LABORATORY TECHNIQUES for the DETECTION of HEREDITARY METABOLIC DISORDERS

V. E. Shih

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Author:

VIVIAN E. SHIH, M.D.
Assistant Professor of Neurology
Massachusetts General Hospital
Boston, Massachusetts

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PREFACE

The application of biochemical techniques for the detection of abnormal compounds in metabolic disorders has become increasingly popular in the past decade. The purpose of this book is to provide a disease-oriented rather than technique-oriented approach, and emphasis is on the detection rather than the extensive study of the disease. Only the commonly used methods are described here. No attempt has been made to cover all the laboratory tests for special problems. There are several books dealing primarily with the techniques of chromatography of physiologic fluid which are referenced in Chapter 1.

I was fortunate to have worked with Mary L. Efron, a most inspiring teacher, who left us a wealth of knowledge, both published and unpublished, and to have been associated with the dynamic Massachusetts metabolic disorder screening program directed by my colleague, Harvey L. Levy. Experiences gained from both these laboratories are reflected in this book.

Over the past few years investigators from various countries who have visited our laboratory have often asked us to write out the routine procedures for screening for metabolic disorders. I hope that this book will be useful to those interested in this field.

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THE AUTHOR

Vivian E. Shih, born on December 27, 1934, in China, graduated from National Taiwan University College of Medicine in 1958. She then came to the United States. After having completed an internship at Edgewater Hospital, Chicago, in 1959 and a residency training in pediatrics at Philadelphia General Hospital, Philadelphia, from 1960 to 1962, she took a fellowship in pediatric neurology at the Children's Hospital of Philadelphia from 1962 to 1965 and furthered her training in the field of amino acid metabolic disorders with Dr. Mary L. Efron at the Massachusetts General Hospital from 1965 to 1967. She has since engaged in the research in inborn errors of metabolism, both the clinical and biochemical aspects. She is now an assistant professor in neurology at Harvard Medical School in charge of the amino acid disorders laboratory, Joseph P. Kennedy, Jr. Memorial Laboratories, Neurology Service, Massachusetts General Hospital, and a consultant to the Massachusetts Metabolic Disorders Program, Institute of Laboratories, Department of Public Health of Massachusetts.

Dr. Shih is certified by the American Board of Pediatrics, and is a member of the Society for Pediatric Research and the Massachusetts Medical Society.

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Chapter 1

INTRODUCTION AND SUMMARY OF CLINICAL FINDINGS IN INBORN ERRORS OF METABOLISM

INTRODUCTION

The study of inborn errors of metabolism is a rapidly growing field. Hereditary metabolic disorders have been discovered by leaps and bounds in recent years; this is largely attributable to the development of simple laboratory techniques. In the mid-1940's Dent introduced paper chromatographic techniques for the study of amino acids in urine[1] to clinical medicine, a milestone in the history of biochemical genetics. Using this technique, Professor Dent's group discovered several amino acid metabolic disorders and aroused interest in these problems. Since the early 1960's there has been a great impetus toward the research of mental retardation. With improvement of the chromatographic techniques and development of new microbiological assays, large-scale screening for amino acid abnormalities became possible. This was conducted initially among the mentally retarded population and patients with neurologic abnormalities, and resulted in the discovery of many previously unknown metabolic defects.

More recent developments in the gas-liquid chromatographic techniques for organic acid analysis have contributed to the discovery of several disorders in organic acid metabolism. Wider application of such techniques will no doubt reveal additional metabolic disorders.

The application of biochemical analyses to the study of metabolic disorders has also helped to distinguish the different types of "variants" of a clinical syndrome as different diseases; the group of mucopolysaccharidoses is a good example.

Techniques for the analysis of lipids and the enzymes in their metabolism are complicated and time-consuming and are, therefore, not suitable for general screening for lipid storage diseases, except when they are performed in a specialized laboratory. Testing for metabolic abnormalities in patients with an undiagnosed disease has become an accepted medical practice. The correct diagnosis of these disorders allows the physician to employ the appropriate therapeutic measures and genetic counseling. Furthermore, the newborn screening programs for the early detection of these metabolic disorders are instrumental in preventing development of brain damage.

This book will deal primarily with the methods of screening for disorders affecting the nervous system and for which treatment is available. Only techniques that can be set up at a "screening laboratory" are described. Those which are complicated and are rarely used at a general screening laboratory will not be included.

There are several books on the basic principles and specific techniques of the various chromatographic methods frequently used in screening; these are given as general references.[2-8]

SUMMARY OF CLINICAL FINDINGS IN INBORN ERRORS OF METABOLISM

Tables 1-1 to 1-6 summarize the main clinical features and abnormal metabolites found in various metabolic disorders.[9,10] It should be cautioned that the clinical picture may be somewhat biased due to the fact that many of these disorders were discovered by screening patients with mental retardation, neurological diseases, or other problems, and may not be representative of the whole spectra of the disorder.

Table 1-7 lists the metabolic disorders that can manifest themselves as "acute" problems in the neonatal period or in the first few weeks of life. Many of the symptoms and signs are nonspecific and may be mistakenly diagnosed as birth injury or sepsis. If the underlying metabolic cause is not recognized and proper treatment not given, a fatal course may result. These metabolic disorders should always be considered in the differential diagnosis in an acutely ill patient.[11]

TABLE 1-1

Amino Acid Metabolic Disorders

Disorder	Clinical findings	Abnormal metabolites
Aromatic amino acids:		
Phenylketonuria (PKU) (classical or typical)	Mainly mental retardation; eczema and a musty odor may be present.	Markedly increased phenylalanine in blood (> 20 mg/dl) and urine. Increased phenylpyruvic acid, phenylacetic acid, phenyllactic acid, and O-hydroxyphenylacetic acid in urine.
Atypical PKU (Severe hyperphenylalaninemia)	Asymptomatic or mental retardation.	Moderate to marked increase in blood phenylalanine (15 to 20 mg/dl). Phenolic acid derivatives may be present.
Persistent mild hyperphenylalaninemia	Asymptomatic.	Mildly increased phenylalanine in blood (< 12 mg/dl) and urine.
Tyrosinosis (Hereditary tyrosinemias)		Increased tyrosine in blood, more marked in the type without hepatorenal dysfunction.
without liver disease	One case with myasthenia gravis. Other cases with mental retardation.	Increased tyrosine and its metabolites (*p*-hydroxyphenylacetic acid, and *p*-hydroxyphenyllactic acid) in urine.
with liver disease and renal tubular dysfunction	Failure to thrive, vomiting, and diarrhea, rickets, hepatosplenomegaly.	Fructosuria may be present in patient with tubular dysfunction.
Tryptophanuria with dwarfism	Developmental retardation, dwarf, photosensitivity, ataxic gait (1 case).	Borderline increased tryptophan in blood. Increased tryptophan in urine.
Hydroxykynureninuria	Bloody diarrhea, hemolytic anemia, hepatosplenomegaly, mental retardation (1 case).	Kynurenine, 3-hydroxykynurenine, xanthurenic acid.
Histidine and imidazole-dipeptide histidinemia	Asymptomatic, also found in mentally retarded patients.	Increased histidine in blood and urine; imidazole derivatives of histidine (imidazole-acetic acid and imidazole-lactic acid) in urine.
Carnosinemia and carnosinuria	Mental retardation, seizures.	Increased carnosine in urine.
Sulfur amino acids:		
Homocystinurias		
Cystathionine synthase deficiency	Dislocated lenses, mental retardation arachnoedactyly, skeletal abnormalities, thromboembolic phenomena.	Increased methionine, homocystine, and homocystine-cysteine disulfide and other derivatives of sulfur amino acids in blood and urine.
B_{12} Coenzyme metabolic defect	Failure to thrive, early death. May be asymptomatic.	Increased homocystine, homocysteine-cysteine disulfide and cystathionine in blood and urine. Normal or low blood methionine. Methylmalonic acid in urine.
$N^{5,10}$-Methylenetetrahydrofolate reductase deficiency	Seizures, muscle weakness (1 case). Episodic schizophrenic attacks (1 case).	Amino acid changes same as above except no methylmalonic aciduria.

TABLE 1-1 (Continued)

Disorder	Clinical findings	Abnormal metabolites
Cystathioninuria (Cystathionase deficiency)	Asymptomatic.	Cystathionine in blood and urine.
β-mercaptolactate-cysteine disulfiduria	Mental retardation (1 case).	β-mercaptolactate-cysteine disulfide and (?) low taurine in urine.
Sulfite oxidase deficiency	Mental retardation, decerebrated posture, dislocated lenses (1 case).	S-sulfocysteine, sulfite, and thiosulfate in urine.
Branched chain amino acids:		
Maple syrup urine disease	Early onset of seizures, feeding difficulties, ketosis, mental retardation, the presence of maple syrup odor. Several variants from mild to severe degree are known. Patients with the intermittent form have episodic occurrences of symptoms.	Increased leucine, isoleucine, alloisoleucine, and valine in blood and urine. Increased branched chain α-keto acids in urine. Above metabolites appear only during "attacks" in the intermittent form.
Hypervalinemia	Vomiting, failure to thrive, nystagmus, and hyperkinesia (1 case).	Increased valine in blood and urine.
Urea cycle and ammonia metabolism:		
Carbamyl phosphate synthetase deficiency	Early onset of vomiting, lethargy, intolerance to milk, acidosis, marked hyperammonemia (1 case).	Markedly increased blood ammonia. Increased blood and urine glutamine.
Ornithine carbamyltransferase deficiency	Early onset of episodic vomiting, lethargy, intolerance to formula, mental retardation, marked hyperammonemia. Death in neonatal period.	Markedly increased blood ammonia. Increased blood and urine glutamine.
Citrullinemia	Mental retardation. Hyperammonemia. Death in neonatal period.	Increased citrulline in blood and urine. Increased blood ammonia.
Argininosuccinic aciduria	Three types: 1. Neonatal onset of seizures, respiratory distress, progressive lethargy, and early death. 2. Enlarging liver, intolerance to formula, seizures, mental and physical retardation, trichorrhexis nodosa. 3. Mental retardation, seizures, and trichorrhexis nodosa.	Argininosuccinic acid in blood and urine. Intermittent postprandial hyperammonemia.
Hyperargininemia	Mental retardation and seizures (2 siblings reported).	Increased arginine and ammonia in blood. Increased urinary arginine, ornithine, lysine, and cystine (a pattern similar to cystinuria).
Hyperornithinemias		
Type I	Myoclonic seizures and mental retardation (1 case).	Markedly increased ornithine and ammonia in blood. Increased homocitrulline in urine.
Type II	Liver disease, renal tubular dysfunction and mental retardation (1 family).	Mildly increased blood ornithine.
Familial protein intolerance	Vomiting, diarrhea, growth failure, hepatosplenomegaly, hyperammonemia associated with a protein-rich diet.	Markedly increased lysine and slightly increased arginine in urine.

TABLE 1-1 (Continued)

Disorder	Clinical findings	Abnormal metabolites
Lysine:		
Hyperlysinemia	Mental retardation and synophrys.	Increased lysine and homoarginine in blood. "Cystinuria" pattern, pipecolic acid, homoarginine and acetyl-lysines in urine.
Congenital lysine intolerance	Early onset of vomiting, convulsion, coma, and intolerance to protein feeding (1 case).	Periodic increase in blood lysine and arginine. Hyperammonemia.
Saccharopinuria	Mental retardation, dwarfism. Spastic diplegia.	Increased saccharopine, homocitrulline, lysine, and citrulline in blood. Increased saccharopine, lysine, homocitrulline, citrulline, homoarginine, and aminoadipic acid in urine.
Hyperpipecolatemia	Feeding problem, hepatomegaly, progressive mental retardation, hypotonia and nystagmus (1 case).	Pipecolate in blood, very little in urine.
Hydroxylysinuria	Mental retardation and seizures.	Hydroxylysine and acetyl-lysines in urine.
Glycine:		
Nonketotic hyperglycinemia	Mental retardation, hyperactivity.	Increased glycine in blood and urine.
Ketotic hyperglycinemia (Propionic acidemia)	Ketosis of early onset, growth and development retardation.	Increased glycine and propionic acid in blood and urine.
Sarcosinemia	Mental retardation.	Sarcosine in blood and urine.
Imino acids:		
Hyperprolinemia		
Type I	Mental retardation, seizures, renal disease.	Increased proline in blood. Increased proline, hydroxyproline, and glycine in urine.
Type II	Mental retardation, seizures.	Increased proline in blood. Increased proline, hydroxyproline, and glycine in urine. In addition, increased Δ-pyrroline-5-carboxylate in urine.
Hydroxyprolinemia	Probably asymptomatic.	Hydroxyproline in blood and urine.
Miscellaneous:		
β-alaninemia	Uncontrollable seizures. Somnolence (1 case).	Increased β-alanine and γ-aminobutyric acid in blood; increased β-alanine, β-aminoisobutyric acid, γ-aminobutyric acid and taurine in urine.
Hypophosphatasia	Rickets or osteomalacia.	Phosphoethanolamine.
Pseudohypophosphatasia	Rickets or osteomalacia.	Phosphoethanolamine.
Hyperalaninemia pyruvate decarboxylase deficiency	Intermittent ataxia, choreoathetosis, mental retardation.	Increased pyruvate, and alanine in blood and urine during attacks.

TABLE 1-1 (Continued)

Disorder	Clinical findings	Abnormal metabolites
Pyruvate carboxylase deficiency	Progressive and relapsing episodes of anorexia, hypotonia, hyporeflexia, ataxia; mental retardation (Leigh's encephalomyelopathy).	Increased lactate and alanine in blood during attacks.
Aspartylglycosaminuria	Mental retardation, coarse features, seizures	Aspartylglycosamine in blood and urine.
Pyroglutamic aciduria	Mental retardation, episodic vomiting, spastic quadriparesis, ataxia (1 case).	Large amount of pyroglutamic acid (not detectable by amino acid screening).

TABLE 1-2

Summary of Amino Acid Transport Disorder

Disorder	Clinical findings	Abnormal metabolites
Hartnup disease (Neutral aminoaciduria)	May be entirely normal. May have intermittent ataxia, photosensitive rashes, mental retardation, psychosis.	Marked increases in urinary neutral amino acids (alanine, threonine, serine, glutamine, histidine, isoleucine, leucine, valine, phenylalanine, tyrosine, tryptophan). Methionine excretion variable. Urinary excretion of indole derivatives and stool amino acids were increased in some cases.
Methionine malabsorption	Mental retardation, convulsions with abnormal electroencephalogram. White hair, an unpleasant odor (2 cases).	Intermittent methioninuria; urinary excretion of α-hydroxybutyric acid; increased methionine in stool.
Blue diaper syndrome (tryptophan malabsorption)	Failure to thrive, recurrent infections, hypercalcemia, nephrocalcinosis, blue diaper (1 family).	Increased excretion of indole derivatives including indole-acetamide, indolactic acid, indole acetylglutamine, and indican.
Cystinuria (Type I to III)	Urinary stone.	Increased cystine, lysine, ornithine, and arginine. Heterozygotes of types II and III have increased cystine lysinuria.
Isolated cystinuria	Hypoparathyroidism hypocalcemic tetany.	Increased urinary cystine only.
Dibasic aminoaciduria	Asymptomatic.	Lysine, arginine, and ornithine.
Iminoglycinuria	Asymptomatic.	Increased urinary excretion of proline, hydroxyproline, and glycine; heterozygotes have increased urinary glycine.
Miscellaneous syndromes		
Fanconi syndrome	Variable, renal tubular acidosis and hypophosphatemic rickets.	Generalized hyperaminoaciduria, glycosuria.
Lowe syndrome (Oculocerebrorenal syndrome)	Mental retardation, cataract and other eye anomalies, renal tubular acidosis.	Generalized hyperaminoaciduria, glycosuria.
Glucoglycinuria	Asymptomatic.	Increased glucose and glycine excretion in urine.

TABLE 1-3

Disorders in Sugar Metabolism

Disorder	Clinical findings	Abnormal metabolites
Galactose:		
Galactosemia (Gal-1-phosphate transferase deficiency)	Failure to thrive, vomiting, intolerance to milk, hepatomegaly, jaundice, cataract, and mental retardation.	Galactose and galactitol in blood and urine. Galactose-1-phosphate in erythrocytes.
Galactokinase deficiency	Cataract.	Galactose and galactitol in blood and urine.
Fructose:		
Hereditary fructose intolerance (fructose-1-phosphate aldolase deficiency)	Two clinical types: (1) Early onset; vomiting, failure to thrive, hypoglycemia, bleeding tendency, renal tubular dysfunction (Fanconi's syndrome), and early death. No rickets. (2) Late onset; less severe, aversion to sweet food, hypoglycemia, hypoprothrombinemia; may be asymptomatic.	Fructosuria; generalized aminoaciduria; in some cases, hypermethioninemia, hypertyrosinemia, and hypertyrosyluria.
Fructose diphosphatase deficiency	Feeding difficulties, failure to thrive, jaundice, hepatomegaly, hypoglycemia, and hypotonia.	Lactic acidemia.
Essential fructosuria (fructokinase deficiency)	Asymptomatic.	Fructosuria after ingestion of fructose-containing food.
Galactose and fructose:		
Familial galactose and fructose intolerance	Severe hypoglycemia induced by fructose and galactose (1 family).	Fructose and galactose, generalized increases in amino acid excretion after fructose and galactose loading.
Pentose:		
Essential pentosuria	Asymptomatic, mainly in Jewish persons.	Large amounts of xylulose, little xylose in urine.
Lactose:		
Lactose intolerance (without lactase deficiency)	Diarrhea, vomiting, cachexia, renal acidosis.	Lactose in urine and generalized aminoaciduria.
Lactose intolerance (lactase deficiency)	Failure to gain weight in infancy; dislike for milk in adults.	Lactose in urine, variable amount.
Sucrose:		
Sucrose intolerance (sucrase deficiency)	Diarrhea, intolerance to table sugar and sweets.	Sucrose in urine and stool.

TABLE 1-4

Disorders in Sugar Transport

Disorder	Clinical findings	Abnormal metabolites
Renal glycosuria	Asymptomatic.	Glucose in urine.
Glucose galactose malabsorption	Severe diarrhea, dehydration, intolerance to all dietary carbohydrates, failure to thrive.	Glucose in stool and mild glycosuria intermittently.
Glucoglycinuria	Asymptomatic.	Increased glucose and glycine in urine.

TABLE 1-5

Disorders in Organic Acids

Disorder	Clinical findings	Biochemical abnormalities
Isovaleric acidemia (isovaleryl CoA dehydrogenase deficiency)	Recurrent vomiting, acidosis, mental retardation, "sweaty feet" odor.	Isovaleric acid and isovalerylglycine in blood and urine; may have hyperglycinemia.
β-Methylcrotonylglycinuria and β-hydroxyisovaleric acidemia (β-methylcrotonyl CoA carboxylase deficiency)	Muscular hypotonia, feeding difficulties, motor retardation, peculiar odor in urine (like that of cat's urine) (1 case).	β-hydroxycrotonyl-glycine and β-hydroxyisovaleric acid in urine.
Propionic acidemia (propionyl CoA carboxylase deficiency)	Recurrent ketoacidosis, protein intolerance, mental retardation, leucopenia, thrombocytopenia, and hypogammaglobulinemia.	Increased propionic acid and glycine in blood and urine; hyperammonemia may be present.
α-Methyl-acetoacetic acidemia (β-ketothiolase deficiency)	Recurrent ketosis, neutropenia, thrombopenia.	Increased α-methyl-acetoacetic acid, α-methyl-B-hydroxybutyric acid in blood and urine; hyperammonemia and hyperglycinemia may be present.
Methylmalonic acidemias:		
Defect in methylmalonyl CoA mutase	Recurrent ketoacidosis, mental retardation, recurrent infection, osteoporosis, neutropenia.	Increased methylmalonic acid and glycine in blood and urine.
Defect in B_{12}-coenzyme metabolism.	Recurrent ketoacidosis, mental retardation, recurrent infection, osteoporosis, neutropenia. Responsive to B_{12} therapy.	Increased methylmalonic acid and glycine in blood and urine.
Defect in methylmalonyl CoA racemase	Metabolic acidosis, coma, and neonatal death (1 case).	Increased methylmalonic acid in blood and urine; hyperammonemia.
Pyruvic acidemia and hyperalaninemia (pyruvate decarboxylase deficiency)	Intermittent ataxia, choreoathetosis, mental retardation.	Increased pyruvate, alanine in blood and urine, more marked during attacks; slight increase in lactic acid; presence of inhibitor of thiamine triphosphate synthesis in blood and urine.
Lactic acidemia and hyperalaninemia (pyruvate carboxylase deficiency)	Subacute necrotizing encephalomyelopathy (Leigh's), progressive and relapsing muscle weakness, hypotonia, hyporeflexia, ataxia, seizures; may be retarded.	Increased lactic acid and alanine in blood and urine; presence of inhibitor of thiamine triphosphate synthesis.
Pyroglutamic aciduria	Mental retardation, episodic vomiting, spastic quadriparesis, ataxia (1 case).	Large amount of pyroglutamic acid.

TABLE 1-6

The Mucopolysaccharidoses (Classification of McKusick)

Disorder		Clinical findings					Abnormal metabolites in urine
Type	Syndrome	Mental retardation	Skeletal deformities	Corneal opacity	Genetics	Hepatosplenomegaly	
I	Hurler's	Severe	Marked	+	Autosomal recessive	+	Dermatan sulfate Heparin sulfate
II	Hunter's	Moderate	Marked	–	Sex-linked recessive	+	Dermatan sulfate Heparin sulfate
III	Sanfilippo's	Severe	Mild	?	Autosomal recessive	+	Heparin sulfate
IV	Morquio's	–	Severe, marked spondyl-epiphyseal dysplasia	±	Autosomal recessive	+	Keratan sulfate
V	Scheie's	–	Mild	Severe	Autosomal recessive	Hepatomegaly	Dermatan sulfate
VI	Maroteaux-Lamy	–	Marked	+	Autosomal recessive	+	Dermatan sulfate
VII		+	Similar to Morquio's	+	Autosomal recessive	+	Keratan sulfate-like material and dermatan sulfate

TABLE 1-7

Metabolic Disorders with Onset of Clinical Manifestations in Neonatal Period or Early Infancy

Disorders	Vomiting, acidosis	Poor feeding FTT	MR	Other neurological abnormality	Liver dysfunction	Renal tubular dysfunction	Odor
Maple syrup urine disease	+	+	+	+	–	–	Maple syrup
Ketotic hyperglycinemia	+	+	+	±	–	–	–
Nonketotic hyperglycinemia	–	–	+	+	–	–	–
Argininosuccinic-aciduria	±	+	+	+	+	–	–
Congenital hyperammonemias	+	+	+	+	+	–	–
Citrullinemia	+	+	+	+	–	–	–
Hyperornithinemia type II	–	+	+	–	+	+	–
Hereditary tyrosinemia	–	+	+	–	+	+	±
Isovaleric acidemia	+	+	+	+	–	–	Sweaty feet
Methylmalonic acidemia	+	+	+	–	–	–	–
Pyruvic and lactic acidemia	+	+	+	+	–	–	–
Galactosemia	+	+	+	+	+	+	–
Fructose intolerance	–	+	+	+	+	+	–

Abbreviations:
FTT = Failure to thrive
MR = Mental retardation

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