

CLINICAL BIOCHEMISTRY

Diseases related to Metabolism

Anjali Manocha and Sabari Das

Department of Biochemistry

Sir Ganga Ram Hospital

Rajendra Nagar

New Delhi – 110060

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Diseases; Metabolism; Glycogen storage diseases; Sphingolipidosis; Lipoprotein; Inborn errors; Aminoacids; Hyperuricemia; Gout.

Hypoglycemia

Hypoglycemia is blood glucose concentration below the fasting level. It is difficult to define a specific limit, however below 45mg/dL (2.5mmol/L) is considered a hypoglycemic condition widely. Hypoglycemia can have many causes - some are transient and relatively insignificant, others can be life threatening.

Symptoms of hypoglycemia include:

- Shakiness
- Dizziness
- Sweating
- Hunger
- Headache
- Pale skin color
- Sudden moodiness or behavioural changes
- Clumsy or jerky movements
- Seizure
- Difficulty in paying attention, or confusion
- Tingling sensation around the mouth
- Nausea
- Rapid pulse
- Light headedness

The symptoms of hypoglycemia are non-specific and vary among individuals on the degree of hypoglycemia, the age of the patient and the rapidity of the decline in blood glucose. They are divided into adrenergic and neuroglycopenic symptoms but they all are the effects of hypoglycemia on the central nervous system (CNS). Restoration of glucose in the circulation usually results in a prompt recovery, but irreversible damage may occur. In patients with diabetes, the most common cause of hypoglycemia is excessive use of insulin or other glucose lowering medications, to lower the blood sugar level.

In healthy subjects the blood glucose is maintained through a tightly controlled balance between glucose products and glucose utilization. Under physiological conditions, the brain is totally dependent on blood glucose for energy production. Very low levels of plasma glucose cause severe CNS dysfunction. During prolonged fasting and hypoglycemia, ketones may be used as an energy source.

Classification of Hypoglycemia

Hypoglycemia can be classified as post-absorptive (fasting) and post-prandial (fed) hypoglycemia. Blood glucose concentrations in the post absorptive and postprandial states are regulated by the interaction between insulin and glucagon. Insulin enhances storage of nutrients by promoting glycogenesis, lipogenesis and protein synthesis. Glucagon, on the other hand, functions primarily to prevent hypoglycemia by stimulating glycogenolysis and glyconeogenesis.

Post-absorptive hypoglycemia: In post-absorptive hypoglycemia an individual is unable to maintain a stable plasma glucose level. It includes non-suppressible insulin like activity in which the glucose level drops below normal fasting level. The patient must be given glucose to relieve symptoms because spontaneous recovery of glucose level does not occur.

Post-absorptive hypoglycemia may be due to:

- β islet cell insulinomas
- Other insulin producing tumors
- Ethanol induced
- Drug induced
- Severe liver diseases
- Enzymatic defects

Symptoms of post-absorptive hypoglycemia are:

- Increased hunger
- Sweating
- Nausea and vomiting
- Dizziness
- Nervousness and shaking
- Blurring of speech and sight
- Mental confusion

Postprandial hypoglycemia: Postprandial hypoglycemia is infrequent and is usually not serious. Its characteristics include an excessive release of insulin that results in glucose levels dropping below normal fasting levels. There is usually a spontaneous recovery of glucose level, as the insulin level returns to normal.

A group of disorders may produce hypoglycemia in the postprandial state. These include:

- Drugs
- Antibodies to insulin or the insulin receptor
- Inborn errors

Hypoglycemia in neonates and infants

Neonatal blood glucose concentrations are much lower than adult and decline shortly after birth when liver glycogen stores are depleted. The more common causes of hypoglycemia in the neonatal period include:

- Prematurity
- Maternal diabetes
- Gestational Diabetes Mellitus
- Maternal toxemia

These are usually transient. Onset of hypoglycemia in infancy is usually less transitory and may be due to inborn errors of metabolism.

Hypoglycemia Unawareness

Some people have no symptoms of hypoglycemia. They may lose consciousness without ever knowing their blood glucose levels were dropping. This problem is called hypoglycemia unawareness.

Hypoglycemia unawareness tends to happen to people who have had diabetes for many years. Hypoglycemia unawareness does not happen to everyone. It is more likely in people who

have neuropathy (nerve damage), people on tight glucose control, and people who take certain heart or high blood pressure medicines.

Hyperglycemia

Hyperglycemia is an increase in blood glucose levels, and it happens when the body has too little or not enough insulin or when the body can't use insulin properly. Insulin is a protein hormone produced by the β cells of the islets of Langerhans in the pancreas. It stimulates the uptake of glucose into fat and muscle, promotes the conversion of glucose to glycogen or fat for storage, inhibits glucose production by the liver, stimulates protein synthesis and inhibits protein breakdown. Hyperglycemia can be a serious problem if not treated.

Causes of Hyperglycemia

Glucose is an essential nutrient that provides energy for the proper functioning of the body cells. Carbohydrates are broken down in the small intestine and the glucose in digested food is then absorbed by the intestinal cells into the blood stream and is carried by the bloodstream to all the cells in the body where it is utilized. However, glucose cannot enter the cells alone and needs insulin to aid in its transport into the cells. Without insulin, the cell becomes starved of glucose energy despite the presence of abundant glucose in the blood stream. The abundant, unutilized glucose is wastefully excreted in the urine.

Insulin in addition to helping glucose enter the cells, also tightly regulates the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the blood stream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down. It is important to note that even in the fasting state there is a low steady release of insulin that fluctuates a bit and helps to maintain a steady blood sugar level during the fasting. In normal individuals, such a regulatory system helps to keep blood glucose levels in a tightly controlled range. However, in patients with diabetes the insulin is either absent, relatively insufficient for the body's needs, or not used properly by the body. All of these factors cause hyperglycemia.

The cardinal manifestation of diabetes mellitus is hyperglycemia, which results from:

- Decreased entry of glucose into cells
- Decreased utilization of glucose by various tissues
- Increased production of glucose (gluconeogenesis) by the liver.

Diabetes Mellitus is divided into two broad categories:

- Type 1 Diabetes Mellitus
- Type 2 Diabetes Mellitus

Type 1 Diabetes Mellitus is characterized by inappropriate hyperglycemia primarily due to pancreatic islet β cell destruction causing an absolute deficiency of insulin secretion and proneness to ketoacidosis.

Type 2 Diabetes in contrast includes hyperglycemia cases that result from insulin resistance with an insulin secretory defect.

Hyperglycemia can be caused by many other different things. Some common causes are:

- Eating more food than is in your diet plan
- Taking less insulin than is prescribed
- Infection or illness
- Injury
- Surgery
- Emotional stress

Depending on the cause, hyperglycemia may develop in hours or days. If the cause is eating too much, the blood sugar will be higher in a few hours. If the cause is infection or illness, hyperglycemia will develop within a few days.

Symptoms of Hyperglycemia

The signs and symptoms of hyperglycemia include:

- Frequent urination
- Increased thirst
- Fatigue, Weakness
- Blurry vision

It is important to treat hyperglycemia as soon as it is detected. If it is not treated, a condition called ketoacidosis (diabetic coma) can occur. Ketoacidosis develops when the body doesn't have enough insulin. Without insulin, the body can not use glucose for fuel. So, the body breaks down fats for energy. When the body breaks down fats, waste products called ketones are produced. Our body cannot tolerate large amounts of ketones and tries to get rid of them through the urine. Unfortunately, the body cannot release all the ketones and they build up in our blood. This can lead to ketoacidosis.

Ketoacidosis is life threatening and needs immediate treatment. Symptoms include:

- Shortness of breath
- Breath that smells fruity
- Nausea and vomiting
- A very dry mouth

Chronic complications of hyperglycemia

Toxicity of glucose is associated with long-term complications of diabetes. Slowly developing changes in small (microangiopathy) and large (macroangiopathy) arteries are part of the diabetic syndrome. In the long term these changes lead to kidney failure (diabetic nephropathy), blindness (caused by diabetic retinopathy) and to the impairment of nerve function (diabetic neuropathy). Diabetic patients also develop lens opacities (cataracts). Owing to macroangiopathy, diabetic individuals are at a two or three times greater risk of myocardial infarction than people who are nondiabetic. Finally, the diabetic peripheral vascular disease is a major cause of foot ulcers and lower limb amputations. Currently, cardiovascular disease is the most prevalent complication, and is the main cause of death among people with diabetes.

Glycosylated Hemoglobin (HbA_{1c})

A draw back of the measurement of plasma glucose is that it changes quickly. Therefore a major advance in the monitoring of diabetic patients has been the measurement of glycosylated hemoglobin or HbA_{1c}. Glycation is the nonenzymatic addition of a sugar residue to amino groups of proteins. Hemoglobin is glycosylated when blood glucose enters the erythrocytes. The fraction of hemoglobin glycosylated is proportionate to blood glucose concentration. Measurement of HbA_{1c} thus provides information useful for the management of diabetes mellitus. Since the mean half-life of an erythrocyte is 60 days, the HbA_{1c} level reflects the average blood glucose concentration over the preceding 6-8 weeks. An elevated HbA_{1c}, which indicates poor control of blood glucose level, can guide the physician in selection of appropriate treatment.

Prevention of Hyperglycemia

The best way to prevent hyperglycemia is to practice good diabetes management. The trick is learning to detect and treat hyperglycemia early before it can get worse.

Glycogen Storage Diseases

Glucose is a major source of energy for the body; it is stored in the form of glycogen and later released with the help of enzymes. Glycogen is found mainly in liver and muscle cells, while the kidneys and intestines are minor storage sites. During fasting, liver glycogen is converted to glucose to provide energy for the whole body.

Persons affected by Glycogen Storage Disease (GSD) have an inherited absence or deficiency of any of the enzymes responsible for forming or releasing glycogen. These enzyme defects lead to abnormal tissue concentrations of glycogen or to its structurally abnormal forms. The liver and skeletal muscle have the highest rate of glycogen metabolism and hence these are the structures which are most affected.

All of the different types of GSD also referred to as “glycogenosis” result in the body not being able to produce sufficient glucose in the blood stream and to utilize glucose as a source of energy. Diagnosis of the type of GSD is made on the basis of an individual’s symptom, the result of a physical examination and of biochemical tests.

GSD is a generic term intended to describe a group of inherited disorders. These are classified by numbers in the chronological sequence in which these defects were identified or by the name of the defective enzyme.

Almost all forms of GSD occur when a child inherits the affected gene from both parents, each of them is a carrier but is not affected themselves.

Type I

Type I GSD also known as von Gierkes disease is the most common and severe form. Patients with Type I GSD are unable to release glucose from glycogen due to the deficiency of glucose-6-phosphatase and hence with time glycogen builds up in the liver. It is characterized by massive enlargement of liver (hepatomegaly), growth retardation, fasting hypoglycemia, increased lactic acid concentrations in the blood (due to excessive glycolysis),

hyperuricemia and hypertriglyceridemia. The diagnosis of Type I GSD should include blood studies, X-rays, and ultrasound of the liver. Glucose-6-phosphatase activity can be assayed in a liver biopsy.

Treatments of Type I GSD are aimed at correcting the biochemical abnormalities and promoting growth and development. Current treatment consists of providing frequent meals and naso-gastric feeding at night to maintain blood glucose concentration.

A variant of the disease, type Ib, has been identified as a defect in the glucose-6-phosphatase transport system. These patients in addition to the problems described above develop frequent bacterial and fungal infection, due to abnormal functioning of the white blood cells. They may also develop chronic pancreatitis, chronic inflammatory bowel disease, and crohn's disease. Other variant forms include a defect in microsomal phosphate or pyrophosphate transport (type Ic) and a defect in microsomal glucose transport (type Id).

Type II

Type II GSD affects predominantly the heart and skeletal muscle producing muscle weakness and cardiomegaly. Liver function is normal and patients do not have hypoglycemia. Every cell in our body contains vesicles called lysosomes that digest the waste products of the cell. Type II GSD is caused by a lack of function of the enzyme acid glucosidase, which is present in lysosomes. Without the proper functioning of this enzyme, the glycogen that comes into the lysosomes is not broken down, but accumulates and disrupts the normal functions of the cell. In muscle tissue, these enlarged lysosomes eventually cause the cells to become dysfunctional and die.

There are at least two forms of Type II GSD, with the most common being the infantile form. **Infantile form** (Pompe's disease): This appears in the first few months of life with weakness and respiratory difficulties. The patients usually die before 12 months of age due to cardiac failure and respiratory weakness.

Juvenile forms: This form of Type II GSD is milder and may present in the second or third decade of life with difficulty in walking. The involvement of muscle weakness progresses slowly over the years.

Diagnosis of Type II GSD is done by determining the activity of the enzyme acid glucosidase in muscle, liver or lenkocytes. This deficiency can be shown with a muscle biopsy or cultured cells from a skin biopsy.

Treatment of Type II GSD is aimed at relieving stress on the muscles. A protein-rich diet is used, along with an intensive daily exercise program.

Type III

Deficiency of Amylo-1, 6-Glucosidase the debranching enzyme results in storage of an abnormal form of glycogen (limit dextrinosis).

Two subtypes of this disorder IIIa & IIIb have been observed. In GSD Type IIIa, the disease involves both liver and muscle tissues, producing hepatomegaly and muscle weakness. Type IIIb involves only the liver without apparent muscle disease.

Clinical and biochemical features resemble those of type I disease. Differentiation from type I is by a hyperglycemic response to galactose, lower concentrations of urate and lactate in the blood and elevated serum transaminase and creatinine kinase activities. Enzyme deficiency is found in muscle or liver and at times in erythrocytes. Treatment of Type III GSD consists of frequent feedings and a high protein diet. Continuous nasogastric feedings similar to those used for Type I GSD are useful.

Type IV

Type IV GSD also known as Andersen's disease is a rare disorder, which is caused due to production of an abnormal form of unbranched glycogen in all tissues. Patients exhibit enlargement of liver and spleen with ascites and liver failure. Abnormal glycogen can be identified in tissues and muscles. Treatment of Type IV GSD has been aimed at the failing liver, which has been symptomatic.

Type V

In the absence of phosphorylase in muscles, glucose cannot be released from the glycogen stored in skeletal muscles for energy. Hence people with Type V GSD, also called McArdle disease, experience problems performing and completing most exercises. These patients experience muscle pain, muscle cramps, muscle fatigue and muscle tenderness. With the breakdown of muscle and the release of myoglobin, myoglobinuria may develop. Patients with type V GSD have increased creatine kinase activities at rest, produce an exaggerated increase in ammonia and myoglobinuria and have diminished activity of muscle phosphorylase activity.

These patients should exercise moderately, for extensive exercise can cause considerable muscle breakdown resulting in great deal of release of myoglobin in the urine. However, patients with type V GSD respond to oral glucose administration or injections of glucagon.

Type VI

Type VI GSD also known as Hers' disease is a rare and relatively benign disorder due to the deficiency of liver phosphorylase or one of the subunits of phosphorylase kinase. Enlargement of liver due to increased deposit of glycogen, growth retardation and mild hypoglycemia are seen. Diagnosis is made by measuring enzyme activity in the liver or in red or white blood cells and also by studying the liver biopsy.

Type VII

Patients with Type VII GSD also known as Tarui's disease have deposits of abnormal glycogen in muscle. With the deficiency of phosphofructokinase, effective glycogen breakdown (glycolysis) during muscle stress cannot be accomplished, resulting in pain, weakness, and cramping in the exercising muscle.

Clinical features of Type VII GSD are exercise intolerance, unresponsiveness of glucose administration and hemolysis (due to decreased glycolysis in erythrocytes). These produces myoglobinuria, hyperbilirubinemia, pigmentation and reticulocytosis. Diagnosis of Type VII GSD is done by muscle biopsy. The different forms of glycogen storage diseases are summarized in table in Table 1.

Table 1: Glycogen Storage Diseases

GSD	Name	Enzyme Defect
Type I	Von Gierke's disease	Deficiency of glucose-6-phosphatase
Type II	Pompe's disease	Deficiency of acid glucosidase
Type III	Limit dextrinosis, Forbes' or Cori's disease	Absence of Amylo-1, 6-glucosidase (debranching enzyme)
Type IV	Andersen's disease	Absence of branching enzyme
Type V	McArdle's syndrome	Absence of muscle phosphorylase
Type VI	Hers' disease	Deficiency of liver phosphorylase
Type VII	Tarui's disease	Deficiency of phosphofructokinase in muscle and erythrocytes.

Lipid malabsorption and steatorrhea

Fat absorption is a complex process, requiring the delivery of bile salts and pancreatic enzymes to the duodenum, together with efficient enterocyte function and normal mixing. Therefore, disease at several levels in the alimentary system can result in fat malabsorption, which is characterized by the presence of steatorrhea. Interference with delivery of bile salts into the duodenum, pancreatic deficiency, failure to achieve near-neutral duodenal pH, mucosal disease and abeta lipoproteinaemia, can all result in steatorrhea.

Steatorrhea is a condition in which the body cannot absorb fat. It causes a build up of fat in the stool and loose, greasy and foul bowel movements. Causes of steatorrhea are:

- malabsorption of fat
- pancreatitis
- celiac disease
- sprue

Symptoms of Steatorrhea are:

- foul smelling feces
- bulky stool
- pale stool
- loose stool
- greasy stool

Impaired fat absorption also leads to vitamin E deficiency because tocopherol is found dissolved in the fat of the diet and is liberated absorbed during fat digestion.

Sphingolipidosis

The Sphingolipidosis are recessively inherited disorders in which there is a deficiency of a specific lysosomal hydrolytic enzyme and consequent deposition of complex lipids in various tissues. They are almost all degenerative diseases with mental retardation and early demise.

Lipid storage disease exhibit several constant features such as:

- In various tissues, there is an accumulation of complex lipids that have a portion of their structure in common – ceramide.
- The rate of synthesis of the stored lipid is comparable to that in normal humans.
- The enzymatic defect in each of these disease is a deficiency due to gene mutation of a specific lysosomal hydrolytic enzyme necessary to breakdown the lipid or of a key activator protein of the enzyme.
- The extent to which the activity of the affected enzyme is decreased is similar in all of the tissues of the affected individuals.

To treat sphingolipidosis enzyme replacement therapy has been tried for many years, but with little success. Recent treatments are aimed at enzymes that have been chemically modified to ensure binding to receptors of target cells. Gene therapy is currently under investigation. The different forms of sphingolipidoses are summarized in the Table 2.

Disorders of Lipoprotein Metabolism

Inherited defects in lipoprotein metabolism lead to the primary condition of either hypo or hyper lipoproteinemia associated with lipoproteins of abnormal composition or an abnormal distribution of the normal lipoprotein classes. Diseases such as diabetes mellitus, hypothyroidism, kidney disease and atherosclerosis show abnormal lipoprotein patterns that are very similar to one or another of the primary inherited conditions. All of these primary conditions are due to a defect at a stage in lipoprotein formation, transport or destruction. Primary disorders of plasma lipoproteins are summarized in the Table 3.

Inborn Errors of Amino Acid Metabolism

Introduction

All polypeptides and proteins are polymers of amino acids. Eight amino acids are essential i.e. they cannot be synthesized in the human body and must be obtained from dietary sources. The rest can be synthesized endogenously. Each amino acid has a unique degradative pathway by which its nitrogen and carbon components are used for the synthesis of other amino acids, carbohydrates and lipids.

More than 70 disorders of amino acid metabolisms are known. Almost all are transmitted as autosomal recessive traits. In general, these disorders are named for the compound that accumulates in the highest concentration in the blood (-emias) or urine (-urias).

Aminoacidurias may be:

1. Primary aminoaciduria -- It is further divided into two groups: -
 - Overflow type
 - Renal type
2. Secondary aminoaciduria

Table 2: Sphingolipidosis

Disease	Signs & Symptoms	Enzyme Defect	Lipidaccumulating
Farber's Disease	Hoarseness, dermatitis, skeletal deformation, mental retardation, fatal in early life.	Ceramidase	Sphingosine Ceramide
Gaucher's Disease	Spleen and liver enlargement, erosion of long bones and pelvis, mental retardation only in infantile form.	β -glucosidase	Glucosylceramide
Niemann-Pick Disease	Liver and spleen enlargement, mental retardation, about 30% with red spot in retina, fatal in early life.	Sphingomyelinase	Sphingomyelin
Krabbe's Disease (globoid)	Mental retardation, almost total absence of myelin.	β -galactosidase	Galactosylceramide
Metachromatic leukodystrophy	Mental retardation, psychological disturbances in adult form, nerves stain yellow-brown with cresyl violet dye, demyelination.	Arylsulfatase A	3-Sulfogalactosylceramide
Fabry's Disease	Reddish purple skin rash, kidney failure, pain in lower extremities	α -galactosidase	Globotriaosylceramide
Tay-Sachs Disease	Mental retardation, red spot in retina, blindness, muscular weakness.	Hexosaminidase A	G _{M2} Ganglioside
Tay-Sachs variant or Sandhoff's Disease	Same as Taysachs, but progresses more rapidly.	Hexosaminidase A & B	Globoside Plus G _{M2} Ganglioside
Generalized gangliosidosis	Mental retardation, liver enlargement, skeletal deformities.	β -Galactosidase	G _{M1} Ganglioside
Fucosidosis	Cerebral degeneration, muscle spasticity, thick skin.	α -Fucosidase	H-Isoantigen
Ceramide lactoside lipidosis	Progressing brain damage, liver and spleen enlargement.	β -galactosidase	Ceramide lactoside

Table 3: Disorders of Lipoprotein Metabolism

Name	Defect
Hypolipoproteinemias Abetalipoproteinemia	Absence chylomicrons, VLDL, or LDL are formed because of defect in triacylglycerol transfer protein (MTP), which prevents the loading of apo B with lipid.
Familial hypobetalipoproteinemia	LDL concentration is 10-60% of normal.
Familial alpha-lipoprotein deficiency Tangier disease Fish-eye disease Apo-A-I deficiencies	Low or near absence of HDL in all.
Hyperlipoproteinemias Familial lipoprotein lipase deficiency (type I)	Deficiency of LPL, or production of abnormal LPL or apo-C-II deficiency
Familial hypercholesterolemia (type II)	Type IIa: Defective LDL receptors or mutation in ligand region of apo B-100. Type IIb: Tendency for VLDL to be elevated in addition.
Wolman's disease (cholesteryl ester storage disease)	Deficiency of cholesteryl ester hydrolase in lysosomes.
Familial type III hyperlipoproteinemia (broad beta disease, remnant removal disease, familial dysbetalipoproteinemia)	Deficiency in remnant clearance by the liver is due to abnormality in apo E, which is normally present in 3 isoforms: E2, E3, and E4. Patients have only E2, which does not react with the E receptor. Truncated apo B species present.
Familial hypertriacylglycerolemia (type IV)	Overproduction of VLDL often associated with glucose intolerance and hyperinsulinemia.
Familial type V hyperlipoproteinemia	Elevated levels of chylomicrons and VLDL, cause unknown.
Familial hyperalphalipoproteinemia	Increased concentrations of HDL.
Hepatic lipase deficiency	Deficiency of the enzyme leads to accumulation of large triacylglycerol-rich HDL and VLDL remnants.
Familial lecithin: cholesterol acyltransferase (LCAT) deficiency	Absence of LCAT leads to block in reverse cholesterol transport. HDL incapable of taking up and esterifying cholesterol.
Familial lipoprotein (a) excess	Lp(a) consists of 1 mol of LDL attached to 1 mol of apo(a). Apo(a) shows structural homologies to plasminogen.

Primary Aminoacidurias

Primary aminoacidurias (summarized in Tables 4 and 5) occur due to an inherited enzyme defect and hence are also known as *Inborn errors of metabolism*. In these, the error is either in the pathway by which a specific amino acid is metabolized or in the specific renal tubular transport system by which the amino acid is reabsorbed.

Table 4: Primary Aminoacidurias

S. No.	Disorder	Defect	Excess in Blood	Excess in Urine
1.	Hyperphenylalaninemia			
(i)	Classic PKU (Type I)	Phenylalanine Hydroxylase (absent)	Phenylalanine	Phenylalanine and its metabolites
(ii)	Variant PKU (Type II)	Phenylalanine Hydroxylase (deficient)	Phenylalanine	Variable
(iii)	Transient neonatal (Type III)	Phenylalanine Hydroxylase (deficient)	Phenylalanine	Phenylalanine metabolites usually not found
(iv)	Type IV	Dihydropteridine reductase (absent)	Phenylalanine	Variable
(v)	Type V	Defect in bipterin synthesis	Phenylalanine	Variable
2.	Tyrosinemia			
(i)	Type I Tyrosinosis	Fumarylacetoacetate hydroxylase ?(absent)	Tyrosine; methionine	Tyrosine and its metabolites
(ii)	Type II	Tyrosine aminotransferase (absent)	Tyrosine	Tyrosine and its metabolites and tyramine
(iii)	Transient Neonatal	Liver immaturity	Tyrosine; phenylalanine	Tyrosine and its metabolites and tyramine
3.	Alkaptonuria	Homogentisic acid oxidase (absent)	Homogentisic acid (Slight)	Homogentisic acid
4.	Histidinemia	Histidase (absent)	Histidine, alanine	Imidazole; pyruvic acid and other histidine metabolites
5.	Branched chain Ketoaciduria (MSUD)	Branched chain keto acid decarboxylase (deficient)	During acute attacks: leucine, isoleucine, alloisoleucine, valine and corresponding ketoacids	During acute attacks as in blood; also odour
6.	Propionic acidemia	Propionyl CoA carboxylase (deficient)	Glycine	Glycine (also in CSF)
7.	Methylmalonic acid acidemia	Methylmalonyl CoA mutase (absent or deficient)	Glycine methylmalonic acid	Glycine; methylmalonic acid

				(also in CSF)
8.	Cystathioninuria	γ - cystathionase (absent or deficient)	Cystathionine	Cystathionine (also in CSF) & cystathionine metabolites
9.	Carnosinemia	Carnosinase (deficient)	Carnosine	Carnosine
10.	Hyperprolinemia			
	Type I	Proline oxidase (deficient)	Proline	Proline, hydroxyproline glycine
	Type II	Δ^5 pyrroline 5- Carboxylic acid dehydrogenase (deficient)	Proline	Proline, hydroxyproline glycine

Table 5: Primary Renal Aminoacidurias

Disorder	Excess in Urine	Inheritance
Cystinuria (classic)	Lysine, ornithine, arginine, cystine	Autosomal recessive
Hypercystinuria	Cystine	Autosomal recessive
Dibasic aminoaciduria and lysinuric protein intolerance	Ornithine, lysine, arginine	Autosomal recessive
Hartnup's disease	All neutral amino acids	Autosomal recessive
Iminoglycinuria	Glycine, proline, hydroxyproline	Autosomal recessive
Dicarboxylic aminoaciduria	Glutamic acid aspartic acid	Autosomal recessive
Methionine malabsorption	Methionine; also tyrosine, phenylalanine, branched chain aminoacids, α - hydroxybutyric acid	Autosomal recessive

Children with inborn errors of metabolism may present with one or more of a large variety of signs and symptoms. These may include high or normal anion gap metabolic acidosis, persistent vomiting, failure to thrive, altered consciousness, seizures, myopathies, developmental delay, hypoglycemia, elevated blood or urinary levels of a particular metabolite (an amino acid, organic acid or ammonia), a peculiar odour or physical changes like dysmorphism, cardiomegaly, rashes, cataract, retinitis, deafness, skeletal dysplasias, macrocephaly or hepatomegaly, jaundice, cirrhosis etc.

In the overflow type of aminoacidurias, the symptoms and the prognoses range from nearly benign as in alkaptonuria to almost lethal as in maple syrup urine disease. There is usually a block in a major catabolic pathway, because of which, the substrates and precursor substrates behind the block accumulate in the blood. When the renal transport mechanisms for these substances are saturated, these substances spill into the urine. Accumulation of ketoacids in maple syrup urine disease and phenylalanines in phenylketonuria are examples of precursors and substrates with toxic effects. After the block, products and intermediates are decreased, e.g. tyrosine in phenylketonuria. In some cases, the substrates do not accumulate in the blood: there is no reabsorption in the kidneys (no threshold aminoacidurias); blood levels are low and urine levels are decreased.

In renal type of primary aminoacidurias as in cystinurias, high levels of aminoacids are not found in the blood because the defective protein coded by the abnormal gene is an element of the tubular reabsorption mechanism for that aminoacid. The transport defect may affect a single aminoacid or a group of aminoacids, e.g. the dibasic aminoacid transport system is impaired in cystinuria.

Inborn errors of metabolism are the result of mutations of DNA base sequence that codes for the specific aminoacid sequence of a particular enzyme protein. The defect in the enzyme that is derived from the altered gene is reflected in diminished or no biological activity. The metabolic defect is expressed as abnormal concentration of normal metabolites or as the appearance of abnormal metabolites associated with chronic symptoms. Theoretically it is possible to diagnose such an inherited disease at three levels: -

1. The DNA abnormality
2. The enzyme defect
3. Metabolic abnormality that is due to the defect.

Once a presumptive diagnosis is made, confirmation is done by direct enzyme assay of extracts of leucocytes, erythrocytes or cultured fibroblasts. DNA based testing is possible for several disorders like PKU, ornithine transcarbamylase deficiency, citrullinemia, etc. Several of these disorders like PKU, branched chain ketoaciduria, homocystinuria, cystinosis, etc. can be diagnosed prenatally by chemical analysis of amniotic fluid, or by chemical, enzymatic or DNA based studies of fresh or cultured amniotic fluid cells.

Secondary Aminoacidurias

Secondary aminoacidurias can also be of overflow or renal types and may affect many aminoacids at a time. Secondary overflow aminoaciduria may occur as a result of fulminant hepatic failure. This appears to be due to production of small molecular, water soluble toxins, both free and bound to protein, which result in the failure of metabolism and detoxification processes normally carried out by the liver.

Secondary renal aminoaciduria occurs as a result of progressive damage to the renal tubules. Causes may be inherited or acquired, all of which cause proximal renal tubular dysfunction. Generalized renal aminoacidurias may be caused by poisons (especially heavy metals), wasting from starvation or disease, acute tubular necrosis or by congenital conditions like galactosemia and Wilson's disease.

Diagnosis of inherited aminoacidurias can be done in

1. Neonatal period and Post neonatal period
2. Prenatal diagnosis

Neonatal and post-neonatal period

Inborn errors of metabolism with clinical manifestations in the neonatal period are usually severe and are often lethal if proper therapy is not initiated promptly.

Since most inherited errors of metabolism are inherited as autosomal recessive traits, a history of consanguinity and/or death in the neonatal period should increase the suspicion of this diagnosis. Physical examination usually is nonspecific with most signs related to central nervous system. Hepatomegaly is a common finding and occasionally an unusual odor may be present. Inherited disorders of metabolism should also be suspected if symptoms appear when feeding is changed or if the condition improves if food is withheld.

Diagnosis requires a variety of specific laboratory studies, e.g. serum concentration of glucose, ammonia, bicarbonate and pH. Elevations of blood ammonia levels are usually caused by defects in urea cycle enzymes. Infants with elevated blood ammonia levels commonly have normal serum pH and bicarbonate value. Elevated ammonia levels have also been observed in infants with certain organic acidemias.

When blood ammonia, pH and bicarbonate values are normal, other aminoacidopathies (e.g. hyperglycinemia) or galactosemia may be considered.

A specific diagnosis is facilitated by direct biochemical assays of their metabolites or their metabolic by-products, or of an enzyme's function. Planar chromatography, also known as thin layer chromatography, a simple and inexpensive technique, is frequently used for this purpose. DNA analysis of the gene, functional tests and neuroradiology are also useful.

Tandem mass spectroscopy and capillary electrophoresis are also useful in neonatal metabolic screening programs, to detect disorders in the presymptomatic stage.

An inborn error of metabolism should be considered in any child with one or more of the following manifestations:

- (1) Unexplained mental retardation, developmental delay or regression, motor deficits or convulsions.
- (2) Unusual odor, particularly during an acute illness.
- (3) Intermittent episodes of unexplained vomiting, acidosis, mental deterioration or coma.
- (4) Hepatomegaly.
- (5) Renal stones
- (6) Muscle weakness or cardiomyopathy.

Prenatal diagnosis

Prenatal diagnosis of an inherited aminoaciduria is particularly desirable when a mother has had a previous child with a severe inherited defect.

Amniotic fluid may be used for prenatal diagnosis; however it is a high-risk procedure to obtain the specimen. Amniocentesis is done at 12-18 weeks of gestation, fetal cells are cultured and the cells are examined for enzyme activity.

Another test done is chorionic villus sampling, which allows direct gene analysis of tissue obtained by biopsy.

Hyperphenylalaninemias

The hyperphenylalaninemias result from impaired conversion of phenylalanine to tyrosine. The most common and clinically important is phenylketonuria, which is characterized by an increased concentration of phenylalanine in blood, increased concentrations of phenylalanine and its by-products (notably phenylacetylglutamine) in urine and severe mental retardation if untreated in infancy.

Types of phenylketonuria (PKU):

- Classical PKU
- PKU Variants
- Transient neonatal hyperphenylalaninemia
- Maternal hyperphenylalaninemia
- Hyperphenylalaninemia due to tetrahydrobiopterin deficiency

Classical PKU (Type I)

In this condition, phenylalanine hydroxylase activity is almost totally absent. As a result phenylalanine accumulates in blood, urine and CSF. This activates minor pathways of phenylalanine metabolism leading to increased production of phenylketones (e.g. phenylpyruvate) and other metabolites. These metabolites are rapidly cleared by the kidneys and excreted into the urine (Fig. 1).

PKU Variants

These are the less severe form and result from partial deficiencies of the enzyme phenylalanine hydroxylase.

Transient Neonatal Hyperphenylalaninemia

This disorder is caused by delayed hepatic maturation of phenylalanine hydroxylase enzyme system. This condition is not an inherited defect and progressively declines towards normal as the neonate matures.

Maternal Hyperphenylalaninemia

This condition is likely to occur in adult phenylketonuric pregnant women who have been successfully treated by dietary control since infancy.

Hyperphenylalaninemia due to Tetrahydrobiopterin deficiency

Caused by a defect in dihydropteridine reductase or one of the enzymatic steps involved in biopterin synthesis.

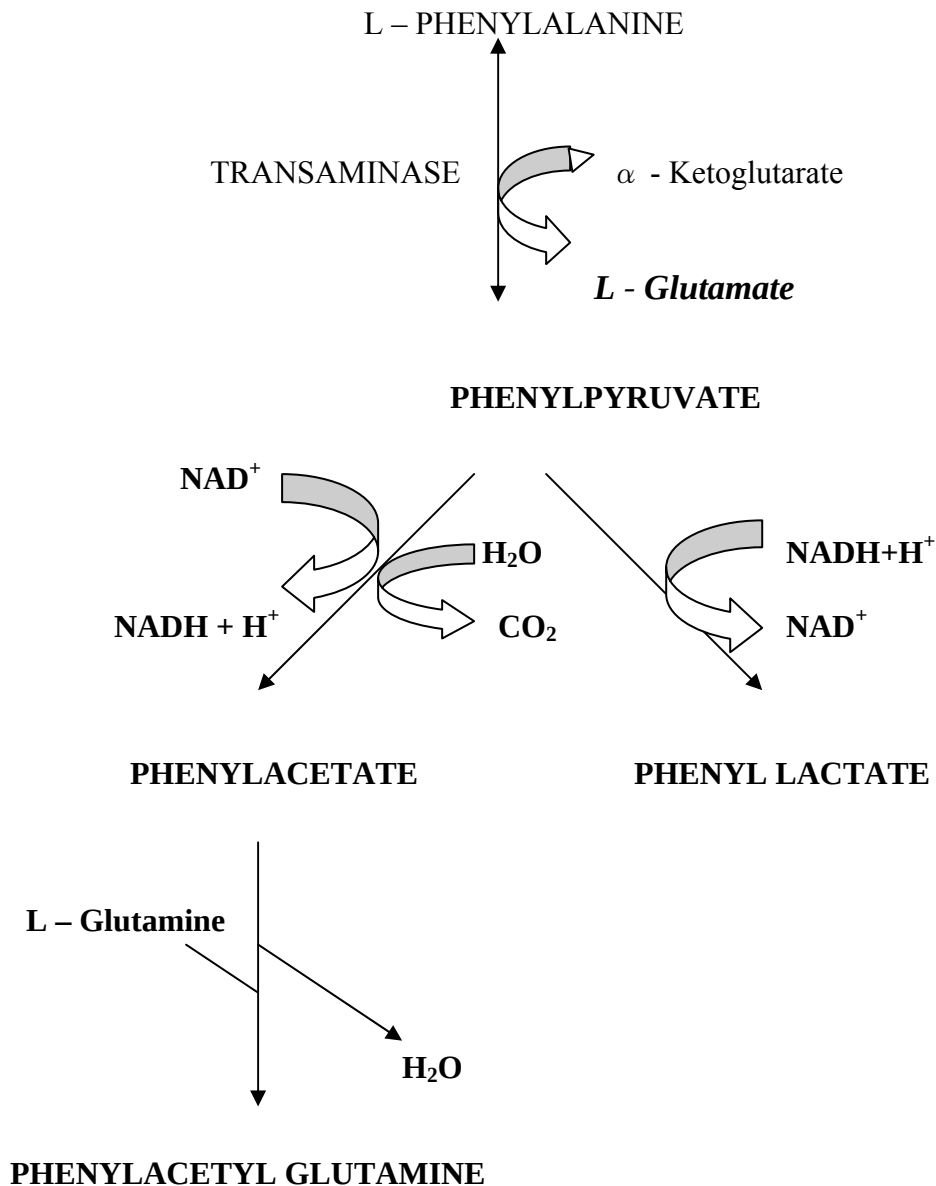


Fig. 1: Alternative pathways of phenylalanine catabolism in phenylketonuria.

Symptoms

Untreated PKU causes severe mental retardation. The affected children appear normal at birth and the earliest symptoms are nonspecific- delayed development, feeding difficulties and vomiting. An unusual but characteristic odor may be noted in urine or sweat due to increased production of phenylpyruvate. Without treatment, irreversible retardation occurs. Older patients may show hypopigmentation, eczema and seizures.

Hypopigmentation in PKU relates to hyperphenylalaninemia, because phenylalanine is a competitive inhibitor of tyrosine, an enzyme that initiates melanogenesis. Accumulation of phenylalanine is also known to reduce synthesis of myelin, norepinephrine and serotonin and may directly or indirectly, cause severe neurological symptoms.

Treatment

Treatment of PKU consists of giving a diet low in phenylalanine and supplemented with tyrosine before the onset of brain damage, so that serum phenylalanine concentrations is kept from exceeding 8 mg/dL. Such a diet therapy should be instituted during the first three weeks of life. Because uncontrolled hyperphenylalaninemia results in brain damage, dietary restriction should be continued and monitored indefinitely.

Diagnosis

Plasma phenylalanine concentrations are usually normal at birth in the hyperphenylalaninemias but rise rapidly after institution of protein feedings. Newborns should be screened by determinations of blood phenylalanine concentration using the Guthrie's bacterial inhibition assay. Identification of phenylketones in urine by ferric chloride may offer a simple test for diagnosis of infants with developmental and neurologic abnormalities. Abnormal values are confirmed using quantitative analysis of plasma aminoacids. Prenatal diagnosis of Type I phenylketonuria is now feasible using DNA based tests that detect specific mutations or polymorphic markers that are linked to phenylalanine hydroxylase gene.

Alkaptonuria

Alkaptonuria is a rare disorder of tyrosine catabolism in which deficiency of the enzyme homogentisate 1, 2-dioxygenase (also known as homogentisic acid oxidase) leads to excretion of large amounts of homogentisic acid in urine and accumulation of oxidized homogentisic acid pigment in connective tissue (ochronosis) (Fig. 2). After many years ochronosis leads to degenerative arthritis and pigmentation of cartilage. Pigment can be first noticed in the ears.

Clinical Manifestations

Alkaptonuria may not be diagnosed till middle age when degenerative joint disease develops. There is a tendency of the patient's urine to darken on standing and there may be slight pigmentation of sclera and ears. Acute arthritis resembles rheumatoid arthritis but small joints may be spared. Some patients may develop pigmented renal or prostatic calculi. Degenerative cardiovascular diseases may occur in older patients.

Diagnosis

Patient's urine darkens on standing and though this feature is characteristic of alkaptonuria, darkening of urine due to other causes should be ruled out.

Diagnosis is usually made from the triad of degenerative arthritis, ochronotic pigmentation and urine turning black on alkalization. Homogentisic acid in urine may be identified. A blue-black colour is observed after addition of ferric chloride or treatment with Benedicts reagent yields a brown colour or a black colour is observed on addition of saturated silver nitrate solution.

The screening tests can be confirmed by chromatographic, enzymatic or spectrophotometric determinations of homogentisic acid. X-rays of lumbar spine show degeneration and dense calcification of intervertebral discs and a bamboo like appearance, i.e. narrowing of intervertebral spaces.

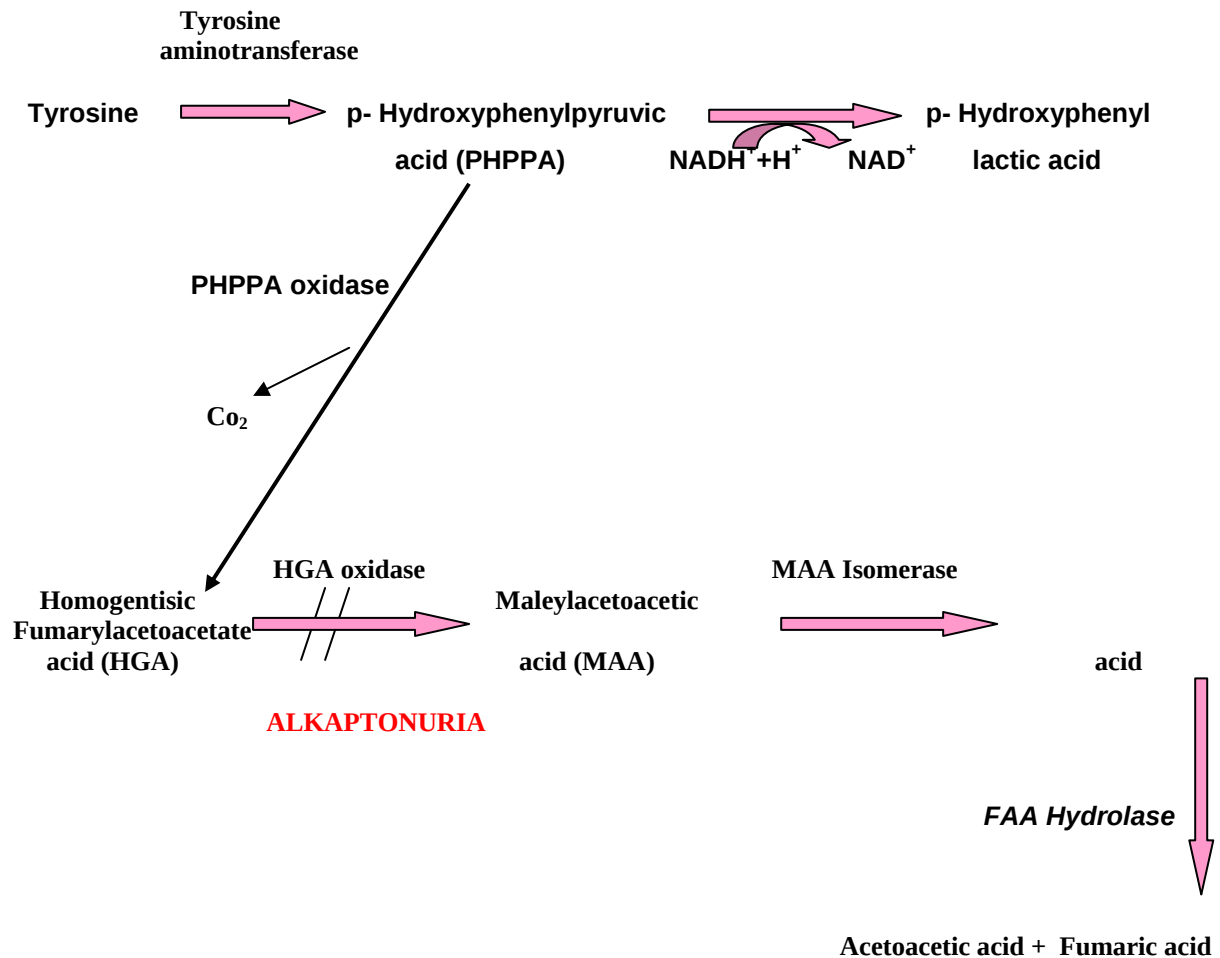


Fig. 2: Pathway of Tyrosine metabolism

Treatment

There is no specific treatment of ochronotic arthritis; symptomatic treatment similar to osteoarthritis is given. Ascorbic acid impedes oxidation and polymerization of homogentisic acid *in vitro*, but the efficacy of this treatment is yet to be established.

Albinism

This condition occurs due to defect in melanin biosynthesis and distribution. The clinical manifestations found to be common to all forms of albinism include depigmentation of the skin, iris and retina. Two late sequelae of albinism may be blindness and skin cancer. The different types of albinism are:

1. Oculocutaneous (generalized albinism)
 - a. Tyrosinase negative (Type I)
 - b. Tyrosinase positive (Type II)
2. Ocular albinism
3. Partial albinism

Oculocutaneous (Generalized Albinism)

This condition has been classified into Tyrosinase negative (Type I) and Tyrosinase positive (Type II) on the basis of the ability of a plucked hair bulb to form melanin when incubated with tyrosine.

Tyrosinase negative (Type I) albinism

It is most severe, caused by deficiency of tyrosinase enzyme and is inherited as autosomal recessive. These patients lack all visual pigment. The hair bulbs fail to convert added tyrosine to pigment, and the melanocytes contain unpigmented melanosomes.

Tyrosinase positive (Type II) albinism

It is the most common form of generalized albinism. It is inherited as autosomal recessive. These patients have some visible pigment and white-yellow to light tan hair. The hair bulb melanocytes may contain lightly pigmented melanosomes, which convert tyrosine to black eumelanin *in vitro*.

Ocular Albinism

In these patients, the eyes are pale blue to light green. Hair colour and skin are within normal limits though lighter than non-affected siblings. Hair bulb tyrosinase is positive in all cases. It occurs as autosomal recessive and as an X-linked trait. Melanocytes of X-linked and heterozygous (but not autosomal recessive) ocular albinos contain macromelanosomes. The retinas of females heterozygous for X-linked ocular albinism (Nettle ship variety) exhibit a mosaic pattern of pigment distribution due to random X chromosome inactivation.

Partial Albinism

Inherited as autosomal dominant trait and is characterized by localized area of skin and hair devoid of pigment.

Gout – Hyperuricemia

Humans convert the major purine nucleosides adenosine and guanosine to the excreted end product uric acid. The intermediates and reactions are shown in the Fig. 3.

Xanthine oxidase thus provides a potential locus for pharmacologic intervention in patients with hyperuricemia and gout.

In hyperuricemia serum urate levels exceed the solubility limit. Resulting crystallization of sodium urate in soft tissues and joints form deposits called Tophi. Tophi lead to an inflammatory reaction, Ac Gouty Arthritis that may progress to a chronic form.

Hyperuricemias may be differentiated based on whether patients excrete normal or excessive quantities (> 600 mg/24hrs) of total urates. While some uricemias reflect specific enzyme defects, several hyperuricemias are secondary to diseases that increase tissue turnover such as cancer or psoriasis. Some of the inherited disorders of purine metabolism with their deficient / absent enzyme are summarized in Table 6.

Gout

Monosodium urate gout is a metabolic disease occurring most often in middle-aged or elderly men. It is typically associated with increased uric acid pool, hyperuricemia, episodic acute

and chronic arthritis, and deposition of monosodium urate crystals in connective tissue tophi and kidneys. Visualization under a polarizing light microscope of needle shaped, intensively negatively birefringent crystals of sodium urate in joint fluid are diagnostic of gout. The crystals appear yellow when their long axis is parallel to the plane of polarized light and blue when perpendicular to it.

ADENOSINE

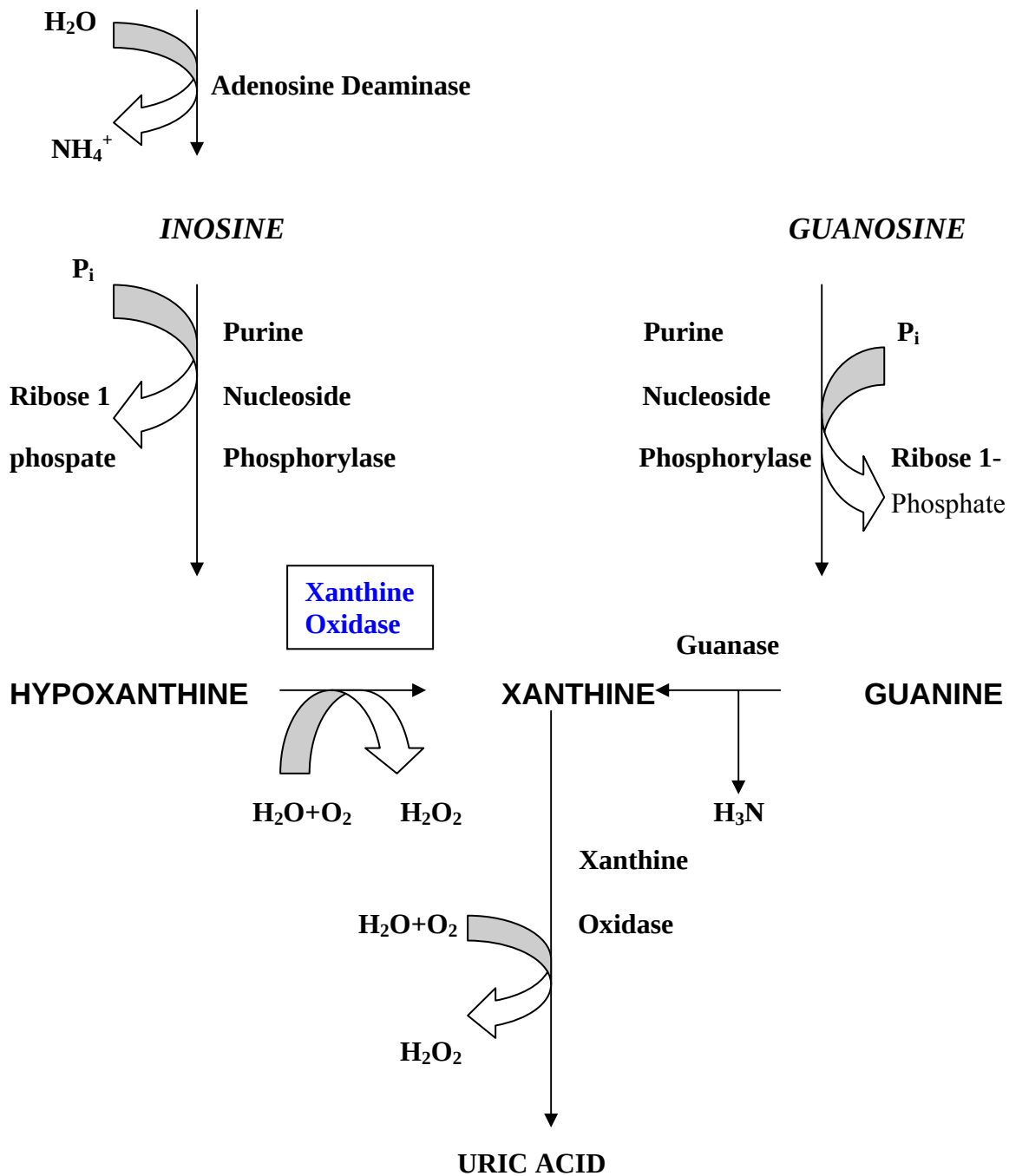


Fig. 3: Formation of uric acid from purine nucleosides

Table 6: Inherited disorders of Purine Metabolism

S. No.	Disorder	Enzyme Deficient / Absent	Inheritance
1.	Gout	PRPP synthetase	X – linked recessive
2.	Gout	HGPRTase	X – linked recessive
3.	Lesch-Nyhan Syndrome	HGPRTase	X – linked recessive
4.	Immuno - deficiency	Adenosine deaminase	Autosomal recessive
5.	Immuno - deficiency	Purine nucleoside Phosphorylase	Autosomal recessive
6.	Renal lithiasis	Adenosine Phosphoribosyl transferase	Autosomal recessive
7.	Xanthinuria	Xanthine Oxidase	Autosomal recessive

Laboratory Diagnosis

Even if clinical appearance strongly suggests gout, diagnosis should be confirmed. Monosodium urate crystals can often be demonstrated in the first metatarsophalangeal joint and in knees not acutely involved in gout.

Serum uric acid can be normal or low at the time of the acute attack due to lowering of uric acid with hypouricemic therapy or other medications. Despite these limitations, serum uric acid is almost always elevated some time and can be used to follow the course of hypouricemic therapy.

A 24-hour urine collection for uric acid is valuable for assessing the risk of stones. Excretion of more than 800mg of uric acid per 24 hours on a regular diet suggests that cause of over-production of purine should be considered.

Urine analysis, BUN, serum creatinine, WBC count and serum lipids should be monitored because of possible pathologic sequelae of gout and other associated diseases requiring treatment.

Suggested Readings

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