Biochemical Genetics

Disorders of Metabolism

Chapter 7

Introduction

- Each metabolic process consists of a sequence Of catalytic steps mediated by enzymes encoded by genes.

- These genes are replicated with high fidelity, and enzymatic systems continue to work effectively from generation to generation.

- Occasionally, mutations reduce the efficiency of encoded enzymes to a level at which normal metabolism cannot occur.

- Such variants of metabolism were recognized by Sir Archibald Garrod at the beginning of the 20th century, based partly on his studies of alkaptonuria (AKU).

- Garrod recognized that these variants illustrated "chemical individualities" and called these disorders "inborn errors of metabolism," thus setting the cornerstone for contemporary biochemical genetics.

TABLE 7-1 Disorders of Metabolism

NAME	PREVALENCE	MUTANT GENE PRODUCT	CHROMOSOMAL LOCATION
Carbohydrate Disorders			
Classic galactosemia	1/35,000 to 1/60,000	Galactose-1-phosphate uridyl transferase	9p13
Hereditary fructose intolerance	1/20,000	Fructose 1,6-bisphosphate aldolase	9q13-q32
Fructosuria	≈1/100,000	Fructokinase	2p23
Hypolactasia (adult)	Common	Lactase	2q21
Diabetes mellitus type 1	1/400 (Europeans)	Multiple	Polygenic
Diabetes mellitus type 2	1/20	Multiple	Polygenic
Maturity-onset diabetes of the young (MODY)	≈1/400	Multiple	Multiple loci
Amino Acid Disorders			
Phenylketonuria	1/10,000	Phenylalanine hydroxylase	12q24
Tyrosinemia (type 1)	1/100,000	Fumarylacetoacetate hydrolase	15q23-25
Maple syrup urine disease	1/180,000	Branched-chain α-ketoacid dehydrogenase (multiple subunits)	Multiple loci
Alkaptonuria	1/250,000	Homogentisic acid oxidase	3q2
Homocystinuria	1/340,000	Cystathionine β-synthase	21q2
Oculocutaneous albinism	1/35,000	Tyrosinase	11q
Cystinosis	1/100,000	CTNS	17p13
Cystinuria	1/7000	SLC3A1 (type 1)	2p
		SLC7A9 (types II & III)	19q13
Lipid Disorders			
MCAD	1/1,000 to 1/15,000	Medium-chain acyl-CoA dehydrogenase	1p31
VLCAD	1/30,000	Very Long-chain acyl-CoA dehydrogenase	17p13.1
SLO	1/10,000	Δ7-sterol reductase	11q12-q13

|			
Organic Acid Disorders			
Methylmalonic acidemia	1/20,000	Methylmalonyl-CoA mutase	6p
Propionic acidemia	Rare	Propionyl-CoA carboxylase	13q32; 3q
Urea Cycle Defects			
Ornithine transcarbamylase deficiency	1/60,000	Ornithine carbamyl transferase	Xp21
Carbamyl phosphate synthetase deficiency	1/300,000	Carbamyl phosphate synthetase I	2p
Argininosuccinic acid synthetase deficiency	1/250,000	Argininosuccinic acid synthetase	9q34
Energy Production Defects			
Cytochrome c oxidase deficiency	Rare	Cytochrome oxidase peptides	Multiple loci
Pyruvate carboxylase deficiency	Rare	Pyruvate carboxylase	11q
Pyruvate dehydrogenase complex (E_1) deficiency	Rare	Pyruvate decarboxylase, $E_1\alpha$	Xp22
NADH-CoQ reductase deficiency	Rare	Multiple nuclear genes	Multiple loci
Heavy Metal Transport Defects			
Wilson disease	1/50,000	ATP7B	13q14
Menkes disease	1/250,000	ATP7A	Xq13
Hemochromatosis	1/200 to 1/400 (Europeans)	HFE	6p21
Acrodermatitis enteropathica	Rare	SLC39A4	8q24

MCAD, Medium-chain acyl-CoA dehydrogenase; *SLO,* Smith-Lemli-Opitz syndrome; *VLCAD,* very long–chain acyl-CoA dehydrogenase.

Introduction

- AKU is a rare disorder in which homogentisic acid (HGA), an intermediate metabolite in phenylalanine and tyrosine metabolism, is excreted in large quantities in urine, causing it to darken on standing. Hence, AKU was classically referred to as "black urine disease."

- Additionally, an oxidation product of HGA is directly deposited in connective tissues, resulting in abnormal pigmentation and debilitating arthritis.
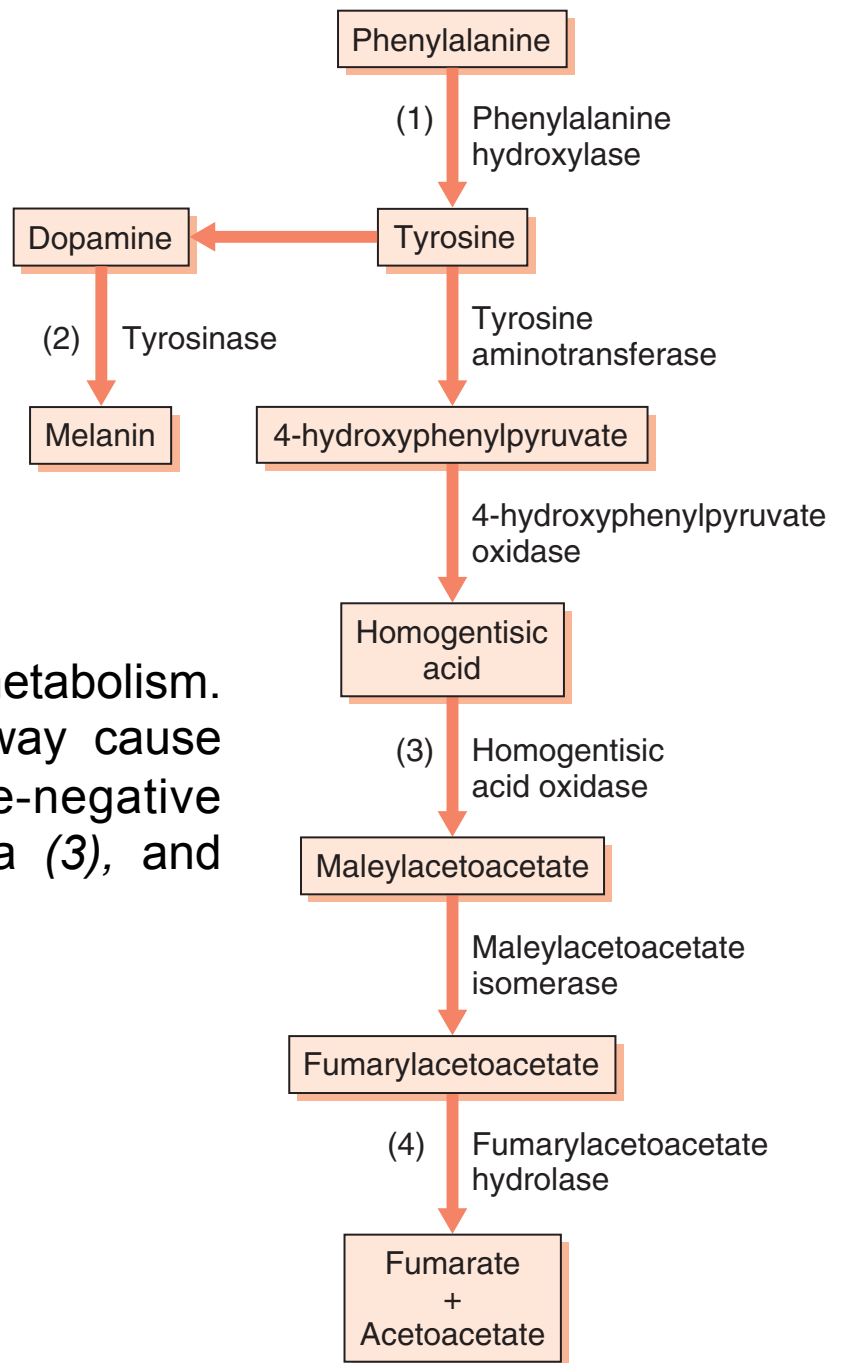
FIG 7-1 Major pathway of phenylalanine metabolism. Different enzymatic defects in this pathway cause classic phenylketonuria *(1),* tyrosinase-negative oculocutaneous albinism *(2),* alkaptonuria *(3),* and tyrosinemia type 1 *(4).*

Introduction

- Garrod proposed in 1902 that AKU was caused by a deficiency of the enzyme that normally splits the aromatic ring of HGA.

- Fifty years later, it was established that AKU is produced by a failure to synthesize homogentisate 1,2- dioxygenase (HGO).

- In 1996, the gene for AKU was cloned.

- Many of the mutations identified in HGO encode proteins that show no HGO activity when expressed in vitro.

- This indicates that AKU is caused by a loss-of-function mutation, confirming the hypothesis put forth by Garrod more than a century ago.

VARIANTS OF METABOLISM

Prevalence of Metabolic Disease

- More than 350 different inborn errors of metabolism have been described to date, and most of these are rare.

- Metabolic disorders account for a substantial percentage of the morbidity and mortality directly attributable to genetic disease.

- The incidence of metabolic disorders is approximately 1 in every 2,500 births, or 10% of all monogenic conditions in children.

Inheritance of Metabolic Defects

- Most metabolic disorders are inherited in an autosomal recessive pattern; only individuals having two mutant alleles are affected.

- Although a mutant allele produces reduced or no enzyme activity, it usually does not alter the health of a heterozygous carrier.

- Since many of the genes encoding disease-related enzymes have been cloned and their mutations characterized, carrier testing and prenatal diagnosis for many metabolic disorders is available.

- Testing samples of dried blood for elevated levels of metabolites in the newborn period (e.g., for phenylketonuria and galactosemia) remains the most commonly used population-based screening test for metabolic disorders.

Types of Metabolic Processes

Metabolic disorders have been classified in many different ways, based on

(1) the pathological effects of the pathway blocked (e.g., absence of end product, accumulation of substrate)

(2) different functional classes of proteins-(e.g., receptors, hormones)

(3) associated cofactors (e.g., metals, vitamins)

(4) pathways affected (e.g., glycolysis, citric acid cycle).

DEFECTS OF METABOLIC PROCESSES

- All biochemical reactions in the human body are controlled by enzymes, which act as catalysts. The catalytic properties of enzymes increase reaction rates by more than a million fold.

- These reactions mediate the synthesis, transfer, utilization, and degradation of biomolecules to build and maintain the internal structures of cells, tissues, and organs.

- Biomolecules can be categorized into four primary groups: nucleic acids, proteins, carbohydrates, and lipids.

- The major metabolic pathways that metabolize these molecules include glycolysis, citric acid cycle, pentose phosphate shunt, gluconeogenesis, glycogen and fatty acid synthesis/storage, degradative pathways, energy production, and transport systems.

Carbohydrate Metabolism
Galactose

- Galactosemia, the most common monogenic disorder of carbohydrate metabolism, affects 1 in every 55,000 newborns.

- It is caused by mutations in the gene encoding galactose-1-phosphate uridyl transferase *(GAL-1-P uridyl transferase)*

- Approximately 70% of galactosemia-causing alleles have a single missense mutation in exon 6.

- Affected individuals cannot effectively convert galactose to glucose; consequently, galactose is alternatively metabolized to galactitol and galactonate.
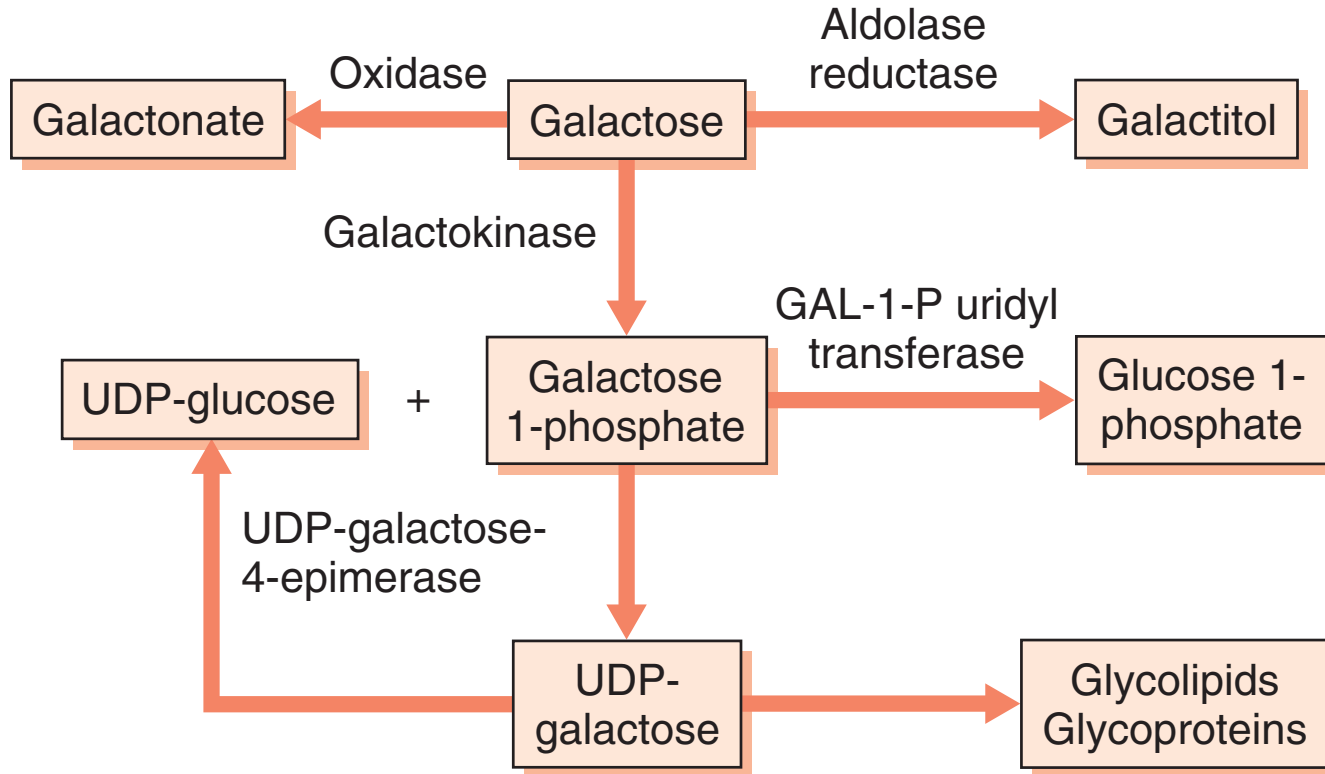
FIG 7-2 Major pathways of galactose metabolism. The most common enzymatic abnormality producing galactosemia is a defect of GAL-1-P uridyl transferase. Defects of galactokinase or of UDP-galactose 4-epimerase are much less common causes of galactosemia. *GAL,* Galactose; *UDP,* uridine diphosphate.
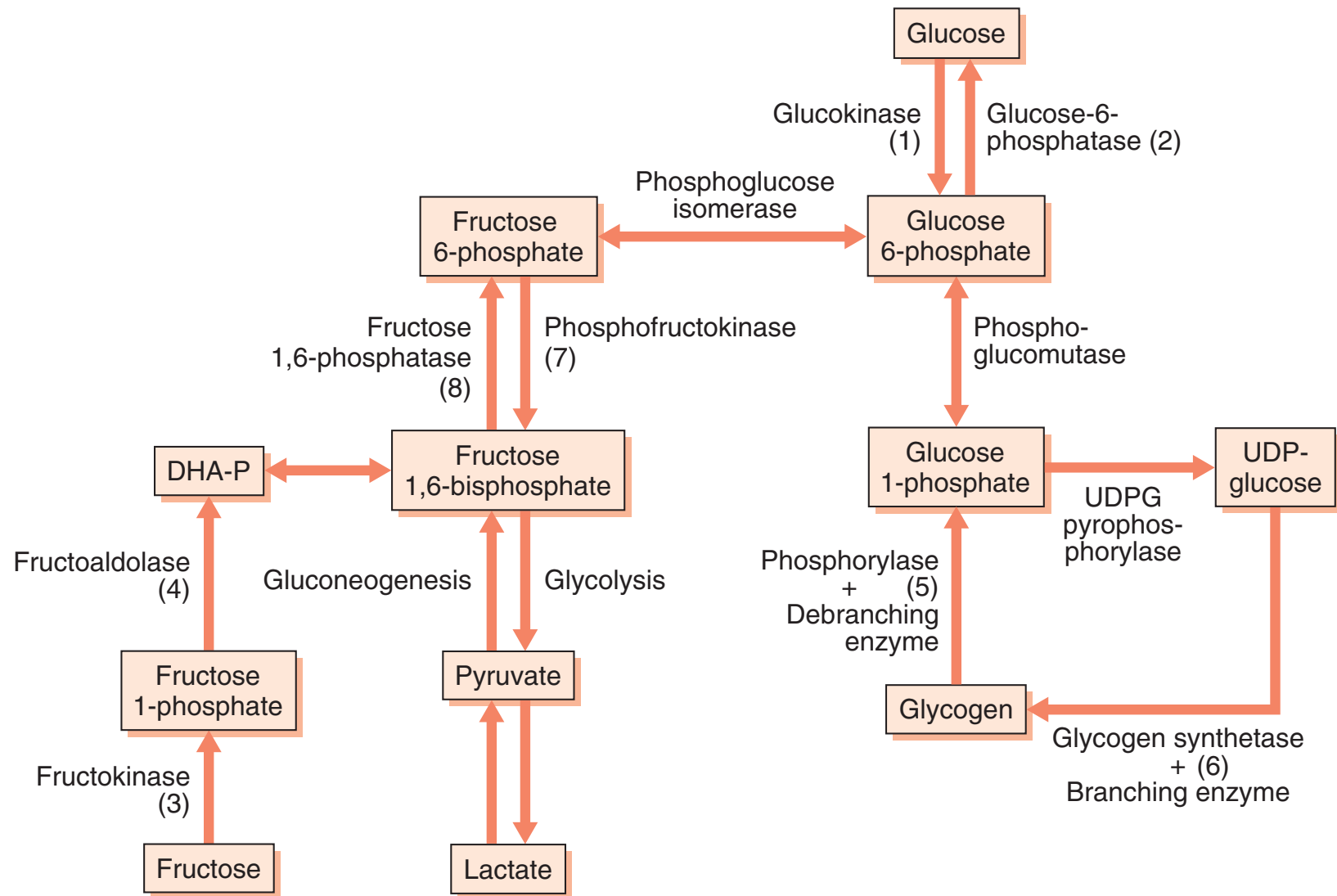
Galactose

- The most common clinical signs of classical galactosemia are failure to thrive, hepatic insufficiency, cataracts (opacification of the lens of the eye) and developmental delay.

- Newborn screening for galactosemia is widespread and is commonly performed by measuring plasma GAL-l-P uridyl transferase activity from a dried drop of blood.

- Early identification affords prompt treatment, which consists largely of eliminating dietary galactose.

Fructose

- Three autosomal recessive defects of fructose metabolism have been described. The most common is caused by mutations in the gene encoding hepatic fructokinase.

- This enzyme catalyzes the first step in the metabolism of dietary fructose, the conversion to fructose-1-phosphate.

- Inactivation of hepatic fructokinase results in asymptomatic fructosuria (presence of fructose in the urine).

FIG 7-3 Summary of glucose, fructose, and glycogen metabolism. Enzymatic defects in this pathway cause hyperglycemia *(1),* Von Gierke disease *(2),* fructosuria *(3),* hereditary fructose intolerance *(4),* Cori disease *(5),* Anderson disease *(6),* Tarui disease *(7),* and fructose 1,6-bisphosphatase (FBPase) deficiency *(8). UDP,* Uridine diphosphate.

Lactose

- The ability to metabolize lactose (a sugar composed of glucose and galactose) depends, in part, on the activity of an intestinal brush-border enzyme called lactase-phlorizin hydrolase (LPH).

- In most mammals, LPH activity diminishes after infants are weaned from maternal milk. The persistence of intestinal LPH activity is a common autosomal recessive trait in human populations, with an incidence ranging from 5% to 90%.

- Lactase nonpersistence (i.e., adult-type hypolactasia or lactose intolerance) is common in most tropical and subtropical countries. Persons with lactase nonpersistence can experience nausea, bloating, and diarrhea after ingesting lactose.

- In many regions where reduced lactase activity is prevalent, the lactose in dairy products is often partially metabolized (e.g., by lactobacilli in the preparation of yogurt) before consumption.

- LPH is encoded by the lactase gene *(LCT)* on chromosome 2. In European populations, adult LPH expression is regulated by a polymorphism located in an upstream gene named *minichromosome maintenance 6 (MCM6)*.

- Insub-Saharan African, Central Asian, and Arabian peninsular populations in which lactase persistence is common, this polymorphism is found at low frequency or is absent.

- Lactase persistence in these populations is caused by different polymorphisms that appear to increase transcription of *LCT.* These polymorphisms appear to have arisen relatively recently in human evolution and have increased in incidence as a result of natural selection independently in populations in Europe and Africa.

- Mutations that abolish lactase activity altogether cause congenital lactase deficiency and produce severe diarrhea and malnutrition in infancy. Such mutations are very rare.

Glycogen

- Carbohydrates are most commonly stored as glycogen in humans.

- Enzyme deficiencies that lead to impaired synthesis or degradation of glycogen are also considered disorders of carbohydrate metabolism.

- Defects of each of the proteins involved in glycogen metabolism have been identified.

- These cause different forms of glycogen storage disorders and are classified numerically according to the chronological order in which their enzymatic basis was described.

TABLE 7-2 Glycogen Storage Disorders

| TYPE	DEFECT	MAJOR AFFECTED TISSUES
Ia (Von Gierke)	Glucose-6-phosphatase	Liver, kidney, intestine
Ib	Microsomal glucose-6-phosphate transport	Liver, kidney, intestine, neutrophils
II (Pompe)	Lysosomal acid β-glucosidase	Muscle, heart
IIIa (Cori)	Glycogen debranching enzyme	Liver, muscle
IIIb	Glycogen debranching enzyme	Liver
IV (Anderson)	Branching enzyme	Liver, muscle
V (McArdle)	Muscle phosphorylase	Muscle
VI (Hers)	Liver phosphorylase	Liver
VII (Tarui)	Muscle phosphofructokinase	Muscle

- The two organs most severely affected by glycogen storage disorders are the liver and skeletal muscle.

- Glycogen storage disorders that affect the liver typically cause hepatomegaly (enlarged liver) and hypoglycemia *(Low* plasma glucose level).

- Glycogen storage disorders that affect skeletal muscle cause exercise intolerance, progressive weakness, and cramping.

- Some glycogen storage disorders, such as Pompe disease (Lysosomal acid α-glucosidase deficiency), can also affect cardiac muscle, causing cardiomyopathy and early death.

Amino Acid Metabolism

- Proteins play the most diverse roles of the major biomol- ecules (e.g., providing mechanical support, coordinating immune responses, generating motion).

- The fundamental structural units of proteins are amino acids.

- Some amino acids can be synthesized endogenously (nonessential), while others must be obtained from the environment (essential).

- Many defects of the metabolism of amino acids have been described.

Hyperphenylalaninemias

- Defects in the metabolism of phenylalanine (an essential amino acid) cause the hyperphenylalaninemias, some of the most widely studied of all metabolic defects.

- These disorders are caused by mutations of the loci encoding components o f the phenylalanine hydroxylation pathway.

- Elevated levels of plasma phenylalanine disrupt essential cellular processes in the brain such as myelination and protein synthesis, eventually producing severe intellectual disability.
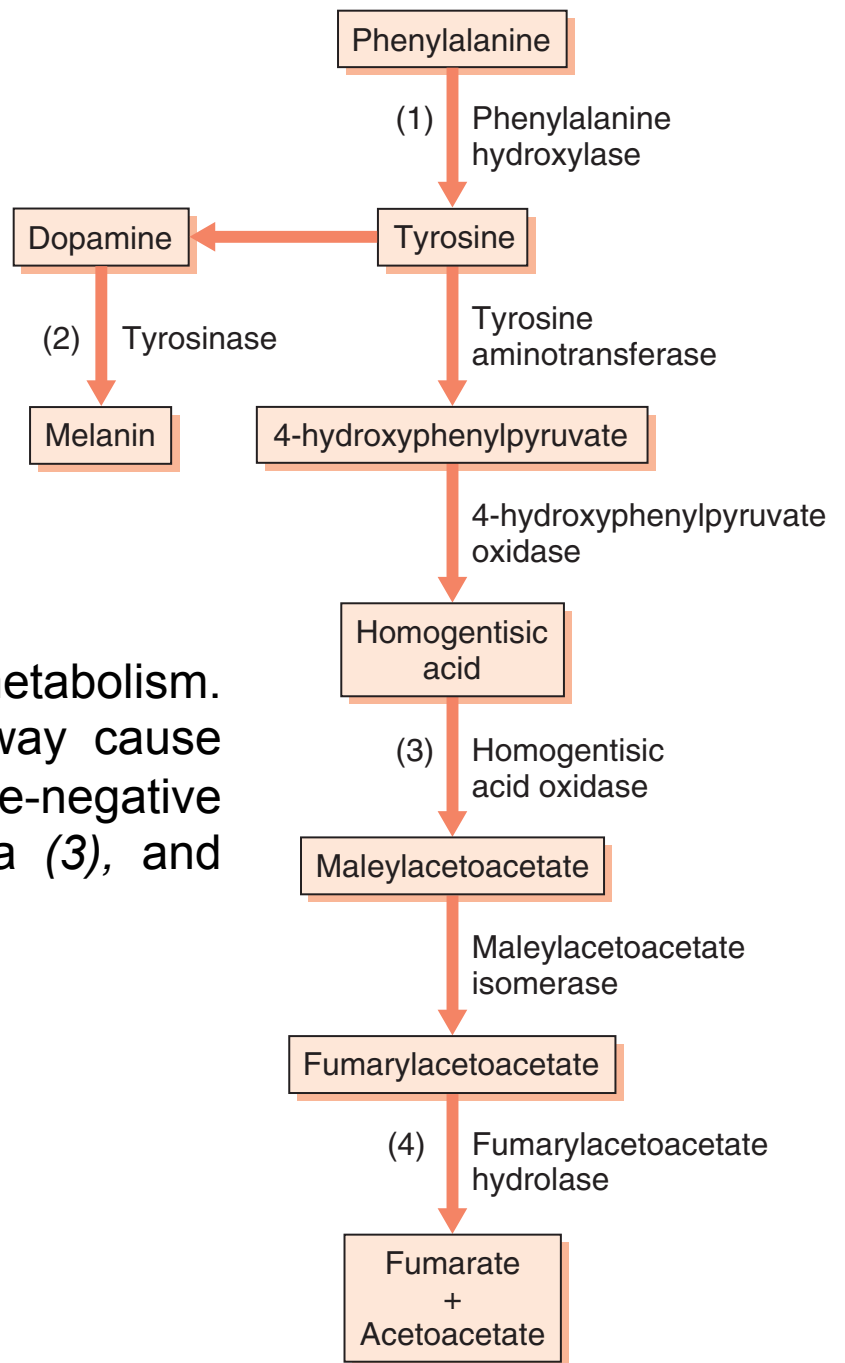
FIG 7-1 Major pathway of phenylalanine metabolism. Different enzymatic defects in this pathway cause classic phenyl- ketonuria *(1),* tyrosinase-negative oculocutaneous albinism *(2),* alkaptonuria *(3),* and tyrosinemia type 1 *(4).*
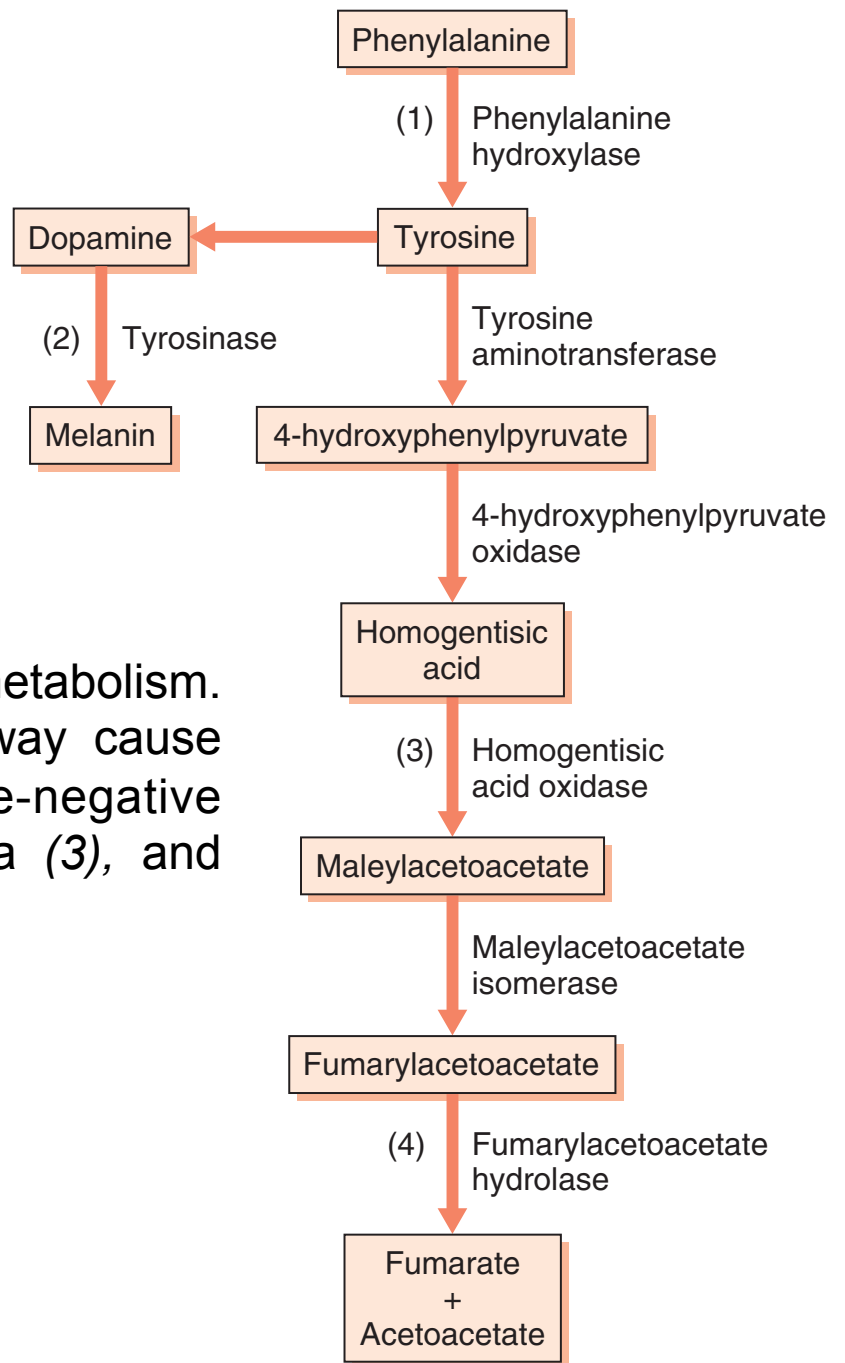
Hyperphenylalaninemias

- Most cases of hyperphenylalaninemia are caused by mutations of phenylalanine hydroxylase (PAH) and produce classical phenylketonuria (PKU).

- Hundreds of different mutations have been identified in PAH, including substitutions, insertions, and deletions.

- The prevalence of hyperphenylalaninemia varies widely among ethnic groups, with PKU ranging from 1 in every 10,000 Caucasians to 1 in every 90,000 in Africans.

- Less commonly, hyperphenylalaninemia is caused by defects in the synthesis of tetrahydrobiopterin, a cofactor necessary for the hydroxylation of phenylalanine, or by a deficiency of dihydropteridine reductase.

- Treatment of most hyperphenylalaninemias is aimed at restoring normal blood phenylalanine levels by restricting dietary intake of phenylalanine-containing foods.

- As phenylalanine is an essential amino acid, and adequate supplies are necessary for normal growth and development. A complete lack of phenylalanine is fatal.

- A fine balance must be maintained between providing enough protein and phenylalanine for normal growth and preventing the serum phenylalanine level from rising too high.

Tyrosine

- The amino acid tyrosine is the starting point of the synthetic pathways leading to catecholamines, thyroid hormones, and melanin pigments, and it is incorporated into many proteins.

- Elevated levels of serum tyrosine can be acquired (e.g., severe hepatocellular dysfunction), or they can result from an inborn error of catabolism, of which there are several.

- Hereditary tyrosinemia type 1 (HT1) is the most common metabolic defect of tyrosine and is caused by a deficiency of fumaryl- acetoacetate hydrolase (FAH), which catalyzes the last step in tyrosine catabolism (see Fig. 7-1).

- Accumulation of FAH's substrate, fumarylacetoacetate, and its precursor, maleylacetoacetate, is thought to be mutagenic and toxic to the liver.

FIG 7-1 Major pathway of phenylalanine metabolism. Different enzymatic defects in this pathway cause classic phenylketonuria *(1),* tyrosinase-negative oculocutaneous albinism *(2),* alkaptonuria *(3),* and tyrosinemia type 1 *(4).*

- Management of HT1 includes supportive care, dietary restriction of phenylalanine and tyrosine, and administration of 2-(nitro-4-trifluoro-methylbenzoyl)-1,3-cyclo- hexanedione (NTBC) or nitisinone, an inhibitor of an enzyme upstream of FAH (4-hydroxyphenylpyruvate dioxygenase).

- Use of NTBC, combined with a low-tyrosine diet, has produced marked improvement in children with HT1. Liver transplantation can be curative but is typically reserved for persons who fail to respond to NTBC or who develop a malignancy. Gene therapy for HT1 in humans might someday replace life-long dietary and pharmacologic treatment.

- Mutations in FAH have been identified in many families. A splice-site mutation is quite common in French-Canadians, and its high frequency is probably the result of founder effect. This mutation results in an in-frame deletion of an exon, which removes a series of critically important amino acids from FAH. **Missense and nonsense** mutations of *FAH* have also been found in persons with HT1.

- Tyrosinemia type 2 (oculocutaneous tyrosinemia) is caused by a deficiency of **tyrosine aminotransferase**. It is mainly characterized by corneal erosions and thickening of the skin on the palms and the soles.

- Tyrosinemia type 3 is associated with reduced activity of **4-hydroxyphenylpyruvate dioxygenase**. Only a few affected persons have been reported.

Branched-Chain Amino Acids

- Approximately 40% of the preformed amino acids required by mammals are branched-chain amino acids (BCAAs) such as valine, leucine, and isoleucine.

- BCAAs can be used as a source of energy through an oxidative pathway that uses an α-ketoacid as an intermediate. Decarboxylation of α-ketoacids is mediated by a multimeric enzyme complex called branched-chain α-ketoacid dehydrogenase (BCKAD).

- The BCKAD complex is composed of at least four catalytic components and two regulatory enzymes, which are encoded by six genes. A deficiency of any one of these six components produces a disorder known as **maple syrup urine disease** (MSUD), so named because the urine of affected persons has an odor reminiscent of maple syrup.

- The prevalence of MSUD in the general population is low, but MSUD is relatively common in the Mennonite community of Lancaster County, Pennsylvania, where approximately 1 in every 7 persons is a heterozygous carrier.

- All of these carriers have the same disease-causing mutation of *E1α,* one of the loci encoding a catalytic component of BCKAD, and all are descendants of a couple who emigrated from Europe in the 18th century. This is another example of founder effect in a small, relatively isolated population.

- Untreated patients with MSUD accumulate BCAAs and their associated ketoacids, leading to progressive neurodegeneration and death in the first few months of life. Treatment of MSUD consists of dietary restriction of BCAAs to the minimum required for normal growth.
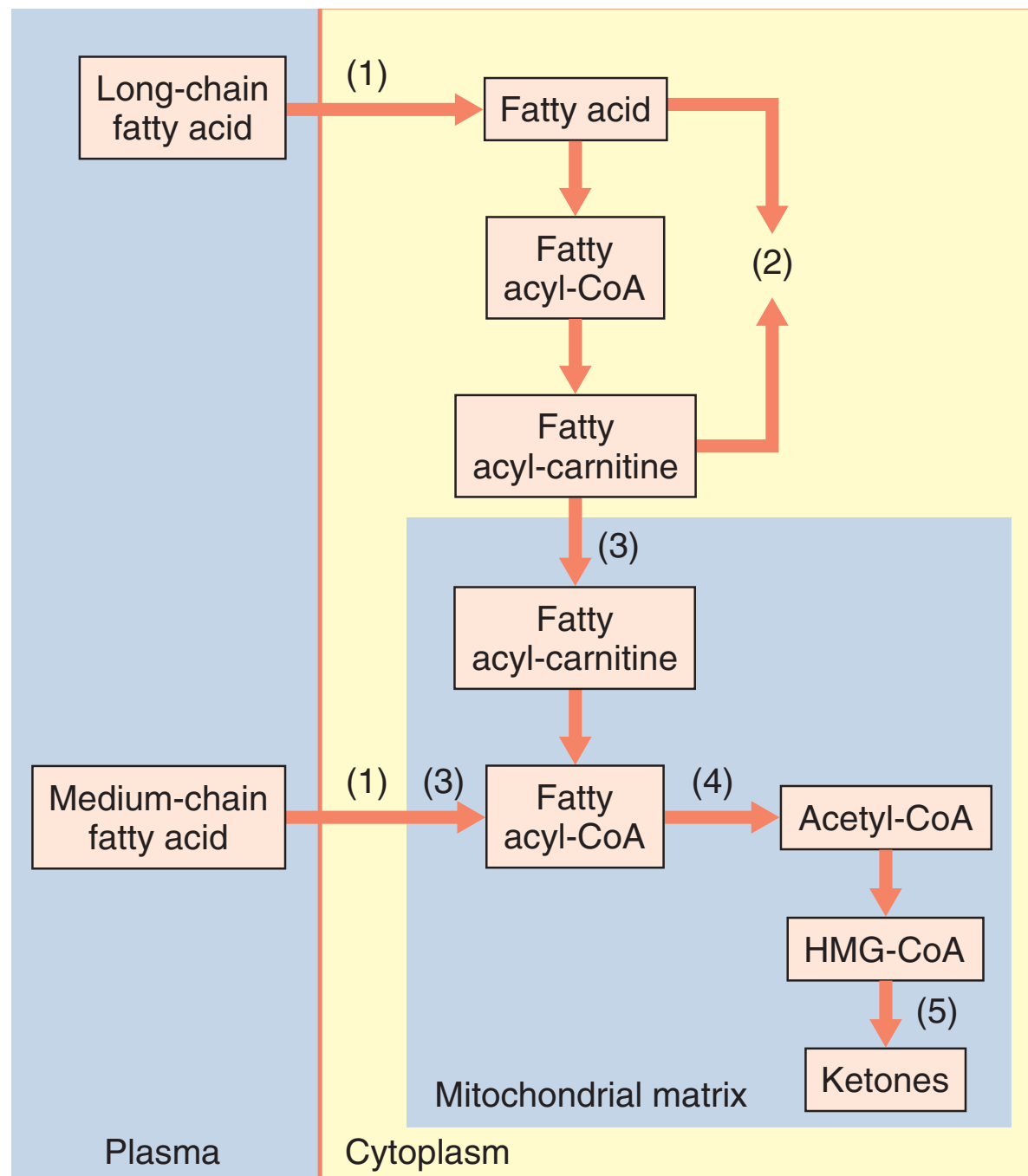
Lipid Metabolism

- Lipids (Greek, *lipos* or fat) are a heterogeneous group of biomolecules that are insoluble in water and highly soluble in organic solvents (e.g., chloroform).

- They provide the backbone for phospholipids and sphingolipids, which are components of all biological membranes.

- Lipids are also constituents of hormones; they act as intracellular messengers and serve as an energy substrate.

- Elevated serum lipid levels (hyperlipidemia) are common and result from defective lipid transport mechanisms.

- Errors in the metabolism of fatty acids (hydrocarbon chains with a terminal carboxylate group) are much less frequent

Lipid Metabolism

- During fasting and prolonged aerobic exercise, fatty acids are mobilized from adipose tissue and become a major substrate for energy production in the liver, skeletal muscle, and cardiac muscle.

- Major steps in this pathway include the uptake and activation of fatty acids by cells, transport across the outer and inner mitochondrial membranes, and entry into the β-oxidation spiral in the mitochondrial matrix.

- Defects in each of these steps have been described in humans, although defects of fatty acid oxidation (FAO) are the most common.

FIG 7-4 Summary of fatty acid metabolism: *(1)* fatty acid entry into a cell, *(2)* activation and transesterification, *(3)* mitochondrial uptake, *(4)* oxidation via β-oxidation, *(5)* formation of ketone bodies. Note that medium-chain fatty acids can traverse the mitochondrial membrane without carnitine-mediated transport. *CoA,* Coenzyme A.

Fatty Acids

- The most common inborn error of fatty acid metabolism results from a deficiency of medium-chain acyl-coenzyme A dehydrogenase (MCAD).

- MCAD deficiency is characterized by episodic hypoglycemia, which is often provoked by fasting.

- Fasting results in the accumulation of fatty acid intermediates, a failure to produce ketones in sufficient quantities to meet tissue demands, and the exhaustion of glucose supplies.

- Cerebral edema and encephalopathy result from the indirect and direct effects of fatty acid intermediates in the central nervous system. Death often follows unless a usable energy source such as glucose is provided promptly.

MCAD Deficiency

- Most of the reported MCAD patients are of northwestern European origin, and 90% of their alleles have an A-to-G missense mutation that results in the substitution of glutamate for lysine.

- Additional substitution, insertion, and deletion mutations have been identified but are much less common.

Cholesterol

- Elevated levels of plasma cholesterol have been associated with various conditions, most notably atherosclerotic heart disease.

- It has been demonstrated that substantially reduced levels of cholesterol can adversely affect growth and development.

- The final step of cholesterol **biosynthesis** is catalyzed by the enzyme Δ7-sterol reductase (DHCR7).

- It has been noted that persons with an autosomal recessive disorder called Smith–Lemli–Opitz (SLO) syndrome have reduced levels of cholesterol and increased levels of 7-dehydrocholesterol (a precursor of DHCR7).

- SLO is characterized by various congenital anomalies of the brain, heart, genitalia, and hands (Fig. 7-5). It is unusual in this respect because most inborn errors of metabolism do not cause congenital malformations.

FIG 7-5 A child with Smith–Lemli–Opitz syndrome. Note the broad nasal root, upturned nasal tip, and inner epicanthal folds that are characteristic of this disorder. (From Jones K. *Smith's Recognizable Patterns of Human Malformation*. 6th ed. Philadelphia: Saunders; 2006:116.)

- In 1998, SLO was discovered to be caused by mutations in the *DHCR7* gene, and to date more than 100 different mutations in *DHCR7* have been found. Most of these are missense mutations that result in substitutions of a highly conserved residue of the protein.

- Population screens for mutant *DHCR7* alleles suggest that the carrier frequency in populations of European ancestry is 3% to 4%. This high frequency suggests the incidence of SLO should be much higher than is com- monly observed. One explanation is that some pregnancies with affected fetuses result in miscarriages or that SLO is undetected in some mildly affected patients.

- Supplementing the diet of SLO children with cholesterol can ameliorate their growth and feeding problems, although its effect on cognitive development is less clear.

Steroid Hormones

- Cholesterol is the precursor for several major classes of steroid hormones including glucocorticoids (e.g., cortisol), mineralocorticoids (e.g., aldosterone), androgens (e.g., testosterone), and estrogens (Fig. 7-6).

- The actions of these steroid hormones are typically mediated by binding to an intracellular receptor, and these receptor–ligand complexes have myriad effects on a wide range of physiological processes. Defects in the synthesis of steroid hormones or their receptors can cause a wide variety of clinical abnormalities.

- Congenital adrenal hyperplasia (CAH) consists of a group of genetically heterogeneous autosomal recessive disorders of cortisol biosynthesis.

- Approximately 95% of cases of CAH are caused by mutations in *CYP21A2,* the gene that encodes 21-hydroxylase, and are characterized by cortisol deficiency, variable deficiency of aldosterone, and an excess of androgens.
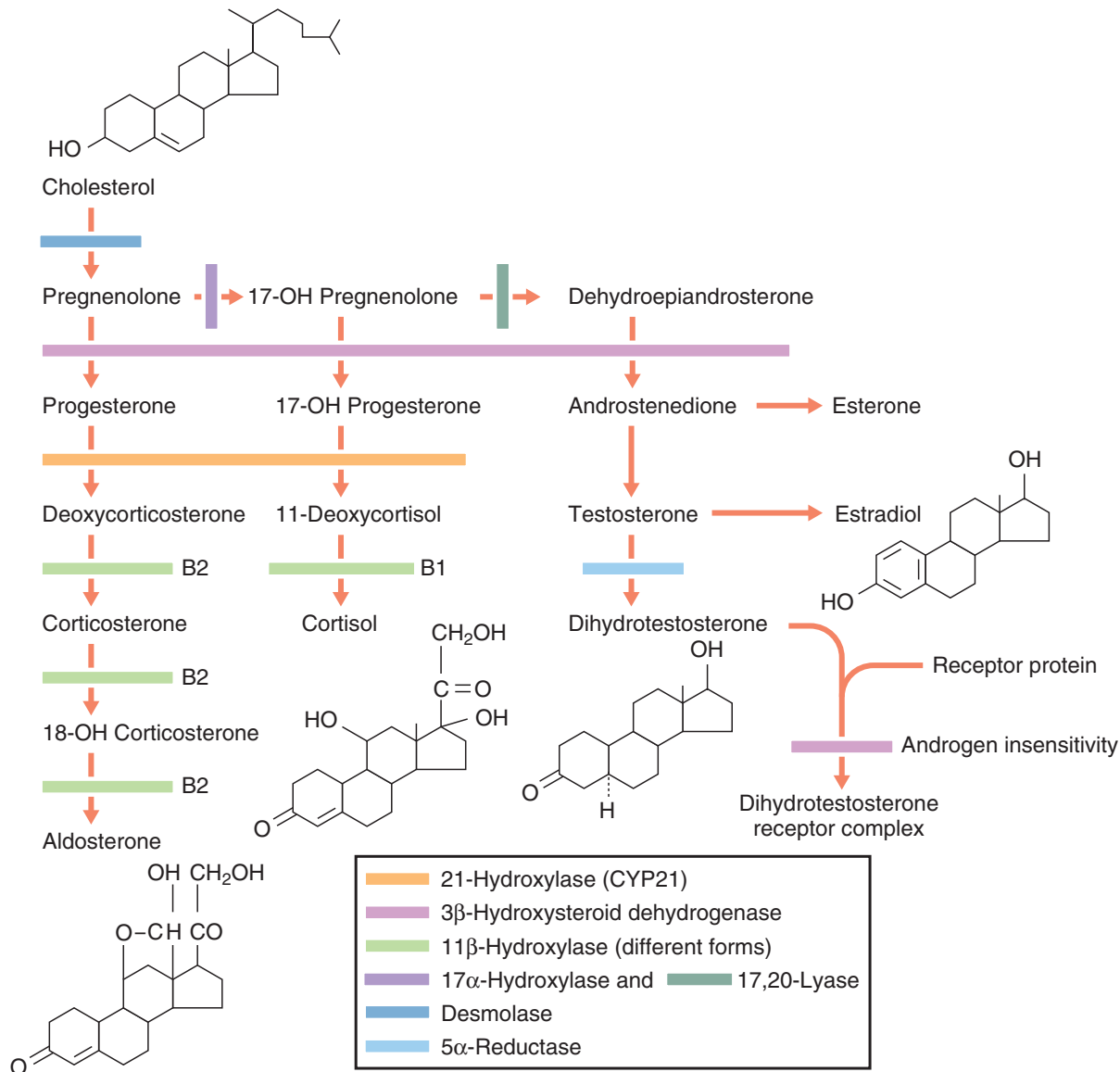
FIG 7-6 Summary of steroid synthesis. Enzymes involved in the production of cortisol, aldosterone, and testosterone are indicated. (Modified from Turnpenny P. *Emery's Elements of Medical Genetics*. 13th ed. Philadelphia: Churchill Livingstone; 2007.)

- The clinical severity of CAH varies widely and depends on the extent of residual 21-hydroxylase activity. The severe or classic form is typified by overproduction of cortisol precursors and thus excess adrenal androgens. In addition, aldoste- rone deficiency leads to loss of salt.

- In the milder forms, sufficient cortisol is produced, but there is still an overproduction of androgens. Female infants with CAH typically have ambiguous genitalia (Fig. 7-7) at birth due to in utero exposure to high concentrations of androgens, and CAH is the most common cause of ambiguous genitalia in 46,XX infants.

- Boys with CAH have normal genitalia at birth, so the age of diagnosis depends on the severity of aldosterone deficiency. Most boys have the salt-losing form of CAH and typically present between 7 and 14 days of age in adrenal crisis manifested as weight loss, lethargy, dehydration, hyponatremia (decreased plasma Na+ concentration), and hyperkalemia (increased plasma K+ concentration).

- Boys who do not have a salt-losing form of CAH present at 2 to 4 years of age with premature virilization.

- Treatment of CAH consists of replacing cortisol, suppressing adrenal androgen secretion, and providing mineralocorticoids to return electrolyte concentrations to normal.

- The surgical management of children born with ambiguous genitalia is complex and somewhat controversial, but most girls with CAH identify as female, and feminizing surgery during the first year of life is standard.

- In pregnancies in which the fetus is at risk for classic CAH, steroids are administered to the mother to suppress the fetal overproduction of androgens and reduce the incidence of ambiguous genitalia in affected female infants.

Lysosomal Storage Disorders

- Enzymes within lysosomes catalyze the stepwise degradation of sphingolipids, glycosaminoglycans (mucopolysaccharides), glycoproteins, and glycolipids.

- Accumulation ("storage") of undegraded molecules results in cell, tissue, and organ dysfunction.

- Most of the lysosomal disorders are caused by enzyme deficiencies, although some are caused by the inability to activate an enzyme or to transport an enzyme to a subcellular compartment where it can function properly.

Lysosomal Storage Disorders
Mucopolysaccharidoses

- The mucopolysaccharidoses (MPS) are a heterogeneous group of disorders caused by a reduced ability to degrade one or more glycosaminoglycans (e.g., dermatan sulfate, heparan sulfate, keratan sulfate, chondroitin sulfate).

- These glycosaminoglycans are degradation products of proteoglycans found in the extracellular matrix.

- Ten different enzyme deficiencies cause six different MPS disorders, which share many clinical features, but these disorders can be distinguished from each other by clinical, biochemical, and molecular analyses.

TABLE 7-3 Mucopolysaccharidoses*

NAME	MUTANT ENZYME	CLINICAL FEATURES
Hurler–Scheie (MPS-I)	α-L-Iduronidase	Coarse face, hepatosplenomegaly, corneal clouding, dysostosis multiplex,[†] intellectual disability
Hunter (MPS-II)	Iduronate sulfatase	Coarse face, hepatosplenomegaly, dysostosis multiplex, intellectual disability, behavioral problems
Sanfilippo A MPS-IIIA	Heparan-N-sulfamidase	Behavioral problems, dysostosis multiplex, intellectual disability
Sanfilippo B MPS-IIIB	α-N-Acetylglucosaminidase	Behavioral problems, dysostosis multiplex, intellectual disability
Sanfilippo C MPS-IIIC	Acetyl-CoA:α-glucosaminide N-acetyltransferase	Behavioral problems, dysostosis multiplex, intellectual disability
Sanfilippo D MPS-IIID	N-Acetylglucosamine-6-sulfatase	Behavioral problems, dysostosis multiplex, intellectual disability
Morquio A MPS-IVA	N-Acetylglucosamine-6-sulfatase	Short stature, bony dysplasia, hearing loss
Morquio B MPS-IVB	β-Galactosidase	Short stature, bony dysplasia, hearing loss
Maroteaux–Lamy MPS-VI	Aryl sulfatase B	Short stature, corneal clouding, cardiac valvular disease, dysostosis multiplex
Sly MPS-VII	β-Glucuronidase	Coarse face, hepatosplenomegaly, corneal clouding, dysostosis multiplex

*Hunter syndrome is an X-linked recessive disorder; the remaining MPS disorders are autosomal recessive.
†Dysostosis multiplex is a distinctive pattern of changes in bone, including a thickened skull, anterior thickening of the ribs, vertebral abnormalities, and shortened and thickened long bones.

- Assays that measure enzyme activity in fibroblasts, leukocytes, serum or dried blood spots are available for each MPS disorder, and prenatal testing after amniocentesis or chorionic villus sampling is possible.

- Except for the X-linked recessive Hunter syndrome, all of the MPS disorders are inherited in an autosomal recessive fashion.

Lysosomal Storage Disorders
Mucopolysaccharidoses

- All MPS disorders are characterized by chronic and progressive multisystem deterioration, which causes hearing, vision, joint, and cardiovascular dysfunction.

- Hurler (α-1-Iduronidase deficiency)(MPS-I), severe Hunter (Iduronate sulfatase deficiency) (MPS-II), and Sanfilippo syndromes (MPS-III) are characterized by intellectual disability, while normal cognition is observed in other MPS disorders.

- Deficiency of iduronidase (MPS I) is the prototypic MPS disorder. It produces a spectrum of phenotypes that have been traditionally delimited into three groups—Hurler, Hurler–Scheie, and Scheie syndromes—which manifest severe, moderate, and mild disease, respectively.

- MPS I disorders cannot be distinguished from each other by measuring enzyme activity; therefore, the MPS I phenotype is usually assigned on the basis of clinical criteria. The iduronidase gene has been cloned, and eventually genotype–phenotype correlations may lead to earlier and more accurate classification.

- Hunter syndrome (MPS II) is caused by a deficiency of iduronate sulfatase. It is categorized into mild and severe phenotypes based on clinical assessment.

- The onset of disease usually occurs between 2 and 4 years of age. Affected children develop coarse facial features, short stature, skeletal deformities, joint stiffness, and intellectual disability.

- The gene that encodes iduronate sulfatase is composed of 9 exons spanning 24 kb. Twenty percent of all identified mutations are large deletions, and most of the remainder are missense and non- sense mutations.

Lysosomal Storage Disorders
sphingolipidoses / Lipid Storage Diseases

- Defects in the degradation of sphingolipids (sphingolipidoses) result in their gradual accumulation, which leads to multiorgan dysfunction.

- Deficiency of the lysosomal enzyme, acid β-glucosidase, causes accumulation of glucosylceramide and Gaucher disease.

- It is characterized by visceromegaly (enlarged visceral organs), multiorgan failure, and debilitating skeletal disease.

- Gaucher disease has traditionally been divided into three subtypes, which can be distinguished by their clinical features.

- Type 1 is most common and does not involve the central nervous system. Type 2 is the most severe, often leading to death within the first 2 years of life. Type 3 Gaucher disease is intermediate between the other two forms.

- In practice, the clinical phenotypes overlap, and the spectrum of Gaucher is so broad that it ranges from death in utero to persons who remain asymptomatic even in old age.

- Gaucher disease is caused by more than 400 different mutations in *GBA,* the gene that encodes glucosylceramidase (β-glucosidase).

- Gaucher disease is rare, although the frequency of disease-causing alleles is greater than 0.03 in Ashkenazi Jews.

- The most common allele is produced by an A-to-G mutation that results in a single amino acid substitution.

- DNA screening for the five most common Gaucher alleles detects 97% of all mutations found in this population.

TABLE 7-4 Lysosomal Storage Disorders*

NAME	MUTANT ENZYME	CLINICAL FEATURES
Tay–Sachs	β-Hexosaminidase A	Hypotonia, spasticity, seizures, blindness
Gaucher (type 1; nonneuropathic)	β-Glucosidase	Splenomegaly, hepatomegaly, bone marrow infiltration, brain usually spared
Niemann–Pick, type 1A	Sphingomyelinase	Hepatomegaly, corneal opacities, brain deterioration
Fabry	α-Galactosidase	Paresthesia of the hands and feet, corneal dystrophy, hypertension, renal failure, cardiomyopathy
G$_{M1}$ gangliosidosis (infantile)	β-Galactosidase	Organomegaly, dysostosis multiplex,[†] cardiac failure
Krabbe	galactosylceramidase	Hypertonicity, blindness, deafness, seizures, (galactosylceramide-specific) atrophy of the brain
Metachromatic leukodystrophy	Aryl sulfatase A	Ataxia, weakness, blindness, brain atrophy (late-infantile)
Sandhoff	β-Hexosaminidase (total)	Optic atrophy, spasticity, seizures
Schindler	α-N-Acetylgalactosaminidase	Seizures, optic atrophy, retardation
Multiple sulfatase deficiency	Aryl sulfatase A, B, C	Retardation, coarse facial features, weakness, hepatosplenomegaly, dysostosis multiplex

*Of the lysosomal storage disorders included in this table, Fabry syndrome is X-linked recessive and the remainder are autosomal recessive.
[†]Dysostosis multiplex is a distinctive pattern of changes in bone, including a thickened skull, anterior thickening of the ribs, vertebral abnormalities, and shortened and thickened long bones.

Urea Cycle Disorders

- The urea cycle consists of five major biochemical reactions that convert nitrogenous waste products to urea, which is subsequently excreted by the kidney.

- Deficiencies of carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (ASA), and argininosuccinase (AS) result in the accumulation of urea precursors such as ammonium and glutamine.

- Enzymatic defects in this pathway lead to the accumulation of urea precursors, progressive neurological impairment, and death if untreated.

- Each of these disorders, except OTC deficiency, is inherited in an autosomal recessive pattern.
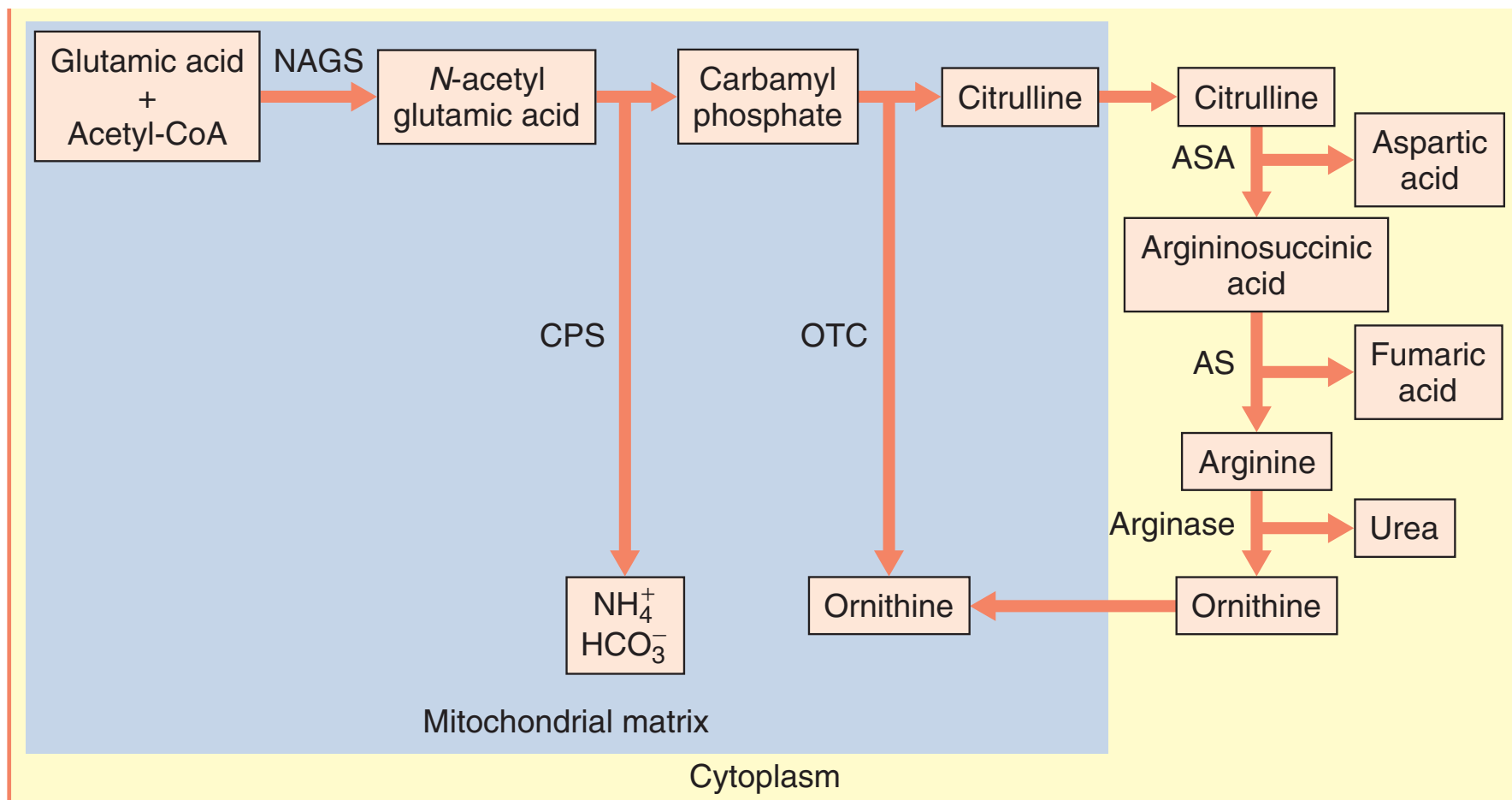
FIG 7-9 Schematic diagram of the urea cycle. *AS,* Argininosuccinase; *ASA,* argininosuccinic acid synthetase; *CoA,* coenzyme A; *CPS,* carbamyl phosphate synthetase; *NAGS, N-*acetylglutamate synthetase; *OTC,* ornithine transcarbamylase.

- The genes for most of these disorders have been cloned, including the most common observed defect, X-linked ornithine transcarbamylase (OTC) deficiency.

- Women can be symptomatic carriers depending, in part, on the fraction of hepatocytes in which the normal allele is inactivated.

- A variety of exon deletions and missense mutations have been described, and mutations that affect RNA processing have been observed.

Diagnosis of a Metabolic Disorder

The presentations of persons with inborn errors of metabolism are highly variable. During gestation, the maternal–placental unit usually provides essential nutrients and prevents the accumulation of toxic substrates. Thus, a fetus is infrequently symptomatic. However, after birth, persons with metabolic disorders can present at ages ranging from the first day of life to adulthood. The presentation may be precipitous and characterized by dramatic alterations in homeostasis and even death. In contrast, the disorder may be insidious, with only subtle changes in function over long periods. For most metabolic disorders, the presymptomatic period and onset of symptoms lie somewhere between these two extremes. The following case illustrates this point.

Anthony is a 9-month-old Latin American boy who comes to the emergency department accompanied by his parents. His parents complain that he has been irritable and vomiting for the last 36 hours, and over the past 12 hours he has become increasingly sleepy. They sought medical attention because it was difficult to awaken Anthony to breast-feed him. Anthony's medical history is unremarkable. He has a healthy 8-year-old sister and had a brother who died in his crib at 7 months of age. An investigation of the brother's death and an autopsy were performed. The findings were consistent with sudden infant death syndrome (SIDS).

Anthony is hospitalized and is noted to be hypoglycemic (low serum glucose level), slightly acidemic (serum pH < 7.4), and hyperammonemic (elevated plasma ammonia). Intravenous infusion of glucose transiently improves his level of alertness, but he becomes comatose and dies 5 days later. An autopsy reveals marked cerebral edema (swelling of the brain) and fatty infiltration of the liver consistent with a diagnosis of Reye syndrome. Anthony's mother is concerned that the boys' deaths are related to each other, especially since she is pregnant again. She is counseled that the causes of death are unrelated and neither disorder is likely to recur in her family.

One year later her 6-month-old daughter, Maria, is hospitalized for the third time because of lethargy and weakness. Laboratory studies reveal moderate hypoglycemia, hyperammonemia, and ketonuria (ketones in the urine). Additional studies, including measurement of urine organic acids,* serum amino acids, and free and esterified plasma carnitines, suggest that Maria has a defect of fatty acid oxidation. Therapy is initiated with intravenous glucose, oral carnitine, and the restriction of fats to no more than 20% of her caloric requirements. More specific biochemical and molecular studies confirm that Maria has medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. Molecular studies from preserved tissues that had been collected at autopsy from Maria's deceased brothers indicate that they also had MCAD deficiency. Maria's asymptomatic older sister is similarly affected. Both girls are healthy 2 years later, eating a low-fat diet and using a carnitine supplement. They have a new baby brother who underwent prenatal testing for MCAD and was found to be unaffected.

The disparate presentations of MCAD deficiency in this family (sudden death, acute illness, chronic illness, and asymptomatic) illustrate the phenotypic variability often observed in persons with inborn errors of metabolism, even those sharing an identical mutation. Thus, there might not be a disease-specific pattern of symptoms and findings. Often it is the heightened index of suspicion of care providers that leads to the testing necessary to identify a metabolic disorder. Supportive therapy can be lifesaving and should be initiated before making a diagnosis. Nevertheless, it is imperative that prudent attempts be made to make a specific diagnosis, because it can have important implications for the family (e.g., prenatal testing, presymptomatic therapy). The treatment of MCAD deficiency is completely effective in preventing early death from the toxic effects of accumulated fatty acid intermediates.

Hereditary Hemochromatosis

The term hemochromatosis refers to all disorders characterized by excessive iron storage. A subgroup of these disorders are hereditary and can be caused by mutations in one of several different genes. The most common form of hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism in which excessive iron is absorbed in the small intestine and then accumulates in a variety of organs such as the liver, kidney, heart, joints, and pancreas. It was described by von Recklinghausen, the same physician who described neurofibromatosis 1, in 1889. Approximately 1 of every 8 northern Europeans is a carrier for HH, and 1 of every 200 to 400 persons is a homozygote. Although the penetrance of the disease-causing genotype is incomplete (as discussed later), HH is one of the most common genetic disorders observed in people of European ancestry. Its prevalence is much lower in Asian and African populations.

The most common symptom of HH is fatigue, although the clinical presentation of patients with HH can vary considerably. Additional findings include joint pain, diminished libido, diabetes, increased skin pigmentation, cardiomyopathy, liver enlargement, and cirrhosis. Abnormal serum iron parameters can identify most men at risk for iron overload, but HH is not detected in many premenopausal women. The most sensitive diagnostic test for HH is a liver biopsy accompanied by histochemical staining for hemosiderin (a form of stored iron).

As early as the 1970s, an increased frequency of the human leukocyte antigen HLA-A3 allele in HH patients indicated that an HH gene might be located near the major histocompatibility region (MHC) on chromosome 6p. Subsequent linkage studies confirmed this hypothesis in the late 1970s, but it was not until 1996 that the HH gene was cloned. The HH gene is a widely expressed HLA class I–like gene, *HFE.* The gene product is a cell-surface protein that binds to the transferrin receptor (transferrin carries iron molecules), overlapping the binding site for transferrin and inhibiting transferrin-mediated iron uptake. However, this does not directly affect iron transport from the small intestine. Instead, this interaction is thought to be involved in a cell's ability to sense iron levels. This function is disrupted in persons with mutations in *HFE,* resulting in excessive iron absorption from the small intestine and iron overload. Thus, hemochromatosis is not caused by a defect of an iron transport protein but rather by a defect in the regulation of transport.

A single missense mutation that results in the substitution of a tyrosine for cysteine in a β2-microglobulin–binding domain accounts for 85% of all HH-causing mutations. A single ancestral haplotype predominates in Europeans, suggesting that there was a selective advantage conferred by having at least one copy of the HH gene. Because iron deficiency affects one third of the world's population and is significantly less common in HH heterozygotes, it is likely that this explains the higher incidence of HH in many populations.

Treatment of HH consists of reducing the accumulated iron in the body. This is accomplished by serial phlebotomy or by the use of an iron-chelating agent such as deferoxamine. Depending on the quantity of iron stored, return to a normal level of iron can take a few years. However, iron reduction prevents further liver damage, cures the cardiomyopathy, returns skin pigmentation to normal, and might improve the diabetes. Persons who have not developed irreversible liver damage have a nearly normal life expectancy.

The estimated penetrance of HH depends on a person's age, sex, and whether the presence of disease is measured by histological findings such as hepatic fibrosis or clinical symptoms. Most men who are homozygous for an HH-causing mutation do not develop clinical symptoms, and those who do are seldom symptomatic before the age of 40 years. An even smaller fraction of homozygous women develop clinical symptoms. If symptoms are seen, they typically occur 20 years or so later than in men because iron accumulation in women is tempered by iron losses during menstruation, gestation, and lactation (Fig. 7-10).
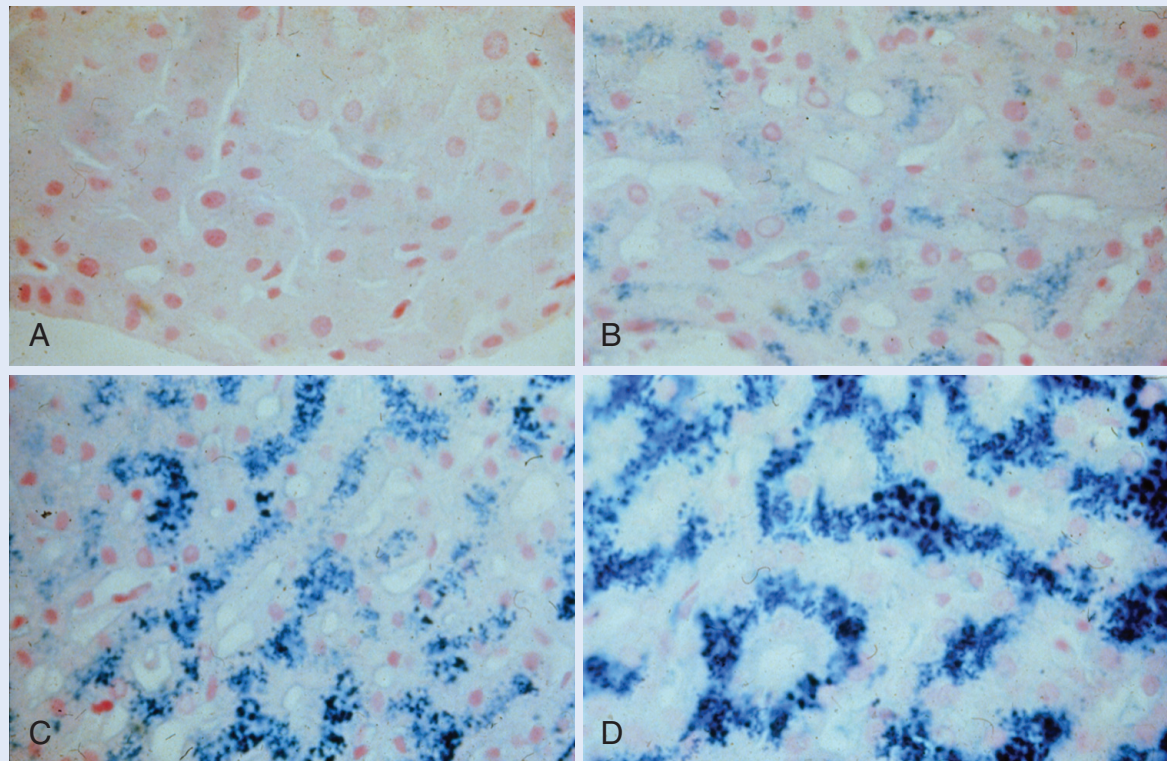
FIG 7-10 Comparison of hemosiderin stain of normal liver **(A)** with hemosiderin stain of livers from persons affected with hemochromatosis **(B, C,** and **D).** Note the varying degree of increased deposition of hemosiderin in livers of HH homozygotes. This damages the liver, impairs its function, and can lead to cirrhosis and liver cancer.