneously.

Neutrophil cell counts usually increase to low to normal values and some but not all neutrophil functions improve. We recommend 5–10 μg GCSF (Amgen, Thousand Oaks, CA, USA)/kg body weight per day subcutaneously during acute infections and the gradual reduction of this dose to 2–3 μg/kg per day for maintenance. The use of this expensive drug should be preferred above that of cotrimoxazole, which has been used as a prophylaxis up till now because of its capacity to penetrate neutrophils. The prognosis may improve markedly due to the new treatment.

Genetics

The inheritance of GSD Ib is autosomal recessive, and this probably also applies to GSD Ic and Id. The relative incidence of GSD Ia to GSD Ib is in the order of 5–10:1. DNA findings are not available.

Debranching Enzyme Deficiency (GSD III)

Clinical Presentation

The hepatic form is characterized by an enormously enlarged liver, protruded abdomen, truncal obesity, doll face, muscle hypotonia, retarded growth, and initially delayed motoric development. The clinical findings may be indistinguishable from GSD I; however, kidney size is usually normal. All these symptoms abate gradually for unknown reasons, and at puberty or adolescence they have usually disappeared completely. Even the liver is normal sized or only slightly enlarged.

The *mixed hepatic–myogenic form* occurs more frequently than the purely hepatic form, and the presence of muscle abnormalities has been underestimated in the past. The muscle function varies: in some patients pronounced hypotonia and weakness improve with aging, but in most patients the muscle symptoms become prominent in adults and can progress from peripheral to proximal. Muscle wasting is often of both a myopathic and neurogenic origin [30]. Electrocardiographic abnormalities are usually present, and cardiomyopathy may develop during the second or third decade. The tendency to hypoglycemia is pronounced at young age in both types of GSD III and abates gradually. Cerebral development is normal except when prolonged severe hypoglycemia after birth has caused damage.

Cirrhosis of the liver is an exceptional complication, as is renal tubular acidosis [31].

Metabolic Derangement

The enzyme defect allows only partial degradation of glycogen during fasting, since it hinders the degradation of the glycogen molecule beyond its outer branches. *Hypoglycemia* results, which, in contrast to GSD I, can be compensated to a certain extent by gluconeogenesis and ketogenesis [6]. Increased gluconeogenesis most likely accounts for the decreased serum levels of alanine and branched chain aminoacids, but drains muscle protein, a process which may contribute to muscle

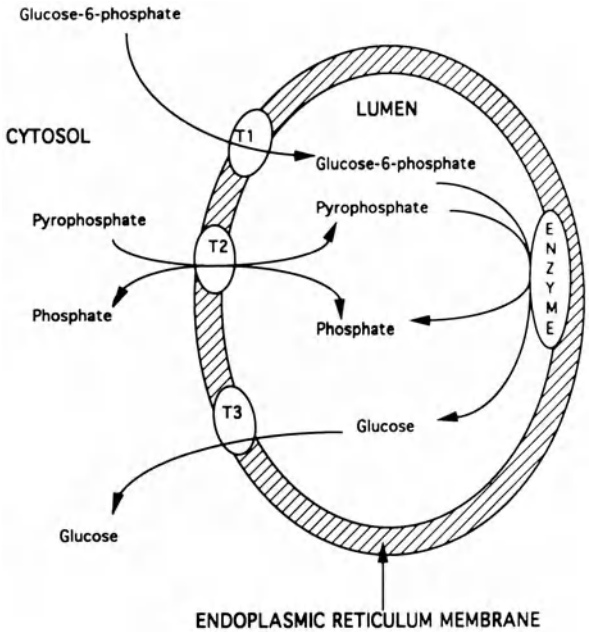

Fig. 2. The hepatic glucose-6-phosphatase system. (From Burchell [28])

weakness and growth retardation [32]. Partial degradation of glycogen results in the formation of limit dextrin, which behaves as a foreign body in liver and muscle. In the liver it leads to leakage of transaminases, periportal fibrosis, and even micronodular cirrhosis. Jaundice may occur. In the muscles it may elicit elevation of creatine kinase and contribute to myopathy. Patients often have ketosis at fasting, high cholesterol levels, and *hyperbetalipoproteinemia* without excessive triglycerides. The abnormalities improve with age.

The finding of different defects of the debranching enzyme system in liver and muscle has considerably improved our understanding of the clinical heterogeneity [33]. Deficiencies exist of one or both catalytic sites of the debranching enzyme protein, i.e., tranferase and debranching enzyme glucosidase. The transferase transfers the three last glucose residues adjacent to a branching point to the end of another chain, after the degradation of the outer chains of the glycogen molecule by phosphorylase. The glucosidase hydrolyzes the remaining glucose molecule off the branch point. Mixed hepatic and myogenic GSD III occurs most frequently and consists of two variants:

- GSD IIIa, in which both transferase and glucosidase are deficient in liver and muscles due to absence of the total debranching protein
- GSD IIId, in which only transferase is deficient in liver and muscles.
- GSD IIIb is a pure liver variant without myopathy and it is caused by the total absence of debranching enzyme protein in the liver, whereas it is normally present and active in the muscles. This occurs in a minority of the patients.

Diagnostic Tests

An oral glucose test is performed to differentiate GSD III (and other liver glycogenoses) from GSD I. A moderate increase of serum lactate is often observed in both GSD III and deficiencies of the phosphorylase system. If the lactate does not rise abnormally, an oral galactose test is performed, which shows a markedly exaggerated lactate increase. A fasting glucagon test might support the tentative diagnosis of GSD III if the glucose curve is flat (see chapter by Fernandes and Saudubray on "Diagnostic Procedures"). The definite diagnosis requires a liver and/or muscle biopsy for confirming debranching enzyme deficiency, biochemically or with a DNA technique. A muscle biopsy should be performed if serum creatine kinase is abnormally increased.

Treatment and Prognosis

Dietary treatment is less demanding than in GSD I. Carbohydrates should be given frequently when the patient is young. Milk products and fruits can be allowed without restriction, as galactose and fructose can be normally converted into glucose. Gastric drip feeding at night and uncooked starch induce catch-up growth, reduce liver size, and decrease serum transaminases [34]. Protein enrichment of meals and drip feeding may counteract the drain from muscle protein and markedly improve muscle function [32]. Thus, the composition of the diet should be approximately 55%–60% carbohydrates, 15%–20% protein, and fat, predominantly polyunsaturated, the remainder. The prognosis, favorable for the purely hepatic GSD IIIb, is less favorable for the other two mixed GSD III types, as severe myopathy and cardiomyopathy may develop even after a long latency.

Genetics

The inheritance is autosomal recessive. The gene for muscle GSD III has been cloned and localized to chromosome 1 at p21 [35]. Antenatal diagnosis is possible by assay of amniotic fluid cells and chorionic villi.

Phosphorylase Deficiency of the Liver (GSD VI) and Phosphorylase b Kinase Deficiency of the Liver (GSD IX)

GSD IX occurs much more frequently and shows more clinical and genetic heterogeneity than GSD VI [36]. Nevertheless, both defects of the phosphorylase system are discussed together because the clinical symptoms and metabolic derangements show much similarity.

Clinical Presentation

Pronounced *hepatomegaly*, a protruded abdomen, and a mild tendency to fasting *hypoglycemia* are the most striking features in early childhood. The liver enlargement decreases slowly and usually disappears at puberty. The spleen and kidney are normal in size. Muscle hypotonia and weakness improve, except in muscle variants of GSD IX. Slightly retarded motor development and retarded growth also normalize gradually. Puberty is delayed in some patients. Mental development is normal. In some cases the course is so mild that hepatomegaly is accidentally found at a routine examination. There are a few exceptions to this usually mild course in GSD IX, of which many variants exist. Rare cases have been described of combinations of hepatic symptoms and myopathy [37], hepatic symptoms and proximal renal tubular acidosis [38], myopathy only [39], and even fatal cardiomyopathy [40].

Metabolic Derangement

Deficiencies of phosphorylase and its activating enzyme phosphorylase b kinase impair the cleavage of glycosyl molecules from the straight chains of glycogen. Since the enzyme defects are usually partial, glycogen degradation is not totally blocked. Also, gluconeogenesis remains intact. Mild fasting hypoglycemia and fasting *ketosis*, moderate *hyperlipidemia* (serum cholesterol more elevated than serum triglycerides), and elevated serum transaminases are evident at a young age, but normalize completely before or at puberty [41].

Diagnostic Tests

An oral glucose test is performed to differentiate deficiencies of the phosphorylase system (and GSD III) from GSD I. A moderate increase of serum lactate is often observed as in GSD III. If the lactate curve does not rise abnormally, an oral galactose test is performed, which shows a markedly exaggerated lactate increase as in GSD III. The glucagon test is of doubtful help, as the increase of glucose is variable. Instead, enzyme assays of erythrocytes should be performed if liver phosphorylase b kinase deficiency is suspected. The enzyme is measured by its activation of inactive phosphorylase b into active phosphorylase a [36]. However, normal phosphorylase b kinase activity in the erythrocytes does not rule out hepatic GSD IX. As the enzyme has many isoenzymes, the diagnosis can be missed without studies of liver and muscle, the latter only in rare cases of muscle involvement.

Enzyme studies of the liver are indicated for diagnosing GSD VI.

Treatment and Prognosis

Dietary treatment, if necessary at all, should be limited to young children. For this age group a late supper should be given if the child has an infectious disease. Prolonged fasting must be prevented. The inclusion of polyunsaturated fat in the diet is a very effective means of suppressing hypercholesterolemia. Retarded growth, which is a worry for many patients, usually normalizes without special treatment.

The prognosis is good for most patients with GSD VI and for hepatic GSD IX, but no prediction is possible for GSD IX with muscle involvement. An adult patient with myopathy and with almost incapacitating loss of muscle strength improved dramatically after intravenous or oral administration of glucose [39].

Genetics

GSD VI has an autosomal recessive mode of inheritance. The gene has been mapped to chromosome 14 [42].

GSD IX is inherited both as a X-linked recessive trait [41] (the majority of the patients) and an autosomal recessive trait [36].

The genes for the X-linked GSD IX (the classical form and a variant) have both been mapped in the Xp22 region of the X chromosome at closely adjacent loci [43].

Branching Enzyme Deficiency (GSD IV)

Clinical Presentation

Marked enlargement of the liver with progressive cirrhosis, splenomegaly, muscle hypotonia and weakness, hypo- or areflexia, retarded motor milestones, and growth retardation are conspicuous features. Fasting hypoglycemia may occur. Most patients die in the first 4 years of life. Exceptionally, muscle involvement and even fatal cardiomyopathy have been reported [44].

Metabolic Derangement

The enzyme defect causes insufficient ramification of the glycogen molecule, and its prolonged inner and outer chains give it the appearance and properties of *amylopectin*. Apparently this abnormal glycogen acts as a foreign body towards the development of *liver cirrhosis*. Furthermore, the glucose release from it is hampered.

Diagnostic Tests

Because of the unspecific liver symptoms, the diagnosis is usually only suspected at the histological examination of a liver biopsy. The hepatocytes contain large deposits that are periodic acid-Schiff (PAS) staining, but partially resistant to diastase digestion. Electromicroscopy shows accumulation of fibrillar aggregations that are typical for amylopectin. The diagnosis is confirmed by enzyme assay of a liver biopsy or fibroblasts. There is no explanation for the different clinical expression of the generalized enzyme defect.

Treatment and Prognosis

Nutritional management with corn starch and gastric drip feeding can improve the clinical condition temporarily, but so far liver transplantation has been the only successful treatment [45]. The clinical course improved remarkably, despite the fact that branching enzyme remained absent in various tissues.

Genetics

The inheritance is autosomal recessive. The gene has been cloned and localized to chromosome 3 [46]. Antenatal diagnosis is feasible by enzyme assay of cultured amniotic fluid cells.

Glycogen Synthase Deficiency (GSD 0)

This rare enzyme defect leads to hypoglycogenosis instead of glycogen storage, because glycogen synthesis is reduced. Nevertheless, it is grouped under the GSD, and this is due to the fact that it shares some metabolic derangements with GSD III, such as *fasting hypoglycemia* and *ketosis* and postprandial hyperlactacidemia. However, there is more resemblance with so-called ketotic hypoglycemia.

Clinically, the patient presents with occasional hypoglycemic convulsions before breakfast or at inadvertent fasting. The liver is not enlarged or only slightly, and its glycogen content is low. Growth lags gradually behind. Oral tolerance tests with glucose, galactose, and fructose are characterized by a marked increase in serum lactate. The glucagon test is of no help. The enzyme defect can only be demonstrated in the liver, not in other tissues.

Treatment with frequent protein-rich meals benefits the clinical course [47].

Inheritance is probably autosomal recessive.

Generalized Glycogen Storage Disease

Lysosomal α-1,4-Glucosidase Deficiency (GSD II, Pompe's Disease)

Clinical Presentation

Three entities exist: an infantile, juvenile, and adult form, with transitional forms in between.

Infantile Form. This is the most severe. It presents with profound hypotonia, muscle weakness, hyporeflexia, and an enlarged tongue. The heart is

extremely enlarged and the electrocardiogram is characterized by huge QRS complexes and shortened PR intervals. There are usually no cardiac murmurs. The liver has a normal size, unless enlarged by cardiac decompensation. The cerebral development is normal. The clinical course is rapidly downward and the child dies from cardiopulmonary failure or aspiration pneumonia in the first year of life.

Juvenile Form. This shows retarded motor milestones, hypotonia, and weakness of limb girdle and truncal muscles, but no overt cardiac disease. Myopathy deteriorates gradually and the patient dies from respiratory failure before adult age.

Adult Form. This mimicks other myopathies with a long latency. Decreased muscle strength and weakness develop in the third or fourth decade of life. Cardiac involvement is minimal or absent. The slow progressive weakness of the pelvic girdle, paraspinal muscles, and diaphragm results in walking difficulty and respiratory insufficiency, but old age can be attained.

Metabolic Derangement

Metabolic abnormalities are not present in either form, because the lysosomal enzyme defect is outside intermediary metabolism.

Diagnostic Tests

In the infantile form, a tentative diagnosis can be based on the typical ECG abnormalities. For confirmation α-1,4-glucosidase should be determined in tissues containing lysosomes. The preferred tissues are fibroblasts or muscle. The enzyme has its pH optimum between 4.0 and 4.5, the acid environment of lysosomes, and the activity of this "acid maltase" must be differentiated from contamination with a nonspecific cytosolic neutral maltase. Although the enzyme defect is generalized in all three GSD II types, the site of glycogen accumulation is different and the amount varies greatly in different organs and even in different muscles. Residual enzyme activity is found in the adult form, whereas the enzyme is absent in the infantile form. At histological examination of a muscle biopsy, large glycogen-laden vacuoles surrounded by a membrane are found next to freely dispersed glycogen outside the lysosomes. Gradually the huge vacuoles lead to impairment of cell function.

Treatment and Prognosis

So far there is no effective treatment. Improvement of muscle function has been obtained by a high-protein diet and, particularly, a high-protein diet fortified with branched-chain aminoacids [48]. This treatment presumably diminishes the catabolism of muscle protein.

Genetics

The mode of inheritance is autosomal recessive.

The clinical heterogeneity of GSD II is due to mutations at the acid α-glucosidase gene locus, which is localized to q21–q23 of chromosome 17 [49].

Antenatal diagnosis is possible by enzymatic investigation of chorionic villi or cultured amniotic cells.

Muscle Glycogen Storage Diseases

Muscle enzyme defects of the glycogenolytic pathway (from glycogen to glucose-6-phosphate) and the glycolytic pathway (from glucose-6-phosphate to pyruvate and lactate) share several clinical and metabolical abnormalities. Therefore, they are discussed under one heading, despite the fact that enzyme defects of the terminal glycolytic pathway do not usually cause glycogen storage in a strict sense.

The most frequently observed enzyme defects are *myophosphorylase deficiency (GSD V)* and *phosphofructokinase deficiency (GSD VII)*. Much more rare are deficiencies of phosphoglycerate kinase, phosphoglycerate mutase, and lactate dehydrogenase (Fig. 1).

Clinical Presentation

The clinical features vary with age and the biochemical heterogeneity of a particular enzyme. Usually the patient starts to complain of *exercise*

intolerance at adult age. The complaints are tiredness, diminished muscle strength, stiffness, and myalgia after exertion. Brief exertion of great intensity and also less intensive but sustained activity, such as climbing stairs, may cause severe muscle cramps, which can be accompanied by muscle tenderness and swelling, myoglobinuria, and even anuria. The complaints subside after resting. Gradually the patient learns to avoid symptoms by adjusting his activities. Other patients have very mild symptoms consisting of tiredness, which may be dismissed as psychogenic. Exceptionally, progressive weakness of late onset (sixth or seventh decade) without a previous history of cramps has been reported, but also early-onset myopathy with a fatal outcome.

Metabolic Derangement

The metabolic derangements are caused by insufficient fuel supply for muscle function. The first provision of energy from glycogen degradation fails and is not timely followed and replenished by energy from fatty acid oxidation, the activation of which proceeds more slowly. An acute shortage of adenosine triphosphate (ATP), the main source of chemical energy, ensues, as it is insufficiently regenerated from adenosine diphosphate (ADP) [50]. Instead, ADP is increasingly converted to adenosine monophosphate (AMP), and this compound is further deaminated to inosine monophosphate and other purines, which are ultimately degraded to uric acid. Thus, *hyperuricemia* is a marker for cell energy crisis. In some patients the hyperuricemia leads to gout or renal calculi.

Serum creatine kinase levels are usually elevated at resting with further increase after exercise, as a sign of increased muscle catabolism and leakage.

Occasionally, a slight and chronic hemolytic jaundice is observed, because the erythrocytes share an isoenzyme of the defective muscle enzyme. This applies to phosphofructokinase deficiency.

Diagnostic Tests

An *exercise test* (semi-ischemic forearm test, a bicycle ergometer test, or a treadmill test) is used to demonstrate the failure of venous lactate and pyruvate to rise and the production of uric acid, inosine, hypoxanthine, and ammonia to increase excessively [51] (see chapter by Fernandes and Saudubray on "Diagnostic Procedures"). Ammonia is produced by the increased deamination of AMP to inosine monophosphate (IMP), which in turn is degraded to inosine, hypoxanthine, and uric acid. The exercise test, which is immediately stopped at the first signs of myalgia or cramps, can be resumed at a lower level of exertion after a short rest, without untoward symptoms. This remarkable "second wind" phenomenon can be explained by the fact that fatty acids become gradually available as an alternative fuel and/or muscle oxygenation improves by vasodilatation.

If the results of the exercise test are abnormal, a metabolic myopathy is probable and should be verified by assaying all enzymes of the glycogenolytic–glycolytic pathway in a muscle biopsy. Histological examination of the muscle biopsy is often nonspecific. Glycogen accumulation is observed between myofibrils (GSD V), or the glycogen is composed of finely fibrillar material like amylopectin (GSD VII).

Treatment and Prognosis

Muscle function may be influenced favorably or unfavorably by the diet.

The effect of protein may be favorable, as it compensates for increased muscle catabolism [52].

The effect of glucose is ambiguous: favorable for phosphorylase deficiency as the localization of the enzyme defect leaves glycolysis intact; unfavorable for enzyme defects distal to glucose-6-phosphate because it does not only overload the blocked glycolytic pathway, but also inhibits lipolysis and thus deprives muscle of free fatty acids and ketone bodies [53].

Genetics

All enzyme defects have an autosomal recessive inheritance except phosphoglycerate kinase deficiency, which is an X-linked disorder (for a review see [54]). Both GSD V and GSD VII genes have been cloned and mutations responsible for the disease identified [55, 56].

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Disorders of Galactose Metabolism

R. Gitzelmann

Whenever you consider a galactose disorder, stop milk feeding first and only then seek a diagnosis

CONTENTS

Galactose forms, together with its 4′-epimer glucose, the disaccharide lactose which is the principal carbohydrate in milk, providing 40% of the energy. Thus, galactose consumption is highest in infancy, and in those who are galactose intolerant toxic effects occur early in life. Therefore, detection after birth, rapid diagnosis, and immediate therapy are mandatory.

Ingested lactose is hydrolyzed to galactose and glucose in the small intestine. After absorption, galactose is converted to galactose-1-phosphate, uridine diphosphate (UDP) -galactose, and UDP-glucose (Fig. 1). UDP-glucose is the glycosyl carrier in several reactions, including the synthesis of glycogen and the metabolism of galactose-1-phosphate. In the latter, glucose-1-phosphate is produced and metabolized to glucose in liver.

Three inborn errors of galactose metabolism are known. Galactokinase deficiency is the most insidious, since it results in the formation of nuclear cataracts without provoking symptoms of intolerance. Galactose-1-phosphate uridyltransferase deficiency exists in two forms. The complete or near-complete deficiency is life-threatening and affects not only the eye lens, but also liver, kidney, and brain. Partial deficiency is usually, if not always, benign. Uridine diphosphate galactose 4′-epimerase deficiency also exists in two forms. The very rare, profound deficiency resembles clinically classical galactosemia. The more frequent, partial deficiency is benign. Review articles are recommended for detailed information [1–3].

Galactokinase Deficiency

Clinical Presentation

Cataracts are the only consistent manifestation of the untreated disorder, though pseudotumor cerebri has been described. Liver, kidney, and brain damage, as seen in transferase deficiency (below), are not features of untreated galactokinase deficiency, and hypergalactosemia and galactose-galactitol-glucose diabetes are the only chemical signs.

Metabolic Derangement

Subjects with galactokinase deficiency lack the ability to phosphorylate galactose (Fig. 1). Consequently, nearly all of the ingested galactose is excreted, either as such or as its reduced metabolite, galactitol, formed by aldose reductase. *Cataracts* result from the accumulation of *galactitol* in the lens, causing osmotic swelling of lens fibers and denaturation of proteins.

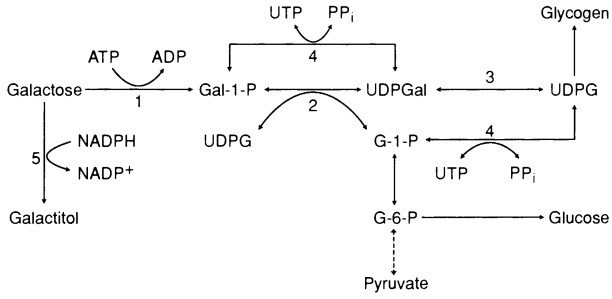

Fig. 1. Galactose metabolism (simplified). *Gal-1-P*, galactose-1-phosphate; *G-1-P*, glucose-1-phosphate; *G-6-P*, glucose-6-phosphate; *UDPG*, uridine diphosphoglucose; *UDPGal*, uridine diphosphogalactose; *NADP*, nicotinamide adenine dinucleotide phosphate; *NADPH*, reduced NADP. *1*, Galactokinase; *2*, galactose-1-phosphate uridyltransferase; *3*, UDP-galactose 4′-epimerase; *4*, UDP-glucose (UDP-galactose) pyrophosphorylase; *5*, aldose reductase

Diagnostic Tests

Newborns with the defect are discovered by mass screening by methods for detecting elevated blood galactose [4], provided they have been fed prior to the test. This is not always the case as today, in many nurseries, babies whose mothers have not yet commenced lactating are tied over by glucose or maltodextrin feeds. Any chance finding of a *reducing substance in urine*, especially in children or adults with nuclear cataracts, calls for the identification of the excreted substance. Besides galactose, galactitol and glucose may be found. Every person with nuclear cataracts ought to be examined for galactokinase deficiency.

Final diagnosis is made by assaying galactokinase activity in heparinized whole blood, red cell lysates, or liver or fibroblasts [4, 5]. Heterozygotes have intermediate activity in erythrocytes [6, 7]. Reports of galactokinase variants have appeared [1, 2].

Treatment and Prognosis

Treatment may be limited to the elimination of milk from the diet. Minor sources of galactose, such as milk products, green vegetables, legumes, drugs in tablet form etc., can probably be disregarded, since it can be assumed that the small amounts of ingested galactose are either metabolized or excreted before significant amounts of galactitol can be formed. When diagnosis is made rapidly and treatment begun promptly, i.e. during the first 2–3 weeks of life, cataracts can clear. When treatment is late and cataracts too dense, they will not clear completely, or not at all, and must be removed surgically. In patients who have had their lenses removed, recurring cataracts may appear, originating from remnants of the posterior lens capsule. This can be avoided by continuing the diet.

The speculation [7] that heterozygosity predisposes to the formation of presenile cataracts remains an open question [1, 2]. Still, it may be reasonable for heterozygotes to restrict their milk intake, though scientific proof of the merits of this measure is lacking.

Genetics

The mode of inheritance is autosomal recessive, the frequency unknown; the disorder is rare. Galactokinase deficiency occurs in many parts of the world. It may be more frequent in the Balkan countries, former Yugoslavia, Rumania, and Bulgaria, where it seems to favor gypsies [8].

Deficiency of Galactose-1-Phosphate Uridyltransferase

Clinical Presentation

Two forms of the deficiency exist. Infants with complete or near-complete deficiency of the enzyme (*classical galactosemia*) have normal weight at birth, but as they start drinking milk lose more weight than their healthy peers and fail to regain birth weight. Symptoms appear in the second half of the first week and include refusal to feed, vomiting, jaundice, lethargy, hepatomegaly, edema, and ascites. Death from liver and kidney failure and sepsis may follow within days. Mental disability may take years to become apparent. Symptoms are milder, and the course is less precipitous when milk is temporarily withdrawn and replaced by intravenous nutrition. Nuclear cataracts appear

within days or weeks and become irreversible within weeks of their appearance.

In many countries, galactosemic newborns are discovered through mass screening for blood galactose or the transferase enzyme, or both, using dried blood spots usually collected between the third and fifth day [4]. At the time of discovery, the first symptoms may have appeared and the infant already have been admitted to hospital, usually for jaundice.

Where newborns are not screened for galactosemia, or when the results of screening are not yet available, diagnosis rests on clinical awareness. It is crucial that milk feeding be stopped as soon as galactosemia is considered and resumed only when a galactose disorder has been excluded. The presence of reducing substance in a routine urine specimen may be the first diagnostic lead. Galactosuria is present, provided the last milk feed does not date back more than a few hours and vomiting has not been excessive. However, owing to the early development of a proximal renal tubular syndrome, the acutely ill galactosemic infant may also excrete some glucose, together with an excess of amino acids. While hyperaminoaciduria may aid in the diagnosis, glucosuria often complicates it. When both reducing sugars, galactose and glucose, are present and two tests are done, i.e. a reduction and a glucose test, and the former is strongly and the latter weakly positive, the discrepancy is easily overlooked, glucosuria is recognized and galactosuria missed. Upon withholding of milk galactosuria ceases, but galactitol and amino acids in excess continue to be excreted for a few days.

Partial transferase deficiency is as a rule asymptomatic. It is more frequent than classical galactosemia and most often discovered in mass newborn screening because of moderately elevated blood galactose and/or low transferase activity [9].

Metabolic Derangement

Individuals with profound deficiency of galactose-1-phosphate uridyltransferase can phosphorylate ingested galactose but fail to metabolize galactose-1-phosphate. As a consequence, *galactose-1-phosphate* and galactose accumulate, and *galactitol* is also formed. As in galactokinase deficiency, cataract formation can be explained by galactitol accumulation. The pathogenesis of the hepatic, renal, and cerebral disturbances is less clear, but is likely related to the accumulation of galactose-1-phosphate and perhaps of galactitol.

Diagnostic Tests

Diagnosis is made by assaying transferase on heparinized whole blood or erythrocyte lysates and/or by measuring abnormally high levels of galactose-1-phosphate in red cells. Where rapid shipment of whole blood is difficult, blood dried on filter paper can also be used for a semiquantitative assay. In patients with classical galactosemia, the deficiency of galactose-1-phosphate uridyltransferase is complete or nearly complete. It should be noted that when an infant has received an exchange transfusion, as is often the case, assays in blood must be postponed for 3–4 months. (In some hospitals, a blood specimen, liquid or dried on filter paper, is collected prior to every exchange transfusion). In this situation, the finding of reduced transferase activity in parental blood may provide welcome preliminary information, since in heterozygotes the enzyme activity in red cells is approximately 50% of normal. Cultured skin fibroblasts can also be used for the enzyme assay, as well as liver or kidney cortex. If taken post mortem, the latter specimens should have been adequately collected and frozen, since in vivo cell damage and/or autolysis may result in decreased enzyme activity. Antenatal diagnosis is possible by measuring transferase activity in cultured aminiotic fluid cells or biopsied chorionic villi, or galactitol in amniotic fluid [10]. Restricting maternal lactose intake does not interfere with a diagnosis based on galactitol measurements in amniotic fluid.

In *partial transferase deficiency*, activities of 10%–50% of normal are measured. As a rule, red cell galactose-1-phosphate is also elevated. Several variants of the partial enzyme defect have been reported, of which the best known is the *Duarte* variant. Some variants can be distinguished by enzyme electrophoresis and, more recently, by DNA analysis. Our own experience and published studies [1, 9] suggest that the Duarte variant is usually, if not always, benign. Each newborn with partial transferase deficiency must nevertheless be observed closely, because allelic variants other than Duarte may be operative [1, 2]. Assessment involves blood galactose, erythrocyte

galactose-1-phosphate, transferase activity, enzyme electrophoresis, aminoaciduria, and investigation of the parents [9]. A pragmatic approach to management is described below.

Galactose tolerance tests are notoriously noxious to the child with classical galactosemia and have no place in evaluating the need for treatment in the partial deficiencies [1].

Treatment and Prognosis

Treatment of the newborn with *classical galactosemia* consists of the exclusion of all galactose from the diet and must be started at once, even before results of diagnostic tests are available. When a galactose-free diet is instituted in time, symptoms disappear promptly, jaundice resolves within days, cataracts may clear, liver and kidney functions return to normal, and liver cirrhosis may be prevented.

For the dietary treatment, the following facts are worthy of consideration:

- From early embryonic life on, man is capable of synthesizing UDP-galactose from glucose through the epimerase reaction [11–13]. Therefore, he does not depend on exogenous galactose. Raising a galactosemic child on a diet completely devoid of galactose would cause it no harm, yet such a diet does not exist.
- Milligram amounts of galactose cause an appreciable rise of galactose-1-phosphate in red blood cells, and one must assume that the same happens in sensitive tissues such as brain, liver, and kidney. It is impossible to define toxic tissue levels of galactose-1-phosphate and, therefore, "safe" amounts of dietary galactose – if they exist at all – cannot be defined either. For these reasons it is advisable to watch out even for traces of the sugar and to eliminate it as much as possible.
- Galactosemics certainly synthesize galactose from glucose [1, 12–15]. In galactosemic newborns, first exposed to milk, then diagnosed and treated properly, erythrocyte galactose-1-phosphate stays high for several weeks. This fact and other observations [13–16] are taken as evidence for the continuous "self-intoxication" [14, 15] by the galactosemic, a matter of concern in view of some late complications such as premature ovarian failure [17] and central nervous system dysfunction.

Minimal amounts of galactose from food and hidden sources probably contribute to erythrocyte galactose-1-phosphate, but only real breaks in the diet cause a rise above 6 mg/dl. Such breaks do not cause any discomfort to the patient, who therefore never develops aversion to galactose-containing food. The measurement of urinary galactitol for monitoring treatment has not been successful, as only large amounts of ingested galactose are reflected, with some delay [18].

Treatment of the Newborn Infant. Treating newborns is comparatively easy, as adequate lactose-free formulae with a meat or soya basis are available. Soya formulae from which raffinose and stachyose have been removed are preferable over others. Elimination of milk and milk products is the mainstay of lifelong [19] treatment.

Spoon-Feeding. When spoon-feeding is started, parents must learn to know all other sources of galactose and need assistance from a pediatrician and dietician, who must have recourse to published recommendations [20–24]. A critical workshop report by Clothier and Davidson [22] is especially helpful. Parents are advised to prepare meals from basic foodstuffs; to avoid canned food, halfproducts, and preserves unless they are certified not to contain lactose or galactose; to read and reread labels and declarations of ingredients which may change without notification; to look out for hidden sources of galactose and lactose from milk powder, milk solids, "hydrolysed whey" (a sweetener labeled as such), in drugs in tablet form, toothpaste, baking additives, as fillers, in sausages etc.; to support campaigns for complete food and drug labeling.

Vegetables and Fruits. Parents must be trained to understand that eliminating all galactose from the diet must remain the goal, although it can never be reached. The reason for this is that galactose is present in a great number of vegetables and fruits [24], as a component of galactolipids and glycoproteins, in the disaccharide melibiose, and in the oligosaccharides raffinose and stachyose [25, 26]. The latter two contain galactose in α-galactosidic linkage not hydrolyzable by human small intestinal mucosa in vitro or in vivo [26]. They are often considered safe for consumption by galactosemics. Yet this may not be the case when the small intestine is colonized by bacteria capable of releasing galactose. Raffinose- and stachyose-rich vege-

tables (beans, peas, lentils etc.) should not be eaten by a galactosemic who has diarrhea. While beans, peas, and lentils should never make up a full meal or a large dish, a few small seeds, e.g., in a dish of young string beans, should not cause concern. Nevertheless, gastroenterologists insist that the small intestine may be colonized in the absence of diarrhea; obviously, the issue is not closed.

Cheese. It is not generally known that Swiss cheeses of the Emmentaler, Gruyères, and Tilsiter types are galactose and lactose free as these sugars are cleared by the fermenting microorganisms [27]. Other hardened cheeses may prove equally safe for galactosemics. Calcium supplements should be prescribed before cheese is introduced to the child's diet; supplements may be needed by older children and young adults as well [28]. Calcium prescriptions containing lactobionate [24] must be avoided, because β-galactosidase of human intestinal mucosa hydrolyzes lactobionate, freeing galactose [29].

Breaks of Discipline. Whether single or repeated breaks of discipline such as an occasional ice cream by a school-age child or adult galactosemic will cause any damage cannot be said. Uridine supplements to galactosemics have been proposed [30]; their therapeutic value has not been demonstrated and therefore they can not be recommended. Dietary treatment of female patients is continued during pregnancy [31].

Complications of Treated Galactosemia. Mild growth retardation, delayed speech development, verbal dyspraxia, difficulties in spatial orientation and in visual perception, and mild intellectual deficit have been variably described as complications of treated galactosemia. The complete set of sequelae is not necessarily present in every patient, and the degree of handicap appears to vary widely. *Ovarian dysfunction*, an almost inescapable consequence of galactosemia which is not prevented even by strict diet, is often signaled early in infancy or childhood by hypergonadotropinism [17]. Treatment consists in the routine administration of estrogen and progesterone and may help in establishing reproductive function during puberty or in reestablishing fertility. Prescription is hampered by the fact that seemingly all drug tablets contain lactose, providing 100 mg or more of the noxious sugar per treatment day.

Treatment of Partial Transferase Deficiency. Because it is impossible to decide whether partial transferase deficiency needs to be treated (see above), some centers have adopted a pragmatic approach, prescribing lactose-free formula to all such infants discovered by newborn screening limited to 4 months [9]. The formula must not be completely free of galactose. When at the end of a 1-week trial with a daily supplement of 2–3 dl cows milk aminoaciduria is normal and erythrocyte galactose-1-phosphate is zero or below 2 mg/dl, the infant is returned to normal nutrition and declared healthy.

Dietary Treatment in Pregnant Women at Risk. Based on the presumption that toxic metabolites deriving from galactose ingested by the heterozygous mother accumulate in the galactosemic fetus, mothers are often counseled to refrain from drinking milk for the duration of pregnancy. Yet, despite dietary restriction by the mother, galactose-1-phosphate and galactitol accumulate in the fetus [1, 13, 21] and in the amniotic fluid [10]. It is hypothesized [12–15] that the affected fetus produces galactose-1-phosphate endogenously from glucose-1-phosphate via the pyrophosphorylase-epimerase pathway (Fig. 1), which also provides for UDP-galactose and thus secures the biosynthesis of galactolipids and galactoproteins indispensable for cell differentiation and growth. Since the affected fetus does not depend on, yet may suffer from, the galactose he receives from his mother via the placenta, galactose restriction is the prudent stance for pregnant mothers. Affected newborns of treated mothers appear healthy at birth.

Genetics

The mode of inheritance is autosomal recessive. In large European screening series, birth incidence of classical galactosemia is 1 in approximately 55 000. The gene is situated on chromosome 9. The gene and its cDNA have been cloned, and mutation analysis is in full progress [32]. Nevertheless, some genotype–phenotype matching is already possible. For instance, homozygosity for the Q188R mutation, unfortunately prevalent, has been associated with unfavorable clinical outcome [32]. Because transferase polymorphism abounds [1, 2, 32], partial transferase deficiency is more frequent than classical galactosemia. Owing to the

high gene frequency of the allelic Duarte variant, compound heterozygosity for galactosemia/Duarte is relatively common, occurring once in approximately 3000–4000 newborns.

Uridine Diphosphate Galactose 4'-Epimerase Deficiency

Clinical Presentation

This disorder [1, 2] exists in two forms, both of which are discovered through newborn screening using suitable tests sensitive to both galactose and galactose-1-phosphate in dried blood [4]. Infants with the mild form appear healthy [11]. The enzyme defect is incomplete; reduced stability and greater than normal requirement for the coenzyme nicotinamine adenine dinucleotide (NAD) have been described [33]. Milk-fed newborns with the mild form singled out in newborn screening are well and have neither hypergalactosemia, nor galactosuria, nor hyperaminoaciduria.

In the only two known children with the severe form of the disorder, the enzyme defect was subtotal. Both newborns presented with vomiting, jaundice, and hepatomegaly reminiscent of untreated classical galactosemia; one was found to have elevated blood methionine on newborn screening, both had galactosuria and hyperaminoaciduria, one had cataracts, and one had sepsis.

Metabolic Derangement

The enzyme deficiency provokes an accumulation of UDP-galactose after milk feeding. This build-up also results in the accumulation of galactose-1-phosphate (Fig. 1).

Diagnostic Tests

The deficiency should be suspected when red cell galactose-1-phosphate is measurable, while galactose-1-phosphate uridyltransferase is normal. Diagnosis is confirmed by the assay of epimerase in erythrocytes. Heterozygous parents have reduced epimerase activity, a finding which usually helps in the evaluation. Diagnosis of the severe form is based on the clinical symptoms, chemical signs, and more marked deficiency of epimerase in red cells.

Treatment and Prognosis

The infants with the mild form of epimerase deficiency thus far described have not required treatment, but it is advisable that the family physician or pediatrican examine one or two urine specimens for reducing substances, and have aminoaciduria excluded, within a couple of weeks after diagnosis while the infant is still being fed with milk. He or she should also watch the infant's psychomotor progress, without, however, causing concern to the parents.

The child with the severe form of epimerase deficiency is unable to synthesize galactose from glucose and is therefore galactose dependent. Dietary galactose in excess of actual biosynthetic needs will cause accumulation of UDP-galactose and galactose-1-phosphate, the latter being one presumptive toxic metabolite in galactosemia. When the amount of ingested galactose does not meet biosynthetic needs, synthesis of galactosylated compounds such as galactoproteins and galactolipids is impaired. As there is no easily available chemical parameter on which to base the daily galactose allowance – e.g., blood phenylalanine in phenylketonuria – treatment is extremely difficult. Both children known to suffer from the disorder have impaired psychomotor development.

Genetics

Epimerase deficiency is inherited as an autosomal recessive trait. The epimerase gene resides on chromosome 1; it is being cloned.

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Disorders of Fructose Metabolism

G. Van den Berghe

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Fructose is one of the main sweetening agents in the human diet. It is found in the free form in honey, fruit, and many vegetables and associated with glucose in the form of the disaccharide sucrose in even more numerous foods and beverages. Sorbitol, also widely distributed in fruit and vegetables, is converted into fructose in the liver by sorbitol dehydrogenase (Fig. 1). Two inborn errors of fructose metabolism are known. Essential fructosuria is a completely harmless anomaly characterized by the appearance of fructose in the urine after the intake of fructose-containing foods. In hereditary fructose intolerance (HFI), fructose provokes prompt gastrointestinal discomfort and hypoglycemia upon ingestion, although sensitivity varies from patient to patient; it may cause liver and kidney failure when taken persistently and becomes life-threatening when given intravenously. Fructose-1,6-bisphosphatase deficiency, sometimes also considered an inborn error of fructose metabolism, will be discussed in Chap. 8. It is manifested by the appearance of hypoglycemia and lactic acidosis during fasting and may also be life-threatening.

Essential Fructosuria

Clinical Presentation

Essential fructosuria is a rare "non-disease" which is detected by routine screening of the urine for reducing sugars [1]. It is caused by the deficiency of fructokinase [2], the first enzyme of the specialized fructose pathway (Fig. 1). Fructokinase is normally only found in liver, kidney, and small intestinal mucosa.

Metabolic Derangement

In the case of deficiency, ingested fructose is in part excreted as such in the urine and in part slowly metabolized by an alternate pathway, namely conversion into fructose-6-phosphate by hexokinase in adipose tissue and muscle.

Diagnostic Tests

Fructose gives a positive test for reducing sugars and a negative reaction with glucose oxidase. It can be identified by various techniques, including thin-layer chromatography [3]. Fructose tolerance tests (see chapter 2) provoke neither an increase in blood glucose as in normal subjects, nor hypoglycemia as in hereditary fructose intolerance and in fructose-1,6-bisphosphatase deficiency.

Treatment and Prognosis

Dietary treatment is not indicated and the prognosis is excellent.

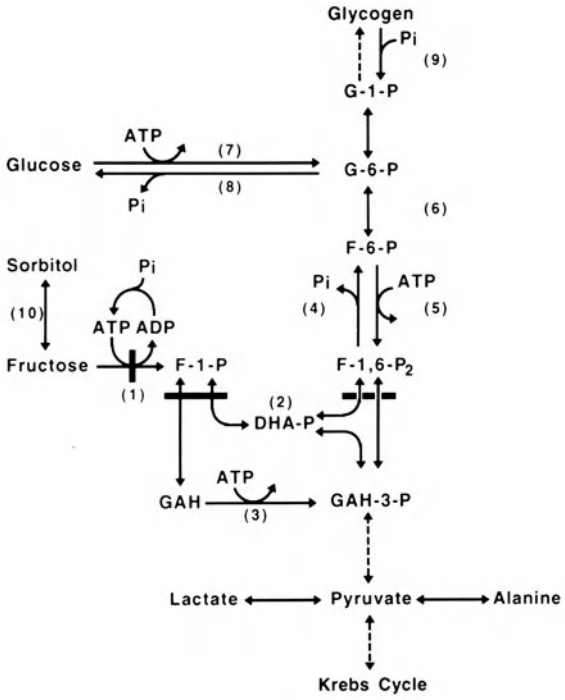

Fig. 1. Fructose metabolism. The specialized pathway of fructose metabolism, found in liver, kidney cortex, and small intestinal mucosa is composed of fructokinase (*1*), aldolase B (*2*), and triokinase (*3*). Aldolase B also intervenes in the glycolytic–gluconeogenic pathway, of which moreover the following enzymes are depicted: fructose-1,6-bisphosphatase (*4*), phosphofructokinase (*5*), glucose-6-phosphate (*G-6-P*) isomerase (*6*), glucokinase and hexokinase (*7*), glucose-6-phosphatase (*8*). Also shown are glycogen phosphorylase (*9*) and sorbitol dehydrogenase (*10*). *F*, fructose; *G*, glucose; *P*, phosphate; *Pi*, inorganic phosphate; F-1, 6-P_2, fructose-1, 6-bisphosphate *DHA-P*, dihydroxyacetone phosphate; *GAH*, glyceraldehyde; *ATP*, adenosine triphosphate; *ADP*, adenosine diphosphate. The two enzyme defects in fructose metabolism are depicted by *solid bars* across the *arrows*; the diminished activity of aldolase B toward F-1,6-P_2 in hereditary fructose intolerance is depicted by a *broken bar*

Genetics

The mode of inheritance is autosomal recessive, and the frequency of the homozygotes has been estimated at 1:130000 [4].

Hereditary Fructose Intolerance

Clinical Presentation

HFI provokes no symptoms as long as the affected subjects do not ingest fructose. Typically, babies do well during breast feeding. Symptoms appear upon introduction of cow's milk formulas sweetened with sucrose, or at weaning when fruits and vegetables are given [5, 6]. Certain patients are very sensitive to fructose, whereas others can tolerate moderate intakes of fructose (up to 250 mg/kg per day, as compared to an average intake of 1–2 g/kg per day in Western societies). Generally, the first signs are gastrointestinal discomfort and hypoglycemia following meals containing fructose. Nausea, vomiting, pallor, sweating, trembling, lethargy, and eventually jerks and convulsions may be observed. If the condition is not recognized and fructose not excluded from the diet, failure to thrive, liver disease manifested by hepatomegaly, jaundice, bleeding tendency, eventually edema and ascites, and proximal renal tubular dysfunction appear. The younger the child and the higher the intake of fructose, the more severe the clinical picture, which, when its cause is not recognized and treated appropriately, may lead to liver and kidney failure and eventually to death.

In young infants, HFI can be suspected if the mother, being aware that her baby does not tolerate certain foods, has excluded them from the diet, so that the infant develops normally. In older children, a distinct aversion toward foods containing fructose develops, which protects them, but is sometimes considered psychotic behaviour. At school age, HFI is occasionally recognized when finding hepatomegaly or growth delay [7]. At adult age, some cases are only diagnosed after life-threatening perfusions with fructose [8] or *sorbitol*. Because approximately half of the adults with HFI are completely free of caries, the diagnosis has also been made by dentists. Although since its recognition as an inborn error of metabolism in 1957 [5] several hundred patients have been identified, these observations indicate that affected subjects remain undiagnosed in the general population.

Metabolic Derangement

HFI is caused by the inability of the second enzyme of the fructose pathway, aldolase B (Fig. 1), to split fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde [9]. In the tissues that possess the specialized fructose pathway, namely liver, kidney cortex, and small intestinal mucosa, fructose can thus not be converted into glucose and lactate. Moreover, as a consequence of the high activity of fructokinase, ingestion and even more intravenous infusion of

fructose results in accumulation of fructose-1-phosphate. This accumulation has two major effects (reviewed in [10]): it inhibits the production of glucose, hence inducing hypoglycemia, and it provokes depletion of adenosine triphosphate (ATP), an essential component for all cellular functions.

Inhibition of Glucose Production. This results from a block of both hepatic glycogenolysis, which maintains blood glucose in the early postprandial phase, and gluconeogenesis, which provides glucose during more prolonged fasting. The block of glycogenolysis results from inhibition by fructose-1-phosphate of phosphorylase, the enzyme that catalyzes the liberation of glucose-1-phosphate from glycogen. The impairment of gluconeogenesis results from inhibition by fructose-1-phosphate of the condensation of glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate into fructose-1,6-bisphosphate, also catalyzed by aldolase B, and of the conversion of fructose-6-phosphate into glucose-6-phosphate, catalyzed by glucose-6-phosphate isomerase.

Depletion of ATP. This (and of the related nucleotide guanosine triphosphate, GTP) in the fructose-metabolizing tissues is a consequence of their utilization in the formation of high amounts of fructose-1-phosphate. It is accompanied by a depletion of inorganic phosphate, which is required to regenerate ATP from adenosine diphosphate (ADP) in the mitochondria (Fig. 1). Because both GTP and inorganic phosphate are inhibitors of liver adenosine monophosphate (AMP) deaminase, a rate-limiting enzyme of the catabolism of the adenine nucleotides, their depletion provokes a degradation of the hepatic adenine nucleotide pool, leading to increased production of uric acid. The depletion of ATP, the energy currency of the cell, induces a series of disturbances, including inhibition of protein synthesis and ultrastructural lesions, which are responsible for gastrointestinal discomfort, and hepatic and renal dysfunction.

It should be noted that the intravenous administration of fructose to normal subjects also induces the metabolic derangements described in the previous paragraph, although higher doses are required than in patients with HFI, as demonstrated by ^{31}P nuclear magnetic resonance spectroscopy [11]. In normal subjects, intravenous fructose raises glycemia, owing to its rapid conversion into glucose. However, the equally rapid conversion of fructose into lactate may provoke metabolic acidosis. For these reasons, the use in parenteral nutrition of fructose, mixtures of glucose and fructose known as invert sugar, and sorbitol has been strongly discouraged [12].

Diagnostic Tests

Whenever HFI is suspected, fructose should be immediately completely withdrawn from the diet. The beneficial effect of withdrawal, usually seen within days, provides a first diagnostic clue. Only after some weeks should an *intravenous fructose tolerance test* be performed (see chapter 2). Oral tests are not recommended because they provoke more ill effects and are less reliable [13]. Laboratory findings in patients with a sustained fructose intake are those of liver disease (elevations of serum transaminases and bilirubin, depletion of blood clotting factors), and of proximal tubular dysfunction (proteinuria, mellituria, generalized hyperaminoaciduria, metabolic acidosis).

To confirm the diagnosis, the activity of aldolase B should be measured in a biopsy of liver, kidney cortex, or intestinal mucosa. In HFI, the capacity of aldolase B to split fructose-1-phosphate is reduced, usually to a few percent of normal [9, 13], although residual activities as high as 30% of normal have been reported [8]. There is also a distinct, but less marked reduction of the activity of aldolase B toward fructose-1,6-bisphosphate. As a consequence, the ratio of the V_{max} toward fructose-1,6-bisphosphate versus the V_{max} toward fructose-1-phosphate, which is approximately 1 in control liver, is increased to 2 to ∞ in the patients. The activity of aldolase is normal in blood cells, muscle, and skin fibroblasts, which contain a different isozyme, aldolase A.

Treatment and Prognosis

Treatment consists in the elimination of all sources of fructose from the diet. This involves suppression of all foods in which fructose and/or sucrose or sorbitol occur naturally or have been added during processing [14]. That fructose may be present in medications and in infant formulas should also be verified. A list of foods to use and to avoid is given to parents (Table 1). Sucrose

Table 1. Sucrose- and fructose-free diet (from [14])

Food group	Foods to use	Foods to avoid
Bread	White and brown bread (ask for composition), soda crackers, saltines	All other bread, crackers, biscuits, cookies
Cereals	Cooked or ready-to-eat cereals (except sugar-coated cereals)	Sugar-coated cereals
Cheese	Any kind	None
Desserts	Natural yoghurt, pudding without sugar, homemade ice cream	All desserts containing sugar (cake, pie, cookies, candy, puddings, jello, ice cream, sherbet), honey, fruit or fruit juice, most of the products for diabetics
Eggs	Any kind	None
Fat	Butter, margarine, oil, homemade mayonnaise and salad dressings made without sugar	Mayonnaise, salad dresings made with sugar
Fruits	None	All fruits and fruit juices, dates
Meat, fish	Beef, chicken, fish, lamb, pork, turkey, veal	Ham, bacon, luncheon meat, and any other meats in which sugar is used in processing
Milk	Any kind	Milk preparations with added sugar
Miscellaneous	Coffee, tea, vegetable juices and soups from allowed vegetables, dietetic beverages with sugar substitutes, cocoa, salt, pepper and other condiments	Ketchup, chili sauces and other sauces containing sugar, carbonated beverages honey, jam, jellies, honey, maple syrup, preserves
Nuts	Any kind	Sugar coated
Potatoes and substitutes	White potatoes, macaroni, noodles, spaghetti, rice	Sweet potatoes
Sweeteners	Glucose, dextrin, maltose, calorie-free sweeteners	Sucrose, fructose, sorbitol
Vegetables	Asparagus, cabbage, cauliflower celery, green beans, green peppers, lettuce, peas, spinach, wax beans, root vegetables except carrots	Carrots, leek, onions, sweet maize, canned vegetables with added sucrose

Labels of all canned, packaged or processed foods, and of medications, should be checked to be sure that sugar or fruit is not used.

should be replaced by glucose, maltose, and/or starch to prevent the fructose-free diet from containing too much fat. After institution of the diet, most abnormalities disappear rapidly, except hepatomegaly, which may persist for months and even years [15]. The reason for this is unclear. An insufficient restriction of fructose has been reported to cause isolated growth retardation, as evidenced by catch-up growth on a stricter diet [7]. The intake of fructose should thus, at least in childhood, not be determined by subjective tolerance.

Needless to say, patients (and their parents) should be made aware of the fact that infusions containing fructose, sorbitol, or invert sugar are life-threatening for them and should report fructose intolerance on any hospital admission.

Genetics

HFI is inherited as an autosomal recessive trait. Studies of the aldolase B gene in 50 predominantly European patients have shown that the most frequent mutation, accounting for 67% of alleles, is a G → C substitution, resulting in an Ala149 → Pro change [16]. This mutation also creates a new recognition site for the restriction enzyme AhaII, which renders it easily detectable. Next in frequency, accounting for 16% of alleles, is a C → A substitution, resulting in an Ala174 → Asp change, which is found in Switzerland and in Southern Europe. Both mutations are believed to each originate from a single ancestor. In the other patients, deletions are found ranging in size from a single base pair to 1.65 kb [17]. In patients from the United States and Canada, the A149P and A174D mutations are found in the same order of prevalence, although at a slightly lower frequency than in Europe [18]. The hetereogeneity of HFI, evidenced by the variability of the sensitivity to fructose and of the residual activity of aldolase B toward fructose-1-phosphate, is thus also apparent at the gene level. The frequency of HFI is estimated at 1:20 000.

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Disorders of Gluconeogenesis

N.R.M. Buist

CONTENTS

Gluconeogenesis is defined as the net formation of glucose from non-carbohydrate precursors such as pyruvate, lactate, glycerol, and certain amino acids. It is crucial for survival because glucose, the primary substrate for a number of tissues, most notably the brain, is only available during feeding, and liver glycogen stores can only provide glucose for a few hours. Gluconeogenesis and glycolysis have many enzymes in common (Fig. 1). Exceptions are the enzymes that catalyze the four irreversible steps which ensure a unidirectional flux from pyruvate to glucose:

- Pyruvate carboxylase (PC)
- Phosphoenolpyruvate carboxykinase (PEPCK)
- Fructose-1,6-bisphosphatase (FBPase)
- Glucose-6-phosphatase (G-6-Pase)

Defects of all four gluconeogenic enzymes are known. Common to all are the tendency to facile *hypoglycemia* induced by fasting and *lactic acidosis* due to failure to recycle lactate to glucose. The deficiency of G-6Pase (Chap. 5) and FBPase usually spare the brain, unless it is damaged by recurrent hypoglycemia. The deficiencies of PEPCK and PC are associated with a progressive neurodegenerative disorder which is usually fatal.

Fructose-1,6-Bisphosphatase Deficiency

Clinical Presentation

Most commonly, the disorder presents in the first hours or days of life with episodes of profound lactic acidosis and hypoglycemia. In about half of the reported cases, the first attack may not occur for weeks or months; in mild variants it may not occur for many years [1]. If the patient survives the acute attack, recurrent episodes can occur following ingestion of fructose or sucrose, any reduction in caloric intake, or periods of increased metabolic stress such as infection. Between episodes, the acidosis may resolve completely or can be mild, intermittent, or chronic. The tendency to these attacks lessens with age. The hypoglycemia is responsible for neuroglycopenic syndromes including seizures and, if not recognized, can result in permanent brain damage. The lactic acidosis causes hyperventilation and can lead to transient neurologic symptoms. Hypotonia and moderate hepatomegaly can be seen. Although the severe episodes can be fatal, in the majority of survivors growth and intellectual development are normal. The chronic progressive neurodegenerative conditions associated with lactic acidosis caused by many of the enzyme defects of energy generation do not occur in FBPase deficiency. When the condition is under good control, most patients are clinically normal [2].

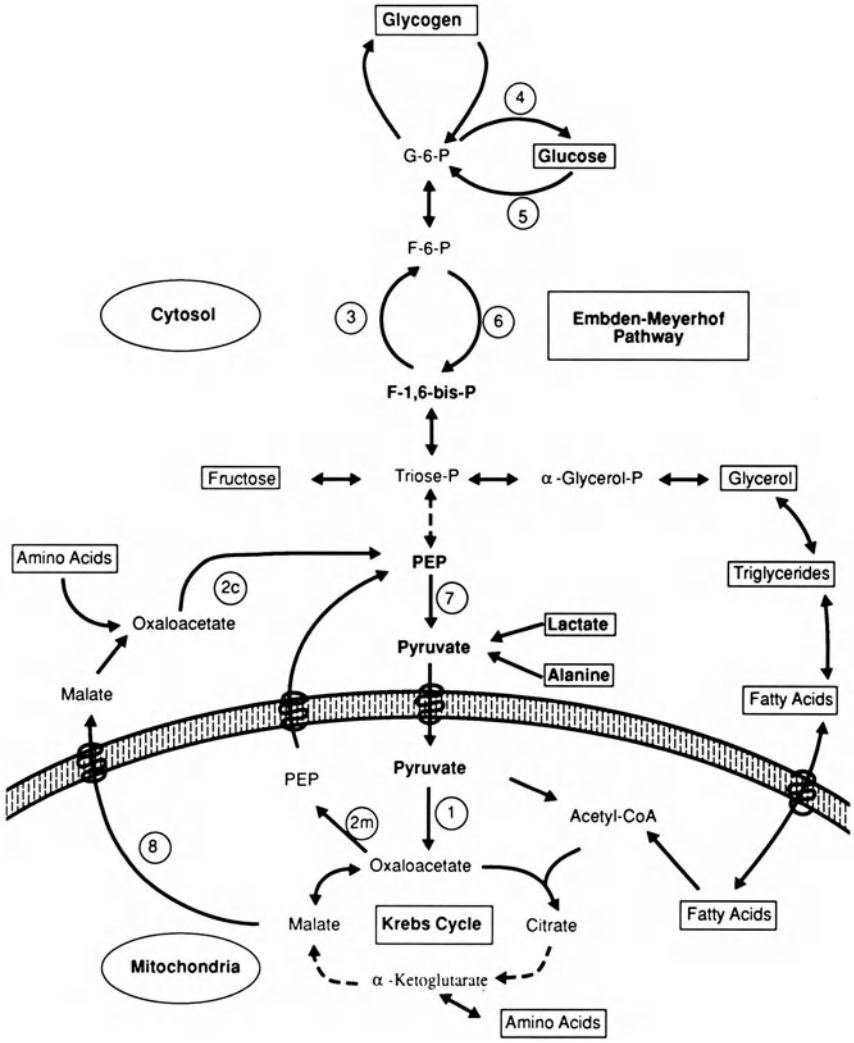

Fig. 1. Pathways relating to fuel homeostasis and gluconeogenesis. *Boxes* indicate fuels. *§*, a mitochondrial membrane transport site. *CoA*, Coenzyme A. ①, Pyruvate carboxylase; ②m, mitochondrial phosphoenolpyruvate (*PEP*) carboxykinase; ②c, cytosolic phosphoenolpyruvate carboxykinase; *3*, fructose-1,6-bisphosphatase (*F-1,6-bis-P*); *4*, glucose-6-phosphatase (*G-6-P*); *5*, hexokinase; *6*, phosphofructokinase; *7*, pyruvate kinase; *8*, the malate shunt

Metabolic Derangement

FBPase deficiency (Fig. 1) results in an impairment of the formation of glucose from all gluconeogenic substrates, including *glycerol*, *lactate*, and *alanine*. Maintenance of normoglycemia thus depends exclusively on glucose intake and on degradation of hepatic glycogen. As a consequence, when the glycogen reserves are limited, as in newborns, or exhausted, as after prolonged fasting, hypoglycemia is likely to occur, accompanied by an elevation of the main gluconeogenic precursors.

Loading tests with fructose (see "Function Tests" in the chapter by Fernandes and Saudubray), glycerol or alanine, provoke hypoglycemia, although, as a rule, higher doses are required than in hereditary fructose intolerance. The mechanism of the effect is similar to that which operates in the latter disorder (see the chapter by Van den Berghe). Owing to the FBPase defect, fructose and sorbitol provoke a build-up of fructose-1-phosphate. The latter inhibits phosphorylase and consequently glycogenolysis, on which the maintenance of normoglycemia depends. The higher tolerance to fructose can be explained by the fact that it can still be metabolized into lactate, hence less fructose-1-phosphate will accumulate. Glycerol loading provokes hypoglycemia because it leads to accumulation of glycerol-3-phosphate, which also inhibits phosphorylase. As in hereditary fructose

intolerance, glucagon does not usually correct the hypoglycemia.

Diagnostic Tests

The standard laboratory findings include facile hypoglycemia and lactate accumulation (which can exceed 25 mmol/l) with concomitant acidosis with pH below 7. The *lactic acidosis* is accompanied by increased plasma (and urine) levels of pyruvate, alanine and ketone bodies, and uric acid and by increased plasma levels of free fatty acids. Gas chromatography of urine organic acids may reveral abnormal levels of glycerol and *glycerol-3-phosphate* and metabolites indicating nonspecific liver damage.

This constellation of clinical and biochemical abnormalities should be differentiated from G-6-Pase deficiency (GSD 1); in the latter, the hepatomegaly is usually more marked and the acidosis usually less profound. Liver biopsy, if performed, can show fatty infiltration, mild fibrosis, and some increase in glycogen.

As discussed in the chapter by Fernandes and Saudubray, diagnostic loading tests with fructose, glycerol, or alanine should not be used in severe cases and, if used at all, should only be done under constant supervision and only in cases that remain diagnostic or management enigmas.

The enzymatic defect can be demonstrated in the liver, intestine, and kidney but not in cultured skin fibroblasts. Whether the defect can be diagnosed in leukocytes remains a matter of debate, owing to the very low activity of the enzyme in these cells. The assay should be done on fresh tissue since the enzyme undergoes rapid inactivation. In the majority of cases, the enzyme activity is absent or reduced to traces. In the others, residual activity can be up to 30% of normal.

Treatment and Prognosis

The acute episodes are life-threatening. Hypoglycemia is treated by providing continuous glucose infusion at a rate higher than the calculated glucose requirement (around 10–12 mg/kg per min for newborn infants). The acidosis can be profound and resistant to standard quantities of sodium bicarbonate; we have used over 200 mEq/24 h in a newborn infant to control the acidosis. Every effort must be made to keep the energy intake as close to normal as possible. Severe persisting lactic acidosis indicates that the gluconeogenic pathway is still being overstimulated and suggests that more glucose and even a glucose–insulin drip might be beneficial. The latter can be set to provide 0.02–0.1 units regular insulin/kg per h and a concomitant solution of 10%–13% dextrose to provide 6–10 g/kg per h. This system is then titrated to maintain normoglycemia; the insulin is given to enhance removal of pyruvate to fatty acids and protein synthesis (see also the chapter 3, this volume).

The basic aim of maintenance therapy is to diminish requirements for gluconeogenesis by frequent feeds and the use of slowly digested carbohydrates such as cornstarch. Most authorities recommend restriction or exclusion of gluconeogenic precursors such as sucrose, fructose, and sorbitol, particularly in small children, and a diet in which fat and protein are reduced to 20%–25% and 10% of energy supply, respectively. It is often necessary to provide chronic bicarbonate therapy, at least in childhood. Ethanol-induced lactic acidosis in adults might be a problem, but this has not yet been well documented. Treatment with folic acid (30 mg/day) has been proposed [2], but its use has not yet been substantiated.

The acute episodes are often severe and can be fatal. Once the diagnosis is established, it should be possible to prevent life-threatening attacks, but recurrent episodes in childhood require hospitalization and i.v. therapy. Once FBPase deficiency has been diagnosed and adequate management established, its course is usually benign. Growth and development are normal, and the tolerance to fasting improves with age [3, 4].

Genetics

The mode of inheritance is autosomal recessive. Heterozygotes are healthy and have intermediate levels of enzyme activity in liver and intestine. Reports of a mother and child with residual activity of 30% and of rare variants with normal activity in leukocytes represent yet further genetic heterogeneity [5]. The enzyme has been cloned and sequenced, but at present DNA diagnosis is not available and prenatal diagnosis is not currently possible.

The enzyme deficiency has been reported once in combination with glucose-6-phosphate dehydrogenase [6], once with G-6-Pase [7], and once with aldolase B deficiency [8]. The chance that

such combined defects are random is low, but they might represent regulatory defects or result from incestuous conceptions.

Phosphoenolpyruvate Carboxykinase Deficiency

Clinical Presentation

At present relatively few patients with PEPCK deficiency have been described. Several have presented shortly after birth, others not for weeks or months. Most patients have recurrent hypoglycemia with concomitant neuroglycopenic manifestations including episodes of lethargy, coma, or convulsions. There is frequently evidence of multisystem damage, namely muscle involvement with hypotonia, *neurologic damage* with developmental delay and failure to thrive, *renal tubular dysfunction*, *hepatomegaly*, and hepatocellular dysfunction with *cardiomyopathy*. The condition is not infrequently fatal in the first year or two of life, but milder cases are known.

During an acute attack, the presentation may resemble Reye syndrome. The multisystem involvement and characteristic neurologic deterioration are reminiscent of the defects of the electron transport chain and call to mind both Leigh and Alper syndromes.

Metabolic Derangement

PEPCK occurs in two isozyme forms: one in the cytosol and the other in the mitochondria. Defects of both isozymes have been reported, but hereditary defects of mitochondrial PEPCK appears to be commoner than defects of the cytosolic isozyme. Both forms of PEPCK deficiency lead to hypoglycemia and *lactic acidosis*, because they hamper flux from pyruvate and other gluconeogenic precursors to glucose. Lactic acidosis might be more severe when mitochondrial PEPCK is deficient. Elevation of oxaloacetate results in increased formation of citrate. Accumulation of fatty acids, can result in steatosis of liver, kidney, and muscle cells [2].

Diagnostic Tests

As with the other gluconeogenic disorders, there is facile hypoglycemia and lactic acidosis; indeed the laboratory findings mimic those of FBPase deficiency. There may also be marked *hyperlipidemia* and biochemical evidence of hepatocellular damage and proximal renal tubulopathy (Fanconi syndrome). Tolerance tests with glucagon, fructose, galactose, or glycerol may show a low to normal response of blood glucose, whereas lactate or alanine infusions usually do not, aggravating the lactic acidosis. Histologic examination shows a varying degree of hepatocellular damage, with steatosis being frequent; it may also be seen in renal tubules, muscle, and heart. Cerebral atrophy is also reported.

While hypoglycemia with lactic acidosis suggests a defect of gluconeogenesis, in truth all these clinical and laboratory findings are hardly diagnostic of PEPCK deficiency and the precise diagnosis must depend upon the detection of a primary enzyme defect. The enzymatic diagnosis is based on finding reduced activity of cytosolic or mitochondrial PEPCK in the liver. The assay must be done on fresh tissue to separate the two forms, since assay of a total homogenate of liver can mask deficiency of either isozyme. For this reason, postmortem analysis in liver can be confusing. The mitochondrial enzyme can be detected in lymphocytes and cultured skin fibroblasts, but the cytosolic enzyme cannot [9]. Cytosolic PEPCK is suppressed in *hyperinsulinism*, so that a low activity in such cases could be confusing, although lactic acid should not accumulate in hyperinsulinemia.

Treatment and Prognosis

In acute conditions, glucose infusion at rates slightly higher than the estimated basal glucose requirements and bicarbonate should easily correct hypoglycemia and acidosis. Poor control of blood glucose may be due to hyperinsulinism, severe hepatic and renal dysfunction, or septicemia; such additional problems must be treated.

Maintenance treatment during infancy and childhood is similar to that to FBPase deficiency with dietary reduction of gluconeogenic substrates and use of corn starch to provide slow-release carbohydrates. In later life special dietary management does not seem necessary.

The majority of patients with proven deficiency of one of these isozymes has died of neurodegenerative disease or recalcitrant hypoglycemia. In the few reported survivors, the

biochemical abnormalities abate, but there may be continuing evidence of muscle, liver, or neurologic damage.

Genetics

The mitochondrial enzyme defects would appear to be inherited as autosomal recessive traits and prenatal diagnosis has been reported. However, at least some of the reported cases of cytosolic PEPCK deficiency were probably due to hyperinsulinism rather than a primary defect of the enzyme. (The cytosolic gene of the rat has been sequenced but not, apparently, the one for the mitochondrial form.)

Pyruvate Carboxylase Deficiency

Clinical Presentation

From what is currently known about cases with proven PC deficiency, it appears that the condition is likely to present in one of two ways. In the first, symptoms develop in the neonatal period with profound neurologic dysfunction associated with hepatomegaly and severe *lactic acidosis*. *Neurologic symptoms* may include seizures, coma, and hypo- or hypertonia with or without dystonia. Such patients rarely survive more than a few months. More frequently, however, the children may appear normal in the neonatal period and then present after some months either with evidence of neurologic damage and developmental delay or with an episode of metabolic (lactic) acidosis during a period of increased metabolic stress. Growth may appear to be normal, but failure to thrive is more usual. The neurologic problems usually include seizures, spasticity or other signs of cerebral palsy, lethargy, microcephaly, and feeding problems and almost always seem to be associated with profound and progressive developmental delay. Some cases have anatomic changes in the brain stem and basal ganglia that are consistent with *Leigh syndrome*, while others do not. Some patients have also had *proximal renal tubular acidosis*, which aggravates the underlying lactic acidosis.

Metabolic Derangement

Biochemically, some patients have had episodes of hypoglycemia, but this is not as frequent nor as profound as in G6Pase nor FBPase deficiency. In severe cases, blood lactate, alanine, and pyruvate are permanently elevated and are associated with continuous metabolic acidosis. In others, they may only be mildly elevated and not noticed until an acute episode of acidosis is precipitated. Urinary ketones, lactate, alpha-ketoglutarate, fumarate, and succinate are frequently elevated, but none of the above findings really help to suggest the precise metabolic defect. In contrast, in the severe forms of the disease, plasma ammonia, citrulline, lysine, and proline are also elevated which might suggest a primary defect of the urea cycle. In PC deficiency however, the mechanism for the accumulation of these latter compounds is likely to be due to depletion of oxaloacetate. This, in turn, should lead to reduced intracellular levels of aspartate, which is a substrate for argininosuccinate synthetase in the urea cycle.

Equally intriguing is the occasional association of PC deficiency with proximal renal tubular acidosis. A proposed mechanism is that PC requires bicarbonate as a substrate for synthesis of oxaloacetate. In the renal tubular cells, this could be one way of capturing the huge quantities of HCO_3 which are reabsorbed [10]. This model would also satisfy the evidence that the renal cortex is gluconeogenic, whereas the distal medulla is a net glucose consumer.

Diagnostic Tests

Neurologic deterioration and lactic acidosis clearly label the patient as having a profound defect of energy metabolism. Leigh syndrome, elevated ketones, and renal tubular acidosis can occur in some patients with disorders of the electron transport chain, so that accurate diagnosis requires enzymatic analysis of tissues.

In generalized defects of the electron transport chain, there is often a ragged red-fiber myopathy, but muscle is not an optimal tissue in which to establish the diagnosis of PC deficiency. The enzyme defect can be demonstrated (in only a few laboratories) in cultured skin fibroblasts, but is probably best studied in the liver. However, the enzyme is quite unstable and special precautions are required for proper handling of the tissue. The most severe defects appear to be associated with total absence of the PC protein (cross-reading material, CRM negative), whereas in others the protein can be detected but is abnormal (CRM

positive). Tissue obtained at a statim (STAT) autopsy (i.e., tissues obtained immediately after death or at least within 2 h) may be helpful, but the results of postmortem analyses should be interpreted with caution. At present, DNA-based diagnosis is not available.

Histologically, the brain may show findings typical of Leigh syndrome, with in addition poor myelination, gliosis, and atrophy of the cortex, basal ganglia, and corpus callosum.

Prenatal diagnosis has been done in some families and heterozygote detection is also sometimes possible.

Treatment and Prognosis

One patient, neurologically normal at 7 years, has been reported [11], but from what we know at present, PC deficiency is usually progressive and fatal and no therapy has been shown to enhance enzyme activity. It is reasonable to try 10–40 mg biotin/day, but this has never been shown to be effective expect in cases of multiple carboxylase deficiency in which the primary defect is one of biotin metabolism (Chap. 23). The diet should be high in carbohydrates, but obsessional attention to the hourly glucose requirements is not necessary. Lactic acidosis is treated with bicarbonate; in one of our infants, who also had renal tubular acidosis, the bicarbonate requirement was 100–300 mEq/day.

The physician's main job is to ensure comfort and that all the appropriate studies for future genetic counseling and testing are obtained.

Genetics

The defect is inherited as an autosomal recessive trait; both forms have been found in many races and communities, such as American Indian, Canadian, and Saudi, in which consanguinity is likely to have occurred.

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Part III
Mitochondrial Energy Metabolism

The Pyruvate Dehydrogenase Complex and Tricarboxylic Acid Cycle

D.S. Kerr and A.B. Zinn

CONTENTS

Defects of the pyruvate dehydrogenase complex (PDHC) and the tricarboxylic acid (TCA) cycle (also referred to as the citric acid or Krebs cycle) invariably affect the central nervous system (CNS), but the severity and developmental pattern of expression varies tremendously among affected patients, and the phenotypes associated with each of these various defects are not specific. The range of expression extends from overwhelming neonatal lactic acidosis with congenital brain malformations and lack of neural regulation of respiration to relatively mild ataxia or mental retardation compatible with otherwise normal adult life. To make matters even more confusing, these same clinical manifestations may be caused by other defects of energy metabolism, especially defects of the electron transport (respiratory) chain. While it is important to appreciate this clinical and genetic heterogeneity, it is critical for the diagnostician to appreciate the limitations of clinical diagnosis. Diagnosis of these disorders depends very heavily on biochemical analyses, which start with metabolite screening tests and should be followed by definitive enzymatic and molecular genetic analyses.

Clinical Presentation

The clinical manifestations of PDHC or TCA cycle enzyme deficiencies have been summarized in previous reviews [1–4]; a current summary is shown in Table 1, along with an indication of the relative frequencies of the different disorders.

PDHC Deficiency. This is by far the most common, with more than 100 cases reported to date [2, 5, 6]. PDHC deficiency usually involves the first component of this enzyme complex, pyruvate dehydrogenase (E_1). The most common features associated with PDHC deficiency in infants and children are delayed development and hypotonia. Seizures and ataxia are less frequent. Less common, but perhaps most characteristic, are defects of CNS respiratory control leading to apnea, dependance on assisted ventilation, or possible sudden unexpected "crib" death. Loss of respiratory control appears to reflect involvement of the basal ganglia and brain stem, which may be visible by computered tomography or magnetic resonance imaging of the brain. The most characteristic degenerative neuropathology described in some of these cases has been the subacute necrotizing encephalomyelopathy described by Leigh in 1951. It should be emphasized that Leigh disease remains a microscopic neuropathological finding, not a radiological diagnosis. Furthermore, this finding is present in only a minority of cases of PDHC deficiency [7, 8] and also may be associated with defects of the electron transport chain (ETC) [9, 10]. By contrast, some newborns who have died with PDHC deficiency have had congenital malformations of the brain, including agenesis of the corpus callosum [3, 6]. Craniofacial dysmorphism suggestive of the fetal alcohol syndrome has been described in association with PDHC deficiency [6], but this observation needs further confirmation. At the other extreme of phenotypic variation, the now oldest reported patient with PDHC deficiency has intermittent ataxia as the only handicap; his cognitive function is normal [5]. Paradoxically, this male has almost unmeasurable PDHC activity in his cultured skin fibroblasts [5, 11].

Five cases of E_1 phosphatase deficiency [12] and five cases of deficiency of the second component of PDHC (dihydrolipoamide dehydrogenase, E_2) or a related component of uncertain function

Table 1. Clinical features of pyruvate dehydrogenase complex (PDHC) and tricarboxylic acid (TCA) cycle defects

Clinical features	Enzyme defect			
	PDHC	KDHC	E_3	Fumarase
Delayed growth and development	+++	+++	+++	+++
Hypotonia	+++	+++	+++	+++
Seizures	++	+	+	++
Ataxia, choreoathetosis	+	++	+	−
CNS degeneration (including. Leigh disease)	++	+++	+	+++
CNS malformations	+	−	−	++
Apnea, hypoventilation	+	−	+	−
Sudden death	+	++	−	−
Skeletal myopathy	−	−	−	−
Cardiomyopathy	−	+	−	−
Hepatic dysfunction	−	++	−	+
Dysmorphic features	+	−	−	+
Number of reported cases	>100	7	7	12

+++, very common (>75%); ++, common (25%–75%); +, uncommon (<25%); −, not noted.
KDHC, α-ketoglutarate dehydrogenase complex; E_3, dihydrolipoamide dehydrogenase.

(component X) have been reported [13]. These other PDHC defects have clinical manifestations which are within the variable spectrum associated with PDHC deficiency due to E_1 deficiency (these are combined in Table 1).

α-Ketoglutarate Dehydrogenase Complex (KDHC) Deficiency. This has been reported in seven children in three unrelated families [14, 15]. As in PDHC deficiency, the primary clinical manifestations are neurological impairment, including developmental delay, hypotonia, ataxia, opisthotonos, and, less commonly, seizures. All patients presented in early childhood, with most presenting in infancy. None of the affected children survived past 10 years of age.

E_3 Deficiency. Seven cases of E_3 deficiency have been described [16, 17]. Since this enzyme is common to all the α-keto acid dehydrogenases, including PDHC, KDHC, and the branched-chain α-keto acid dehydrogenase complex, E_3 deficiency results in multiple α-keto acid dehydrogenase deficiency and should be thought of functionally as a combined PDHC and TCA cycle defect (see next section). The clinical manifestations of E_3 deficiency are similar to those shown for PDHC (E_1) deficiency (Table 1).

Fumarase Deficiency. Approximately 12 patients with fumarase deficiency have been reported [18, 19], and we are aware of three unreported cases. The clinical features of this disorder are summarized in Table 1. Three patients have had prenatal onset of cerebral dysgenesis (hydrocephalus or agenesis of the corpus callosum or both), sometimes associated with polyhydramnios. All patients have shown poor postnatal neurological dysfunction; the most severely affected patients generally develop seizures and respiratory control difficulties and die in early childhood, whereas less severely affected patients develop a static encephalopathy and survive into adolescence and adulthood.

Abnormal Succinate Oxidation. Several patients with abnormal succinate oxidation have been reported [1, 2]. It appears that most of these are not specifically defects of succinate dehydrogenase (SDH), but are more complex disorders involving ETC components, documented by impaired activity of succinate-coenzyme Q oxidoreductase (ETC complex II) or succinate-cytochrome C reductase (ETC complexes II and III; Chap. 10). In addition, the associated clinical and ultrastructural findings are more consistent with other ETC defects than with TCA defects. For example, one patient presented with the clinical picture of Kearns-Sayre syndrome, while another presented with a clinical picture resembling Leigh syndrome. One exception appears to be a young man with lifelong exercise intolerance, lactic acidosis with a normal or increased lactate to pyruvate (L/P) ratio at rest but a decreased L/P ratio during exercise, and decreased succinate dehydrogenase activity documented histochemically, biochemically, and immunologically [20]. Interestingly, this patient also has aconitase deficiency and, as shown more

recently, ETC complex I and III deficiencies. The basis of this apparent combined TCA deficiency is not known, although the authors speculate that the defect could involve iron–sulfur clusters present in all these enzymes.

Electron Transport Chain Defects. None of these clinical features is unique, since they also can be manifestations of defects of the electron transport chain or other metabolic disorders. However, the opposite is not true: defects of the electron transport chain may be associated with clinical features that are *not* associated with PDHC or TCA cycle deficiencies. Specifically, it is very unusual for patients with PDHC or TCA cycle defects to develop primary skeletal myopathy (as opposed to hypotonia on a CNS basis) or cardiomyopathy. Skeletal muscle biopsies do not show evidence of "ragged red fibers" (indicating mitochondrial proliferation) or fatty infiltration. Therefore, the presence of these findings in a patient with lactic acidemia should point to a possible defect of the electron transport chain, rather than a PDHC or TCA cycle defect. Liver dysfunction is also not a characteristic manifestation of PDHC or TCA cycle defects; an enlarged liver in association with fasting hypoglycemia and elevated blood lactate would suggest a possible defect of one of the enzymes of gluconeogenesis (see the chapter by Fernandes and Chen and the chapter by Buist, this volume).

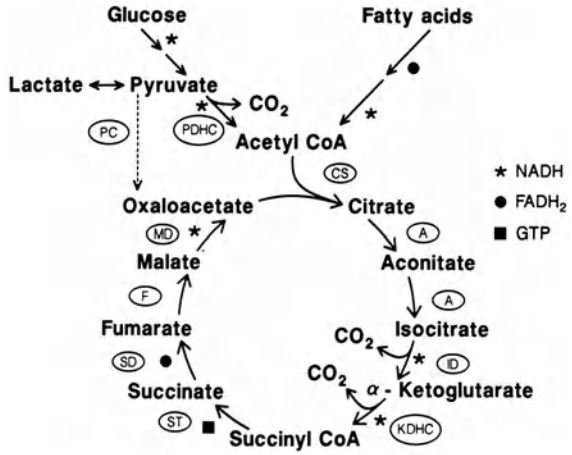

Fig. 1. Overview of glucose and fatty acid oxidation via pyruvate dehydrogenase complex (PDHC) and the tricarboxylic acid (TCA) cycle. *CS*, citrate synthase; *A*, aconitase; *ID*, isocitrate dehydrogenase; *KDHC*, α-ketoglutarate dehydrogenase complex; *ST*, succinyl-CoA transferase; *SD*, succinate dehydrogenase; *F*, fumarase; *MD*, malate dehydrogenase; *NADH*, reduced nicotinamide adenine dinucleotide; $FADH_2$, reduced flavin adenine dinucleotide; *GTP*, guanosine triphosphate; *PC*, pyruvate carboxylase. Amino acids enter the TCA cycle at several points, as discussed in the text. Intermediates which transfer energy for oxidation via the electron transport chain are shown by their indicated symbols

Metabolic Derangement

The TCA cycle is the final common pathway of oxidation of all nutrient fuels, including carbohydrates, fatty acids, and amino acids (Fig. 1). Carbohydrate or glucose oxidation depends initially on glycolysis, which results in formation of two moles of pyruvate from each mole of glucose and occurs in the cytoplasm of cells. Without mitochondrial oxidation, pyruvate is reduced to lactate, yielding less than one tenth of the total available adenosine triphosphate (ATP) which would be derived from complete oxidation of glucose via pyruvate. In the presence of oxygen and normal mitochondria, pyruvate can be oxidized to acetyl-coenzyme A (acetyl-CoA) via PDHC. PDHC is therefore the "gateway" for complete oxidation of carbohydrate via the TCA cycle. By yielding one CO_2 for each half O_2 consumed, the PDHC reaction accounts for the higher respiratory quotient for carbohydrate compared to fat. Mitochondrial β-oxidation of fatty acids, on the other hand, yields acetyl-CoA directly without a requirement for PDHC. Subsequent oxidation of acetyl-CoA via the TCA cycle requires a continuing source of oxaloacetate, which is derived from TCA recycling, "anapleurotic" replenishment via pyruvate carboxylase, or degradation of certain amino acids. Different amino acids enter the Krebs cycle at different points, including pyruvate (alanine and serine), acetyl-CoA ("ketogenic" amino acids), and α-ketoglutarate, succinyl-CoA, or oxaloacetate ("gluconeogenic" amino acids).

Hence, defects of PDHC are specifically defects of carbohydrate oxidation, and the clinical manifestations are likely to be aggravated by consumption of carbohydrate. Provision of alternative sources of acetyl-CoA from fat oxidation (via β-hydroxybutyrate, acetoacetate, and fatty acids) would therefore appear to be a logical therapeutic strategy (see below). In contrast, defects of the TCA cycle are combined defects of carbohydrate, fat, and protein oxidation. The clinical manifestations of these disorders may vary with the prevalent dietary energy source, but are not likely to be

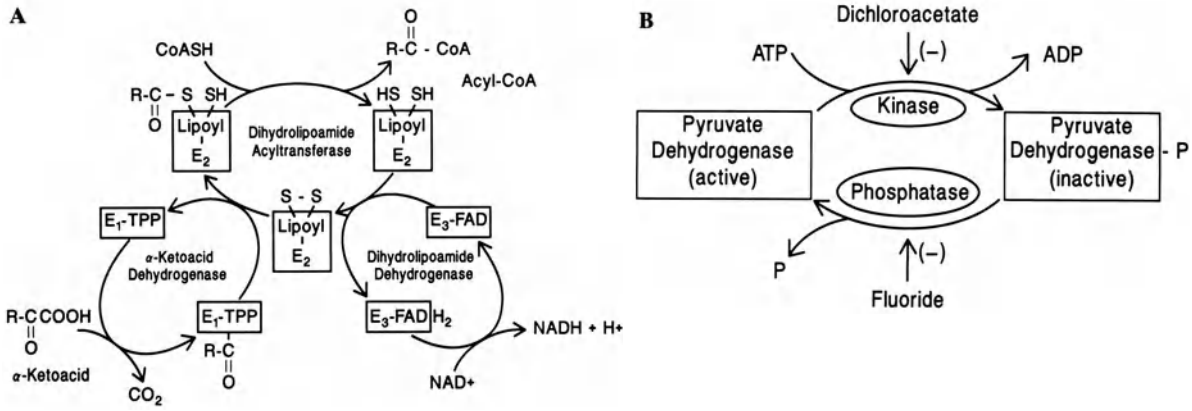

Fig. 2. **A** The reactions which are common to the three α-keto acid dehydrogenase complexes, including PDHC, pyruvate dehydrogenase complex (PDHC), α-ketoglutarate dehydrogenase complex (KDHC), and the branched-chain α-keto acid dehydrogenase complex. The first and second catalytic components, E_1 and E_2, are specific for each complex, while E_3 is the same protein in all three complexes. *R*, methyl group (for pyruvate) or the corresponding moiety for α-ketoglutarate and the branched-chain α-keto acids; *TPP*, thiamine pyrophosphate; *CoA*, coenzyme A; *FAD*, flavin adenine dinucleotide; *NAD*, nicotinamide adenine dinucleotide. **B** The phosphorylation–dephosphorylation reactions which render the pyruvate dehydrogenase component of PDHC (E_1) active or inactive. Dichloroacetate is an inhibitor of the kinase, and fluoride is an inhibitor of the phosphatase. *ATP*, adenosine triphosphate; *ADP*, adenosine diphosphate

aggravated or relieved by a change in dietary composition.

PDHC and *KDHC* are similar in structure and analogous to the branched-chain α-keto acid dehydrogenase complex (see Ogier et al., "Branched-Chain Organic Acidurias," this volume), and they all share the same E_3 component (Fig. 2A) [2]. These three enzyme complexes all utilize thiamine pyrophosphate as part of the first enzyme component (E_1), which in each case is a substrate-specific dehydrogenase composed of two different subunits (α and β). The E_1 reaction results in decarboxylation of the α-keto acid, providing a convenient means of assaying enzyme activity in cells and tissue samples. For PDHC, the E_1 component is rate limiting and is regulated in part by phosphorylation/dephosphorylation catalyzed by two specific enzymes, E_1 kinase and E_1 phosphatase, which are intrinsic to the overall PDHC complex (Fig. 2B). The second enzyme (E_2) is a transacetylase that utilizes covalently bound lipoic acid and is also unique for each substrate-specific complex; E_2 serves as the structural core of the complex. The third catalytic component, dihydrolipoyl dehydrogenase (E_3), is a flavoprotein common to all three α-keto acid dehydrogenases (as well as the glycine cleavage enzyme). Another component of PDHC, identified as component "X," has no apparent direct catalytic function, but appears to be critical for binding of the subunits comprising the overall complex.

In addition to PDHC and KDHC, three other enzymes of the Krebs cycle produce either reduced pyridine nucleotide (NADH) or flavin adenine dinucleotides ($FADH_2$) (Fig. 1), which are in turn substrates for oxidation via the electron transport chain. NADH and $FADH_2$ enter the electron transport chain via complex I or coenzyme Q, respectively (see Munnich, this volume). The complete oxidation of pyruvate to CO_2 accounts for more than 90% of the energy yield from glucose metabolism; altogether complete oxidation of one mole of glucose via pyruvate and the Krebs cycle produces 38 moles of ATP, whereas anerobic oxidation of glucose to lactate produces only two moles of ATP.

Fumarase is a homotetramer that catalyses the reversible interconversion of fumarate and malate (Fig. 1). No cofactors are required for this enzyme. The gene that encodes for fumarase is part of a gene family that encodes for other enzymes that catalyze reactions involving fumarate, namely argininosuccinate lyase and adenylosuccinate lyase (see Kvittingen et al. and Leonard, this volume). There are two isoforms of fumarase, mitochondrial and cytosolic, which have the same primary amino acid sequence except for the amino terminal residue. Both proteins are encoded by the same gene and the same mRNA, but the fuma-

rase transcript is alternately translated to generate the two isoforms.

Succinate dehydrogenase is part of a larger enzyme unit, which is complex II of the ETC (see Munnich, this volume). Succinate dehydrogenase is composed of two subunits, a flavoprotein and an iron–sulfur-containing protein. The remaining two subunits of complex II are thought to attach succinate dehydrogenase to the inner mitochondrial membrane and the other complexes of the electron transport chain.

Differentiation of PDHC, TCA Cycle and ETC Defects. PDHC, TCA cycle, and ETC deficiencies may be distinguished from each other by the degree to which they impair NADH production or oxidation. The L/P ratio is a reflection of the NADH to NAD ratio in the cytosol, and the β-hydroxybutyrate to acetoacetate (B/A) ratio is a reflection of the NADH to NAD ratio within the mitochondrion. PDHC deficiency is characterized by a normal or low L/P ratio and a normal B/A ratio, whereas ETC deficiencies (at least those of complexes I, III, and IV) are generally characterized by high L/P and B/A ratios because of impaired NADH oxidation. In theory, TCA cycle defects may impair intramitochondrial production of NADH. This reduction, in turn, may decrease the gradient for transporting cytosolic NADH to the mitochondrion via the malate–aspartate shuttle. Thus, TCA cycle defects may be associated with lower NADH to NAD ratios in both the cytosol and the mitochondrion compared to ETC defects. In the small number of patients with KDHC deficiency or SDH deficiency who have been examined, TCA cycle defects appear to be associated with normal L/P and B/A ratios [14, 15, 20].

The absolute concentration of ketone bodies may also be a useful tool for discriminating between PDHC, TCA cycle, and ETC defects. While PDHC deficiency is associated with normal ketone production and ETC defects are associated with decreased ketone body production (due to impairment of fatty acid oxidation), TCA cycle defects would be expected to be associated with enhanced ketogenesis during the fed state (because acetyl-CoA, but not fatty acid oxidation, is impaired) [14]. Thus TCA defects may be associated with a unique phenotype: decreased L/P ratios during exercise and a normal B/A ratio with increased ketones during the fed state. Clearly, additional patients need to be studied to confirm the validity and reliability of this generalization. In addition, the clinical utility of these distinctions need to be interpreted cautiously, because very ill patients with impaired ventilation, circulation, or perfusion may have insufficient peripheral oxygenation, resulting in an increased L/P ratio, and may receive intravenous glucose, which suppresses ketogenesis. Furthermore, pyruvate and acetoacetate are less stable than lactate and β-hydroxybutyrate, and artifacts of sample preparation or delayed processing may result in spuriously increased ratios.

All of the proteins that are components of PDHC and enzymes of the TCA cycle are encoded by nuclear genes. They are initially synthesized as precursor proteins, which include a leader sequence of amino acids that is critical for mitochondrial import. Once inside the mitochondrion, these leader sequences are cleaved off by specific proteases. In contrast to the enzyme complexes of the ETC (except for complex II), PDHC and the TCA cycle enzymes do not include subunits encoded by mitochondrial DNA. The genetic implications for these disorders are discussed below.

Diagnostic Tests

Metabolite Assays in Blood, Cerebrospinal Fluid, and Urine. The two most important laboratory tests for initial recognition of disorders of PDHC and enzymes of the TCA cycle are measurement of blood lactate and pyruvate and analysis of urinary organic acids. Measurement of cerebrospinal fluid (CSF) lactate and pyruvate and quantitative analysis of plasma amino acids are also useful in recognition of some PDHC defects, especially E_3 deficiency (Table 2).

It must be emphasized that these abnormalities are quite variable and may not be apparent at the particular time of testing. For example, blood lactate and pyruvate and plasma alanine can be intermittently normal in patients with PDHC deficiency, but an increase is expected after an oral carbohydrate load. While the L/P ratio is usually normal, a high ratio does not exclude these deficiencies, for the reasons mentioned above. Similarly, elevated blood lactate and increased excretion of various TCA cycle intermediates is variable in patients with deficiency of TCA cycle enzymes. Most patients with KDHC deficiency

Table 2. Metabolic abnormalities in pyruvate dehydrogenase complex (PDHC) and tricarboxylic acid (TCA) cycle defects

Metabolic abnormality (increased)	Enzyme defect			
	PDHC	KDHC	E_3	Fumarase
Blood lactate	+++	+++	+++	+
Lactate/pyruvate ratio	+	+++	+	+
Plasma alanine	+++	−	+++	−
Plasma branched-chain amino acids	−	−	+++	−
Plasma glutamate, glutamine	−	++	+	−
Urine lactate	++	+	++	+
Urine α-ketoglutaric acid	+	++	+++	+
Urine branched-chain α-keto/hydroxy acids	−	−	++	−
Urine fumaric acid	−	+	−	+++
Other urine TCA cycle acids	+	+	+	++
Hypoglycemia	−	+	+	−

+++, very common (>75%); ++, common (25%–75%); +, uncommon (<25%); −, not noted.
KDHC, α-ketoglutarate dehydrogenase complex; E_3, dihydrolipoamide dehydrogenase.

have increased blood lactate with a normal (but sometimes increased) L/P ratio and increased urinary excretion of α-ketoglutaric acid as well as other TCA cycle intermediates. Plasma glutamate and glutamine may be increased. The predicted pattern of increased plasma branched-chain amino acids (leucine, isoleucine, valine) and their corresponding urinary 2-keto- and 2-hydroxyacids is not always seen in E_3 deficiency. Mild lactic acidosis and mild hyperammonemia are sometimes seen in infants with fumarase deficiency, but generally are not seen in older children. The key finding in this disorder is increased urinary fumaric acid, which is associated in some cases with increased succinic and α-ketoglutaric acids.

The practical solution to these variations is to obtain several samples of blood and urine for thorough screening, including samples collected under different dietary conditions (e.g., during an acute illness, after fasting, and postprandially after a high-carbohydrate and/or high-protein meal). In contrast to deficiencies of pyruvate carboxylase or other gluconeogenic enzymes, fasting hypoglycemia is not common in PDHC or TCA cycle enzyme deficiencies, and blood lactate and pyruvate usually decrease after fasting. Formal glucose tolerance or carbohydrate loading tests have been suggested for detection of PDHC deficiency or other defects associated with impaired pyruvate oxidation [21]. However, these challenge tests should be performed with caution, as acute deterioration has been observed in PDHC deficient patients after a glucose load. A practical and probably safer solution for routine screening is to obtain a blood sample 1–2 h after an ordinary carbohydrate-containing meal.

Failure to find elevated blood lactate may be an indication to obtain CSF for measurement of lactate and pyruvate (and possibly organic acids), because there may be a disequilibrium between blood and CSF metabolites in patients with primary CNS disease. Conversely, finding increased blood lactate can be misleading if there was a significant struggle involved in obtaining the sample, since blood lactate increases during strenuous exercise. If necessary, blood sampling from an indwelling line may resolve this type of problem.

Specific Enzyme Assays in Cells and Tissues. The most commonly utilized samples for assays of specific enzyme activity are cultured skin fibroblasts. In addition, some assays can be performed in fresh mononuclear blood leukocytes, which saves significant time and expense compared to tissue culture, but has the disadvantage of not permitting follow-up testing from the same sample [7]. If available, skeletal muscle and/or liver biopsies are very useful; these are optimally done in a setting where facilities are available for preparation of intact mitochondria, which permit functional polarographic assays that may help pinpoint an unknown defect [15,18]. Additionally, isolated mitochondria can be frozen for subsequent specific enzyme assays [7, 18]. If a patient with a suspected but unproven metabolic defect dies, then it is essential to obtain samples of skeletal muscle, heart muscle, liver, and, if possible, brain as soon as possible postmortem. These specimens should

be obtained within 2–4 h after death, immediately frozen, and stored at −70°C. A skin biopsy and bladder aspiration of urine can also be obtained postmortem, if these samples were not obtained earlier. The advantage of obtaining multiple samples is that many defects are expressed in a tissue-specific pattern; this may be especially true for PDHC deficiency [22].

Although not routinely available in most laboratories, functional assays in whole cells are useful screening tests for some of these disorders. In particular, cultured skin fibroblasts may be incubated with glucose and the formation of lactate and pyruvate determined [23]. An increase in lactate formation associated with a normal L/P ratio can be expected in PDHC-deficient cells. This result can be distinguished from that found with cells deficient in the ETC, which produce an increased L/P ratio [23]. Analogous studies might be useful in patients with TCA cycle defects, but have not been reported.

Specific assays can be applied to homogenates of cells and tissues or isolated mitochondria. It is always important to relate the activity of the enzyme in question to concurrent controls and to measure the activity of other mitochondrial enzymes that serve as internal controls for the quality of the specimen and its preparation. These precautions are essential to correct for assay variability, sample deterioration, and dilution of mitochondria with nonmitochondrial protein.

The two α-keto acid dehydrogenase complexes, PDHC and KDHC, can in theory be assayed by measuring rates of formation of any of the products of the overall reaction (CO_2, the respective acyl-CoA, or NADH) (Fig. 2A). For practical purposes, release of $^{14}CO_2$ from the appropriate [1-^{14}C]-labeled substrate has proved most useful for assaying these complexes in crude homogenates of cells and tissues [7, 11]. PDHC and KDHC deficiency have been detected in cultured skin fibroblasts, skeletal muscle, isolated muscle and liver mitochondria, and, in the case of PDHC, mononuclear blood leukocytes and several other tissues [7]. PDHC must be activated (dephosphorylated) prior to assay, which can be done by preincubation of whole cells or mitochondria with dichloroacetate (DCA, an inhibitor of the kinase) or by preincubation of freeze-thawed cells or tissues with E_1 phosphatase (Fig. 2B). E_1 phosphatase deficiency is implicated if PDHC cannot be activated in cells or mitochondria preincubated with DCA, but can be activated by addition of E_1 phosphatase [12]. The three catalytic components of PDHC can be assayed separately, but the reactions utilized for these assays do not employ physiological substrates. The rate of the E_1 component, when assayed separately, is extremely low, and the results must be considered more qualitative than quantitative. Assay of the E_3 component is a straightforward spectrophotometric method utilizing free D,L-lipoamide or reduced D,L-lipoamide as the substrate [7, 16]. Western immunoblotting of these components can help distinguish whether a particular protein is missing. This is critical if there is no catalytic assay for the defective component, as is the case with component X [13]. Caution is required in interpreting the significance of western immunoblotting, since mutations that result in the absence or instability of one of the subunits of E_1 are almost always accompanied by loss of the other subunit [2, 24].

Standard spectrophotometric assays are available for all of the TCA cycle enzymes [18]. Fumarase is generally measured in mononuclear blood leukocytes, cultured skin fibroblasts, skeletal muscle, or liver by monitoring the formation of fumarate from malate or, more sensitively, by coupling the reaction with malate dehydrogenase and monitoring production of NADH. Assay of succinate dehydrogenase activity requires spectrophotometric measurement of succinate dehydrogenase itself as well as the overall succinate–coenzyme Q oxidoreductase reaction (which includes all four components of complex II; see Munnich, this volume).

Treatment and Prognosis

PDHC Deficiency. The general prognosis for these disorders is poor and treatment is usually not effective or claimed benefits not well documented. Experience with early prospective treatment to prevent irreversible brain injury is lacking. Perhaps the most rational strategy for treating any of these defects is use of a ketogenic diet for PDHC deficiency [8, 25]. As described above, provision of fatty acids, β-hydroxybutyrate, and acetoacetate should, in principle, replenish acetyl-CoA that is not derived from pyruvate. A few cases of PDHC deficiency have been treated with ketogenic diets and have shown laboratory improvement (i.e., lowering of blood lactate), but limited or no long-term clinical benefit. We are aware of two male patients with PDHC deficiency due to $E_1\alpha$ muta-

tions who have been treated from infancy with ketogenic diets and in whom the clinical outcome has been favorable compared to other male patients with the same mutations [22, 26] (and unpublished observations). Maintenance of ketosis is an important part of the treatment strategy because fatty acids do not cross the blood–brain barrier. However, sustaining significant ketosis over an extended period of time is difficult, as has been learned from experience in use of ketogenic diets for treatment of epilepsy. Furthermore, infants appear to be somewhat resistant to development of ketosis. It is not known what degree of ketosis is required in this situation, but we attempt to keep blood β-hydroxybutyrate levels above 2 m*M*. This requires restricting dietary carbohydrate to less than 10% and protein to less than 15% of energy, i.e., at least 75% of energy should be derived from fat. Dietary fat can be provided partly as medium-chain triglycerides to enhance ketogenesis.

Other therapies attempted for PDHC deficiency have included use of large doses of various B vitamins, especially thiamine. There is little evidence for any benefit from thiamine therapy, which is widely used, except perhaps in rare cases in which the mutation is associated with altered affinity for thiamine pyrophosphate. Use of biotin or riboflavin makes little sense in PDHC deficiency, since these cofactors are not involved in the E_1 reaction, which is the most commonly affected component. One case of E_3 deficiency reportedly improved after supplementation with D,L-lipoic acid, and skin fibroblasts from another case showed improvement when cultured with supplemental lipoic acid [17]. Again, it is not clear why lipoic acid was helpful in these cases, since it is not part of the defective E_3 protein. If lipoic acid served as an alternative substrate for the E_2 component (replacing E_3), then it might be expected that very large (i.e., substrate level) quantities of lipoic acid would be required, equivalent to the amount of pyruvate, 2-ketoglutarate, and branched-chain 2-keto acids being oxidized. However, we do not recommend the use of large amounts of the nonphysiological D,L-lipoic acid mixture, since in other situations (e.g., D,L-carnitine) use of racemic mixtures has been harmful.

Another potential treatment strategy for PDHC deficiency is to maintain any residual PDHC in its active form by preventing phosphorylation and inactivation. This can be done with DCA, an inhibitor of E_1-kinase (Fig. 2B), and it is possible to give sufficient amounts of DCA to achieve a significant inhibitory concentration in vivo without apparent toxicity (about 50 mg/kg per day) [27]. Over two dozen cases of congenital lactic acidosis due to various defects have been treated with DCA, most of whom appeared to benefit [27]. A multicenter, controlled clinical trial of DCA in congenital lactic acidosis has not been performed.

TCA Cycle Defects. Although dietary restriction of both carbohydrate and protein might, in theory, reduce the substrate load for PDHC and the branched-chain α-keto acid dehydrogenase complex, there is no nutritional solution that would bypass α-ketoglutarate dehydrogenase. For similar reasons, dietary treatment of fumarase deficiency or other TCA cycle defects is not likely to be of benefit. Efforts to devise potential therapies for defects of the TCA cycle must take into account two potential mechanisms of toxicity:

- Impaired energy production caused by interrupting the flow of the TCA cycle.
- Accumulation of metabolites proximal to the primary enzyme deficiency.

The first mechanism limits the number of enzymatic steps at which reducing equivalents can be generated and transferred to the electron transport chain. In addition, this mechanism may lead to depletion of oxaloacetate, preventing continued influx of acetyl-CoA via citrate synthase. The second mechanism inhibits other pathways of oxidative metabolism. An example is fumarase deficiency, in which secondary inhibition of succinate dehydrogenase and glutamate dehydrogenase was shown in isolated skeletal muscle mitochondria [18]. As yet, effective strategies to compensate for these pathogenetic mechanisms have not been developed. Protein restriction was reportedly of no benefit for one patient with fumarase deficiency. While removal of certain amino acids which are precursors of fumarate could be beneficial, removal of exogenous aspartate might deplete a potential source of oxaloacetate. At present, the prognosis for these disorders appears to depend more on the severity of the mutation than on the mode of medical intervention, which is largely supportive.

Genetics

Mode of Inheritance. Most of the genes that encode the various subunits of PDHC and enzymes of the TCA cycle and the corresponding defects are inherited in an autosomal recessive manner. However, defects of the X-linked $E_1\alpha$ subunit of PDHC are the most frequent and account for the higher recognition of PDHC deficiency in males. There are two $E_1\alpha$ genes, one on the X-chromosome (in region Xp22.13) that is expressed in somatic cells [28, 29] and a second processed (intronless) gene on chromosome 4 that is expressed only in developing and mature sperm [30]. In the majority of cases, PDHC deficiency appears to be the consequence of new X-linked $E_1\alpha$ mutations (see below) [26, 31, 32]. PDHC deficiency due to defects of $E_1\alpha$ are also expressed in females, but the range of clinical severity is considerably greater due to skewed inactivation of the X-chromosome [33]. For example, we are aware of identical twin girls with $E_1\alpha$ deficiency with very different degrees of clinical manifestations.

Fumarase deficiency is inherited as an autosomal recessive trait and the carrier status is identifiable by enzyme assay. The fumarase gene had been mapped to chromosome 1q42 before the deficiency state was discovered; recently, this gene localization has been confirmed using molecular genetic techniques [34]. The gene for E_3 is located on chromosome 7 [29], and deficiency of this enzyme also is inherited as an autosomal recessive trait and the carrier status is detectable. α-Ketoglutarate dehydrogenase deficiency also appears to be inherited as an autosomal recessive trait, but the gene has not yet been mapped.

Mutations. To date, some 35 mutations of the $E_1\alpha$ subunit of PDHC have been characterized [4, 26, 35]. About half of the mutations are small deletions or insertions, and half are point mutations. While the consequences of these mutations on enzyme structure and function need further definition, some appear to affect highly conserved amino acids that are critical for subunit interaction, binding of thiamine pyrophosphate, dephosphorylation, or catalysis at the active site. The molecular basis of PDHC-$E_1\beta$, PDHC-E_2, KDHC, and SDH deficiencies has not been reported. In one case of E_3 deficiency, the patient was found to be a compound heterozygote for two missense point mutations affecting conserved amino acids [36]. The molecular basis of fumarase deficiency has recently been shown to be caused by different missense mutations in cases from two unrelated families [34].

Experience with Prenatal Testing. To date there has been little success with prenatal diagnosis of these disorders. In light of the high frequency of new mutations, the benefit of prenatal diagnosis of PDHC-$E_1\alpha$ deficiency may be very limited. We have monitored eight pregnancies in seven mothers who previously had an affected child by measuring PDHC activity in chorionic villus samples and/or cultured amniocytes and found normal PDHC activity in all cases [32]. In two unpublished cases prenatal testing of cultured amniocytes had indicated normal PDHC activity, but testing after birth indicated PDHC deficiency. The biological basis for this discrepancy is not known, but we have found that PDHC deficiency may not always be expressed in cultured fibroblasts [22]. In theory, prenatal diagnosis of KDHC, E_3, or fumarase deficiency should be possible by measurement of the corresponding enzyme activity in chorionic villus samples or cultured amniocytes, but this has not been reported.

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The Respiratory Chain

A. Munnich

CONTENTS

Respiratory chain deficiencies have long been regarded as neuromuscular diseases. In fact, *oxidative phosphorylation*, i.e., adenosine triphosphate (ATP) synthesis by the respiratory chain does not only occur in the neuromuscular system. Indeed, a number of non-neuromuscular organs and tissues are dependent upon mitochondrial energy supply. For this reason, a respiratory chain deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age with any mode of inheritance, due to the twofold genetic origin of respiratory enzymes (nuclear DNA and mitochondrial DNA, mtDNA).

In the past few years, it has become increasingly clear that genetic defects of oxidative phosphorylation account for a large variety of clinical symptoms in childhood. Among 100 patients with respiratory chain deficiencies identified in our center, 56% presented with an extra neuromuscular symptom and 44% were referred for a neuromuscular problem. It appears that the diagnosis of a respiratory chain deficiency is difficult to consider initially when solely the presenting symptom is present. In contrast, this diagnosis is easier to consider when two seemingly unrelated symptoms are observed.

Clinical Presentation

Due to the ubiquitous nature of oxidative phosphorylation, a defect of the mitochondrial respiratory chain should be considered in patients presenting with (a) an unexplained association of neuromuscular and/or non-neuromuscular symptoms, (b) with a rapidly progressive course, and (c) involving seemingly unrelated organs or tissues.

Although the disease may begin at virtually any age, it is worth noting that onset before 1 month of age occurred in 36% of our series, between 1 month and 2 years of age in 44%, and after 2 years of age in 20%. Table 1 summarizes the most frequently observed symptoms. Whatever the age of onset and the presenting symptom, the major feature is the increasing number of tissues affected in the course of the disease. This progressive organ involvement is constant and the central nervous system is almost consistently involved in the late stage of the disease.

While the initial symptoms usually persist and gradually worsen, they may occasionally improve or even disappear as other organs become involved. This is particularly true for bone marrow and gut. Indeed, remarkable remissions of pancytopenia or watery diarrhea have been reported in infants who later developed other organ involvement. Moreover, several patients whose disease apparently started in childhood or adulthood were retrospectively shown to have experienced transient sideroblastic anemia, neutropenia, chronic watery diarrhea or failure to thrive of unexplained origin in early infancy. Similarly, a «benign» reversible infantile myopathy, with hypotonia, weakness, macroglossia, respiratory distress and spontaneous remission within 1–2 years has been described.

Certain clinical features or associations are more frequent at certain ages and have been occasionally identified as distinct entities, suggesting that these associations are not fortuitous. Yet, considerable overlap in clinical features leads to difficulties in the classification of many patients, and this questions the usefulness of subclassification. In fact, attempts to ascribe clinical profiles to particular syndromes or to delineate boundaries between syndromes is useless and unrewarding,

Table 1. The most frequently observed symptoms in respiratory chain deficiencies

Age	Affected Organ	Symptoms
Neonatal period (0–1month)	Central nervous system	Iterative apnea, lethargy, drowsiness, near-miss sudden infant death, limb and trunk hypotonia, congenital lactic acidosis, ketoacidotic coma
	Muscle	Myopathic presentation, muscular atrophy, hypo-, hypertonia, stiffness, recurrent myoglobinuria, poor head control, poor spontaneous movement
	Liver	Hepatic failure, liver enlargement
	Heart	Hypertrophic cardiomyopathy (concentric ++)
	Kidney	Proximal tubulopathy (De Toni Debré Fanconi syndrome)
Infancy (1 month–2 years)	Central nervous system	Recurrent apneas, near-miss, recurrent ketoacidotic coma, poor head control, limb spasticity, psychomotor regression, mental retardation, cerebellar ataxia, stroke-like episodes, myoclonus, generalized seizures, subacute necrotizing encephalomyopathy (Leigh syndrome), progressive infantile poliodystrophy (Alpers syndrome)
	Muscle	Myopathic features, muscular atrophy, limb weakness, hypotonia, myalgia, exercise intolerance, recurrent myoglobinuria
	Liver	Progressive liver enlargement, hepatocellular dysfunction, Valproate-induced hepatic failure
	Heart	Hypertrophic cardiomyopathy (concentric)
	Kidney	Proximal tubulopathy (De Toni Debré Fanconi syndrome), tubulo-interstitial nephritis (mimicking nephronophtisis), nephrotic syndrome, renal failure, hemolytic uremic syndrome
	Gut	Recurrent vomiting, chronic diarrhea, villous atrophy, exocrine pancreatic dysfunction, failure to thrive, chronic interstitial pseudo-obstruction
	Endocrine	Short stature, retarded skeletal maturation, recurrent hypoglycemia, multiple hormone deficiency
	Bone marrow	Sideroblastic anemia, neutropenia, thrombopenia, myelodysplastic syndrome, dyserythropoiesis
	Ear	Hearing loss, sensorineural deafness (brainstem or cochlear origin)
	Eye	Optic atrophy, diplopia, progressive external ophthalmoplegia, limitation of eye movements (all directions, upgaze ++), ⟨⟨salt and pepper⟩⟩ retinopathy, pigmentary retinal degeneration, lid prosis cataract,
	Skin	Mottled pigmentation of photo exposed areas, trichothiodystrophy, dry, thick, and brittle hair
Childhood (> 2 years) and adulthood	Central nervous system	Myoclonus, seizures (generalized, focal, drop attacks, photosensitive, tonicoclonus), cerebellar ataxia, spasticity, psychomotor regression, dementia, mental retardation, stroke-like episodes, hemicranial headache, migraine, recurrent hemiparesis, cortical blindness or hemianopsia, leukodystrophy, cortical atrophy, peripheral neuropathy
	Muscle	Progressive myopathy, limb weakness (proximal), myalgia, exercise intolerance, recurrent myoglobinuria
	Heart	Concentric hypertrophic or dilated cardiomyopathy, different types of heart block
	Endocrine	Diabetes mellitus (insulin-dependent and non-insulin dependent), growth hormone deficiency, hypoparathyroidism, hypothyroidism, adrenocorticotrophin deficiency, hyperaldosteronism, infertility (ovarian failure or hypothalamic dysfunction)
	Eye	Lid ptosis, diplopia, progressive external ophthalmoplegia, limitation of eye movements (all directions, upgaze ++), pigmentary retinal degeneration, cataract, corneal opacities, Leber hereditary optic neuroretinopathy
	Ear	Sensorineural deafness, aminoglycoside-induced ototoxicity (maternally inherited)

especially as the nature, clinical course and severity of symptoms vary among (and even within) affected individuals. It is more useful to bear in mind that the diagnosis of respiratory chain deficiency should be considered when presented with an unexplained association of signs with a progressive course involving seemingly unrelated organs or tissues, regardless of the age of onset and the nature of the presenting symptom. The non-exhaustive list of clinical profiles listed below illustrates the diversity of presentations (see also Table 1).

Neonates. In the neonate (below 1 month), the following clinical profiles are met:

- Ketoacidotic coma with recurrent apneas, seizures, severe hypotonia, liver enlargement and proximal tubulopathy in the neonatal period, with or without symptom-free period [1].
- Severe neonatal sideroblastic anemia (with or without hydrops fetalis) with neutropenia, thrombopenia and exocrine pancreatic dysfunction of unexplained origin (Pearson marrow pancreas syndrome) [2].
- Concentric hypertrophic cardiomyopathy and muscle weakness with an early onset and a rapidly progressive course (dilated cardiomyopathies are exceptional) [3].
- Concentric hypertrophic cardiomyopathy with profound central neutropenia and myopathic features in males (Barth syndrome) [4].
- Hepatic failure with lethargy, hypotonia and proximal tubulopathy of unknown origin with neonatal onset [1].

Infants. In infancy (1 month–2 years), the clinical profiles seen include the following:

- Failure to thrive with or without chronic watery diarrhea and villous atrophy, unresponsive to gluten-free and cow milk protein-free diet [5].
- Recurrent episodes of acute myoglobinuria, hypertonia, muscle stiffness and elevated plasma levels of enzymes unexplained by an inborn error of glycolysis, glycogenolysis, fatty acid oxidation or muscular dystrophy [6].
- Proximal tubulopathy (de Toni Debré Fanconi syndrome) with recurrent episodes of watery diarrhea, rickets and mottled pigmentation of photo-exposed areas; or a tubulo-interstitial nephritis mimicking nephronophtisis with the subsequent development of renal failure and encephalomyopathy with leukodystrophy [7].
- Severe trunk and limb dwarfism, unresponsive to growth hormone administration with subsequent hypertrophic cardiomyopathy, sensorineural deafness and retinitis pigmentosa.
- Early-onset insulin dependent diabetes mellitus with diabetes insipidus, optic atrophy and deafness (Wolfram syndrome) [8].
- Rapidly progressive encephalomyopathy with hypotonia, poor sucking, weak crying, poor head control, cerebellar ataxia, pyramidal syndrome, psychomotor regression, developmental delay, muscle weakness, respiratory insufficiency, occasionally associated with proximal tubulopathy and/or hypertrophic cardiomyopathy.
- Subacute necrotizing encephalomyopathy (Leigh's disease). This is a devastating encephalopathy characterized by recurrent attacks of psychomotor regression with pyramidal and extra pyramidal symptoms, leukodystrophy and brainstem dysfunction (respiratory abnormalities). The pathological hallmark consists of focal, symmetrical and necrotic lesions in the thalamus, brainstem, and the posterior columns of the spinal cord. Microscopically these spongiform lesions show demyelination, vascular proliferation and astrocytosis [9].

Children and Adults. In childhood (above 2 years) and adulthood, the neuromuscular presentation is the most frequent:

- Muscle weakness with myalgia and exercise intolerance with or without progressive external ophthalmoplegia [9].
- Progressive sclerosing poliodystrophy (Alpers disease) associated with hepatic failure [10].
- Encephalomyopathy with myoclonus, ataxia, hearing loss, muscle weakness and generalized seizures (myoclonus epilepsy, ragged red fibers, MERRF) [9].
- Progressive external ophthalmoplegia (PEO) ranging in severity from pure ocular myopathy to Kearns-Sayre syndrome (KSS). KSS is a multisystem disorder characterized by the triad: onset before age 20, PEO and pigmentary retinal degeneration, plus at least one of the following: complete heart block, cerebrospinal fluid (CSF) protein levels above 100 mg/dl, or cerebellar ataxia [9].
- Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS).

This syndrome is characterized by onset in childhood with intermittent hemicranial headache, vomiting, proximal limb weakness, and recurrent neurological deficit resembling strokes (hemiparesis, cortical blindness, hemianopsia), lactic acidosis and ragged red fibers in the muscle biopsy. Computed tomography (CT) brain scanning shows low-density areas (usually posterior) which may occur in both white and gray matter, but does not always correlate with the clinical symptoms or the vascular territories The pathogenesis of stroke-like episodes in MELAS has been ascribed to either cerebral blood flow disruption or acute metabolic decompensation in biochemically deficient areas of the brain [9].

- Leber's hereditary optic neuroretinopathy (LHON). This disease is associated with rapid loss of bilateral central vision due to optic nerve death. Cardiac dysrythmia is frequently associated with the disease, but no evidence of skeletal muscle pathology or gross structural mitochondrial abnormality has been documented. The median age of vision loss is 20–24 years, but it can occur at any age between adolescence and late adulthood. Expression among maternally related individuals is variable and there is a bias toward males being affected [9].
- Neurogenic muscle weakness, ataxia, retinitis pigmentosa and variable sensory neuropathy with seizures and mental retardation or dementia (NARP) [11].
- Mitochondrial myopathy and peripheral neuropathy, encephalopathy and gastro-intestinal disease manifesting as intermittent diarrhea and intestinal pseudo-obstruction (myo-neurogastro intestinal encephalopathy, MNGIE) [9].

Metabolic Derangement

The respiratory chain is divided into five functional units or complexes, embedded in the inner mitochondrial membrane (Fig. 1). *Complex I (NADH-coenzyme Q reductase)* carries reducing equivalents from NADH to coenzyme Q (CoQ) and consists of 25–28 different polypeptides, seven of which are encoded by mtDNA. *Complex II (succinate-CoQ reductase)* carries reducing equivalents from $FADH_2$ to CoQ and contains five polypeptides, including the FAD-dependent succinate dehydrogenase, and a few non-heme iron sulfur centers. This is the only complex that

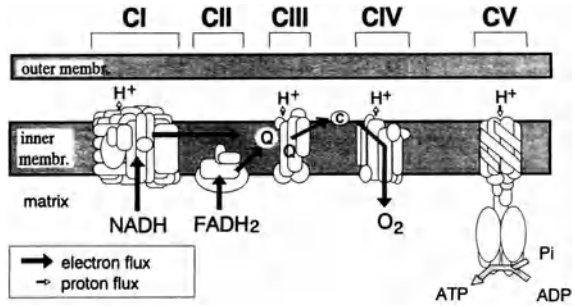

Fig. 1. The mitochondrial respiratory chain. *CI* complex I (NADH-CoQ reductase); *CII*, complex II (succinate-CoQ reductase); *CIII*, complex III (ubiquinol-cytochrome c reductase); *CIV*, complex IV (cytochrome c oxidase); *CV* complex V (ATPase)

does not contain any mtDNA-encoded protein. *Complex III (reduced CoQ-cytochrome c reductase)* carries electrons from CoQ to cytochrome c. It contains 11 subunits, one of which (the apoprotein of cytochrome b) is encoded by mtDNA. *Complex IV (cytochrome c oxidase, COX)*, the last component of the respiratory chain, catalyzes the transfer of reducing equivalents from cytochrome c to molecular oxygen. It is composed of two cytochromes (a and a_3), two copper atoms, and 13 different protein subunits, three of which are encoded by mtDNA [12].

The mitochondrial respiratory chain catalyzes the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP. During the oxidation process, electrons are transferred to oxygen via the energy-transducing complexes of the respiratory chain: complexes I, III and IV for NADH-producing substrates; complexes II, III and IV for succinate; complexes III and IV for $FADH_2$ derived from the β-oxidation pathway, via the electron transfer flavoprotein (ETF) and the ETF-CoQ oxidoreductase system. CoQ (a lipoidal quinone) and cytochrome c (a low molecular weight hemoprotein) act as «shuttles» between complexes.

The free energy generated from the redox reactions is converted into a transmembrane proton gradient. Protons are pumped through the mitochondrial inner membrane at three coupling sites (represented by complexes I, III and IV) which creates a charge differential. *Complex V (ATP synthase)* allows protons to flow back into the mitochondrial matrix and uses the released energy to synthesize ATP. Three ATP molecules are made for each NADH oxidized.

As the respiratory chain transfers electrons to oxygen, a disorder of oxidative phosphorylation

should result in an increase in the concentration of reducing equivalents in both mitochondria and cytoplasm and in the functional impairment of the citric acid cycle, due to the excess of NADH and the lack of NAD. Therefore, an increase in the ketone body (3-OH butyrate/acetoacetate) and lactate/pyruvate molar ratios (L/P) with a secondary elevation of blood lactate might be expected in the plasma of affected individuals. This is particularly true in the postabsorptive period, when more NAD is required to adequately oxidize glycolytic substrates.

Similarly, as a consequence of the functional impairment of the citric acid cycle, ketone body synthesis increases after meals due to the chanelling of acetyl CoA towards ketogenesis. The elevation of the total level of ketone bodies in a fed individual is paradoxical, as it should normally decrease after meals, due to insulin release (paradoxical hyperketonemia).

Yet, the position of the block might differentially alter the metabolic profile of the patient. A block at the level of complex I impairs the oxidation of the 3 mol of NADH formed in the citric acid cycle. In theory, at least, oxidation of $FADH_2$ derived from succinate-producing substrates (methionine, threonine, valine, isoleucine and odd number fatty acids) should not be altered, because it is mediated by complex II. Similarly, oxidation of $FADH_2$ derived from the first reaction of the β-oxidation pathway should occur normally because it is mediated by the ETF-CoQ reductase system. On the other hand, complex II deficiency should not markedly alter the redox status of affected individuals fed a carbohydrate-rich diet. A block at the level of complex III should impair the oxidation of both NAD-linked and FAD-linked substrates. Finally, given the crucial role of complex IV in the respiratory chain, it is not surprising that severe defects of COX activity should cause severe lactic acidosis and markedly alter redox status in plasma.

Diagnostic Tests

Screening Tests. Screening tests include the determination of lactate, pyruvate, ketone bodies and their molar ratios in plasma, as indices of oxidation reduction status in cytoplasm and mitochondria, respectively (Table 2). Determinations should be made before and 1 h after meals throughout the day. Blood glucose and nonesterified fatty acids should be simultaneously monitored (see Fernandes and Saudubray, this volume).

The observation of a *persistent hyperlactatemia* (>2.5 m*M*) with elevated L/P and ketone body molar ratios (particularly in the postabsorptive period) is highly suggestive of a respiratory chain deficiency. In addition, investigation of the redox status in plasma can help discriminate between the different causes of congenital lactic acidosis based on L/P and ketone body molar ratios in vivo. Indeed, an impairment of oxidative phosphorylation usually results in L/P ratios above 20 and ketone body ratios above 2, whereas a defect of the pyruvate dehydrogenase (PDH) complex results in low L/P ratios (below 10). Although little is known regarding tricarboxylic acid cycle disorders, it appears that these diseases also result in high L/P ratios, but ketone body molar ratios are lower in these conditions (<1) than in respiratory chain defects (as also observed in pyruvate carboxylase deficiency) [13, 14] (see Kerr and Zinn, this volume). Yet, the above diagnostic tests may fail to detect any disturbance of the redox status in plasma. Pitfalls in the metabolic screening are the following:

- Hyperlactatemia may be latent in basal conditions and revealed by a glucose loading test only (2 g/kg orally) or by determination of the redox status in the CSF. The measurement of lactatorrachia and/or L/P ratio in the CSF is useless when the redox status in plasma is altered.
- Proximal tubulopathy may lower blood lactate and increase urinary lactate. In this case, gas chromatography-mass spectrometry can detect urinary lactate and citric acid cycle intermediates.
- Diabetes mellitus may hamper entry of pyruvate into the citric acid cycle;
- Tissue-specific isoforms may be selectively impaired, barely altering the redox status in plasma (this may be particularly true for hypertrophic cardiomyopathies);
- The defect may be generalized but partial: the more those tissues with higher dependencies on oxidative metabolism such as brain and muscle suffer, the more the oxidation reduction status in plasma is impaired;
- The defect may be confined to complex II, barely altering (in principle) the redox status in plasma.

Table 2. Screening tests

Type of test	Parameters tested
Standard screening tests[a]	Plasma lactate
	Lactate/pyruvate molar ratio (redox status in cytoplasm)
	Ketonemia (‹‹paradoxical›› elevation in fed individuals)
	β-OH butyrate/acetoacetate molar ratio (redox status in the mitochondria)
	Blood glucose and free fatty acids
	Urinary organic acids (GC-MS) (lactate, ketone bodies, citric acid cycle intermediates)
Provocative tests[b]	Glucose test (2 g/kg, orally) in fasted individuals with determination of blood glucose, lactate pyruvate, ketone bodies and their molar ratios at *t* 15 min, 30 min, 45 min, 60 min, 90 min
	Lactate/pyruvate molar ratios in the CSF (only when no elevation of plasma lactate is observed)
	Redox status in plasma following exercise
Screening for multiple organ involvement	Liver: hepatocellular dysfunction?
	Kidney: proximal tubulopathy, distal tubulopathy, proteinuria, renal failure?
	Heart: hypertrophic cardiomyopathy heart block? (ultrasound, EKG)
	Muscle: myopathic features? (CK, ALAT, ASAT, histological anomalies, RRF?)
	Brain: leukodystrophy, poliodystrophy, hypodensity of the cerebrum, cerebellum and the brainstem, multifocal areas of hyperintense signal (MELAS), bilateral symmetrical lesions of the basal ganglia and brainstem (Leigh)? (EEG, MRI, CT scan)
	Peripheral nerve: distal sensory loss, hypo- or areflexia, distal muscle wasting (usually subclinical), reduced motor NCV and denervation features? (NCV, EMG, peripheral nerve biopsy showing axonal degeneration and myelinated fibre loss)
	Pancreas: exocrine pancreatic dysfunction?
	Gut: villous atrophy?
	Endocrine: hypoglycemia, hypocalcemia, hypoparathyroidism, growth hormone deficiency? (stimulation tests)
	Bone marow: anemia, neutropenia, thrombopenia, pancytopenia, vacuolization of marrow precursors?
	Eye: PEO, ptosis, optic atrophy, retinal degeneration? (fundus, ERG, visual evoked potentials)
	Ear: sensorineural deafness? (auditory evoked potentials, brainstem evoked response)
	Skin: trichothiodystrophy, mottled pigmentation of photo exposed areas?

[a] At least four determinations per day before and 1 h after meals.
[b] When standard tests are inconclusive.
EKG, electrocardiogram; CK, creating kinase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GC-MS, gas chromatography mass spectrometry; RRF, ragged red fiber; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; EEG, electroencephalogram; MRI, magnetic resonance imaging; CT, computed tomography; NCV, nerve conduction velocity; EMG, electromyogram; PEO, progressive external ophthalmoplegia; ERG, electroretinogram.

When diagnostic tests are negative, the diagnosis of a respiratory chain deficiency may be missed, especially when solely the onset symptom is present. By contrast, the diagnosis is easier to consider when seemingly unrelated symptoms are observed. For this reason, the investigation of patients at risk (whatever the onset symptom) includes the systematic screening of all target organs, as multiple organ involvement is an important clue to the diagnosis of this condition (Table 2).

Enzyme Assays. The observation of an abnormal redox status in plasma and/or the evidence of multiple organ involvement prompts one to carry out further enzyme investigations. These investigations include two entirely distinct diagnostic procedures which provide independent clues to respiratory chain deficiencies: polarographic studies and spectrophotometric studies.

Polarographic studies consist of the measurement of oxygen consumption by mitochondria-enriched fractions in a Clarck electrode in the

presence of various oxidative substrates (malate + pyruvate, malate + glutamate, succinate, palmitate, etc.). In the case of complex I deficiency, polarographic studies show impaired respiration with NADH-producing substrates, whilst respiration and phosphorylation are normal with FADH-producing substrates (succinate). The opposite is observed in the case of complex II deficiency, whereas a block at the level of complexes III or IV impairs oxidation of both NADH- and FADH-producing substrates. In complex V deficiency, respiration is impaired with various substrates, but adding the uncoupling agent 2,4-dinitrophenol or calcium ions returns the respiratory rate to normal, suggesting that the limiting step involves phosphorylation rather than the respiratory chain [15].

It is worth remembering that polarographic studies detect not only disorders of oxidative phosphorylation but also PDH deficiencies, citric acid cycle enzyme deficiencies and genetic defects of carriers, shuttles and substrates (including cytochrome c, cations and adenylate), as these conditions also impair the production of reducing equivalents in the mitochondrion. In these cases, however, respiratory enzyme activities are expected to be normal.

While previous techniques required gram amounts of muscle tissue, the scaled-down procedures available now allow the rapid recovery of mitochondria-enriched fractions from small skeletal muscle biopsies (100–200 mg, obtained under local anesthetic), thus making polarography feasible in infants and children [16]. Polarographic studies on intact circulating lymphocytes (isolated from 10 ml blood on a Percoll cushion) or detergent-permeabilized cultured cells (lymphoblastoid cell lines, skin fibroblasts) are also feasible and represent a non-invasive and easily reproducible diagnostic test [17]. The only limitation of these techniques is the absolute requirement of fresh material: no polarographic studies are possible on frozen material.

Spectrophotometric studies consist of the measurement of respiratory enzyme activities separately or in groups, using specific electron acceptors and donors. They do not require the isolation of mitochondrial fractions and can be carried out on tissue homogenates. For this reason, the amount of material required for enzyme assays (1–20 mg) is very small and can be easily obtained by needle biopsies of liver and kidney, and even by endomyocardial biopsies [3]. Similarly, a 25 ml flask of cultured skin fibroblasts or a lymphocyte pellet derived from a 10 ml blood sample are sufficient for extensive spectrophotometric studies. Samples should be frozen immediately and kept dry in liquid nitrogen (or at −80°C).

Since conclusive evidence of respiratory chain deficiency is given by enzyme assays, the question of which tissue should be investigated deserves particular attention. In principle, the relevant tissue is the one which clinically expresses the disease. When the skeletal muscle expresses the disease, the appropriate working material is a microbiopsy of the deltoid. When the hematopoietic system expresses the disease (i.e., Pearson syndrome), tests should be carried out on circulating lymphocytes, polymorphonuclears or bone marrow. However, when the disease is predominantly expressed in liver or heart, gaining access to the target organ is far less simple. Yet, a needle biopsy of the liver or an endomyocardial biopsy are usually feasible. If not, or when the disease is mainly expressed in a barely accessible organ (brain, retina, endocrine system, smooth muscle), peripheral tissues should be extensively tested (including skeletal muscle, cultured skin fibroblasts, circulating lymphocytes). Whichever the expressing organ, it is essential to take skin biopsies from such patients (even post mortem) for subsequent investigations on cultured fibroblasts.

It should be borne in mind, however, that the in vitro investigation of oxidative phosphorylation remains difficult regardless the tissue tested. Several pitfalls should be considered:

- A normal respiratory enzyme activity does not preclude mitochondrial dysfunction even when the tissue tested, clinically expresses the disease. One might be dealing with a kinetic mutant, tissular heterogeneity or cellular mosaicism (heteroplasmy, see below). In this case, one should carry out extensive molecular genetic analyses, test other tissues, and possibly repeat investigations later.
- A deficient respiratory enzyme activity does not imply that oxidative phosphorylation is primarily impaired. We are now aware of deficient respiratory enzyme activities secondary to inborn errors of intramitochondrial β-oxidation (long chain and 3-hydroxy-long chain acyl CoA dehydrogenase deficiency). It is important to

carry out in vitro investigations of β-oxidation when the clinical presentation is also compatible with an inborn error of fatty acid oxidation (cardiomyopathy, hepatic failure).

- The scattering of control values occasionally hampers the recognition of enzyme deficiencies as normal values frequently overlap those found in the patients. It is helpful to express results as ratios, especially as the normal functioning of the respiratory chain requires a constant ratio of enzyme activities [18]. Under these conditions, patients whose absolute activities are in the low normal range can be unambiguously diagnosed as enzyme deficient, although this expression of results may fail to recognize generalized defects of oxidative phosphorylation.
- No reliable method for the assessment of complex I activity in circulating or cultured cells is presently available, because oxidation of NADH-generating substrates by detergent-treated or freeze-thawed control cells is variable and the rotenone-resistant NADH-cytochrome c reductase activity is very high in this tissue.
- The phenotypic expression of respiratory enzyme deficiencies in cultured cells is unstable and activities return to normal values when cells are grown in a standard medium [19]. The addition of uridine ($200\mu M$) to the culture medium avoids counterselection of respiratory enzyme deficient cells and allows them to grow normally, thereby stabilizing the mutant phenotype (uridine which is required for nucleic acid synthesis is probably limited by the secondary deficiency of the respiratory chain-dependent dihydro-orotate dehydrogenase activity) [20].
- Discrepancies between control values may indicate faulty experimental conditions. Activities dependent on a single substrate should be consistent when tested under non-rate limiting conditions. For example, normal succinate-cytochrome c reductase (SCCR) activity should be twice as high as normal succinate-quinone-dichlorophenolindophenol reductase (SQDR) activity (because one e^- is required to reduce cytochrome c, while two are required to reduce dichlorophenolindophenol, DCPIP).
- Incorrect freezing may result in a rapid loss of quinone-dependent activities, probably due to peroxidation of membrane lipids. Tissue samples fixed for morphological studies are inadequate for subsequent respiratory enzyme assays.

Histopathological Studies. The histological hallmark of mitochondrial myopathy is the ragged red fiber (RRF), which is demonstrated using the modified Gomori trichrome stain, and contains peripheral and inter myofibrillar accumulations of abnormal mitochondria. Although the diagnostic importance of pathological studies is undisputed, the absence of RRFs does not rule out the diagnosis of mitochondrial disorder [9]. Different histochemical stains for oxidative enzymes are used to analyze the distribution of mitochondria in the individual fibers and to evaluate the presence or absence of the enzymatic activities. Histochemical staining permits an estimation of the severity and heterogeneity of enzyme deficiency in the same muscle section. Myofibrillar integrity and the predominant fibre type and distribution can be evaluated with the myofibrillar ATPase stain. Studies using polyclonal and monoclonal antibodies directed against COX subunits are carried out in specialized centers. For analysis, the muscle specimen taken under local anesthetic must be frozen immediately in liquid nitrogen-cooled isopentane.

Magnetic Resonance Spectroscopy (MRS) of Muscle and Brain. Phosphorus MRS allows the study of muscle and brain energy metabolism in vivo. Inorganic phosphate (Pi), phosphocreatine (PCr), adenosine monophosphate (AMP), diphosphate (ADP) or ATP and intracellular pH may be measured. The Pi/PCr ratio is the most useful parameter and may be monitored at rest, during exercise, recovery and at rest. An increased ratio is found in most patients, and MRS is becoming a useful tool in the diagnosis of mitochondrial diseases as well as in the monitoring of therapeutic trials. Yet, the observed anomalies are not specific to respiratory enzyme deficiencies and no correlation between MRS findings and the site of the respiratory enzyme defect can be made [9].

Treatment and Prognosis

No satisfactory therapy is presently available for respiratory chain deficiency. Treatment remains largely symptomatic and does not significantly alter the course of the disease. It includes avoidance of drugs and procedures known to have a detrimental effect, symptomatic treatments, supple-

mentation with cofactors, prevention of oxygen radical damage to mitochondrial membranes and dietary recommendations.

It is advisable to avoid sodium valproate and barbiturates which inhibit the respiratory chain and have occasionally been shown to precipitate hepatic failure in respiratory enzyme-deficient children [10]. Tetracyclines and chloramphenicol should be avoided as well, as they inhibit mitochondrial protein synthesis. Due to the increasing number of tissues affected in the course of the disease, it is recommended that organ transplantations (bone marrow, liver, heart) be avoided.

► Symptomatic treatments include slow infusion of sodium bicarbonate during acute exacerbation of lactic acidosis, pancreatic extract administration in the case of exocrine pancreatic dysfunction and repeated transfusions in cases of anemia or thrombopenia.

► Sustained improvement has been reported in cases of complex III deficiency given menadione (vit K_3, 40–160 mg/day) or Coenzyme Q10 (80–300 mg/day). Treatment with riboflavine (100 mg/day) has been associated with improvement in a few patients with complex I deficiency myopathy. A combination of menadione (or Coenzyme Q10) and riboflavine is usually given to the patients. Carnitine is suggested in patients with secondary carnitine deficiency. The prevention of oxygen radical damage is the rationale for ascorbate administration (2–4 g/day). Dichloroacetate or 2-chloropropionate administration have been proposed to stimulate pyruvate dehydrogenase activity and have occasionally reduced the level of lactic acid [21] but detrimental effects of dichloroacetate have been recently reported (reversible peripheral neuropathy).

► Dietary recommendations are a high lipid-low carbohydrate diet in patients with complex I deficiency. Indeed, a high glucose diet is a metabolic challenge for patients with impaired oxidative phosphorylation, especially as glucose oxidation is largely aerobic in the liver. Based on our experience, we suggest avoiding a hypercaloric diet and parenteral nutrition and recommend a low carbohydrate diet in addition to the symptomatic treatment. Succinate (6 g/day), succinate-producing aminoacids or propionyl carnitine have occasionally been given to patients with complex I deficiency, as these substrates enter the respiratory chain via complex II.

Genetics

Any mode of inheritance may be observed in mitochondrial diseases: autosomal recessive, dominant, X-linked, maternal, or sporadic. This variability is due to the high number of genes that encode the respiratory chain proteins, of which most are located in the cell nuclei, and 13 in the mitochondria. mtDNA molecules are small (16.5 kb), double-stranded, circular, and contain no introns (Fig. 2). mtDNA has a number of unique genetic features [22]:

- It is maternally inherited and its mutations are, therefore, transmitted by the mother
- It has a very high mutation rate, involving both nucleotide substitutions and insertions-deletions
- During cell division, mitochondria are randomly partitioned into daughter cells.

This means that if normal and mutant mtDNA molecules are present in the mothers cells (heteroplasmy), some lineages will have only abnormal mtDNA (homoplasmy), others only normal mtDNA (wild type), and still others again both normal and abnormal mtDNA. In the latter cells, the phenotype will reflect the proportion of abnormal mtDNA.

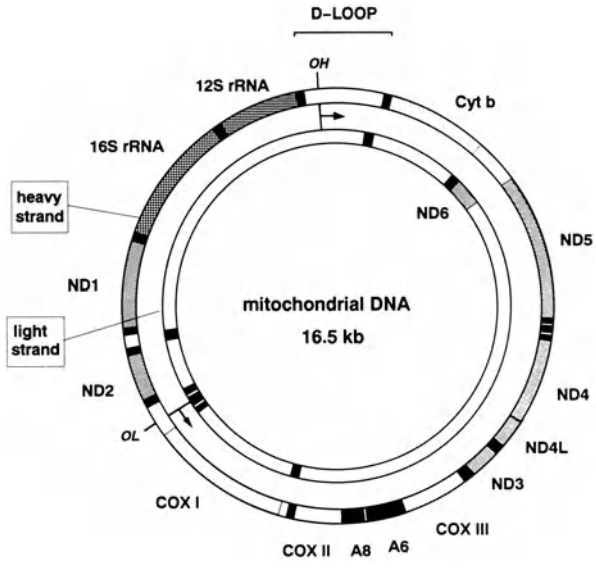

Fig. 2. Map of the mitochondrial genome. Regions encoding cytochrome b (*Cyt b*), various subunits of NADH-coenzyme Q reductase (*ND*), cytochrome oxidase (*COX*), and ATPase (*A*), and ribosomal RNAs (*rRNA*) are indicated. Replication of the heavy strand starts in the displacement (*D*)-loop at the heavy strand origin (*OH*), and that of the light strand at *OL*

Mutations of Mitochondrial DNA. Pathological alterations of mtDNA fall into three major classes: point mutations, rearrangements, and depletions of the number of copies.

Point mutations result in amino acid substitutions and modifications of mRNA and tRNA. Most are heteroplasmic, maternally inherited, and associated with a striking variety of clinical phenotypes (LHON, MERRF, MELAS, NARP, Leigh syndrome, diabetes, and deafness) [23].

Rearrangements comprise deletions-duplications which markedly differ in size and position from patient to patient but usually encompass several coding and tRNA genes. They are usually sporadic, heteroplasmic and unique, and probably arise de novo during oogenesis or in early development (KSS, Pearson syndrome, PEO, diabetes, and deafness [23]). Occasionally maternally transmitted mtDNA rearrangements are found [7]. In other cases, autosomal dominant transmission of multiple mtDNA deletions occurs, suggesting mutation of a nuclear gene essential for the function of the mitochondrial genome [24].

Depletions of the number of copies of mtDNA, consistent with autosomal recessive inheritance, have been reported in rare cases of lethal infantile respiratory, muscle, liver or kidney failure [25].

Mutations in Nuclear DNA. Little is known about the molecular basis of the disorders with mendelian nuclear inheritance. Barth disease has been mapped to chromosome Xq28 [4].

Genetic Analysis of Respiratory Chain Deficiencies. An extensive pedigree with documentation of minor signs in relatives is of paramount importance to recognize the mode of inheritance and to decide on the molecular studies. Maternal inheritance points toward mtDNA mutations, autosomal dominant inheritance toward multiple mtDNA deletions, sporadic cases and autosomal recessive inheritance (consanguineous parents) toward mtDNA deletions-duplications and depletions, respectively.

Investigations require a highly specialized, experienced laboratory and should take into account that:

- The distribution of mutated mtDNA molecules may differ widely among tissues, accounting for the variable clinical expression and requiring investigation of the tissue which actually expresses the disease
- mtDNA rearrangements are unstable and gradually disappear in cultured cells unless uridine is included in the culture medium, thus precluding growth under standard conditions [19]
- Negative investigations neither rule out an mtDNA mutation nor provide a clue that a nuclear mutation is involved.

Genetic Counseling and Prenatal Diagnosis. The identification of certain clinical phenotypes, listed above, allows some prediction with respect to their inheritance. Moreover, it should be borne in mind that in the case of maternal inheritance of a mtDNA mutation, risk is absent for the progeny of an affected male, but high for that of a carrier female. In this case, determination of the proportion of mutant mtDNA on chorion villi or amniotic cells is a rational approach. Nevertheless, its predictive value remains uncertain owing to incomplete knowledge with respect to tissue distribution of abnormal mtDNA, its changes during development, and quantitative relationship to disease severity.

Unfortunately, in the majority of cases, the inheritance of mtDNA rearrangements remains unknown and no reliable genetic counseling can be given. Indeed, dealing with an isolated mtDNA deletion, it is impossible to determine whether a de novo event has occurred or if a heritable mutation is involved.

In the absence of detectable mtDNA mutations, the measurement of the activities of respiratory enzymes in cultured amniocytes or choriocytes provides the only possibility of prenatal diagnosis, particularly since no nuclear mutations have been identified hitherto. Taking advantage of COX deficiencies in cultured skin fibroblasts of probands, we have been able to carry out successful prenatal diagnoses in three unrelated families. Unfortunately, relatively few enzyme deficiencies are expressed in cultured fibroblasts of probands, even when grown with uridine.

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Disorders of Fatty Acid Oxidation

C.A. Stanley

CONTENTS

The oxidation of fatty acids in mitochondria plays an important role in energy production. During late stages of fasting, fatty acids are used for hepatic ketone synthesis and for oxidation in muscle to provide 80% of total body energy needs. Fatty acids are the preferred fuel for the heart and also serve as an essential source of energy for skeletal muscle during sustained exercise. Free fatty acids are released from adipose tissue triglyceride stores and circulate bound to albumin. Their oxidation to CO_2 and H_2O by peripheral tissues, such as muscle, spares glucose consumption and the need to convert body protein to glucose. The use of fatty acids by the liver provides energy for gluconeogenesis and ureagenesis. Equally important, the liver uses fatty acids to synthesize ketones, which serve as a fat-derived fuel for the brain and, thus, further reduce the need for glucose utilization.

A dozen genetic defects in the fatty acid oxidation pathway are currently known. Nearly all of these defects present in early infancy as acute life-threatening episodes of hypoketotic, hypoglycemic coma induced by fasting (for recent reviews, see [1–3]). In some of the disorders there also may be chronic skeletal muscle weakness or acute exercise-induced rhabdomyolysis and acute or chronic cardiomyopathy. Recognition of the fatty acid oxidation disorders is often difficult, because patients can appear well until exposed to prolonged fasting.

As shown in Fig. 1, the pathway for oxidizing fatty acids can be divided into four components:

- The carnitine cycle
- The β-oxidation cycle
- The electron transfer path
- The ketone synthesis path.

Typical endogenous fatty acids are long-chain compounds containing 16–18 carbons. These long-chain fatty acids are activated to their coenzyme A (CoA) esters in the cytosol and then must be shuttled across the barrier of the inner mitochondrial membrane as acylcarnitine esters. (Medium- and short-chain fatty acids from the diet can enter mitochondria directly.) Within the mitochondrial matrix, the four steps of the β-oxidation cycle sequentially shorten the fatty acid by two carbons until it is completely converted to acetyl-CoA. There are two or more chain length-specific isoenzymes for each of these β-oxidation steps. The electron transfer path transfers some of the energy released in β-oxidation to adenosine triphosphate (ATP) production. In the liver, most of the acetyl-CoA from fatty acid β-oxidation is used to synthesize the ketones β-hydroxybutyrate and acetoacetate. The ketones are then exported for terminal oxidation chiefly by the brain. In other tissues, such as muscle, the acetyl-CoA directly enters the Krebs cycle for oxidation and ATP production.

Clinical Presentation

The clinical phenotypes of most of the disorders of fatty acid oxidation are very similar [1, 2]. Table 1 presents the three major types of presentation: a form with signs mainly of hepatic involvement, one with predominantly cardiac involvement, and one chiefly involving skeletal muscle. The indi-

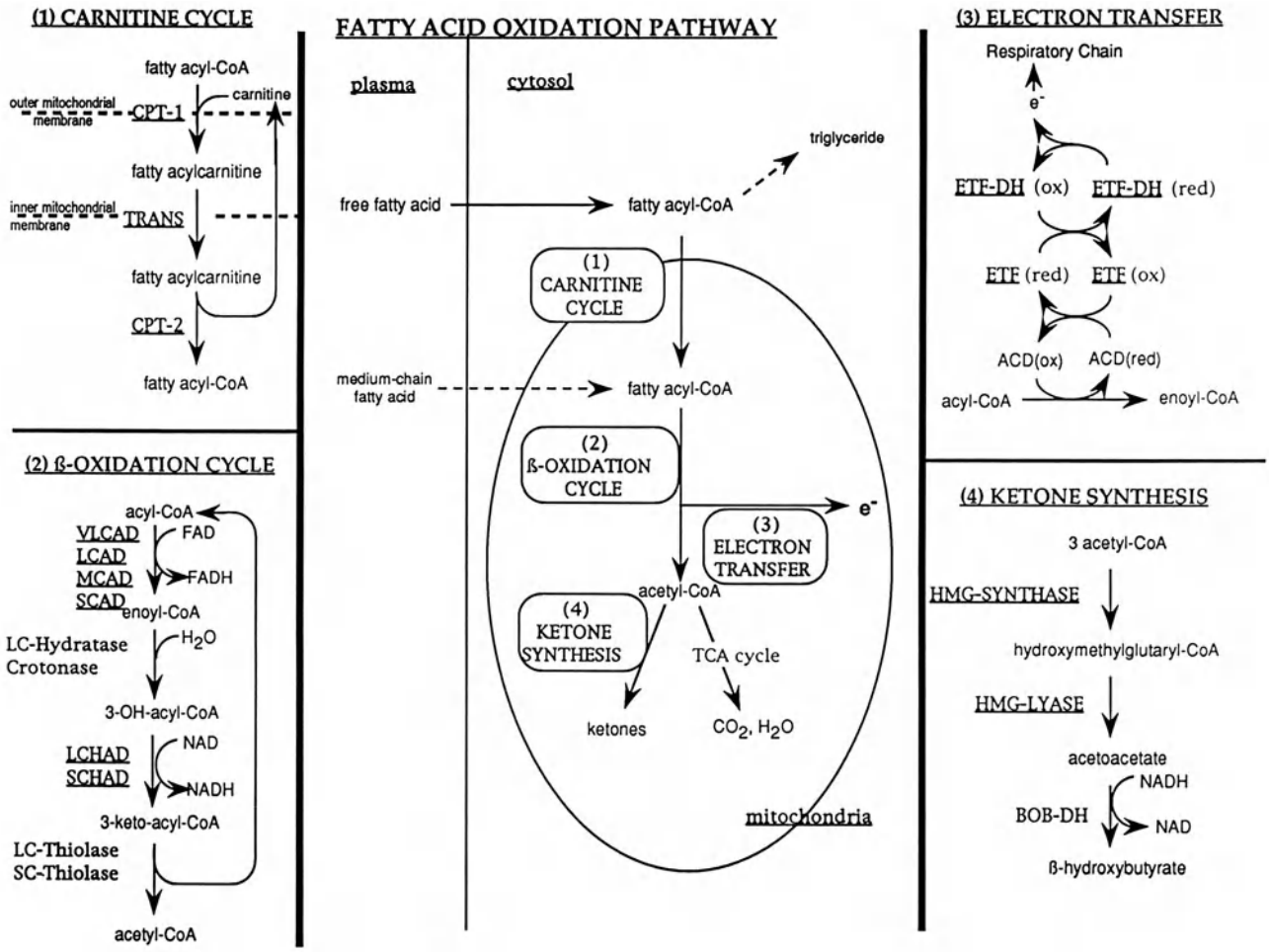

Fig. 1. Mitochondrial fatty acid oxidation pathway. In the center panel, the pathway is subdivided into its four major components, which are shown in detail in the side panels. Sites of identified defects are *underscored*. *CoA*, Coenzyme A; *CPT*, carnitine palmitoyl transferase; *TRANS*, carnitine/acylcarnitine translocase; VLCAD, very long chain acyl-CoA dehydrogenase; *LCAD*, long-chain acyl-CoA dehydrogenase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *SCAD*, short-chain acyl-CoA dehydrogenase; *FAD*, flavin adenine dinucleotide; *FADH*, reduced FAD; *NAD*, nicotinamide adenine dinucleotide; *NADH*, reduced NAD; *TCA*, tricarboxylic acid; *ETF* electron transfer flavoprotein; *ETF-DH*, ETF dehydrogenase; *HMG*, 3-hydroxy-3-methylglutaryl

vidual defects are discussed below under the four components of the fatty acid oxidation pathway outlined in Fig. 1 and Table 1.

Carnitine Cycle. The following defects and deficiencies occur in the carnitine cycle.

Carnitine Transporter Defect. Although most of the fatty acid oxidation disorders affect the heart and skeletal muscle as well as the liver, cardiac failure is seen as the major presenting manifestation only in carnitine transporter defect (CTD) [4]. In this disorder, sodium-dependent transport of carnitine across the plasma membrane is absent in muscle and kidney. This leads to severe reduction (<2%–5% of normal) of carnitine in plasma and in heart and skeletal muscle. These levels of carnitine are low enough to impair fatty acid oxidation. Over half of the 20–30 known cases of CTD first presented with progressive heart failure and generalized muscle weakness. The age of onset of cardiomyopathy or skeletal muscle weakness ranged from 12 months to 7 years. The cardiomyopathy in CTD patients is most evident on echocardiography, which shows poor contractility and thickened ventricular walls. Electrocardiograms may be normal or show increased T-waves. Without carnitine treatment, the cardiac failure can progress rapidly to death by 2–4 years of age.

During the first year, extended fasting stress may provoke an attack of hypoketotic, hypo-

Table 1. Inherited disorders of mitochondrial fatty acid oxidation

Enzyme/transporter deficiency	Clinical manifestations of defect			
	Hepatic	Cardiac	Skeletal muscle	
			Acute	Chronic
Carnitine Cycle				
Carnitine transporter (CTD)	+	+		
Carnitine palmityl transferase-1 (CPT-1)	+			
Carnitine/acylcarnitine translocase (TRANS)	+	+		+
Carnitine palmityl transferase-2 (CPT-2)	+	+	(+)	+
β-Oxidation cycle				
Acyl-CoA dehydrogenases				
Very long-chain (VLCAD)	+	+		+
Long-chain (LCAD)	+	+		+
Medium-chain (MCAD)	+			
Short-chain (SCAD)				+
3-Hydroxyacyl-CoA dehydrogenases				
Long-chain (LCHAD)	+	+		
Short-chain (SCHAD)				+
2,4-Dienoyl-CoA reductase (DER)				+
Electron transfer				
Electron transfer flavoprotein (ETF)	+	+		+
ETF dehydrogenase (ETF-DH)	+	+		+
Ketone synthesis				
HMG-CoA synthase	+			
HMG-CoA lyase	+			

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

glycemic coma with or without evidence of cardiomyopathy. This hepatic presentation occurs less frequently than the myopathic forms, because the liver has a separate transporter for carnitine and can usually maintain sufficient levels of carnitine to support ketogenesis.

Carnitine Palmitoyl Transferase-1 Deficiency. In patients with carnitine palmitoyl transferase-1 (CPT-1) deficiency, only the nonmuscle isoenzyme of CPT-1 which is expressed in liver and kidney is affected. Patients with this defect have usually presented during the first 2 years of life with attacks of fasting hypoketotic coma [5–7]. They do not have cardiac or skeletal muscle involvement. CPT-1 deficiency is the only fatty acid oxidation disorder with elevated plasma total carnitine levels (see below) [6]. The defect is also noteworthy for unusually severe abnormalities in liver function tests during and for several weeks after acute episodes of illness, including massive increases in serum transaminases and hyperbilirubinemia. Transient renal tubular acidosis has also been described in a patient with CPT-1 deficiency, probably reflecting the importance of fatty acids as fuel for the kidney [8].

Carnitine/Acylcarnitine Translocase Deficiency. Four cases of this defect are known. All of the patients were severely affected with onset in the neonatal period. Presentations included fasting hypoketotic coma, cardiopulmonary arrest, and ventricular arrhythmias. The single patient who survived the newborn period had persistent muscle weakness. He died at 3 years of age with progessive liver failure and muscle weakness, despite intensive feeding [9, 10]. This defect as well as the severe forms of CPT-2 and electron transfer flavoprotein/ETF dehydrogenase (ETF/ETF-DH) deficiencies appear to have a very poor prognosis.

Carnitine Palmitoyl Transferase-2 Deficiency. Two forms of this defect are known, a mild adult onset form characterized by exercise-induced attacks of rhabdomyolysis and a severe neonatal onset form which presents with life-threatening coma, cardiomyopathy, and weakness [11–14]. Neonatal-onset CPT-2 deficiency as well as the severe form of ETF/ETF-DH deficiency have been associated with congenital brain and renal malformations.

Patients with the milder adult form of CPT-2 deficiency begin to have attacks of rhabdomyolysis in the second and third decades of life. These attacks are triggered by catabolic

stresses such as prolonged exercise, fasting, or cold exposure. Episodes are associated with aching muscle pain, elevated plasma creatine phosphokinase (CPK) levels, and myoglobinuria, which may lead to renal shutdown [11].

β-Oxidation Enzymes. Deficiencies can be divided into acyl-CoA and 3-hydroxy acyl-CoA dehydrogenase deficiencies

Acyl CoA Dehydrogenases. Long-chain and very long chain acyl-CoA dehydrogenase (LCAD/VLCAD) deficiencies are considered together here because there has been difficulty in distinguishing which of these related enzymes is defective. A few patients labeled as LCAD deficient based on assays of activity with palmitoyl-CoA have recently been proven to have VLCAD deficiency [15, 16]. Of two dozen patients in this category, most have had severe clinical manifestations with chronic cardiomyopathy and weakness in addition to episodes of fasting coma [17, 18]. Several have presented in the newborn period with severe life-threatening coma similar to patients with carnitine/acylcarnitine translocase (TRANS) or severe CPT-2 deficiency.

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the single most common fatty acid oxidation disorder [1, 19, 20]. It is also one of the mildest, with no evidence of chronic muscle or cardiac involvement. In addition, it is unusually homogeneous, because 90% of patients are homozygous for a single A985G missense mutation originating in northwestern Germany and the British Isles [21]. The estimated incidence in Britain is 1 in 10 000 births [22].

As shown in Table 1, patients with MCAD deficiency have an exclusively "hepatic" type of presentation. Affected individuals appear to be entirely normal until an episode of illness is provoked by an excessive period of fasting. This may occur with any infection that interferes with normal feeding or simply because breakfast is delayed. The first episode typically occurs between 3–24 months of age, after nocturnal feedings have ceased. A few neonatal cases have been reported in which attempted breast-feeding was sufficient fasting stress to cause illness. Attacks become less frequent after childhood, because fasting tolerance improves with increasing body mass.

The response to fasting in MCAD deficiency identifies many of the pathophysiologic features of the hepatic presentation of the fatty acid oxidation disorders (Fig. 2). No abnormalities occur during the first 12–14 h, because lipolysis and fatty acid oxidation have not yet been activated. By 16 h, plasma levels of free fatty acids have risen dramatically, but ketones remain inappropriately low, reflecting the defect in hepatic fatty acid oxidation. Hypoglycemia develops shortly thereafter, probably because of excessive glucose utilization due to the inability to switch to fat as a fuel. Severe symptoms of lethargy and nausea develop in association with the marked increase in plasma fatty acids. It should be stressed that patients with fatty acid oxidation defects can become dangerously ill *before* plasma glucose falls to hypoglycemic values. This may be due to several factors, including absence of ketones, failure of energy supply to heart and skeletal muscle, and toxic effects of elevated plasma free fatty acids or tissue fatty acid intermediates. An acute attack in MCAD deficiency usually features lethargy, nausea, and vomiting which rapidly progresses to coma within 1–2 h. Seizures may occur and patients may die suddenly from acute cardiorespiratory arrest.

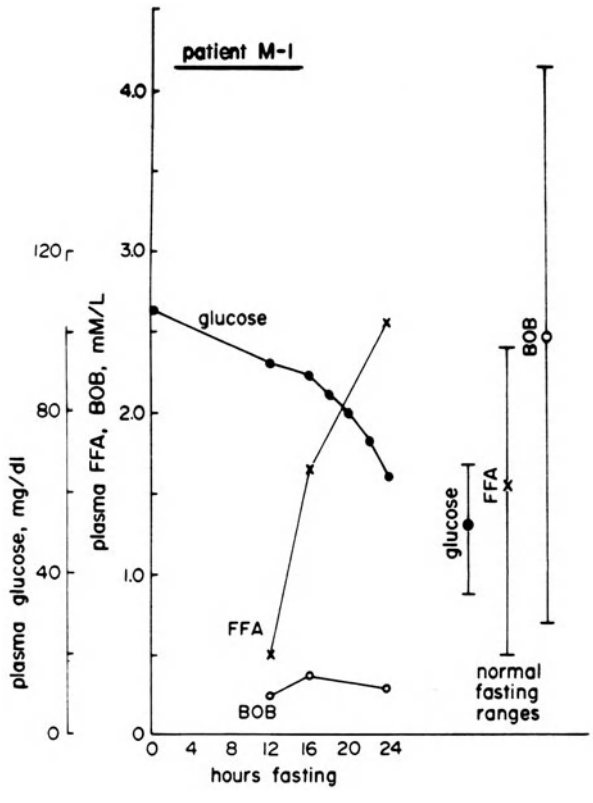

Fig. 2. Response to fasting in a patient with MCAD deficiency. Shown are plasma levels of glucose, free fatty acids (*FFA*), and β-hydroxylbutyrate (*BOB*) in the patient and the mean and range of values in normal children fasted for 24 h. The patient became ill with pallor, lethargy, nausea, and vomiting, beginning at 14–16 h of fasting

They may also die or suffer permanent brain damage from cerebral edema. Up to 25% of MCAD patients die during their first attack. Because there is no forewarning, the first episode may be misdiagnosed as Reye syndrome, sudden infant death syndrome (SIDS) etc. MCAD deficiency can be considered as one cause of Reye syndrome, although the age of onset is younger than usual, and the liver biopsy shows steatosis but not mitochondrial swelling.

At the time of an acute attack in MCAD deficiency, the liver may be slightly enlarged or it may become enlarged during the first 24 h of treatment. Chronic cardiac and skeletal muscle abnormalities are not seen in MCAD deficiency, perhaps because the block in fatty acid oxidation is incomplete. However, the enzyme defect is probably expressed in cardiac and skeletal muscle and these organs are probably responsible for the sudden death which may occur during attacks of illness in MCAD deficient infants and children.

Only six or seven cases of *short-chain acyl-CoA dehydrogenase* (SCAD) deficiency are known and the clinical phenotype remains very unclear [23]. Most have presented in early infancy with acidemia, failure to thrive, muscle weakness, and/or progressive developmental delay. None have been reported to have fasting hypoglycemia and, in the one case studied, fasting ketogenesis was not impaired.

3-Hydroxy Acyl-CoA Dehydrogenases. Recent work indicates that the activities of long-chain enoylCoA hydratase, 3HO-acyl-CoA dehydrogenase, and β-keto-thiolase are combined in a single trifunctional protein. Some patients have isolated *long-chain 3-hydroxy acyl-CoA dehydrogenase* (LCHAD) deficiency, while others are also deficient in long-chain enoyl-CoA hydratase and long-chain β-keto acyl-CoA thiolase activities [24–28]. The clinical phenotype ranges from a mild disorder resembling MCAD deficiency to more severe involvement of heart and skeletal muscle similar to LCAD/VLCAD deficiency. Some patients have had retinal degeneration or peripheral neuropathy, suggesting a toxicity effect. Several cases have been reported of heterozygote mothers developing acute fatty liver of pregnany (AFLP) syndrome when carrying affected fetuses [29]. This reinforces the suggestion of peculiar toxicity effects in LCHAD deficiency.

Only one case of *short-chain 3-hydroxy acyl-CoA dehydrogenase* (SCHAD) deficiency is known. The presentation was recurring episodes of myoglobinuria and hypoglycemic coma. Enzyme deficiency was found in muscle, but not in fibroblasts [30].

Only a single case of *2,4-dienoyl-CoA reductase* (DER) deficiency has been reported in the pathway required for oxidation of unsaturated fatty acids [31]. The patient was hypotonic from birth and died at 4 months of age. The disorder was suspected based on low plasma total carnitine levels and urinary excretion of an unusual unsaturated fatty acylcarnitine in urine.

Electron Transfer. *ETF/ETF-DH* deficiencies in the pathway for transferring electrons from the first step in β-oxidation to the electron transport system are grouped together [32]. They are also known as *glutaric aciduria type 2* or *multiple acylCoA dehydrogenation deficiencies*. These defects block not only fatty acid oxidation, but also the oxidation of branched-chain amino acids, lysine, and glutaric acid. Patients with severe or complete deficiencies of the enzymes present with hypoglycemia, acidosis, hypotonia, cardiomyopathy, and coma in the neonatal period. Some neonates with ETF/ETF-DH deficiencies have had congenital anomalies (polycystic kidney, midface hypoplasia). Partial deficiencies of ETF/ETF-DH are associated with milder disease, resembling MCAD or LCAD deficiency. Some patients have been reported to respond to supplementation with riboflavin, a co-factor for these enzymes. The urine organic acid profile is usually diagnostic, especially in the severe form of these deficiencies.

Ketone Synthesis. The single case of *3-hydroxy-3-methylglutaryl-CoA synthase* deficiency that has been identified presented in mid-childhood with fasting hypoketotic, hypoglycemic coma [33]. There was no evidence of myopathy and there was no impairment in fibroblast fatty acid oxidation. These findings are consistent with the fact that the mitochondrial synthase enzyme is expressed only in organs capable of making ketones, the liver and kidney. In contrast to all of the other fatty acid oxidation defects, plasma and tissue total carnitine levels were normal. This may prove to be a useful clue in identifying other patients with the synthase defect.

Although often classified with the amino acid oxidation disorders, *3-hydroxy-3-methylglutaryl-CoA lyase* deficiency is included here because it is the last step in ketone synthesis from fatty acids, as well as being the last step in the leucine oxida-

tion path. Most reported patients have presented with episodes of hypoketotic, hypoglycemic coma induced by fasting [34]. The illness mimics MCAD deficiency, except that lyase deficiency is associated with a marked metabolic acidosis due to accumulation of hydroxymethylglutaric acid.

Metabolic Derangement

The metabolic derangements associated with different defects in the mitochondrial fatty acid oxidation and ketone synthesis pathway vary significantly depending on the exact site of defect and are therefore discussed in the section on "Diagnostic Tests" below.

Diagnostic Tests

Tests of Overall Pathway. These include in vivo fasting study, in vitro fatty acid oxidation, and histology.

In Vivo Fasting Study. In diagnosing the fatty acid oxidation disorders, it is frequently useful to first demonstrate an impairment in the overall pathway before attempting to identify the specific site of defect. Blood and urine samples collected immediately prior to treatment of an acute episode of illness can be used for this purpose, e.g., by showing elevated plasma free fatty acid but inappropriately low ketone levels at the time of hypoglycemia. A carefully monitored study of fasting ketogenesis can provide this information (Fig. 2). This provocative test can put the patient at risk and should only be done under controlled circumstances with careful supervision. Some investigators prefer using fat-loading as an alternative means of testing hepatic ketogenesis [35] (Chap. 2).

In Vitro Fatty Acid Oxidation. Cultured skin fibroblasts or lymphoblasts from patients can also be used to demonstrate a general defect in fatty acid oxidation using ^{14}C or ^{3}H-labeled substrates. In addition, different chain-length fatty acid substrates can be used with these cells to localize the probable site of defect. The utility of in vitro oxidation assays depends on the site of defect. Very low rates of labeled fatty acid oxidation are found in CPT-1, TRANS, CPT-2, and ETF/ETF-DH deficiencies. However, high residual rates of oxidation (50%–80% or more of normal) frequently make identification of the β-oxidation enzyme defects difficult. In CTD, oxidation rates are normal unless special steps are taken to grow cells in carnitine-free media. The in vitro oxidation assays do not detect the defects in ketone synthesis 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase and HMG-CoA lyase.

Histology. The appearance of increased triglyceride droplets in affected tissues sometimes provides a clue to the presence of a defect in fatty acid oxidation. In the hepatic presentation of any of the fatty acid oxidation disorders, a liver biopsy obtained during an acute episode of illness shows an increase in neutral fat deposits which may have either a micro- or macrovesicular appearance. Between episodes, the amount of fat in liver may be normal. More severe changes, including hepatic fibrosis, have been seen in LCAD/VLCAD patients who where ill for prolonged periods [36]. This damage appears to reflect persistent efforts to metabolize fatty acids, since it may resolve as patients are adequately nourished. On electron microscopy, mitochondria do not show the severe swelling described in Reye syndrome, but may show minor changes such as crystalloid inclusion bodies. The fatty acid oxidation disorders which are expressed in muscle may be associated with increased fat droplet accumulation in muscle fibers and demonstrate the appearance of "lipoid myopathy" on biopsy.

Disease-Related Metabolites. In general, efforts to identify fatty acid oxidation disorders by the identification of disease-related metabolites have not been as successful as in other groups of inborn errors, such as the amino acid oxidation or urea cycle defects. This is particularly true since any abnormal metabolites which may be formed are unlikely to be present except during fasting or other stressed conditions.

Urinary Organic Acids. The urinary organic acid profile is usually normal in patients with fatty acid oxidation disorders when they are well. During times of fasting or illness, all of the disorders are associated with an "inappropriate" *dicarboxylicaciduria*, i.e., urinary medium chain dicarboxylic acids are elevated, while urinary ketones are not. This reflects the fact that dicarboxylic acids, derived from partial oxidation of fatty acids in microsomes and peroxisomes, are produced

Table 2. Fatty acid oxidation disorders with distinguishing metabolic markers

Disorder	Organic acids (urine)	Acylcarnitines	Acylglycines (urine)
Very long chain/long-chain acyl-CoA dehydrogenase (VLCAD)		Tetradecenoyl (P)	
Medium-chain Acyl-CoA dehydrogenase (MCAD)		Octanoyl Decenoyl (P, U)	Hexanoyl Suberyl Phenylpropionyl
Short-chain acyl-CoA dehydrogenase (SCAD)	Ethylmalonic	Butyryl (P, U)	Butyryl
Long-chain 3-hydroxy-acyl-CoA dehydrogenase (LCHAD)	3-Hydroxy dicarboxylic		
2,4-Dienoyl-CoA reductase (DER)		Dodecadienoyl (P)	
Electron transfer flavoprotein (ETF) and ETF dehydrogenase (ETF/ETF-DH)	Ethylmalonic Glutaric	Isovaleryl Glutaryl (U)	Isovaleryl Hexanoyl
HMG-CoA Lyase	3-Hydroxy-3-methylglutaryl (plus others)	Methylglutaryl (U)	

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; P, plasma; U, urine.

whenever plasma free fatty acid concentrations are elevated. In MCAD deficiency, the amounts of dicarboxylic acids excreted are two- to fivefold greater than in normal fasting children. However, in other defects, only the ratio of ketones to dicarboxylic acids is abnormal. In a few of the disorders, specific abnormalities of urine organic acid profiles may be present (Table 2), but are not likely to be found except during fasting stress.

Plasma Fatty Acids. In MCAD deficiency, specific increases in plasma concentrations of the medium-chain fatty acids octanoate and cis-4-decenoate have been identified which can be useful for diagnosis. Abnormally elevated plasma concentrations of these fatty acids are most apparent during fasting. It is not known whether specific abnormalities in plasma fatty acid profiles might be found in other disorders.

Urinary Acylglycines. In MCAD deficiency, the urine contains increased concentrations of the glycine conjugates of hexanoate, suberate (C-8 dicarboxylic acid), and phenylpropionate, which are derived from their coenzyme A esters [37]. When these are quantitated by isotope dilution-mass spectrometry, specific diagnosis of MCAD deficiency is possible even using random urine specimens. Abnormal glycine conjugates are present in urine from patients with some of the other disorders of fatty acid oxidation (Table 2). A *loading test of phenylpropionate* followed by assay of urinary phenylpropionylglycine excretion can be used as a test specifically for MCAD deficiency [38].

Urine/Plasma Acylcarnitines. Since acylCoA intermediates proximal to blocks in the fatty acid oxidation pathway can be transesterified to carnitine, some of the fatty acid oxidation disorders can be detected by analysis of either plasma or urinary acylcarnitine profiles (Table 2) [1].

Plasma and Tissue Total Carnitine Concentrations. A peculiar feature of the fatty acid oxidation disorders is that all but one are associated with either decreased or increased concentrations of total carnitine in plasma and tissues [9]. In CTD, plasma total carnitine levels are severely decreased (<5% of normal) [4]. In CPT-1 deficiency, total carnitine levels are increased (150%–200% of normal) [6]. In all of the other defects, except HMG-CoA synthase deficiency, total carnitine levels are reduced to 25%–50% of normal ("secondary carnitine deficiency"). Thus, simple measurement of plasma total carnitine is often helpful to determine the presence of a fatty acid oxidation disorder. It should be emphasized that samples must be taken in the well-fed state with normal dietary carnitine intake, because patients with disorders of fatty acid oxidaion may show acute increases in plasma total carnitine during prolonged fasting or during attacks of illness.

The basis of the carnitine deficiency in CTD has been shown to be a defect in the plasma membrane carnitine transporter activity. The reason for the increased carnitine levels in CPT-1 defi-

ciency and the decreased carnitine levels in other fatty acid oxidation disorders has been unclear. Recently, we have demonstrated that both phenomena can be explained by the competitive inhibitory effects of long-chain and medium-chain acylcarnitines on the carnitine transporter [39]. Thus, in patients with MCAD or TRANS deficiency, the blocks in acyl-CoA oxidation lead to accumulation of acylcarnitines which inhibit renal and tissue transport of free carnitine and result in lowered plasma and tissue concentrations of carnitine. Conversely, the inability to form long-chain acyl-CoA in CPT-1 deficiency results in less inhibition of carnitine transport from long-chain acylcarnitines than normal and therefore increases renal carnitine thresholds and plasma levels of carnitine to values greater than normal.

Assay of Enzyme Activity. Cultured skin fibroblasts or cultured lymphoblasts have become the preferred material in which to measure the in vitro activities of specific steps in the fatty acid oxidation pathway. All of the known defects, except HMG-CoA synthase, are expressed in these cells and results of assays in cells from both control and affected patients have been reported. Because these assays are not widely available, they are most usefully applied to confirm a site of defect that is suggested by other clinical and laboratory data.

Treatment and Prognosis

The following sections focus on treatment of the hepatic presentation of fatty acid oxidation disorders, since this is the most life-threatening aspect of these diseases. Although there is a high risk of mortality or long-term disability during episodes of fasting-induced coma, with early diagnosis and treatment patients with most of the disorders have an excellent prognosis. The mainstay of therapy is to prevent recurrent attacks by adjusting the diet to minimize fasting stress.

► **Management of Acute Illnesses.** When patients with fatty acid oxidation disorders become ill, treatment with intravenous glucose should be given immediately. Delay may result in sudden death or permanent brain damage. The goal is to provide sufficient glucose to stimulate insulin secretion to levels that will not only suppress fatty acid oxidation in liver and muscle, but also block adipose tissue lipolysis. Solutions of 10% dextrose, rather than the usual 5%, should be used at infusion rates of 10 mg/kg per min or greater to maintain high to normal levels of plasma glucose, above 100 mg/dl (5.5 mmol/l). Resolution of coma may not be immediate, perhaps because of the toxic effects of fatty acids noted above, but may take 2–4 h in mildly ill patients or as long as 1–2 days in severely ill patients.

Long-Term Diet Therapy. It is essential to prevent any period of fasting which would require the use of fatty acids as a fuel. This can be done by simply ensuring that patients have a carbohydrate feeding at bedtime and do not fast for more than 12 h overnight. During intercurrent illnesses, when appetite is diminished, care should be taken to give extra feedings of carbohydrate during the night. In a few patients with very severe defects in fatty acid oxidation who had developed weakness and/or cardiomyopathy, we have gone further to completely eliminate fasting by the addition of continuous nocturnal intragastric feedings. The use of uncooked corn starch at bedtime might be considered as a slowly released form of glucose (for details see Fernandes and Chen, this volume), although this has not been formally tested in these disorders. Some authors recommend restricting fat intake. Although this seems reasonable in patients with severe defects, we have not routinely restricted dietary fat in milder defects such as MCAD deficiency.

Carnitine Therapy. In patients with CTD, treatment with carnitine improves cardiac and skeletal muscle function to nearly normal within a few months [4]. It also corrects any impairment in hepatic ketogenesis which may be present [4]. With oral carnitine at doses of 100 mg/kg per day, plasma carnitine levels can be maintained in the low to normal range and liver carnitine levels may be normal. However, muscle carnitine concentrations remain less than 5% of normal. Since these low levels are adequate to reverse myopathy in CTD, it appears that the threshold for defining carnitine deficiency is a tissue concentration less than approximately 5% of normal. ◄

A possible role for carnitine therapy in those disorders of fatty acid oxidation which are associated with secondary carnitine deficiency remains controversial. Since these disorders involve blocks at specific enzyme steps that do not involve carnitine, it is obvious that carnitine treatment cannot

correct the defect in fatty acid oxidation. It has been proposed that carnitine might help to remove toxic metabolites in these disorders, because the enzyme defects might be associated with accumulation of acyl-CoA intermediates. However, there has been no direct evidence that this is true. In addition, as noted above, the mechanism of the secondary carnitine deficiency is not a direct one, via loss of acylcarnitines in urine, but appears to be indirect, via inhibition of the carnitine transporter in kidney and other tissues by medium or long-chain acylcarnitines. It should also be noted that the secondary carnitine deficiency could be a protective adaptation, since there is data showing that long-chain acylcarnitines may have toxic effects. Our current practice is not to recommend the use of carnitine except as an investigational drug in fatty acid oxidation disorders other than CTD.

Other Therapies. Since medium-chain fatty acids bypass the carnitine cycle (Fig. 1) and enter the midportion of the mitochondrial β-oxidation spiral directly, it is possible that they might be used as fuels in defects which block either the carnitine cycle or long-chain β-oxidation. For example, dietary MCT was suggested to be helpful in a patient with LCHAD deficiency. The benefits of MCT have not been thoroughly investigated, but MCT clearly must not be used in patients with MCAD, SCAD, SCHAD, ETF/ETF-DH, HMG-CoA synthase, or HMG-CoA lyase deficiencies. Some patients with mild variants of ETF/ETF-DH and SCAD deficiencies have been reported to respond to supplementation with high doses of riboblavin (100 mg/day), the cofactor for these enzymes.

Prognosis. Although acute episodes carry a high risk of mortality or permanent brain damage, many patients with disorders of fatty acid oxidation can be easily managed by avoidance of prolonged fasts. These patients have an excellent long-term prognosis. Patients with chronic cardiomyopathy or skeletal muscle weakness have a more guarded prognosis, since they seem to have more severe defects in fatty acid oxidation. For example, TRANS or the severe variants of CPT-2 and ETF/ETF-DH deficiencies frequently lead to death in the newborn period. On the other hand, the mild form of CPT-2 deficiency may remain silent as long as patients avoid extreme exercise stress.

Genetics

All of the genetic disorders of fatty acid oxidation which have been identified are inherited in autosomal recessive fashion. Heterozygote carriers are clinically normal (with the possible exception, noted above, of the occurrence of AFLP in LCHAD heterozygote mothers carrying an affected fetus). Carriers of the fatty acid oxidation disorders show no biochemical abnormalities except for CTD, in which carriers have half normal levels of plasma total carnitine concentrations. Since some of the disorders, such as MCAD deficienty, may be present without having caused an attack of illness, siblings of patients with fatty acid oxidation disorders should be investigated to determine whether they might be affected.

Prenatal diagnosis by assay of labeled fatty acid oxidation and/or enzyme activity in amniocytes or chorionic villi should theoretically be possible for those disorders which are expressed in cultured skin fibroblasts, i.e., all of the currently known defects except HMG-CoA synthase deficiency. This has been done in a few instances in MCAD deficiency, although newer molecular methods are now available for this particular defect. Metabolite screening of amniotic fluid has not been useful for most defects. No general newborn screening test has been developed for the fatty acid oxidation disorders, although filter paper blood spots can be used to diagnose MCAD deficiency by analysis of fatty acid or acylcarnitine profiles or demonstration of the common A985G mutation. Analysis of acylcarnitine profiles from newborn blood spots might also prove useful in neonatal detection of other fatty acid oxidation disorders, although only a few have yet been associated with specific abnormalities (Table 2).

Rapid progress has been made in establishing the molecular basis for several of the defects in fatty acid oxidation (see [1]). This has become especially useful clinically in MCAD deficiency. Over 80% of MCAD patients are homozygous for a single missense mutation, an A to G substitution at cDNA position 985 which changes a lysine residue to glutamate. This probably represents a "founder" effect and explains why most MCAD patients share a northwestern European ethnic background. Simple polymerase chain reaction (PCR) assays have been established to detect the A985G mutation using DNA from many different sources, including newborn blood spot cards. This method has been used to diagnose MCAD defi-

ciency in a variety circumstances including prenatal diagnosis, postmortem diagnosis, diagnosis of affected siblings, and for surveys of disease incidence. It has been suggested that this would also provide a possible method for newborn screening.

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Part IV
Aminoacids

Hyperphenylalaninaemias

I. Smith and D.P. Brenton

CONTENTS

Normally a high proportion of ingested phenylalanine is converted to tyrosine in the liver by the enzyme phenylalanine hydroxylase. This is the controlling step of *phenylalanine* homeostasis (Fig. 1) [1]. Primary forms of *hyperphenylalaninaemia* are a group of recessively inherited disorders in which the rate of this reaction is reduced. As a result, concentrations of phenylalanine increase relative to tyrosine in blood and other body fluids. A parallel increase occurs in the production and excretion of phenylketones and phenylamines (due to a combination of transamination, decarboxylation and deamination). The term "*phenylketonuria*" (or PKU) is often reserved, rather illogically, for more severe forms of deficiency in the enzyme phenylalanine hydroxylase in which urinary phenylketones are easy to detect by simple chemical methods.

How do we define "hyperphenylalaninaemia"? Normally fasting plasma phenylalanine concentrations range from 35 μmol/l to 100 μmol/l. Following a protein-rich meal, concentrations rise by as much as two to three times, reaching a peak 2–3 h after the meal. Tyrosine concentrations are generally at or just above those of phenylalanine, giving a ratio of between 0.6 and 1.5. In pregnant women or those taking the pill, the ratio tends to be at the higher end of the range, and in heterozygous carriers the ratio is usually between 1.2 and 2.5 with blood phenylalanine levels occasionally above 200 μmol/l. A phenylalanine to tyrosine ratio of 3 or more provides us with the most useful definition of "hyperphenylalaninaemia".

Disorders causing primary hyperphenylalaninaemia will be discussed under three main headings (Table 1):

- Phenylalanine hydroxylase deficiency
- Maternal phenylketonuria
- Defects of biopterin metabolism

Phenylalanine Hydroxylase Deficiency

Clinical Presentation

In late-detected subjects with more severe forms of hyperphenylalaninaemia (plasma phenylalanine concentrations greater than 1200 μmol/l), retarded development and intellectual impairment are the most constant clinical features. Infantile spasms with a pattern of hypsarrythmia on electroencephalography (EEG) begins after the first few months in around a third of infants, often with microcephaly, increased limb tone and mousy odor due to excretion of phenylacetic acid. A majority have lightly pigmented eyes, hair and skin in comparison to parents and unaffected siblings, and eczema occurs in 20%–40%. In older patients disturbed behaviour is common with hyperactivity, destructiveness, self-injury and episodes of excitement. Schizophrenia-like symptoms have been described. About 25% have seizures of grand mal-type at some time, and abnormalities in the EEG are almost invariable.

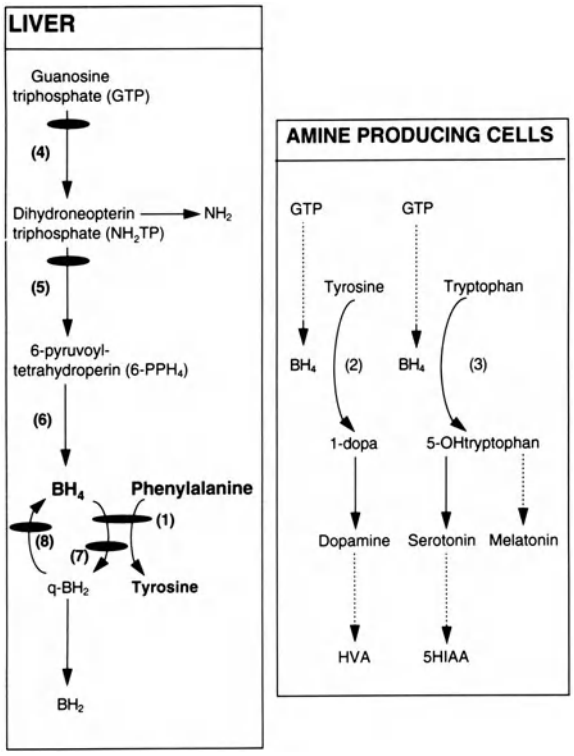

Fig. 1. Hydroxylation phenylalanine to tyrosine with synthesis and recycling of pterins in liver and in amine producing cells. *BH_4*, tetrahydrobiopterin; *BH_2*, dihydrobiopterin; *q-BH_2*, quinonoid BH_2; *NH_2*, dihydroneopterin. Known inborn errors ⬬. Enzymes: *1*, phenylalanine hydroxylase; *2*, tyrosine hydroxylase; *3*, tryptophan hydroxylase; *4*, guanosine triphosphate (GTP) cyclohydrolase; *5*, 6-pyruvoyltetrahydropterin (6-PBH_4) synthase; *6*, sepiapterin reductase; *7*, tetrahydropterin carbinolamine dehydratase; *8*, dihydropteridine reductase (DHPR)

Pyramidal tract signs (notably hyperreflexia and increased tone) are common. Tremor, abnormalities of gait, posturing and ticks also occur, and overt parkinsonism has been described. Generally the level of disability is stable beyond early childhood, although progressive intellectual regression and increasing neurological features are described.

It should be stressed that even in biochemically severe forms of the disorder there is wide variation in clinical phenotype. Within a sibship one individual may exhibit a typical array of symptoms, whilst another shows only minor impairment. It has been estimated that without treatment one in six to seven subjects achieves an intellectual status adequate for education in a normal school [2].

Subjects with milder disease have a lower risk of mental handicap. Blood phenylalanine concentrations below a certain threshold (often less than 1000 μmol/l, sometimes less than 600 μmol/l) have been regarded as harmless (hence the term "benign hyperphenylalaninaemia"). However, handicap has been observed in such subjects and subtle intellectual impairment of diminishing degrees can be detected down to phenylalanine levels of around 400 μmol/l [3].

Table 1. Causes of hyperphenylalaninaemia

Primary inherited
Phenylalanine hydroxylase deficiency
Severe, less than 1% enzyme activity ("classical" or "typical" phenylketonuria)
Moderate, 1%–5% enzyme activity ("non-classical" or "atypical" phenylketonuria)
Mild, more than 5% enzyme activity (mild phenylketonuria, "mild" or "benign" hyperphenylalaninaemia)
Maternal phenylketonuria
Defects of biopterin metabolism
Guanosine triphosphate cyclohydrolase deficiency
6-Pyruvoyltetrahydropterin synthase deficiency
Dihydropteridine reductase deficiency
Tetrahydropterin carbinolamine dehydratase deficiency
Secondary
Without hypertyrosinaemia
Transient neonatal, often with prematurity
Drug related (methotrexate, trimethoprim)
Severe inflammatory response
Renal disease
With hypertyrosinaemia
Transient neonatal, often with prematurity
High protein intake
Liver disease including galactosaemia and tyrosinaemia

Metabolic Derangement

Subjects with phenylalanine hydroxylase deficiency present a continuum of biochemical abnormality and blood phenylalanine concentrations ranging from just above the normal range to more than 30 times normal. Besides the phenylamine and phenylketone increases, hyperphenylalaninaemia competitively inhibits transport of aromatic and other amino acids across cell membranes (in both directions) including the blood–brain barrier. High brain phenylalanine concentrations inhibit the rate of protein synthesis, which may affect early dendritic proliferation and myelinization and increase myelin breakdown due to inhibition of adenosine triphosphate (ATP) sulphurylase [4]. Synthesis of *seroto-*

nin, *dopamine* and *norepinephrine* is reduced due to competitive inhibition of tyrosine and tryptophan hydroxylation and reduced intraneuronal substrate concentrations. Which of the above events (or others) are critical for brain development and function remains uncertain [3]. Synthesis of dopamine in the prefrontal cortex (in contrast to the basal ganglia) is uniquely sensitive to very small changes in tyrosine concentration [5]. This may explain how impaired intellect and neuropsychological performance can occur in subjects with relatively mild hyperphenylalaninaemia.

In a foetus with phenylalanine hydroxylase deficiency (and mother an obligate heterozygote), blood phenylalanine concentrations are about double those in the mother. It has been suggested that these small amino acid changes damage the foetus. Changes in the metaphyses of the long bones soon after birth [6] and a reported increase in the frequency of cardiac anomalies [7] support this view.

Diagnostic Tests

Detection by Screening and Confirmation of Diagnosis. Screening depends on detection of raised blood phenylalanine concentrations. Where testing is done within 48 h of birth, the chosen limit is likely to be 120–150 μmol/l, and it is recommended that those tested within 24 h of birth should be re-tested before 3 weeks of age. Where the recommended time of testing is relatively late (6–14 days after birth in the UK), the chosen limit will be higher (180–240 μmol/l) [8].

Routine diagnostic investigation consists of general clinical evaluation, review of milk intake (to identify unusually high or low protein or energy intake), quantitative measurement of plasma *phenylalanine* and *tyrosine* and exclusion of defective biopterin metabolism (Table 2). If tyrosine concentrations are elevated the infant is most unlikely to have a defect in hydroxylation. Except in premature or sick infants (see Table 1), transient forms of hyperphenylalaninaemia without tyrosinaemia are uncommon. All infants with an initial phenylalanine of more than 300 μmol/l and normal tyrosine require diagnostic investigation as well as all with a second positive screening test.

The place of molecular genetics in diagnosis is still being explored to see whether mutation detection is a good predictor of phenotype [9]. Enzyme studies (requiring liver biopsy) or protein or phenylalanine loading tests may also be undertaken as part of a planned research protocol, but are not necessary for good routine care. In subjects presenting with symptoms, the degree of neurological damage, EEG and developmental assessment will be needed to assess prognosis and as a basis for judging response to treatment.

Counselling Parents. Identification of hyperphenylalaninaemia in a screening programme is a crisis for the parents. The way in which the positive result is explained and the style and promptness of subsequent diagnostic investigations, counselling and advice on management are of vital importance to the family's well-being. This is so in all families, including those whose child has very mild or transient hyperphenylalaninaemia. Parents want to know everything about their child's disorder and are most secure when given full information with written material to support oral communications. It takes time (hours) over several sessions to provide the support required to implement treatment successfully.

Treatment and Prognosis

A recent Report of Medical Research Council Working Party on Phenylketonuria [10] recommends that if diagnostic phenylalanine concentrations are greater than 360 μmol/l (tyrosine concentrations less than 120 μmol/l), a low-phenylalanine diet should be introduced. Other subjects with blood phenylalanine concentrations between 120 and 360 μmol/l and/or a phenylalanine to tyrosine ratio of 3 or less should be monitored for at least the first year of life, as phenylalanine concentrations may increase substantially at weaning, especially in babies with relatively low tyrosine concentrations at diagnosis. Treatment consists of a low-phenylalanine diet. Generally low-protein diets have no place in management, neither in infancy nor at any other age.

Low-Phenylalanine Diet. The diet replaces a measured proportion of phenylalanine-containing foods with synthetic, phenylalanine-low substitutes, of which a wide range are now available. The nutrient content of the diet should provide full (but not excessive) nutrient intakes recommended for the patient's age (amino acids, vita-

Table 2. Protocol of diagnostic biochemical studies; normal values for children

Investigation	Approximate normal ranges
1. Plasma phenylalanine and tyrosine concentrations (μmol/l) on a normal protein intake (3 g/kg in infancy)	Phenylalanine 35–180 Tyrosine 50–180 Ratio ≤1.5
2. Dihydropteridine reductase (DHPR) activity in dried blood spots or red cells or white cells	Depends on assay
3. Urine total biopterin and neopterin concentrations (mmol/mol creatinine; neonates have higher neopterin values)	Biopterin 0.4–2.5 Neopterin 0.1–5.0 BNCR 9–200 % Biopterin 20–80
4. BH_4 load; 10 mg/kg orally, plasma phenylalanine and tyrosine at baseline, 1, 2, 4, 6 and 24 h. Baseline phenylalanine should be over 200 μmol/l	No change (or small fall) in phenylalanine and tyrosine
5. Plasma (or dried blood spot) total biopterins (ng/ml; Crithidia fasciculata)	Plasma biopterin 1.4–3 Blood biopterin <2.4–6
6. CSF concentrations of HVA and 5HIAA (nmol/ml; neonates have the higher values)	HVA 400–1000 5HIAA 200–400
7. CSF total biopterin and neopterin (nmol/l; neonates have the higher values)	Biopterin 12–40 Neopterin 10–30
8. Percentage of total biopterins as BH_4 in urine and CSF	Urine 60–80 CSF 90–98
9. Total folates (ng/ml; *Lactobacillus casei*) in serum, red cells and CSF (DHPR deficiency only)	Serum 3–12 RBC 150–500 CSF 25–50
10. Phenylalanine load; 100 mg/kg orally, plasma phenylalanine, tyrosine and total biopterins, urine total biopterins, neopterins at baseline, 1, 2, 4 and 6 h. Reserve for subjects with baseline phenylalanine 200 μmol/l or less	Phenylalanine 4–5 times Tyrosine 2–3 times Biopterin 4–5 times Baseline values by 6 h
11. Combined phenylalanine/biopterin load; BH_4 given 1 h after phenylalanine	Compare phenylalanine response to that for 10.

1, 2 and either 3, 4 or 5 are required for all subjects with hyperphenylalaninaemia, 1–11 for those with suspected biopterin defects.
BNCR, biopterin neopterin creatinine ratio; HVA, homovanillic acid; 5HIAA, 5-hydroxyindoleacetic acid; RBC, red blood cells.

mins, minerals, fat and carbohydrate). Manufacturers have done a great deal to incorporate new knowledge into the manufacture of their special food products, but nutrient deficiency and imbalance is a constant risk. Regular review of nutritional status, of new knowledge of requirements and of nutrient intake is essential. Wide variation in the severity of hyperphenylalaninaemia, eating patterns, culture, the composition of manufactured foods and the provision of health services means that management has to be tailored to individual circumstances and practical implementation is far from easy. Families require continuing education and specialist support from a knowledgeable team equipped to deliver the services required.

Control of Blood Phenylalanine Concentrations and Monitoring. The amount of dietary phenylalanine required to control blood phenylalanine concentrations depends on the chosen "safe" range and the severity of the underlying defect. It has been recommended that in children under 5 years of age the aim should be to keep blood phenylalanine levels between 120 and 360 μmol/l [10]. The slightly elevated lower limit is based on the need to avoid phenylalanine deficiency. Subjects with severe types of hyperphenylalaninaemia will need around 200 mg phenylalanine/day (equivalent to approximately 4 g natural protein). Subjects whose initial phenylalanine concentration was less than 1200 μmol/l almost invariably tolerate more than 300 mg phenylalanine/day. Rate of growth also has some influence on phenylalanine requirements, but body size practically none.

At the start of treatment in those with severe disease, a period on phenylalanine-free milk brings blood levels down at a rate of 300–400 μmol/l per day. Once levels are close to the therapeutic range, phenylalanine is added at 50 mg/kg per day (1 g protein/kg per day) and the prescription is adjusted according to serial blood phenylalanine levels (at least weekly for the first year or two of life). All those looking after such

patients are familiar with their brittle phenylalanine control and low phenylalanine tolerance. Levels rise in response to minor events such as intercurrent illness, changes in energy intake and growth rate. In subjects with milder hyperphenylalaninaemia, blood levels fall more readily, less rigorous phenylalanine restriction is required and smooth control is much easier to achieve.

Given the scale of intervention required to sustain a strict low-phenylalanine diet, it is not surprising that many clinics still choose to relax or stop treatment before or around adolescence. However, opinions are changing in the light of new knowledge (see "Prognosis"). In the UK it has been recommended that patients should be appraised of current findings and offered the opportunity to remain on a diet into adulthood aiming to keep blood phenylalanine concentrations below 700 μmol/l [10]. In severe disease this requires restriction of phenylalanine to less than 600 mg/day (12 g natural protein). Even this degree of restriction may not avoid the neuropsychological consequences of phenylalanine accumulation.

Prescription of Phenylalanine-Containing Foods. Phenylalanine (and in hyperphenylalaninaemia, tyrosine) is an essential amino acid and should only be excluded from the diet when trying to reduce high phenylalanine levels rapidly (e.g., at the start of treatment). The protein and, to a lesser extent, the amino acid contents of foods have been fairly well studied [11], but the very approximate nature of the figures should be born in mind. Generally between 4%–6% of amino acid in protein is phenylalanine and 1 g protein is approximately equivalent to 50 mg phenylalanine.

In bottle-fed infants, phenylalanine is prescribed and adjusted according to blood levels in measured amounts of milk powder (or volumes of liquid milk), which is then divided between four or five feeds, spread over the 24 h and given first at each feed. The phenylalanine-low milk is given, to appetite, in a separate bottle. The total amount of phenylalanine-low milk offered in each 24 h is estimated to provide energy and protein needs with "a little to spare" so that appetite can dictate total quantity of phenylalanine-free milk. In breast-fed infants the pattern is reversed: the phenylalanine-low milk is prescribed and adjusted in measured amounts and given first at each of four or five feeds, and breast-feeding is allowed to appetite.

The prescription and adjustment of phenylalanine intake once weaning begins (at the usual time) is based on lists of foods providing information on the average phenylalanine content of foods. One approach is to list the measures of most foods equivalent to 15 mg phenylalanine, to calculate total daily intake on this basis and adjust by 15 mg portions according to blood levels. Another approach (devised in the UK to simplify the arithmetic, make communication easier and take account of the approximate nature of the measures being used) is to divide foods into the following:

- *High-phenylalanine foods* (e.g., meat, fish, cheese, egg, some wheat products, pulses), which are either totally excluded or are prescribed in useful portions equivalent to 300 mg phenylalanine (approximately equivalent to 6 g protein).
- *Medium-phenylalanine foods* (e.g., potato, cream, milk, rice, corn, yoghurt, wholewheat cereals), which provide convenient interchangeable portions (one exchange), equivalent to 25 mg or 50 mg phenylalanine (approximately 0.5 or 1 g protein). Exchanges are calculated daily and adjusted according to blood phenylalanine concentrations.
- *Low-phenylalanine foods* (e.g., refined fat and carbohydrate, fruit and many vegetables) which provide less than 25 mg phenylalanine in meal-appropriate portions; these are ignored in adjusting phenylalanine intake. When the "usual" portions increase to sizes which causes significant variation day to day, these foods can be incorporated into the calculation of exchanges.

Prescription of Phenylalanine-Low Foods. Manufactured, phenylalanine-low food substitutes vary in composition from "complete" infant milks (other than phenylalanine) to single-category nutrient mixtures (minerals, vitamins, amino acids, fats or carbohydrates). Amino acid substitutes are provided in "pharmaceutical" form, energy in the form of protein-free flour, bread, biscuits, milk substitute, chocolate bars, pasta, rice etc. These "foods" are very deficient in amino acids, minerals and vitamins. Although the newer amino substitutes are adequately supplemented with tyrosine, vitamins and minerals (including selenium), there remains some concern about nutritional balance and adequacy [12]. Recent

work has focused on deficiencies in long-chain, polyunsaturated fat [13]. Phenylalanine-low natural foods (fats, sugar, starches, fruit and some vegetables) are a valuable source of fibre, energy, vitamins and minerals.

It is of fundamental importance that those caring for subjects with PKU have a full understanding of nutrient requirements, the composition of the products being used, the limitations in nutrient content and the consequences of changes in intake. It is our practice to maintain amino acid intake at the equivalent of 3g/kg per day to the end of the first year and 2g/kg to at least mid-childhood and to continually stress the importance of taking adequate amounts of protein substitute, taking a careful history of the pattern of consumption at regular intervals. This helps avoid the problem of inadvertent or covert reduction in intake. The need to spread the foods as equally as possible over the 24h is stressed. All this will go some way to keep blood levels of amino acids other than phenylalanine, including tyrosine, in a range which will help enhance the transport of amino acids across the blood–brain barrier. Whether an even higher amino acid intake would be beneficial needs further examination. The use of pharmacological amounts of branched-chain amino acids, tyrosine or trytophan has been studied on a small scale. This does limit phenylalanine uptake across the blood–brain barrier, but the questions on long-term use remain unanswered.

All phenylalanine-free amino acid substitutes are expensive and relatively unpalatable. There is a temptation to reduce the prescription as children get older, especially if phenylalanine restrictions have been relaxed deliberately or if blood phenylalanine levels are regularly high due to non-compliance. If minerals and vitamins are combined with amino acids, and if natural foods are chosen from a restricted range of vegetable foods, certain nutrients may become deficient even though overall protein intake, energy intake and general nutrition is well maintained. Vitamin B_{12}, selenium and calcium, being largely confined to animal products, are particularly prone to become deficient under these circumstances.

Managing Illness and Feeding Problems. During illness most children cannot take their prescribed diet. High-energy fluids (e.g., fruit-flavoured glucose polymer with or without fat emulsion) will help reduce catabolism and are more acceptable to sick children than protein supplements, which are usually refused until recovery begins. As anabolism takes over, it is important to reintroduce phenylalanine to avoid low blood levels as diet is re-established. Parents should be instructed *never* to force their children to take the diet during illness (or at other times); children recover rapidly from illness, but take months or years to recover from force feeding.

Diets always risk causing feeding problems, especially is parents are anxious or angry about the diagnosis, are poorly supported or if the diet prescription is inappropriate (e.g., too high energy, too low amino acid). Prescriptions too high in energy, inflexible instructions (he *must* eat all of it!), parents blamed when problems arise and failure to acknowledge the immense pressure the family is under are all potent causes of feeding problems. When problems do occur, careful analysis of the areas of difficulty followed by modification in small graded steps within a "desensitization" framework usually works very well. The aid of psychologists experienced in managing feeding problems and phobias is invaluable.

Prognosis. In severe forms of phenylalanine hydroxylase deficiency, early diagnosis has reduced the frequency of mental handicap from around 80%–90% to 6%–8% (compared with a general population risk of around 2%) [3]. However, signs of subtle neurological impairment persist in early-treated subjects, the scale depending on the quality of phenylalanine control during the pre-school years (age at start of treatment, average phenylalanine concentrations, and duration of low phenylalanine levels) and current phenylalanine status. An overall reduction in mean IQ of around 0.5 standard deviations, behavioural disturbance such as hyperactivity, delay in acquisition of speech, poor concentration and educational difficulties are well documented. Quality of phenylalanine control is highly dependent on the severity of the biochemical defect. As a result, those with the more severe disorder tend to do less well than those with relatively mild disease. The possible contribution of prenatal influences to outcome requires further study.

Stopping treatment before mid-childhood is associated with further intellectual impairment, and at all ages raising blood phenylalanine concentrations is associated with reversible worsening of neuropsychological performance, in particular planning skills (executive function) dependent on pre-frontal lobe function. Subjects with severe

biochemical disease on normal or very relaxed diet commonly exhibit unusually brisk tendon jerks (including ankle clonus, finger and jaw jerks) and intention tremor. In addition, there have been a few reports of overt neurological deterioration with limb weakness and increased tone, which may improve when strict diet is re-implemented. In the great majority of subjects with blood phenylalanine concentrations regularly greater than 700 μmol/l, magnetic resonance imaging (MRI) studies of brain reveal changes in the white matter indicative of an increased water content. These changes are particularly marked in subjects with overt neurological symptoms. Overall the extent of the MRI changes are linearly proportional to blood phenylalanine concentrations, and the changes diminish when phenylalanine control is improved. The long-term clinical implication of these findings is still undecided, although these have to be considered alongside the biochemical effects of phenylalanine on myelin, the older literature showing demyelination in some subjects and the new work on the neuropsychology of hyperphenylalaninaemia. The authors consider that patients should be fully appraised of current knowledge and do not advise individual patients to stop treatment unless the risks and problems of continuing clearly outweigh any possible benefits. It is more difficult to decide whether patients already receiving a normal diet should be advised to return to treatment, since most are well and only a small minority (<1%) have overt neurological problems.

Genetics

In most populations of European origin, phenylalanine hydroxylase deficiency is by far the commonest form of inherited hyperphenylalaninaemia, with a prevalence of between 1 in 4000 and 1 in 40000 births, Iceland and Ireland having the highest and Finland the lowest frequency.

Over 100 mutations in the phenylalanine hydroxylase gene have already been described [14]. Although different groups of mutations and polymorphisms tend to predominate in certain populations, there are only a few instances where a single mutation accounts for almost all affected subjects (for example, Yemenite Jews). Most subjects with hyperphenylalaninaemia are compound heterozygotes rather than homozygotes, explaining the wide and continuous spectrum of biochemical phenotype long observed in clinical practice [15].

Using a combination of mutation analysis and polymorphisms in the hydroxylase gene, it is now possible to undertake reliable *prenatal diagnosis* in the vast majority of couples (or inbred families) with an affected child. It is still not easy to detect carriers in the general population. Simple biochemical tests such as a phenylalanine to tyrosine ratio produce a broad overlap between normal subjects and carriers. Intravenous loading tests with labelled phenylalanine produces much better separation (1% overlap) [16], and combined with testing for the commonest mutations a much better analysis of risk could be developed. As yet, however, such services are not widely available.

Maternal Phenylketonuria

Clinical Presentation

Typical features (Table 3) [17], present in over 80% of infants born to mothers with hyperphenylalaninaemia, include low birth weight, microcephaly, dysmorphic facies, slow development, slow postnatal growth and congenital malformations, most often in heart or great vessels (20% of subjects). Other malformations are less common, but have included oesophageal atresia and tracheo-oesophogeal fistula, bowel malrotation, bladder extrophy and other urogenital anomalies, coloboma or cataract of eye and cleft lip and palate. Dysmorphic features resemble those of the fetal alcohol syndrome, with small

Table 3. Percentage of subjects with abnormalities in the offspring of mothers with phenylketonuria PKU

	Maternal phenylalanine concentrations (mg/100 ml; ×60 μmol/l)			
	20	16–19	11–15	3–10
Mental retardation	92 (172)	73 (37)	22 (23)	21 (29)
Microcephaly	73 (138)	68 (44)	35 (23)	24 (21)
Congenital heart disease	12 (225)	15 (46)	6 (33)	0 (44)
Birth weight <2500 g	40 (89)	52 (33)	56 (9)	13 (16)

Numbers of subjects on which frequency is based in brackets [17].

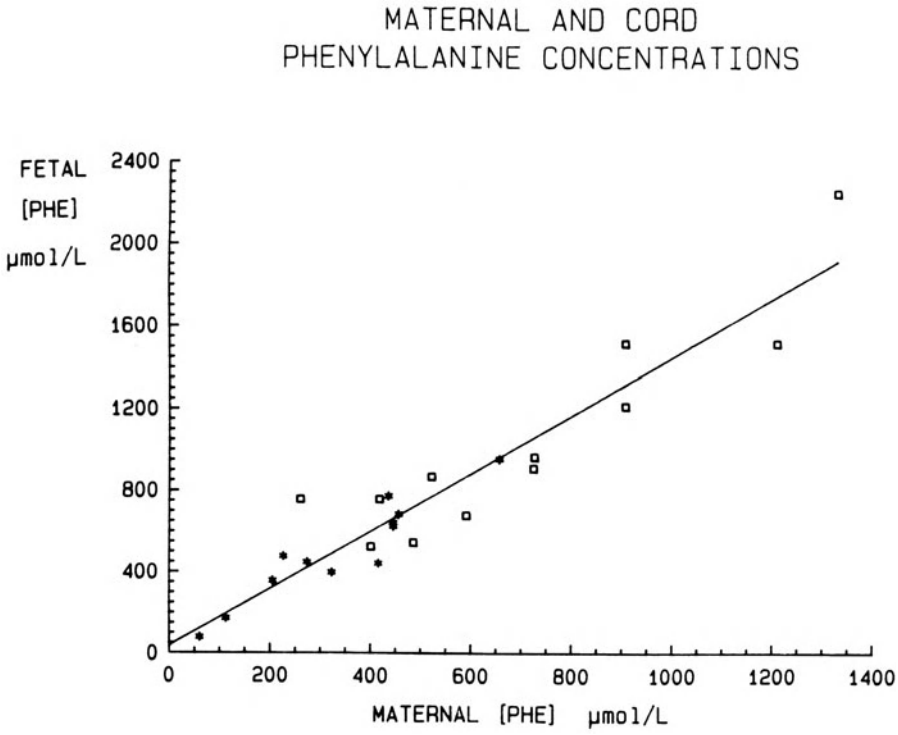

Fig. 2. Simultaneously measured maternal and cord blood phenylalanine concentrations. *Asterisks*, the author's patients; *squares*, patients from the literature

palpebral fissures, epicanthic folds, short or absent philtrum and reduced upper lip. Wide variations in phenotype do occur, and even in mothers with phenylalanine concentrations greater than 1200μmol/l the foetus may escape significant damage. The prevalence of abnormalities in the offspring of women with mild to moderate hyperphenylalaninaemia is lower, but by no means zero. The offspring of men with phenylketonuria appear to be entirely normal.

Metabolic Derangement

In all species foetal amino acid concentrations are higher than maternal (with the exception of total cysteine) due to active net transport from mother to fetus [18, 19]. A high foetal to maternal ratio for phenylalanine is present from early pregnancy and persists even at high maternal phenylalanine concentrations (Fig. 2). Phenylalanine very probably competes with other neutral amino acids for placental transport and may thus contribute to slower foetal growth and interfere with major organ development. The biochemical effects of raised plasma phenylalanine concentrations in foetal brain are likely to be rather similar to those occurring after birth, from the earliest weeks to the end of pregnancy, no doubt causing the high incidence of microcephaly and mental retardation.

Treatment and Prognosis

Counselling. Counselling begins with the parents of newborns with phenylketonuria and continues at intervals during the course of long-term management. A girl as young as 4 can understand a simple explanation of maternal phenylketonuria. By the teens, counselling should be directed to the girls themselves in order to:

- Ensure basic understanding of conception and phenylketonuria
- Explain that a baby in utero is exposed to the mother's high phenylalanine and is likely to develop abnormally from conception onwards, thus the necessity for strict diet before conception
- Distinguish the very high risk of foetal damage during pregnancy from the relatively small chance of a baby actually inheriting phenylketonuria

- Explain that the scale of risk to the foetus will depend on mother's blood phenylalanine control.

Contraception should be discussed with older teenage girls and the risks of unprotected intercourse emphasized. When appropriate, the patient should bring her partner to the clinic so that they share the counselling.

Re-starting a Strict Diet. The patient who has not been on diet for several years may well need to be admitted to hospital or receive very close supervision in her own home to ensure that she is able to consume, and knows how to use, the measures of phenylalanine and the dietary substitutes. This also ensures that blood phenylalanine concentrations fall rapidly, giving a sense of achievement and encouragement.

It is a medical emergency when a woman with phenylketonuria presents already pregnant. Women who have conceived with a persistently high phenylalanine (>800 μmol/l) have a strong case for termination. The risk of intellectual impairment is high if there is no dietary treatment during pregnancy. Present evidence does not accurately define the risk if diet is successfully introduced within a few weeks of conception, although, as microcephaly can still occur, the risk is likely to be considerable. The fact that it is too late to reduce the risks to other organs, particularly the heart, must be explained. Foetal ultrasound examination of heart, other organs and skull diameter may provide further information on the health of the foetus. The patient who requires time to reach a decision on termination should re-start a diet without delay.

Control of Blood Phenylalanine Concentrations. Keeping foetal phenylalanine below 500 μmol/l will require maternal concentrations below 300 μmol/l. It has been recommended [10] that treatment should be introduced in all women with blood phenylalanine concentrations greater than 300 μmol/l and that maternal phenylalanine values should be maintained between 120 and 250 μmol/l from conception, with monitoring at least twice weekly. In subjects with severe disease, natural protein intake will need to be reduced to a total of 6 g/day or less and at least 70 g supplemental amino acids per day will be required to cover nutritional requirements. We use extra tyrosine 3–4 g/day from around 18 weeks. However, raising plasma tyrosine values may further impair the transport of other neutral amino acids across the placenta and the blood/brain barrier. This could be damaging if it is not tyrosine which is rate limiting for growth and brain development, but some other amino acid(s).

Phenylalanine tolerance increases progressively from around 20–22 weeks' gestation (often to around 30 g protein per day), which presumably relates to the increased requirements for growth of foetus, foetal membranes and uterus and the increase in phenylalanine hydroxylase activity in fetal liver.

Antenatal and Obstetric Care. The patient is seen monthly for nutritional monitoring, antenatal care, ultrasound examination of the foetus and consultation with clinician and dietitian. Admission may be required for poor weight gain, poor phenylalanine control, vomiting during pregnancy or other problems. There is nothing to suggest that the birth of the baby needs anything other than normal obstetric considerations. One would not expect the additional phenylalanine which will be present in the mother's milk, if she returns to a free diet, to be harmful unless the neonate is homozygous for PKU.

Prognosis. In untreated pregnancies in women with severe PKU, the risk to the foetus is high (Table 3). In women with milder disease (phenylalanine values of less than 1100 μmol/l), among 24 offspring of untreated pregnancies only one child had an IQ of less than 70. However, the same study observed a graded effect of maternal phenylalanine on the IQ of the offspring down to maternal blood phenylalanine levels of 600 μmol/l [20].

Treatment with a well-controlled phenylalanine-low diet from before conception greatly reduces the risk of growth retardation, microcephaly and malformations. In a study of 94 infants, 67 from the UK and 27 from the rest of Europe and Australia [21], 28 infants were born to mothers whose phenylalanine had been controlled by diet from before conception to 600 μmol/l or less. Mean birth weight corrected for sex and gestation (3421 g) and head circumference (34.7 cm) in these 28 did not differ significantly from (although were slightly lower than) population norms and they had no malformations. When the diet was

poorly controlled (phenylalanine >600 μmol/l) or had started in the first trimester, mean birth weight was lower (mean, 3178 g and 2865 g, respectively) and so were head circumferences (33.7 and 32.8 cm, respectively). Foetal malformations were found in 12 out of 66 pregnancies (19%) treated after conception or not at all, including six with congenital heart disease. Birth weight and head circumference were both inversely and linearly related to maternal phenylalanine around the time of conception. The relationship extended down to near normal blood phenylalanine levels, suggesting adverse effects of even moderately elevated maternal phenylalanine values from the earliest weeks of pregnancy.

The authors have personal experience of cognitive development (Griffiths Developmental Scale) in 23 children now over 1 year of age whose mothers were treated by strict diet from before conception. Apart from one who was severely abnormal from birth (gross hypotonia, developmental delay and frequent fits, probably unrelated to maternal PKU), all have scores within the normal range and 20 have scores greater than 100. However, strict neurological assessment has shown that minor neurological impairments can be detected in the majority, which may indicate a risk of cognitive deficits later.

Defects of Biopterin Metabolism

Tetrahydrobiopterin (BH_4) is the cofactor required by phenylalanine, tyrosine and tryptophan hydroxylases [22]. Defects in cofactor metabolism are generally very much rarer than phenylalanine hydroxylase deficiency, occurring in around 1 in 500000 to 1 in 1000000 births.

Clinical Presentation

In the most severe types of disorder *microcephaly*, *developmental delay* and progressive *neurological deterioration* leading to death in childhood occurs commonly, although some patients stabilize or make slow developmental progress. In those with severe disease, the neurological features are highly characteristic and include profound infantile parkinsonism (hypokinesis, drooling, swallowing difficulty, sweating, pinpoint pupils, oculogyric spasms, truncal hypotonia, increased limb tone, blank facies with relative preservation of smiling) accompanied by myoclonus, choreic or dystonic limb movements, very brisk tendon jerks and sometimes infantile spasms, grand-mal fits, hyperpyrexia and disturbance of sleep pattern.

Less severe symptoms may occur, consisting of minor developmental delay, movement disorder or epilepsy. Symptoms may be absent or may be precipitated by a phenylalanine load, acute infection or even just day to day changes in blood phenylalanine levels. It has been suggested that *Segawa's syndrome* (dystonia with marked diurnal variation responsive to L-dopa) may be due to defective BH_4 synthesis.

In subjects with defective BH_4 synthesis birth weight is reduced, and prenatal damage contributes to the neurological impairment. There is no evidence that prenatal damage occurs in *dihydropteridine reductase (DHPR) deficiency* but progressive dysmyelination and demyelination may develop, causing progressive microcephaly, paraplegia, bulbar palsy, long-tract sensory loss and deterioration in cortical function. Changes in brain include myelin loss with microvascular calcification in the basal ganglia and white matter, visible on MRI and computed tomography (CT). These changes closely resemble the effects of methotrexate toxicity [2].

Metabolic Derangement

Disturbance in the synthesis or recycling of BH_4 causes not only phenylalanine accumulation, but also defective synthesis of dopamine, serotonin, noradrenaline and adrenalin (Fig. 1) [1, 22]. Reduced concentrations of these amines and metabolites are found in body fluids; symptoms are linked to these *neurotransmitter deficiencies*. It is probable that melatonin turnover is also reduced. Concentrations in cerebrospinal fluid (CSF) of homovanillic acid (HVA) and 5-hydroyindoleacetic acid (5HIAA), the major metabolites of dopamine and serotonin in the human nervous system, provide a useful measure of amine turnover in vivo and the severity of the phenotype. Phenylalanine accumulation, which may be very mild, further inhibits any residual amine turnover. The reduction in BH_4 concentrations, and the phenylalanine excess, stimulate guanosine triphosphate (GTP) cyclohydrolase (unless a defect of this enzyme is present) and increase the production of abnormal pterins (Fig. 1). Failure of biopterins to rise in response to hyper-

phenylalaninaemia is characteristic of defects of synthesis. By contrast, in DHPR deficiency and phenylalanine hydroxylase deficiency and in normal individuals, total biopterins and neopterins increase in proportion to the hyperphenylalaninaemia.

BH_4 is also required for synthesis of nitric oxide and for oxidation of the ether bond in plasmalogens (cell membrane lipids which are oxidized in peroxisomes). However, dysfunction relating to disturbed nitric oxide or plasmalogen turnover has not been identified, indicating either that the requirement for BH_4 is small or that additional mechanisms exist to sustain BH_4 turnover in nitric oxide producing cells and in peroxisomes.

Defects of Synthesis. In addition to the changes in amine metabolism in GTP cyclohydrolase deficiency, concentrations of biopterins and neopterins are low in urine, blood and CSF; these pterins are also low in Segawa's syndrome. In *defects of 6-PBH$_4$ synthase* biopterins are low in body fluids, but neopterins rise with a marked fall in the ratio of total biopterins to neopterins. Administration of BH_4 corrects the phenylalanine accumulation, increases plasma tyrosine concentrations and restores the pterin profile towards normal. This response to BH_4 forms the basis of one of the diagnostic tests for defective BH_4 synthesis (Table 2). Obligate heterozygotes may also exhibit persistent neopterin accumulation. Subjects with "partial" defects with less marked changes in pterin concentration have smaller or intermittent reductions in CSF amine metabolites. Those in whom central amine and pterin turnover are entirely normal are said to have "peripheral" defects. Whether or not some of these infants are heterozygotes is not clear. The pterin and amine changes after birth are mirrored in the amniotic fluid of affected foetuses.

Defects of Recycling. Phenylalanine hydroxylase requires two enzymes to successfully recycle BH_4 in the liver, DHPR and tetrahydropterin carbinolamine dehydratase (Fig. 1). A defect in either enzyme may cause hyperphenylalaninaemia.

A defect of DHPR blocks the normal recycling of q-BH_2 to BH_4, leading to accumulation of BH_2 in blood, urine and CSF. BH_4 concentrations in CSF are reduced to about half normal, but remain well above the levels in defects of synthesis. However, BH_2 is a potent inhibitor of aromatic amino acid hydroxylases and this, rather than simple BH_4 deficiency, may well be the cause of the hyperphenylalaninaemia and amine deficiency in this disease. Abnormal enzyme protein can be detected in cultured fibroblasts and lymphocytes from some subjects but not others, and these two groups differ in their response to BH_4. A fall in plasma phenylalanine concentrations occurs in those without detectable enzyme protein but is seen only with very large doses of BH_4 in those with detectable enzyme protein. Subjects with partial defects of DHPR have been reported. Defective *folate metabolism* with progressive neurological damage is an important feature of DHPR deficiency, probably due to the effects of BH_2 (or other abnormal pterins) on folate metabolism. Megaloblastic changes are unusual despite low folate concentrations in serum, red cells, CSF and brain. Histological and neuroradiological changes in the brain are similar to those due to congenital folate malabsorption, 5, 10-methylene tetrahydrofolate reductase deficiency or methotrexate toxicity (see "Clinical Presentation").

Subjects with dehydratase deficiency excrete an excess of primapterin, oxo-primapterin and anapterin in the urine as well as neopterin; so far, all subjects have had a peripheral type of defect.

Diagnostic Tests

Routine Testing of Infants with Hyperphenylalaninaemia. All subjects with hyperphenylalaninaemia should be screened for disorders of biopterin metabolism (Table 2). To achieve maximum discrimination testing for defects of synthesis must be carried out before phenylalanine concentrations have been brought under control. DHPR deficiency can be excluded by an enzyme assay, defects of synthesis by means of either a BH_4 load, or pterin measurements on urine (total biopterins and neopterins) or total biopterins in dried blood spots (biopterins fail to rise when synthesis is defective). Use of dried blood spots to measure both DHPR activity and total biopterins has proved a reliable method of routine testing in the UK [8] and has the great advantage that specimens are easy to obtain and can be posted to the specialist laboratory.

Diagnostic Investigation. All infants with positive routine tests for biopterin defects require a full

diagnostic investigation (Table 2) including CSF pterins and amine metabolites. Transient increase in the neopterin to biopterin ratio may occur in neonates. Currently MILUPA (Friedreichsdorf, Germany) holds a licence for the use of BH_4 as a test substance. A phenylalanine load (which may precipitate acute symptoms) should be used in combination with a BH_4 load and reserved for patients without very obvious hyperphenylalaninaemia. Diagnosis should be confirmed by enzyme studies whenever possible. All the enzymes can be measured in liver tissue. Otherwise GTP cyclohydrolase assay requires stimulated white cells; DHPR activity can be measured in dried blood spots, whole blood red cells, white cells or fibroblasts, 6-PBH_4 synthase white cells or fibroblasts. Tetrahydropterin carbinolamine dehydratase is not yet assayable with any ease.

Prenatal Diagnosis. DHPR and 6-PBH_4 deficiency can be detected using enzyme assay in foetal fibroblasts, which can now be supplemented by molecular genetic methods in an increasing proportion of families [22]. It is possible to confirm 6-PBH_4 synthase deficiency using analysis of amine metabolite and pterins in amniotic fluid. GTP cyclohydrolase deficiency can also be detected by similar analysis of amniotic fluid, though how reliably is not clear.

Treatment and Prognosis

As experience has increased, treatment has proved rather straightforward compared with many metabolic disorders and, within the limits imposed by existing neurological damage, remarkably effective. In most patients treatment is likely to be required throughout life.

► **Control of Phenylalanine Accumulation.** There are sound arguments for maintaining strict control of plasma phenylalanine concentrations (60–180 μmol/l). Residual amine synthesis is present in all affected subjects and is likely to be further inhibited by phenylalanine excess (see "Metabolic Derangement"). When L-dopa and 5-hydroxytryptophan are given to correct the amine disturbance (see below), plasma phenylalanine concentrations will influence the rate of uptake of these compounds across the blood–brain barrier. In DHPR deficiency hyperphenylalaninaemia stimulates production of BH_2. In defects of synthesis phenylalanine control is best achieved by administration of a single dose of BH_4 daily (1–3 mg/kg) but has to be given on a "named patient" basis. The cost is comparable to that of a low-phenylalanine diet. BH_4 is not recommended for DHPR deficiency because the doses required are large and such therapy increases accumulation of potentially harmful dihydropterins. A low-phenylalanine diet is effective in both groups of disorder; phenylalanine tolerance is usually high and dietary control is relatively easy to achieve.

Correction of Amine Deficiency. Administration of dihydroxyphenylalanine (L-dopa) in ratio of 1 in 10 or 1 in 4 to decarboxylase inhibitor (carbidopa) and combined with 5-hydroxytryptophan has been widely used to treat the amine deficiency. Doses of 10–12 mg/kg per day L-dopa and 8–10 mg/kg per day of 5-hydroxytryptophan are required to restore CSF amine metabolite concentrations to the normal range in subjects with severely defective amine synthesis. Very low doses (1–2 mg/kg) are introduced and then increased stepwise every 4–5 days to avoid vomiting, the main side effect of L-dopa in young children. Fidgetiness and/or abnormal movements can also occur. The precursors are given together, divided into *at least* four doses 30 min before meals. Smaller final doses may be adequate in subjects with milder disorders, and in some just controlling the hyperphenylalaninaemia is sufficient to restore amine metabolite concentrations to normal. This probably explains the beneficial effects of BH_4 administration seen in some patients. Large doses of BH_4 (20 mg/kg) are required to penetrate the blood–brain barrier and such doses have no place in routine treatment. Once treatment has been stabilized, it is important to measure CSF amine metabolites at intervals. Final adjustment of the timing and balance of L-dopa/carbidopa/5-hydroxytryptophan will need to be made according to individual response and doses need to be regularly reviewed and updated (every 3–6 months in early childhood) according to response, weight and CSF amine metabolite concentrations. The "on/off" phenomenon, familiar in adult Parkinson's disease, may occur but in our experience is uncommon. Adjustment of the proportion of L-dopa to 5-hydroxytryptophan and decarboxylase inhibitor, consistent control of phenylalanine concentrations and attention to the drug schedule in relation to meals usually re-

established smooth symptom control. The use of dopamine agonists such as bromocriptine and inhibitors of monoamine oxidase-B, such as selegiline, to replace or supplement L-dopa requires further exploration.

Folate Therapy in DHPR Deficiency. Although not all subjects with DHPR deficiency develop folate deficiency, in those who do there is an urgent need for treatment. Administration of tetrahydrofolate (5-formyltetrahydrofolate, folinic acid) in sufficient amounts to keep CSF concentrations in the high to normal range (30–40 ng/ml) prevents demyelination and halts the demyelinating process in those who already show abnormalities. As in subjects with methotrexate toxicity, use of folic acid may cause acute neurological deterioration and should be avoided. If control of plasma phenylalanine concentrations is strictly maintained, 15 mg folinic acid orally each day will maintain CSF folate concentrations in the normal range during infancy and early childhood; 3 mg/day is insufficient. Larger doses are needed in older subjects, and in those with poorly controlled phenylalanine concentrations. In view of the cost, folinic acid treatment should probably be confined to subjects with low or falling CSF folate concentrations and dose should be similarly adjusted. This means that careful monitoring using lumbar puncture is required in all patients with DHPR deficiency.

Prognosis. In infants detected by routine testing, changes in feeding, expression, tone, posture and mobility may be observed within days of starting treatment even when, initially, symptoms seemed to be minor or absent. Infants with defective synthesis, particularly if they are of low birth weight and exhibit neurological symptoms early in life, may show developmental delay even when treatment is started early, whereas others make near normal developmental progress. Those with DHPR deficiency also appear to make good progress if treatment is started early and if folate status is maintained. The long-term prognosis of these disorders is still unknown, but at least up to the teens progress is well maintained. In patients who present with neurological disease the response to therapy varies but, in our experience, marked clinical improvement occurs in most and can be dramatic.

Genetics

Genes have been cloned for DHPR, 6-PBH_4 synthase, GTP cyclohydrolase and tetrahydropterin carbinolamine dehydratase [22]. Mutations have been identified for the first two enzymes. These and polymorphisms within the genes are being exploited in prenatal diagnosis.

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Tyrosine

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CONTENTS

Three well-defined inborn errors of tyrosine catabolism are known:

- Hereditary tyrosinaemia type I (hepatorenal type)
- Hereditary tyrosinaemia type II (oculocutaneous type or Richner-Hanhart syndrome)
- Alcaptonuria, characterized by ochronosis and arthritis in advanced age.

Other inborn errors of tyrosine metabolism include oculocutaneous albinism caused by a deficiency of tyrosinase which converts tyrosine into dopa quinone, and aromatic L-amino acid decarboxylase deficiency. Only tyrosinaemia types I and II will be discussed in this chapter.

Hereditary Tyrosinaemia Type I (Hepatorenal Type)

Clinical Presentation

The clinical heterogeneity of tyrosinaemia is wide and the patients may present at almost any age from infancy to adulthood.

In the most acute forms of the disease, the patients present within weeks of birth with severe liver failure, often with vomiting, diarrhoea, jaundice, hypoglycaemia, edema, ascites and particularly a bleeding diathesis. Sepsis is common and the patients may also have early hypophosphataemic bone disease secondary to renal tubular dysfunction. Later in infancy and childhood, the patients may present with liver failure, rickets or more non-specific problems such as bleeding tendency, failure to thrive or hepatosplenomegaly. Very occasionally the children may present with neurological crisis, hypotonia secondary to rickets or a relapsing polyneuropathy [1]. The neurological problems often complicate the course of the disease and the attacks resemble those of acute intermittent porphyria with abdominal pain, peripheral neuropathy with muscle weakness and hypertension [2].

In the chronic forms of tyrosinaemia, the symptoms and signs are more subtle. There may be only slight enlargement of the liver, mild growth retardation and sub-clinical rickets. A history of bruising may be apparent on careful inquiry. In some patients, symptoms of liver disease are minimal and the liver involvement only evident on careful biochemical investigation or imaging. Computed tomography (CT) and ultrasound of the liver usually demonstrate abnormal structure [3], but the techniques do not detect all abnormalities, even with overt regeneration nodules. Most, but not all, patients with chronic tyrosinaemia have renal tubular dysfunction and rickets. Nephromegaly and nephrocalcinosis may also be observed. Some develop renal failure that requires renal transplant [4]. In the absence of rickets and with few, if any symptoms of liver disease, some patients with chronic disease easily elude diagnosis until liver cirrhosis is evident or hepatocellular carcinoma has developed. Hepatocellular carcinoma is a major complication of all types of tyrosinaemia [5], even those with few symptoms and signs of liver disease. Carcinoma may develop

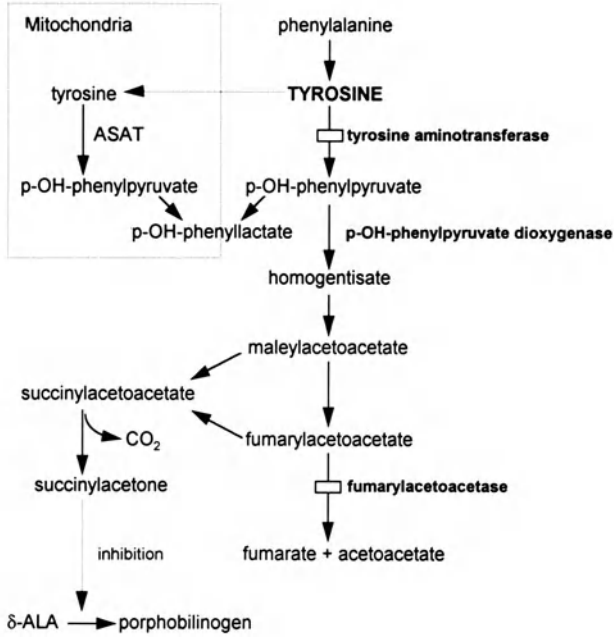

Fig. 1. Tyrosine degradation pathway (in cytosol). A deficiency of cytosol tyrosine aminotransferase causes tyrosinaemia type II. High urinary excretion of *p*-OH-phenylpyruvate and -lactate in this disorder may be caused by transamination by mitochondrial (aspartate) aminotransferase (*ASAT*) in tissues not containing enzyme systems for further metabolism. A deficiency of fumarylacetoacetase causes tyrosinaemia type I. Due to the enzyme block, fumaryl- and maleylacetoacetate accumulate and are reduced and decarboxylated to succinylacetone, which is a strong inhibitor of δ-aminolevulinate (δ-*ALA*) dehydratase (forming porphobilinogen)

early (from 1 year of age), but is more common in late childhood or adolescence. The incidental finding of any abnormality of kidneys and/or liver function or of a bone disorder in a child or an adolescent should prompt investigation for tyrosinaemia type I.

Metabolic Derangement

Tyrosinaemia type I is due to a deficiency of fumarylacetoacetase (FAH; Fig. 1). The enzyme block results in accumulation of fumaryl- and possibly maleylacetoacetate, which are alkylating agents thought to cause the hepatorenal damage. These metabolites are reduced and decarboxylated to succinylacetone (SA), which accumulates in plasma and urine of the patients. SA is a potent inhibitor of δ-amino-levulinate dehydratase. This results in elevation of δ-amino-levulinate and may explain the porphyria-like symptoms which occur in some patients. Other intermediates located between tyrosine and the enzyme defect also accumulate, namely *p*-hydroxyphenylpyruvic acid and its derivative *p*-hydroxyphenyllactic acid.

Diagnostic Tests

The hallmark of the diagnosis is demonstration of elevated levels of SA (and precursors). The method employed should be sufficiently sensitive to detect SA in healthy individuals [6]. Some patients with tyrosinaemia type I apparently excrete only low levels of SA and, if urinary excretion is only marginally elevated, the diagnosis should be confirmed by assay of FAH in lymphocytes or fibroblasts. The diagnosis of tyrosinaemia should not be based on FAH assay alone, because of a genetic variant in healthy individuals that is responsible for low activity of FAH in fibroblasts and lymphocytes, close to the range of the affected patients [7]. Enzyme diagnosis in liver tissue of tyrosinaemia patients may also be misleading because of a peculiar phenomenon: in some areas of the liver, the genetic defect may have "reverted" and in these areas high FAH activity is present [8].

Apart from the diagnostic tests as discussed above a number of unspecific biochemical abnormalities are present. Serum tyrosine is elevated, and in acutely ill patients methionine as well. Urinary excretion of *p*-hydroxyphenylpyruvic and -lactic acid and often δ-aminolevulinate is high. If tubulopathy is present, a full Fanconi syndrome (hyperaminoaciduria, glucosuria and hyperphosphaturia) may be present. Biochemical findings of liver cell damage and failure of protein synthesis are present to varying degrees. Vitamin K-dependent coagulation factors are highly abnormal in acute cases and usually abnormal also in patients with the chronic form of the disease.

γ-Glutamyltransferase may be elevated in patients in whom other liver enzymes are within the reference range. The α-fetoprotein level is often highly elevated in acute patients, but may be normal in chronic patients.

Treatment and Prognosis

The treatment of tyrosinaemia type I has traditionally been with tyrosine- and phenylalanine-restricted diet and later on by liver transplantation. Recently, a new drug has been introduced, 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclo-hexanedione (NTBC) that is a potent inhibitor of *p*-hydroxyphenylpyruvate dioxygenase (Fig. 1) [9]. It appears very effective and may prove to be the treatment of choice for tyrosinaemia type I. It will be discussed first, as other forms of therapy will be influenced by this new approach.

NTBC Treatment. As a result of the inhibition of *p*-hydroxyphenylpyruvate dioxygenase (Fig. 1), the formation of the metabolites distally from the enzyme is markedly reduced. In five patients treated with an oral NTBC dose of 0.6 mg/kg body weight [9], all the biochemical abnormalities of the disorder normalized with the exception of *p*-hydroxyphenylpyruvate and tyrosine, which increased. Since elevated tyrosine may be associated with various symptoms (see tyrosinaemia type II), the patients were kept on a tyrosine- and phenylalanine-restricted diet to keep the tyrosine level below 800 μmol/l. The clinical conditions of the patients, of whom one had been seriously ill, improved dramatically. α-Fetoprotein concentrations decreased, except in one of the patients, in whom liver transplantation for hepatocellular carcinoma was required. NTBC treatment has been reported to abolish porphyria-like neurological crises in one patient [1]. The long-term value of NTBC treatment has yet to be determined, particularly with respect to the risk of development of liver cancer. This may largely depend on when the treatment is started as liver cell damage is reversible only up to a certain point. Liver biopsy in patients identified on neonatal screening programmes and started on NTBC at an early age have not shown any serious abnormalities. NTBC is not yet commercially available, but a multicenter study is being conducted from Gothenburg. At the time of writing, about 60 patients from different countries are included in this study, and the results confirm those published [9]. In some patients treated for more than 2 years, the liver size is reduced and CT scans normalized, and no side-effects of NTBC have been seen (personal communication, S. Lindstedt and E. Holme).

Liver Transplantation. Liver transplantation for tyrosinaemia has been performed for a decade [10, 11]. Since the recipient retains the genotype of the donor, the patients no longer have the enzyme defect in liver tissue. The blood biochemical abnormalities are corrected as are the renal tubular defects. Transplanted patients do not need dietary treatment. However, increased urinary excretion of SA persists, presumably originating from the kidneys. The long-term prognosis of kidney function after liver transplantation remains unclear.

The immediate prognosis of tyrosinaemia patients after liver transplantation is related to the procedure itself and the immunosuppressive treatment, and in most centres the survival rate is high (70%–90%) [10, 11]. The timing of liver transplantation has been a matter of concern. In patients with end-stage liver failure, not amenable to other treatments, liver transplantation may be life-saving. In chronic patients with few symptoms, the timing of liver transplantation is difficult. Postponing liver transplantation until hepatocellular carcinoma is evident may prove too late [12]. Neither α-fetoprotein nor hepatic imaging are reliable for detection of carcinoma and generally underestimate the severity of the liver disease. Asymptomatic malignancies may be found at the time of the transplantation. However, three Norwegian patients were transplanted due to rise of α-fetoprotein at age 7, 12 and 14 years, all had hepatocellular carcinoma and all are alive without complications between 2 and 5 years after the transplantation.

For children with more severe disease, transplantation has been done electively in early childhood, but the promising results of NTBC treatment should reduce the need for liver transplantation. Presumably some patients will have developed malignant change at the time of diagnosis, so a liver transplant will still be required. Patients presenting with acute liver failure, not responding to NTBC, will also need a transplant urgently.

Dietary Treatment. Dietary treatment with restriction of tyrosine and phenylalanine intake has been employed in tyrosinaemia type I for 30 years [13]. The principle for dietary treatment is to keep intake of tyrosine and phenylalanine as low as possible whilst still meeting the needs for normal growth. The diet is based on a protein hydrolysate or amino acid mixture free of phenylalanine and tyrosine. A small amount of natural protein is given in addition. Undetectable levels of succinylactone were achieved in one patient by an intake of 15–20 mg/kg body weight of each amino acid [14]. On dietary treatment, the renal tubular dysfunction almost completely resolves, but the liver disease is not cured and malignant change is not prevented. Alternative treatment is eventually needed (e.g., liver transplantation).

Supportive Treatment. In the acutely ill patient supportive treatment is essential. The patient is often depleted of potassium and phosphate. Clotting factors, albumin, calcium, phosphate, electrolytes and acid – base balance should be closely monitored and corrected as necessary. Tyrosine/phenylalanine intake should be kept to a minimum during acute decompensation. Addition of vitamin D, preferably 1,25-hydroxy vitamin D_3 or an analogue, may be required to heal the rickets. Infections should be treated intensively.

Genetics

Hereditary tyrosinaemia type I is inherited as an autosomal recessive trait. The gene for FAH is located at 15q23–25. Prenatal diagnosis is available by determination of SA in amniotic fluid supernatant [6] and by determination of FAH in cultured amniotic fluid cells and in chorionic villus material [15]. In families in which either parent has a compound genotype for tyrosinaemia and a genetic variant ("pseudo-deficiency"), prenatal diagnosis by enzyme determination may not be feasible. Before embarking on prenatal diagnosis, the enzyme activity of both parents should be determined, to decide whether either carries the genetic variant. Several mutations causing tyrosinaemia have been reported [16–18]. Diagnosis using molecular genetics may prove especially useful in prenatal diagnosis in families with complex genotypes.

Hereditary Tyrosinaemia Type II (Oculocutaneous Tyrosinaemia, Richner-Hanhart Syndrome)

Clinical Presentation

The disorder is characterized by eye lesions (about 75% of the cases), skin disease (80%) and neurological complications (60%) or any combination of these [19]. The disorder usually presents in infancy, but may become manifest at any age.

Eye symptoms are often the presenting problem and may start in the first months of life [20]. The patient usually has photophobia and lacrimation, and intense burning pains are common. The conjunctivae are inflamed, and on split-lamp examination herpetic-like corneal ulcerations are found. However, in contrast to herpetic ulcers, which are usually unilateral, the lesions in tyrosinaemia type II are bilateral. The lesions stain poorly with fluorescein. Neovascularization may be prominent. If untreated, serious damage may occur with corneal scarring, visual impairment, nystagmus and glaucoma.

The skin lesions are limited to the palms and soles and especially affect the pressure areas [21]. They begin as blisters or erosions with crusts and become hyperkeratotic with an erythematous rim. The lesions are painful and may range in diameter from 2 mm to 3 cm.

The neurological complications are highly variable. Some patients are normal, whilst others have defects of fine coordination and language. The problems may also be more severe with microcephaly, self-mutilation and gross retardation. As the eye and/or skin problems may be the only symptoms, any patient with bilateral keratitis, particularly if it does not respond to treatment, or hyperkeratotic skin lesions of palms and soles should be investigated for tyrosinaemia type II.

Metabolic Derangement

Tyrosinaemia type II is due to a defect of hepatic cytosol tyrosine aminotransferase. As a result of the metabolic block, the tyrosine concentration in serum and cerebrospinal fluid is markedly elevated. The accompanying increased production of the phenolic acids *p*-hyroxyphenylpyruvate, -lactate and -acetate may be a consequence of direct deamination of tyrosine in the kidneys or of

metabolism of tyrosine by mitochondrial aminotransferase (Fig. 1). The eye and skin damage probably results from the intracellular formation of tyrosine crystals as concentrations exceed saturation. The crystals may resolve when plasma tyrosine concentrations fall. The aetiology of the neurological problems is not known.

Diagnostic Tests

The plasma tyrosine concentration is diagnostic in this disorder. In untreated patients it is usually well above 1000 μmol/l, higher in younger than older patients. Urinary excretion of the phenolic acids *p*-hydroxyphenylpyruvate, -lactate and -acetate is highly elevated and *N*-acetyltyrosine and *p*-tyramine are also increased. Liver biopsy with enzyme determination is not justified, as the clinical findings together with high tyrosine concentrations are diagnostic. There should be a rapid resolution of the eye and skin symptoms on treatment (see below).

Treatment and Prognosis

Diet with restriction of phenylalanine and tyrosine intake alleviates the eye disorder within a week, and the skin lesions after a few months. If the patient has general symptoms such as failure to thrive, improvement is evident within days of treatment. Traditionally, the diet is based on a tyrosine/phenylalanine-free amino acid mixture. The intake of tyrosine and phenylalanine is adjusted to allow for appropriate growth. A diet based on low protein intake (1.5 g/kg body weight) has also proved successful and is easier to manage [21]. Generally, there are no eye and skin lesions at tyrosine levels less than 800 μmol/l. Neurological development has also been satisfactory with this treatment [22]. However, since the basis for the neurological damage is not understood, a safe tyrosine level cannot be fixed unambiguously and may have to be decided on an individual basis.

As the impact of hypertyrosinaemia on the developing brain is not known, strict dietary control may be indicated in pregnancy of women with tyrosinaemia type II [23].

Genetics

Tyrosinaemia type II is of autosomal recessive inheritance. The gene is located at 16q22.1–q22.3. Several mutations leading to enzyme defect are known [24].

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Urea Cycle Disorders

J.V. Leonard

CONTENTS

Five inherited disorders of the *urea cycle* are now well described. These are characterised by *hyperammonaemia* and disordered amino acid metabolism. The presentation is highly variable: those presenting in the newborn period usually have an overwhelming illness, but the presentation may be subtle in those who present later in childhood or adult life.

Clinical Presentation

Patients with urea cycle disorders may present at almost any age. However, there are certain times at which they are more likely to develop symptoms because of metabolic stress such as infection, precipitating protein catabolism. These are:

- The neonatal period.
- During late infancy. Children are vulnerable during this period because of the slowing of growth, the changing to cow's milk and weaning foods as well as declining maternal antibody with the development of intercurrent infections.
- Puberty. The changing growth rate and psychosocial factors may precipitate decompensation.

However, it must be emphasised that many patients may present outside these periods. The patterns of the clinical presentation of hyperammonaemia are rather characteristic and broadly similar for all the disorders except arginase deficiency, which is discussed separately. The early symptoms are often non-specific and therefore initially the diagnosis is easily overlooked. The most important point about diagnosing hyperammonaemia is to think of it and measure the plasma ammonia concentration.

Neonatal Presentation. Most babies with urea cycle disorders are of normal birth weight and are initially healthy, but then after a short interval that can be less than 24 h they become unwell. Common early symptoms are poor feeding, vomiting, lethargy and/or irritability and tachypnoea. The initial working diagnosis is almost invariably sepsis. Rather characteristically, these babies may have a transient mild respiratory alkalosis at this stage, which can be a useful diagnostic clue. Usually they deteriorate rapidly with more obvious neurological and autonomic problems, including changes of tone with loss of normal reflexes, vasomotor instability and hypothermia, apnoea and fits. The baby may soon become totally unresponsive and requiring full intensive care. Untreated, most babies will die, often with complications such as cerebral or pulmonary haemorrhage, the underlying metabolic cause for which may not be recognised. Some survive neonatal hyperammonaemia, but are invariably handicapped to some degree.

Infantile Presentation. In infancy, the symptoms are generally rather less acute and more variable than in the neonatal period and include anorexia, lethargy, vomiting and failure to thrive with poor developmental progress. Irritability and behavioural problems are also common. The liver is often enlarged, but as the symptoms are rarely specific the illness is initially attributed to many different causes that include gastrointestinal disorders (e.g., gastro-oesophageal reflux, cow's milk protein intolerance), food allergies, behavioural problems or hepatitis. The correct diagnosis is often only established when the patient develops a more obvious encephalopathy with

changes in conscious level and neurological signs (see below).

Children and Adults. These patients present with the following illnesses.

Acute Encephalopathy. Older patients often present with episodes of acute metabolic encephalopathy, although they may also have chronic symptoms. Usually symptoms develop following metabolic stress such as infection, anaesthesia or protein catabolism, for example that produced by the rapid involution of the uterus in the puerperium [1]. However, an obvious trigger is not always apparent. The patients first become anorexic, lethargic and unwell. Sometimes they are agitated and irritable with behavioural problems or confusion. Vomiting and headaches may be prominent, suggesting migraine or cyclical vomiting. Others may be ataxic as though intoxicated. On examination, hepatomegaly may be present, particularly in those with argininosuccinic aciduria. The patients may then recover completely, but if not they may then develop neurological problems including a fluctuating level of consciousness, fits and sometimes focal neurological signs such as hemiplegia [2] or cortical blindness. Untreated, they continue to deteriorate, becoming comatose and may die or alternatively recover with a significant neurological deficit. The cause of death is usually cerebral oedema.

In between episodes, the patients are usually relatively well, although some, particularly young ones, may continue to have problems such as vomiting or poor developmental progress. Some patients may voluntarily restrict their protein intake. In addition to those disorders already mentioned, the illness may be attributed to a wide variety of other disorders including Reye's syndrome, encephalitis, poisoning and psychosocial problems.

Chronic Neurological Illness. Learning difficulties or more obvious mental retardation are common and some patients, particularly those with argininosuccinic aciduria, may present with relatively few symptoms apart from mental retardation and fits. About half the patients with arginosuccinic acid have brittle hair (trichorrhexis nodosa). Patients may present with chronic ataxia, which is worse with intercurrent infections [3].

Arginase Deficiency. Arginase deficiency usually presents with a spastic diplegia, and initially a diagnosis of cerebral palsy is almost always suspected. However, the neurological abnormalities are slowly progressive, although it may be difficult to distinguish this from an evolving cerebral palsy. During the course of the disease fits, ataxia and dystonia may develop. Occasional patients may present with an acute encephalopathy or anticonvulsant-resistant fits [4].

Metabolic Derangement

The urea cycle is the final common pathway for the excretion of waste nitrogen in mammals. The steps in the urea cycle are shown in Fig. 1. Ammonia is probably derived principally from glutamine and glutamate and is converted to carbamoylphosphate by *carbamoylphosphate synthetase*. This enzyme requires an allosteric activator *N*-acetylglutamate for full activity. This compound is formed by the condensation of acetylcoenzyme A (acetyl-CoA) and glutamate in a reaction catalysed by *N-acetylglutamate synthetase*. The carbamoyl phosphate condenses with ornithine to form citrulline in a reaction catalysed by *ornithine carbamoyl transferase*. The product, citrulline, condenses with aspartate to produce argininosuccinate catalysed by *argininosuccinate synthetase*, and the argininosuccinate is then split into arginine and fumarate. The arginine is itself cleaved by *arginase*, releasing urea with ornithine being reformed. Within the urea cycle itself, ornithine is neither formed nor lost. Each molecule of urea contains two molecules of waste nitrogen, one derived from ammonia and the other from aspartate. Regulation of the urea cycle is not fully understood and it is likely that there are several mechanisms controlling the flux through this pathway [5]. These include enzyme induction, the concentrations of substrates, intermediates and *N*-acetylglutamate as well as hormonal effects. Defects of each step have now been described and are listed in Table 1.

The plasma ammonia concentration is raised as a result of the metabolic blocks in the urea cycle. The degree to which it is elevated depends on several factors, including the enzyme involved and its residual activity and the rate of endogenous protein catabolism, particularly if this is increased because of infection, fever and other metabolic stress. The values may also be falsely elevated if the specimen is not collected and handled correctly.

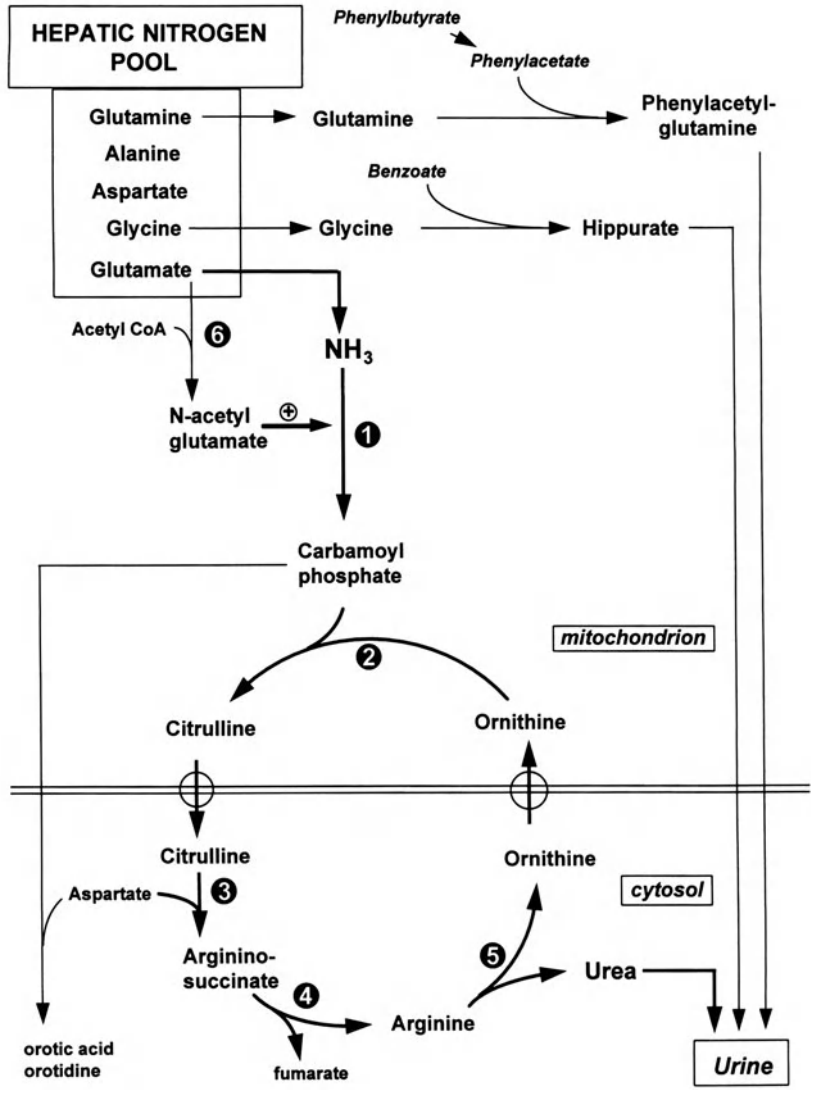

Fig. 1. Pathways for the disposal of waste nitrogen: the urea cycle and alternative pathways of nitrogen excretion. *1*, Carbamoylphosphate synthetase; *2*, ornithine carbamoyltransferase; *3*, argininosuccinate synthetase; *4*, argininosuccinate lyase; *5*, arginase; *6*, *N*-acetylglutamate synthetase; *CoA*, coenzyme A

The concentrations of the amino acids in the metabolic pathway immediately proximal to the enzyme defect will increase and those beyond the block decrease (Table 1). In addition, plasma alanine and particularly *glutamine* accumulate in all the disorders. The concentration of *citrulline* is often helpful diagnostically, but it may not always be reliable in the newborn period [6].

Orotic acid and orotidine are excreted in excess in the urine if there is a metabolic block distal to the formation of carbamoylphosphate, as in ornithine carbamyltransferase (OCT) deficiency, citrullinaemia, argininosuccinic aciduria and arginase deficiency (Fig. 1). In these disorders, carbamolyphosphate accumulates, leaves the mitochondrion and once in the cytosol enters the pathway for the de novo synthesis of pyrimidines. The urea cycle is also closely linked to many other pathways of intermediary metabolism, particularly the citric acid cycle.

Toxicity. Ammonia increases the transport of tryptophan across the blood–brain barrier, which then leads to an increased production and release of serotonin [7]. Some of the symptoms of hyperammonaemia can be explained on this basis, and the dietary tryptophan restriction has reversed anorexia in some patients with urea cycle disorders [8]. Ammonia induces many other electrophysiological, vascular and biochemical

Table 1. Urea cycle disorders: biochemical and genetic details

Disorder	Alternative names	Plasma amino acid concentrations	Urine orotic acid	Tissue for enzyme diagnosis	Genetics (chromosome localisation)
Carbamyl phosphate synthetase deficiency (CPSD)	CPS deficiency	↑ Glutamine ↑ Alanine ↓ Citrulline ↓Arginine	N	Liver	AR (chromosome 2p)
Ornithine carbamyl-transferase deficiency (OCTD)	OCT deficiency	↑ Glutamine ↑ Alanine ↓ Citrulline ↓ Arginine	↑↑	Liver	X-Linked (Xp 21.1)
Argininosuccinic synthetase deficiency (ASSD)	Citrullinaemia	↑↑ Citrulline ↓ Arginine	↑	Liver	AR (chromosome 9q)
Argininosuccinate lyase deficiency (ASLD)	Argininosuccinic aciduria	↑ Citrulline ↑ Argininosuccinic acid ↓ Arginine	†	RBC Liver	AR (chromosome 7q)
Arginase deficiency	Hyperargininaemia	↑↑ Arginine	↑	RBC Liver	AR (chromosome 6q)
N-acetylglutamate sythetase deficiency	NAGS deficiency	↑ Glutamine ↑ Alanine	N	Liver	AR (not confirmed)

AR, autosomal recessive; RBC, red blood cells; N, normal.

changes in experimental systems, but it is not known to what extent these are relevant to the problems of clinical hyperammonaemia in man [9].

Glutamine can also be shown to accumulate at high concentrations both in experimental models and also in man in vivo using proton nuclear magnetic resonance spectroscopy [10]. The concentrations are such that the increase in osmolality could be responsible for cellular swelling and cerebral oedema.

Diagnostic Tests

Biochemical Tests. Routine tests are not helpful for establishing the diagnosis of hyperammonaemia. Plasma transaminases may be elevated, which combined with hepatomegaly may lead to the erroneous diagnosis of hepatitis.

The most important diagnostic test in urea cycle disorders in measurement of the plasma ammonia concentration. Normally this is less than 50 μmol/l, but may be slightly raised as a result of a high protein intake, exercise, struggling or not separating the blood at once. Generally, patients who are acutely unwell with urea cycle disorders have plasma ammonia concentrations greater than 150 μmol/l and often significantly higher. However, the concentrations may be near normal when patients are well or if they have been on a low-protein, high-carbohydrate intake for some time.

Healthy neonates have slightly higher values [11]. If they are ill (sepsis, perinatal asphyxia, etc.), plasma ammonia concentrations may increase up to 180 μmol/l. Patients with inborn errors presenting in the newborn period usually have concentrations greater than 200 μmol/l and often very much greater. In that case, further investigations, particularly of the plasma amino acids and urine organic acids are urgent. The following investigations for raised plasma ammonia concentrations should be performed:

- Blood pH and gases
- Plasma chemistry: urea and electrolytes, glucose and creatinine
- Liver function tests and clotting studies
- Full blood count
- Plasma amino acids
- Urine organic acids, orotic acid and amino acids
- Plasma free and acylcarnitine

In all urea cycle disorders, there is accumulation of glutamine and alanine, and in citrullinaemia, arginosuccinic aciduria and arginase deficiency the changes in the amino acids are usually diagnostic (Table 1). Orotic aciduria with raised plasma glutamine and alanine concentrations suggests OCT deficiency. The diagnosis of this and the other disorders can be confirmed by measuring enzyme activity in appropriate tissue (Table 1). The enzyme diagnosis of *N*-acetylglutamate synthetase deficiency is not straightforward, and the response to a load of *N*-carbamylglutamate, an orally active analogue of *N*-acetylglutamate, may be helpful both diagnostically as well as for treatment.

Imaging. Patients who present with an acute encephalopathy commonly have brain imaging at an early stage. This may show no abnormality, a localised area of altered signal or, if the patient is very seriously ill, widespread cerebral oedema [12].

If focal areas of altered signal are identified, they should be distinguished from herpes simplex encephalitis. A careful history revealing previous episodes of encephalopathy, albeit mild, may provide vital clues. Imaging in patients who have recovered from a severe episode of hyperammonaemia usually shows cerebral atrophy that may be focal, particularly in those areas in which there were altered signal during the acute illness.

Differential Diagnosis. The differential diagnosis of hyperammonaemia is wide and the most common conditions are summarised in Table 2. In the neonatal period, the most common differential diagnoses are organic acidaemias, particularly propionic acidaemia. Patients with this disorder may have had marked hyperammonaemia with minimal metabolic acidosis or ketosis. Although babies with transient hyperammonaemia of the newborn are often born prematurely with early onset of symptoms [13], it may be difficult to distinguish between urea cycle disorders and transient hyperammonaemia of the newborn.

All patients in whom a tentative diagnosis of Reye's syndrome is made should have detailed investigations for inherited metabolic disorders, including those of the urea cycle.

Table 2. Differential diagnosis of hyperammonaemia

Inherited disorders
Urea cycle enzyme defects
Carbamyl phosphate synthetase deficiency
Ornithine carbamyltransferase deficiency
Argininosuccinate synthetase deficiency (citrullinaemia)
Argininosuccinate lyase deficiency (argininosuccinic aciduria)
Arginase deficiency
N-Acetylglutamate synthetase deficiency
Transport defects of urea cycle intermediates
Lysinuric protein intolerance
Hyperammonemia–hyperornithinemia–homocitrullinuria syndrome
Organic acidurias
Propionic acidaemia
Methylmalonic acidaemia and other organic acidaemias
Fatty acid oxidation disorders
Medium-chain acyl-CoA dehydrogenase deficiency
Systemic carnitine deficiency
Long-chain fatty acid oxidation defects and other related disorders
Other inborn errors
Pyruvate carboxylase deficiency (neonatal form)
Acquired
Transient hyperammonemia of the newborn
Reyes syndrome
Liver failure, any cause, both acute and chronic
Valproate therapy
Infection with urease-positive bacteria (particular with stasis in the urinary tract)
Leukaemia therapy, including treatment with asparaginase
Severe systemic illness particularly in neonates

Treatment and Prognosis

The aim of treatment is to correct the biochemical disorder and yet ensure that all the nutritional needs are met. The major strategies used are to reduce protein intake, to utilise alternative pathways of nitrogen excretion and to replace nutrients that are deficient.

Low-Protein Diet. Most patients with urea cycle disorders require a low-protein diet. The exact quantity will depend mainly on the age of the patient and the severity of the disorder. Many published regimens suggest severe protein restriction, but in early infancy patients may need 1.8–2 g/kg per day or sometimes even more during phases of very rapid growth. The protein intake usually decreases to approximately 1.2–1.5 g/kg per day during pre-school years and 1 g/kg per day in late childhood. After puberty, the quantity of natural protein may be less than 0.5 g/kg per day. However, it must be emphasised that there is considerable variation in the needs of individual patients.

Essential Amino Acids. In the most severe variants it may not be possible to achieve good metabolic control and satisfactory nutrition with restriction of natural protein alone. Other patients will not take their full protein allowance. In both these groups of patients some of the natural protein may be replaced with an essential amino acid mixture, up to 0.7 g/kg per day. Using this, essential amino acid requirements can still be met, but in addition waste nitrogen is re-utilised to synthesise non-essential amino acids, hence reducing the waste nitrogen load.

Alternative Pathways for Nitrogen Excretion. In many patients additional therapy is necessary. A major advance in this field has been the development of compounds that are conjugated to amino acids and rapidly excreted [14]. The effect of the administration of these substances is that nitrogen is excreted as compounds other than urea and hence the load on the urea cycle is reduced (Fig. 1). The first compound introduced was sodium benzoate. *Benzoate* is conjugated with glycine to form hippurate which is rapidly excreted. For each mole of benzoate given, 1 mol nitrogen is removed. Sodium benzoate is usually given in doses up to 250 mg/kg per day, but in acute emergencies this can be increased to 500 mg/kg per day. The major side-effects are nausea, vomiting and irritability.

The next drug used was phenylacetate, but this has now been superceded by *phenylbutyrate*, because the former has a peculiarly unpleasant clinging mousey odour. Phenylbutyrate is oxidised in the liver to phenylacetate, which is then conjugated with glutamine. The resulting phenylacetylglutamine is rapidly excreted by the urine and hence 2 mol nitrogen are lost for each mole of phenylbutyrate given. Phenylbutyrate is usually given as the sodium salt in doses of 250 mg/kg per day, but has been given in doses of up to 650 mg/kg per day [15]. The only side-effect appears to be vomiting and possibly constipation. However, patients are often reluctant to take the medicine and great ingenuity is sometimes needed to ensure that the patient takes it.

Arginine and Citrulline. Arginine is normally a non-essential amino acid because it is synthesised

within the urea cycle. For this reason, all patients with urea cycle disorders except those with arginase deficiency are likely to need a supplement of arginine to replace that which is not synthesised [16]. The aim should be to maintain plasma arginine concentrations between 50 and 200 μmol/l. For OCT and carbamylphosphate synthetase (CPS) deficiences a dose of 100–150 mg/kg per day appears to be sufficient for most patients. However, in severe variants of OCT and CPS, citrulline may be substituted for arginine in doses up to 170 mg/kg per day, as this will utilise additional nitrogen. Patients with citrullinaemia and argininosuccinic aciduria have a higher requirement, because ornithine is lost as a result of the metabolic block and this is replaced by giving arginine. Doses of up to 700 mg/kg per day may be needed, but this does have the disadvantage of increasing the concentrations of citrulline and argininosuccinic acid, respectively. The consequences of this are thought to be less important than those caused by the accumulation of ammonia and glutamine.

Other Medication. *Citrate* has long been used to provide a supply of Krebs cycle intermediates [17]. It is known to reduce postprandial elevation of ammonia and may be particularly important in the management of argininosuccinic aciduria [18]. N-carbamylglutamate can be used in *N*-acetylglutamate synthetase deficiency to replace the missing compound, as it is active orally. The dose is 100–300 mg/kg per day [19]. Patients who respond may require treatment only with this compound. Anticonvulsants may be needed for patients with urea cycle disorders, but sodium valproate should *not* be used, as this drug may precipitate fatal decompensation in OCT patients in particular [20].

General Aspects of Therapy. All treatment must be monitored with regular quantative estimations of plasma ammonia and amino acids, paying particular attention to the concentration of glutamine and essential amino acids. The aim is to keep plasma ammonia less than 80 μmol/l and plasma glutamine less than 800 μmol/l [21] with concentrations of essential amino acids within the normal range (see the algorithm, Fig. 2). All diets must, of course, be nutritionally complete and meet the requirements of vitamins, energy and trace minerals for growth and normal development.

The concept of balance of diet and medicines is important. The protein intake of the patients varies considerably and the figures that have been given should be regarded only as a guide. The variation does not just reflect the residual enzyme activity, but also many other factors including appetite and growth rate. Some patients have an aversion to protein, so it can be difficult to get them to take even their recommended intake. Consequently, they are likely to need smaller doses of sodium benzoate and phenylbutyrate. Others prefer to take more protein and this has to be balanced by an increase in the dosage of benzoate and phenylbutyrate. Some will not take adequate quantities of sodium benzoate or sodium phenylbutyrate, and therefore their protein intakes necessarily have to be stricter than would be needed if they took the medicines. Hence, for each patient a balance must be found between the protein intake and the dose of their medicines.

Acute Decompensation and Emergency Treatment. All patients with urea cycle disorders are at risk of acute decompensation with acute hyperammonaemia. This can be precipitated by different kinds of metabolic stress such as fasting, a large protein load, infection, anaesthesia and surgery. For this reason, all patients should have detailed instructions of what to do when they are at risk. We routinely use a three-stage procedure [22]. If the patient is off colour, the protein intake is reduced and more carbohydrate given. If symptoms continue, protein should be stopped and a high energy intake given together with their medication both day and night. However, if they cannot tolerate oral drinks and the medicines, are vomiting or becoming progressively encephalopathic, then they the patient should go to hospital for assessment and intravenous therapy without delay. For further practical details see [22]. Patients should also have a high carbohydrate intake to cover any anaesthesia or surgery.

For patients who are seriously ill with hyperammonaemia treatment is very urgent. The steps are listed in the Appendix and early treatment is essential (see also Ogier and Saudubray, this volume).

Prognosis. The prognosis in these disorders is closely related to the age of the patient and their condition at the time of diagnosis. For those patients who present with symptomatic hyperammonaemia in the newborn period, the outlook is very poor. Even with the most aggressive treatment the majority of the survivors will be handi-

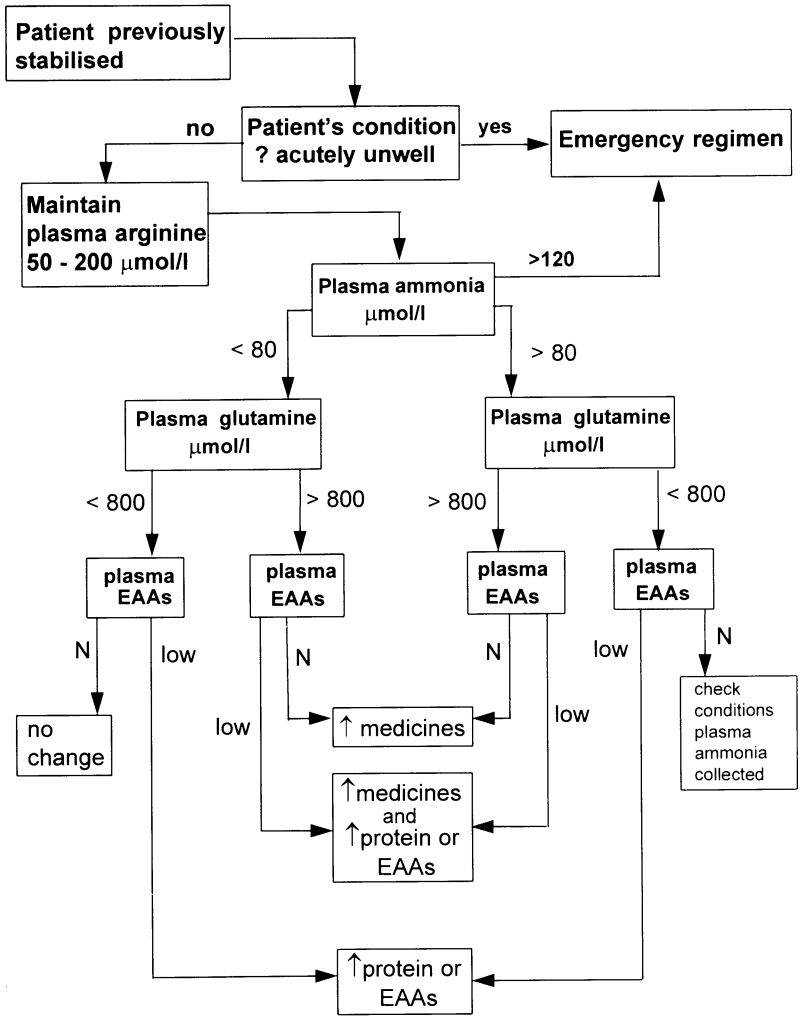

Fig. 2. Guidelines for the management of patients with urea cycle disorders (except arginase deficiency). This is intended for use in patients who have been stabilised previously and should only be regarded as a guide, as some patients may have individual requirements. For more detail and information about doses, please refer to the text. *EAA*, essential amino acid; *N*, normal

capped. Those who are treated prospectively do much better, but there may still be significant complications [23]. For these patients there remains a serious risk of decompensation, and careful consideration should be given to early liver transplantation, which may offer the hope of a better long-term outlook [24]. Of those who present later, their neurological problems at the time of diagnosis are critical, as most will have already suffered neurological damage. At best this may apparently resolve, but almost all are left with some degree of learning and neurological disability. Patients who have widespread cerebral oedema almost all die or survive severely handicapped. By contrast, those who are treated prospectively have a much better outcome.

Genetics and Prenatal Diagnosis

The genes for urea cycle enzymes, except *N*-acetylglutamate synthetase, have been mapped, isolated and fully characterised. The most common urea cycle disorder is OCT deficiency, which is an X-linked disorder. When the diagnosis of OCT deficiency is established, it is necessary to take a careful family history and for mother's carrier status to be assessed. Currently, the most convenient investigation is the allopurinol test, which is used to detect increased de novo synthesis of pyrimidines (see "Metabolic Derangement"). It appears to have good sensitivity and specificity [25, 26]. This is easier than the protein or alanine loading tests and carries no risk of hyper-

ammonaemia. Molecular genetic studies may also be highly informative. Prenatal diagnosis for OCT deficiency can be This can be done using a gene probe for mutation detection or to identify informative polymorphisms and can help a substantial proportion of families. For those in whom these studies are not possible, then prenatal liver biopsy can also be used. Whilst the phenotype of the males can be predicted, that of the females cannot because of the random inactivation of the X chromosome. This presents a problem when counselling families, but the prognosis for females who are treated prospectively from birth is good.

All the other conditions have autosomal recessive inheritance, and prenatal diagnosis is possible for all disorders except *N*-acetylglutamate synthetase deficiency. For carbamoylphosphate synthetase deficiency, prenatal diagnosis using closely linked gene markers is now possible for a substantial proportion of families. If the molecular genetic studies are uninformative, prenatal liver biopsy is a possible alternative. Citrullinaemia and argininosuccinic aciduria can both be diagnosed on chorionic villus biopsy. Arginase deficiency can be diagnosed either with molecular genetic studies or, if they are not informative, on a foetal blood sample.

Appendix

Emergency treatment of hyperammonaemia

- Stop protein intake
- Give a high energy intake: either (a) orally (10%–20% soluble glucose polymer or protein-free formula) or (b) intravenously (10% glucose by peripheral infusion or 10%–25% glucose by central venous line)

The volumes which are given are related to age and the condition of the patient. Fluid volumes should be restricted if there is any concern about cerebral oedema.

- Give:
  - Sodium benzoate up to 500 mg/kg per day (orally or intravenously)
  - Sodium phenylbutyrate up to 600 mg/kg per day
  - L-arginine
    In citrullinaemia and arginosuccinic aciduria up to 700 mg/kg per day
    In OCT deficiency and CPS deficiency up to 150 mg/kg per day

  Note: For the emergency treatment of hyperammonaemia before diagnosis is known, this step may be replaced by:
  - L-arginine (300 mg/kg per 24 h)
  - L-carnitine (200 mg/kg per 24 h)

  Both can be given orally or intravenously.
- Dialysis. If hyperammonaemia is not controlled, haemofiltration (or haemodialysis) should be started. Alternatively, peritoneal dialysis can be used, but this is a much less effective method for reducing hyperammonaemia.
- Treat other conditions, e.g. shock, sepsis, fits etc.
- Monitor intracranial pressure with the usual measurements to reduce raised pressure and maintain perfusion pressure.

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Homocystinuria Due to Cystathionine β-Synthase Deficiency and Related Disorders

G. Andria and G. Sebastio

CONTENTS

Several enzyme defects have been ascertained in the conversion of the sulfur-containing amino acid methionine into cysteine (Fig. 1). Some of these enzyme defects have been detected in asymptomatic subjects and are therefore not described here. Others are associated with inherited disorders of vitamins (see Rosenblatt and Shevell, this volume). *Cystathionine β-synthase deficiency* (CBS) [1] is the only enzymatic defect associated with severe clinical abnormalities and is described in this chapter.

In methionine metabolism, homocysteine represents the connection between the transsulfuration pathway (Fig. 1, right) and the transmethylation cycle (Fig. 1, left). In the latter, about 50% of the available homocysteine is remethylated to methionine through two alternative reactions. In the first, the methyl group is donated by betaine and the reaction is catalyzed by betaine-homocysteine methyltransferase (enzyme 3); the alternative methyl donor is 5-methyl tetrahydrofolate, formed from 5,10-methylenetetrahydrofolate, and the enzyme involved is 5-methyltetrahydrofolate-homocysteine methyltransferase, requiring methylcobalamin as a cofactor (enzyme 2). (For more details on the transmethylation cycle, see Rosenblatt and Shevell, this volume.)

The transsulfuration pathway is the main metabolic route from methionine to cysteine and inorganic sulphate. The first step is the formation of *S*-adenosylmethionine, catalyzed by methionine adenosyltransferase. *S*-Adenosylhomocysteine is produced from the subsequent transmethylation reaction. It is cleaved by a hydrolase to homocysteine and adenosine. Homocysteine can be either remethylated to methionine, as already mentioned, or condensed with serine, yielding cystathionine in an irreversible reaction catalyzed by CBS, an enzyme requiring pyridoxal phosphate as a cofactor. CBS can alternatively catalyze serine sulfhydration to form cysteine using H_2S rather than homocysteine. Cystathionine is finally cleaved to cysteine and α-ketobutyrate by another pyridoxal phosphate-dependent enzyme, γ-cystathioninase. The sulfur atom of methionine, transferred into the cysteine molecule through the transsulfuration pathway, ends up mainly as inorganic sulfate in the urine. Two molecules of homocysteine may be oxidized chemically or enzymatically to homocystine, the metabolite actually found in body fluids. The −SH group of homocysteine can easily react with the −SH groups of other molecules, leading to the formation of compounds such as homocysteine–cysteine mixed disulfide or protein-bound homocysteine.

Clinical Presentation

Four systems are involved in the typical presentation of homocystinuria due to cystathionine β-synthase deficiency: the eye, the skeleton, the central nervous system, and the vascular system. The patient is normal at birth and, if left untreated, progressively develops the full-blown clinical picture.

Eye. *Ectopia lentis*, the dislocation of the ocular lens, myopia, and glaucoma, sometimes with pupil entrapment of the dislocated lens, are frequent,

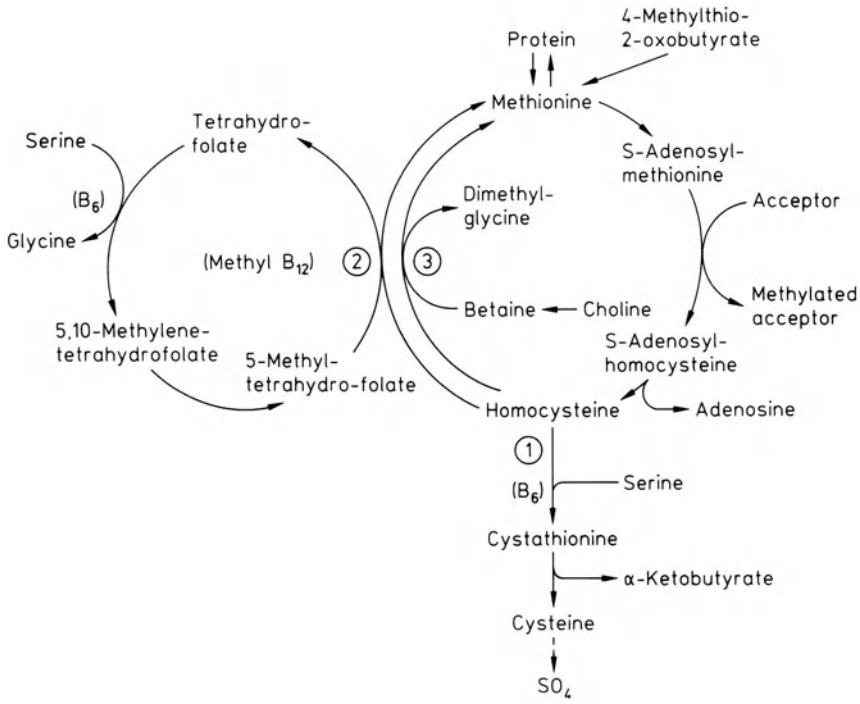

Fig. 1. The methionine transsulfuration pathway and transmethylation cycle. 1, Cystathionine β-synthase; 2,5-methyltetrahydrofolate-homocysteine methyltransferase; 3, betaine-homocysteine methyltransferase

severe, and characteristic complications. Retinal detachment and degeneration, optical atrophy, and cataracts eventually appear. Myopia may precede subluxation of the lens and worsens afterwards. Ectopia lentis is detected in most untreated patients after a few years during the first decade of life and in nearly all by the end of the fourth decade. It can often be the clue to the diagnosis. The dislocation is generally downward, whereas it is more often upward in the Marfan syndrome. Once ectopia lentis has occurred, it is possible to observe a peculiar trembling of the iris (iridodonesis) when the eyes or the head move.

Skeleton. *Osteoporosis* is almost invariably detected, at least after childhood. Frequent consequences of osteoporosis are scoliosis, as well as a tendency to pathological fractures and vertebral collapse. As in the Marfan syndrome, homocystinuric patients tend to be tall, with thinning and elongation (dolichostenomelia) of long bones near puberty, and enlarged metaphyses and epiphyses, especially at the knees. *Arachnodactyly*, defined as a metacarpal index (the average ratio of length to breadth of metacarpals II–V) above 8.5, is present in about half the patients. Other *bone deformities* include genu valgum with knobby knees, pes cavus, pectus carinatum, or excavatum. Restricted joint mobility, particularly at the extremities, contrasts with the joint laxity observed in the Marfan syndrome.

Peculiar roentgenographic findings are biconcavity and flattening of the intervertebral discs, growth arrest lines in the distal tibia, metaphyseal spicules in the hands and feet, enlarged carpal bones, retarded lunate development, and shortening of the fourth metacarpal.

Central Nervous System. Developmental delay and *mental retardation* affect about 50% of patients to a variable degree of severity. Seizures, electroencephalogram (EEG) abnormalities, and psychiatric disturbances have been also reported in approximately 50% of cases. Focal neurologic signs may be a consequence of cerebrovascular accidents (see below).

Vascular System. *Thromboembolic complications*, occurring in any arterial and venous district of the body, represent the major cause of morbidity and mortality. The prognosis is influenced by the site and the extent of the vascular occlusion. Noninvasive methods, such as echo Doppler techniques, can detect abnormalities of the vessels in a presymptomatic stage [2]. Even mild hyperhomocyst(e)inemia (basal or after methionine

loading test) has been related with higher risk of premature occlusive vascular disease (see [3] for review).

Clinical Variability and Natural History. Clinical variability is present and mild cases may not be recognized until severe complications, such as thromboembolic accidents, occur. Those patients are more frequently responsive to pharmacological doses of pyridoxine, the cofactor of CBS.

Mudd et al. carried out an international questionnaire survey and collected data on 629 patients [4]. Time-to-event curves were calculated for all main clinical manifestations, including mental retardation, lens dislocation, first episode of thromboembolism, radiologic detection of spinal osteoporosis, and mortality. Each abnormality occurred significantly earlier and at a higher rate in untreated pyridoxine-nonresponsive individuals than in untreated pyridoxine-responsive ones. Another question concerned the risk of thromboembolic accidents in patients undergoing surgery. Following 586 operations, complications were only recorded in 25 patients, six of which were lethal.

Pyridoxine-responsive women can carry out pregnancies without a significant risk of malformations for the offspring. However, ocular abnormalities, mental retardation, and psychiatric disorders have been observed in the offspring of two pyridoxine-nonresponsive women. It is not yet clear whether an excess of fetal loss may affect pyridoxine-responsive women.

Metabolic Derangement

CBS activity can be found in many organs: liver, from which it has been purified, brain, pancreas, and cultured fibroblasts [5]. Pyridoxal phosphate is the cofactor required for activation of CBS. It binds to two other ligands, *S*-adenosylmethionine and a heme moiety [6]. Fibroblasts from patients with homocystinuria can be classified into three groups:

- No residual activity
- Reduced activity
- Normal affinity for pyridoxal phosphate [7].

In vivo responsiveness to pharmacological doses of pyridoxine is demonstrated in approximately 50% of homocystinuric patients. The presence of at least a small residual enzymatic activity seems to be necessary for pyridoxine responsiveness, though some conflicting results have been reported.

The pathophysiology of homocystinuria due to CBS deficiency has not yet been completely elucidated. Accumulation of homocyst(e)ine probably plays a major role in determining some of the most relevant clinical manifestations, including generalized vascular damage and thromboembolic complications. This view is supported by the observation that patients affected by homocystinuria due to defects in the remethylation pathway of homocysteine, with low or normal plasma methionine, show similar lesions in blood vessels [8]. Thromboembolism has been suggested to be the end point of homocyst(e)ine-induced abnormalities of platelets, endothelial cells, and coagulation factors.

Homocysteine may cause an abnormal cross-linking of collagen, leading to abnormalities of skin, joints, and skeleton in the patients [9].

Diagnostic Tests

The amino acid profile is generally typical of CBS deficiency and includes *homocystinuria* and *hyperhomocyst(e)inemia, hypermethioninemia*, and low plasma cystine and cystathionine. As 70%–80% of homocysteinyl moieties are bound to protein, treatment of the plasma sample with reducing agents is required before deproteination. Normal plasma total homocyst(e)ine values are 5.7–16.5 μM for females and 6.5–15.8 μM for males. Untreated CBS patients may have total homocysteine levels higher than 200 μM.

The cyanide-nitroprusside test is used as a screening test, but it also gives a positive reaction for other conditions, such as cystinuria. A modified nitroprusside reagent is quite specific for homocyst(e)ine [10]. Column chromatography is the method of choice for measuring homocyst(e)ine and other sulfur-containing compounds, such as the mixed cysteine–homocysteine disulfide, in plasma and urine. As some responsive patients are extremely sensitive to very low doses of pyridoxine, contained in multivitamin tablets, false-negative results may be obtained.

Other genetic causes of *hyperhomocyst(e)inemia* include deficiency of 5,10-methylenetetrahydrofolate reductase and defects in cytosolic cobalamin metabolism, leading to impaired synthesis of methylcobalamin and conse-

quent deficiency of 5-methyltetrahydrofolate-homocysteine methyltransferase (see Rosenblatt and Sherell, this volume).

Nongenetic causes of hyperhomocyst(e)inemia are renal insufficiency and administration of 6-azauridine triacetate, methotrexate, isonicotinic acid hydroxide, and colestipol plus niacin.

Hypermethioninemia is highly specific for CBS deficiency, since in other genetic forms of homocystinuria, e.g., the disorders of homocysteine remethylation, plasma methionine levels are normal or low. In some older patients with CBS deficiency who develop a folate depletion and a consequent defect in remethylation, methionine concentrations may return to normal. Other biochemical sequelae include a slight increase in plasma concentrations of ornithine, copper, and ceruloplasmin.

CBS activity has to be greatly reduced to confirm the diagnosis. The enzyme is usually assayed in cultured skin fibroblasts [5, 7] and phytohemagglutinin-stimulated lymphocytes [11], but the deficiency may be demonstrated in liver biopsy as well [12]. Exceptional patients may have significant residual activity of CBS in fibroblast extracts and yet have the typical abnormalities of the disease.

Screening mass newborn programs have been implemented in many countries by microbiologically determining methionine levels in dried blood spots collected on filter paper, but they yield an unacceptably high rate of false-negative results, particularly among pyridoxine-responsive patients [13].

Genetic and acquired deficiencies of hepatic methionine *S*-adenosyltransferase and CBS cause an accumulation of plasma methionine. Other rarer causes of hypermethioninemia have been reported, but their mechanisms are not well established.

Prenatal Diagnosis. Prenatal diagnosis of homocystinuria has been performed in some pregnancies at risk by assaying CBS in extracts of cultured amniocytes [14]. CBS activity can be measured in chorionic villi. However, the enzymatic activity in control samples is only detectable after culturing the biopsy [15].

Heterozygotes. Heterozygosity for CBS deficiency can be diagnosed by enzymatic assay in liver biopsy, cultured skin fibroblasts, and phytohemagglutinin-stimulated lymphocytes. With the methods so far described few obligate heterozygotes have values overlapping those at the lower limit of the control range.

Indirect methods for the identification of heterozygotes have also been proposed, such as the measurement of sulfur-containing compounds after an oral methionine load [16], provided the results are compared with those of age- and sex-matched controls.

A significant number of vascular patients with hyperhomocyst(e)inemia displays a CBS activity compatible with a heterozygous condition [17, 18]. On the other hand, obligate heterozygotes for CBS deficiency showed early vascular lesions, detected by noninvasive ultrasounds methods in a presymptomatic stage, at a significantly higher rate than controls [2].

Treatment and Prognosis

About half the patients with homocystinuria due to CBS deficiency respond to large doses of *pyridoxine* given orally. In some cases only partial response is observed. After a variable period (up to a few weeks) homocystinuria disappears, hypermethioninemia decreases, and cystinemia increases to values within the control range. To monitor the effect of megavitamin treatment, it is advisable to measure total plasma homocyst(e)ine levels, which also include, apart from the free homocysteine disulfides (homocystine plus homocysteine–cysteine mixed disulfide) protein-bound homocysteine.

Some responsive patients need a few milligrams of pyridoxine daily, others up to 1000 mg/day. Response to the vitamin is also influenced by folate depletion, which may be due to pyridoxine administration itself: therefore folic acid (10 mg/day) should be added to the treatment [19].

Megadoses of pyridoxine, taken for other disorders, may exceptionally cause sensory neuropathy. Therefore, the patient is considered nonresponsive if 500–1000 mg/day of pyridoxine do not correct the biochemical abnormalities after several weeks of treatment. In this case, a low-methionine, high-cystine diet is started [20]. A less rigid methionine-restricted diet is probably advisable for pyridoxine-responsive patients too. Synthetic methionine-free amino acid mixtures are commercially available and especially useful for infants (Analog RVHB, Maxamaid RVHB, Maxamum RVHB, Albumaid methionine-low, methionine-free amino acid mix, Scientific Hospi-

tal Supplies, LTD. England; HOM 1 and HOM 2, Milupa AG, Germany). The requirement for methionine is met by small amounts of infant formula; supplements of essential fatty acids and carbohydrates are also required. After infancy, foods relatively low in methionine (when related to their protein content) can be introduced: these include gelatin, pulses, such as lentils and soybeans. However, it should be realized that soya-modified formulas are usually enriched with methionine. In homocystinuria, a stricter regimen is not necessary during infections and catabolism, as in other amino acid disorders.

The parameters to be monitored during the diet are normal growth rate, methioninemia ($<40\,\mu M$), and normal levels of total homocyst(e)ine in blood and urine. Plasma cystine should be maintained within the normal range ($67 \pm 20\,\mu M$) and supplemented accordingly (up to 200 mg/kg per day), since it becomes an essential amino acid in methionine-restricted diets.

Beside pyridoxine and folate, the usual vitamin and mineral supplements are recommended.

In pyridoxine-nonresponsive patients, especially when treatment is started late, it is difficult to obtain good compliance to the diet. In these patients *betaine* has been added (4–6 g/day) as a methyl donor [21, 22]. The concentration of methionine increases consequently, but apparently it does not influence the pathophysiology of the disease.

Dipyridamole (100 mg four times per day) either alone or (100 mg daily) combined with aspirin (1 g/day) has been proposed to prevent thromboembolic complications, but no clear-cut evidence has been obtained so far as to the effectiveness of these therapeutic approaches. As the suggested aspirin dose is potentially dangerous, a rationale for testing the efficacy and safety of a low-dose aspirin has been reported [23].

The results of the international survey, reported by Mudd et al. [4], provided a firmly established baseline for the evaluation of past and future therapeutic regimens. When the low-methionine diet was started in the newborn period, mental retardation was prevented, the start and progression of lens dislocations were delayed, and the incidence of seizures probably decreased. After pyridoxine treatment, the first thromboembolic episode occurred at lower rates in late-diagnosed responsive subjects.

Since the treatment is more successful when the diagnosis is made early, mass newborn screening programs should probably be implemented as soon as the present technical pitfalls are solved. It is still unsettled whether preventive treatment with vitamins is indicated in heterozygotes for homocystinuria or in other subjects with homocyst(e)inemia from various causes.

Genetics

Homocystinuria due to CBS deficiency is inherited as an autosomal recessive trait. Genetic subtype, e.g., pyridoxine responsiveness, is also genetically determined and probably related to mutations of specific regions of the protein.

CBS is a tetramer of identical 63-kDa subunits. The CBS gene is located on chromosome 21 (21q22.3), and CBS cDNA has been cloned and sequenced; the open reading frame predicts a polypeptide with 551 amino acid residues [6]. Molecular studies on CBS patients have led to the characterization of more than ten mutations [6]. Two of these appear to be of epidemiologic relevance. The first one, G919A (G307S as amino acid substitution), has been found in groups of Celtic origin, mainly in Ireland. The second, T833C (I278T), has been found in different ethnic groups (Italian, Jewish, French, German, Norwegian, English). G919A seems to be incompatible with pyridoxine responsiveness, which is not the case for T833C, even when this mutation is present in homozygosity. A rapid strategy to analyze these two more frequent mutations, both localized in exon 8 of the CBS gene, is already available.

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Ornithine

V.E. Shih

CONTENTS

Ornithine is an important intermediary metabolite derived almost entirely from arginine (Fig. 1). Excess ornithine generated from arginine is catabolized via ornithine-δ-aminotransferase (OAT), a pyridoxal phosphate requiring enzyme, which converts ornithine and α-ketoglutarate to Δ^1-pyrroline-5-carboxylate and then to proline and glutamate. Ornithine also plays a major role in the urea cycle: it accepts the transfer of the carbamoyl group of carbamoyl phosphate, formed from CO_2 and ammonia, and is converted to citrulline by ornithine transcarbamylase (OTC). Since both OTC and OAT are mitochoondrial matrix enzymes, ornithine produced in the cytoplasm must be transported to the mitochondrial matrix by a specific energy-requiring transport system [1]. A small fraction of ornithine is decarboxylated in the cytosol to form putrescine for polyamine biosysnthesis.

The two clinically different disorders of ornithine metabolism that result in hyperornithinemia are discussed in this chapter. The deficiency of OTC is discussed in the chapter by Leonard (this volume).

Hyperornithinemia Due to Ornithine Aminotransferase Deficiency (Gyrate Atrophy of the Choroid and Retina)

Clinical Presentation

Night blindness and myopia are usually the first symptoms in early childhood. Ocular findings include myopia, constricted visual fields, elevated dark adaptation thresholds, and very small or nondetectable electroretinographic (ERG) responses. Retinopathy can be detected before visual disturbances. Fundoscopic appearances of the choreoretinal atrophy are illustrated in Fig. 2A, B. Patients develop posterior subcapsular *cataracts* by the late teens and usually become virtually blind between the ages of 40 and 55 due to extensive chorioretinal atrophy. Pyridoxine-responsive patients often have a milder course and maintain adequate visual acuity at older ages. Considerable heterogeneity exists in the appearance of the fundus even within the same family, and siblings at the same age can show substantial differences in the severity of the ocular disease. Vitreous hemorrhage causing sudden loss of vision is a rare complication [2].

Histopathologic study of the eye obtained postmortem from a pyridoxine-responsive patient showed focal areas of photoreceptor atrophy with adjacent retinal pigment epithelial hyperplasia [3]. Electron microscopic studies revealed abnormal mitochondria in the corneal endothelium and the nonpigmented ciliary epithelium and similar, but less severe, abnormalities in the photoreceptors.

In addition to the ocular findings, systemic abnormalities have been reported in some patients. Most patients have normal intelligence. The com-

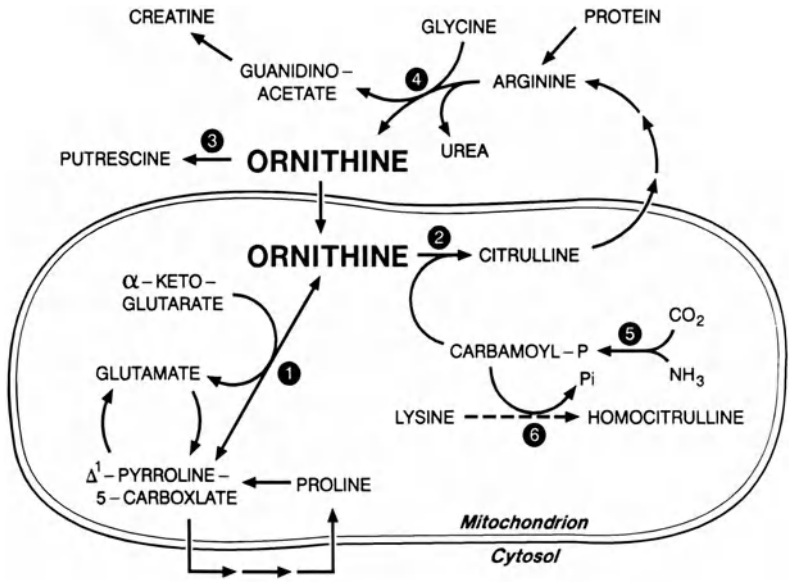

Fig. 1. Ornithine metabolic pathways. *Pi*, inorganic phosphate; *1*, ornithine-δ-aminotransferase (OAT); *2*, ornithine transcarbamylase (OTC); *3*, ornithine decarboxylase; *4*, glycine transamidinase; *5*, carbamoylphosphate synthetase; *6*, lysine transcarbamylase. The step indicated by the *broken line* is not well defined

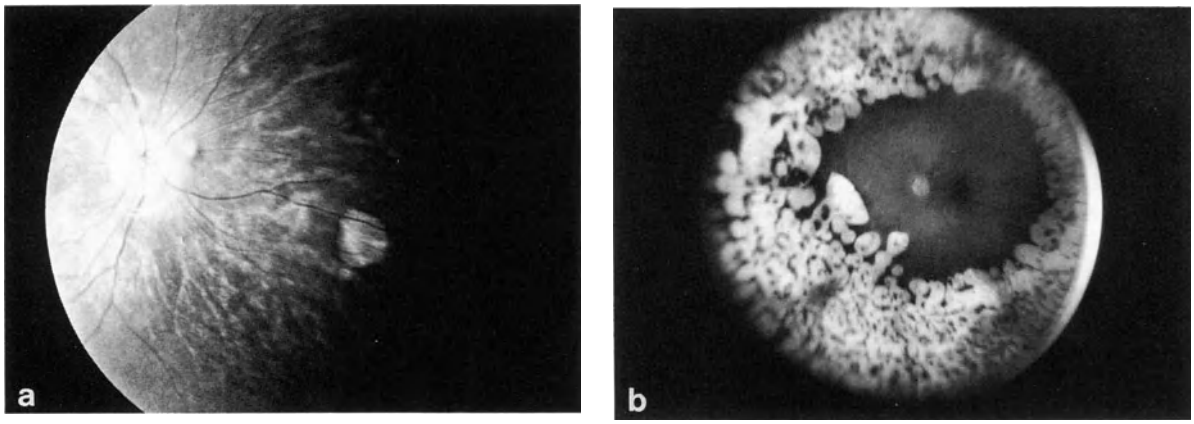

Fig. 2a, b. Gyrate atrophy of the choroid and retina. **a** Fundus of a $5^{10}/_{12}$-year-old girl showing early changes. There is an overall thinning of the retinal pigment epithelium, and the large choroid blood vessels are exposed. One discrete spot of atrophy can be seen midway between the disc and the ora serrata. Multiple spots of similar atrophic area are in the far periphery (not shown: courtesy of Dr. Tatsuo Hirose). **b** Fundus of a 10-year-old girl showing an advanced characteristic chorioretinal atrophy distributed circumferentially around the peripheral fundus. (Courtesy of Dr. Eliot L. Berson)

mon finding on electroencephalogram has been diffuse slowing. Muscle pathology includes tubular aggregates and type 2 fiber atrophy [4], but only a small number of patients have clinical evidence of muscle weakness. Abnormal ultrastructure of hepatic mitochondria has been described [5]. Peculiar fine, sparse, straight hair with microscopic abnormalities has been found in some patients [6].

Metabolic Derangement

Patients with gyrate atrophy of the choroid and retina (GA) have marked hyperornithinemia due to a deficiency of OAT activity (also known as ornithine keto acid transaminase, OKT) [7]. The enzyme deficiency has been demonstrated in liver, muscle, hair roots, cultured skin fibroblasts, and lymphoblasts. The pathophysiological mechanism of the retinal degeneration is unclear.

Table 1. Differential diagnosis of disorders involving ornithine metabolism

	OAT deficiency	HHH syndrome	OTC deficiency
Major clinical findings	Gyrate atrophy of the choroid and retina	Mental retardation Episodic lethargy and ataxia Neonatal coma (rare)	Severe form: Neonatal onset of coma Mental retardation Early death Milder form: Aversion to protein foods Episodic lethargy and ataxia
Inheritance	Autosomal recessive	Autosomal recessive	X-linked
Blood ammonia	Normal	Increased	Increased
Major amino acid changes	Increased blood ornithine	Increased blood ornithine Increased urine homocitrulline	Normal blood ornithine Decreased blood citrulline
Orotic acid	Normal	Increased	Increased

HHH, hyperornithinema–hyperammonemia–homocitrullinuria.

OAT requires pyridoxal phosphate (PLP) as a cofactor. In a small number of patients, the OAT activity increased substantially when measured in the presence of high concentrations of PLP. Most of these patients showed a partial reduction of plasma ornithine when given pharmacological doses of pyridoxine (vitamin B_6). In rare cases, the in vivo response is in discord with the in vitro response to pyridoxine. On the basis of in vitro and in vivo responses, at least two variants – pyridoxine responsive and pyridoxine nonresponsive – have been described.

GA patients have low creatine and its precursor, guanidinoacetate, in the blood and urine, as well as low creatine and creatine phosphate in skeletal muscle [8]. The most likely explanation for these findings is that the biosynthesis of creatine from guanidinoacetate is reduced as a result of ornithine inhibition of glycine transamididase (Fig. 1).

Diagnostic Tests

The main biochemical finding is a plasma ornithine concentration 5- to 20-fold above normal. Patients with the pyridoxine-responsive variant tend to have lower plasma ornithine levels than those with the pyridoxine-nonresponsive variant. Urinary excretion of ornithine as well as that of lysine, arginine, and cystine is often increased when the concentration of plasma ornithine is 400μmol/l or greater. These changes are secondary to competitive inhibition by ornithine of the common renal transport shared by these amino acids. Small amounts of ornithine methyl ester and gamma glutamylornithine are sometimes detected in the urine. The absence of hyperammonemia and homocitrullinuria differentiate this disorder from the hyperornithinemia–hyperammonemia–homocitrullinura (HHH) syndrome (Table 1).

For confirmation of OAT deficiency, skin fibroblasts and lymphoblasts are suitable. Direct assay of OAT activity is performed in cell extracts and the production of pyrroline-5-carboxylate from ornithine is monitored by either a colorimetric or radioisotopic technique. An indirect assay measures the isotopic incorporation from ^{14}C-labeled L-ornithine into macromolecules by intact cells [9]. Since OTC is not expressed in cultured cells, cells lacking OAT activity cannot convert ^{14}C-L-ornithine to protein amino acids (i.e., ^{14}C-L-proline and ^{14}C-L-glutamate) for incorporation. Cells from patients with the pyridoxine-responsive variant may incorporate as much as 45% of the control value. This incorporation assay uses fewer cells than the direct assay and is applicable to genetic complementation analysis and prenatal diagnosis, but is not useful for heterozygote identification.

Treatment and Prognosis

There are several approaches to treatment. Since the pathophysiologic mechanism responsible for the retinal degeneration in this disorder is unknown, the goal of treatment has been to correct the amino acid abnormalities. Megavitamin and/or diet therapy have been used to reduce

hyperornithinemia. In addition, the administration of proline or creatine has been tried.

Pharmacological dosage of pyridoxine HCl has resulted in plasma ornithine reduction in a small number of patients. Doses between 15 and 600 mg a day lowered plasma ornithine levels from 25% to 60% [10–12]. A 2-week trial of *pyridoxine treatment* (300–600 mg/day) is recommended for all newly diagnosed patients to determine their responsiveness. Reduction of hyperornithinemia can also be achieved by restriction of protein intake and arginine (a precursor of ornithine in foods) [8, 13, 14]. On average, food proteins contain 4%–6% arginine (nuts and seeds have higher arginine contents). The low-protein diet may be supplemented with a synthetic mixture of essential amino acids providing up to one half of the nitrogen intake. Commercial products containing no arginine have been developed for this purpose. Products designated for patients with urea cycle disorders can also be used. Severe arginine depletion, however, can result in hyperammonemic complication [14].

Compliance to diet restriction can be a major problem with this form of treatment and long-term commitment and motivation are important factors. At least 30 patients have been given a low-arginine diet, some in combination with pharmacological doses of pyridoxine, for periods up to 10 years. Most of these patients were teenagers or adults with already advanced retinal disease. Despite good control of plasma ornithine to near normal levels, progression in the retinal atrophy and/or in the loss of visual functions occurred in most patients, including three patients whose treatment was initiated in the first decade of life [8, 13, 15, 16].

Stabilization of visual function has been reported in two adult patients during 10 years of dietary treatment with good biochemical control [16]. The results of an arginine-restricted diet during 5–7 years in two sets of siblings are encouraging and showed that the younger and earlier-treated sibling in each pair had less retinal atrophy [17].

It has been hypothesized that insufficient de novo formation of proline from ornithine in retinal pigment epithelium and ciliary body rather than ornithine toxicity is responsible for the development of retinal degeneration [11]. Five patients with GA were placed on *proline supplements* of 2–10 g a day for periods of 2–4 years. The youngest patient had been detected by routine amino acid screening and had only minimal fundoscopic changes without visual complaints. He was started on 3 g proline daily at age 4 years, and at age 8 he had remained asymptomatic. Stabilization of the ocular disease was reported in one patient and varying degrees of progression in three other patients. In five other patients, creatine administration at 0.75–1.5 g per day corrected the muscle histopathology, but did not halt the progression of the retinal degeneration [8].

Although GA is a progressive degeneration, the natural history of this disease in the short-term period among patients of different age needs further clarification. The matter of defining natural history should take confounding factors into consideration: age, intrafamilial variability, and reproducibility of visual function tests. The long-term effects of the above therapeutic measures have yet to be assessed.

Genetics

GA is an autosomal recessive disorder and has been described in patients from various ethnic backgrounds, but its incidence is highest in the Finnish population [18]. Intermediate levels of OAT activity have been observed in skin fibroblasts from obligate heterozygotes for both pyridoxine-nonresponsive and pyridoxine-responsive variants. Heterozygotes for the pyridoxine-responsive variant can be distinguished by a doubling of OAT activity when assayed with and without PLP.

The human gene for OAT has been mapped to chromosome 10 with pseudogenes on the X-chromosome. Many different mutations in the OAT structural gene have been defined in GA patients of varied ethnic origins [19].

Hyperornithinemia, Hyperammonemia, and Homocitrullinuria Syndrome

Clinical Presentation

The HHH syndrome [20] is rare and only approximately 40 cases are known. There is a wide spectrum of clinical manifestations, most of which are related to hyperammonemia. Ocular abnormalities are notably absent. Intolerance to protein feeding, vomiting, seizures, and developmental delay from infancy are common complaints. Neo-

natal onset of lethargy, hypotonia, seizures with progression to coma, and death has been observed in the most severe form [21]. Progressive spastic paraparesis is often a late complication. Abnormal cranial computed tomography (CT) showing diffuse white matter, low-density, and cerebellar vermis atrophy was described in one patient. Coagulopathy, especially factor VII and X deficiencies, has been reported in several patients [22].

Mildly affected adult patients may present with hepatitis-like hyperammonemia and apparently normal intelligence. One adult man with the HHH syndrome came to attention because of episodic ataxia and personality change, and his sister was diagnosed by family survey [23]. She had only a history of protein intolerance and refusal of high-protein foods in childhood and poor school performance.

Electron microscopic examination of biopsied hepatic tissue showed an increased number of mitochondria and many were large or bizarre shaped with unusually long tubular internal structure [24]. The structural changes seen in fibroblast mitochondria are in some respects similar to those found in hepatic mitochondria.

Metabolic Derangement

Patients with the HHH syndrome have a marked elevation of plasma ornithine associated with hyperammonemia and increased urinary excretion of homocitrulline, a derivative of lysine. Activities of the two major ornithine metabolizing enzymes, OTC and OAT measured in liver homogenate and fibroblast extracts, respectively, are normal [25]. In contrast, the utilization of ornithine by intact fibroblasts and lymphoblasts is impaired [9]. These findings suggest that the HHH syndrome is a disorder of compartmentation and its defect is in the import of ornithine into the mitochondrion, resulting in a functional deficiency of both OTC and OAT activities (Fig. 1). Results of in vitro studies using isolated mitochondria support this concept [26, 27]. The clinical consequences of this metabolic derangement are those associated with OTC deficiency (Table 1).

Diagnostic Tests

The HHH syndrome can be differentiated from other hyperammonemic syndromes including the urea cycle enzymopathies by laboratory findings (Table 1). The triad of hyperornithinemia, hyperammonemia, and homocitrullinuria is pathognomonic. Plasma ornithine concentration is three to ten times elevated and tends to be somewhat lower than that seen in GA patients. Despite a functional deficiency of OTC activity, the plasma citrulline is often normal in the HHH syndrome.

Urine amino acid screening shows increased ornithine and homocitrulline when the plasma ornithine concentration is above 400 μmol/l; ornithine methyl ester and γ-glutamylornithine are sometimes increased. At lower plasma ornithine concentrations, homocitrullinuria may be the only urine amino acid abnormality. Excessive homocitrulline excretion is known to occur in infants on canned formula due to transformation of lysine to homocitrulline during manufacture. Persistent homocitrullinuria without dietary source is abnormal. Increased urinary homocitrulline has also been detected in hyperlysinemia. Homocitrulline may escape detection when present in small quantities, since it coelutes with methionine in some amino acid analyzer buffer systems. However, its presence can be revealed by a pink color reaction with Ehrlich reagent (2% acidic *p*-dimethylaminobenzaldehyde in acetone) when used to overstain isatin- or ninhydrin-treated thin-layer or paper chromatogram. *Orotic aciduria* is common and increased excretion of orotic acid and orotidine can be induced by allopurinol challenge [23], as in patients with primary OTC deficiency (see Leonard, this volume).

The metabolic defect can best be confirmed by ^{14}C-L-ornithine incorporation assay using fibroblast monolayers [9]. Frozen tissue is not suitable for this study. The compartmentation of ornithine in HHH fibroblasts prevents the conversion of ornithine to proline and glutamate (Fig. 1) and results in very low incorporation of radioactivity into trichloroacetic acid (TCA)-precipitable macromolecules. This test is also abnormal in GA fibroblasts. These two hyperornithinemic disorders can be distinguished biochemically by direct measurement of OAT activity in cell extracts (normal in HHH, deficient in GA). The ^{14}C ornithine incorporation assay is a sensitive test that has been used for prenatal diagnosis of the HHH syndrome [21], but it is not useful for heterozygote identification.

Treatment and Prognosis

Treatment is aimed at preventing ammonia toxicity and follows the principles outlined for the urea cycle disorders (Leonard, this volume). Based upon the observation that *ornithine* given with protein loading prevented hyperammonemic response, several HHH patients were put on oral administration of ornithine in the range of 0.5–1.0 mmol/kg per day and were found to show lower blood ammonia levels and increased nitrogen tolerance. However, not all patients benefited from ornithine supplementation. A low dose of *arginine* and *citrulline* supplementation in combination with a low-protein diet has had similar effects [22, 28]. These supplements also corrected low creatine excretion in the two patients. In general, a low-protein diet has been effective in achieving biochemical control for most patients. This treatment results in improved growth and development, but has not prevented the development of spastic gait.

The reports of women with urea cycle disorders who developed hyperammonemic coma after child birth [29] suggest that women with HHH syndrome may also be at risk for such complications. It is thus advisable to exercise caution in the postpartem dietary management of HHH patients whose protein tolerance may be lower than during pregnancy. Offspring from both women and men with the HHH syndrome have been apparently normal.

Genetics

Inheritance of the HHH syndrome is autosomal recessive. The mode of clinical presentation and responses to treatment suggest heterogeneity among HHH patients. Obligate heterozygotes are clinically normal and cannot be identified by biochemical studies.

Hyperornithinemia Associated with Creatine Deficiency

A single case of a new disorder associated with hyperornithinemia has very recently been described [30].

Clinical Presentation

The patient was considered to be normal until 5 months of age, when he was noted to have developmental arrest. He gradually developed severe extrapyramidal movements, hypotonia, frequent vomiting and difficulties in handling secretions. His electroencephalogram showed very slow backgound activity and multifocal spike slow waves. Magnetic resonance imaging (MRI) revealed bilateral abnormalities of the globus pallidus as hypointensities in T1-weighted images and as hyperintensities in T2-weighted images.

Metabolic Derangement

The observation that administration of arginine to this patient raised the guanidinoacetate but not the creatine level in the brain suggests that the defect is in the final step of creatine biosynthesis, mediated by guanidinoacetate methyltransferase (Fig. 1). Confirmation of the enzyme defect is in progress.

Diagnostic Tests

Routine chemistry showed a marked reduction of both serum creatinine (7–10 μmol/L; normal, 25–100 μmol/L) and urine creatinine, and mild hyperammonemia. Amino acid analysis showed a two- to four-fold increase in plasma ornithine and a low plasma arginine but no detectable urine homocitrulline. Urinary organic acid analysis showed some nonspecific increases in dicarboxylic acids, 3-methylglutaconic acid, and ethylmalonic acid. The values of these organic acids might have been exaggerated because they were expressed in reference to urine creatinine.

Proton and phosphorus magnetic resonance spectroscopy confirmed a complete deficiency of creatine and creatine phosphate in gray and white matter, basal ganglia, and cerebellum. In addition, a new resonance normally not found in the brain was detected and identified as guanidinoacetate. This metabolite is an intermediary in the biosynthetic pathway of creatine from arginine (Fig. 1).

Treatment and Prognosis

After 12 weeks administration of creatine monohydrate at 400 mg/kg per day, the patient showed significant clinical improvement of muscle tone and extrapyramidal symptoms. He became more alert and gained motor skills. His electroencephalogram (EEG), MRI, and metabolite

patterns all improved. Creatine (and creatine phosphate) in gray and white matter increased to almost 50% of its normal concentration, guanidinoacetate normalized, and guanidinoacetate phosphate disappeared.

Genetics

Only a single case has been reported.

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Nonketotic Hyperglycinemia

K. Tada

CONTENTS

Hyperglycinemia represents a group of disorders characterized by elevated concentrations of glycine in body fluids. Two types exist, the nonketotic and the ketotic type [1]. Nonketotic hyperglycinemia (NKH) is a disorder of glycine degradation due to a primary defect in the glycine cleavage system (GCS). NKH is a relatively frequent metabolic cause of overwhelming illness in infancy. In the ketotic type, the most striking feature is ketoacidosis, which begins early in life and in which hyperglycinemia is secondarily associated with organic acidemia [1].

Clinical Presentation

NKH is usually classified into two types from a clinical point of view: neonatal type and late-onset type.

The neonatal type is the common type. Most affected infants appear normal at birth. After a short interval, seldom longer than 48 h, the patient develops rapidly progressing neurological symptoms such as muscular hypotonia, depressed Moro response, seizures, apneic attacks, and lethargy or coma. Most patients die within a few weeks, the survivors showing severe psychomotor retardation. Convulsive seizures range from myoclonic seizures to grand-mal convulsions. Hiccupping is often seen. During the first few weeks of life, a characteristic electroencephalogram (EEG) pattern is seen with bursts of high complex waves of 1–3 s, arising periodically from a hypoactive background (Fig. 1). This so-called burst–suppression pattern disappears at the end of the first month and changes to hypsarrhythmia. Muscular hypotonia is prominent in the neonatal period, but thereafter spasticity develops gradually.

In the late-onset type the patient has no abnormal symptoms or signs in the neonatal period, and thereafter neurological symptoms develop in variable degrees. Onset ranges from infancy to adolesence. In our experience of 30 cases, 26 (87%) were of the neonatal type. Among them, 22 died between 6 days and 5 years of life. The remaining four patients survived, but were severely retarded [2].

Metabolic Derangement

The defect of NKH is in the GCS, which catalyzes the transformation of glycine and tetrahydrofolate into CO_2, NH_3, and methylene-tetrahydrofolate (Fig. 2) [3]. GCS is a multienzyme complex, which is composed of four protein components: P-protein (a pyridoxal phosphate-dependent glycine decarboxylase), H-protein (a lipoic acid-containing protein), T-protein (a tetrahydrofolate-requiring enzyme), and L-protein (lipoamide dehydrogenase) [4]. The overall activity of GCS in liver from 30 patients was not detectable or extremely low in the neonatal type, whereas in the late-onset type some residual activity was seen [2, 5]. Thus, the clinical phenotypes do seem to relate with the degree of the defect in the GCS.

The glycine content of the brain is elevated in NKH in contrast to that in ketotic hyperglycinemia [1, 6]. Consistent with this, the activity of GCS in the brain is undetectable in NKH and normal in ketotic hyperglycinemia [1]. The majority of the NKH patients (26 out of 30, 87%) appeared to have a specific defect in P-protein and the remaining a specific defect in T-protein. The component analysis was also made in the brain from seven autopsied cases. The sites of the defect in these cases were identical in both the

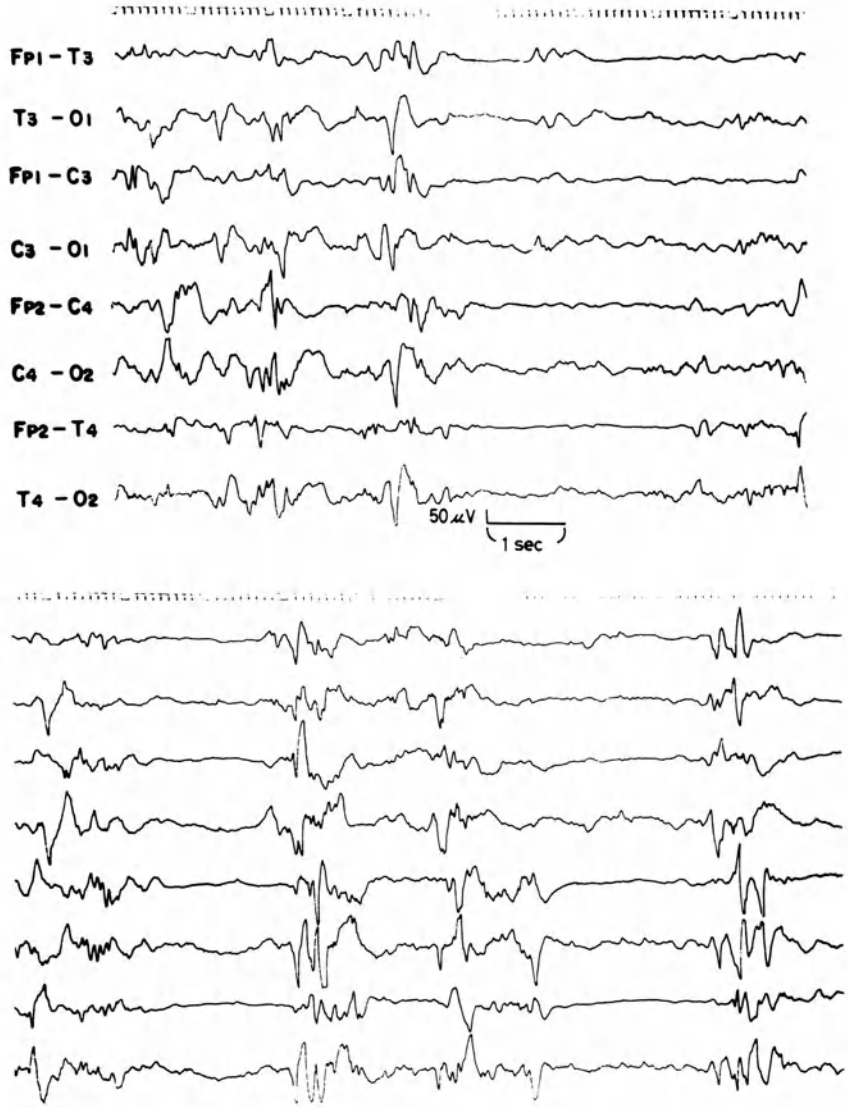

Fig. 1. Electroencephalogram (EEG) of a 6-week-old patient with nonketotic hyperglycinemia showing a "burst–suppression" pattern

brain and liver. This suggests that the GCS proteins in liver and brain are controlled by the same gene. With respect to *secondary ketotic hyperglycinemia*, GCS activity was normal in liver obtained at biopsy from a patient with propionic acidemia which was under adequate dietary treatment, with a normal plasma glycine level [5, 7]. In contrast, GCS activity was significantly reduced in livers from patients with propionic acidemia or methylmalonic acidemia who died in a hyperglycinemic state. This suggests that in ketotic hyperglycinemia, the elevation of glycine occurs as a result of a secondary suppression of GCS, by the excess of some organic acids or their coenzyme A derivatives [8].

Recently, several observations have provided a new insight into the relationship of glycine and excitotoxicity of the brain. Whereas glycine has been so far considered to act as an inhibitory neurotransmitter at a strychnine-sensitive receptor, glycine has also been found to have an excitatory property which potentiates the glutaminergic *N*-methyl-D-aspartate (NMDA) receptors [9]. It is suggested that glycine enhances NMDA-mediated responses at a site closely associated with the NMDA receptor. Glycine administration enhanced NMDA-induced seizures in mice whose classic glycine receptor had been blocked with strychnine [10]. Furthermore, it was shown that the developing brain has heightened susceptibility

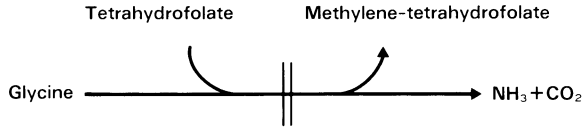

Fig. 2. The site of metabolic defect in nonketotic hyperglycinemia: glycine cleavage system

to NMDA-mediated brain injury, and high levels of glycine may be particularly devastating to the central nervous system of the neonate [11]. Also, the sites of GCS were concurrent with the region rich in NMDA receptors in rat brain by immunohistochemical study [12]. Thus, the elevated concentrations of glycine in the brain may contribute to the pathophysiology of NKH by overstimulating NMDA receptors via an action at the associated glycine modulatory site.

Diagnostic Tests

When infants develop seizures, muscular hypotonia, and somnolence or lethargy, and these symptoms can not readily be explained by infection, trauma, hypoxia, or other commonly encountered pediatric problems, NKH should be considered and plasma amino acids analyzed. Differentiation from ketotic hyperglycinemia is sometimes not easy. Absence of ketoacidosis, as reflected by normal plasma bicarbonate levels, normal arterial, or capillary blood pH, and exclusion of organic acidemia by gas chromatographic analyses of urine or plasma are crucial. In NKH the glycine level in CSF is elevated, and the ratio of CSF to plasma glycine concentration is above 0.09, whereas under normal circumstances and in ketotic hyperglycinemia it is below 0.04 [1].

As GCS is expressed in liver, kidney, and brain, liver biopsy is performed for the *enzymatic diagnosis* of NKH. However, a new method has recently been developed using peripheral blood. It is based on the fact that GCS is induced in B lymphocytes by infection and transformation using Epstein-Barr virus (EBV) [13]. This method is useful for differential diagnosis between NKH and ketotic hyperglycinemia and for carrier detection of NKH.

Prenatal Diagnosis. There is a strong demand for it, since no effective treatment is available for NKH. Cultured amniotic cells do not have GCS activity and are, therefore, not useful. However, we found the existence of GCS in chorionic villi of placenta and suggested prenatal diagnosis of NKH by chorionic villi sampling [14]. Accordingly, 20 pregnant women who had children with NKH were investigated at the eighth–16th week of gestation [15]. In 15 out of 20 cases, GCS activity was found to be normal. Their pregnancies were continued and healthy babies were born after full term pregnancies. In the remaining five cases, GCS activity was undetectable, suggesting that the fetus was affected with NKH. The pregnancies were terminated by the parents' desire. The GCS activities in the liver and brain from the aborted fetuses were nearly undetectable. DNA diagnosis, too, is possible when the mutation of the family is known [16].

Treatment and Prognosis

To lower the glycine concentration in NKH patients, many therapeutic approaches have been attempted, including protein restriction, a synthetic diet devoid of glycine and its precursor serine, promotion of renal clearance by benzoate, administration of ursodeoxycholic acid which conjugates with glycine and is excreted in bile, and exchange transfusion. These treatments were effective for lowering plasma levels, but not CSF levels of glycine, and did not alter appreciably the clinical course of the disease. Tentative treatment with strychnine (see under "Metabolic Derangement") [17], diazepam, a competitor of glycine receptors, or one-carbon donors, such as methionine, leucovorin, choline, or formate, did not or only ambiguously improve the clinical symptoms.

Recently a tentative treatment using NMDA antagonists, in keeping with the excitotoxicity hypothesis by glycine, has been tried [18]. Oral administration (8 mg/kg per day, in four divided doses) of ketamine, a NMDA receptor antagonist, to a patient with NKH at 7 months of age brought a partial improvement of neurological symptons and EEG findings. Hamosh et al. [19] reported a 1-year experience with combined therapy with dextromethorphan, a blocker of NMDA channels and high-dose benzoate. Therapy with benzoate, 500 mg/kg per day, was started on day 5 of life and the dosage was increased to 750 mg/kg per day on day 8. Dextromethorphan (7.5 mg/kg per day, in three divided doses) was added to the regimen on day 12. Physical examination and growth were normal at 12 months of age, although DQ lagged

behind at 60. These findings indicate a possibility that early treatment with NMDA receptor antagonist may prevent brain damage in NKH.

Genetics

NKH is transmitted as an autosomal recessive trait. The prevalence of NKH is not firmly known. In northern Finland it is estimated to be 1:12,000.

cDNAs encoding human P-protein and T-protein of GCS were cloned by our group [2, 16, 20–22] and point as well as frame shift mutations, due to one- or three-base deletions [16, 20], have been identified. The majority of NKH patients in Finland, was found to carry a common G→T substitution, resulting in a serine to isoleucine change.

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Part V
Peptide Metabolism

Disorders of the Gamma Glutamyl Cycle

A. Larsson

CONTENTS

The gamma glutamyl cycle involves six enzymes (Fig. 1). The biosynthesis of glutathione is catalyzed by gamma glutamyl cysteine synthetase and glutathione synthetase. The breakdown of the tripeptide is mediated by four enzymes. The initial step is catalyzed by gamma glutamyl transpeptidase. The gamma glutamyl residue is then released as 5-oxoproline, which is converted to glutamate by 5-oxoprolinase. The biosynthesis of glutathione is feedback regulated, i.e., glutathione acts as an inhibitor to gamma glutamyl cysteine synthetase.

Glutathione has been postulated to participate in several fundamental functions, such as free radical scavenging, redox reactions, formation of deoxyribonucleotides, xenobiotic metabolism, and amino acid transport [1]. Patients with genetic defects in the metabolism of glutathione are therefore likely to exhibit a variety of symptoms.

In diagnostic work it is essential to remember that erythrocytes contain an incomplete gamma glutamyl cycle; they lack both gamma glutamyl transpeptidase and 5-oxoprolinase.

Of all disorders of the gamma glutamyl cycle, glutathione synthetase deficiency occurs most frequently and is therefore discussed first. The other enzyme defects, which are much rarer, are discussed subsequently.

Glutathione Synthetase Deficiency

Two forms can be distinguished [2]. One is generalized, and the other is expressed only in erythrocytes. The generalized form is due to mutations affecting the catalytic properties of the enzyme, the erythrocyte form to a mutation which affects the stability of the enzyme.

Generalized Glutathione Synthetase Deficiency

Clinical Presentation

Generalized deficiency of glutathione synthetase has been recognized in approximately 30 patients. The clinical condition is variable and presumably correlated to the extent of the enzyme defect. Most patients show symptoms within the first few days of life: metabolic acidosis, jaundice, and hemolytic anemia. After the neonatal period the condition is usually stabilized. During episodes of gastroenteritis and other infections, however, the patients may become critically ill due to pronounced acidosis and electrolyte imbalance. Several patients have died during such episodes. The majority of the patients has progressive central nervous system (CNS) damage, including mental retardation, ataxia, spasticity, and seizures. One patient died at the age of 28 years and autopsy revealed atrophy of the granule cell layer of the cerebellum as well as focal lesions of the cortex. Two patients had increased susceptibility to bacterial infections due to defective granulocyte function.

Metabolic Derangement

As a result of the enzyme defect, glutathione concentrations in erythrocytes and other tissues are very low, while gamma glutamyl cysteine, the substrate before the enzyme defect, is produced in

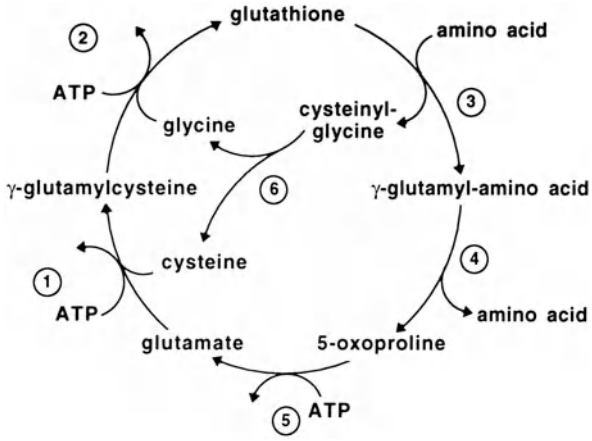

Fig. 1. The gamma glutamyl cycle. *1*, Gamma glutamylcysteine synthetase; *2*, glutathione synthetase; *3*, gamma glutamyltranspeptidase; *4*, gamma glutamylcyclotransferase; *5*, 5-oxoprolinase; *6*, dipeptidase; *ATP*, adenosine triphosphate

excess due to a lack of feedback inhibition of gamma glutamyl cysteine synthetase. Gamma glutamyl cysteine is converted by gamma glutamyl cyclotransferase into *5-oxoproline* and cysteine. 5-Oxoproline is transferred to glutamate by 5-oxoprolinase, which is the rate-limiting enzyme of the gamma glutamyl cycle in many tissues. The excessive formation of 5-oxoproline exceeds the capacity of 5-oxoprolinase. Therefore, 5-oxoproline accumulates in the body fluids, causing metabolic acidosis and 5-oxoprolinuria. Up to 30 g 5-oxoproline is excreted daily.

Diagnostic Tests

The diagnosis is usually established in a newborn infant with severe metabolic acidosis. In the urine, massive excretion of L-5-oxoproline (up to 1 g/kg body weight per day) can be demonstrated by gas liquid chromatography. Note that 5-oxoproline is ninhydrine negative. The distinction between the D and L forms can be made after acid hydrolysis and analysis of L-glutamate by L-glutamic acid dehydrogenase. Decreased activity of glutathione synthetase can be demonstrated in, for instance, erythrocytes, leukocytes, or cultured skin fibroblasts.

Treatment and Prognosis

This involves acidosis correction, using parenteral administration of sodium bicarbonate initially and oral maintenance doses of sodium bicarbonate or citrate (up to 10 mmol/kg body weight per day). During episodes of acute infections, higher doses may be required. Vitamin E (alpha-tocopherol) has been shown to correct the defective granulocyte function. Therefore, vitamin E should be given in doses of about 10 mg/kg body weight per day. Recently, treatment with ascorbate (100 mg/kg body weight per day) has been postulated to be of benefit. Drugs which precipitate hemolytic crises in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency should be avoided to prevent such crises. Therapeutic trials have been made in order to substitute for the lack of glutathione. Oral administration of glutathione, mercaptopropionylglycine, and N-acetylcysteine have been tested. The effect has mainly been monitored by cellular glutathione levels and excretion of 5-oxoproline. None of these trials has been successful. Dietary manipulations – including adjustment of the protein intake – have not affected the excretion of 5-oxoproline.

The prognosis of the patients depends on the measures taken during acute episodes. Especially during the neonatal period it is essential to correct the metabolic acidosis and electrolyte imbalance, treat anemia, and prevent excessive hyperbilirubinemia. Central nervous system damage is progressive and cannot yet be prevented. It is, however, essential to remember that generalized glutathione synthetase deficiency is a heterogeneous condition, and it is difficult to predict the outcome for individual patients.

Genetics

The defective gene is transmitted by autosomal recessive inheritance.

Erythrocyte Glutathione Synthetase Deficiency

Several families with hereditary erythrocyte glutathione synthetase deficiency have been reported. The characteristic symptom is mild hemolytic anemia. Some patients had splenomegaly. No other clinical symptoms have been reported. Glutathione concentration and glutathione synthetase activity in the erythrocytes were decreased. Urinary levels of 5-oxoproline were normal. No treatment has been proposed. It seems reasonable, however, that the patients

should avoid those drugs which cause hemolytic crises in patients with G6PD deficiency.

The mode of inheritance seems to be autosomal recessive.

Gamma Glutamyl Transpeptidase Deficiency

Five patients with gamma glutamyl transpeptidase deficiency have been reported or are under investigation.

Three of them have CNS involvement, though two siblings have apparently no signs of CNS damage. This may reflect the fact that the first three patients were identified by screening for amino acid defects in populations of mentally retarded patients.

The patients have increased glutathione concentrations in plasma and urine, but the cellular levels are normal. In addition to glutathionuria, urinary levels of gamma glutamyl cysteine and cysteine are also increased.

The patients are often identified by urinary screening for amino acid disorders using thin layer or paper chromatography and ninhydrine detection. This reveals glutathionuria (up to 1 g per day). Decreased activity of gamma glutamyl transpeptidase can be demonstrated in leukocytes or cultured skin fibroblasts, but not in erythrocytes, which lack this enzyme under normal conditions.

No specific treatment has been postulated. The prognosis must be considered as serious if the patient presents with psychiatric or neurologic symptoms [3]. On the other hand, two siblings aged 11 and 13 years so far have no signs of CNS involvement.

Gamma glutamyl transpeptidase deficiency is transmitted by autosomal recessive inheritance.

5-Oxoprolinase Deficiency

Five patients with hereditary defects in 5-oxoprolinase have been described. The clinical symptoms which led to the discovery of the presently known patients are not necessarily related to their metabolic defect. Two brothers were investigated because of renal stone formation [4]. They also had chronic enterocolitis, but no signs of hemolytic anemia (except after salazosulfapyridine in one patient) or CNS damage. The third patient was a woman with mild mental retardation who had given birth to children with congenital malformations. Two brothers with 5-oxoprolinase deficiency were recently reported. One had neonatal hypoglycemia, whereas the other had not shown any apparent symptom of his metabolic disorder. The patients were identified because of 5-oxoprolinuria, excreting 4–10 g L-5-oxoproline per day. They had normal cellular levels of glutathione and normal acid-base balance. 5-Oxoprolinase is not present in erythrocytes and therefore leukocytes or other tissues must be used for final diagnosis. No specific treatment has been proposed, and prognosis remains to be established.

The mode of inheritance is autosomal recessive.

Gamma Glutamyl Cysteine Synthetase Deficiency

Two siblings have been reported with gamma glutamyl cysteine synthetase deficiency [5]. They had mild hemolytic anemia. Cerebellar involvement, peripheral neuropathy, and myopathy developed subsequently. Both patients had generalized aminoaciduria, but no other renal function defect. Treatment with sulfonamide precipitated psychosis and pronounced hemolytic anemia.

Gamma glutamyl cysteine synthetase activity was low in erythrocytes, as were the levels of glutathione in erythrocytes, leukocytes, and skeletal muscle. In one adult patient hemolytic anemia and modest decreases in the amount of glutathione in cultured lymphoblasts and fibroblasts were the only abnormalities [6]. Apparently, the clinical symptoms are not necessarily related to the metabolic defect.

The diagnosis is established by analysis of the relevant enzyme in erythrocytes or other tissues.

The prognosis, treatment, and genetics in gamma glutamyl cysteine synthetase deficiency remain to be established.

5-Oxoprolinuria Without an Inborn Error of the Gamma Glutamyl Cycle

5-Oxoprolinuria has been described in conditions other than generalized glutathione synthetase deficiency and 5-oxoprolinase deficiency. Patients with severe burns or Stevens-Johnson syndrome and infants fed formula based on acid-hydrolyzed protein have been found to excrete increased

amounts of 5-oxoproline, usually in the range of a few milligrams per day. Patients with homocystinuria have increased excretion of 5-oxoproline (up to 1 g per day). Likewise, patients suffering from metabolic crises due to urea cycle defects, e.g., ornithine carbamyltransferase deficiency, have been found to excrete a few grams of 5-oxoproline. This seems to occur as a consequence of lack of adenosine triphosphate (ATP) in critical organs such as liver and kidney.

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Disorders of Small Peptides

J. Jaeken

CONTENTS

This chapter deals with disorders in the catabolism of the dipeptides carnosine (carnosinemia) and homocarnosine (homocarnosinosis) and of the imidodipeptides (imidodipeptiduria, prolidase or peptidase D deficiency). Diseases due to defects in the metabolism of the tripeptide glutathione are discussed in Larsson (this volume).

Carnosinemia

Clinical Presentation

Some 30 individuals have been reported with this disorder, first described in 1967 [1]. The majority of them showed mental retardation to a variable degree. Some patients had seizures and one congenital myopathy. A few had no symptoms at all, making the relationship between the biochemical abnormalities and the clinical picture uncertain [2].

Metabolic Derangement

Carnosine is a dipeptide consisting of histidine and β-alanine and is present in high concentrations in skeletal muscle. On a meat-free diet, it is increased in serum and urine of carnosinemic persons. *Carnosinase* activity is reduced in liver, kidney, and serum. Several variants have been described with abnormal kinetic properties of the enzyme. In cerebrospinal fluid (CSF) of patients *homocarnosine* was also increased.

Diagnostic Tests

The diagnosis is made by quantitative amino acid analysis of serum and/or urine after exclusion of meat from the diet and is confirmed by measuring carnosinase activity in serum or tissues.

Treatment and Prognosis

No efficient treatment is available. In view of the above remarks it is uncertain whether treatment would be necessary. There is no reason to withhold meat from the diet because the accumulating carnosine is primarily endogenous. Prognosis is variable and does not seem to correlate with the degree of enzyme deficiency.

Genetics

Inheritance is autosomal recessive.

Homocarnosinosis

Clinical Presentation

This condition was described in 1976 in a Norwegian family (three of four siblings and their

mother) [3]. The three offspring showed progressive spastic diplegia, mental retardation, and retinitis pigmentosa, with onset between 6 and 29 years of age. The mother, on the other hand, was symptom free. As in carnosinemia, this makes it uncertain whether there is a relationship between the biochemical defect and the clinical symptoms.

Metabolic Derangement

Homocarnosine is a dipeptide consisting of histidine and GABA; it is only found in brain and CSF. In the CSF of the three siblings as well as in that of their clinically normal mother, homocarnosine was about 20 times the mean of control levels. Deficiency of homocarnosinase activity was found in brain and deficiency of carnosinase activity in serum [4]. This raises the question whether homocarnosinosis and carnosinemia could be the same (or a similar) disorder.

Diagnostic Tests

The diagnosis is made by quantitative amino acid analysis of the CSF.

Treatment and Prognosis

The same remarks apply to this disorder as for carnosinemia.

Genetics

Inheritance in the Norwegian family seems to be autosomal dominant.

Prolidase Deficiency

Clinical Presentation

At least 30 individuals with prolidase deficiency have been reported since 1968 [5]. About a quarter of them were asymptomatic at the time of the report. The others had their first symptoms between birth and 22 years. All patients showed skin lesions, either mild (face, palms, soles) or severe, and recalcitrant ulceration particularly on the lower legs. A majority of patients exhibited impaired motor or cognitive development and an increased frequency of infections.

Metabolic Derangement

The hallmark biochemical finding is massive hyperexcretion of a large number of imidodipeptides (dipeptides with a N-terminal proline or hydroxyproline, particularly glycylproline). This is due to a deficiency of the exopeptidase prolidase (or peptidase D).

Diagnostic Tests

The hyperimidodipeptiduria can be detected and quantified by partition and elution chromatography and by direct chemical ionization mass spectrometry. The finding of low or absent prolidase activity in hemolysates or in homogenates of leukocytes or fibroblasts confirms the diagnosis.

Treatment and Prognosis

Due to the rarity of the disease, experience with treatment is scarce. The skin ulcers improved with oral ascorbate, manganese (cofactor of prolidase), and an inhibitor of collagenase in one patient and with local applications of L-proline- and glycine-containing ointments in another patient. Skin grafts have been unsuccessful [6, 7].

As to prognosis, age of onset and severity of clinical expression are unpredictable.

Genetics

Inheritance is autosomal recessive. The prolidase locus has been assigned to the proximal long arm of chromosome 19.

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Part VI
Organic Acids

Branched-Chain Organic Acidurias

H. Ogier de Baulny, U. Wendel, and J.-M. Saudubray

CONTENTS

Branched chain organic acidurias are a group of disorders that result from an inherited abnormality of specific enzymes mainly involving the catabolism of branched-chain amino acids (BCAA). Collectively, maple syrup urine disease (MSUD), isovaleric aciduria (IVA), 3-methylcrotonylglycinuria (3-MCG), propionic aciduria (PA), and methylmalonic aciduria (MMA) represent the most commonly encountered abnormal organic acidurias. Beside these disorders, 3-methylglutaconic aciduria and 3-hydroxyisobutyric aciduria due to leucine and valine catabolism defects, respectively, are rare diseases without any effective treatment (Fig. 1).

Clinical Presentation

Children with MSUD, IVA, 3-MCG, PA, and MMA have many clinical and biochemical symptoms in common. They can be divided into three schematic presentations:

- A severe neonatal-onset form with metabolic distress
- A chronic, intermittent, late-onset form
- A chronic, progressive form presenting as hypotonia, failure to thrive, and developmental delay.

In addition, prospective data gathered by some newborn screening programs and the systematic screening of siblings have demonstrated the relative frequency of asymptomatic forms [1].

Severe Neonatal-Onset Form. The general presentation of this form can be summarized as a neurologic distress of the intoxication type with either ketosis or ketoacidosis; it belongs to type I or II in the classification of the neonatal inborn errors of metabolism (see the chapter by Saudubray et al. on the "Clinical Approach to Inherited Metabolic Diseases"). An extremely evocative clinical setting is that of a full-term baby born after a normal pregnancy and delivery who, after an initial symptom-free period, undergoes relentless deterioration which has no apparent cause and which is unresponsive to symptomatic therapy. The interval between birth and clinical symptoms may range from hours to weeks, depending on the nature of the defect and is not necessarily linked to the protein content of the feeding. Typically, the first reported signs are poor feeding and drowsiness, after which the newborn sinks into an unexplained progressive coma. At a more advanced stage, neurovegetative dysregulation with respiratory distress, hiccups, apneas, bradycardia, and hypothermia may appear. In the comatose state, most patients have characteristic changes in muscle tone and involuntary movements. Generalized hypertonic episodes with opisthotonus, boxing, or pedaling movements as well as slow limb elevations, spontaneously or upon stimulation, are frequently observed. Another pattern is that of axial hypotonia and limb hypertonia with large-amplitude tremors and myoclonic jerks, which are often mistaken for convulsions. In contrast, true convulsions occur late and inconsistently. The electroencephalogram (EEG) may show a burst–supression pattern [1].

The newborn with MSUD may display cerebral edema with bulging fontanel, arousing suspicion of central nervous system (CNS) infection. Concomitantly with the onset of the symptoms, he or she emits an intensive (sweety, malty, caramel-like) maple syrup-like odor. Neonatal MSUD does not display pronounced dehydration or metabolic acidosis, and the main laboratory ab-

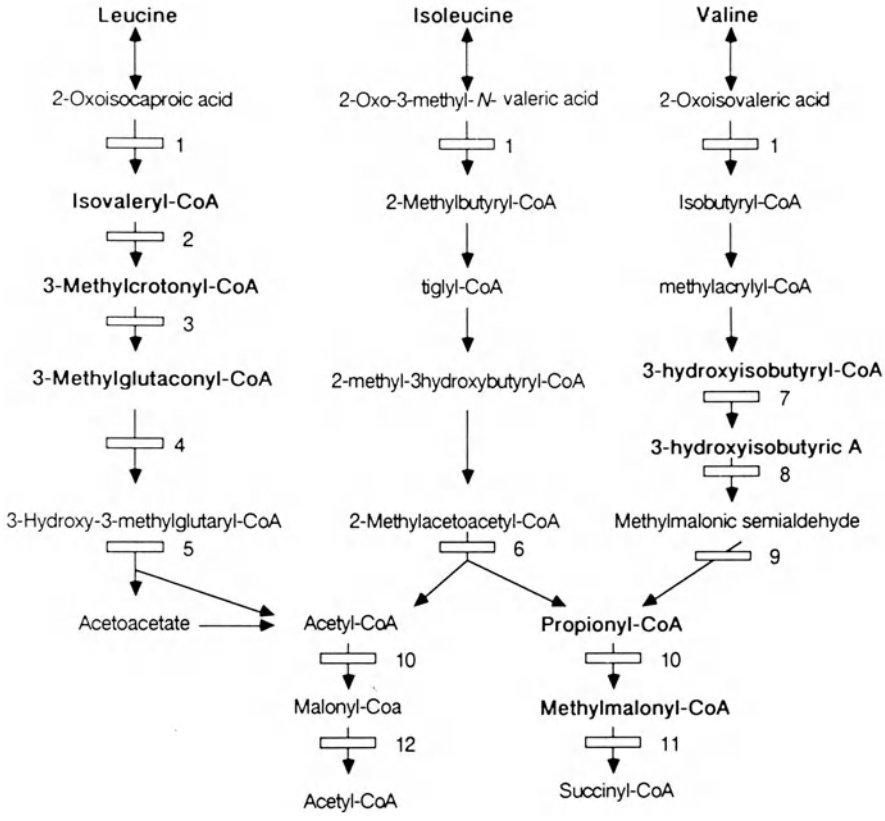

Fig. 1. Metabolism of branched-chain amino acids, showing the position of the known metabolic disorders. *1*, Branched-chain oxo acid dehydrogenase; *2*, isovaleryl-CoA dehydrogenase; *3*, 3-methyl-crotonyl-CoA carboxylase; *4*; 3-methylglutaconyl-CoA hydratase; *5*, 3-hydroxy-3-methylglutaryl-CoA lyase; *6*, 2-methylacetoacetyl-CoA thiolase; *7*, 3-hydroxyisobutyryl-CoA deacylase; *8*, 3-hydroxyisobutyric dehydrogenase; *9*, methylmalonyl-semialdehyde dehydrogenase; *10*, propionyl-CoA carboxylase; *11*, methylmalonyl-CoA mutase; *12*, malonyl-CoA decarboxylase

normality in urine is the presence of 2-oxo acids detected with the 2,4-dinitrophenylhydrazine (DNPH test). In contrast, dehydration is a frequent finding in patients with PA and MMA. Moderate hepatomegaly may be observed in all conditions. In some cases, the combination of vomiting, abdominal distension, and constipation may suggest gastrointestinal obstruction. In IVA, an unpleasant "sweaty feet" odor of urine and skin is constantly present. Generally, in organic acid disorders metabolic acidosis (pH $<$ 7.30) with increased anion gap and associated ketonuria (Acetest 2–3+) is observed. However, ketoacidosis can be moderate and transient and is often responsive to symptomatic therapy. *Hyperammonemia* is a constant finding in IVA, 3-MCG, PA, and MMA. When it is very high ($>$800 μmol/l), it can induce respiratory alkalosis and lead to the erroneous diagnosis of an urea cycle disorder. Moderate hypocalcemia ($<$1.7 mmol/l) and hyperlactacidemia (3–6 mmol/l) are frequent symptoms. The physician should be wary of attributing marked neurologic dysfunction merely to these findings. Blood glucose can be reduced or elevated and can even reach 20 mmol/l or more. If associated with glycosuria, ketoacidosis, and dehydration, it may mimic neonatal diabetes [2]. Neutropenia, thrombocytopenia, nonregenerative anemia, and pancytopenia are findings that can be confused with sepsis.

Intermittent Late-Onset Form. In approximately one third of the patients, the disease presents with late onset after a symptom-free period commonly longer than 1 year or even in adolescence or adulthood. Recurrent attacks are frequent and in between the child may seem entirely normal. Onset of an acute attack may arise during catabolic stress, such as in infections or following increased intake of protein-rich foods, but sometimes without overt cause. Recurrent attacks of coma, or

lethargy with ataxia are the main presentation of these late-onset acute forms. The most frequent variety of coma is that presenting with *ketoacidosis*. Exceptionally, ketosis may be absent. Hypoglycemia is frequent in patients with MSUD and 3-MCG, while in other disorders blood glucose levels are low, normal, or high. Hyperammonemia is frequently encountered during recurrent episodes of 3-MCG, while in other organic acidurias it is inconstant and less severe than during the neonatal period. Unlike patients with PA and MMA, patients with IVA are mostly normoglycinemic.

Although most recurrent comas are not accompanied by focal neurologic signs, MSUD, PA, and MMA patients may present with acute hemiplegia, hemianopsia, or symptoms and signs of cerebral edema mimicking cerebrovascular accident or cerebral tumor [3]. These disorders must therefore be considered in the diagnostic list of metabolic strokes after respiratory chain disorders and urea cycle defects.

Some patients, especially those affected with 3-MCG, may present with a Reye syndrome-like illness characterized by onset of coma, cerebral edema, hepatomegaly, liver dysfunction, hyperammonemia, and even macro- or microvesicular fatty infiltration of the liver. These observations stress the importance of complete metabolic investigations in such situations [4, 5].

The initial manifestations mentioned have frequently been preceded by other premonitory symptoms which had been missed or misdiagnosed. They include acute ataxia, unexplained episodes of dehydration, persistent and selective anorexia, chronic vomiting with failure to thrive, hypotonia, and progressive developmental delay.

Severe *hematologic manifestations* are frequent, mostly concomitant with ketoacidosis and coma, sometimes as the presenting problem. Neutropenia is regularly observed in both neonatal- and late-onset forms of IVA, PA, and MMA. Thrombocytopenia occurs only in infancy and anemia only in the neonatal period. Neutropenia and thrombocytopenia appear to be the major contributing factors to the cause of death in uncontrolled IVA [5].

Chronic, Progressive Forms. Persistent anorexia, chronic vomiting, failure to thrive, and osteoporosis are frequent revealing signs. This presentation is easily misdiagnosed as gastroesophageal reflux, cow's milk protein intolerance, coeliac disease, late-onset chronic pyloric stenosis, or fructose intolerance, particularly as these symptoms start after weaning and diversifying food intake [6].

Some patients present with severe hypotonia, muscular weakness, and poor muscle mass and can simulate congenital neurologic disorders or myopathies [5, 7]. Nonspecific developmental delay and progressive psychomotor retardation or dementia as well as seizures and movement disorders can also be observed during the course of the disease. However, these rather nonspecific symptoms are rarely the only presenting symptom [8].

Recurrent infections are common, and there appears to be a special relationship with *Candida*. The infections in these children may be related to their poor nutritional status [9].

Complications

Neurologic Complications. Cerebral edema and its sequelae are well-recognized complications of untreated MSUD in the newborn period [10]. Later, during acute metabolic decompensation, cerebral edema and brain stem compression may cause unexpected death in the hours following intemperate rehydration [3, 11]. Increased intracranial pressure may also develop slowly due to longstanding elevations of BCAA [12]. Additionally, chronic dysmyelination proportional to the average plasma leucine values have been found by computed tomography (CT) and magnetic resonance (MR) brain imaging in patients on a relaxed treatment. These changes mainly involve the periventricular areas, the deep cerebellar white matter, the dorsal part of the brain stem, the cerebral pedoncles, the dorsal limb of the internal capsule, and basal ganglia [10, 11, 13].

Symmetrical necrosis of the globus pallidus is increasingly recognized in patients with MMA. In most cases, these lesions, with corresponding extrapyramidal signs, result from acute stroke-like events [14]. In other cases, progressive symptoms over several years appear to be the result of long-term poor metabolic control [15]. In patients with PA and MMA, spongiosis in the globus pallidus and internal capsules have already been observed on neuroradiological examinations or in autopsied cases [16]. For both MSUD and organic acidurias, these dramatic complications are arguments to

aim at adequate lifelong dietary control, even if the patient is free of symptoms.

Renal Complications. Chronic renal impairment is increasingly recognized in patients with MMA [17]. The renal lesion is a tubulointerstitial nephritis with type 4 tubular acidosis and adaptative changes secondary to the reduced glomerular filtration rate [18]. Renal function varies with the severity of the disease. In the subacute form of the disorder, renal failure may be a presenting symptom which improves with metabolic control. If this nephritis is the complication of a chronic glomerular hyperfiltration secondary to excessive MMA excretion, prevention of renal injury requires strict metabolic control.

Skin Disorders. Frequently, large superficial desquamation and alopecia may develop in the course of late and severe PA or MMA decompensations. More specifically, among our patients two with MMA and two with PA developed a staphylococcal scalded skin syndrome with epidermolysis leading to death despite supportive measures. This complication, potentially due to nutrient or essential amino acid deficiency, has been recently described as acrodermatitis enteropathica-like syndrome [19].

Pancreatitis. Pancreatitis has been described in patients affected with either MSUD or various organic acidurias. It has been the revealing symptom in two patients with the late-onset form of IVA. In other cases, pancreatic involvement in the course of acute episodes has been associated with hyperglycemia and hypocalcemia. The increase of serum lipase and amylase is variable. Enlargement and even calcification or hemorrhage of the gland can be visualized by abdominal ultrasonography [20].

Cardiomyopathy. Acute cardiac failure, due to cardiomyopathy, may be responsible for rapid deterioration or death in MMA and PA. This complication may develop as part of the presenting illness or occur during an intercurrent metabolic decompensation [21]. The pathogenesis is unclear. Diverse factors may play additional roles, such as carnitine or micronutrient deficiencies or intercurrent viral infections. Acute energy deprivation due to the propionyl-CoA oxidation defect is another possibility.

Metabolic Derangement

The three essential and chemically similar BCAA L-leucine, L-isoleucine, and L-valine are unique among the amino acids in that they undergo oxidation to a greater extent in the peripheral tissues than in the liver. They are metablized initially by a common pathway, transamination followed by oxidative decarboxylation (Fig. 1). Subsequent to decarboxylation, the degradative pathways of BCAA diverge. Leucine is further metabolized to acetoacetate and acetylcoenzyme A (acetyl-CoA), which enters the Krebs cycle. Specific enzyme deficiencies at every stage of this metabolic pathway are known. In contrast, only a few disorders of L-isoleucine and L-valine metabolism have been identified. Nevertheless, they are of particular interest, since both amino acids are major precursors of propionyl-CoA and methylmalonyl-CoA, which enter the Krebs cycle via succinyl-CoA.

BCAA Common Pathway. Transamination is reversible and converts amino acids to their 2-oxo acid derivatives. Hypervalinemia and hyperleucine-isoleucinemia have been identified as disorders of BCAA transamination in extremely rare cases and are thus not described in detail. All three branched-chain 2-oxo acids are decarboxylated to the corresponding acyl-CoA derivatives by a single mitochondrial multienzyme complex called *branched-chain 2-oxo acid dehydrogenase* (BCODH). This complex has three catalytic components:

- A decarboxylase composed of E1α and E1β subunits in $\alpha_2\beta_2$ conformation
- A dihydrolipoyl acyltransferase (E2)
- A dihydrolipoamide dehydrogenase (E3)

Thiamine pyrophosphate is the coenzyme for the decarboxylation reaction. The reaction is regulated by phosphorylation and dephosphorylation of the E1α subunit by a kinase and a phosphatase. The E1 and E2 components are specific for BCODH, whereas E3 is identical to the dihydrolipoamide dehydrogenase associated with the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes. The BCODH activity is deficient in patients with MSUD with mutations affecting the E1 and E2 genes [22]. A deficiency of E3 produces a syndrome with features of congenital lactic acidosis, branched chain 2-oxoaciduria, and 2-oxoglutaric aciduria.

Maple Syrup Urine Disease. In MSUD, the impaired catabolic pathway produces marked increases in plasma, urinary, cerebrospinal fluid (CSF) branched-chain 2-oxo acids, and, due to reversible transamination, also in BCAA (Fig. 1, step 1). Smaller amounts of the respective 2-hydroxy acids, especially of 2-hydroxyisovaleric acid, are formed by reduction of the oxo acids. L-Alloisoleucine is invariably found in the blood of MSUD patients.

The leucine/2-oxoisocaproic acid pair appears to be the most toxic of the branched-chain metabolites. They are always present in plasma in about equimolar concentrations. Whereas high leucine/2-oxoisocaproate concentrations (>1 mmol/l plasma) may cause acute brain dysfunction, valine and isoleucine are of less clinical significance. Their 2-oxo acid to amino acid ratios favor the less toxic amino acids, and cerebral symptoms do not occur even when the blood levels of both amino acids are extremely high [23].

Leucine Metabolism. Three deficiencies will be discussed, namely IVA, 3-MCG, and 3-methylglutaconic aciduria.

Isovaleric Aciduria. Isovaleryl-CoA produced by the oxidative decarboxylation of leucine is metabolized by a specific mitochondrial flavin adenine dinucleotide electron-transferring flavoprotein (FAD-ETF) – dependent dehydrogenase to 3-methylcrotonyl-CoA (Fig. 1, step 2). A defect in this dehydrogenase activity may occur due to an apoenzyme mutation (IVA) or to FAD-ETF system dysfunction (glutaric aciduria type II).

IVA is caused by a defect in IV-CoA-DH, leading to an accumulation of IV-CoA and its byproducts. The disease is characterized by a greatly increased excretion in the urine of *N*-isovalerylglycine (IVG) and 3-hydroxyisovaleric acid, which are diagnostic. The concentration of free isovaleric acid is usually increased both in plasma and urine, although normal levels have been reported. The majority of the accumulating IV-CoA is conjugated with glycine to IVG by the action of glycine *N*-acylase, which shows a high affinity for IV-CoA. IVG thus formed is rapidly excreted in the urine. IV-CoA accumulation also favors isovalerylcarnitine (IVC) synthesis by carnitine *N*-acylase, which leads to high IVC urinary excretion [24]. These two alternate pathways are of therapeutic interest, since they allow the transformation of the highly neurotoxic isovaleric acid into nontoxic byproducts with a high renal clearance.

3-Methylcrotonylglycinuria. 3-Methylcrotonyl-CoA is carboxylated by a specific biotin-dependent acyl-CoA carboxylase (3-methylcrotonyl-CoA carboxylase, 3-MCC) to form 3-methylglutaconyl-CoA (Fig. 1, step 3). Deficient activity of this enzyme leads to 3-MCG. Variants are known that include *defects in biotin metabolism* which are responsible for *multiple carboxylase deficiency* (MCD). Isolated biotin-resistant 3-MCC deficiency is much less common than MCD. In contrast to the biotin-responsive MCD, the disorder is resistant to biotin supplementation in vivo as well as in vitro. Theoretically, an isolated biotin-responsive form of 3-MCC may exist. However, most of the initial reports have later been demonstrated to be MCD.

Secondary to the block, accumulated 3-methylcrotonyl-CoA is mainly metabolized to 3-hydroxyisovalerate (3-HIVA) by the action of an enoyl-hydratase with subsequent hydrolysis of the thioester. In addition, conjugation with glycine and direct hydrolysis of 3-methylcrotonyl-CoA form 3-methylcrotonylglycine and 3-methylcrotonic acid, respectively. Acylation with carnitine probably is operative as most patients have *secondary carnitine deficiency* with accumulation of short-chain acylcarnitine [25]. Theoretically, the diagnosis relies on a very characteristic profile of organic acids without any excretion of lactate, methylcitrate, or tiglylglycine. However, during acute decompensation a huge amount of 3-HIVA excretion, even in the absence of accumulated 3-methylcrotonylglycine, seems to be the best clue for diagnosis.

3-Methylglutaconic Aciduria. 3-Methylglutaconyl-CoA is metabolized to 3-hydroxy-3-methyl-glutaryl-CoA by 3-methylglutaconyl-CoA hydratase (Fig. 1, step 4). Defective activity leads to 3-methylglutaconic (3-MGC) aciduria type I characterized by urinary excretion of 3-methylglutaconic and 3-methylglutaric acids [26]. Both metabolites derive from accumulated 3-methylglutaconyl-CoA through hydrolysis and dehydrogenation, respectively. The metabolic pattern also includes 3-HIVA and normal amounts of 3-hydroxy-3-methylglutaric acids. However, there are many distinct phenotypes of 3-MGC aciduria with normal hydratase activity in tissues [27]. 3-MGC aciduria type II is X-linked and described in male

patients with cardiomyopathy, neutropenia, and growth retardation [28]. Type III is reported in Iraqi Jewish patients who showed early bilateral optic atrophy, pyramidal dysfunction, with later development of spasticity, ataxia, and cognitive deficit [29]. Finally, among a large heterogeneous group of patients suffering from a variable multisystemic disease (3-MGC aciduria type IV, unspecified), some respiratory chain disorders can be distinguished [30].

In patients with documented 3-methylglutaconyl-CoA hydratase deficiency, the combined urinary excretion of 3-MGC and 3-methylglutaric acids ranges from 500 to 1000 mmol/mol creatinine, of which 3-methylglutaric acid represents about 1%. Conversely, the urinary excretion of 3-MGC and 3-methylglutaric acid in patients with normal hydratase activity is much lower (10–200 mmol/mol creatinine) without elevation of 3-HIVA and 3-hydroxy-3-methylglutaric acids. This pattern is not significantly altered by leucine administration. Persistent excretion of ethylhydracrylic acid, lactate, and abnormal amounts of citric acid cycle intermediates could be a sign of respiratory chain dysfunction.

Valine Metabolism. Isobutyryl-CoA, the product of valine decarboxylation, is converted to 3-hydroxyisobutyryl-CoA via two enzymatic steps. A specific deacylase hydrolyzes 3-hydroxyisobutyryl-CoA to 3-hydroxyisobutyric acid (Fig. 1, step 7). 3-Hydroxyisobutyric acid is then oxidized to form methylmalonic semialdehyde, which in turn is converted to propionyl-CoA in a complex reaction catalyzed by methylmalonic semialdehyde dehydrogenase (Fig. 1, step 8).

3-Hydroxyisobutyric Aciduria. The only patient identified with 3-hydroxyisobutyryl-CoA deacylase deficiency did not disclose any organic aciduria but cysteine and cysteamine conjugates of methylacrylic acid: *S*-(2-carboxypropyl)-cysteine and *S*-(2-carboxypropyl)-cysteamine [31].

Seven additional patients from five families have been described with 3-hydroxyisobutyric aciduria. Two of them possibly had combined methylmalonic, malonic, and ethylmalonic semialdehyde dehydrogenase deficiencies, characterized by high urinary excretion of 3-hydroxyisobutyrate associated with abnormal excretion of 3-hydroxypropionate, 2-ethylhydracrylate, 3-aminoisobutyrate, and β-alanine. These latter compounds may derive from accumulated methylmalonic, malonic, and ethylmalonic acid semialdehyde via reduction and transamination and may be altered following L-valine load. In some patients, 3-hydroxyisobutyric aciduria in association with brain dysgenesis and various malformations raises the question of the teratogenic role of accumulated byproducts and especially of methacrylyl-CoA [32].

Propionyl-CoA Metabolic Pathway. The final step in the metabolism of L-isoleucine involves the cleavage of 2-methylacetoacetyl-CoA to acetyl-CoA and propionyl-CoA (Fig. 1, step 6). L-Valine is also metabolized ultimately to propionyl-CoA, and thus these two BCAA form the major precursors of propionyl-CoA. Threonine and methionine are also metabolized to this intermediate via 2-oxobutyryl-CoA. The β-oxidation of fatty acids containing an odd number of carbons, which are minor components of dietary fats and body lipids, yields propionyl-CoA, too. Through the peroxisomal β-oxidation, the side chain of cholesterol is also a minor precursor of propionate synthesis. Finally, propionate synthesis by gut bacteria may also be of interest in disorders of propionate metabolism [33].

Propionyl-CoA is carboxylated to methylmalonyl-CoA by a mitochondrial biotin-dependent enzyme (Fig. 1, step 10). This carboxylation is readily reversible. A defect in propionyl-CoA carboxylase activity results in propionyl-CoA accumulation and, hence, PA. Propionyl-CoA accumulation also occurs in MCD due to defective activities of all three mitochondrial biotin-dependent carboxylases.

Methylmalonyl-CoA is converted to succinyl-CoA by the vitamin B_{12}-dependent methylmalonyl-CoA mutase (Fig. 1, step 11). Succinyl-CoA subsequently enters the Krebs cycle. Deficient activity of the apomutase enzyme leads to MMA, and because of the adenosylcobalamin (AdoCbl) requirement of the apomutase, abnormal B_{12} metabolism leads to variant forms of MMA.

Defects of the metabolism of propionyl-CoA and MM-CoA have been recorded at the two steps of their metabolic pathway. They may also be caused by disorders in the synthesis of the AdoCbl cofactor required by MM-CoA mutase, and of propionyl-CoA holocarboxylase due to deficient activity of holocarboxylase synthetase or biotinidase. Both defects share some characteris-

tic features due to accumulation of propionyl-CoA, which is responsible for several metabolic inhibitions.

Propionic Acidemia. Isolated PA is secondary to a defect in the synthesis or structure of propionyl-CoA carboxylase (PCC). The enzyme has the structure $\alpha_4\beta_4$ with the α chain containing a biotin prosthetic group. Theoretically, beside the common biotin-nonresponsive PA, and independently of combined carboxylase deficiency, a biotin-responsive form of PA may exist. PA is characterized by greatly increased concentrations of free propionate in blood and urine. However, this major sign may be absent, and in that case the diagnosis is based upon the presence of multiple organic acid by products. Methylcitrate and 3-hydroxypropionate are major diagnostic metabolites. The latter compound is formed either by β- or ω-oxidation of propionyl-CoA. Methylcitrate arises via condensation of propionyl-CoA with oxaloacetate by the action of citrate synthase. Furthermore, organic aciduria could include low levels of tiglylglycine, tiglic acid, and propionylglycine. During ketotic episodes, 3-hydroxyisovaleric acid is formed via propionyl-CoA and acetyl-CoA condensation and reduction. Some lactate, 3-hydroxybutyrate, methylmalonate, 2-methyl-3-hydroxy-butyrate, and several other organic acids may be present [34].

Methylmalonic Acidemia. Deficient activity of methylmalonyl-CoA apomutase, and because of the requirement by the apomutase for AdoCbl, defects at any step of AdoCbl metabolism, can lead to MMA. Impairment of mutase activity leads to accumulation of MM-CoA and secondary of propionyl-CoA which is reflected by the presence of greatly increased amounts of MMA and PA in blood and urine. Propionyl-CoA metabolites such as methylcitrate, 3-hydroxypropionate, 3-hydroxyisovalerate are usually also found in the urine. Vitamin B_{12} deficiency must be excluded when excessive amounts of urinary MMA are found, especially in infants who are breastfed by a mother who is either a strict vegetarian or suffering from subclinical pernicious anemia [35]. Through neonatal screening, some "healthy" patients have been identified with MMA 10–50 times the normal urinary level without any excretion of other metabolites derived from propionyl-CoA. In contrast, patients screened with high urinary excretion of MMA associated with propionate derivatives are probably at risk in case of catabolic stress, even if they have been asymptomatic for several years [36].

Secondary Metabolic Disturbances. Propionyl-CoA and its metabolites are known to produce a variety of metabolic disturbances which have major effects on intermediary metabolism. These include inhibition of Krebs cycle activity resulting in reduced adenosine triphosphate (ATP) synthesis. Propionyl-CoA also inhibits the pyruvate dehydrogenase complex, *N*-acetyl-glutamate synthetase, and the glycine cleavage system. In addition, MM-CoA inhibits pyruvate carboxylase activity. These inhibitions may explain some clinical features common to both disorders such as *hypoglycemia*, mild *hyperlactacidemia*, *hyperammonemia*, and *hyperglycinemia* [34]. Patients affected with PA or MMA have increased acylcarnitines in blood and urine, in which propionyl-carnitine is the major metabolite. It results from carnitine *N*-acylase activity, which can remove propionyl-CoA from the mitochondria and restore a pool of essential free CoA. Thus, a relative insufficiency of L-carnitine may occur in a state of continual propionyl-CoA accumulation, as in PA and MMA. L-Carnitine supplements may further increase propionyl-CoA removal and relieve all intramitochondrial inhibitions due to accumulation of propionyl-CoA [37].

Odd carbon number fatty acids are precusors of propionyl-CoA, but propionyl-CoA can replace acetyl-CoA as a "primer" for de novo long-chain fatty acids synthesis, leading to the formation of odd-numbered fatty acids. MM-CoA, by competition with malonyl-CoA, is responsible for the accumulation of methyl-branched long-chain fatty acids. Measurement of odd-numbered fatty acids in erythrocytes is a potentially useful means for long-term assessment of these disorders. These abnormal fatty acids can be incorporated into lipids all along pre- and postnatal life, providing certain amounts of toxic propionyl-CoA if lipolysis occurs during catabolic conditions [38].

Diagnostic Tests

In this group of disorders, the final diagnosis is made by identifying specific abnormal metabolites using amino acid chromatography and gas-liquid chromatography and mass spectrometry (GLC-MS). Only two disorders can be diagnosed by us-

ing amino acid chromatography alone: MSUD and the exceptional 3-hydroxyisobutyric deacylase deficiency. All other organic acidurias are diagnosed by GLC-MS, amino acid chromatography being irrelevant as it displays nonspecific abnormalities such as hyperglycinemia and hyperalaninemia. Some patients with PA and MMA may present with pseudo-cystinuria–lysinuria. Whatever the acute or chronic clinical presentation, the diagnosis can be made by sending to an experienced laboratory fresh or frozen urine samples, 5 ml fresh heparinized whole blood, or 1–2 ml fresh or frozen plasma. Specific loading tests are not necessary even in case of chronic intermittent forms, which most often display a sufficiently evocative biochemical pattern to allow diagnosis. Only for the ill-defined 3-MGC and 3-hydroxyisobutyric acidurias could leucine and valine loading tests be useful.

Enzymatic studies are useful for diagnostic confirmation, for a better delination of the enzymatic group and combined with molecular analysis, to determine phenotype–genotype relationships. In each disease, these studies can be performed in cultured fibroblasts. For rapid diagnosis, fresh peripheral leukocytes can be used with the possibility of evaluating the response to vitamin in vitamin-reponsive disorders such as MSUD, 3-MCC, and PA.

Reliable and fast *prenatal diagnosis* of IVA, PA, and MMA can be performed through the direct measurement of metabolites in amniotic fluid collected at the 12th week of gestation, using GLC-MS, stable isotope dilution technique, or fast atom bombing – mass spectrometry (FAB-MS) [39]. Direct enzymatic assay can also be performed in fresh or cultured chorionic villi or in cultured amniotic cells. Prenatal diagnosis of MSUD is performed by enzymology and relies on enzymatic assay in cultured amniocytes or on chorionic villi.

Treatment and Prognosis

Over the past few decades, several hundred patients have been treated. Evidence is accumulating that the CNS dysfunction can be prevented by early diagnosis and emergency treatment, followed by compliance to the restricted diet. This aspect is important in view of more radical therapeutic approaches such as liver transplantation and gene therapy, which represent a real hope for patients with the most severe PA and MMA who have to deal with recurrent life-threatening episodes of metabolic acidosis.

The heterogeneity of clinical manifestations is reflected in the different management strategies. Neonatal-onset forms require early toxin removal. Thereafter, the restricted food pattern essential to limit formation of organic acid byproducts is applied to survivors of the difficult newborn period as well as to the patients affected with the late-onset form. In both, prevention and early treatment of recurrent episodes of metabolic imbalance is crucial. At any age, each metabolic derangement is potentially life-threatening, and parents must be taught to recognize early warning signs and have an immediate plan for intervention.

General Principles of Long-Term Treatment. Long-term dietary treatment is aimed at reducing accumulated toxic metabolites, while at the same time maintaining normal development and nutritional status and preventing catabolism. Variability of treatment has been reported. Some patients tolerate normal foods, and others need only minimal restriction or can even regulate the diet themselves. On the other hand, many need very specific food allowances, implying stringent dietary restrictions, which will likely be a life-long necessity.

The treatment involves limiting one or more essential amino acids which, if present in excess, are toxic or precursors of organic acids. This means that protein is highly restricted, which might interfere with normal growth if the diet is not otherwise enriched. Therefore, the amount of protein in relation to total nutrient requirements, energy intake, and the distribution of protein must be carefully considered.

Precise prescriptions are established for the daily intake of amino acids, protein, and energy. The diet is checked for the recommended daily allowance (RDA) and for the estimated safe and adequate daily dietary intake of minerals and vitamins. In order to prevent dehydration in infants, the osmolality of synthetic or semisynthetic formulas must be estimated.

Amino Acid Intake. Requirements for BBCA vary widely from patient to patient and in the same patient, depending on the nature and severity of the disorder, any other therapy prescribed (e.g., use of an alternate pathway), growth rate, state of

health, and feeding difficulties. Individual requirements must be estimated for each child by frequent monitoring of clinical and metabolic status. The daily amino acid supply should be provided in a quantity sufficient to promote normal growth and nutritional status, without accumulation of toxic levels of metabolites. Balancing between protein malnutrition and metabolic disequilibrium is difficult, especially in children with PA and MMA, who need regular control especially after an acute intercurrent imbalance or after change of the diet.

The prescribed amounts of amino acids are provided by natural foods. Infant formula is used in young infants. For toddlers or children, solids are introduced, using specific serving lists. Apart from milk, high-protein foods (e.g., eggs, meat, dairy products) are generally avoided, because lower percentage in amino acids in vegetable protein, as compared with animal protein, makes it easier to satisfy the appetite of children.

Protein Intake. Limitation of essential amino acids to or even below the minimum requirements necessitates the use of synthetic amino acids mixtures which do not contain any of the potentially toxic amino acid(s). Although still controversial, the goal is to supply some additional nitrogen and other essential and nonessential amino acids in order to promote a protein-sparing anabolic effect. Some studies performed in PA or MMA patients demonstrate that this effect can be obtained with alanine supplements [40]. Others show that the addition of a special amino acid mixture to a severely restricted diet has no effect on growth and metabolic status and that these amino acids are mostly broken down and excreted as urea. However, in MSUD patients normal growth requires the use of an amino acid mixture. Special formulas have been developed by food companies (Milupa's formulas OS1, OS2, MSUD1, MSUD2; Weyth's formula S14; SHS' Maxamaid X).

In theory, an amino acid mixture is added to the natural protein in an amount sufficient to meet protein RDA for the patient's age. From a practical point of view, this is rarely possible. First, because of the bad taste of these synthetic mixtures, and second, because a satisfactory nutritional status can be reached with much less protein than the RDA suggests. In patients with high tolerance, it is even possible to restrict protein in the diet without adding amino acid mixtures.

Energy Intake. Requirements vary widely and may be greater than normal to ensure that essential amino acids are not degraded to provide energy or nitrogen for the biosynthesis of nitrogenous metabolites. Reduction in energy intake below the individual's requirements results in a decreased growth rate and a metabolic imbalance. The energy requirement is met through natural foods, special amino acid formulas, and additional fat and carbohydrates from other sources. Distribution of energy intakes from protein, carbohydrates, and lipids should approach the recommended percentages.

Micronutrient Intake. The diet must be checked for minerals, vitamins, and trace elements. If incomplete, the diet must be supplemented with an appropriate commercial preparation.

Water Intake. Enough water must be added to prevent dehydration of these patients, who may have a low renal concentrating capacity and may not tolerate hyperosmolar formulas. The appropriate concentration of formula mixtures is about 0.7–0.9 kCal/ml; measured or calculated osmolarity should be less than 450 mosmol/kg.

Design of a Low-Protein Diet. The design of a low-protein diet is illustrated by the following guidelines:

- Calculate the amount of infant formula and/or servings of solid food required to meet the desired intake of the amino acid concerned.
- Calculate the amount of protein provided by each natural food used, substract it from the protein prescription, and provide the remainder with a special amino acid mixture.
- Calculate the energy provided by natural foods and the amino acid mixture and fill the energy prescription with carbohydrates, fat, and/or specialized low-protein products, or use Mead-Johnson 80056 formula.
- Check the diet for other nutrients, particularly minerals and vitamins.
- Add sufficient water to meet liquid requirements.

For young infants, total alimentation is provided by the prescribed formula. Introduction of solid foods must be planned carefully, depending on the infant's appetite and metabolic stability. During this introduction, parents are taught how to use serving lists in order to introduce variety in

the diet and promote appetite. Progressively, milk intake is reduced, whereas fruits and vegetables are increased. Because of its high biologic value, maintenance of a certain percentage (25%) of total protein intake as dairy protein is recommended.

Evaluation of Nutritional Status. This comprises monthly evaluation of height, weight, and head circumference, which should remain in growth percentiles appropriate for age. Nutritional status is also judged by blood cell count, hemoglobin and hematocrit, plasma protein and albumin, iron and ferritin, calcemia, phosphatemia, and alkaline phosphatase. Beside the metabolic evaluation, the nutritional status is evaluated weekly during the first month of therapy, then every 3–6 months depending on the clinical status.

Apart from treatment of the disease, regular developmental assessments are essential in monitoring progress. Moreover, this practice makes it possible to provide the necessary psychologic support, as social and emotional needs are major elements of the overall therapy of the affected child and of the well-being of the family.

Maple Syrup Urine Disease. Specific adjustments are discussed below.

Toxin Removal Procedures. In order to protect the neonatal brain from permanent damage, the acutely ill newborn needs an emergency treatment in the form of exogenous toxin removal, because a high-energy enteral or parenteral nutrition alone is ineffective to rapidly lower plasma leucine levels [41, 42]. Continuous blood exchange transfusion, hemodialysis, or hemofiltration are efficient methods which allow a high-energy dietary treatment within hours as soon as the plasma leucine level is reduced to 1 mmol/l or less [23, 43]. During the recovery interval, the BCAA intake has to be adjusted according to the plasma levels, which are monitored every day until the balance is attained. During this stage, plasma concentrations of isoleucine and valine may decrease too much and become rate limiting for protein synthesis, a situation which requires supplements in doses of 100–200 mg/day.

Dietary Therapy. The objective of lifelong maintenance therapy is to maintain 2- to 3-h postprandial plasma BCAA to near normal concentrations (leucine, 80–200 μmol/l; isoleucine, 40–90 μmol/l; valine, 200–425 μmol/l). Because leucine is the most toxic precursor, the diet can be based on leucine requirement, isoleucine and valine being provided in proportion. In the classical severe form, the leucine requirement is 300–400 mg/day, which is about 50%–60% of the leucine intake in the healthy newborn. Minimum isoleucine and valine requirements are about 150–200 mg/day and 240–280 mg/day, respectively. Intakes must frequently be titrated against plasma concentrations. Occasionally, small amounts of free valine and isoleucine must be added to the amounts provided by natural protein, because the tolerance for leucine is lower than for the other two [23]. Under conditions with high leucine and low valine and isoleucine levels, a rapid fall in plasma leucine can be achieved only by combining a reduced leucine intake with a temporary supplementation of valine and isoleucine. Apart from considerable interindividual variations, the growing child with the classical form of MSUD tolerates about 500–700 mg leucine per day. Individuals with variant forms tolerate higher amounts, and some do well on a low-protein diet. Nevertheless, constant care is indicated, especially during intercurrent episodes.

Treatment is assessed weekly, every 2 weeks, and then monthly up to the age of 1–2, 2–4, and over 4–5 years, respectively, by plasma amino acid chromatography. In parallel, dietary intakes are checked on a diet calendar, which is filled in by parents for 1–3 days prior to each blood test.

Vitamin Therapy. Pharmacologic doses of *thiamine* (5 mg/kg per day) for a minimum of 3 weeks may improve BCAA tolerance in some patients. However, normal leucine tolerance has never been restored, and the degree of response is often difficult to assess [44]. The original thiamin-responsive patient has a normal long-term outcome without obvious cerebral impairment on MR imaging [11].

Prognosis. Patients with MSUD are now expected to survive; they are generally healthy between episodes of metabolic imbalance, and some attend regular schools and have a normal IQ score. However, on the whole, the average intellectual performance is far below the normal. This intellectual outcome is inversely related to the time after birth that plasma leucine levels remained above 1 mmol/l and is dependent on the quality of long-term metabolic control [45]. These results grossly parallel those of chronic brain damage described

earlier, and both are arguments for rapid toxin removal in the neonatal period and meticulous long-term management throughout life. In addition, timely evaluation and intensive treatment of minor illnesses at any age is essential, as death attributed to recurrence of metabolic crises with infections has occurred [11].

Isovaleric Acidemia. The following specific adjustments are made.

Toxin Removal Procedures. Exogenous toxin removal, such as blood exchange transfusion, may be needed in newborns with IVA, who often present in poor clinical condition, precluding the effective use of alternate pathways [46]. L-Glycine therapy is one specific and effective means of treatment. Oral supplementation of 250–600 mg/kg per day increases IVG excretion during acute metabolic episodes, while 150–250 mg/kg per day is an effective dose in the steady state [47]. *Glycine* can be provided as a 100-mg/ml water solution, delivered in four to eight separate doses. L-*Carnitine* is another effective agent that, in addition to other common effects, stimulates the excretion of IVG [48].

Dietary Therapy. Goals of nutritional support are to keep the urine free of IVA and 3-OHIVA. A special amino acid mixture free of leucine is useful as long as a stringent protein-restricted diet is maintained. In comparison to MSUD, IVA patients supplemented with glycine and carnitine have a high leucine tolerance. During the first year of life, leucine intake can be gradually increased to 800 mg/day, which represents an important provision of natural proteins (8 g/day). Subsequently, higher amounts can be tested, and the strict low-leucine diet may be replaced by a low-protein diet, which requires less monitoring. Most children can tolerate about 20–30 g protein per day, which is sufficient to assure normal growth and development without amino acid mixture supplements.

Oral L-glycine and L-carnitine are employed in long-term therapy. There is no evidence that both supplementations are needed. L-Carnitine (50–100 mg/kg per day) along with mild protein restriction appears to be adequate [49]. The therapeutic measures must be evaluated monthly in infants and every 3–6 months in older children. Maintenance of the fasting plasma glycine concentration between 200–500 μmol/l and of total plasma carnitine concentration within the normal range is a reasonable goal.

Prognosis. Once they have passed the neonatal period, patients need careful nutrition support. However, prognosis is better than in any other organic aciduria. Intellectual prognosis depends on early diagnosis and treatment and, subsequently, on long-term compliance.

3-Methylcrotonylglycinuria. At onset and during intercurrent episodes, isolated 3-MCC-deficient patients need symptomatic correction of hypoglycemia, acidosis, and dehydration associated with a low-protein and high-energy diet. In addition, oral *glycine* (±150 mg/kg) and L-*carnitine* (50–100 mg/kg) may be beneficial. Long-term treatment based on a mildly restricted protein diet along with L-carnitine supplements and avoidance of catabolic stress appear to be effective in preventing life-threatening episodes and further cerebral injury.

Propionic and Methylmalonic Acidemias. Specific adjustments made include the following.

Toxin Removal Procedures. The urinary excretion of propionic acid is negligible, and no alternate pathway is sufficient to effectively detoxify newborns with PA who need exogenous toxin removal procedures. In contrast, one efficient removal of toxin in MMA is urinary excretion. The high clearance of methylmalonic acid (22 ± 9 ml/min per 1.73 m^2) allows excretions as high as 4–6 mmol/day, which is much higher than quantities removed by a 24-h peritoneal dialysis (2.2 ± 0.4 mmol). Thus, the emergency treatment of the MMA newborn mainly comprises rehydration and promotion of anabolism. Simultaneously, most neonatal cases of MMA benefit from a rapid toxin removal such as a blood exchange transfusion, which is successful in the ensuring a partial removal of methylmalonic acid accumulated in the blood.

Dietary Therapy. Special amino acid mixtures for PA and MMA patients are available. They are free of isoleucine, valine, methionine, and threonine. Because valine is one of the more direct precusors of propionyl-CoA, the diet can be based on valine intake, other amino acids being provided in proportion. In the neonatal period during the refeeding phase, valine intake is progressively

increased from 25–50 mg/day to 240–280 mg/day over a period of 5–10 days, depending on clinical status, weight gain, and biochemical results. Thereafter, the individual child's tolerance should be tested. Subsequently, the valine intake for the following years is quite homogenous between 350 and 500 mg/day, which represents 5.5–7.5 g natural protein per day. The stringent protein restriction may require additional intake of special amino acid mixtures to prevent protein deficiency. In general, the entire artificial diet supplement must be delivered during nocturnal gastric feeding. Apart from the natural foods which provide the required amounts of valine, low-protein products may be offered during the day, more for social, psychologic, and developmental than for nutritional ones. By preventing chronic malnutrition, catabolism, and prolonged fasting periods, this practice has improved the average prognosis of these severe cases. Also, it allows a more rapid and effective adaptation in case of intercurrent crises.

Most patients with a late-onset form are easier to manage. Individual tolerance is quite high, and the diet may be based on the protein intake rather than on the daily valine intake. By the age of 2 years, they can tolerate more than 12 g natural protein per day, and supplementary amino acid mixtures are no longer necessary. Even though their individual tolerance allows a less rigid protein restriction and leads to lower risks of malnutrition, these patients must be taught to immediately reduce their protein intake during intercurrent illness in order to prevent metabolic imbalance.

Vitamin Therapy. Some late onset forms and more rarely neonatal onset forms of MMA are vitamin B_{12} responsive, thus parenteral vitamin therapy must be carefully tried, starting with 1000–2000 μg/day for a few days. Generally, hydroxocobalamin is preferred to cyanocobalamin and to deoxyadenosylcobalamin [50]. During this period, 24-h urine samples are collected for an organic acid analysis. Vitamin B_{12} responsiveness leads to a prompt and sustained decrease of propionyl-CoA byproducts. However, as biochemical results may be difficult to assess, they must later be confirmed by in vitro studies. Most of the B_{12}-responsive patients need only mild protein restriction or none at all, while vitamin B_{12} is either given orally once a day or intramuscularly administered once a week (1000–2000 μg). In some cases i.m. vitaminotherapy can be kept in reserve for intercurrent infections.

Metronidazole Therapy. Gut bacteria have been implicated as an important source of propionate in children with inborn errors of propionate metabolism [33]. The microbial propionate production can be suppressed by antibiotics. *Metronidazole*, an antibiotic which inhibits colonic flora, has been found specifically effective in reducing urinary excretion of propionate metabolites by 40% in MMA and PA patients [51]. Long term metronidazole therapy at a dose of 10–20 mg/kg once daily for 10 consecutive days monthly may be of significant clinical benefit [36]. This alternate administration may prevent the known side effects of the drug such as leukopenia, peripheral neuropathy, and pseudomembranous colitis.

Prognosis. In both neonatal- and late-onset forms, the prognosis depends on the individual tolerance to catabolic stress, as most patients with the early-onset forms survive the neonatal period if they are promptly and carefully managed. However, the late-onset form has a better survival rate than the early-onset type. The long-term treatment of MMA and PA still raises considerable problems, and the degree of developmental delay varies as does neurological impairement [52].

Genetics

MSUD is inherited in an autosomal recessive mode, with an incidence of 1 in 120 000 to 1 in 500 000. About 75% of those affected suffer from a severe classical form, and the remainder from intermediate or intermittent variants. Various mutations of E1α, E1β, and E2 genes have been identified that are responsible for the classical type of MSUD [22]. Despite the implication of the E1α subunit in thiamine-dependent decarboxylation, the E2 subunit is mutated in the thiamine-responsive patient. This mutation either alters the thiamine binding by E1α or causes instability of the complex that thiamine stabilizes [53].

Isovaleric acidemia is an autosomal recessive-inherited disorder. Despite the absence of complementation groups, five distinct classes of molecular variants have been described which correspond to various point mutations or deletions of the gene located on chromosome 15 [54].

Propionic acidemia is an autosomal recessive disorder with a low incidence (less than 1 in 100 000). Irrespective of the clinical phenotype, severe reduction but not complete absence of PCC activity (1%–5%) has been found in cultured fibroblasts. Two distinct genotypic forms are distinguished by cell complementation: pccA, resulting from defects in the α (PCCA) gene, and pccB, resulting from defects in the β (PCCB) gene. PCCA and PCCB cDNA clones have been obtained, and mutations in both PCCA and PCCB have been identified [55]. In addition, DNA-mediated gene transfer developments allow us to consider somatic gene therapy in the near future [56].

Methylmalonic acidemias are autosomal recessive disorders. The incidence of benign and severe forms are each about 1 in 50 000. Genetic defects are categorized by somatic cell complementation as either mut defects, due to mutations in the gene encoding the methylmalonyl-CoA mutase (MCM) or Cbl defects, due to mutations in genes required for provision of the cobalamin cofactors. Approximately half of the patients have a mutase apoenzyme defect further divided into mut° and mut$^-$ groups. Mut° lines show no detectable enzyme activity even if OH-Cbl is provided in excess, mut$^-$ lines have an enzyme with detectable activity when stimulated by a high concentration of OH-Cbl. Recent cloning and sequencing of human MCM has provided new complementation delineation and molecular characterization of mutations at this locus and may enable somatic gene therapy to be undertaken [57].

The remaining patients are cobalamin variants. Among them, CblA and CblB types implicate AdoCbl synthesis. Heterokaryons [mut/Cbl] and [CblA/CblB] have normal methylmalonate metabolism. CblA is due to a defect in mitochondrial cobalamin reductase and CblB to defective AdoCbl transferase. All CblA patients and 40% of CblB are vitamin B_{12} responsive (see Baumgartner and Suormala, this volume).

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Ketolysis Defects

J.-M. Saudubray, N. Specola, and C. Charpentier

CONTENTS

The ketone bodies acetoacetate (AA), 3-hydroxybutyrate (3OHB) and acetone, are formed in the liver from fatty acids and ketogenic amino acids. Acetoacetate and 3-OHB are maintained in equilibrium by 3OHB dehydrogenase, whereas acetone, formed nonenzymatically, is eliminated in breath. Ketone bodies serve as fuels for a variety of extrahepatic tissues such as cardiac and skeletal muscle, particularly when cellular glucose is in short supply, as in fasting and in insulin deficiency. In normal children aged 1–7 years, blood ketone levels reach 3–6 mmol/l after a 24-h fast (see Chapter 2) [1]. Ketoacidosis, defined as ketonemia above 7 mmol/l, is observed in a number of inborn errors of carbohydrate metabolism (see Chapters 5 and 8), in which it is caused by excess of ketone body production, secondary to hypoglycemia [2]. Defects of ketolysis, in contrast, are primary defects of enzymes that utilize ketone bodies and related metabolites and are characterized by severe ketoacidosis with normal or even elevated blood glucose levels. Defects of five ketolytic enzymes have been described in man (Fig. 1):

- Succinyl-CoA 3-keto acid CoA transferase (also termed succinyl-CoA acetoacetate transferase or thioacyl transferase)
- Methyl-acetoacetyl-CoA thiolase (commonly named 3-ketothiolase)
- Mitochondrial acetoacetyl-CoA thiolase
- Cytosolic acetoacetyl-CoA thiolase
- Peroxisomal B- (or 3-) ketoacyl-CoA thiolase

Owing to its cellular localization, the latter will be considered in the chapter 39 (this volume). In addition, the B-ketoacyl-CoA thiolase involved in the fourth step of mitochondrial fatty acid B-oxidation, which has a broad specificity, is presented in the chapter 11.

Clinical Presentation

Succinyl-CoA 3-Keto Acid CoA Transferase Deficiency. The first patient affected by this very rare disorder S-CoA transferase was extensively described by Tildon in 1972 [3]. More patients have since been described [4, 5]. They presented with a stereotypic clinical and biochemical picture of recurrent attacks of polypnea, lethargy and hypotonia with normal or elevated blood glucose, and massive ketoacidosis in the neonatal period [3, 5] or infancy [4]. Our two patients displayed a tendency toward hyperglycemia during attacks even before therapeutic intervention. Normal or low blood lactate and ammonia levels were noted. They completely recovered with parenteral serum bicarbonate and glucose. Gas-liquid chromatography mass spectrometry (GCMS) of urinary organic acids during attacks displayed the presence of high concentrations of 3OHB, AA, and 3-hydroxyisovalerate without any other abnormality. The clinical diagnosis rests on the demonstration of a permanent ketosis both in urine (Acetest +) and plasma (3OHB + AA levels greater than 0.2 mmol/l). In three patients [4, 5], the ketonemia was not only increased after an overnight fast reaching 4–5 mmol/l with a low free fatty acid to ketone body ratio, but also during the day, when the patients were normally fed with a moderately restricted protein diet. In our first patient during the fasting test performed under

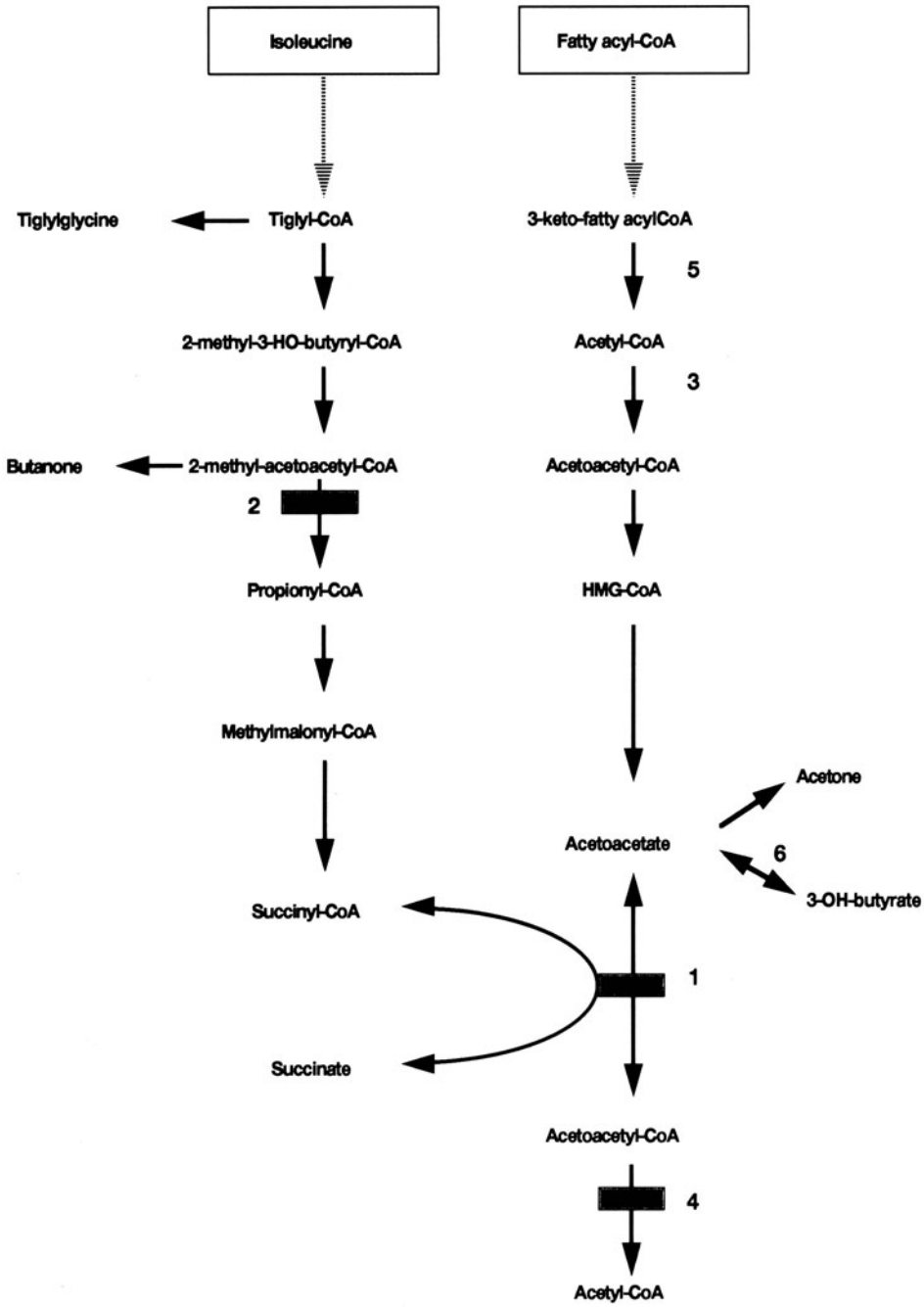

Fig. 1. Biochemical pathways of ketone bodies. *1*, Succinyl-CoA 3-keto acid CoA (acetoacetate) transferase (S-CoA transferase); *2*, Methyl-acetoacetyl-CoA thiolase (3-keto thiolase (MAA-CoA thiolase)); *3*, acetoacetyl-CoA thiolase (AA-CoA Thiolase), hepatic; *4*, acetoacetyl-CoA thiolase, extrahepatic; *5*, B-ketoacyl-CoA thiolase; *6*, B-hydroxybutyrate dehydrogenase. *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A

careful supervision, blood AA and 3OHB concentrations rose to 3.7 and 6 mmol/l, respectively, with a very low free fatty acid to ketone body ratio of 0.3 and a normal blood glucose level at the end of the fast.

Methyl-Acetoacetyl-CoA Thiolase and Acetoacetyl-CoA Thiolase Deficiencies. The first patient was described by Daum et al. [6, 7] as a "new" disorder of isoleucine catabolism. Since this first description, more than 20 further affected families have been

reported under various denominations, including 2-methyl-acetoacetyl aciduria, 3-ketothiolase deficiency, and acetoacetyl-CoA thiolase AA-CoA thiolase deficiency [4, 8–12].

Most of the cases present a late-onset form in childhood. Only few start before 6 months. The only patient with a neonatal onset [19] had a very unusual presentation similar to methylmalonic or propionic aciduria. MAA-CoA thiolase was not determined in this patient. The most frequent presentation is a history of recurrent ketoacidotic and vomiting episodes with lethargy and dehydration or recurrent frequent headaches, triggered by catabolic events such as intercurrent infections, fast, or a high protein intake. During attacks, blood glucose levels are usually normal or elevated. This hyperglycemia associated with severe ketoacidosis can mimic diabetic ketoacidosis. High levels of acetoacetate in blood and urine can erroneously suggest the presence of salicylate on routine testing and thus simulate salicylism [16]. Blood lactic acid and ammonia levels are generally normal or low [4], as is the plasma carnitine. Amino acids are normal. Between attacks physical and mental development are normal. Various central nervous system (CNS) symptoms including encephalopathy, hypotonia, weak reflexes, ataxia, diplegia, irritability, and speech problems have been reported in 12 patients [18, 20], but as a whole the disease can be considered a rather benign disorder if the condition is recognized early at the first attack. In four recently described cases, there was neurologic involvement and on magnetic resonance imaging (MRI) an increased T2 intensity bilaterally within the posterior lateral part of the putamen [21]. Our first patient diagnosed at 7 years is now 27 years old and has had no more attacks [9]. At least two more patients are totally asymptomatic [17, 20]. In most patients, the excretion of 2-methyl-3-hydroxybutyrate and tiglylglycine (TG) is greatly increased in the urine. 2-Methyl-acetoacetate, butanone (volatile decarboxylation product of 2 methyl acetoacetate) [7, 9], and 6-methyluracil [22] may also be present, especially in acute phases when the patients are ketotic and acidotic. However, the excretion of these organic acids is largely dependent on the protein intake. During remission phases, it can be elicited by an acute or chronic isoleucine or protein load [7, 9, 23].

Cytosolic Acetoacetyl-CoA Thiolase Deficiency. Only five patients with this disorder have been reported [24, 25]. All but one presented with severe mental retardation, hypotonia, and loss of psychomotor abilities. A ketogenic diet induced a severe ketoacidotic attack in one case [25]; a permanent excretion of excessive amounts of 3OHB and AA with a persistent elevation of blood ketones was found in other cases. There were no other specific abnormalities in organic acid excretion. This defect can be easily missed by physicians and biochemists, as its clinical presentation is nonspecific. Persistent ketosis may be the only initial abnormality.

Metabolic Derangement

The pathways involved in the ketolytic defects are depicted schematically in Fig. 1. Succinyl-CoA 3-keto acid CoA transferase (step 1) is only found in extrahepatic tissues, where it is located in the mitochondria and catalyzes the first, key step of the utilization of ketone bodies, namely the transfer of CoA from succinyl-CoA to acetoacetate, producing acetoacetyl-CoA. The subsequent conversion of acetoacetyl-CoA into acetyl-CoA, catalyzed by extrahepatic mitochondrial AA-CoA thiolase (step 4) allows energy production from the ketone bodies. The deficiency of succinyl-CoA acetoacetate transferase explains the accumulation of blood ketones.

MM-CoA thiolase (step 2), also localized in the mitochondria, cleaves methyl-acetoacetyl-CoA, an intermediate of isoleucine catabolism, into propionyl-CoA and acetyl-CoA. Owing to the similarity of this reaction with those catalyzed by hepatic and extrahepatic mitochondrial AA-CoA thiolase (steps 3 and 4), the enzyme is often also referred to as AA-CoA thiolase or 3-ketothiolase. The accumulation of intermediates of the isoleucine catabolic pathway – 2-methyl-acetoacetate, 2-methyl-3-hydroxytbutyrate, TG, and butanone – accords with the localization of the enzyme defect. That of acetoacetate and resulting B-OH-butyrate is explained by competition of elevated methyl-acetoacetyl-CoA with acetoacetyl-CoA for cleavage into acetyl-CoA. In two patients, clinical and enzymatic data were in keeping with defective ketone body utilization but normal isoleucine catabolism [8, 13]. This suggests a specific defect in extrahepatic mitochondrial AA-CoA thiolase (step 4, Fig. 1).

Cytosolic AA-CoA thiolase (not presented in Fig. 1), which can be differentiated from the

hepatic and extrahepatic mitochondrial acetyl-CoA thiolases by the fact that it is not stimulated by K^+, is presumed to play a role in the synthesis of cholesterol in extrahepatic tissues. The severe neurologic symptoms produced by the enzyme defect might result from deficient synthesis of essential isoprene-derived substances such as dolichols, impairing myelination and membrane function.

Diagnostic Tests

Succinyl-CoA 3-Keto Acid CoA Transferase Deficiency. Because there is no specific metabolite excretion, the diagnosis must be confirmed by the demonstration in cultured fibroblasts of a defective 14C acetoacetate oxidation and of absent [3] or low residual [4] S-CoA transferase activity measured by acetoacetyl-CoA consumption. The in vitro synthesis of fatty acids is normal. In addition, U[^{14}C]glucose oxidation by patients' fibroblasts shows a significant reduction of $^{14}CO_2$ production [3, 4]. The latter data suggest that absence of S-CoA transferase causes accumulation of an inhibitor of glycolysis in cells in vitro.

Methyl-Acetoacetyl-CoA Thiolase and Acetoacetyl-CoA Thiolase Deficiencies. All patients have total or partial deficiencies of mitochondrial K-dependent AA-CoA thiolase in cultured fibroblasts [14] or leukocytes [12].

The urinary excretion of TG, 2-methyl-3-hydroxybutyrate, and 2-methyl-acetoacerate is not specific for MAA-CoA thiolase deficiency. These compounds may occur in patients with normal activity of this enzyme [18]. Absence of the key urinary metabolites is rare and should raise the suspicion of a variant enzyme defect. In two such patients, a deficiency of the cytosolic enzyme was inferred [24, 25]. Loading with isoleucine [9, 23] may be a useful procedure in patients in whom the pattern of organic aciduria leaves doubt to the diagnosis. Evidence has been presented that the concentration of 2-methyl-acetoacerate in the urine after an isoleucine load increases only in 3-ketothiolase deficiency [23]. 6-Methyluracil excretion could also be a specific marker of the disease [22].

Whatever the basic defect may be, K-dependent mitochondrial thiolase deficiencies show a wide variation in clinical severity, which cannot be explained only by biochemical heterogeneity, since the AA-CoA thiolase has a nearly zero activity toward 2-methyl-acetoacetatyl-CoA [14, 18]. Furthermore, Yamaguchi et al. [26] reported the absence of the mitochondrial MAA-CoA thiolase protein from fibroblasts of their patient. Heterogeneity must be due to variable compensation of the thiolysis of AA-CoA by other cellular thiolases.

Cytosolic Acetoacetyl-CoA Thiolase Deficiency. There is no specific metabolite excretion. Enzyme analysis in cultured fibroblasts [24] and in liver [25] revealed a probable 50% deficiency of cytosolic AA-CoA thiolase, with normal mitochondrial thiolase activity. Reduced sterol synthesis was found in one case [24].

Treatment and Prognosis

Succinyl-CoA 3 Keto Acid CoA Transferase Deficiency. Treatment of this rare but potentially fatal condition consists in avoiding prolonged fasting and in a moderately protein-restricted diet (1.5–2 g/kg). Special care must be taken during infections and catabolic states: in such circumstances, acetonuria must be carefully monitored with the Acetest. When the Acetest is 3+, 1 g sodium bicarbonate should be given every 8 h accordingly.

Methyl-Acetoacetyl-CoA Thiolase Deficiency. If the diagnosis is made timely, mitochondrial thiolase deficiency is a relatively easy disorder to treat. As for S-CoA transferase deficiency, catabolic states should be carefully monitored by the Acetest, and sodium bicarbonate should be given accordingly. The additional administration of carnitine (200 mg/kg) during acute attacks has been proposed [23].

Cytosolic Acetoacetyl-CoA Thiolase Deficiency. No effective treatment is presently available.

Genetics

Succinyl-CoA 3-Keto Acid CoA Transferase Deficiency. Family pedigrees suggest an autosomal recessive mode of inheritance. Prenatal diagnosis should be possible. The human liver S-CoA transferase cDNA has recently been cloned and should allow molecular investigations very soon.

Methyl-Acetoacetyl-CoA Thiolase Deficiency. Inheritance is autosomal recessive and prenatal diagnosis should be possible. By genetic complementation analysis of cultured fibroblasts, evidence was obtained of three complementation groups in seven cell strains analysed [27]. Yamaguchi et al. [26, 28] studied the enzyme deficiency by immunochemical methods and pulse-chase experiments. In one patient the lack of MAA-CoA thiolase activity could be attributed to a defect of enzyme biosynthesis in fibroblasts. Four cell lines were capable of synthesizing small amounts of enzyme protein [28]. Subsequently, the Japanese group has cloned and sequenced cDNA for human MAA-CoA thiolase [29, 30]. The cDNA was 1518 bp in length and encoded a 427-amino acid precursor of the enzyme. With this information, a number of molecular lesions have been described, highlighting a considerable genetic and biochemical heterogeneity of this disorder.

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Glutaric Aciduria Type I and Related Cerebral Organic Acid Disorders

G.F. Hoffmann

CONTENTS

Neurological manifestations are very common in organic acid disorders and sometimes the leading and/or presenting feature [1]. One group presents exclusively with characteristic (progressive) neurological symptoms of ataxia, myoclonus, extrapyramidal symptoms, metabolic stroke, and macrocephaly [2]. These "cerebral" organic acid disorders include glutaric aciduria type I, 2-oxoglutaric aciduria, L-2-hydroxyglutaric aciduria, and mevalonic aciduria. Strikingly, in all these disorders the pathological compound is a five-carbon organic acid.

Cerebral organic acid disorders often remain undiagnosed. Abnormalities such as hypoglycemia, metabolic acidosis, or lactic acidemia, the usual concomitants of disorders of organic acid metabolism, are generally absent. Also, elevations of diagnostic metabolites may be very small and be missed on routine organic acid analysis, e.g., in glutaric aciduria type I. Therefore, the correct diagnosis requires an increased awareness of these disorders. Additional important diagnostic clues can be derived from neuroimaging findings (Fig. 1). Disturbances (progressive) 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 [3].

Glutaric Aciduria Type I (Glutaryl-CoA Dehydrogenase Deficiency)

Clinical Presentation

When there is *megalencephaly* in an infant together with progressive atrophic changes on computed tomography (CT) or nuclear magnetic resonance (NMR; Fig. 1a,b,c) and/or acute profound *dyskinesia* or subacute motor delay accompanied by increasingly severe *choreoathetosis* and dystonia, glutaric aciduria type I should have a high priority in the differential diagnosis [4–8].

In many patients macrocephalus has been present at or shortly after birth and preceded the severe neurological disease. An important clue to early diagnosis is not so much the finding of macrocephalus at birth, but the observation of a pathologically increasing skull circumference crossing the percentiles and peaking at the age of 3–6 months. Affected babies often present additional "soft" neurological symptoms of hypotonia, irritability, and jitteriness. Retrospectively, neuroimaging has been performed in a number of patients during this presymptomatic period, revealing the characteristic findings of *frontotemporal atrophy* and delayed myelination (Fig. 1a). Additional findings in presymptomatic infants

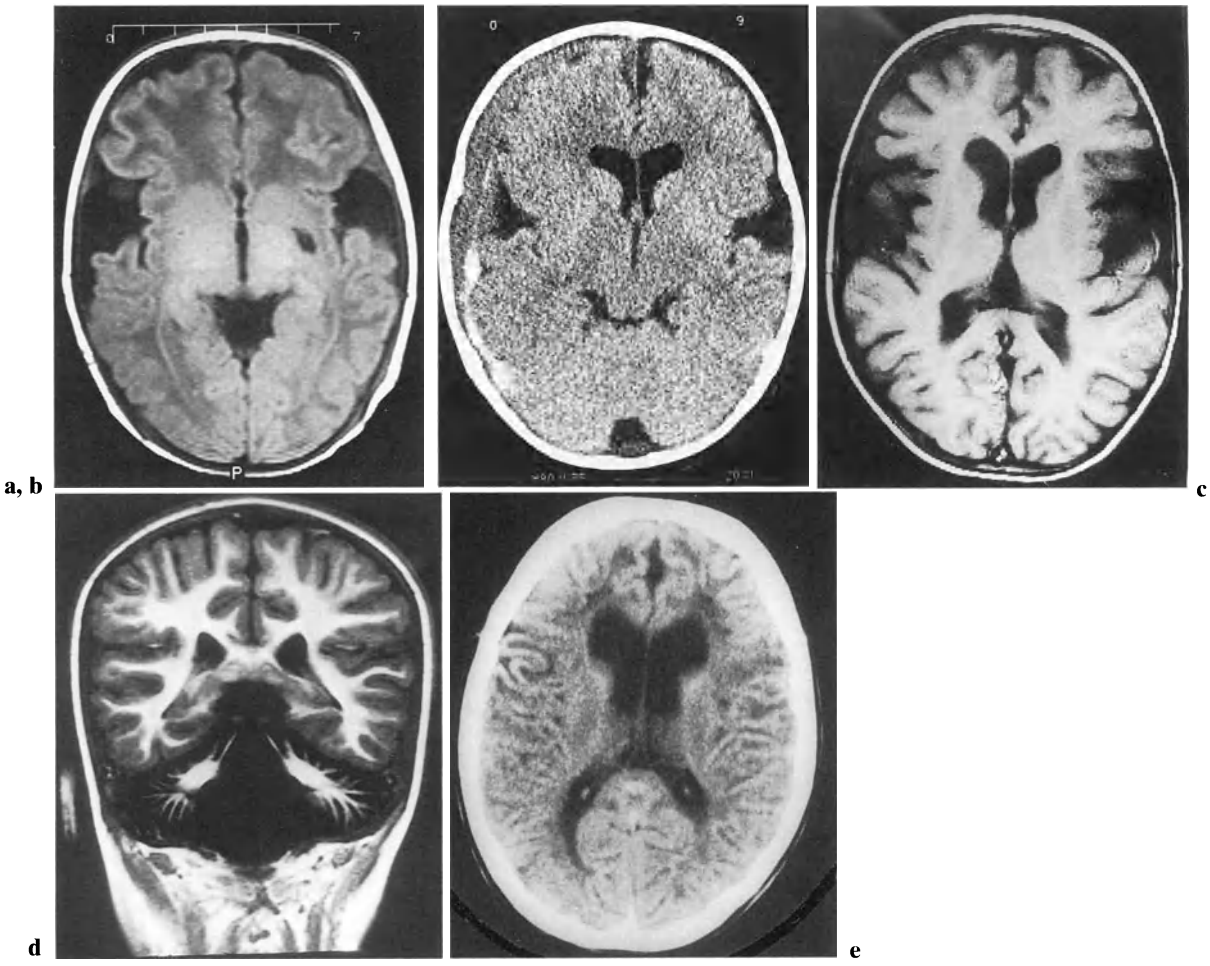

Fig. 1a–e. Neuroimaging findings which are suggestive of cerebral organic acid disorders. **a** Transversal nuclear magnetic resonance (NMR) image of a 2-month-old presymptomatic boy with glutaryl-CoA dehydrogenase deficiency showing enlargement of cerebral spinal fluid (CSF) spaces anterior to the temporal lobes with marked extension of Sylvian fissures (frontotemporal atrophy). Spin echo technique (1.0 T): time of repetition, 660 ms; time of echo, 20 ms; slice thickness, 5.5 mm. **b** Computed tomography (CT) scan of a 9-month-old presymptomatic boy with glutaryl-CoA dehydrogenase deficiency. In addition to frontotemporal atrophy, subdural effusions and hematomas causing midline shift are visible. There is no pathology of the basal ganglia, and the child continued to develop normally. **c** CT scan of a 2-year-old boy with glutaryl-CoA dehydrogenase deficiency who suffered an encephalopathic crisis at the age of 13 months, showing severe frontotemporal atrophy as well as diffuse loss of white matter. **d** Coronal section of the central nervous system (CNS) of a 6-year-old girl with mevalonic aciduria by NMR. There was marked atrophy of the hemispheres of the cerebellum. Spin echo technique (1.0 T): time of repetition, 2460 ms; time of echo, 30 ms; time of inversion, 600 ms; slice thickness, 9 mm. **e** CT scan of a 15-year-old girl with L-2-hydroxyglutaric aciduria, showing the involvement of the subcortical white matter and ventricular enlargement

are *chronic subdural effusions* and *hematomas* (Fig. 1b). Unfortunately, at this point metabolic investigations are generally not undertaken. At an average age of 14 months, approximately 75% of patients suffer an acute encephalopathic crisis, mostly associated with an infection. Generally, there are no or only mild metabolic symptoms. After recovery, the children have lost most motor skills and function at a 1- to 2-months-old level. A severe (dys)hypotonic movement disorder develops. At this point the very distinctive clinical picture of a severe *dystonic–dyskinetic syndrome* in alert-looking children with relatively well preserved intellectual functions and a *prominent forehead* may be recognized. If the underlying metabolic disorder remains undiagnosed, additional cerebral systems are slowly and progressively affected. A generalized cerebral atrophy emerges (Fig. 1c), consistent with the clinical development of pyramidal tract signs and mental retardation.

Although the majority of patients presents with

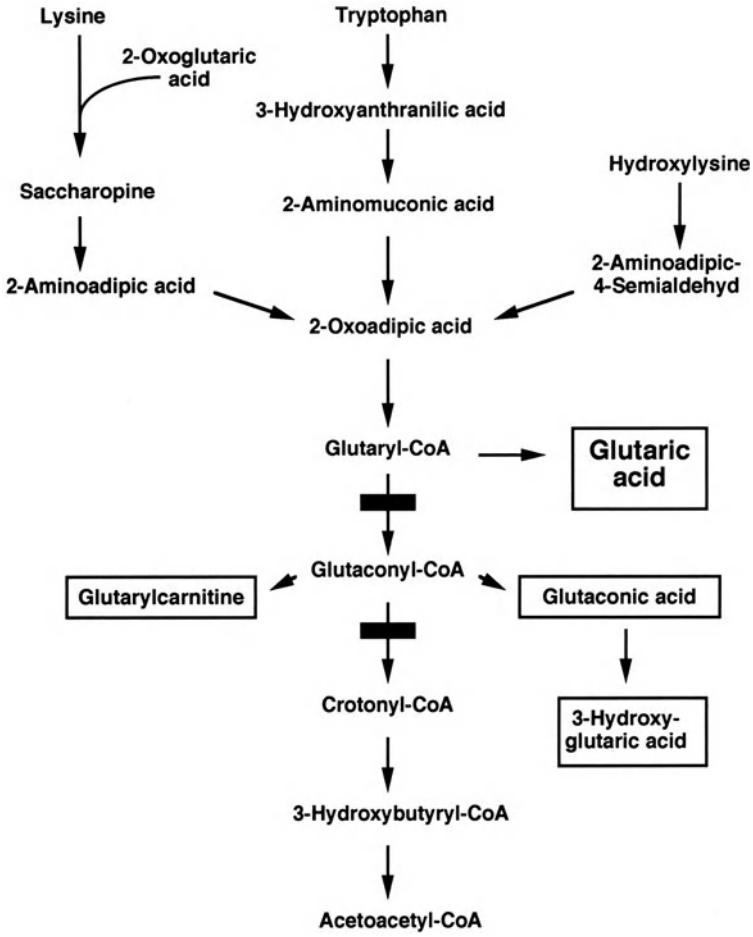

Fig. 2. Catabolic pathways involving glutaric acid. Glutaryl-CoA dehydrogenase catalyzes the dehydrogenation of glutaryl-CoA as well as the subsequent decarboxylation of glutaconyl-CoA to crotonyl-CoA. Glutaryl-CoA dehydrogenase deficiency (GDD) results in characteristic increases in several metabolites, most notably glutaric acid, which are marked by *squares* (modified from [6])

characteristic symptoms and disease course, the natural history of glutaric aciduria type I can be remarkably variable even within families [1, 5, 7]. A minority of patients (approximately 25%) present with developmental delay from birth and (progressive) dystonic cerebral palsy. Finally, there have been four cases reported who were older than 6 years of age or even adults and never developed neurological disease [4, 5].

Metabolic Derangement

Glutaric aciduria type I is an inborn error of lysine, hydroxylysine, and tryptophan catabolism due to deficiency of glutaryl-CoA dehydrogenase, a mitochondrial flavin adenine dinucleotide-requiring enzyme, which catalyzes the dehydrogenation of glutaryl-CoA as well as the next decarboxylation of glutaconyl-CoA to crotonyl-CoA (Fig. 2). Part of the accumulating glutaryl-CoA is esterified with carnitine by carnitine acyltransferase and excreted as glutarylcarnitine leading to severe *secondary carnitine deficiency* and a pathologically increased ratio of acylcarnitines to free carnitine in plasma and urine. The patients often show increased urinary excretion of *dicarboxylic acids*, indicative of disturbed fatty acid oxidation and increased excretion of 2-oxoglutaric acid and succinic acid [6, 9]. Secondary carnitine deficiency is a causative factor of acute metabolic crises, which are infrequent, but do occur in glutaric aciduria type I. Metabolic crises (14% in our series) manifest with severe hypoglycemia and variable metabolic acidosis and can progress to a Reye-like syndrome. Such crises are different from the above-described encephalopathic crises characteristic for this disorder. They can develop at any age and respond well to intravenous therapy with glucose, car-

Table 1. Therapeutic recommendations for patients with glutaric aciduria type 1

Measures	Infants	Children (<6 years)	Children (>6 years)	Adults
Emergency measures:				
Stop protein supply				
Glucose infusion (10–20 mg/kg per min, if necessary with insulin)				
Carnitine (200 mg/kg per day intravenously)				
Supplementations				
L-Carnitine (mg/day)	100	50–100	50–100	50
Riboflavine (mg/day)	100–200	100–200	100–200	100–200
Diet				
Natural protein (g/kg per day)	1.0–1.2	0.8–1.0	1.0–1.5	0.8–1.0
Amino acid mixture (g/kg per day)	1.1–1.3	0.6–1.0	n.a.	n.a.
Lysine (mg/kg per day)	90–100	60–80	n.a.	n.a.
Tryptophan (mg/kg per day)	⩾20	⩾12–20	n.a.	n.a.
Energy (kCal/kg per day)	95–112	90–102	46–73	35–50
Neuropharmaceutical agents (for patients with neurological disease):				
Baclofen (1–2 mg/kg per day), vigabatrin, clonazepam, diazepam, triheyphenidyl, memantine, haloperidol, L-dopa, levodopa, 5-hydroxytryptophan, glutamine				
Do not use valproic acid				
Multiprofessional support of patient and family				

Therapeutic measures with convincingly demonstrated benefit are written in bold type.
n.a., not applicable.

nitine, and bicarbonate. If treated early and vigorously (Table 1), there will be no neurological sequelae [6, 8].

The pathophysiology of the *encephalopathic crises* in glutaric aciduria type I is unknown. The basal ganglia are specifically affected. Neurons of the caudate nuclei and the putamen may be especially sensitive to glutaric acid, glutaconic acid, or 3-hydroxyglutaric acid, which in vitro inhibit glutamic acid decarboxylase. This may cause a decreased production of gamma aminobutyric acid (GABA) in the basal ganglia in vivo [10]. Data on concentrations of glutaric acid and GABA in cerebrospinal fluid (CSF) and postmortem brain tissue are inconsistent. Most investigators found levels of glutaric acid of below 40 μmol/l, far too low to inhibit glutamic acid decarboxylase, and normal GABA in CSF [6, 7, 11].

Diagnostic Tests

Patients with glutaric aciduria type I are generally diagnosed by urinary organic acid analysis. However, individuals with complete deficiency of glutaryl-CoA dehydrogenase and characteristic neurological disease, but without (or with inconsistent) elevations of glutaric acid in the urine, have been diagnosed in increasing numbers [7, 11, 12]. Therefore, repeated and quantitative urinary organic acid analysis may be necessary for diagnosis. Additional diagnostic hints can be obtained by finding carnitine in serum reduced and a pathologically increased ratio of acylcarnitines to free carnitine in serum and urine. In most patients with borderline urinary excretion of free glutaric acid, excretion of glutarylcarnitine is unequivocally increased [12]. This can be confirmed by quantitation of acylcarnitines in the urine or, more simply, by quantitation of total glutaric acid (after hydrolysis) in a 24-h urine sample. As even the quantification of acylcarnitines in the urine can fail to reveal the diagnosis, ultimately enzyme assays of glutaryl-CoA dehydrogenase in lymphocytes or cultured fibroblasts must be undertaken [13]. This procedure is indicated for family studies or when clinical suspicion is strong [5, 12]. Enzymatic confirmation of the enzyme defect should also be done as a prerequisite for prenatal diagnosis as well as to exclude a deficiency of glutaryl-CoA oxidase as a cause of glutaric aciduria [14].

Treatment and Prognosis

Rational therapy is hampered by our lack of understanding the natural history of the disease, especially the pathogenesis of encephalopathic crises. Five different therapeutic approaches exist (Table 1):

- Emergency treatment during intercurrent illnesses, especially gastrointestinal infections, consists of a high dose of intravenous glucose and carnitine, followed by frequent feedings with a high-carbohydrate intake and low-protein intake. All patients with glutaric aciduria type I should have an emergency card.
- Oral supplementations with *carnitine* and *riboflavin* are widely used. Carnitine should be supplemented in any patient who is carnitine deficient (free carnitine less than 15 μmol/l). The rationale for carnitine supplementation is not to enhance the elimination of glutaric acid, but to prevent secondary metabolic crises. The amount of glutaric acid, which is excreted as glutarylcarnitine, cannot usually be raised higher than 5% [6]. Riboflavin responsiveness is an extreme rarity and should be carefully investigated by giving riboflavin in increasing doses from 50 to 300 mg and monitoring total glutaric acid in 24-h urine samples. In evaluating the response, unrelated marked variations of the daily urinary excretion of glutaric acid must be taken into account [6, 7].
- The patient is treated with a diet low in protein which restricts the intake of lysine to its requirement. This is supplemented with an lysine-free amino acid mixture. Usually the intake of tryptophan is reduced as well. However, tryptophan contributes only 20% or less to total body glutaric acid production, and plasma levels cannot be monitored by regular amino acid analysis. Furthermore, concentrations of tryptophan directly modulate production of serotonine in the central nervous system (CNS). When using diets low in lysine and tryptophan, we observed tryptophan deficiency with normal plasma levels of lysine, resulting in side effects such as sleeplessness, ill temper, irritability, and loss of appetite, which could be improved by additional tryptophan supplementation [6]. Dietary treatment should therefore consist of a diet low in protein adjusted to the requirement of lysine with supplementation of an amino acid mixture, which must contain tryptophan (6–7 mg/g protein).
- In neurologically symptomatic patients, treatment results in no major clinical improvement. All patients are left severely handicapped [6, 8]. Nevertheless, the combination of a low-protein diet with carnitine supplementation at least halts the course of disease. A number of presymptomatic patients who were diagnosed by family studies or children with macrocephaly diagnosed and treated before the onset of encephalopathic crises have developed normally up to examination at 2–7 years of age [15].
- Several *neuropharmaceutical agents* have been tried to ameliorate neurological symptoms. Baclofen (Lioresal) reduces involuntary movements and improves motor function. In some patients its use and dosage is limited by worsening of truncal hypotonia. Valproic acid should not be given. In one patient valproic acid appeared to aggravate symptoms [16]. It competes with glutaric acid for esterification with L-carnitine and may promote disturbances in the mitochondrial acetyl-CoA to CoA ratio [6]. Surprisingly, little information is available on the effects of other neuropharmaceutical agents and all medications listed in Table 1 could be empirically employed.
- Nonspecific multiprofessional support is most important in the care of the patients. It must be kept in mind that, given the severe motor handicap, intellectual functions are well preserved until late in the course of the disease. Using Bliss boards and especially new language computers, the social integration of patients can be greatly improved. As involuntary movements of orofacial muscles may be especially severe, feeding difficulties can become a major problem. In addition, increased muscle tonus and sweating require a high intake of energy and water. A percutaneous gastrostomy, which allowed high-energy feeding, caused a dramatic improvement of nutritional status, a marked relief of psychological tension and care load in the families, and even a reduction of the dystonic–dyskinetic syndrome [8]. Neurosurgical interventions of subdural hygromas and hematomas in infants and toddlers with glutaric aciduria type I should be avoided, if possible (Fig. 1c).

Our current treatment schedule of patients with glutaric aciduria type I is specified in Table 1.

Although the nature of the encephalopathic crises is not understood, the risk for these crises seems to subside after the age of 4–5 years. Patients with neurological disease should not be treated with severe protein restriction beyond 6 years of age. No recommendations can be given about discontinuation of dietary treatment in presymptomatic patients until we know more about the pathophysiology of the disorder. Carnitine supplementation and emergency measures during intercurrent illnesses, especially gastrointestinal infections, are a lifelong necessity.

Genetics

Glutaric aciduria type I is an autosomal recessive disorder. The deficiency of glutaryl-CoA dehydrogenase can be reliably demonstrated in tissues, white blood cells, cultured fibroblasts, amniotic fluid cells, and chorionic villi [13]. Residual enzyme activity does not correlate with disease severity. Carrier detection is possible by enzyme assay, though the results are sometimes equivocal [13]. *Prenatal diagnosis* can be offered by enzyme assay [13] as well as by determination of glutarate by stable isotope dilution gas chromatography mass spectrometry (GCMS) assay in amniotic fluid [17].

L-2-Hydroxyglutaric Aciduria

Clinical Presentation

L-2-Hydroxyglutaric aciduria was only recently recognized [18]. This was followed by a number of reports of patients from all over the world illustrating previous mis- and underdiagnosis. From the initial report of eight patients from five families with L-2-hydroxyglutaric aciduria, a very characteristic clinical picture evolved [18, 19]. During the first 2 years the mental and psychomotor development appears normal or only slightly retarded. Thereafter, progressive *ataxia*, slight *extrapyramidal signs*, and progressive *mental retardation* become the most obvious clinical findings. The IQ at teenager age is about 40–50. The oldest known patients are over 30 years of age. They are bedridden and severely mentally retarded. The neuroimaging findings are very specific. A progressive loss of arcuate fibers (a spongiform encephalopathy) is combined with a severe *cerebellar atrophy* (Fig. 1d).

Metabolic Derangement

No specific biochemical function or pathway involving L-2-hydroxyglutaric acid is known in humans. Recently, the enzyme L-2-hydroxyglutaric acid dehydrogenase was tentatively identified in human liver [19]. It remains to be established whether this enzyme is defective in L-2-hydroxyglutaric aciduria.

Diagnostic Tests

On organic acid analysis, L-2-hydroxyglutaric acid is found to be highly elevated in all body fluids [18–20]. In addition, lysine concentration is slightly elevated in cerebrospinal fluid, and protein concentration in cerebrospinal fluid is increased in the absence of pleocytosis. Differentiation between the two isomers of 2-hydroxyglutaric acid is indispensable for diagnosis. Recently, D-2-hydroxyglutaric aciduria was described in a patient who manifested an early-onset, severe seizure disorder with hypotonia and cortical blindness [21], possibly another cerebral organic acid disorder.

Treatment and Prognosis

No specific therapy exists. Epilepsy can generally be controlled by standard medications.

Genetics

The observation of two affected offsprings in three families, of three affected offsprings in one family, and a similar clinical picture in men and women strongly supports an autosomal recessive mode of inheritance [19]. Heterozygotes display no detectable clinical or biochemical abnormalities related to L-2-hydroxyglutaric aciduria. *Prenatal diagnosis* may be possible utilizing accurate determination of L-2-hydroxyglutaric acid by stable isotope dilution GCMS assay in amniotic fluid [21].

Mevalonic Aciduria (Mevalonate Kinase Deficiency)

Clinical Presentation

Mevalonic aciduria is an inherited disorder of cholesterol and nonsterol isoprene biosynthesis mani-

festing severe symptoms in infancy or early childhood [22]. Although patients with mevalonic aciduria have a recognizable phenotype of serious clinical manifestations, some are likely to remain undiagnosed. The most severely affected died in infancy with severe failure to thrive, profound developmental delay, dysmorphic features, cataracts, hepatosplenomegaly, lymphadenopathy, and anemia, as well as diarrhea and malabsorption. The presenting picture may suggest congenital infections or chromosomal abnormalities. Less severely affected patients may be classified among patients with psychomotor retardation, hypotonia, myopathy, and ataxia of unknown etiology. All patients have had recurrent crises with fever, lymphadenopathy, increase in size of liver and spleen, arthralgia, edema, a morbilliform rash, and high white cell counts, but always without metabolic acidosis or hypoglycemia, pointing to an infectious or autoimmune etiology.

Metabolic Derangement

Mevalonic aciduria is a consequence of the deficiency of mevalonate kinase, the first enzyme after 3-hydroxy-3-methylglutaryl-CoA reductase in the biosynthesis of cholesterol and nonsterol isoprenes [22, 23]. In contrast to defects in the catabolic pathway, symptoms are thought to be due to an underproduction of the end products of the biosynthetic pathway.

Diagnostic Tests

Serum levels of creatine kinase and transaminases are elevated in most patients. The only diagnostic biochemical abnormality is the gross elevation of mevalonic acid in all body fluids, detectable by organic acid analysis. The diagnosis needs to be confirmed by assay of mevalonate kinase in white blood cells or cultured fibroblasts [22, 23].

Treatment and Prognosis

Treatment is still experimental [22]. Dietary supplementations of bile acids and cholesterol had to be discontinued because of worsening diarrhea and general malaise. Trials of corticosteroid therapy during clinical crises (2 mg prednisone/kg per day) resulted in positive responses in two children. Symptoms of acute crisis disappeared within 24–48 h. Both children showed a significant improvement of their somatic and psychomotor development over the following year of intermittent therapy with corticosteroids.

The most severely affected patients died in infancy with profound developmental delay, dysmorphic features, cataracts, hepatosplenomegaly, lymphadenopathy, and anemia, as well as diarrhea and malabsorption. In the other patients the clinical course was progressive during the first years of life with considerable phenotypic heterogeneity. Later on, they displayed a stable clinical picture of borderline mental retardation, cerebellar ataxia, and muscular hypotonia.

Genetics

Mevalonic aciduria is an autosomal recessive disorder. The gene for mevalonate kinase is localized on chromosome 12. In one patient a disease-causing mutation has been identified (A⇒C at nucleotide 902 of the open reading frame, resulting in an asparagine to threonine substitution) [24].

Carrier detection is possible by enzyme assay, though the results are sometimes equivocal [23]. Heterozygotes were also found to show a significantly increased excretion of mevalonic acid in their urine [23]. *Prenatal diagnosis* is possible by assaying mevalonate kinase activity in amniocytes and in chorionic villus cells and by direct assy of mevalonic acid in amniotic fluid by GCMS [23].

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Part VII
Vitamin-Responsive Disorders

Biotin-Responsive Multiple Carboxylase Deficiency

R. Baumgartner and T. Suormala

CONTENTS

Four carboxylases, propionyl-coenzyme A carboxylase (PCC), 3-methylcrotonyl-coenzyme A carboxylase (MCC), pyruvate carboxylase (PC), and acetyl-coenzyme A carboxylase (ACC) require the vitamin biotin for their activity. Biotin is bound covalently to the four inactive apocarboxylases (Fig. 1) by holocarboxylase synthetase (HCS). It is released again by biotinidase after proteolytic degradation of the active holocarboxylases into short biotinyl peptides and biocytin (biotinyl-lysine), thus enabling recyling of biotin. By the same mechanism biotinidase liberates biotin from dietary sources.

Inherited defects of HCS [1, 2] and biotinidase [3] are known. Both result in multiple carboxylase deficiency (MCD). Owing to the role of the carboxylases in gluconeogenesis, fatty acid synthesis, and the catabolism of several amino acids (Fig. 2), their deficiency provokes multiple, profound metabolic derangement, eliciting neurologic symptoms and a characteristic organic aciduria. MCD can also be caused by acquired biotin deficiency.

Clinical Presentation

The characteristic manifestation of MCD is metabolic acidosis associated with neurologic abnormalities and skin disease.

The expression of the clinical and biochemical features is variable in both inherited disorders [1]. While patients with HCS deficiency commonly present with the characteristic manifestations of MCD, patients with biotinidase deficiency show a less consistent clinical picture, particularly during the early stage of the disease. The onset in biotinidase deficiency may be insidious, and the manifestation is usually very variable, neurologic symptoms being prominent often without markedly abnormal organic acid excretion or metabolic acidosis. Later-onset forms of HCS deficiency cannot be distinguished clinically from biotinidase deficiency, so that the diagnosis must be confirmed by enzyme assay.

Holocarboxylase Synthetase Deficiency. Although HCS deficiency was initially termed early-onset MCD, recent experience shows that the age of onset of symptoms varies widely from a few hours after birth to 6 years of age. Nevertheless, most patients have presented acutely in the first days of life with symptoms very similar to those observed in other severe organic acidurias, i.e., lethargy, hypotonia, vomiting, seizures, and hypothermia. The most common initial clinical features consist in respiratory difficulties such as tachypnoea or Kussmaul breathing. Increasingly severe metabolic acidosis, ketosis, and hyperammonaemia may lead to coma and early death. Patients with a less severe defect and later onset may also present with recurrent life-threatening attacks of metabolic acidosis and typical organic aciduria [4]. Surviving early-onset patients and patients with a less severe defect may develop, in addition to the above-mentioned symptoms, psychomotor retardation, hair loss, and skin lesions. The latter include an erythematous, scaly skin rash spreading over the whole body, but particularly prominent in the diaper and intertriginous areas, or may resemble seborrheic dermatitis or ichthyosis. Superinfection with *Candida* may occur. Disorders of immune function have been observed with decreased T cell count and impaired in vitro and in vivo response to *Candida* antigen. Episodes of acute illness are often precipitated by catabolism

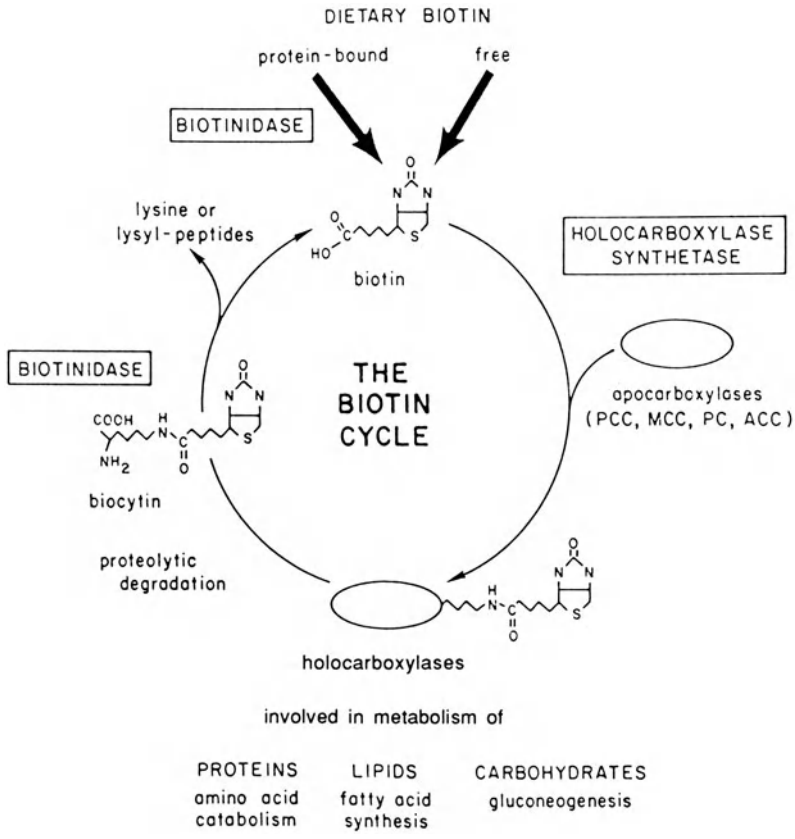

Fig. 1. The biotin cycle. *PCC*, propionyl-CoA carboxylase; *MCC*, 3-methylcrotonyl-CoA carboxylase; *PC*, pyruvate carboxylase; *ACC*, acetyl-CoA carboxylase. (From [32])

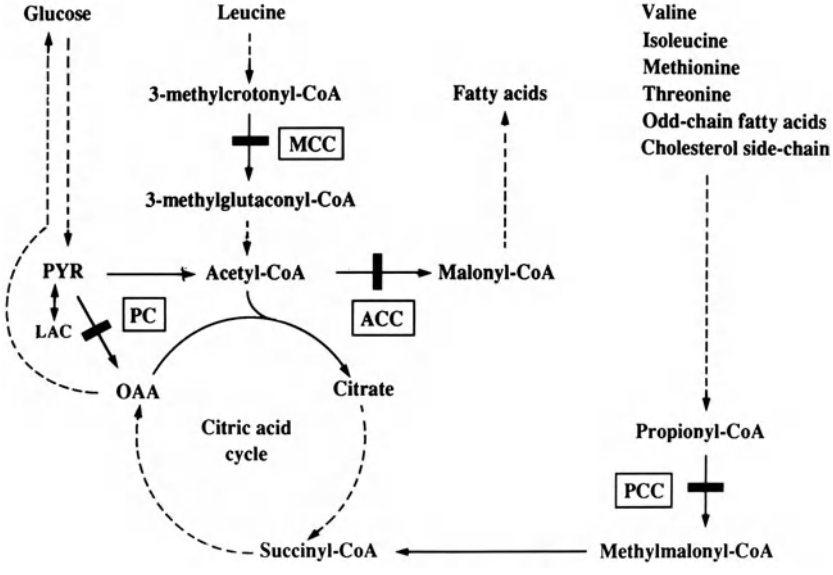

Fig. 2. The carboxylase deficiencies. Sites of enzyme defects are indicated by *black bars*. *Full lines* indicate one enzyme, and *dotted lines* indicate that several enzymes are involved. *pyr*, pyruvate: *lac*, lactate; *OAA*, oxaloacetate; *PCC*, propionyl-CoA carboxylase; *MCC*, 3-methylcrotonyl-CoA carboxylase; *PC*, pyruvate carboxylase; *ACC*, acetyl-CoA carboxylase

during intercurrent infections or by a higher protein intake.

Biotinidase Deficiency. Important features are the gradual development of symptoms and episodes of remission, which may be related to increased free biotin in the diet. The full clinical picture has been reported as early as 7 weeks [5], but discrete neurologic symptoms may occur much earlier, even in the neonatal period [6]. *Neurologic manifestations* (lethargy, muscular hypotonia, grand mal and myoclonic seizures, ataxia) are the most frequent initial symptoms. In addition, respiratory abnormalities such as stridor, episodes of hyperventilation, and apnea frequently occur and may also be of neurologic origin [7]. Skin rash and/or alopecia are hallmarks of the disease. However, they may develop late or not at all [8, 9]. *Skin lesions* are usually patchy, erythematous–exsudative, and typically localized periorificially. Eczematoid dermatitis or an erythematous rash covering large parts of the body as well as keratoconjunctivitis have also been observed. *Hair loss* is usually discrete, but may in severe cases become complete, including the eyelashes and eyebrows. Immunologic dysfunction may occur in acutely ill patients. Because of the variability and nonspecificity of clinical manifestations there is a great risk of a delay in diagnosis [10]. Late-diagnosed patients often have *psychomotor retardation* and permanent neurologic deficits, such as hearing loss and optic atrophy, which may be irreversible [8–11]. The outcome may even be fatal. One patient died at the age of 22 months with features of Leigh's syndrome proven by histopathology [7].

Metabolic acidosis and the characteristic organic aciduria of MCD are frequently lacking in the early stages of the disease. Plasma lactate and 3-hydroxyisovalerate may be only slightly elevated, whereas in cerebrospinal fluid their levels may be significantly higher [12, 13]. This fact, as well as the finding of severely decreased carboxylase activities in brain but moderately deficient activities in liver and kidney in a patient with lethal outcome [7], are in accordance with the predominance of neurologic symptoms and show that in biotinidase deficiency the brain is affected earlier and more severely than other organs. The threat of irreversible brain damage requires this disorder to be considered in all children with neurologic problems even without obvious organic aciduria and/or cutaneous findings.

Metabolic Derangement

In the HCS defect, a decreased affinity of the enzyme for biotin and/or a decreased maximal velocity leads to reduced formation of the four active holocarboxylases from their corresponding, inactive apocarboxylase [2, 14].

In biotinidase deficiency, biotin cannot be released from biocytin and biotinyl peptides. Patients with biotinidase deficiency are thus unable to either recycle endogenous biotin or to use protein-bound dietary biotin [3]. Consequently, biotin is lost in the urine, mainly in the form of biocytin [15, 16], and progressive biotin depletion occurs. Depending on the amount of free biotin in the diet and the severity of the enzyme defect, the disease becomes clinically manifest in the first few months or later in infancy or childhood.

The deficient activity of the carboxylases (Fig. 2) results in the accumulation of lactic acid and of derivatives of 3-methylcrotonyl-CoA and propionyl-CoA (for details see "Diagnostic tests" below).

Isolated inherited deficiencies of the three mitochondrial carboxylases are also known: PCC, the most common (see Ogier et al., "Branched-Chain Organic Acidurias," this volume), MCC (see Tada, this volume), and PC (see Buist, this volume). A single patient with an isolated defect of hepatic and fibroblast ACC (cytosolic) has been reported [17]. These isolated deficiencies are due to the absence or abnormal structure of the apoenzyme and do not respond to biotin therapy.

Acquired biotin deficiency is rare. It occurs under the following conditions: excessive consumption of raw egg white (binding to avidin makes biotin unavailable), malabsorption due to a short bowel, long-term total parenteral nutrition, or hemodialysis without biotin supplementation, long-term anticonvulsant therapy [1].

Diagnostic Tests

Characteristic organic aciduria due to systemic deficiency of the carboxylases is the key feature of MCD. In severe cases an unpleasant odor of the urine (cat's urine) may even suggest the defect. MCD is reflected in elevated urinary and plasma concentrations of organic acids as follows:

- Deficiency of PCC: methylcitrate, 3-hydroxypropionate, propionylglycine, tiglylglycine, propionic acid in small to moderate amounts

- Deficiency of MCC: 3-hydroxyisovaleric acid in high concentrations, 3-methylcrotonylglycine in smaller amounts
- Deficiency of PC: lactate in high concentrations, pyruvate in smaller amounts

The majority of HCS-deficient patients excrete all of the typical organic acids in elevated concentrations, provided that the urine sample has been taken during an episode of acute illness, whereas in biotinidase deficiency only elevated excretion of 3-hydroxyisovalerate may be found.

The measurement of carboxylase activities in lymphocytes provides direct evidence of MCD [18]. These activities are low in HCS deficiency, but may be low or normal in biotinidase deficiency depending on the degree of biotin deficiency.

The two inherited disorders can easily be distinguished by assay of biotinidase in serum. Today this assay is included in neonatal screening programs in many countries worldwide.

Holocarboxylase Synthetase Deficiency. Biotin concentrations in plasma and urine are normal. Carboxylase activities in lymphocytes are deficient and cannot be activated by in vitro preincubation with biotin. Direct measurement of HCS activity requires an apocarboxylase as one of the substrates [2, 14] and is therefore not routinely performed.

HCS deficiency can be diagnosed indirectly by demonstrating severely decreased carboxylase activities in fibroblasts cultured in medium with low biotin concentration (10^{-10}mol/l) and by normalization or at least some increase in the activities in culture media supplemented with high biotin concentrations (10^{-6}–10^{-5}mol/l) [1, 18]. It must be noted that fibroblasts of some later-onset patients may exhibit normal levels of carboxylase activities when cultured in standard media supplemented with 10% fetal calf serum, which results in a final biotin concentration of about 10^{-8}mol/l provided by the fetal calf serum.

The response to oral biotin reflected by an increase of carboxylase activities in lymphocytes and by an improvement of organic acid excretion is variable [19]. All patients respond to some extent to 10–20 mg/day, but complete normalization of these parameters may require even higher doses (40–80 mg/day) or may not be achieved [19, 20].

Biotinidase Deficiency. Biotinidase activity in plasma is absent or decreased [3, 21, 22]. Many patients have measurable residual activity and should be evaluated for the presence of K_m mutations (see below).

Symptomatic patients usually have decreased biotin concentrations in plasma and urine [6, 15, 22] and decreased carboxylase activities in lymphocytes which are normalized within hours after a single dose of oral biotin [6]. This rapid increase of carboxylase activities is also found by in vitro preincubation of lymphocytes with biotin [18], in contrast to a lack of an in vitro response in HCS deficiency.

Patients excrete biocytin in urine [16], the concentration being dependent on the level of residual biotinidase activity [22]. Carboxylase activities in fibroblasts cultured in low-biotin medium are similar to those in control fibroblasts and always normal in fibroblasts cultured in standard medium.

Acquired Biotin Deficiency. Biotinidase activity is normal in plasma, and biotin concentrations are low in plasma and urine. Carboxylase activities in lymphocytes are decreased and are promptly normalized after a single dose of oral biotin or after preincubation with biotin in vitro.

Treatment and Prognosis

Both inherited disorders can be treated effectively with pharmacologic doses of *biotin*. Restriction of protein intake is not necessary, except in very severe cases of HCS deficiency. Acutely ill patients with metabolic decompensation require general emergency treatment in addition to biotin therapy.

Holocarboxylase Synthetase Deficiency. The required dose of biotin is dependent on the severity of the enzyme defect and has to be assessed individually. Most patients have shown a good clinical response to 10–20 mg/day, although some may require higher doses, i.e., 40 mg/day [19, 20, 23]. In spite of apparently complete recovery, some patients continue to excrete abnormal metabolites (particularly 3-hydroxyisovalerate), a finding which correlates inversely with the actual level of carboxylase activities in lymphocytes. Persistent clinical and biochemical abnormalities have been observed exceptionally despite treatment with very high doses of biotin [20]. One severely retarded patient, showing some biochemical but no clinical response to 20 mg biotin per day, died at

the age of 6 years [24]. Mutations causing totally unresponsive HCS deficiency are probably lethal in utero.

So far the prognosis of most surviving patients with HCS deficiency seems to be good if the initial response to biotin therapy is adequate and sufficient biotin is given regularly. However, careful follow-up studies are needed to judge the long-term outcome. In one patient, followed up for 9 years and treated prenatally and from the age of 3.5 months with 6 mg biotin/day, some difficulties in fine motor tasks were obvious at the age of 9 years [25]. Severe residual neurologic deficits have rarely been reported, in contrast to the findings in biotinidase deficiency.

Successful prenatal treatment (10 mg daily) has been reported in two pregnancies, preventing acute neonatal symptoms [26, 27].

Biotinidase Deficiency. Introduction of neonatal screening programs has resulted in the detection of asymptomatic patients with residual biotinidase activity. Based on measurement of plasma biotinidase activity with the natural substrate biocytin, we have classified the patients in three groups [22]:

- Patients with undetectable biotinidase activity, i.e., classical biotinidase deficiency
- Patients with detectable activity from 0.06% to 30% of mean normal and a normal K_m for biocytin
- Patients with decreased affinity of biotinidase for biocytin, i.e., K_m variants

Group 1. In early diagnosed children, 5–10 mg oral biotin/day promptly reverses or prevents all clinical and biochemical abnormalities. Under careful clinical and biochemical control it may be possible to reduce the daily dose of biotin to 2.5 mg. In one patient 1 mg per day was insufficient during infections [9]. Biotin has to be given lifelong and regularly each day since biotin depletion develops rapidly. For chronic treatment a daily dose of 5–10 mg is recommended and no adverse effects have been observed. Under such treatment there is no accumulation of biocytin in body fluids [15, 16], which was previously suspected to be a possible risk.

In patients who are diagnosed late, irreversible brain damage may have occurred before the commencement of treatment. In particular, auditory and visual defects often persist in spite of biotin therapy [8–11], and intellectual impairment and ataxia have been observed as long-term complications [9]. Neonatal screening for biotinidase deficiency [28] allows early diagnosis and effective treatment. In such patients the diagnosis must be confirmed by quantitative measurement of biotinidase activity. Treatment should be instituted without delay, since patients may become biotin deficient within a few days after birth [15].

Group 2. According to our experience with 82 patients, those with activities below 8% show a great risk of becoming biotin deficient and should be treated with biotin [22]. We, as well as others [21], recommend that all patients with less than 10% biotinidase activity, i.e., profound biotinidase deficiency, be treated with biotin, e.g., 2.5 mg/day. Some mild biochemical abnormalities, such as slightly elevated excretion of 3-hydroxyisovalerate in urine and slightly low plasma biotin concentration, were found intermittently in a few of the patients with residual activity of 16%–18%. One infant with about 30% enzyme activity developed hypotonia, skin rash, and hair loss during an episode of gastroenteritis at 6 months of age. This was reversed by biotin therapy [29]. It is therefore also necessary to regularly check patients with 10%–30% residual activity and to supplement patients with borderline abnormalities with small doses of biotin, e.g., 2.5–5 mg/week. Such a therapy seems justified, since there is evidence that the central nervous system is affected earlier than the rest of the body [7].

Group 3. We found five patients with a K_m mutation among 113 patients. These patients have a high risk of becoming biotin deficient and therefore must be treated with biotin.

Genetics

Both disorders are inherited as an autosomal recessive trait. HCS deficiency seems to be rarer than biotinidase deficiency. The patients with HCS deficiency evaluated so far all belonged to a single complementation group [30]. However, there is considerable biochemical evidence for heterogeneity within this group. Heterozygote detection has not been accomplished.

The incidence of profound biotinidase deficiency (less than 10% residual activity) is on average 1 in 110 000, and the incidence of partial biotinidase deficiency (residual activity, 10%–30%) 1 in 130 000 [21]. Heterozygotes show about

50% of the mean control activity. Patients with partial biotinidase deficiency and K_m variants indicate heterogeneity within this disorder.

Prenatal diagnosis of HCS deficiency is possible by enzymatic studies in cultured chorionic villi or amniotic fluid cells or by organic acid analysis of amniotic fluid [26, 31]. It allows rational prenatal therapy preventing severe metabolic derangement in the early neonatal period [27]. Biotinidase can be measured in chorionic villi or cultured amniotic fluid cells, but this in our opinion is not warranted, because prenatal treatment is not necessary.

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Inherited Disorders of Cobalamin and Folate Absorption and Metabolism

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CONTENTS

Cobalamin (Cbl, vitamin B_{12}) is a widely distributed, cobalt-containing, water-soluble vitamin that is not found in higher plants. Cbl is needed for only two reactions in man, but its metabolism involves a complex absorption and transport system and intricate intracellular conversions. As methylcobalamin (MeCbl), it is a cofactor for the cytoplasmic enzyme methionine synthase, which synthesizes the essential amino acid methionine from homocysteine and thereby recycles the crucial folate derivative tetrahydrofolate (THF). As 5′-deoxyadenosylcobalamin (AdoCbl), it is the cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase, which is involved in the catabolism of valine, threonine, and odd-chain fatty acids into succinyl-CoA, an intermediate of the Krebs cycle.

Patients with inherited disorders affecting Cbl absorption or metabolism show elevations of homocysteine or methylmalonic acid, either alone or in combination. For those disorders that affect MeCbl formation, the major manifestations include megaloblastic anemia secondary to folate deficiency and neurological abnormalities, presumably secondary to methionine deficiency. For those disorders that affect AdoCbl formation, the main findings relate to metabolic acidosis.

Inherited disorders of cobalamin metabolism are classified as disorders of absorption and transport and disorders of intracellular utilization [1–5].

Disorders of Absorption and Transport of Cobalamin

Absorption of dietary Cbl involves first binding to a glycoprotein (R binder, haptocorrin, TCI) in the stomach. In the intestine, the R binder is digested by proteases, allowing the Cbl to bind to intrinsic factor (IF), which is produced in the stomach by parietal cells. Via specific ileal receptors, the IF–Cbl complex enters the enterocyte. After release, Cbl slowly enters into the portal vein bound to transcobalamin II (TCII), the physiologically important circulating Cbl-binding protein. Inherited defects at several of these steps are known.

Clinical Presentation

Hereditary Intrinsic Factor Deficiency. Megaloblastic anemia is the principal symptom [6, 7]. It appears only after depletion of the fetal hepatic Cbl stores, usually after the first year of life but before the age of 5 years. In cases of partial deficiency, clinical presentation has appeared as late as at the age of 12 years. The patients present with failure to thrive, often with vomiting and alternating diarrhea and constipation, anorexia, and irritability. On examination they are anemic,

have stomatitis or atrophic glossitis, developmental delay, and myelopathy. Hepatosplenomeglay may be present.

Defective Transport of Cobalamin by Enterocytes (Imerslund-Gräsbeck Syndrome). Megaloblastic anemia usually also presents once fetal hepatic Cbl stores have been depleted, mostly between the ages of 1 and 5 years, although it can appear later [8–12]. Most patients have associated proteinuria, and in a few cases this is of the tubular type with all species of protein represented rather than albumin alone. The literature on the renal pathology has been reviewed recently [13]. Patients who excrete protein during childhood continue to excrete protein in adulthood. The renal lesions are not progressive [9]. Neurological abnormalities such as spasticity, truncal ataxia, and cerebral atrophy may also be present [12].

Transcobalamin II Deficiency. TCll-deficient patients usually develop symptoms much earlier than patients with other causes of Cbl malabsorption, mostly within the first few months of life. Nevertheless, even though the only TCII in cord blood is of fetal origin, patients are not clinically deficient at birth. Symptoms include pallor, failure to thrive, diarrhea, and weakness. Although the anemia is usually megaloblastic, patients have been described with pancytopenia or isolated erythroid hypoplasia. Because of the presence of immature white cell precursors in an otherwise hypocellular marrow, leukemia may be mistakenly diagnosed. Approximately 6–30 months following the onset of symptoms, neurological findings appear, including developmental retardation, neuropathy, myelopathy, and encephalopathy. Defective granulocyte function has been seen along with severe immunological deficiency with both defective humoral and cellular immunity.

R-Binder Deficiency. This defect has been described in the plasma, saliva, and leukocytes of a small number of patients [14] with predominantly neurological, specifically myelopathic symptoms, rather than hematological findings.

Metabolic Derangement

Hereditary Intrinsic Factor Deficiency. IF is either absent or nonfunctional. It may be detectable immunologically. There has been a report of an IF which is more sensitive to destruction by acid and pepsin and has a low affinity for Cbl [7].

Defective Transport of Cobalamin by Enterocytes. In a number of patients an apparent absence of the ileal receptor has been described. In one sibship, however, homogenates of ileal biopsies bound IF–Cbl normally, suggesting that the defect does not lie in the absence of receptors.

Transcobalamin II Deficiency. Functional TCII, as determined by an ability to bind Cbl, was absent in all but one patient. Nevertheless, immunologically reactive TCII was found in three patients. TCII from another patient was able to bind Cbl, but the TCII–Cbl complex did not mediate Cbl incorporation into cells.

R-Binder Deficiency. Serum Cbl levels are low because most circulating Cbl is bound to TCI. However, TCII–Cbl levels are normal and the patients do not have Cbl deficiency. It is therefore unclear whether the TCI deficiency is the cause of disease in these patients. A role for TCI has been postulated in the scavenging of potentially toxic Cbl analogues and in protecting the brain from the effects of these analogues.

Diagnostic Tests

The hematological abnormalities in defects of Cbl absorption and transport will be picked up by measurement of red blood cell indices along with a complete blood count and a bone marrow examination. A deoxyuridine suppression test on marrow cells is useful, but not easily available in most clinical laboratories. This test measures the incorporation of label from thymidylate into a tricholoroacetic acid precipitate before and after an incubation of washed bone marrow cells in an excess of deoxyuridine. In the presence of folate or Cbl deficiency, this preincubation reduces incorporation to only 30%–40% of that observed in the absence of the preincubation in deoxyuridine, as compared to about 10% when there is no folate or Cbl deficiency. Serum Cbl and folate levels are useful, and measurement of homocyst(e)ine and methylmalonic acid in urine or plasma is essential. Even in the presence of normal serum Cbl levels, specific measurement of serum TCII must be

done. A Schilling test performed both with and without intrinsic factor is also important.

Inherited Intrinsic Factor Deficiency. Megaloblastic anemia is present, associated with a low serum total Cbl level. Homocystinuria and methylmalonic aciduria may be present. There is normal gastric acid excretion and normal gastric cytology. No or inactive IF is found. Cbl absorption, as measured by the Schilling test, is abnormal, but is normalized when the Cbl is mixed with a source of normal IF, such as human gastric juice from an unaffected individual.

Defective Transport of Cobalamin by Enterocytes. The Schilling test for Cbl absorption is also abnormal, but is not corrected by exogenous IF. Gastrointestinal morphology, pancreatic function, and IF are normal and no IF autoantibodies are found.

Transcobalamin II Deficiency. Homocystinuria and methylmalonic aciduria have been incidentally found. Total serum Cbl is not decreased. The Schilling test of Cbl absorption is usually abnormal [15]. In those cases where the Schilling test was normal, immunoreactive TCII was found.

Treatment and Prognosis

Hereditary Intrinsic Factor Deficiency. This can be initially treated with daily hydroxycobalamin (OH-Cbl) supplementation to replete body stores until biochemical and hematological values normalize. The subsequent dose of OH-Cbl required to maintain normal values may then be as low as 0.25 mg (250 μg) every 3 months. While biochemical and hematological parameters may completely normalize, neurological findings may not be completely corrected if treatment is delayed.

Defective Transport of Cobalamin by Enterocytes. Treatment with systemic OH-Cbl corrects the anemia and neurologic findings, but not the associated proteinuria. As with intrinsic factor deficiency, once Cbl stores are replete, low doses of systemic OH-Cbl may be sufficient to maintain normal biochemical and hematological values.

Transcobalamin II Deficiency. To treat successfully, it is necessary to maintain very high serum Cbl levels, in the range of 1–10 ng/ml (1000–10 000 pg/ml). These have been achieved with doses of oral or systemic OH-Cbl or cyanocobalamin (CN-Cbl) of 0.5–1.0 mg twice weekly. Initially, it is not unreasonable to begin with a dose of 1 mg OH-Cbl per day intramuscularly and then to reduce the dose to once or twice weekly once a hematological response is obtained. Folate in the form of folic acid or formyltetrahydrofolate (folinic acid) has been used in large doses (up to 15 mg orally four times daily) to reverse the hematological findings. Because folate does not correct the neurological findings and may result in hematological relapse, it should not be given without giving Cbl concurrently.

Genetics

The inheritance of the disorders of Cbl transport is autosomal recessive. The Imerslund-Gräsbeck syndrome is most commonly found in Finland and among North African (Sephardic) Jews, although it is not restricted to these ethnic groups and approximately 150 cases have been reported [12–13]. TCII deficiency has been found in 28 patients [1, 16, 17]. There are five normal electrophoretic isoforms of TCII and three others which are associated with TCII deficiency. Using the segregation pattern of these isoforms, it has been possible to genetically link TCII to the P blood group locus on chromosome 22 [18]. The DNA for human TCII has been cloned and deficiency may be due to deletions or point mutations [19, 20].

Disorders of Intracellular Utilization of Cobalamin

Intracellular metabolism of Cbl (Fig. 1) is initiated by uptake of the TCII–Cbl complex by receptor-mediated endocytosis. The complex enters lysosomes, where TCII is degraded. Free Cbl is transported from the lysosomes into the cytoplasm. Once in the cytoplasm, the central cobalt of Cbl is reduced from a 3^+ to a 2^+ oxidation state. Divalent cobalamin is then in part reduced and methylated to yield MeCbl in the cytoplasm and in part transported into the mitochondria, where it is reduced and adenosylated to yield AdoCbl [1–5]. Inherited defects at several of these steps are known which, depending on their location, result in combined deficiency of AdoCbl and MeCbl or of isolated deficiency of AdoCbl or MeCbl. The

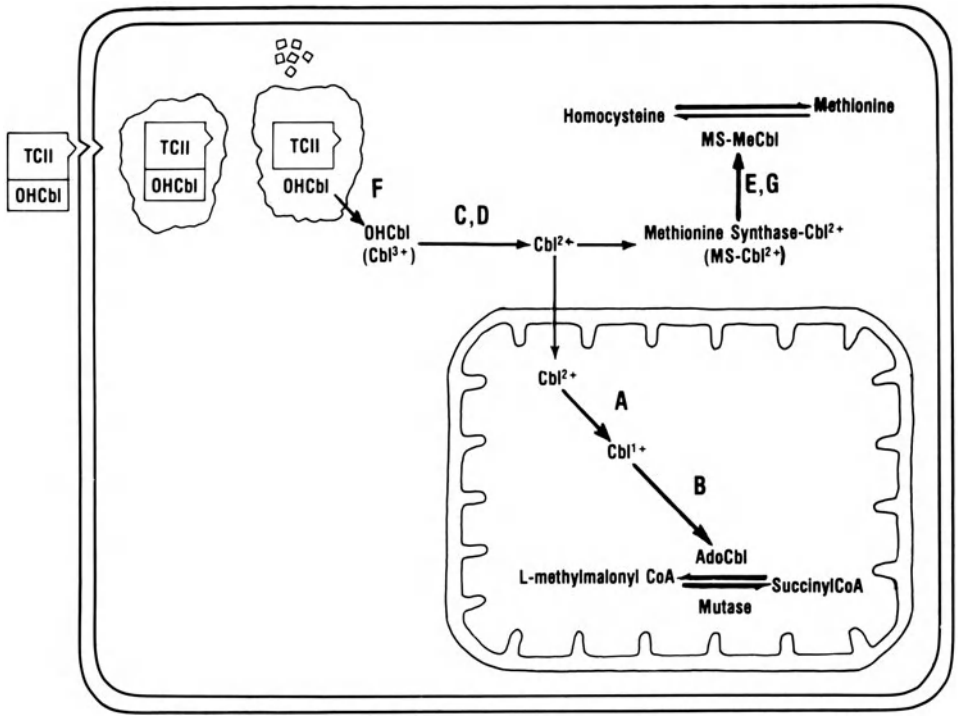

Fig. 1. Intracellular cobalamin (*Cbl*) endocytosis and metabolism. *Letters* refer to the presumed sites of blocks that have been defined by complementation analysis. $^{1+}$, $^{2+}$, $^{3+}$ refer to the oxidation state of the central cobalt of Cbl. The cytoplasmic, mitochondrial, and lysosomal compartments are indicated. *TC*, transcobalamin; *MeCbl*, methylcobalamin; *AdoCbl*, 5-deoxyadenosylcobalamin. (Reprinted with permission from [4])

defects are lettered according to the order of their discovery.

Clinical Presentation

Combined Deficiency of 5-Deoxyadenosylcobalamin and Methylcobalamin. Three disorders, cblC, cblD, and cblF, result in functional defects of both methylmalonyl-CoA mutase and methionine synthase and are therefore characterized by both *methylmalonic acidemia* and *homocyst(e)inemia.*

cblC. This is the most frequent form, with more than 75 patients known [21–23]. Many are acutely ill in the first month of life and most are diagnosed within the first year. Major findings in this early-onset group included feeding difficulties, failure to thrive, developmental delay, microcephaly, seizures, and hypotonia. Some patients showed macrocytic anemia and leukopenia. Less frequent findings included dementia, myelopathy, ventricular septal defects, thrombocytopenia, microcytic anemia, and microthrombotic disease. An unusual retinopathy has been described, consisting of perimacular hypopigmentation surrounded by a hyperpigmented ring and a salt and pepper retinopathy more peripherally. Nystagmus may be present. Hydrocephalus, hepatic dysfunction, and hemolytic uremic syndrome have been reported. A small number of cblC patients are not diagnosed until after the first year of life and as late as the end of the second decade of life. The earlier diagnosed patients in this group have overlapping findings with those found in the first year of life. Major clinical findings in this late-onset cblC group include confusion and disorientation and gait abnormalities resulting from myelopathy. Macrocytic anemia is seen in only about one third of the older patients, thus serum metabolite levels are essential for accurate diagnosis.

cblF. Of the five known patients with cblF disease, four had clinical findings in the first year of life. The original infant girl had glossitis and stomatitis in the first week of life [24, 25]. She had severe feeding difficulties requiring tube feeding. Tooth abnormalities and dextrocardia were present.

Other clinical findings in cblF have included anemia, failure to thrive, recurrent infections, developmental delay, lethargy, hypotonia, aspiration pneumonia, hepatomegaly, and encephalopathy. One infant suddenly died at home in the first year of life. One boy developed juvenile rheumatoid arthritis at the age of 4 years and a pigmented skin abnormality at 10 years.

cblD. There is only one reported sibship of two males with cblD [26–28]. The elder sibling was diagnosed at the age of 14 years with behavioral problems and mild mental retardation. Thromboembolic cerebrovascular disease later developed. Homocystinuria, methylmalonic aciduria, and hyperglycinemia was noted. Although there was no megaloblastic anemia, the deoxyuridine suppression test was abnormal.

Adenosylcobalamin Deficiency. This comprises two disorders, cblA and cblB, characterized by Cbl-responsive methylmalonic aciduria. The phenotype resembles methylmalonyl-CoA mutase deficiency. Most patients have an acidotic crisis in the first year of life, many in the neonatal period. Symptoms are related to methylmalonic acid accumulation and include vomiting, dehydration, tachypnea, lethargy, failure to thrive, developmental retardation, hypotonia, and encephalopathy. The toxic levels of methylmalonic acid may also result in bone marrow abnormalities and produce anemia, leukopenia, and thrombocytopenia. Hyperammonemia, hyperglycinemia, hypoglycemia, and ketonuria may also be found.

Methylcobalamin Deficiency. The comprises two disorders, cblE and cblG, characterized by homocyst(e)inemia and homocystinuria in the absence of methylmalonic acid acidemia [29–32]. Only one cblE patient had transient methylmalonic aciduria. Hypomethioninemia and cystathioninemia may be present and there may be increased serine in the urine. Symptoms begin early (70% prior to 3 months of age) and findings include poor feeding, vomiting, failure to thrive, developmental delay, nystagmus, hypotonia or hypertonia, ataxia, seizures, and blindness. Cerebral atrophy may be seen on imaging studies of the central nervous system, and at least one cblE patient showed a spinal cord cystic lesion on autopsy. One cblG patient was diagnosed much later, at the age of 21 years, and carried a misdiagnosis of multiple sclerosis [33]. Neuropsychiatric symptoms have been noted in another patient who was not diagnosed until he was in his thirties.

Metabolic Derangement

Combined Deficiency of 5-Deoxyadenosylcobalamin and Methylcobalamin. In the cblC and cblD disorders, patients are thought to have a defect in the reduction of the central cobalt of Cbl from a 3^+ to a 2^+ oxidation state after efflux of the Cbl from the lysosome. If this reduction does not occur, methylation or adenosylation does not proceed and neither AdoCbl nor MeCbl is formed.

In cblF, the defect appears to be due to a failure of Cbl to be transported across the lysosomal membrane following degradation of the TCII in the lysosome. As a result, Cbl cannot be converted to either AdoCbl or MeCbl. The inability of CblF patients to absorb oral Cbl suggests that IF–Cbl also has to pass through a lysosomal stage in the enterocyte before Cbl is released into the portal circulation.

5-Deoxyladenosylcobalamin Deficiency. In cblA, the defect presumably lies in the reduction of the central cobalt of Cbl from the 2^+ to the 1^+ oxidation state in the mitochondria. The defect in cblB is in the adenosyltransferase-catalyzed reaction, the final intramitochondrial step in the synthesis of AdoCbl [34].

Methylcobalamin Deficiency. In cblE, the defect appears to be in a reducing system for Cbl that is associated with methionine synthase, whereas the defect in cblG may lie in the methionine synthase apoenzyme itself.

Diagnostic Tests

Diagnosis of disorders of intracellular Cbl metabolism is based first on determinations of homocyst(e)ine, methylmalonic acid, Cbl, and methionine in plasma. Precise diagnosis requires tests in cultured fibroblasts. The incorporation of [^{14}C]propionate in macromolecules is a good screen for integrity of the methylmalonyl-CoA mutase reaction, and the incorporation of [^{14}C]methyl-tetrahydrofolate is a good screen for the function of methionine synthase. The conver-

sion of labeled CN–Cbl to both MeCbl and AdoCbl can be measured following hot ethanol extraction using high-performance liquid chromatography. Complementation analysis is used to define the specific mutant class. Cells from the undiagnosed patient are cocultivated with cells from patients with known defects, and replicate cultures are either treated or not with polyethylene glycol, which acts as a cell-fusing agent. If cells belong to the same class, incorporation of labeled substrates (methyl-THF or propionate) will not increase in fused cells, but if the cell lines belong to different classes fusion results in partial correction of the incorporation defect, implying mutations at distinct genetic loci.

Combined Deficiency of 5-Deoxyadenosylcobalamin and Methylcobalamin. In cblC, homocyst(e)inemia and methylmalonic acidemia are the biochemical hallmarks. In general, levels are lower than in methylmalonyl-CoA mutase deficiency, but higher than in the Cbl transport defects. Plasma methionine levels are either normal or decreased.

In cblD, the biochemical findings in cblD are identical to those seen in cblC. The total incorporation of [^{57}Co]CN–Cbl by fibroblasts can differentiate cblC and cblD cells (low incorporation) from cblF cells (high incorporation). In addition, cblC and cblD cell lines form distinct complementation classes on somatic cell hybridization.

In cblF, both homocyst(e)inemia and methylmalonic aciduria are expected. The original cblF patient was never demonstrated to have elevated homocysteine, and methylmalonic aciduria was picked up on newborn screening. Macrocytic anemia, neutropenia, and thrombocytopenia may be found. The Schilling test has been abnormal in all patients tested, and serum Cbl may be low.

5-Deoxyadenosylcobalamin Deficiency. Total serum Cbl is usually normal and there is massive methylmalonic aciduria (0.8–1.7 mmol/day; normal, <0.04 mmol/day) but no homocystinuria. The differentiation of cblA and cblB from methylmalonyl-CoA mutase deficiency may be made by biochemical studies in cultured cells, by complementation analysis, or by the failure of cblA or cblB cells to increase propionate incorporation following transfection by a vector containing cloned mutase cDNA. A patient has been described with all the clinical and biochemical features of cblA but cells from this patient complemented those from other cblA patients. This implies that more than one step may be involved in the intramitochondrial reduction of Cbl.

Methylcobalamin Deficiency. Homocyst(e)inemia and homocystinuria are found in the absence of methylmalonic acidemia. Differential diagnosis between cblE and cblG may be made by assay of methionine synthase in fibroblast extracts or by complementation analysis. On incubation with labeled Cbl, both cblE and CblG cell lines fail to synthesize MeCbl and both have decreased incorporation into macromolecules of label from [^{14}C]methyl-THF.

Treatment and Prognosis

Combined Deficiency of 5-Deoxyadenosylcobalamin and Methylcobalamin. In cblC, treatment of early-onset patients with 1 mg/day OH-Cbl parenterally decreases the elevated metabolite levels, but these are usually not completely normalized. In one comprehensive study, oral OH-Cbl was found not to be sufficient and both folic acid and carnitine were ineffective. Daily oral betaine (250 mg/kg per day) along with twice-weekly OH-Cbl (1 mg/day) resulted in normalization of methionine and homocysteine levels and decreased methylmalonic aciduria [35].

On follow-up of a group of 44 early-onset patients, 11 had died, and of the surviving patients only one was well, whereas the others had severe or moderate neurological impairment. Of the six late-onset patients on whom we have follow-up data, only one is minimally impaired neurologically.

In cblF, treatment with parenteral OH-Cbl first daily and then biweekly at a dose of 1 mg/day seems to be effective in correcting both metabolic and clinical findings. Despite the fact that two Schilling tests showed an inability to absorb Cbl with or without IF, the original patient responded to oral Cbl before being switched to parenteral Cbl.

5-Deoxyadenosylcobalamin Deficiency (cblA and cblB). These patients respond to protein restriction and to OH-Cbl supplementation. Some patients appear to become resistant to Cbl treatment. Therapy with AdoCbl has been attempted in cblB with and without success, and it may be that AdoCbl does not reach the target

enzyme intact. There has been one report of prenatal therapy with Cbl in AdoCbl deficiency. Most (90%) cblA patients improve on Cbl therapy with 70% doing well long term. Only 40% of cblB patients respond to Cbl, and the long-term survival is poorer [36].

Methylcobalamin Deficiency (cblE and cblG). Both these disorders are usually treated with OH-Cbl, at first daily and then once or twice weekly. Although the metabolic abnormalities are nearly always corrected, it is difficult to reverse the neurological findings once they have developed. Treatment with betaine (250 mg/kg per day) and MeCbl has also been attempted. One cblG patient has been treated with L-methionine (40 mg/kg per day) and has had neurological improvement. Many patients with cblG do not do well despite therapy.

Genetics

Both males and females have been described with cblC and cblF and inheritance is probably autosomal recessive. Prenatal diagnosis of cblC has been made using cultured amniocytes and excluded using chorionic villus biopsy and cultured cells. As both siblings with cblD are male, the possibility of sex linkage has not been ruled out. Autosomal recessive inheritance is presumed in both cblA and cblB, since male and female patients have been described and parents of cblB patients have decreased adenosyltransferase activity. Only two of nine cblE patients have been male, but there have been roughly equal numbers of male and female cblG patients. It is therefore thought that both are autosomal recessive disorders. Decreased methyltetrahydrofolate incorporation has been detected in fibroblasts from both of the parents of the original proband with cblE. Prenatal diagnosis was performed on amniocytes in this family, and the mother was treated with OH-Cbl supplementation beginning in the second trimester and the baby was subsequently treated from birth on with good results.

Folate

The conversion of the vitamin folic acid, especially plentiful in liver, yeast, and green plants, into reduced folates is essential for the endogenous synthesis of purines, thymidylate, and DNA and in the metabolism of glycine, serine, and methionine (Fig. 2). Defects of folate absorption and metabolism thus decrease purine and pyrimidine synthesis, the presumed reason for the hematologic findings in these disorders. The common hematologic findings in folate and cobalamin disorders are the consequence of the fact that MeCbl is an obligatory cofactor for methionine synthase. Operation of the latter reaction is essential for recycling of THF into its reduced derivatives required for the synthesis of thymidylate and purines.

There are only three confirmed inborn errors of folate absorption and metabolism [3, 37, 38]:

- Hereditary folate malabsorption
- Methylene-THF reductase deficiency
- Glutamate formiminotransferase deficiency

Patients present with megaloblastic anemia and/or global developmental delay. Reports of patients with deficiencies of dihydrofolate reductase, methenyl-THF cyclohydrolase, and methionine synthase without homocystinuria (not cblG) have not been confirmed and should be considered with caution.

Clinical Presentation

Hereditary Folate Malabsorption. Patients present in the first few months of life with severe megaloblastic anemia, failure to thrive, gastrointestinal manifestations such as stomatitis and diarrhea, and usually progressive neurological deterioration with frequent seizures.

Glutamate Formiminotransferase Deficiency. Over a dozen patients with this disorder have been reported, but its clinical significance is still unclear [3]. Two different phenotypes have been described. In the severe form of glutamate formiminotransferase deficiency there is both mental and physical retardation, seizures, and dilatations of cerebral ventricles with cortical atrophy. Several of the patients had a folate-responsive megaloblastic anemia with macrocytosis and hypersegmentation of neutrophils. In the mild form of glutamate formiminotransferase deficiency, there is no mental retardation but mild developmental disability or speech delay. Age at diagnosis has ranged from 3 months to 42 years.

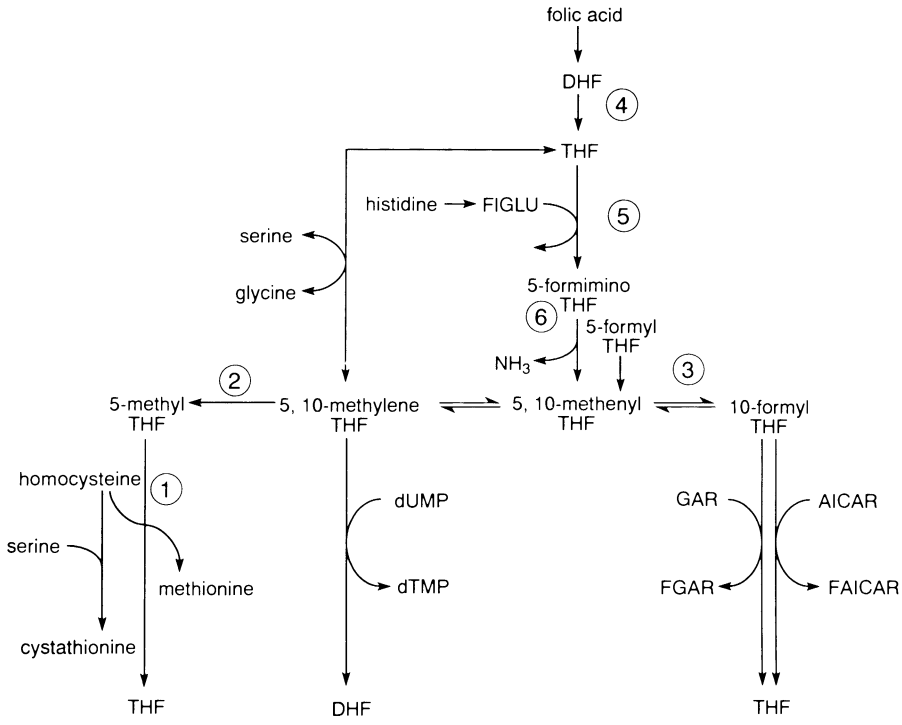

Fig. 2. Folate metabolism. *1*, Methionine synthase; *2*, methylenetetrahydrofolate reductase; *3*, methenyltetrahydrofolate cyclohydrolase; *4*, dihydrofolate reductase; *5*, Glutamate formiminotransferase; *6*, formiminotetrahydrofolate cyclodeaminase. *THF*, tetrahydrofolate; *FIGLU*, formiminoglutamate; *dUMP*, deoxyuridine monophosphate; *dTMP*, deoxythymidine monophosphate; *DHF*, dihydrofolate; *GAR*, glycinamide ribotide; *AICAR*, aminoimidazole carboxamide ribotide; *FGAR*, formylglycineamide ribotide; *FAICAR*, formyl aminoimidazole carboxamide ribotide

Methylenetetrahydrofolate Reductase Deficiency. More than 30 patients are known with methylene-THF reductase deficiency [37, 39, 40]. Most commonly the patients are diagnosed in infancy and more than half present in the first year of life. The most frequent clinical manifestation is developmental delay with recurrent apneic episodes, microcephaly, and seizures. Patients may become symptomatic at any time from infancy to adulthood and in the older patients psychomotor delay, gait abnormalities, psychiatric disorders (schizophrenia), and symptoms related to cerebrovascular events have been reported. At least one adult with severe enzyme deficiency has been completely asymptomatic. Autopsy findings have included dilated cerebral vessels, microgyria, and hydrocephalus as well as perivascular changes, demyelination, gliosis, astrocytosis, and macrophage infiltration. In some patients thrombosis of both cerebral arteries and veins were the major causes of death. Findings similar to those seen in subacute degeneration of the spinal cord due to Cbl deficiency have been reported. Of note is the fact that methylene-THF reductase deficiency is not associated with megaloblastic anemia.

Metabolic Derangement

Hereditary Folate Malabsorption. All patients have severely decreased absorption of oral folic acid or reduced folates such as formyl-THF (folinic acid) or methyl-THF. The patients provide the best evidence for the existence of a single transport system for folate at the level of both the intestine and the choroid plexus. Indeed, cerebrospinal fluid folate levels remained low even when treatment resulted in blood levels high enough to correct the megaloblastlc anemla, except in one case [41]. Transport of folates across other cell membranes is not affected as the hematological and gastrointestinal manifestations are corrected by relatively low levels of folate, and folate metabolism in cultured fibroblasts is not abnormal.

Glutamate Formiminotransferase Deficiency. Histidine catabolism is associated with a for-

mimino group transfer to THF with the subsequent release of ammonia and the formation of 5,10-methenyl-THF (Fig. 2). A single octameric enzyme catalyzes two different activities, glutamate formiminotransferase and formimino-THF cyclodeaminase. These activities are found only in liver and kidney and defects in either of these activities will result in formiminoglutamate (FIGLU) excretion. It has been suggested, without any direct enzyme measurements, that the severe form of this disease is due to a block in the cyclodeaminase activity, whereas the mild form is due to a block in the formiminotransferase activity.

Methylenetetrahydrofolate Reductase Deficiency. The block in the conversion of 5,10-methylene-THF to 5-methyl-THF (Fig. 2) does not result in trapping of folates as methyl-THF and does not interfere with the availability of reduced folates for purine and pyrimidine synthesis. This explains the absence of megaloblastic anemia in methylene-THF reductase deficiency. Nevertheless, because methyl-THF is the major circulating form of folate, serum folate levels may sometimes be low. Moreover, methyl-THF is the methyl donor for the conversion of homocysteine to methionine. The deficiency thus results in an elevation of homocysteine and a decrease of methionine. It is not clear whether the neuropathology in this disease results from the elevated homocysteine levels, from decreased methionine and a resulting interference with methylation reactions, or from some other metabolic effect.

Diagnostic Tests

Hereditary Folate Malabsorption. Measurements of serum, red blood cell, and cerebrospinal fluid folate should be performed along with a complete study of erythrocyte indices and a bone marrow examination if necessary. The most important diagnostic features are the severe megaloblastic anemia in the first few months of life together with low serum folate levels. Excretion of FIGLU and of orotic acid may be seen. Folate absorption may be looked for directly by measuring serum folate following an oral dose of folic acid (doses used range between 5 and 100 mg).

Glutamate Formiminotransferase Deficiency. Megaloblastic anemia is found, with normal to high serum levels of folate. Elevated FIGLU excretion as well as elevated levels of FIGLU in the blood following a histidine load have been reported. Although plasma amino acid levels were usually normal, on occasion hyperhistidinemia and histidinuria have been reported. Two other metabolites that may be found in urine are hydantoin-5-propionate, a stable oxidation product of the FIGLU precursor 4-imidazolone-5-propionate, and 4-amino-5-imidazolecarboxamide, an intermediate of purine synthesis. Glutamate formiminotransferase activity is expressed only in liver. It is not expressed in cultured fibroblasts, and there is considerable debate as to whether it is expressed in red blood cells. The residual activity that has been measured in the livers of five patients have varied from 14% to 54% of control values, and these levels are higher than would be expected for an enzymatic block causing disease.

Methylenetetrahydrofolate Reductase Deficiency. Homocystinuria is seen in all patients with a mean of 130 μmol/24 h and a range of 15-667 μmol/24 h. These values are much lower than those seen in cystathionine synthase deficiency. Plasma methionine levels have ranged from 0 to 18 μM (mean, 12 μM; normal range, 23–35 μM). Neurotransmitter levels, measured in only a few patients, are usually low. Methylene-THF reductase activity can be measured in liver, leukocytes, lymphocytes, and cultured fibroblasts. In cultured fibroblasts, the specific activity is highly dependent on the stage of the culture cycle, with activity highest in confluent cells. There is a rough inverse correlation between specific activity of the reductase in cultured fibroblasts and clinical severity and an even better inverse correlation between clinical severity and either the proportion of total cellular folate which is methyl-THF or the extent of labeled formate incorporation into methionine. The clinical heterogeneity in reductase deficiency can be seen at the biochemical level. Some of the patients have residual enzyme, which is more thermolabile than the control enzyme [42].

Observations on thermolabile reductase have been carried into the adult population, and it has been postulated that a thermolabile allele of reductase without severe enzyme deficiency and without any of the clinical findings in reductase deficiency may be an independent risk factor for vascular disease and coronary heart disease. Some of these patients may be ascertained because they

have elevated levels of total plasma homocysteine [43, 44].

Treatment and Prognosis

Hereditary Folate Malabsorption. In some patients pharmacologic doses of oral folate (2–40 mg/day) corrected the hematologic findings. Parenteral folates reverse the anemia in these patients, but are less effective in correcting the neurological findings and in raising the level of folate in the cerebrospinal fluid. Both methyl-THF and folinic acid may be more effective in getting across the blood – brain barrier. The clinical response to folates has varied among patients and in some cases seizures were made worse after folate therapy was started. It is important to maintain blood and cerebrospinal fluid folate in the normal range. Oral doses of folate may be increased to 100 mg/day if necessary, and if oral therapy does not rise folate levels parenteral therapy can be used. Consideration of the use of intrathecal folate therapy has been suggested if cerebrospinal fluid levels of folate cannot be raised by other treatments. A number of patients have done well on parenteral folic acid or folinic acid treatment.

Glutamate Formiminotransferase Deficiency. It is not clear whether reducing FIGLU excretion is of any clinical value since the degree of excretion does not correspond to clinical severity. Although two patients in one family responded to folate therapy by reducing excretion of FIGLU, six others did not. One of two patients responded to methionine supplementation. Pyridoxine and folic acid have been used to correct the megaloblastic anemia in one infant.

Methylenetetrahydrofolate Reductase Deficiency. It is important to diagnose the enzyme defect early because in the infantile forms the only patients that have done well have been those who have been treated essentially from birth. Early treatment with betaine following prenatal diagnosis has resulted in the best outcome to date [45, 46]. Betaine is a substrate for betaine methyltransferase, an enzyme which converts homocysteine to methionine, but which is mainly active in the liver (see Andria and Sebastio, this volume). Betaine may then be expected to have the doubly beneficial effect of lowering homocysteine levels and raising methionine levels. Because betaine methyltransferase is not present in the brain, the central nervous system (CNS) effects must be mediated through the effects of the circulating levels of metabolites. Other therapeutic agents include folic acid or reduced folates methionine, pyridoxine, Cbl, and carnitine. Most of the treatment protocols omitting betaine have not been effective.

Genetics

Hereditary Folate Malabsorption. Of the 12 known patients with hereditary folate malabsorption, all but one have been girls. The one male has atypical findings, including no mental retardation and rapid correction of cerebrospinal fluid folate levels on correction of serum folate levels. Consanguinity has been noted in four families, and the father of one of the patients had intermediate levels of folate absorption, making autosomal recessive inheritance likely. The defect in hereditary folate malabsorption is not expressed in amniocytes or chorionic villus cells and thus this disorder cannot be presently diagnosed prenatally.

Glutamate Formiminotransferase Deficiency. Glutamate formiminotransferase deficiency has been found in both male and female children of unaffected parents. The disease is presumably inherited in an autosomal recessive manner. Because of the lack of expression of the enzyme in cultured cells, prenatal diagnosis has not been possible, but it may be possible to look directly at FIGLU levels in amniotic fluid. This has not been reported. A cDNA has been cloned for the glutamate formiminotransferase, formimino-THF cyclodeaminase, and molecular analysis of the patients should be possible once the human gene structure has been determined.

Methylenetetrahydrofolate Reductase Deficiency. The deficiency is inherited as an autosomal recessive disorder. There have been multiple affected children of both sexes with unaffected parents and affected families with consanguinity. Prenatal diagnosis had been reported using amniocytes and the enzyme is present in chorionic villi. Recently, a partial cDNA clone (1.2 kb) has been isolated and the gene coding for methylene-THF reductase has been localized to chromosome 1p36.3. Two missense mutations in residues that are conserved in bacteria and one nonsense mutation have been identified, confirming heterogeneity at the molecular level.

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Part VIII
Lipids

Abbreviations

ABL:	abetalipoproteinemia
ACAT:	acyl-Coenzyme A: cholesterol aclytransferase
apo:	apolipoprotein
CE:	cholesteryl esters
CESD:	cholesteryl ester storage disease
CETP:	cholesteryl ester transfer protein
CHD:	coronary heart disease
CM:	chylomicron
CMR:	chylomicron remnant
FCH:	familial combined hyperlipidemia
FDB:	familial defective apo B
FED:	fish-eye disease
FFA:	free fatty acids
FHALP:	farnilial hypoalphallpoproteinemia
FHC:	familial hypercholesterolemia
FHTG:	familial hypertriglyceridemia
HBL:	hypobetalipoproteinemia
HC:	hypercholesterolemia
HDL:	high density lipoproteins
HDL-C:	HDL-cholesterol
HL:	hepatic lipase
HLP:	hyperlipoproteinemia
HMG-CoA:	3-hydroxymethylglutaryl Coenzyme A
HTG:	hypertriglyceridemia
IDL:	intermediate density lipoproteins
IEF:	isoelectric focusing
LAL:	lysosomal acid lipase
LCAT:	lecithin:cholesterol acyltransferase
LDL:	low density lipoproteins
LDL-C:	LDL-cholesterol
Lp(a):	lipoprotein(a)
LPL:	lipoprotein lipase
MI:	myocardial infarction
MTTP:	microsomal triglyceride transfer protein
PCR:	polymerase chain reaction
TG:	triglycerides
TGRL:	triglyceride-rich lipoproteins
UC:	unesterified cholesterol
VLDL:	very low density lipoproteins

Dyslipidemias

G. Assmann, A. von Eckardstein, and P. Cullen

CONTENTS

Genetic dyslipidemias are caused by defects of structural components of lipoproteins, enzymes, or cell surface receptors which affect the formation, modification, and/or removal of lipoproteins. To better understand the pathophysiology of the dyslipidemias and the approaches to their treatment, we shall briefly summarize the major features of lipoprotein metabolism using the classical concept of Goldstein and Brown [1].

Overview of Lipoprotein Metabolism

Transport of Exogenous Lipids

Epithelial cells of the small intestine synthesize apolipoproteins (apo) A-I, A-IV, and B-48 as well as triglyceride (TG) from glycerol and fatty acids derived from dietary fat. These components, together with small amounts of dietary cholesterol, phospholipids, and cholesteryl esters (CE), are assembled into chylomicrons (CM). These large triglyceride-rich lipoproteins (TGRL) with a density below 0.95 g/ml are secreted into lymph, which reaches the venous circulation via the thoracic duct. In both lymph and the systemic circulation, CM become further enriched with water-soluble apolipoproteins including apo C-II, apo C-III, and apo E. In the capillaries, especially those of skeletal muscle, the heart, and adipose tissue, TG of CM are hydrolyzed by lipoprotein lipase (LPL). This enzyme is mainly synthesized and secreted by myocytes and adipocytes, crosses the endothelial cells, and is bound by heparan sulfate on the luminal side of the vascular endothelium. The presence of apo C-II is an absolute requirement for activation of lipoprotein lipase. Lipolysis of CM gives rise to two particles:

- Core remnants which are rich in CE and which bear apo B-48, apo C, apo E, and small amounts of LPL on their surface

- Phospholipid-rich surface remnants which carry apo A-I, apo A-IV, and apo C and which contribute to the formation of HDL

The presence of apo E and LPL makes core remnants available for receptor-mediated uptake into the liver. Two receptors appear to be involved in the catabolism of core remnants, the low-density lipoprotein (LDL; apo B, E) receptor and a chylomicron remnant (apo E) receptor, which very likely corresponds to the LDL receptor-related protein (LRP). LPL appears to facilitate the binding of remnants to the receptor by interaction with proteoglycans on liver cells, whereas apo E is the specific ligand of both receptors. In normal individuals lipolysis of CM and remnant removal is completed approximately 12 h after the ingestion of dietary fat (reviewed in [1, 2]).

Transport of Endogenous Lipids

Ninety percent of the TG in fasting blood is synthesized by the liver and secreted as a component of very low density lipoproteins (VLDL). VLDL have a density of 0.95–1.006 g/ml and consist of 60%–70% TG, 10%–15% cholesterol and phospholipids, and 10% protein. Each mole of VLDL contains 1 mol apo B-100 and several moles of apo C-I, C-II, C-III, and E. In the circulation, lipolysis of VLDL by LPL forms intermediate-density lipoproteins (IDL; density, 1.006–1.019 g/ml), which are analogous to CM core remnants. IDL are either taken up by hepatic remnant and apo B,E receptors or further lipolyzed by hepatic lipase (HL) to LDL. The CE-rich LDL have a density of 1.019–1.063 g/ml and contain as their sole apolipoprotein apo B-100, by means of which they are recognized by the LDL receptor and catabolized. LDL receptors are expressed by all cells, but are most abundant on hepatocytes. Thus, in a normal individual, 80% of LDL are catabolized by the liver. Bound LDL and LDL receptor are internalized and enter the endosomal–lysosomal pathway. Lysosomal acid lipase (LAL) hydrolyzes CE. The released cholesterol is an important regulator of cellular cholesterol homeostasis. First, it downregulates the key enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase; second, it downregulates de novo synthesis and recycling of LDL receptors; third, it activates the intracellular cholesterol-esterifying enzyme acyl-CoA:cholesterol-acyltransferase (ACAT). Together these regulatory processes prevent an unlimited increase in the intracellular cholesterol concentration. In the extracellular compartment these negative feedback mechanisms contribute to the pathogenesis of hypercholesterolemia (HC), e.g., when uptake of excess dietary cholesterol decreases LDL receptor expression or when defective LDL – LDL receptor interactions increase the hepatic synthesis of cholesterol [1].

Both the lipid and protein moieties of LDL can be oxidatively modified. This process takes place in the arterial wall and forms epitopes on apo B which are recognized by scavenger receptors on macrophages. Since these receptors are not down regulated by increasing intracellular cholesterol concentration, macrophages can accumulate large amounts of CE, which changes them into foam cells. The uptake of modified LDL by macrophages of the arterial wall also leads to the synthesis of a series of cytokines, growth factors, and adhesion molecules as well as of substances which are toxic for the endothelium and which together contribute to atherogenesis [3, 4].

High-Density Lipoproteins and Reverse Cholesterol Transport

The reverse cholesterol transport model describes the transport of cholesterol from peripheral cells to the liver by high-density lipoproteins (HDL). HDL precursors are generated through lipolysis of CM and VLDL as well as through direct secretion by the liver. Different subfractions can take up excess cellular cholesterol in a stepwise process. It is generally accepted that unesterified cholesterol (UC) diffuses from the cell membrane to HDL via nonspecific lipid–lipid interactions and that specific HDL apolipoproteins are not necessary for this step. After uptake into solely apo A-I- or solely apo E-containing lipoproteins, UC is transferred to α-HDL_3, where it is esterified by the plasma enzyme lecithin:cholesterol acyltransferase (LCAT). The generation of CE and the acceptance of surface remnants of CM and VLDL increase the size of α-HDL_3 with formation of α-HDL_2. From α-HDL, CE can be removed by at least four mechanisms to be directed to the liver, where cholesterol is utilized for bile acid synthesis

- Cholesterol ester transfer protein (CETP) exchanges CE of HDL with the TG of VLDL, IDL, LDL, CM, and CM remnants (CMR)

- A subpopulation of HDL acquires apo E and is recognized by hepatic apo E receptors
- HDL without apo E can also be endocytozed by hepatocytes, possibly after binding to hepatic apo A-I receptors
- CE of some HDL appear to be selectively taken up by hepatocytes without any receptor-mediated interaction [5, 6]

Metabolism of HDL and TG is closely linked, since lipolysis of CM and VLDL produces surface remnants, which are one of the precursors of HDL, and since CETP catalyzes the exchange of TG and CE between HDL and TGRL. Hence, hypertriglyceridemia (HTG) depletes HDL of CE and, since TG in HDL are easily hydrolyzed by HL, leads to a reduction in the level of larger HDL_2 with predominance of HDL_3. On the other hand, TGRL become more atherogenic due to the accumulation of CE. In this scheme, the effectiveness of postprandial lipid metabolism assumes great importance. Several investigators have in fact demonstrated a strong correlation between the degree of postprandial HTG and the plasma concentration of HDL_2 cholesterol [5, 7].

Deficiencies of Apolipoprotein B

Apo B is present in two isoforms, a larger isoform termed apo B-100, which is produced in the liver, and a smaller isoform, termed apo B-48, 48% the size of apo B-100, which is synthesized in the intestine. Both apo B species are encoded by a single gene on chromosome 2. The truncation of apo B-48 results from mRNA editing which converts a cytosine in the apo B mRNA to a uracil and thus introduces a premature stop codon. Apo B-48 is a protein component of CM and their remnants; apo B-100 is a protein component of VLDL, IDL, LDL, and Lp(a). Deficiency of apo B-containing lipoproteins occurs in at least three genetically distinct inherited disorders which resemble one another in their clinical presentation:

- Abetalipoproteinemia (ABL)
- Homozygous hypobetalipoproteinemia (HBL)
- Anderson's disease

All these diseases are very rare [8].

Abetalipoproteinemia

Clinical Presentation

The clinical picture of ABL was first described by Bassen and Kornzweig [9]. The main clinical manifestations are in the gastrointestinal tract, the neuromuscular system, the eyes, and hemopoietic system. *Acanthocytes*, bizarrely spiculated red blood cells, are seen in the peripheral blood smear. The inability to synthesize apo B-48 and to form CM in the intestine results in malabsorption of fat and fat-soluble vitamins. Symptoms of *fat malabsorption* manifest themselves in infancy and dominate the clinical picture in early childhood. Steatorrhea together with poor appetite and vomiting are the first symptoms of fat intolerance and bring the patients to the attention of pediatric gastroenterologists. Malabsorption is commonly associated with poor weight gain and growth retardation. Since the absorption of both vitamin E and A is dependent on the formation of CM, ABL is characterized by very low plasma concentrations of these vitamins. The malabsorption of vitamins D and K is less affected. The development of vitamin E deficiency seems to be a critical factor in the manifestation of neurological, muscular, and ocular symptoms and abnormalities. The earliest signs of neurological involvement appear during the first decade if the disorder remains undiagnosed and untreated. The first sign is loss of tendon reflexes followed by loss of vibration sensation and proprioception. Several patients also exhibit positive extensor plantar responses and Romberg's sign. The neurological symptoms include clumsiness with progressive unsteadiness in gait. Because of ataxia in the trunk and limbs, the patients are unable to walk by their mid-twenties. In addition, they commonly show some signs of cerebellar disease: dysarthria, dysmetria, dysdiadochokinesis, and intention tremor. Spinocerebellar degeneration and lack of physical activity result in muscle wasting and weakness, which worsens still the ability to move. The cardiac muscle may also be involved, and arrhythmias and/or cardiomyopathy have been observed in some patients. The observed axonopathy has been related to the deficiency of vitamin E.

Metabolic Derangement

ABL is caused by the deficiency of the microsomal triglyceride transfer protein (MTTP), which is responsible for the transfer of TG into the endoplasmic reticulum of enterocytes and hepatocytes, where they are assembled with apo B to CM and VLDL, respectively. The lack of MTTP thus results in a failure to produce apo B-containing lipoproteins [11, 12]. The clinical symptoms probably

result from a failure to adsorb dietary lipids and fat-soluble vitamins A, D, E, and K in the intestine and to transport them in the circulation. The deficiency in vitamin E is thought to be responsible for the impaired functions of the nervous system and the retina [10].

Diagnostic Tests

Extremely low levels of cholesterol (<1.5 mmol/l, 60 mg/dl) and TG (<0.5 mmol/l, 80 mg/dl) in infants with either fat malabsorption or fat intolerance, spinocerebellar degeneration, peripheral neuropathy, retinitis pigmentosa, or acanthocytosis are indicative of ABL and should lead to the determination of the apo B serum concentration. Absence of apo B strongly supports the diagnosis. ABL is differentiated from homozygous hypobetalipoproteinemia by the finding of normal serum concentrations of apo B, cholesterol, and TG in the parents of the former group [10].

Treatment and Prognosis

Early treatment from the neonatal period onwards may prevent the progression of *neuropathy* and *retinitis pigmentosa*. The supplementation of vitamin E at a dose of 100 mg/kg body weight per day is sufficient to replenish tissue levels, although the plasma level of tocopherol remains below 10% of normal. Restriction of dietary fat is required to control steatorrhea, but in spite of this adequate nutritional care is critical to guarantee beneficial effects. Replacement therapy of vitamins A, K, and D is necessary. It seems to be less effective if started in adults with manifest neurological problems and retinopathy.

Genetics

ABL is inherited as an autosomal recessive trait and caused by defects in the gene for MTTP [12].

Familial Hypobetalipoproteinemia

Clinical Presentation

The clinical presentation of homozygous HBL closely resembles ABL, but is milder in most cases, Heterozygotes are clinically normal, but have low levels of LDL-C and apo B [8, 13].

Metabolic Derangement

HBL results from mutations in the apo B gene which prevent the synthesis of full-length apo B molecules. The ability of the truncated isoforms of apo B to bind lipid depends on their length, with longer isoforms present in lipid-rich particles to a proportionately greater extent. Very small isoforms are absent from the plasma compartment (e.g., apo B-25 and apo B-29), longer ones are present in HDL (e.g., apo B-31), and others predominate in VLDL and LDL (e.g., apo B-86). The failure to form TGRL leads to impairment in the absorption and transport of fat and fat-soluble vitamins, which is the main cause of the clinical phenotype [8, 13].

Diagnostic Tests

Similar to ABL, the diagnosis of homozygous HBL is based on the clinical presentation together with the finding of very low serum concentrations of TG, cholesterol, and apo B. Family studies should be performed to detect relatives with low levels of LDL cholesterol (LDL-C) and apo B. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of plasma and isolated lipoproteins and subsequent immunoblotting may lead to the identification of anti-apo B-immunoreactive protein(s) with unusually low molecular weight and abnormal lipoprotein distribution. Vertical transmission of this protein polymorphism confirms the diagnosis [8, 13].

Treatment and Prognosis

In homozygous HBL, treatment is symptomatic as described for ABL. Heterozygotes are healthy and do not need treatment [8, 13].

Genetics

The mode of inheritance is autosomal codominant. Approximately 40 nonsense mutations in the apo B gene have been identified as the genetic basis of HBL. These mutations prevent the trans-

lation of full-length apo B. The heterozygote frequency of truncated apo B is estimated to range between 1 in 500 and 1 in 1000 [8, 13].

Anderson's Disease (Chylomicron Retention Disease)

Clinical Presentation

In 1961, Anderson reported the case of an infant suffering from fat malabsorption associated with the presence of fat droplets in the enterocytes [14]. Also, no CM were detectable in the child's plasma after a fatty meal. Concentrations of LDL-C and HDL cholesterol (HDL-C) were low. Thereafter, several patients were identified who presented with severe diarrhea or steatorrhea, malabsorption of fat and fat-soluble vitamins, growth retardation, and the presence of fat-laden enterocytes [15].

Metabolic Derangement

Since LDL are present but CM are absent, and because lipid accumulates in enterocytes, Anderson's disease is believed to result from a selective failure of enterocytes to form lipoproteins.

Diagnostic Tests

As with ABL and homozygous HBL, Anderson's disease should be suspected in infants and children with steatorrhea and/or diarrhea, fat malabsorption, and growth retardation who have low levels of cholesterol, TG, LDL-C, HDL-C, and apo A-I and B. Absence of CM after a fat load makes the diagnosis more likely. Definitive diagnosis depends on the finding of fat-laden enterocytes in intestinal biopsies.

Treatment and Prognosis

Treatment is based on restriction of dietary fat and dietary supplementation with fat-soluble vitamins.

Genetics

An autosomal recessive mode of inheritance is likely, since in some families several members were symptomatic while the parents were asymptomatic. Linkage analysis has excluded the apo B gene in Anderson's disease [16].

Hypertriglyceridemias

Chylomicronemia – Lipoprotein Lipase Deficiency and Apolipoprotein C-II Deficiency

Clinical Presentation

Typically, patients with either LPL deficiency or apo C-II deficiency present with a history of recurrent acute pancreatitis or attacks of abdominal pain, hepatosplenomegaly, lipemia retinalis, and eruptive xanthomas over the buttocks, shoulders, and extensor surfaces of the extremities. Fasting TG are extremely high and vary between 10 and 100 mmol/l. However, chylomicronemia and/or defects in the LPL gene are often also found in individuals who have never experienced *abdominal crises*. The risk for pancreatitis appears to correlate with the magnitude of elevated TG. In the neonate, LPL deficiency may cause periodic abdominal colics and failure to thrive. LPL-deficient infants and children usually have hepatosplenomegaly without xanthomas.

Metabolic Derangement

Fasting chylomicronemia occurs either with a normal serum concentration of VLDL in the very rare type I HLP or together with increased serum VLDL in the more common type V HLP. Various genetic defects in the LPL gene have been identified which prevent the synthesis and secretion of the enzyme or abolish its ability to hydrolyze TG in CM and VLDL. Typically, patients are homozygotes or compound heterozygotes for these mutations. However, heterozygosity for functionally relevant LPL defects may also be the basis of chylomicronemia if the patient has additional permissive factors such as diabetes mellitus or alcohol intake. Due to the obligatory role of apo C-II in LPL activation, deficiency of apo C-II also causes chylomicronemia [17–19].

Diagnostic Tests

Chylomicronemia is defined as the presence of CM in plasma after 12 h fasting. CM become

visible as a creamy layer on serum or plasma after over-night storage at 4°C. A clear infranatant in the absence of HC indicates type I HLP, while a turbid infranatant in the presence of HC indicates type V HLP. Secondary causes for severe HTG such as diabetes mellitus or alcoholism in adults and glycogen storage diseases in children should be ruled out before performing expensive diagnostic tests for LPL deficiency and apo C-II deficiency. LPL can be measured in postheparin plasma either by immunological determination of the protein concentration or by functional assessment of the hydrolysis of radiolabeled triolein or tributyrate. In LPL-deficient patients, the activity is typically reduced by at least two standard deviations below the mean of a healthy control population. However, the interindividual variation of LPL activity is very high. This is due not only to the genetic heterogeneity of the LPL gene, but also to its extensive hormonal regulation [20]. Therefore, low LPL activity does not necessarily imply hereditary LPL deficiency. Moreover, because of the multifunctional character of LPL, normal lipolytic activity in postheparin plasma does not exclude a dysfunctional enzyme. Therefore, a definite diagnosis of LPL deficiency should be based on sequence information of the LPL gene. Although the LPL gene is multiallelic, allele-specific approaches to some frequent mutations may facilitate the diagnosis, especially in ethnically homogenous populations such as French Canadians [21]. Very often, however, the gene must be sequenced. Apo C-II deficiency can be sought by quantifying apo C-II or by means of isoelectric focusing (IEF) of plasma and subsequent anti-apo C-II immunoblotting. The latter method helps to identify structural variants of apo C-II which may also cause HTG. Due to the small size of the apo C-II gene, analysis of its sequence is reasonable when the clinical and biochemical presentation strongly indicates a defect in apo C-II [19].

Treatment and Prognosis

Patients coming to medical attention because of acute pancreatitis firstly require symptomatic treatment. After this acute phase, the patient should receive a fat-free diet (<5 g fat per day) for several days. The permanent therapy of chylomicronemic patients is based on the restriction of dietary fat to 10%–20% of total energy intake and withdrawal of alcohol. The elimination of fat from the diet requires an increase in carbohydrates, which may cause some increase of VLDL TG. Therefore, it is sometimes necessary to increase the percentage of protein in the diet. The use of medium-chain TG may help to balance the energy intake. The diet should be administered in the form of several small meals spread throughout the day. Physical activity will also help to treat HTG. If necessary, body weight should be normalized and therapy of diabetes mellitus optimized. In most cases the use of lipid-lowering drugs is not beneficial. However, if dietary intervention is not successful or when the high carbohydrate content of the diet causes a severe increase of VLDL TG, therapy with fibrates, nicotinic acid, or omega-3 fatty acids can be tried under strict control. Drugs causing HTG such as estrogens, thiazide diuretics, and some beta blockers should be avoided. Pregnancy may aggravate chylomicronemia [17].

The prognosis of chylomicronemia depends on the frequency and extent of acute pancreatitis and the degree to which the endocrine and exocrine functions of the pancreas are maintained.

Genetics

A broad variety of defects in the LPL gene have been identified, not only in patients with pure chylomicronemia but also in a considerable proportion of patients with type V hyperlipoproteinemia (HLP) or HTG without chylomicronemia (i.e., type IV HLP) [17–19]. Most patients are homozygotes or compound heterozygotes. Heterozygosity for one of these defects may also lead to chylomicronemia if other factors such as diabetes mellitus are present. Apo C-II deficiency is an extremely rare autosomal recessive disease. Approximately ten defects in apo C-II have been identified [19].

Familial Hypertriglyceridemia

Clinical Presentation

Familial HTG (FHTG) is a heterogeneous disorder which is characterized by increased serum concentrations of VLDL, sometimes accompanied by fasting chylomicronemia. Thus, FHTG encompasses Fredrickson type IV and type V HLP. Usu-

ally, this condition is asymptomatic and discovered on routine lipid screening. In most patients, serum concentrations of TG are only moderately increased, i.e., they range between 2.3 and 4.6 mmol/l (200 and 400 mg/dl). Frequently, HTG is accompanied by low levels of HDL-C (i.e., below 0.9 mmol/l or 35 mg/dl) [22]. A considerable proportion of hypertriglyceridemic patients also suffer from obesity, impaired glucose tolerance, or non-insulin-dependent diabetes mellitus, uricemia, and/or arterial hypertension and may hence belong to an entity of diseases which has been termed "syndrome X," the "metabolic syndrome," or "dyslipidemic hypertension" [23–25].

Over the years, HTG may cause fatty liver which may be detected on abdominal ultrasound examination. Some patients with episodes of fasting chylomicronemia may experience abdominal crises and acute pancreatitis.

Metabolic Derangement

FHTG is probably heterogeneous in its origin. Some patients are homozygous, compound heterozygous, or heterozygous for defects in the LPL gene [17]. In other patients, the liver appears to synthesize TG and VLDL at increased rates. In some patients the increased production of VLDL may be caused by peripheral insulin resistance, either directly by insulin itself or indirectly via the increase of free fatty acids (FFA) which accompanies insulin resistance [23].

Diagnostic Tests

As FHTG probably encompasses a broad variety of heterogeneous disorders, there may also be some overlap with other dyslipidemias, e.g., familial combined hyperlipidemia (FCH) and familial hypoalphalipoproteinemia (FHALP). Due to the lack of distinct genetic markers, FHTG is diagnosed by finding elevated TG in several first-degree relatives of the index case. Secondary causes for HTG such as diabetes mellitus, alcoholism, renal, hepatic, endocrine, inflammatory, and hematological or oncological diseases, as well as the intake of a series of HTG-inducing drugs must be excluded. HTG also occurs in a broad variety of genetic disorders in lipid metabolism, including defects of LPL and apo C-II, HL, apo E, apo A-I, LCAT, Tangier disease, and various lipid storage diseases. Diagnostic tests for these conditions are described in the respective sections.

Treatment and Prognosis

The prognosis of HTG depends on the associated risks of acute pancreatitis and coronary heart disease (CHD). The risk of acute pancreatitis depends on the presence and extent of chylomicronemia. The possible association of HTG with CHD is still a matter of debate. In most prospective epidemiological studies, HTG was not an independent risk marker for CHD when HC, low HDL-C, diabetes mellitus, arterial hypertension, and smoking were taken into account. In the Prospective Cardiovascular Münster (PROCAM-) Study, the simultaneous presence of an LDL to HDL ratio greater than 5, HDL-C less than 0.9 mmol/l (35 mg/dl), and TG greater than 2.3 mmol/l (200 mg/dl) predicted 25% of myocardial infarctions occurring during 6 years of follow-up of more than 4000 men aged 40–60 [26]. Thus, (F)HTG may indicate the presence of a complex metabolic syndrome which puts these patients at increased risk for atherosclerosis.

HTG usually responds to dietary and physical therapy. Normalization of body weight, withdrawal of alcohol, and regular physical exercise frequently help to reduce fasting triglyceride levels to below 2.3 mmol/l (200 mg/dl). Dietary ◄ recommendations include restriction of energy intake to normalize body weight, reduction of fat to less than 30% of energy intake, and avoidance of simple sugars (i.e., sucrose and fructose). Fat should be poor in saturated and rich in monounsaturated fatty acids. Where possible, drugs known to aggravate HTG such as estrogens, corticosteroids, thiazide diuretics, and some beta blockers should be avoided. Drugs which lower TG are fibrates, nicotinic acid, and omega-3 fatty acids (for details on drugs, dosage, adverse effects, and contraindications see Table 1) [27].

Genetics

FHTG is inherited as an autosomal dominant trait. The penetrance varies. Its genetic origin appears to be heterogeneous. Defects in the LPL gene may account for a considerable proportion of cases, although the extent of the contribution of

Table 1. Essential characteristics of lipid-lowering drugs

Drug	Daily dosage	Contraindications[a]	Adverse effects[b]	Drug interactions[c]
Cholestyramine	2–6 × 4 g	A 9, 10, 15	B 1	C 1, 3, 4, 5, 6, 7, 8, 9, 10
Colestipol	2–6 × 5 g	A 9, 10, 15	B 1	C 1, 3, 4, 5, 6, 7, 8, 9, 10
Lovastatin	20–80 mg	A 1, 3, 4, 14	B 1, 2, 4, 8, 11	
Simvastatin	20–40 mg	A 1, 3, 4, 14	B 1, 2, 4, 8, 11	
Nicotinic acid	3 × 1–2 g	A 1, 6, 7, 8, 16	B 1, 2, 7, 8, 12, 13, 14	C 2
Probucol	2 × 500 mg	A 1, 3, 4, 12, 17	B 1, 5, 8, 11, 16	
Bezafibrate	3 × 200 mg or 1 × 400 mg	A 1, 2, 3, 4, 5, 14	B 1, 2, 3, 4, 5, 6	C 1
Ciprofibrate	100–200 mg	A 1, 2, 3, 4, ?5, 14	B 1, 2, 3, 4, 5, 8	C 1
Clofibrate	3–4 × 500 mg	A 1, 2, 3, 4, 5, 14	B 1, 2, 3, 4, 5, 6, 8	C 1
Fenofibrate	3 × 100 mg	A 1, 2, 3, 4, 5, 14	B 1, 2, 3, 4, 5, 6	C 1
Gemfibrozil	2 × 600 mg	A 1, 2, 3, 4, 5, 14	B 1, 2, 3, 4, 5, 6	C 1, 2

Based upon "Physician's Desk Report," "Rote Liste," and [77].

[a] Contraindications (A): 1, hepatic dysfunction, hepatitis; 2, severe renal dysfunction; 3, pregnancy; 4, lactation; 5, gallbladder disease; 6, congestive heart failure; 7, recent myocardial infarction; 8, acute haemorrhage; 9, nephrolithiasis; 10, hyperparathyroidism; 11, hypothyroidism; 12, cholestasis; 13, inflammatory bowel disease; 14, hypersensitivity; 15, acute peptic ulcer; 16, gout; 17, arrhythmia.

[b] Adverse reactions (B): 1, gastrointestinal disorders; 2, hepatic dysfunction, increased liver enzymes; 3, cholelithiasis or increased lithogenicity; 4, myositis or increased creatine phosphokinase; 5, disturbance of potency; 6, hair loss; 7, severe flushing; 8, pruritus, urticaria, or rash; 9, feeling of anxiety; 10, tachycardia; 11, transient headache; 12, hyperacidity; 13, hypotension; 14, impaired glucose tolerance; 15, cardiac arrhythmias; 16, QT prolongation in the ECG.

[c] Drug interactions (C): 1, coumarin anticoagulants; 2, oral hypoglycemics; 3, digitalis glycosides; 4, thyroid hormones; 5, tetracyclines; 6, phenylbutazone; 7, propranolol; 8, chlorothiazide; 9, phenobarbitone; 10, penicillin G.

LPL is not clear [17–19]. Unidentified genes regulating the synthesis of TG and VLDL may possibly also contribute to FHTG.

Hypercholesterolemias

Familial Hypercholesterolemia (Low-Density Lipoprotein Receptor Deficiency)

Clinical Presentation

Patients heterozygous for familial HC (FHC) come to medical attention either due to greatly elevated LDL-C levels detected during carrier screening in affected families or routine lipid screening in the population or because of clinical symptoms developing in the third and fourth decades of life. The most important clinical features are premature *atherosclerosis* and *xanthomatosis*. Typically *xanthomas* occur in the Achilles tendon and the tendons about the elbows, knees, and the backs of the hands. Xanthelasmas of the soft connective tissue especially on the eyelids also occur. In heterozygous FHC, xanthomatosis rarely develops before adulthood (less than 10%) and is completely absent in a considerable proportion of patients. Moreover, xanthomatosis is not restricted to FHC. Corneal arcus, another classical symptom, is also not specific. FHC heterozygotes are at greatly increased risk of developing CHD. A 30-year-old male FCH heterozygote has a 5% risk of CHD events, such as myocardial infarction or angina pectoris, requiring aortocoronary bypass surgery or angioplasty. This risk increases to 20% by age 40, to 50% by age 50, and to 75% by age 60. In general, women develop CHD symptoms 10 years later.

Serum concentrations of LDL-C in heterozygous FHC patients are usually above the 95th percentile of sex- and age-matched controls, i.e., in practical terms above 3.3 mmol/l (130 mg/dl) in childhood and above 5.4 mmol/l (210 mg/dl) in adulthood. TG are either normal or moderately increased. HDL-C levels are frequently below the risk threshold value of 0.9 mmol/l (35 mg/dl). In FCH, severe HC typically occurs independently of secondary factors such as obesity, diabetes mellitus, hypothyroidism, renal and hepatic diseases, or intake of drugs.

Homozygous FHC is extremely rare and characterized by manifestation of the above-mentioned clinical features at a very young age and cholesterol levels of between 12 and 25 mmol/l (500 and 1000 mg/dl). Xanthomatosis may be present at birth and usually develops before the

age of 2 years. Atherosclerosis of the coronary arteries usually appears before the age of 10 years in LDL receptor-negative FHC homozygotes and before the age of 20 years in LDL receptor-defective FHC homozygotes.

Metabolic Derangement

FHC is caused by defects in the LDL receptor gene which either prevent the synthesis of LDL receptors (type I defects) or lead to the synthesis of dysfunctional receptors. The latter are either defective in binding (type 2) or internalization of LDL (type 3). The reduced number of functional LDL receptors blocks the catabolism of LDL and leads to their accumulation in the plasma compartment. Moreover, the reduced uptake of LDL into liver cells, where most (90%) LDL receptors are expressed, upregulates hepatic cholesterol synthesis and may thereby increase the production of VLDL. Due to the lack of functional LDL receptors, the removal of VLDL and its conversion products IDL is impaired and the formation of LDL is enhanced. Thus, LDL-HC in FHC reflects both impaired LDL catabolism and LDL overproduction. Some LDL, probably after oxidative modification, is removed by scavenger receptors on macrophages. Uptake of (modified) LDL transforms the macrophages into foam cells and mediates a series of autocrine and paracrine mechanisms which together have been made responsible for the pathogenesis of atherosclerosis and xanthomatosis in FHC [1, 3, 4].

Diagnostic Tests

The diagnosis should be considered in any patient whose LDL-C level exceeds the 95th percentile of sex- and age-matched controls in the absence of hypothyroidism or renal or hepatic disease who clinically presents with premature CHD and/or xanthomatosis and whose family history indicates a high prevalence of HC and premature myocardial infarction. Definitive diagnosis is made on the basis of functional or genetic tests.

The classical test is the measurement of the ability of cultured skin fibroblasts to bind and internalize radioiodinated LDL. The test, however, is laborious and time consuming. LDL receptor activity can also be measured in peripheral mononuclear cells by the use of monoclonal anti-LDL receptor antibodies and fluorescence-assisted flow cytometry [28]. Due to its practicability, this procedure may help to establish the diagnosis of FHC more frequently than was possible in the past based upon the clinical presentation alone.

Due to the presence of more than 100 different LDL receptor alleles, the genetic approach to the diagnosis of FHC in most cases is based on cosegregation analysis using indirect markers of the LDL receptor gene. Allele-specific tests are recommended in families with known defects and in ethnically and socioculturally homogenous populations in which founder effects have increased the prevalence of defined alleles [29]. Family analysis using indirect markers is informative in large pedigrees (at least five first-degree relatives for a 95% probability). However, drawbacks result from the fact that some FHC heterozygotes may have relatively low LDL-C levels and that some family members may also suffer from HC because of other reasons (phenocopy) [29, 30].

Treatment and Prognosis

FHC puts affected individuals at a greatly elevated risk of premature CHD. This can be substantially reduced by lowering cholesterol levels. Therefore, attempts should be made to identify patients with FHC as early as possible to assess potential coronary atherosclerosis and to start primary or secondary preventive treatment of HC. The National Cholesterol Education Program in the USA (NCEP) and the Task Force for the Prevention of CHD in Europe have recently published guidelines for the prevention of CHD [27, 31]. For FHC patients, these guidelines ◄ recommend LDL-C levels below 135 mg/dl (3.5 mmol/l) as the therapeutic goal. In patients with manifest CHD, LDL-C below 3 mmol/l (115 mg/dl) should be reached. Moreover, additional cardiovascular risk factors including smoking, arterial hypertension, diabetes mellitus, and obesity worsen the prognosis of FHC and should be corrected.

Heterozygous FHC. The choice of lipid-lowering therapy in *heterozygous FHC* must take into account the patient's age and the presence of coronary artery disease (CAD) or cardiovascular risk factors. Dietary intervention is recommended for ◄ both children and adults. In a first step, total fat

should be reduced to less than 30% of energy intake with equal amounts of saturated, mono-, and polyunsaturated fatty acids; cholesterol should be reduced to less than 300 mg per day. In a second step, total fat should be reduced by a further 5% and cholesterol to less than 200 mg per day [27, 31]. In children, care must be taken to ensure that energy intake is sufficient for normal growth and development, i.e., reductions in fat intake must be adequately replaced by complex carbohydrates.

Usually, dietary intervention alone will not sufficiently reduce cholesterol levels in FHC and additional drug therapy is necessary. To sufficiently decrease LDL-C, it is generally necessary to treat adult patients with HMG-CoA reductase inhibitors (statins). Lovastatin is given at a dosage of 20, 40, 60, or 80 mg per day, and simvastatin and pravastatin at a dosage of 10, 20, or 40 mg per day. Treatment should be started with the lowest dosage. After 4–6 weeks, therapeutic effectiveness and any adverse effects should be monitored. If necessary, the dosage should be increased in a stepwise fashion with monitoring of lipids, creatinine kinase, and liver enzymes every 4–6 weeks. When the optimal dosage is found, biochemical analyses should be performed every 6 months. If one statin is not well tolerated, it should be replaced by another. Administration of statins at the highest dosage will decrease LDL-C by up to 50%. If monotherapy with statins is not successful, combination with bile acid sequestrants (resins, i.e., colestipol or cholestyramine) should be tried. This combination can lower LDL-C levels by 60%. Because of possible liver toxicity, combination of statins and fibrates is not recommended. If statins are not tolerated, combination of resins and fibrates or of resins and nicotinic acid can be tried. However, these regimens will only decrease LDL-C by 25%–30% [27]. If drug therapy does not sufficiently lower LDL-C, extracorporeal elimination of LDL should be considered, especially for those patients who already have CHD. Current procedures include the adsorption of LDL to anti-apo B-antibodies, to dextran sulfate, and to heparin. The frequency of the treatments varies from once a week to once every 2 weeks. In addition, the patient should be treated with drugs, usually with statins [32].

Homozygous FHC. The excessive HC in *homozygous FHC* must be treated vigorously. Unfortunately, statins, the most potent lipid-lowering drugs, are not effective in homozygous FHC, since their lipid-lowering effect depends on the presence of functional LDL receptors. Despite this, it is worth trying therapy with statins, beginning with either 20 mg lovastatin or 10 mg pravastatin or simvastatin per day, since some patients with apparent homozygous FHC may in fact have residual LDL receptor activity. The dosage can be increased in a stepwise manner. Especially in smaller children high dosages require very careful monitoring of side effects with particular reference to hepatic and muscle toxicity. Resins have little or no effect in homozygous FHC. In some patients nicotinic acid effectively reduces LDL-C when given at high dosages (1–5 g per day). Again, careful monitoring of liver function is necessary. Fibrates are also ineffective. Although it does not lower LDL-C, probucol has been reported to cause reduction of tendon and skin xanthomas. However, the clinical benefit is not clear.

Because drug therapy is ineffective, patients with homozygous FHC generally need treatment with extracorporeal LDL apheresis by adsorption to anti-apo B-immunoglobulins, heparin, or dextran sulfate. In most cases the extracorporeal elimination of LDL is performed every 2 weeks. In addition, patients should receive medication as outlined before [32].

Liver transplantation has been performed in several patients. This resulted in near normal LDL-C values when additional treatment with HMG-CoA reductase inhibitors was given. A major disadavantage of this approach is the necessity of immunosuppression. Somatic gene therapy of LDL receptor deficiency has been performed in animal models and very recently for the first time in a human subject. However, lowering of LDL-C was achieved in the animal model only. Future research will show whether this therapy is superior to transplantation and will also determine the most efficient method of introducing functional LDL receptor genes into human liver cells [33].

Treatment in Children

Whereas the use of drug therapy in FHC in adults is well established, it is a matter of debate whether children should receive medication and, if so, at which age treatment should begin. It is generally held that children under 16 years of age should receive resins in preference to statins. Drug therapy should be considered in children above the age of 6 years with LDL-C levels of greater than 4.2 mmol/l (160 mg/dl). Treatment should be

started with low dosages of resins (4 g cholestyramine or 5 g colestipol per day) or sitosterol (3 g per day), which can be increased to the limits of the child's tolerance. Trials performed in the last few years to analyze the therapeutic benefit of statins in children have observed no serious adverse effects; thus, in the future, treatment of children with statins may become more widely accepted [34, 35]. Children with homozygous FHC are at risk of developing myocardial infarction before the age of 20 years. Therefore, they need careful and regular cardiological examinations including resting and stress electrocardiograms, thallium scintigraphy, and coronary angiography. The latter should be performed at the age of 10 years, or earlier if the child has developed clinical signs of myocardial ischemia. Depending on the severity of CHD, revascularization by either patch angioplasty or ostial endarterectomy should be performed. In children, bypass surgery should be delayed as much as possible. As a final choice, heart transplantation may be necessary, possibly in combination with *liver transplantation.*

Genetics

At an estimated frequency of one heterozygote in 500 of the general population, FHC is one of the most common autosomal dominant disorders. The disease is caused by a large number of mutations in the LDL receptor gene on chromosome 19q13. In general, individual mutations are very rare. However, due to founder effects the frequency of some alleles is considerably increased in some populations such as French Canadians, Afrikaners, Lebanese, Icelanders, and Finlanders [29].

Familial Defective Apolipoprotein B

Clinical Presentation

Patients heterozygous for familial defective apo B (FDB) may be clinically indistinguishable from heterozygotes for FHC, although xanthomas occur less frequently. The family history frequently indicates an increased prevalence of HC and myocardial infarction (MI) [36]. To date, two homozygotes for FDB have been identified. In contrast to the excessive HC and the very early onset of cardiovascular disease in FHC homozygotes, cholesterol levels in FDB homozygotes were not higher than in FDB heterozygotes and cardiovascular risk did not appear to be further increased, since these patients were 32 and 54 years old at the time of their identification [37].

Metabolic Derangement

FDB is caused by a missense mutation in codon 3500 of the apo B gene which causes a replacement of a glutamine by an arginine. This defect resides in the LDL receptor-binding domain of apo B and markedly decreases the affinity with which LDL binds to the LDL receptor. Consequently, LDL accumulate in the plasma. Disturbance of the cholesterol homeostasis of hepatocytes also results. Moreover, the accumulating LDL which contain apo B3500:Gln→Arg resembles small, dense LDL, which is considered to be especially atherogenic [36, 38].

The absence of massive HC in FDB homozygotes has been attributed to the fact that, in contrast to FHC, the patients' LDL receptors still recognize and eliminate VLDL and IDL [37].

Diagnostic Tests

Like FHC, FDB should be considered in any patient with HC. The diagnosis is most easily and specifically made by allele-specific polymerase chain reaction (PCR) tests of the apo B gene [36, 39].

Treatment and Prognosis

The frequency of FDB in patients from cardiological rehabilitation centers is 1 in 220 as compared to 1 in 700 in a random population. This clearly indicates the increased risk for MI in FDB, which is probably due to HC. Therefore, the FDB patient requires the same treatment and follow-up as FHC heterozygotes. In most cases this includes drug treatment [27, 31]. It has been reported that the HC of FDB is more sensitive to treatment with resins than that of FHC. Statins also effectively lower cholesterol in FDB. Combination of both resins and statins may be beneficial.

Clinical follow-up includes cardiological examination to assess progression of CHD as early as possible. Diagnosis of FDB by means of PCR is not difficult. Therefore, families of FDB patients should be screened for carriers of the mutation.

Genetics

FDB is caused by a missense mutation in codon 3500 of the apo B gene on chromosome 2 which has been identified in several Caucasian populations. The frequency of heterozygotes is approximately 1 in 500. HC in these patients is inherited as an autosomal codominant trait. The penetrance of the phenotype is variable [36].

Lysosomal Acid Lipase Deficiency – Wolman Disease and Cholesteryl Ester Storage Disease

Clinical Presentation

Patients with lysosomal acid lipase (LAL) deficiency present with a massive accumulation of TG and CE in most tissues of the body. Depending on the age of onset and the severity of symptoms, two phenotypes are differentiated. Wolman disease occurs in infancy and is fatal in most cases before the age of 1 year. The infants suffer from hepatosplenomegaly, steatorrhea, and failure to thrive. X-ray examination reveals *calcification of the adrenal glands*. Cholesteryl ester storage disease (CESD) is more benign and may not be diagnosed until adulthood. Although lipids accumulate in many organs, the patients frequently present with hepatomegaly only. Patients with CESD have profound hypercholesterolemia, HDL-C levels in the region of 0.5 mmol/l (20 mg/dl), and a very high risk of premature atherosclerosis.

Metabolic Derangement

LAL is a crucial enzyme in cellular cholesterol homeostasis, since it hydrolyzes CE which have been internalized by cells either via cell surface receptors for various lipoproteins or by phagocytosis with entry into the endosomal – lysosomal route. Due to the regulatory effect of the released cholesterol on the de novo synthesis of cholesterol, the expression of LDL receptors, and the activity of ACAT, the deficiency of LAL in Wolman disease or CESD leads not only to lipid accumulation in lysosomes, but also to increased synthesis of cholesterol, increased hypercholesterolemia, and a rise in the concentration of circulating apo B-containing lipoproteins [40].

Diagnostic Tests

Wolman disease should be considered in infants with hepatomegaly, gastrointestinal symptoms, and failure to thrive. These symptoms should lead to X-ray examination of lungs, bones, and abdomen. Although not pathognomonic, the finding of bilateral calcifications of the adrenals favors the diagnosis. Definitive diagnosis is based on the measurement of LAL activity in either cultured skin fibroblasts or lymphocytes from peripheral blood using either radioactive or fluorescent tracers [40]. CESD should be considered in patients who present with unexplained hepatosplenomegaly, severely elevated LDL-C levels, and decreased levels of LDL-C. Adult patients may come to medical attention with premature atherosclerosis. Final diagnosis also depends on the finding of strongly reduced LAL activity in lymphocytes or cultured skin fibroblasts.

Possible differential diagnoses to be considered in Wolman disease are adrenal insufficiency, adrenoleukodystrophy, and other lysosomal storage disease. In CESD, the differential diagnosis includes biliary cirrhosis, glycogen or lipid storage diseases, Niemann-Pick disease type B, and mucopolysaccharidoses [40].

Treatment and Prognosis

There is no specific treatment for either phenotype of LAL deficiency. Hypercholesterolemia in patients with CESD can be treated with HMG-CoA reductase inhibitors. Patients with Wolman disease usually die before the age of 1 year. Patients with CESD may also die during childhood. Adult patients are at great risk of atherosclerosis.

Genetics

Both Wolman disease and CESD are inherited as autosomal recessive traits and arise from mutations in the LAL gene on chromosome 10.

Sitosterolemia

Clinical Presentation

Patients with sitosterolemia are characterized by the occurrence of tendon and tuberous xanthomas

in childhood and by premature atherosclerosis. Some patients also present with hemolysis. Cholesterol levels can be normal, but are often raised and may reach very high levels.

Metabolic Derangement

The disease appears to result from increased intestinal absorption of plant or shell fish sterols. Decreased biliary excretion of both plant and animal sterols is also found.

Diagnostic Tests

The diagnosis is based on finding increased serum concentrations of campesterol, stigmasterol, cholestanol, or 5α-derivatives of plant sterols on gas chromatographic examination of serum.

Treatment and Prognosis

Sitosterolemic patients should consume a diet which avoids plant foods with high fat content such as oils, margarine, nuts, seed, chocolate, olives, or avocados. Shell fish should also be avoided. Food from animal sources, fruits and vegetables, and cereals without germ are allowed. Drug therapy with cholestyramine or neomycin helps to lower serum concentrations of plant sterols. The prognosis is determined by the extent of atherosclerosis.

Genetics

Sitosterolemia is a very rare autosomal, recessively inherited disorder of unknown origin.

Polygenic Hypercholesterolemia

Clinical Presentation

Polygenic HC presents with mild to moderate elevations of LDL-C (>4.5 mmol/l, 175 mg/dl) on repeated measurements in the absence of one of the primary or secondary causes of HC discussed above. The patients do not present with any specific clinical phenotype. The disorder is common. Its prevalence among MI survivors has been estimated to be 15% [41].

Metabolic Derangement

Polygenic HC is the result of the interaction of several genetic and nongenetic factors which modulate lipid metabolism [41].

Diagnostic Tests

Polygenic HC is a diagnosis of exclusion. The patient displays persistent HC despite following steps I and II of the American Heart Association diet. Other primary hyperlipidemias must be ruled out. Secondary causes for HC including renal, hepatic, and thyroid diseases as well as the intake of HC-inducing drugs must also be excluded.

Treatment and Prognosis

Polygenic HC is very frequent in the population and thus is a major contributor to morbidity and mortality from CHD in the general population. The intensity of therapeutic intervention varies and depends on the patient's sex and age, the presence of CHD or other risk factors for CHD, the severity of the HC, and the family history [27, 31].

Genetics

As indicated by its name, polygenic HC is caused by a combination of defective and polymorphic alleles in a number of genes. Each defect of itself has little influence on lipid levels and does not cause vertical transmission of the phenotype. A typical example of this is the apo E-4 allele, which in many horizontal population studies has been found to be associated with levels of LDL-C and apo B 10% greater than those in persons with the apo E-3 allele. The prevalence of apo E-4 was also found to be increased in patients with MI and to be associated with reduced life expectancy. Moreover, carriers of the apo E-4 allele experience MI at a younger age and have a lower life expectancy. Finally, a recent intriguing finding is the associa-

tion of apo E-4 with increased risk of the sporadic and late-onset forms of Alzheimer's disease [42, 43].

Mixed Hyperlipidemias

Remnant Hyperlipidemia (Type III Hyperlipidemia)

Clinical Presentation

Type III HLP is usually not diagnosed at a young age because hyperlipidemia and clinical symptoms do not become manifest before the ages of 30 and 40 years, respectively. The patients present either with palmar and plantar *xanthomas*, which appear as orange or yellow striae on the palmar and digital creases and which are characteristic of this disorder, or with tuberoeruptive xanthomas, especially over the knees and elbows, which can reach the size of a lemon. *Xanthelasmas* of the eyelids and xanthomas of the Achilles tendon are frequently present, but are also found in other forms of primary hyperlipidemia. The patients also suffer from severe *atherosclerosis* of the coronary, cerebral, and peripheral arteries. For this reason, they frequently come to medical attention because of MI, stroke, or intermittent claudication.

Remnant hyperlipidemia is characterized by elevation of cholesterol to above 7 mmol/l (300 mg/dl) and TG to above 3.5 mmol/l (300 mg/dl). Lipoprotein electrophoresis shows a typical pattern with a broad beta band located between beta- and prebetalipoproteins. HDL-C levels are generally below 0.8 mmol/l (30 mg/dl).

Metabolic Derangement

Type III HLP is caused by the defective removal of remnants of CM and VLDL (i.e., IDL) due to impaired binding and internalization of these remnants by hepatic apo E and apo B, E receptors. In most cases, this impairment is caused by homozygosity for the apo E-2 allele, which occurs with a frequency of approximately 1% in Caucasian populations. However, only 2% of apo E-2 homozygotes develop type III HLP, which may appear if the patient also develops another primary hyperlipidemia, hypothyroidism, diabetes mellitus, obesity, or hepatic or renal disease. Moreover, several rare apo E variants have been identified which cause remnant hyperlipidemia with high penetrance in heterozygotes [2].

Diagnostic Tests

Remnant hyperlipidemia is likely to be present in patients who present with characteristic xanthomatosis and with profound mixed hyperlipidemia. The diagnosis is supported by the finding of the typical broad beta band upon lipoprotein electrophoresis (type III pattern). Definitive diagnosis is based on biochemical proof of remnant accumulation and/or the presence of apo E2/2 homozygosity or apo E variants. Biochemical investigation requires the isolation of VLDL by ultracentrifugation at a density of 1.006 g/ml. In remnant hyperlipidemia, the ratio of VLDL-C to plasma TG typically exceeds 0.3. On lipoprotein electrophoresis, VLDL of type III hyperlipidemic patients exhibit beta mobility instead of normal prebeta mobility. Homozygosity for the apo E-2 phenotype can be demonstrated by IEF of VLDL apolipoproteins or of delipidated serum with subsequent anti-apo E immunoblotting. Homozygosity for the apo E-2 genotype can be shown with allele-specific DNA amplification by means of PCR. The possibility that type III HLP is due to rare apo E variants must be considered if IEF shows an atypical banding pattern of apo E isoforms or if apo E-2 homozygosity is not found despite marked clinical and biochemical findings. In the latter case, both the apo E genotype and phenotype should be determined. Discrepancies may indicate the presence of apo E variants. Definitive diagnosis of rare apo E variants is based on sequence analysis of the apo E gene [39].

Treatment and Prognosis

Since remnant hyperlipidemia puts affected individual at a greatly increased risk of atherosclerosis but can easily be corrected by adequate therapy, the disorder should never escape medical attention. The treatment of type III HLP should firstly focus on a search for and treatment of precipitating disorders such as hypothyroidism and diabetes mellitus. If necessary, energy intake must be restricted to normalize body weight. Type III HLP is frequently sensitive to the content and composition of fat in the diet. The patients' diet should ◄

therefore restrict fat to 30% of energy with less than 300 mg cholesterol per day and a ratio 1:1:1 for saturated to monounsaturated to polyunsaturated fatty acids, as recommended by the American Heart Association (step I diet). If unsuccessful, a step II diet should be tried, in which total fat is reduced by a further 5% at the expense of saturated fatty acids and cholesterol (less than 200 mg/day). Reduction or withdrawal of alcohol are also important interventions. The drugs of first choice for the treatment of type III HLP are the fibrates, which in most cases correct lipid levels when their use is accompanied by normalization of body weight, cessation of alcohol consumption, and reduction of dietary fat intake. In patients who show no response to fibrates or cannot tolerate them, nicotinic acid or HMG-CoA reductase inhibitors should be tried. Bile acid sequestrants are not useful and actually aggravate the disorder [27, 31].

Genetics

The prevalence of type III HLP in Caucasian populations is 1 in 5000. The disorder is caused by mutations in the apo E gene on chromosome 19. In all investigated populations, the apo E gene exhibits a nonsynonymous polymorphism with three alleles: e2, e3, and e4 code for apo E-2(112:Cys, 158:Cys), apo E-3(112:Cys, 158:Arg), and apo E-4(112:Arg, 158:Arg). Furthermore, various rare apo E variants have been identified. Depending on the molecular defect, type III HLP is inherited by two modes of inheritance:

- In most cases, remnant hyperlipidemia is inherited as an autosomal recessive trait which is expressed with a low penetrance. This is usually caused by homozygosity for apo E(112:Cys, 158:Cys; i.e., apo E-2). This structural change is close to the receptor-binding domain of apo E. Two percent of apo E-2 homozygotes express the hyperlipidemic phenotype, usually when they are also affected by other primary dyslipidemias or by endocrine, metabolic, renal, or hepatic diseases.
- Very rarely, type III HLP is inherited as an autosomal dominant trait with high penetrance. In these cases, the underlying defects are rare apo E variants whose structural changes are located within the receptor-binding domain of apo E [2].

Hepatic Lipase Deficiency

Clinical Presentation

Hepatic lipase (HL) deficiency has been diagnosed in only four families so far. The clinical presentation of these patients closely resembled type III HLP with premature atherosclerosis, severe mixed hyperlipidemia with levels of cholesterol and TG above the 95th percentile, and the presence of β-VLDL. Unlike type III HLP, HDL-C also exceeded the 95th percentile in most cases. More detailed lipid analyses revealed the presence of high levels of apo B and TG in all lipoproteins, including LDL and HDL [44].

Metabolic Derangement

HL is bound by proteoglycans to the endothelium of hepatic capillaries. The enzyme hydrolyses TG especially in IDL and HDL and thereby contributes to the formation of LDL and small HDL (HDL_3). Deficiency of HL thus leads to the enrichment of triglycerides in both apo B-containing lipoproteins and HDL [44, 45].

Diagnostic Tests

HL activity should be determined in patients with the above-mentioned phenotype in whom type III HLP has been excluded. The activity of HL is measured in postheparin plasma by hydrolysis of radiolabeled triolein in the presence of excess sodium chloride or anti-LPL antibodies to inhibit LPL. Plasma of HL-deficient patients does not hydrolyze triolein under these conditions. Genetic diagnosis of HL deficiency by sequence analysis is difficult, since the HL gene is polymorphic in the population and several nonsynonymous mutations are functionally irrelevant. Cosegregation analysis is frequently not helpful to prove cause – effect relationships of mutations in the HL gene, since heterozygotes for HL deficiency are clinically and biochemically indistinguishable from unaffected family members [44].

Treatment and Prognosis

One report described that hyperlipidemia in HL deficiency is sensitive to treatment with lovastatin, but not gemfibrozil [44].

Genetics

HL deficiency is an autosomal recessive disease. The HL gene is polymorphic in the Caucasian population, with several nonsynonymous mutations. Heterozygotes are clinically and biochemically indistinguishable from unaffected family members [44].

Familial Combined Hyperlipidemia

Clinical Presentation

Familial combined hyperlipidemia (FCH) was first identified by Goldstein et al. in 1973 when investigating families of survivors of premature MI [46]. These authors defined FCH as a familial dyslipidemia which presents either as HTG, HC, or mixed hyperlipidemia. The clinical presentation is bland. Xanthomas, xanthelasmas, and arcus corneae are much less frequent than in FHC. The lipid phenotype is said to vary over time. Recent studies suggest that a considerable proportion, if not all patients are characterized by high serum concentrations of apo B or an increased ratio of apo B to LDL-C (so-called hyperapobetalipoproteinemia, hyperapo B) and the presence of small, dense LDL [47]. An evaluation of a large number of families, including those originally reported by Goldstein et al., found evidence for a major gene effect on TG levels, but not on cholesterol levels in FCH [48]. FCH is thought to be a very frequent disorder, especially in patients with premature CHD. In young MI survivors (<60 years), its frequency has been estimated to range between 15% and 20%.

Metabolic Derangement

The metabolic basis of FCH is unknown. It is probably a heterogeneous disorder both in presentation and origin [49]. Involvement of the LDL receptor is unlikely. Metabolic studies have found increased synthesis of apo B and VLDL [50]. This hepatic overproduction of apo B again may be heterogeneous in origin. One explanatory model is based on the assumption that FFA and incompletely hydrolyzed CMR are diverted from peripheral cells to the liver, where they stimulate synthesis and secretion of VLDL. In this context, impaired interactions of so-called basic proteins with adipocytes and fibroblasts have been held responsible for the diversion of FFA from peripheral to hepatic cells [51]. Another protein putatively involved in the pathogenesis of FCH is LPL, whose activity was found to be decreased in postheparin plasma of FCH patients and their relatives [52]. Finally, FCH may be caused by defects in hepatic proteins which regulate the synthesis of lipids or apo B or the assembly and secretion of apo B-containing lipoproteins.

Diagnostic Tests

In the absence of a pathognomonic clinical or biochemical presentation in index patients and without detailed understanding of the genetic and metabolic basis, FCH can only be diagnosed on the basis of family studies. Thus screening should identify several first-degree relatives of the presenting subject with either HTG, (>2.3 mmol/l, 200 mg/dl), LDL-HC (>3.8 mmol/l, 150 mg/dl), or both, together with an increased prevalence of premature CHD in the family history. Increased levels of apo B, especially if disproportionately higher than LDL-C or TG, or increased proportions of small, dense (so-called pattern B) LDL support the diagnosis. Secondary causes of hyperlipidemia must be ruled out in any patient and relative for whom the diagnosis FCH is considered. FHC and FDB should be excluded in patients and relatives with HC, type III HLP in patients with mixed hyperlipidemia, and defects in LPL and apo C-II in patients with HTG.

Treatment and Prognosis

Patients and relatives with FCH should be encouraged to modify their diet with respect to energy intake and fat content, since many patients with FCH are obese and respond well to reduction in body weight and dietary fat intake. Generally, FCH patients should follow the step I guidelines which have been outlined above. If dietary intervention alone is not successful, drug therapy must be chosen on the basis of the dyslipidemic phenotype. Patients with pure HC should receive HMG-CoA reductase inhibitors and/or resins. These drugs, however, tend to increase TG. Therefore, fibrates and nicotinic acid should be administered to patients who present with HTG or mixed hyperlipidemia.

Children from FCH families are usually normolipidemic and do not need treatment. Nevertheless, they should be encouraged to follow a moderate-fat and cholesterol-reduced diet and to perform physical exercise to maintain optimal body weight and to learn a healthy life-style.

Genetics

FCH is believed to be an autosomal dominant disorder which occurs at a frequency of 1%–2% in the population. Its genetic basis is unknown, but is very likely to be heterogeneous. Candidate genes or proteins include LPL on chromosome 8 [51], the apo A-I/C-III/A-IV-gene cluster on chromosome 11 [53], and basic proteins whose structures and genes are as yet unknown [49].

Lipoprotein(a)

Clinical Presentation

Lp(a) was first identified as an antigen with a higher level in patients with MI than in control populations. Numerous clinical studies later demonstrated an association between elevated Lp(a) levels and risk of CHD, stroke, reocclusion after aortocoronary bypass surgery, and graft atherosclerosis in heart transplant patients [54–56]. Lp(a) levels are increased by renal diseases and the menopause; they are decreased by LPL defects and estrogens.

Lp(a) values exceeding threshold values of 30 mg/dl are thought to indicate an increased risk of CHD events independently of other risk factors, including other dyslipidemias [57]. However, recent nested prospective studies have questioned the role of elevated Lp(a) levels as a cardiovascular risk predictor in a random population [58, 59]. Patients with elevated Lo(a) levels cannot be identified on the basis of symptoms. Lp(a) should be quantified in every patient at risk for MI, i.e., in men and postmenopausal women who have CHD or additional risk factors for CHD.

Metabolic Derangement

Lp(a) resembles LDL both in lipid composition and in the presence of 1 mol apo B-100 per mol lipoprotein. In addition, Lp(a) contains a glycoprotein, apo(a), which is covalently linked to apo B by a disulfide bridge. Apo(a) contains three structural motifs that are also present in plasminogen. Because of the homology between apo(a) and plasminogen it has been hypothesized that Lp(a) exerts its atherogenic effect through interference with fibrinolysis. It has been observed that Lp(a) is bound to fibrin and that the generation of plasmin at the plasmin – fibrin interface is decreased. Lp(a) is synthesized and secreted by hepatocytes. The catabolic fate of Lp(a) is unknown, but removal from the circulation via LDL receptors and macrophage scavenger receptors has been suggested. The latter pathway may contribute to the atherogenicity of Lp(a) [54–56, 60].

Diagnostic Tests

Lp(a) levels can be quantified in serum by commercially available immunoassays. Problems arise in the comparison of values obtained by different laboratories due to the absence of standardization of Lp(a) measurements [61]. Analysis of apo(a) phenotype was not of use in predicting cardiovascular risk [62]. The diagnostic value of genotype analysis is unknown.

Treatment and Prognosis

Lp(a) levels are little influenced by diet, physical activity, or drugs. In postmenopausal women, estrogen replacement was found to decrease Lp(a) levels and is also recommended because of other beneficial effects on coronary risk [63]. Bezafibrate and nicotinic acid have also been reported to decrease Lp(a) levels. However, these reports are not conclusive, and in most cases the decrease in Lp(a) was not sufficient to produce a reduction of coronary risk [64]. Reports on the effect of HMG-CoA reductase inhibitors are also contradictory. Due to the presence of apo B in Lp(a), LDL apheresis also eliminates Lp(a). Very recently, specific Lp(a) immunoapheresis has been introduced. The implications of extracorporeal Lp(a) elimination for the progression and regression of CHD are unclear [32].

Genetics

The apo(a) gene is localized on chromosome 6q26–27 in a linkage group with the plasminogen gene [65].

Disturbances of High-Density Lipoprotein Metabolism

Familial Hyperalphalipoproteinemia (Cholesteryl Ester Transfer Protein Deficiency)

Clinical Presentation

Patients with homozygous CETP deficiency were first identified in Japan. HDL-C levels were very high and ranged between 3.9 mmol/l (150 mg/dl) and 7.8 mmol/l (300 mg/dl) with low levels of LDL-C and TG. These individuals are clinically normal. They have no signs of atherosclerotic diseases and have an increased life expectancy. Heterozygotes are also clinically normal. Their HDL-C levels (especially HDL_2-C) are frequently above the 90th percentile for sex- and age-matched controls [5, 66].

Metabolic Derangement

Because CETP activity is absent, CE formed by LCAT accumulate in HDL instead of being transferred to VLDL and LDL. HDL becomes enriched with apo E and comes to resemble HDL_c or HDL_1 and is eliminated via LDL receptors [5, 67].

Diagnostic Tests

CETP deficiency should be suspected in individuals with HDL-C levels above the 95th percentile in whom secondary causes of raised HDL-C have been excluded. Estrogen treatment in particular can considerably increase HDL-C levels. Definitive diagnosis is based on the measurement of CETP activity or CETP plasma concentration.

Treatment and Prognosis

CETP deficiency does not need treatment. Life expectancy may be increased in hyperalphalipoproteinemic subjects [66].

Genetics

In Japan, due to the high frequency of a G→A transversion in the 5′-splice donor site in exon 14 of the CETP gene, allele-specific genetic tests are advisable [66]. This mutation has been estimated to contribute 20% of the regulation of HDL-C levels in Japan. Other defects in the CETP gene have also been identified in Europe. Some of these occur commonly and appear to increase HDL-C levels [5, 66].

Apolipoprotein A-I Deficiency and Apolipoprotein A-I Variants

Clinical Presentation

To date, apo A-I deficiency has been diagnosed in ten patients from seven families. The homozygous patients came to medical attention for different reasons. This symptoms included premature CHD, planar xanthomas, and corneal opacifications. The latter were present in all subjects and were visible on gross physical examination in some patients, while others required slit-lamp examination. The biochemical presentation consisted of absent or markedly reduced levels of HDL-C and apo A-I.

Heterozygous relatives of these patients and heterozygotes for other apo A-I variants, who were detected by screening of the population and hypoalphalipoproteinemic patients had levels of HDL-C and apo A-I below the 5th percentile for age and sex (HDL-C < 0.78 mmol/l, 30 mg/dl; apo A-I < 110 mg/dl). Levels in heterozygotes were significantly lower than in unaffected family members. In most cases, heterozygous apo A-I variant carriers were asymptomatic, but heterozygosity for two apo A-I variants – apo A-I(26:Gly→Arg) and apo A-I(60:Leu→Arg) – was associated with familial amyloidosis [68].

Metabolic Derangement

Apo A-I, the major protein component of HDL, activates LCAT and is a major contributor to the ability of plasma to promote cholesterol efflux from cells. Absence of apo A-I, or the presence of apo A-I variants with grossly changed primary and/or secondary structure, prevents the formation of normal HDL. This derangement does not necessarily prevent reverse cholesterol transport, since not all patients suffered from atherosclerosis and apo A-I-free lipoproteins can also stimulate cholesterol efflux [5, 6, 68].

Diagnostic Tests

Apo A-I deficiency should be suspected in patients who present with planar xanthomas, corneal opacities, and atherosclerosis and/or with HDL-C levels below 0.13 mmol/l (5 mg/dl) in the absence of secondary causes of HDL deficiency such as liver failure, acute intestinal disease, acute inflammation, hemato-oncological disease, or intake of HDL-C-lowering drugs (anabolic steroids, probucol). Apo A-I serum concentrations in homozygotes are below 5 mg/dl. It is usually possible to identify first-degree relatives with HDL-C below 0.78 mmol/l (30 mg/dl) and apo A-I levels below 100 mg/dl. IEF may lead to the identification of abnormal banding patterns in both homozygous patients and heterozygous relatives, indicating the presence of structural apo A-I variants. Finally, sequencing of the apo A-I gene can be used to identify missense or nonsense mutations which interfere with the synthesis of normal apo A-I.

Treatment and Prognosis

The prognosis depends on the presence of CHD. This was present in only some of the cases identified so far. Therefore, thorough cardiological examination, including coronary angiography, should be performed in patients older than 30 years. When present, CHD should be treated symptomatically. Since apo A-I deficiency cannot be treated causally, it is necessary to eliminate and treat all other cardiovascular risk factors such as smoking, HC, hypertension, and diabetes mellitus. Despite having HDL-C levels below the risk threshold value of 0.91 mmol/l (35 mg/dl), heterozygotes for nonsense or missense mutations in the apo A-I gene are apparently not at increased cardiovascular risk and hence do not need special treatment. Corneal opacities found in apo A-I-deficient patients did not impair vision and did not require treatment. Specific treatments do not exist for the amyloid neuropathy and nephropathy found in some patients heterozygous for certain apo A-I variants.

Genetics

To date approximately 50 different mutations in the apo A-I gene on chromosome 11 which interfere with the synthesis of normal apo A-I have been identified. Screening studies have identified structural apo A-I variants at a heterozygosity frequency of approximately 1 in 1000. Most of these structural variants do not affect HDL-C levels, however. In most cases, only homozygotes were clinically manifest. HDL-C levels were close to zero in homozygotes and half-normal in heterozygotes [68].

Lecithin: Cholesterol Acyltransferase Deficiency and Fish-Eye Disease

Clinical Presentation

Absence of LCAT activity due to mutations in the LCAT gene leads to two different clinical conditions, namely familial LCAT deficiency and fish-eye disease (FED). Both syndromes are characterized by the development of corneal opacifications. Above the age of 30 years, the corneal opacities are visible on physical examination; slit-lamp examination is required for younger subjects. Patients with familial LCAT deficiency, but not those with FED, also develop renal disease with proteinuria, hematuria, and ultimately renal failure. An additional clinical hallmark of familial LCAT deficiency is the presence of hypochromic anemia with target cells. Patients with LCAT deficiency and FED have very low levels of HDL-C (<0.13 mmol/l, 5 mg/dl) and apo A-I (30–40 mg/dl). Frequently, the patients are moderately hypertriglyceridemic. The pathognomonic feature of familial LCAT deficiency is the very low ratio of CE to total cholesterol. This ratio is 0.7 in normal individuals. By contrast, in FED-impaired esterification of cholesterol can only be recognized by measurement of LCAT activity.

Metabolic Derangement

LCAT is the only cholesterol-esterifying enzyme in the plasma compartment. It transfers fatty acids from the sn-2 position of phosphatidyl-choline (i.e., lecithin) to the 3β-hydroxy group of cholesterol and thereby produces lysolecithin and CE. In normal plasma this process takes place in HDL. In familial LCAT deficiency, LCAT is either absent or lacks catalytic activity. As a result, there is very little CE in the plasma. In FED, however, LCAT esterifies cholesterol abnormally in VLDL and

LDL, but produces near normal ratios of CE to total cholesterol.

Diagnostic Tests

The presence of *corneal opacifications* and/or *nephropathy* in patients with HDL deficiency should lead to the differential quantification of cholesterol and CE. A free cholesterol to total cholesterol ratio above 0.7 establishes the diagnosis of familial LCAT deficiency. Diagnosis of FED requires measurement of the ability of plasma to esterify radiolabeled cholesterol in exogenous HDL or exogenous apo A-I-containing lipoproteins (α-LCAT activity) and in exogenous apo B-containing lipoproteins (β-LCAT activity) or in endogenous lipoproteins (cholesterol esterification rate). In FED, α-LCAT activity is absent, but β-LCAT activity and the cholesterol esterification rate are normal. By contrast, in familial LCAT deficiency all three activities are severely impaired. Since the LCAT gene is not polymorphic, identification of nonsense or missense mutations in this gene also helps to diagnose LCAT deficiency.

Treatment and Prognosis

In most cases corneal opacifications do not impair vision and do not need treatment. Moreover, since little experience exists of corneal transplantation under these special conditions, the benefit of this procedure in LCAT deficiency of FED is not clear. Renal failure in patients with familial LCAT deficiency is life-threatening. The age of onset of renal failure appears to depend on the degree of residual LCAT activity, i.e., on the nature of the particular mutation. However, the prognosis may also be determined by other as yet unidentified factors, since identical mutations have resulted in different clinical sequelae. End-stage renal failure is treated with chronic dialysis. Kidney transplantation is not recommended, since the transplanted organ is rapidly destroyed. Since LCAT is almost exclusively synthesized in the liver, it may be helpful to transplant both liver and kidney in LCAT-deficient patients who have developed terminal renal failure. However, no experience with this procedure has been reported. It is unclear whether CHD risk is increased in FED and LCAT deficiency. Some FED patients developed symptoms of CAD after the age of 50 years. In familial LCAT deficiency it is difficult to decide whether atherosclerosis has resulted from the dyslipidemia or from the renal disease. Since causal treatment is not possible in either case, special attention must be paid to other coronary risk factors.

Genetics

Familial LCAT deficiency and FED are extremely rare autosomal, recessively inherited disorders. To date approximately 20 mutations in the LCAT gene on chromosome 16 have been identified, two of which cause FED: LCAT(123:Thr→Ile) and LCAT(10:Pro→Leu) [69, 70]. Compound heterozygosity for a mutation causing FED and a mutation causing LCAT deficieny was associated with the clinical presentation of FED [71]. Heterozygotes for mutations in the LCAT gene have levels of HDL-C which are significantly lower than unaffected members of the same family, but which are too high to allow definitive identification in the family or population. α-LCAT activity in heterozygotes, however, is two standard deviations below the mean of controls and is a helpful marker for the identification of heterozygotes.

Tangier Disease

Clinical Presentation

Since its initial description in two siblings from Tangier Island (Virginia, USA) in 1961, Tangier disease has been diagnosed in a total of approximately 50 patients worldwide. The major clinical signs are *hyperplastic orange tonsils, neuropathy*, and *hepatosplenomegaly*. Each of these symptoms has been observed in two thirds of the patients described. However, the pathognomonic tonsil anomalies may be overlooked in cases where the adenoid tissue has been removed during childhood. Peripheral neuropathy may be asymptomatic or relapsing and occasionally devastating. In general, patients with Tangier disease present with three types of neurologic abnormalities:

- The mononeuropathic or asymmetric type is frequently transient or relapsing and sometimes includes isolated cranial nerve deficits.
- The polyneuropathic or symmetric type mostly affects the lower extremities and is slowly progressive.
- The syringomyelia-like type is also slowly progressing and presents with early loss of pain and thermal sensation, atrophy, and paresis, especially in the distal parts of the upper extremities. Sensory loss may progress to global anesthesia.

Symptoms of neuropathy include weakness, paresthesia, dysesthesia, increased sweating, diplopia, reduced strength, ptosis, ocular muscle palsies, diminished or absent deep-tendon reflexes, muscular atrophy, and loss of pain and temperature sensation. In most patients with neuropathy, nerve conduction velocities were normal; the only finding was occasional prolongation of distal latency.

Other clinical signs include corneal opacifications which are only visible on slit-lamp examination, abnormal erythrocytes termed stomatocytes, and the presence of a yellowish rectal mucosa due to foam cell deposition.

Biochemically, Tangier disease is characterized by severe deficiency or absence of normal HDL and extremely low plasma concentration of apo A-I. Typically, the serum concentration of cholesterol is also very low and the TG level moderately increased.

Metabolic Derangement

The molecular basis is unknown. Because the key features of Tangier disease are HDL deficiency and CE accumulation in histiocytes and because HDL are important in the removal of cellular cholesterol, it is likely that cellular cholesterol deposition results from an imbalance in cellular cholesterol homeostasis. In vitro, Tangier monocytes bind slightly more HDL than control cells and internalize them normally. However, instead of assembling internalized HDL with intracellular lipids prior to resecretion of cholesterol-enriched HDL, as occurs in normal cells, Tangier monocytes degrade internalized HDL in lysosomes. It has therefore been concluded that Tangier disease is a disorder of intracellular trafficking.

Diagnostic Tests

Since the etiology is unknown, there is no definite diagnostic test available. The diagnosis should be considered in any patient with unexplained hepatic or splenic enlargement and/or neuropathy and should lead to a close examination of the oropharynx and rectal mucosa and quantification of serum concentrations of cholesterol, TG, and HDL-C. HDL-C concentrations below 0.13 mmol/l (5 mg/dl) using precipitation techniques, total cholesterol levels below 3.25 mmol/l (125 mg/dl), and elevated TG levels are characteristic. There should be no lipoproteins with alpha mobility on lipoprotein electrophoresis. Serum concentrations of apo A-I and apo A-II are usually both below 5 mg/dl. Analysis of apo A-I isoproteins by IEF will reveal the increase in proapo A-I characteristic of hypercatabolic forms of HDL deficiency. Family studies are important to demonstrate that HDL deficiency is of genetic origin, since the Tangier phenotype is mimicked in some secondary forms of HDL deficiency such as hepatic diseases, acute inflammations, and hemato-oncological disorders. Other forms of familial HDL deficiency should be excluded.

Treatment and Prognosis

There is no specific treatment. Consideration may be given to reducing the fat content of the diet, since remnants of TGRL appear to accumulate in the plasma in association with fat intake.

Genetics

The clinical phenotype of Tangier disease is inherited in an autosomal recessive fashion. Inheritance of the biochemical phenotype is autosomal codominant, since obligate heterozygotes have half-normal levels of HDL-C and apo A-I. The disease appears to be extremely rare. The gene responsible for the defect in Tangier disease remains to be identified. Gene sequencing and family studies using RFLP as indirect markers in our laboratory have excluded the candidate genes apo A-I, apo A-IV, apo C, apo E, and the LDL receptor.

Familial Hypoalphalipoproteinemia

Clinical Presentation

Several epidemiological and clinical studies have revealed an inverse correlation between low plasma concentrations of HDL-C and apo A-I and increased risk of MI [72]. Family and twin studies suggested partial heredity of low HDL-C levels and indicate that 35%–50% of the variance in HDL is explained by genetic factors. Frequently, individuals with low HDL-C report a high prevalence of CHD in their families. However, the genetic trait which is transmitted in these families is often not low HDL-C, but some other form of dyslipidemia. In a recent study of patients with low HDL-C and premature CHD, first-degree relatives of the proband most frequently suffered from a combination of HTG and hypoalphalipoproteinemia or from mixed hyperlipidemia. Pure hypoalphalipoproteinemia was the least commonly transmitted trait [73].

Metabolic Derangement

The metabolic basis of FHALP is unknown. This syndrome is probably heterogeneous. A small proportion of cases is accounted for by heterozygotes for variants of apo A-I and LCAT and for Tangier disease. It is very likely that some overlap occurs between FHTG and FCH.

Diagnostic Tests

The diagnosis requires family studies which identify first-degree relatives of the index case with HDL-C levels below the 10th percentile.

Treatment and Prognosis

Low HDL-C is a common cardiovascular risk factor. To date, no interventional trial has been performed to test whether increasing HDL-C levels reduces CHD risk. Several clinical studies which primarily intended to lower LDL-C levels provide indirect evidence for this conclusion. In some of these studies, therapeutic intervention led to an increase of HDL-C, which was accompanied by a reduction in coronary events and with regression or slowed progression of atherosclerosis. For this reason, life-style factors known to decrease HDL-C levels such as smoking, obesity, and physical inactivity should be corrected. HDL-C-lowering drugs such as beta blockers and thiazide diuretics should be avoided. If HC and/or HTG are present and if dyslipidemia does not respond sufficiently to life-style changes, drug therapy with fibrates or nicotinic acid should be given [27]. In postmenopausal women, estrogen replacement therapy increases HDL-C levels. Estrogens alone may cause some increase in TG, which can be prevented by the addition of gestagens [74].

Genetics

Apart from rare mutations in the genes of LCAT and apo A-I, the genetic basis of FHALP is not known and is probably heterogeneous. Some studies have implicated the apo A-I/C-III/A-IV gene cluster, but these results have been contradicted by others [75, 76]. Moreover, some family studies indicate that hypoalphalipoproteinemia may not be the only phenotype transmitted. The disorder may also appear as HTG or mixed hyperlipidemia. Hence, some overlap with FHTG and FCH is likely.

Guidelines for the Treatment of Hyperlipidemia

Cardiovascular disease is the greatest single cause of morbidity and mortality in industrialized countries. For this reason, and because of the high prevalence of risk factors for atherosclerosis in these populations, consensus conferences in both America and Europe have developed guidelines for the prevention of cardiovascular disease [27, 31]. In addition to general recommendations for the correction of risk factors in the general population (population strategy), the European guidelines present specific recommendations for the treatment of hyperlipidemia, hypertension, and diabetes mellitus [27]. Conservative treatment of hyperlipidemia includes the treatment of underlying disease, correction of obesity, the intake of a lipid-lowering diet, and ensuring sufficient physical activity. The decision to administer drug treatment depends on whether cardiovascular disease or additional cardiovascular risk factors are present. Table 2 summarizes the levels to which LDL-C should be lowered [27].

Table 2. Therapeutic goals for lowering Low-Density Lipoprotein Cholesterol (LDL-C)

Examples of cardiovascular risk	Reduce LDL-C to:
Little elevated risk Cholesterol before treatment 5.2–7.8 mmol/l (200–300 mg) No additional risk factor Ratio of cholesterol to HDL-C, 4.5–5	4–4.5 mmol/l (155–175 mg/dl)
Moderately elevated risk Cholesterol before treatment 5.2–7.8 mmol/l (200–300 mg) *and* one additional risk factor *or* HDL-C less than 1 mmol/(39 mg/dl)	3.5–4 mmol/l (135–155 mg/dl)
High risk Presence of atherosclerotic cardiovascular diseas *or* familial hypercholesterolemia or cholesterol greater than 7.8 mmol/l (>300 mg) *or* cholesterol before treatment 5.2–7.8 mmol/l *and* two additional risk factors	3–3.5 mmol/l (115–135 mg/dl) Some experts suggest 2.5–3 mmol/l (95–115 mg/dl)

Additional risk factors are male sex, age, family history positive for cardiovascular events, arterial hypertension, smoking, diabetes mellitus, and overweight.
Therapy means intervention by diet and/or drugs.
Summarized from [34].
HDL-C, high-density lipoprotein cholesterol.

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Part IX
Nucleic Acids

Disorders of Purine and Pyrimidine Metabolism

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CONTENTS

Purine and pyrimidine nucleotides are essential cellular constituents which intervene in energy transfer, metabolic regulation and the synthesis of DNA and RNA. Both purine (see Fig. 1) and pyrimidine (see Fig. 3) metabolism can be divided into three pathways:

- The biosynthetic pathway, often termed de novo, synthesizes purine and pyrimidine nucleotides from small molecules.
- The catabolic pathway of the purines produces uric acid, whereas that of the pyrimidines yields citric acid cycle intermediates.
- The salvage pathway recovers purine and pyrimidine bases, which are provided by food intake or by the catabolic pathway.

Table 1 lists the major presenting signs and laboratory findings in the inborn errors which have been detected along these various pathways. With the exception of muscle adenosine monophosphate (AMP) deaminase deficiency, all these enzyme defects are very rare.

Inborn Errors of Purine Metabolism

Inborn errors of purine metabolism comprise errors in the synthesis of purine nucleotides – phosphoribosyl pyrophosphate synthetase superactivity and adenylosuccinase deficiency – and in purine catabolism – muscle AMP deaminase deficiency, adenosine deaminase (ADA) deficiency, purine nucleoside phosphorylase (PNP) deficiency, and xanthine oxidase deficiency – and purine salvage – hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency and adenine phosphoribosyltransferase (APRT) deficiency.

Phosphoribosyl Pyrophosphate Synthetase Superactivity

Clinical Presentation

The disorder is mostly manifested by the appearance, in young adult males, of gouty arthritis and/or uric acid lithiasis, potentially leading to renal insufficiency [1, 2]. *Uricemia* can be very high, reaching 10–15 mg/dl (normal adult values, 2.9–5.5 mg/dl). The urinary excretion of uric acid is also increased, reaching up to 2400 mg/24 h (normal adult values, 500–800 mg/24 h).

A few patients have been reported in whom clinical signs of uric acid overproduction already appeared in infancy and were accompanied by neurologic abnormalities, mainly sensorineural deafness, particularly for high tones, but also hypotonia, locomotor delay, ataxia and autistic features [2].

Metabolic Derangement

The enzyme forms phosphoribosyl pyrophosphate (PRPP) from ribose-5-phosphate and adenosine

Table 1. Main presenting clinical signs and laboratory data in inborn errors of purine and pyrimidine metabolism

	Diagnostic possibilities
Clinical signs	
Psychomotor delay	PRPP synthetase superactivity Adenylosuccinase deficiency Combined xanthine and sulfite oxidase deficiency HGPRT deficiency (complete) UMP synthase deficiency Dihydropyrimidine dehydrogenase deficiency
Ataxia	HGPRT deficiency (complete)
Autistic features	PRPP synthetase superactivity Adenylosuccinase deficiency Dihydropyrimidine dehydrogenase deficiency
Self-mutilation	HGPRT deficiency (complete)
Deafness	PRPP synthetase superactivity
Growth retardation	Adenylosuccinase deficiency ADA deficiency UMP synthase deficiency
Infections (recurrent)	ADA deficiency PNP deficiency
Arthritis	PRPP synthetase superactivity HGPRT deficiency (partial)
Kidney stones	PRPP synthetase superactivity (uric acid) Xanthine oxidase deficiency (xanthine) HGPRT deficiency (uric acid) APRT deficiency (2,8-dihydroxyadenine)
Renal insufficiency	PRPP synthetase superactivity HGPRT deficiency APRT deficiency
Muscle cramps	Myoadenylate deaminase deficiency
Muscle wasting	Adenylosuccinase deficiency
Laboratory data	
Anemia	UMP synthase deficiency
Lymphopenia	
– B and T cells	ADA deficiency
– T cells	PNP deficiency
Hyperuricemia	PRPP synthetase superactivity HGPRT deficiency (complete or partial)
Hypouricemia	PNP deficiency Xanthine oxidase deficiency (isolated or combined with sulfite oxidase deficiency)
Orotic aciduria	UMP synthase deficiency

ADA, adenosine deaminase; APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PNP, purine nucleoside phosphorylase; PRPP, phosphoribosyl pyrophosphate; UMP, uridine monophosphate.

triphosphate (ATP; Fig. 1). PRPP is the first intermediate of the de novo synthesis of purine nucleotides (not shown in detail in Fig. 1), which leads to the formation of inosine monosphosphate (IMP), from which the other purine compounds are derived. PRPP synthetase is highly regulated. Various genetic regulatory and catalytic defects [1, 2] lead to superactivity, resulting in increased generation of PRPP. Because PRPP amidotransferase, the first enzyme of the de novo pathway, is physiologically not saturated by PRPP, the synthesis of purine nucleotides increases, and

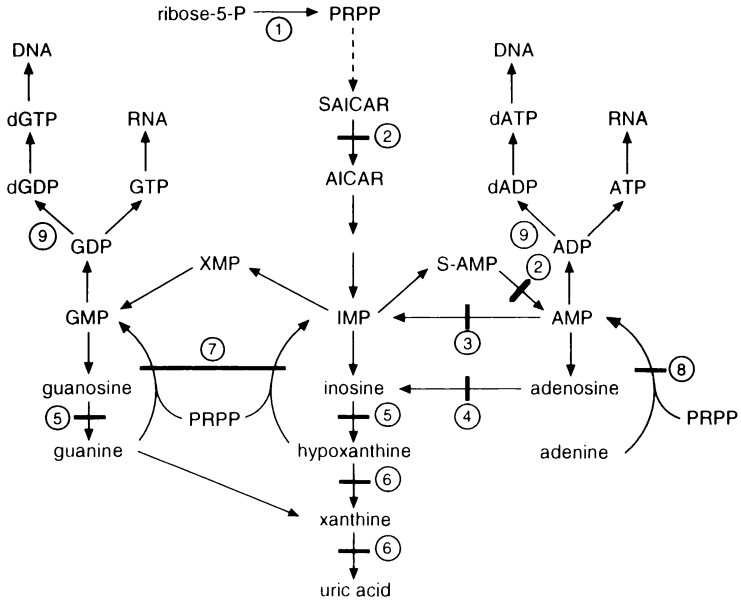

Fig. 1. Pathways of purine metabolism. *1*, Phosphoribosyl pyrophosphate, (*PRPP*) synthetase; *2*, adenylosuccinase; *3*, adenosine monophosphate (*AMP*) deaminase; *4*, adenosine deaminase; *5*, purine nucleoside phosphorylase; *6*, xanthine oxidase (dehydrogenase); *7*, hypoxanthine-guanine phosphoribosyltransferase; *8*, adenine phosphoribosyltransferase; *9*, ribonucleotide reductase. *GTP*, guanosine triphosphate; *GDP*, guanosine diphosphate; *GMP*, guanosine monophosphate; *ATP*, adenosine triphosphate; *ADP*, adenosine diphosphate; *SAICAR*, succinylaminoimidazole carboxamide ribotide; *IMP*, inosine monophosphate; *S-AMP*, adenylosuccinate

hence so does the production of uric acid. PRPP synthetase superactivity is one of the few known examples of an hereditary anomaly of an enzyme which enhances its activity. The mechanism of the neurologic symptoms is unresolved.

Diagnostic Tests

Diagnosis requires extensive kinetic studies of the enzyme, which are performed on erythrocytes and cultured fibroblasts in a few laboratories in the world. The disorder should be differentiated from partial HGPRT deficiency, which gives similar clinical signs.

Treatment and Prognosis

Patients should be treated with allopurinol, which inhibits xanthine dehydrogenase, the last enzyme of purine catabolism (Fig. 1). This results in a decrease in the production of uric acid and in its replacement by hypoxanthine, which is about tenfold more soluble, and xanthine, which is slightly more soluble than uric acid. Initial dosage of allopurinol is 10–20 mg/kg per day in children and 2–10 mg/kg per day in adults. It should be adjusted to the minimum required to maintain normal uric acid levels in plasma and reduced in subjects with renal insufficiency. In rare patients with a considerable increase in de novo synthesis, xanthine calculi can be formed during allopurinol therapy [3]. Additional measures to prevent crystallization are thus recommended. These include a low-purine diet (free of organ meats, sardines, dried beans and peas), high fluid intake, and, since uric acid and xanthine are more soluble at alkaline than at acid pH, administration of sodium bicarbonate, potassium citrate, or citrate mixtures to bring urinary pH to 6.0–6.5. Adequate control of the uricemia prevents gouty arthritis and urate nephropathy, but does not correct the neurological symptoms.

Genetics

The various forms of PRPP synthetase superactivity are inherited as sex-linked traits. In the families in which the anomaly is associated with sensorineural deafness, heterozygous females have also

been found with gout and/or hearing impairment [2].

Adenylosuccinase Deficiency

Clinical Presentation

Severe psychomotor retardation is the principal symptom in most patients diagnosed hitherto. The majority of these children also have epilepsy. Autistic features (failure to make eye-to-eye contact, repetitive behavior, temper tantrums) are found in about half of the patients [4, 5]. Some affected children display in addition profound growth retardation, associated with muscular wasting [5].

Strikingly, one patient, a girl, is only slightly retarded [5]. Whereas adenylosuccinase deficiency with profound mental retardation is often referred to as type I, this variant is termed type II. The marked clinical heterogeneity justifies systematic screening for the deficiency in unexplained psychomotor retardation and neurological disease.

Metabolic Derangement

Adenylosuccinase (also named adenylosuccinate lyase) catalyzes two steps in purine synthesis (Fig. 1):

- The conversion of succinylamino-imidazole carboxamide ribotide (SAICAR) into AICAR, along the de novo pathway
- The conversion of adenylosuccinate (S-AMP) into AMP.

Its deficiency results in accumulation in cerebrospinal fluid, plasma, and urine of the succinylpurines, SAICA riboside, and succinyladenosine, the products of the dephosphorylation by cytosolic 5′-nucleotidase of the two substrates of the enzyme [4]. Strikingly, the mild type II patient has markedly higher succinyladenosine levels than the profoundly retarded type I patients, the concentrations of SAICA riboside being similar in both types. The adenylosuccinase defect is marked in liver and kidney, not found in erythrocytes and granulocytes, but also expressed in muscle of the patients with growth retardation and muscle wasting [5]. The higher levels of succinyladenosine in type II might be explained by a more profound loss of activity of the enzyme toward adenylosuccinate than toward SAICAR, as compared with a parallel deficiency in type I [6]. The symptoms of the deficiency remain unexplained, but positron emission tomography reveals a marked decrease in the uptake of fluorodeoxyglucose in the cortical brain areas [7].

Diagnostic Tests

Diagnosis is based on the presence in cerebrospinal fluid and urine of SAICA riboside and succinyladenosine, which are normally undetectable. These can be recognized by various techniques. For systematic screening, a modified Bratton-Marshall test [8], performed on urine, appears most practical. False-positive results are, however, recorded in patients who receive sulphonamides, for the measurement of which the test was initially devised. Final diagnosis thus requires high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection [4]. The activity of adenylosuccinase should be measured on fresh biopsy specimens, owing to the sensitivity of the enzyme to freezing and thawing.

Treatment and Prognosis

With the aim of replenishing hypothetically decreased concentrations of adenine nucleotides in adenylosuccinase-deficient tissues, some patients have been treated for several months with oral supplements of adenine (10 mg/kg per day) and allopurinol (5–10 mg/kg per day). Adenine can be incorporated into the adenine nucleotides via APRT (Fig. 1). Allopurinol is required to avoid conversion of adenine by xanthine dehydrogenase into minimally soluble 2,8-dihydroxyadenine, which forms kidney stones. No clinical or biochemical improvement was recorded, with the exception of weight gain and some acceleration of growth [5].

The prognosis for survival of type I adenylosuccinase-deficient patients seems poor. The oldest child with the defect has now reached 17 years of age, but two others have died at the ages of 8 and 13 years, respectively.

Genetics

The deficiency is transmitted as an autosomal recessive trait [4, 5]. Studies of the adenylosuccinase

gene, localized on chromosome 22, suggest that each affected family carries a different mutation. In a first family, a T→C substitution was detected, resulting in a Ser413→Pro change [9].

Muscle Adenosine Monophosphate Deaminase Deficiency

Clinical Presentation

The deficiency of muscle AMP deaminase (frequently referred to as *myoadenylate deaminase* in the clinical literature) can be either a primary genetic defect or secondary to another neuromuscular disease. The primary defect typically presents with isolated muscular weakness, fatigue, cramps, or myalgias following moderate to vigorous exercise, sometimes accompanied by an increase in serum creatine kinase and minor electromyographic abnormalities. Muscular wasting or histologic abnormalities are absent [10]. Primary AMP deaminase deficiency was initially detected in young adults, but later wide variability was observed with respect to the age of onset of the symptoms (1.5–70 years) [11, 12]. Moreover, the enzyme defect has been detected in asymptomatic family members of individuals with the disorder. Secondary muscle AMP deaminase deficiency is found in several diseases, including amyotrophic lateral sclerosis, fascioscapulohumeral myopathy, Kugelberg-Welander syndrome, polyneuropathies, and Werdnig-Hoffmann disease [11, 12].

Metabolic Derangement

AMP deaminase, adenylosuccinate synthetase, and adenylosuccinase form the purine nucleotide cycle (Fig. 2). Numerous functions have been proposed for this cycle in muscle (reviewed in [13]):

- Removal of AMP formed during exercise, in order to favor the formation of ATP from adenosine diphosphate (ADP) by myokinase (adenylate kinase)
- Release of NH_3 and IMP, both stimulators of glycolysis and hence of energy production
- Production of fumarate, an intermediate of the citric acid cycle, which also yields energy.

It has therefore been proposed that the muscle dysfunction observed in primary myoadenylate deaminase deficiency is caused by impairment of energy production for muscle contraction. However, this does not tally with the existence of asymptomatic myoadenylate deaminase-deficient individuals in families with the deficiency and with the frequent occurrence of the enzyme defect in other muscle disorders.

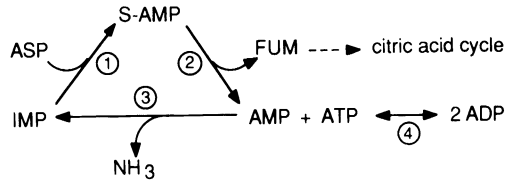

Fig. 2. The purine nucleotide cycle. *1*, Adenylosuccinate synthetase; *2*, adenylosuccinase; *3*, adenosine monophosphate (*AMP*) deaminase; *4*, also shown is myokinase. *IMP*, inosine monophosphate; *S-AMP*, adenylosuccinate; *FUM*, fumarate; *ATP*, adenosine triphosphate; *ADP*, adenosine diphosphate; *ASP*, aspartate

It should be noted that muscle, liver, and erythrocytes contain different isoforms of AMP deaminase. A regulatory mutation of liver AMP deaminase has been proposed as a cause of primary gout with overproduction of uric acid [14]. Individuals with a complete, although totally asymptomatic deficiency of erythrocyte AMP deaminase have been detected in Japan, Korea, and Taiwan [15].

Diagnostic Tests

Screening for the defect can be performed by an *exercise test* (see Chapter 2). A severalfold elevation of venous plasma ammonia, seen in normal subjects, is absent in myoadenylate deficiency. Final diagnosis is established by histochemical or biochemical assay in a muscle biopsy. In the primary defect, the activity of myoadenylate deaminase is below 2% of normal, and little or no immunoprecipitable enzyme is found. In the secondary defect, the activity is between 2% and 15% of normal, and usually appreciable immunoreactivity is present [16]. Remarkably, in several large series of muscle biopsies for diagnostic purposes, low enzyme activities were found in about 2% of all specimens [11, 12].

Treatment and Prognosis

Patients may display a gradual progression of their symptoms, which may lead to the point that even dressing and walking a few steps lead to

fatigue and myalgias. They should be advised to exercise with caution to prevent rhabdomyolysis and myoglobinuria. Administration of ribose (2–60 g per day orally in divided doses) has been reported to improve muscular strength and endurance [17].

Genetics

Primary myoadenylate deaminase deficiency is apparently transmitted as an autosomal recessive trait. AMPD1, the gene encoding muscle AMP deaminase, is located on chromosome 1. In all patients with the primary deficiency investigated hitherto, the defect is caused by a C→T substitution at nucleotide 34, resulting in a stop codon [18]. Population studies suggest that this mutant allele is found with a high frequency in Caucasians. This accords with the finding that about 2% of diagnostic muscle biopsies are myoadenylate deaminase deficient and suggests that the mutation arose in a remote Western European ancestor.

Adenosine Deaminase Deficiency

Clinical Presentation

The majority of patients display within the first weeks or months after birth a profound impairment of both humoral and cellular immunity, known as *severe combined immunodeficiency disease* (SCID). Multiple, recurrent infections rapidly become life-threatening [19, 20]. A few cases with delayed (up to 3 years of age) and late (5–8 years of age) onset have, nevertheless, been reported. Caused by a broad variety of organisms, infections are mainly localized in the skin and the respiratory and gastrointestinal tract. In the latter, they often lead to intractable diarrhea and malnutrition. In affected children over 6 months of age, the hypoplasia or apparent absence of lymphoid tissue constitutes a suggestive sign. Bone abnormalities, clinically evident as prominence of the costochondral rib junctions and radiologically as cupping and flaring thereof, are found in about half of the patients. In a few affected children neurologic abnormalities are found, including spasticity, head lag, movement disorders, nystagmus, and inability to focus.

SCID can be confirmed by relatively simple laboratory tests: lymphopenia (usually less than 500 total lymphocytes per mm^3), involving both B and T cells, and hypogammaglobulinemia are almost invariably present. Whereas the IgM deficiency may be detected early, the IgG deficiency becomes manifest only after the age of 3 months, when the maternal supply has been exhausted. More elaborate tests show a deficiency of antibody formation following specific immunization and an absence or severe diminution of the lymphocyte proliferation induced by mitogens. The disease is progressive, since residual B and T cell function which may be found at birth disappears later on.

Metabolic Derangement

The deficiency results in the accumulation in body fluids of adenosine (Fig. 1) and also of deoxyadenosine (not shown in Fig. 1), another substrate of the enzyme, derived from the catabolism of DNA. Both compounds are normally nearly undetectable. Among the mechanisms which have been proposed to explain the immunodeficiency, two are considered most important, namely inhibition of ribonucleotide reductase and impairment of the transmethylation reactions [21].

Ribonucleotide reductase is an essential enzyme for the synthesis of DNA (Fig. 1), which has to proceed at a high rate during lymphocyte development and differentiation. Its inhibition in ADA-deficient lymphocytes is explained by the accumulation of excess dATP, formed from deoxyadenosine. Transmethylation reactions are vital in a number of physiological processes, including lymphocyte function. Accumulation of deoxyadenosine has been shown to inactivate *S*-adenosylhomocysteine hydrolase, an essential enzyme of the transmethylation reaction sequence.

Diagnostic Tests

The diagnosis is mostly performed on red blood cells. Affected individuals display less than 1% residual ADA activity [19, 20]. It should be noted that only about 20%–30% of the patients with the clinical and hematologic picture of inherited SCID are ADA deficient. In the other patients, SCID is caused by unknown mechanisms. A few subjects have been described with ADA deficiency in red

blood cells but normal immunocompetence [20]. This is explained by the presence of residual ADA activity in their lymphocytes. A hereditary, approximately 50-fold elevation of red cell ADA has been shown to cause nonspherocytic hemolytic anemia [22].

Treatment and Prognosis

► *Bone marrow transplantation*, the first choice provided an histocompatible donor is available, gives a good chance for complete cure, both clinically and immunologically [23]. The graft provides stem cells, and hence T and B cells, which have sufficient ADA activity to prevent accumulation of adenosine and deoxyadenosine. Survival is, however, much lower with HLA-mismatched transplants. If no histocompatible bone marrow donor is found, enzyme replacement therapy can be accomplished with normal erythrocytes, irradiated before use to prevent graft-versus-host disease. Repeated partial exchange transfusions are performed at 2- to 4-week intervals. In some patients marked clinical and immunological improvement is obtained, but in most response is poor or not sustained [23]. In addition, this therapy carries the risks inherent to repeated transfusions.

► A much more effective enzyme replacement therapy can be achieved with polyethylene glycol-modified ADA (PEG-ADA). Covalent attachment of PEG to bovine ADA results in marked extension of its half-life and reduction of immunogenicity [24]. Weekly to biweekly intramuscular injections of 15–30 units of PEG-ADA per kg resulted in mostly marked clinical improvement in a series of 29 patients. In vitro immune function also significantly improved.

The first approved clinical trial of *gene therapy* was performed in 1990 in a 4-year-old girl with ADA deficiency and shortly thereafter in a second affected child [25]. Peripheral blood T cells of the patients were collected, cultured with interleukin-2, corrected by insertion of the ADA gene by means of a retroviral vector, and reinfused. Both patients improved, but the treatment has to be repeated at regular time intervals because lymphocytes live only a few months. Future trials are therefore aimed at the technically more demanding gene transfer into stem cells, which in theory have an unlimited life span.

Enzyme replacement therapies have significantly improved the prognosis of ADA deficiency. Untreated, the defect invariably led to death, usually within the first year of life, unless drastic steps were taken, such as rearing in strictly sterile conditions from birth on.

Genetics

Approximately one third of the cases of inherited SCID are X-linked, whereas two thirds are autosomal recessive. ADA deficiency is found only in the latter group, where it accounts for about 50% of the patients. The frequency of the deficiency is estimated at 1 per 100 000–500 000 births. Studies of the ADA gene, located on chromosome 20, have hitherto revealed approximately 20 mutations, the majority of which are single nucleotide changes, resulting in an either inactive or unstable enzyme [21]. Most patients carry two different mutations on each chromosome 20, but others, mainly from inbred communities, are homozygous for the mutation.

Purine Nucleoside Phosphorylase Deficiency

Clinical Presentation

Recurrent infections are usually of later onset, starting from the end of the first year up to 5–6 years of age, and are initially less severe than in ADA deficiency [20, 26, 27]. A strikingly enhanced susceptibility to viral diseases, such as varicella, measles, cytomegalovirus, and vaccinia has been reported, but severe pyogenic infections also occur. One third of the patients have anemia, and two thirds display neurologic symptoms, including spastic tetra- or diplegia, ataxia, and tremor. Immunological studies reveal an increasing *deficiency of cellular immunity*, reflected by a marked reduction in the number of T cells. B lymphocyte function is deficient in about one third of the patients.

Metabolic Derangement

The deficiency provokes an accumulation in body fluids of the four normally nearly undetectable substrates of the enzyme, namely guanosine, inosine (Fig. 1), and their deoxycounterparts (not shown in Fig. 1), the latter derived from DNA breakdown. Formation of uric acid is thus severely

hampered. The profound impairment of cellular immunity, characterizing the disorder, has been explained by an accumulation, particularly in T cells, of excess dGTP. It is formed from deoxyguanosine and inhibits ribonucleotide reductase and hence cell division.

Diagnostic Tests

Patients display a striking decrease of the production of uric acid: plasma uric acid is usually below 1 mg/dl. The urinary excretion of uric acid is also markedly diminished. Enzymatic diagnosis is mostly performed on red blood cells.

Treatment and Prognosis

Most patients have died, although at a later age than untreated ADA-deficient children, from overwhelming viral or bacterial infections. Treatments consisted of bone marrow transplantation and repeated transfusions of normal, irradiated erythrocytes [20, 23, 27]. Gene therapy might become available in the near future.

Genetics

The deficiency is inherited in an autosomal recessive fashion. Studies performed in a few patients of the PNP gene, located on chromosome 14, have revealed a number of point mutations.

Xanthine Oxidase Deficiency

Clinical Presentation

Two types of xanthine oxidase (or dehydrogenase) deficiency (also termed hereditary *xanthinuria*) are known: an isolated form [28] and a combined xanthine oxidase and *sulfite oxidase deficiency* [29]. Isolated xanthine oxidase deficiency can be completely asymptomatic, although in about one third of the cases kidney stones are formed. Most often not visible on X-ray, they may appear at any age [28]. Myopathy may be present, associated with crystalline xanthine deposits. In combined xanthine oxidase and sulfite oxidase deficiency, the clinical picture of sulfite oxidase deficiency (which is also found as an isolated defect [30]), overrides that of xanthine oxidase deficiency. The symptoms include neonatal feeding difficulties and seizures, myoclonia, increased or decreased muscle tone, eye lens dislocation, and severe mental retardation.

Metabolic Derangement

The deficiency results in the near total replacement of uric acid by hypoxanthine and xanthine as the end products of purine catabolism (Fig. 1). In combined xanthine oxidase and sulfite oxidase deficiency, there is in addition an accumulation of sulfite and of sulfur-containing metabolites and a diminution in the production of inorganic sulfate. The combined defect is caused by the deficiency of a *molybdenum* cofactor, which is required for the activity of both xanthine oxidase and sulfite oxidase.

Diagnostic Tests

In both forms of the deficiency, plasma concentrations of uric acid below 1 mg/dl are measured; they may decrease to virtually undetectable values on a low-purine diet. Urinary uric acid is reduced to a few percent of normal and replaced by hypoxanthine and xanthine. In the combined defect, these urinary changes are accompanied by an excessive excretion of sulfite and other sulfur-containing metabolites, such as *S*-sulfocysteine, thiosulfate, and taurine. The enzymatic diagnosis requires liver or intestinal mucosa, the only human tissues which normally contain appreciable amounts of xanthine oxidase. Sulfite oxidase and the molybdenum cofactor can be assayed in liver and fibroblasts.

Treatment and Prognosis

Isolated xanthine oxidase deficiency is mostly benign. A low-purine diet should be prescribed and fluid intake increased. Allopurinol has been advocated in subjects with residual xanthine oxidase activity to completely block the conversion of hypoxanthine into about tenfold less soluble xanthine. The prognosis of combined xanthine oxidase and sulfite oxidase deficiency is very poor. So far, all therapeutic attempts, including low-sulfur diets, the administration of sulfate and molyb-

denum [29], and trials to bind sulfite with thiol-containing drugs, have been unsuccessful.

Genetics

The inheritance of both isolated xanthine oxidase deficiency and combined xanthine oxidase and sulfite oxidase deficiency is autosomal recessive.

Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency

Clinical Presentation

The disorder can present under two forms. Patients with complete or near-complete deficiency of HGPRT display the *Lesch-Nyhan syndrome* [31]. Affected children generally appear normal during the first few months of life. At 3–4 months of age, a neurologic syndrome evolves, including delayed motor development, choreoathetoid movements, and spasticity with hyperreflexia and scissoring. Over the years, the patients develop a striking, compulsive *self-destructive behavior*, involving biting of their fingers and lips, which leads to mutilating loss of tissue. Speech is hampered by athetoid dysarthria. Whereas most patients have an IQ of around 50, some display normal intelligence. Approximately 50% of the patients have seizures. Sooner or later they form uric acid stones. Mothers of Lesch-Nyhan patients have reported the finding of orange crystals on diapers during the first few weeks after birth. Untreated, the *uric acid* nephrolithiasis progresses to obstructive uropathy and renal failure during the first decade of life. The latter clinical picture may, exceptionally, also be observed in early infancy.

Partial HGPRT deficiency is found in rare patients with gout. Most of them are normal on neurologic examination, but occasionally spasticity, dysarthria, and a spinocerebellar syndrome are found [32]. Whereas most patients with the Lesch-Nyhan syndrome do not develop gouty arthritis, this finding is common in partial HGPRT deficiency.

Metabolic Derangement

The considerable increase in the production of uric acid is explained as follows: PRPP, which is not utilized at the level of HGPRT (Fig. 1), is available in increased quantities for the first, rate-limiting enzyme of the de novo synthesis, PRPP amidotransferase (not shown in Fig. 1). Since the latter is normally not saturated with PRPP, its activity increases and the ensuing acceleration of the de novo synthesis results in the overproduction of uric acid.

The pathogenesis of the neurologic symptoms is still not satisfactorily explained. Postmortem studies of the basal ganglia, the most affected area according to the neurologic dysfunction, have shown marked decreases in the concentration of dopamine, in the enzymes required for its synthesis, and in its major metabolite, homovanillic acid [33]. How the HGPRT defect leads to the deficit of the dopaminergic system remains to be clarified.

Diagnostic Tests

Patients excrete excessive amounts of uric acid, ranging from 25 to 140 mg/kg body weight per 24 h, as compared to an upper limit of 18 mg/kg per 24 h in normal children. Determination of the ratio of uric acid to creatinine (mg/mg) in morning samples of urine provides a screening test. This ratio is much higher in HGPRT deficiency than the normal upper limits of 2.5, 2.0, 1.0, and 0.6 for infants, children aged 2 years and 10 years, and adults, respectively [34]. Increased ratios are also found in other disorders with uric acid overproduction, such as PRPP synthetase superactivity, glycogenosis type I, and lymphoproliferative diseases. The overproduction of uric acid is as a rule accompanied by an increase of serum urate, which may reach concentrations as high as 18 mg/dl. Occasionally, however, particularly before puberty, uricemia may be in the normal or high normal range.

Patients with the Lesch-Nyhan syndrome display nearly undetectable HGPRT activity in red blood cells [35]. In partial deficiencies, similarly low or higher values may be found [32]. Rates of incorporation of hypoxanthine into the adenine nucleotides of intact fibroblasts correlate better with the clinical symptomatology than HGPRT activities in erythrocytes: patients with the complete Lesch-Nyhan syndrome incorporated less than 1.2% of normal, those with gout and neurologic symptoms 1.2–10% of normal, and those with isolated gout, 10%–55% of normal [36].

Treatment and Prognosis

Allopurinol, as detailed under PRPP synthetase superactivity, is indicated to prevent urate nephropathy. Allopurinol, even when given from birth, has, however, no effect on the neurologic symptoms, which have so far resisted all therapeutics attempts. Adenine has been administered, together with allopurinol, with the aim of correcting a possible depletion of purine nucleotides. However, no or minimal changes in neurologic behaviour were recorded [37]. Patients should be made more comfortable by appropriate restraints, including elbow splints, and even tooth extraction, to diminish self-mutilation. Diazepam, haloperidol, and barbiturates may sometimes improve choreoathetosis.

In a 22-year-old patient, bone marrow transplantation restored erythrocyte HGPRT activity to normal, but did not change neurologic symptoms [38].

Genetics

Both the Lesch-Nyhan syndrome and the partial deficiencies of HGPRT are transmitted in a sex-linked recessive manner. Studies of the HGPRT gene in large groups of unrelated patients have revealed a variety of defects, ranging from point mutations provoking single amino acid substitutions, and henceforth enzymes with altered stability and/or kinetic properties, to extensive deletions resulting in suppression of enzyme synthesis [39]. These studies have contributed a great deal to the understanding of the clinical variation observed in human inherited disease and provided support for the concept that, in X-linked disorders, new mutations constantly appear in the population.

Adenine Phosphoribosyltransferase Deficiency

Clinical Presentation

The deficiency may become clinically manifest in childhood [40], even from birth [41], but may remain silent for several decades. Symptoms include urinary passage of gravel, small stones, and crystals, frequently accompanied by abdominal colic, dysuria, hematuria, and urinary tract infection. Some patients may even present with acute anuric renal failure [42]. The urinary precipitates are composed of 2,8-dihydroxyadenine, are radiotranslucent, and are not distinguishable from uric acid stones by routine chemical testing.

Metabolic Derangement

The deficiency results in suppression of the salvage of adenine (Fig. 1) provided by food and by the polyamine pathway (not shown in Fig. 1). Consequently, adenine is oxidized by xanthine dehydrogenase into *2,8-dihydroxyadenine*, a very poorly soluble compound (solubility in urine, at pH 5 and 37°C, is about 0.3 mg/dl as compared to 15 mg/dl for uric acid).

The deficiency can be complete or partial. The partial deficiency is only found in the Japanese, among whom it may be quite common [43]. Activities range from 10%–30% of normal at supraphysiological concentrations of PRPP, but a 20- to 30-fold decrease in the affinity for PRPP results in near inactivity under physiological conditions.

Diagnostic Tests

Identification of 2,8-dihydroxyadenine requires complex analyses, including UV and infrared spectrography, mass spectrometry, and X-ray crystallography [40,41]. It is therefore usually easier to measure APRT activity in red blood cells.

Treatment and Prognosis

In patients with symptoms, allopurinol should be given, as detailed under PRPP synthetase superactivity, to inhibit the formation of 2,8-dihydroxyadenine. Both in patients with stones and in those without symptoms, dietary purine restriction and high fluid intake are recommended. Alkalinization of the urine is, however, not advised: unlike that of uric acid, the solubility of 2,8-dihydroxyadenine does not increase up to pH 9 [40].

Ultimate prognosis depends on renal function at the time of diagnosis: late recognition may result in irreversible renal insufficiency, requiring chronic dialysis, and early treatment in the prevention of stones.

Genetics

APRT deficiency is inherited as an autosomal recessive trait. Approximately 80% of the Japanese patients carry the same T→C substitution, resulting in a Met136→Thr change [43]. In Caucasians about a dozen mutations have been identified so far, some of which seem more common, also suggesting founder effects [44].

Inborn Errors of Pyrimidine Metabolism

Hereditary Orotic Aciduria
(Uridine Monophosphate Synthase Deficiency)

Clinical Presentation

Megaloblastic anemia, which appears a few weeks or months after birth, is usually the first manifestation [45,46]. Peripheral blood smears often show anisocytosis, poikilocytosis, and moderate hypochromia. Bone marrow examination reveals erythroid hyperplasia and numerous megaloblastic erythroid precursors. Characteristically, the anemia does not respond to iron, folic acid, vitamin B_{12} etc. Unrecognized, the disorder leads to failure to thrive and to retardation of growth and psychomotor development.

Metabolic Derangement

Uridine monophosphate (UMP) synthase is a bifunctional enzyme of de novo pyrimidine synthesis (Fig. 3), which sequentially converts orotic acid into OMP (orotate phosphoribosyltransferase, OPRT) and decarboxylates OMP into UMP (orotate decarboxylase, ODC). The defect provokes a massive overproduction of *orotic acid* and a deficiency of pyrimidine nucleotides [46]. The overproduction is attributed to the ensuing decrease of the feedback inhibition exerted by the pyrimidine nucleotides on the first enzyme of their de novo synthesis, cytosolic carbamoyl phosphate synthetase II (Fig. 3). The deficiency of pyrimidine nucleotides leads to impairment of cell division, which results in megaloblastic anemia and in retardation of growth and development.

Diagnostic Tests

Urinary analysis reveals a massive overexcretion of orotic acid, reaching in infants 200- to 1000-fold

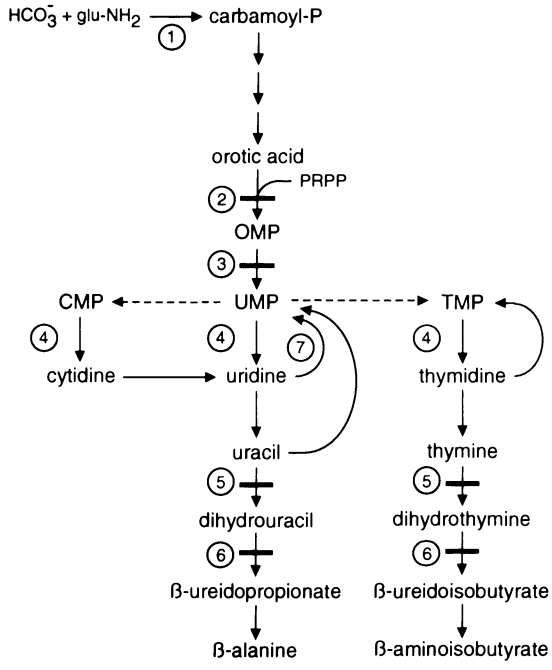

Fig. 3. Pathways of pyrimidine metabolism. *1*, Carbamoylphosphate synthetase; *2*, orotate phosphoribosyltransferase; *3*, orotidine decarboxylase (2 and 3 form uridine monophosphate, *UMP*, synthase); *4*, pyrimidine 5′-nucleotidase; *5*, dihydropyrimidine dehydrogenase; *6*, dihydropyrimidinase; *7*, uridine kinase, *PRPP*, phosphoribosyl pyrophosphate; *CMP*, cytidine monophosphate; *OMP*, orotidine monophosphate; *UMP*, uridine monophosphate; *TMP*, thymidine monophosphate

the normal adult value of 1–1.5 mg per 24 h. Occassionally, orotic acid crystalluria is noted, particularly upon dehydration. Enzymatic diagnosis can be performed on red blood cells. In all patients reported hitherto, except one, both OPRT and ODC activities were deficient. This defect is termed type I. In a single patient (type II), only the activity of ODC was initially deficient, although that of OPRT also decreased later on [46].

Treatment and Prognosis

The enzyme defect can be bypassed by the administration of *uridine*, which is converted into UMP by uridine kinase (Fig. 3). An initial dose of 100–150 mg/kg, divided over the day, induces prompt hematologic response and acceleration of growth. Further dosage should be adapted to obtain the lowest possible output of orotic acid. In some cases normal psychomotor development was

achieved, but not in others, possibly owing to delayed onset of therapy.

Genetics

Hereditary orotic aciduria is inherited as an autosomal recessive trait. The genetic lesion results in synthesis of an enzyme with reduced stability [47].

Dihydropyrimidine Dehydrogenase Deficiency

Clinical Presentation

Two clinical forms occur. In children, neurologic symptoms vary from epilepsy with normal intelligence but autistic features to severe mental retardation accompanied by generalized hypertonia and hyperreflexia [48]. In asymptomatic female adults, the deficiency was only revealed by 5-fluorouracil therapy for breast carcinoma [49, 50]. This caused severe toxicity, manifested by cytopenia, stomatitis, diarrhea, and neurologic symptoms, including ataxia, paralysis, and stupor.

Metabolic Derangement

Dihydropyrimidine dehydrogenase catalyzes the catabolism of uracil and thymine into, respectively, dihydrouracil and dihydrothymine (Fig. 3). The defect leads to the accumulation of uracil and thymine [48]. It also blocks the catabolism of pyrimidine analogues, such as the anticancer drug 5-fluorouracil. This results in a marked potentiation of its action and henceforth of its toxicity [49]. Why the defect becomes manifest during infancy in some patients, and only upon administration of 5-fluorouracil in others, and how it leads to neurologic symptoms is not known.

Diagnostic Tests

Patients excrete high amounts of *uracil* (2–10 mmol per g creatinine, as compared to less than 0.3 mmol per g creatinine in control urine) and of *thymine* (2–7 mmol per g creatinine, normally undetectable). Uracil and thymine are also elevated in plasma and cerebrospinal fluid.

The enzyme defect can be demonstrated in the patients' fibroblasts, leukocytes [48], liver, and blood mononuclear cells [50].

Treatment and Prognosis

In most pediatric patients symptoms remained the same, but a more severely affected child died in early infancy. In the adult cancer patients, discontinuation of 5-fluorouracil results in slow resolution of the toxic symptoms [49, 50].

Genetics

Both the infantile and the adult form seem inherited as autosomal recessive traits.

Dihydropyrimidinase Deficiency

Clinical Presentation

This disorder has been reported in a single male baby of consanguineous parents, presenting with convulsions and metabolic acidosis [51].

Metabolic Derangement

The enzyme catalyzes the cleavage of dihydrouracil and dihydrothymine into β-ureidopropionate and β-ureidoisobutyrate, respectively (Fig. 3). Consequently, considerable amounts of dihydrouracil and dihydrothymine, both normally undetectable, are excreted in urine [51].

Diagnostic Tests

Urinary *dihydrouracil* and *dihydrothymine* can be detected by chromatography. Enzyme assay requires liver tissue, since dihydropyrimidinase activity seems absent in normal blood cells and fibroblasts [51].

Treatment and Prognosis

The patient recovered completely and apparently displays normal physical and mental development.

Genetics

The defect is probably inherited as an autosomal recessive trait.

Pyrimidine 5′-Nucleotidase Deficiency

This defect, restricted to erythrocytes, provokes chronic *hemolytic anemia* with basophilic stippling [52].

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Part X
Neurotransmitters

Disorders of Neurotransmitters

J. Jaeken and C. Jakobs

CONTENTS

Although there is a large number of (established and putative) neurotransmitters, the number of known diseases due to hereditary defects in the metabolism of these substances is rather small. The established neurotransmitter systems can be divided into aminoacidergic (mainly gamma aminobutyrate, (GABA), glycine, aspartate, and glutamate), cholinergic (acetylcholine), monoaminergic (mainly adrenaline, dopamine, noradrenaline, and serotonin), and purinergic (adenosine and adenosine monophosphate, diphosphate, and triphosphate, AMP, ADP, and ATp), while a rapidly growing list of peptides are considered putative neurotransmitters. Possibly involved in neurotransmission and/or neuromodulation are *N*-acetyl amino acids and *N*-acetyl peptides.

This review deals with hereditary diseases in the metabolism of GABA, the monoamines, and *N*-acetylaspartate. Disorders in the metabolism of glycine are treated in the chapter by Tada (this volume). The recent discovery of a glycine receptor defect in hyperekplexia [1] indicates that the scope of this chapter should be broadened to disorders of *neurotransmission* in the next edition of this book!

Inborn Errors of Gamma Aminobutyrate Metabolism

Three genetic diseases due to a defect in brain GABA metabolism have been reported: glutamic acid decarboxylase deficiency and two defects in GABA catabolism, GABA transaminase deficiency and succinic semialdehyde dehydrogenase (SSADH) deficiency (Fig. 1).

Pyridoxine-Responsive and -Unresponsive Glutamic Acid Decarboxylase Deficiency

Pyridoxine-responsive glutamic acid decarboxylase deficiency (*pyridoxine-responsive convulsions*) was first reported in 1954 [2]. It is a rare cause of convulsions in early childhood [3]. Recently, indirect evidence was presented for pyridoxine-unresponsive glutamic acid decarboxylase deficiency in infants with a "stiff baby-like" syndrome and convulsions. This is not further discussed in this chapter.

Clinical Presentation

The clinical picture of typical pyridoxine-responsive convulsions has to be differentiated from the more recently identified atypical presentation. The typical form satisfies the following criteria:

- Onset of convulsions before or shortly after birth
- Rapid response to pyridoxine
- Refractoriness to other anticonvulsants
- Dependence on a maintenance dose
- Absence of pyridoxine deficiency

The disease may start as intrauterine convulsions as early as in the fifth month of pregnancy. Some patients suffered from peripartal asphyxia probably as a consequence of this disorder. The seizures are intermittent at onset, but may proceed to status epilepticus. All types of seizures can be

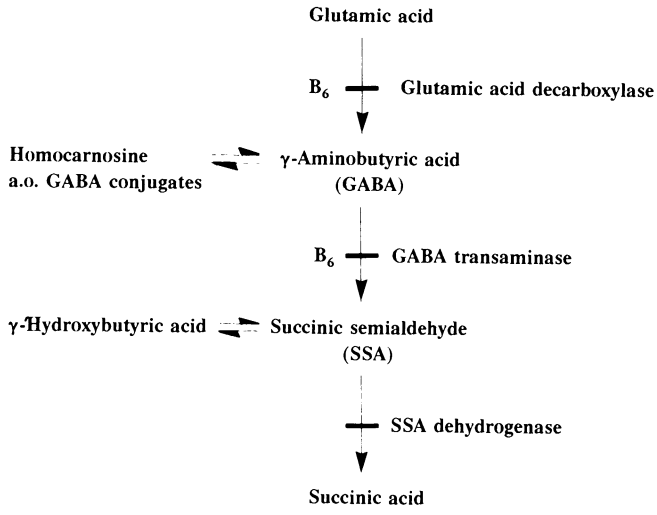

Fig. 1. Brain metabolism of gamma aminobutyrate (*GABA*). B_6, pyridoxal phosphate coenzyme. *Dotted arrows* indicate reactions postulated

observed, mostly long-lasting seizures and repeated status epilepticus, but also brief convulsions (generalized or partial), atonic attacks, and infantile spasms. There is pronounced hyperirratibility that can alternate with flaccidity. Abnormal eye movements are often reported (nystagmus, "rolling" eyes, miosis, and/or poor reaction of the pupils to light).

The atypical presentation [4] differs from the typical one as follows:

- Later onset of the attacks (up to the age of about 15 months)
- Prolonged seizure-free intervals without pyridoxine (as long as 5 months)
- Need of larger pyridoxine doses in some patients
- Higher incidence

Metabolic Derangement

Pyridoxine-responsive convulsions are considered to be due to brain GABA deficiency resulting from a genetic defect at the pyridoxal phosphate coenzyme-binding site of glutamic acid decarboxylase, the rate-limiting enzyme in GABA synthesis. Brain and cerebrospinal fluid (CSF) GABA have only rarely been measured and were found to be low [5, 6].

Diagnostic Tests

The diagnosis rests on the clinical response to *pyridoxine*. A trial of pyridoxine should be performed in all unclear seizure disorders with onset before the age of about 15 months. Results of CSF free GABA determinations and investigations at the DNA level should not be waited for, as these are mostly not readily available.

Treatment and Prognosis

The disease promptly responds to pyridoxine, but is refractory to other antiepileptic medications. The minimum effective daily dose is at least ten times the minimum daily amount recommended for healthy infants and usually varies between 2 and 15 mg. Treatment with isoniazid increases the minimum effective dose. The convulsions cease within a few minutes when pyridoxine is administered parenterally and within a few hours when it is given orally. The effect of a single dose remains constant in the same patient (mostly 2–5 days). When treatment is interrupted, the seizures return, although there might be exceptions to this rule (delayed maturation of enzyme activity?) [6]. In the case of (suspected) *intrauterine convulsions*, treatment of the mother with pyridoxine is effective (around 100 mg/day). In the later-onset pre-

sentation, doses of 100–200 mg may be necessary to control the seizures. Here, too, the minimum effective maintenance dose has to be determined individually.

In the absence of early appropriate treatment severe psychomotor retardation is the rule, and if untreated the disease runs a fatal course, at least in the neonatal form.

Genetics

In its typical form the disease has an autosomal recessive inheritance, and there is evidence that this also holds true for the later-onset presentation.

Gamma Aminobutyric Acid Transaminase Deficiency

Gamma aminobutyric acid transaminase deficiency was first reported in 1984 in a brother and sister from a Flemish family [7]. No other patients seem to have been described since.

Clinical Presentation

Both patients showed feeding difficulties from birth, often necessitating gavage feeding. They had a pronounced axial hypotonia and generalized convulsions. A high-pitched cry and hyperreflexia were present during the first 6–8 months. Further evolution was characterized by lethargy and psychomotor retardation (the developmental level of 4 weeks was never attained). Corneal reflexes and reaction of the pupils to light remained normal. A remarkable, continued acceleration of length–growth was noted from birth to death. This was explained by increased fasting plasma growth hormone levels (8–39 ng/ml; normal, <5); these could be suppressed by oral glucose. In one of the patients, head circumference showed a rapid increase during the last 6 weeks (from the 50th to the 97th percentiles). Postmortem examination of the brain showed a spongiform leukodystrophy.

Metabolic Derangement

The CSF and plasma concentrations of GABA, GABA conjugates, and β-alanine are shown in Table 1. Liver GABA and β-alanine concentrations were normal. This metabolite pattern could be explained by a decrease in GABA transaminase activity in the liver (and lymphocytes). Intermediate levels were found in the healthy sibling, the father, and the mother. It can be assumed that the same enzymatic defect exists in the brain, since GABA transaminases of human brain and of peripheral tissues have the same kinetic and molecular properties. β-Alanine seems to be an alternative substrate for GABA transaminase, hence its increase in this disease.

In this context it can be mentioned that the antiepileptic drug γ-vinyl GABA (vigabatrin) causes an irreversible inhibition of GABA transaminase, leading to two- to threefold increases in CSF free GABA. Interestingly, we have noted that this drug also constantly and significantly decreases serum glutamic pyruvic transaminase, but not glutamic oxaloacetic transaminase activity.

Diagnostic Tests

The diagnosis requires amino acid analysis of the CSF. Due to enzymatic homocarnosine degradation, free GABA levels in the CSF show artifactual increases unless samples are deep-frozen (at −20°C) within a few minutes if analysis is performed within a few weeks and at −70°C if time until analysis is longer. Control CSF free GABA levels range from about 40 to 150 nmol/l after the age of 1 year and are lower in younger children. Because of these low levels, sensitive techniques have to be used such as ion-exchange chromatography and fluorescence detection [8] or a stable isotope dilution technique [9].

Enzymatic confirmation can be obtained on lymphocytes, lymphoblasts, and liver. As for prenatal diagnosis, GABA transaminase activity is not expressed in fibroblasts, but activity is present in chorionic villus tissue [10].

Treatment and Prognosis

We obtained no clinical or biochemical response after administration of pharmacological doses of pyridoxine, the precursor of the coenzyme of GABA transaminase, nor with picrotoxin, a potent noncompetitive GABA antagonist.

Table 1. Range of cerebrospinal fluid (CSF) and plasma concentrations of gamma aminobutyrate (GABA), GABA- conjugates, and β-alanine (μmol/l) in a patient (four determinations) with GABA transaminase deficiency, compared with control values ($n = 20$). (From [7])

	CSF			Plasma		
	Patient	Controls		Patient	Controls	
		Mean	Range		Mean	Range
Total GABA	32.8–51.3	9.2	5.3–10.1	nd		
Free GABA	1.8–4.8	0.08	0.04–0.12	1.6–2.9	0.31	0.12–0.5
Homocarnosine	23.4–36.8	7.6	4–8.7	nd		
"Unidentified" GABA conjugates	7.6–9.7	0.5	0–2	nd		
β-Alanine	0.33–0.48	0.05	0.02–0.06	12.3–22.8	5.8	8–11.7

nd, not determined.

Both children died at the ages of 1 year and 2 years and 7 months, respectively.

Genetics

Inheritance is autosomal recessive.

Succinic Semialdehyde Dehydrogenase Deficiency

SSADH deficiency was first reported as *γ-hydroxybutyric aciduria* in 1981 [11]. It has been documented in at least 32 patients [12].

Clinical Presentation

The clinical presentation varies from mild to severe and comprises psychomotor retardation, delayed speech development, hypotonia, ataxia, and, less frequently, hyporeflexia, convulsions, aggressive behavior, hyperkinesis, oculomotor apraxia, choreoathetosis, and nystagmus. Ataxia, when present, may resolve with age.

Metabolic Derangement

The key feature is an accumulation of γ-hydroxybutyrate in urine, plasma, and CSF (Fig. 1). γ-Hydroxybutyrate is a neuropharmacologically active compound. Its accumulation in the body fluids tends to decrease with age. Metabolites indicative of the β- and α-oxidation of γ-hydroxybutyric acid may be variably detected in the urine of SSADH-deficient patients. The identification of other metabolites in the urine of SSADH-deficient patients related to pathways of fatty acid, pyruvate, and glycine metabolism suggests that the deficiency has metabolic consequences beyond the pathway of GABA metabolism.

Diagnostic Tests

Diagnosis is made by organic acid analysis of urine, plasma, and/or CSF. γ-Hydroxybutyrate can be higher in CSF than in plasma and even be extremely increased. The enzyme deficiency can be demonstrated in lymphocytes and lymphoblasts. Patients typically show 0%–19% of residual activity in isolated lymphocytes or 0%–12% in cultured lymphocytes (0%–6% in lymphoblasts and 4%–12% in intact lymphoblasts), and parents have intermediate levels of enzyme activity [12]. SSADH activity is expressed in normal human liver, kidney, and brain, and SSADH deficiency in these tissues was recently demonstrated. The prenatal diagnosis of an affected fetus has been reported: γ-hydroxybutyrate was elevated in amniotic fluid, and SSADH activity was absent from cultured amniocytes and autopsied fetal brain, liver, and kidney [13].

Treatment and Prognosis

In an attempt to reduce the accumulation of γ-hydroxybutyrate, we introduced a novel treatment principle, namely inhibition of the preceding enzymatic step GABA transaminase. This was realized ◄ by giving *γ-vinyl GABA (vigabatrin)*, an irreversible inhibitor of this enzyme, in doses of 50–100 mg/kg per day (divided into two daily doses)

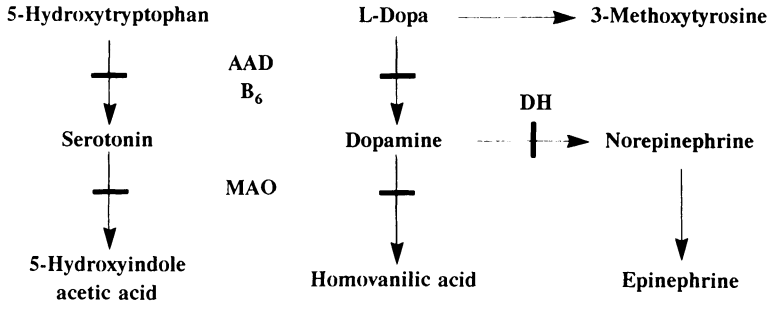

Fig. 2. Synthesis and catabolism of dopamine and serotonin. B_6, pyridoxal phosphate coenzyme; *AAD*, aromatic L-amino acid decarboxylase; *MAO*, monoamine oxidase; *DH*, dopamine β-hydroxylase

[14]. This treatment was shown to reduce CSF γ-hydroxybutyrate levels and improved cerebellar signs in five out of six patients [15]. However long-term administration of vigabatrin should be monitored closely because this drug increases CSF (and probably also brain) GABA levels.

As for the prognosis, this disease can manifest a mild to severe neurological course. Some patients have died, although there was no evidence for metabolic acidosis or decompensation.

Genetics

The mode of inheritance is autosomal recessive.

Inborn Errors of Monoamine Metabolism

The following disorders are discussed: dopa-responsive dystonia, aromatic L-amino acid decarboxylase deficiency, and monoamine oxidase A deficiency (MAO-A) [16]. Dopamine β-hydroxylase deficiency is not dealt with because it has only been diagnosed in adults (orthostatic hypotension), and defects of tetrahydrobiopterin are discussed in the chapter by Smith and Brenton (Fig. 2).

Dopa-Responsive Dystonia

Dopa-responsive dystonia was first reported in 1976 [17]. At least 130 patients are presently known. Nygaard et al. have reported 66 personally examined patients [18].

Clinical Presentation

Age at onset of the dystonia is mostly between 1 and 10 years. There is a great variability in the severity of the disorder. The dystonia starts in the lower extremities, mostly with gait difficulties, and often remains limited to the extremities (e.g., writer's cramp) with no or minimal axial dystonia. About 25% of affected children have clinical signs suggestive of spastic diplegia. In most patients there is a marked diurnal fluctuation of symptoms characterized by worsening of symptoms and increasing fatigue throughout the day and marked benefit of sleep. Symptoms noted in a minority of patients are scoliosis, opisthotonus, dysarthria, dysphagia, postural tremor, and/or intermittent abnormal eye movements. The disorder may be expressed as pure *parkinsonism* in some adults without any dystonia in childhood.

Metabolic Derangement

The precise biochemical defect is still unknown. Most evidence points to a defect in striatal dopamine production. CSF levels of homovanillic acid, a dopamine metabolite, tend to be reduced. Levels of neopterin and its metabolite biopterin (a co-factor for tyrosine hydroxylase, the rate-limiting enzyme in dopamine production) are reduced.

Diagnostic Test

The only diagnostic test is the dramatic responsiveness to levodopa which distinguishes dopa-responsive dystonia from other causes of childhood-onset dystonia–parkinsonism.

Treatment and Prognosis

Low doses of L-dopa (5–30 mg/kg per day), associated with a decarboxylase inhibitor such as in ◄

prolopa or sinemet, cause a marked improvement with complete or almost complete remission of symptoms usually within days or weeks. Progressive improvement continues to occur for months in some cases without increase in dosage. On withdrawal of L-dopa, there is immediate recurrence of symptoms. The effect of levodopa is sustained and free from the complications which occur in Parkinson disease, such as wearing off and unpredictable dose response [19].

Genetics

Available data strongly suggest autosomal dominant inheritance with sex-related reduced penetrance. Girls are more frequently and severely affected than boys. Moreover, it seems that the age of onset is earlier in girls. The disorder occurs worldwide. Recently, the gene for dopa-responsive dystonia has been mapped to chromosome 14q by linkage analysis [20].

Aromatic L-Amino Acid Decarboxylase Deficiency

Aromatic L-amino acid decarboxylase deficiency was reported in 1990 in one family (male twins) [20].

Clinical Presentation

Generalized hypotonia, developmental delay, and paroxysmal movements with *oculogyric crises* were noted at 2 months of age. At 9 months there was also a fine chorea of the distal limbs and temperature instability with excessive sweating. The electroencephalogram (EEG) was normal, but brain imaging showed cerebral atrophy.

Metabolic Derangement

Deficiency of aromatic L-amino acid decarboxylase leads to accumulation of L-dopa, its metabolite 3-methoxytyrosine, and of 5-hydroxytryptophan in CSF, plasma, and urine, as L-amino acid decarboxylase has both L-dopa decarboxylase and 5-hydroxytryptophan decarboxylase activities. In urine, there is also a gross elevation of vanillactic acid, a metabolite of 3-methoxytyrosine.

On the other hand, blood concentrations of serotonin and catecholamines, and CSF concentrations of their metabolites 5-hydroxyindoleacetic acid and homovanillic acid, are decreased. Very low activity of L-dopa decarboxylase in plasma and of 5-hydroxytryptophan decarboxylase in a liver biopsy sample confirmed the diagnosis. The parents of the affected index patient had plasma aromatic L-aminoacid decarboxylase activity of 16% and 19% of control values, respectively, and yet they had a totally normal phenotype [21].

Diagnostic Tests

The diagnosis can be made by finding the characteristic profile in CSF of very high levels of 3-methoxytyrosine and very low levels of 5-hydroxyindoleacetic acid and homovanillic acid. This suggests a systematic investigation of monoamine metabolism in unexplained neurological disease. Very low activity of aromatic L-amino acid decarboxylase in plasma and/or liver tissue confirms the diagnosis. The feasibility of prenatal diagnosis is not yet known.

Treatment and Prognosis

The index patients were treated with *pyridoxine* (cofactor of the defective enzyme), *bromocriptine* (dopamine agonist), and *tranylcypromine* (monoamine oxidase inhibitor). Pyridoxine (50mg b.d.) caused a drop in CSF 3-methoxytyrosine and L-dopa and an increase in homovanillic acid. However, there was no clinical effect. Bromocriptine alone stopped the oculogyric crises, while tranylcypromine improved muscle tone, increased spontaneous movements, and reduced sweating. Growth, which had stopped, resumed during treatment. Anticonvulsants were ineffective.

Genetics

Inheritance is most probably autosomal recessive.

Monoamine Oxidase-A Deficiency

MAO-A has been identified very recently in five generations of a large Dutch kindred [22].

Clinical Presentation

Only males were affected. They showed borderline mental retardation (IQ scores around 85) with prominent behavioral disturbances including aggressive and sometimes violent behavior, arson, attempted rape, and exhibitionism. Aggressive behavior was usually triggered by anger and tended to cluster in periods of 1–3 days. During this time the affected male would experience frequent night terror. Several affected males were reported to suddenly grasp or hold female relatives. All patients displayed a tendency toward stereotyped hand movements such as hand wringing, plucking, or fiddling. Growth and morphology were normal. All of the females functioned normally.

Metabolic Derangement

Marked elevations were noted in the MAO substrates such as serotonin, normetanephrine, 3-methoxytyramine, and tyramine in the urine. Levels of the MAO products vanillylmandelic acid, homovanilic acid, 5-hydroxyindoleacetic acid, and 3-methoxy-4-hydroxyphenylglycol, however, were reduced. As platelet MAO-B activity was found to be normal, these results are consistent with a deficiency of MAO-A, the isozyme found in neural tissue.

Diagnostic Tests

The discovery of this disorder suggests that it might be worthwhile to perform systematic urinary monoamine analysis in unexplained significant *behavior disturbances*, particularly when occurring in several male family members.

Treatment and Prognosis

No efficient treatment is known at the present time. Both the borderline mental retardation and the behavior abnormalities seem to have a stable evolution. No patient has been institutionalized because of mental retardation.

Genetics

The locus for this disease has been assigned to the Xp11–21 region. A point mutation was identified in the eighth exon of the MAO-A structural gene which changes a glutamine to a termination codon.

Canavan Spongiform Leukodystrophy: Aspartoacylase Deficiency

Kvittingen et al. first described patients with cerebral atrophy and leukodystrophy with *N*-acetylaspartic aciduria. It was the merit of Matalon et al. and Divry et al. to link this disorder with Canavan disease [23].

Clinical Presentation

The diagnosis of *Canavan disease* can be suggested by clinical features including leukodystrophy, megaloencephaly, mental retardation, and optic atrophy. Death usually occurs in the first decade of life. Computed tomography (CT) scan or magnetic resonance imaging (MRI) show white matter attenuation. Brain histopathology shows spongy degeneration of the myelin, astrocytic swelling, and elongated mitochondria; neurons are normal. Three clinical variants of the disease have been described [24]:

- A congenital form in which the disease is apparent from birth
- An infantile form, which is the most common and which becomes manifest after the first 6 months of life (this form is frequently seen among Jewish patients)
- A juvenile form in which symptoms appear after the first 5 years of life.

Metabolic Derangement

The key feature is accumulation of *N-acetylaspartic acid* in the body fluids, levels in the CSF being much higher than in the serum. The enzymatic defect can be confirmed by assay of aspartoacylase in fibroblasts. Aspartoacylase is also deficient in brain, liver, and kidney [23, 25].

N-Acetylaspartic acid is abundant in brain, where its concentration is second only to glutamic acid in the free amino acid pool and is higher than that of GABA. Brain is the only organ where biosynthesis of *N*-acetylaspartic acid has been demonstrated [26]. Its normal function is not well understood. It has been referred to as an essential

component in a series of reactions required for the conversion of lignoceric acid to cerebronic acid, a component of myelin, and the formation of glutamic acid and has been assigned a possible role in neurotransmission and in the production of *N*-acetylaspartylglutamate. It may also serve as a chemical compartment so that aspartate is released only by the action of aspartoacylase and the released aspartate may be channeled to form arginine [23].

Diagnostic Tests

The diagnosis is based on gas chromatography mass spectrometry (GCMS) of urine or other body fluids showing increased *N*-acetylaspartic acid levels and is confirmed by aspartoacylase assay on cultured fibroblasts and/or by restriction fragment length polymorphism (RFLP) or mutation analysis of DNA. For prenatal diagnosis the measurement of aspartoacylase in fresh chorionic villi samples [27] or the quantitation of *N*-acetylaspartic acid in amniotic fluid supernatant [28] are the methods of choice. Obviously, molecular diagnosis using RFLP or mutation analysis within given families will provide prenatal diagnostic facilities and carrier detection in at risk populations in the near future [29].

Treatment and Prognosis

It has been proposed that *N*-acetylaspartic acid serves as a transporter of acetyl groups from mitochondria to the cytosol for lipogenesis. Therefore, in an attempt to supply alternative substrate for lipogenesis in the brain, a ketogenic diet was given to one patient for 5 months. No improvement was seen [25]. No other treatment is available. Progressive deterioration leads to a decorticate condition and death within a few years.

Genetics

Genetic transmission is autosomal recessive. The human aspartoacylase gene is localized on chromosome 17 p13-ter [30]. A full-length human cDNA for aspartoacylase has been isolated and a predominant point mutation 856 A > C was identified in Jewish patients [29].

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Part XI
Metals

Copper: Wilson and Menkes Diseases

D.M. Danks

CONTENTS

Wilson and Menkes diseases epitomise the fundamental biological problem of the transport and utilisation of copper in the human body – coping with an element which is essential, but toxic. We must have evolved very efficient systems for transporting copper safely to the sites within the body cells where it is needed. The two diseases represent failures of this sophisticated system due to faults in two different genes which encode two of the proteins involved in the copper transport system. In Wilson disease we see toxic consequences of copper accumulation, and in Menkes disease we meet most of the effects that have ever been ascribed to copper deficiency in other species. Recently, the genes have been cloned in Wilson disease [1] and Menkes disease [2]. They are so similar that sequences from the Menkes disease gene were used to isolate Wilson disease cDNA. More detailed analyses must be awaited before complete explanations of pathogenesis can be given.

Wilson Disease

This is a disorder of hepatic copper transport leading to toxic effects in liver and brain (basal ganglia) hepatolenticular degeneration. More detailed reviews provide the sources of unreferenced statements [3].

Clinical Presentation

Hepatic and neurological presentations are approximately equally frequent, but are encountered at different ages. Hepatic symptoms are most frequent between the ages of 8 and 18 years, but can first occur at any age, even as late as 60 years [4]. Neurological presentation is rare before 15 years and most frequent between 20 and 40 years of age. Other presentations are much less frequent.

Almost any symptom of *liver disease* may be seen, but acute presentation is surprisingly frequent, considering the gradual accumulation of copper in the organs. Sadly, some patients have several episodes of *jaundice* before diagnosis – a tragic situation if the final episode is fulminant and fatal. *Haemolysis* may dominate the presentation and the underlying liver disease may not be recognised. Wilson disease is the commonest cause of chronic liver disease in childhood (after infancy) and should be the assumed diagnosis until disproved. A similar attitude can be justified in adults, because it is one of the few treatable causes.

Dysarthria and deterioration of coordination and voluntary movement are the most frequent *neurological symptoms*, with involuntary movements and disorders of posture and tone. Some disturbance of intellectual function and/or behaviour may occur early and often develops later. Untreated, these symptoms progress to bulbar palsy and death. Wilson disease is one of the very few really treatable causes of these symptoms and it must always be remembered. Asymptomatic hepatomegaly and liver disease is usually present.

Kayser-Fleischer rings (golden brown granular pigmentation at the limbus) provide the most

valuable diagnostic clinical sign, but are not always present in cases with liver disease, especially children. They may develop in copper retention secondary to cholestatic liver disease. Slit-lamp examination is required.

Osteoarthropathy, renal tubular acidosis, renal calculi, cardiomyopathy, anaemia, cataracts and hypoparathyroidism are occasional features. Wilson disease is not a cause of mental retardation.

Metabolic Derangement

The two major disturbances of copper transport are reduced excretion of copper in the bile and reduced incorporation of copper into caeruloplasmin. Presumably a single basic defect must account for both of these disturbances. The very recently cloned gene encodes a protein which has all of the characteristics of a P-type ATPase membrane channel with classical transmembrane domains, ion channel, adenosine triphosphate (ATP)-binding site, phosphorylation site and six metal-binding domains [1]. The closely related Menkes disease gene product is clearly involved in extrusion of copper from most cells other than liver cells. It seems probable that the Wilson disease gene product may prove responsible for extrusion of copper from liver cells into bile canaliculi. Reduction in this function could explain the reduced biliary copper excretion, which is the cardinal feature of the disease.

Another theory of excretion of copper as degradation products of caeruloplasmin with failure of copper incorporation in caeruloplasmin, the primary defect in Wilson disease [5], now seems unlikely, unless the protein has a specific role in transport into the Golgi apparatus.

All patients with Wilson disease show a gross reduction in the rate of incorporation of radioactive copper into caeruloplasmin, although the levels of caeruloplasmin in plasma may range from barely detectable to normal levels. Conversely, the non-caeruloplasmin copper content of plasma is increased and correlates with deposition of copper in organs other than the liver and increased urinary copper excretion.

Copper accumulates progressively in the liver and levels are generally 20–50 times normal in symptomatic patients. Accumulation in lysosomes seems to be relatively harmless, but cytoplasmic accumulation correlates quite closely with liver damage. Copper deposition in the renal tubules and in the basal ganglia of the brain damages these tissues. The rate of deposition of copper in the liver may be important, slow accumulation causing only minimal injury and allowing time for brain damage. Viral infections which injure liver cells precipitate acute haemolytic/hepatic crises, as may infarction of hyperplastic liver nodules. The acute hepatic crisis comprises a vicious cycle of liver cell damage, releasing ionic copper into the plasma, which damages further liver cells, red blood cells, renal tubular cells and brain cells, just as in acute copper poisoning. In a crisis, non-caeruloplasmin copper in the plasma is greatly elevated.

Diagnostic Tests

The classical biochemical findings (Table 1) of a moderate to severe reduction in plasma caeruloplasmin levels, with only slightly reduced serum copper (indicating an increased non-caeruloplasmin copper level), are found in nearly all patients presenting with neurological symptoms, in most (but not all) adults with hepatic symptoms, but in only about 50% of children with hepatic symptoms. Excessive reliance upon these tests is a major reason for failure to diagnose Wilson disease. Exclusion of the diagnosis requires physical/chemical analysis of liver copper or a kinetic study with radioactive copper.

Urinary excretion of copper is generally increased (Table 1), with a large increase after penicillamine. These tests are of marginal use when positive and are unreliable when negative. Although some features of liver pathology may suggest Wilson disease, their absence does not exclude the diagnosis. Copper stains are useful when strongly positive, but unreliable when negative.

It is essential to remember Wilson disease in all children and adults with liver disease and in adolescents or adults with neurological features of basal ganglia disease. Assay of copper in a liver biopsy is the best method of proving or excluding the diagnosis. A copper isotope study is most useful in distinguishing Wilson disease from secondary copper retention in other liver diseases. Intravenous administration of radioactive ^{64}Cu or ^{67}Cu is followed by plasma sampling 10 min, 1 h, 2 h, 4 h, 24 h and 48 h after injection [3]. The levels of radioactivity should fall to a nadir between 1 and 4 h and rise again to greater than double this level by 48 h. (If using ^{64}Cu, with low specific activ-

Table 1. Copper measurements of diagnostic use in Wilson disease and Menkes disease

	Birth		2 weeks		3–6 months		Child or abult	
	MD	N	MD	N	MD	N	WD	N
Serum copper (mmol/l)	8–12	3–16	<6	10–12	2–6	11–24	3–10	11–24
Serum caeruloplasmin (mg/l)	130–150	80–120	<40	60–75	0–50	200–400	0–200	200–400
Liver copper (mg/g dry weight)	<20	120–200	<20	NE	0–20	50–120	300–3000	20–50
Duodenal copper (mg/g dry weight)	NE	NE	NE	NE	50–80	7–29	NE	NE
Urinary copper (μg/24 h)	NE	NE	NE	NE	NE	NE	100–1000	<40
Urinary copper after penicillamine (μg/24 h)	NE	NE	NE	NE	NE	NE	1500–3000	100–600

MD, Menkes disease; WD, Wilson disease; NE, not examined; N, normal.

ity and short half-life, all samples must be counted immediately.) In Wilson disease, the plasma radioactivity goes on falling throughout the testing period. Intermediate results may be encountered in patients with other forms of liver disease or in some heterozygotes.

All siblings require exclusion of the diagnosis by liver biopsy and/or radio copper studies, unless simpler tests are clearly diagnostic.

Treatment and Prognosis

Penicillamine has been the standard treatment of Wilson disease for over 30 years, and there are hundreds of healthy individuals alive today after more than 20 years of treatment. However, it does have some shortcomings. About 5%–10% of patients develop serious side-effects: bone marrow depression, renal tubular damage, allergic reactions or elastosis perforans [3]. It takes several months to have a clinical effect, and liver disease or neurological damage may progress dangerously during this interval. Acute neurological deterioration may occur in some patients started on a full therapeutic dose. Alarmingly rapid development of irreversible liver damage has been observed in some patients who have taken themselves off treatment after many years, probably because some copper retention may persist. Its mode of action is not certain. Nonetheless, it is probably still the drug of choice in most patients today.

Triethylamine tetramine (Trientene) has similar decoppering capacity, but fewer side-effects [6]. General availability has been a problem.

Treatment with high doses of zinc salts orally (50 mg zinc, as sulphate or acetate, before each meal) is an alternative treatment introduced in the early 1970s. Zinc induces high levels of metallothionein in gut mucosal cells; copper displaces zinc from the metallothionein and remains complexed until the cells are shed. The zinc that is absorbed also induces excess metallothionein in liver, kidney and other tissues, which binds copper and prevents cell damage. When used in newly diagnosed patients, zinc causes an initial rise in liver copper (bound to metallothionein) which persists for 1–2 years before falling below pretreatment levels [7]. Nonetheless, inflammatory changes in the liver disappear soon after treatment is started.

The final agent which has been used in a small number of centres is ammonium tetrathiomolybdate (TTM). It is much the most potent copper chelator yet used and can cause severe copper deficiency. It has been used extensively and safely in the treatment of copper toxicosis in sheep. It is likely to have an important place in the initial treatment of Wilson disease, particularly in patients with severe symptoms, neurological or hepatic. We have used it intravenously to control the massive excess of "free" copper in acute hepatic crises, but have not seen healing of the liver damage in these cases. It has controlled severe, acutely progressive brain symptoms [8]. Others have also used it successfully as the initial treatment in neurological cases [9].

Liver transplantation cures Wilson disease, but leaves the patient with the prognosis of a transplanted liver. The degree of neurological improvement has been remarkable in some patients.

If treatment of Wilson disease is started before there is irreversible damage to liver or brain and is maintained, long-term prognosis is excellent – better than the outlook for the recipient of a liver

transplant. However, the medical treatments cannot reverse severe established cirrhosis, and the haemodynamic consequences such as portal hypertension may continue or even increase. Neurological damage is also only partially reversible. Serious side-effects may limit the effectiveness of treatment even after trying several different agents. Liver transplantation has an important part to play in some of these situations and, especially, in acute fulminant cases.

In acute fulminant hepatic failure, the immediate problem is acute copper poisoning with very high levels of free ionic copper circulating in the plasma. These can be brought under control within hours by haemofiltration or by intravenous TTM treatment. Maintenance of control is easier (and much cheaper) with TTM. Less perfect control can be achieved with plasmaphoresis or peritoneal dialysis. Haemodialysis is of no value. Unfortunately, even the best of biochemical control is rarely followed by regeneration of the liver and so most of these patients need liver transplantation, once stabilised.

► In a patient diagnosed with moderate hepatic or neurological illness, most experts would probably still recommend penicillamine, starting with 1 g per day in divided doses in a hepatic case or with 250 mg a day and working up to 1 g a day over 2–3 weeks in a neurological case. The dose should be increased until urinary excretion is over 3 mg per 24 h or until a dose of 3 g per day has been reached in an adult (or an equivalent dose in a child). Pyridoxine supplements (50 mg daily) should be given. A few people would propose that Trientene would be preferable.

The two groups who introduced zinc therapy would strongly argue that this should be the initial treatment and I would agree with them, provided I was dealing with a patient who was very reliable. A sudden cessation of treatment in the first 3 years might release the copper bound to metallothionein and cause an acute crisis.

I believe that further experience will show that the best treatment of Wilson disease is with TTM, until the liver is decoppered, and then with zinc.

Genetics

Wilson disease is inherited as an autosomal recessive trait. The gene is located at 13q14 and its properties were described above. Mutations have been identified in a small number of patients, but no extensive surveys of mutations have yet been reported. It is too early to be absolutely certain that this locus is involved in every family, but there is no particular reason to suspect other loci. Unusual patterns of illness in certain ethnic groups, e.g., late-onset neurological disease in Polish Jews in New York and the early-onset hepatic disease in an inbred island population in Japan, will probably turn out to be explained by different alleles and/or cultural difference in copper intake. There are too many instances of both the hepatic presentation and neurological presentation in siblings to propose that the difference between these two major presentations will be allele specific.

Menkes Disease

A defect in a membrane copper transport channel interferes with the absorption of copper from the food and its distribution within cells, with the consequence of generalised copper deficiency. It is, in fact, a family of diseases to which the names classical Menkes disease (kinky hair disease, steely hair disease, tricho polio dystrophy), mild Menkes disease and occipital horn syndrome (X-linked cutix laxa, Ehlers-Danlos syndrome type IX) have been given. Mild Menkes disease was recognised as a variant of the classical disorder immediately, whereas there was delay in recognising the relationship of occipital horn syndrome to Menkes disease. Preliminary studies of the cloned gene indicate that they are all probably allelic mutations. The description given here will be of the classical Menkes disease, with additional comments where necessary to cover the other two variants. Support for statements not referenced can be found in reviews [9].

Clinical Presentation

Classical Menkes Disease. The first abnormality may be premature rupture of the membranes. The newborn baby frequently suffers hypothermia and unconjugated hyperbilirubinaemia. Central nervous system (CNS) function may not be noticeably abnormal. The characteristic facies and brittle hair may be apparent or may not appear for several months. The diagnosis is usually made at 3–6 months because of arrested development, loss of skills and/or convulsions, which may prove dif-

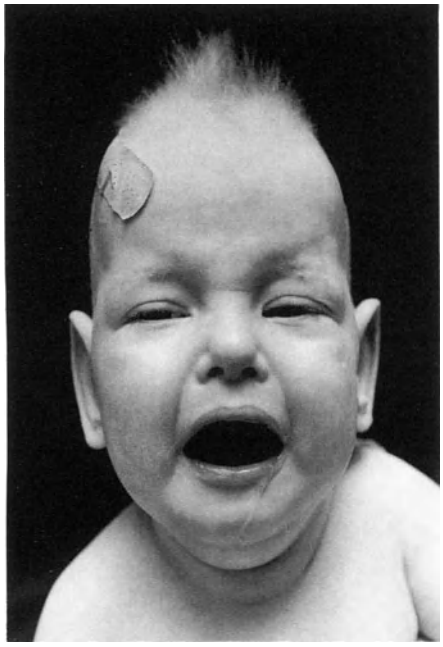

Fig. 1. Menkes' disease

ficult to control. The face is then diagnostic with pudgy, sagging jowls and lips which have an exaggerated cupid's bow (Fig. 1), and abnormal hair and eyebrows. Hair breaks to leave a stubble around the occipital and parietal regions. When grown longer it is grey-brown and lustreless, with shafts tangled around one another (steely hair). Microscopically, there is pili torti. Hypothermia may occur in older babies, especially during an infection. Skin and joint laxity, urinary tract infection, rupture of a diverticulum of the bladder or ureter or subdural haemorrhage are other features.

X-rays of skull and long bones show Wormian bones, osteoporosis and flared metaphyses with corner fractures. Cranial ultrasound or computed tomography (CT) scan may show a subdural haematoma. Child maltreatment may be erroneously diagnosed. Arteriography shows elongated, tortuous arteries with dilated and narrowed regions. Plasma and cerebrospinal fluid lactate and pyruvate levels may be elevated.

The remarkable combination of features makes clinical diagnosis easy.

The natural history is of progressive neurological deterioration to death by 2–3 years. Occasional patients have lived on to 14–15 years with profound brain damage; these are long-lived classical cases, not mild cases. Arterial rupture, urinary tract rupture or chest infection secondary to inhalation may prove lethal. Some babies are irritable, while others are placid, as in other progressive neurogenerative conditions. Susceptibility to infection is not greater than in other brain-damaged babies.

Mild Menkes Disease. The two patients known with this form of the disease showed a mild phenotype from birth. Both presented at about 2 years of age, one because of delayed motor development due mainly to severe ataxia, and the other with hernias, bladder diverticuli and mildly delayed development. Both had typical hair changes and facial appearance and both are now teenagers progressing slowly through normal school.

Occipital Horn Syndrome. This is a name used to describe some older children and young adults with widespread connective tissue abnormalities and calcification in the occipital insertion of the paraspinal muscles [10]. Inguinal hernias, bladder diverticula, skin ligamentous laxity and bony abnormalities are remarkable. The full clinical features and natural history of this condition remain very poorly documented in literature, although the basic defect in cellular copper transport has been more thoroughly examined and is indistinguishable from that in Menkes disease.

Metabolic Derangement

The clinical features are explained by deficient activity of the copper-requiring enzymes (Table 2). The basic problem is not simply whole-body copper deficiency. Rather there is *maldistribution of copper* between organs and within cells. The levels of copper in the intestinal mucosa and kidney are very greatly elevated, whereas the levels in most other organs are reduced at the time of symptomatic presentation or when tissues are examined at autopsy. Although the supply of copper across the placenta is probably reduced during intra-uterine life (as judged by studies in mice with homologous mutations), it is still relatively abundant compared with that available postnatally when the combination of deficient intestinal absorption and excessive urinary excretion deprive the body of copper. Prenatally, all organs except the liver have increased levels of copper. The postnatal copper deprivation reduces the tissue levels of copper to normal, or below, except in those organs which have a special access to

Table 2. Relationship of clinical features to copper enzymes

Clinical features	Enzyme deficiency
Premature rupture of membrances	Lysyl oxidase (elastin and collagen cross-linking)
Facies	
Skin and joint laxity	
Urinary tract diverticulae	
Arterial stretching and rupture	
Hernias	
Retinal tears	
Abnormal hair – pili torti	Unknown copper-dependent keratin cross-linking enzyme
Depigmentation	Tyrosinase
Muscle weakness	Cytochrome C oxidase
Brain damage and epilepsy	Cytochrome C oxidase, superoxide dismutase, dopamine β-hydroxylase
Hypothermia	Cytochrome C oxidase
Neonatal jaundice	Cytochrome C oxidase (probably)
Hypotension	Dopamine β-hydroxylase
Effects uncertain	Caeruloplasmin

copper – intestinal mucosal and renal tubular epithelium.

Studies in cultured cells have shown excessive accumulation of copper which is the result of a normal rate of uptake combined with a greatly reduced rate of release. Despite the increased levels of copper in cells, some copper enzymes are defective in their function, indicating disturbed intracellular transport. The increased copper in the cells is bound to metallothionein. Liver cells appear not to be affected.

The recent isolation of the Menkes disease gene has explained the copper accumulation. The gene encodes a protein which is a member of the P-type ATPase ion transporter family of membrane proteins [2]. In common with other members of this protein family it has transmembrane regions, an ion channel, an ATPase-binding site and a phosphorylation site. It differs from previously described proteins by having six metal-binding motifs. (Bacterial proteins of this family which are involved in copper, cadmium and mercury excretion have only a single metal binding motif.) Amplification of this gene in mutant strains of Chinese hamster ovary (CHO) cells selected for copper resistance confirms its important role in the egress of copper from cells (J.F.B. Mercer, M. Petris, J. Camakaris 1993, personal communication). It may also be involved in transport of copper into some intracellular organelles for incorporation into copper enzymes. It is expressed in all organs except the liver.

The clinical features of the disease are clearly explained by the known functions of copper enzymes, with the full range of these functions severely disturbed in classical Menkes disease (Table 2) and, to a lesser degree, in the mild cases. In occipital horn syndrome (and in blotchy mice), the emphasis seems to be upon lysyl oxidase deficiency, but the other enzymes have not been adequately assessed. Studies of the mutations in patients and mice support involvement of multiple alleles at one locus [11].

Diagnostic Tests

It should be possible to make a confident diagnosis clinically. Measurement of serum copper and caeruloplasmin is usually sufficient to confirm the diagnosis (Table 1). In mild cases and in the occipital horn syndrome, the reduction of copper and caeruloplasmin may be less marked. A low level of copper in a liver biopsy or high level in an intestinal mucosal biopsy can resolve any doubt. The final arbiter is analysis of uptake and release of ^{64}Cu or ^{67}Cu by cultured fibroblastic cells. The role of direct detection of the mutation in the gene is yet to be assessed. So far only a minority of patients have shown easily recognised deletions.

A particular difficulty arises in newborn babies in whom low levels of copper and caeruloplasmin are normal (Table 1). Another measurement 2

weeks later will resolve the problem, because levels rise quite rapidly in normal babies and continue to fall in affected babies.

Prenatal diagnosis has been available for many years using cultures of amniotic fluid cells or chorion villus cells. High copper levels, a greatly increased accumulation of ^{64}Cu over 24 h in culture and a greatly reduced loss of ^{64}Cu in a subsequent 24 h of culture in a medium without isotope are the classical features. The very high levels of copper present in uncultured chorion villus samples can also be used to identify affected males and even to identify heterozygous females prenatally. However, there is some risk of copper contamination of these tiny samples, and errors have been made using this technique. These tests need to be performed by very experienced laboratories because each has pitfalls. Presumably, direct detection of mutation will replace these techniques.

Detection of female heterozygotes is unsatisfactory by present methods of examining cultured fibroblasts because X inactivation can frequently give normal results in women who are carriers. This will be the principal application of direct detection of mutations.

Treatment and Prognosis

Daily injections of copper, best given as copper histidinate (formulation described in [12]), can quickly restore levels of copper and caeruloplasmin in the plasma to normal. Other injectable preparations are less satisfactory and oral administration is ineffective. In classical cases presenting at 3–6 months of age with profound neurological damage, such a treatment does not seem to alter the course of neurological disease significantly. It can cause some improvement in physical growth and strength, but this may not be a useful achievement. We do not recommend copper treatment in these cases.

Copper injections have been used with reasonable success in four babies who were diagnosed prenatally or neonatally and started on treatment soon after birth. All four are showing borderline normal intellectual development at ages between 5 and 15 years. Many features of residual deficiency of copper enzymes are seen – bone changes as in occipital horn syndrome, skin and joint laxity, severe postural hypotension (treatable with L-threo-3,4-dihydroxy phenylserine, L-DOPS) and hair abnormalities. In two of these babies, premature delivery (deliberate in one case) allowed treatment to be initiated at 35–36 weeks' gestation. They have not progressed significantly better than the other two cases. In brindled mice which have a similar neurological disease, there is a critical difference between starting treatment at 7 days and starting at 10 days. The equivalent stage of human development is probably 30 weeks' gestation. We describe this therapy to parents as one which diminishes the effects of the disease, but does not restore normal health and do not present it as a serious alternative to termination of affected pregnancies.

The initial dosage in newborn babies is of the order of 100–200 μg copper daily. Older children require 1 mg copper daily. Less frequent injections are not satisfactory because of a high rate of urinary excretion. Monitoring serum copper can give an indication of undertreatment, but cannot warn of overtreatment. Liver biopsies are needed to check that dose is not excessive. Caution is needed because cultured cells from Menkes disease patients show an increased susceptibility to copper toxicity as well as an increased requirement for copper. The window of acceptable copper levels in the cells of patients is presumably quite narrow.

Better methods of monitoring need to be developed if other patients are to be treated. Perhaps measurement of white cell enzymes such as cytochrome oxidase, superoxide dismutase or dopamine β-hydroxylase might be useful.

Genetics

The inheritance is X-linked recessive. The gene is located at Xq13 and was cloned in 1992 from cells of a girl with Menkes disease caused by an X:2 translocation which disrupted the 120-kb gene [2]. The features of the protein it encodes have been described above. Many patients have no detectable mRNA, but few of these have deletions. The other mutations have not been determined.

The reason for particularly severe deficiency of lysyl oxidase in the occipital horn syndrome and in blotchy mice, which otherwise show a less severe disturbance of copper transport than classical Menkes disease and brindled mice, has not been resolved. All seem to be the result of mutations of the same (or homologous) locus [11].

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Genetic Defects Related to Metals Other Than Copper

I. Lombeck

CONTENTS

Magnesium

Two inherited disorders are known, primary hypomagnesemia and magnesium-losing kidney.

Primary Hypomagnesemia

Clinical Presentation

Since the first description in 1956 [1], more than 30 infants from different parts of the world have been observed. Most affected infants, born at term, are apparently healthy until the initial symptoms start, usually between the third week and fourth month of life. The infants become irritable, develop sleeping and feeding difficulties, jitteriness, hyperreactivity, tetany with facial twitching, and carpopedal spasm and the signs of Chvostek and Trousseau are positive. Generalized convulsions then develop. Opisthotonus, hypotonicity, and areflexia may be present. Occasionally peripheral edema occurs and raised intracranial pressure, bulging fontanels, and increased occipitofrontal circumference was observed once.

Metabolic Derangement

Primary or chronic hypomagnesemia can be caused by impaired intestinal absorption or inadequate Mg handling by the kidney. Tracer doses of ^{28}Mg (half-life time, 21.3 h) reveal Mg malabsorption, whereas the secretion is normal [2, 3]. A reduced Mg retention is also proven by Mg balance studies. The biochemical substrate for the malabsorption has not yet been found, but a defect or absence of a specific protein facilitating the active Mg transport in the gut is probable. Hypocalcemia is considered to be secondary due to impaired synthesis, secretion, or end-organ response to parathormone (PTH). Moderate Mg deficiency is believed to stimulate, severe to inhibit PTH release and cause end-organ resistance to PTH.

Diagnostic Tests

The main laboratory findings in these infants are hypomagnesemia with Mg values from 0.15 to 0.30 mmol/l (normal range, 0.70–1.00 mmol/l) and hypocalcemia with calcium values from 1.2 to 1.6 mmol/l (normal range, 2.2–2.7 mmol/l). Urinary Mg excretion is markedly reduced during hypomagnesemia. The estimation of reduced Mg levels in erythrocytes and/or leukocytes is not well established, and in most centers normal values for cells of different ages are lacking. Serum inorganic phosporus is elevated and alkaline phosphatase normal. Values of circulatory PTH are variable. All other biochemical parameters, including glomerular filtration or tubular reabsorption tests, do not reveal any dysfunction. Neither hypocalcemia nor clinical symptoms respond to calcium, vitamin D, or PTH.

Treatment and Prognosis

Without treatment, primary hypomagnesemia usually leads to death within the first year of life. ◄ Adequate treatment consists of high – dose Mg supplementation. During the acute phase, Mg

must be administered parenterally as Mg sulphate, gluconate, or chloride. Usually $MgSO_4$ 10% is used at a dose of 0.4–1.0 mmol/kg (1 mol $MgSO_4 \times 7H_2O$ = 246 g). This produces a rapid clinical remission. After Mg supplementation alone, a spontaneous return of plasma calcium to normal values occurs. Mg should be given intravenously only by slow infusion (6–24 h). Intramuscular injections are also effective. Subsequent oral therapy is adequate in a dose which must be adjusted to the clinical response and side effects (diarrhea). Supplements of 1.5–2.0 (−5) mmol Mg/kg body weight are needed. Besides $MgSO_4 \times 7H_2O$, $MgCl_2 \times 6H_2O$ (1 mol = 203 g), $MgCH_3(COO^-)2 \times 4H_2O$ (1 mol = 214 g), trimagnesium dicitrate $\times 14H_2O$ (1 mol = 703 g), or more seldom Mg hydroxide, lactate, gluconate, aspartate, and glycerophosphate are used. The oral supplementation should be given in three to five divided doses to prevent diarrhea. The treatment must be given lifelong. Interruption results in a recurrence of hypomagnesemia and hypocalcemia with tetanic convulsions within 1–4 weeks, depending on body Mg stores. Usually it is difficult to achieve normal plasma Mg values.

The prognosis is good. A continuous Mg supplementation results in normal growth and psychomotor development. In some patients caries was observed, and because of the relatively small number of published patients it is not clear whether epilepsy occurs more frequently.

Genetics

The rare disease is most frequently observed in boys (ratio of boys to girls, 3:1). Despite some other suggestions, primary hypomagnesemia appears to be an autosomal recessive disorder. Parenteral consanguinity was observed in four families. The condition was diagnosed three times among siblings. In at least three other families older siblings had died from similar clinical disorders. One female was found to also have multiple congenital abnormalities and balanced translocation 46 − XX − t(9; x)(q12; p22).

Magnesium-Losing Kidney

Clinical Presentation

The clinical symptoms differ markedly. Many patients [4–6] already suffer from tetany during childhood, but the diagnosis is often delayed until adulthood. Then about 50% present with nephrocalcinosis. Chondrocalcinosis or osteochondrosis were also observed. Patients with additional hypokalemia complained about muscular weakness. This type of hypomagnesemia due to renal wasting is difficult to distinguish from Bartter syndrome. Occasionally dermatitis, sensoneural deafness, schizoid behavior, and oligospermia were reported. Four patients were asymptomatic and were discovered during family surveys.

Metabolic Derangement

The primary defect is a tubular Mg reabsorption defect, which can also be proven by isotope studies or intravenous Mg loading [4]. Hypomagnesemia of hereditary renal origin comprises different congenital disorders. Some patients also suffer from primary or secondary defects of other tubular transport systems.

Diagnostic Tests

Hypomagnesemia varies from 0.29 to 0.60 mmol/l. Moderate hypocalcemia is present in about 40% and hypokalemia in about 50% of cases. The urinary Mg excretion is inappropriately high. During Mg depletion, an Mg excretion above 0.46 mmol/24 h is considered to be high in adults. Renal Mg wasting may be associated with other tubular dysfunctions, incomplete tubular acidosis or intermittent glucosuria, increased levels of amino acids in blood and urine, hypercalciuria, and hyperkaliuria. Three patients had elevated PTH levels. Creatinine clearance was moderately reduced in 50%. Renal biopsies reveal various pictures; some show patchy interstitial fibrosis, but most are normal.

Treatment and Prognosis

Mg supplementation was tried in similar doses as in patients with intestinal Mg malabsorption. An amelioration of the tetanic symptoms was achieved in less than 50% of the patients. Hypercalciuria was either reduced or increased by Mg therapy. In spite of Mg supplementation, the renal function of two patients with renal Mg wasting nephrocalcinosis and incomplete

tubular acidosis deteriorated to end – stage renal failure.

Genetics

Familial Mg-losing kidney probably comprises different tubular absorption defects. Parental consanguinity and diseases in siblings of both sexes have been described. A recessive mode of inheritance is postulated, but also an autosomal dominant inheritance [7].

Zinc

Two inherited disorders are known: acrodermatitis enteropathica (AE) [8] and hereditary hyperzincemia, a non-disease without symptoms.

Acrodermatitis Enteropathica (Danboldt-Closs Disease)

Clinical Presentation

The most dramatic clinical feature is *skin rash*, which has a characteristic symmetrical, circumorificial, retroauricular, and acral distribution. The skin lesions are erythematous in acute stages; then vesicobullous, pustular, or hyperkeratotic changes may become prominent. Secondary infection is common, usually with candida or staphylococci, which may lead to a wrong diagnosis. Mucosal lesions include gingivitis, stomatitis, and glossitis. Symptoms usually present in infancy. Onset is delayed in breast – fed infants until after weaning, whereas babies fed on infant formula develop the syndrome as early as the first 2–4 weeks of life. During early infancy, frequent passage of watery stool, anorexia, and failure to thrive often precede the skin lesions without being recognized as typical symptoms of this genetic disorder. Total *alopecia*, e.g., loss of scalp and superciliary hair occurs frequently. Nail deformities and ophthalmological problems including blepharitis, conjunctivitis, photophobia, and impaired dark adaption may also occur. Mood changes, irritability, lethargy, or depression also belong to the early features of zinc deficiency, as do recurrent infections. All clinical features are aggravated during infections and physiological stress, at growth spurts in early childhood and puberty. After puberty, men are less vulnerable to zinc deficiency than women. One third of pregnancies in untreated patients ended in spontaneous abortion or in congenital defects of the skeletal or central nervous system [9]. Although fluctuation in the clinical course occurred, it usually went progressively downhill before the advent of zinc therapy. A few patients have been described [10] who suffered from a variant of AE with severe diarrhea, occasional cheilosis, growth failure, and normal plasma zinc concentration. Their symptoms were exacerbated after withdrawal of zinc therapy.

Metabolic Derangement

The disturbance of zinc homeostasis results from a partial block in the intestinal absorption [11]. This was first demonstrated in vivo after oral application of tracer doses of zinc-65 [12] or zinc-69m [13]. Atherton et al. [14] also showed that in vitro zinc-65 accumulation by jejunal mucosal biopsies is markedly reduced. Ultrastructural studies of duodenal biopsies revealed characteristic inclusion bodies in Paneth cells. Reduced zinc absorption due to a transport defect results in a severe zinc deficiency state with an impairment of the function of many zinc metalloenzymes. All major metabolic pathways are regulated by zinc metalloenzymes. The function of these enzymes include catalytic, structural, and regulatory roles. The clinical picture of severe zinc deficiency results from a defective metabolism in many tissues.

Diagnostic Tests

In most patients plasma zinc (serum zinc is about 15% higher) is reduced to 20%–40% of the values for age – matched controls (3–6μmol/l; normal range, 9–20μmol/l). Blood samples for zinc analysis should be taken in the morning in the fasting state and the patient should preferably be without infection. The sample should be centrifuged within 2h avoiding hemolysis and contamination. By analyzing plasma zinc, the diagnosis of AE can never be proven beyond doubt. In some plasma, zinc may be normal because of zinc released from catabolized tissues. On the other hand, plasma zinc may be low because of acquired zinc deficiency or redistribution of zinc in other body pools during stress and infection. For practical reasons neutrophils, lymphocytes, or red cells are unsuitable for zinc determination. Usually urinary zinc excretion is decreased if plasma zinc is lowered, and sometimes plasma copper is at the upper

range. Plasma alkaline phosphatase parallels in general plasma zinc during severe zinc deficiency. Hair zinc is unreliable in AE because hair growth is often impaired. Raised blood ammonia, hypo-β-lipoproteinemia, and altered fatty acid pattern also occur. In many patients impaired immune responses are associated with zinc deficiency states, pointing to depressed humoral and cell-mediated immunity.

So far the diagnosis in patients with the characteristic clinical picture can be suspected if markedly reduced plasma zinc values are found or, preferably, if absorption tests reveal a defective intestinal absorption. The diagnosis is established if after successful zinc therapy and clinical remission a withdrawal of zinc leads to a relapse. This is the best way to differentiate the recessively inherited defect AE with low plasma zinc and its variant form with normal zinc, from acquired zinc deficiency.

Treatment and Prognosis

Before zinc deficiency was known to cause the clinical symptoms of AE, treatment mainly consisted of feeding human milk and giving hydroxylated quinolines. This resulted in partial or total remission in several patients. Nowadays we know that the bioavailability of zinc from human milk is higher than from other dietary sources and that hydroxylated quinolines enhance zinc absorption in the gut. The drug therapy caused serious side effects, especially irreversible optic atrophy. More than 90% of the patients died. Since 1973 zinc supplementation has been used. Usually within 1 week after zinc therapy has ben started skin lesions disappear and plasma zinc and alkaline phosphatase [15] increase to normal values, as does urinary zinc excretion. The usual therapeutic dose is between 30 and 50 (−100) mg zinc/day (10–30 μmol Zn/kg per day). Zinc is not very toxic, thus higher amounts of 50–200 mg do not cause side effects. Plasma copper should be monitored to avoid hypocupremia. Zinc salts used are gluconate and acetate. More often zinc sulfate ($ZnSO_4 \times 7H_2O$, 200 mg, about 45 mg Zn) is used. It is administered in individually prepared capsules, tablets (Solvezinc), or as sweet solutions. If gastric problems occur it should be given in at least three divided doses per day. In many patients the total dose per day remains constant during their whole childhood. A higher dose (100 mg Zn/day) is needed during growth spurts and especially during pregnancy and lactation. By this management uncomplicated pregnancies were achieved and the zinc content of human milk from AE mothers was kept normal. Due to zinc supplementation the prognosis of AE is now good. In patients with the variant form of AE, similar zinc doses were used and lead to an amelioration of the clinical symptoms.

Genetics

AE is a recessively inherited defect. Up to now heterozygous carriers cannot be detected. The HLA pattern was only investigated in a few patients. It has not been established whether a specific pattern exists.

Hyperzincemia

In 1976 Smith et al. [16] reported a dominantly inherited defect with elevated plasma zinc levels (2500–4350 ng/ml). There were no clinical and biochemical abnormalities. The zinc contents of erythrocytes and hair were normal.

Selenium

Selenium (selenocysteine) is an integral part of the three (or four) different glutathione peroxidases (cellular, plasma, and phospholipid hydroperoxide (and intestinal)) which protect intra- and extramembranous compartments of tissues against oxidative damage. It is also a constituent of type I 5′-iodothyronine deiodinase [17], which converts T_4 to T_3.

Two genetic defects concerning glutathione peroxidase were described about 20 years ago. Necheles et al. [18] reported moderate or severe erythrocyte glutathione peroxidase deficiency resulting in compensated hemolytic disease, drug – induced hemolysis, and neonatal jaundice. Extensive studies on more patients with hemolytic diseases from different ethnic groups are lacking. Karpatkin and Weiss [19] reported a reduced activity of platelet glutathione peroxidase with high levels of reduced glutathione in three patients with Glanzman thrombasthenia. The inheritance is assumed to be autosomal recessive. The heterozygous state is usually asymptomatic. In China

two endemic diseases, Keshan disease [20] (a cardiomyopathy) and probably Kaschin – Beck disease [21] (an osteochondroarthropathy) are observed in remote areas with low selenium content in food. They can be prevented by Se supplementation. It is still speculative which other factors – genetics, nutrition, environment, or infection – may play a role. People from other countries with a low Se state during parenteral or semisynthetic feeding [22] seldom reveal signs of Se – responsive myopathy or skeletal or cardiac abnormalities.

Other Metals

Manganese-related disease (prolidase deficiency) is discussed in the chapter by Jaeken; *molybdenum*-related disease (combined deficiency of sulfite oxidase and xanthine oxidase) is discussed in the chapter by Van den Berghe and Vincent.

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Part XII
Porphyrins and Heme

Porphyrias

Y. Nordmann

CONTENTS

The porphyrias are a group of inherited disorders of heme biosynthesis [1, 2]. Each enzyme defect (Table 1) produces a specific pattern of overproduction of intermediates of the pathway, associated with characteristic clinical features. Porphyrias are classified as erythropoietic or hepatic, depending on the main site of overproduction. Most porphyrias are caused by partial enzyme defects, resulting in approximately 50% of normal activity, and are inherited as autosomal dominant traits. Exceptions are the rarer congenital erythropoietic porphyria and Doss porphyria (DP), with autosomal recessive inheritance and profoundly decreased enzyme activity.

Hepatic Porphyrias

Acute Hepatic Porphyrias

Clinical Presentation

Mechanism of the Acute Attack

It is generally accepted that the symptoms of acute attacks of porphyria are due to the effect of the disease on the nervous system. Several hypotheses have been put forward, two of which are presently favoured: one involves the deficiency of heme synthesis in neural tissues, while the other involves the neurotoxicity of the precursors of heme, mainly δ-aminolevulinic acid (ALA); typical neurological manifestations such as DP, lead intoxication, or tyrosinemia have been observed in patients who overproduce ALA but not porphobilinogen (PBG).

Typical, identical attacks of *acute abdominal pain* with vomiting and constipation occur in the four acute hepatic porphyrias: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), and DP. The attacks are sometimes associated with psychiatric manifestations such as anxiety, depression, disorientation, confusion, and delirium. *Neurological symptoms* such as peripheral neuropathy with occasional cranial nerve involvement, respiratory distress, and grand mal seizures are most often caused by treatment errors. Despite the autosomal inheritance of the acute porphyrias, more than 80% of the patients are 18 to 45-year-old women, in accordance with the fact that sex steroids are among the most important eliciting factors of the attacks.

Darkening of urine (which may look like port wine) is attributed to non-enzymatic conversion of PBG to a porphyrin-like compound and is common to all acute porphyrias. Cutaneous abnormalities (which are described under "Porphyria Cutanea Symptomatica"), though sometimes concurring with abdominal pain in HCP and/or VP, may be absent in these acute porphyrias; they are never observed in AIP.

Metabolic Derangement

Precise identification of the type of acute porphyria by clinical examination alone is very difficult. Diagnosis is therefore primarily based on analysis of heme precursors in urine and stool.

In AIP, large amounts of ALA and PBG are excreted in the urine. Values are 20- to 200-fold above normal and provide specific diagnosis. Uro- and coproporphyrin are usually moderately in-

Table 1. Defects of heme biosynthesis

Pathway intermediates	Enzymes	Porphyrias	Metabolites								
			In urine			In feces			In erythrocytes		
			ALA PBG	Uro	Copro	Uro	Copro	Proto	Uro	Copro	Proto
Glycine + succinyl-CoA											
↓	ALA synthase										
ALA		Doss porphyria	+++ (ALA)	++	+++						++
	ALA dehydrase	(DP)	± (PBG)								
↓											
PBG		Acute intermittent porphyria	+++	++	++	++	+				
	PBG deaminase	(AIP)	(++)		(+)						
↓											
Uroporphyrinogen I (Uroporphyrin I)		Congenital erythropoietic porphyria		+++	+++	++	+++	+	+++	++	++
	Urocosynthase	(CEP)									
↓											
Uroporphyrinogen III (Uroporphyrin III)		Porphyria cutanea symptomatica		+++	+	++	++	+			
	Urodecarboxylase	(PCS)					(+)				
↓		Hepatoerythropoietic porphyria		+++	+	++	++				++
		(HEP)									
Coproporphyrinogen III (Coproporphyrin)		Hereditary coproporphyria	+++	++	+++	++	++++	+			
	Copro-oxidase	(HCP)	(+)		(+)		(+++)				
↓											
Protoporphyrinogen IX (Protoporphyrinogen)		Variegate porphyria	+++	++	+++	+	++	+++			
	Proto-oxidase	(VP)	(+)		(+)		(+)				
↓											
Protoporphyrin IX (Protoporphyrin)		Erythropoietic protoporphyria					±	+++		+	++++
	Ferrochelatase	(EPP)									
↓											
Heme											

Parentheses under "Metabolites" refer to data during remission.
ALA, δ-aminolevulinic acid; PBG, porphobilinogen; Uro, uroporphyrin; Copro, coproporphyrin; Proto, protoporphyrin.

creased in urine and feces, owing to nonenzymatic conversion of PBG. In about one third of the clinically asymptomatic carriers, PBG excretion is slightly increased.

In HCP, the profile of urinary heme precursors during acute attacks is similar to that in AIP, although coproporphyrin may sometimes be dramatically increased. Stool porphyrins are usually diagnostic: characteristically there is a large excess of coproporphyrin, associated with normal protoporphyrin. Approximately 20%–30% of asymptomatic carriers show this typical fecal profile after puberty, often with a normal urinary profile.

In VP, urinary findings during acute attacks are identical to those in AIP and HCP. Carriers with only chronic cutaneous manifestations or even without symptoms often show a slight increase in ALA and PBG. The diagnostic finding is elevated fecal protoporphyrin, resulting from nonenzymatic oxidation of protoporphyrinogen and usually visible as an isolated peak on high-performance liquid chromatography (HPLC). When differential extraction is used, a less marked elevation of coproporphyrin can also be evidenced. A plasma fluorescence emission maximum at 626 nm has been described as a diagnostic marker for VP.

DP (ALA dehydrase deficiency) was described in two young adults [3] presenting with a large increase in the urinary excretion of ALA and coproporphyrin. PBG was only moderately elevated. Fecal excretion of porphyrins was normal, but erythrocyte porphyrins were slightly increased. This pattern of overproduction of heme precursors closely resembles that of severe lead poisoning.

Diagnostic Tests

Enzyme assays are now widely used not only to confirm the type of porphyria, but also to detect clinically latent individuals.

In AIP, PBG deaminase deficiency (50% of normal activity) has been demonstrated in all examined tissues, including erythrocytes. PBG deaminase measurement has to be done carefully: several sources of error have been reported, such as an increased activity during or just after an acute attack, in liver disease, in lymphoproliferative disorders, but also in normal babies under 6 months of age. In case of doubt, PBG deaminase can be measured in mitogen-stimulated lymphocytes, in which overlap between normal and decreased activities is less than in erythrocytes. AIP is genetically heterogeneous and more than 30 mutations have been reported in the PBG deaminase gene [4]. Most mutations have been found in only one or two families, except in Sweden and in Holland, where a few mutations are common to several families, indicating founder effects.

In HCP, coproporphyrinogen oxidase (copro-oxidase) is decreased to around 50% of normal in liver, fibroblasts, lymphocytes, and leukocytes. Considerable excess of copro-oxidase activity in liver cells probably explains why patients with acute attacks are rare, whereas several asymptomatic carriers are found in their kindred when the enzyme is assayed. Besides the dominant heterozygotes, two clinically different types of homozygotes have been described, both with a copro-oxidase activity of about 10% of normal. Different mutations are found, underlining the genetic heterogeneity of the disorder.

In VP, several studies have shown a 50% deficiency of the activity of protoporphyrinogen IX oxidase (proto-oxidase). Homozygous patients of VP have also been described who display severe, lifelong photosensitivity, slightly decreased growth rate, an increased erythrocyte protoporphyrin, the mechanism of which remains unexplained.

In DP, with autosomal recessive inheritance, ALA dehydrase activity is profoundly deficient, to approximately 1% of normal. Compound heterozygotes exist for different missense mutations in the ALA dehydrase gene.

Treatment and Prognosis

As soon as an attack has been diagnosed, a careful search for precipitating factors should be undertaken, especially for drugs, including oral contraceptives (see the Appendix), underlying infections, and/or hypocaloric diets, particularly with poor carbohydrate intake. Agitation and other psychiatric manifestations are usually controlled with chlorpromazine. Treatment of severe pain requires morphine-like drugs such as pethidine, despite the danger of addiction. We usually combine chlorpromazine with pethidine and give the patient a quiet room.

► Drug treatment is now superseded by glucose and hematin. Adequate administration of glucose (300–400 g per day, usually by slow intravenous infusion) frequently leads to a reduction in the urinary excretion of heme precursors by still undefined mechanism(s). Treatment of porphyric attacks has been greatly improved by the introduction of hematin, which is superior to glucose since most patients respond favorably to hematin, whereas several do not respond favorably to glucose. Hematin is given intravenously in doses of up to 3–4 mg/kg body weight per 24 h, usually during 4 days. Hematin probably replenishes the depleted hepatic pool of heme. The fact that urinary ALA and PBG decrease dramatically within 2–3 days shows that it exerts feedback on ALA synthase. Although the side effects of hematin, such as coagulopathy or phlebitis, have never been associated with real hemorrhage, hematin should not be used in conjunction with anticoagulant therapy, and the vein for infusion has to be changed each day. Stable preparations of hematin are now available (i.e., Heme-arginate, Leiras, Finland) with negligible side effects.

Recently, women with cyclical perimenstrual acute attacks have benefitted from administration of luteinizing hormone-releasing hormone (LHRH) agonists, which inhibit ovulation. It should be noted that all treatments have to be used early in the attack, before any nervous or respiratory complications arise, otherwise a biochemical response without concomitant clinical amelioration may be the only result.

Porphyria Cutanea Symptomatica (Porphyria Cutanea Tarda)

Clinical Presentation

Porphyria cutanea symptomatica (PCS) is the most common form of porphyria. *Cutaneous photosensitivity* is the predominating clinical feature. Acute attacks with abdominal pain and psychiatric and/or neurological manifestations are never observed. PCS is heterogeneous and includes at least two types:

- The sporadic type (or type 1), more often seen in 40- to 50-year-old men without a family history of the disease, which appears related to some inducing compounds such as alcohol
- The rarer familial type with earlier onset, sometimes before puberty, which is equally frequent in both sexes

The lesions of photosentivity involve the light-exposed areas such as face and neck, backs of the hands, and, in women, legs and backs of the feet. *Skin fragility* is perhaps the most specific feature: a minimal trauma is followed by a superficial erosion, soon covered by a crust. Bullae (vesicles) usually appear after sun exposure and take several weeks to heal, leaving hypo- or hyperpigmented atrophic scars. White papules (milia) may develop in areas of the bullae, particularly the backs of the hands. *Hypertrichosis* is often found in the upper part of the cheeks (malar area) and sometimes also on the auricles of the ears and the arms. Increased, uniform pigmentation of sun-exposed areas is common. Alopecia and hypopigmented scleroderma-like lesions of the skin are rarer. All skin lesions are similar to those seen in VP and HCP. The incidence of hepatic cancer among PCS patients seems to be variably increased, from 74% in Czechoslovak series to 0% in Italy.

Among the precipitating factors, alcohol, estrogens, and iron are most frequently incriminated. The mechanisms of exacerbation are unclear. It should be emphasized that most of the drugs classified as porphyrinogenic (Appendix) may have been used for several years before PCS develops. Abnormal iron metabolism appears to be another precipitating factor: serum iron is frequently elevated, and a mild hepatic siderosis has been described in at least 80% of the patients. Although some authors found a normal total body iron store, iron removal by phlebotomy is a highly effective treatment. The deleterious iron effect might be explained by induction of a free radical system.

Pathogenesis of Skin Lesions of Porphyrias

The high levels of porphyrins present in the extracellular fluid of the skin can be stimulated to an excited state by absorption of light energy. The excited porphyrins may either destroy cellular components directly or may react with molecular oxygen species, which can be highly damaging to cellular components.

Metabolic Derangement

Urine contains increased concentrations of uroporphyrin (mainly isomer I[1]) and 7-carboxylic porphyrin (mainly isomer III[1]); 6- and 5-carboxylic porphyrins and coproporphyrin (the successive products of the four steps of decarboxylation of uroporphyrinogen III) are moderately elevated. The accompanying liver disease may lead to a minor increase in ALA excretion. In feces, the dominant porphyrin is often a coproporphyrin derivative, isocoproporphyrin. However, excretion of coproporphyrin, 7-carboxylic porphyrin, and uroporphyrin may also be enhanced.

Diagnostic Tests

In sporadic PCS, uroporphyrinogen III decarboxylase (urodecarboxylase) activity is only deficient in liver, where a 50% reduction of activity is measured. In familial PCS, there is a 50% reduction of urodecarboxylase activity in all tissues, including erythrocytes. The familial type accounts for 15%–20% of the cases of PCS. It is not uncommon to find several patients in the same family, in addition to latent carriers with normal excretion of porphyrins, but revealed by enzyme assay.

Treatment and Prognosis

Patients should first of all be advised to avoid all precipitating factors (alcohol, medications, and exposure to sunlight) until treatment has resulted in clinical and biological remission. Presently, phlebotomy is the treatment of choice in PCS, even when serum iron or ferritin levels are not increased. Venesections of 300 ml, at 10- to 12-day intervals, are performed during 2 months, until serum iron decreases to 60%–70% of its initial value. Urinary porphyrin patterns should be followed monthly. Clinical remission and normalization of urinary porphyrins are usually obtained within 4–6 months, but in the feces copro- and isocoproporphyrin may remain elevated for a long time.

When phlebotomy is contraindicated (anemia, cardiac, or pulmonary disorders, age), low-dose chloroquine therapy (250 mg weekly) is the favored alternative therapy. Duration of treatment and relapse rate are only marginally higher than with venesection. High-dose chloroquine has to be avoided because it causes a hepatitis-like syndrome in PCS patients.

Hepatoerythropoietic Porphyria

Hepatoerythropoietic porphyria (HEP) is a very rare porphyria which is clinically very similar to congenital erythropoietic porphyria (CEP), with a severe *photosensitivity* usually beginning in early infancy. However, patterns of urinary and fecal porphyrins are similar to those found in PCS, and a severely deficient activity of urodecarboxylase (around 7% of normal) suggests that these patients are homozygous for the gene that causes PCS [5]. Nevertheless, although several point mutations and a deletion of the urodecarboxylase gene have been identified in patients with HEP, these mutations have not been found as yet in patients with familial PCS.

Erythropoietic Porphyrias

Congenital Erythropoietic Porphyria

CEP, or Gunther's disease, is one of the rarer inherited porphyrias. It was, however, both the first porphyria described and the first in which a specific enzyme defect could be demonstrated, namely the deficiency of uroporphyrinogen III cosynthase (urocosynthase).

Clinical Presentation

In infants, the first symptom is usually the excretion of red urine, resulting in pink staining of the diapers. The red discoloration depends on the amount of porphyrins excreted and is subject to large fluctuations, ranging from pink to dark reddish brown.

Photosensitivity is manifested when the child is exposed to sunlight. A vesicular or bullous eruption develops on the face and the back of the hands. The vesicles contain a serous fluid that may exhibit a red fluorescence under ultraviolet (UV)

[1] There are four isomers of uroporphyrin and coproporphyrin: only the I and III isomers occur in nature. In isomer I, the side chains are arranged symmetrically around the ring; in isomer III the substituents on one of the four pyrroles are reversed.

light. Often the vesicles lead to erosions and impetiginous infection and heal slowly, leaving pigmented or even depigmented scars. Scarring due to severe infection may give the skin a pinched appearance. The occurrence of minor injuries, bullous eruptions, and infections may lead to severe mutilations of ears, nose cartilage, and digits. Fortunately, careful antibiotic treatment can greatly reduce secondary infections and severe scarring. Nevertheless, all exposed or already affected areas remain more sensitive to slight injury, and a diffuse thickening of the skin (pseudoscleroderma) is a common feature after several years. Hypertrichosis appears in most patients; it also affects the exposed areas, especially the upper arm and the face on the temporal and malar region. The additional hair is typically blond, downy, and of lanugo type, but may be coarse and dark. There may also be loss of cranial hair that occurs as a "scarring alopecia" in the scalp. Fingernails are often dystrophically altered (koilonychia).

Erythrodontia is reported in almost all cases and, if present, is pathognomonic of CEP. The teeth, deciduous and/or permanent, may exhibit a red or usually dirty-brown discoloration under normal light and a red fluorescence under UV light. This is due to the deposit of large amounts of porphyrins in the dentine. The discoloration of the deciduous teeth suggests that porphyrin overproduction begins in the fetus. The eyes may show severe ulcerations, ectropion, or cataracts, with ensuing blindness.

Splenomegaly is another common feature. It has been found at, or soon after birth in a few cases, but usually appears as the disease progresses, in most cases in relation to hemolysis, which is found in the majority of the patients. In most of them, anemia is slight owing to compensation by increased production of erythrocytes. In some cases, however, anemia is at times very severe, requiring multiple transfusions. Early death due to anemia has been reported.

Since 1965, five cases of late-onset CEP have been described with clinical features similar to sporadic porphyria cutanea, but presenting in adulthood. It should finally be noted that CEP patients never exhibit the abdominal or neuropsychiatric symptoms observed in the acute hepatic porphyrias.

Metabolic Derangement

Although there are daily and seasonal fluctuations, the urine always contains large amounts of uroporphyrin and, to a lesser extent, coproporphyrin. Smaller amounts of 7-, 6-, and 5-carboxylic porphyrins can also be extracted, but their excretion pattern is quite different from that found in PCS. The major fraction (>80%) of urinary uroporphyrin and coproporphyrin is of isomeric series I, but there is an absolute increase in uroporphyrin III in all patients. ALA is usually within normal limits or only slightly increased. PBG is usually normal.

Feces contain large amounts of coproporphyrin and little uroporphyrin. As in urine, most of the fecal porphyrins belong to the isomeric series I. Protoporphyrin excretion is usually within the normal range.

Red blood cells contain variably increased concentrations of uroporphyrin and coproporphyrin I. Protoporphyrin is usually not higher than in other hemolytic conditions.

Diagnostic Tests

Erythrocyte urocosynthase is decreased to 10%–30% of normal, with higher residual activities in the milder phenotypes. Prenatal diagnosis is possible. Studies of the urocosythase gene have revealed rare insertions and deletions but mostly point mutations, among which a Cys73→Arg change accounts for 20%–25% of cases.

Treatment and Prognosis

General treatment includes minimal exposure to the sun, avoidance of trauma to the skin, and careful treatment of any skin infection. Packed erythrocyte transfusions markedly reduce excessive hemolysis, reduce erythropoiesis, and decrease porphyrin excretion. Nevertheless, multiple transfusions may be harmful. Splenectomy is not necessarily recommended. In the absence of other therapeutic advances, bone marrow transplantation might represent an effective treatment.

Erythropoietic Protoporphyria

Clinical Presentation

Photosensitivity is the major clinical manifestation: short exposures to sunlight induce painful burning sensations in the sun-exposed skin areas, followed by edema and erythema. Vesicles, bullae, and crusting rarely occur. Chronic skin changes may develop, which consist of thickening of skin areas that are most exposed to sunlight (backs of the hands, face). Quite typically, facial skin appears normal, although it bears a few shallow, circular scars often scattered over the bridge of the nose, forehead, and cheeks. Onset of cutaneous symptoms is usually in early childhood (3–5 years). Nevertheless, penetrance of erythropoietic protoporphyria (EPP) is variable: subjects carrying the abnormal gene may remain asymptomatic, whereas others show only a mild photosensitivity. EPP is therefore sometimes detected in late adulthood.

EPP is generally a benign disease, although a number of patients with liver abnormalities and/or cholelithiasis, often requiring cholecystectomy, have been reported. In rare cases, fatal liver disease with cirrhosis develops.

Metabolic Derangement

Elevated protoporphyrin levels in erythrocytes and plasma are characteristic. Fecal protoporphyrin may be increased, but urinary porphyrin excretion is normal. Nevertheless, in the rare cases with impaired liver function, urinary porphyrins (mostly coproporphyrin) are elevated. Chemical analysis of the gallstones reveals high levels of protoporphyrin.

Diagnostic Tests

Deficiency (50% of normal activity) of the mitochondrial enzyme ferrochelatase (heme synthetase) can be demonstrated in all examined tissues. The first homozygous case of EPP was described in 1986: lymphocyte ferrochelatase activity of the patient was only 6% of normal, whereas that of both parents and several of their family members was around 50%. Noteworthy, several asymptomatic patients are detected only by measurement of ferrochelatase activity.

Recent studies of the ferrochelatase gene have revealed several mutations, confirming the heterogeneity of EPP. Although inheritance is mostly autosomal dominant, a few mutations appear recessive.

Treatment and Prognosis

Oral administration of β-carotene has been shown to provide photoprotection and hence enhanced tolerance to the sun, although about 20% of patients experience no improvement. Since porphyrin concentrations remain unchanged, it is thought that β-carotene prevents the photosensitivity reaction by quenching the singlet oxygen or the triplet states of protoporphyrin. Therapy of liver disease in EPP includes ingestion of the resin cholestyramine to try to interrupt the enterohepatic recirculation of protoporphyrin and the administration of bile salts to mobilize protoporphyrin directly from the liver. Liver transplantation is the therapy of last resort in irreversible liver damage.

Appendix: Unsafe Drugs

Mefenamic acid
Nalidixic acid
Pipemidic acid
Piromidic acid
Acitretine
Alcohol
Alcuronium
Alfadolone-alfaxolone
Alizapride
Allopurinol
Alminoprofen
Alpidem
Alprazolam
Alverine
Amidopyrine
Amineptine
Amiodarone
Amisulpride
Amobarbital
Androgens
Articaine
Astemizole
Baclofen
Barbiturates
Benfluorex
Benzbromarone
Benzylthiouracile
Bepridil
Beta histine
Bromocriptine
Bupivacaine
Buspirone
Busulfan
Captopril
Carbamazepine
Carisoprodol
Cefaclor
Cefuroxime
Chloramphenicol
Chlormezanone
Chloroquine
Cibenzoline
Cicletanine
Ciprofibrate
Clobazam
Clofibrate
Clometacin
Clomethiazole
Clomifene
Clonidine

Clorazepate
Clotiazepam
Cyclophosphamide
Cyproterone
Danazol
Dapsone
Dexfenfluramine
Dextromoramide
Dextropropoxyphene
Diazepam
Dihydralazine
Dimenhydrinate
Disopyramide
Dosulepin
Econazole
Enalapril
Enflurane
Ergotamine and derivatives
Erythromycin
Estro-progestatives
Estrogens
Etamsylate
Ethenzamide
Ethosuximide
Etidocaine
Etomidate
Famotidine
Fenfluramine
Fenofibrate
Fenoprofen
Fenoverine
Fipexide
Floctafenine
Fluconazole
Flumequine
Flunarizine
Flurbiprofene
Fluvoxamine
Gemfibrozil
Glutethimide
Griseofulvin
Halofantrine
Halothane
Hexapropymate
Hyd roxyzine
Hydantoins
Hydralazine
Ibuprofen
Ifosfamide
Imao
Isoniazid
Isradipine
Ketamine
Ketoconazole
Lidocaine
Loflazepate
Loprazolam
Loxapine
Mebeverine
Mebubarbital
Medifoxamine
Mefloquine
Mephenesine
Mepivacaine
Meprobamate
Mesna
Metapramine
Methyldopa
Methylergometrine
Methyprylone
Metronidazole
Mexiletine
Mianserine
Miconazole
Nifedipine
Nitrazepam
Nitrendipine
Nizatidine
Noramydopyrine
Nordazepam
Ondansetron
Ornidazole
Oxetorone
Oxybutynine
Paracetamol
Pargyline
Pentamidine
Pentazocine
Pentoxifylline
Phenacetin
Phenazone
Phenobarbital
Phenoxybenzamine
Phenylbutazone
Phenytoin
Pipamperone
Pipebuzone
Piribedil
Pirprofen
Pravastatine
Prazepam
Prenylamine
Prilocaine
Primidone
Probenecid
Progabide
Progestatives
Propafenone
Propantheline
Pyrazinamide
Pyrrocaine
Quinapril
Quinine and derivatives
Ranitidine
Rilmenidine
Sotalol
Roxithromycin
Secobarbital
Simvastatine
Spironolactone
Succinimides
Sulphonamides
Sulfinpyrazone
Sulpiride
Sultopride
Tamoxifen
Tetrazepam
Theophylline
Thioridazine
Tiadenol
Tiapride
Ticlopidine
Tilbroquinol
Tiliquinol
Tinidazole
Tolbutamide
Toloxatone
Trazodone
Triazolam
Trimethadione
Trimipramine
Tritoqualine
Urapidil
Valproate
Valpromide
Veralipride
Vigabatrin
Viloxazine
Zolpidem

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Inborn Errors of Bile Acid Synthesis

P.T. Clayton

CONTENTS

The conversion of cholesterol to bile acids in the liver requires modifications to the sterol nucleus and oxidation of the side chain, as shown in Fig. 1 [1, 2]. Two defects in the nuclear modification reactions have been described:

- 3β-Hydroxy-Δ⁵-C_{27}-steroid dehydrogenase (3β-dehydrogenase) deficiency
- 3-Oxo-Δ⁴-steroid 5β-reductase (5β-reductase) deficiency

Defective side chain oxidation occurs in cerebrotendinous xanthomatosis (sterol 27-hydroxylase deficiency) and in peroxisomal disorders [3]. The peroxisomal disorders are discussed in Poll Thé and Saudubray (this volume).

3β-Hydroxy-Δ⁵-C_{27}-Steroid Dehydrogenase Deficiency

Clinical Presentation

Most patients with 3β-dehydrogenase deficiency have presented with prolonged neonatal jaundice (conjugated bilirubin greater than 40μM at the age of 2 months or older) associated with steatorrhoea. The stools have been pale but not acholic. Rickets (due to malabsorption of vitamin D) was often apparent before the age of 6 months, and one patient developed a bleeding diathesis due to vitamin K deficiency at the age of 9 months [3–5]. Routine investigations performed at age 2–6 months have not been very helpful in distinguishing 3β-dehydrogenase deficiency from other causes of giant cell hepatitis. The biochemical evidence of fat-soluble vitamin malabsorption is perhaps more striking, e.g., plasma vitamin E concentration consistently less than 4μM (normal range, 11.5–35μM) and the γ-glutamyl transpeptidase may be normal or only minimally elevated (despite a significantly elevated aspartate aminotransferase). The liver biopsy shows a periportal inflammatory infiltrate (often including eosinophils), giant cells, some hepatocellular necrosis and bridging fibrosis or even early cirrhosis. In untreated patients, pruritus often becomes apparent from the age of 6 months, and the problems of steatorrhoea and malabsorption of fat-soluble vitamins continue. Presentation in the second decade with chronic hepatitis has been described [6].

Metabolic Derangement

3β-Dehydrogenase catalyses the second reaction in the major pathway for synthesis of bile acids – the conversion of 7α-hydroxycholesterol to 7α-hydroxycholest-4-en-3one. When the enzyme is deficient, the accumulating 7α-hydroxycholesterol can undergo side-chain oxidation ± 12α-hydroxylation to produce 3β,7α-dihydroxy-5-cholenoic acid and 3β,7α,12α-trihdroxy-5-cholenoic acid. These unsaturated C_{24} bile acids are sulphated in the C_3 position, a proportion is conjugated to glycine and they can be found in high concentrations in the urine. Concentrations of bile acids in the

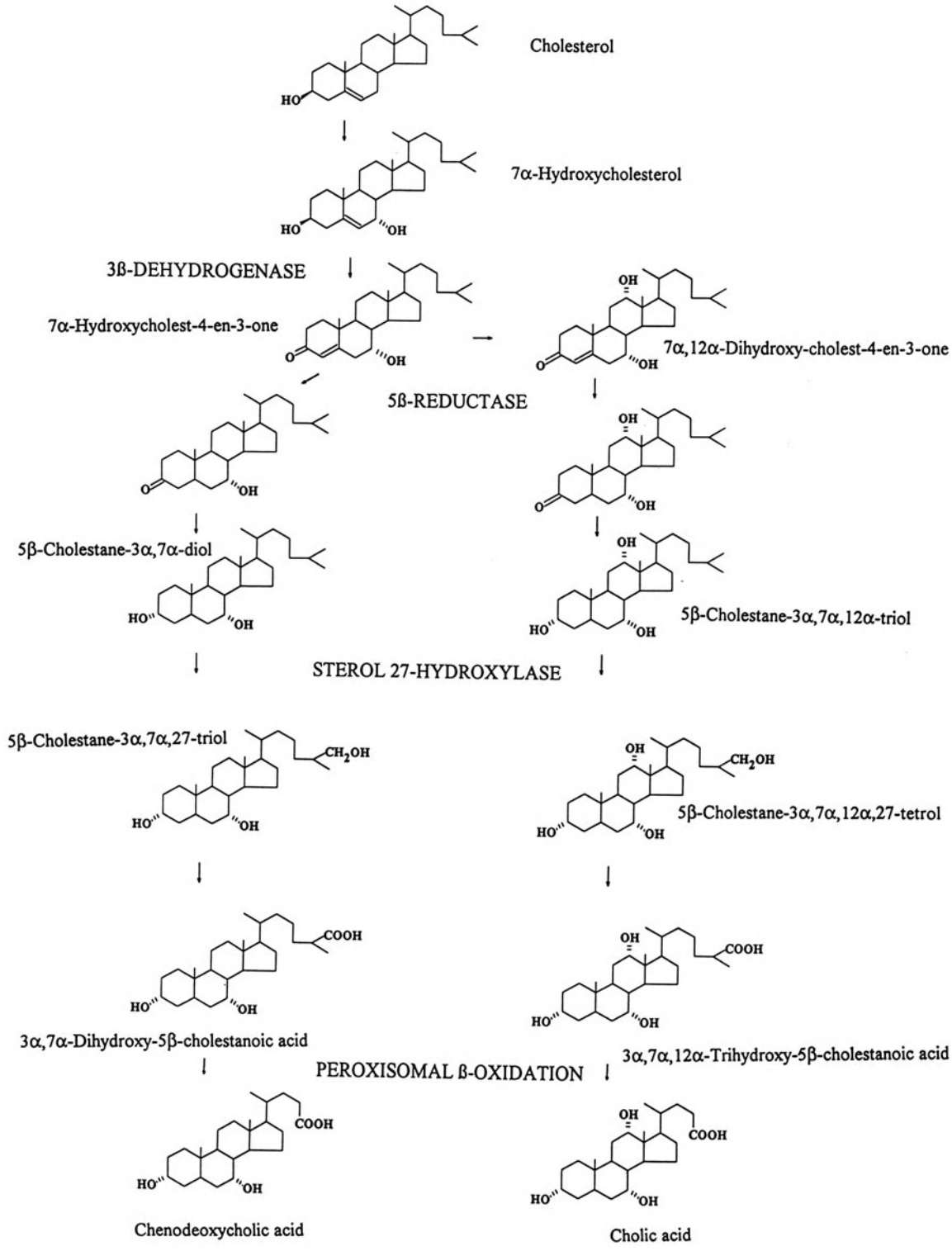

Fig. 1. A simplified version of the major pathways for the conversion of cholesterol to bile acids highlighting the steps which are blocked in the inborn errors discussed in the text. *3β-DEHYDROGENASE*, 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase; *5β-REDUCTASE*, 3-oxo-Δ^4-steroid 5β-reductase. Sterol 27-HYDROXYLASE deficiency causes cerebrotendinous xanthomatosis (CTX)

bile are low [7]. It is probable that the sulphated Δ^5 bile acids cannot be secreted into the bile canaliculi and fuel bile flow in the same way as occurs with the normal bile acids. There are at least two possible ways in which this sequence of events might lead to damage to hepatocytes and ultimately to cirrhosis:

- The abnormal metabolites produced from 7α-hydroxycholesterol may be hepatotoxic.
- Failure of bile acid-dependent bile flow may lead to hepatocyte damage, perhaps as a result of accumulation of toxic compounds normally eliminated in the bile.

Diagnostic Tests

The diagnosis is established by demonstrating the presence of the characteristic Δ^5 bile acids in plasma or urine. It is important to remember that bile acids with a Δ^5 double bond and a 7-hydroxy group are acid labile. They may be destroyed by some of the methods that are used for solvolysis of sulphated bile acids prior to chromatographic analysis. Solvolysis is best performed using tetrahydrofuran-methanol-trifluoroacetic acid (900:100:1 v/v) [7]. Analysis by fast atom bombardment mass spectrometry (FAB-MS) overcomes the problem of lability [4, 8].

Plasma. If plasma bile acids are analysed using a gas chromalography (GC)-MS method which does not include a solvolysis step, the profile of non-sulphated bile acids which is obtained shows concentrations of cholic and chenodeoxycholic acid which are extremely low for an infant with *cholestasis*. The concentration of 3β,7α-dihydroxy-5-cholestenoic acid is increased. Inclusion of a solvolysis step reveals the presence of high concentrations of 3β,7α-dihydroxy-5-cholenoic acid (3-sulphate) and 3β,7α,12α-trihydroxy-5-cholenoic acid (3-sulphate). These can also be detected when plasma is analysed by FAB-MS.

Urine. Two rapid tests can be used to make a diagnosis from a urine sample; in both cases the first step is extraction of the bile acids using a C_{18} (octadecylsilane-bonded silica) cartridge. The dried urine extract will give an intense purple colour with Lifschütz reagent (glacial acetic acid/concentrated H_2SO_4, 10:1 v/v). Alternatively, reconstitution of the extract in glycerol/methanol and analysis by FAB-MS will show the characteristic ions of the diagnostic unsaturated bile acids: mass/charge ratio (m/z)469, 485, 526 and 542. Very occasionally, significant-sized ions with m/z values 469 and 485 (steroid sulphates?) are seen in normal urine; if there is any uncertainty the urine should be analysed by GC-MS following careful solvolysis.

Fibroblasts. 3β-Dehydrogenase can be assayed in cultured skin fibroblasts using tritiated 7α-hydroxycholesterol [9]. Patients show very low activity.

Treatment and Prognosis

Untreated 3β-dehydrogenase deficiency has led to death from complications of cirrhosis before the age of 5 years; patients with milder forms of the disorder may survive with a chronic hepatitis into their second decade. The response to treatment depends upon the severity of the liver disease at the time of starting treatment. In patients with a ◄ bilirubin level less than 120μM and aspartate amino transferase (AST) less than 260 U/l, *chenodeoxycholic acid therapy* has led to a dramatic improvement in symptoms and in liver function tests within 4 weeks and to an improvement in the liver biopsy appearances within 4 months. The dose of chenodexycholic acid that has been used is 12–18 mg/kg per day initially (for 2 months) followed by 9–12 mg/kg per day maintenance. In one infant with severe disease, chenodeoxycholic acid (15 mg/kg per day) led to a rise in bilirubin and AST. Her treatment regime was changed to 7 mg chenodeoxycholic acid/kg per day plus 7 mg cholic acid/kg per day. Over the course of 15 months, her bilirubin and transaminases returned to normal and a repeat liver biopsy showed a more normal parenchyma and less inflammation. The combination of cholic acid and chenodeoxycholic acid is probably the treatment of choice for patients with severe liver damage. Bile acid replacement therapy may work in one of two ways:

- By fuelling bile acid-dependent flow (hence directly relieving cholestasis)
- By suppressing the activity of cholesterol 7α-hydroxylase (thereby reducing the accumulation of potentially toxic metabolites of 7α-hydroxycholesterol)

Genetics

3β-Dehydrogenase deficiency is probably inherited as an autosomal recessive trait. Siblings have been affected without the parents having any evidence of liver disease, and there is a high incidence of consanguinity among the parents. Levels of 3β-dehydrogenase in fibroblasts from the parents is at the lower end of or below the normal range [9]. The cDNA encoding 3β-dehydrogenase has not yet been characterised.

3-Oxo-Δ⁴-Steroid 5β-Reductase Deficiency

Clinical Presentation

In 1988, Setchell et al. [10] described male twins who presented with neonatal cholestatic jaundice associated with failure to thrive but no hepatosplenomegaly. Transaminases and alkaline phosphatase were mildly elevated and the prothrombin time was slightly prolonged (and not responsive to vitamin K). Plasma tyrosine and methionine were slightly elevated. Liver biopsies showed lobular disarray as a result of giant cell and pseudoacinar transformation of hepatocytes, hepatocellular and canalicular bile stasis and a minimal lobular and portal cellular infiltrate. Electron microscopy showed small canaliculi with slit-like structure and few or absent microvilli. Setchell et al. showed that the twins were excreting in their urine large amounts of two 3-oxo-Δ⁴ bile acids: 7α-hydroxy-3-oxo-4-cholenoic acid and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid; they postulated that these infants had a primary genetic deficiency of 5β-reductase. Others had previously urged caution in interpreting substantial excretion of 3-oxo-Δ⁴ bile acids in this way [11].

Metabolic Derangement

There is general agreement that excretion of 3-oxo-Δ⁴ bile acids is likely to result from reduced activity of the hepatic enzyme which brings about the 5β(H) saturation of the Δ⁴ double bond of 7α-hydroxy-cholest-4-en-3-one and 7α,12α-dihydroxy-cholest-4-en-3-one. These intermediates may then undergo side-chain oxidation to produce the corresponding C_{24} bile acids. Alternatively, they may be reduced by 3-oxo-Δ⁴-steroid 5α-reductase prior to side-chain oxidation, thus giving rise to 5α(H) bile acids (allochenodeoxycholic and allocholic acid). In some patients, defective 5β-reductase activity is clearly secondary to a known cause of hepatocyte damage such as severe hepatitis B infection, and the 3-oxo-Δ⁴ bile acids disappear when the hepatocytes recover from the primary insult [3, 11]. This observation creates considerable difficulty in the diagnosis of primary genetic deficiency of the 5β-reductase. Russell and Setchell [2] have demonstrated a reduced concentration of immunoreactive enzyme protein in the liver of the twins described above; however, this does not represent proof of a defective gene. Details on the other 12 patients thought to have primary deficiency of 5β-reductase [2] have not been published. The mechanism of hepatocyte damage and cholestasis in 5β-reductase deficiency is unknown; as with 3β-dehydrogenase deficiency, toxicity of unsaturated intermediates and unsaturated bile acids and loss of bile acid-dependent bile flow have been postulated.

Diagnostic Tests

Plasma. GC-MS analysis of plasma bile acids from the twins described by Setchell et al. revealed a high concentration of chenodeoxycholic acid, a normal or low concentration of cholic acid, the two characteristic 3-oxo-Δ⁴ bile acids (10%–20% of the total bile acid mixture) and allo bile acids (25%–30% of the total). This pattern is qualitatively similar to that seen in patients with secondary 5β-reductase deficiency, but in these patients the proportion of allo bile acids does not usually reach 25%–30%.

Urine. Analysis of urine by FAB-MS shows the presence of major ions attributable to the glycine and taurine conjugates of 7α-hydroxy-3-oxo-4-cholenoic acid (m/z 444 and 494) and 7α,12α-dihydroxy-4-cholenoic acid (m/z 460 and 510). These identities can be confirmed by GC-MS analysis following enzymatic deconjugation. In the patients who are considered to have primary 5β-reductase deficiency, the 3-oxo-Δ⁴ bile acids have comprised more than 70% of the total urinary bile acids; a lower percentage is found in most children whose excretion of 3-oxo-Δ⁴ bile

acids is secondary to liver damage of other aetiology.

Treatment and Prognosis

Untreated, the sibling of the twins described by Setchell et al. died at 4 months of hepatic failure [10]. The twins were treated with ursodeoxycholic acid (200 mg/day) followed by chenodeoxycholic acid and cholic acid (100 mg/day of each) and then by ursodeoxycholic acid and cholic acid (100 mg/day of each). The latter combination appeared to be the most successful – synthesis of 3-oxo-Δ^4 bile acids and allo bile acids was suppressed and liver function tests and bile canalicular morphology normalised [6, 12].

Genetics

The putative cases of primary 5β-reductase defeiciency have shown a pattern of inheritance compatible with an autosomal recessive trait.

Cerebrotendinous Xanthomatosis

Clinical Presentation

The first symptom of cerebrotendinous xanthomatosis (CTX) is often mental retardation detected during the first decade. *Cataracts* may also be present as early as 5 years. Wevers et al. [13] have documented four Dutch patients in whom persistent diarrhoea was present from early childhood. Motor dysfunction (spastic paresis, ataxia, expressive dysphasia) develops in approximately 60% of patients in the second or third decade. Tendon xanthomata may be detectable during the second decade, but more usually appear in the third or fourth decade. The Achilles tendon is the commonest site; other sites include the tibial tuberosities, the extensor tendons of the fingers and the triceps. *Premature atherosclerosis* leading to death from myocardial infarction occurs in some patients. In others, death is caused by progression of the *neurological disease* with increasing spasticity, tremor and ataxia and pseudobulbar palsy. It is important to recognise that the neurological deterioration is very variable [14]. For example, some patients are normal intellectually but suffer from a neuropathy or mild spastic paresis; others have no neurological signs but present with psychiatric symptoms resembling schizophrenia. Magnetic resonance imaging of the brain may show diffuse cerebral atrophy and increased signal intensity in the cerebellar white matter on T2-weighted scans [15]. *Osteoporosis* is common in CTX and may produce pathological fractures; it is associated with low plasma concentrations of 25-hydroxyvitamin D and 24,25-dihydroxy-vitamin D [16].

Metabolic Derangement

It is now firmly established that CTX is caused by a defect in the gene for *sterol 27-hydroxylase*, the mitochondrial enzyme which catalyses the first step in the process of side-chain oxidation which is required to convert a C_{27} sterol into a C_{24} bile acid [17]. 5β-Cholestane-3α,7α,12α-triol cannot be hydroxylated in the C_{27} position and accumulates in the liver. As a result, it is metabolised by an alternative pathway starting with hydroxylation in the C_{25} position (in the endoplasmic reticulum). Further hydroxylations, e.g. in the C_{22} or C_{23} position, result in the synthesis of the characteristic bile alcohols which are found (as glucuronides) in the urine. Bile acid precursors other than 5β-cholestane-3α,7α,12α-triol also accumulate. Some of these (e.g., 7α-hydroxy-cholest-4-en-3-one) are probably converted to cholestanol by a pathway involving 7α-dehydroxylation. Because patients with CTX have a reduced rate of bile acid synthesis, the normal feedback inhibition of cholesterol 7α-hydroxylase by bile acids is disrupted. This further enhances the production of bile alcohols and cholestanol from bile acid precursors. The major symptoms of CTX are produced by accumulation of cholestanol (and cholesterol) in almost every tissue in the body, but in particular in the nervous system, in atherosclerotic plaques and in tendon xanthomata.

Diagnostic Tests

Plasma. The concentration of cholestanol in plasma can be determined by GC or high-performance liquid chromatography (HPLC). Patients with CTX have plasma concentrations in the range of 30–400μM (normal range, 2.6–16μM).

The plasma cholestanol to cholesterol ratio may be a better discriminant than the absolute cholestanol concentration. The following bile acid precursors have been detected at increased concentration in plasma:

- 7α-Hydroxycholesterol
- 7α-Hydroxy-cholest-4-en-3-one
- 7α,12α-dihydroxy-cholest-4-en-3-one

Plasma concentrations of bile acids are low; plasma concentrations of bile alcohol glucuronides are elevated.

Urine. Rapid diagnosis of CTX can be achieved using FAB-MS. The major cholanoids in the urine are cholestane pentol glucuronides, giving rise to an ion of m/z ratio 627 [3, 18]. The full bile alcohol profile can be produced by analysing urine by GC-MS following treatment with Helix pomatia glucuronidase / sulphatase; the major alcohols are 3α,7α,12α,23,25-pentols and 3α,7α,12α,22,25-pentols. Increased bile alcohol concentrations in the urine can be detected using a simple enzymatic assay based on 7α-hydroxysteroid dehydrogenase [19]. The urinary bile alcohol excretion following cholestyramine administration has been used as a test for carriers of CTX [20].

Fibroblasts. 27-Hydroxylation of C_{27} sterols can be measured in cultured skin fibroblasts and the enzyme activity is virtually absent in fibroblasts from patients with CTX [21].

DNA. In certain populations in which one or two common mutations predominate, DNA analysis may prove to be a rapid method for diagnosis of both homozygotes and carriers of CTX (see below).

Treatment and Prognosis

Patients with untreated CTX usually die from progressive neurological dysfunction or myocardial infarction between the ages of 30 and 60 years. The results of treatment with chenodeoxycholic acid were first reported in 1984 [22]. The rates of synthesis of cholestanol and cholesterol were reduced and plasma cholestanol concentrations fell. A significant number of patients showed reversal of their neurological disability with clearing of the dementia, improved orientation, a rise in IQ and enhanced strength and independence. Urinary excretion of bile alcohol glucuronides was markedly suppressed. Chenodeoxycholic acid almost certainly works by suppressing cholesterol 7α-hydroxylase activity; ursodeoxycholic acid, which does not inhibit the enzyme, is ineffective. Adults have usually been treated with a dose of chenodeoxycholic acid of 750 mg/day. Other treatments which have been used in CTX include hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins such as lovastatin) [23] and low-density lipoprotein apheresis [24]. There is insufficient information available to assess these forms of treatment at the present time.

Genetics

CTX is inherited as an autosomal recessive trait. The cDNA encoding the 27-hydroxylase enzyme has recently been characterised and the gene localised to chromosome 2 q33-qter [17]. CTX can be caused by point mutations which lead to a production of an inactive enzyme (e.g. Arg→Cys at positions 362 and 446) [25]. In Moroccan Jews there appear to be two common mutations which both lead to failure of production of sterol 27-hydroxylase mRNA One is a frameshift mutation, the other a splice junction mutation [26].

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Bilirubin

N. Yazigi and W.F. Balistreri

CONTENTS

Bilirubin is the end product of the degradation of heme and heme proteins [1, 2]. The majority of the circulating bilirubin (80%–85%) is derived from the breakdown of hemoglobin, originating in senescent red blood cells, in the reticuloendothelial system. The remaining proportion derives from the destruction of maturing erythroid cells in the bone marrow (ineffective erythropoiesis) and from nonerythroid components involving the turnover of heme and heme proteins; these components constitute the "early-labeled" fraction of bilirubin. In adults up to 400 mg (4–5 mg/kg per day) of unconjugated bilirubin (UCB) is formed each day through enzymatic degradation of heme derived from hemoglobin (35 mg hemoglobin yields 350 mg UCB). UCB is metabolized by the liver to conjugated bilirubin (CB) via a multistep process, which can be divided into three distinct phases:

- Uptake by the hepatocyte, which involves the dissociation of the UCB–albumin complex, followed by internalization and binding to the cytoplasmic anionic binding protein Ligandin, which prevents the reflux of UCB back into the circulation.
- Conjugation, predominantly with glucuronic acid, which converts the bilirubin molecule into the water-soluble form (CB). The conjugation reaction occurs in the endoplasmic reticulum, catalyzed by specific isoforms of a group of membrane-bound enzymes known as uridine diphosphogluconurate glucuronosyltransferase (UGT), also called UDP glucuronyl transferase. This is a two-step process, producing bilirubin monoglucuronide (BMG) and then bilirubin diglucuronide (BDG), both of which are then excreted by the hepatocyte. The normal proportion of these conjugates in bile is 85% BDG and 15% BMG.
- Excretion into bile, an energy-dependent step, is rate limiting in bilirubin metabolism. Only CB can be excreted in the bile, primarily as BMG and BDG. Photoisomers of the naturally occurring Z-Z isomeric form of bilirubin are water soluble and thus can be excreted in the bile; this constitutes the rationale for phototherapy in the treatment of unconjugated hyperbilirubinemia. Any alteration in the excretion process results in a regurgitation of bilirubin back into the circulation, manifested as jaundice.

Upon reaching the intestinal lumen, CB can be excreted as such in the stool or metabolized by the bacterial flora to urobilinogen and related products. Urobilinogen is partially excreted in the stool; a significant portion is reabsorbed to either be excreted in the urine or undergo an enterohepatic cycle. Most CB circulating in serum will bind loosely to albumin; 5% of CB remains free to be filtered by the kidneys. CB can also irreversibly bind to albumin, forming complexes which cannot be filtered by the kidneys. This is observed in patients with severe prolonged cholestasis. Under normal circumstances the daily urinary excretion of urobilinogen is 4 mg, and the fecal excretion is less than 280 mg.

The liver has a large reserve capacity to clear an increased bilirubin load; this capacity must be greatly exceeded before any significant bio-

chemical or clinical sign of hyperbilirubinemia is detectable [3]. Jaundice (hyperbilirubinemia) is a frequent manifestation of altered bilirubin metabolism due to a large number of hepatic and systemic disorders. Evaluation of the patient with jaundice most often begins with the fractionation of the serum total bilirubin into the direct-reacting component (CB) and the indirect fraction (UCB).

It should be noted that routine automated procedures may significantly overestimate the direct-reacting fraction at relatively low total bilirubin concentration. In particularly puzzling cases, discrimination can be made by separation and quantitation of bilirubin and conjugates by high-performance liquid chromatography (HPLC) or by the exclusion of cholestasis using serum bile acid determinations.

Defects in each of the steps in bilirubin metabolism and transport have been described (Table 1). The most frequent cause of altered bilirubin metabolism are in fact *acquired*, as seen in patients with liver disease (hepatitis or cholestasis) or in the presence of hemolysis. Inborn errors in bilirubin metabolism, such as specific *congenital* enzymopathies or transport defects, are less common.

Unconjugated Hyperbilirubinemia (Table 2)

Gilbert's Syndrome

Clinical Presentation

This disease was first described in 1907 by Gilbert et al. as a mild familial jaundice, which they termed "simple congenital cholemia" [4]. In 1947 Meulengracht reported a series of cases with mild jaundice and lassitude occurring at the time when jaundice was most prominent [5]. With the increased understanding of bilirubin metabolism and transport, it is possible to discern a subset of patients with a benign form of congenital unconjugated hyperbilirubinemia that is now commonly called Gilbert's Syndrome. It is characterized by a mild fluctuating jaundice (serum UCB levels approximately 2–5 mg/dl), the only abnormal physical sign, usually noted in the second decade of life, often as an incidental finding [6, 7]. The degree of unconjugated hyperbilirubinemia is exacerbated by intercurrent infections, fasting, or stress. A large proportion of patients have associated constitutional and vague gastrointestinal complaints, but there is no relation to the degree of hyperbilirubinemia. In all, 3%–7% of the adult population is affected, and there is a 4:1 male preponderance.

Metabolic Derangement

The pathogenesis of this disorder, which was postulated to result from a reduction in hepatic bilirubin clearance to 30% of normal, is undefined and presumably heterogeneous. It appears that several steps in the transport of bilirubin from blood to bile may be variably defective and present as Gilbert's Syndrome:

- Early studies suggested the presence of a reduced amount of the enzyme UGT (glucuronyl transferase).
- A total of 50%–60% of Gilbert's patients are described as having an increased bilirubin turnover due to a slight reduction in the red cell survival.
- There is increasing evidence of subgroups of patients with Gilbert's syndrome who have defects in carrier proteins involved in hepatic uptake of organic anions (defective hepatic clearance of bilirubin) [8].

Diagnostic Tests

The diagnosis of Gilbert's syndrome is one of exclusion; there is no specific test. The diagnosis could be made in an otherwise asymptomatic patient if there is documented recurrent unconjugated hyperbilirubinemia, no evidence of hemolysis or inefficient erythropoiesis, and normal serum aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and bile acid levels. As a research tool, determination of the profile of bilirubin conjugates by HPLC would confirm the diagnosis; affected patients would have an elevation of UCB and a decreased proportion of bilirubin diconjugates in the bile and duodenal juice. A liver biopsy is not indicated. Fasting (a low-lipid, 400-calorie diet for 48 h) will increase the serum UCB level by two- to threefold, possibly from an increase in the hepatic heme turnover. This increase is promptly reversed by refeeding and can be prevented by prefast admin-

Table 1. Classification of hyperbilirubinemic conditions

Disorder	Defect in Bilirubin Physiology
Unconjugated hyperbilirubinemia	
Hemolysis, hemolytic anemias and hematomas	Increased production (1l blood = 150mg hemoglobin = 5g bilirubin = 20 × normal daily production)
Ineffective erythropoiesis	Increased production
Neonatal (physiologic) jaundice	Increased production (decreased UGT activity; decreased cytosolic ligandin; increased intestinal bilirubin absorption)
Breast-milk jaundice	Inhibition of UGT activity (?); increased intestinal absorption
Drugs	Reduced hepatic uptake
Crigler-Najjar syndrome type I	Absent bilirubin UGT activity
Crigler-Najjar syndrome type II	Markedly decreased bilirubin UGT activity
Gilbert's syndrome	Decreased bilirubin UGT activity; decreased hepatic uptake (?); hemolysis or dyserythropoiesis (?)
Fasting hyperbilirubinemia (marked response in Gilbert's syndrome)	Increased production; decreased hepatic clearance; decreased uptake and conjugation (?)
Sepsis	Decreased uptake and conjugation (?)
Conjugated hyperbilirubinemia	
Hepatocellular disease (hepatocellular, canalicular, or ductular damage)[a]	Decreased biliary secretion; bilirubin deconjugation leads to increased plasma unconjugated bilirubin
Recurrent intrahepatic cholestasis	Decreased biliary secretion
Cholestatic jaundice of pregnancy	Decreased biliary secretion
Extrahepatic cholestasis (mechanical obstruction)	Decreased biliary secretion; bilirubin deconjugation
Dubin-Johnson syndrome	Impaired biliary secretion (canalicular membrane defect?)
Rotor's syndrome	Decreased hepatic uptake and storage (?); decreased biliary secretion (?)
Drugs	Decreased biliary secretion
Sepsis	Decreased biliary secretion

[a] In patients with hepatocellular disease (hepatitis and cirrhosis), there is usually interference in several steps of bilirubin metabolism (uptake, conjugation, and excretion). Since excretion is the rate-limiting step, this is usually impaired to the greatest extent and, as a result, conjugated hyperbilirubinemia predominates.
Modified from [1].
UGT, glucuronosyl transferase.

istration of phenobarbital. However, care must be taken in the interpretation of the results of the fast, since a slight rise in the serum bilirubin level is also seen in patients with hemolysis and certain forms of liver disease. The UGT activity, measured in liver tissue, is decreased; so is the bilirubin clearance, measured after the injection of a tracer or a loading bilirubin dose.

Treatment and Prognosis

Gilbert's syndrome, a benign condition, requires no treatment; affected patients have an excellent prognosis. Reassurance is the mainstay of therapy. In those patients in whom icterus presents a cosmetic problem, phenobarbital (60–180mg/day) will clear the jaundice and is used as needed.

Genetics

The mode of transmission is not known, but there is a suggestion of autosomal dominant transmission.

Crigler-Najjar Syndrome

Clinical Presentation

Seven infants, all from consanguineous marriages, were noted by Crigler and Najjar in 1952 to have marked unconjugated hyperbilirubinemia without evidence of altered liver function and no significant hepatic histologic changes. They termed the condition "congenital familial nonhemolytic jaundice with kernicterus." It is now known that the Crigler-Najjar Syndrome is due to a deficiency of

Table 2. Chronic nonhemolytic unconjugated hyperbilirubinemic syndromes

	Gilbert's syndrome	Crigler-Najjar type I syndrome	Crigler-Najjar type II syndrome
Incidence	7% of population	Rare	Uncommon
Mode of inheritance	Autosomal dominant (?)	Autosomal recessive	Autosomal dominant with variable penetrance (?)
Plasma bilirubin	<6 mg/dl; usually <3 mg/dl	17–50 mg/dl; usually >20 mg/dl	6–22 mg/dl; usually <20 mg/dl
Icterus	Limited to fluctuating mild scleral icterus, recognized in early adulthood; may remain undetected	Severe jaundice, beginning 3–4 days after birth	Jaundice beginning shortly after birth; occasionally unrecognized until childhood
Clinical features	Onset usually early adulthood; may be first recognized with fasting	Jaundice and kernicterus in infants or young adults	Kernicterus rare; usually asymptomatic jaundice
Plasma bilirubin turnover	Moderately increased in a significant proportion of individuals	Normal	Normal
Plasma bilirubin clearance	30% of normal	<50% of normal	<50% of normal
Bilirubin UGT activity	Decreased (5%–50% of controls)	Undetectable	Markedly decreased
Associated defects	Mild hemolysis (≤50% of patients); decreased hepatic uptake of bilirubin, BSP (≤40% of patients) and ICG (≤20% of patients)?	None	None
Bile	Yellow; relative decrease in BDG and increase in BMG	Yellow; trace of unconjugated bilirubin and BMG	Yellow; mostly BMG
Effect of phenobarbital on serum bilirubin concentration	Decreased to normal	None	Marked decrease

Modified from [1].
UGT, glucuronosyltransferase; BMG, bilirubin monoglucuronide; BDG, bilirubin diglucuronide, BSP, sulfobromophthalein; ICG, indocyanine green.

hepatic microsomal bilirubin UGT activity. Arias et al. [9] subdivided the syndrome into two apparent disparate clinical phenotypes, types I and II (Table 2).

Congenital complete absence of the enzyme glucuronyl transferase (called the Crigler-Najjar Syndrome, type I) is a rare condition which is manifested during the first days of life [10]. The unconjugated bilirubin reaches extreme levels, up to 45 mg/dl. Kernicterus is the major, almost universal complication of this disorder; rarely have patients survived past 18 months of age. The use of home phototherapy has improved the outcome, but severe neurological disease and death may occur at any age. In partial deficiency of UGT (Crigler-Najjar, type II), jaundice is noted in the second decade of life in an otherwise healthy patient. Neurologic complications are possible, but rare.

Metabolic Derangement

The molecular mechanisms of these two disorders are only partially understood and are quite complex due, in part, to the fact that the activities of multiple UGT isoforms are deficient in various Crigler-Najjar syndromes [11]. Recent studies have somewhat clarified these molecular mechanisms by which a single mutation causes complete deficiency of several UGT isoforms [12]. Other studies have suggested that Crigler-Najjar type II

is caused by a homozygous mutation in UGT isoform 1 activity [13]. Crigler-Najjar type II and Gilbert's syndromes have been described in the same family, leading to the suggestion that two allelic genes may be involved in the Crigler-Najjar variant and that Gilbert's disease results when only one of the genes is present [14]. Further work is still needed to confirm that theory.

Diagnostic Tests

Diagnosis of *Crigler-Najjar type I* is based on the early age of onset, the level of bilirubin elevation in the absence of any hemolysis, the absolute lack of conjugated bilirubin, the presence of pale yellow, almost colorless bile and the normal liver aminotransferase levels and histology. Studies by Schmid and Hammaker [15], using radiolabeled bilirubin, indicated that the UCB pigment in patients with this syndrome (or in the animal model, the Gunn rat) is mainly excreted in the urine and feces, in the form of metabolites, including hydroxyl derivatives of bilirubin and diazo-negative compounds, all products of the alternate bilirubin metabolism pathway. This maintains the serum UCB level at a relatively constant, albeit elevated, level.

In *Crigler-Najjar type II* patients, there is a high UCB level and a normal liver biochemical profile and biopsy. Although the enzyme is undetectable by available techniques, there is a documentable response to phenobarbital treatment in affected patients, with a drop in the serum UCB from approximately 25 mg/dl to 4–6 mg/dl. The responsiveness to phenobarbital is a diagnostic tool distinguishing this variant from type I (Table 2) [16]. The bile is pigmented, but its predominant pigment is BMG. The bilirubin level rises with fasting and during stress, a feature shared with Gilbert's syndrome.

Treatment and Prognosis

Various therapeutic approaches have been attempted, all with the aim of preventing bilirubin encephalopathy. These have included the following:

- Metalloporphyrin therapy to limit bilirubin production through inhibition of heme oxygenase [17]
- Avoiding agents that can displace the bilirubin from its albumin bond (sulfa drugs, salicylates)
- Maintaining adequate albumin levels
- Preventing excessive hemoglobin breakdown, preventing infections, fasting, and strenuous exercise, all of which increase bilirubin production, cholestyramine, and agar to bind bilirubin

Other treatment modalities to decrease the plasma bilirubin include phlebotomy, plasmapheresis, and phototherapy; the latter modality has proven particularly effective for maintenance treatment [18], but needs to be used for a minimum of 12 h a day. The long-term side effects of chronic *phototherapy* are unknown, and some patients have a poor response. Phenobarbital (60–180 mg/day) is useful only for patients with Crigler-Najjar type II; phenobarbital has been shown to increase UGT activity in the heterozygous Gunn rats, but not in humans with type II.

At best, a reduction of less than 50% in the bilirubin level is possible, forestalling the onset or diminishing the severity of neurologic disease. Liver transplantation should be an early consideration for patients with type I; it remains their best option [19]. *Liver transplantation* might be considered for patients with type II if phototherapy is ineffective at maintaining UCB levels within an acceptable range or if it is incompatible with a decent quality of life. Genetically engineered enzyme replacement is a promising treatment for the future.

Genetics

Type I is transmitted as a autosomal recessive trait. The inheritance mode in type II is generally accepted to be autosomal dominant with incomplete penetrance; however, an autosomal recessive inheritance is possible [13].

Conjugated Hyperbilirubinemia (Table 3)

Dubin-Johnson Syndrome

Clinical Presentation

This is a syndrome of chronic, benign recurrent hyperbilirubinemia, of a mixed component. It was first described in 1954 by Dubin and Johnson as a "chronic idiopathic jaundice" in patients with

Table 3. Congenital conjugated hyperbilirubinemic syndromes

	Dubin-Johnson syndrome	Rotor's syndrome
Incidence	Uncommon	Rare
Mode of inheritance	Autosomal recessive	Autosomal recessive
Plasma bilirubin concentration	Usually 2–5 mg/dl (60% conjugated), increase by estrogens and pregnancy	Usually 2–5 mg/dl (60% conjugated)
Clinical features	Usually asymptomatic jaundice in early adulthood; occasionally hepatosplenomegaly	Asymptomatic jaundice
Serum bile acid levels	Normal	Normal
Gross and histologic appearance of liver	Black–brown color; pigment granules in centrilobular areas	Normal
Oral cholecystography	Gallbladder not usually visualized	Normal
^{99m}Tc-HIDA	Prolonged retention in hepatocyte (impaired excretion)	No visualization
Kinetic parameters		
BSP removal from plasma	Initially slow; secondary "late" increase at >90 min	Initially very slow; no secondary increase
BSP transport maximum	Markedly decreased	Moderately decreased
BSP hepatic storage capacity	Normal	10% of normal[a]
Urinary coproporphyrin excretion and isomer pattern (coproporphyrin III is normally 75% of total)	Normal total; coproporphyrin I (>80% of total)	Increased total; coproporphyrin I increased to <80% of total

Modified from [1].
[a] This abnormality resembles that seen in the so-called hepatic storage disease.
BSP, sulfobromophthalein; HIDA, hepatic imino diacetic acid.

fluctuating hyperbilirubinemia, epigastric distress, and fatigability with or without hepatomegaly [20, 21]. They also noted conspicuous dark-brown iron-free pigment in centrilobular liver cells. The patients are usually asymptomatic; however, some have vague systemic signs such as fever, fatigue, or gastrointestinal symptoms. The liver is often enlarged and is tender in approximately 25% of cases. There have been reports of cholelithiasis associated with this syndrome.

Metabolic Derangement

The Dubin-Johnson Syndrome is characterized a defect in the liver cell secretion of bilirubin glucuronide and other organic anions [22, 23]. This syndrome in humans is phenotypically similar to the defect described in mutant Corridale sheep and in mutants rats (TR-) with defective adenosine triphosphate ATP-dependent bile canalicular transport and inherited conjugated hyperbilirubinemia [24]. The functional defect in TR-rats involves a virtual absence of ATP-dependent transport of organic anions, including BDG [25].

Diagnostic Tests

The diagnosis is based on the clinical presentation of conjugated hyperbilirubinemia in the presence of apparent good health and normal liver function tests, including normal serum alkaline phosphatase and serum bile acids levels. Acquired hepatobiliary disease, such as hepatitis or bile duct obstruction (cholestasis), must first be excluded. The serum bilirubin is usually mildly elevated, but may reach levels as high as 27 mg/dl [26]. There is a reversed serum ratio of monoconjugates to diconjugates, with the latter predominating. Oral and intravenous cholangiography fail to visualize the biliary tract. The ^{99m}Tc-imino diacetic acid (HIDA) scan while allowing for visualization of the liver and gallbladder at 90 min, fails to show the intrahepatic bile ducts; there is intestinal dye excretion. Historically, the extended sulfobromophthalein (BSP) test was of diagnostic value, with Dubin-Johnson syndrome characterized by an initial fall followed by a late rise in the plasma BSP level (due to regurgitation of the anionic dye out of the liver); therefore the BSP level is greater after 120 min than at 45 min [27]. The liver pathology is also characteristic, with the typi-

cal feature being the presence of a brown pigment in the hepatocytes (centrilobular area); it is presumed to be melanin. The presence of this pigment does not correlate with the severity of the jaundice. On visual examination of a needle biopsy specimen from adults, the section appears to be greenish-black, due to this pigment. The age of onset of this pigmentary change remains unclear; however, specific pigment granules have been found in the liver cell in a patient as young as 6 years of age [28]. Electron microscopy has been used to document the presence of enlarged and irregular lysosomes with pigment in dense clusters. Normally, coproporphyrins are excreted mainly in bile and partly in urine, coproporphyrin I being the most abundant in bile and coproporphyrin III the predominant form in urine. Increased urinary porphyrin excretion is a common finding in patients with cholestasis. In Dubin-Johnson syndrome, the urinary coproporphyrin excretion is quantitatively normal, but abnormal in distribution, with predominance of coproporphyrin I. The increase in urinary coproporphyrin I excretion could be a consequence of a reduced biliary clearance of the compound.

Treatment and Prognosis

The prognosis of this syndrome is excellent and no treatment is required. Many patients remain asymptomatic. Phenobarbital, if used, decreases the hyperbilirubinemia and partially corrects the abnormal BSP kinetics.

Genetics

The transmission of the Dubin-Johnson Syndrome is now recognized as autosomal recessive. Cases have been reported from all over the world, in particular amongst the Ashkenazi and Sephardic Jews, Arabs, and Japanese.

Rotor's Syndrome

Clinical Presentation

It was first described in 1948 by Rotor et al., who reported several cases of nonhemolytic jaundice, with a direct Van den Bergh reaction occurring in families [29]. These patients had concomitant abdominal pain. Like Dubin-Johnson Syndrome, Rotor's syndrome is characterized by chronic, benign familial conjugated hyperbilirubinemia, but is less frequent. Affected patients are otherwise asymptomatic and maintain a bilirubin in the 10 mg/dl range [30]. As compared with patients with the Dubin-Johnson Syndrome, the intensity of the jaundice varies with stress and intercurrent infections.

Metabolic Derangement

Functional dye studies suggest that the defect causing CB (and BSP) retention involves the hepatic uptake of organic anions. There is no visualization of the hepatobiliary axis during ^{99m}Tc-HIDA studies; instead, the isotope is eliminated by the kidneys.

Diagnostic Tests

The diagnostic tests include the following:

- Normal liver function tests
- Normal oral cholecystography
- Failure of the ^{99m}Tc-HIDA scan to visualize the liver, gallbladder, or biliary tract
- Normal liver histology
- Abnormal BSP elimination curve (impaired initial slope, suggesting an uptake defect with no secondary rise)

The BSP transport maximum capacity is slightly reduced, but the relative storage capacity is reduced by 90% [31]. The total urinary coproporphyrin concentration is increased, as in patients with the Dubin-Johnson syndrome, but the pattern is similar to that noted in other cholestatic liver diseases (coproporphyrin I, approximately 60%–65% of the total); coproporphyrin III excretion is low [32]. The major differentiation from the Dubin-Johnson syndrome is the absence of intrahepatic brown pigment, the inability to visualize the gallbladder on cholecystography, and the absence of a secondary rise in BSP level.

Treatment

The condition is benign, the prognosis is excellent, and no specific treatment is needed.

Genetics

Transmission is autosomal recessive [23].

Conclusion

With the recent advances in our understanding of bilirubin metabolism, the mechanisms of inborn errors in bilirubin metabolism have been clarified. Although rare, it is important to identify affected patients, since it will soon be possible to develop targeted therapies using molecular and cellular biology techniques. At present, further work is needed to validate the use of metalloporphyrins. Hepatocyte transplantation, enzyme replacement, and genetic manipulation are the therapeutic approaches of the future.

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Part XIII
Membrane Transport

Alpha-1-Antitrypsin Deficiency

D.J.F. Feist

CONTENTS

A relationship between the development of *emphysema* and low serum levels of alpha-1-antitrypsin (AAT) was first noted in 1963 by Laurell and Eriksson [1]. A few years later, Sharp et al. [2] reported liver damage in AAT deficiency (AATD). AAT, also referred to as α_1-protease inhibitor or protein inhibitor (PI), is a 52-kDa glycoprotein synthesized in the liver and secreted into plasma, where it accounts for 90% of serum α_1-globulin. Although inhibitory of trypsin (hence its name), the major function of AAT is to inhibit neutrophil elastase at inflammation sites, e.g., within the alveoles. Whereas normal AAT (termed M) readily diffuses throughout interstitial fluids, the defective molecules formed in AATD aggregate inside the hepatocytes, resulting in poor secretion into blood and the fluids of the respiratory tract. Several defective variants of AAT have been identified, of which the most frequently encountered is Z.

Clinical Presentation

In infancy and childhood, AATD may cause a great variety of liver symptoms, but also remain asymptomatic. AATD-associated liver disease is mainly found in subjects expressing the Z variant of the inhibitor (see "Genetics"), primarily the *PI ZZ homozygotes*, but may also be seen in PI MZ and MS heterozygotes during their first 6 months of life [3]. The earliest and most frequent hepatic disturbance is *neonatal cholestasis*. This condition, which affects 10% of PI ZZ infants [4], must be differentiated from biliary atresia and other diseases causing conjugated hyperbilirubinemia. In the infantile manifestation of AATD, liver biopsy provides evidence of hepatocyte necrosis, cholestasis, proliferation or hypoplasia of interlobular bile ducts, portal infiltration, mild or severe portal fibrosis, and the presence of diastase-resistant periodic acid-Schiff (PAS)-positive granules of abnormal AAT within the hepatocytes. In the majority of these infants, the hepatocellular damage remits with the return of normal hepatic function, but in approximately 10% cirrhosis develops and progresses to portal hypertension and liver failure in the second decade [5]. About 50% of apparently healthy PI ZZ children have subclinical liver disease, reflected by elevated serum levels of aminotransferases and gamma glutamyl transpeptidase. *Liver cirrhosis*, however, has also been reported in adults who have had no history of neonatal cholestasis [6]. These cirrhoses have been associated with the PI types ZZ, MZ, and SZ [7]. Moreover, *hepatocellular carcinoma* has been described in MZ and ZZ individuals [8].

In the adult patient the earliest symptom of AATD is usually dyspnea on exertion. Approximately 50% of PI ZZ individuals develop *chronic bronchitis* or recurrent pulmonary infections. Recurrent inflammation of the respiratory tract in childhood is usually not a leading symptom of AATD. Adult ZZ patients suffering from *emphysema*, however, seemed to have been more prone to bronchial asthma in their youth [9]. Usually the characteristic panacinar destructive emphysema manifests itself in the third to fourth decade. Emphysema develops earlier in smokers and can probably be prevented in a nonsmoking environment [10].

Metabolic Derangement

Mutations in the PI gene result in the loss of a carbohydrate side chain on the AAT molecule.

Due to this change in the molecular structure, the hepatocyte cannot secrete AAT. There is still debate as to the mechanism responsible for the liver damage. Two theories have been advanced. The first proposes that AATD of the plasma renders the liver more susceptible to the unopposed action of proteolytic enzymes. The second view holds that it is the accumulation of AAT in hepatocytes that causes the damage. The blockage in the transport of the Z variant is proposed to enhance lysosomal activity and elicit proteolytic processes within the cell with ensuing hepatocellular damage and death. However, the observation that some PI ZZ individuals possess cytoplasmatic AAT globules without accompanying liver damage suggests that additional hormonal, metabolic, or environmental factors are involved to cause liver disease.

The alveolar destruction with ensuing emphysema is due to an imbalance between proteolytic enzymes, particularly neutrophil elastase, present inside the alveoles during an inflammation, and the opposing inhibitor AAT. So far the typical pattern of pulmonary damage has only been proved in the ZZ and SZ types. The MZ type, however, seems to aggravate other lung diseases such as idiopathic pulmonary fibrosis, chronic bronchitis, or asthma [11].

Furthermore, the phenotype PI MZ was significantly more prevalent in patients with *chronic pancreatitis* than in a control group, suggesting that this deficiency might render the pancreas more vulnerable to the effects of alcohol or other toxic agents [12].

Diagnostic Tests

The normal level of AAT in serum is between 150 and 350 mg/dl. AATD can be diagnosed by inspecting the original serum protein electrophoresis, since it lacks the α_1-globulin peak. The serum level of AAT itself is measured by electroimmunoassay. If it is decreased below 100 mg/dl, the PI type has to be determined in the patient and the patient's parents by starch gel electrophoresis or isolectric focusing. AATD is confirmed by the presence of one or two of the main deficiency alleles Z or S.

Treatment and Prognosis

So far it has been impossible to treat liver damage effectively. However, it is possible to prevent the development of emphysema by intravenous infusion or inhalation of recombinant or pooled AAT. A total of 60 mg AAT per kg body weight infused once per week achieves serum levels which are likely to prevent the lung from being destroyed by neutrophil elastase, if emphysema has not yet developed [13]. Gene therapy, already performed in animals, might become available in the near future [14]. At the moment *liver transplantation* is the only therapy eliminating both liver damage and the underlying genetic condition. It is indicated in juvenile patients if they are in the terminal stage of AATD-associated liver disorder, but have not yet suffered from pulmonary emphysema.

Genetics

AAT is encoded at the PI locus on chromosome 14 [15]. AAT is a genetically polymorphic protein, of which at least 33 variants, encoded by codominant PI alleles, have been identified. The nomenclature of the variants refers to relative mobility in acid starch gel electrophoresis. The most frequent PI allele encodes type M. About 90% of Caucasians are MM homozygotes and display "normal" mobility and serum level of AAT. The most frequently encountered abnormal PI allele encodes type Z, which differs by a single Glu342→Lys substitution and results in a slow-moving AAT with a serum level of only 15% of normal. Type S differs by a single Glu264→Val substitution, resulting in a serum AAT level of 60% of normal. The classical homozygous AATD variant ZZ and the PI type SS are found in less than 1% of Caucasians. Some 4% belong to the heterozygous types MS or MZ [6, 16, 17], whereas the deficiency alleles P, W, and zero are so rare that their clinical importance is negligible. The fact that PI-"zero" individuals do not display the characteristic granules in the hepatocytes suggests that they lack the PI gene and that AAT is not synthesized.

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Transport Defects of Amino Acids at the Cell Membrane: Cystinuria, Hartnup Disease, and Lysinuric Protein Intolerance

O. Simell and K. Parto

CONTENTS

Inherited defects in amino acid transport at the cell membrane are usually expressed as selective renal aminoaciduria, i.e., the concentration of affected amino acids is high in the urine, but normal or low in plasma. Intestinal absorption of the affected amino acids is almost always impaired as well. The clinical symptoms may be due to substrate excess in the urine or deficiency of the substrates in the body. Consequently, the symptoms in cystinuria are caused by formation of renal stones, as cystine is poorly soluble in urine; the pellagra-like skin disease and ataxia in Hartnup disease are due to deficiency of tryptophan and, subsequently, of niacin; and hyperammonemia in lysinuric protein intolerance is the result of deficiency of arginine and ornithine, which are obligatory urea cycle intermediates.

Cystinuria

Clinical Presentation

Subjects with cystinuria have a high risk for *urolithiasis* [1]. Occasional patients never develop any symptoms, but others already have recurrent symptomatic stones in early childhood. Severe acute episodes of abdominal or low-back pain, hematuria, pyuria, or spontaneous passing of stones may be the presenting sign. Cystine stones are usually radiopaque and visible in ultrasonography.

Metabolic Derangement

Several transport systems have been described at the cell membrane for cystine and the structurally related dibasic amino acids lysine, arginine, and ornithine [2]. The defect in cystinuria is expressed on the luminal membrane of the epithelial cells; the defect leads to poor absorption of cystine in the intestine and the renal tubules. Tubular cystine concentration may then exceed cystine solubility: crystals and stones are formed.

Amino acids are absorbed in the intestine not only as free amino acids, but also as small peptides; the peptides are then cleaved to free amino acids inside the epithelial cell and exported through the antiluminal membrane of the cell to the body. In cystinuria, sufficient amounts of cystine are apparently absorbed as cystine-containing oligopeptides from the intestinal lumen to fulfill the needs of the body, as no definite signs of cystine deficiency have been described.

Diagnostic Tests

Nitroprusside test of the urine and analysis of urinary amino acids lead to the diagnosis. Homozygotes excrete more than 0.1 mmol cystine/mmol

creatinine (250 mg cystine/g creatinine) or more than 800 μmol cystine/24 h per 1.73 m^2 (>200 mg cystine/24 h per 1.73 m^2) in the urine, but excretion varies markedly also in the homozygotes. Chemical analysis of the stones alone may be misleading, because mixed stones are not uncommon in patients with homozygous cystinuria and some stones contain no cystine at all. Levels of cystine and the dibasic amino acids in plasma are normal or slightly decreased. Plasma cystine concentration remains unchanged in type I and II cystinuria after an oral cystine load, but increases slowly in type III cystinuria [3]. In *type I*, intestinal biopsy specimens do not transport cystine, lysine, or arginine; in *type II*, there is no transport of lysine, and cystine transport is also markedly reduced; and in *type III*, cystine transport is either normal or variably reduced, and lysine transport is reduced. The obligate heterozygotes in types II and III cystinuria show moderate cystine-lysinuria, but in type I urinary amino acids are within the reference range.

Treatment and Prognosis

Crystallization of cystine in the urine can be prevented by excessive hydration. Adults should consume more than 3000 ml fluid/24 h, 500 ml of this before bedtime and, if possible, 500 ml during the night. Because cystine is much more soluble in alkaline urine, permanent alkalinization of urine by sodium bicarbonate (about 100 mmol/24 h for adults) or potassium and sodium citrate should be encouraged. Regular emotional support is necessary for compliance. Restriction of methionine intake to limit endogenous cystine synthesis is difficult, but may be helpful [4]. Decreased sodium intake may also decrease cystine excretion [5, 6]. Thiol compounds form water-soluble cysteine disulfides with cystine and are thus suitable for stone dissolving [7, 8]. Daily D-penicillamine, 2 g/1.73 m^2, is well tolerated by most patients, but may cause hypersensitivity reactions and lead to glomerulopathy or nephrotic syndrome and to production of antinuclear antibodies. Alpha mercaptopropionylglycine is better tolerated than D-penicillamine, but it has occasionally led to glomerulopathy or hyperlipidemia [9, 10]. Recently, captopril has been proposed for use and is obviously well tolerated [11–13]. Percutaneous nephrolithotripsy is seldom effective in stone removal because cystine stones are extremely hard. Surgical procedures are often needed at some point in life, but should always be combined with conservative preventive therapy. Regular follow-up of the patients is mandatory [14].

Genetics

The mode of inheritance of the three major genetic subtypes of cystinuria is autosomal recessive, and the total incidence varies from 1 in 2000 to 1 in 15000. A renal membrane protein, encoded by the rBAT (originally called D2H) gene on the short arm of chromosome 2, has been cloned [15–18]. It is a 90-kDa type II glycoprotein with transport kinetics similar to those in the renal brush border; it is capable of transporting neutral amino acids as well. The gene is in linkage disequilibrium with cystinuria, and six different mutations have so far been found which segregate with the cystinuria phenotype; the patients were either homozygotes or compound heterozygotes for the mutations.

Hartnup Disease

Clinical Presentation

Since the first description of the syndrome in several members of the Hartnup family in 1956 [19], an extensive number of patients who fulfill the biochemical diagnostic criteria have been described.

The classical clinical symptoms resemble closely those of nutritional *niacin deficiency* and probably reflect deficient production of the essential tryptophan metabolites, particularly of nicotinamide. However, most patients never show any clinical symptoms of the disease, possibly because the *tryptophan* needs are fulfilled by absorption of tryptophan from tryptophan-containing oligopeptides or because the niacin content of the food is large enough. The developmental outcome has shown no clear difference from normal in growth or IQ scores, although low academic scores were found in the Hartnup patients, and transient skin lesions occurred in five of the 21 Hartnup subjects studied [20]. The skin lesions as well as the neurological symptoms, if expressed, usually occur in early childhood. *Pellagra-like skin changes* are found on the light-exposed areas of the face, neck, forearms, and dorsal aspects of the hands and legs.

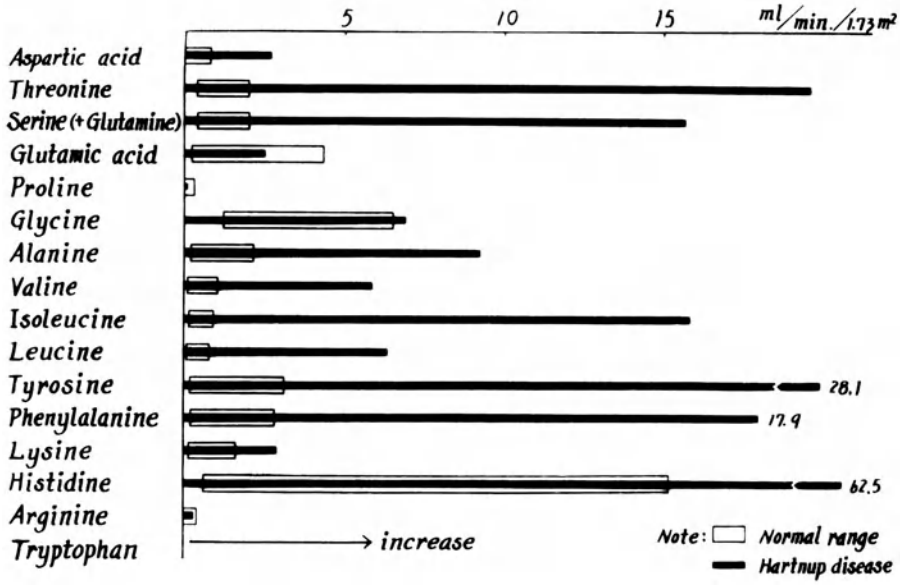

Fig. 1. Renal clearance of amino acids in Hartnup disease

The skin hardens and becomes scaly, rough, and hyperpigmented.

Cerebellar ataxia, attacks of headache, muscle pain, and weakness may resemble symptoms seen in porphyria or in the porphyria crises of type I tyrosinemia. Occasional patients have presented with mental retardation or seizures. Psychiatric symptoms from emotional instability to delirium may occur. Exposure to sunlight, fever, diarrhea, inadequate diet, or psychological stress may precipitate symptoms. Usually symptoms become milder with increasing age.

Metabolic Derangement

The *hyperaminoaciduria* in Hartnup disease is characteristic and differs from findings in the renal Fanconi syndrome. The affected amino acids share a common transporter which is expressed only at the luminal border of the epithelial cells in the renal tubuli and intestinal epithelium. Alanine, serine, threonine, asparagine, glutamine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine, and citrulline are excreted in excess in the urine, and their plasma concentrations are decreased or low-normal. The renal clearance values of the other amino acids are almost within the normal range (Fig. 1) [21].

The transport defect is expressed also in the intestine [22]. Stools contain increased amounts of free amino acids, and the pattern reflects closely that in the urine. Oral tryptophan loads lead to lower plasma tryptophan peaks in the patients than in the controls, and the patients excrete less kynurenine and *N*-methyl-nicotinamide (tryptophan metabolites produced in the body) than the controls [23]. The unabsorbed amino acids in the colon are exposed to bacterial degradation; degradation of tryptophan produces large amounts of indican, indole acetic acid, indole acetylglutamine, indolylacyloylglycine, and several other compounds, which are then excreted in the urine. Urinary indole excretion may be normal but is always increased after tryptophan loads; the response can be decreased by oral antibiotics. Oral loads with histidine-containing dipeptides and incubations of intestinal biopsy specimens with histidine and with glycyl-histidine show that tryptophan and other affected amino acids are readily absorbed as short oligopeptides, but not as free amino acids [24]. Histidine accumulation in the biopsy specimen was markedly decreased, but incubation with glycyl-histidine led to normal accumulation of histidine [25].

Diagnostic Tests

The characteristic excess of neutral monoamino-monocarboxylic amino acids in the urine and normal or low-normal concentration of the affected amino acids in plasma confirm the diagnosis. An oral load of L-tryptophan (100 mg/kg) leads to a supranormal increase in indole excretion. Excretion rates of indole compounds may be within the

normal range if the child is on a normal or low-protein diet.

Treatment and Prognosis

► Dermatitis and neurological symptoms usually disappear with oral nicotinamide treatment, 40–200 mg daily, in 1–2 weeks. Oral neomycin delays intestinal tryptophan degradation and decreases indole production; however, the role of the indole compounds in the disease has been poorly characterized. Sunlight should be avoided and exposed areas protected by sun-blocking agents. Psychosis, ataxia, or other neurological symptoms disappear rapidly during nicotinamide supplementation. A patient who presented with severe intermittent dystonia failed to improve with nicotinic acid, but the condition improved with trihexyphenidyl, 1–2 mg/kg per day [26]. Adequate supply of high-quality protein is probably important for prevention of symptoms in patients detected in newborn screening. Recently, tryptophan ethyl ester has been successfully used in Hartnup patients for the circumvention of the tryptophan transport defect [27].

Genetics

Hartnup disease follows an autosomal recessive pattern of inheritance. Heterozygotes excrete normal amounts of amino acids in the urine, but tests of intestinal absorption in heterozygotes have been inconclusive. The incidence in newborns screened for aminoaciduria has varied from 1 in 18000 in Massachusetts to 1 in 200000 in Australia; the mean number of these studies is 1 in 24000 [28, 29]. The gene responsible for the defect and the transporter have not been characterized.

Lysinuric Protein Intolerance

Clinical Presentation

Infants with lysinuric protein intolerance (LPI) are symptom free if breast-fed but may develop postprandial episodes of *hyperammonemia* after weaning when the protein content of the diet increases [30, 31]. Hyperammonemia may cause eating difficulties, vomiting, stupor, coma, and finally death (caveat: forced tube feeding). Strong aversion to high-protein foods usually develops around the age of 1 year. The infant fails to thrive. The liver and spleen are moderately enlarged.

In toddlers and school-aged children the presenting signs have most often been growth failure and *hepato- and splenomegaly*. Because of severe osteoporosis, the children often have fractures caused by minor trauma. The bone maturation is retarded [32, 33]. In childhood some patients have anemia and leukopenia and the platelet count may be decreased. The red cells show poikilocytosis, anisocytosis, and mild macrocytosis [34]. Serum ferritin concentration is constantly two to 20 times higher than the reference values and may exceed the normal range by 50- to 100-fold during severe infections [34]. The children are usually hypotonic and muscular endurance is decreased. The neurological development is normal if severe or prolonged hyperammonemia has been avoided.

The clinical heterogeneity of LPI is obvious in adults. Most patients are moderately stunted, but some are of normal height. They have abundant subcutaneous fat on a square-shaped trunk, but the extremities are thin. They may have marked hepatomegaly with or without splenomegaly. Two thirds of adults have *skeletal abnormalities*: osteoporosis, abnormal cortices of the long bones, scoliosis, vertebral end-plate deformities, early cartilage destruction, and metacarpal cortical thickening [33]. Pathologic fractures seldom occur in adults.

Radiological signs of pulmonary fibrosis are common, but only a few have had clinical signs of interstitial lung disease [35]. Mental capacity varies from high-normal to moderate retardation depending on the severity of the previous periods of hyperammonemia.

About 100 patients have been described or are known to us, 42 of them from Finland. The mean age of the latter is 24 years (range, 2–49 years). Eight patients have moderate and one severe mental retardation. The pregnancies have been complicated by toxemia and deliveries by bleeding problems. The Finnish patients have various complications such as hypothyreosis, systemic lupus erythematosus, arthritis, bronchiolitis obliterans, high blood pressure, and a history of stroke. Six have died, four as children. One adult died in an accident; the other adult had, in addition to LPI, signs of neuropathy and myopathy and died suddenly, probably because of cardiac arrythmia. The deaths in children have always occurred after a

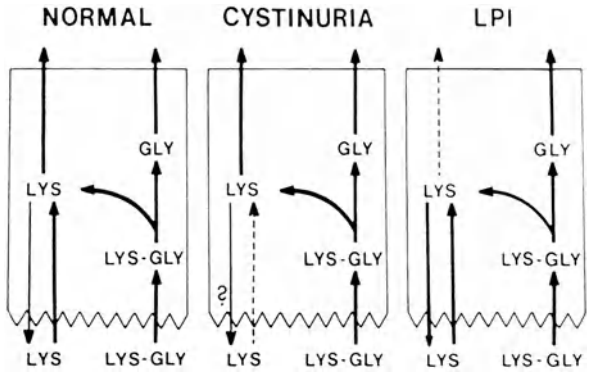

Fig. 2. Absorption of diamino acids (here lysine in free and dipeptide form) by brush-border cells of jejunal mucosa, and sites of defect in cystinuria and lysinuric protein intolerance (*LPI*). Decreased fluxes are shown by *dashed arrows*. (From [41], by permission of Lancet). *LYS*, lysine; *GLY*, glycine; *LYS-GLY*, lysyl-glycine

very similar course: the patients develop progressive multiple organ system failure; they have alveolar proteinosis, progressive glomerulonephritis which leads to renal insufficiency, and severe bleeding diathesis [36]. Patients in the USA, Israel, Italy, France, and Japan have shown similar complications [37, 38].

Metabolic Derangement

The transport of the cationic (dibasic) amino acids *lysine*, *arginine* and *ornithine* is imparied at the cell membrane. Massive amounts of lysine and smaller amounts of the other dibasic amino acids are excreted in the urine, whereas plasma concentration of the cationic amino acids are low or low-normal. Lysine is an essential amino acid, and arginine and ornithine are intermediates in the urea cycle. The symptoms and signs are secondary to the decreased availability of these three amino acids in the body. Cyst(e)ine transport is normal, although cystine concentration may be slightly increased in the urine.

In the normal intestine, free amino acids and short oligopeptides first cross the luminal membrane of the epithelial cells to reach the cytoplasm (Fig. 2). Oligopeptides are hydrolyzed to free amino acids during this passage and in the cytoplasm, and only free amino acids cross the antiluminal (basolateral) membrane of the cell. In LPI, the cationic amino acids pass poorly through the antiluminal membrane of the epithelial cells and reach the body in inadequate amounts [39–41]. Reclamation of the cationic amino acids by the kidney tubuli is also decreased, resulting in massive loss of lysine and moderate losses of arginine and ornithine in the urine [42]. The plasma membrane of cultured skin fibroblasts and apparently of other parenchymal cells functions like the antiluminal cell membrane of the epithelial cells and expresses the transport defect as a defect in efflux. More specifically, the trans-stimulated efflux of the cationic amino acids is impaired in the parenchymal cells [43]. The efflux defect leads to increased intracellular concentration of the cationic amino acids in epithelial and parenchymal cells.

Poor intestinal absorption and increased renal loss of the cationic amino acids lead to negative net balance in the body. In the liver cell, the function of the urea cycle is impaired, hyperammonemia develops, and clinical symptoms occur which resemble those of urea cycle enzyme deficiencies.

Diagnostic Tests

The diagnosis of LPI is based on the following:

- Urinary excretion of the cationic amino acids, especially of lysine, is increased. The excretion rates of the cationic amino acids have in a few instances been within the reference range when the dietary protein load has been extremely low, and plasma concentrations of the cationic amino acids have decreased to exceptionally low values. Cyst(e)ine excretion may be slightly increased as in type II or type III cystinuria heterozygotes. Glutamine, alanine, serine, proline, glycine, and citrulline are excreted in slight excess because their plasma concentrations in patients with LPI are slightly higher than in healthy subjects.
- Plasma lysine concentration is usually less than 80 μmol/l, arginine less than 40 μmol/l, and ornithine less than 30 μmol/l. Plasma glutamine, alanine, serine, proline, citrulline, and glycine are increased to values 1.5 to ten times the upper reference limits.
- Blood ammonia concentration is increased after protein meals or an i.v. L-alanine load (6.6 mmol/kg during 90 min as a 5% aqueous solution; samples at 0, 120, 270, and 360 min). The concentration of urea in serum rises slowly. Orotic aciduria occurs in the first 2-h collections of urine after the loads.

- The activity of lactate dehydrogenase and the concentration of ferritin and thyroxin-binding globulin in serum are increased.
- Skeletal radiographs often show marked osteoporosis, abnormal cortices of the long bones, scoliosis, and vertebral end-plate deformities. Some adults have early cartilage destruction and the second to fourth metacarpal bones may be short with abnormally thick cortices.

Treatment and Prognosis

The treatment of LPI aims at prevention of hyperammonemia and sufficient supply of protein and essential amino acids for normal metabolism and growth.

► The protein tolerance can be improved if citrulline, a neutral amino acid and intermediate in the urea cycle, is given as a daily supplement (0.1–0.5 g/kg before or during three to five meals) [44]. Citrulline is readily absorbed and converted in the cells partly to arginine and ornithine, and all three amino acids then reach the liver and improve the function of the urea cycle. Children usually tolerate 1.0–1.5 g/kg and adults 0.5–0.8 g/kg of protein daily without hyperammonemia or increases in orotic acid excretion if suppemented with citrulline. A patient's protein tolerance may vary markedly, and variations between patients are even larger. Infections, pregnancy, and lactation may alter tolerance extensively. Frequent blood ammonia measurements at home are necessary for optimization of the diet; our patients all use dry chemistry ammonia checkers for home monitoring of blood ammonia values.

Oral supplementation of lysine or lysine-containing oligopeptides does not correct lysine deficiency, because poor absorption of lysine leads to osmotic diarrhea. Experimentally, a neutral lysine analogue, ∈-*N*-acetyl-lysine, increased plasma lysine concentration, but homocitrulline, another potential lysine "supplier," had no influence [45]. Because of price and poor availability, ∈-*N*-acetyl-lysine has not been used in long-term supplementation. In one patient plasma lysine concentration increased during carnitine substitution; further clinical trials of the effects of carnitine are warranted [46].

► In acute hyperammonemic crisis all protein and nitrogen-containing substances should be removed from the nutrition and sufficient energy supplied as intravenous glucose. Infusion of ornithine, arginine, or citrulline i.v., beginning with a priming dose of 0.5–1.0 mmol/kg in 5–10 min and continuing with 0.5–1.0 mmol/kg per h, clears hyperammonemia rapidly. Sodium benzoate and sodium phenylacetate may also be used to stimulate alternate pathways (see Ogier and Saudubray, this volume) [47].

Hyperammonemia and mental retardation are avoidable with the current therapy. However, several of the features and complications of LPI are not caused by hyperammonemia but are most likely due to deficiency of the essential amino acid lysine.

Genetics

LPI is an autosomal recessive disease. The defective gene has not been localized or cloned, and the molecular background of the transport defect is unknown.

With an incidence of 1 in 60000 in 5 million inhabitants, Finland has a relatively rich LPI gene pool. Another is located close to Naples, Italy. Elsewhere the disease is rare, but patients of black and white American, French, Dutch, Irish, Norwegian, Swedish, Russian, Turkish, Maroccan, and Japanese origin have been described.

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Part XIV
Organelle Disorders:
Lysosomes, Golgi and Pre-Golgi Systems, Peroxisomes

Sphingolipids

P.G. Barth

CONTENTS

Sphingolipids are complex membrane lipids composed of one molecule of the C_{18} amino alcohol sphingosine, one molecule of a long-chain fatty acid attached to the C-2 amino group of sphingosine, and various polar head groups attached by a β-glycosidic linkage to the OH group at C-1 (Fig. 1). There are three classes of sphingolipids, all derivatives of ceramide:

- Cerebrosides
- Sphingomyelins
- Gangliosides

Cerebrosides have a single sugar, either glucose or galactose, and an additional sulfate group on galactose in the sulfatides. Sphingomyelins contain phophorylcholine or phosphorylethanolamine. Gangliosides, the most complex sphingolipids, contain several sugar units and one (monosialogangliosides, G_M) or more (disialogangliosides, G_D etc.) sialic acid residues (*N*-acetyl neuraminic acid, NANA). Two monosialogangliosides, G_{M1} and G_{M2}, are primarily involved in lysosomal storage.

The catabolism of the sphingolipids requires lysosomal hydrolases. Their deficiency results in sphingolipidoses, which are classified according the compound stored (Table 1).

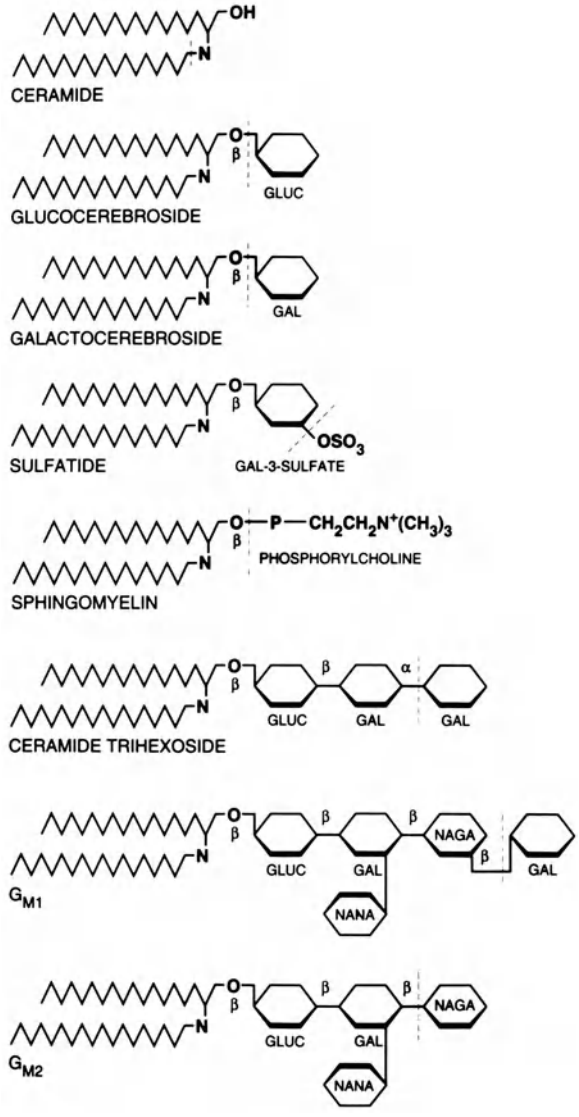

Fig. 1. Structure of the sphingolipids which accumulate in the sphingolipidoses. The uppermost formula belongs to ceramide, which is composed of the C_{18} amino alcohol sphingosine (*top of the ceramide figure*). A long-chain fatty acid is attached to the amino group at C-2 (*bottom of the ceramide figure*). All sphingolipids are derived from ceramide and distinguished by their different polar groups at C-1. *NANA*, *N*-acetyl neuraminic acid; G_M, monosialoganglioside; *NAGA*, *N*-acetyl galactosamine

Table 1. Classification of sphingolipidoses according to the compound stored

	Enzyme deficiency	Gene localization[a]	Assay
Niemann-Pick A,B	Sphingomyelinase	17	L, F, A, C
Niemann-Pick C	Defect in intracellular cholesterol transport		F, C
Gaucher type I, II, III	Glucocerebrosidase	1q21	L, F, A, C
Fabry	Ceramide trihexosidase	Xq21.3–q22	S, L, F, A, C
Farber	Ceramidase		L, F, A
G_{M1} gangliosidosis	β-Galactosidase	3pter–3p21	L, F, A
G_{M2} gangliosidosis B variant (Tay-Sachs)	β-Hexosaminidase A	15q23–q24	L, F, A, C
G_{M2} gangliosidosis O variant (Sandhoff)	β-Hexosaminidase A, B	5q13	L, F, A, C
G_{M2} gangliosidosis AB variant	G_{M2} activator protein	5	F
G_{M2} gangliosidosis B1 variant	β-Hexosaminidase A	15q23–q24	L, F, A, C
Metachromatic leukodystrophy (sulfatidosis)	Sulfatidase	14	L, F, A, C
Globoid cell leukodystrophy (Krabbe)	Galactocerebrosidase	14q24.3–q32.1	S, L, F, A, C

[a] Rosenberg [34].
S, serum; L, leukocytes; F, fibroblasts; C, chorionic villus; A, aminocytes.

Clinical Presentation

Niemann-Pick Disease. Clinically widely different disorders with storage of sphingomyelin were originally grouped together under the eponym Niemann-Pick disease. Originally types A, B, C, and D were defined [1]. However, only types A and B are deficient in sphingomyelinase (enzyme activity 10% or less). Types C and D are due to impaired intracellular cholesterol transport with concomitant storage of sphingomyelin, but with normal or slightly decreased sphingomyelinase. In a recent classification the former types A and B were regrouped as type I, while types C and D were regrouped as type II [2].

Type IA (acute, sphingomyelinase deficient) is the infantile type. Onset is insiduous with feeding difficulties and dystrophy in the first months of life. Respiratory infections are common, and X-ray examination of the thorax often shows a mottled pattern, resembling miliary tuberculosis. Widening of the medullary cavities and cortical thinning are seen in long bones. Hepatomegaly is constant and predominates over splenomegaly in contrast with Gaucher's disease. Concomitant with visceral and skeletal signs neurological deterioration takes place, with loss of visual contact and hearing. In about half of the cases a cherry-red spot is seen in the macula region. Foam cells (Niemann-Pick cells) in the bone marrow are often present, as are vacuolated lymphocytes or monocytes in peripheral blood. In the brain and spinal cord neuronal storage is widespread, leading to cytoplasmic swelling together with atrophy of the cerebellum. The progressive course leads to death in early childhood.

Type IS (subacute, sphingomyelinase deficient) has an early onset with hepatosplenomegaly and signs of bone marrow involvement similar to type IA. Neurological deterioriation develops more gradually.

Type IC (chronic, sphingomyelinase deficient) presents largely with asymptomatic hepatosplenomegaly, which may be discovered by accident in an otherwise normal-appearing person. Splenic rupture, liver cirrhosis, and occasionally cerebellar ataxia may be present.

Type II (sphingomyelinase normal, defective intracellular cholesterol transport) includes the former Crocker type C and probably type D (Nova Scotia type). Niemann-Pick type II causes neonatal conjugated hyperbilirubinemia in about half the cases. This symptom usually resolves spontaneously, to be followed by neurological symptoms later in childhood. In some cases, however, early liver involvement in type II has a rapidly fatal course and is often misdiagnosed as fetal hepatitis. Age at onset of neurological deterioration in Niemann-Pick type II may be throughout childhood. In the severe infantile form of type II hepatosplenomegaly is usual, accompanied by hypotonia and mental regression by the age of 1–1.5 years. This is often followed by spasticity. Supranuclear palsy and epileptic seizures are rare. In the juvenile form of type II early onset supranuclear upward gaze paralysis is a characteristic symptom. Hepatosplenomegaly may be mild or absent. Dementia, cerebellar ataxia, epileptic seizures, and in some cases dystonia are found. Dystonia appearing in childhood should alert the clinician to the possibility of Niemann-Pick disease type II. Death usually occurs in the second

decade. Clinical staging has led to the delineation of two subgroups, characterized as preschool onset and school-age onset types with a higher mortality in the former [3].

Gaucher's Disease. Gaucher's disease (all types) results from storage of glucocerebroside in visceral organs; the brain is affected in two of three types [4].

Type 1 is most common in the Ashkenazi Jewish population. It causes hepatosplenomegaly, anemia, thrombocytopenia, occasionally leukopenia, and skeletal changes. The bleeding tendency causes epistaxis and bleeding gums. Subcapsular splenic infarctions may cause attacks of acute abdominal pain. Bleeding tendency may be enhanced by deficiency of liver-dependent clotting factors. Frank cirrhosis is unusual. The lungs may be involved by diffuse infiltration, requiring treatment with oxygen. Skeletal involvement causes the typical Erlemeyer deformity of the femur. This is seen only in a minority of patients. Aseptic necrosis of the femoral head is more common. Medullary infarction of long bones may cause severely painful crises that have to be treated by powerful analgesics. Osteopenia may cause spontaneous fractures and involves long bones as well as the vertebral column. The central nervous system (CNS) is not clinically affected by storage. However, spontaneous fractures and coagulopathy may cause neurological damage.

Type 2 causes hepatosplenomegaly and severe CNS involvement in infancy, with death in early childhood. Hepatosplenomegaly appears in the course of the first 6 months. CNS involvement causes spastic quadriplegia. Characteristic for Gaucher's disease is involvement of the bulbar motor centers. It causes regurgitation and aspiration of food. Convergent squinting and horizontal gaze palsy are also characteristic. Early death before the first birthday is usual.

Type 3 has been subdivided in types 3a and 3b. Type 3a causes slowly progressive neurological disease and hepatosplenomegaly. In this type early horizontal gaze paralysis is usual. (Notice that in Niemann-Pick type II vertical gaze palsy is seen.) Type 3b causes splenomegaly and hepatomegaly which may lead to esophageal varices.

Fabry's Disease. Fabry's disease results from storage of glycolipids, mainly ceramidetrihexoside (globotriaosylceramide, Gb3), in endothelial, perithelial, and smooth muscle cells of blood vessels, cardiac myocytes, the autonomic spinal ganglia, glomerular endothelium, and epithelial cells of glomeruli and tubuli of the kidney. The disorder is X-linked. Its typical presentation is in a young male during puberty or adolescence, with crises of severe pain in the extremities (acroparesthesia) provoked by exertion or temperature changes. Unexplained bouts of fever and hypohidrosis occur. Characteristic skin lesions, called *angiokeratoma*, consist of clusters of small dark-red angiectases, on the lower part of the abdomen, buttocks, and scrotum. Although angiokeratoma may be found in some other lysosomal storage disorders, such as fucosidosis and galactosialidosis, its finding in a mentally normal young male or female who complains of acroparesthesia or has renal or cardiovascular disease is most helpful in the diagnostic workup. Other symptoms that may be encountered alone or in combination are single or recurrent stroke [5], arthralgia, priapism, hypertrophic cardiomyopathy, diarrhea, weight loss, and disturbed temperature sensation, especially for cold, in the extremities. In the absence of a positive family history, the diagnosis may be easily missed. Because of the random inactivation of the x-chromosome, female carriers of the gene may express the deficiency to the extent of clinical symptoms of the disease, especially acroparesthesiae and angiokeratoma.

Farber's Lipogranulomatosis. This disease results mainly from storage of ceramide in various organs. It presents as an early-onset disease with joint swelling, swellings over bone prominences, and hoarseness due to laryngeal involvement and causes early death in most cases. There is variable involvement of the brain due to storage of ceramide and gangliosides. Lymph nodes, heart, and lungs may be affected. Most patients die before their first year. However, a later-onset type has been recognized with progressive neurological involvement. Six types have been delineated [6].

Gangliosidoses. Ganglioside storage diseases comprise two main groups: G_{M1} and G_{M2} gangliosidoses. In all gangliosidoses storage in neurons causes displacement of the nucleus towards the periphery and a characteristic swelling of the proximal part of the axon, the so-called meganeurite. The storage material is contained in lysosomes that take the aspect of concentric

lamellated bodies or membranous cytoplasmic bodies (MCB).

G_{M1} Gangliosidoses. There are three main types. An infantile type, a juvenile type and an adult or chronic type. *Infantile G_{M1} gangliosidosis* or Landing's disease [7, 8] causes delayed head control from birth. Babies learn to make eye pursuit movements and usually learn to grasp, although insufficiently. Most develop hepatosplenomegaly, a puffy face, wide upper lip, maxillary hyperplasia, hypertrophied gums, large, low-set ears, and moderate macroglossia. The wrists are thickened. At the age of half a year striking Hurler-like bone changes are seen with vertebral beaking in the thoracolumbar zone, broadening of the shafts of the long bones with distal tapering, and widening of the metacarpal shafts with proximal pinching of the four lateral metacarpals. About half have a cherry-red spot in the macular region similar to Tay-Sachs disease (see below). Rapid neurological regression is usual after the first year of life, with generalized seizures, swallowing disorder, decerebrate posturing, and death usually before the second birthday. Beside the infantile type a severe neonatal onset type has been described with cardiomyopathy [9].

Juvenile G_{M1} gangliosidosis has no external distinguishing features. Patients are normal until 1 year of age, then lose manipulative skills and become dull with autistiform behavior. Ataxia, epilepsy, and spastic pareses develop progressively after the first year. *Adult G_{M1} gangliosidosis* has its onset during childhood, mainly as an extrapyramidal disorder or atypical as spinocerebellar ataxia. There are no external distinguishing symptoms, and skeletal changes are minimal or absent.

G_{M2} Gangliosidoses. There are four biochemical variants known as *variant B* or *Tay-Sachs disease*, *variant O* or *Sandhoff's disease*, *variant AB* or *activator protein deficiency* and *variant B_1*, with altered substrate specificity to artificial substrate. Tay-Sachs disease and Sandhoff's disease are largely similar in neurological expression. Tay-Sachs disease is mostly, although not exclusively, seen in Ashkenazi Jews. Sandhoff's disease appears to be ubiquitous. Hepatosplenomegaly or storage macrophages are sometimes found in Sandhoff's disease, but not in Tay-Sachs disease. The infantile type of both disorders becomes manifest during the first 6 months with exaggerated response to loud noises [10]. Some functions such as manual reaching and even sitting may be achieved, but are subsequently lost before the first birthday. Loss of visual attentiveness with roving eye movements is also seen early. A typical cherry-red spot in the macula region is seen on fundoscopy in all patients with Tay-Sachs or Sandhoff disease. After the first year spasticity, disordered swallowing, and seizures develop, the latter usually amenable to standard treatment. Typically, progressive macrocephaly appears during the second year and is due to swelling of cerebral white matter. Death, often due to aspiration pneumonia, is between 2 and 4 years of age. Later-onset G_{M2} gangliosidoses are mainly due to deficiency of hexosaminidase A. In juvenile G_{M2} gangliosidosis due to hexosaminidase A deficiency, onset of ataxia and dementia is during the second year or later. Cherry-red spots are only seen in a minority. An adult type of the disease also exists. Presentations of the adult type include psychosis [11, 12], dystonia [13], and a syndrome of supranuclear ophthalmoplegia, cerebellar ataxia, and spinal anterior horn disease [14]. Adult-onset hexosaminidase AB deficiency may present as spinocerebellar degeneration [15]. The B_1 variant may be similar in its presentation to classic Tay-Sachs disease or may present as a juvenile progressive neurodegenerative disorder. The cherry-red spot, characteristic of the infantile-onset forms, may appear late in the late-onset forms [16, 17]. The G_{M2} activator protein deficiency may also take the form of classical Tay-Sachs disease or present at a later age [18].

Metachromatic Leukodystrophy. Storage of sulfatide results in metachromatic leukodystrophy (MLD) or sulfatidosis. The storage entails both central and peripheral demyelination. The typical presentation is in an infant about to walk unsupported who starts with weak valgus feet. At this stage tendon reflexes may be absent due to concomitant peripheral neuropathy. Walking then rapidly becomes more difficult and in the course of the second year impossible. The next stage is dementia and spastic quadriplegia with retained ability to perform some voluntary acts, followed by a vegetative stage and death, usually between 3 and 6 years of age.

A detailed description by Hagberg [19] is based on a large personal experience. Storage products may lead to radiculopathy with severe pains that require analgesic treatment. Peripheral nerve conduction is strongly delayed due to demyelination,

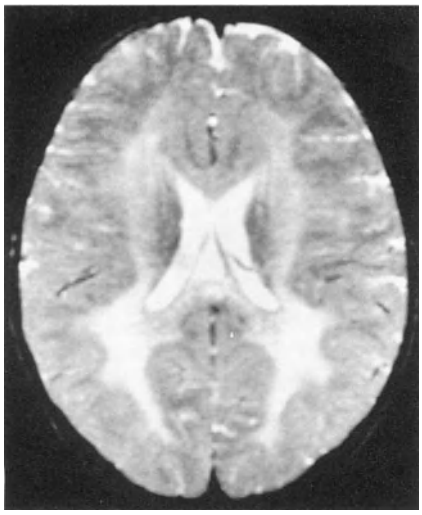

Fig. 2. Magnetic resonance imaging (MRI), axial T2-weighted section, from a patient with infantile metachromatic leukodystrophy. Increased signal in central white matter with subcortical sparing. Abnormalities predominate in the posterior halves of the hemispheres

and cerebrospinal fluid (CSF) protein content is elevated. Computed tomography (CT), or preferably magnetic resonance imaging (MRI; Fig. 2), shows central demyelination and often cerebellar atrophy.

Juvenile MLD starts at school age, usually between 6 and 8 years. Patients may be "dull" before deterioration sets in. Initial symptoms may be ataxia, spasticity, and absent or low tendon reflexes together with positive Babinski signs. Spastic quadriplegia and dementia will ensue within a few years after onset. Dystonic features may occasionally prevail. In adult MLD, psychiatric symptoms are often the presenting symptom.

Krabbe's Disease. Krabbe's disease or globoid cell leukodystrophy is caused by storage of galactocerebroside and, like MLD, causes both central and peripheral demyelination. The denomination "globoid cell leukodystrophy" is derived from the presence of large numbers of multinuclear macrophages in cerebral white matter.

The disease usually starts within the first 3–6 months with irritable behavior, crying, tonic spasms upon light or noise stimulation, and symptoms of early-onset cerebral palsy, together with blindness, optic atrophy, and deafness. Periods of unexplained fever may occur. This stage is followed by permanent optisthotonic posturing with flexed upper extremities and extended lower extremities. Hyperpyrexia, hypersalivation, frequent seizures, and loss of all social contact is seen at this stage. This is followed by loss of bulbar functions and hypotonia and ultimately death from hyperpyrexia, respiratory complications, or aspiration at a median age of 13 months [20]. CSF protein is elevated and nerve conduction velocities are delayed, because of coincident segmental demyelination of peripheral nerve [21]. Later onset is described in patients whose disease starts between 6 months and 3 years or even later during childhood. In the late-infantile form, presentation may be failing vision, irritablity, motor deterioraton, ataxia, and stiffness. In the late-childhood form, failing vision appears to be most common, but, surprisingly, hemiparesis may be the first symptom [22]. While peripheral neuropathy is always seen in the early infantile form, normal peripheral nerve findings in the late childhood and adult forms, together with a hemiplegic presentation, may cause diagnostic delay. CT scanning or preferably MRI will display cerebral white matter degeneration. Occasionally calcifications are seen in basal ganglia, thalamus, or white matter [23].

Metabolic Derangement

Niemann-Pick Disease. Types IA and IS (former types A and B) are due to deficiency of lysosomal *sphingomyelinase*, which catalyzes the breakdown of sphingomyelin to ceramide and phosphorylcholine. Residual activity is higher in the chronic type than in the acute type. Mutation analysis indicates the same deficiency in types IA and IS, the type of mutation being responsible for residual enzyme activity [24]. The basic defect in Niemann-Pick type II is in the intracellular transport of exogenous low-density lipoprotein (LDL) derived cholesterol, leading to impaired esterification and trapping of unesterified cholesterol in lysosomes. Therefore, Niemann-Pick disease type II is not a sphingolipidosis in the strict sense.

Sphingomyelinase activity is normal or elevated in most tissues, but partially deficient (60%–70%) in fibroblasts from the majority of patients by conventional methods. Indeed, storage of sphingomyelin in tissues is much less than in Niemann-Pick types IA and IS and is accompanied by additional storage of unesterified cholesterol, and bis(monacylglycero)phosphate.

Gaucher's Disease. All types of Gaucher's disease are caused by deficiency of the lysosomal enzyme *glucocerebrosidase*, which splits glucose from cerebroside, yielding ceramide and glucose. About 36 mutations have been identified in the same gene, but the three most prevalent mutations together are responsible for 80% of the patients [25]. A minority of the patients with Gaucher's disease have a deficiency of saposin C, a cohydrolase required by glucocerebrosidase.

Fabry's Disease. Fabry's disease is caused by the deficiency of the lysosomal enzyme *α-galactosidase A*, which splits the terminal α-galactoside binding, releasing galactose from ceramide trihexoside (globotriaosylceramide). Five point mutations in one single gene have each been associated with Fabry's disease [26].

Farber's Lipogranulomatosis. Farber's lipogranulamotosis is caused by deficiency of the lysosomal enzyme *ceramidase*.

G_{M1} Gangliosidoses. All G_{M1} gangliosidoses are caused by the deficiency of the lysosomal enzyme *β-galactosidase*. The structural mutation affects the 85-kDa precursor so that it is secreted instead of being compartmentalized to the lysosome [27].

G_{M2} Gangliosidoses. All G_{M2} gangliosidoses are due to deficient cleavage of a terminal β-hexoside bond on G_{M2} ganglioside by *β-hexosaminidase*. β-Hexosaminidase A (Hex A) and β-hexosaminidase B (Hex B) are isoenzymes with overlapping specificities. Both enzymes are constituted from two polypeptide subunits. Subunit composition is $\alpha\beta$ in the case of Hex A and $\beta\beta$ in the case of Hex B. Four G_{M2} gangliosidoses have been defined biochemically:

- Variant B (Tay-Sachs disease), Hex A deficient, is caused by impaired synthesis of subunit α.
- Variant O (Sandhoff's disease), Hex A and B deficient, is caused by impaired synthesis of subunit β.
- Variant AB is caused by a defect in the activator protein while both β-hexosaminidases are structurally present; β-hexosaminidase activity is deficient due to deficiency of the activator protein.
- Variant B_1, Hex A variant with impaired affinity to the natural substrate, has normal affinity to the artificial substrate 4-methyl-umbelliferyl-β-*N*-acetylglucosaminide and diminished activity against the sulfated artificial substrate.

Metachromatic Leukodystrophy. The lysosomal enzyme *cerebroside sulfatase* has two portions, of which arylsulfatase A is the heat-labile portion. Adequate function requires the presence of a heat-stable activator protein, sphingolipid activator protein or SAP-1. Therefore, MLD can be caused by either of two deficiencies: arylsulfatase A or SAP-1. The common cause is deficiency of arylsulfatase A. SAP-1 deficiency [28] appears to be very rare, but this may be due in part to the difficult diagnosis. The deposition of sulfatide in central and peripheral nerve tissue in MLD results in staining properties which lent the disease its original name. The metachromasy of sulfatide masses in MLD tissues, seen after staining with aniline dyes such as cresyl violet, marks a break with the past, when diagnostic confirmation was entirely by neuropathological means. A large number of allelic mutations may cause arylsulfatase A-deficient MLD, with age of onset related to residual activity. Infantile, juvenile, and adult types exist. A double homozygous mutation in the arylsulfatase A gene is associated with low arylsulfatase A activity against the artificial substrate *p*-nitrocatechol sulfate, but its activity against the natural substrate is sufficient to prevent clinical MLD [29]. The gene is known as pseudodeficiency (PD) gene. It occurs in MLD heterozygotes (MLD/PD) or in PD homozygotes (PD/PD) [30]. In both conditions the *p*-nitrocatechol sulfate-based arylsulfatase A assay will demonstrate decreased activity that may be in the homozygote MLD range. The impaired turnover of radiolabeled sulfatide in fibroblasts distinguishes true MLD from the PD state. This test will also detect SAP-1 deficiency [31]. This auxiliary test is also particularly important in prenatal diagnosis.

Diagnostic Tests

Oligosaccharide excretion patterns in the urine are abnormal in G_{M1} gangliosidosis and in the O variant of G_{M2} gangliosidosis (Sandhoff's disease). Mucopolysaccharide excretion is increased in G_{M1} gangliosidosis (keratan sulfate) and in multiple sulfatase deficiency (which includes arylsulfatases A, B, and C, steroid sulfatase, and diverse sulfatases involved in mucopolysaccharide catabolism). The storage of sulfatide in MLD can be

demonstrated by its excretion in urine, its presence as metachromasy in peripheral nerve biopsies, or the presence of typical inclusions in peripheral nerve biopsies on electronmicroscopy. The demonstration of storage products in this way may aid in distinguishing true MLD, including SAP-1 deficiency, from the PD state in symptomatic patients. MLD causes demyelinating polyneuropathy which can be demonstrated by delayed conduction velocities in peripheral nerves. Delayed peripheral nerve conduction is also an important symptom in Krabbe's disease. All sphingolipidoses cause storage in extracerebral tissues. Routine morphological examination of peripheral blood may reveal vacuolated lymphocytes in G_{M1} gangliosidosis and in Niemann-Pick disease type I. Examination of the bone marrow may reveal storage macrophages in Gaucher's disease and in Niemann-Pick disease types I and II. Small skin biopsies or conjunctival biopsies prepared for electron microscopy can demonstrate specific storage within lysosomes, which may help in doubtful cases [16, 32]. Enzymatic tests making use of artificial substrates are available for all sphingolipidoses, except those caused by deficient activator proteins. (The problem of PD in MLD is addressed under "Metabolic Derangement.")

Treatment and Prognosis

Treatment now exists in the form of enzyme replacement in the case of ceredase in Gaucher's disease, types II and III [4], with hopeful prospects. Splenectomy in Gaucher's disease may help in correcting thrombocytopenia and anemia, but enhances the risk of serious infections and may accelerate the progression of the disease at other sites. This may also apply to Niemann-Pick type IC, where huge splenomegaly can cause serious problems.

In the case of metachromatic leukodystrophy, bone marrow transplantation has been attempted in early stages, but its efficacy has yet to be settled.

In Fabry's disease involvement of the cardiovascular system, including cerebral and renal vessels, is the cause of limited life expectancy, which used to be 40 years or less. Dialysis and renal transplantation are the main therapeutic instruments to have improved the outlook.

The alleviation of pain is important in Fabry's disease (crises), chronic Gaucher's disease (bone pain), and in MLD and Krabbe's disease (radiculopathy and spasms). In the case of MLD and Krabbe's disease, part of the problem may be caused by a secondary deficiency of gamma aminobutyric acid (GABA). Administration of the glutamic acid transaminase inhibitor vigabatrine, better known as an antiepileptic drug, has been of particular use in a small number of patients with these leukodystrophies [33].

Genetics

Genetic patterns of all the known sphingolipidoses are autosomal recessive, with the exception of Fabry's disease, which is X-linked. Chromosomal mapping of sphingolipidoses is indicated in Table 1. Mutation analysis has become an instrument to identify heterozygotes in a susceptible population, e.g., communities of Ashkenazi Jews who have an increased risk of Gaucher's disease type 1 or Tay-Sachs disease.

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Mucopolysaccharides and Oligosaccharides

M. Beck and J. Spranger

CONTENTS

Mucopolysaccharides and oligosaccharides are essential constituents of the organism. They occur in connective tissue, parenchymal organs, cartilage and the nervous system. Mucopolysaccharides (for which the preferred term is glycosaminoglycans) are complex heterosaccharides consisting of long sugar chains rich in sulphate groups. The polymeric chains are bound to specific proteins (core proteins). In glycoproteins, oligosaccharide chains are attached to a peptide core via a *N*-glycosidic or *O*-glycosidic linkage. There is extensive diversity in the composition and structure of oligosaccharides.

The degradation of mucopolysaccharides and glycoproteins takes place inside the lysosomes. Genetic defects of enzymes that are involved in the intralysosomal degradation of these macromolecules lead to chronic and progressive storage disorders that share many clinical features such as organomegaly, dysostosis multiplex and abnormal facies. The diseases show a broad range of clinical manifestations ranging from lethal foetal hydrops to an almost normal phenotype and life expectancy.

Clinical Presentation

Mucopolysaccharidoses (Table 1)**.** Deficiency of the lysosomal enzyme α-L-iduronidase is the cause of *mucopolysaccharidosis type I* (MPS I). The spectrum of clinical phenotypes ranges from the severe form (Hurler disease) to the adult variant (Scheie disease). Hurler disease represents the prototype of all mucopolysaccharidoses: in the first year of life, a delay in motor and mental development is observed. Thereafter, the patient develops the full clinical picture with coarse facial features, macrocephaly, thick skin and corneal clouding (Fig. 1). Liver and spleen are enlarged. The bone dysplasia leads to growth retardation and severe deformities of the trunk and extremities (Fig. 2). The patients die within the first two decades. Patients with Scheie disease are of almost normal height and do not show mental retardation. Typical symptoms are stiff joints, corneal opacities, carpal tunnel syndrome and mild skeletal changes. In most cases the heart is involved in the storage process. Some patients with α-iduronidase (IDUA) deficiency exhibit symptoms that are intermediate between Hurler and Scheie disease. The different phenotypes are caused by allelic mutations of the IDUA gene and various states of double heterozygocity.

Hunter disease (MPS II) differs from all other known mucopolysaccharidoses by an X-linked mode of inheritance. It is caused by the deficiency of iduronate sulphatase. The clinical picture resembles that of Hurler disease, but corneal clouding is absent. There are Hunter patients who have only moderate or no mental retardation and a higher life expectancy (Hunter disease type B). The severe type A and the mild type B represent extremes of a broad phenotypic spectrum expressing numerous allelic mutations of the iduronate sulphatase (IDS) gene [1].

The cause of all four subtypes of *mucopolysaccharidosis type III* (Sanfilippo disease) is a disturbance in the intralysosomal degradation of heparan sulphate. The clinical appearance is dominated by central nervous symptoms, whereas the somatic features are moderate. After normal development in the first 3–4 years, patients with Sanfilippo disease lose acquired skills such as speech, normal gait, and toilet training. Later, aggressive behaviour and hyperactivity occur. The hair is coarse, often blond, and

Table 1. The genetic mucopolysaccharidoses

Disorder	Eponym	Defective enzyme	Gene localization	Assay
MPS I-H	Hurler	α-Iduronidase	4p16.3	L, F, AC, CV
MPS I-S	Scheie	α-Iduronidase	4p16.3	L, F, AC, CV
MPS I-H/S	Variants	α-Iduronidase	4p16.3	L, F, AC, CV
MPS II	Hunter	Iduronate-2-sulphatase	Xq28	S, F, AF, AC
MPS III A	Sanfilippo A	Sulphamidase	?	L, F, AC, CV
MPS III B	Sanfilippo B	α-*N*-Acetylglucosaminidase	?	S, F, AC, CV
MPS III C	Sanfilippo C	AcCoA: α-Glucosaminide-*N*-Ac-transferase	?	F, AC
MPS III D	Sanfilippo D	Glucosamine-6-sulphatase	12q14	F, AC
MPS IV A	Morquio A	Galactosamine-6-sulphatase	16q24	L, F, AC
MPS IV B	Morquio B	β-Galactosidase	3	L, F, AC, CV
MPS VI	Maroteaux-Lamy	*N*-Acetylgalactosamine-4-sulphatase	5q13	L, F, AC
MPS VII	Sly	β-Glucuronidase	7q21–q22	S, F, AC

MPS, Mucopolysaccharidosis; L, leucocytes; S, serum; F, cultured fibroblasts; AC, cultured amniotic cells; AF, amniotic fluid; CV, chorionic villi.

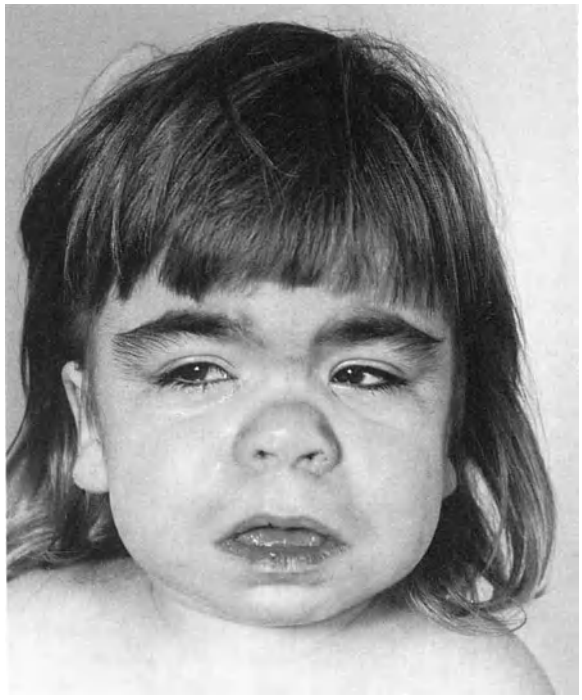

Fig. 1. Four-year-old girl with mucopolysaccharidosis (MPS) I (Hurler disease). Coarse facial features, depressed nasal bridge, macroglossia

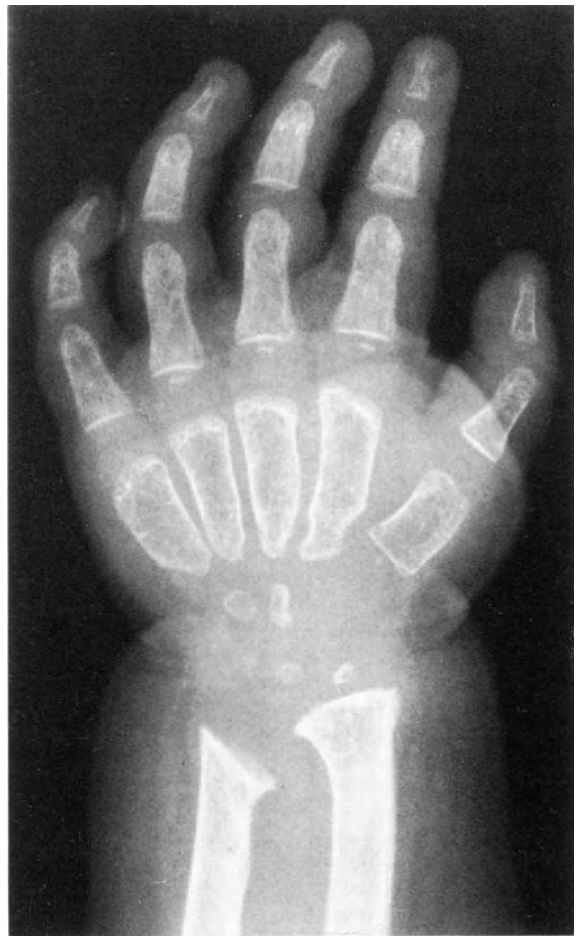

Fig. 2. Dysostosis multiplex (Hurler disease, 4-year-old patient). Short, deformed metacarpals and phalanges. Ulna and radius are slanted towards each other

hirsutim may be present. Bone changes are mild (Fig. 3). MPS III patients are of normal height, sometimes have seizures and become tetraspastic in the first or second decade of their life; they usually die from aspiration pneumonia.

Morquio disease (*mucopolysaccharidosis type IV*) is caused by a genetic defect in the degradation of keratan sulphate. Patients with Morquio disease type A (*N*-acetylgalactosamine-6-sulphatase deficiency) are characterized by disproportionate dwarfism, joint contractures, kyphoscoliosis and corneal clouding. In Morquio disease type B β-galactosidase activity is absent and the course is milder. An identical enzyme defect is responsible for G_{M1} gangliosidosis, a

Table 2. Glycoproteinoses

Disorder	Defective enzyme	Gene localization	Assay
α-Mannosidosis	α-Mannosidase	19p13.2–q12	S, L, F, AC,
β-Mannosidosis	β-Mannosidase	?	S, L, F, AC
α-Fucosidosis	α-Fucosidases	1p34–p36	S, L, F, AC
Sialidosis, type I	α-Neuraminidase	10pter–q23	F, AC
Sialidosis, type II	α-Neuraminidase	10pter–q23	F, AC
Sialidosis, congenital	α-Neuraminidase	10pter–q23	F, AC
Galactosialidosis	Protective protein	20	F, AC
Aspartylglucosaminuria	Aspartylglycosaminidase	4q33–q35	F, AC
Schindler disease	α-*N*-Ac-Galactosaminidase	22q13–qter	F
Free neuraminic storage disorder	Proton-driven carrier	?	F
Mucolipidosis II (I-cell disease)	*N*-Acetylglucosamine-1-phosphotransferase	?	S, F, AF, AC
Mucolipidosis III (pseudo-Hurler)	*N*-Acetylglucosamine-1-phosphotransferase	?	S, F, AF, AC

L, leucocytes; S, serum; F, cultured fibroblasts; AC, cultured amniotic cells; AF, amniotic fluid; CV, chorionic villi.

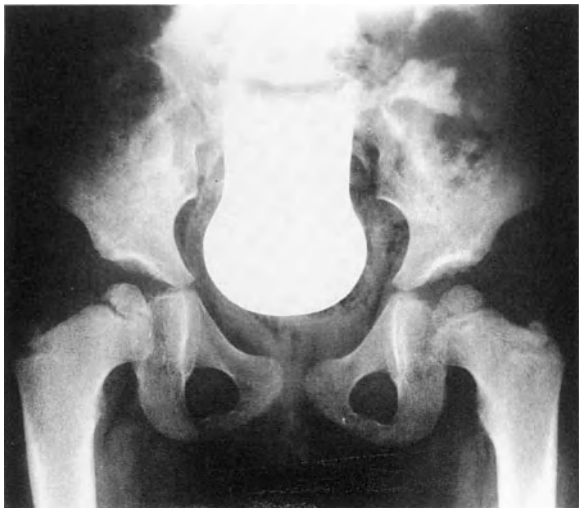

Fig. 3. Dysostosis multiplex (Sanfilippo disease, 4-year-old patient). Hypoplastic basilar portions of the iliac bones, small capital femoral epiphyses

neurodegenerative disorder with predominant involvement of the central nervous system. Patients with *mucopolysaccharidosis type VI* (Maroteaux-Lamy disease) have somatic features resembling those of Hurler patients, but without neurological impairment. Sly disease (*mucopolysaccharidosis type VII*) shows the broadest phenotypic spectrum of mucopolysaccharidoses ranging from lethal foetal hydrops to almost normal individuals. Most MPS VII patients exhibit symptoms almost identical to those of Hurler disease, such as coarse facial features, dysostosis multiplex and hepatosplenomegaly.

Glycoprotein Storage Disorders (Table 2). Glycoprotein storage disorders resemble clinically the

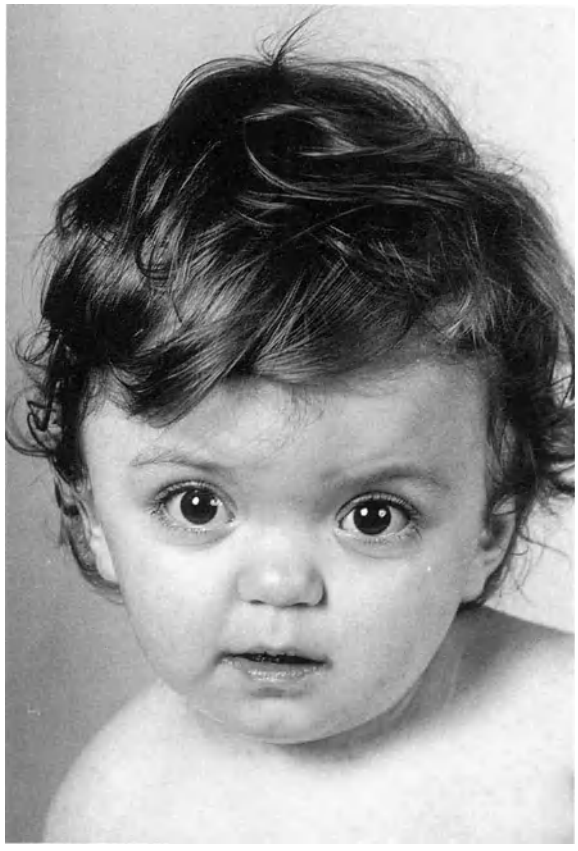

Fig. 4. Eighteen-month-old boy with α-mannosidosis. Slightly coarse facial features with flat nasal bridge and small nose

mucopolysaccharidoses, but urinary mucopolysaccharide excretion is normal. In *α-mannosidosis* mild skeletal deformities, coarse facial features and moderate to marked mental retardation are present (Fig. 4). Patients often are deaf and have an increased susceptibility to infections. Only a

few patients with *β-mannosidosis* have been reported. All of them showed a relatively mild course without major skeletal lesions or facial dysmorphism, but with prominent hearing loss [2]. Clinical symptoms of *fucosidosis* include progressive neurological degeneration and mental impairment, seizures, angiokeratoma and mild skeletal dysplasia. A wide, continuous spectrum of phenotypes reflects different mutations of the α-fucosidosis gene [3]. The clinical spectrum of *sialidosis* (neuraminidase deficiency) ranges from a congenital form with foetal hydrops to the comparatively mild cherry-red spot myoclonus syndrome with survival beyond 30 years.

In *galactosialidosis* two lysosomal enzymes (β-galactosidase and neuraminidase) are deficient due to a malfunction of a common protective protein with cathepsin-A like activity. Patients with the early-infantile form have symptoms similar to those with G_{M1} gangliosidosis and sialidosis; they are hypotonic from birth on, with facial oedema, coarse features and cherry-red retinal spots. In juvenile galactosialidosis, skeletal dysplasia, corneal opacities and mental retardation are the leading symptoms.

Aspartylglucosaminuria has a high prevalence in Finland and is rare in other countries. Clinical findings include progressive psychomotor retardation, coarse facial features and mild skeletal dysplasia.

Sialic storage diseases are characterized by abnormal accumulation of free (unbound) sialic acid in lysosomes of different tissues. The two main phenotypes are the "Finnish" Salla disease, presenting with mental retardation, ataxia and near-normal life span, and an infantile form without any ethnic prevalence which presents with severe visceral involvement, skeletal dysplasia, psychomotor retardation and early death.

Two original patients with α-*N*-acetylgalactosaminidase (α-NAGA) deficiency (Schindler disease) had progressive psychomotor deterioration, blindness and seizures [4]. Kanzaki [5] reported an adult female with α-NAGA deficiency who appeared mentally and physically normal except for disseminated angiokeratoma. Wevers [6] described two siblings with a α-NAGA defect; the girl presented with convulsions, whereas the younger brother was asymptomatic. Patients with *mucolipidosis II* (I-cell disease) resemble those with severe Hurler disease and rarely survive the first decade of life. Symptoms of *mucolipidosis III* are less severe, with variable dysostosis multiplex, growth retardation and joint stiffness. Mental retardation is moderate or may be absent.

Metabolic Derangement

Lysosomal Storage Diseases. These are caused by the deficient activity of one or more lysosomal enzymes (Tables 1, 2). This leads to the accumulation of the substrates (glycosaminoglycans or oligosaccharides) that are ordinarily degraded by the enzyme(s). The undegraded or partially degraded substances are stored in lysosomes and excreted in urine. The clinical phenotype partially depends on the type and amount of storage substance. For example, the accumulation of heparan sulphate, an essential component of the nervous cell membrane, results in progressive mental deterioration (e.g., MPS III, Sanfilippo disease), whereas in Morquio disease (MPS IV) a defect in keratan sulphate degradation causes severe skeletal deformities. Other symptoms such as organomegaly or coarse face are non-specific storage phenomena.

The primary defects in most of the lysosomal storage disorders are mutations of genes that encode single lysosomal enzymes. Multiple lysosomal enzyme deficiencies in mucolipidosis II and mucolipidosis III result from post-translational defects; after their synthesis in the rough endoplasmic reticulum, lysosomal enzymes are subjected to a series of post-translational modifications. Thus, *N*-acetylglucosamine-1-phosphotransferase transfers phosphate groups into oligosaccharide units of lysosomal enzyme precursors. In its absence the common phosphomannosyl recognition marker of acid hydrolases is not generated, and multiple enzymes are not targeted to the lysosomes. As a consequence the enzymes are secreted in the extracellular space, where high activities are found, while inside the cells the enzyme levels are considerably reduced. The combined neur-/β-gal- defect is caused by a lack of a 32-kDa glycoprotein ("protective protein") that is responsible for the stabilization of an α-neuraminidase/β-galactosidase complex. The protective protein is identical to a multifunctional protein that exhibits esterase/deamidase/carboxypeptidase activities [7].

Sialic Acid Storage Disorders. These are produced by a defect of a proton-driven carrier that is responsible for the efflux of sialic acid (and other

acidic monosaccharides) from the lysosomal compartment [8]. Because the carrier has a wide substrate specifity, storage of different compounds may be involved in the pathogenesis of these disorders. Sialic storage disorders must be distinguished from other forms of sialuria, in which lysosomal storage is not observed [9].

Diagnostic Tests

Because most patients with a storage disorder excrete increased amounts of oligosaccharides or mucopolysaccharides (glycosaminoglycans), analysis of urine for the presence of these substances is recommended as the first diagnostic step [10]. For the qualitative and quantitative measurement of glycosaminoglycans many methods have been described. Spot tests are quick and inexpensive, but may produce false-negative and false-positive results. In particular, MPS III and MPS IV are often missed. For quantitative measurement of urinary glycosaminoglycans several tests are available (e.g., determination of uronic acid based on the carbazol method or the spectrophotometric assay using the dye dimethylmethylene blue). One- or two-dimensional electrophoresis allows discrimination between classes of glycosaminoglycans (heparan, keratan and dermatan sulphate). To detect abnormal urinary excretion of oligosaccharides thin-layer chromatography is available. Free sialic acid is excreted in increased amounts in free sialic acid storage disorders and can be determined in urine by thin-layer chromatography or spectrophotometric assay [11].

All urinary tests that have been described for the diagnosis of lysosomal storage disorders can give false-negative results, especially in older patients with a mild clinical manifestation. Patients with mucolipidosis II and mucolipidosis III are usually missed. Therefore the definitive diagnosis has to be confirmed by enzymatic assay in serum, leucocytes and/or fibroblasts [12].

Treatment and Prognosis

Presently only palliative treatment is available, but supportive management can improve the patient's quality of life. Examples are the treatment of sleep disturbances and hyperactivity in MPS III by appropriate medication [13], corneal transplantation in MPS VI, cervical fusion to prevent atlanto-axial subluxation, especially in Morquio disease, release operation in carpal tunnel syndrome and shunt operation to relieve hydrocephalus.

Attempts to correct the metabolic defect by the administration of missing enzymes have failed. Bone marrow has been transplanted to more than 150 patients with various mucopolysaccharidoses [14]. After successful engraftment leucocyte enzyme activity normalized, organomegaly decreased and joint mobility increased. Skeletal abnormalities remained unchanged. Whether the brain function can be improved in patients with mental retardation remains questionable. Some patients maintained their learning capability or intelligence quotient, while others continued to deteriorate.

Gene transfer using retroviral vectors corrected the metabolic defect in human MPS VII fibroblasts [15]. Identical results have been obtained in fibroblasts of MPS I [16] and MPS VI [17]. Kyle [18] reported phenotypic correction of murine mucopolysaccharidosis VII by gene therapy.

Genetics

With the exception of mucopolysaccharidosis type II (Hunter disease) and Fabry disease (α-galactosidase deficiency), the lysosomal storage disorders are inherited in an autosomal recessive manner. The genes coding for most of the lysosomal enzymes have been localized (Tables 1, 2). Numerous allelic mutations have been detected explaining the clinical variability of these disorders. Recent findings in molecular genetics of mucopolysaccharidoses have been reviewed [19].

Gene analysis enables the reliable identification of carriers of Hunter disease [20]. Prenatal diagnosis is possible in all lysosomal storage disorders by measurement of enzyme activity in chorionic villi, cultured trophoblasts or cultured amniotic cells [21].

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Cystinosis

M. Broyer

CONTENTS

Cystinosis is a generalized lysosomal storage disease of unknown etiology. The lysosomal cystine accumulation leads to cellular dysfunction of many organs, the most serious being renal involvement. Three phenotypic forms are discerned: the nephropathic or infantile form, an intermediate or juvenile-onset form, and a benign or adult form. The infantile form is the most frequent and is the main subject of this chapter.

Infantile Cystinosis

Clinical Presentation

First Stage. Usually the first 3–6 months of life are symptom free. The first symptoms mostly develop before the age of 1 year [1]. They include anorexia, vomiting, polyuria, and failure to thrive. If the diagnosis is delayed, severe *rickets* develops after 10–18 months in spite of correct vitamin D supplementation. The diagnosis can be immediately suspected if both glucose and protein are found in the urine. When the disease has become symptomatic, the full expression of Fanconi's syndrome is generally present at first examination. It includes normoglycemic glycosuria, generalized aminoaciduria, tubular proteinuria (with massive excretion of β_2-microglobulin and lysozyme), phosphaturia with hypophosphatemia, and excessive loss of potassium and sodium bicarbonate leading to hypokalemia, hyponatremia, and acidosis. Hypercalciuria is also massive and hypouricemia is constantly found. Tubular loss of carnitine may cause carnitine depletion. A severe concentrating defect develops simultaneously, leading rapidly to a *polyuria* of 2–5 l/day. Urine of cystinotic patients is characteristic, being pale and cloudy with a peculiar odor, probably due to aminoaciduria.

The biological symptoms are related to a general reabsorptive defect of the proximal tubule. This may explain severe hydroelectrolyte imbalance, which may be life-threatening. Episodes of fever probably related to dehydration are also commonly noted. Lithiasis has been reported in rare cases, related to the high urinary excretion of urate, calcium, and organic acids. Blond hair and a fair complexion with difficulty of tanning after exposure to the sun are often noted in white cystinotic children.

Involvement of the eye is a primary symptom of cystinosis, starting with *photophobia*, which usually appears at 2–3 years of age and is more or less marked. Ophthalmological examination with a slit lamp and a biomicroscope reveals cystine crystal deposits. There are also fundus abnormalities with typical retinopathy and subsequent alteration of the retinogram.

End-Stage Renal Failure. The natural history of the disease includes severe stunting of growth and a progressive decrease of the glomerular filtration rate, leading to end-stage renal failure (ESRF) between 6 and 12 years of age. This evolution may be delayed by cysteamine treatment, especially when started in the first months of life. This treatment also improves growth velocity. During the course of renal deterioration the decrease in glomerular filtration is reflected by an improvement of urinary losses and a spurious regression of Fanconi's syndrome. At ESRF, severe renal hypertension may have developed. Repeated nasal bleeding is sometimes observed in cystinotic pa-

tients on dialysis [2]. After kidney transplantation there is no recurrence of Fanconi's syndrome, even if cystine crystals are seen in the graft, where they are carried inside macrophages or leukocytes. Tubular symptoms in grafted patients are in fact due to a rejection reaction.

Late Symptoms. The advent of renal replacement therapy and transplantation has uncovered the continued cystine accumulation in extrarenal organs and has emphasized the multisystemic nature of cystinosis, which may additionally involve the eyes, thyroid, liver, spleen, pancreas muscle, and central nervous system (CNS) [2, 3].

Late Ocular Complications. The severity of eye involvement differs from one patient to another [4, 5]. Corneal deposits accumulate progressively in the stroma of the cornea and iris in all patients and on the surface of the anterior lens and retina in some. Photobia, watering, and blepharospasm may become disabling; these symptoms are often associated with erosion of the corneal epithelium, leading eventually to keratopathy. Photophobia may improve after kidney grafting. Sight may be progressively reduced, leading to blindness in a few patients who already had major ocular symptoms at an early age.

Endocrine Disturbances. These include hypothyroidism and disturbances of the pituitary gonadal function and endocrine pancreas.

Hypothyroidism. Thyroid dysfunction usually appears between 8 and 12 years of age, but it may be earlier or later [6]. Some patients do not develop hypothyroidism even after 20 years of age. It is rarely overt with clinical symptoms, but rather discovered by systematic assessment of thyroid function [2, 6], and it may be partly responsible for the growth impairment. Pituitary resistance with a persistently high level of thyroid-stimulating hormone (TSH) has been reported in one series of patients [7], but not in another [2].

Pituitary Gonadal Function. Abnormalities in the pituitary testicular axis with a low plasma testosterone and high follicle-stimulating hormone/luteinizing hormone (FSH/LH) level [8] seem common in male patients with cystinosis. They may preclude full pubertal development being attained. Female patients seem to have more normal gonadal functions.

Endocrine Pancreas. Postoperative hyperglycemia and permanent insulin-dependent diabetes have been reported in several series of cystinotic patients after kidney transplantation [9]. Exocrine pancreas is not usually affected, except in one reported case with steatorrea [10].

Liver and Spleen Involvement. Hepatomegaly and splenomegaly occur in one third to one half of patients after 15 years of age [2]. Hepatomegaly is related to enlarged Kupffer's cells that transform into large foam cells containing cystine crystals. This enlargement may be the cause of portal hypertension with gastroesophageal varices bleeding. Splenomegaly is also related to the development of foam cells in the red pulp. Hematological symptoms of hypersplenism may be noted.

Muscle. A distinctive myopathy was reported in some patients with generalized muscle atrophy and weakness of both proximal and distal muscles of all limbs with more severe involvement of the interossei muscles and the muscles of thenar eminence [11].

Central Nervous System. Several kinds of neurologic complications have been reported in cystinosis. Convulsions may occur at any age, but it is difficult to evaluate the direct responsibility of cystinosis in this complication as opposed to uremia, electrolyte desequilibrium, drug toxicity etc. A subtle and specific visuoperceptual defect and lower cognitive performances with sometimes subtle impairment of visual memory were reported recently [12, 13]. More severe central nervous system (CNS) abnormalities with various defects have also been reported [2, 3, 14, 15]. The clinical symptoms include hypotonia, swallowing and speech difficulties, development of bilateral pyramidal signs and walking difficulties, cerebellar symptoms, and a progressive intellectual deterioration. In other cases acute ischemic episodes may occur with hemiplegia or aphasia. This cystinotic *encephalopathy* was only observed above 19 years of age, and at present it is not possible to state its actual incidence. The effectiveness of cysteamine treatment for the prevention of CNS involvement is not known either. Cysteamine treatment was associated in some cases with an improvement of neurologic symptoms. Brain imaging in cystinosis may show several types of abnormalities. Brain atrophy,

calcifications, and abnormal features of white matter on magnetic resonance imaging (MRI) examination are commonly observed after 15–20 years of age [16–18].

Metabolic Derangement

The primary defect which causes the lysosomal accumulation of cystine in many tissues including kidney, bone marrow, conjunctiva, thyroid, muscle, choroid plexus, brain parenchyma, and lymph nodes is not yet known. Extensive searches for an enzyme defect of cystine breakdown have remained negative. However, it has been shown that the movement of cystine out of cystinotic lysosomes was significantly decreased in comparison with that of normal lysosomes [19]. This abnormality is probably related to a molecular defect of the proteins involved as transport carriers across the lysosomal membrane [20]. Why lysosomal cystine accumulation leads to cellular dysfunction is not known. Recently it was shown that cystine loading of proximal tubular cells in vitro was associated with adenosine triphosphate (ATP) depletion [21]. This partial knowledge has led to the use of cysteamine, which is supposed to increase the transport of cystine out of the lysosome.

Diagnostic Tests

Cystinosis is ascertained by the assay of the free cystine content, usually in leukocytes, which in patients with nephropathic cystinosis is about 50–100 times the normal content [22]. The assay using a protein-binding technique on polymorphonuclear cells is very sensitive and enables even heterozygous carriers to be detected [23]. This assay may also be carried out on fibroblasts, conjunctiva, and muscle. S-Labeled cystine incorporation in fibroblasts cultured from the skin, amniotic cells, or chorionic villi enables a *prenatal diagnosis* in the first trimester to be made [24].

Treatment and Prognosis

The therapy of nephropathic cystinosis is both symptomatic and specific. The main purpose of symptomatic treatment is to replace the tubular losses due to Fanconi's syndrome. Several abnormalities have to be corrected:

- Water. Intake must be adjusted to diuresis, short-term weight variation, and if necessary the plasma protein concentration. Fluid requirement increases with external temperature and with fever. It is also increased by the required mineral supplements.
- Acid-base equilibrium. Sodium and potassium bicarbonate or citrate have to be given in order to obtain a plasma bicarbonate level between 21 and 24 mmol/l. This is sometimes difficult and may need large amounts of buffer of up to 10–15 mmol/kg.
- Sodium. Losses sometimes remain uncompensated after achieving acid-base equilibrium. This point is documented by a persistent hyponatremia with failure to thrive.
- Potassium. Hypokalemia requires potassium supplements in order to maintain serum potassium above 3 mmol/l. Four to 10 mmol/kg are usually necessary to achieve this goal. Prescription of amiloride at a dose of 2–5 mg/day may help in some cases.
- Phosphorus. Hypophosphatemia must also be corrected with a supplement of sodium/potassium phosphate at a dose of 0.5–2 g/day. The aim is to obtain a plasma phosphate just above 1.0–1.2 mmol/l. This supplement may be gradually withdrawn after a period of some months or years.
- Calcium and magnesium. These may also be prescribed in order to replace losses, while avoiding administrating the phosphate salt simultaneously. Calcium is given as calcium gluconate or carbonate (1–2 g/day) separately from phosphates and magnesium as the chloride salt.
- Vitamin D supplementation. 25-OHD3, which is lost in urine, has to be replaced (10–25 μg/day). Since tubular 1α-hydroxylation is diminished in this disease, it is also justified to give 1α- or 1α25-OHD3 (0.10–0.50 μg/day), especially in the case of symptomatic rickets. These prescriptions must be carefully adjusted by regular follow-up of serum calcium.
- Carnitine supplementation 100 mg/kg per day in four parts has been proposed in order to correct muscle carnitine depletion [25].

All these supplements have to be given regularly in order to replace the losses which are permanent. A good way to achieve this goal is to prepare

in advance all the supplements, except calcium, in a bottle containing the usual amount of water for the day. Calcium and magnesium may be given during the meals. Loss of water, potassium, and sodium may be drastically reduced by the prescription of *indomethacin* at a dose of 1.5–3 mg/kg in two separate doses [26]. When renal deterioration progresses and the glomerular filtration rate decreases, tubular losses also decrease and the mineral supplements must be adjusted and progressively tapered off in order to avoid overload, especially with sodium and potassium. At the dialysis stage, mineral supplements are no longer necessary.

Renal Replacement Therapy. There is no specific requirement for cystinotic children at this stage. Hemodialysis or CAPD/CCPD (continuous ambulatory/cyclic peritoneal dialysis) are both effective and applied according to the circumstances. As for any child with ESRF, kidney transplantation is considered the best approach. Results of kidney transplantation in the European Dialysis and Transplant Association (EDTA) pediatric registry were better than in any other primary renal disease in children [2, 27].

Symptomatic Treatment of Extrarenal Complications. Hypothyroidy has to be compensated by L-thyroxine supplementation, even if it is asymptomatic. Growth stunting, one of the most striking complications of nephropathic cystinosis, was reported to be improved by administration of recombinant growth hormone at a dose of 1 U/kg per day [29]. Portal hypertension may lead to ascites and bleeding esophageal varices, rendering a portal bypass necessary. Hypersplenism with permanent leukopenia and/or thrombopenia may be an indication for splenectomy. Regarding ophthalmological treatment, photophobia and watering may be improved by local symptomatic therapy such as artificial tears, topical lubricants, and thin-bandage soft contact lenses. It has been shown that eye drops containing 0.5% cysteamine were able to prevent corneal deposits [28] and may decrease the deposits already present. A corneal graft has occasionally been performed, with variable results.

Specific Therapy. Several attempts have been made to suppress lysosomal cystine storage, which is the basic lesion of cystinosis. Dietary restriction of sulfur amino acids has no effect; ascorbic acid, which is able to reduce cystine accumulation in vitro, is of no clinical value and even worsens the renal prognosis. Dithiotreitol was also ineffective. Only one drug, *cysteamine* ($HS\text{-}CH_2\text{-}NH_2$), has been employed in cystinosis with apparent benefit, as shown in a prospective study [30]. Nevertheless, the prescription of cysteamine raises some problems, since its odor and taste make its administration unattractive. It is also responsible for an unpleasant breath smell. Phosphocysteamine would have the same efficacy with a better odor and taste [31]. Cysteamine is an orphan drug currently available through hospital pharmacies as cysteamine chlorhydrate, either in capsule form or as powder ready for solution. The dose is progressively increased from 10 to 50 mg/kg per day. Cysteamine is rapidly absorbed and its maximum effect assessed by cystine assay in leukocytes occurs after 1–2 h; it generally lasts no longer than 5–8 h. Therefore, it has to be given in three or four separate doses (one every 6–8 h) in order to obtain the best prevention of cystine accumulation. Careful monitoring of polymorphonuclear cystine content is essential, since the response to cysteamine is variable. Leukocyte cystine content should be determined just prior to the next dose: the aim is to keep this content under 2 or better under 1 μmol half cystine per mg protein. The drug should be started as soon as the diagnosis is confirmed [32, 33]. The good results obtained on nephropathy suggest that cysteamine could also be given to patients who are at risk of developing extrarenal complications.

Adolescent Cystinosis

Adolescent cystinosis corresponds to a milder form of the disease with a later clinical onset and delayed evolution to ESRF [34]. The first symptom appears usually after 6–8 years of age. Proteinuria may be misleading because its severity is sometimes in the nephrotic range. Fanconi's syndrome is usually moderate, and tubular losses are less important than in infantile cystinosis. The same is true for extrarenal symptoms. ESRF usually develops around 15 years of age in most patients.

The diagnosis is ascertained by the assessment of the cystine content of leukocytes, which has been found to be similar to that of infantile cases.

Adult Benign Cystinosis

Adult or benign cystinosis was first reported by Cogan et al. in 1957 [35]. This autosomal recessive disorder is characterized by the presence of cystine crystals in the eye and the bone marrow. Crystals in the cornea are usually found by chance examination. The level of cystine in leukocytes is intermediate between that of heterozygotes and homozygotes of nephropathic cystinosis. The affected patients are asymptomatic.

Genetics

Nephropathic cystinosis is an autosomal recessive disorder. Adolescent and adult forms have the same mode of inheritance. Lack of complementation in somatic cell hybrids between fibroblasts from patients with nephropathic and benign cystinosis supports the hypothesis that the genes of these three diseases are alleles on the same locus [36]. However, adult and infantile forms have never been observed in the same family.

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Carbohydrate-Deficient Glycoprotein Syndromes

J. Jaeken

CONTENTS

The carbohydrate-deficient glycoprotein (CDG) syndromes or hypoglycosylation syndromes are genetic, multisystemic disorders characterized by deficiencies of the carbohydrate moiety of glycoproteins. The first patients were reported in 1980 [1], and as of January 1994 at least 130 patients, including some 20 adults, were known worldwide. Data on 71 patients have been published (see reviews in [2–4]). Three types or variants have been isolated; only two patients have been reported with type II and two with type III [5–7].

Clinical Presentation

Symptomatology is present very early, at least in type I; for those familiar with the disease it can be recognized shortly after birth. The nervous system is affected in all patients, and most other organs are involved in a variable way. The neurologic picture comprises alternating internal strabism and other abnormal eye movements, axial hypotonia, psychomotor retardation (IQ mostly between 40 and 60), ataxia, hyporeflexia and, after infancy, retinitis pigmentosa, often stroke-like episodes, and sometimes epilepsy. As a rule there is no regression. During the first year, there are feeding problems (anorexia, vomiting, diarrhea) resulting not infrequently in severe failure to thrive. Other features are a variable dysmorphy, in particular large ears and an abnormal adipose tissue distribution (fat pads, tallow), mild to moderate hepatomegaly, skeletal abnormalities, hypogonadism, and proteinuria. Some infants develop pericardial effusion and/or cardiomyopathy. The patients have often an extrovert and happy appearance. About 20% have died during the first years of life due to severe infection, liver failure, or cardiac insufficiency. Neurologic investigation reveals (olivoponto)cerebellar hypoplasia, variable cerebral hypoplasia, and peripheral neuropathy. Liver pathology is characterized by fibrosis, and electron microscopy shows myelin-like and granular lysosomal inclusions and abnormalities of the endoplasmic reticulum in hepatocytes.

The two patients with type II differ from type I patients in that they have no cerebellar hypoplasia, no peripheral neuropathy, and no lysosomal inclusions in the liver. In type III, special features are infantile spasms and pigmentary skin changes. Very recently we identified a patient with a CDG syndrome comprising neonatal cataracts and involvement of the spinal cord [8]. Possibly this represents a new variant.

Metabolic Derangement

Glycoproteins contain sugar molecules, attached covalently to an asparagine (N-linked) or a serine or threonine (O-linked) residue. Glycosylation occurs in the endoplasmic reticulum and the Golgi apparatus. Most glycoproteins are secreted from cells and include transport proteins, glycoprotein hormones, complement factors, enzymes, and enzyme inhibitors. Abnormalities of all these classes of glycoproteins have been identified in CDG syndromes (Table 1). CDG syndrome type II is due to a deficiency of the Golgi enzyme *N*-acetylglucosaminyltransferase II and has thus to be classified as a Golgi disease [9]. The basic defects of types I and III are still unclear; recent findings suggest that the endoplasmic reticulum is the site of the defect in type I [10].

Table 1. Serum glycoproteins reported to be abnormal[a] in carbohydrate-deficient glycoprotein (CDG) syndrome type I and/or type II

Transport proteins	Enzymes
Haptoglobin	Clotting factors and inhibitors
Ceruloplasmin	Factor IX
Transferrin	Factor XI
Ferritin	Factor XII
α_2-Macroglobulin	Antithrombin III
Transcobalamin II	Heparin cofactor II
Vitamin D-binding globulin	Protein C
Thyroxine-binding globulin	Protein S
Apoprotein B	Lysosomal
Apoprotein CII	Arylsulphatase A
Apoprotein E	*N*-Acetyl-β-hexosaminidase
Transcortin	α-Fucosidase
Sex hormone-binding globulin	β-Galactosidase
	β-Glucuronidase
Glycoprotein hormones	Other
Follicle-stimulating hormone (FSH)	Carboxypeptidase N
Luteinizing hormone (LH)	Cholinesterase
Prolactin	Glutamic oxaloacetic transaminase
Insulin-like growth factor-1 (IFG_1)	Glutamic pyruvic transaminase
	Enzyme inhibitors
Complement factors	α_1-Antitrypsin
Complement CH50	α_1-Antichymotrypsin
Complement C1 esterase inhibitor	Other glycoproteins
Complement C2	α_1-Acid glycoprotein
Complement C3a	α_1-B Glycoprotein
Complement C4A	Peptide PLS:29
	Peptide PLS:34

[a] Abnormal pattern (isoelectric focusing or high-resolution two-dimensional polyacrylamide gel electrophoresis) and/or increased/decreased concentration or enzymatic activity.

Diagnostic Tests

Although a number of serum glycoprotein abnormalities (see Table 1) are suggestive of a CDG syndrome, the diagnosis is usually made by isoelectric focusing and immunofixation of serum transferrin. Normal serum transferrin is mainly composed of tetrasialotransferrin besides small amounts of mono-, di-, tri-, penta- and hexasialotransferrins. The partial deficiency of sialic acid (a negatively charged sugar) in CDG syndromes causes a cathodal shift, resulting in a marked increase of both asialo- and disialotransferrin, and a pronounced decrease of tetra-, penta-, and hexasialotransferrins. These changes can be measured by densitometry. The *carbohydrate-deficient transferrin* (CDT) assay enables the quantification of the total sialic acid-deficient serum transferrin. It has to be noted that similar transferrin changes, though usually less pronounced, are found in chronic alcoholism (secondary CDG syndrome). Galactosemia has also recently been identified as a secondary CDG syndrome.

One of these tests should be performed in any unexplained neurologic syndrome, including psychomotor retardation when associated with various combinations of the above-mentioned clinical and biochemical abnormalities. These syndromes should be considered in neonatal olivopontocerebellar hypoplasia, in atypical presentations of leprechaunism, lipodystrophy, and Smith-Lemli-Opitz syndrome (after exclusion of a cholesterol synthesis defect [11]) as well as in unexplained nonimmunological hydrops fetalis. Due to their thyroxine-binding globulin (TBG) deficiency (low total T_4) as well as a slight but significant increase in thyroid-stimulating hormone (TSH), these patients can be detected through *neonatal screening for congenital hypothyroidism.*

Prenatal diagnosis is not yet possible; in the fetus with CDG syndrome type I, the tests used for the detection of the glycosylation defect show normal results.

Treatment and Prognosis

No specific treatment is known. Prognosis is described under "Clinical Presentation."

Genetics

Inheritance in CDG syndrome type I is generally considered to be autosomal recessive. Both sexes are equally affected. However, dominant inheritance should also be considered for the following reasons. Although no family has been reported with clinical expression in successive generations, some healthy parents of patients with CDG syndrome type I show a partial biochemical expression, mainly decreased serum TBG and cholesterol, and slightly increased sialo-deficient transferrin. The absence of clinically affected offspring of patients with this syndrome could be attributed to infertility as a consequence of

hypogonadism and to the fact that most patients are severely retarded. Other arguments for, or compatible with, dominant inheritance are the high prevalence of affected siblings, the great variability of clinical expression even within families, and the rarity of consanguinity within and between families. Of special interest are two families with possible dominant inheritance and genomic imprinting: one with two patients who are female cousins (their fathers are brothers) and the other with two patients who are female second cousins (the mothers of their fathers are sisters). The chromosomal localization will most probably be elucidated this year (1994), opening the way to prenatal diagnosis and heterozygote detection.

Inheritance in CDG syndrome type II is most probably autosomal recessive. The defective gene, *N*-acetylglucosaminyltransferase II, has been localized to chromosome 14q21 [12].

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Peroxisomal Disorders

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CONTENTS

The peroxisomal disorders constitute a clinically and biochemically heterogeneous group of disease states sharing an impairment of one or more peroxisomal functions [1]. Up to now at least 21 disorders have been found which are linked to peroxisomal dysfunction. Most of them show severe central nervous system (CNS) involvement [2].

Clinical Presentation

A great clinical heterogeneity exists in the expression of diseases with similar biochemical defects. This variability comprises the type and severity of symptoms, as well as the age of onset of symptoms. On the other hand, similar clinical phenotypes may also be associated with different biochemical lesions. This absence of correlation between clinical expression and biochemical defects is illustrated by the classical Zellweger syndrome (CZ) and infantile Refsum's disease (IRD), which have similar multiple enzyme defects; it is also evident in the dissimilar enzyme defects underlying one clinical syndrome, e.g., rhizomelic chondrodysplasia punctata (RCDP) [3]. Given the diversity of clinical and biochemical abnormalities, an arbitrary approach has been made to divide peroxisomal disorders into two groups: those with a deficient assembly of peroxisomes, and those with one single deficient peroxisomal enzyme (Table 1). However, this biochemical "Classification" is not useful for clinicians who are faced with clinical symptoms and not with biochemical phenotypes. In general, the onset of symptoms is not accompanied by an acute metabolic event or abnormal routine laboratory tests indicating metabolic derangement. In most disorders, the presentation is more likely to be associated with chronic encephalopathy from infancy/early childhood or a progressive neurologic manifestation from the school-age period. Peroxisomal disorders should be considered in various clinical conditions which depend on the age of the patient (Table 2): predominant errors of morphogenesis, predominant neurologic presentation, and predominant hepatodigestive presentation. All these presentations are possible in the neonatal period.

General features of recognition are dysmorphia, neurologic dysfunction, and hepato-digestive manifestations.

Polymalformative Syndrome and Dysmorphia. Cranio facial abnormalities including large fontanelles, high forehead, epicanthus, and abnormal ears may be mistaken for chromosomal aberrations such as Down syndrome. They are frequently associated with other abnormalities: rhizomelic shortening of limbs in RCDP; stippled calcifications of epiphyses in RCDP and CZ; renal cysts in CZ; abnormalities in neuronal migration (gyral abnormalities, neuronal heterotopias) and cerebral myelination. These congenital manifestations point to dysmorphogenesis during the prenatal period, as observed in some inborn errors affecting energy-producing pathways of the fetus and in metabolic dysfunctions of the mother during pregnancy [4].

Neurologic Dysfunction. At birth, the predominant symptom is often a severe *hypotonia* with areactivity which can be mistaken for a neuromuscular disorder, a disorder of the CNS and autonomic nervous system, and malformation syndromes. An increasing number of inborn errors of metabolism without evident biochemical abnormalities by routine laboratory screening

Table 1. Classification of peroxisomal disorders

Peroxisome assembly deficiencies		
Classical Zellweger syndrome Neonatal adrenoleukodystrophy Infantile Refsum disease	Absent	Generalized
Pseudo-infantile Refsum disease	Absent	Generalized
Zellweger-like syndrome	Present	VLCFA oxidation, THCA oxidation, DHAPAT, Phytanic acid oxidation
Rhizomelic chondrodysplasia punctata (classical/atypical phenotype)	Abnormal	DHAPAT, alkyl DHAP synthase, phytanic acid oxidase, unprocessed peroxisomal thiolase
Atypical Refsum disease		Phytanic acid oxidase Pipecolic acid oxidase
Single peroxisomal enzyme deficiencies		
Rhizomelic chondrodysplasia punctata		Isolated DHAPAT or alkyl DHAP synthase
Rhizomelic chondrodysplasia punctata		Phytanic acid oxidase
X-Linked adrenoleukodystrophy	Normal	VLCFA-CoA synthetase transport
Pseudoneonatal adrenoleukodystrophy	Enlarged	Acyl-CoA oxidase
Bifunctional enzyme deficiency	Normal	Bi(tri)functional enzyme
Pseudo-Zellweger syndrome	Enlarged	Peroxisomal thiolase
Trihydroxycholestanoic acidemia		THCA-CoA oxidase
Isolated pipecolic acidemia	Abnormal	Pipecolic acid oxidase
Mevalonic aciduria		Mevalonate kinase
Classical Refsum disease		Phytanic acid oxidase
Glutaric aciduria type III	Normal	Peroxisomal glutaryl-CoA oxidase
Hyperoxaluria type I	Normal	Alanine: glyoxylate amino-transferase
Hyperoxaluria type I		Mistargeting
Acatalasemia	Normal	Catalase

DHAPAT, dihydroxyacetone phosphate acyltransferase; DHAP, dihydroxyacetone phosphate; VLCFA, very long chain fatty acids; THCA, trihydroxycholestanoic acid; CoA, coenzyme A.

should also be considered [4]. Severe axial hypotonia may be associated with a neurologic distress with hypertonia of the limbs, and seizures. It may be difficult to differentiate between a mitochondrial respiratory chain disorder and a peroxisomal disease. An important difference is that peroxisomal disorders are not associated with an acute metabolic derangement or abnormal routine laboratory tests, such as metabolic acidosis or lacticacidemia.

Hepatodigestive Manifestations. The predominant manifestations may be hepatomegaly, cholestasis, hyperbilirubinemia, and prolonged jaundice, especially in isolated di- and trihydroxycholestanoic acidemia [5].

Specific Disorders. Two prototypes of neonatal presentation are CZ, which is the most severe condition, and RCDP. Their phenotypes are distinct from the other disorders and should not cause difficulties in the differential diagnosis.

Classical Zellweger Syndrome. CZ is characterized by the association of the following:

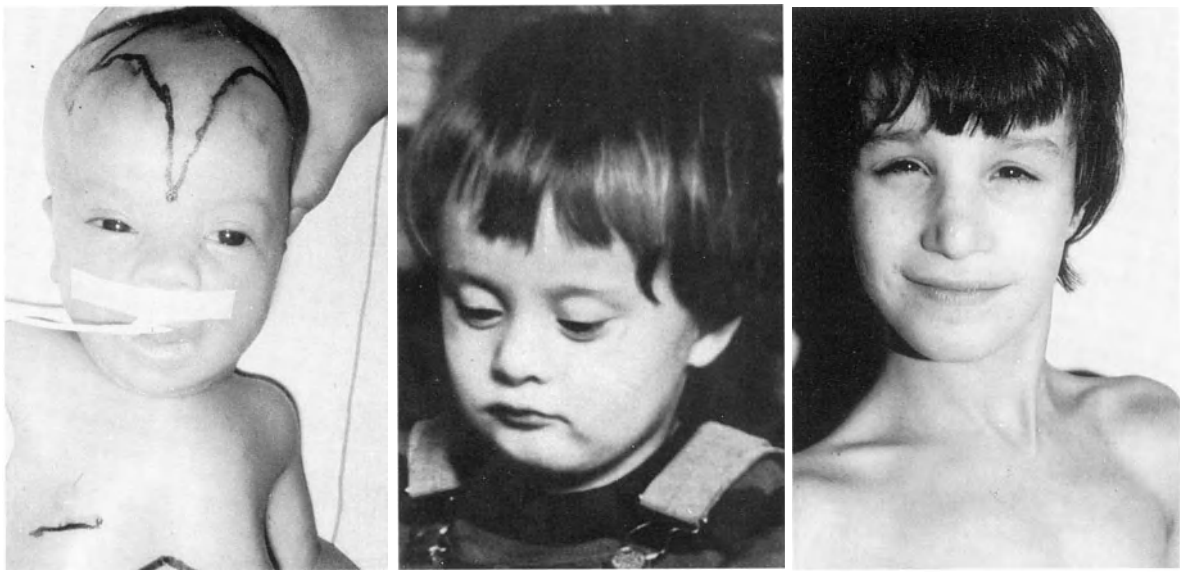
- Errors of morphogenesis
- Severe neurologic dysfunction
- Sensorineural hearing loss
- Ocular abnormalities
- Degenerative changes
- Hepatodigestive involvement with failure to thrive

- Absence of recognizable hepatic peroxisomes (presence of peroxisomal "ghosts")
- Death usually in the first year

The patients show typical facial dysmorphia (Fig. 1a), which may become less characteristic if the patient survives beyond the first year of life. Although certain milestones develop, only some "older" CZ patients attain the ability to sit without support and subsequently develop peripheral hypertonia.

Classical Rhizomelic Chondrodysplasia Punctata. Another typical phenotype is the classical RCDP, which is characterized by the presence of shortened proximal limbs, facial dysmorphia, cataracts, psychomotor retardation, coronal clefts of vertebral bodies, and stippled foci of calcification of the epiphyses in infancy, which may disappear after the age of 2 years. The chondrodysplasia punctata is more widespread than in CZ and may involve extraskeletal tissues. Some patients have ichthyosis. Peroxisomal structures appear to be intact in fibroblasts, whereas in liver these organelles may be fewer or absent in some hepatocytes and enlarged in size in others [6]. Patients were described with a new variant of chondrodysplasia punctata associated with the characteristic peroxisomal defects observed in classical RCDP, but without the rhizomelic shortening of the limbs [7, 8]. Conversely, patients were identified with the typical clinical phenotype of classical RCDP, but with a single enzyme deficiency [3]. Classical RCDP and its variants must be distinguished from other forms of chondrodysplasia punctata such as the Conradi-Hünermann syndrome and the X-linked dominant and recessive forms of chondrodysplasia punctata.

Neonatal adrenoleukodystrophy. These patients are somewhat less severely affected than CZ [9]. Facial dysmorphia is not always present, and patients may show some development before their progressive deterioration sets in, followed usually by death before the age of 6 years. Cerebral demyelination is more prominent than dysmyelination and gray matter heterotopia. Computed tomography (CT) scan of the brain may show abnormal contrast enhancement around demyelination areas. Chondrodysplasia punctata and renal cysts are absent. Several patients have been described with a single enzyme defect, but with clinical manifestations resembling those of neonatal adrenoleukodystrophy (NALD; pseudo-NALD, acyl-CoA oxidase deficiency [10]; bi(tri) functional enzyme deficiency [11]) or those of the CZ (pseudo-Zellweger syndrome, peroxisomal thiolase deficiency [12]). Liver peroxisomes were normal (bi(tri) functional enzyme deficiency) or appeared to be enlarged in size (acyl-CoA oxidase and peroxisomal thiolase deficiency), whereas in



a–b c

Fig. 1a–c. Three patients with multiple enzyme defects and defective peroxisome assembly. **a** Classical Zellweger syndrome at 2 weeks. **b** Infantile Refsum disease at 2 years; note facial dysmorphism resembling Down syndrome. **c** Infantile Refsum at 11 years

CZ and NALD they are morphologically absent or severely decreased in number.

Hyperpipecolic Acidemia. This term was assigned to patients on the basis of the observation of an accumulation of pipecolic acid prior to the discovery of the generalized peroxisomal defects. However, hyperpipecolic acidemia should only be assigned to patients with solely elevated pipecolic acid values in body fluids. Hyperpipecolic acidemia associated with a Joubert syndrome has been observed in three siblings.

Di- and Trihydroxycholestanoic Acidemia. This has been reported in patients with predominantly hepatic manifestations associated with neurologic involvement [5].

Mevalonic Acidura. This is a disorder with dysmorphic features and cataracts and probably should be considered a peroxisomal disorder, since mevalonate kinase is predominantly localized in peroxisomes [13, 14] (see Hoffmann, this volume).

First Six Months of Life. During this period of life, the predominant symptoms may be hepatomegaly associated or not with prolonged jaundice, liver failure, and nonspecific digestive problems (anorexia, vomiting, diarrhea) leading to failure to thrive and osteoporosis. Hypocholesterolemia, hypolipoproteinemia, and decreased values of fat-soluble vitamins which resemble a malabsorption syndrome are frequently present (Table 2). Most CZ patients develop hepatomegaly and seizures and do not survive beyond this period.

Table 2. Clinical symptoms of peroxisomal disorders related to age

Symptoms	Disorder
Neonatal period	
Hypotonia, areactivity, seizures	ZS, ZS variants
Craniofacial dysmorphia	Neonatal ALD
Skeletal abnormalities	Pseudoneonatal ALD
Conjugated hyperbilirubinemia	(acyl-CoA oxidase deficiency)
	Bifunctional enzyme deficiency
	RCDP (typical/atypical)
	THC acidemia
	Pipecolic acidemia
	Mevalonic aciduria
First 6 months of life	
Failure to thrive	IRD, pseudo-IRD
Hepatomegaly, prolonged jaundice	Pipecolic acidemia, neonatal ALD, milder forms of ZS
Digestive problems, hypocholesterolemia	
Vitamin E deficiency	Atypical chondrodysplasia
Visual abnormalities	Mevalonic aciduria
Six months to 4 years	
Failure to thrive	IRD, pseudo-IRD
Neurologic presentation	Pipecolic acidemia, neonatal ALD, milder forms of ZS
Psychomotor retardation	
Visual and hearing impairment (ERG, BAEP)	Atypical chondrodysplasia
Osteoporosis	DHC and THC acidemia
Beyond 4 years of age	
Behavior changes	X-Linked ALD
Deterioration of intellectual functions	
White matter demyelination	
Visual and hearing impairment	Classical Refsum
Peripheral neuropathy, gait abnormality	

ZS variants include ZS-like disease and pseudo-ZS (peroxisomal thiolase).
ZS, Zellweger syndrome; ALD, adrenoleukodystrophy; DHC, dihydroxy-cholestanoic acid; RCDP, rhizomelic chondrodysplasia punctata; THC, trihydroxycholestanoic acid; IRD, infantile Refsum disease; ERG, electroretinogram; BAEP, brain auditory-evoked potentials.

IRD is similar to CZ biochemically as well as in the absence or with a significantly decreased number of liver peroxisomes [9]. However, IRD patients differ clearly from CZ with respect to age of onset, initial symptoms, degree of CNS involvement, and duration of survival. Only minor or no facial dysmorphia is noted in early childhood (Fig. 1b,c). Early developmental milestones are usually normal, before slowing sets in between the age of 1 and 3 years. This is followed by completely arrested development associated with autistic behavior in some patients. Most patients walk independently before the age of 3 years. Recently a patient with pseudo-IRD has been described with clinical similarity to IRD, but with somewhat different biochemical abnormalities [15].

Between Six Months and Four Years. In this period of life severe psychomotor retardation become evident (Table 2). Sensorineural hearing loss is associated with abnormal brain stem auditory-evoked responses. Various ocular abnormalities can be observed, including cataract, retinitis pigmentosa, optic nerve atrophy, glaucoma, and brush-field spots. The electroretinogram and visual-evoked responses are frequently disturbed, and this may precede the fundoscopic abnormalities. Retinitis pigmentosa associated with hearing loss, developmental delay, and dysmorphia may be mistaken for other diseases including malformative syndromes [16]. In this respect, it has to be realized that the limits between malformative syndromes and inborn errors are not well delineated. This fact is confirmed by the recent finding of a defective cholesterol biosynthesis in the Smith-Lemli-Opitz syndrome [17]. Most NALD patients do not survive beyond this period.

Beyond Four Years of Age. *X-linked adrenoleukodystrophy* (ALD) is the most common peroxisomal disorder. Considerable clinical variability exists even within the same kindred [18]. The childhood form is the most common and the most severe phenotype with onset of neurologic involvement usually between 5–10 years of age, leading to a vegetative state and death in a few years. The affected males may present with school failure, attention deficit disorder, or behavior changes as first manifestations, followed by visual impairment and quadriplegia, whereas seizures are usually a late symptom. Hypoglycemic episodes and a dark discoloration of the skin may reflect adrenal insufficiency, which may precede, coincide with, or follow the onset of neurologic involvement. Most childhood patients show characteristic symmetric cerebral lesions on CT or magnetic resonance imaging (MRI) involving the periventricular white matter in the posterior and occipital lobes. Following intravenous injection of contrast material, a garland contrast enhancement adjacent to hypodense lesions is shown by CT. The CNS demyelination has a caudorostral progression. Liver peroxisomes are normal.

Behavior changes associated with visual impairment may initially be mistaken for psychiatric manifestations. Intellectual deterioration in this period of life may be related to various other regressive encephalopathies including Sanfilippo disease, Niemann-Pick type C, Wilson's disease, subacute sclerosing panencephalitis, multiple sclerosis, and ceroid lipofuscinosis.

Classical Refsum disease is another peroxisomal disorder with a clinical onset in the school-age period. Retinitis pigmentosa, peripheral polyneuropathy, cerebellar ataxia, and elevated cerebrospinal fluid protein level are the main features. Less constant are nerve deafness, anosmia, ichthyosis, and skeletal and cardiac abnormalities. Mental retardation, liver dysfunction, and dysmorphia are absent. The onset of clinical manifestations varies from childhood to the fifth decade. The disorder is associated with a deficiency of phytanic acid α-oxidation.

Recently, there has been a report of four related patients, three with classical Refsum disease and the fourth who died from a progressive neurologic disorder with clinical and neuropathologic abnormalities unusual for classical Refsum disease. In addition to increased plasma levels of phytanic acid, those of pipecolic acid were also increased in two of these patients [19].

Metabolic Derangements

Peroxisomes are present in all human cells except in mature erythrocytes. They are paticularly abundant in liver and kidney and have an average diameter of 0.2–1 μm. In other tissues, including the brain and cultured skin fibroblasts, peroxisomes are less abundant and smaller. Patients with a peroxisomal disorder may show a discrepancy between the decreased peroxisomal number in hepatocytes and that in fibroblasts.

The most important peroxisomal functions are discussed below [20] (Table 1, Fig. 2).

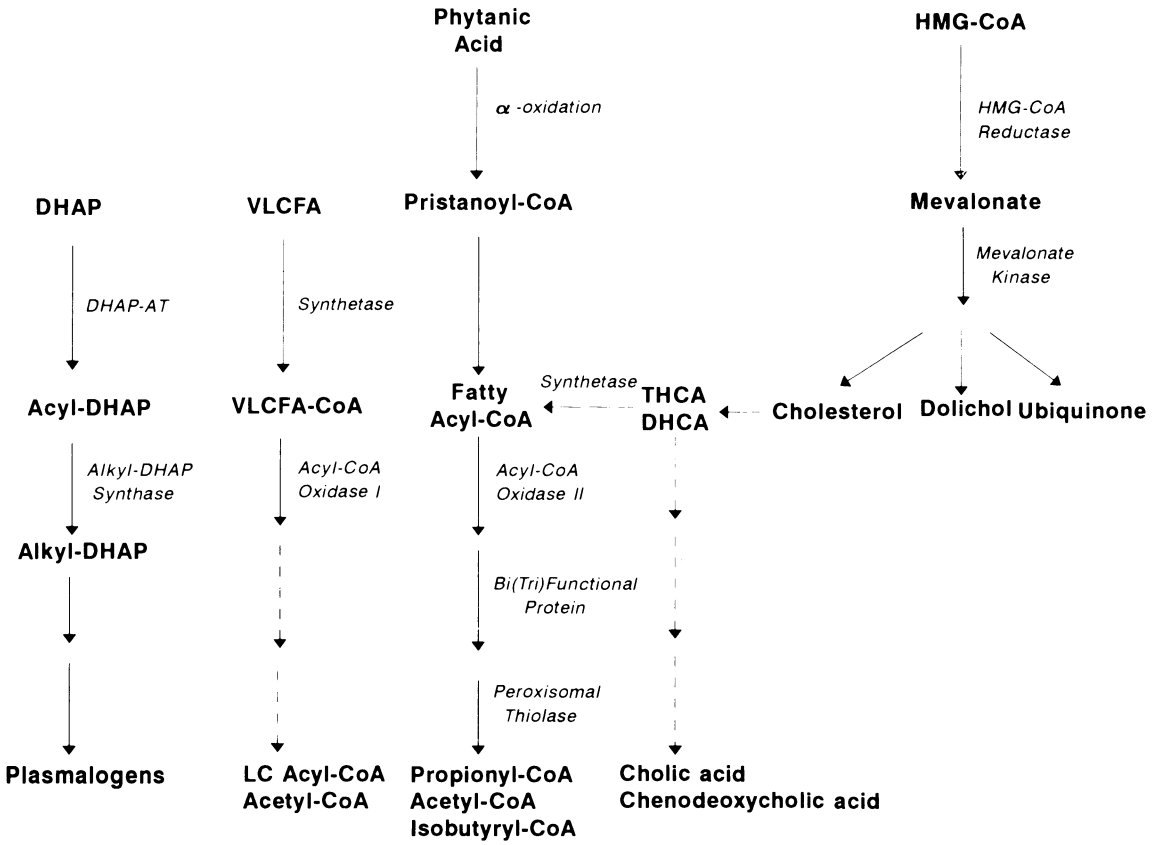


Fig. 2. The most important peroxisomal functions. See text for details. *DHAP*, dihydroxyacetone phosphate; *DHAP-AT*, DHAP acyltransferase; *VLCFA*, very long chain fatty acid; *CoA*, coenzyme A; *LC*, long chain; *THCA*, trihydroxycholestanoic acid; DHCA, dihydroxycholestanoic acid; *HMG-CoA*, 3-hydroxy-3-methylglutaryl-CoA

Plasmalogen Biosynthesis. The enzymes acyl-CoA: dihydroxyacetone phosphate acyltransferase (DHAP-AT) and alkyl-dihydroxyacetone phosphate (alkyl-DHAP) synthase are essential in the biosynthesis of ether phospholipids known as plasmalogens. Plasmalogens are present in cell membranes and are particularly abundant in nervous tissue. Their physiological function has not yet been clarified, although they are known to be involved in platelet activation and in the scavenging of free radicals.

Bile Acids Biosynthesis. The β-oxidation of di-trihydroxy cholestanoic acid, normal intermediates in the biosynthesis of the primary bile acids, is carried out in peroxisomes.

Cholesterol Biosynthesis. Peroxisomes are not only involved in cholesterol oxidation (biosynthesis of bile acids), but also in cholesterol biosynthesis. Peroxisomes contain aceto-acetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl-CoA reductase, mevalonate kinase, and the sterol carrier protein-2, at least in rat liver [14].

β-Oxidation of Fatty Acids. The peroxisomal β-oxidation system is involved in the chain shortening of a distinct group of compounds which includes saturated very long chain fatty acids (VLCFA; 22 carbons or more), long-chain dicarboxylic acids, polyunsaturated fatty acids, and branched-chain fatty acids such as pristanic acid [21]. Following activation to their corresponding acyl-CoA esters via membrane-bound acyl-CoA synthetases, fatty acyl-CoA esters are degraded in the peroxisomal matrix via the β-oxidation cycle, which consists of acyl-CoA oxidase, bi(tri) functional protein, and peroxisomal 3-oxoacyl-CoA thiolase. Acyl-CoA oxidase is only active towards fatty acyl-CoA esters containing six carbons or more; therefore, VLCFA are only chain shortened in the peroxisome and subsequently further oxidized in the mitochondrial matrix. Human liver peroxisomes contain two acyl-CoA oxidases: a palmitoyl-CoA

oxidase and a branched-chain acyl-CoA oxidase which oxidizes pristanoyl-CoA as well as di- and trihydroxycoprostanoyl-CoA [22]. The enzyme known as the bifunctional protein appears to be a trifunctional protein as it also includes an enoyl-CoA isomerase in additon to the hydratase and the dehydrogenase activities [23].

Phytanic Acid α-Oxidation. Phytanic acid, which is exclusively of dietary origin, is primarily oxidized to pristanic acid in peroxisomes in humans and in mitochondria in rodents [24].

Glutaric Acid Oxidation. Glutaric acid is an intermediate in the catabolism of lysine as well as pipecolic acid. Apart from the mitochondrial glutaryl-CoA dehydrogenase, there probably also exists a peroxisomal glutaryl-CoA oxidase [25, 26].

Pipecolic Acid Oxidation. The first step in the degradation of L-pipecolic acid, an intermediate in lysine catabolism, is catalyzed by L-pipecolic acid oxidase present in human liver peroxisomes.

Glyoxylate Metabolism. Glyoxylate, the most important precurser of oxalate (See Gagnadoux and Broyer, this volume), can be transaminated to glycine in a reaction catalyzed by alanine: glyoxylate aminotransferase [27].

Diagnostic Tests

Independently of the clinical symptoms and age of onset, most peroxisomal disorders can be clinically screened by neurophysiological investigations (electroretinogram, visual-evoked responses, brain auditory-evoked potentials), which are almost constantly abnormal.

Summarizing the possible clinical manifestations, peroxisomal disorders should be considered in patients showing one or more of the following abnormalities:

- Craniofacial abnormalities and/or other dysmorphic features
- Skeletal abnormalities, including calcific stippling and shortened proximal limbs
- Neurologic abnormalities, including encephalopathy, hypotonia, seizures, hearing loss, and cerebral abnormalities
- Ocular abnormalities, including retinopathy, cataract, optic nerve dysplasia, and abnormal electroretinogram and/or visual-evoked potentials
- Hepatological abnormalities, including hepatomegaly, liver dysfunction, cholestasis, and fibrosis/cirrhosis

Assays. Table 3 lists a variety of assays which are available for the diagnosis of peroxisomal disorders. Only urinary pipecolic acid excretion, medium- and long-chain dicarboxylic aciduria, hyperoxaluria, and mevalonic aciduria can be detected by an overall metabolic screening. Nine of the 17 peroxisomal disorders with neurologic involvement are associated with an accumulation of VLCFA, which suggests that an assay of plasma VLCFA should be used as a primary test. However, assays of plasma phytanic acid and plasma/urine bile acid intermediates should also be performed in view of the recent reports of atypical chondrodysplasia variants (without rhizomelic shortening) and isolated trihydroxycholestanoic aciduria. The clinical presentation of the typical phenotype of RCDP (phytanic acid, plasmalogens) and classical Refsum (phytanic acid) are distinct from the other disorders and should not cause difficulties in their diagnosis. In order to elucidate whether the accumulation of VLCFA in a patient's plasma results from a defect in peroxisome biogenesis or is caused by a defect in one of the peroxisomal β-oxidation enzyme activities, additional assay procedures must be carried out, in particular plasmalogen levels and immunoblotting of peroxisomal β-oxidation proteins. Therefore, it should be stressed that it is no longer possible to screen all peroxisomal disorders only by measuring plasma VLCFA. It would be advisable to carry out assays of plasma bile acid intermediates, phytanic, pristanic, and pipecolic acid, plasmalogens in red blood cells, and DHAPT and alkyl DHAP synthase in cultured skin fibroblasts. In some patients with variant forms, the enzymatic deficit(s) are only expressed in liver and not in cultured fibroblasts. Extensive peroxisomal investigations are necessary (even when the clinical phenotype is very typical), since some disorders may be associated with very atypical biochemical phenotypes.

Although in some cases, levels of metabolites in CSF from patients exceed the control range, measurements of VLCFA, bile acids, pristanic acid, and phytanic acid do not seem to provide a diagnostic advantage, as all measurements can be performed more conveniently in plasma [28]. For

Table 3. Diagnostic assays in peroxisomal disorders

Disease	Material	Type of assay
	Plasma	VLCFA, bile acids, phytanic acid, pristanic acid, pipecolic acid, polyunsaturated fatty acids
Classical ZS Neonatal ALD Infantile Refsum Zellweger-like Pseudo-infantile Refsum	RBC Fibroblasts	Plasmalogens Plasmalogens biosynthesis, DHAPAT, alkyl DHAP synthase, Particle bound catalase, VLCFA β-oxidation, Immunoblotting β-oxidation proteins Phytanic acid oxidation
Rhizomelic chondrodysplasia punctata (classical/atypical phenotypes)	Plasma RBC Fibroblasts	Phytanic acid Plasmalogens DHAPAT, alkyl DHAP synthase, Phytanic acid oxidation
Isolated peroxisomal β-oxidaton defects	Plasma Fibroblasts	VLCFA, Bile acids VLCFA β-oxidation, immunoblotting β-oxidation proteins
Isolated defect of bile acid synthesis	Plasma Liver	Bile acids THCA-CoA oxidase
Isolated pipecolic acidemia	Plasma Liver	Pipecolic acid Pipecolic acid oxidase
Mevalonic aciduria	Plasma Urine Fibroblasts Lymphocytes	Organic acids Mevalonate kinase
Classical Refsum	Plasma Fibroblasts	Phytanic acid Phytanic acid oxidation
Glutaric aciduria type III	Urine Liver	Organic acids Glutaryl-CoA oxidase
Hyperoxaluria type I	Urine Liver	Organic acids AGT
Acatalasemia	RBC	Catalase

VLCFA, very long chain fatty acids; DHAPAT, dihydroxyacetone phosphate acyltransferase; DHAP, dihydroxyacetone phosphate; THCA, trihydroxycholestanoic acid; AGT, alanine: glyoxylate aminotransferase; RBC, red blood cells.

some disorders, a retrospective diagnosis can be obtained by analyzing stored blood spots collected during neonatal screening [29, 30].

Histological Detection. Using the diaminobenzidine staining procedure, which reacts with the peroxisomal marker enzyme catalase, and immunochemical techniques with antibodies against matrix and membrane peroxisomal proteins facilitates the histological detection of peroxisomes [31]. The abundance, size, and structure of liver peroxisomes should be studied. When peroxisomes are lacking, virtually all of the catalase is present in the cytosolic fraction, instead of the particulate fraction.

Prenatal Diagnosis. A variety of techniques are available. Almost all peroxisomal disorders can be identified prenatally, either by using (cultured) chorion villous samples or amniocytes or by direct analysis of levels of VLCFA and bile acid intermediates in amniotic fluid. Measurement of VLCFA and/or assay of plasmalogen synthesis are the most useful methods today, except in the case of trihydroxycholestanoic acid (THCA)-CoA oxidase deficiency, isolated pipecolic acidemia, glutaric aciduria type III, and hyperoxaluria type I (fetal liver biopsy). Another approach is the cytochemical staining of peroxisomes in chorion villus samples.

Heterozygote Identification. This is only available for X-linked ALD using VLCFA analysis or restriction fragment polymorphism [32].

Treatment and Prognosis

In classical Refsum disease, reduction of plasma phytanic acid levels by a low-phytanate diet (especially proscription of ruminant meats and ruminant fats), combined or not with plasmapheresis, has been successful in arresting the progress of the peripheral neuropathy. The stored phytanic acid is exclusively of exogenous origin. However, when the diet is too strict, it may lead to a reduction of the energy intake, weight loss, and a paradoxical rise in plasma phytanic acid levels followed by clinical deterioration. This is due to the mobilization of phytanate from lipids stored in the adipose tissue.

In X-linked ALD patients, it has been demonstrated that it is possible to normalize the plasma VLCFA levels by a regimen that combines dietary restriction of VLCFA (from seafish) with the oral intake of monounsaturated fatty acids (oleic and erucic acid from olive oil and rapeseed oil, respectively). However, the clinical benefit of the dietary therapy has been rather disappointing, and final conclusions will hopefully be available in the near future [18]. The same consideration must be applied to the usefulness of bone marrow transplantation, which has been encouraging in a clinically mildly affected patient with the childhood form of X-ALD [33].

For patients with abnormal peroxisomal assembly and defects that originate in fetal life, the possibilities for treatment are very poor. Supplementation of docosahexaenoic acid [34] or other regimens [8, 35, 36] are now being tested in patients with the milder forms of multiple peroxisomal dysfunction or atypical chondrodysplasia punctata.

Genetics

With the exception of X-linked ALD, the pattern of inheritance is autosomal recessive. There may exist a subset of NALD with an X-linked inheritance.

In three CZ patients, genetic mutations have been shown to exist in two peroxisomal integral membrane proteins [37, 38]. Somatic cell fusion experiments with fibroblasts from different patients with multiple defects suggest that there are at least nine genes involved in the formation of normal peroxisomes and in the transport of peroxisomal enzymes [39–43]. The lack of correlation in these complementation groups between genotype and clinical phenotype suggests that the currently used clinical denominations are not sufficiently distinctive.

As in CZ, the peroxisomal thiolase protein is present in its 44-kDa, unprocessed form in liver and fibroblasts from patients with classical RCDP. Patients with the clinical and biochemical phenotype of classical RCDP belong to a single complementation group, whereas the patient with an isolated DHAP-AT deficiency belongs to a different complementation group, indicating that mutations in at least two different genes can lead to the clinical phenotype of RCDP [44].

In X-linked ALD, a defect has been suggested in a protein involved in the transport of VLCFA-CoA synthetase into the peroxisomal membrane or in a protein which is associated with the VLCFA-CoA synthetase in the membrane [45].

Mistargeting of a peroxisomal protein to mitochondria has been demonstrated in some patients with hyperoxaluria type I [27].

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Oxalosis (Primary Hyperoxaluria)

M.F. Gagnadoux and M. Broyer

CONTENTS

Oxalosis is the systemic deposition of calcium oxalate crystals, caused by primary hyperoxaluria type I (PH I), an autosomal recessive peroxisomal defect involving glyoxylate metabolism in the liver and leading to an excesssive synthesis of oxalate. Primary hyperoxaluria type II (or L-glyceric aciduria) is a much rarer disease caused by a different enzymatic defect and does not seem to induce oxalosis [1].

Primary Hyperoxaluria Type I

Clinical Presentation

PH I is a rare cause of nephrolithiasis and renal failure in children.

Initial Symptoms. The first manifestations usually occur in early childhood, before 5 years in 50% of cases, and even in the first year as a "malignant" variant. A total of 90% of patients are symptomatic before 25 years [2, 3]. The first organ affected by oxalate deposits is the kidney and the initial symptoms are usually those of *urolithiasis*: renal colic, hematuria, pyuria, and urinary tract obstruction. X-ray films show very dense stones of various sizes and localizations, often bilateral, and associated with *nephrocalcinosis* in most cases, which is very suggestive of the diagnosis. The presentation of infantile cases is somewhat different [4]. Urolithiasis is usually absent and the symptoms are those of advanced renal failure. The presence of nephrocalcinosis on ultrasonography or X-rays suggests the diagnosis.

Evolution. The urolithiasis is highly recurrent and renal failure ultimately develops, leading to end stage before 15 years in 50% of cases and before 30 years in 80% [2].

As the glomerular filtration rate (GFR) decreases, a systemic retention of oxalate occurs, approximately from a GFR lower than 40 ml/mn per 1.73 m^2, though usually not apparent until the dialysis stage. The first visible localization of the extrarenal deposits is *bone tissue* [5]. The calcium oxalate crystals accumulate first in the metaphyseal areas and form dense *supra-metaphyseal bands* on X-rays, which are almost pathognomonic. Later on, the whole bones become dense and spontaneous fractures occur, ultimately leading to a severe disability.

The other main sites of crystal deposits are as follows:

- The conducting system of the heart, with a risk of sudden death
- Joints
- Media of arteries, sometimes causing gangrene of the extremities in older patients
- Retina (without visual impairment) in approximately one third of patients
- Skin and oral mucosa with subcutaneous or submucosal crystalline tophi
- Peripheral nerves

After some years on dialysis, the quality of life is usually miserable.

Metabolic Derangement

The disease is due to a defect in the liver peroxisomes of the enzyme *alanine:-glyoxylate aminotransferase* (AGT), which catalyses the intraperoxisomal transamination of glyoxylate to

glycine using alanine as the amine donor (Fig. 1) [6]. The noncatabolized glyoxylate is oxidized to oxalate or reduced to glycolate, which both are excreted in excess in urine. The pathology is due to the insolubility of excess calcium oxalate at physiological pH.

Among PH I patients a large heterogeneity is observed at the enzymological level, as regards both AGT catalytic activity and AGT immunoreactivity [3]. Three groups of patients may be defined: 50% have no detectable levels of either AGT enzymatic activity or AGT immunoreactive protein (defined as enz^-/crm^-), about 20% are enz^-/crm^+, and 30% are enz^+/crm^+. Nevertheless, no close correlation can be found between the level of residual activity (3%–40%) and the clinical course. It may be because in the enz^+ patients, the intracellular distribution of AGT is mistargeted, 90% being localized in the mitochondria instead of the peroxisomes, the normal site of activity [7]. There are no other peroxisomal deficiencies and liver peroxisomes are normal in number and appearance.

Diagnostic Tests

The diagnosis must be considered in every child with urolithiasis, especially if there are recurrences, associated nephrocalcinosis, mild renal failure, or parental consanguinity. The measurement of oxalate excretion in the urine is sufficient to establish the diagnosis in most cases. The upper limit of normal value of oxaluria, usually measured enzymatically, is 50 mg or 550 μmol/1.73 m^2 per day. Oxaluria exceeds frequently 1000 μmol (90 mg) per day, even in children, before advanced renal failure. This hyperoxaluria is associated in two thirds of cases with an increased glycolaturia, less commonly assessed (normal value, <80 mg or 900 μmol/1.73 m^2 per day) [8]. When 24-h urine output is not available, in infants for example, the oxalate to creatinine ratio must be used (normal value, <0.26 mmol/mmol in infants, <0.08 mmol/mmol in adults) [9]. In end-stage renal failure, measurement of urinary oxalate is no longer reliable. In this situation, measurement of oxalemia may be useful, but is technically more difficult. Also, non-PH I anuric patients may have an oxalate retention, with increased oxalemia. Only when the plasma oxalate to creatinine ratio exceeds 0.07 mmol/mmol (normal, 0.03 mmol/mmol $\pm$ 0.01) can the diagnosis of PH I be considered [9].

The crystallographic analysis of renal stones may confirm the diagnosis by showing the almost exclusive presence of "whewellite," the calcium oxalate monohydrate crystal [10].

The measurement of AGT in a liver biopsy is performed in only few laboratories in the world [6]. This assay can be omitted if massive hyperoxaluria and glycolaturia are present. It is principally necessary in anuric patients whose initial disease was misdiagnosed. It is also the only procedure which could allow antenatal diagnosis, but it requires a fetal liver biopsy [11]. Indeed, oxalate and glycolate levels in amniotic fluid are normal, as they may be in the urine in the first days of life [12, 13].

Nephrolithiasis and even progressive renal insufficiency may also be caused by secondary hyperoxaluria [10] due to primary bowel disease, massive intakes of oxalate, or potential precursor (ethylene glycol, ascorbic acid). Hyperoxaluria in

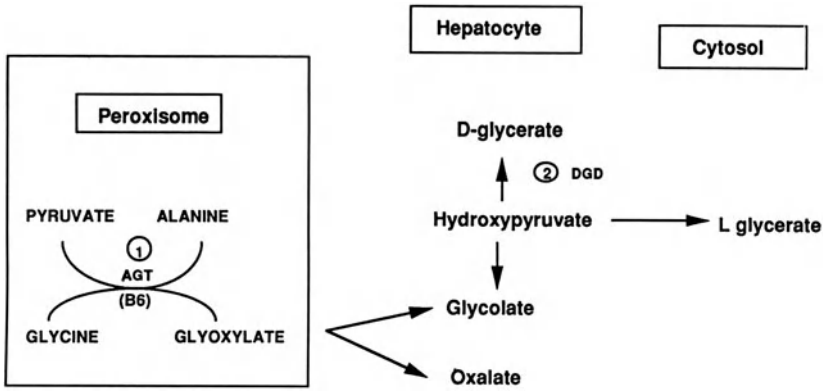

Fig. 1. Enzymatic defects in primary hyperoxalurias (PH). *1*, PH I: defect of alanine glyoxylate aminotransferase (*AGT*); *2*, PH II: defect of D-glycerate dehydrogenase (*DGD*)

these cases is never associated with increased glycolate excretion.

Treatment and Prognosis

Conservative Management. As long as renal function is normal, only preventive measures aimed at slowing down the deposition of calcium oxalate crystals in kidney are indicated. In order to reduce oxalate production, a treatment with *pyridoxine*, which is a cofactor of AGT, deserves to be attempted in every patient, at several dosages (25–1000 mg/m^2). However, only a few patients are pyridoxine sensitive [2, 13]. Dietary restriction of oxalate-rich food (rhubarb, spinach, chocolate, tea) is of limited efficacity.

The most effective measures are aimed at increasing the solubility of calcium oxalate in urine. The first one is to ensure a copious *fluid intake* (>2 l/m^2 per day); if necessary, it must be given intravenously, for example in case of general anesthesia. Among the inhibitors of calcium oxalate saturation, the most efficient seems to be *sodium citrate* (0.15 g/kg orally) [14]. Magnesium oxide and orthophosphate, once advocated, have a less convincing efficacity; large doses of phosphate may actually be harmful by increasing the formation of sodium oxalate, which is more easily absorbed [14].

The treatment of urolithiasis must be the least aggressive possible. Only obstructive stones must be removed, by limited interventions and with a very cautious perioperative management [15].

Dialysis and Transplantation. At the end stage of renal failure, *dialysis* is not a satisfactory mode of treatment. Indeed, no form of dialysis is able to remove sufficient amounts of oxalate to avoid its extrarenal accumulation. Hemodialysis seems to be more efficient [16]. *Renal transplantation* is advocated by some groups with a specific perioperative management in order to limit the toxicity of oxalate on the graft and the recurrence of nephrocalcinosis [17]. Nevertheless, this recurrence remains a long-term threat, and graft survival is poor as compared to patients with other primary renal disease [18]. Only *liver transplantation* allows definite correction of the metabolic defect and prevents the occurence of systemic oxalosis, if performed early enough. However, very few isolated liver grafts have been performed until now in this indication [19]. Combined liver and kidney transplantation is currently considered as the treatment of choice, especially in infants and young children [20]. Oxalemia normalizes immediately after grafting, but hyperoxaluria lasts until all accumulated oxalate is eliminated. Therefore, the earlier the transplantation is performed after the onset of renal failure, the better are the results, and it should be planned before dialysis becomes necessary.

Genetics

PH I is a recessive autosomic disorder related to mutation(s) in the AGT gene located on chromosome 2q36–q37. This gene has been cloned and the coding sequence is distributed among 11 exons covering 10 kb [21]. Several mutations have been reported, but in only 50% of the patients.

There is also a polymorphism of the AGT gene in the normal population, 80% exhibiting the "major" allele and 10%–20% the "minor" allele with two or three point mutations. The molecular basis of AGT mistargeting to mitochondria is not yet completely understood. It has nevertheless been associated with several point mutations. The most frequent is one base substitution in exon 4, always associated with the minor allele [22]. Other points mutations associated with an enzymatically inactive AGT in the peroxisome have also been reported associated with the major allele.

Except in families with well defined mutation there is no possibility to perform a genetic diagnosis on fetuses by molecular biology.

Primary Hyperoxaluria Type II

Primary hyperoxaluria type II, probably a rarer disease than type I, is characterized by excessive urinary excretion of L-glycerate and oxalate (Fig. 1). Clinical symptoms are due to renal stone formation caused by the hyperoxaluria; renal failure seems exceptional [1]. The defect concerns D-glycerate dehydrogenase, an enzyme which intervenes in the conversion of hydroxypyruvate (produced from serine) into 2-*P*-glycerate, an intermediate of the glycolytic-gluconeogenic pathway. The enzyme can be assayed in red cells and also shows a glyoxylate reductase activity. Both activities have been found defective in the liver of patients.

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Part XV
New Trends of Treatment

Liver Transplantation

O. Bernard

CONTENTS

Current results of liver transplantation indicate that 70%–80% of children treated are alive, most of them leading normal lives [1]. Liver transplantation is carried out for liver diseases (inborn or acquired) which result in death through liver cell failure and for inherited disorders expressed exclusively or mostly in the liver without the risk of liver cell failure, but with the risk of severe extrahepatic damage.

This chapter reviews briefly the main surgical and postsurgical aspects of liver transplantation in children and describes the results of liver transplantation performed so far in patients with inborn errors of metabolism.

Procedure

The development of liver transplantation and the extension of the indications in adults and in children have resulted, over the past few years, in a shortage of donors for children. This has been partially overcome by the use of liver splitting [2] in brain-dead patients as well as by the use of the left lobe of related living donors [3], with results similar to those of whole liver transplantation. Except for emergency situations, ABO blood group identity or compatibility is necessary.

Orthotopic liver transplantation is a major procedure, comprising hepatectomy in the donor, hepatectomy in the recipient, and engraftment of the donor's liver with vascular and biliary anastomoses. With the UW (University of Wisconsin) preservation solution, cold ischemia time may be extended to 10–15 h. A first impression of the graft function is provided by its early production of bile.

The postoperative follow-up can be separated into three periods.

Early Postoperative Period. This lasts an average of 10 days and takes place in the intensive care unit. Supportive measures are taken as after any major surgical procedure; it is especially important to monitor adequate diuresis, as a good renal function will ease the use of cyclosporine. An attempt at lowering the incidence or severity of cytomegalovirus (CMV) infections is made by specific anti-CMV immunoglobulin infusions or with acyclovir. In high-risk situations, heparin is used to prevent hepatic artery thrombosis. Immunosuppression is started with i.v. methylprednisolone (10 mg/kg) and azathioprine (2 mg/kg) during the operation; i.v. cyclosporine is started postoperatively at increasing doses as soon as the hemodynamics and diuresis are satisfactory; azathioprine is discontinued when stable blood levels of cyclosporin are reached; methylprednisolone is progressively decreased down to 1 mg/kg per day at the end of the first week.

A rise in serum transaminases is normally observed on days 1–3, followed by a rapid decrease to normal or near-normal values. Prothrombin time, often somewhat prolonged on day 1, also decreases regularly; a proaccelerin level above 50% is an early indicator of good liver function.

Four major *complications* may be observed during this period:

- Primary graft nonfunction is rare, but of such severity that it requires emergency retransplantation.
- Infections, mostly bacterial, are very frequent, but usually controlled by antibiotics.
- Rejection is also frequent and usually occurs at the end of the first week. Liver needle biopsy is

carried out as soon as rejection is suspected on clinical or biochemical grounds; histological signs of rejection include portal tract inflammatory infiltrates, endotheliitis of the portal and centrilobular veins, and/or periductular infiltrate with interlobular bile duct damage. Urgent therapy is needed, usually using three i.v. injections of methylprednisolone. Whenever this fails to control rejection, OKT3 monoclonal antilymphocyte preparation or FK-506 is given after checking the signs of persisting rejection by a repeat biopsy. Refractory rejection can lead to rapid or progressive liver cell failure and/or to a vanishing bile duct syndrome, which ultimately requires retransplantation.

- Hepatic artery thrombosis is a major risk in children; it occurs in 10%–15% of cases with a much higher incidence when both donor and recipient are very young. Thrombosis is looked for daily by Duplex ultrasound and confirmed by arteriography. Emergency surgery may make it possible to remove the obstruction of the artery and to prevent necrosis of the liver or biliary complications secondary to ischemia of the biliary epithelium.

Intermediary Follow-Up. The second period of follow-up takes place in conventional hospital settings and lasts an average of 6 weeks. Monitoring of liver and kidney function tests and blood cyclosporine trough levels is carried out daily. Prednisone is given orally and its dosage diminished to 0.5 mg/kg per day at 1 month; cyclosporine is progressively switched from i.v. to oral.

Three main *complications* are observed in this period:

- Rejection is always possible and is managed as described above.
- CMV infection must be searched for by repeated blood and urine cultures; the combination of prevention with specific immunoglobulins, early detection, and DHPG (dehydroxyphenylguanine, Gancyclovir) treatment of the symptomatic forms has made the severe forms of CMV infections very rare.
- Biliary complications occur in 10%–15% of cases and may be of two types: either stenosis at the level of the anastomosis, unrelated to hepatic artery thrombosis and cured by endoluminal dilation or surgical repair, or as a delayed consequence of hepatic artery thrombosis; treatment of the latter is difficult, using various types of interventional radiology techniques, reoperation on the bile ducts, and sometimes retransplantation.

Long-Term Follow-Up. In the majority of cases the children can resume a life close to normal when they leave the hospital and can attend school within 2–3 months of surgery. Clinical and biochemical monitorings are carried out in outpatient clinics; a salt-restricted diet is recommended, at least initially, because of the hypertensive effect of cyclosporine. Since the risk of rejection persists, immunosuppression must be pursued indefinitely, with the goal of reaching the lowest possible doses compatible with normal liver function tests in order to lower the risk of kidney damage due to cyclosporin. Prednisone is given on an alternate day basis, allowing normal growth and a significant increase in height velocity in most children who displayed growth retardation prior to transplantation [4]. *Complications* at this stage include late biliary stenosis, opportunistic infections, anemia and gastrointestinal bleeding due to portal vein stenosis, and Epstein-Barr virus (EBV)-related lymphoproliferative syndrome [5]. The latter seems in part related to the cumulative degree of immunosuppression; with early diagnosis, lowering or interruption of immunosuppression, resection of the proliferative zones when limited and solitary, and careful supervision, regression may occur, but retransplantation may later be necessary because of chronic rejection.

Medium-range follow-up studies indicate that 80% of surviving children have normal serum bilirubin levels and serum transaminase activity below twice normal. Eighty percent of surviving children attend a school level normal for their ages or only 1 year below normal. A significant prognostic factor is the condition of the child before transplantation, both in term of nutrition and liver cell function. For instance among 284 children treated in our group between 1986 and 1993, survival was 57% in the children whose pretransplant prothrombin time was below 50%, and 93% in the children whose prothrombin time was equal to or above 50%. In particular all children transplanted for an inborn error of metabolism without severe liver damage are currently alive.

Inborn Metabolic Disorders Treated by Liver Transplantation

In the European Liver Transplant Registry issued in October 1993 a metabolic disorder was recorded as the indication for transplantation in 18% of 1248 children treated by liver transplantation from May 1988 to June 1993. Two groups can be distinguished, as discussed below.

Liver Disorders Associated with Severe Liver Damage

The majority of liver transplantations reported in the literature were performed in children with hereditary liver diseases resulting in liver cell failure, cirrhosis, or a risk of hepatocellular carcinoma and for which there was no other effective treatment available.

Alpha-1-Antitrypsin Deficiency [6]. Liver transplantation is the only effective therapy for children with cirrhosis and the protein inhibitor (PI) Z phenotype. Serum levels of alpha-1-antitrypsin (AAT) return to normal; the PI phenotype of the donor's liver becomes detectable in the recipient's serum within 24 h of transplantation. It may be hoped that this will prevent pulmonary emphysema during adulthood.

Hereditary Tyrosinemia [7]. For several years, liver transplantation has proven extremely useful in children with this otherwise lethal disease. The recent introduction of the enzyme inhibitor NTBC (see chapter by Kvittington, Clayton, and Leonard, this volume) [8] may diminish the need for liver transplantation. So far, however, liver transplantation remains indicated in children with cirrhosis above the age of 2 because of the high risk of hepatocellular carcinoma after this age and of the lack of data concerning the efficacy of NTBC for its prevention.

Wilson's Disease [9]. This is usually treated successfully with copper-chelating agents; in a few cases, however (e.g., acute liver failure with or without hemolysis, acute relapse after discontinuation of therapy), liver transplantation is necessary and results not only in the elimination of liver pathology and normalization of copper metabolism, but also in the regression of the neurological signs if already present.

Type IA and IB Glycogen Storage Diseases [10]. Beside severe hypoglycemia, growth failure, and acidosis, they also carry a risk of liver adenoma, possibly complicated by intratumoral hemorrhage or liver cell carcinoma. Liver transplantation may be recommended in patients with adenomas or failure of medical therapy; it allows restoration of a normal glucose metabolism and significant improvement in growth [11, 12]. In type IB, polymorphonuclear dysfunction is not corrected by liver transplantation and requires permanent treatment with granulocyte colony-stimulating factor (G-CSF) [13].

Type IV Glycogen Storage Disease [14]. Liver transplantation eliminates cirrhosis and may even improve the degree of amylopectin storage in the heart. Careful follow-up is, however, necessary in this respect [15].

Hemophilia A. [16]. Four patients who had severe post-transfusion hepatitis with cirrhosis underwent liver transplantation. In the three surviving patients, the plasma coagulating activity of factor VIII was normal, confirming that the liver is the main source of factor VIII synthesis and secretion.

Protoporphyria [17]. Five adult and adolescent patients with cirrhosis underwent liver transplantation. This resulted in normal liver function and disappearance of or significant improvement in skin photosensitivity. Careful follow-up remains necessary because of the important role of erythropoietic tissue in protoporphyrin production in such patients, which may result in relapsing accumulation of protoporphyrin in the transplanted liver.

Cholesterol Ester Storage Disease [18]. Cirrhosis is a possible complication and was successfully treated by liver transplantation in a 14-year-old child.

Neonatal Hemochromatosis [19, 20]. A few infants received liver transplantation with success. The recent availability of medical antioxidant therapy may limit the need for transplantation in the future [21].

Gaucher's Disease Type I and Niemann-Pick Disease Type A [22–24]. Four patients with Gaucher's or Niemann-Pick disease and cirrhosis received a

successful liver transplant. Reaccumulation of glucocerebroside or sphyngomyelin was not detected or was minimal in the transplanted liver.

Liver Disorders Associated with Severe Extrahepatic Complications

A small number of patients underwent liver transplantation for a genetic disorder of a liver-specific or liver-predominant function that would not result in severe permanent liver damage but rather in severe lesions of extrahepatic organs.

Type I Hyperoxaluria [25]. This is due to a deficiency of liver peroxisomal alanine:glyoxylate aminotransferase and results in renal lithiasis, nephrocalcinosis, and kidney failure. Current recommendations are that transplantation should be planned as soon as the glomerular filtration rate (GFR) is between 25 and 65 ml/mn per 1.73 m^2. Liver transplantation alone could be considered if the decline in kidney function is rapid. Alternatively combined kidney–liver transplantation should be carried out as soon as GFR decreases below 25 ml/mn per 1.73 m^2.

Crigler-Najjar Disease Type I [26]. The lack of detectable activity of liver glycuronyltransferase results in a permanently raised serum level of unconjugated bilirubin which is usually maintained at acceptable levels by home phototherapy; serum bilirubin may increase suddenly and unexpectedly with a risk of kernicterus. Liver transplantation is thus recommended before such neurological accidents occur. The treated patients display normal serum bilirubin levels and lead normal lives.

Familial Hypercholesterolemia [27]. This was treated by combined liver–heart transplantation in a few patients. Liver transplantation alone was successfully carried out in a 4-year-old boy with less than 2% low-density lipoprotein (LDL) receptor activity and serum cholesterol levels close to 700 mg/dl and resulted in long-term normalization of serum cholesterol and presumably prevention of atherosclerosis [28].

Homozygous Protein C Deficiency [29]. A 20-month-old child with severe thromboses was successfuly treated by liver transplantation.

Urea Cycle Disorders [30, 31]. A few patients with partial ornithine carbamyltransferase, carbamoylphosphate synthetase, or argininosuccinate synthetase deficiency were successfully treated with liver transplantation. Ammonemia remained normal on a normal diet.

Conclusions

These results indicate that liver transplantation can cure not only genetic disorders that present in the liver, but also liver disorders associated with extrahepatic pathology. Furthermore, a genetic defect present in all body cells such as familial hypercholesterolemia can be compensated by the mass of normal cells provided by liver transplantation. Moreover, the surprising decrease of extrahepatic deposits of amylopectin in glycogen storage disease type IV and of glucocerebroside in Gaucher's disease type I after liver transplantation opens the possibility that the microchimerism due to migration of lymphocytes/macrophages from the donor's liver to the host's extrahepatic sites result in cell-to-cell enzyme transmission [24]; this may not be true in all instances, however, as shown by some lipid storage diseases, where neurological involvement may not be prevented by liver transplantation [32].

In diseases that do not result in severe liver damage, gene therapy may provide a less risky solution in the future; auxiliary transplantation leaving the recipient's right lobe in place might prove a satisfactory way of awaiting the availability of gene therapy [33].

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Bone Marrow Transplantation

P.M. Hoogerbrugge and J.M.J.J. Vossen

CONTENTS

Allogeneic bone marrow transplantation (BMT) has been used successfully during recent decades for the treatment of patients suffering from hematologic malignancies and other diseases of cells of the hemopoietic system, e.g., aplastic anemia and severe combined immune deficiency (SCID) disease. Following appropriate conditioning of the recipient of an allogeneic bone marrow graft, his or her hemopoietic system is replaced by that of the bone marrow donor and a chimeric state is achieved. Engraftment of hemopoietic stem cells from an allogeneic donor requires intensive cytoreduction and immunosuppression of the immunologically competent recipient in order to create space, achieve engraftment, and prevent rejection of the graft. Pretreatment of children for BMT for inherited metabolic disorders consisted mostly of intensive pretreatment, e.g., by cyclophosphamide (50 mg/kg per day, ×4) and busulfan (4 mg/kg per day, ×4) [1]. Alternatively, total body irradiation or total lymph node irradiation was used. After allogeneic BMT a reverse rejection of the recipient tissue by the donor bone marrow graft, the so-called graft-versus-host disease (GvHD), must be prevented. Matching of donor and recipient for the major histocompatibility complex antigens (HLA antigens), T cell depletion of the graft, and total gastrointestinal decontamination and administration (post-transplantation) of methotrexate and/or cyclosporin A are effective in preventing or mitigating GvHD [2].

The majority of patients treated for inborn errors of metabolism suffered from SCID, which is a lethal disease in untreated children. Allogeneic BMT is the treatment of choice in children suffering from SCID. Survival is approximately 90% if marrow from HLA-identical sibling is available and approximately 50% if marrow from a non-HLA-identical sibling donor is used [3]. In contrast to the situation in patients suffering from SCID, the role of BMT in patients suffering from metabolic diseases which do not primarily affect the hemopoietic system is less clear. In the following pages, we will focus on the latter diseases. As for the patients, with SCID, the success of the BMT procedure in other inherited diseases largely depends on the type of bone marrow donor. Table 1 presents the outcome of BMT in a group of 76 patients reported to the files of the European Group for Bone Marrow Transplantation (EBMT). Following allogeneic BMT in a conditioned recipient, the engrafted hemopoietic stem cells give rise to progeny in all hemopoietic lineages, including the monocyte lineage, as precursors of tissue macrophages in the skin, lung, and liver tissue, for example [2]. Based on that experience, Hobbs [1] proposed that a bone marrow graft might serve as a permanent source for the missing enzyme α-iduronidase in the treatment of patients with Hurler disease.

Hypothetically, at least four different mechanisms may contribute to a beneficial effect of allogeneic BMT in the treatment of lysosomal storage disorders. In the first place, replacement of the enzymatically deficient cells by enzymatically normal cells may be important, especially in those diseases in which the mononuclear phagocytic cell system is primarily affected, e.g., Gaucher's disease [4].

A second mechanism by which BMT may be effective is the transfer of enzyme from the enzymatically normal, bone marrow-derived cells to deficient cells by direct cell–cell contact [5]. It can be expected that this effect of enzyme transfer by cell–cell interaction will be limited to tissues rich in bone marrow-derived cells, e.g., spleen, liver, lung, and skin.

Thirdly, BMT may be effective by release of enzyme into plasma, e.g., by disintegration of donor-derived white blood cells, which then may be taken up by enzymatically deficient cells [6].

Finally, a concentration gradient of storage product between the tissues and the plasma compartment may result from the breakdown of circulating substrate by the lysosomal enzymes present in white blood cells and tissue macrophages of donor origin. Such a gradient may improve clearance of the storage product.

One of the major issues concerning the role of BMT in metabolic diseases is whether metabolic correction in brain tissue occurs and whether this results in alleviation or prevention of symptoms. Circulating enzyme cannot pass the blood–brain barrier to enter brain tissue, but infiltration of donor-derived cells into brain tissue may contribute to metabolic correction [7]. Circulating enzyme can reach the leptomeninges, resulting in a decrease of storage in these structures [8], which may lead to a diminution of the hydrocephalus and associated neurological impairment.

Results in Patients Suffering from Lysosomal Storage Diseases

Since the first report on BMT in a patient suffering from Hurler disease (mucopolysaccharidosis I, MPS I) by Hobbs et al. [1], more than 150 patients with lysosomal storage diseases have been grafted. The majority suffered from MPS. Sixty-eight of these patients reported to the EBMT registry have been presented recently [9].

Mucopolysaccharidoses

Following bone marrow engraftment in MPS patients, the enzyme levels in white blood cells rose rapidly to donor levels, indicating stable hemopoietic engraftment. Another common phenomenon in all successfully grafted MPS patients was the rapid reduction of urinary excretion of glycosaminoglycans (GAG). Apart from these biochemical improvements, a marked regression of hepatosplenomegaly was found. Most patients transplanted for MPS suffered from MPS IH (Hurler disease; see Beck and Spranger, this volume).

Table 1. Outcome of bone marrow transplantation (BMT) for metabolic disease (see also [9])

Type of BMT	Patients (*n*)	Deaths from BMT complications (*n*)	Rejection (*n*)	Stable engraftment (*n*)
HLA=	53	5	6	42[b]
MUD	8	2	3	3
Haplo	15	3	9	3
Total	75	10	18[a]	47

HLA=, donor was a HLA-identical sibling; MUD, donor was a matched unrelated donor; Haplo, donor was haploidentical donor.
[a] Eleven patients still alive, one lost from follow-up, and six died.
[b] Four patients died as a result of disease progression and two were lost from follow-up.

In a long-term follow-up report from the Westminster group [10] on BMT in seven children with *MPS IH*, only minimal effect on bone deformities was reported: the lumbar vertebrae remained deformed and spinal growth was impaired. In the long-term surviving patients, psychomotor development has been studied by serial assessments. These tests showed deterioration in two patients, stabilization in four, and marked improvement in one patient [10]. Corneal clouding, although reduced, had not completely cleared in all patients after several years of follow-up. Although follow-up must be quite long to determine the effect of BMT in *MPS I*, a similar pattern was seen by others [9, 11]. The data of the EBMT indicate that BMT seldom results in improvement of skeletal or neurological symptoms that are already present at BMT. Whether BMT, if performed before the onset of clinical symptoms, can prevent the occurrence of these symptoms is still open to study.

The Minnesota BMT group reported that in ten of 13 surviving children transplanted for Hurler disease, the intellectual gain continues to fall below normal [12].

In nine children with Hurler disease reported by the EBMT neurological examination and brain imaging studies (magnetic resonance imaging, MRI; computed tomography, CT) revealed no or only minor abnormalities, except for a developmental quotient (DQ) below 70 in two children with MPS IH and the child with MPS IHS. The DQ did not change significantly after BMT in two

and improved dramatically from 70 to 96 in one child. Except for a decrease in dysostosis multiplex and a decrease of thoracolumbar gibbus in one child, stabilization of the skeletal symptoms occurred, but there was no improvement. A carpal tunnel syndrome was documented in four children; in none of them was clinical improvement seen after BMT. However, follow-up in these children ranges from 1.8 to 2.6 years, which is relatively short for definite conclusions.

The results of BMT in five long-term (>1 year) surviving patients with *Hunter disease (MPS II)* and in 14 patients with *Sanfilippo's syndrome (MPS III)* have been reviewed recently [13]. Stabilization of neurological symptoms was documented in two patients with Hunter disease. Most other patients were already severely retarded at BMT and showed progression of their disease thereafter.

In five patients transplanted for *Morquio's disease (MPS IV)*, the skeletal abnormalities did not improve despite proven donor cell engraftment [13].

One well-documented patient transplanted for *Maroteaux-Lamy syndrome (MPS VI)* in a progressive, life-threatening stage of the disease showed dramatic improvement of cardiopulmonary function [14]. Remodeling of skeletal lesions was not observed. At least six other patients have been treated, but the disease stages varied considerably and conclusions on the outcome cannot be drawn yet [13].

Lipidoses

As glucocerebroside is predominantly stored in the macrophages of patients with *Gaucher's disease*, it can be expected that replacement of macrophages results in diminishing of symptoms. Bone marrow aspirates and liver biopsies showed that the clearance of glucocerebroside-laden macrophages is a slow process. It has been clearly demonstrated that following successful BMT, physical anomalies, e.g., hepatosplenomegaly and growth retardation, diminish [15, 16]. In a follow-up period of more than 9 years, the growth rate improved and Gaucher cells disappeared from the bone marrow at 3 years after transplantation. The issue of splenectomy prior to BMT is not yet resolved. Better results have been reported in patients grafted following splenectomy than in patients grafted without prior splenectomy [17]. On the other hand, Ceredase, a modified, macrophage-targeted form of glucocerebrosidase, also results in the reduction of hepatosplenomegaly and improvement of hematologic parameters [18]. This drug may be used to decrease the splenomegaly prior to HLA-identical BMT. If no HLA-identical donor is available, Ceredase may be preferable to non-HLA-identical BMT. So far, beneficial effects have only been reported in patients with Gaucher's disease without neurological symptoms, both following BMT and lifelong Ceredase treatment. No data on neurological forms are available, but in a recent report of a patient who died of septicemia 2 years after BMT for non-neurological Gaucher's disease, no enzyme was found in brain tissue [19].

Two long-term surviving patients treated for *metachromatic leukodystrophy (MLD)* have been reported by Krivit et al. [20] and by Ladisch et al. [21, 22]. In the first patient, treated at 4 years of age, the brain stem evoked potentials and nerve conduction velocity have stabilized since the transplantation at levels that are slightly abnormal. The child's condition has not deteriorated. In the other long-term surviving patient, no detailed follow-up was presented after BMT. Included in the files of the EBMT registry are 11 patients who received a bone marrow graft for MLD. All six patients with clinical follow-up of more than 2 years were more or less severely neurologically affected at BMT. In these patients relief of symptoms was not reported. It is not known whether the occurrence of symptoms can be prevented by BMT before the onset of symptoms.

So far, two patients have been reported in detail following transplantation for *Niemann-Pick disease*. One patient with Niemann-Pick type B disease had hepatosplenomegaly without further symptoms. Following BMT, the size of liver and spleen decreased [24]. A patient with Niemann-Pick type IA disease had minimal developmental delay at transplantation, performed at 4 months of age. One year later, neurodevelopmental impairment progressed, and no decrease in hepatosplenomegaly had occurred; this patient died 30 months after BMT. Enzyme activity was practically undetectable in brain and liver tissue at autopsy [25].

Shapiro et al. [23] mentioned disease progression a stably engrafted patient with infantile *globoid cell leukodystrophy (Krabbe's disease)* who died 3 years after BMT due to an overwhelming infection. Stabilization and slight improvement was reported in a patient transplanted for

juvenile globoid cell leukodystrophy (follow-up more than 1 year).

Other Lysosomal Storage Diseases

Despite proper engraftment. BMT failed to halt neurological deterioration in patients with G_{M1} gangliosidosis [26], Sandhoff's, Tay-Sachs, and Farber's disease at various stages of the disease process [9]. Three patients suffering from *Pompe's disease* died shortly after successful engraftment was obtained [26–28]. In the patient reported by Harris et al. [28], who died at day 148 after BMT due to progression of the disease, enzyme activity in muscle tissue was not detectable at autopsy and accumulation of glycogen had not diminished, indicating that enzyme transfer had not occurred.

Conclusions

Despite the relatively large number of BMT performed in patients with lysosomal storage diseases, it is still unclear whether this experimental form of treatment is of any benefit. In contrast to inbred animal models, the natural course of the disease in humans suffering from lysosomal storage diseases is variable, sometimes even within one family. This calls for caution in evaluating the possible effects of BMT in individual patients.

Despite these caveats, various common patterns emerge from the data reported so far (Table 2):

- Enzyme activity determined in white blood cells and liver tissue after transplantation merely reflects stable engraftment and replacement of tissue macrophages in most patients. A reduction of storage material in liver and spleen indicates replacement of storage product-laden host macrophages by enzymatically competent donor-derived cells. These cells may also induce clearing of stored material from neighboring cells, e.g., hepatocytes. This may improve the well-being of patients with non-neurological forms of Gaucher's and Niemann-Pick disease, but is insufficient to improve the quality of life of patients with severe musculoskeletal or neurological involvement.
- Most authors agree that improvement of skeletal deformities in lysosomal storage diseases is absent following BMT. Detailed studies are necessary to evaluate whether transplantation very early in life, preferably before the onset of GAG accumulation, may prevent skeletal deformities.
- Neurological involvement is a major problem in the majority of patients with lysosomal storage diseases. So far, clear improvement of neurological symptoms has not been observed, but stabilization or prevention of neurological lesions by early BMT cannot be excluded. Studies in animal models indicate that BMT before the onset of neurological symptoms can prevent or delay the occurrence of symptoms, whereas no clear beneficial effect occurs when BMT is performed at the time symptoms are already present [29–31].
- The mortality and engraftment rate of allogeneic BMT depends largely on the type of donor used. This has to be considered when discussing whether or not a BMT is to be performed, especially if no HLA-identical sibling donor is available. It is hoped that with the increase in the knowledge of gene transfer into hematopoietic stem cells, somatic gene therapy may be available for these patients lacking an HLA-identical marrow donor to avoid the high mortality related to non-HLA-identical BMT.

Table 2. Effect of stable bone marrow engraftment on physical parameters (see also [9])

Disease	Symptom[a]	Patients (*n*)	Patients with improvement or stabilization (*n*)
MLD	Neurological	6	0
MPS 1H(S)	Chest deformities	4	3
	Dysostosis multiplex	6	4
	Gibbus	7	4
MPS III	Neurological	3	0
Gaucher	Bone pain	2	2
	Growth delay	4	4

[a] See text for details.

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Somatic Gene Therapy

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CONTENTS

Inborn errors of metabolism, like most diseases, have both genetic and environmental components. Current therapy for inborn errors of metabolism focuses on the diagnosis of a genetic defect before the onset of clinical symptoms, which allows changes to be made in the environment of the patient to prevent the progression of the pathological disease process. Dietary therapy and drug therapy are not directed at altering the genetic component of the inborn error of metabolism, but rather its environmental component. The prototype for this approach to treating inborn errors of metabolism is dietary therapy for phenylketonuria, in which the body's genetic inability to metabolize phenylalanine is treated by eliminating this amino acid from the environment. Such therapies are effective for some inborn errors of metabolism but not others, as described elsewhere in this volume. In contrast, organ transplantation seeks to alter the body's inherent (genetic) incapacity by replacing cells or organs which are genetically defective with a normal graft. While organ transplantation may be effective for many inborn errors of metabolism, the clinical risks and costs associated with transplantation have made this an uncommon therapy [1].

Somatic gene therapy for an inborn error of metabolism is directed at altering the intrinsic metabolic capacity of the body and modifying the genetic component of the disease. Gene therapy involves the use of genes as therapeutic molecules which may be introduced into the body to provide functions necessary to preserve health or treat disease. In the case of most inborn errors of metabolism, in which the inheritance of abnormal alleles for an essential enzyme leads to deficiency of certain metabolic functions, the purpose of somatic gene therapy is to introduce into the body a copy of a gene capable of expressing the essential enzyme and restoring the requisite metabolic function.

The process of gene therapy involves identifying and cloning genes involved in inherited metabolic disease, introducing these genes into the proper cell within the body where the metabolic function is required, and controlling the expression of the gene within therapeutic and safe levels. With the progress of the human genome project, genes for most inborn errors of metabolism have been, or are being, identified. Many different methods have been described for introducing genes into the body, and gene therapy has been performed successfully in many animal models of inherited disease. Clinical trials are currently underway for several model disorders, including adenosine deaminase deficiency, familial hypercholesterolemia, cystic fibrosis, and Gaucher's disease, and it is likely that clinical trials of gene therapy for other metabolic diseases will be proposed in the next several years [2–4].

With the advent of clinical trials, the promise of gene therapy is increasingly assessed against the criteria of real clinical need and clinical practicality. It is likely that gene therapy will become part of routine clinical care for indi-

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viduals with certain inborn errors of metabolism. For this to happen, however, gene therapies must be effective, safe, and superior to conventional pharmaceutical or surgical therapeutics; gene therapies must map to established clinical practice and build on existing clinical expertise, and the methods and products used for gene therapy must be acceptable to physicians, patients, regulatory authorities, and reimbursement agencies. Many methods for gene therapy which have been proposed may not meet these standards. This chapter reviews the current status of gene therapy in light of the clinical issues which will direct the application and acceptance of this important new technology in clinical practice.

Principles

The principle of gene therapy for inherited metabolic disease is that a gene can be introduced into cells in a patient to express an essential gene product (usually an enzyme) and restore a genetically defective metabolic function. A successful gene therapy requires three components:

- A *gene* which encodes a gene product capable of reversing the pathological progression of the disease
- A *vector* which causes the gene to be expressed by cells within the body
- A *vector delivery* method for introducing the vector into select cells within the body

Therapeutic Genes

In its simplest form, gene therapy for inherited metabolic diseases involves introducing a gene into a patient to express an enzyme which the patient lacks because of an inherited genetic defect. Examples of such therapy would be the replacement of phenylalanine hydroxylase in patients with phenylketonuria or the low-density lipoprotein (LDL) receptor in patients with familial hypercholesterolemia. This approach to gene therapy would be applicable to most autosomal recessive diseases and many X-linked diseases where one copy of the normal gene or in many cases even a fraction of normal enzymatic activity is known to prevent the pathological phenotype of the disorder. Autosomal dominant disorders would be more difficult targets for gene therapy, since simple replacement of a defective gene would not be effective, and methods to circumvent the toxic effects of the dominant products will be required.

In considering gene therapy for some metabolic diseases, however, it may not be technically feasible to introduce the normal gene into the patient or it may not be possible to introduce the therapeutic gene into enough cells in the particular tissue where enzyme activity is required. This may be particularly true when successful therapy requires genes to be placed into large number of cells within the body or into sequestered sites such as the brain. Thus, various alternative approaches to gene therapy may be considered. One alternative would be to constitute novel genetic functions to circumvent an inherited genetic defect. There are precedents for this approach in the administration of betaine to treat homocystinuria by activating alternative metabolic pathways. Another alternative would be to use gene therapy to alter the regulation of genes which are present in the body. For example, autosomal dominant diseases might be treated by inhibiting expression of a mutant allele, polygenic (multifactorial) diseases may be treated by restoring a balance between various genetic functions, and certain metabolic diseases might be treated by preventing the synthesis of metabolic precursors or inducing the elimination of toxic products. A precedent for this approach to therapy is the administration of Lovastatin, which causes an increase in LDL receptor levels and, consequently, the lowering of cholesterol levels. This approach to therapy may be increasingly important in the future as the genetic factors which regulate gene expression come to be more completely understood. Another alternative would involve using gene transfer as a form of enzyme replacement therapy. This would involve constituting expression of a gene product in one cell type with the expectation that the recombinant gene product would be secreted from these cells and taken up elsewhere in the body.

It should be noted that somatic gene therapy, as currently conceived, is not directed at repairing or replacing a defective gene in the body's cells or in the inherited genetic material. While methods for such gene replacement have been described in select animal models using techniques for homologous recombination, these technologies currently have little practical application in clinical practice.

It is apparent that the various approaches to gene therapy require in-depth understanding of metabolism and the role of metabolic pathways in health and disease. Gene therapy represents a form of metabolic engineering, the success of which will be dependent upon both metabolic and genetic functions. For example, certain enzymes will only function in cells that provide complementary subunits, substrates, or cofactors. Thus, phenylalanine hydroxylase may only function effectively in cells that provide the tetrahydrobiopterin cofactor [5], and the function of one component of a multicomponent enzyme such as propionyl-CoA carboxylase-α (PCCα) requires the concomitant presence of other components such as the β subunit (PCCβ) to constitute the active multimer [6]. One issue which will be particularly important in developing gene therapies is the presence of rate-limiting steps on multistep pathways. For example, overexpression of methylmalonyl-CoA mutase (MCM) does not result in higher than normal rates of propionate metabolism due to limits in the rate of substrate production [7]. It is likely that many metabolic pathways will have quantitative limitations introduced by rate-limiting processes, feedback regulation, and metabolic rigidity. It is this understanding of the metabolic consequences of gene expression that will determine how much enzyme needs to be expressed in how many cells, and which organs are the appropriate target for gene therapy. These are difficult issues. It is not necessarily true, for example, that expressing low-level enzyme activity in a large number of cells (as is the case in many mild forms of metabolic disease) will produce the same effect as expressing high levels of enzyme activity in a small population of cells. For certain metabolic diseases, it may not be true that expressing an enzyme in a heterotopic site, distant to the site of primary pathology, will have the same biological effect as expressing the enzyme in a site where the enzyme normally functions.

Thus, the identification of genes for gene therapy requires not only cloning of disease-related genes, but also an essessment of the deficient biological function and the consequences of introducing a recombinant enzyme into metabolic pathways. It is likely that considerable basic and clinical research in metabolism as well as molecular genetics will be required to fully understand the consequences of even the most simple forms of gene therapy.

Vectors

The function of a vector is to control how much of the therapeutic product is produced and where in the body it is produced. Effective gene therapy requires that a therapeutic gene be introduced into cells of the body in a form which allows the gene (DNA) to be transcribed into mRNA, for the mRNA to be properly processed within the cell, for the mRNA to be translated into a protein, and for this protein to be properly modified, to reach the appropriate compartment, and to properly function. This is achieved by constructing an *expression vector* which contains the genetic sequences encoding the therapeutic protein combined with genetic elements that control the processes of transcription, RNA splicing, translation, and post-translational modification of the protein. Often it is desirable to have expression of a gene controlled by its normal genetic elements to achieve "normal" patterns of expression. For example, it is likely that gene therapy for congenital hypothyroidism will require gene replacement into thyroid follicular cells as well as highly regulated expression to establish normal levels of thyroid hormone. In such cases the vector can be constructed using genetic elements that normally direct expression of a particular gene. Other times, however, it is desirable to express supraphysiological amounts of protein in a smaller number of cells or to express the protein in a heterotopic location where it is not normally expressed. For example, gene therapy for type I diabetes will require expression of insulin from cells other than the B cell. This requires the recombination of the gene with novel control elements in a chimeric vector containing genetic control elements derived from different genes.

The limiting factor in gene therapy is often achieving sufficient amounts of the product to exert a therapeutic effect. For this reason, many of the vectors currently being considered as potential therapeutics use genetic elements from viruses that are known to provide high levels of expression during viral infections. Another important factor is often the location of gene expression. Many genes in the body are normally expressed in a tissue-specific manner. The genetic elements that normally cause a gene to be expressed in one tissue, for example the liver, and not other tissues, do so by controlling the transcription of the DNA into mRNA. Thus, there are elements associated with tissue-specific genes such as phenylalanine

hydroxylase or ornithine transcarbamylase that restrict transcription of these genes to the liver and others which restrict the expression of hemoglobin to red blood cell precursors. These genetic elements, and others, have been identified and may be combined with virtually any gene to achieve restricted expression in certain tissues. Restricting gene expression to certain tissues and cells may be important to achieve a reproducible effect of gene transfer, since different tissues have different metabolic pathways and may respond differently to expression of a therapeutic protein.

It is also possible to design vectors to control the duration of gene expression. While gene therapy is often perceived to be a means for achieving a permanent cure for disease, it is increasingly recognized that permanent genetic manipulations may introduce unnecessary clinical risk in light of the inevitable complications of clinical care such as misdiagnosis, poor compliance, and reports of adverse events. Some vectors are designed to permanently integrate genes into the chromosomes of the host cell to achieve indefinite expression of the therapeutic product. This is usually accomplished by incorporating viral elements into the vector that allow the vector to be inserted into the chromosomes of the host cell in a random fashion. Other vectors may be designed to maintain the therapeutic gene as an extrachromosomal (episomal) element within the nucleus that can be replicated and repaired like a normal gene without integrating into the host's chromosome. It is possible in certain cell and animal models to insert genes into select locations using a technique termed homologous recombination based on the fact that a gene sequence introduced into a cell will preferentially integrate into cells at locations having matching (homologous) sequences. The low frequency of these events makes this approach to therapy currently impractical for gene therapy in vivo.

Increasingly, attention has turned away from the development of permanent gene therapies to the use of genes which do not persist indefinitely within the body, but rather will be eliminated from the body like conventional medicines, providing a finite duration of action and predictable pharmacokinetic properties. Such therapies might be administered repetitively, allowing the physician to adjust the frequency or dose of therapy or even change or terminate therapy, based on the patient's individual needs over time. For such therapies to be effective, they must have relatively low toxicity and be nonimmunogenic and the frequency as well as the route of administration must be compatible with achieving reasonable compliance.

Vector Delivery

Viral Vector Delivery. The initial strategies for gene therapy involved the construction of *viral vectors* in which the expression vector was packaged into a virus particle that was capable of infecting cells. The premise of developing viral vectors was that it might be possible to exploit, for therapeutic purposes, the highly evolved ability of viruses to introduce their genes into certain cells. To do this, strategies were developed for constructing attenuated or defective viral vectors that were capable of carrying therapeutic genes into cells but were incapable of further propagation in patients, and were incapable of inducing viral pathogenesis. Many different viruses have been proposed as vehicles for vector delivery, and most of the clinical trials performed to date employ viral vectors.

The viruses which have been most extensively studied for gene therapy are *retroviral vectors* derived from the murine leukemia virus [8]. To date, more than 100 patients have been treated with such viruses in clinical trials. These viruses have several properties that have been exploited for gene therapy. The most important is that it is possible to construct completely *defective* viruses which carry therapeutic genes but do not encode any viral proteins. This is done using a packaging cell line that provides all of the proteins necessary to assemble a viral particle. If a packaging signal is incorporated into the vector and the vector is introduced into the packaging cell line, then the vector will be packaged inside the otherwise empty particle produced by the cell line. The defective retrovirus produced by these cell lines is still capable of infecting cells and stably integrating the vector into the chromosomes of the host cell. The methods for producing defective retrovirus have been described in detail elsewhere [8].

Because retroviral vectors are commonly produced in relatively low concentrations and have a relatively low infectivity, these viruses are commonly employed in ex vivo strategies for gene therapy. Ex vivo therapy involves removing cells from a patient by a surgical procedure, growing these cells in the laboratory, infecting them with the retroviral vector, and then returning these

cells to the patient by autologous transplantation. While the ex vivo strategy is well suited for certain targets such as bone marrow or lymphocytes where autologous transplantation is well established, it is less well suited to many solid organs in which clinically tested methods for cellular transplantation are incompletely developed [9]. The recent work of Wilson and colleagues demonstrates that this method may be used to introduce genes into the liver of animals [10] and patients [11] at low frequency and may have a metabolic effect.

There is longstanding concern about the safety of retroviral vectors due to concern that the defective, therapeutic vectors may spontaneously recombine with naturally occurring retrovirus in the environment to give rise to new pathogens and to the risk associated with inserting a novel gene randomly into the genome, where it may disburb essential functions or activate proto-oncogenes [12]. An additional limitation of retroviral gene therapy is the requirement for replication of the target cell. Nondividing cells of many target organs are thus not appropriate targets for this form of gene therapy unless it is possible to safely stimulate replication. Nevertheless, retroviral vectors are currently important vehicles for vector delivery, and it is likely that additional clinical trials will begin to further evaluate their therapeutic potential.

Another vector which has been studied extensively and has entered clinical trials is the adenovirus. Adenoviruses have become an important research tool since it is possible to generate large numbers of viral particles, infect virtually any cell (dividing or nondividing) by direct administration in vivo, and achieve high levels of gene expression [13, 14]. Unlike retroviral vectors, adenoviral vectors are not completely defective, but are merely attenuated by removing certain pathogenic determinants. Also unlike retroviruses, genes delivered by adenoviruses do not persist indefinitely within the cell, but commonly exhibit a half-life of several weeks to months. The limitation of adenoviruses for clinical use relates to the fact that the current generation of adenoviral vectors exhibits substantial cytopathicity and immunogenicity, which may provide a narrow therapeutic index and make them unsuitable for routine clinical use. Moreover, the immunogenicity observed after i.v. administration of adenovirus may prohibit the repetitive dosing which would be required to treat chronic disease [14].

Many other viruses exhibit selective properties that are attractive for gene therapy and have been proposed as potential vehicles for vector delivery. These include the adeno-associated virus, which may be able to insert its genes into a select location in the genome rather than a random location like the retrovirus [15], as well as herpesvirus, which may be able to achieve latency in various cells providing an extended period of gene expression [16]. Research in many centers is aimed at reengineering these viruses into safe vehicles for vector delivery.

Cell Based Therapy. Organ transplantation is itself a form of gene therapy in which allogenic cells containing a normal gene are implanted into the body. Genetic manipulation may be used to enhance transplantation therapy, both, by allowing genetic correction of autologous cells which can then be transplanted into patients, or by allowing modification of allogenic or xenogeneic cells to make them less antigenic. These methods continue to be limited by the lack of established clinical methods for cellular transplantation.

DNA Vector Delivery. It is also possible to deliver DNA vectors directly to cells without the use of viruses. This involves the application of pharmaceutical methods for drug delivery to target DNA vectors to specific cells after direct administration to patients and to enhance the process by which DNA vectors are taken into cells and traffick to the nucleus where transcription can take place. In particular, pharmaceutical experience with liposomes and other particulate drug carriers may be employed in drug delivery [17].

Various different approaches have been described for DNA delivery, including the use of purified DNA administered directly into the body by intramuscular injection [18], the use of ballistic particles coated with DNA [19], the use of cationic lipids [20], and the use of various ligands to target DNA to specific cell types [21] and enhance the entry of DNA into the cell [22]. Many different methods for direct DNA delivery are currently being explored. For example, direct intramuscular injection leads to expression of genes by muscle cells and might be used to express proteins such as dystrophin [18]. Asialoglycoproteins may be coupled to DNA and used to direct DNA expression vectors to liver cells to treat diseases such as familial hypercholesterolemia [23] or methylmalonic acidemia [24]. Several clinical trials are

currently underway using drug delivery methods for gene therapy of cancer and cystic fibrosis. These nonviral methods may offer a greater margin of safety and clinical acceptance than viral vectors. The progressive pharmaceuticalization of vector delivery may also result in products that can be manufactured, distributed, and administered in routine clinical practice like conventional medicines.

Clinical Applications

Current Clinical Trials

Since the commencement of the first clinical trial involving gene transfer into human subjects in 1989, more than 100 patients have been treated and more than 70 clinical trials are underway. Many of the ongoing clinical trials are *gene-marking* trials, in which genes are introduced into cells prior to transplantation to study their fate and function. The majority of current clinical trials are aimed at gene therapy for cancer. Others are aimed at exploring the potential of gene therapy for inborn errors of metabolism including adenosine deaminase deficiency, familial hypercholesterolemia, Gaucher's disease, and cystic fibrosis.

The first therapeutic trials involved the introduction of a gene for adenosine deaminase into the cultured lymphocytes from children with severe combined immunodeficiency secondary to adenosine deaminase deficiency. These cells were subsequently transfused into patients to provide essential immunological function. A second trial involved the introduction of a gene for the LDL receptor into cultured hepatocytes from patients with familial hypercholesterolemia and subsequent transplantation of these genetically reconstituted cells into the liver. Both of these trials employed retroviral vectors. More recently, trials for cystic fibrosis have begun using several adenoviral vectors as well as DNA vectors instilled directly into the trachea to achieve expression of the CFTR gene in the lung. Several centers have also proposed therapy for Gaucher's disease using an ex vivo procedure in which bone marrow is harvested from patients with severe forms of the disorder, infected with retroviral vectors to replace the defective gene, and then transplanted back into the patient.

Clinical Indications

The first clinical trials using retroviral vectors demonstrated the feasibility and social and regulatory acceptance of gene transfer into human subjects. The initial trials have greatly expanded interest in gene therapy and led to a more focused analysis of the potential clinical indications for gene therapy. To date, the number of patients treated is still too small to fully assess the safety or efficacy of either retroviral vectors or any specific gene therapy.

Gene replacement is now commonly performed in research laboratories studying inborn errors of metabolism, and more than 100 diseases have been "cured" in experiments performed in cells cultured from affected patients. The list of potential diseases for gene therapy now encompasses virtually any inborn error of metabolism for which the gene has been cloned, the necessary level of expression of the therapeutic gene is achievable, and the target cell is accessible. Gene delivery to the liver and certain bone marrow-derived cells has been achieved, and a variety of metabolic diseases which affect these organs may be considered realistic candidates for gene therapy. At the present time, however, the fraction of cells in any organ that can be effectively targeted by vector delivery is limited, and the levels of expression that have been achieved in vivo are relatively low.

With the increased understanding of, both, the potential and limitations of gene therapy, several diseases which were initially considered attractive candidates for gene therapy are now considered less suitable. For example, current methods may not produce sufficiently high expression of hemoglobin to treat thalassemia, and the difficulty of vector delivery to the central nervous system makes gene therapy for Lesch-Nyhan disease more distant. At the same time, there is a growing realization that gene therapy may be relatively safe and that such therapy does not need to be restricted to end-stage diseases or disorders in which there is no other approach to therapy. Gene therapy may be considered for any disease where the potential of gene therapy is considered to be favorable relative to the risk, cost, and benefit of conventional therapeutics or other experimental therapies involving conventional drugs, advanced drug delivery methods, or biological products. With the development of increasingly safe and

effective methods for vector delivery, it is likely that there will be clinical trials for a large number of inherited metabolic diseases rather than a select list of model disorders.

Regulatory Considerations

The intense social discourse concerning the propriety and ethical implications of gene therapy has been largely resolved with the realization that somatic gene therapy is in principle no different than replacing deficient functions with enzyme replacement therapy or organ transplantation. Because of intense social concern generated by gene therapy, clinical trials in the United States are reviewed in public by the Recombinant Advisory Committee (RAC) of the National Institute of Health as well as by the Food and Drug Administration (FDA) through the traditional IND (Investigational Drug) process [25, 26]. A similar review process has been established by many European countries and the European Union. It should be noted that genetic manipulation of the inherited germ line (sperm or eggs) continues to raise profound social and ethical questions, and clinical trials of such technologies are explicitly excluded under current regulations in the United States and many other countries.

As public concern about gene therapy in general has abated, there has been increased focus on the clinical and regulatory issues involved in developing gene therapies for clinical application [27–29]. The initial clinical trials have been largely academic efforts to assess the feasibility of gene transfer into human subjects. These trials have used various vector delivery technologies and have focused on end-stage diseases such as cancer. Such trials provide little information about the pharmacology and toxicology of gene therapies, issues which have to be studied in a more normal physiological context. Most of these trials have also not had control groups to allow any significant assessment of effectiveness.

Future clinical trials will assume a different focus. These trials will increasingly resemble trials of conventional pharmaceuticals, designed to provide a critical assessment of therapeutic products leading to regulatory approval for marketing and clinical use [26]. Such studies must be rigorous, ethical, controlled, statistically significant, and cost-effective and must be coupled with the development of methods for manufacture of gene therapy products. These trials will follow the conventional guidelines of investigation and regulatory review.

Towards Clinical Applications

The efficacy of gene therapy will ultimately need to be assessed and demonstrated relative to conventional therapeutics. While it is widely believed that many inborn errors of metabolism may come to be best treated by gene therapy, there will also be significant advances in the development of conventional pharmaceutical products, biological products such as enzyme replacement, and transplantation, including the potential for inducing tolerance to allogeneic or xenogeneic grafts. The question "Why gene therapy?" must be answered by showing that proposed gene therapies are more effective and safer than other therapies, provide adequate compliance, and are cost-effective relative to other therapies. The present focus of gene therapy is on demonstrating that gene replacement is feasible and can be effective. Extensive work remains to be done to demonstrate the safety and practicality of gene therapy. This requires increased attention to the pharmacology, toxicity, manufacture, and economics of gene therapy in addition to the biology.

It is already apparent that the economics of gene therapy of inherited metabolic diseases need to be considered in detail in developing a gene therapy. While gene therapy has been proposed for many inborn errors of metabolism, such therapies require the development of products which are clinically validated and manufactured for clinical use. Moreover, once gene therapy is commenced, there will be an ethical imperative to make these products available on a continuous basis to treat current and future patients. Academic centers which are currently involved in gene therapy research may have a limited capacity for production and a limited commitment to do so indefinitely once such therapies move beyond the research phase. The "orphan drug" law in the United States was designed to encourage industrial interest in rare diseases. Even this incentive is unlikely to encourage the development of gene therapies for more than a handful of the hundreds of genetic disorders potentially amenable to gene therapy. It is unlikely that gene therapies will be-

come available on a commercial or ongoing basis for most of the rare inborn errors of metabolism for which it has been proposed.

Conclusion

Gene therapies are currently being studied in more than 50 clinical trials. This method holds considerable promise for many inborn errors of metabolism. Gene therapy for inborn errors of metabolism requires the development of biologically sound strategies for correcting metabolic defects, vectors for controlling the expression of therapeutic genes, and methods for delivering vectors safely to patients. The clinical applicability of gene therapy will require increased attention not only to the potential effectiveness of gene delivery as a therapeutic modality, but to clinical issues including safety, pharmacology, toxicology, compliance, and economics. Extensive clinical investigation will be required to assess the role of gene therapy relative to conventional and evolving pharmaceutical, biological, and surgical methods.

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