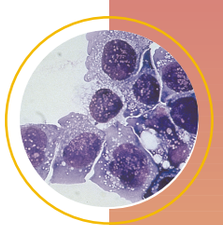
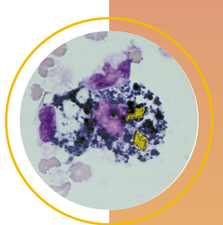
CHAPTER 9





Urine Screening for Metabolic Disorders

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1. Explain the abnormal accumulation of metabolites in the urine in terms of overflow and renal disorders.
2. Name the metabolic defect in phenylketonuria, and describe the clinical manifestations it produces.
3. Discuss the performance of the Guthrie and ferric chloride tests and their roles in the detection and management of phenylketonuria.
4. State three causes of tyrosyluria and the recommended screening test for its presence.
5. Name the abnormal urinary substance present in alkaptonuria, and tell how its presence may be suspected.
6. Discuss the appearance and significance of urine that contains melanin.
7. Describe a basic laboratory observation that has relevance in maple syrup urine disease.
8. Discuss the significance of ketonuria in a newborn.
9. Differentiate between the presence of urinary indican owing to intestinal disorders and Hartnup disease.
10. State the significance of increased urinary 5-hydroxyindoleacetic acid.
11. Differentiate between cystinuria and cystinosis, including the differences that are found during analysis of the urine and the disease processes.
12. Name the chemical screening test for cystine.
13. Describe the components in the heme synthesis pathway, including the primary fluids used for their analysis.
14. Briefly discuss the major porphyrias with regard to cause and clinical significance.
15. Differentiate between the Ehrlich reaction and fluorescent testing with regard to the testing of porphyrin components.
16. Describe the appearance of urine that contains increased porphyrins.
17. Define mucopolysaccharides, and name three syndromes in which they are involved.
18. List three screening tests for the detection of urinary mucopolysaccharides.
19. State the significance of increased uric acid crystals in newborns' urine.
20. Explain the reason for performing tests for urinary-reducing substances on all newborns.

KEY TERMS

alkaptonuria
cystinosis
cystinuria
galactosuria
homocystinuria
inborn error of metabolism
indicanuria
Lesch-Nyhan disease
maple syrup urine disease
melanuria
melituria
mucopolysaccharidoses
organic acidemias
phenylketonuria
porphyrinuria
tyrosinuria

As has been discussed in previous chapters, many of the abnormal results obtained in the routine urinalysis are related to metabolic rather than renal disease. Urine as an end product of body metabolism may contain additional abnormal substances not tested for by the routine urinalysis. Often, these substances can be detected by additional screening tests that can also be performed in the urinalysis laboratory. Positive screening tests can then be followed up with more sophisticated procedures performed in other sections of the laboratory.

The need to perform additional tests may be detected by the observations of alert laboratory personnel during the performance of the routine analysis or from observations of abnormal specimen color and odor by nursing staff and patients (Table 9–1). In other instances, clinical symptoms and family histories are the deciding factors. Several metabolic screening tests are routinely performed on all newborns.[3]

Overflow Versus Renal Disorders

The appearance of abnormal metabolic substances in the urine can be caused by a variety of disorders that can generally be grouped into two categories, termed the overflow type and renal type. Overflow disorders result from the disruption of a normal metabolic pathway that causes increased plasma concentrations of the nonmetabolized substances. These chemicals either override the reabsorption ability of the renal tubules or are not normally reabsorbed from the filtrate because they are only present in minute amounts. Abnormal accumulations of the renal type are caused by malfunctions in the tubular reabsorption mechanism as discussed in Chapter 8.

The most frequently encountered abnormalities are associated with metabolic disturbances that produce urinary overflow of substances involved in protein and carbohydrate metabolism. This is understandable when one considers the vast number of enzymes used in the metabolic pathways of proteins and carbohydrates and the fact that their function is essential for complete metabolism. Disruption of enzyme function can be caused by failure to inherit the gene to produce a particular enzyme, referred to as an ***inborn error of metabolism***,[7] or by organ malfunction from disease or toxic reactions. The most frequently encountered abnormal urinary metabolites are summarized in Table 9–2 and their appearance is classified according to functional defect. This table also includes those substances and conditions that are covered in this chapter.

TABLE 9–1 **Abnormal Metabolic Constituents or Conditions Detected in the Routine Urinalysis**

Color	Odor	Crystals
Homogentisic acid Melanin Indican Porphyrins	Phenylketonuria Maple syrup urine disease Isovaleric acidemia Cystinuria Cystinosis Homocystinuria	Cystine Leucine Tyrosine Lesch-Nyhan disease

TABLE 9–2 **Major Disorders of Protein and Carbohydrate Metabolism Associated with Abnormal Urinary Constituents Classified as to Functional Defect**

	Overflow	
Inherited	**Metabolic**	**Renal**
Phenylketonuria Tyrosinemia Alkaptonuria Maple syrup urine disease Organic acidemias Cystinosis Porphyria Mucopolysaccharidoses Melituria (galactosuria) Lesch-Nyhan disease	Tyrosinemia Melanuria Indicanuria 5-Hydroxyindoleacetic acid Porphyria	Hartnup disease Cystinuria

Amino Acid Disorders

The amino acid disorders with urinary screening tests include ***phenylketonuria*** **(PKU)**, ***tyrosinuria***, ***alkaptonuria***, ***melanuria***, ***maple syrup urine disease***, ***organic acidemias***, ***indicanuria***, ***cystinuria***, and ***cystinosis***.

PHENYLALANINE-TYROSINE DISORDERS

Many of the most frequently requested special urinalysis procedures are associated with the phenylalanine-tyrosine metabolic pathway. Major inherited disorders include PKU, tyrosyluria, and alkaptonuria. Metabolic defects cause production of excessive amounts of melanin. The relationship of these varied disorders is illustrated in Figure 9–1.

Phenylketonuria

The most well known of the **aminoacidurias**, PKU is estimated to occur in 1 of every 10,000 to 20,000 births and, if undetected, results in severe mental retardation. It was first identified in Norway by Ivan Følling in 1934, when a mother with other mentally retarded children reported a peculiar mousy odor to her child's urine. Analysis of the urine showed increased amounts of the keto acids, including phenylpyruvate. As shown in Figure 9–1, this will occur when the normal conversion of phenylalanine to tyrosine is disrupted. Interruption of the pathway also produces children with fair complexions even in dark-skinned families, owing to the decreased production of tyrosine and its pigmentation metabolite melanin.

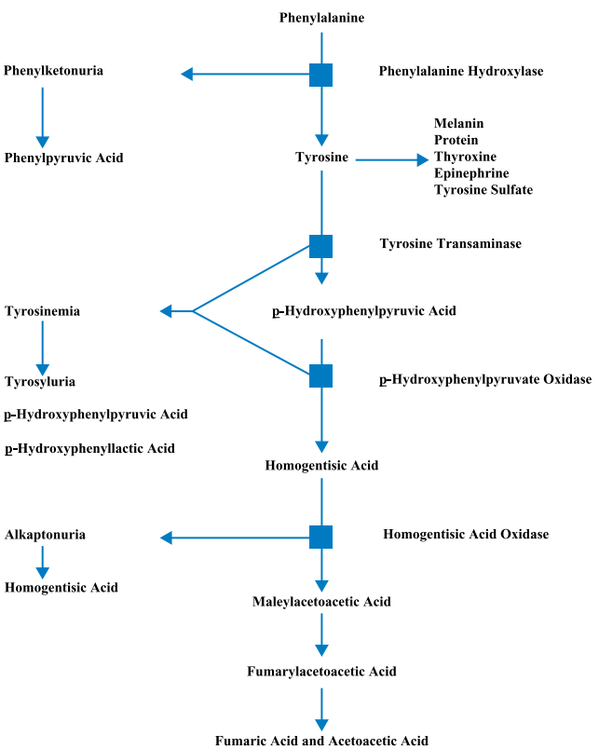


FIGURE 9–1 Phenylalanine and tyrosine metabolism. (Adapted from Frimpton,[6] and Kretchmer and Etzwiler.[15])

PKU is caused by failure to inherit the gene to produce the enzyme phenylalanine hydroxylase. The gene is inherited as an autosomal recessive trait with no noticeable characteristics or defects exhibited by heterozygous carriers. Fortunately, screening tests are available for early detection of the abnormality, and all states have laws that require the screening of newborns.[26] Once discovered, dietary changes that eliminate phenylalanine, a major constituent of milk, from the infant's diet can prevent the excessive buildup of serum phenylalanine and can thereby avoid damage to the child's mental capabilities. As the child matures, alternate pathways of phenylalanine metabolism develop, and dietary restrictions can be eased. Many products that contain large amounts of phenylalanine, such as aspartame, now have warnings for people with phenylketonuria.

The initial screening for PKU does not come under the auspices of the urinalysis laboratory, because increased blood levels of phenylalanine must, of course, occur prior to the urinary excretion of phenylpyruvic acid, which may take from 2 to 6 weeks. Blood samples must be obtained before the newborn is discharged from the hospital. The increasing tendency to release newborns from the hospital as early as 24 hours after birth has caused concern about the ability to detect increased phenylalanine levels at that early stage. Studies have shown that in many cases phenylalanine can be detected as early as 4 hours after birth and, if the cutoff level for normal results is lowered from 4 mg/dL to 2 mg/dL, the presence of PKU should be detected. Tests may need to be repeated during an early visit to the pediatrician.[5] More girls than boys escape detection of PKU during early tests because of slower rises in blood phenylalanine levels.[6]

Urine testing can be used as a follow-up procedure in questionable diagnostic cases, as a screening test to ensure proper dietary control in previously diagnosed cases, and, more recently, as a means of monitoring the dietary intake of pregnant women known to lack phenylalanine hydroxylase.

The most well-known blood test for PKU is the bacterial inhibition test developed by Guthrie.[9] In this procedure, blood from a heelstick is absorbed into filter paper circles. The circle must be completely saturated with a single layer of blood. The blood-impregnated disks are then placed on culture media streaked with the organism *Bacillus subtilis*. If increased phenylalanine levels are present in the blood, phenylalanine will counteract the action of beta-2-thienylalanine, an inhibitor of *B. subtilis* that is present in the media, and growth will be observed around the paper disks. Notice that in Figure 9–2 the bacterial

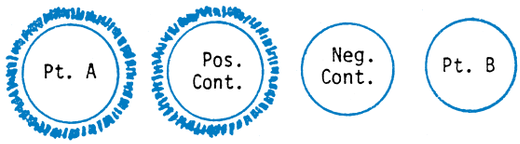


FIGURE 9–2 Guthrie's test.

PROCEDURE

Ferric Chloride Tube Test

1. Place 1 mL of urine in a tube.
2. Slowly add five drops of 10% ferric chloride.
3. Observe color.

growth around the disk from patient A corresponds to the positive control, indicating an increased level of phenylalanine. Modifications of the Guthrie test also will detect maple syrup urine disease, ***homocystinuria***, tyrosinemia, histidinemia, valinemia, and galactosemia.[23] Chemical and immunologic tests for many other substances including thyroid hormones, trypsin, and biotinidase can also be performed from dried blood collected by heel stick.[3] Additional methods are available for measuring serum levels of phenylalanine, including an automated technique that measures the fluorescence of phenylalanine when it is heated in the presence of Ninhydrin and L-leucyl-L-alanine or glycyl-L-leucine.[13]

Urine tests for phenylpyruvic acid are based upon the ferric chloride reaction performed by tube test. As will be seen in other discussions in this chapter, the ferric chloride test is a nonspecific reaction and will react with many other amino acids and commonly ingested medications (see Table 9–4 later in the chapter). Some brands of disposable diapers also produce false-positive reactions for PKU when tested with ferric chloride.[14] The addition of ferric chloride to urine containing phenylpyruvic acid produces a permanent blue-green color.

Tyrosyluria

The accumulation of excess tyrosine in the plasma (tyrosinemia) producing urinary overflow may be due to several causes and is not well categorized. As can be seen in Table 9–2, disorders of tyrosine metabolism may result from either inherited or metabolic defects. Also, because two reactions are directly involved in the metabolism of tyrosine, the urine may contain excess tyrosine or its degradation products *p*-hydroxyphenylpyruvic acid and *p*-hydroxyphenyllactic acid.

Most frequently seen is a transitory tyrosinemia in premature infants, which is caused by underdevelopment of the liver function necessary to complete the tyrosine metabolism. This condition seldom results in permanent damage, but it may be confused with PKU when urinary screening tests are performed, because the ferric chloride test will produce a green color. This reaction can be distinguished from the PKU reaction in the ferric chloride tube test because the green color fades rapidly when tyrosine is present.

Acquired severe liver disease also will produce tyrosyluria resembling that of the transitory newborn variety and, of course, is a more serious condition. In both instances, rarely seen tyrosine and leucine crystals may be observed during microscopic examination of the urine sediment.

Hereditary disorders in which enzymes required in the metabolic pathway are not produced present a serious and usually fatal condition that results in both liver and renal disease and in the appearance of a generalized aminoaciduria.

PROCEDURE

Nitroso-Napthol Test

1. Place five drops of urine in a tube.
2. Add 1 mL of 2.63N nitric acid.
3. Add one drop of 21.5% sodium nitrite.
4. Add 0.1 mL 1-nitroso-2-napthol.
5. Mix.
6. Wait 5 minutes.
7. Observe color.

The recommended urinary screening test for tyrosine and its metabolites is the nitroso-naphthol test. Like the ferric chloride test, the nitroso-naphthol test is nonspecific and, as shown in Table 9–4, will react with compounds other than tyrosine and its metabolites. However, the presence of an orange-red color shows a positive reaction and indicates that further testing is needed.

Alkaptonuria

Alkaptonuria was one of the six original inborn errors of metabolism described by Garrod in 1902. The name alkaptonuria was derived from the observation that urine from patients with this condition darkened after becoming alkaline from standing at room temperature. Therefore, the term "alkali lover," or alkaptonuria, was adopted. This metabolic defect is actually the third major one in the phenylalanine-tyrosine pathway and occurs from failure to inherit the gene to produce the enzyme homogentisic acid oxidase. Without this enzyme, the phenylalanine-tyrosine pathway cannot proceed to completion, and homogentisic acid accumulates in the blood, tissues, and urine. This condition does not usually manifest itself clinically in early childhood but observations of brown-stained or black-stained cloth diapers and reddish-stained disposable diapers have been reported.[21] In later life, brown pigment becomes deposited in the body tissues (particularly noticeable in the ears). Deposits in the cartilage eventually lead to arthritis. A high percentage of persons with alkaptonuria develop liver and cardiac disorders.[23]

Homogentisic acid will react in several of the routinely used screening tests for metabolic disorders, including the ferric chloride test, in which a transient deep blue color is produced in the tube test. A yellow precipitate is produced in the Benedict's test or Clinitest, indicating the presence of a reducing substance. A more specific screening test for urinary homogentisic acid is to add alkali to freshly voided urine and to observe for darkening of the color; however, large amounts of ascorbic acid will interfere with this reaction.[24] The addition of silver nitrate and ammonium hydroxide also will produce a black urine. A spectrophotometric method to obtain quantitative measurements of both urine and plasma homogentisic acid is available, as are chromatography procedures.

PROCEDURE

Homogentisic Acid Test

1. Place 4 mL of 3% silver nitrate in a tube.
2. Add 0.5 mL of urine.
3. Mix.
4. Add 10% NH_4OH by drops.
5. Observe for black color.

Melanuria

The previous discussion has focused on the major phenylalanine-tyrosine metabolic pathway illustrated in Figure 9–1; however, as is the case with many amino acids, a second metabolic pathway also exists for tyrosine. This pathway is responsible for the production of melanin, thyroxine, epinephrine, protein, and tyrosine-sulfate. Of these substances, the major concern of the urinalysis laboratory is melanin, the pigment responsible for the dark color of hair, skin, and eyes. Deficient production of melanin results in **albinism**.

Like homogentisic acid, increased urinary melanin will produce a darkening of urine. The darkening appears after the urine is exposed to air. Elevation of urinary melanin is a serious finding that indicates the overproliferation of the normal melanin-producing cells (melanocytes), producing a malignant melanoma. These tumors secrete a colorless precursor of melanin, 5,6-dihydroxyindole, which oxidizes to melanogen and then to melanin, producing the characteristic dark urine. Differentiation between the presence of melanin and homogentisic acid must certainly be made.

Melanin will react with ferric chloride, sodium nitroprusside (nitroferricyanide), and Ehrlich's reagent. In the ferric chloride tube test, a gray or black precipitate will form in the presence of melanin and is easily differentiated from the reactions produced by other amino acid products. The sodium nitroprusside test provides an additional screening test for melanin. A red color is produced by the reaction of melanin and sodium nitroprusside. Interference due to a red color from acetone and creatinine can be avoided by adding glacial acetic acid, which will cause melanin to revert to a green-black color, whereas acetone turns purple, and creatinine becomes amber.[2]

Summary of Urine Screening Tests for Disorders of the Phenylalanine-Tyrosine Pathway

Phenylketonuria
Ferric chloride tube test

Tyrosyluria
Ferric chloride tube test
Nitroso-naphthol test

Alkaptonuria
Ferric chloride tube test
Benedict's test or Clinitest
Alkalization of fresh urine

Melanuria
Ferric chloride tube test
Sodium nitroprusside test
Ehrlich's test

BRANCHED-CHAIN AMINO ACID DISORDERS

The branched-chain amino acids differ from other amino acids by having a methyl group that branches from the main aliphatic carbon chain. Two major groups of disorders are associated with errors in the metabolism of the branched-chain amino acids. In one group, accumulation of one or more of the early amino acid degradation products occurs as is seen in maple syrup urine disease. Disorders in the other group are termed organic acidemias and result in accumulation of organic acids produced further down in the amino acid metabolic pathway.

A significant laboratory finding in branched-chain amino acid disorders is the presence of ketonuria in a newborn.

Maple Syrup Urine Disease

Although maple syrup urine disease is rare, a brief discussion is included in this chapter because the urinalysis laboratory can provide valuable information for the essential early detection of this disease.

Maple syrup urine disease is caused by an inborn error of metabolism, inherited as an autosomal recessive trait. The amino acids involved are leucine, isoleucine, and valine. The metabolic pathway begins normally, with the transamination of the three amino acids in the liver to the keto acids (α-ketoisovaleric, α-ketoisocaproic, and α-keto-β-methylvaleric). Failure to inherit the gene for the enzyme necessary to produce oxidative decarboxylation of these keto acids results in their accumulation in the blood and urine.[6]

Newborns with maple syrup urine disease begin to exhibit clinical symptoms associated with failure to thrive after approximately 1 week. The presence of the disease may be suspected from these clinical symptoms; however, many other conditions have similar symptoms. Personnel in the urinalysis laboratory or in the nursery may detect the disease through the observation of a urine specimen that produces a strong odor resembling maple syrup, which is caused by the rapid accumulation of keto acids in the urine. Even though a report of urine odor is not a part of the routine urinalysis, notifying the physician about this unusual finding can prevent the development of severe mental retardation and even death. Studies have shown that if maple syrup urine disease is detected by the 11th day, dietary regulation and careful monitoring of urinary keto acid concentrations can control the disorder.[4]

The screening test most frequently performed for keto acids is the 2,4-dinitrophenylhydrazine (**DNPH**) reaction. The DNPH test can also be used for home monitoring of

PROCEDURE

2,4-Dinitrophenylhydrazine (DNPH) Test

1. Place 1 mL of urine in a tube.
2. Add 10 drops of 0.2% 2,4-DNPH in 2N HCl.
3. Wait 10 minutes.
4. Observe for yellow or white precipitate.

diagnosed patients. Adding DNPH to urine that contains keto acids will produce a yellow turbidity or precipitate. Large doses of ampicillin will interfere with the DNPH reaction. Like many other urinary screening tests, the DNPH reaction is not specific for maple syrup urine disease, inasmuch as keto acids are present in other disorders, including PKU. In addition, all specimens with a positive reagent strip test result for ketones will produce a positive DNPH result. However, treatment can be started on the basis of odor, clinical symptoms, and a positive DNPH test while confirmatory procedures using amino acid chromatography are being performed.

Organic Acidemias

Generalized symptoms of the organic acidemias include early severe illness, often with vomiting accompanied by metabolic acidosis; hypoglycemia; ketonuria; and increased serum ammonia.[8] The three most frequently encountered disorders are isovaleric, propionic, and methylmalonic acidemia.

Isovaleric acidemia may be suspected when urine specimens, and sometimes even the patient, possess a characteristic odor of "sweaty feet." This is caused by the accumulation of isovalerylglycine due to a deficiency of isovaleryl coenzyme A in the leucine pathway. There is no screening test for isovalerylglycine, and its presence is identified using chromatography.

Propionic and methylmalonic acidemias result from errors in the metabolic pathway converting isoleucine, valine, threonine, and methionine to succinyl coenzyme A. Propionic acid is the immediate precursor to methylmalonic acid in this pathway.

A screening test is available for methylmalonic aciduria. The procedure uses *p*-nitroaniline to produce an emerald green color in the presence of methylmalonic acid.[24]

TRYPTOPHAN DISORDERS

The major concern of the urinalysis laboratory in the metabolism of tryptophan is the increased urinary excretion of the metabolites indican and 5-hydroxyindoleacetic acid (**5-HIAA**). Figure 9–3 shows a simplified diagram of the metabolic pathways by which these substances are produced. Other metabolic pathways of tryptophan are not included because they do not relate directly to the urinalysis laboratory.

Indicanuria

Under normal conditions, most of the tryptophan that enters the intestine is either reabsorbed for use by the body in the production of protein or is converted to indole by the

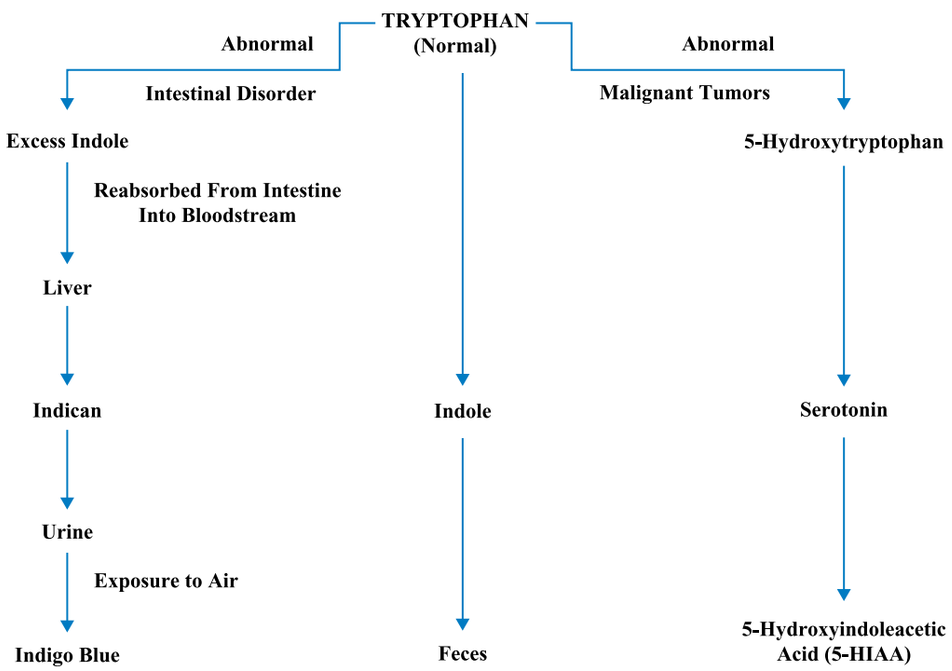


FIGURE 9–3 Tryptophan metabolism. (Adapted from Meister.[17])

PROCEDURE

p-Nitroaniline Test

1. Place one drop of urine in a tube.
2. Add 15 drops of 0.1% *p*-nitroaniline in 0.16 M HCl.
3. Add five drops of 0.5% sodium nitrite.
4. Mix.
5. Add 1 mL of 1 M sodium acetate buffer at pH 4.3.
6. Boil for 1 minute.
7. Add five drops of 8N NaOH.
8. Observe for emerald green color.

intestinal bacteria and excreted in the feces. However, in certain intestinal disorders (including obstruction; the presence of abnormal bacteria; malabsorption syndromes; and **Hartnup disease**, a rare inherited disorder) increased amounts of tryptophan are converted to indole. The excess indole is then reabsorbed from the intestine into the bloodstream and circulated to the liver, where it is converted to indican and then excreted in the urine. Indican excreted in the urine is colorless until oxidized to the dye indigo blue by exposure to air. Early diagnosis of Hartnup disease is sometimes made when mothers report a blue staining of their infant's diapers, referred to as the "blue diaper syndrome." Urinary indican will react with acidic ferric chloride to form a deep blue or violet color that can subsequently be extracted into chloroform.

Except in cases of Hartnup disease, correction of the underlying intestinal disorder will return urinary indican levels to normal. The inherited defect in Hartnup disease affects not only the intestinal reabsorption of tryptophan but also the renal tubular reabsorption of other amino acids, resulting in a generalized aminoaciduria (Fanconi's syndrome). The defective renal transport of amino acids does not appear to affect other renal tubular functions. Therefore, with proper dietary supplements, including niacin, persons with Hartnup disease have a good prognosis.[11]

5-Hydroxyindoleacetic Acid

As shown in Figure 9–3, a second metabolic pathway of tryptophan is for the production of serotonin used in the stimulation of smooth muscles. Serotonin is produced from tryptophan by the argentaffin cells in the intestine and is carried through the body primarily by the platelets. Normally, the body uses most of the serotonin, and only small amounts of its degradation product 5-HIAA are available for excretion in the urine. However, when carcinoid tumors involving the argentaffin (enterochromaffin) cells develop, excess amounts of serotonin are produced, resulting in the elevation of urinary 5-HIAA levels.

The addition of nitrous acid and 1-nitroso-2-naphthol to urine that contains 5-HIAA causes the appearance of a purple to black color, depending on the amount of 5-HIAA present. The normal daily excretion of 5-HIAA is 2 to 8 mg, and argentaffin cell tumors will produce from 160 to 628 mg per 24 hours.[22] Therefore, the test is usually performed on a random or first morning specimen because there can be little chance of false-negative results. If a 24-hour sample is used, it must be preserved with hydrochloric or boric acid. Patients must be given explicit dietary instructions prior to the collection of any sample to be tested for 5-HIAA, because serotonin is a major constituent of foods such as bananas, pineapples, and tomatoes. Medications, including phenothiazines and acetanilids, will also cause interference. Patients should be requested to withhold medications for 72 hours prior to specimen collection.

CYSTINE DISORDERS

There are two distinct disorders of cystine metabolism that exhibit renal manifestations. Confusion as to their relationship existed for many years following the discovery by Wollaston in 1810 of renal calculi consisting of cystine. It is now known that, although both disorders are inherited, one is a defect in the renal tubular transport of amino acids (cystinuria) and the other is an inborn error of metabolism (cystinosis). A noticeable odor of sulfur may be present in the urine in disorders of cystine metabolism.

Cystinuria

As the name implies, the condition is marked by elevated amounts of the amino acid cystine in the urine. The presence of increased urinary cystine is not due to a defect in the metabolism of cystine but, rather, to the inability of the renal tubules to reabsorb cystine filtered by the glomerulus. The demonstration that not only cystine but also lysine, arginine, and ornithine are not reabsorbed has ruled out the possibility of an error in metabolism even though the condition is inherited.[20] The disorder has two modes of inheritance: one in which reabsorption of all four amino acids—cystine, lysine, arginine, and ornithine—is affected, and the other in which only cystine and lysine are not reabsorbed. The primary clinical consideration in cystinuria is the tendency of persons with defective reabsorption of all four amino acids to form calculi. Approximately 65 percent of these people can be expected to produce calculi early in life.

Because cystine is much less soluble than the other three amino acids, laboratory screening determinations are based on the observation of cystine crystals in the sediment of concentrated or first morning specimens. Cystine is also the only amino acid found during the analysis of calculi from these patients. Elevations in the other three amino acids must be determined separately using chromatography procedures. A chemical screening test for urinary cystine can be performed using cyanide-nitroprusside. Reduction of cystine by sodium cyanide followed by the addition of nitroprusside will produce a red-purple color in a specimen that contains excess cystine. False-positive reactions will

PROCEDURE

Cyanide-Nitroprusside Test

1. Place 3 mL of urine in a tube.
2. Add 2 mL sodium cyanide.
3. Wait 10 minutes.
4. Add five drops 5% sodium nitroprusside.
5. Observe for red-purple color.

occur in the presence of ketones and homocystine, and additional tests may have to be performed.

Cystinosis

Regarded as a genuine inborn error of metabolism, cystinosis can occur in three variations, ranging from a severe fatal disorder developed in infancy to a benign form appearing in adulthood. The incomplete metabolism of cystine results in crystalline deposits of cystine in many areas of the body, including the cornea, bone marrow, lymph nodes, and internal organs. A major defect in the renal tubular reabsorption mechanism (Fanconi's syndrome) also occurs. Routine laboratory findings include polyuria, generalized aminoaciduria, positive test results for reducing substances, and lack of urinary concentration. In severe cases, there is a gradual progression to total renal failure. Renal transplants and the use of cystine-depleting medications to prevent the buildup of cystine in other tissues are extending lives.

Homocystinuria

Defects in the metabolism of homocystine can result in failure to thrive, cataracts, mental retardation, thromboembolic problems, and death. As mentioned previously, increased urinary homocystine gives a positive result with the cyanide-nitroprusside test. Therefore, an additional screening test for homocystinuria must be performed by following a positive cyanide-nitroprusside test result with a silver-nitroprusside test, in which only homocystine will react. The use of silver nitrate in place of sodium cyanide will reduce homocystine to its nitroprusside-reactive form but will not reduce cystine. Consequently, a positive reaction in the silver-nitroprusside test confirms the presence of homocystinuria.[24] Fresh urine should be used when testing for homocystine. The screening tests for cystine and homocystine have been converted to spectrophotometric procedures, which provide better detection of low levels.[27]

PROCEDURE

Silver Nitroprusside Test

1. Place 1 mL of urine in a tube.
2. Add two drops concentrated NH_4OH.
3. Add 0.5 mL 5% silver nitrate.
4. Wait 10 minutes.
5. Add five drops sodium nitroprusside.
6. Observe for red-purple color.

Porphyrin Disorders

Porphyrins are the intermediate compounds in the production of heme. The basic pathway for heme synthesis presented in Figure 9–4 shows the three primary porphyrins (uroporphyrin, coproporphyrin, and protoporphyrin) and the porphyrin precursors (α-aminolevulinic acid **[ALA]** and porphobilinogen). As can be seen, the synthesis of heme can be blocked at a number of stages. Blockage of a pathway reaction will result in the accumulation of the product formed just prior to the interruption. Detection and identification of this product in the urine, bile, feces, or blood can then aid in determining the cause of a specific disorder.

The solubility of the porphyrin compounds varies with their structure. ALA, porphobilinogen, and uroporphyrin are the most soluble and readily appear in the urine. Coproporphyrin is less soluble but is found in the urine, whereas protoporphyrin is not seen in the urine. Fecal analysis has usually been performed for the detection of cop-

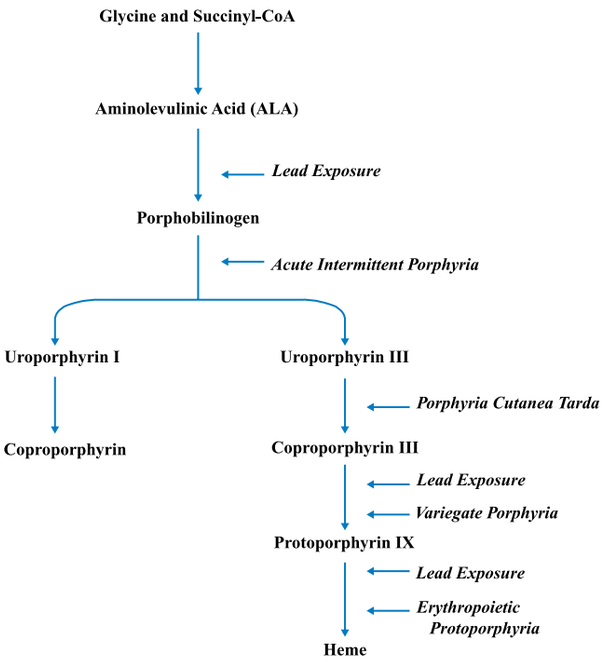


FIGURE 9–4 Pathway of heme formation, including stages affected by the major disorders of porphyrin metabolism. (Adapted from Miale.[18])

roporphyrin and protoporphyrin. However, to avoid false-positive interference, bile is a more acceptable specimen.[19] The Centers for Disease Control and Prevention recommends analysis of whole blood for the presence of free erythrocyte protoporphyrin (**FEP**) as a screening test for lead poisoning.

Disorders of porphyrin metabolism are collectively termed **porphyrias**. They can be inherited or acquired from erythrocytic and hepatic malfunctions or exposure to toxic agents. Common causes of acquired porphyrias include lead poisoning, excessive alcohol exposure, iron deficiency, and liver and renal disease. Inherited porphyrias are much rarer than acquired porphyrias. They are caused by failure to inherit the gene that produces an enzyme needed in the metabolic pathway. In Figure 9–4, the enzyme deficiency sites for some of the more common porphyrias are shown. The inherited porphyrias are frequently classified by their clinical symptoms, either neurologic/psychiatric or cutaneous photosensitivity or a combination of both (Table 9–3).

An indication of the possible presence of ***porphyrinuria*** is the observation of a red or port wine color to the urine. As seen with other inherited disorders, the presence of congenital porphyria is sometimes suspected from a red discoloration of an infant's diapers.

The two screening tests for porphyrinuria use the Ehrlich reaction and fluorescence under ultraviolet light in the 550 to 600 nm range. The Ehrlich reaction can be used only for the detection of ALA and porphobilinogen. Acetylacetone must be added to the specimen to convert the ALA to porphobilinogen prior to performing the Ehrlich test. The fluorescent technique must be used for the other porphyrins. The Ehrlich reaction, including the Watson-Schwartz test for differentiation between the presence of urobilinogen and porphobilinogen and the Hoesch test, were discussed in detail in Chapter 5. Testing for the presence of porphobilinogen is most useful when patients exhibit symptoms of an acute attack. Increased porphobilinogen is associated with acute intermittent porphyria. A negative test result will be obtained in the presence of lead poisoning unless ALA is first converted to porphobilinogen.

Fluorescent screening for the other porphyrins uses their extraction into a mixture of glacial acetic acid and ethyl acetate. The solvent layer is then examined. Negative reactions have a faint blue fluorescence. Positive reactions will fluoresce as violet, pink, or red, depending on the concentration of porphyrins. If the presence of interfering substances is suspected, the organic layer can be removed to a separate tube, and 0.5 mL of hydrochloric acid added to the tube. Only porphyrins will be extracted into the acid layer, which will then produce a bright orange-red fluorescence. The fluorescence method will not distinguish among uroporphyrin, coproporphyrin, and protoporphyrin, but it will rule out porphobilinogen and ALA. The identification of the specific porphyrins requires additional extraction techniques and the analysis of fecal and erythrocyte samples. Increased protoporphyrin is best measured in whole blood.

Mucopolysaccharide Disorders

Mucopolysaccharides, or glycosaminoglycans, are a group of large compounds located primarily in the connective tissue. They consist of a protein core with numerous polysaccharide branches. Inherited disorders in the metabolism of these compounds prevent the complete breakdown of the polysaccharide portion of the compounds, resulting in accumulation of the incompletely metabolized polysaccharide portions in the lysosomes of the connective tissue cells and their increased excretion in the urine. The products most frequently found in the urine are dermatan sulfate, keratan sulfate, and heparan sulfate, with the appearance of a particular substance being determined by the specific metabolic error that was inherited. Therefore, identification of the specific degradation product present may be necessary to establish a specific diagnosis.[16]

There are many types of ***mucopolysaccharidoses***, but the best known are Hurler's syndrome, Hunter's syndrome, and Sanfilippo's syndrome. In both Hurler's and Hunter's syndromes, the skeletal structure is abnormal and there is severe mental retardation; in Hurler's syndrome, mu-

TABLE 9–3 **Summary of Most Common Porphyrias**

Porphyria	Elevated Compound(s)	Clinical Symptoms	Laboratory Testing
Acute intermittent porphyria	ALA Porphobilinogen	Neurologic/psychiatric	Urine/Ehrlich's reaction
Porphyria cutanea tarda	Uroporphyrin	Photosensitivity	Urine fluorescence
Congenital erythropoietic porphyria	Uroporphyrin Coproporphyrin	Photosensitivity	Urine or feces fluorescence
Variegate porphyria	Coproporphyrin	Photosensitivity/neurologic	Bile or feces fluorescence
Erythropoietic protoporphyria	Protoporphyrin	Photosensitivity	Blood FEP Bile or feces fluorescence
Lead poisoning	ALA Protoporphyrin	Neurologic	Urine porphobilinogen/Ehrlich's reaction Blood FEP

PROCEDURE

Cetytrimethylammonium Bromide (CTAB) Test

1. Place 5 mL of urine in a tube.
2. Add 1 mL 5% CTAB in citrate buffer.
3. Read turbidity in 5 minutes.

copolysaccharides accumulate in the cornea of the eye. Both syndromes are usually fatal during childhood, whereas in Sanfilippo's syndrome, the only abnormality is mental retardation.[25]

Urinary screening tests for mucopolysaccharides are requested either as part of a routine battery of tests performed on all newborns or on infants who exhibit symptoms of mental retardation or failure to thrive. The most frequently used screening tests are the acid-albumin and cetyltrimethylammonium bromide (**CTAB**) turbidity tests and the metachromatic staining spot tests. In both the acid-albumin and the CTAB tests, a thick, white turbidity will form when these reagents are added to urine that contains mucopolysaccharides. Turbidity is usually graded on a scale of 0 to 4 after 30 minutes with acid-albumin and after 5 minutes with CTAB.[12] Metachromatic staining procedures use basic dyes to react with the acidic mucopolysaccharides. Papers can be prepared by dipping Whatman No. 1 filter paper into a 0.59 percent azure A dye in 2 percent acetic acid and letting it air dry.[1] Urine that contains mucopolysaccharides will produce a blue spot that cannot be washed away by a dilute acidified methanol solution.

Purine Disorders

A disorder of purine metabolism known as ***Lesch-Nyhan disease*** that is inherited as a sex-linked recessive results in massive excretion of urinary uric acid crystals. Failure to inherit the gene to produce the enzyme hypoxanthine guanine phosphoribosyltransferase is responsible for the accumulation of uric acid throughout the body. Patients suffer from severe motor defects, mental retardation, a tendency toward self-destruction, gout, and renal calculi. Development is usually normal for the first 6 to 8 months with the first symptom often being the observation of uric acid crystals resembling orange sand in diapers.[21] Laboratories should be alert for the presence of increased uric acid crystals in pediatric urine specimens.

PROCEDURE

Lactose Screening Test

1. Mix 3 g lead acetate with 15 mL of urine.
2. Filter.
3. Add 2 mL of concentrated NH_4OH.
4. Boil and observe for a brick red precipitate.

PROCEDURE

Mucopolysaccharide (MPS) Paper Test

1. Place one drop of urine on dry MPS paper.
2. Dry.
3. Wash 5 minutes (in 1 mL acetic acid + 200 mL methanol diluted to a liter).
4. Dry.
5. Observe for blue spot.

Carbohydrate Disorders

The presence of increased urinary sugar (***melituria***) is most frequently due to an inherited disorder. In fact, **pentosuria** was one of Garrod's original six inborn errors of metabolism.[7] Fortunately, the majority of meliturias cause no disturbance to body metabolism.[10] However, as discussed in Chapter 5, pediatric urine should be routinely screened for the presence of reducing substances using the Benedict's or Clinitest copper reduction tests. The finding of a positive copper reduction test result combined with a negative reagent strip glucose oxidase test result is strongly suggestive of a disorder of carbohydrate metabolism. Of primary concern is the presence of ***galactosuria***, indicating the inability to properly metabolize galactose to glucose. The resulting galactosemia with toxic intermediate metabolic products results in infant failure to thrive, combined with liver disorders, the presence of cataracts, and severe mental retardation. Early detection of galactosuria followed by removal of lactose (the precursor of galactose) from the diet can prevent these symptoms.

Other causes of melituria include lactose, fructose, and pentose. **Lactosuria** may be seen during pregnancy and lactation. **Fructosuria** is associated with parenteral feeding and pentosuria with ingestion of large amounts of fruit. Whenever a nonglucose-reducing substance is encountered in pediatric urine, it should be further identified using chromatography. Urine screening tests for metabolic disasters are summarized in Table 9–4.

PROCEDURE

Fructose Screening Test

1. Place 5 mL of urine in a tube.
2. Add 5 mL of 25% HCl.
3. Boil 5 minutes.
4. Add 5 mg resorcinol.
5. Boil 10 seconds.
6. Observe for a red precipitate.

TABLE 9–4 **Comparison of Urinary Screening Tests**

Test	Disorder	Observation
Color	Alkaptonuria	Black
	Melanuria	Black
	Indicanuria	Dark blue
	Porphyrinuria	Port wine
Odor	Phenylketonuria	Mousy
	Maple syrup urine disease	Maple syrup
	Isovaleric acidemia	Sweaty feet
	Cystinuria	Sulphur
	Cystinosis	Sulphur
	Homocystinuria	Sulphur
Crystals	Tyrosyluria	Sheaths of fine needles
	Cystinuria	Colorless hexagonal plates
	Lesch-Nyhan disease	Yellow-brown crystals
Ferric chloride tube test	Phenylketonuria	Blue-green
	Tyrosyluria	Transient green
	Alkaptonuria	Transient blue
	Melanuria	Gray-black
	Maple syrup urine disease	Green-gray
	Indicanuria	Violet-blue with chloroform
	5-HIAA	Blue-green
Nitroso-naphthol	Tyrosyluria	Red
	Maple syrup urine disease	Red
	5-HIAA	Violet with nitric acid
2,4-Dinitrophenylhydrazine	Phenylketonuria	Yellow
	Tyrosyluria	Yellow
	Maple syrup urine disease	Yellow
	Isovaleric acidemia	Yellow
	Propionic acidemia	Yellow
	Methylmalonic acidemia	Yellow
Acetest	Maple syrup urine disease	Purple
	Isovaleric acidemia	Purple
	Propionic acidemia	Purple
	Methylmalonic acidemia	Purple
	Melanuria	Red
p-Nitroaniline	Methylmalonic acidemia	Emerald green
Cyanide-nitroprusside	Cystinuria	Red-purple
	Cystinosis	Red-purple
	Homocystinuria	Red-purple
Silver nitroprusside	Homocystinuria	Red-purple
	Alkaptonuria	Black
Ehrlich's reaction	Porphyrinuria	Red
	Melanuria	Red
Cetytrimethylammonium bromide	Mucopolysaccharidoses	White turbidity
Mucopolysaccharide paper	Mucopolysaccharidoses	Blue spot
Clinitest	Melituria	Orange-red
	Cystinosis	Orange-red
	Alkaptonuria	Orange-red

REFERENCES

1. Bordon, M: Screening for metabolic disease. In Nyhan, WL: Abnormalities in Amino Acid Metabolism in Clinical Medicine. Appleton-Century-Crofts, Norwalk, CT, 1984.
2. Bradley, M, and Schumann, GB: Examination of urine. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1984.
3. Buist, NRM: Laboratory aspects of newborn screening for metabolic disorders. Lab Med 19(3):145–150, 1988.
4. Clow, CL, Reade, TH, and Scriver, CR: Outcome of early and long-term management of classical maple syrup urine disease. Pediatrics 68(6):856–862, 1981.
5. Doherty, LB, Rohr, FJ, and Levy, HL: Detection of phenylketonuria in the very early newborn specimen. Pediatrics 87(2):240–244, 1991.
6. Frimpton, GW: Aminoacidurias due to inherited disorders of metabolism. N Engl J Med 1289:835–901, 1973.
7. Garrod, AE: Inborn Errors of Metabolism. Henry Froude & Hodder & Stoughton, London, 1923.
8. Goodman, SI: Disorders of organic acid metabolism. In Emery, AEH,

and Rimoin, DL: Principles and Practice of Medical Genetics. Churchill Livingstone, New York, 1990.
9. Guthrie, R: Blood screening for phenylketonuria. JAMA 178(8):863, 1961.
10. Hiatt, HH: Pentosuria. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
11. Jepson, JB: Hartnup's disease. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
12. Kelly, S: Biochemical Methods in Medical Genetics. Charles C.Thomas, Springfield, IL, 1977.
13. Kirkman, H, et al: Fifteen year experience with screening for phenylketonuria with an automated fluorometric method. Am J Hum Genet 34(5):743–752, 1982.
14. Kishel, M, and Lighty, P: Some diaper brands give false-positive tests for PKU. N Engl J Med 300(4):200, 1979.
15. Kretchmer, N, and Etzwiler, DD: Disorders associated with the metabolism of phenylalanine and tyrosine. Pediatrics 21:445–475, 1958.
16. McKusick, VA, and Neufeld, EF: The mucopolysaccharide storage diseases. In Stanbury, JB, Wyngaarden, JB, and Fredrickson, DS (eds): The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
17. Meister, A: Biochemistry of the Amino Acids. Academic Press, New York, 1965.
18. Miale, JB: Laboratory Medicine: Hematology. CV Mosby, St. Louis, 1982.
19. Nuttall, KL: Porphyrins and disorders of porphyrin metabolism. In Burtis, CA, and Ashwood, ER: Tietz Fundamentals of Clinical Chemistry. WB Saunders, Philadelphia, 1996.
20. Nyhan, WL: Abnormalities in Amino Acid Metabolism in Clinical Medicine. Appleton-Century-Crofts, Norwalk, CT, 1984.
21. Nyhan, WL, and Sakati, NO: Diagnostic Recognition of Genetic Disease. Lea & Febiger, Philadelphia, 1987.
22. Sjoerdsma, A, Weissbach, H, and Udenfriend, S: Simple test for diagnosis of metastatic carcinoid (argentaffinoma). JAMA 159(4):397, 1955.
23. Stanbury, JB: The Metabolic Basis of Inherited Diseases. McGraw-Hill, New York, 1983.
24. Thomas, GH, and Howell, RR: Selected Screening Tests for Metabolic Diseases. Yearbook Medical Publishers, Chicago, 1973.
25. Thompson, JS, and Thompson, MW: Genetics in Medicine. WB Saunders, Philadelphia, 1978.
26. Waber, L: Inborn errors of metabolism. Pediatr Ann 19(2):105–118, 1990.
27. Wu, JT, Wilson, LW, and Christensen, S: Conversion of a qualitative screening test to a quantitative measurement of urinary cystine and homocystine. Ann Clin Lab Sci 22(1):18–29, 1992.

STUDY QUESTIONS

1. State two reasons for the appearance of overflow metabolites in the urine.
2. Name two physical characteristics of urine that can alert medical personnel to the possibility of a metabolic disorder.
3. List four metabolic disorders associated with the phenylalanine-tyrosine metabolic pathway.
4. Why are laws present that require PKU testing of all newborns?
5. Why are the original PKU newborn tests performed on blood rather than urine?
6. Name the enzyme lacking in persons with PKU.
7. When performing the Guthrie test, does the presence of increased phenylalanine inhibit the growth of *B. subtilis*? Why or why not?
8. What is the purpose for testing urine from people with PKU with ferric chloride?
9. State three possible causes of tyrosyluria. Which is usually the least serious?
10. What is the significance of an orange-red color in the nitroso-naphthol test?
11. Why is increased urinary homogentisic acid called alkaptonuria?
12. Why does the presence of homogentisic acid produce a positive Clinitest result?
13. What is the significance of a urine that turns black following exposure to air and reacts with sodium nitroprusside and Ehrlich's reagent? Why is this of medical importance?
14. Describe the ferric chloride tube test in PKU, tyrosyluria, alkaptonuria, and melanuria.
15. How did maple syrup urine disease get its name?
16. What chemical test in the routine urinalysis is associated with a positive DNPH reaction?
17. Which organic acidemia produces urine with an odor of "sweaty feet"? Which reacts with *p*-nitroaniline?
18. Why are intestinal disorders associated with blue urine? How does the significance of a blue diaper differ from that of a blue urine specimen in an adult?
19. What is the significance of a urine that turns purple upon addition of nitrous acid and 1-nitroso-2-naphthol? How could this be a false-positive result?
20. Why is cystinosis considered an inborn error of metabolism and cystinuria is not?
21. Why are cystine crystals and not lysine crystals found in the urine in cystinuria?
22. How can cystinuria be differentiated from homocystinuria in the laboratory?
23. List three heme precursor substances that are tested for in urine, two in feces or bile, and one in blood.
24. Name an inherited porphyria with neurologic symptoms, one with photosensitivity, and one with both symptoms.
25. What is the most common cause of acquired porphyria?
26. Name three heme precursor substances elevated in lead poisoning.
27. How could you determine if porphobilinogen or uroporphyrin is the cause of a port wine–colored urine?

28. What is the signficance of a blue spot on paper containing azure A dye?

29. What is the characteristic urine abnormality in Lesch-Nyhan disease?

30. What is the primary concern when melituria is present in a newborn?

CASE STUDIES AND CLINICAL SITUATIONS

1. A premature infant develops jaundice. Laboratory tests are negative for hemolytic disease of the newborn, but the infant's bilirubin level continues to rise. Abnormal urinalysis results include a dark yellow color, positive Ictotest, and needle-shaped crystals seen on microscopic examination.

a. What is the most probable cause of the infant's jaundice?
b. How will urine from this infant react in the ferric chloride test?
c. Could these same urine findings be associated with an adult? Explain your answer.
d. What kind of crystals are present? Name another type of crystal with a spherical shape that is associated with this condition.
e. When blood is drawn from this infant, what precaution should be taken to ensure the integrity of the specimen?

2. A newborn develops severe vomiting and symptoms of metabolic acidosis. Urinalysis results are positive for ketones and negative for glucose and other reducing substances.

a. State a urinalysis screening test that would be positive in this patient.
b. If the urine had an odor of "sweaty feet," what metabolic disorder would be suspected?
c. If the newborn was producing dark brown urine with a sweet odor, what disorder would be suspected?
d. State an additional urinalysis screening test that might be ordered on the infant. If this test produces an emerald green color, what is the significance?
e. The urine produces a green-gray color when tested with ferric chloride. Is this an expected result? Why or why not?
f. For the most accurate diagnosis of the newborn's condition, what additional testing should be performed?

3. A 13-year-old boy is awakened with severe back and abdominal pain and is taken to the emergency room by his parents. A complete blood count is normal. Family history shows that both his father and uncle are chronic kidney stone formers. Results of a urinalysis are as follows:

COLOR: Yellow	KETONES: Negative
APPEARANCE: Hazy	BLOOD: Moderate
SP. GRAVITY: 1.025	BILIRUBIN: Negative
pH: 6.0	UROBILINOGEN: Normal
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTE: Negative

Microscopic

>15–20 RBCs/hpf	Few squamous epithelial cells
0–3 WBCs/hpf	Many cystine crystals

a. What condition does the patient's symptoms represent?
b. What is the physiologic abnormality causing this condition?
c. If amino acid chromatography was performed on this specimen, what additional amino acids would you expect to find?
d. Why are they not present in the microscopic constituents?
e. What chemical test could be performed to confirm the identity of the cystine crystals?
f. What is the significance of the family history?

4. An 8-month-old boy is admitted to the pediatric unit with a general diagnosis of failure to thrive. The parents have observed slowness in the infant's development of motor skills. They also mention the occasional appearance of a substance resembling orange sand in the child's diapers. Urinalysis results are as follows:

COLOR: Yellow	KETONES: Negative
APPEARANCE: Slightly hazy	BLOOD: Negative
SP. GRAVITY: 1.024	BILIRUBIN: Negative
pH: 5.0	UROBILINOGEN: Normal
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTE: Negative

Microscopic
Many uric acid crystals

a. Is the urine pH consistent with the appearance of uric acid crystals?
b. Is there any correlation between the urinalysis results and the substance observed in the child's diapers? Explain your answer.
c. What disorder do the patient's history and the urinalysis results indicate?
d. Is the fact that this is a male patient of any significance? Explain your answer.
e. Name the enzyme that is missing.

5. Shortly after arriving for the day shift in the urinalysis laboratory, a technician notices that an undiscarded urine has a black color. The previously completed report indicates the color to be yellow.

a. Is this observation significant? Explain your answer.
b. What two reactions might be seen with the ferric chloride test?
c. Which ferric chloride reaction would correlate with a positive Clinitest result?
d. The original urinalysis report showed the specimen to be positive for ketones. Is this significant? Why or why not?

6. Bobby Williams, age 8, is admitted through the emergency department with a ruptured appendix. Although surgery is successful, Bobby's recovery is slow, and the physicians are concerned about his health prior to the ruptured appendix. Bobby's mother states that he has always been noticeably underweight despite a balanced diet and strong appetite and that his younger brother exhibits similar characteristics. A note in his chart from the first postoperative day reports that the evening nurse noticed a purple coloration on the urinary catheter bag.
   a. Is the catheter bag color significant?
   b. What additional tests should be run?
   c. What condition can be suspected from this history?
   d. What is Bobby's prognosis?

7. A Watson-Schwartz test is performed on an anemic patient who is exhibiting signs of severe photosensitivity. The test result is negative.
   a. What metabolic disorder was suspected in this patient?
   b. Was sufficient testing performed to rule out this disorder? Why or why not?
   c. Can the Watson-Schwartz test be used to detect lead poisoning? Explain your answer.

8. The laboratory receives a request for a resorcinol test.
   a. What substance will be detected?
   b. What treatment might this patient be receiving?