MINISTRY OF HIGHER AND SECONDARY SPECIAL EDUCATION

MINISTRY OF HEALTH OF THE REPUBLIC

UZBEKISTAN

ANDIJAN STATE MEDICAL
INSTITUTE

" Approved "
Vice-Rector for Academic Affairs
ASMI
_____ B.R. Abdullajonov
<< 29 >> 08 2022

DEPARTMENT OF BIOLOGICAL CHEMISTRY

# TRAINING AND METODOLOGY COMPLEX

by subject

# BIOLOGICAL CHEMISTRY

for 2nd year students

(1 semester)

Knowledge Area: 500000-Health and Welfare

Field of education: 510000-Health

Direction of training: 60910100-Dentistry

Andijan - 2022 / 23

The educational methodological complex was developed on the basis of the curriculum approved by the minutes of the meeting of the Council of Andijan State Medical Institute №. ___ dated <<29>> 08 2022.

Representative of the educational council: D.Sh.Tulanov

**Compiled by:**

I.Yu.Mamatova - Head of the Department of Andijan State Medical Institute, Doctor of Chemical Sciences

I.R.Askarov - professor. Department of Chemistry, Andijan State University, Doctor of Chemical Sciences.

G.A. Muminova - Associate professor of the Department of Biological Chemistry , Andijan State Medical Institute , PhD .

M.M. Bokiev - Assistant of the Department of Biological Chemistry, Andijan State Medical Institute

M.M.Muminjonov - Senior teacher, Department of Chemistry, Andijan State University, DSc .

**Reviewer:**

Saidullev T.S. - Associate Professor of the Department of Medical Biology, Histology, ASMI

Isaev Yu.T. - Associate Professor, Department of Chemistry, Andijan State University, DSc .

The report was approved by the decision of the Central Methodological Council by Decree №. ___ dated <<29>> 08 2022

<< Agreed>>
educational and methodological department: K.M. Yusupov

## Contents

# Digestion and Absorption of Nutrients

The Gastrointestinal Tract

**Learning objectives**

**After reading this chapter you should be able to:**

Describe the main stages of digestion.

Discuss mechanisms involved in the absorption of nutrients from the digestive tract.

Discuss the role of digestive enzymes.

Discuss digestion of the main classes of nutrients: carbohydrates, proteins and fats.

Identify compounds arising from the digestion of carbohydrates, proteins and fats that become substrates for further metabolism.

## Introduction

**The gastrointestinal tract and the organs functionally associated with it, are responsible for digestion and absorption of food**

All organisms require sources of energy and other materials to enable function and growth. Their survival depends on the ability to extract and assimilate these resources from the ingested food. Also, the intestinal epithelium and the tight junctions between the enterocytes form the most important barrier between the organism and its external environment. The barrier has selective absorption and secretion functions and also may become a scene of immune (or autoimmune) response.

The gastrointestinal (GI) tract and the organs functionally associated with it, principally the liver and pancreas, are responsible for digestion and absorption of food. Digestion is the process by which food is broken down into components simple enough to be absorbed in the intestine. Absorption is the uptake of the products of digestion by intestinal cells (enterocytes) and their delivery to blood or lymph. Digestion and absorption of nutrients are closely linked and are regulated by the nervous system, hormones and paracrine factors. The physical presence of food particles in the GI tract also stimulates these processes.

Importantly, the absorption and secretion of ions such as sodium, chloride potassium, and bicarbonate, and the absorption of water, are also essential functions of the gastrointestinal tract. Therefore many clinical problems associated with digestion and absorption are closely linked to fluid and electrolyte disorders (Chapter 24). It would be a mistake to regard these two fields as separate: a clinician must see them in an integrated way.

Impairment of digestion and absorption results in maldigestion and malabsorption syndromes, respectively. **Maldigestion** denotes impaired breakdown of nutrients to their absorbable products. **Malabsorption** is the defective absorption, uptake and transport of nutrient products that were adequately digested.

The key clinical signs of signs of malabsorption and/or maldigestion are diarrhea, steatorrhea and loss of weight. In children there is failure to thrive. While acute diarrhea carries a risk of fast dehydration and electrolyte depletion, chronic diarrhea is associated with progressive malnutrition. Worldwide, diarrheal disease is, according to the WHO data (2011), a 5th leading cause of death. Malabsorption and maldigestion may also develop as consequences of surgical intervention such as gastrectomy, small bowel resection or colectomy.

The overall function of the GI tract is to break down food into components that can be absorbed and utilized by the body (Fig. 10.1), and then to excrete the nonabsorbable material. Its different anatomical segments have specific functions relating to digestion and absorption:

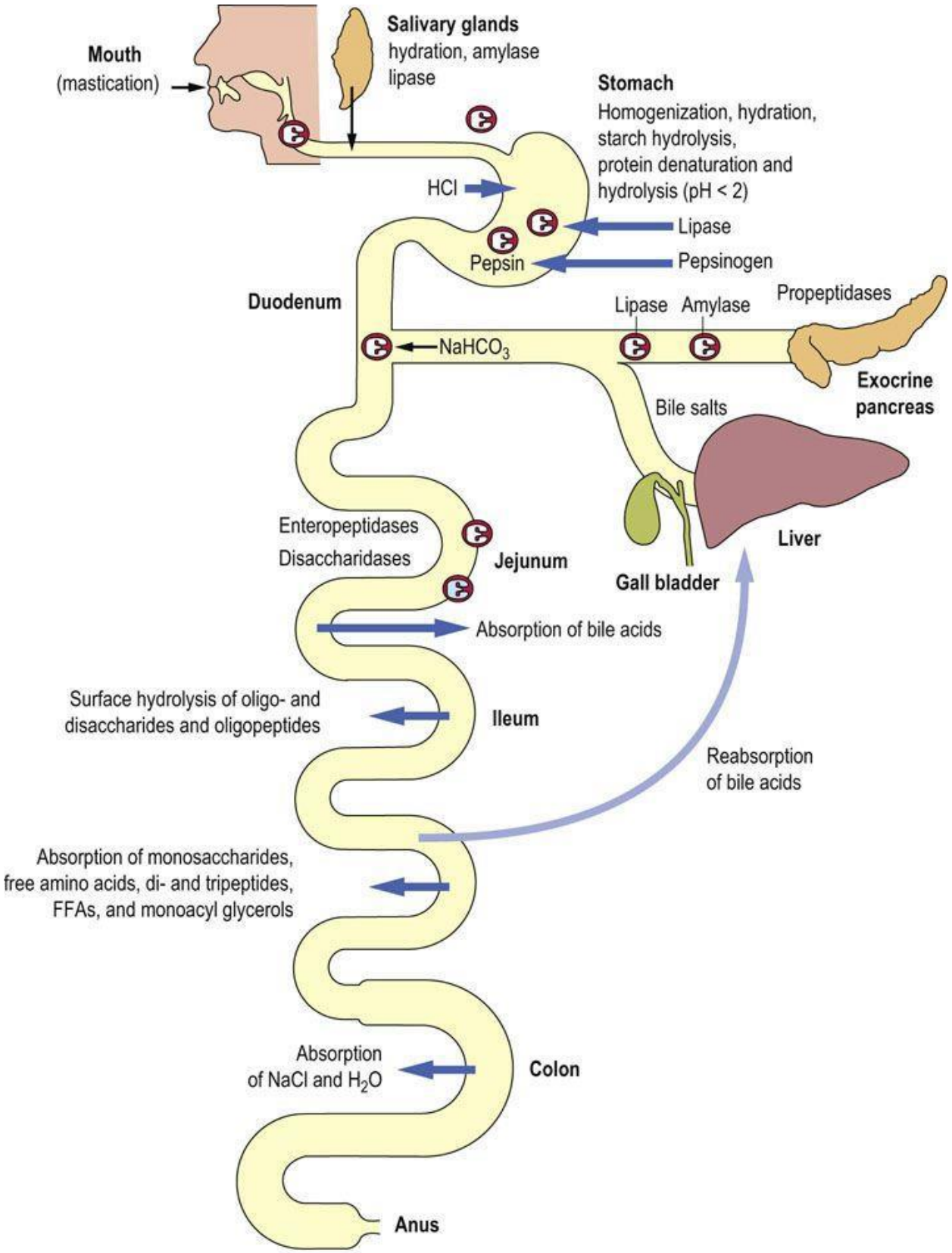

Mouth
(mastication)
Salivary glands
hydration, amylase
lipase
Stomach
Homogenization, hydration,
starch hydrolysis,
protein denaturation and
hydrolysis (pH < 2)
HCl
Lipase
Pepsin
Pepsinogen
Duodenum
Lipase
Amylase
Propeptidases
NaHCO3
Exocrine
pancreas
Bile salts
Liver
Gall bladder
Enteropeptidases
Disaccharidases
Jejunum
Absorption of bile acids
Surface hydrolysis of oligo- and
disaccharides and oligopeptides
Ileum
Reabsorption
of bile acids
Absorption of monosaccharides,
free amino acids, di- and tripeptides,
FFAs, and monoacyl glycerols
Absorption
of NaCl and H2O
Colon
Anus

**FIG. 10.1** The gastrointestinal (GI) tract. Digestion and absorption of nutrients require integrated function of several organs. Mixing of food and initiation of digestion take place in the stomach. The absorptive processes start in the jejunum. However, the bulk of nutrients are absorbed in the ileum. The large intestine is involved in the absorption of water and electrolytes, and participates in the recirculation of the bile acids to the liver (Chapter 30). Taking into account all intake and secretions, a large amount of fluid (approximately 10 liters) passes through the GI tract every day. FFA, free fatty acids. Reproduced from Dominiczak MH. Medical Biochemistry Flash Cards. London: Elsevier, 2012, Card 34.

The mouth, stomach and duodenum deal with the initial process of mixing ingested food and initiating digestion.

In the duodenum, bile and pancreatic secretions enter through the common bile duct.

The small intestine is the main digestive area: in the jejunum digestive processes continue and absorption is initiated; it continues in the ileum.

The large intestine (cecum, colon and rectum; primarily the colon) is involved in the absorption and secretion of electrolytes and water.

## Water and electrolyte handling in the gastrointestinal tract

### *Handling of electrolytes and water by the GI tract is one of its main functions*

Handling of electrolytes and water by the GI tract is one of its main functions, and includes not only their absorption and secretion but also cell volume maintenance, and affects cell proliferation and differentiation, as well as apoptosis and carcinogenesis.

### Large volume of fluid is secreted and reabsorbed by the GI tract

In a 24 h period, around 10.0 L of fluid enter and leave the GI tract. One liter of saliva is secreted per day; it contains electrolytes, protein and mucus. Intestinal secretions total about 7.0 liters every day, over and above an average water intake of about 2.0 liters. Most of this fluid is reabsorbed by the small intestine. The colon is particularly active in reabsorption: it absorbs around 90% of the fluid that passes through it, and only about 150–250 mL of water are normally contained in the stool.

### Electrolytes are secreted by the salivary glands, stomach and the pancreas

Several secretory processes take place in the GI tract. Salivary glands, stomach and pancreas secrete digestive enzymes in the form of inactive zymogens. There is the hydrogen ion secretion in the stomach. Secretion of the bicarbonate ion takes place throughout the GI tract, with particularly large amounts being secreted in the pancreatic juice. Potassium secretion occurs predominantly in the colon and is regulated by aldosterone.

### Impaired intestinal function leads to fluid-electrolyte and acid–base disorders

All this has clinical implications: diseases of the GI tract, and surgical removal of large segments of small and large intestine, carry a risk of major water and electrolyte disorders. Before treatment for cholera was known, a person with

fulminant diarrhea caused by *Vibrio cholerae* infection could die of dehydration within a few hours. Acidosis due to bicarbonate loss can also be a feature of bowel disease (Chapter 25).

**Clinical box Causes of fluid and electrolyte loss from the gastrointestinal tract**

Prolonged **vomiting** causes loss of water, hydrogen and chloride ions, and a further loss of potassium due to the body's compensatory mechanisms. **Diarrhea** may be caused by increased intestinal secretion due to, for instance, inflammation, or may be caused by malabsorption of nutrients. Severe diarrhea, leading to the loss of alkaline intestinal contents may lead to dehydration and metabolic acidosis. It also results in the loss of sodium, potassium and other minerals (Chapters 11, 23 and 24). Patients with a short gut syndrome resulting from extensive small bowel resection, may develop severe fluid balance problems due to inability to reabsorb water.

***Mechanisms of water and electrolyte transport in the intestine***

**Sodium-potassium ATPase is the driving force for transport processes in the enterocytes**

Enterocytes possess an array of transporters and ion channels (Fig. 10.2). The sodium-potassium ATPase, described in more detail in Chapter 24, is located on the basolateral membrane (the ‘blood side’) and transports the sodium ion outside the cell in exchange for the potassium (3Na+ to every 2K+ ions). This creates a sodium concentration gradient, and hyperpolarizes the membrane, increasing the intracellular negative potential and driving the passive transport systems (and thus transcellular ion transport). Moreover, transport of sodium (and chloride) is accompanied by passive transport of water, which is both paracellular (through the tight junctions) and transcellular (by membrane water transporters, the aquaporins).

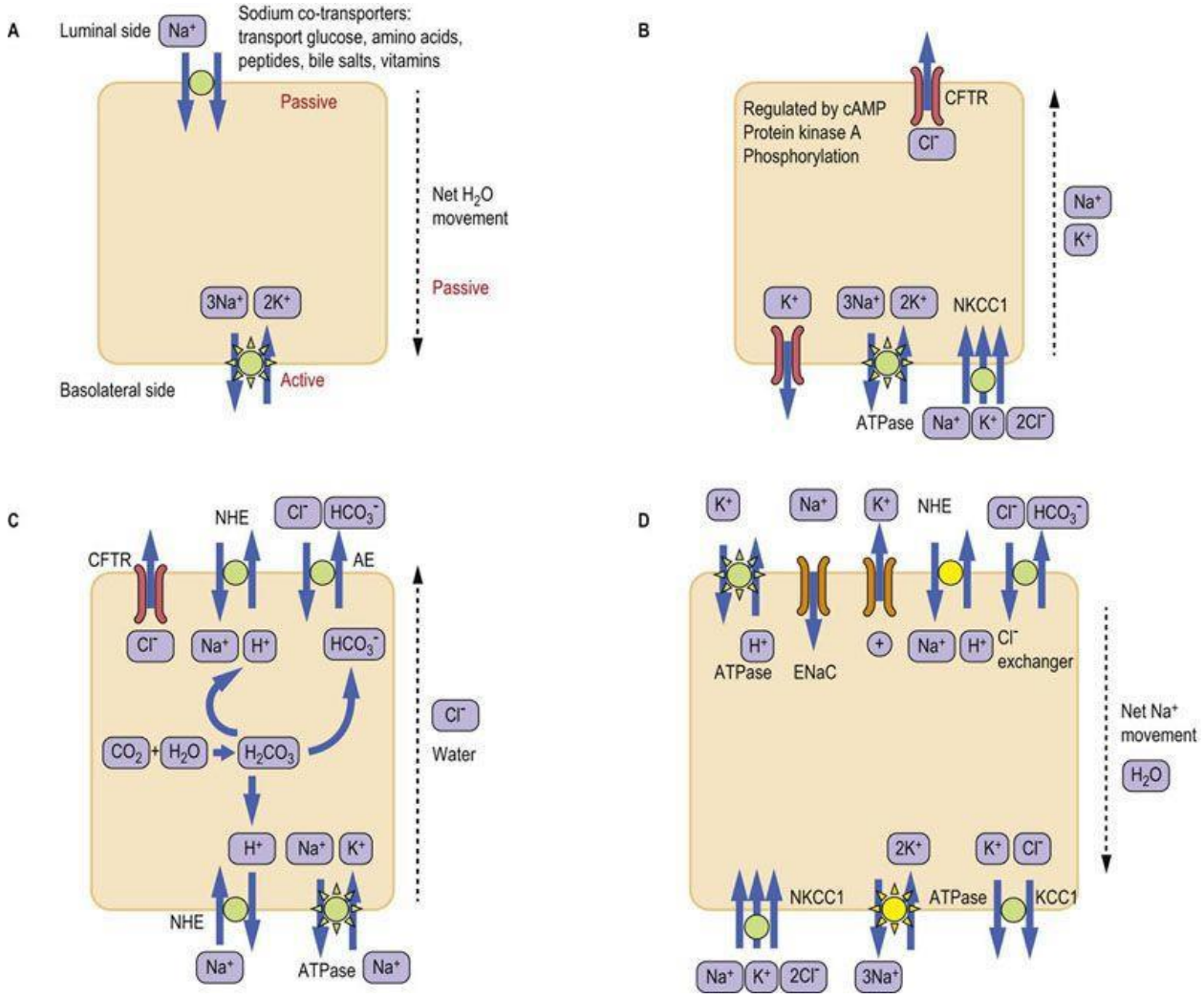

A
Luminal side
Na+
Sodium co-transporters:
transport glucose, amino acids,
peptides, bile salts, vitamins
Passive
Net H2O
movement
Passive
3Na+
2K+
Basolateral side
Active
B
Regulated by cAMP
Protein kinase A
Phosphorylation
CFTR
Cl-
Na+
K+
K+
3Na+
2K+
NKCC1
ATPase
Na+
K+
2Cl-
C
NHE
Cl-
HCO3-
CFTR
AE
Cl-
Na+
H+
HCO3-
CO2
+
H2O
H2CO3
Cl-
Water
H+
Na+
K+
NHE
Na+
ATPase
Na+
D
K+
Na+
K+
NHE
Cl-
HCO3-
H+
ATPase
ENaC
+
Na+
H+
Cl-
exchanger
Net Na+
movement
H2O
2K+
K+
Cl-
NKCC1
ATPase
KCC1
Na+
K+
2Cl-
3Na+

**FIG. 10.2** Intestinal electrolyte transport systems. **(A)** Sodium co-transporters transport a wide range of substrates, including glucose. Sodium gradient is created by the Na+/K+-ATPase located in the basolateral membrane. **(B)** CFTR transporter secretes chloride ion and is regulated by the cAMP-PKA signaling cascade. Sodium and potassium may also be secreted as counterions. Note the potassium 'leak' channel in the basolateral membrane. The NKCC1 transporter supplies chloride to the cell. **(C)** The electroneutral sodium absorption and the secretion of bicarbonate. **(D)** Electrogenic sodium absorption and potassium secretion in the distal colon. Transporters marked **yellow** are regulated by **aldosterone** in the distal colon. See text for details. CFTR, cystic fibrosis transmembrane conductance regulator; NHE, sodium/hydrogen exchanger; ENaC, epithelial sodium channel; AE, anion exchanger (chloride/bicarbonate exchanger); NKCC1, Na+ K+ Cl− co-transporter; KCC1, K+ Cl− co-transporter. **Sodium co-transporters are a common mode of intestinal transport**

Sodium co-transporters transport the sodium ion together with another molecule (Fig. 10.2A). For instance, glucose is absorbed together with sodium by the sodium-glucose co-transporter present in the luminal membrane, known as **SGLT-1** (Sodium/Glucose Linked Transport-1). Glucose is subsequently extruded into plasma at the basolateral membrane by the**GLUT2** transporter (Fig. 10.7 below). The discovery of the link between intestinal transport of sodium and glucose had enormous clinical consequences. It was during a cholera epidemic in Manila, in the late 1960s, that researchers observed that patients who had been dehydrated because of diarrhea did not absorb oral sodium chloride well during attempts at oral rehydration. However, they started to do so when glucose was also provided. This observation led to the formulation of the **WHO oral rehydration solution**, which subsequently saved the lives of millions of children affected by severe diarrhea worldwide.

**Other modes of sodium transport are the electroneutral (Fig. 10.2B) and electrogenic transport**

The electroneutral sodium transport is through sodium/hydrogen exchanger (**NHE**), usually combined with chloride transport via the chloride/bicarbonate exchanger known as AE exchanger (Fig. 10.2C). The exchangers are present on both luminal and basolateral membranes. This type of transport is responsible for most of the sodium chloride reabsorption in the colon. In the distal colon the NHE exchanger is upregulated by glucocorticoids.

The electrogenic absorption of sodium occurs through the epithelial sodium channels (**ENaCs**, also known as amiloride-sensitive sodium channels), which are present on the luminal side of the epithelium (Fig. 10.2D). ENaCs are regulated by steroids (aldosterone) and are important particularly in the distal colon. Absorption of Na+ is accompanied by Cl− following through a chloride channel (which could be the CFTR – see below). Aldosterone also upregulates the Na+/K+-ATPase.

**Chloride transport: the cystic fibrosis transmembrane conductance regulator (CFTR)**

Luminal secretion of chloride occurs via the cystic fibrosis transmembrane conductance regulator (CFTR; Fig. 10.2B). The CFTR is a single-polypeptide

membrane ion channel. It is also present in the epithelia of the lung and sweat glands. Its function is controlled by the G-protein–cAMP-protein kinase A (PKA) signaling cascade (Chapter 40). Because the CFTR is activated by cAMP, prostaglandin E2 (PGE2), serotonin, as well as the cholera toxin and the *E. coli* heat-stable enterotoxin, all activate chloride secretion. On the other hand, the loss-of-function mutations of CFTR are the cause of **cystic fibrosis**, where the chloride transport is impaired or inhibited. CFTR also has regulatory function: its phosphorylation inhibits the NHE exchanger, thus decreasing Na+ absorption. Interestingly CFTR is also able to transport chloride in the opposite direction, aiding chloride reabsorption (above).

The basolateral C1− uptake occurs through the Na+ K+ C1− co-transporter (known as NKCC1) and through chloride/bicarbonate exchangers.

**Clinical box Cystic fibrosis**

Cystic fibrosis, a monogenic autosomal recessive disorder, involves inhibition of chloride transport due to the absence of the CFTR. Different mutations of the *CFTR* gene lead to either complete absence of the transporter or impair its functionality.

The prevalence of CF is 1 : 3000 live births in the USA and northern Europe. In the USA, cystic fibrosis is the No. 1 cause of **malabsorption**. It manifests itself predominantly in childhood. The main problems are usually respiratory. Chloride secretion is decreased and the Na+ reabsorption is accelerated. This results in decreased hydration of epithelial secretions. In the respiratory tract there is **decreased hydration of the airway mucus** and thus failure of its clearance, with ensuing bacterial infections. Gastrointestinal problems include **meconium ileus** and **intestinal obstruction**. The absence of the CFTR also affects functioning of the Cl−/HCO3− exchanger (and thus the passive secretion of Na+) – this impairs pancreatic enzyme secretion. Thickened biliary secretions may be a cause of focal **biliary cirrhosis** and **chronic cholelithiasis**. There also is impairment of mucus secretion in the colonic crypts, with enhanced Na+ reabsorption through Na+ channels and Na+/H+ transporters.

**Potassium absorption and potassium secretion in the colon is aided by a several potassium channels**

Potassium absorption is mediated by **H+/K+ ATPases** (belonging to the family of P-type ATPases) in the luminal membrane. On the other hand, the basolateral potassium transport is by potassium channels and the K+ and Cl− cotransporter (**KCC1**). Both basolateral and luminal K+ channels are necessary to hyperpolarize the membrane to establish a driving force for the ENaC transporter.

K+ secretion through luminal K+ channels parallels the Cl− secretion through CFTR, and is similarly stimulated by cAMP, cGMP and protein kinase C (PKC). The expression of luminal K+ channels is also stimulated by aldosterone and glucocorticoids.

**Reabsorption of short-chain fatty acids occurs together with bicarbonate secretion**

The colon reabsorbs short-chain fatty acids (SCFAs) derived from bacterial fermentation of fiber, and this is combined with secretion of bicarbonate.

Bicarbonate is secreted using luminal anion exchangers SCFA/HCO3− or C1−/HCO3−.

**Aquaporins control colonic water reabsorption**

Water reabsorption in the colon is mediated by ion channels known as aquaporins (AQP). AQP1,3 and 4 are located on basolateral and AQP8 on luminal membranes. The intestinal electrolyte transport systems are summarized in Figure 10.2.

**The pH of intestinal secretions vary**

Hydrogen ion concentration varies widely in different parts of the GI tract. This is essential for the digestive process and for protection of the underlying tissues in the stomach and intestines. Saliva secreted into the mouth is alkaline due to its bicarbonate content. On the other hand, the contents of the stomach are strongly acidic, but the mucus protecting its walls is alkaline. Thus while the parietal cells of the stomach secrete large amounts of hydrogen ion (principally through the action of the luminal H+/K+-ATPase), the gastric surface cells secrete mucus containing bicarbonate ion; they employ the Cl−/HCO3− exchanger. On entry to the duodenum the acidic content of the stomach is neutralized by strongly alkaline (due to the bicarbonate content) pancreatic secretions.

**Digestion**

There is a significant mechanical component to digestion. Chewing breaks down food to enable it to be swallowed, whilst the addition of saliva in the mouth begins the digestive process and acts as lubrication to facilitate swallowing. The food is then moved into the esophagus by a process driven by the esophageal reflex. As it transfers into the stomach, it is broken down into smaller particles. The presence of the digest triggers peristalsis, which further helps mixing and stimulates digestive secretions. Major stimuli to peristalsis are mediated through the parasympathetic nervous system. Absorption of nutrients depends on the rate of transit: thus, increased motility may lead to malabsorption.

The stomach and intestines are lined by epithelium, which has an invaginated surface that greatly increases its area. The small intestine is lined by enterocytes arranged in intestinal villi, and in addition each cell contains microvilli. The total absorptive surface area of the intestine is approximately 250 m2, roughly the area of a junior basketball court.

**Advanced concept box Digestive function of the stomach**

There are different cell types in the mucosal wall of the stomach, performing different digestive functions. Cells called ‘chief cells’ secrete **pepsinogen**, which is a precursor of **pepsin**. Pepsinogen is activated to pepsin in the acidic environment of the stomach lumen. Parietal cells generate **hydrogen ions** through the action of carbonic anhydrase and then pump them into the lumen by an ATP-dependent proton pump on the luminal membrane. The H+ secretion is dependent on the parallel export of K+through luminal K+ channel.

Parietal cell activity is stimulated by the action of **histamine** acting on H2 receptors. (Chapter 41), produced by histamine-secreting cells. The hormone **gastrin** is secreted by G-cells, and is triggered by food entering the stomach. Stomach cells also secrete the **intrinsic factor (IF)**, which facilitates absorption of

vitamin B12 in the intestine (Chapter 11). Last, but not least, epithelial cells secrete alkaline mucus, which protects the stomach lining from the effects of the strong acid.

Damage to the lining of the stomach or duodenum leads to **ulceration**. Treatment of acid-related symptoms such as **dyspepsia** or **gastroesophageal reflux**, can be achieved with **antacids** which simply neutralize the pH, **H2 antagonists**, e.g. cimetidine or ranitidine, which prevent histamine release, or **proton pump inhibitors**, e.g. omeprazole, which block H+ secretion by the parietal cells.

**Digestion is a sequential, ordered series of processes**

The carbohydrates, proteins and fats are split to absorbable products. Some ingested material, such as complex carbohydrates of plant origin, is indigestible and constitutes fiber.

The process of digestion is characterized by a number of stages that occur in a sequence, allowing the interaction of fluid, pH, emulsifying agents and enzymes. This, in turn, requires concerted secretory action from the salivary glands, liver and gall bladder, the pancreas, and the intestinal mucosa. The processes involved are outlined in Figure 10.1 and can be summarized as follows:

Lubrication and homogenization of food with fluids secreted by glands of the intestinal tract, starting in the mouth.

Secretion of enzymes that break down macromolecules to a mixture of oligomers, dimers and monomers.

Secretion of electrolytes, hydrogen ions and bicarbonate within different parts of the GI tract to optimize the conditions for enzymic hydrolysis.

Secretion of bile acids to emulsify dietary lipid, facilitating enzymic hydrolysis and absorption.

Further hydrolysis of oligomers and dimers by membrane-bound enzymes (jejunum).

Specific transport of digested material into enterocytes and thence to blood or lymph.

Recycling of bile acids. Absorption of the SCFAs produced by colonic bacterial fermentation.

Reabsorption of water and electrolytes.

***Spare capacity in the intestinal tract***

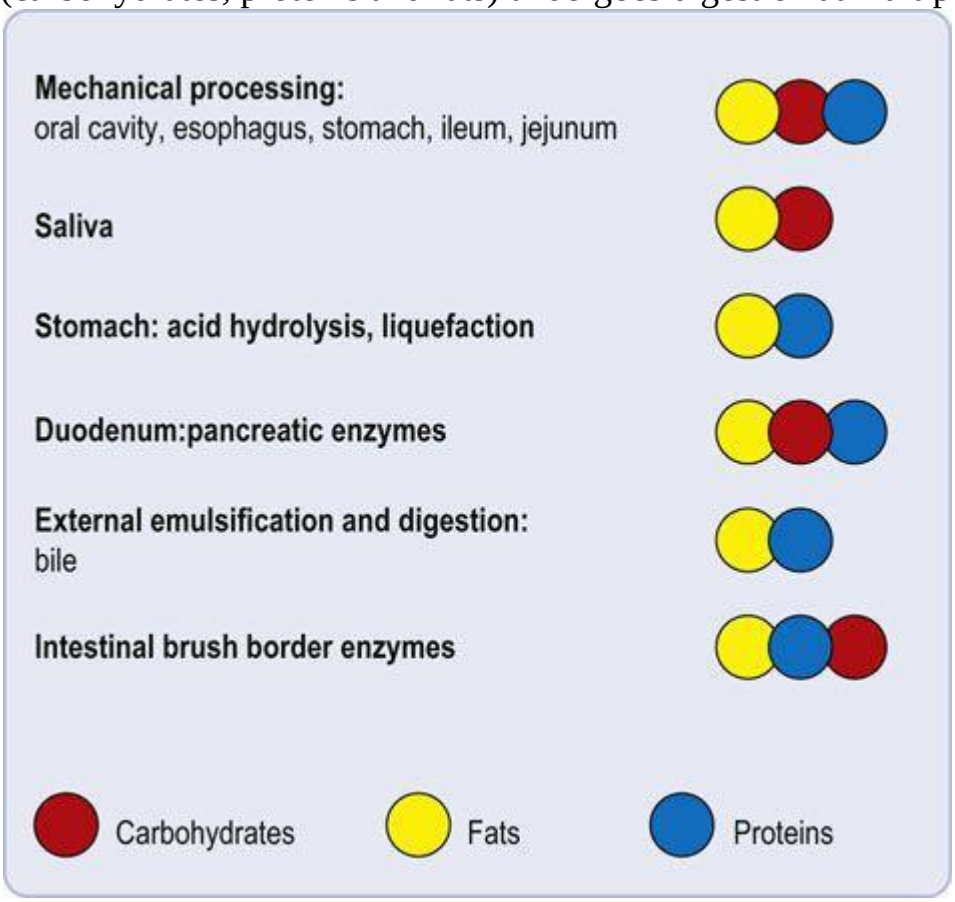
**There is considerable functional reserve in all aspects of digestion and absorption**

Because if this, minor functional loss may go unnoticed, allowing pathology to progress for some time before being diagnosed. A considerable impairment of structure/function needs to be present before signs and symptoms of GI maldigestion or malabsorption occur. Each of the organs involved in digestion and absorption has the capacity to increase its activity several fold in response to specific stimulation; this adds to the reserve capacity. For example, pancreatic disease manifests itself only after 90% of the pancreatic function is destroyed.

Note also that digestion of a particular nutrient takes place at several points in the GI tract. Lipids, carbohydrates and proteins can be digested at multiple points

along the GI tract. Therefore disruption of digestive mechanisms at a single point is unlikely to cause a complete inability to digest a nutrient group (Fig. 10.3).

**FIG. 10.3** Digestion as a multiorgan process. Each of the main group of nutrients (carbohydrates, proteins and fats) undergoes digestion at multiple points.




The GI tract can also accommodate loss of function of one particular constituent organ. For example, if the stomach is surgically removed, the pancreas and small intestine can compensate for the loss of gastric digestion. On the other hand, in pancreatic disease, lingual lipases can accommodate, some loss of pancreatic lipase.

**Digestive enzymes and zymogens Most digestive enzymes are secreted as inactive precursors**

With the exception of salivary amylase and lingual (associated with the tongue; hence oral) lipases, digestive enzymes are secreted into the gut lumen as inactive precursors termed zymogens (Chapter 6). Secretion of enzymes is similar in the salivary glands, gastric mucosa and pancreas. These organs contain specialized cells for the synthesis, packaging and transport of zymogen granules to the cell surface and thence to the intestinal lumen. These secretions are termed exocrine, i.e. ‘secreting to the outside’, as opposed to the endocrine secretion of hormones.

Enzymes involved in digestion of protein (proteases) and fat (lipase: phospholipase A2) are synthesized as inactive zymogens and are only activated on their release to the gut lumen. In general, these enzymes, once in their active form, can activate their own precursors. Activation of the precursors can also occur by change in pH (e.g. pepsinogen in the stomach is converted at pH below 4.0 into pepsin) or by the

action of specific enteropeptidases bound to the mucosal membrane of the duodenum (Fig. 10.1).

**All digestive enzymes are hydrolases**

All digestive enzymes hydrolyze their substrates. The products of such hydrolytic procedures are oligomers, dimers and monomers of the parent macromolecule. Thus, carbohydrates are hydrolyzed into a mixture of dissacharides and monosaccharides. Proteins are broken down to a mixture of di- and tripeptides and amino acids. Lipids are broken down to a mixture of fatty acids (FAs), glycerol and mono- and diacylglycerols (Fig. 10.4).

**FIG. 10.4** Digestion of dietary polymers. Polysaccharides are digested to yield di- and monosaccharides, and proteins to yield the component amino acids. Fat (triacylglycerols) digestion is a stepwise removal of fatty acid molecules, yielding di- and monoacylglycerols.

**Digestion and absorption of carbohydrates**

**Dietary carbohydrates enter the GI tract as mono-, di- and polysaccharides**

Dietary carbohydrates consist of mainly plant and animal starches (polysaccharides), the disaccharides sucrose and lactose, and the monosaccharides (Fig. 10.5). Monosaccharides include glucose, fructose and galactose, which are either present in the diet or are generated by digestion of di- and polysaccharides. Lactose, for instance, is a disaccharide derived from dairy products, and is hydrolyzed to the monosaccharides glucose and galactose by lactase and β-galactosidase. These sugar monomers can then be absorbed from the GI tract.

**FIG. 10.5** Structure of the key dietary carbohydrates. Starch and amylopectin are polysaccharides, and only two component sugar molecules are shown for each to illustrate the intermolecular linkages. Sucrose and lactose are the most common disaccharides, and fructose and glucose the most common monosaccharides. Refer to the glucose molecule for the standard numbering of carbon atoms (see also Chapter 3).

**Advanced concept box Role of amylase, α-glucosidases and isomaltase in polysaccharide digestion**

During eating, homogenization of food occurs by chewing. It is aided by contractions of the stomach wall muscles and gastric folds. One consequence of this is that dietary polysaccharides become hydrated. This is necessary for the action of **amylase**, which is specific for internal α-1,4-glycosidic linkages and not the α-1,6 linkages. Amylase also does not act on α-1,4 linkages of glycosyl residues serving as branching units. Thus, the cleaved units formed by its action are the disaccharide maltose, the trisaccharide maltotriose, and an oligosaccharide with one or more α-1,6 branches and containing on average eight glycosyl units, termed the ‘α-limit dextrin’. These compounds are further cleaved to glucose by **oligosaccharidase** and **α-glucosidase**, the latter removing single glucose residues from α-1,4-linked oligosaccharides (including maltose). A **sucrase–isomaltase** complex is secreted as a single polypeptide precursor molecule and is activated into two separate enzymes, one of which (isomaltase) is responsible for the hydrolytic cleavage of α-1,6-glycosidic bonds. Thus the final product of digestion

of starches is glucose. The amylase occurs free in the lumen, whereas α-glucosidases and **isomaltase** are attached to the membrane of the enterocyte.

**Disaccharides, and polysaccharides such as starch and glycogen, require hydrolytic cleavage into monosaccharides before absorption**

Disaccharides are broken down by membrane-bound disaccharidases present on the intestinal mucosal surface. Starch and glycogen require additional hydrolytic capacity of the enzyme amylase found in the secretions of the salivary glands and pancreas. Hydrolytic cleavage of polysaccharides. Enzymatic hydrolysis is the mechanism of digestion of polysaccharides and disaccharides. The arrows illustrate points of cleavage and the type of hydrolyzed bond. Note that α-limit dextrin still contains both α-1,4 and α-1,6 bonds.

Starch is a plant polysaccharide and glycogen is its animal equivalent. Both contain a mixture of linear chains of glucose molecules linked by α-1,4-glycosidic bonds (amylose) and by branched glucose chains with α-1,6 linkages (amylopectin). Glycogen has a far more branched structure than starch. Digestion of these polysaccharides is promoted by the endosaccharidases, and amylase.

The products of hydrolysis of starch are the disaccharide maltose, the trisaccharide maltotriose and a branched unit, termed the α-limit dextrin. These products are further hydrolyzed by enzymes bound to the enterocytes, finally yielding the monosaccharide glucose.Digestion and absorption of dietary carbohydrates. **(A)** Monosaccharides are released as a result of hydrolysis of different polysaccharides. Note that preliminary digestion occurs in the gut lumen, and the final stage takes place on the mucosal surface. **(B)** Links between absorption of monosaccharides and sodium, and their relationship to the activity of Na+/K+-ATPase. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pi, inorganic phosphate. Compare with Figure 8.5.

Dietary disaccharides such as lactose, sucrose and trehalose (a disaccharide made up of two glucose molecules joined by an α-1,1 linkage) are hydrolyzed to their constituent monosaccharides by specific disaccharidases attached to the small intestinal brush border membrane. The catalytic domains of these enzymes project into the gut lumen to react with their specific substrates, whilst their noncatalytic, structural domain(s) are attached to the enterocyte membrane.

**Disaccharidases are inducible, with the exception of lactase**

The greater the amount of a disaccharide (e.g. sucrose) present in the diet or produced by digestion, the greater is the amount of the relevant specific disaccharidase (e.g. sucrase) produced by the enterocytes. The rate-limiting step in the absorption of dietary disaccharides is thus the transport of the resultant monosaccharides. Lactose, on the other hand, is a noninducible brush border disaccharidase, and therefore the rate-limiting factor in lactose absorption is its hydrolysis.

**Clinical box Types of diarrhea**

Diarrhea can be caused by the nonabsorbable solutes present in the gut (osmotic diarrhea), by the failure to either digest or absorb nutrients, and also by the secretory agonists (secretory diarrhea).

**Osmotic diarrhea** may be caused by malabsorption, digestive enzyme deficiencies or short bowel, and inflammatory disease.
**Secretory diarrhea** may be caused by infections, malabsorption of bile salts, malabsorption of fat or by endocrine causes such as carcinoid syndrome or Zollinger–Ellison syndrome. Main causes of inflammatory diarrhea are Crohn's disease, ulcerative colitis, and irritable bowel syndrome.
Absorption can be impaired, and secretion increased, in conditions that lead to the inflammation of the bowel (**inflammatory diarrhea**). Characteristically, the secretory diarrhea but not osmotic one, persists on fasting.

**Active and passive transport systems transfer monosaccharides across the brush border membrane**

The process of digestion results in a large increase in the number of osmotically active monosaccharide particles within the gut lumen. This leads to water being drawn into the lumen from the GI tract mucosa and vascular compartment. Increased brush border hydrolysis increases the osmotic load, while increased monosaccharide transport across the brush border enterocyte decreases it. For most oligo- and disaccharidases, the transport of the resulting monomers is rate limiting. As monomeric sugar concentrations (and osmolality) increase in the gut lumen, there is a compensatory decrease in the activity of brush border disaccharidases. This controls the osmotic load and prevents fluid shifts.
Glucose, fructose and galactose are the primary monosaccharides resulting from the digestion of dietary carbohydrates. Absorption of these sugars and other minor monosaccharides occurs by means of specific carrier-mediated mechanisms (Fig. 10.7B), all of which demonstrate substrate specificity and stereospecificity, show saturation kinetics and can be specifically inhibited. In addition, all monosaccharides can cross the brush border membrane by a simple diffusion, although this is extremely slow.

**Clinical box A boy with abdominal discomfort, bloating and diarrhea: lactose intolerance**

A 15-year-old African-American boy came across to the UK on an exchange visit for 2 months. After 2 weeks in the UK, he complained of abdominal discomfort, a feeling of being bloated, increased passage of urine and, more recently, the development of diarrhea. His only change in diet noted at the time was the introduction of milk. He had developed a considerable liking for milk and was consuming 1–2 large cartons per day. A lactose tolerance test was performed, whereby the young man was given 50 g lactose in an aqueous vehicle to drink. Plasma glucose levels did not rise by more than 1 mmol/L (18 mg/dL) over the next 2 hours, with sampling at 30-minute intervals. A diagnosis of lactose intolerance was made.

**Comment.**

Lactose intolerance results from acquired lactase deficiency. Lactase activity decreases with increasing age in children, but the extent of the decline in activity is genetically determined and demonstrates ethnic variation. Lactase deficiency in the adult black population varies from 45% to 95%. If symptoms of malabsorption occur after the introduction of milk to adult diets, the diagnosis of acquired lactase

deficiency should be considered. The diagnosis is made by challenging the small bowel with lactose and monitoring the rise in plasma glucose. An increase of more than 1.7 mmol/L (30 mg/dL) is considered normal. A rise of less than 1.1 mmol/L (20 mg/dL) is diagnostic of lactase deficiency. A rise of 1.1–1.7 mmol/L (20–30 mg/dL) is inconclusive.

**There are at least two carrier-mediated transport mechanisms for monosaccharides**

At the brush border membrane both glucose and galactose are transported by the sodium-dependent glucose transporter mentioned above. This membrane-linked protein binds with glucose (or galactose) and Na+ at separate sites, and transports both into the enterocyte cytosol. Na+ is transported down its concentration gradient (the concentration within the gut lumen exceeding that inside cells), and carries glucose along *against* the glucose concentration gradient. This transport is linked to Na+/K+-ATPase). The transport of glucose or galactose is thus an indirect active process.

Fructose is transported across the brush border membrane by a sodium-independent facilitated diffusion involving the membrane-associated glucose transporter GLUT-5 present on the brush border side of the enterocyte, and GLUT-2, which transfers monosaccharides out of the enterocyte into the circulation (Table 8.2).

An incomplete digestion of carbohydrates (the components of fiber) leads to their conversion to short-chain fatty acids (acetate, propionate, butyrate) by the colonic bacteria.

**Clinical box A young man with weight loss, diarrhea, abdominal bloating and anemia: celiac disease**

A 22-year-old man presented with a history of weight loss, diarrhea, abdominal bloating and anemia. He described his stools as pale and bulky. Laboratory features included hemoglobin of 90 g/L (9 g/dL) (reference range 130–180 g/L; 13–18 g/dL). Biopsy of his small bowel demonstrated flattening of the mucosal surface, villous atrophy and disappearance of microvilli. A diagnosis of gluten-induced enteropathy (celiac disease) was made. All wheat products were removed from the patient's diet and the symptoms resolved.

**Comment.**

Celiac disease is an autoimmune condition precipitated by sensitivity to gluten resulting in inflammation of the small bowel mucosa. Gluten is a storage protein of wheat, barley and rye. It is actually a mixture of proteins, which includes the gliadins (the alcohol-soluble fraction of gluten) and glutelins. The gliadins pass through the intestinal barrier during, for instance, infections, and trigger the immune response. The inflammatory reaction ensues. The result is villous athrophy and hyperplasia of the crypts. Since the absorptive surface is markedly reduced, the resulting malabsorption can be severe.

Circulating antibodies to wheat gluten and its fractions are frequently present in cases of celiac disease. The diagnosis involves duodenal biopsy and testing the response to a gluten-free diet. The autoantibodies are tested for are **antigliadin antibodies** and/or tissue **transglutaminase antibodies** (transglutaminase is an

enzyme that deamidates gliadin in the intestinal wall). Celiac disease is underdiagnosed, especially in patients with unexplained anemia.
For hematology reference values, refer to Table 5.2.

**Advanced concept box Short-chain fatty acids are produced in the large bowel from undigested carbohydrates.** Decreased absorption of dietary starch leads to the bacterial production of short-chain fatty acids (SHFAs) by the colonic bacteria. SHFAs can be produced from fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs). Animal studies showed the presence of the short chain fatty acids receptor 2 (FFA2), a G-protein-coupled receptor present on intestinal endocrine cells., Binding of SCFA releases serotonin, leading to the increase in intestinal motility.

**Digestion and absorption of lipids**

Approximately 90% of fat in the diet is **triacylglycerol (TAG; also termed triglyceride)**. The remainder consists of cholesterol, cholesteryl esters, phospholipids and nonesterified fatty acids (NEFAs).

**Fats need to be emulsified before digestion**

The hydrophobic nature of fats prevents the access of water-soluble digestive enzymes. Furthermore, fat globules present only a limited surface area for enzyme action. These issues are overcome by the emulsification process. The change in the physical nature of lipids begins in the stomach: the core body temperature helps to liquefy dietary lipids, and the peristaltic movements of the stomach facilitate formation of a lipid emulsion. The emulsification process is also aided by the acid-stable salivary and gastric lipases. The initial rate of hydrolysis is slow, due to the separate aqueous and lipid phases and limited lipid–water interface. Once hydrolysis begins, however, the water-immiscible TAGs are degraded to fatty acids, which act as surfactants. They confer a hydrophilic surface to lipid droplets and break them down into smaller particles, thus increasing the lipid–water interface and facilitating hydrolysis. The lipid phase disperses throughout the aqueous phase as an emulsion. Dietary phospholipids, fatty acids and monoacyl glycerols also act as surfactants.

**Bile salts and pancreatic enzymes act on the lipid emulsion in the duodenum**

The lipid emulsion passes from the stomach into the duodenum where the further digestion occurs, driven by enzymes secreted by the pancreas. Solubilization is aided by the release of bile salts from the gall bladder, stimulated by the hormone cholecystokinin.

The major enzyme secreted by the pancreas is pancreatic lipase. Lipase, however, remains inactive in the presence of bile salts normally secreted into the small intestine. This inhibition is overcome by the concomitant secretion of co-lipase by the pancreas. Co-lipase binds to both the water–lipid interface and to pancreatic lipase, simultaneously anchoring and activating the enzyme. As shown in Figure 10.8, only a small proportion of dietary TAGs become completely hydrolyzed to glycerol and fatty acids. The ‘second’ and ‘third’ fatty acids in TAGs are hydrolyzed with increasing difficulty: the pancreatic lipase produces mainly 2-monoacyl glycerols (2-MAGs), which are absorbed into enterocytes. Digestion and absorption of dietary lipids. Dietary triglycerides undergo variable degrees of

hydrolysis in the intestinal lumen. Subsequently, medium- and short-chain fatty acids are absorbed into the portal blood. However, long-chain fatty acids are resynthesized into triacylglycerols and then are incorporated into chylomicrons. The fatty acids are activated by acetyl-CoA before the synthesis of acylglycerols can take place. Note that enterocytes do not possess glycerol kinase: formation of glycerol phosphate requires the presence of glucose. TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; CoA, coenzyme A. Reproduced from Dominiczak MH. Medical Biochemistry Flash Cards. London: Elsevier, 2012, Card 38.

**Bile salts are essential for solubilizing lipids during the digestive process**

Bile acids (which are bile salts at the alkaline pH of the intestine) act as detergents and reversibly form lipid aggregates (micelles). Micelles are considerably smaller than lipid emulsion droplets. Their size depends on the bile acid concentration and the ratio of bile acids to lipids.

Thus, the lipid digest changes from fat emulsion droplets into micellar structures. The micelles transport the lipids to the brush border of the enterocyte.

The absorption of lipids into the epithelial cells lining the small intestine occurs by diffusion through the plasma membrane. Almost all the fatty acids and 2-MAGs are absorbed, as both are water soluble. Water-insoluble lipids are poorly absorbed: only 30–40% of dietary cholesterol is absorbed. The bile salts pass into the ileum, where they themselves are reabsorbed and transferred back to the liver. This is called the enterohepatic circulation (Chapter 17).

**Clinical box An alcoholic man with central abdominal pain: pancreatitis**

A 56-year-old man with a long history of alcohol abuse presented with chronic central abdominal pain, weight loss and diarrhea. He described his bowel motions as pale and greasy, and difficult to flush away. The abdominal radiograph revealed epigastric calcification in the area of the pancreas, and CT scanning revealed an atrophic calcified pancreas. The stool sample sent for fecal elastase quantification revealed this to be significantly reduced. Treatment was initiated with pancreatic enzyme supplements, resulting in resolution of his diarrhea and weight gain.

**Comment.**

Acute pancreatitis is a serious and life-threatening illness, caused by gallstones blocking the pancreatic duct, alcohol abuse, or more rarely by drugs such as azathioprine, viruses such as mumps, or hypertriglyceridemia. Patients present with severe abdominal pain, nausea and vomiting. The most important biochemical marker of pancreatitis is **increased amylase activity in serum**. Increased activity of lipase and a decrease in serum calcium can also occur.

Chronic pancreatitis is a consequence of long-term inflammation and leads to malnutrition and **steatorrhea** (excessive fecal fat). It is also associated with failure of endocrine pancreatic function, leading to hyperglycemia.

**Advanced concept box Exocrine function of the pancreas**

The pancreas has two distinct functional roles: an **exocrine** function, i.e secretion of digestive enzymes via the pancreatic duct; and an **endocrine** function, i.e secretion of insulin, glucagon and other hormones by the islets of Langerhans.

(Chapter 21) These hormones are responsible for glycemic control and aspects of gastrointestinal function.

**Exocrine secretions** flow into the pancreatic duct, which empties into the duodenum along with the common bile duct from the liver and the gall bladder. Food entering the duodenum stimulates the secretion of cholecystokinin, and this in turn stimulates pancreatic enzyme production and secretion. The acidity of the stomach contents entering the duodenum stimulates the release of another hormone, secretin, which triggers the secretion of bicarbonate-rich pancreatic fluid, which neutralizes the acidity in the duodenum.

The pancreas secretes enzymes which digest carbohydrates, lipids and proteins. Pancreatic **amylase** digests carbohydrates to oligo- and monosaccharides; **lipase** digests triacylglycerols while **cholesteryl esterase** yields free cholesterol and fatty acids; finally, **proteases and peptidases** break down proteins and peptides. To prevent the powerful proteases breaking down the pancreas itself (autodigestion), they are secreted as proenzymes (Chapter 6) and are activated in the intestinal lumen.

**The fate of fatty acids depends on their chain length**

Medium- and short-chain fatty acids (less than 10 carbon atoms) pass directly through the enterocytes into the hepatic portal system. In contrast, fatty acids containing more than 12 carbon atoms are bound to a fatty acid-binding protein within the cell, and are transferred to the rough endoplasmic reticulum for resynthesis into TAGs. The glycerol required for this process is obtained from the absorbed 2-MAGs (the MAG pathway; Fig. 10.8), from the hydrolysis of 1-MAG (which yields free glycerol), or from the glycerol-3-phosphate obtained from glycolysis (the phosphatidic acid pathway). Glycerol produced in the intestinal lumen is not used in the enterocyte for TAG synthesis, and passes directly to the portal vein.

**Triacylglycerol synthesis requires activation of fatty acids**

All absorbed long-chain fatty acids are reutilized to form TAG before being transferred to chylomicrons. Fatty acid activation is accomplished by the acyl-CoA synthase. Chylomicrons are assembled within the rough endoplasmic reticulum before being released by exocytosis into the intercellular space. They leave the intestine via lymph (Chapter 18).

**Digestion and absorption of proteins**

The gut receives 70–100 g dietary proteins per day and 35–200 g of endogenous proteins. The latter (mostly enzymes) are either secreted into the gut or shed from the epithelium as a result of cell turnover. The digestion and absorption of protein is extremely efficient: of this large load, only 1–2 g of nitrogen, equivalent to 6–12 g of protein, are lost in the feces daily.

**proteins are hydrolyzed by peptidases** Proteins are broken down by hydrolysis of peptide bonds by peptidases. These enzymes can either cleave internal peptide bonds (endopeptidases) or cleave off one amino acid at a time from either the –COOH (carboxypeptidases) or –NH2 (aminopeptidases) terminal of the polypeptide. Endopeptidases break down large polypeptides to smaller oligopeptides, which can subsequently be acted upon by the exopeptidases to

produce amino acids and di- and tripeptides, the final products of protein digestion which are absorbed by the enterocytes. Depending on the source of the peptidases, the protein digestion can be divided into gastric, pancreatic and intestinal phases (Fig. 10.9).

**FIG. 10.9** Digestion and absorption of dietary proteins. The preliminary stage is protein denaturation, which takes place in the stomach. The peptide bonds between amino acids are hydrolyzed by endo- and exopeptidases. Single amino acids and di- and tripeptides are absorbed using specific transport systems located in the enterocyte membrane.

**Protein digestion begins in the stomach**

In the stomach, the secreted HCl reduces the pH to 1–2, with consequent denaturation of dietary proteins. Denaturation unfolds polypeptide chains, making proteins more accessible to proteases. In addition, the chief cells of the gastric mucosa secrete pepsin. It is released as inactive precursor-, pepsinogen, and is activated by either an intramolecular reaction (autoactivation) at pH below 5.0 or by active pepsin (autocatalysis). At pH above 2.0 the liberated peptide remains bound to pepsin and acts as an inhibitor of its activity. This inhibition is removed by either a drop in pH below 2.0 or by further pepsin action. The products of digestion of proteins by pepsin are large peptide fragments and some free amino acids. They stimulate cholecystokinin release in the duodenum, in turn triggering the release of the main digestive enzymes by the pancreas, as well as the contraction of the gallbladder to release bile. **Proteolytic enzymes are released from the pancreas as inactive zymogens, in a manner similar to pepsinogen**

Duodenal enteropeptidase converts trypsinogen to the active trypsin. This enzyme is then capable of autoactivation. It also activates all other pancreatic zymogens (chymotrypsin, elastase and carboxypeptides A and B). Because of this prime role of trypsin in activating other pancreatic enzymes, its activity is controlled within the pancreas and pancreatic ducts by a low-molecular-weight inhibitory peptide.

**Pancreatic proteases cleave peptide bonds in different locations in a protein**

**Trypsin** cleaves proteins at arginine and lysine residues, **chymotrypsin** at aromatic amino acids, and **elastase** at hydrophobic amino acids. The combined effect is to produce an abundance of free amino acids and low-molecular-weight peptides of 2–8 amino acids in length. Alongside protease secretion, the pancreas also produces copious amounts of **sodium bicarbonate**. This neutralizes the stomach as they pass into the duodenum, thus promoting pancreatic protease activity.

**Final digestion of peptides is dependent on small intestinal peptidases**

The final digestion of di- and oligopeptides is carried out in the small intestine by membrane-bound endopeptidases, dipeptidases and aminopeptidases. The end-products of this are free amino acids, and di- and tripeptides, which are then absorbed across the enterocyte membrane by specific carrier-mediated transport. Within the enterocyte, di- and tripeptides are further hydrolyzed to their constituent amino acids. The final step is the transfer of free amino acids out of the enterocyte into the portal blood.

**Clinical box Diagnostic approaches to malabsorption**

Malabsorption can be caused by cystic fibrosis, and lactase or other specific digestive enzyme deficiencies. The most common cause of carbohydrate malabsorption is lactose deficiency. Pancreatic insufficiency is also an important cause, as is inadequate amount of bile. Malabsorption can also result from the damage to the intestinal wall by, for instance, lymphoma, inflammatory bowel disease or radiotherapy. Important causes are surgical interventions: gastrectomy, pancreatectomy and resection of large fragments of the small bowel.

Rare endocrine causes include Zollinger–Ellison syndrome and abetalipoproteinemia (a rare disorder of lipoprotein metabolism in which chylomicron assembly is impaired).

The signs of malabsorption are **chronic diarrhea, steatorrhea and loss of weight**, and-in children – **failure to thrive**. Its complications result from the inadequate intake of nutrients, vitamins or trace metals (Chapter 11).

**Diagnosis of malabsorption syndromes** involves the conventional hematology and biochemistry tests and testing for active inflammatory processes (C-reactive protein), as well as stool examination and stool culture. Specialist tests include testing for vitamin deficiencies. Imaging-based investigations such as abdominal ultrasound and CT scan can be performed, and portions of the GI tract can be visualized through esophago-gastro-duodenoscopy. Biopsies can be taken from the stomach, duodenum and the small bowel.

The hydrogen breath tests are used in the diagnosis of carbohydrate malabsorption. Older tests for malabsorption include the xylose absorption test and lactose absorption test. If pancreatic insufficiency is suspected, the fecal excretion of enzymes such as elastase and lipase can be assessed, and endoscopic retrograde pancreatography (ERCP) performed.

**Advanced concept box Active transport of amino acids into intestinal epithelial cells**

Mechanisms of active transport of amino acids and di- or tripeptides into intestinal epithelial cells are similar to those described for glucose. At the brush border membrane, Na+-dependent symporters mediating amino acid uptake are linked to ATP-dependent pumping out of Na+ at the basolateral membrane. A similar H+-dependent symporter is present on the brush border surface for di- and tripeptide transport into the cell. Na+-independent transporters are present on the basolateral surface, allowing facilitated transport of amino acids into the portal vein.

At least six specific symporter systems have been identified for the uptake of L-amino acids from the intestinal lumen:

Neutral amino acid symporter for amino acids with short or polar side chains (Ser, Thr, Ala).

Neutral amino acid symporter for aromatic or hydrophobic side chains (Phe, Tyr, Met, Val, Leu, Ileu).

Imino acid symporter (Pro, OH-Pro).

Basic amino acid symporter (Lys, Arg, Cys).

Acidic amino acid symporter (Asp, Glu).

β-amino acid symporter (β-Ala, Tau).

These transport systems are also present in the renal tubules and defects in their molecular structure can lead to disease (e.g. **Hartnup disease**, an inherited disorder with defects of intestinal amino acid absorption and urinary loss of neutral amino acids described in the box on p. 84).

**Summary** Digestion is a series of processes which prepare food for absorption.
Digestion and absorption of foods make the metabolic fuels available to the organism.
Carbohydrates are digested to simple sugars.
Fats are hydrolyzed to di- and monoglycerides.
Proteins are hydrolyzed to di- and tripeptides and free amino acids.
Defects in these mechanisms result in a variety of malabsorption and food intolerance syndromes.

**Active learning**

1. Describe the process of digestion of starch.
2. Discuss the possible complications of persistent vomiting.
3. Which hormones aid digestion?
4. List the secretory products of the stomach.
5. Outline the mechanisms of sugar transport in the small intestine.
6. What is the role of micelles in the digestion of fat?

## Biological membranes. Structure, properties, functions

### Abstract

*Biological membranes*, together with *cytoskeleton*, form the structure of living cell. *Cell* or
*cytoplasmic* membrane surrounds every cell. The nucleus is surrounded by two nucleus membranes
- external and internal. All the intracellular structures (mitochondria, endoplasmic reticulum, Goldi'
apparatus, lisosomes, peroxisomes, phagosomes, synaptosomes, etc.) represent closed *membrane*
*vesicles*. Each membrane type contains a specific set of proteins - receptors and enzymes but the
base of every membrane is a bimolecular layer of lipids (*lipid bilayer*) that performs in each
membrane two principal functions: (1) a *barrier* for ions and molecules, and (2) structural base
(matrix) for functioning of receptors and enzymes.

### Introduction

Studying an electronic microscopic picture of the ultrafine section of living tissue, after its
fixation and proper staining, fine double lines can be clearly seen that «pattern» the shape of cell
and intracellular organelles (See Fig. 1). These are sections through biological membranes - finest

films consisting of a double layer of lipid molecules and proteins built in to this layer. As a matter
of fact, it is membranes, together with *cytoskeleton*, who forms the structure of living cells. *Cellular*
or *cytoplasmic* membrane surrounds each cell. The nucleus is surrounded by two *nucleus*
membranes: outer and inner. All the intracellular structures (mitochondria, endoplasmic reticulum,
Golgi.s apparatus, lisosomes, peroxisomes, phagosomes, synaptosomes, etc.) represent closed
*membrane vesicles*.

**History of studies on the properties and structure of membranes**

The term «membrane» as an invisible film that surround a cell and serves as a barrier between
cell contents and the invironment and at the same time as a semipermeable partition through which
water and some substances dissolved in it can pass, was first used obviously by botanists von Mol
and independently K. Von Negeli (1817-1891) in 1855 for explanation of plasmolytic phenomena.
Botanist W. Pfeffer (1845-1920) published his paper «Investigations of osmos» (1877, Leipzig)
where he postulated the existence of cell membranes basing on the similarity between cells and
osmometers having artificial semipermeable membranes that had been prepared not long before by
M. Traube. Further investigation of osmotic phenomena in vegetable cells by Danish botanist
Ch. De Friz (1848-1935) laid the basis in the creation of physical chemical theories of osmotic
pressure and electrolytic dissociation by Danish scientist J. Vant-Hoff (1852-1911) and by Swedish
scientist V. Arrenius (1859-1927). In 1888, German physicist and chemist W.Nernst (1864-1941)
deduced the equation of diffusion potential. In 1890, German physicist, chemist and phylosophist
W. Ostwald (1853-1932) drew attention to a possible role of membranes in bioelectrical processes.
Between 1895 and 1902, E. Overton (1865-1933) measured cell membrane permeability for many
compounds and showed a direct relationship between the ability of these compounds to penetrate
through membranes and their solubility in lipids. It was a clear indication that it is lipids who forms

the film through which substances from surrounding solution pass to cell. In 1902, Yu. Bernstein
(1839-1917) used the membrane hypothesis for explanation of the electric properties of living cells.
Gorter and Grendel showed in 1925 that the area of the monolayer of lipids extracted from
erythrocyte membranes is two times larger than the total area of erythrocytes. They extracted lipids
from hemolysed erythrocytes with/by acetone, evaporated the solution on the surface of water, and
measured the area of the formed monomolecular lipid film. The results of these investigations
suggested that lipids in membrane are arranged as a bimolecular layer. This supposition was
verified by investigations of the electrical parameters of biomembranes (Cole & Curtis, 1935): high
electrical resistance (approx. 107 Ohm ≥□m2) and high electrical capacitance (0.51 F/m2).
2
At the same time, there were experimental data that testified to the fact that biological
membranes contained protein molecules as part of their composition. These contradictions in
experimental results were removed by Danielli & Dawson who proposed in 1935 the so-called
«sandwich»(butterbrod/bread-and-butter) model of biological membranes. composition that had
been used in membranology, though with some small variations, for almost forty years. According
to this model, proteins are located/disposed in membranes on the surface of phospholipid layer.

## Functions of biological membranes

Functions of cytoplasmic and some intracellular membranes are listed in Table 1.
In all living cells, biological membranes carry out the function of «*barrier*» that divides the cell
from the environment and the internal cell volume into comparably isolated «compartments».
Partitions dividing cells into compartments are built of a double layer of lipid molecules (which is often called «*bilayer*») and are practically impermeable for ions and polar water-soluble molecules.
But this lipid bilayer includes numerous built-in protein molecules and molecular complexes one of those have/possess the properties of selective «*channels*» for ions and molecules, and others – those of «*pumps*» capable to pump/transfer actively ions through membrane. The barrier properties of membranes and working of membrane pumps cause irregular/disbalanced distribution of ions between the

cell and extracellular medium, which lies in the basis of the processes of intracellular regulation and signal transfer in the form of electrical impulse between cells. A second function, common for all membranes, is the function of «mounting plate», or *matrix* on which there are proteins and protein groups that are disposed in a definite order and form/create systems of electron transfer, energy accumulation in the form of ATP, regulation of intracellular processes by hormones coming in from outside and intracellular mediations, recognizing of other cells and foreign proteins, light reception, mechanical effects, etc.
A flexible and elastic film which lay in the basis of all membranes also plays a definite *mechanical function* keeping the cell intact under mild mechanical loads and disturbances in/upsets of osmotic balance between the cell and environment.
Common for all membranes functions of barrier for ions and molecules and matrix for protein
groups are mainly provided by the lipid bilayer that has in principle/general the same structure in all membranes. Nevertheless, the set of proteins is individual/unique for each membrane type which allows membranes to take part in carrying out different/various functions in different cells and cell structures.

**Cells Membranes Function**

All cells Cell (cytoplasmic) Active transport of K+,Na+,Ca2+,
maintaining of osmotic equilibrium
Majority of cells Cell membranes Binding of hormones and switching
on of mechanisms of intracellular
signalling
Nerve and muscle cells Cell membranes Generation of potentials of peace
and action, distribution of action
potential
Majority of cells (except
erythrocytes)
Inner membrane of
mitochondria
Transfer of electrons on oxygen and
synthesis of ATP (oxidative
phosphorylation)
Majority of cells (except
erythrocytes)
Endoplasmic reticulum Transfer of Ca2+ from cell juice into
vesicles
Eye epythelium cells Membranes of eye disks Absorption of light quanta and
generation of intracellular signal
3

## Membrane srtucture

### *General scheme of membrane structure*

According to modern information, all cell and intracellular membranes have similar structure:

the base of the membrane is composed of a lipid double molecular layer (lipid bilayer) on the
surface and inside of which proteins are disposed.
Integral protein
Hydrocarbon
Peripheral proteins
Cytosceleton
Lipid
bilayer

## Membrane lipids

### *Membrane lipids*

Lipid bilayers are formed by amphiphilic molecules of phospholipids and sphingomyelin in
water phase. These molecules are called amphiphilic because they are composed of two parts which differ by their solubility in water: (1) polar «head» possessing high affinity for water, i.e.
hydrophilic, and (2) .tail» that is formed by non-polar carbohydrate chains of fatty acids; this part of the molecule has low affinity for water, i.e. it is hydrophobic.
Membrane lipids are mainly composed of phospholipids, sphingomyelins, and cholesterol. For
example, in human erythrocyte membranes their contents are 36, 30, and 22%, respectively; 12%
are glycolipids (A.Kotyk & K.Yanachek «Membrane Transport», Moscow, «Mir», 1980, p.45).
Phosphatidylethanolamine molecule, whose structure is shown in **Fig.2**, can serve as an example of amphiphilic molecule. Phosphatidylethanolamine, like other phospholipids, represents chemically the esters of three-atom glycerol with two fatty acids; orthophosphate is bound to the third hydroxyl group; and a small arganic molecule characteristic of each type phospholipids is bound to orthophosphate. In this very case it is ethanolamine, but it can also be choline, inositol, serine, and some other molecules.
The composition of membrane lipid layer also includes cholesterol and sphingomyelins, the
latter are close to phospholipids by chemical structure and physical properties.

## Amphyphilic molecules

Lipid bilayers are formed by *amphiphilic* molecules of phospholipids and sphingomyelin in
water phase. These molecules are called amphiphilic because they are composed of two parts which differ by their solubility in water: (1) polar «head» possessing high affinity for water, i.e.
*hydrophilic*, and (2) «tail» that is formed by non-polar carbohydrate chains of fatty acids; this part of the molecule has low affinity for water, i.e. it is *hydrophobic*.
Membrane lipids are mainly composed of phospholipids, sphingomyelins, and cholesterol. For

example, in human erythrocyte membranes their contents are 36, 30, and 22%, respectively; 12% are glycolipids (A. Kotyk & K. Yanachek «Membrane Transport», Moscow, «Mir», 1980, p. 45).

Phosphatidylethanolamine molecule, whose structure is shown in Fig. 2, can serve as an example of amphiphilic molecule. Phosphatidylethanolamine, like other phospholipids, represents chemically the esters of three-atom glycerol with two fatty acids; orthophosphate is bound to the 4 third hydroxyl group; and a small arganic molecule characteristic of each type phospholipids is bound to orthophosphate.

## Membrane proteins

Membrane proteins are usually divided into integral and peripheral. *Integral proteins* have vast *hydrophobic* areas on the surface and are insoluble in water. They are connected with membrane lipids by/with *hydrophobic interactions* and partially immersed into lipid bilayer, and they often pierce the bilayer leaving on its surface comparatively small hydrophilic areas. It is possible to separate these proteins from the membrane only with the help of detergents such as dodecyl sulfate or cholines which destroy lipid bilayer and transfer protein to soluble form (*solubilize* it) creating associates with it. All further operations on purifying integral proteins are also carried out in the presence of *detergents*.

*Peripheral proteins* are connected to the surface of lipid bilayer by electrostatic forces and can be washed out of the membrane by saline solutions.

## Membrane lipid layer

The data of X-ray analysis and some other show that phospholipid molecules have a specific shape, namely, they resemble a cylinder, in which the diameter of the polar head is close to that of hydrophobic tale.

A peculiar property of an amphiphilic molecules, including those of phospholipids, results in formation of a lipid bilayer and then liposomes in aqueous solutions. In the membrane, "fatty tails" of the molecules are hidden inside, while polar "heads" of the molecules are exposed to water environment.

In aqueous environment amphiphilic molecules are spontaneously gathering together to form lipid bilayer, that in its turn is closing up, so making a vesicle (liposome).

## Model membranes

Studies on the physical properties of membrane lipid layer are carried out mainly on artificial membrane structures of two types formed by synthetic phospholipids or lipids extracted from biological sources: (1) liposomes and (2) bilayer lipid membranes (BLM).

### *Liposomes*

*Liposomes* are lipid vesicles that are formed from phospholipids in water solutions. In order to obtain liposomes, a phospholipid dissolved in alcohol is injected into a large-volume water solution;

insoluble in water phospholipids create small vesicles whose walls are composed of lipid bilayer (***unilayer liposomes***).

The phospholipid solution can first be dried from a solution in an organic solvent (for example, in chloroform) in a tube. Then water solution should be added into

the tube and shaken well. Lipids pass into the water solution, now in the form of ***multilayer*** liposomes. Liposome suspensions are usually used in studies on the physical properties of lipid bilayer such as viscosity, surface charge, or dielectric permittivity, as well as for investigation of permeability for uncharged molecules.

***Bilayer Lipid Membranes (BLM)***

In studies on ion permeability of the lipid layer of membranes BLM are used.
For preparation of BLM (see Fig. 6), an electrolyte-containing glass was used into which a teflon cup with an orifice (D = 1mm) in its wall was put.

A small drop of a solution of phospholipid in liquid hydrocarbon, heptane or hexane is introduced/injected into the orifice using a capillary tube.
The polar heads of phospholipids are directed into water phase, and nonpolar hydrocarbonchains of fatty acids merge into a homogeneous viscous phase in the inner side of lipid membranes.
This film is alike by many properties with the lipid layer of biomembranes.

**The mobility of hydrophobic tails of phospholipid molecules in the lipid bilayer of membranes**

The carbon atoms in hydrocarbon backbone of the phospholipid fatty acids are connected to each other by ordinary bounds, around which, as on an axis, the different sites of the molecule can rotate. This rotation results in that the hydrophobic chain can be in the most various configurations. As a result of such rotation, the fatty acid chains seem to be flexible, though actually they could not be bent in common sense of this word: they only can turn around of the bonds between atoms, which results in a bend of a molecule as a whole.

***Kinks***

The ability of fatty acids to change their configuration is of primary importance for dissolution of various molecules and ions in a lipid layer and for their diffusion through membrane lipid phase.Sometimes two adjacent loops of fatty acid chains may form a sort of the cave, named a *kink*. The *kinks* are formed as a result of thermal movement of phospholipid molecules, and ions can diffuse through the lipid layer of the membrane, jumping from one kink to another.

## TOPIC: BIOLOGICAL OXIDATION 1

Bioenergetics and Oxidative Phosphorylation

Bioenergetics describes the transfer and utilization of energy in biologic systems. It concerns the initial and final energy states of the reaction components, not the reaction mechanism or how much time it takes for the chemical change to occur. Bioenergetics makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Because changes in free energy provide a measure of the energetic feasibility of a chemical reaction, they

allow prediction of whether a reaction or process can take place. In short, bioenergetics predicts if a process is possible, whereas kinetics measures the action rate.

II.FREE ENERGY

The direction and extent to which a chemical reaction proceeds are determined by the degree to which two factors change during the reaction. These are enthalpy (ΔH, a measure of the change [Δ] in heat content of the reactants and products) and entropy (ΔS, a measure of the change in randomness or disorder of the reactants and products), as shown in. Neither of these thermodynamic quantities by itself is sufficient to determine whether a chemical reaction will proceed spontaneously in the direction it is written. However, when combined mathematically, enthalpy and entropy can be used to define a third quantity, free energy (G), which predicts the direction in which are action will spontaneously proceed.

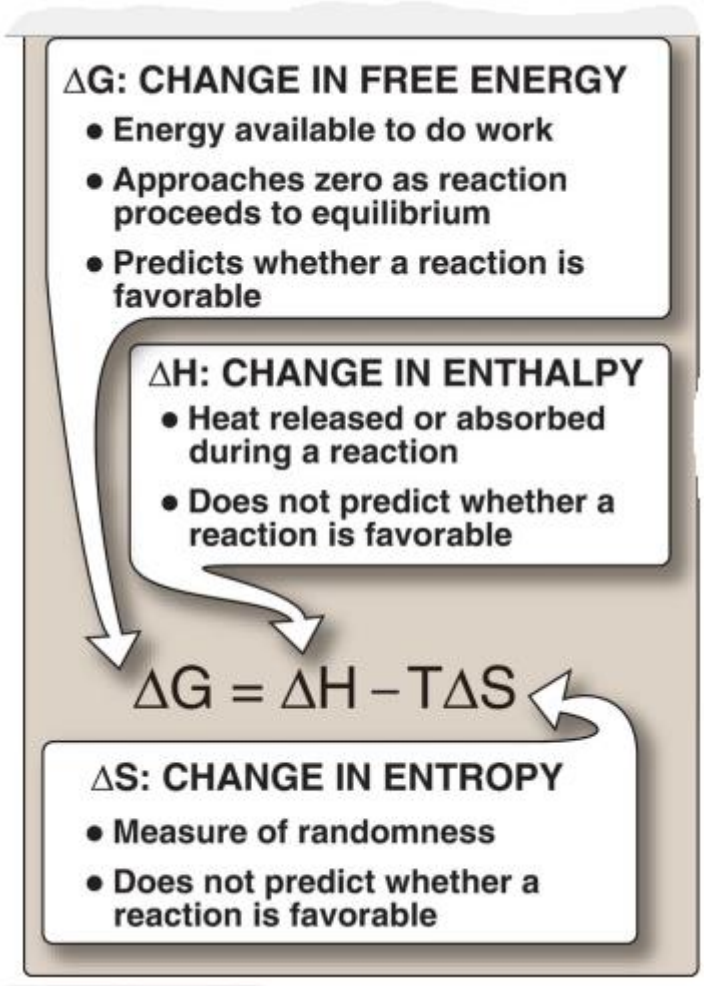



Figure 6.1 Relationship between changes in free energy (G), enthalpy (H), andentropy (S). T is the absolute temperature in Kelvin (K), where K = °C + 273.III.

**FREE ENERGY CHANGE**The change in free energy is represented in two ways, ΔG and ΔG0. The first, ΔG (without the superscript "0"), represents the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. ΔG, then, is a variable. This contrasts with the standard free energy change, ΔG0 (with the superscript "0"), which is the energy change when reactants and products are at a concentration of 1 mol/l. [Note: The concentration of protons (H+) is assumed to be 10-7 mol/l (that is, pH = 7). This may be shown by a prime sign (′), for example, ΔG0′.] Although ΔG0, a constant, represents energy changes at these non physiologic concentrations of reactants and products, it is nonetheless useful in comparing the energy changes of different reactions. Furthermore, ΔG0 can readily be determined from measurement of the equilibrium constant. [Note: This section outlines the uses of ΔG, and ΔG0 is described in D. below.]

**A. ΔG and reaction direction**. The sign of ΔG can be used to predict the direction of a reaction at constant temperature and pressure. Consider the reaction:

$$A \rightleftarrows B$$

1. Negative ΔG: If ΔG is negative, then there is a net loss of energy, and the reaction goes spontaneously as written (that is, A is converted into B) as shown in. The reaction is said to be exergonic.

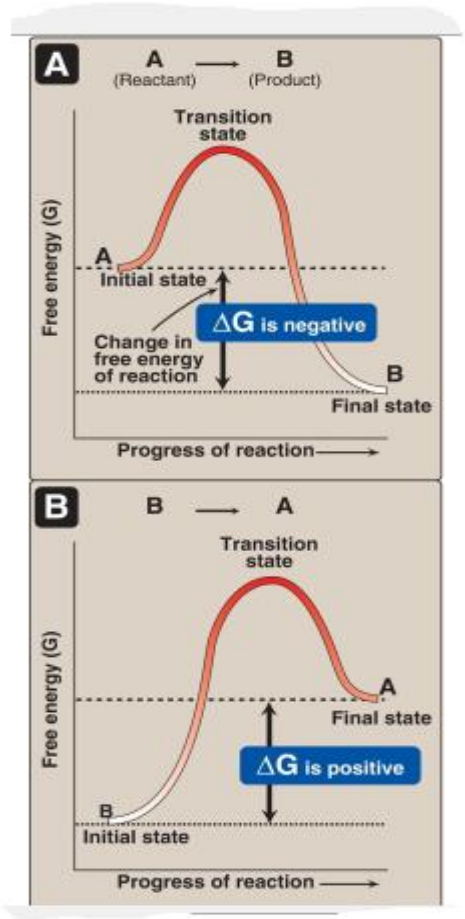



Fig.: Change in free energy (ΔG) during a reaction. A. The product has a lower free energy (G) than the reactant. B. The product has a higher free energy than the reactant.

2. Positive ΔG: If ΔG is positive, then there is a net gain of energy, and the reaction does not go spontaneously from B to A. Energy must be added to the system to make the reaction go from B to A. The reaction is said to be endergonic.

3. Zero ΔG: If ΔG = 0, then the reaction is in equilibrium. [Note: When are action is proceeding spontaneously (that is, ΔG is negative), there action continues until ΔG reaches zero and equilibrium is established.]. ΔG of the forward and back reactions The free energy of the forward reaction (A → B) is equal in magnitude but opposite in sign to that of the back reaction (B → A). For example, if ΔG of the forward reaction is -5 kcal/mol, then that of the back reaction is +5kcal/mol. [Note: ΔG can also be expressed in kilojoules per mole or kJ/mol(1 kcal = 4.2 kJ).]

C. ΔG and reactant and product concentrations The ΔG of the reaction A → B depends on the concentration of the reactant and product. At constant temperature and pressure, the following relationship can be derived:

$$\Delta G = \Delta G^0 + RT \ln \frac{[B]}{[A]}$$

where ΔG0 is the standard free energy change (see D. below)R is the gas constant (1.987 cal/mol K)T is the absolute temperature (K)[A] and [B] are the actual concentrations of the reactant and product ln represents the natural logarithm.A reaction with a positive ΔG0 can proceed in the forward direction if the ratio of products to reactants ([B]/[A]) is sufficiently small (that is, the ratio of reactants to products is large) to make ΔG negative. For example, consider the reaction:

Glucose 6-phosphate ⇄ fructose 6-phosphate

Figure A shows reaction conditions in which the concentration of reactant, glucose 6-phosphate, is high compared with the concentration of product, fructose 6-phosphate. This means that the ratio of the product to reactant is small, and RT ln ([fructose 6-phosphate]/[glucose 6-phosphate])is large and negative, causing ΔG to be negative despite ΔG0 being positive. Thus, the reaction can proceed in the forward direction.

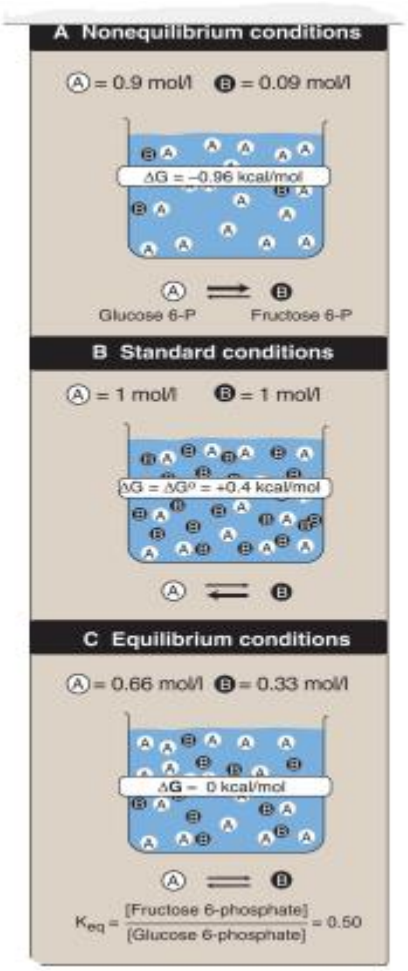



Figure: Free energy change (ΔG) of a reaction depends on the concentration of reactant and product. For the conversion of glucose 6-phosphate to fructose6-phosphate, ΔG is negative when the ratio of reactant to product is large (top, panel A), is positive under standard conditions (middle, panel B), and is zero atequilibrium (bottom, panel C). ΔG0 = standard free energy change.

**D. Standard free energy change.** The standard free energy change, ΔG0, is so called because it is equal to the free energy change, ΔG, under standard conditions (that is, when reactants and products are at 1 mol/l concentrations;). Under the seconditions, the natural logarithm of the ratio of products to reactants is zero(ln1 = 0), and, therefore, the equation shown at the bottom of the previous page becomes:

$$\Delta G = \Delta G^0 + 0$$

1. ΔG0 and reaction direction: Under standard conditions, ΔG0 can be used to predict the direction a reaction proceeds because, under these conditions, ΔG0 is equal to ΔG. However, ΔG0 cannot predict the direction of a reaction under physiologic conditions because it is composed solely of constants (R, T, and Keq [see 2. below]) and is not,

therefore, altered by changes in product or substrate concentrations. 2. Relationship between ΔG0 and Keq: In a reaction A ⇄B, a point of equilibrium is reached at which no further net chemical change takes place (that is, when A is being converted to B as fast as B is being converted to A). In this state, the ratio of [B] to [A] is constant, regardless of the actual concentrations of the two compounds:

$$K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

where K eq is the equilibrium constant, and [A]eq and [B]eq are the concentrations of A and B at equilibrium. If the reaction A ⇄B is allowed to go to equilibrium at constant temperature and pressure, then, at equilibrium, the overall ΔG is zero. Therefore,

$$\Delta G = 0 = \Delta G^0 + RT \ln \frac{[B]_{eq}}{[A]_{eq}}$$

where the actual concentrations of A and B are equal to the equilibrium concentrations of reactant and product ([A]eq and [B]eq), and their ratio is equal to the Keq. Thus,

$$\Delta G^0 = -RT \ln K_{eq}$$

This equation allows some simple predictions:

| | |
|---|---|
| If $K_{eq} = 1$, then $\Delta G^0 = 0$ | A ⇌ B |
| If $K_{eq} > 1$, then $\Delta G^0 < 0$ | A ⇀ B |
| If $K_{eq} < 1$, then $\Delta G^0 > 0$ | A ↼ B |

ΔG0s of two consecutive reactions: The ΔG0s are additive in any sequence of consecutive reactions, as are the ΔGs. For example:

| | | |
|---|---|---|
| Glucose + ATP | → glucose 6-phosphate + ADP | $\Delta G^0 = -4{,}000$ cal/mol |
| Glucose 6-phosphate | → fructose 6-phosphate | $\Delta G^0 = +400$ cal/mol |
| Glucose + ATP | → fructose 6-phosphate + ADP | $\Delta G^0 = -3{,}600$ cal/mol |

ΔGs of a pathway: The additive property of ΔG is very important in biochemical pathways through which substrates (reactants) must pass in a

particular direction (for example, A → B → C → D → …). As long as the sum of the ΔGs of the individual reactions is negative, the pathway can proceed as written, even if some of the individual reactions of the pathway have a positive ΔG. However, the actual rates of the reactions depend on the lowering of activation energies (Ea) by the enzymes that catalyze the reactions.

AN ENERGY CARRIER

Reactions or processes that have a large positive ΔG, such as moving ionsagainst a concentration gradient across a cell membrane, are made possible by coupling the endergonic movement of ions with a second, spontaneous process with a large negative ΔG such as the exergonic hydrolysis of ATP.[Note: In the absence of enzymes, ATP is a stable molecule because its hydrolysis has a high Ea.]shows a mechanical model of energy coupling. The simplest example of energy coupling in biologic reactions occurs when the energy-requiring and the energy-yielding reactions share a common intermediate.

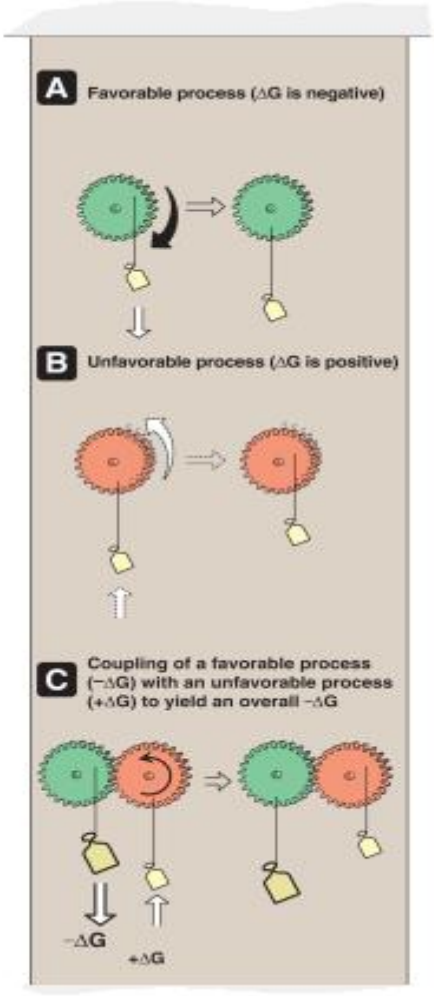



Figure: Mechanical model of the coupling of favorable and unfavorable processes. A. Gear with weight attached spontaneously turns in the direction that

achieves the lowest energy state. B. The reverse movement is energetically unfavorable (not spontaneous).

**The energetically favorable movement can drive the unfavorable one**. ΔG = change in free energy. A. Common intermediates Two chemical reactions have a common intermediate when they occur sequentially in that the product of the first reaction is a substrate for the second. For example, given the reactions D is the common intermediate and can serve as a carrier of chemical energy between the two reactions.

$$A + B \rightarrow C + D$$

$$D + X \leftrightarrow Y + Z$$

[Note: The intermediate may be linked to an enzyme.] Many coupled reactions use ATP to generate a common intermediate. These reactions may involve the transfer of a phosphate group from ATP to another molecule. Other reactions involve the transfer of phosphate from an energy-rich intermediate to adenosine diphosphate (ADP), forming ATP.

Energy carried by ATP ATP consists of a molecule of adenosine (adenine + ribose) to which three phosphate groups are attached. Removal of one phosphate produces ADP, and removal of two phosphates produces adenosine monophosphate (AMP). For ATP, the ΔG0 of hydrolysis is approximately – 7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large negative ΔG0 of hydrolysis, ATP is called a high-energy phosphate compound. [Note: Adenine nucleotides are inter converted (2 ADP ⇄ATP + AMP) by ***adenylate kinase***.].

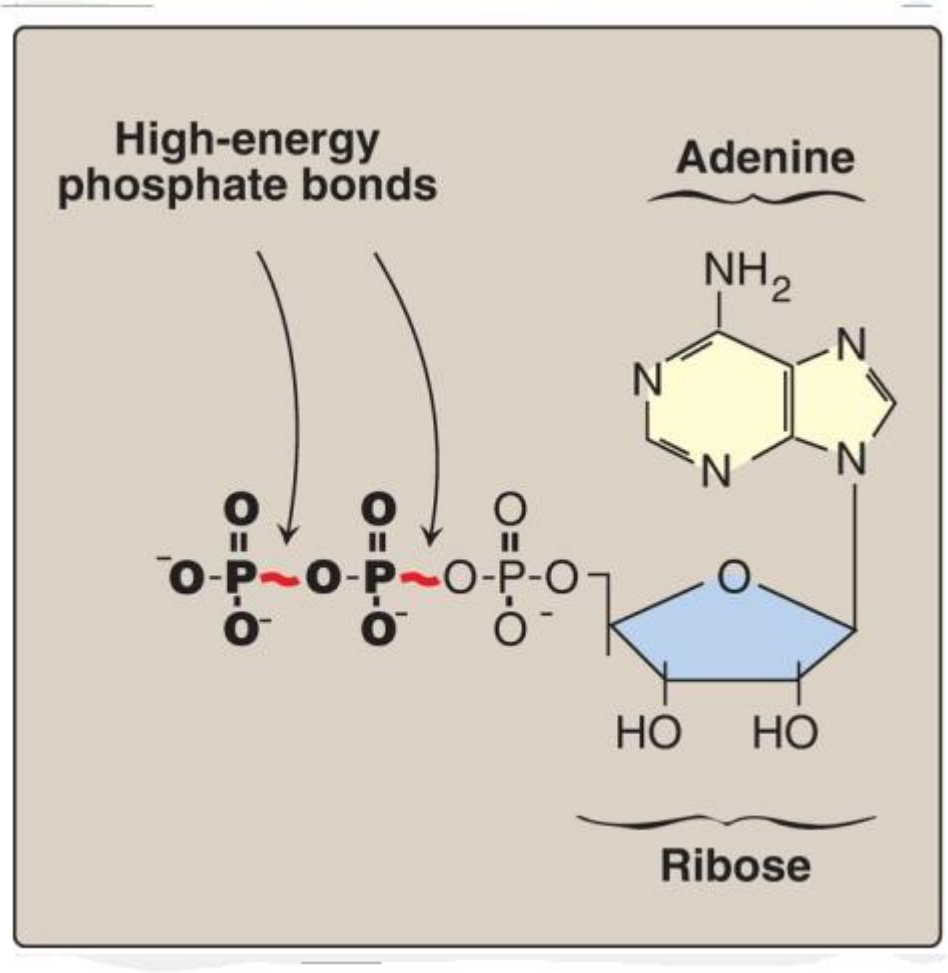



Figure: Adenosine triphosphate (ATP).

ELECTRON TRANSPORT CHAIN

Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding carbon dioxide and water (H2O), as shown in. The metabolic intermediates of these reactions donate electrons to specific coenzymes, nicotinamide adenine dinucleotide (NAD+) and Flavinadenine dinucleotide (FAD), to form the energy-rich reduced forms, NADH andFADH2. These reduced coenzymes can, in turn, each donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain (ETC), described in this section. As electrons are passed down the ETC, they lose much of their free energy. This energy is used to move H+ across the inner mitochondrial membrane, creating a H+ gradient that drives the production of ATP from ADP and inorganic phosphate (Pi), described on p. The coupling of electron transport with ATP synthesis is called oxidative phosphorylation, sometimes denoted as OXPHOS. It proceeds continuously inall tissues that contain mitochondria. [Note: The free energy not trapped as ATP is used to drive ancillary reactions such as transport of calcium ions into mitochondria and to generate heat.]

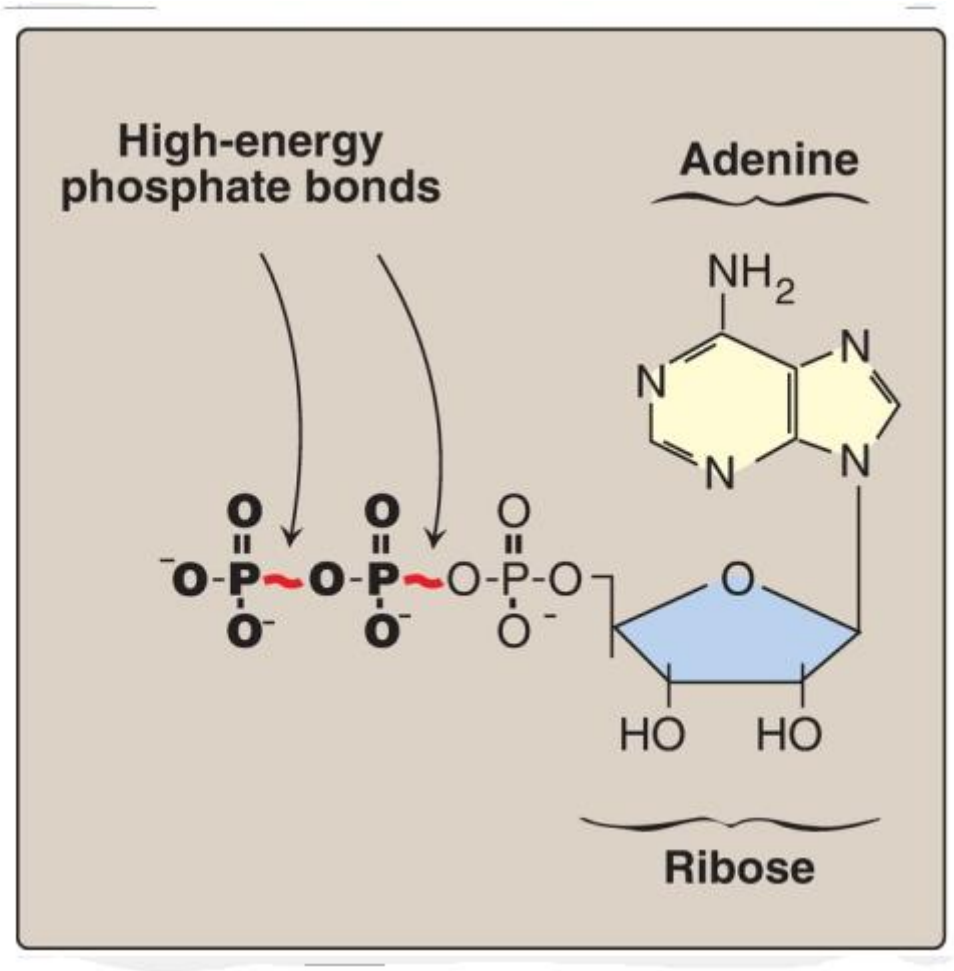



Figure: The metabolic breakdown of energy-yielding molecules. NAD(H) =nicotinamide adenine dinucleotide; FAD(H2) = flavin adenine dinucleotide;ADP = adenosine diphosphate; Pi = inorganic phosphate; CO2 = carbon dioxide.

A. Mitochondrial electron transport chain The ETC (except for cytochrome c,) is located in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen (O2), reducing it to H2O .1. Mitochondrial membranes: The mitochondrion contains an outer and an inner membrane separated by the intermembrane space. Although the outer membrane contains special channels (formed by the protein porin), making it freely permeable to most ions and small molecules, the inner membrane is a specialized structure that is impermeable to most small ions, including H+, and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function. Specialized carriers or transport systems are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually

rich in proteins, over half of which are directly involved in oxidative phosphorylation. It also contains convolutions, called cristae, which greatly increase its surface area.

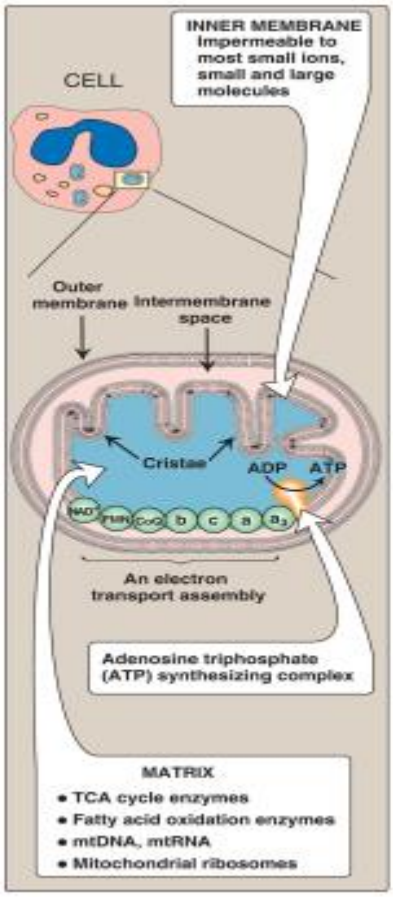



Figure: Structure of a mitochondrion showing schematic representation of the electron transport chain and the ATP synthesizing complex on the inner membrane. [Note: Unlike the inner membrane, the outer membrane is highly permeable, and the milieu of the intermembrane space is like that of the cytosol.] mt = mitochondrial; RNA = ribonucleic acid; ADP = adenosine diphosphate; TCA=tricarboxylic acid.

2. Mitochondrial matrix: The gel-like solution of the matrix (interior) of mitochondria is also rich in proteins. These include the enzymes responsible for the oxidation of pyruvate, amino acids, and fatty acids (by β-oxidation) as well as those of the tricarboxylic acid (TCA) cycle. The synthesis of glucose, urea, and heme occurs partially in the matrix of mitochondria. In addition, the matrix contains NAD+ and FAD (the oxidized forms of the two coenzymes that are required as electron acceptors), and ADP and Pi, which are used to produce ATP. [Note: The matrix also

contains mitochondrial deoxyribonucleic acid (mtDNA), ribonucleic acid (mtRNA), and ribosomes.] B. OrganizationThe inner mitochondrial membrane contains four separate protein complexes, called Complexes I, II, III, and IV that each contain part of the ETC. These complexes accept or donate electrons to the relatively mobile electron carrier coenzyme Q (CoQ) and cytochrome c. Each carrier in the ETC can receive electrons from an electron donor and can subsequently donate electrons to the next acceptor in the chain. The electrons ultimately combine with O2 and H+ to form H2O. This requirement for O2 makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of O2.

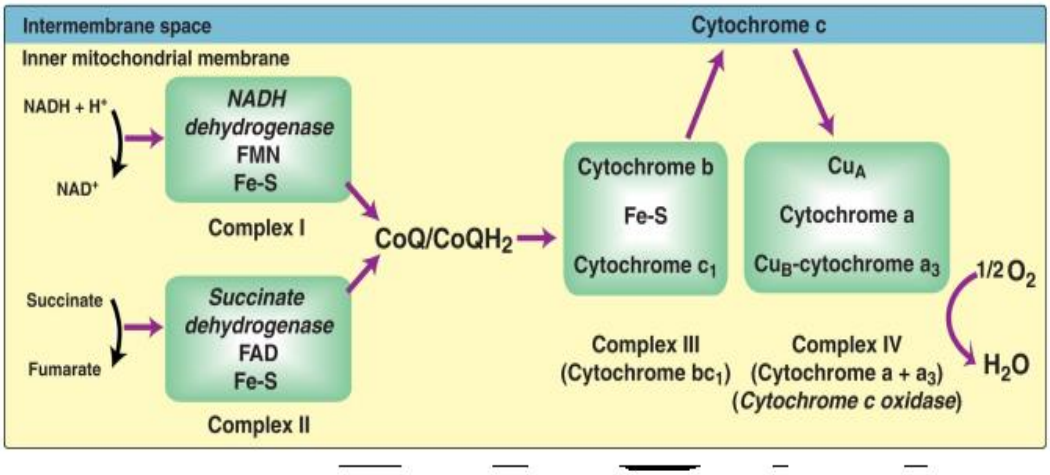



Figure: Electron transport chain. Electron flow is shown by magenta arrows. NAD(H) = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; Fe-S = iron-sulfur; CoQ = coenzyme Q; Cu =copper.

C. Reactions With the exception of CoQ, which is a lipid-soluble quinone, all members of the ETC are proteins. These may function as enzymes as is the case with the flavin-containing ***dehydrogenases***, may contain iron as part of an ironsulfur (Fe-S) center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper (Cu) as does the cytochrome a + a3 complex.

1. NADH formation: NAD+ is reduced to NADH by ***dehydrogenases*** that remove two hydrogen atoms from their substrate. [Note: For examples of these reactions, see the discussion of the ***dehydrogenases*** of the TCA cycle, p. 112.] Both electrons but only one H+ (that is, a hydride ion [:H-]) are transferred to the NAD+, forming NADH plus a free H+.

2. NADH dehydrogenase: The free H+ plus the hydride ion carried by NADH are transferred to ***NADH dehydrogenase***, a protein complex (Complex I) embedded in the inner mitochondrial membrane. Complex I has a tightly bound molecule of flavin mononucleotide (FMN), a coenzyme structurally related to FAD that accepts the two hydrogen atoms (2 electrons + 2 H+), becoming FMNH2. ***NADH dehydrogenase*** also contains peptide subunits with Fe-S centers. At Complex I, electrons move from NADH to FMN to the iron of the Fe-S centers and then to CoQ. As electrons flow, they lose energy.This energy is used to pump four H+ across the inner mitochondrial membrane, from the matrix to the intermembrane space.

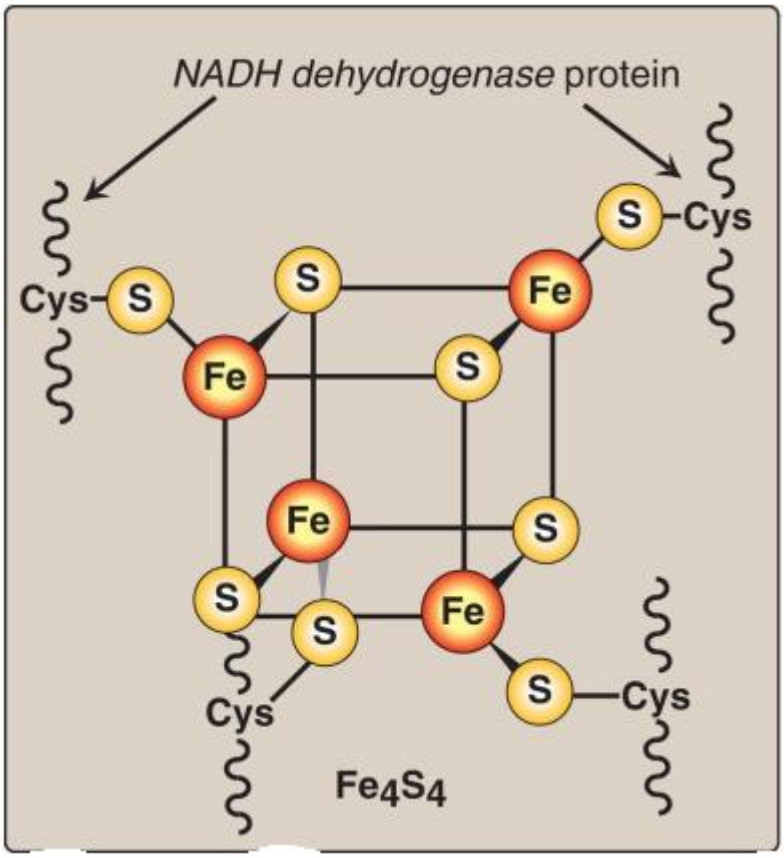



Figure: Iron-sulfur (Fe-S) center of Complex I. [Note: Complexes II and III also contain Fe-S centers.] NADH = nicotinamide adenine dinucleotide; Cys = cysteine.

3. Succinate dehydrogenase: At Complex II, electrons from the ***succinate dehydrogenase***–catalyzed oxidation of succinate to fumarate move from the coenzyme, FADH2, to an Fe-S protein, and then to CoQ. [Note: Because no energy is lost in this process, no H+ are pumped at Complex II.]

4. Coenzyme Q: CoQ is a quinone derivative with a long, hydrophobic isoprenoid tail. It is made from an intermediate of cholesterol synthesis. [Note: It is also called ubiquinone because it is ubiquitous in biologic systems.] CoQ is a mobile electron carrier and can accept electrons from ***NADH dehydrogenase*** (Complex I), from ***succinate dehydrogenase*** (Complex II) and from other mitochondrial ***dehydrogenases***, such as ***glycerol 3-phosphate dehydrogenase*** and ***acyl CoA dehydrogenases***. CoQ transfers electrons to Complex III (cytochrome bc1). Thus, a function of CoQ is to link the flavoprotein ***dehydrogenases*** to the cytochromes.

5. Cytochromes: The remaining members of the ETC are cytochrome proteins. Each contains a heme group (a porphyrin ring plus iron). Unlike the heme groups of hemoglobin, the cytochrome iron is reversibly converted from its ferric (Fe3+) to its ferrous (Fe2+) form as a normal part of its function as an acceptor and donor of electrons. Electrons are passed along the chain from cytochrome bc1 (Complex III), to cytochrome c, and then to cytochromes a + a3 ([Complex IV]). As electrons flow, four H+ are pumped across the inner mitochondrial membrane at Complex III and two at Complex IV. [Note: Cytochrome c is located in the inter membrane space, loosely associated with the outer face of the inner membrane. As seen with CoQ, cytochrome c is a mobile electron carrier.]

6. Cytochrome a + a3: Because this cytochrome complex (Complex IV) is the only electron carrier in which the heme iron has an available coordination site that can react directly with O2, it also is called ***cytochrome c oxidase***. At Complex IV, the transported electrons, O2, and free H+ are brought together, and O2 is reduced to H2O. [Note: Four electrons are required to reduce one molecule of O2 to two molecules of H2O.] ***Cytochrome c oxidase*** contains Cu atoms that are required for this complicated reaction to occur. Electrons move from CuA to cytochrome a to cytochrome a3 (in association with CuB) to O2. 7. Site-specific inhibitors: Inhibitors of specific sites in the ETC have been identified and are illustrated in. These respiratory inhibitors prevent the passage of electrons by binding to a component of the chain, blocking the oxidation-reduction reaction. Therefore, all electron carriers before the block are fully reduced, whereas those located after the block are oxidized. [Note: Inhibition of the ETC inhibits ATP synthesis because these processes are tightly coupled.]

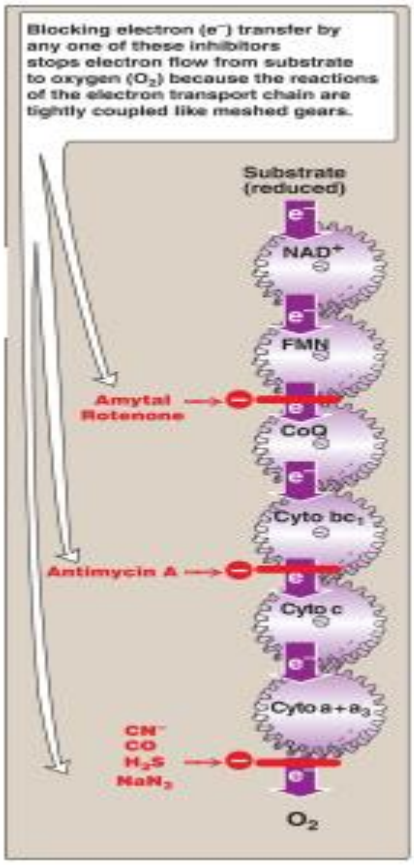



Figure: Site-specific inhibitors of electron transport shown using a mechanical model for the coupling of oxidation-reduction reactions. [Note: Normal direction of electron flow is illustrated.] NAD+ = nicotinamide adenine dinucleotide; FMN = flavin mononucleotide; CoQ = coenzyme Q; Cyto = cytochrome; CN- = cyanide; CO = carbon monoxide; H2S = hydrogen sulfide; NaN3 = sodium azide.

Leakage of electrons from the ETC produces reactive oxygen species (ROS), such as superoxide (O2-·), hydrogen peroxide (H2O2), and hydroxyl radicals (OH·). ROS damage DNA and proteins and cause lipid peroxidation. Enzymes such as ***superoxide dismutase*** (***SOD***), ***catalase***, and ***glutathione peroxidase*** are cellular defenses against ROS (see p. 148). D. Free energy release during electron transport The free energy released as electrons are transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump H+ at Complexes I, III, and IV. [Note: The electrons can be transferred as hydride ions to NAD+; as hydrogen atoms to FMN, CoQ, and FAD; or as electrons to cytochromes.]1. Redox pairs: Oxidation (loss of electrons) of one substance is always accompanied by reduction (gain of electrons) of a second. For example, figure shows the oxidation of NADH to

NAD+ by ***NADH dehydrogenase*** at Complex I, accompanied by the reduction of FMN, the prosthetic group, to FMNH2. Such redox reactions can be written as the sum of two separate half reactions, one an oxidation and the other a reduction. NAD+ and NADH form a redox pair, as do FMN and FMNH2. Redox pairs differ in their tendency to lose electrons. This tendency is a characteristic of a particular redox pair and can be quantitatively specified by a constant, E0 (the standard reduction potential), with units in volts.

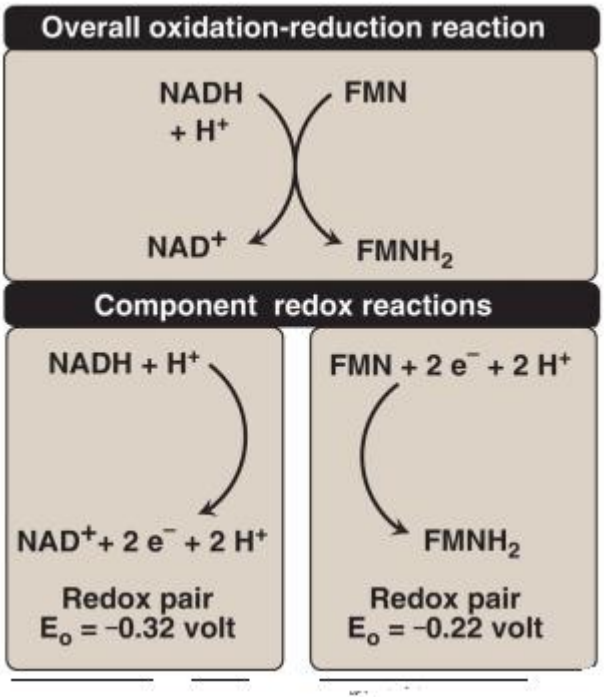



Figure: Oxidation of NADH by FMN, separated into two component half reactions. NAD(H) = nicotinamide adenine dinucleotide; FMN(H2) = Flavin mononucleotide; e- = electron; H+ = proton; E0 = standard reduction potential.

2. Standard reduction potential: The E0 of various redox pairs can be ordered from the most negative E0 to the most positive. The more negative the E0 of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E0, the greater the tendency of the oxidant member of that pair to accept electrons. Therefore, electrons flow from the pair with the more negative E 0 to that with the more positive E0. The E0 values for some members of the ETC are shown in. [Note: The components of the chain are arranged in order of increasingly positive E0 values.]

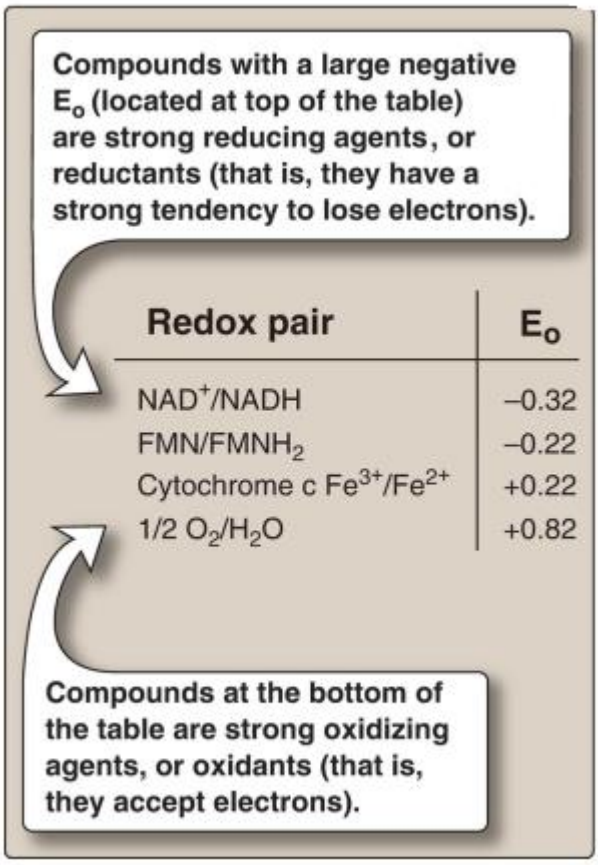



Figure: Standard reduction potentials (E0) of some reactions. NAD(H) = nicotinamide adenine dinucleotide; FMN(H2) = flavin mononucleotide; Fe = iron.

3. Relationship of ΔG0 to ΔE0: The ΔG0 is related directly to the magnitude of the change in E0: where n = number of electrons transferred (1 for a cytochrome, 2 for NADH, FADH2, and CoQ) F = Faraday constant (23.1 kcal/volt mol) ΔE 0 = E0

$$\Delta G^0 = -n F \Delta E_0,$$

of the electron-accepting pair minus the E0 of the electrondonating pair ΔG0 = change in the standard free energy

4. ΔG0 of ATP: The ΔG0 for the phosphorylation of ADP to ATP is +7.3 kcal/mol. The transport of a pair of electrons from NADH to O2 through the ETC releases 52.6 kcal. Therefore, more than sufficient energy is available to produce three ATP from three ADP and three Pi (3 × 7.3 = 21.9 kcal/mol), sometimes expressed as a P/O ratio (ATP made per O atom reduced) of 3:1. The remaining calories are used for ancillary reactions or

released as heat. [Note: The P:O for FADH2 is 2:1 because Complex I is bypassed.]

PHOSPHORYLATION OF ADP TO ATP

The transfer of electrons down the ETC is energetically favored because NADHis a strong electron donor and O2 is an avid electron acceptor. However, the flowof electrons does not directly result in ATP synthesis. A. Chemiosmotic hypothesis. The chemiosmotic hypothesis (also known as the Mitchell hypothesis)explains how the free energy generated by the transport of electrons by theETC is used to produce ATP from ADP + Pi.1. Proton pump: Electron transport is coupled to ADP phosphorylation by the pumping of H+ across the inner mitochondrial membrane, from the matrix to the inter membrane space, at Complexes I, III, and IV. For each pair of electrons transferred from NADH to O2, 10 H+ are pumped. This rates an electrical gradient (with more positive charges on the cytosolicside of the membrane than on the matrix side) and a pH (chemical)9gradient (the cytosolic side of the membrane is at a lower pH than the matrix side), as shown in. The energy (proton-motive force)generated by these gradients is sufficient to drive ATP synthesis. Thus,the H+ gradient serves as the common intermediate that couplesoxidation to phosphorylation.

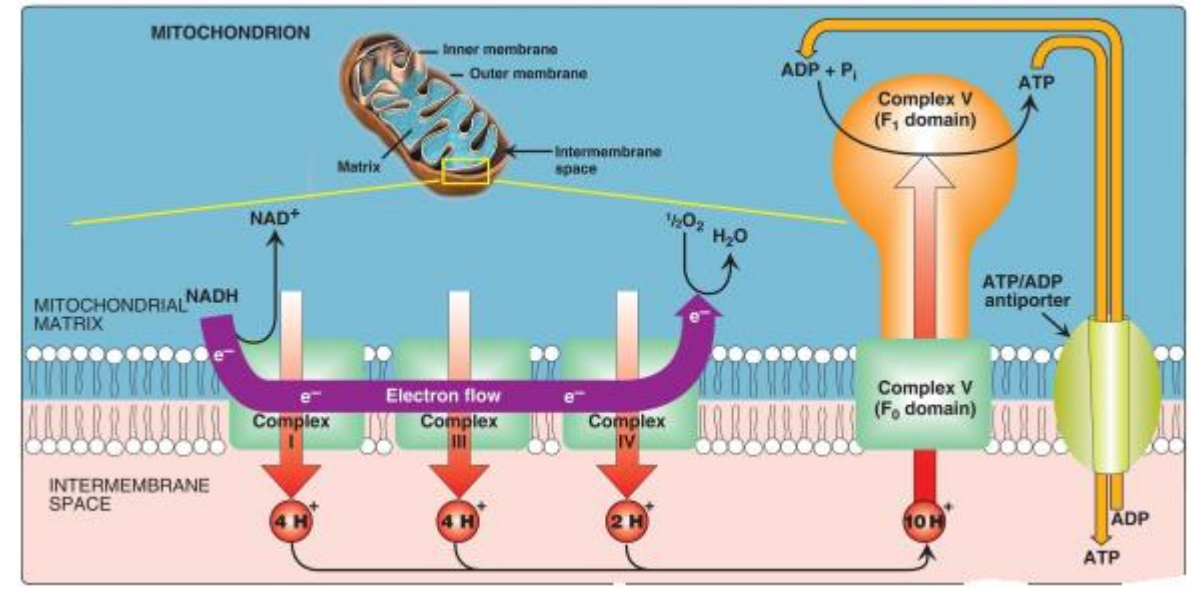



Figure: Electron transport chain shown in association with proton (H+)pumping. Ten H+ are pumped for each nicotinamide adenine dinucleotide (NADH) oxidized. [Note: H+ are not pumped at Complex II.] e- = electron;Complex V = ***ATP synthase***.2. ATP synthase: The multi subunit enzyme ***ATP synthase*** ([Complex V]Fig. 6.14) synthesizes ATP using the energy of the H+

gradient. It on tains a membrane domain (Fo) that spans the inner mitochondrial membrane and an extra membranous domain (F1) that appears as a sphere that protrudes into the mitochondrial matrix (see Fig. 6.13). The chemiosmotic hypothesis proposes that after H+ have been pumped to thecytosolic side of the inner mitochondrial membrane, they reenter thematrix by passing through a H+ channel in the Fo domain, drivingotation of the c ring of Fo and, at the same time, dissipating the pH and electrical gradients. Rotation in Fo causes conformational changes in the three βsubunits of F1 that allow them to bind ADP + Pi, phosphorylate ADP to ATP, and release ATP. One complete rotation of the c ringproduces three ATP. [Note: ***ATP synthase*** is also called ***F1/Fo-ATPase*** because the enzyme can also catalyze the hydrolysis of ATP to ADP and Pi.]

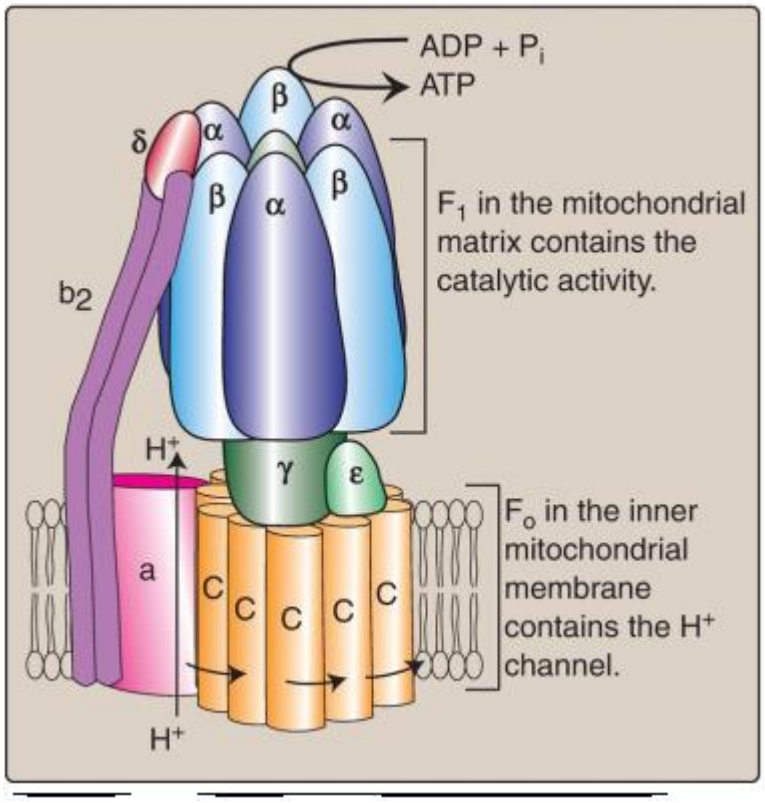



Figure:***ATP synthase*** (***F1Fo-ATPase***). [Note: The c ring of vertebrates contains eight subunits. One complete turn of the ring is driven by eight H+(protons) moving through the Fo domain. The resulting conformational changes in the three β subunits of the F1 domain allow phosphorylation of three adenosine diphosphates (ADP) to three ATP.] Pi = inorganic phosphate.

a. Coupling in oxidative phosphorylation: In normal mitochondria, ATPsynthesis is coupled to electron transport through the H+ gradient.Increasing (or decreasing) one process has the same effect on the other. For example, hydrolysis of ATP to ADP and Pi in energy requiring reactions increases the

availability of substrates for ***ATPsynthase*** and, thus, increases H+ flow through the enzyme. Electront ransport and H+ pumping by the ETC increase to maintain the H+gradient and allow ATP synthesis.b. Oligomycin: This drug binds to the Fo (hence the letter "o") domain of***ATP synthase***, closing the H+ channel and preventing reentry of H+into the matrix, thereby inhibiting phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this phosphorylation inhibitor, electron transport stops because of the difficulty of pumping any more H+ against the steep gradient. This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control and is the consequence of the tight coupling of these processes. Uncoupling proteins: Uncoupling proteins (UCP) occur in the inner +mitochondrial membrane of mammals, including humans. Theseproteins form channels that allow H+ to reenter the mitochondrial matrix without energy being captured as ATP. The energy is released as heat, and the process is called nonshiveringthermogenesis. UCP1, also called thermogenin, is responsible for heat production in the mitochondria-rich brown adipocytes of mammals.[Note: Cold causes catecholamine-dependent activation of USP expression.] In brown fat, unlike the more abundant white fat, ~90% of its respiratory energy is used for thermogenesis in infants in response to cold. Thus, brown fat is involved in energy expenditure, whereas white fat is involved in energy storage. [Note: Brown fat depots have recently been shown to be present in adults.]

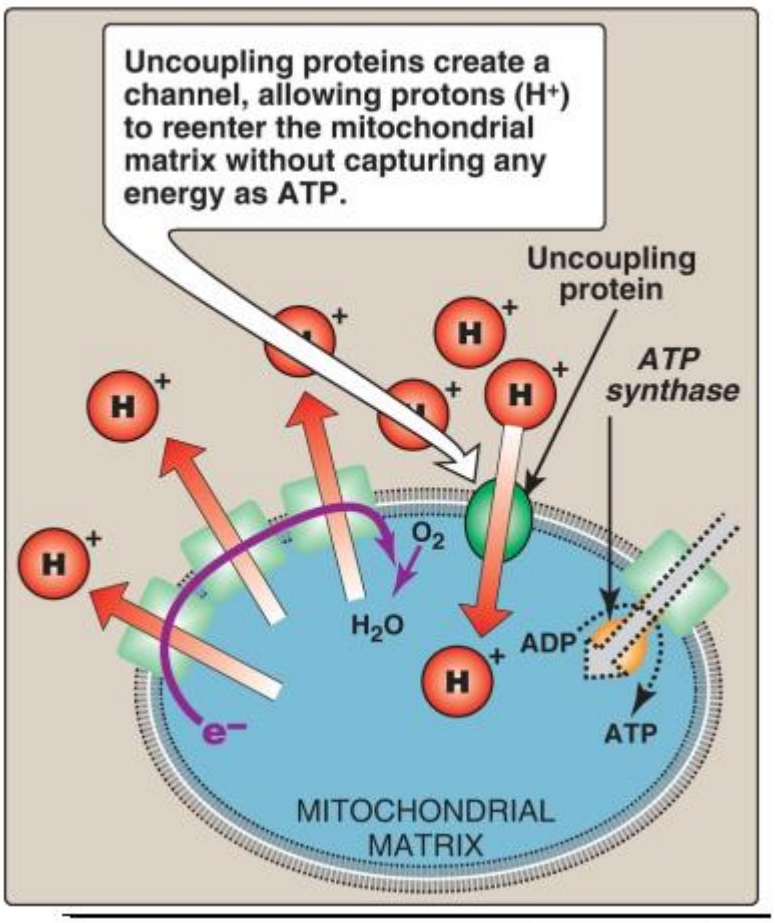



Figure: Transport of protons across the mitochondrial membrane by a uncoupling protein. ADP = adenosine diphosphate; e- = electrons.

d. Synthetic uncouplers: Electron transport and phosphorylation of ADPcan also be uncoupled by compounds that shuttle H+ across the inner mitochondrial membrane, dissipating the gradient. The classic example is 2,4-dinitrophenol, a lipophilic H+ carrier (ionophore) thatreadily diffuses through the mitochondrial membrane. Thisncoupler causes electron transport to proceed at a rapid rate withoutestablishing a H+ gradient, much as do the UCP. Again, energy isreleased as heat rather than being used to synthesize ATP. [Note: Inhigh doses, aspirin and other salicylates uncouple oxidativephosphorylation. This explains the fever that accompanies toxicoverdoses of these drugs.].

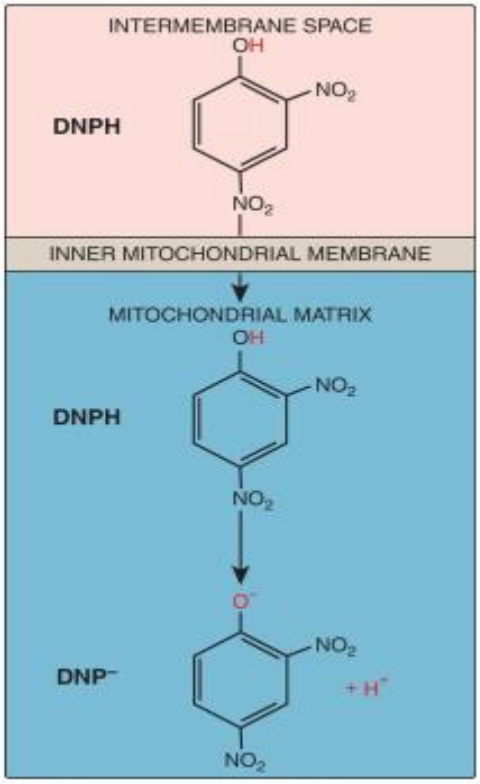



Figure: 2,4-Dinitrophenol (DNP), a proton (H+) carrier, shown in its reduced(DNPH) and oxidized (DNP–) forms.

B. Membrane transport systemsThe inner mitochondrial membrane is impermeable to most charged orhydrophilic substances. However, it contains numerous transport proteinsthat permit passage of certain molecules from the cytosol to themitochondrial matrix.

1. ATP and ADP transport: The inner membrane requires specializedcarriers to transport ADP and Pi from the cytosol (where ATP ishydrolyzed to ADP in many energy-requiring reactions) intomitochondria, where ATP can be resynthesized. An adenine nucleotideantiporter imports one ADP from the cytosol into the matrix, whileexporting one ATP from the matrix into the cytosol. A symporter cotransports Pi and H+ from the cytosol into the matrix.

2. Reducing equivalent transport: The inner mitochondrial membrane lacksan NADH transporter, and NADH produced in the cytosol (for example,in glycolysis; see p. 101) cannot directly enter the mitochondrial matrix.However, reducing equivalents of NADH are transported from thecytosol into the matrix using substrate shuttles. In the glycerol 3-phosphate shuttle, two electrons are transferred from NADHto dihydroxyacetone phosphate by cytosolic ***glycerol 3-phosphatedehydrogenase***. The glycerol 3-phosphate produced is oxidized by themitochondrial isozyme as FAD is reduced to FADH2. CoQ of the ETCoxidizes

the FADH2. Therefore, the glycerol 3-phosphate shuttle results in the synthesis of two ATP for each cytosolic NADH oxidized. This contrasts with the malate-aspartate shuttle, which producesNADH (rather than FADH2) in the mitochondrial matrix, therebyyielding three ATP for each cytosolic NADH oxidized by ***malatedehydrogenase*** as oxaloacetate is reduced to malate. A transport protein moves malate into the mitochondrial matrix.

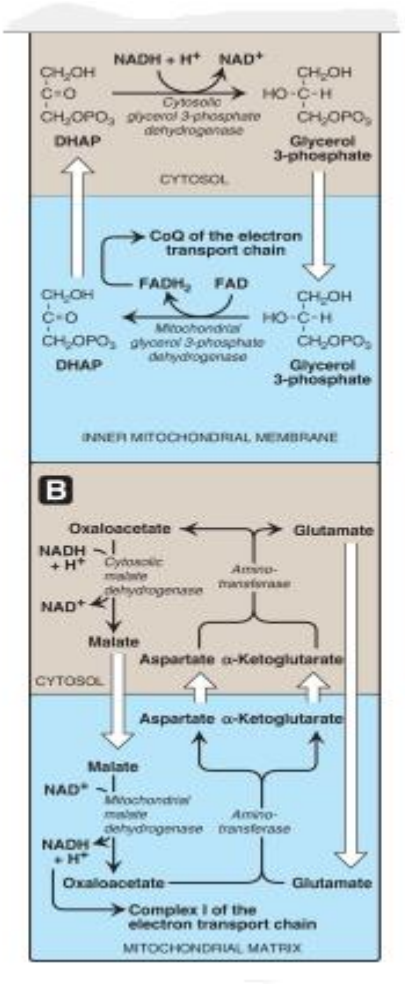



Figure: Substrate shuttles for the transport of reducing equivalents across theinner mitochondrial membrane. A. Glycerol 3-phosphate shuttle. B. Malateaspartate shuttle. DHAP = dihydroxyacetone phosphate; NAD(H) =nicotinamide adenine dinucleotide; H+ = proton; FAD(H2) = flavin adeninedinucleotide; CoQ = coenzyme Q.

C. Inherited defects in oxidative phosphorylationThirteen of the ~90 polypeptides required for oxidative phosphorylation areencoded by mtDNA and synthesized in mitochondria, whereas theremaining proteins are encoded by nuclear DNA, synthesized in the cytosol,and then transported into mitochondria. Defects in oxidativephosphorylation are more likely a result of alterations in mtDNA, which has mutation rate about 10 times greater than that of nuclear DNA.

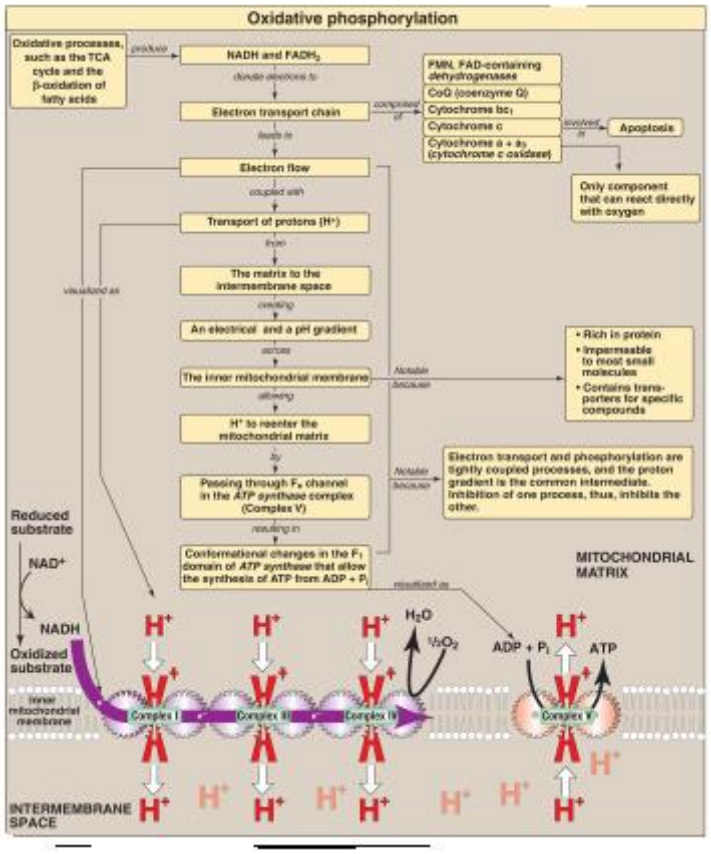
Tissueswith the greatest ATP requirement (for example, the central nervoussystem, skeletal and heart muscle, and the liver) are most affected bydefects in oxidative phosphorylation. Mutations in mtDNA are responsiblefor several diseases, including some cases of mitochondrial myopathies,and Leber hereditary optic neuropathy, a disease in which bilateral loss ofcentral vision occurs as a result of neuroretinal degeneration, includingdamage to the optic nerve. [Note: mtDNA is maternally inherited becausemitochondria from the sperm cell do not enter the fertilized egg.].

D. Mitochondria and apoptosisThe process of apoptosis (programmed cell death) may be initiated throughthe intrinsic (mitochondrial-mediated) pathway by the formation of pores inthe outer mitochondrial membrane. These pores allow cytochrome c toleave the intermembrane space and enter the cytosol. There, cytochrome c,in association with proapoptotic factors, activates a family of proteolyticenzymes (the ***caspases***), causing cleavage of key proteins and resulting inthe morphologic and biochemical changes characteristic of apoptosis.

The change in free energy (ΔG) occurring during a reaction predicts thedirection in which that reaction will spontaneously proceed. If ΔG isnegative (that is, the product has a lower free energy than the substrate),then the reaction is spontaneous as written. If ΔG is positive, then thereaction is not spontaneous. If ΔG = 0, then the reaction is in equilibrium.The ΔG of the forward reaction is equal in magnitude but opposite in signto that of the back reaction. The ΔG are additive in any sequence ofconsecutive reactions, as are the standard free energy changes (ΔG0).Therefore, reactions or processes that have a large, positive ΔG are madepossible by coupling with those that have a large, negative ΔG such as ATPhydrolysis. The reduced coenzymes nicotinamide adenine dinucleotide(NADH) and flavin adenine dinucleotide (FADH2) each donate a pair ofelectrons to a specialized set of electron carriers, consisting of flavinmononucleotide (FMN), iron-sulfur centers, coenzyme Q, and a series ofheme-containing cytochromes, collectively called the electron transportchain. This pathway is present in the inner mitochondrial membrane(impermeable to most substances) and is the final common pathway byhich electrons derived from different fuels of the body flow to oxygen(O2), which has a large, positive reduction potential (E0), reducing it towater. The terminal cytochrome, ***cytochrome c oxidase***, is the onlycytochrome able to bind O2. Electron transport results in the pumping ofprotons (H+) across the inner mitochondrial membrane from the matrix tothe intermembrane space, 10 H+ per NADH oxidized. This process createslectrical and pH gradients across the inner mitochondrial membrane. AfterH+ have been transferred to the cytosolic side of the membrane,

theyeenter the matrix by passing through the Fo H+ channel in ***ATP synthase***(Complex V), dissipating the pH and electrical gradients and causingconformational changes in the F1 β subunits of the ***synthase*** that result inthe synthesis of ATP from ADP + inorganic phosphate. Electron transportand phosphorylation are tightly coupled in oxidative phosphorylation ([OXPHOS]). Inhibition of one process inhibits the other. Theseprocesses can be uncoupled by uncoupling protein-1 of the innermitochondrial membrane of brown adipocytes and by synthetic compoundsuch as 2,4-initrophenol and aspirin, all of which dissipate the H+gradient. In uncoupled mitochondria, the energy produced by electrontransport is released as heat rather than being used to synthesize ATP.Mutations in mitochondrial DNA, which is maternally inherited, areresponsible for some cases of mitochondrial diseases such as Leberhereditary optic neuropathy. The release of cytochrome c into thecytoplasm and subsequent activation of proteolytic ***caspases*** results inapoptotic cell death. Key concept map for oxidative phosphorylation (OXPHOS). [Note:Electron (e-) flow and ATP synthesis are shown as sets of interlocking gears toemphasize coupling.] TCA = tricarboxylic acid; NAD(H) = nicotinamideadenine dinucleotide; FAD(H2) = flavin adenine dinucleotide; FMN = flavinononucleotide; ADP = adenosine diphosphate.Study QuestionsChoose the ONE best answer.6.1. 2,4-Dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, wasused as a weight-loss agent in the 1930s. Reports of fatal overdoses led toits discontinuation in 1939. Which of the following would most likely berue concerning individuals taking 2,4-DNP?A. ATP levels in the mitochondria are greater than normal.B. Body temperature is elevated as a result of hypermetabolism.C. Cyanide has no effect on electron flowD. The proton gradient across the inner mitochondrial membrane isgreater than normal.E. The rate of electron transport is abnormally low.Correct answer = B. When phosphorylation is uncoupled from electron flow, adecrease in the proton gradient across the inner mitochondrial membrane and,therefore, impaired ATP synthesis are expected. In an attempt to compensatefor this defect in energy capture, metabolism and electron flow to oxygen areincreased. This hypermetabolism will be accompanied by elevated bodytemperature because the energy in fuels is largely wasted, appearing as heat.The electron transport chain will still be inhibited by cyanide6.2. Which of the following has the strongest tendency to gain electrons?A. Coenzyme Q. Cytochrome cC. Flavin adenine dinucleotideD. Nicotinamide adenine dinucleotideE. OxygenCorrect answer = E. Oxygen is the terminal acceptor of electrons in theelectron transport chain (ETC). Electrons flow down the ETC to oxygenbecause it has the highest (most positive) reduction potential (E0). The otherchoices precede oxygen in the ETC and have lower E0 values.6.3. Explain why and how the malate-aspartate shuttle moves

nicotinamidedenine dinucleotide reducing equivalents from the cytosol to themitochondrial matrix.There is no transporter for nicotinamide adenine dinucleotide (NADH) in theinner mitochondrial membrane. However, cytoplasmic NADH can be oxidizedto NAD+ by malate dehydrogenase as oxaloacetate (OAA) is reduced tomalate. The malate is transported across the inner membrane to the matrixwhere the mitochondrial isozyme of malate dehydrogenase oxidizes it to OAAas mitochondrial NAD+ is reduced to NADH. This NADH can be oxidized byComplex I of the electron transport chain, generating three ATP through thecoupled processes of oxidative phosphorylation.6.4. Carbon monoxide (CO) binds to and inhibits Complex IV of the electrontransport chain. What effect, if any, should this respiratory inhibitor haveon phosphorylation of adenosine diphosphate (ADP) to ATP?Inhibition of electron transport by respiratory inhibitors such as CO results inan inability to maintain the proton (H+) gradient. Therefore, phosphorylation ofADP to ATP is inhibited, as are ancillary reactions such as calcium uptake bymitochondria, because they also require the H+ gradient.



Key concept map for oxidative phosphorylation (OXPHOS). [Note:Electron (e-) flow and ATP synthesis are shown as sets of interlocking gears toemphasize coupling.] TCA = tricarboxylic acid; NAD(H) = nicotinamideadenine dinucleotide; FAD(H2) = flavin adenine dinucleotide; FMN = flavinmononucleotide; ADP = adenosine diphosphate.

## CARBOHYDRATE METABOLISM AND FUNCTIONS.

Some tissues, such as the brain, red blood cells (RBC), kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for <  hours in the absence of dietary intake of carbohydrate. During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is made from noncarbohydrate precursors. The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation (that is, the change in standard free energy [ΔG] is negative). Instead, glucose is synthesized de novo by a special pathway, gluconeogenesis, which requires both mitochondrial and cytosolic enzymes. [Note: Deficiencies of gluconeogenic enzymes cause hypoglycemia.] During an overnight fast, ~ % of gluconeogenesis occurs in the liver, with the remaining ~ % occurring in the kidneys. However, during prolonged fasting, the kidneys become major glucoseproducing organs, contributing ~ % of the total glucose production. [Note: The small intestine can also make glucose.] shows the relationship of gluconeogenesis to other essential pathways of energy metabolism.

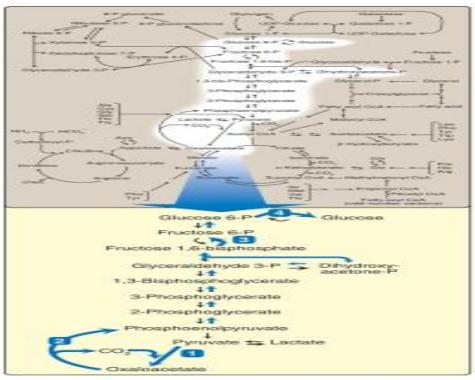



Figure: Gluconeogenesis shown as one of the essential pathways of energy metabolism. The numbered reactions are unique to gluconeogenesis. [Note: See, for a more detailed map of metabolism.] P = phosphate; CO=carbon dioxide.

SUBSTRATES Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. The most important gluconeogenic precursors are glycerol, lactate, and α-keto acids obtained from the metabolism of glucogenic amino acids. [Note: All but two amino acids (leucine and lysine) are glucogenic A. Glycerol Glycerol is released during the hydrolysis of triacylglycerols (TAG) in adipose tissue and is delivered by the blood to the liver. Glycerol is phosphorylated by *glycerol kinase* to glycerol -phosphate, which is oxidized by ***glycerol*** - *phosphate dehydrogenase* to dihydroxyacetone phosphate, an intermediate of glycolysis and gluconeogenesis. B. Lactate Lactate from anaerobic glycolysis is released into the blood by exercising skeletal muscle and by cells that lack mitochondria such as RBC. In the Cori cycle, this lactate is taken up by the liver and oxidized to pyruvate that is converted to glucose, which is released back into the circulation.

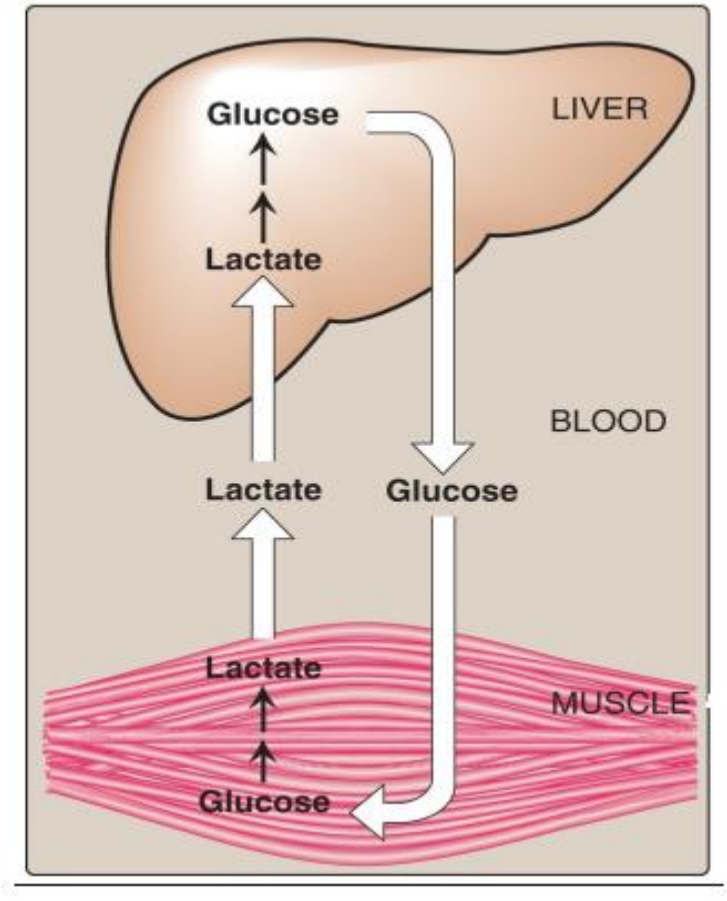



Figure: The intertissue Cori cycle links gluconeogenesis with glycolysis. [Note: Diffusion of lactate and glucose across membranes is facilitated by transport proteins.]

C. Amino acids Amino acids produced by hydrolysis of tissue proteins are the major sources of glucose during a fast. Their metabolism generates α-keto

acids, such as pyruvate that is converted to glucose, or α-ketoglutarate that can enter the tricarboxylic acid (TCA) cycle and form oxaloacetate (OAA), a direct precursor of phosphoenolpyruvate (PEP). [Note: Acetyl coenzyme A (CoA) and compounds that give rise only to acetyl CoA (for example, acetoacetate, lysine, and leucine) cannot give rise to a net synthesis of glucose. This is because of the irreversible nature of the *pyruvate dehydrogenase complex* (*PDHC*), which converts pyruvate to acetyl CoA These compounds give rise instead to ketone bodies and are termed ketogenic.]

**REACTIONS** Seven glycolytic reactions are reversible and are used in the synthesis of glucose from lactate or pyruvate. However, three glycolytic reactions are irreversible and must be circumvented by four alternate reactions that energetically favor the synthesis of glucose. These irreversible reactions, which together are unique to gluconeogenesis, are described below. A. Pyruvate carboxylation The first roadblock to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by *pyruvate kinase (PK).* In gluconeogenesis, pyruvate is carboxylated by *pyruvate carboxylase (PC)* to OAA, which is converted to PEP by *PEPcarboxykinase* (*PEPCK*)

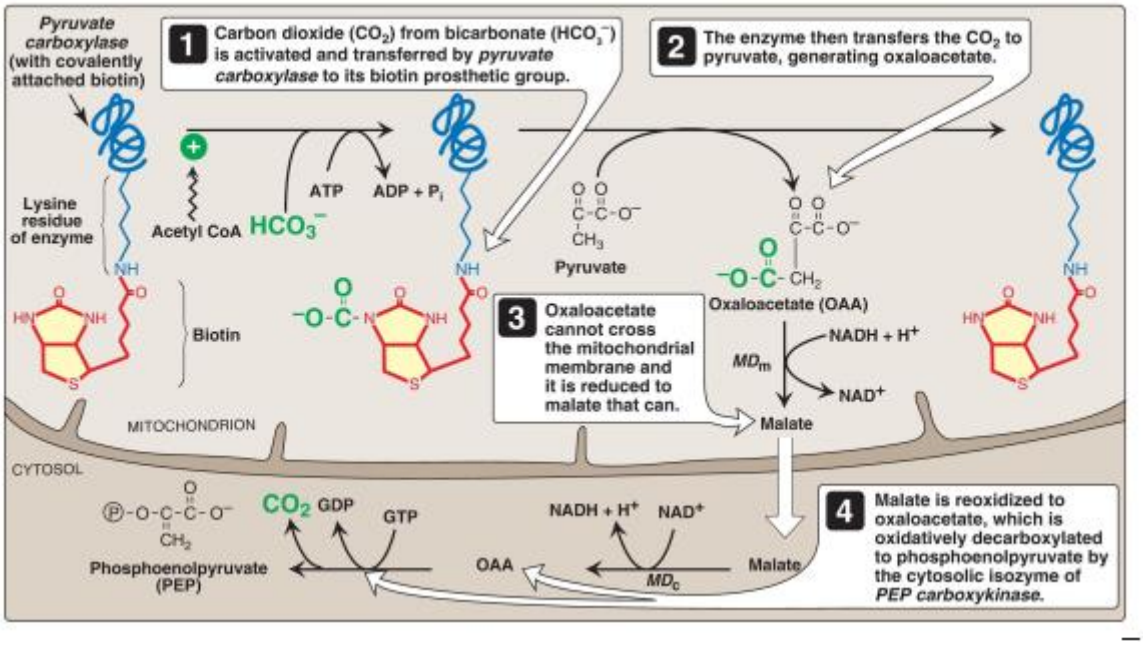



Figure: PEP synthesis in the cytosol. [Note: The process moves nicotinamide adenine dinucleotide (NADH) reducing equivalents required for gluconeogenesis out of mitochondria into the cytosol.] *MD*m and *MD*c = mitochondrial and cytosolic isozymes of *malate dehydrogenase*; GTP and GDP= guanosine tri- and diphosphates; ADP = adenosine diphosphate.

1.Biotin: ***PC*** requires the coenzyme biotin covalently bound to the ε-amino group of a lysine residue in the enzyme ATP hydrolysis drives formation of an enzyme–biotin–carbon dioxide (CO) intermediate, which then carboxylates pyruvate to form OAA. [Note: HCO - provides the CO.] The ***PC*** reaction occurs in the mitochondria of liver and kidney cells and has two purposes: to allow production of PEP, an important substrate for gluconeogenesis, and to provide OAA that can replenish the TCA cycle intermediates that may become depleted. Muscle cells also contain ***PC*** but use the OAA product only for the replenishment (anaplerotic) purpose and do not synthesize glucose. [Note: Pyruvate carrier protein moves pyruvate from the cytosol into mitochondria.]

***PC*** **is one of several** ***carboxylases*** **that require biotin. Others include** ***acetyl CoA carboxylase, propionyl CoA carboxylase,*** **and** ***methylcrotonyl CoA carboxylase***

2.Allosteric regulation: ***PC*** is allosterically activated by acetyl CoA. Elevated levels of acetyl CoA in mitochondria signal a metabolic state in which increased synthesis of OAA is required. For example, this occurs during fasting, when OAA is used for gluconeogenesis in the liver and kidneys. Conversely, at low levels of acetyl CoA, ***PC*** is largely inactive, and pyruvate is primarily oxidized by the ***PDHC*** to acetyl CoA that can be further oxidized by the TCA cycle.

**B. Oxaloacetate transport** to the cytosol gluconeogenesis to continue, OAA must be converted to PEP by ***PEPCK***. PEP production in the cytosol requires transport of OAA out of mitochondria. However, there is no OAA transporter in the inner mitochondrial membrane, and OAA is first reduced to malate by mitochondrial ***malate dehydrogenase*** (***MD***). Malate is transported into the cytosol and reoxidized to OAA by cytosolic ***MD*** as nicotinamide adenine dinucleotide (NAD+) is reduced to NADH. The NADH is used in the reduction of, -bisphosphoglycerate to glyceraldehyde -phosphate by ***glyceraldehyde -phosphate dehydrogenase***, a reaction common to glycolysis and gluconeogenesis. [Note: When abundant, lactate is oxidized to pyruvate as NAD+ is reduced. The pyruvate is transported into mitochondria and carboxylated by ***PC*** to OAA, which can be converted to PEP by the mitochondrial isozyme of ***PEPCK***. PEP is transported to the cytosol. OAA can also be converted to aspartate that is transported into the cytosol.].

**C. Cytosolic oxaloacetate decarboxylation** OAA is decarboxylated and phosphorylated to PEP in the cytosol by ***PEPCK***. The reaction is driven by

hydrolysis of guanosine triphosphate ([GTP] The combined actions of ***PC*** and ***PEPCK*** provide an energetically favorable pathway from pyruvate to PEP. PEP is then acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose, -bisphosphate. The pairing of carboxylation with decarboxylation drives reactions that would otherwise be energetically unfavorable. This strategy is also used in fatty acid (FA) synthesis

**D. Fructose -bisphosphate dephosphorylation** Hydrolysis of fructose -bisphosphate by ***fructose, -bisphosphatase***, found in the liver and kidneys, bypasses the irreversible ***phosphofructokinase-*** (***PFK-***) reaction of glycolysis and provides an energetically favorable pathway for the formation of fructose-phosphate. This reaction is an important regulatory site of gluconeogenesis.

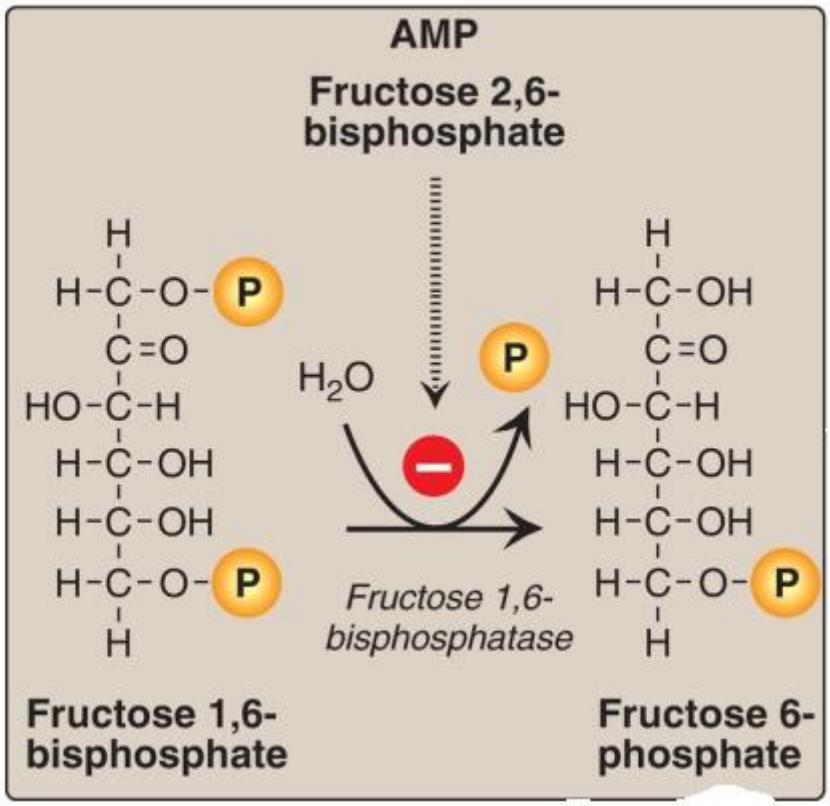



Figure: Dephosphorylation of fructose, -bisphosphate.AMP = adenosine monophosphate; = phosphate.

1.Regulation by intracellular energy levels: ***Fructose, -bisphosphatase*** is inhibited by a rise in the adenosine monophosphate (AMP)/ATP ratio, which signals a low-energy state in the cell. Conversely, low AMP and high ATP levels stimulate gluconeogenesis, an energy-requiring pathway.

2.Regulation by fructose, -bisphosphate: ***Fructose, - bisphosphatase*** is inhibited by fructose, -bisphosphate, an allosteric effector whose concentration is influenced by the insulin/glucagon ratio. When glucagon is high, the effector is not made by hepatic ***PFK-***, and thus, the ***phosphatase*** is active. [Note: The signals that inhibit (low energy, high fructose, -bisphosphate) or activate (high energy, low fructose, -bisphosphate) gluconeogenesis have the opposite effect on glycolysis, providing reciprocal control of the pathways that synthesize and oxidize glucose.

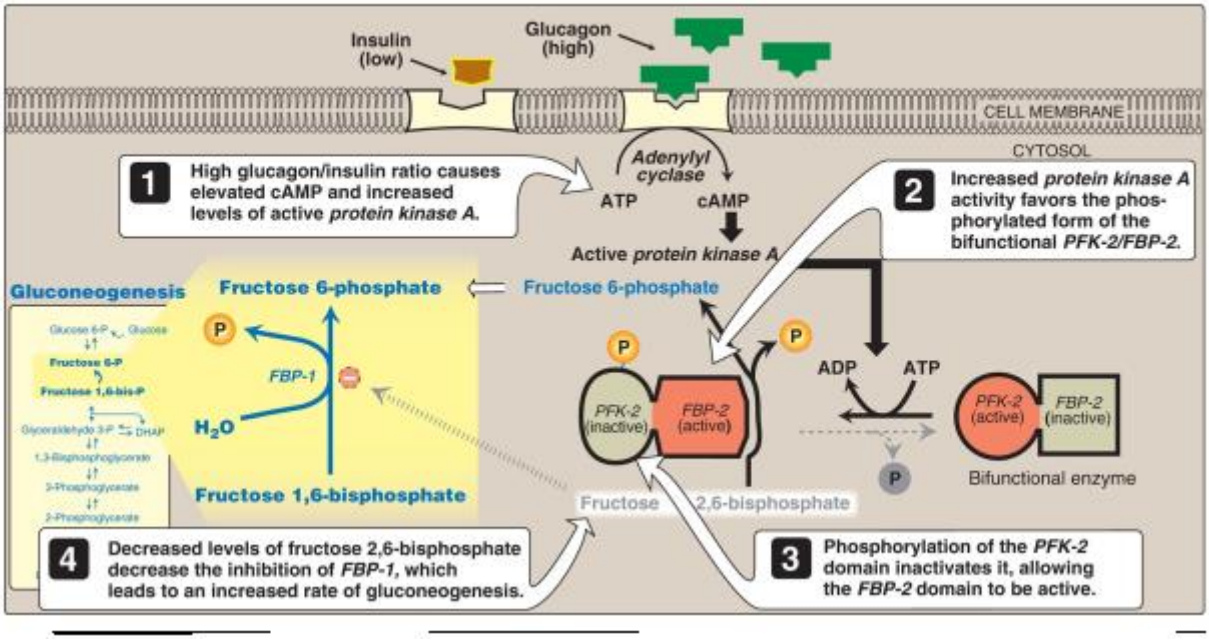



Figura: Effect of elevated glucagon on the intracellular concentration of fructose -bisphosphate in the liver. AMP and ADP = adenosine mono- and diphosphates; c AMP = cyclic AMP; ***PFK-*** = ***phosphofructokinase- FBP-*** = ***fructose, -bisphosphatase***; ***FBP-*** = ***fructose, -bisphosphatase***; and = phosphate.

E. Glucose-phosphate dephosphorylation Glucose-phosphate hydrolysis by ***glucose-phosphatase*** bypasses the irreversible ***hexokinase/glucokinase*** reaction and provides an energetically favorable pathway for the formation of free glucose. The liver is the primary organ that produces free glucose from glucose-phosphate. This process requires a complex of two proteins found only in gluconeogenic tissue: glucose-phosphate translocase, which transports glucose-phosphate across the endoplasmic reticular (ER) membrane, and ***glucose -phosphatase***, which removes the phosphate, producing free glucose. [Note: These ER membrane proteins are also required for the final step of glycogen degradation. Glycogen storage diseases Ia and Ib, caused by deficiencies in the ***phosphatase*** and the translocase, respectively, are characterized by severe fasting hypoglycemia,

because free glucose is unable to be produced from either gluconeogenesis or glycogenolysis.] Specific transporters are responsible for moving the free glucose into the cytosol and then into blood.

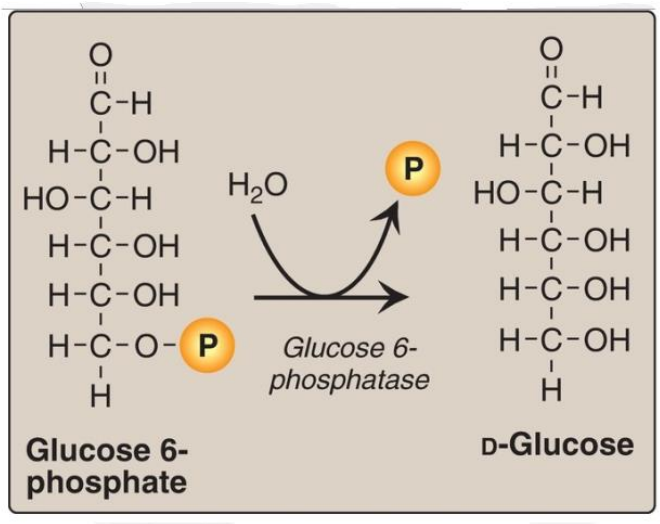



Figure: Dephosphorylation of glucos-phosphate allows release of free glucose from gluconeogenic tissues (primarily the liver) into blood. = phosphate.

F. Summary of the reactions of glycolysis and gluconeogenesis Of the reactions required to convert pyruvate to free glucose, are catalyzed by reversible glycolytic enzymes. The irreversible reactions (catalyzed by ***hexokinase/glucokinase***, ***PFK-***, and ***PK***) are circumvented by reactions catalyzed by ***glucose-phosphatase***, ***fructose , -bisphosphatase***, ***PC, and PEPCK.*** In gluconeogenesis, the equilibria of the reversible glycolytic reactions are pushed toward glucose synthesis as a result of the essentially irreversible formation of PEP, fructose-phosphate, and glucose by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from two pyruvate molecules couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of one glucose molecule]

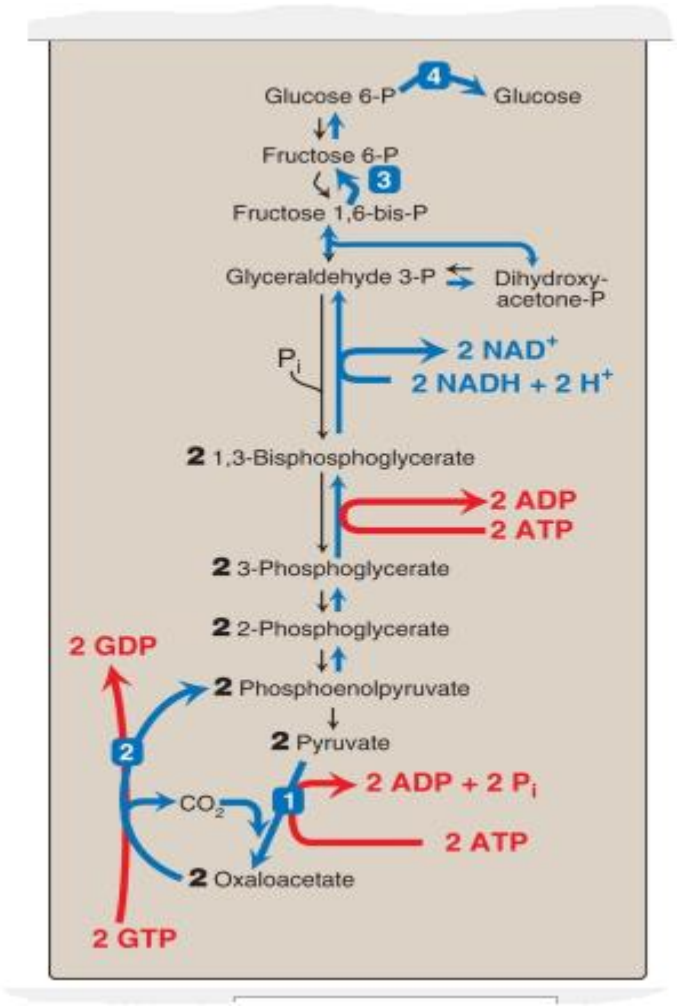



Figure: Summary of the reactions of glycolysis and gluconeogenesis, showing the energy requirements of gluconeogenesis. The numbered reactions are unique to gluconeogenesis. P = phosphate; GDP and GTP = guanosine diand triphosphates; NAD(H) = nicotinamide adenine dinucleotide; ADP =adenosine diphosphate.

**REGULATION** The moment-to-moment regulation of gluconeogenesis is determined primarily by the circulating level of glucagon and by the availability of gluconeogenic substrates. In addition, slow adaptive changes in enzyme amount result from an alteration in the rate of enzyme synthesis or degradation or both. [Note: Hormonal control of the glucoregulatory system is presented in]

**Glucagon** This peptide hormone from pancreatic islet α cells stimulates gluconeogenesis by three mechanisms.

1.Changes in allosteric effectors: Glucagon lowers hepatic fructose -bisphosphate, resulting in ***fructose, -bisphosphatase*** activation and ***PFK-*** inhibition, thereby favoring gluconeogenesis over glycolysis. [Note: for the role of fructose, -bisphosphate in glycolysis regulation.].

2.Covalent modification of enzyme activity: Glucagon binds its G protein–coupled receptor and, via an elevation in cyclic AMP (c AMP) level and ***c AMP-dependent protein kinase A*** activity, stimulates the conversion of hepatic ***PK*** to its inactive (phosphorylated) form. This decreases PEP conversion to pyruvate, which has the effect of diverting PEP to gluconeogenesis.

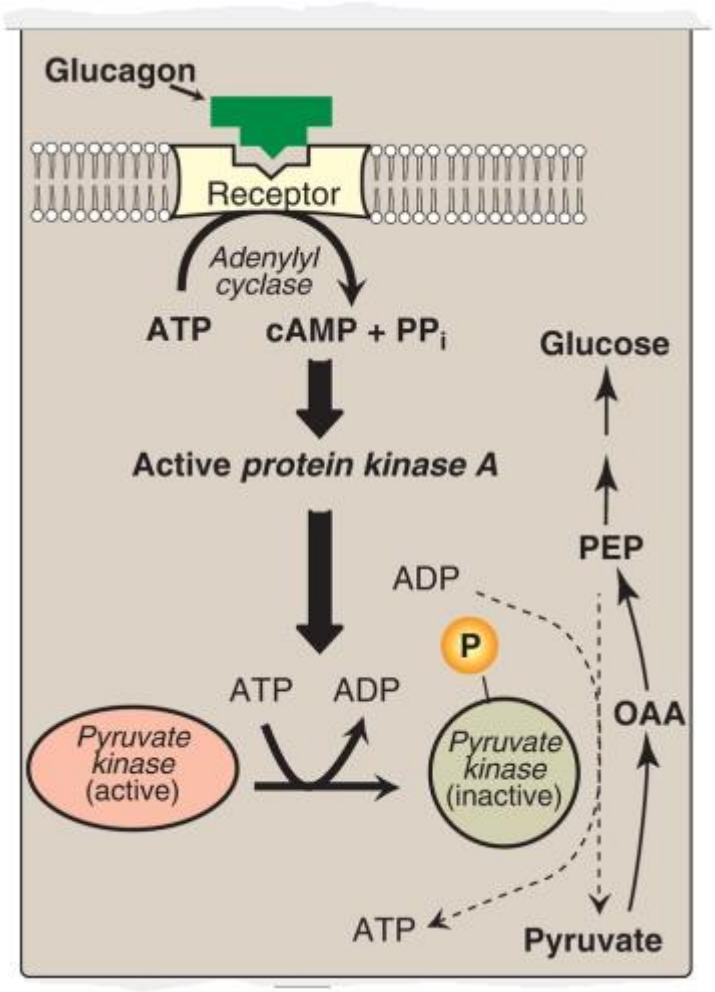



Figure: Covalent modification of ***pyruvate kinase*** results in inactivation of the enzyme. [Note: Only the hepatic isozyme is subject to covalent regulation.] OAA = oxaloacetate; PEP = phosphoenolpyruvate; P Pi = pyrophosphate; =phosphate; AMP and ADP = adenosine mono- and diphosphates; c AMP = cyclic AMP.

3.Induction of enzyme synthesis: Glucagon increases transcription of the gene for ***PEPCK*** via the transcription factor c AMP response element–binding (CREB) protein, thereby increasing the availability of this enzyme as levels of its substrate rise during fasting. [Note: Cortisol (a glucocorticoid) also increases expression of the gene, whereas insulin decreases expression.]

**B. Substrate availability** The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of glucose

synthesis. Decreased insulin levels favor mobilization of amino acids from muscle protein to provide the carbon skeletons for gluconeogenesis. The ATP and NADH coenzymes required for gluconeogenesis are primarily provided by FA oxidation.

**C. Allosteric activation by acetyl CoA**. Allosteric activation of hepatic ***PC*** by acetyl CoA occurs during fasting. As a result of increased TAG hydrolysis in adipose tissue, the liver is flooded with FA. The rate of formation of acetyl CoA by β-oxidation of these FA exceeds the capacity of the liver to oxidize it to CO and water. As a result, acetyl CoA accumulates and activates ***PC***. [Note: Acetyl CoA inhibits the ***PDHC*** (by activating ***PDH kinase***; see p. Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle.]

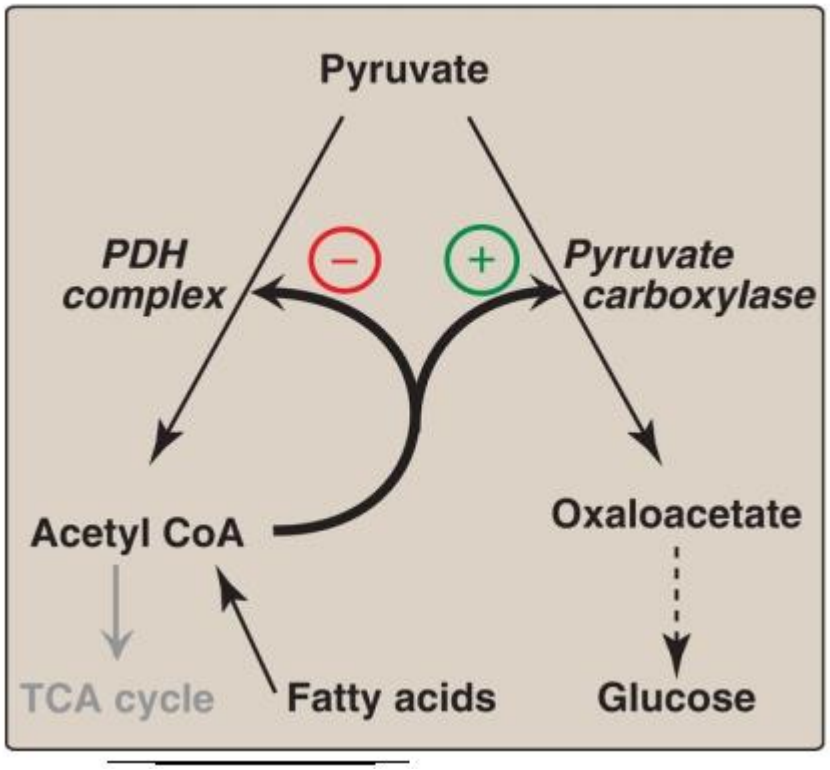



Figure: Acetyl coenzyme A (CoA) diverts pyruvate away from oxidation and toward gluconeogenesis. ***PDH*** = ***pyruvate dehydrogenase***; TCA =tricarboxylic acid.

**D. Allosteric inhibition by AMP** ***Fructose, -bisphosphatase*** is inhibited by AMP, a compound that activates ***PFK-*** This results in reciprocal regulation of glycolysis and gluconeogenesis seen previously with fructose, -bisphosphate. [Note: Thus, elevated AMP stimulates energy-producing pathways and inhibits energy-requiring ones.]

**CHAPTER SUMMARY** Gluconeogenic precursors include glycerol released during triacylglycerol hydrolysis in adipose tissue, lactate released by

cells that lack mitochondria and by exercising skeletal muscle, and α-keto acids (for example, α-ketoglutarate and pyruvate) derived from glucogenic amino acid metabolism. Seven of the reactions of glycolysis are reversible and are used for gluconeogenesis in the liver and kidneys. Three reactions, catalyzed by ***pyruvate kinase***, ***phosphofructokinase***, and ***glucokinase*/*hexokinase***, are physiologically irreversible and must be circumvented. Pyruvate is converted to oxaloacetate and then to phosphoenolpyruvate (PEP) by ***pyruvate carboxylase*** (***P C***) and ***PEPcarboxykinase*** (***PEPCK*** ). ***PC*** requires biotin and ATP and is allosterically activated by acetyl coenzyme ***PEPCK*** requires guanosine triphosphate. Transcription of its gene is increased by glucagon and cortisol and decreased by insulin. Fructose, -bisphosphate is converted to fructose-phosphate by ***fructose, -bisphosphatase***. This enzyme is inhibited by a high adenosine monophosphate (AMP)/ATP ratio. It is also inhibited by fructose, -bisphosphate, the primary allosteric activator of glycolysis. Glucose-phosphate is dephosphorylated to glucose by ***glucose-phosphatase***. This enzyme of the endoplasmic reticular membrane catalyzes the final step in gluconeogenesis and in glycogen degradation. Its deficiency results in severe, fasting hypoglycemia.

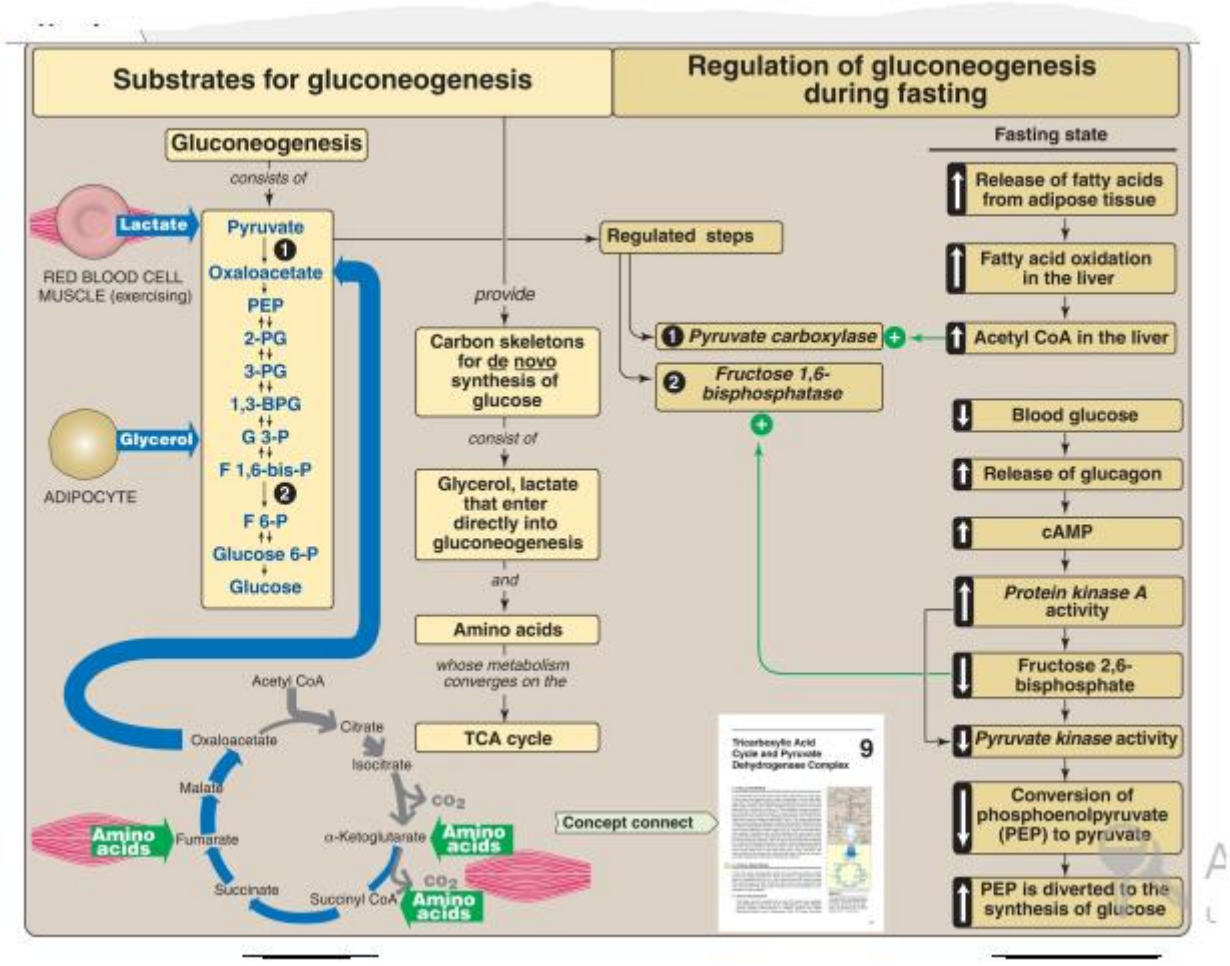


Figure: Key concept map for gluconeogenesis. TCA = tricarboxylic acid. CoA = coenzyme A; cAMP = cyclic adenosine monophosphate; P = phosphate; (B)PG = (bis)phosphoglycerate; G = glyceraldehyde; F = fructose; CO= carbon dioxide.

**Glucose catabolism and gluconeogenesis**.

A constant source of blood glucose is an absolute requirement for human life.Glucose is the greatly preferred energy source for the brain and the requiredenergy source for cells with few or no mitochondria such as mature red bloodcells. Glucose is also essential as an energy source for exercising muscle, whereit is the substrate for anaerobic glycolysis. Blood glucose can be obtained fromthree primary sources: the diet, glycogen degradation, and gluconeogenesis.Dietary intake of glucose and glucose precursors, such as starch (apolysaccharide), disaccharides, and monosaccharides, is sporadic and, dependingon the diet, is not always a reliable source of blood glucose. In contrast,gluconeogenesis can provide sustained synthesis of glucose, but it issomewhat slow in responding to a falling blood glucose level. Therefore, thebody has developed mechanisms for storing a supply of glucose in a rapidlymobilized form, namely, glycogen. In the absence of a dietary source of glucose,this sugar is rapidly released into the blood from liver glycogen. Similarly,muscle glycogen is extensively degraded in exercising muscle to provide thattissue with an important energy source. When glycogen stores are depleted,specific tissues synthesize glucose de novo, using glycerol, lactate, pyruvate, andamino acids as carbon sources for gluconeogenesis shows the reactions of glycogen synthesis and degradation as part of theessential pathways of energy metabolism.

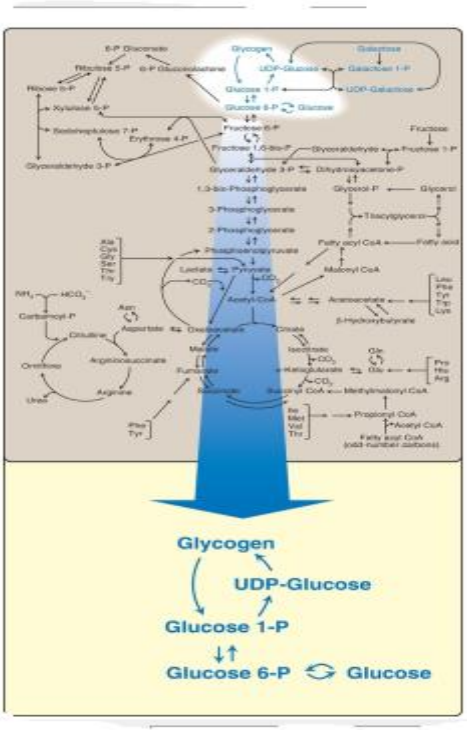



Figure: Glycogen synthesis and degradation shown as a part of the essentialpathways of energy metabolism. [Note: for a more detailedmap of metabolism.] P = phosphate; UDP = uridine diphosphate.

STRUCTURE AND FUNCTIONThe main stores of glycogen are found in skeletal muscle and liver, althoughmost other cells store small amounts of glycogen for their own use. The functionof muscle glycogen is to serve as a fuel reserve for the synthesis of ATP duringmuscle contraction. That of liver glycogen is to maintain the blood glucoseconcentration, particularly during the early stages of a fast. [Note: Liver glycogen can maintain blood glucose for <24 hours.]

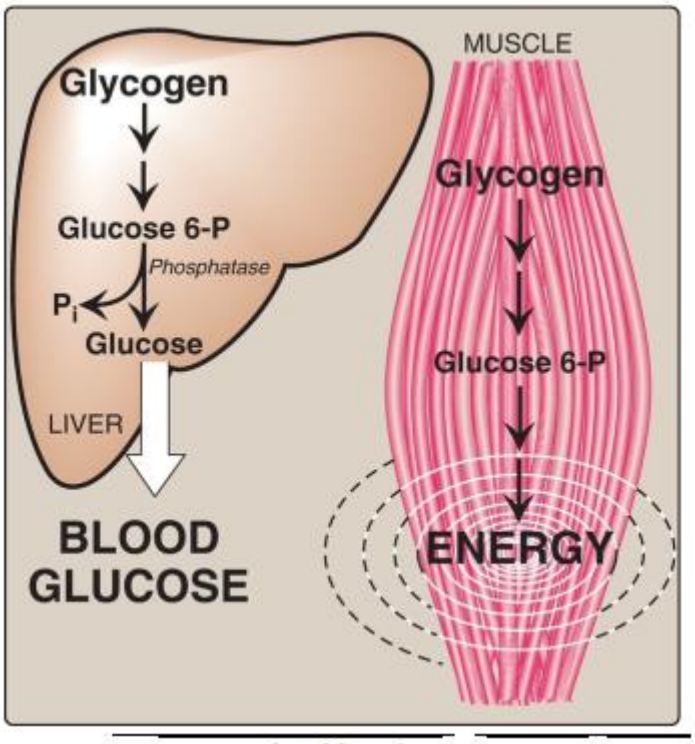



Figure: Functions of muscle and liver glycogen. [Note: The presence of***glucose 6-phosphatase*** in liver allows release of glucose into blood.] P =phosphate; Pi = inorganic phosphate.

**A. Amounts in liver and muscle**Approximately 400 g of glycogen make up 1%–2% of the fresh weight ofresting muscle, and ~100 g of glycogen make up to 10% of the fresh weightof a well-fed adult liver. What limits the production of glycogen at theselevels is not clear. However, in some glycogen storage diseases (GSD), the amount of glycogen in the liver and/or muscle can besignificantly higher. [Note: In the body, muscle mass is greater than livermass. Consequently, most of the body's glycogen is found in skeletalmuscle.]

**B. Structure**Glycogen is a branched-chain polysaccharide made exclusively from α-Dglucose. The primary glycosidic bond is an α(1→ 4) linkage. After anaverage of 8–14 glucosyl residues, there is a branch containing an α(1→ 6)linkage. A single glycogen molecule can contain up to 55,000glucosyl residues. These polymers of glucose exist as large, spherical,cytoplasmic granules (particles) that also contain most of the enzymesnecessary for glycogen synthesis and degradation.

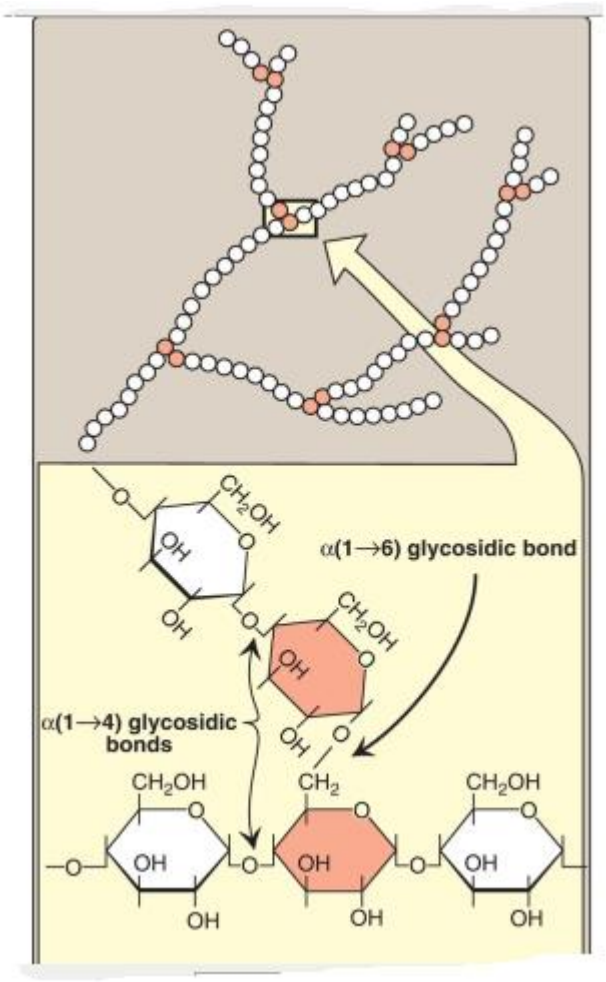



Figure 11.3 Branched structure of glycogen, showing α(1→ 4) and α(1→ 6)glycosidic bonds.

**C. Glycogen store fluctuation**Liver glycogen stores increase during the well-fed state (see p. 323) and aredepleted during a fast. Muscle glycogen is not affected by shortperiods of fasting (a few days) and is only moderately decreased inprolonged fasting (weeks). Muscle glycogen is synthesized to replenishmuscle stores after they have been depleted following strenuous exercise.[Note: Glycogen synthesis and degradation go on continuously. Thedifference between the rates of these two processes determines the levels ofstored glycogen during specific physiologic states.].

**SYNTHESIS (GLYCOGENESIS)**Glycogen is synthesized from molecules of α-D-glucose. The process occurs inthe cytosol and requires energy supplied by ATP (for the phosphorylation ofglucose) and uridine triphosphate (UTP).A. Uridine diphosphate glucose synthesisα-D-Glucose attached to uridine diphosphate (UDP) is the source of all theglucosyl residues that are added to the growing glycogen molecule. UDP glucose is synthesized from glucose 1-phosphate and UTP by***UDP–glucose pyrophosphorylase***. Pyrophosphate (PPi), thesecond product of the reaction, is hydrolyzed to two inorganic phosphates(Pi) by ***pyrophosphatase***. The hydrolysis is exergonic, insuring that the***UDP–glucose pyrophosphorylase***reaction proceeds in the direction ofUDP-glucose production. [Note: Glucose 1-phosphate is generated fromglucose 6-phosphate by

***phosphoglucomutase.*** Glucose 1,6-bisphosphate isan obligatory intermediate in this reversible reaction.]

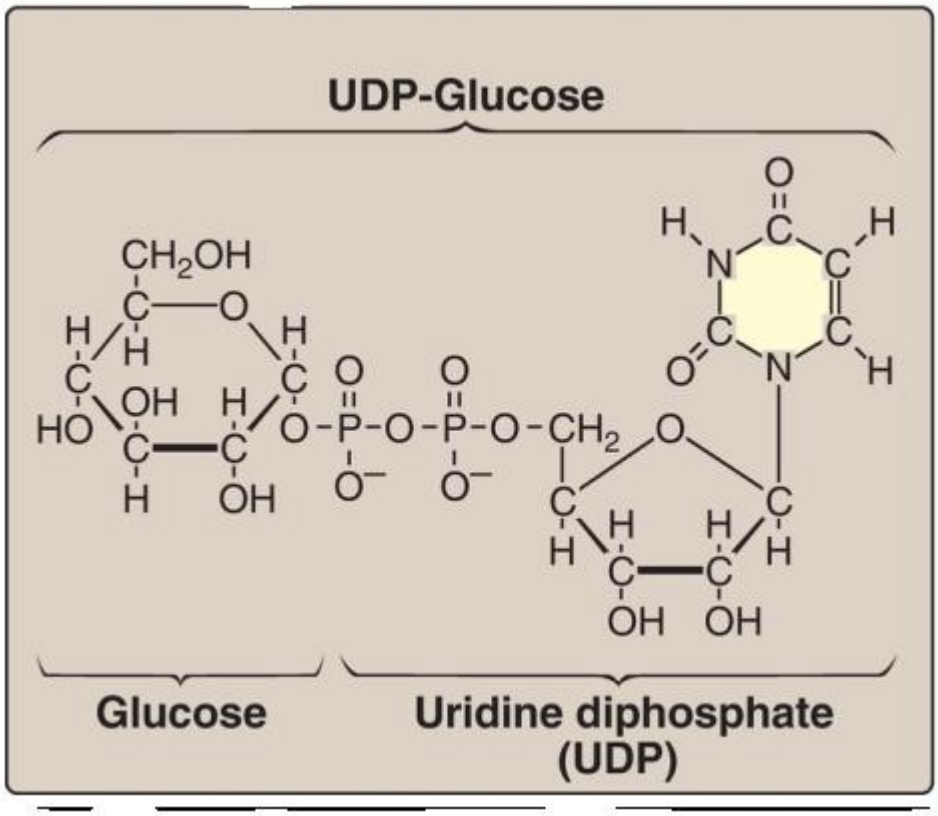



Figure: The structure of UDP-glucose, a nucleotide sugar.

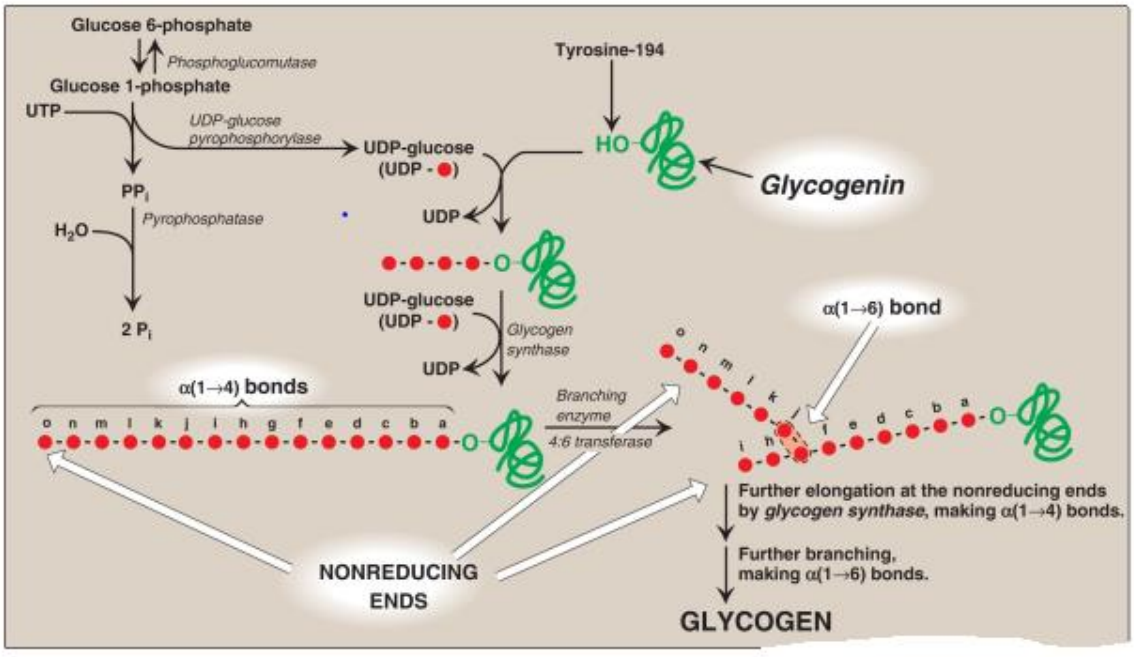



Figure 11.5 Glycogen synthesis. UDP and UTP = uridine di- and triphosphates;PPi = pyrophosphate; Pi = inorganic phosphate.

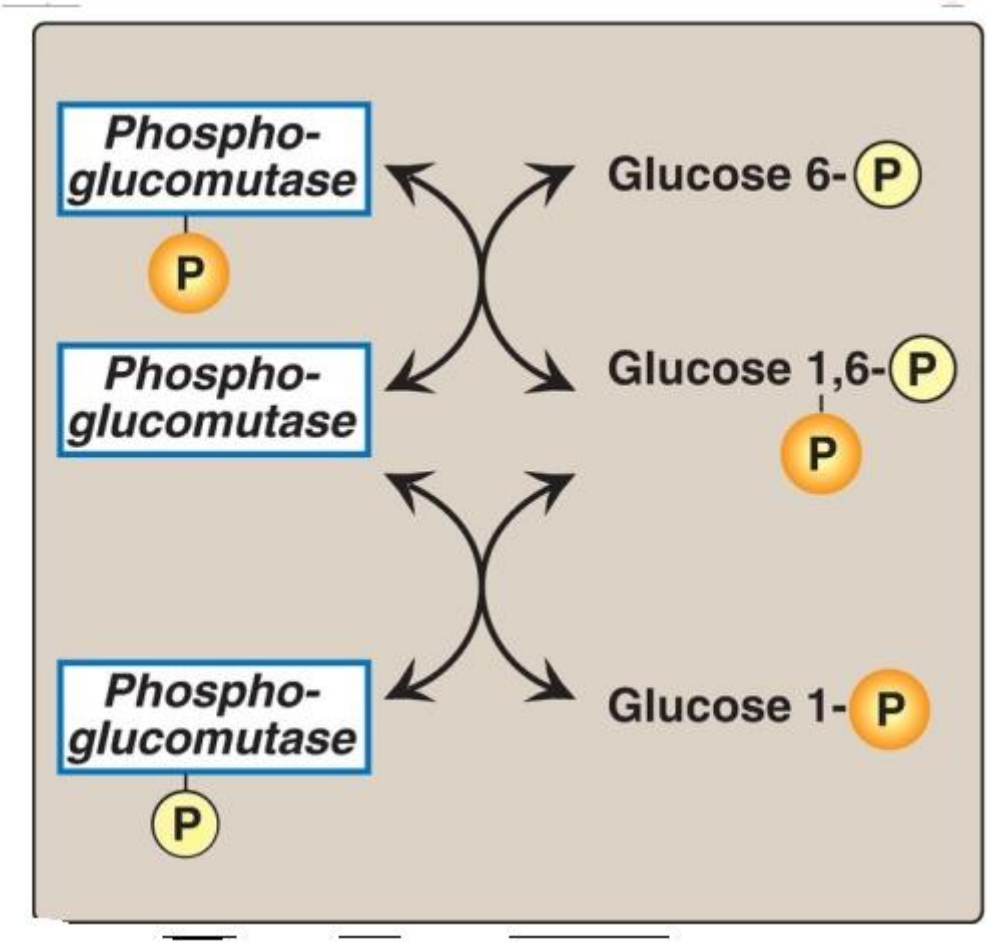



Figure: Interconversion of glucose 6-phosphate and glucose 1-phosphate by***phosphoglucomutase***. and = phosphate.

**B. Primer requirement and synthesis*Glycogen synthase*** makes the α(1→ 4) linkages in glycogen. This enzymecannot initiate chain synthesis using free glucose as an acceptor of amolecule of glucose from UDP-glucose. Instead, it can only elongatealready existing chains of glucose and, therefore, requires a primer. Afragment of glycogen can serve as a primer. In the absence of a fragment,the homodimeric protein ***glycogenin***can serve as an acceptor of glucosefrom UDP-glucose. The side-chain hydroxyl group oftyrosine-194 in the protein is the site at which the initial glucosyl unit isattached. Because the reaction is catalyzed by ***glycogenin***itself viaautoglucosylation, ***glycogenin***is an enzyme. ***Glycogenin***then catalyzes thetransfer of at least four molecules of glucose from UDP-glucose, producinga short, α(1→ 4)-linked glucosyl chain. This short chain serves as a primerthat is able to be elongated by ***glycogen synthase***, which is recruited by***glycogenin***, as described in C. below. [Note: ***Glycogenin***stays associatedwith and forms the core of a glycogen granule.]C. Elongation by glycogen synthaseElongation of a glycogen chain involves the transfer of glucose from UDPglucose to the nonreducing end of the growing chain, forming a newglycosidic bond between the anomeric hydroxyl group of carbon 1 of theactivated glucose and carbon 4 of the accepting glucosyl residue. [Note: The nonreducing end of a carbohydrate chain is one in whichthe anomeric carbon of the terminal sugar is linked by a glycosidic bond toanother molecule, making the

terminal sugar nonreducing.] Theenzyme responsible for making the α(1→ 4) linkages in glycogen is***glycogen synthase***. [Note: The UDP released when the new α(1→ 4)glycosidic bond is made can be phosphorylated to UTP by ***nucleosidediphosphate kinase*** (UDP + ATP ⇄UTP + ADP.]D. Branch formationIf no other synthetic enzyme acted on the chain, the resulting structurewould be a linear (unbranched) chain of glucosyl residues attached byα(1→ 4) linkages. Such a compound is found in plant tissues and is calledamylose. In contrast, glycogen has branches located, on average, eightglucosyl residues apart, resulting in a highly branched, tree-like structure that is far more soluble than the unbranched amylose.Branching also increases the number of nonreducing ends to which newglucosyl residues can be added (and also, as described in IV. below, fromwhich these residues can be removed), thereby greatly accelerating the rateat which glycogen synthesis can occur and dramatically increasing the sizeof the glycogen molecule.

1. Branch synthesis: Branches are made by the action of the branchingenzyme, ***amylo-α(1→ 4)→ α(1→ 6)-transglycosylase***. This enzymeremoves a set of six to eight glucosyl residues from the nonreducing endof the glycogen chain, breaking an α(1→ 4) bond to another residue onthe chain, and attaches it to a nonterminal glucosyl residue by an α(1→ 6)linkage, thus functioning as a ***4:6 transferase***. The resulting new,nonreducing end, as well as the old nonreducing endfrom which the six to eight residues were removed,can now be further elongated by ***glycogen synthase***.

2. Additional branch synthesis: After elongation of these two ends has beenaccomplished, their terminal six to eight glucosyl residues can beremoved and used to make additional branches.

**DEGRADATION (GLYCOGENOLYSIS**)The degradative pathway that mobilizes stored glycogen in liver and skeletalmuscle is not a reversal of the synthetic reactions. Instead, a separate set ofcytosolic enzymes is required. When glycogen is degraded, the primary productis glucose 1-phosphate, obtained by breaking α(1→ 4) glycosidic bonds. Inaddition, free glucose is released from each α(1→ 6)–linked glucosyl residue(branch point).

Chain shortening***Glycogen phosphorylase*** sequentially cleaves the α(1→ 4) glycosidic bondsbetween the glucosyl residues at the nonreducing ends of the glycogenchains by simple phosphorolysis (producing glucose 1-phosphate) until fourglucosyl units remain on each chain at a branch point. Theresulting structure is called a limit dextrin, and ***phosphorylase*** cannotdegrade it any further. [Note: ***Phosphorylase*** requires pyridoxalphosphate (a derivative of vitamin B6; as a coenzyme.]

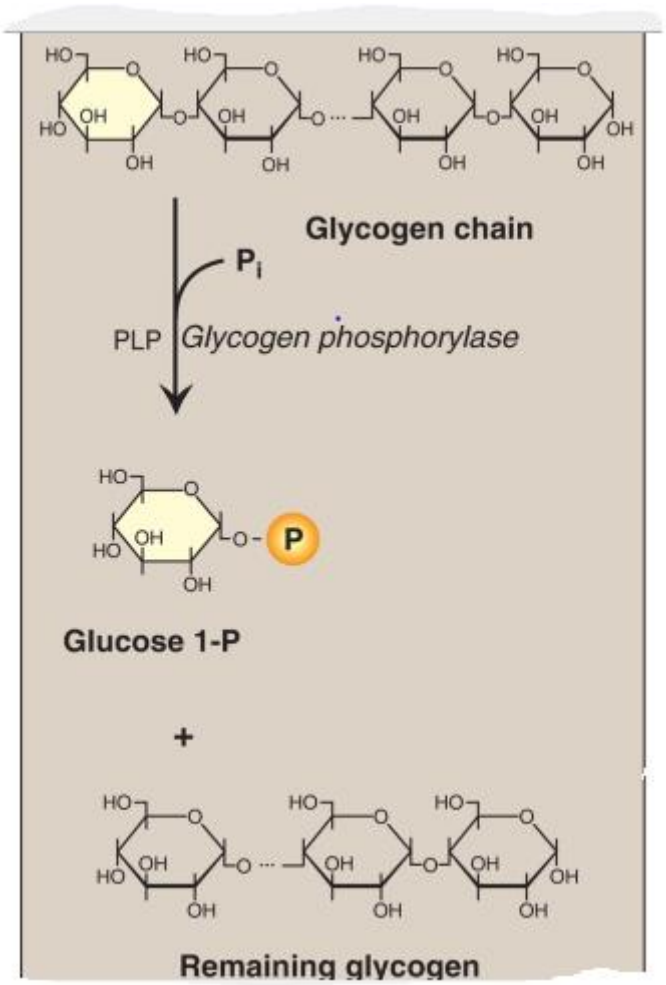



Figure 11.7 Cleavage of an α(1→ 4)-glycosidic bond. PLP = pyridoxalphosphate; Pi = inorganic phosphate; = phosphate.

B. Branch removalBranches are removed by the two enzymic activities of a single bifunctionalprotein, the debranching enzyme. First, ***oligo-α(1→ 4)→ α(1→ 4)-glucantransferase***activity removes the outer three ofthe four glucosyl residues remaining at a branch. It next transfers them tothe nonreducing end of another chain, lengthening it accordingly. Thus, anα(1→ 4) bond is broken and an α(1→ 4) bond is made, and the enzymefunctions as a ***4:4 transferase***. Next, the remaining glucose residue attachedin an α(1→ 6) linkage is removed hydrolytically by ***amylo-α(1→ 6)-glucosidase*** activity, releasing free (nonphosphorylated) glucose. Theglucosyl chain is now available again for degradation by ***glycogenphosphorylase*** until four glucosyl units in the next branch are reached.

**C. Glucose 1-phosphate isomerization to glucose 6-phosphate**Glucose 1-phosphate, produced by ***glycogen phosphorylase***, is isomerizedin the cytosol to glucose 6-phosphate by ***phosphoglucomutase***. In the liver, glucose 6-phosphate is transported into the endoplasmicreticulum (ER) by glucose 6-phosphate translocase. There, it isdephosphorylated to glucose by ***glucose 6-phosphatase*** (the same enzymeused in the last step of gluconeogenesis; see p. 121). The glucose is thentransported from the ER to the cytosol. Hepatocytes release glycogenderived glucose into the blood to help maintain blood glucose levels untilthe gluconeogenic

pathway is actively producing glucose. [Note: Musclelacks ***glucose 6-phosphatase***. Consequently, glucose 6-phosphate cannot bedephosphorylated and sent into the blood. Instead, it enters glycolysis,providing energy needed for muscle contraction.].

**D. Lysosomal degradation**A small amount (1%–3%) of glycogen is degraded by the lysosomalenzyme, ***acid*** α(***1→ 4***)***-glucosidase*** (***acid maltase***). The purpose of thisautophagic pathway is unknown. However, a deficiency of this enzymecauses accumulation of glycogen in vacuoles in the lysosomes, resulting inthe serious GSD type

**II: Pompe disease**. [Note: Pompedisease is the only GSD that is a lysosomal storage disease.]

**Lysosomal storage diseases are genetic disorders characterized by theaccumulation of abnormal amounts of carbohydrates or lipids primarily dueto their decreased lysosomal degradation resulting from decreased activityor amount of lysosomal *acid hydrolases***.

**GLYCOGENESIS ANDGLYCOGENOLYSIS REGULATION**Because of the importance of maintaining blood glucose levels, the synthesis anddegradation of its glycogen storage form are tightly regulated. In the liver,glycogenesis accelerates during periods when the body has been well fed,whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle,glycogenolysis occurs during active exercise, and glycogenesis begins as soon asthe muscle is again at rest. Regulation of synthesis and degradation isaccomplished on two levels. First, ***glycogen synthase*** and ***glycogenphosphorylase*** are hormonally regulated (by covalentphosphorylation/dephosphorylation) to meet the needs of the body as a whole.Second, these same enzymes are allosterically regulated (by effector molecules)to meet the needs of a particular tissue.A. Covalent activation of glycogenolysisThe binding of hormones, such as glucagon or epinephrine, to plasmamembrane G protein–coupled receptors ([GPCR] signals the needfor glycogen to be degraded, either to elevate blood glucose levels or toprovide energy for exercising muscle.

1. Protein kinase A activation: Binding of glucagon or epinephrine to theirspecific hepatocyte GPCR, or of epinephrine to a specific myocyteGPCR, results in the G protein–mediated activation of ***adenylyl cyclase***.This enzyme catalyzes the synthesis of cyclic adenosine monophosphate(cAMP), which activates ***cAMP-dependent protein kinase A*** (***PKA***).cAMP binds the two regulatory subunits of tetrameric ***PKA***, releasingtwo individual catalytic subunits that are active. ***PKA*** then phosphorylates several enzymes of glycogen metabolism,affecting their activity. [Note: When cAMP is removed, the inactivetetramer reforms.]

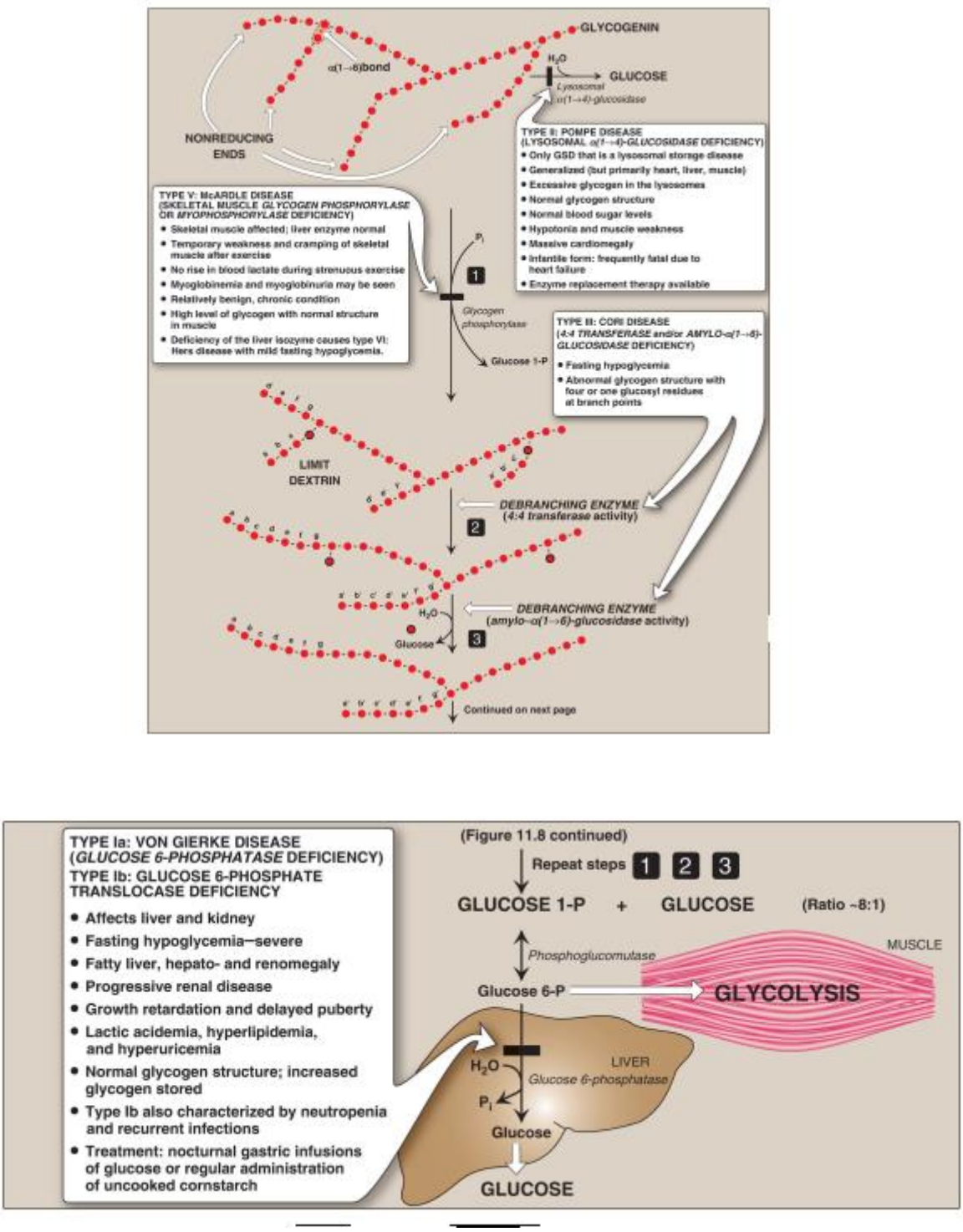






Figure: Glycogen degradation, showing some of the glycogen storage diseases (GSD). [Note: GSD type IV: Andersen disease is caused by defects in ***branching enzyme***, an enzyme of synthesis, resulting in liver cirrhosis that can be fatal in early childhood.] Pi = inorganic phosphate; P = phosphate. (Continued on next page.)

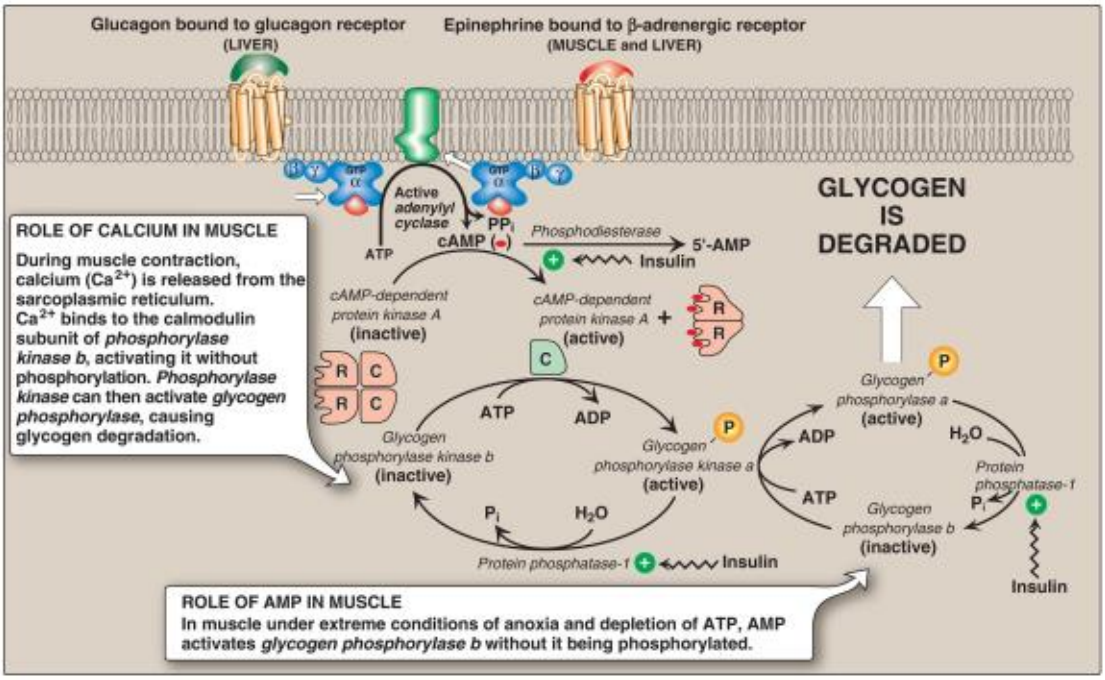



Figure 11.9 Stimulation and inhibition of glycogen degradation. AMP =adenosine monophosphate; cAMP = cyclic AMP; GTP = guanosinetriphosphate; = phosphate; PPi = pyrophosphate; R = regulatory subunit; C =catalytic subunit.

2. Phosphorylase kinase activation: ***Phosphorylase kinase*** exists in twoforms: an inactive "b" form and an active "a" form. Active ***PKA***phosphorylates the inactive "b" form of ***phosphorylase kinase***,producing the active "a" form.

3. Glycogen phosphorylase activation: ***Glycogen phosphorylase*** also existsin a dephosphorylated, inactive "b" form and a phosphorylated, active"a" form. ***Phosphorylase kinase a*** is the only enzyme thatphosphorylates ***glycogen phosphorylase b*** to its active "a" form, whichthen begins glycogenolysis.

4. Signal amplification: The cascade of reactions described above activatesglycogenolysis. The large number of sequential steps serves to amplifythe effect of the hormonal signal (that is, a few hormone moleculesbinding to their GPCR result in a number of ***PKA*** molecules beingactivated that can each activate many ***phosphorylase kinase*** molecules).This causes the production of many active ***glycogen phosphorylase a***molecules that can degrade glycogen.

5. Phosphorylated state maintenance: The phosphate groups added to***phosphorylase kinase*** and ***phosphorylase*** in response to cAMP aremaintained because the enzyme that hydrolytically removes thephosphate, ***protein phosphatase-1*** (***PP1***), is inactivated by inhibitorproteins that are also phosphorylated and activated in response to cAMP. [Note: ***PP1*** is activated by a signal cascade initiated byinsulin. Because insulin also activates the***phosphodiesterase*** that degrades cAMP, it opposes the effects ofglucagon and epinephrine.]

**B. Covalent inhibition of glycogenesis**The regulated enzyme in glycogenesis, ***glycogen synthase***, also exists intwo forms, the active "a" form and the inactive "b" form. However, incontrast to ***phosphorylase kinase*** and ***phosphorylase***, the active form of***glycogen synthase*** is dephosphorylated, whereas the inactive form isphosphorylated at several sites on the enzyme, with the level of inactivationproportional to the degree of phosphorylation. Phosphorylationis catalyzed by several different ***protein kinases*** in response to cAMP (forexample, ***PKA*** and ***phosphorylase kinase***) or other signaling mechanisms(see C. below). ***Glycogen synthase b*** can be reconverted to the "a" form by***PP1***summarizes the covalent regulation of glycogenmetabolism.

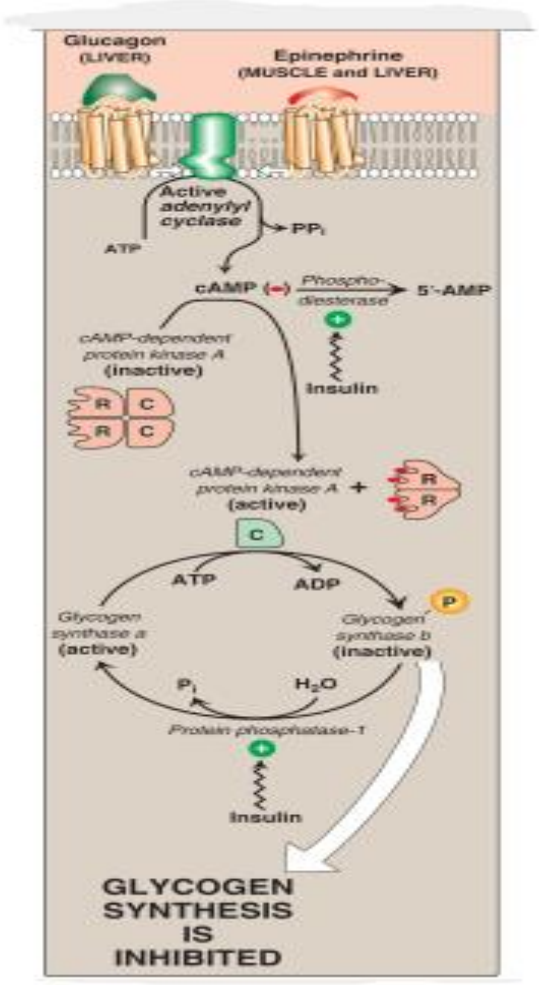



Figure 11.10 Hormonal regulation of glycogen synthesis. [Note: In contrast to***glycogen phosphorylase, glycogen synthase*** is inactivated by phosphorylation.]cAMP = cyclic adenosine monophosphate; = phosphate; PPi = pyrophosphate; R= regulatory subunit; C = catalytic subunit; ADP = adenosine diphosphate.

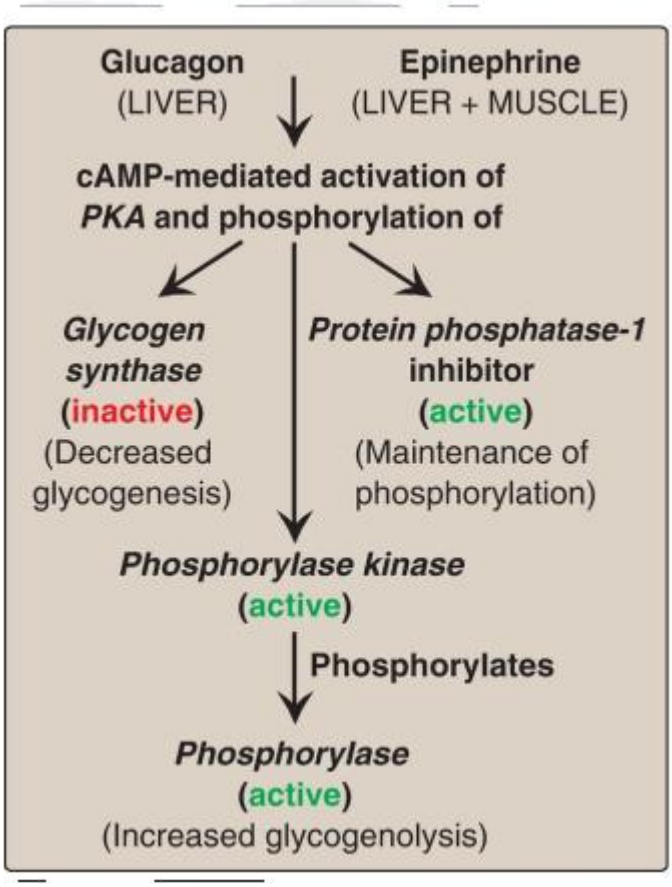



Figure: Summary of the hormone-mediated covalent regulation ofglycogen metabolism. cAMP = cyclic adenosine monophosphate; ***PKA*** = ***proteinkinase A***.

**C. Allosteric regulation of glycogenesis andglycogenolysis.**In addition to hormonal signals, ***glycogen synthase*** and ***glycogenphosphorylase*** respond to the levels of metabolites and energy needs of thecell. Glycogenesis is stimulated when glucose and energy levels are high,whereas glycogenolysis is increased when glucose and energy levels arelow. This allosteric regulation allows a rapid response to the needs of a celland can override the effects of hormone-mediated covalent regulation.[Note: The "a" and "b" forms of the allosteric enzymes of glycogenmetabolism are each in an equilibrium between the R (relaxed, more active)and T (tense, less active) conformations. The binding ofeffectors shifts the equilibrium and alters enzymic activity without directlyaltering the covalent modification.]

1. Regulation in the well-fed state: In the well-fed state, ***glycogen synthaseb*** in both liver and muscle is allosterically activated by glucose 6-phosphate, which is present in elevated concentrations. Incontrast, ***glycogen phosphorylase a*** is allosterically inhibited by glucose6-phosphate, as well as by ATP, a high-energy signal. [Note: In liver, butnot muscle, free glucose is also an allosteric inhibitor of ***glycogenphosphorylase a***.]

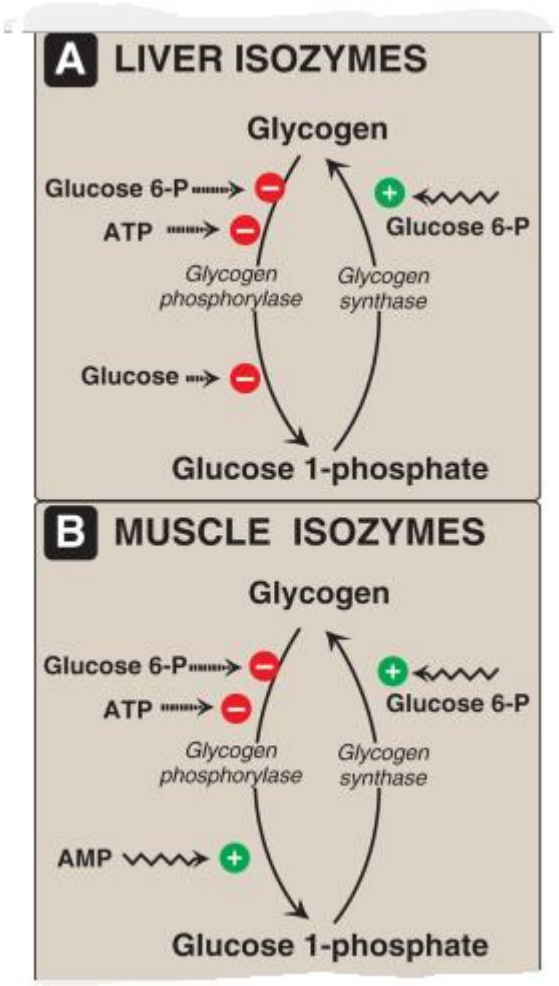



Figure 11.12 Allosteric regulation of glycogenesis and glycogenolysis in liver(A) and muscle (B). P = phosphate; AMP = adenosine monophosphate.

**2. Glycogenolysis activation by AMP**: Muscle ***glycogen phosphorylase***(***myophosphorylase***), but not the liver isozyme, is active in the presenceof the high AMP concentrations that occur under extreme conditions ofanoxia and ATP depletion. AMP binds to ***glycogen phosphorylase b***,causing its activation without phosphorylation. [Note:Recall that AMP also activates ***phosphofructokinase-1*** of glycolysis, allowing glucose from glycogenolysis to be oxidized.]

**3. Glycogenolysis activation by calcium**: Calcium ($Ca^{2+}$) is released intothe sarcoplasm in muscle cells (myocytes) in response to neuralstimulation and in the liver in response to epinephrine binding to α1-adrenergic receptors. The $Ca^{2+}$ binds to calmodulin (CaM), the mostwidely distributed member of a family of small, $Ca^{2+}$-binding proteins.The binding of four molecules of $Ca^{2+}$ to CaM triggers a conformationalchange such that the activated $Ca^{2+}$–CaM complex binds to and activatesprotein molecules, often enzymes, that are inactive in the absence of thiscomplex. Thus, CaM functions as an essential subunit ofmany complex proteins. One such protein is the tetrameric***phosphorylase kinase***, whose "b" form is activated by the binding of$Ca^{2+}$ to its δ subunit (CaM) without the need for the ***kinase*** to bephosphorylated by ***PKA.*** [Note: Epinephrine at β-adrenergic receptorssignals through a rise in cAMP, not $Ca^{2+}$.]

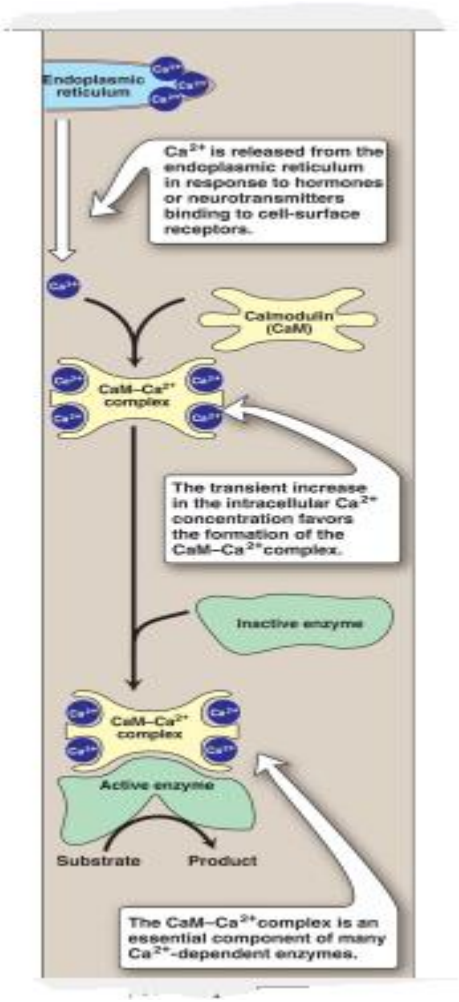



Figure 11.13 Calmodulin mediates many effects of intracellular calcium (Ca2+).[Note: Ca2+ activates ***phosphorylase kinase*** in liver and muscles.]

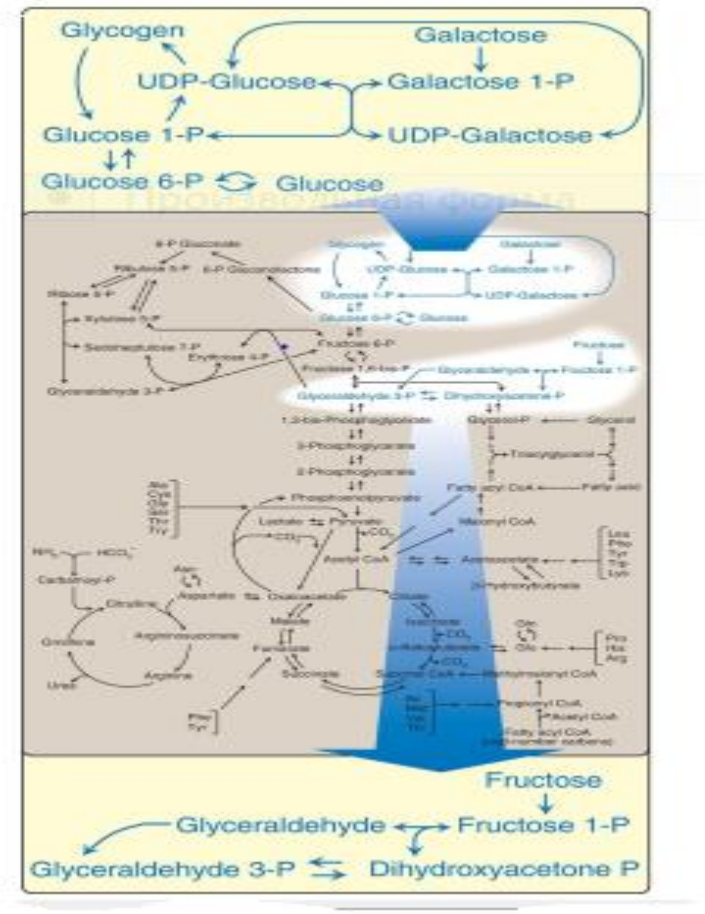
**a. Muscle phosphorylase kinase activation**: During muscle contraction,there is a rapid and urgent need for ATP. It is supplied by thedegradation of muscle glycogen to glucose 6-phosphate, which entersglycolysis. Nerve impulses cause membrane depolarization, whichpromotes Ca2+ release from the sarcoplasmic reticulum into thesarcoplasm of myocytes. The Ca2+ binds the CaM subunit, and thecomplex activates muscle ***phosphorylase kinase b***.

**b. Liver phosphorylase kinase activation**: During physiologic stress,epinephrine is released from the adrenal medulla and signals the needfor blood glucose. This glucose initially comes from hepaticglycogenolysis. Binding of epinephrine to hepatocyte α1-adrenergicGPCR activates a phospholipid-dependent cascade thatresults in movement of Ca2+ from the ER into the cytoplasm. A Ca2+–CaM complex forms and activates hepatic ***phosphorylase kinase b***.[Note: The released Ca2+ also helps to activate ***protein kinase C*** thatcan phosphorylate (therefore, inactivate) ***glycogen synthase a***.]

**Fructose and galactose metabolism. Significance of pentose phosphate pathway.**

Glucose is the most common monosaccharide consumed by humans, and itsmetabolism has already been discussed. Two other monosaccharides, fructoseand galactose, also occur in significant amounts in the diet (primarily indisaccharides) and make important contributions to energy metabolism. Inaddition, galactose is an important component of glycosylated proteins. Shows the metabolism of fructose and galactose as part of the essentialpathwaysof energy metabolism.

Galactose and fructose metabolism as part of the essential pathwaysof energy metabolism.

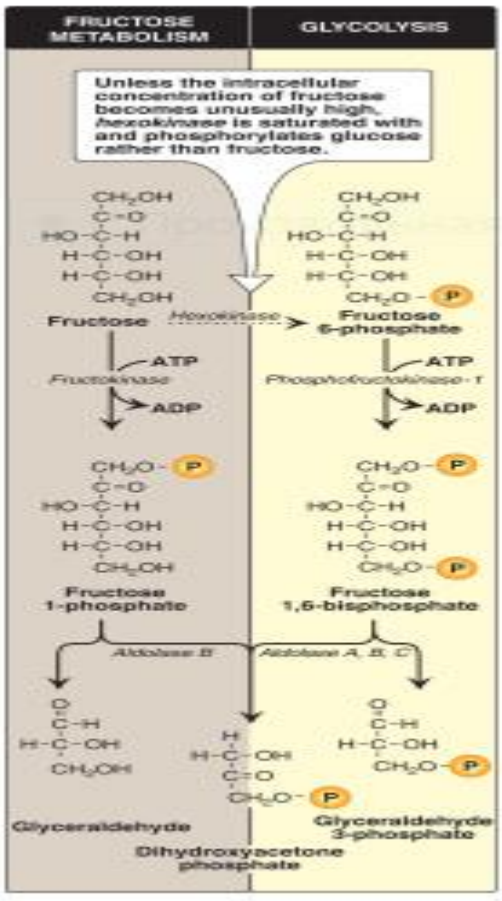



FRUCTOSE METABOLISM

About 10% of the calories in the Western diet are supplied by fructose (~55 g/day). The major source of fructose is the disaccharide sucrose, which, when cleaved in the intestine, releases equimolar amounts of fructose and glucose. Fructose is also found as a free monosaccharide in many fruits, in

honey,andinhigh-fructose corn syrup (typically, 55% fructose and 45% glucose), which isused to sweeten soft drinks and many foods (see p. 364). Fructose transport intocells is not insulin dependent (unlike that of glucose into certain tissues; and, in contrast to glucose, fructose does not promote the secretion ofinsulin.

**Phosphorylation**For fructose to enter the pathways of intermediary metabolism, it must first be phosphorylated. This can be accomplished by either***hexokinase*** or ***fructokinase***. ***Hexokinase*** phosphorylates glucose in most cells of the body and several additional hexoses can serve assubstrates for this enzyme. However, it has a low affinity (that is, a highMichaelis constant [Km]; for fructose. Therefore, unless theintracellular concentration of fructose becomes unusually high, the normalpresence of saturating concentrations of glucose means that little fructose isphosphorylated by ***hexokinase***. ***Fructokinase***provides the primary mechanism for fructose phosphorylation.The enzyme has alow Km for fructose and a high V max([maximal velocity]. It isfound in the liver (which processes most of the dietary fructose), kidneys,and the small intestine and converts fructose to fructose 1-phosphate, usingATP as the phosphate donor. [Note: These three tissues also contain***aldolase B***, discussed in section B.]




**Fructose 1-phosphate cleavage**

Fructose 1-phosphate is not phosphorylated to fructose 1,6- bisphosphate as is fructose 6-phosphate but is cleaved by ***aldolase B*** (also called ***fructose 1-phosphate aldolase***) to two trioses, dihydroxyacetone phosphate (DHAP) and glyceraldehyde. [Note: Humans express three ***aldolase*** isozymes (the products of

three different genes): ***aldolase A*** in most tissues; ***aldolase B*** in the liver, kidneys, and small intestine; and ***aldolase C*** in the brain. All cleave fructose 1,6-bisphosphate produced during glycolysis to DHAP and glyceraldehyde 3-phosphat but only ***aldolase B*** cleaves fructose 1-phosphate.] DHAP can be used in glycolysis or gluconeogenesis, whereas glyceraldehyde can be metabolized by a number of pathways, as illustrated in.

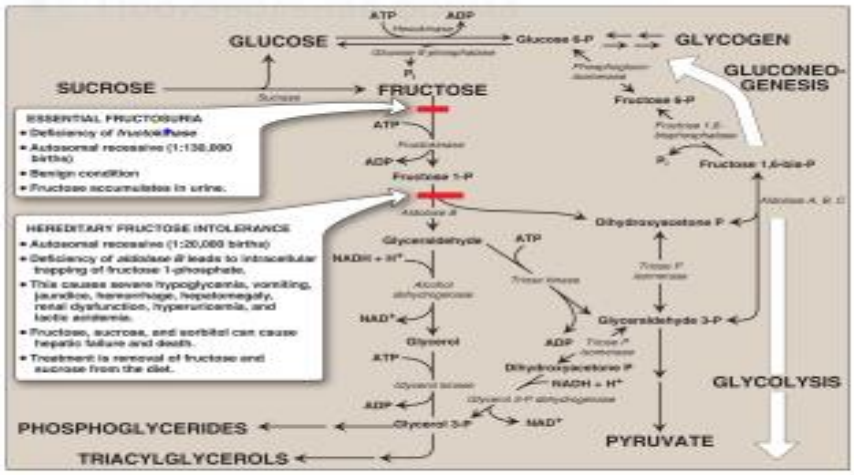



Figure 12.3 Summary of fructose metabolism. P = phosphate; $P_i$ = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ADP = adenosine diphosphate.

**Kinetics**

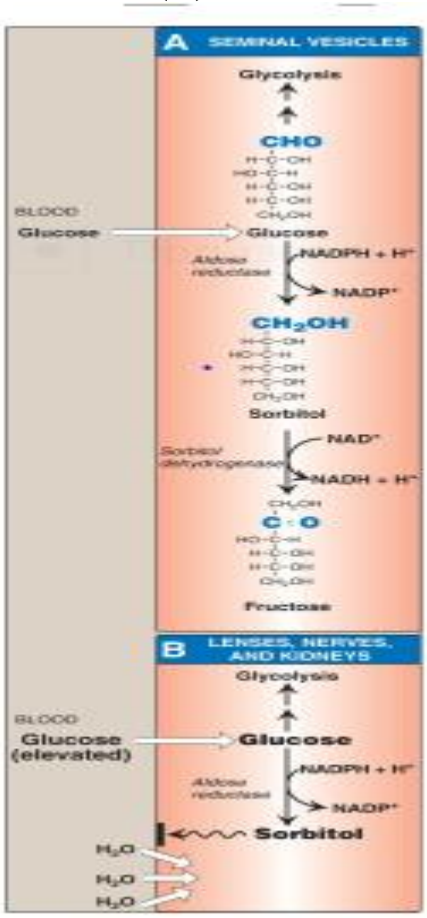
The rate of fructose metabolism is more rapid than that of glucose because triose production from fructose 1-phosphate bypasses ***phosphofructokinase-1,*** the major rate-limiting step in glycolysis

**Disorders**

A deficiency of one of the key enzymes required for the entry of fructose into metabolic pathways can result in either a benign condition as a result of ***fructokinase***deficiency (essential fructosuria) or a severe disturbance of liver and kidney metabolism as a result of ***aldolase B*** deficiency (hereditary fructose intolerance [HFI]), which occurs in ~1:20,000 live births. The first symptoms of HFI appear when a baby is weaned from lactose-containing milk and begins ingesting food containing sucrose or fructose. Fructose 1-phosphate accumulates, resulting in a drop in the level of inorganic phosphate (Pi) and, therefore, of ATP production. As ATP falls, adenosine monophosphate (AMP) rises. The AMP is degraded, causing hyperuricemia (and lactic acidemia; see p. 299). The decreased availability of hepatic ATP decreases gluconeogenesis (causing hypoglycemia with vomiting) and protein synthesis (causing a decrease in blood-clotting factors and other essential proteins). Renal reabsorption of Pi is also decreased. [Note: The drop in Pi also inhibits glycogenolysis. Diagnosis of HFI can be made on the basis of fructose in the urine, enzyme assay using liver cells, or by DNA-based testing. With HFI, sucrose, as well as fructose, must be removed from the diet to prevent liver failure and possible death. [Note: Individuals with HFI display an aversion to sweets and, consequently, have an absence of dental caries.] E. Mannose conversion to fructose 6-phosphate Mannose, the C-2 epimer of glucose (see p.

84), is an important component of glycoproteins (see p. 166). ***Hexokinase*** phosphorylates mannose, producing mannose 6-phosphate, which, in turn, is reversibly isomerized to fructose 6-phosphate by ***phosphomannose isomerase***. [Note: Most intracellular mannose is synthesized from fructose or is preexisting mannose produced by the degradation of glycoproteins and salvaged by ***hexokinase***. Dietary carbohydrates contain little mannose.] F. Glucose conversion to fructose via sorbitol Most sugars are rapidly phosphorylated following their entry into cells Therefore, they are trapped within the cells, because organic phosphates cannot freely cross membranes without specific transporters. An alternate mechanism for metabolizing a monosaccharide is to convert it to a polyol (sugar alcohol) by the reduction of an aldehyde group, thereby producing an additional hydroxyl group.

Sorbitol synthesis: ***Aldose reductase*** reduces glucose, producing sorbitol (or, glucitol;), but the Km is high. This enzyme is found in many tissues, including the retina, lens, kidneys, peripheral nerves ovaries, and seminal vesicles. A second enzyme, ***sorbitol dehydrogenase***, can oxidize sorbitol to fructose in cells of the liver, ovaries, and seminal vesicles. The two-reaction pathway from glucose to fructose in the seminal vesicles benefits sperm cells, which use fructose as a major carbohydrate energy source. The pathway from sorbitol to fructose in the liver provides a mechanism by which any available sorbitol is converted into a substrate that can enter glycolysis. Sorbitol metabolism. NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate.



Hyperglycemia and sorbitol metabolism: Because insulin is not required for the entry of glucose into cells of the retina, lens, kidneys, and peripheral nerves, large amounts of glucose may enter these cells during times of hyperglycemia (for example, in uncontrolled diabetes). Elevated intracellular glucose concentrations and an adequate supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) cause ***aldose reductase*** to produce a significant increase in the amount of sorbitol, which cannot pass efficiently through cell membranes and, therefore,remains trapped inside the cell. This is exacerbated when***sorbitol dehydrogenase*** is low or absent (for example, in cells of theretina, lens, kidneys, and peripheral nerves). As a result, sorbitolaccumulates in these cells, causing strong osmotic effects and cellswelling due to water influx and retention. Some of the pathologicalterations associated with diabetes can be partly attributed to thisosmotic stress, including cataract formation, peripheral neuropathy, andmicrovascular problems leading to nephropathy and retinopathy. [Note: Use of NADPH in the ***aldose reductase*** reaction decreasethe generation of reduced glutathione, an important antioxidant, and may be related to diabetic complications.]

GALACTOSE METABOLISM

The major dietary source of galactose is lactose (galactosylβ-1,4-glucose)obtained from milk and milk products. [Note: The digestion of lactose by ***β-galactosidase*** (***lactase***) of the intestinal mucosal cell membrane was discussed. Some galactose can also be obtained by lysosomal degradation ofglycoproteins and glycolipids. Like fructose (and mannose), the transport ofgalactose into cells is not insulin dependent.PhosphorylationLike fructose, galactose must be phosphorylated before it can be furthermetabolized. Most tissues have a specific enzyme for this purpose,***galactokinase***, which produces galactose 1-phosphateAs withother ***kinases***, ATP is the phosphate donor.Metabolism of galactose. UDP and UTP = uridine di- andtriphosphates; P = phosphate; PPi = pyrophosphate; NADP(H) = nicotinamideadenine dinucleotide phosphate; ADP = adenosine diphosphate.

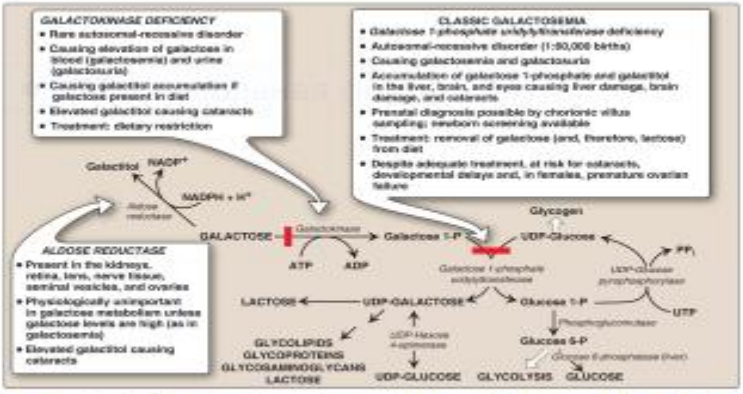



Figure 12.5 Metabolism of galactose. UDP and UTP = uridine di- and triphosphates; P = phosphate; $PP_i$ = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate; ADP = adenosine diphosphate.

B.**Uridine diphosphate–galactose formation**

Galactose 1-phosphate cannot enter the glycolytic pathway unless it is firstconverted to uridine diphosphate (UDP)-galactose This occursin an exchange reaction, in which UDP-glucose reacts with galactose 1-phosphate, producing UDP-galactose and glucose 1-phosphate. The reaction is catalyzed by ***galactose 1-phosphateuridylyltransferase***(***GALT***). [Note: The glucose 1-phosphate product canbe isomerized to glucose 6-phosphate, which can enter glycolysis or bedephosphorylated.]Structure of UDP-galactose. UDP = uridine diphosphate.

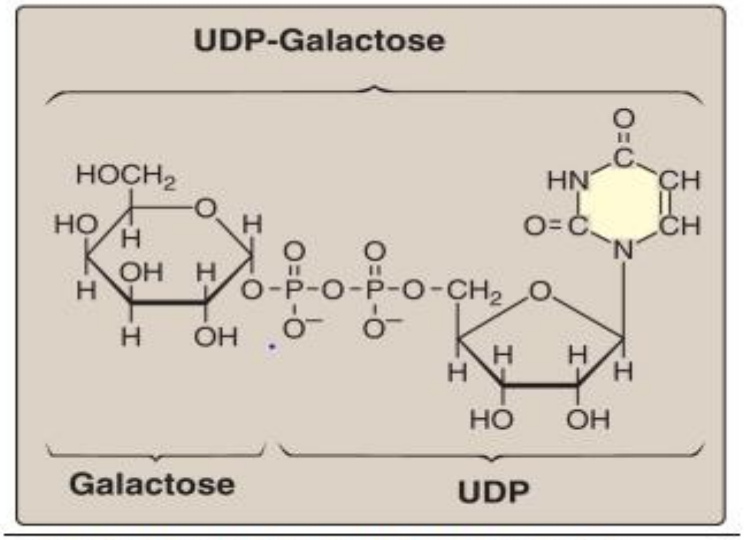
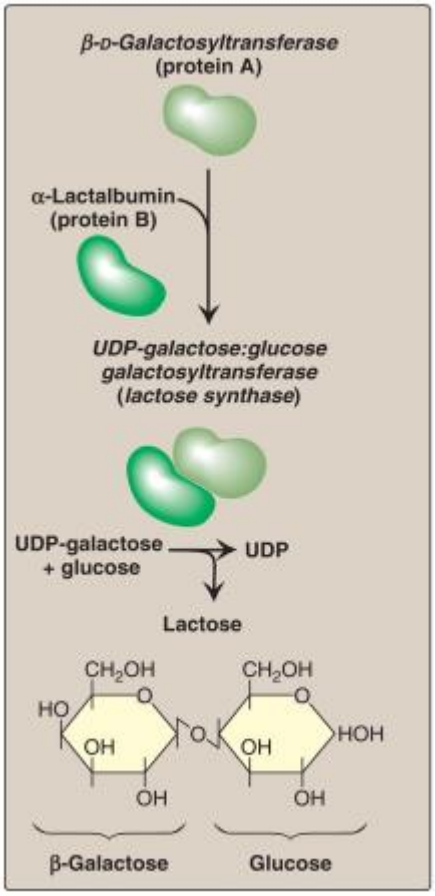



Figure 12.6 Structure of UDP-galactose. UDP = uridine diphosphate.

C. UDP-galactose conversion to UDP-glucoseFor UDP-galactose to enter the mainstream of glucose metabolism, it mustfirst be isomerized to its C-4 epimer, UDP-glucose, by ***UDP-hexose 4-epimerase.*** This "new" UDP-glucose (produced from the original UDPgalactose) can participate in biosynthetic reactions (for example,glycogenesis) as well as in the ***GALT*** reaction. [Note: for asummary of the interconversions.]D. UDP-galactose in biosynthetic reactionsUDP-galactose can serve as the donor of galactose units in a number ofsynthetic pathways, including synthesis of lactose, glycoproteins, glycolipids, andglycosaminoglycans. [Note: If galactose is not provided by thediet (for example, when it cannot be released from lactose owing to a lackof ***β-galactosidase*** in people who are lactose intolerant), all tissuerequirements for UDP-galactose can be met by the action of ***UDP-hexose4-epimerase*** on UDP-glucose, which is efficiently produced from glucose1-phosphate and uridine triphosphate. E. Disorders***GALT*** is severely deficient in individuals with classic galactosemia. In this disorder, galactose 1-phosphate and, therefore, galactoseaccumulate. Physiologic consequences are similar to those found in HFI, but a broader spectrum of tissues is affected. The accumulatedgalactose is shunted into side pathways such as that of galactitol production.This reaction is catalyzed by ***aldose reductase***, the same enzyme that reduces glucose to sorbitol. ***GALT*** deficiency is part of thenewborn screening panel. Treatment of galactosemia requires removal ofgalactose and lactose from the diet. [Note: Deficiencies in ***galactokinase***and the ***epimerase*** result in less severe disorders of galactose metabolism,although cataracts are common.

LACTOSE SYNTHESIS

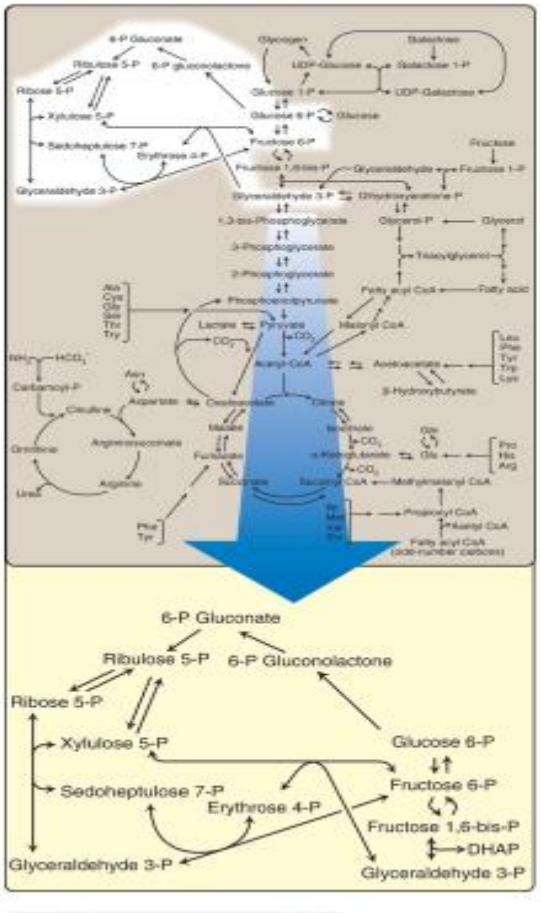
Lactose is a disaccharide that consists of a molecule of β-galactose attached by aβ(1→ 4) linkage to glucose. Therefore, lactose is galactosylβ(1→ 4)-glucose.Because lactose (milk sugar) is made by lactating (milk-producing) mammaryglands, milk and other dairy products are the dietary sources of lactose. Lactoseis synthesized in the Golgi by ***lactose synthase*** (***UDP-galactose:glucosegalactosyltransferase***), which transfers galactose from UDP-galactose toglucose, releasing UDP. This enzyme is composed of two proteins, Aand B. Protein A is a ***β-D-galactosyltransferase*** and is found in a number ofbody tissues. In tissues other than the lactating mammary gland, this enzymetransfers galactose from UDP-galactose to N-acetyl-D-glucosamine, forming thesame β(1→ 4) linkage found in lactose, and producing N-acetyllactosamine, acomponent of the structurally important N-linked glycoproteins. Incontrast, protein B is found only in lactating mammary glands. It is α-lactalbumin, and its synthesis is stimulated by the peptide hormone prolactin.Protein B forms a complex with the enzyme, protein A, changing the specificityof that ***transferase*** (by decreasing the Km for glucose) so that lactose, rather thanN-acetyllactosamine, is produced.




**Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate.**

The pentose phosphate pathway (or, hexose monophosphate shunt) occurs in the cytosol. It includes an irreversible oxidative phase, followed by a series of reversible sugar–phosphate interconversions. In the oxidative phase, carbon 1 of a glucose 6-phosphate molecule is released as carbon dioxide (CO2), and one pentose sugar-phosphate plus two reduced nicotinamide adenine dinucleotide

phosphates (NADPH) are produced. The rate and direction of the reversible reactions are determined by the supply of and demand for intermediates of the pathway. The pentose phosphate pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant. It also produces ribose 5-phosphate, required for nucleotide biosynthesis, and provides a mechanism for the conversion of pentose sugars to triose and hexose intermediates of glycolysis. No ATP is directly consumed or produced in the pathway. Pentose phosphate pathway shown as a component of the metabolic map P = phosphate; DHAP = dihydroxyacetone phosphate.




## IRREVERSIBLE OXIDATIVE REACTIONS

The oxidative portion of the pentose phosphate pathway consists of three irreversible reactions that lead to the formation of ribulose 5-phosphate, CO2, and two molecules of NADPH for each molecule of glucose 6-phosphate oxidized. This portion of the pathway is particularly important in the liver, lactating mammary glands, and adipose tissue for the NADPH-dependent biosynthesis of fatty acids; in the testes, ovaries, placenta, and adrenal cortex for the NADPH-dependent biosynthesis of steroid hormones; and in red blood cells (RBC) for the NADPH-dependent reduction of glutathione Reactions of the pentose phosphate pathway. Enzymes numbered above are: (1, 2) ***glucose 6-phosphate dehydrogenase*** and ***6-phosphogluconolactone hydrolase***, (3) ***6-phosphogluconate dehydrogenase***, (4) ***ribose 5-phosphate isomerase***, (5) ***phosphopentose epimerase***, (6, 8) ***transketolase***(coenzyme: thiamine pyrophosphate), and (7) ***transaldolase***. Δ2C = two carbons are transferred from a ketose donor to an aldose

acceptor in ***transketolase***reactions; Δ3C = three carbons are transferred in the ***transaldolase***reaction. This can be represented as: 5C sugar + 5C sugar 7C sugar + 3C sugar 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate;= phosphate; CO2 = carbon dioxide.

Glucose 6-phosphate dehydrogenation.

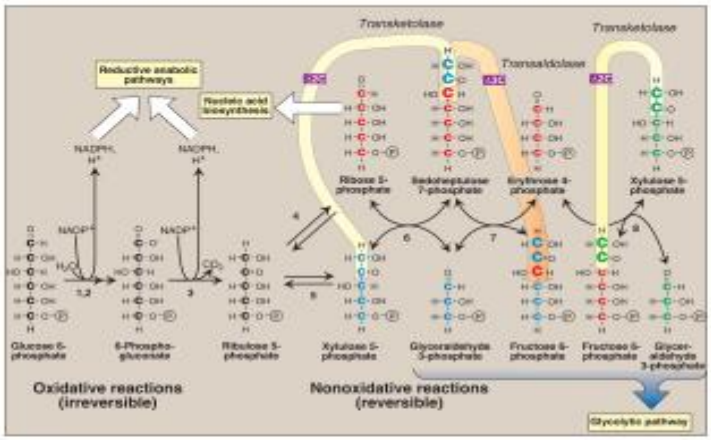



Figure 13.2 Reactions of the pentose phosphate pathway. Enzymes numbered above are: (1, 2) ***glucose 6-phosphate dehydrogenase*** and ***6-phosphogluconolactone hydrolase***, (3) ***6-phosphogluconate dehydrogenase***, (4) ***ribose 5-phosphate isomerase***, (5) ***phosphopentose epimerase***, (6, 8) ***transketolase*** (coenzyme: thiamine pyrophosphate), and (7) ***transaldolase***. Δ2C = two carbons are transferred from a ketose donor to an aldose acceptor in ***transketolase*** reactions; Δ3C = three carbons are transferred in the ***transaldolase*** reaction. This can be represented as: 5C sugar + 5C sugar 7C sugar + 3C sugar 4C sugar + 6C sugar. NADP(H) = nicotinamide adenine dinucleotide phosphate; = phosphate; $CO_2$ = carbon dioxide.

***Glucose 6-phosphate dehydrogenase*** (***G6PD***) catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone as the coenzyme NADP+gets reduced to NADPH. This initial reaction is the committed, ratelimiting, and regulated step of the pathway. NADPH is a potent competitive inhibitor of ***G6PD***, and the ratio of NADPH/NADP+ is sufficiently high to substantially inhibit the enzyme under most metabolic conditions. However, with increased demand for NADPH, the ratio of NADPH/NADP+ decreases, and flux through the pathway increases in response to the enhanced activity of ***G6PD***. [Note: Insulin upregulates expression of thegene for ***G6PD***, and flux through the pathway increases in the absorptive state.

Ribulose 5-phosphate formation6-Phosphogluconolactone is hydrolyzed by ***6-phosphogluconolactonehydrolase*** in the second step. The oxidative decarboxylation of the product,6-phosphogluconate, is catalyzed by ***6-phosphogluconate dehydrogenase.***This third irreversible step produces ribulose 5-phosphate (a pentose sugar–phosphate), CO2 (from carbon 1 of glucose), and a second molecule of NADPH.

**REVERSIBLE NONOXIDATIVEREACTIONS**

The nonoxidative reactions of the pentose phosphate pathway occur in all celltypes synthesizing nucleotides and nucleic acids. These reactions catalyze theinterconversion of sugars containing three to seven carbons These reversible reactions permit ribulose 5-phosphate (produced by theoxidative portion of the pathway) to be converted either to ribose 5-phosphate(needed for nucleotide

synthesis; see p. 293) or to intermediates of glycolysis(that is, fructose 6-phosphate and glyceraldehyde 3-phosphate). For example,many cells that carry out reductive biosynthetic reactions have a greater need forNADPH than for ribose 5-phosphate. In this case, ***transketolase***(which transferstwo-carbon units in a thiamine pyrophosphate [TPP]-requiring reaction) and***transaldolase***(which transfers three-carbon units) convert the ribulose 5-phosphate produced as an end product of the oxidative phase to glyceraldehyde3-phosphate and fructose 6-phosphate, which are glycolytic intermediates. Incontrast, when the demand for ribose for nucleotides and nucleic acids is greaterthan the need for NADPH, the nonoxidative reactions can provide the ribose 5-phosphate from glyceraldehyde 3-phosphate and fructose 6-phosphate in the absence of the oxidative steps. Formation of ribose 5-phosphate from intermediates of glycolysis. P= phosphate; DHAP = dihydroxyacetone phosphate.In addition to ***transketolase***, TPP is required by the multienzymecomplexes ***pyruvate dehydrogenase***, ***α-ketoglutaratedehydrogenase***of the tricarboxylic acid cycle, and ***branchedchainα-keto acid dehydrogenase*** of branched-chain amino acid catabolism

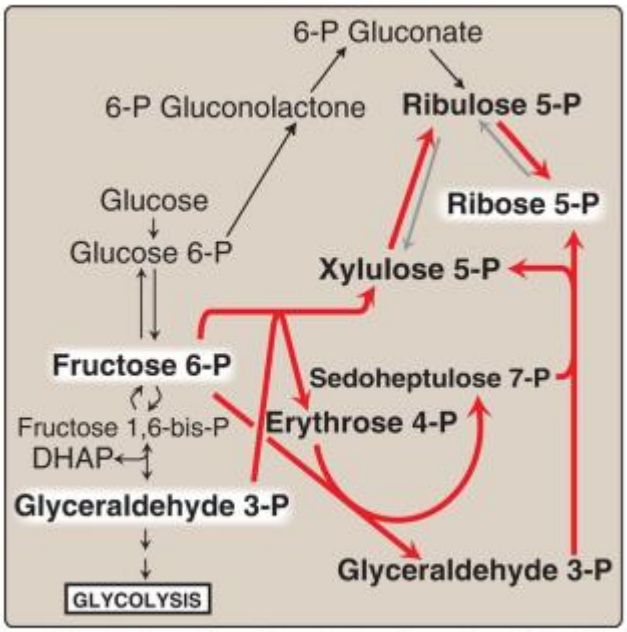



Figure 13.3 Formation of ribose 5-phosphate from intermediates of glycolysis. P = phosphate; DHAP = dihydroxyacetone phosphate.

**NADPH USES**

The coenzyme NADPH differs from nicotinamide adenine dinucleotide (NADH)only by the presence of a phosphate group on one of the ribose units This seemingly small change in structure allows NADPH to interact withNADPH-specific enzymes that have unique roles in the cell. For example, in thecytosol of hepatocytes, the steady-state NADP+/NADPH ratio is ~0.1, whichfavors the use of NADPH in reductive biosynthetic reactions. This contrasts withthe high NAD+/NADH ratio (~1,000), which favors an oxidative role for NAD+.This section summarizes some important NADPH-specific functions in reductivebiosynthesis and detoxification reactions.Structure of reduced

nicotinamide adenine dinucleotide phosphate(NADPH).Reductive biosynthesisLike NADH, NADPH can be thought of as a high-energy molecule. However, the electrons of NADPH are used for reductive biosynthesis,rather than for transfer to the electron transport chain as is seen with NADH. Thus, in the metabolic transformations of the pentose phosphatepathway, part of the energy of glucose 6-phosphate is conserved inNADPH, a molecule with a negative reduction potential, that,therefore, can be used in reactions requiring an electron donor, such as fatty acid, cholesterol, and steroid hormone synthesis.

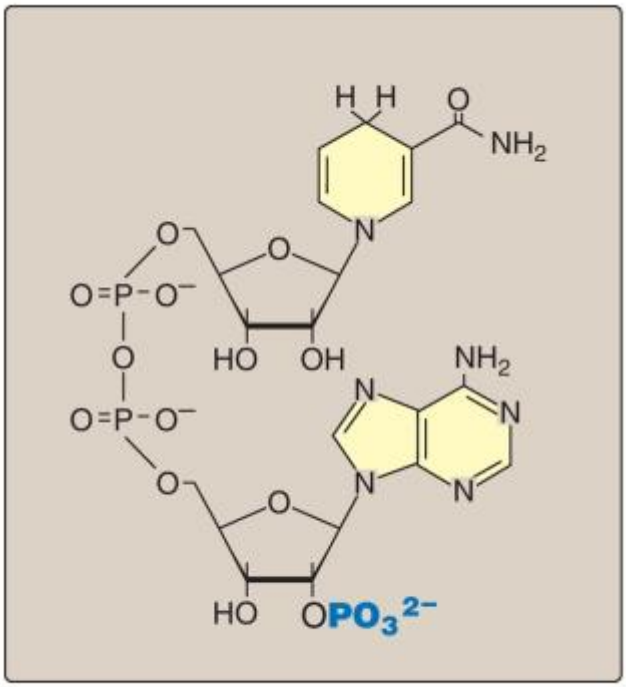



Figure 13.4 Structure of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

**Structure, function and metabolism of lipids**.

Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules. Because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane associated lipids or droplets of triacylglycerol in adipocytes, or transported in blood in association with protein, as in lipoprotein particles or on albumin. Lipids are a major source of energy for the body, and they also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures. Lipids serve additional functions in the body (for example, some fat-soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis). Deficiencies or imbalances of lipid metabolism can lead to some of the major clinical problems encountered by physicians, such as atherosclerosis, diabetes, and obesity.

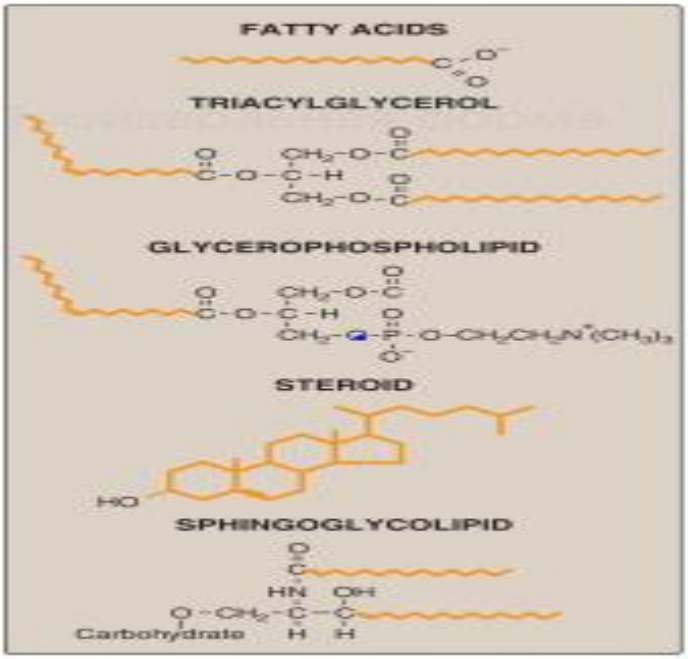



Figure 1. Structures of some common classes of lipids. Hydrophobic portions of the molecules are shown in orange.

DIGESTION, ABSORPTION,SECRETION, AND UTILIZATION: The average daily intake of lipids by U.S. adults is ~78 g, of which >90% is triacylglycerol ([TAG], formerly called triglyceride [TG]), that consists of three fatty acids (FA) esterified to a glycerol backbone (see Fig.2 ). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and nonesterified (free) FA (FFA). The digestion of dietary lipids begins in the stomach and is completed in the small intestine. The process is summarized in Figure 3

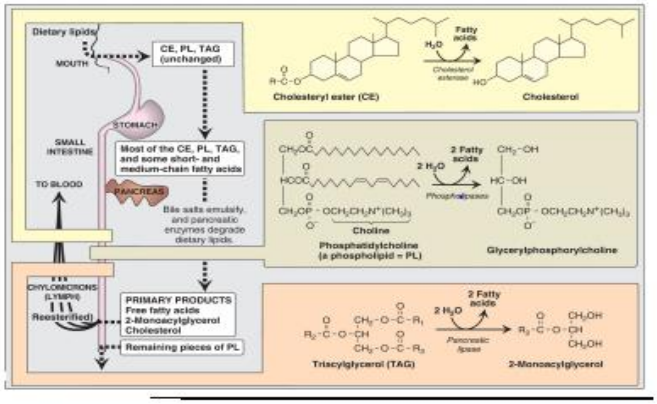



Figure 3 Overview of lipid digestion

**A.Digestion in the stomach:**

Lipid digestion in the stomach is limited. It is catalyzed by ***lingual lipase*** that originates from glands at the back of the tongue and ***gastric lipase*** that is secreted by the gastric mucosa. Both enzymes are relatively acid stable,with optimal pH values of 4 to 6. These ***acid lipases*** hydrolyze FA from TAG molecules, particularly those containing short- or medium-chainlength (≤12 carbons) FA such as are found in milk fat. Consequently, these ***lipases*** play a particularly important role in lipid digestion in infants for whom milk fat is the primary source of calories. They also become important digestive enzymes in individuals with pancreatic insufficiency such as those with cystic fibrosis (CF). ***Lingual*** and ***gastric lipases*** aid these patients in degrading TAG molecules (especially those with short- to medium-chain FA) despite a near or complete absence of ***pancreatic lipase*** (see Section D.1. below).

**B. Cystic fibrosis**

CF is the most common lethal genetic disease in Caucasians of Northern European ancestry and has a prevalence of ~1:3,300 births in the United States. CF is an autosomal-recessive disorder caused by mutations to the gene for the CF transmembrane conductance regulator (CFTR) protein that functions as a chloride channel on epithelium in the pancreas, lungs, testes, and sweat glands. Defective CFTR results in decreased secretion of chloride and increased uptake of sodium and water. In the pancreas, the depletion of water on the cell surface results in thickened mucus that clogs the pancreatic ducts, preventing pancreatic enzymes from reaching the intestine, thereby leading to pancreatic insufficiency. Treatment includes replacement of these enzymes and supplementation with fat-soluble vitamins. [Note: CF also causes chronic lung infections with progressive pulmonary disease and male infertility.]

**C. Emulsification in the small intestine**

The critical process of dietary lipid emulsification occurs in the duodenum. Emulsification increases the surface area of the hydrophobic lipid droplets so that the digestive enzymes, which work at the interface of the droplet and the surrounding aqueous solution, can act effectively. Emulsification is accomplished by two complementary mechanisms, namely, use of the detergent properties of the conjugated bile salts and mechanical mixing due to peristalsis. Bile salts, made in the liver and stored in the gallbladder, are amphipathic derivatives of cholesterol (see p. 4). Conjugated bile salts consist of a hydroxylated sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage (Fig.5). These emulsifying agents interact with the dietary lipid droplets and the aqueous duodenal contents, thereby stabilizing the droplets as they

become smaller from peristalsis and preventing them from coalescing.[Note: See p.6 for a more complete discussion of bile salt metabolism.]

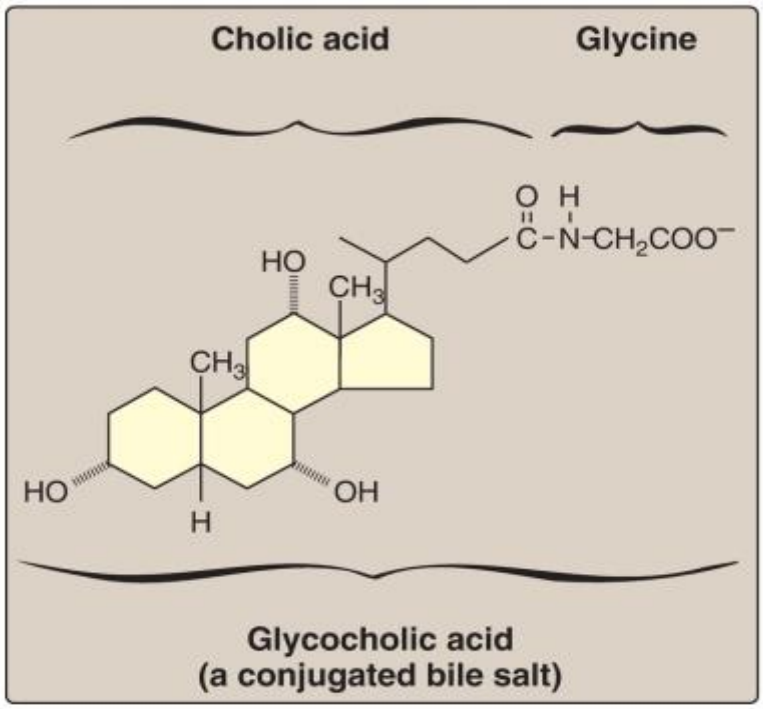



Figure 4 Structure of glycocholic acid

**D. Degradation by pancreatic enzymes**

The dietary TAG, cholesteryl esters, and phospholipids are enzymaticallydegraded (digested) in the small intestine by pancreatic enzymes, whosesecretion is hormonally controlled.1. Triacylglycerol degradation: TAG molecules are too large to be taken upefficiently by the mucosal cells (enterocytes) of the intestinal villi.Therefore, they are hydrolyzed by an ***esterase***, ***pancreatic lipase***, whichpreferentially removes the FA at carbons 1 and 3. The primary productsof hydrolysis are, thus, a mixture of 2-monoacylglycerol (2-MAG) and FFA (see Fig.2). [Note: ***Pancreatic lipase*** is found in high concentrations in pancreatic secretions (2%–3% of the total protein present), and it is highly efficient catalytically, thus insuring that only severe pancreatic deficiency, such as that seen in CF, results in significant malabsorption of fat.] A second protein, ***colipase***, also secreted by the pancreas, binds the ***lipase*** at a ratio of 1:1 and anchors it at the lipid–aqueous interface. ***Colipase*** restores activity to ***lipase*** in the presence of inhibitory substances like bile salts that bind the micelles. [Note: ***Colipase*** is secreted as the zymogen, procolipase, which is activated in the intestine by ***trypsin***.] Orlistat, an antiobesitydrug,inhibits***gastric*** and ***pancreatic lipases***,

thereby decreasing fat absorption,resulting in weight loss.2. Cholesteryl ester degradation: Most dietary cholesterol is present in the free (nonesterified) form, with 10%–15% present in the esterified form.Cholesteryl esters are hydrolyzed by pancreatic ***cholesteryl ester hydrolase*** (***cholesterol esterase***), which produces cholesterol plus FFA (see Fig.2). Activity of this enzyme is greatly increased in the presence of bile salts. 3. Phospholipid degradation: Pancreatic juice is rich in the proenzyme of ***phospholipase A2*** that, like procolipase, is activated by ***trypsin*** and, like ***cholesteryl ester hydrolase***, requires bile salts for optimum activity ***Phospholipase A2*** removes one FA from carbon 2 of a phospholipid,leaving a lysophospholipid. For example, phosphatidylcholine (the predominant phospholipid of digestion) becomes lysophosphatidylcholine. The remaining FA at carbon 1 can be removed by ***lysophospholipase***, leaving a glycerylphosphoryl base (for example, glycerylphosphorylcholine, see Fig.2) that may be excreted in the feces, further degraded, or absorbed.4. Control: Pancreatic secretion of the hydrolytic enzymes that degrade dietary lipids in the small intestine is hormonally controlled (Fig.4).Cells in the mucosa of the lower duodenum and jejunum produce the peptide hormone cholecystokinin (CCK), in response to the presence of lipids and partially digested proteins entering these regions of the upper small intestine. CCK acts on the gallbladder (causing it to contract and release bile, a mixture of bile salts, phospholipids, and free cholesterol) and on the exocrine cells of the pancreas (causing them to release digestive enzymes). It also decreases gastric motility, resulting in a slower release of gastric contents into the small intestine (see p. 4). Other intestinal cells produce another peptide hormone, secretin, in response to the low pH of the chyme entering the intestine from the stomach. Secretin causes the pancreas to release a solution rich in bicarbonate that helps neutralize the pH of the intestinal contents,bringing them to the appropriate pH for digestive activity by pancreatic enzymes.

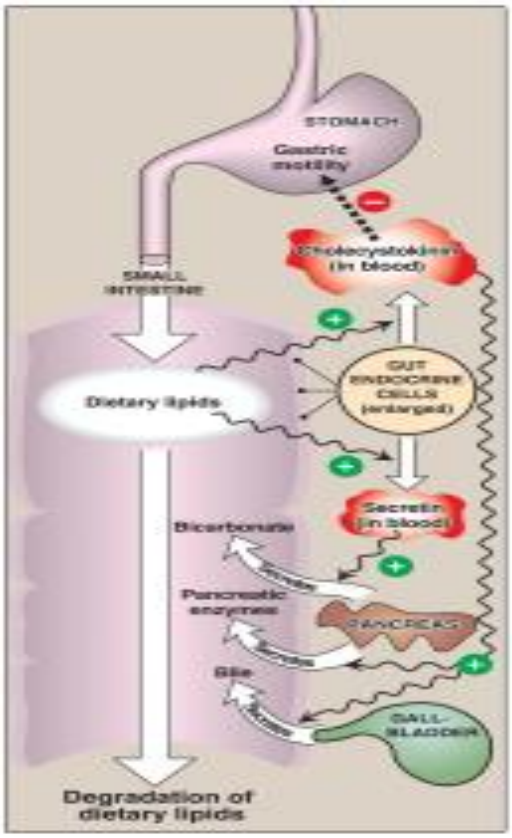



Figure.4 Hormonal control of lipid digestion in the small intestine. [Note: The small intestine is divided into three parts: the duodenum (upper 5%), the jejunum, and the ileum (lower 55%).]

**E. Absorption by enterocytes**

FFA, free cholesterol, and 2-MAG are the primary products of lipid digestion in the jejunum. These, plus bile salts and fat-soluble vitamins (A,D, E, and K), form mixed micelles (that is, disc-shaped clusters of a mixture of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside). Therefore, mixed micelles are soluble in the aqueous environment of the intestinal lumen (Fig.5). These particles approach the primary site of lipid absorption,the brush border membrane of the enterocytes. This microvilli-rich apical membrane is separated from the liquid contents of the intestinal lumen by an unstirred water layer that mixes poorly with the bulk fluid. The hydrophilic surface of the micelles facilitates the transport of the hydrophobic lipids through the unstirred water layer to the brush border membrane where they are absorbed. Bile salts are absorbed in the terminal ileum, with <5% being lost in the feces. [Note: Relative to other dietary lipids, cholesterol is only poorly absorbed by the enterocytes. Drug therapy (for example, with ezetimibe) can further reduce cholesterol absorption in the small intestine.] Because short- and medium-chain FA

are water soluble, they do not require the assistance of mixed micelles for absorption by the intestinal mucosa.

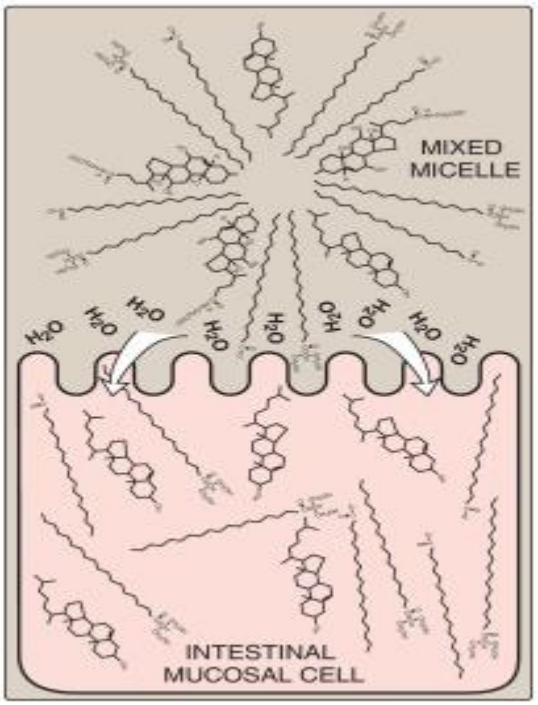



Figure.5 Absorption of lipids contained in a mixed micelle by an intestinal mucosal cell. The micelle itself is not absorbed. [Note: Short- and mediumchain-length fatty acids do not require incorporation into micelles.]

**F. Triacylglycerol and cholesteryl ester resynthesis**

The mixture of lipids absorbed by the enterocytes migrates to the smoothendoplasmic reticulum (SER) where biosynthesis of complex lipids takes place. The long-chain FA are first converted into their activated form by ***fatty acyl coenzyme A*** (***CoA***) ***synthetase***(***thiokinase***), as shown in Figure.6. Using the fatty acyl CoA derivatives, the 2-MAG absorbed by the enterocytes are converted to TAG through sequential reacylations by two ***acyltransferases***, ***acyl CoA:monoacylglycerol acyltransferase*** and ***acyl CoA:diacylglycerol acyltransferase***. Lysophospholipids are reacylated to form phospholipids by a family of ***acyltransferases***, and cholesterol is acylated primarily by ***acyl CoA:cholesterol acyltransferase*** [Note: Virtually all long-chain FA entering the enterocytes are used in this fashion to form TAG, phospholipids, and cholesteryl esters. Short- and medium-chain FA are not converted to their CoA derivatives and are not reesterified to 2-MAG. Instead, they are released into the portal circulation,where they are carried by serum albumin to the liver.]

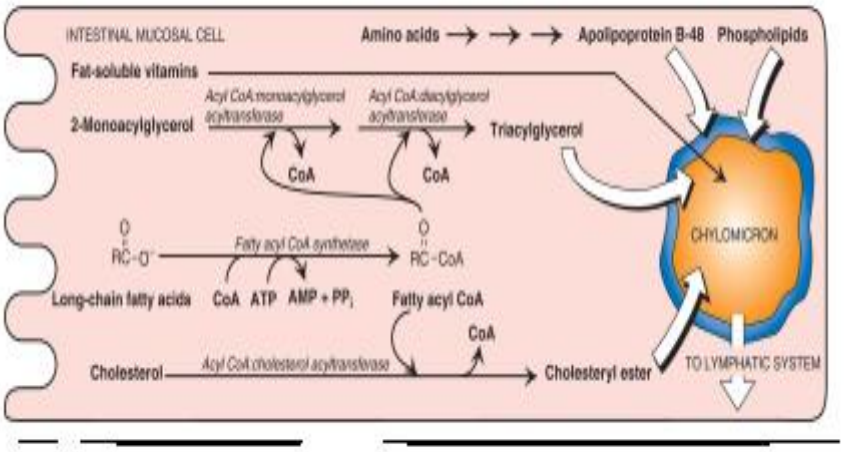



Figure 6 Assembly and secretion of chylomicrons by intestinal mucosal cells.[Note: Short- and medium-chain-length fatty acids do not require incorporation into chylomicrons and directly enter into the blood.] CoA = coenzyme A; AMP = adenosine monophosphate; PPi = pyrophosphate.

## G. Lipid malabsorption

Lipid malabsorption, resulting in increased lipid (including the fat-soluble vitamins and essential FA, in the feces, a condition known assteatorrhea, can be caused by disturbances in lipid digestion and/or absorption (Fig.7). Such disturbances can result from several conditions, including CF (causing poor digestion) and short bowel syndrome (causing decreased absorption)

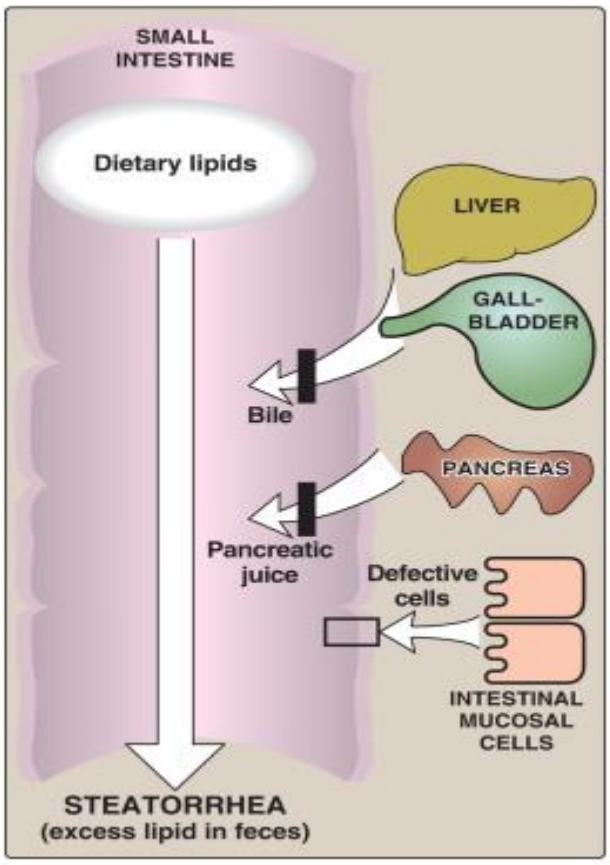



Figure 7 Possible causes of steatorrhea

The ability of short- and medium-chain FA to be taken up by enterocyteswithout the aid of mixed micelles has made them important in medicalnutrition therapy for individuals with malabsorption disorders.

**H. Secretion from enterocytes**

The newly resynthesized TAG and cholesteryl esters are very hydrophobicand aggregate in an aqueous environment. Therefore, they must bepackaged as particles of lipid droplets surrounded by a thin layer composedof phospholipids, nonesterified cholesterol, and a molecule of the proteinapolipoprotein (apo) B-48. This layer stabilizes the particle andincreases its solubility, thereby preventing multiple particles fromcoalescing. [Note: Microsomal triglyceride transfer protein is essential forthe assembly of all TAG-rich apo B–containing particles in the ER.] The lipoprotein particles are released by exocytosis from enterocytesinto the lacteals (lymphatic vessels in the villi of the small intestine). Thepresence of these particles in the lymph after a lipid-rich meal gives it a milky appearance. This lymph is called chyle (as opposed to chyme, the name given to the semifluid mass of partially digested food that passes from the stomach to the duodenum), and the particles are named chylomicrons. Chylomicrons follow the lymphatic system to

the thoracic duct and are then conveyed to the left subclavian vein, where they enter the blood. The steps in the production of chylomicrons are summarized in Figure 6. [Note: Once released into blood, the nascent (immature) chylomicrons pick up apolipoproteins E and C-II from high-density lipoproteins and mature. (For a more detailed description of chylomicron structure and metabolism. I. Use by the tissues Most of the TAG contained in chylomicrons is broken down in the capillary beds of skeletal and cardiac muscle and adipose tissue. The TAG is degraded to FFA and glycerol by ***lipoprotein lipase*** (***LPL***). This enzyme is synthesized and secreted primarily by adipocytes and muscle cells. Secreted ***LPL*** is anchored to the luminal surface of endothelial cells in the capillaries of muscle and adipose tissues. [Note: Familial chylomicronemia (type I hyperlipoproteinemia) is a rare, autosomal-recessive disorder caused by a deficiency of ***LPL*** or its coenzyme apo C-II (see p. 228). The result is fasting chylomicronemia and severe hypertriacylglycerolemia, which can cause pancreatitis.] 1. Fate of free fatty acids: The FFA derived from the hydrolysis of TAG may either directly enter adjacent muscle cells and adipocytes or be transported in the blood in association with serum albumin until they are taken up by cells. [Note: Human serum albumin is a large protein secreted by the liver. It transports a number of primarily hydrophobic compounds in the circulation, including FFA and some drugs.] Most cells can oxidize FA to produce energy. Adipocytes can also reesterify FFA to produce TAG molecules, which are stored until the FA are needed by the body. 2. Fate of glycerol: Glycerol released from TAG is taken up from the blood and phosphorylated by hepatic ***glycerol kinase*** to produce glycerol 3-phosphate, which can enter either glycolysis or gluconeogenesis by oxidation to dihydroxyacetone phosphate or be used in TAG synthesis. 3. Fate of chylomicron remnants: After most of the TAG has been removed,the chylomicron remnants (which contain cholesteryl esters,phospholipids, apolipoproteins, fat-soluble vitamins, and a small amount of TAG) bind to receptors on the liver (apo E is the ligand;) and are endocytosed. The intracellular remnants are hydrolyzed to their component parts. Cholesterol and the nitrogenous bases of phospholipids (for example, choline) can be recycled by the body. [Note: If removal of remnants by the liver is decreased because of impaired binding to their receptor, they accumulate in the plasma. This is seen in the rare type III hyperlipoproteinemia (also called familial dysbetalipoproteinemia or broad beta disease).]

## CHAPTER SUMMARY

Dietary lipid digestion begins in the stomach and continues in the small intestine (Fig.8). Cholesteryl esters, phospholipids, and triacylglycerols (TAG) containing long-chain-length fatty acids (FA) are degraded in the small intestine by pancreatic

enzymes. The most important of these enzymes are ***cholesterol esterase***, ***phospholipase A2***, and ***pancreatic lipase***.In cystic fibrosis, thickened mucus prevents these enzymes reaching the intestine. In contrast, TAG in milk fat contain short- to medium-chainlength FA and are degraded in the stomach by ***acid lipases*** (***lingual lipase*** and ***gastric lipase***). The hydrophobic nature of lipids requires that dietary lipids be emulsified for efficient degradation. Emulsification occurs in the small intestine using peristaltic action (mechanical mixing) and bile salts (detergents). The primary products of dietary lipid degradation are 2-onoacylglycerol, nonesterified (free) cholesterol, and free FA. These compounds, plus the fat-soluble vitamins, form mixed micelles that facilitate dietary lipid absorption by intestinal mucosal cells (enterocytes).These cells use activated long-chain FA to regenerate TAG and cholesteryl esters and also synthesize protein (apolipoprotein [apo] B-48), all of which are then assembled with the fat-soluble vitamins into lipoprotein particles called chylomicrons. Short- and medium-chain FA enter blood directly. Chylomicrons are first released into the lymph and then enter the blood,where their lipid core is degraded by ***lipoprotein lipase*** (with apo C-II as the coenzyme) in the capillaries of muscle and adipose tissues. Thus,dietary lipids are made available to the peripheral tissues. Fat maldigestion or malabsorption causes steatorrhea (lipid in the feces). A defic

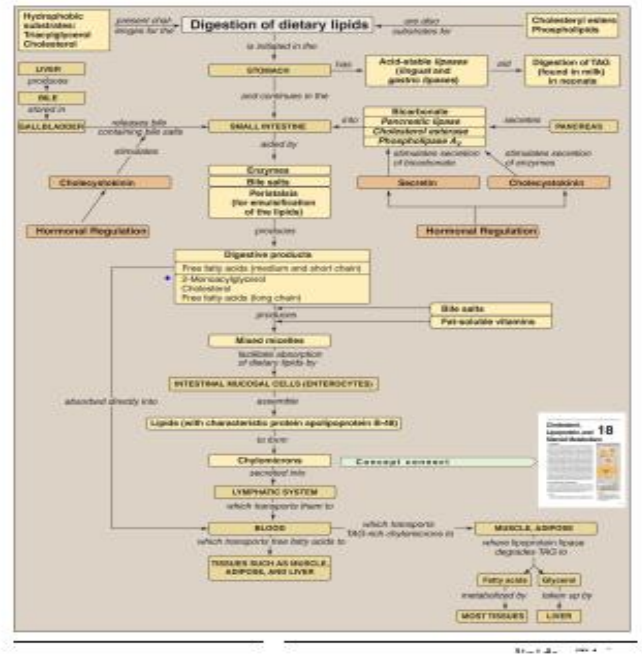



Figure 8 Key concept map for metabolism of dietary lipids. TAG =triacylglycerols.

***<u>Topic:</u>*** **Intermediate exchange of fats**.

Glycerophospholipid synthesis involves either the donation of PA from cytidine diphosphate (CDP)-DAG to an alcohol or the donation of the phosphomonoester of

the alcohol from CDP-alcohol to DAG (Fig1). In both cases, the CDPbound structure is considered an activated intermediate, and cytidine monophosphate (CMP) is released as a side product. Therefore, a key concept in glycerophospholipid synthesis is activation, of either DAG or the alcohol to be added, by linkage with CDP. [Note: This is similar in principle to the activation of sugars by their attachment to uridine diphosphate (UDP).] The FA esterified to the glycerol alcohol groups can vary widely, contributing to the heterogeneity of this group of compounds, with saturated FA typically found at carbon 1 and unsaturated ones at carbon 2. Most phospholipids are synthesized in the smooth endoplasmic reticulum (SER). From there, they are transported to the Golgi and then to membranes of organelles or the plasma membrane or are secreted from the cell by exocytosis. [Note: Ether lipid synthesis from dihydroxyacetone phosphate begins in peroxisomes.]

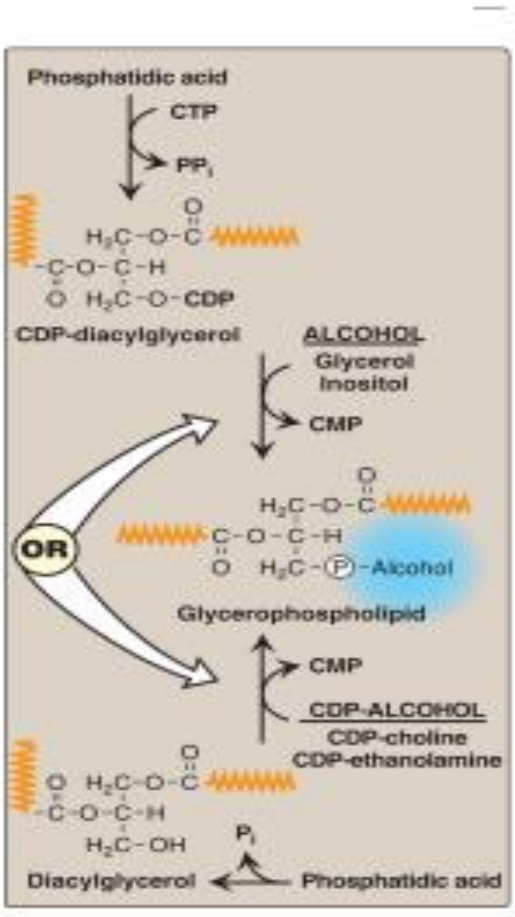



Figure 1 Glycerophospholipid synthesis requires activation of either diacylglycerol or an alcohol by linkage to cytidine diphosphate (CDP). CMP and CTP = cytidine mono- and triphosphates; Pi = inorganic phosphate; PPi = pyrophosphate. ( is a fatty acid hydrocarbon chain.)

**A.Phosphatidic acidPA is the precursor of other glycerophospholipids**.

The steps in its synthesisfrom glycerol 3-phosphate and two fatty acyl coenzyme A (CoA) moleculeswere illustrated in Figure 2, p. 189, in which PA is shown as aprecursor of triacylglycerol (TAG).Essentially all cells except mature erythrocytes can synthesizephospholipids, whereas TAG synthesis occurs essentially only in the liver,adipose tissue, lactating mammary glands, and intestinal mucosal cells.B. Phosphatidylcholine and phosphatidylethanolamineThe neutral phospholipids PC and PE are the most abundant phospholipidsin most eukaryotic cells. The primary route of their synthesis uses cholineand ethanolamine obtained either from the diet or from the turnover of thebody's phospholipids. [Note: In the liver, PC also can be synthesized fromPS and PE (see 2. below).]1. Synthesis from preexisting choline and ethanolamine: These syntheticpathways involve the phosphorylation of choline or ethanolamine by***kinases***, followed by conversion to the activated form, CDP-choline orCDP-ethanolamine. Finally, choline phosphate or ethanolaminephosphate is transferred from the nucleotide (leaving CMP) to a molecule of DAG (Fig. 3).a. Significance of choline reutilization: The reutilization of choline isimportant because, although humans can synthesize choline de novo,the amount made is insufficient for our needs. Thus, choline is anessential dietary nutrient with an adequate intake of 550mg for men and 425 mg for women. [Note: Choline is also used for thesynthesis of acetylcholine, a neurotransmitter.]b. Phosphatidylcholine in lung surfactant: The pathway described aboveis the principal pathway for the synthesis ofdipalmitoylphosphatidylcholine (DPPC or, dipalmitoyl lecithin). InDPPC, positions 1 and 2 on the glycerol are occupied by palmitate, asaturated LCFA. DPPC, made and secreted by type II pneumocytes, isa major lipid component of lung surfactant, which is the extracellularfluid layer lining the alveoli. Surfactant serves to decrease the surfacetension of this fluid layer, reducing the pressure needed to reinflatealveoli, thereby preventing alveolar collapse (atelectasis). [Note:Surfactant is a complex mixture of lipids (90%) and proteins (10%),with DPPC being the major component for reducing surface tension.]Fetal lung maturity can be gauged by determining the DPPC/sphingomyelinratio, usually written as L (for lecithin)/S, in amniotic fluid. A value ≥2 isevidence of maturity, because it reflects the shift from sphingomyelin toDPPC synthesis that occurs in pneumocytes at ~32 weeks' gestation.c. Lung maturity: Respiratory distress syndrome (RDS) in preterm infantsis associated with insufficient surfactant production and/or secretionand is a significant cause of all neonatal deaths in Western countries.Lung maturation can be accelerated by giving the motherglucocorticoids shortly before delivery to induce expression of specificgenes. Postnatal administration of natural or synthetic surfactant (byintratracheal instillation) is also used. [Note: Acute RDS, seen in allage groups, is the result of alveolar damage (due to infection, injury, or aspiration) that causes

fluid to accumulate in the alveoli, impeding the exchange of oxygen (O2) and carbon dioxide (CO2).] 2. Phosphatidylcholine synthesis from phosphatidylserine: The liver requires a mechanism for producing PC, even when free choline levels are low, because it exports significant amounts of PC in the bile and as a component of plasma lipoproteins. To provide the needed PC, PS is decarboxylated to PE by ***PS decarboxylase***. PE then undergoes three methylation steps to produce PC, as illustrated in Figure 4. Sadenosylmethionine is the methyl group donor .

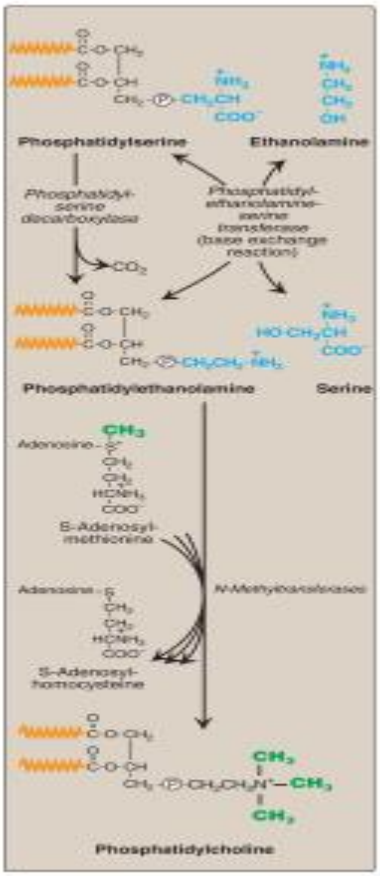



Figure 2 Synthesis of phosphatidylcholine from phosphatidylserine in the liver. ( is a fatty acid hydrocarbon chain.) = phosphate; CO2 = carbon dioxide.

## B.Phosphatidylserine

PS synthesis in mammalian tissues is provided by the base exchange reaction, in which the ethanolamine of PE is exchanged for free serine. This reaction, although reversible, is used primarily to produce the PS required for membrane synthesis. PS has a net negative charge. (See onlinefor the role of PS in clotting.) D. Phosphatidylinositol PI is synthesized from free inositol and CDP-

DAG, as shown in. PI is an unusual phospholipid in that it most frequently contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol.Therefore, PI serves as a reservoir of arachidonic acid in membranes and,thus, provides the substrate for prostaglandin synthesis when required. Like PS, PI has a net negative charge. [Note: There is asymmetry in the phospholipid composition of the cell membrane. PS and PI, for example, are found primarily on the inner leaflet. Asymmetry is achieved by ATP-dependent enzymes known as "flippases" and "floppases."] 1. Role in signal transduction across membranes: The phosphorylation of membrane-bound PI produces polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate ([PIP2]; The cleavage of PIP2 by ***phospholipase C*** occurs in response to the binding of various neurotransmitters, hormones, and growth factors to G protein–coupled receptors (GPCR), such as the α1 adrenergic receptor, on the cell membrane and activation of the G q α-subunit The products of this cleavage, inositol 1,4,5-trisphosphate (IP3) and DAG, mediate the mobilization of intracellular calcium and the activation of ***protein kinase C***, which act synergistically to evoke specific cellular responses. Signal transduction across the membrane is, thus, accomplished.

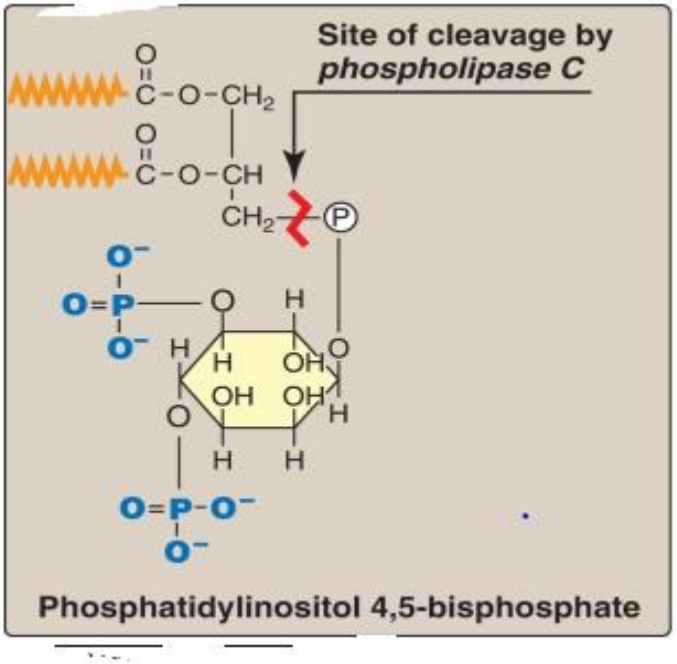



Figure 3 Structure of phosphatidylinositol 4,5-bisphosphate (PIP2). Cleavage by ***phospholipase C*** produces inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. ( is a fatty acid hydrocarbon chain.) = phosphate.

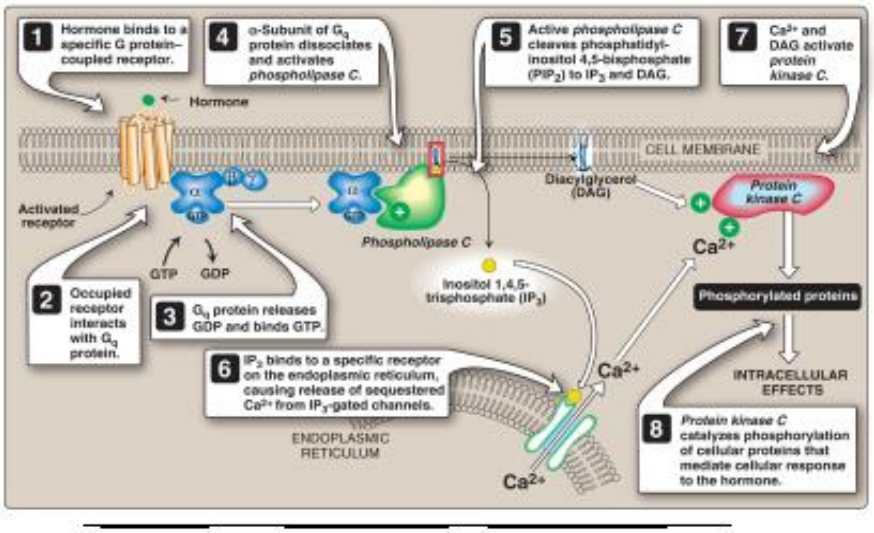



Figure 4 Role of inositol triphosphate and diacylglycerol in cell signaling GDP and GTP = guanosine di- and triphosphates; Ca2+ = calcium.

Role in membrane protein anchoring: Specific proteins can be covalently attached through a carbohydrate bridge to membrane-bound PI. For example, ***lipoprotein lipase***, an enzyme that degrades triacylglycerol in lipoprotein particles, is attached to capillary endothelial cells by a glycosyl phosphatidylinositol (GPI) anchor. [Note: GPI-linked proteins are also found in a variety of parasitic protozoans, such as trypanosomes and leishmania.] Being attached to a membrane lipid (rather than being an integral part of the membrane) allows GPI-anchored proteins increased lateral mobility on the extracellular surface of the plasma membrane. The protein can be cleaved from its anchor by the action of ***phospholipase C*** [Note: A deficiency in the synthesis of GPI in hematopoietic cells results in the hemolytic disease paroxysmal nocturnal hemoglobinuria, because GPI-anchored proteins protect blood cells from complement-mediated lysis.]

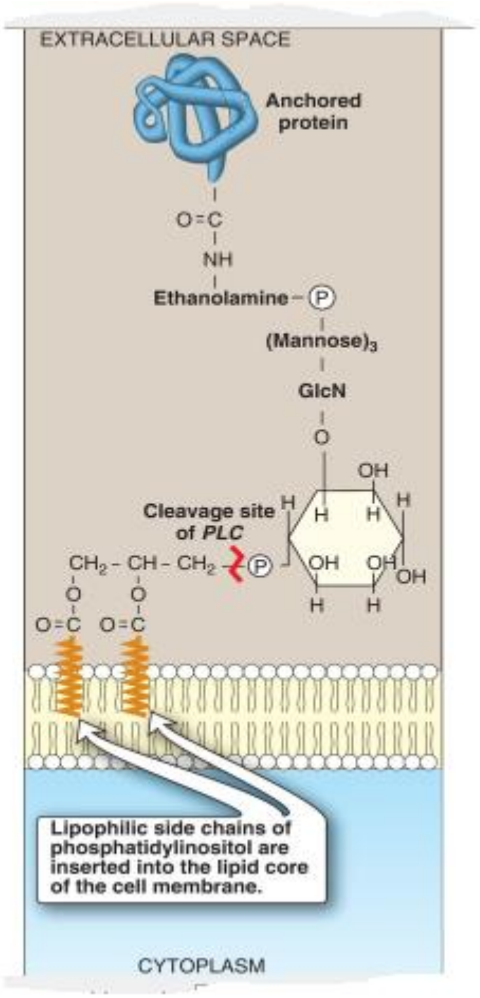



Figure 5 Example of a glycosyl phosphatidylinositol (GPI) membrane protein anchor. GlcN = glucosamine; = phosphate; ***PLC*** = ***phospholipase C***

**E. Phosphatidylglycerol and cardiolipin**

Phosphatidylglycerol occurs in relatively large amounts in mitochondrial membranes and is a precursor of cardiolipin (diphosphatidyglycerol). It is synthesized from CDP-DAG and glycerol 3-phosphate. Cardiolipin is synthesized by the transfer of DAG 3-phosphate from CDPDAG to a pre-existing molecule of phosphatidylglycerol.

**F. Sphingomyelin**

Sphingomyelin, a sphingosine-based phospholipid, is found in cell membranes and in the myelin sheath. The synthesis of sphingomyelin is shown in Briefly, palmitoyl CoA condenses with serine, as CoA and the carboxyl group (as CO2) of serine are lost. [Note: This reaction, like the decarboxylation reactions involved in the synthesis of PE from PS and of regulators from amino acids for example, the catecholamines from tyrosine, requires pyridoxal phosphate (a derivative of vitamin B6) as a coenzyme.] The product is reduced in a nicotinamide adenine dinucleotide phosphate (NADPH)-requiring reaction to

sphinganine (dihydrosphingosine). The sphinganine is acylated at the amino group with one of a variety of LCFA and then desaturated to produce a ceramide, the immediate precursor of sphingomyelin and other sphingolipids, as described on p.

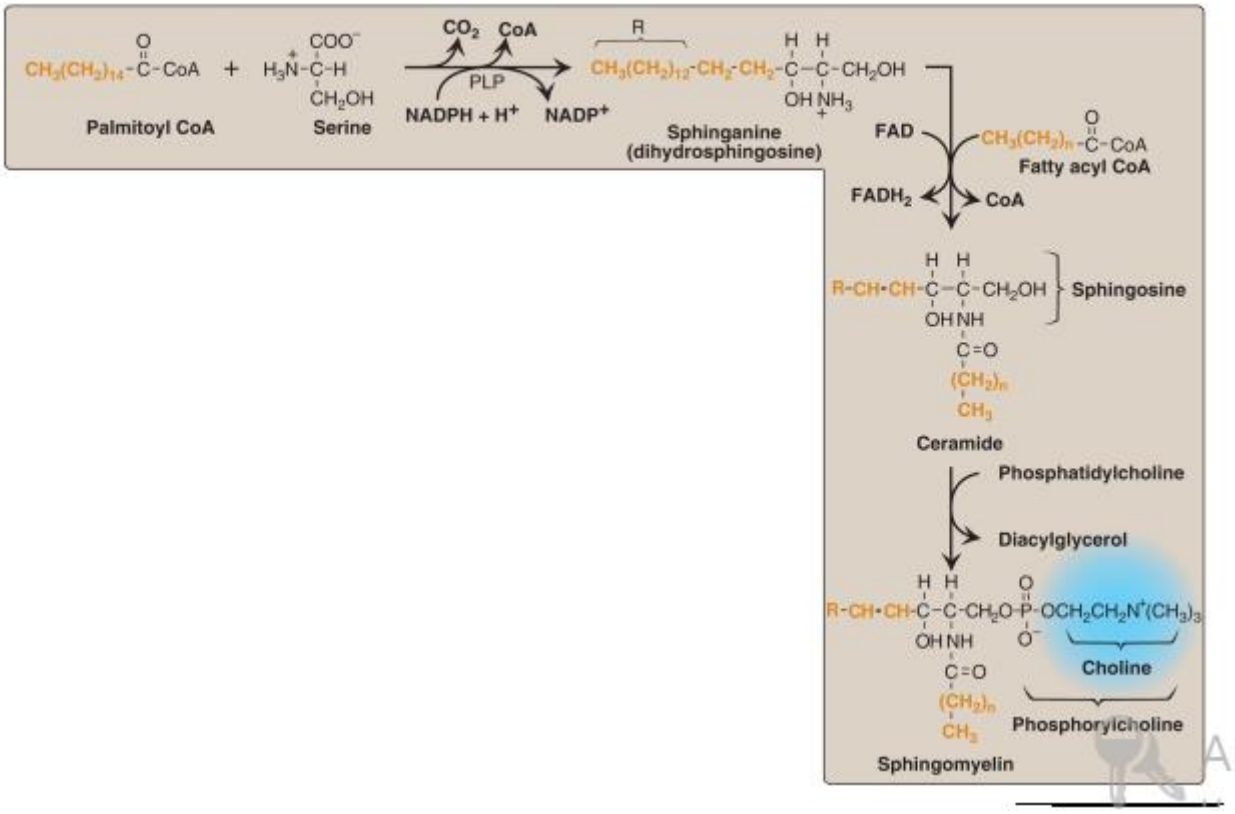



Figure 6 Synthesis of sphingomyelin. PLP = pyridoxal phosphate;NADP(H) = nicotinamide adenine dinucleotide phosphate; FAD(H2) = flavin adenine dinucleotide; CoA = coenzyme A.

Ceramides play a key role in maintaining the skin's water-permeability barrier. Decreased ceramide levels are associated with a number of skin diseases. Phosphorylcholine from PC is transferred to the ceramide, producing sphingomyelin and DAG. [Note: Sphingomyelin of the myelin sheath contains predominantly longer-chain FA such as lignoceric acid and nervonic acid, whereas gray matter of the brain has sphingomyelin that contains primarily stearic acid.]

PHOSPHOLIPID DEGRADATION

The degradation of phosphoglycerides is performed by ***phospholipases*** found in all tissues and pancreatic juice. [Note: For a discussion of phospholipid digestion. A number of toxins and venoms have ***phospholipase*** activity, and several pathogenic bacteria produce ***phospholipases*** that dissolve cell membranes

and allow the spread of infection. Sphingomyelin is degraded by the lysosomal ***phospholipase***, ***sphingomyelinase*** (see B. below).

Phosphoglycerides

***Phospholipases*** hydrolyze the phosphodiester bonds of phosphoglycerides,with each enzyme cleaving the phospholipid at a specific site. The major ***phospholipases*** are shown in. [Note: Removal of the FA from carbon 1 or 2 of a phosphoglyceride produces a lysophosphoglyceride,which is the substrate for ***lysophospholipases***.] ***Phospholipases*** release molecules that can serve as second messengers (for example, DAG and IP3) or that are the substrates for synthesis of messengers (for example,arachidonic acid). ***Phospholipases*** are responsible not only for degrading phospholipids but also for remodeling them. For example, ***phospholipases A1*** and ***A2*** remove specific FA from membrane-bound phospholipids, which can be replaced with different FA using ***fatty acyl CoA transferase***. This mechanism is used as one way to create the unique lung surfactant DPCC and to insure that carbon 2 of PI (and sometimes of PC) is bound to arachidonic acid. [Note: Barth syndrome, a rare X-linked disorder characterized by cardiomyopathy, muscle weakness, and neutropenia, is the result of defects in cardiolipin remodeling.]

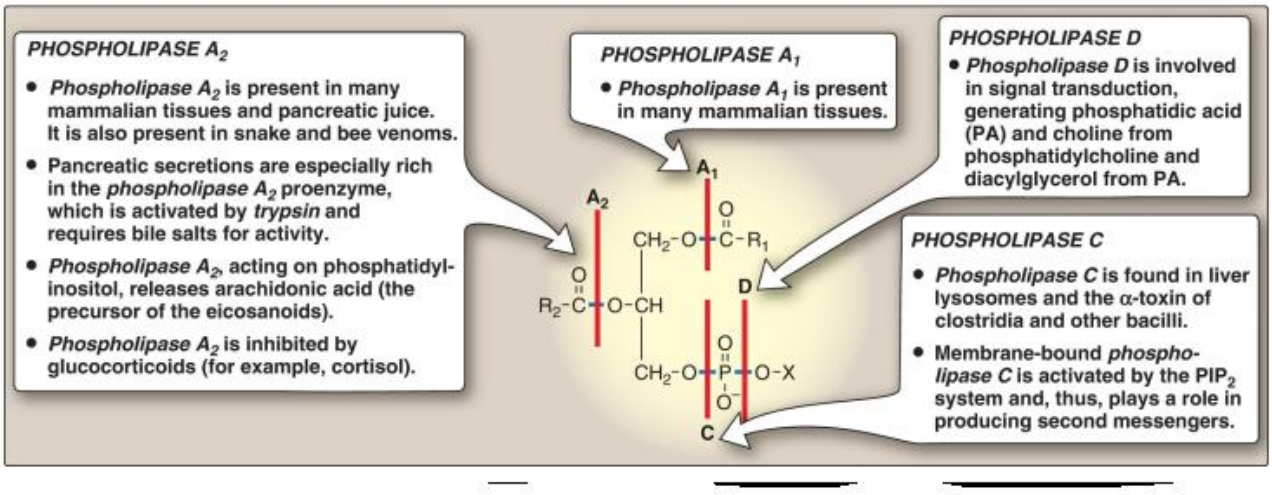



Figure 7 Degradation of glycerophospholipids by ***phospholipases***. PIP2 = phosphatidylinositol 4,5-bisphosphate; R1 and R2 = fatty acids; X = an alcohol.

**B. Sphingomyelin**

Sphingomyelin is degraded by ***sphingomyelinase***, a lysosomal enzyme that removes phosphorylcholine, leaving a ceramide. The ceramide is, in turn,cleaved by ***ceramidase*** into sphingosine and a free FA. [Note:The released ceramide and

sphingosine regulate signal transduction pathways, in part by influencing the activity of ***protein kinase C*** and, thus,the phosphorylation of its protein substrates. They also promote apoptosis.] Niemann-Pick disease (types A and B) is an autosomal-recessive disorder caused by the inability to degrade sphingomyelin due to a deficiency of ***sphingomyelinase***, a type of ***phospholipase C***. In the severe infantile form (type A, which shows <1% of normal enzymic activity), the liver and spleen are the primary sites of lipid deposits and are, therefore, greatly enlarged. The lipid consists primarily of the sphingomyelin that cannot be degraded. Infants with this lysosomal storage disease experience rapid and progressive neurodegeneration as a result of deposition of sphingomyelin in the CNS, and they die in early childhood. A less severe variant (type B, which shows up to 10% of normal activity) with a later age of onset and a longer survival time causes little to no damage to neural tissue, but lungs, spleen, liver, and bone marrow are affected, resulting in a chronic form of the disease. Although Niemann-Pick disease occurs in all ethnic groups, type A occurs with greater frequency in the Ashkenazi Jewish population.

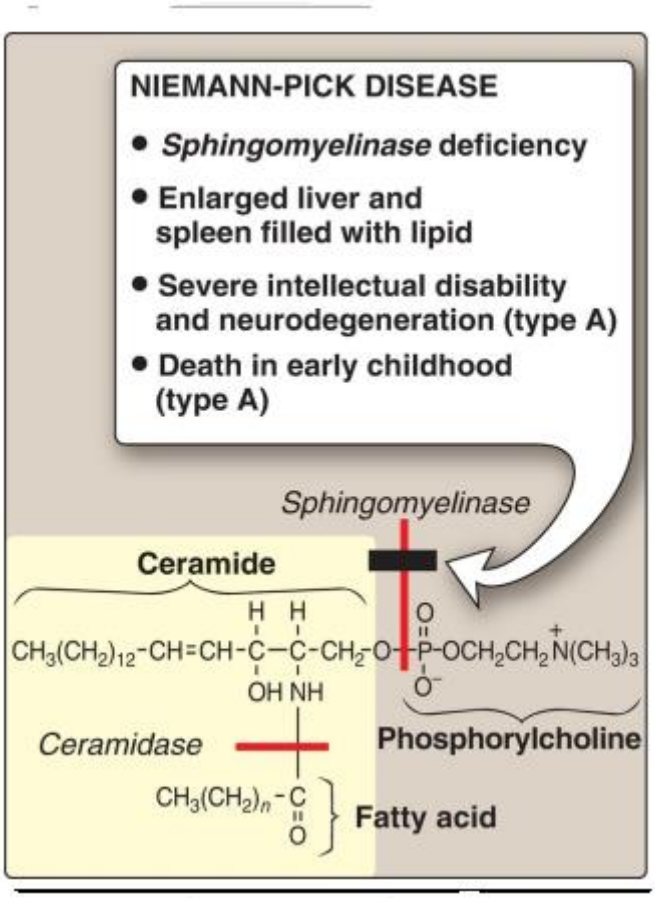



Figure 8 Degradation of sphingomyelin. [Note: Type B is the nonneuropathic form. It has a later age of onset and a longer survival time than type A.]

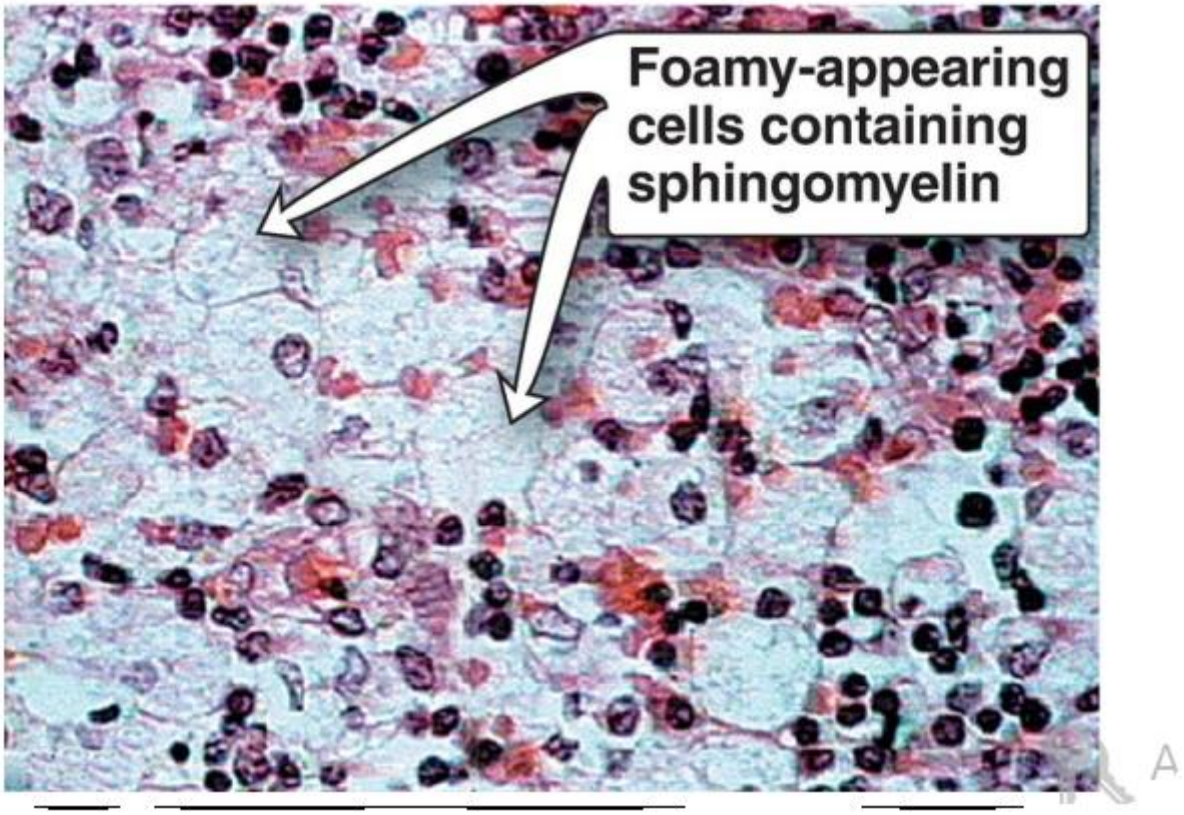



Figure 9 Accumulation of lipids in spleen cells from a patient with Niemann-Pick disease.

GLYCOLIPID OVERVIEW

Glycolipids are molecules that contain both carbohydrate and lipid components.Like the phospholipid sphingomyelin, glycolipids are derivatives of ceramides inwhich a LCFA is attached to the amino alcohol sphingosine. Therefore, they aremore precisely called glycosphingolipids. [Note: Thus, ceramides are theprecursors of both phosphorylated and glycosylated sphingolipids.] Like thephospholipids, glycosphingolipids are essential components of all membranes inthe body, but they are found in greatest amounts in nerve tissue. They are locatedin the outer leaflet of the plasma membrane, where they interact with theextracellular environment. As such, they play a role in the regulation of cellularinteractions (for example, adhesion and recognition), growth, and development.

**Membrane glycosphingolipids associate with cholesterol and GPI-anchoredproteins to form lipid rafts, laterally mobile microdomains of the plasmamembrane that function to organize and regulate membrane signaling andtrafficking functions**.

Glycosphingolipids are antigenic and are the source of ABO blood group antigens, various embryonic antigens specific for particular stages offetal development, and some tumor antigens. [Note: The carbohydrate portion ofa glycolipid is the antigenic determinant.] They have been coopted for use as cellsurface receptors for cholera and tetanus toxins as well as for certain viruses andmicrobes. Genetic disorders associated with an inability to properly degrade theglycosphingolipids result in lysosomal accumulation of these compounds. [Note:Changes in the carbohydrate portion of glycosphingolipids (and glycoproteins)are characteristic of transformed cells (cells with dysregulated growth).] GLYCOSPHINGOLIPID STRUCTURE

The glycosphingolipids differ from sphingomyelin in that they do not containphosphate, and the polar head function is provided by a monosaccharide oroligosaccharide attached directly to the ceramide by an O-glycosidic bond. The number and type of carbohydrate moieties present determine thetype of glycosphingolipid.

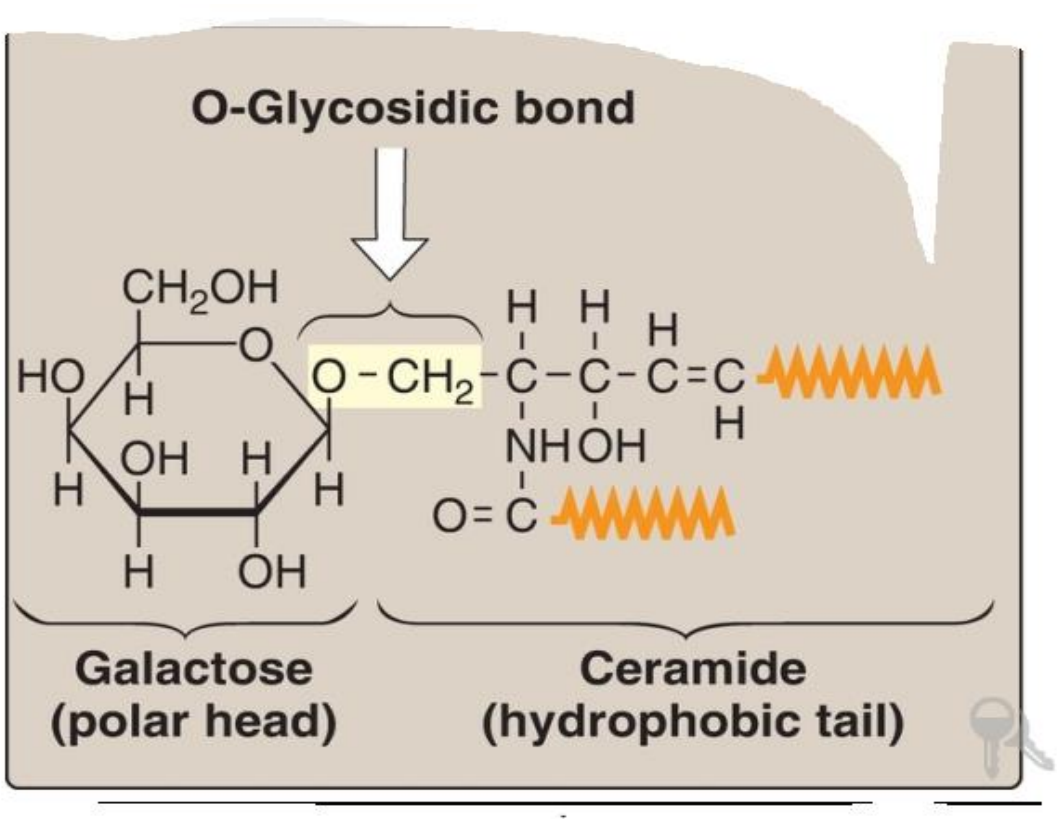



Figure 10 Structure of a neutral glycosphingolipid, galactocerebroside. ( is a hydrophobic hydrocarbon chain.)

**A.Neutral glycosphingolipids**

The simplest neutral glycosphingolipids are the cerebrosides. These are ceramide monosaccharides that contain either a molecule of galactose (forming

ceramide-galactose or galactocerebroside, the most common cerebroside found in myelin, as shown in or glucose (forming ceramide-glucose or glucocerebroside, an intermediate in the synthesis and degradation of the more complex glycosphingolipids). [Note: Members of a group of galacto- or glucocerebrosides may also differ from each other in the type of FA attached to the sphingosine.] As their name implies,cerebrosides are found predominantly in the brain and peripheral nerves, with high concentrations in the myelin sheath. Ceramide oligosaccharides (or globosides) are produced by attaching additional monosaccharides to a glucocerebroside, for example, ceramide-glucose-galactose (also known as lactosylceramide). The additional monosaccharides can include substituted sugars such as ***N***-acetylgalactosamine. B. Acidic glycosphingolipids Acidic glycosphingolipids are negatively charged at physiologic pH. The negative charge is provided by N-acetylneuraminic acid ([NANA], a sialic acid, as shown in gangliosides or by sulfate groups in sulfatides.

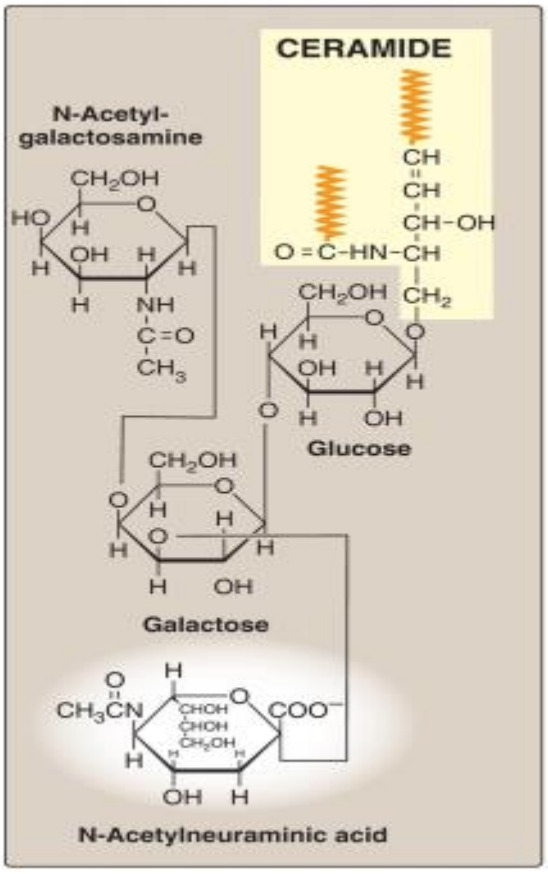



Figure 11 Structure of the ganglioside GM2. is a hydrophobic hydrocarbon chain.

**Gangliosides:** These are the most complex glycosphingolipids and are found primarily in the ganglion cells of the CNS, particularly at the nerve endings. They are derivatives of ceramide oligosaccharides and contain one or more molecules of NANA (from CMP-NANA). The notation for these compounds is G (for

ganglioside) plus a subscript M, D, T, or Q to indicate whether there is one (mono), two (di), three (tri), or four (quatro) molecules of NANA in the ganglioside, respectively. Additional numbers and letters in the subscript designate the monomeric sequence of the carbohydrate attached to the ceramide. (for the structure of GM2.) Gangliosides are of medical interest because several lipid storage disorders involve the accumulation of NANA-containing glycosphingolipids in cells. Sulfatides: These sulfoglycosphingolipids are sulfated galactocerebrosides that are negatively charged at physiologic pH.Sulfatides are found predominantly in the brain and kidneys. GLYCOSPHINGOLIPID SYNTHESIS AND DEGRADATION

Synthesis of glycosphingolipids occurs primarily in the Golgi by sequential addition of glycosyl monomers transferred from UDP-sugar donors to the acceptor molecule. The mechanism is similar to that used in glycoprotein synthesis

A. Enzymes involved in synthesis

The enzymes involved in the synthesis of glycosphingolipids are ***glycosyltransferases*** that are specific for the type and location of the glycosidic bond formed. [Note: These enzymes can recognize both glycosphingolipids and glycoproteins as substrates.]

B. Sulfate group addition

A sulfate group from the sulfate carrier 3′-phosphoadenosine-5′- phosphosulfate ([PAPS], is added by a ***sulfotransferase*** to the 3′-hydroxyl group of the galactose in a galactocerebroside, forming the sulfatidegalactocerebroside 3-sulfate . [Note: PAPS is also the sulfur donor in glycosaminoglycan synthesis and steroid hormone catabolism (see p. 240).] An overview of the synthesis of sphingolipids is shown in.

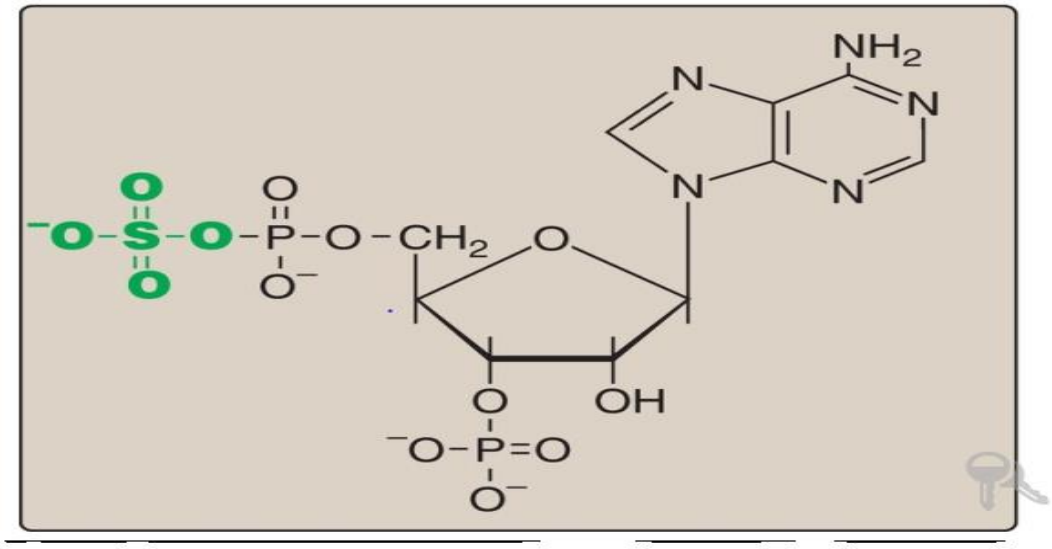


Figure 12 Structureof 3′-phosphoadenosine-5′-phosphosulfate

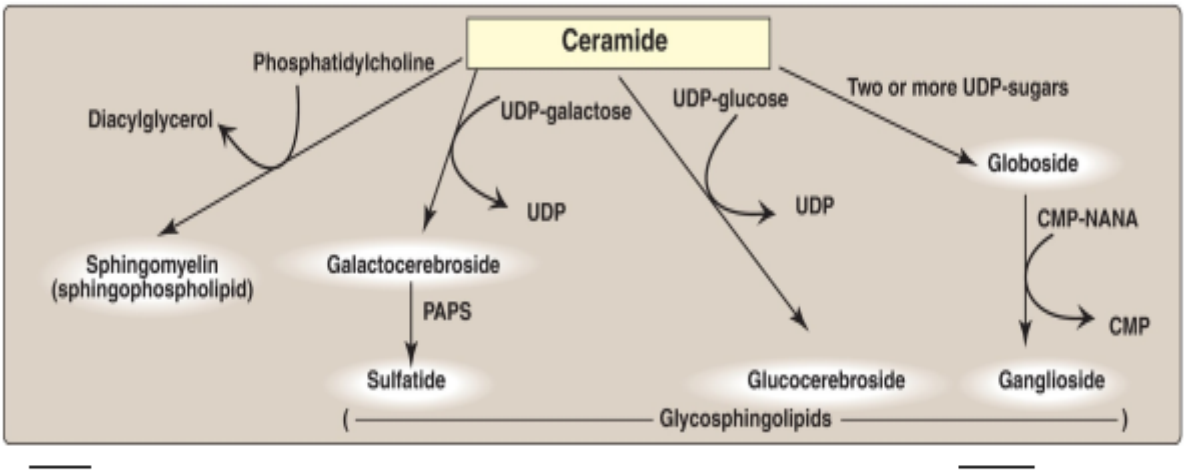



Figure 13 Overview of sphingolipid synthesis. UDP = uridine diphosphate;CMP = cytidine monophosphate; NANA = N-acetylneuraminic acid; PAPS = 3′-phosphoadenosine-5′-phosphosulfate.

**C. Glycosphingolipid degradation**

Glycosphingolipids are internalized by phagocytosis as described for the glycosaminoglycans. All of the enzymes required for the degradative process are present in lysosomes, which fuse with the phagosomes. The lysosomal enzymes hydrolytically and irreversibly cleave specific bonds in the glycosphingolipid. As seen with the glycosaminoglycans and glycoproteins, degradation is a sequential process following the rule “last on, first off,” in which the last group added during synthesis is the first group removed in degradation. Therefore, defects in the degradation of the polysaccharide chains in these three glycoconjugates result in lysosomal storage diseases.

## D. Sphingolipidoses

In a normal individual, synthesis and degradation of glycosphingolipids are balanced, so that the amount of these compounds present in membranes is constant. If a specific lysosomal ***acid hydrolase*** required for degradation is partially or totally missing, a sphingolipid accumulates. Lysosomal lipid storage diseases caused by these deficiencies are called sphingolipidoses.The result of a specific ***acid hydrolase*** deficiency may be seen dramatically in nerve tissue, where neurologic deterioration can lead to early deathprovides an outline of the pathway of sphingolipid degradation and descriptions of some sphingolipidoses. [Note: Some sphingolipidoses can also result from defects in lysosomal activator proteins (for example, the saposins) that facilitate access of the ***hydrolases*** to short carbohydrate chains as degradation proceeds.]

1. Common properties: A specific lysosomal hydrolytic enzyme is deficient in the classic form of each disorder. Therefore, usually, only a single sphingolipid (the substrate for the deficient enzyme) accumulates in the involved organs in each disease. [Note: The rate of biosynthesis of the accumulating lipid is normal.] The disorders are progressive and, although many are fatal in childhood, extensive phenotypic variability is seen leading to the designation of different clinical types, such as types A and B in Niemann-Pick disease. Genetic variability is also seen because a given disorder can be caused by any one of a variety of mutations within a single gene. The sphingolipidoses are autosomal-recessive disorders,except for Fabry disease, which is X linked. The incidence of the sphingolipidoses is low in most populations, except for Gaucher and Tay-Sachs diseases, which, like Niemann-Pick disease, show a high frequency in the Ashkenazi Jewish population. [Note: Tay-Sachs also has a high frequency in Irish American, French Canadian, and Louisiana Cajun populations.]

2. Diagnosis and treatment: A specific sphingolipidosis can be diagnosed by measuring enzyme activity in cultured fibroblasts or peripheral leukocytes or by analyzing DNA. Histologic examination of the affected tissue is also useful. [Note: Shell-like inclusion bodies are seen in Tay-Sachs, and a crumpled tissue paper appearance of the cytosol is seen in Gaucher disease. Prenatal diagnosis, using cultured amniocytes or chorionic villi, is available. Gaucher disease, in which macrophages become engorged with glucocerebroside, and Fabry disease, in which globosides accumulate in the vascular endothelial lysosomes of the brain, heart, kidneys, and skin,are treated by recombinant human enzyme replacement therapy, but the monetary cost is extremely high. Gaucher has also been treated by bone marrow transplantation (because macrophages are derived from hematopoietic

stem cells) and by substrate reduction therapy through pharmacologic reduction of glucosylceramide, the substrate for the deficient enzyme

## EICOSANOIDS:PROSTAGLANDINS,THROMBOXANES,AND LEUKOTRIENES

Prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT) are collectively known as eicosanoids to reflect their origin from ω-3 and ω-6 polyunsaturated FA with 20 carbons (eicosa = 20). They are extremely potent compounds that elicit a wide range of responses, both physiologic (inflammatory response) and pathologic (hypersensitivity). They insure gastric integrity and renal function,regulate smooth muscle contraction (the intestine and uterus are key sites) and blood vessel diameter, and maintain platelet homeostasis. Although they have been compared to hormones in terms of their actions, eicosanoids differ from endocrine hormones in that they are produced in very small amounts in almost all tissues rather than in specialized glands and act locally rather than after transport in the blood to distant sites. Eicosanoids are not stored, and they have an extremely short half-life, being rapidly metabolized to inactive products.Their biologic actions are mediated by plasma membrane GPCR which are different in different organ systems and typically result in changes in cyclic adenosine monophosphate production. Examples of eicosanoid structures are shown.

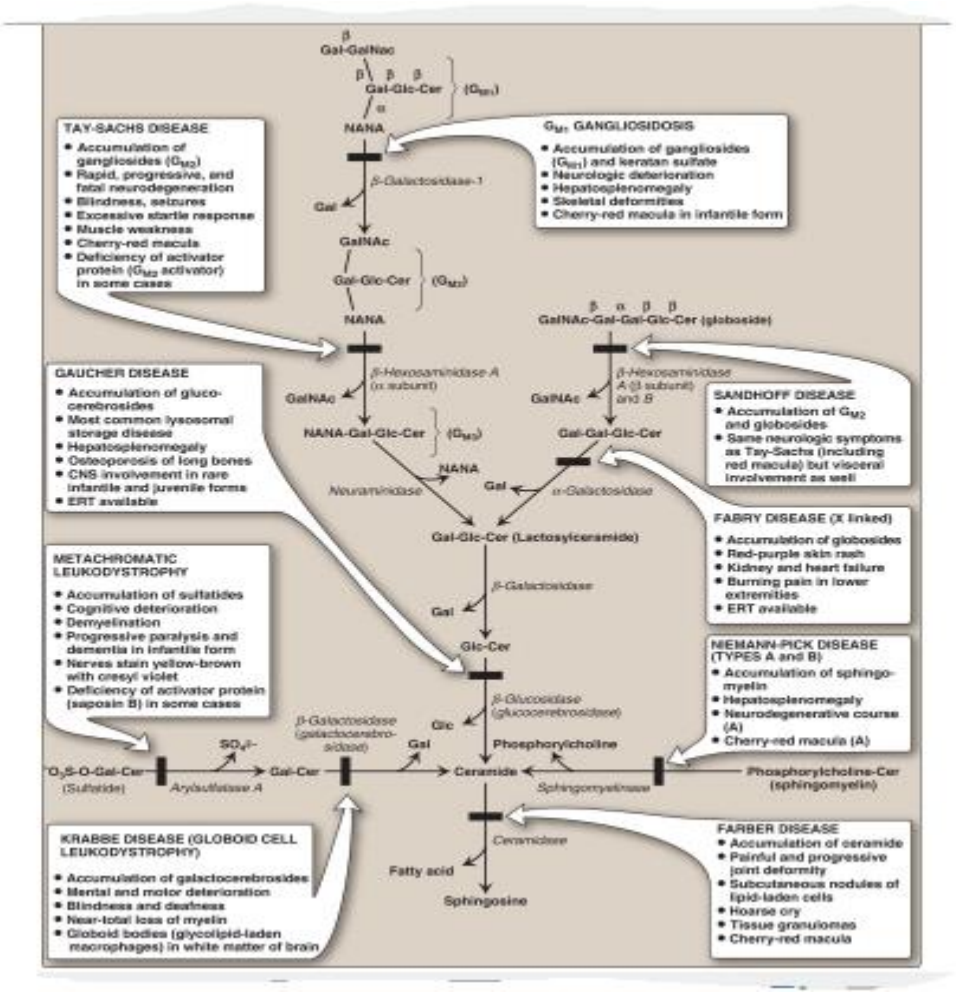



Figure 14 Degradation of sphingolipids showing the lysosomal enzymes affected in related genetic diseases, the sphingolipidoses. All are autosomalrecessive diseases except Fabry, which is X linked, and all can be fatal in early life. Cer = ceramide; Gal = galactose; Glc = glucose; GalNAc = Nacetylgalactosamine; NANA = N-acetylneuraminic acid; CNS = central nervous system. = sulfate; ERT = enzyme replacement therapy.

A.Prostaglandin and thromboxane synthesis

Arachidonic acid, an ω-6 FA containing 20 carbons and four double bonds (an eicosatetraenoic FA), is the immediate precursor of the predominant type of human PG (series 2 or those with two double bonds, as shown in. It is derived by the elongation and desaturation of the essential FA linoleic acid, also an ω-6 FA. Arachidonic acid is incorporated into membrane phospholipids (typically PI) at carbon 2, from which it is released by ***phospholipase A2*** in response to a variety of signals, such as a rise in calcium. [Note: Series 1 PG contain one double bond and are derived from an ω-6 eicosatrienoic FA, dihomo-γ-linolenic acid, whereas series 3 PG contain three double bonds and are derived from eicosapentaenoic acid (EPA), an ω-3 FA.]

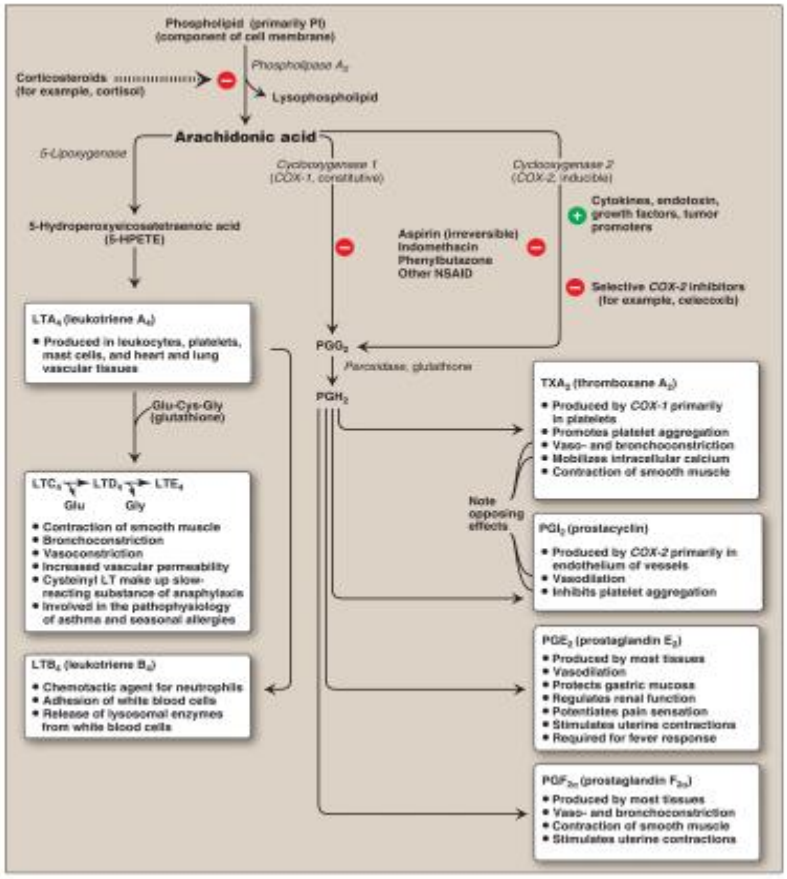



Figure 15 Overview of the biosynthesis and function of some important prostaglandins (PG), leukotrienes (LT), and a thromboxane (TX) from arachidonic acid. [Note: The arachidonic acid in the membrane phospholipid was derived from the ω-6 essential fatty acid (FA), linoleic, also an ω-6 FA.] PI = phosphatidylinositol; NSAID = nonsteroidal anti-inflammatory drugs; Glu = glutamate; Cys = cysteine; Gly = glycine.

Prostaglandin H2 synthase: The first step in PG and TX synthesis is the oxidative cyclization of free arachidonic acid to yield PGH2 by ***PGH2 synthase*** (or, ***prostaglandin endoperoxide synthase***). This enzyme is an ER membrane-bound protein that has two catalytic activities: ***fatty acid cyclooxygenase*** (***COX***), which requires two molecules of O2, and ***peroxidase***, which requires reduced glutathione (see p. 148). PGH2 is converted to a variety of PG and TX, as shown in, by cellspecific***synthases***. [Note: PG contain a five-carbon ring, whereas TX contain a heterocyclic six-membered oxane  Two isozymes of ***PGH2 synthase***, usually denoted as ***COX-1*** and ***COX-2***, are known. ***COX-1*** is made constitutively in most tissues and is required for aintenance of healthy gastric tissue, renal homeostasis, and platelet aggregation. ***COX-2*** is inducible in a limited number of tissues in response to products of activated immune and inflammatory cells. [Note:The increase in PG synthesis subsequent to the induction of ***COX-2***mediates

the pain, heat, redness, and swelling of inflammation and thefever of infection.]2. Synthesis inhibition: The synthesis of PG and TX can be inhibited byunrelated compounds. For example, cortisol (a steroidal antiinflammatory agent) inhibits ***phospholipase A2*** activity and, therefore, arachidonic acid, the substrate for PG and TX synthesis, isnot released from membrane phospholipids. Aspirin, indomethacin, andphenylbutazone (all nonsteroidal anti-inflammatory drugs [NSAID])inhibit both ***COX-1*** and ***COX-2*** and, thus, prevent the synthesis of theparent molecule, PGH2. [Note: Systemic inhibition of ***COX-1***, withsubsequent damage to the stomach and the kidneys and impaired clottingof blood, is the basis of aspirin's toxicity.] Aspirin (but not other NSAID)also induces synthesis of lipoxins (anti-inflammatory mediators madefrom arachidonic acid) and resolvins and protectins (inflammationresolving mediators made from EPA). Inhibitors specific for ***COX-2*** (thecoxibs, for example, celecoxib) were designed to reduce pathologicinflammatory processes mediated by ***COX-2*** while maintaining thephysiologic functions of ***COX-1***. However, their use has been associatedwith increased risk of heart attacks, likely as a result of decreased PGI2synthesis (see B. below), and some have been withdrawn from themarket.

B. Thromboxanes and prostaglandins in platelethomeostasis

Thromboxane A2 (TXA2) is produced by ***COX-1*** in activated platelets. Itpromotes platelet adhesion and aggregation and contraction of vascularsmooth muscle, thereby promoting formation of blood clots (thrombi). Prostacyclin (PGI2), produced by ***COX-2*** in vascularendothelial cells, inhibits platelet aggregation and stimulates vasodilationand, so, impedes thrombogenesis. The opposing effects of TXA2 and PGI2limit thrombi formation to sites of vascular injury. [Note: Aspirin has anantithrombogenic effect. It inhibits TXA2 synthesis by ***COX-1*** in plateletsand PGI2 synthesis by ***COX-2*** in endothelial cells through irreversible acetylation of these isozymes. ***COX-1*** inhibition cannot beovercome in platelets, which lack nuclei. However, ***COX-2*** inhibition canbe overcome in endothelial cells because they have a nucleus and,therefore, can generate more of the enzyme. This difference is the basis oflow-dose aspirin therapy used to lower the risk of stroke and heart attacksby decreasing formation of thrombi.]

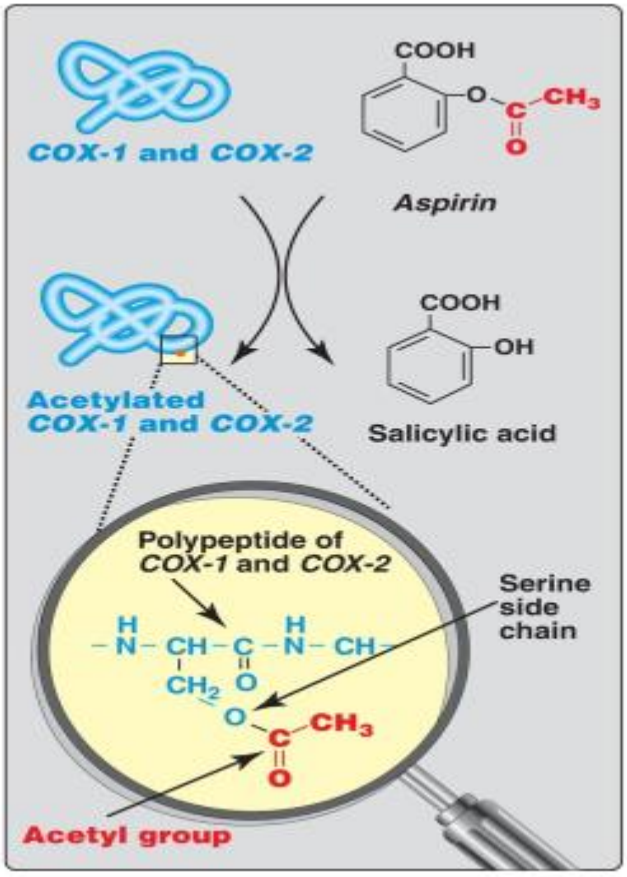



Figure 16 Irreversible acetylation of ***cyclooxygenase*** (***COX*)-*1 and COX-2*** by aspirin.

C. Leukotriene synthesis Arachidonic acid is converted to a variety of linear hydroperoxy (–OOH)acids by a separate pathway involving a family of ***lipoxygenases*** (***LOX***).For example, ***5-LOX*** converts arachidonic acid to 5-hydroperoxy-6,8,11,14eicosatetraenoic acid ([5-HPETE]; 5-HPETE is convertedto a series of LT containing four double bonds, the nature of the finalproducts varying according to the tissue. LT are mediators of allergicresponse and inflammation. Inhibitors of ***5-LOX*** and LT-receptorantagonists are used in the treatment of asthma. [Note: LT synthesis isinhibited by cortisol and not by NSAID. Aspirin-exacerbated respiratorydisease is a response to LT overproduction with NSAID use in ~10% ofindividuals with asthma.]

CHAPTER SUMMARY

Phospholipids are polar, ionic compounds composed of an alcohol (forexample, choline or ethanolamine) attached by a phosphodiester bondeither to diacylglycerol (DAG), producing phosphatidylcholine orphosphatidylethanolamine, or to the amino alcohol sphingosine. Addition of a long-chain fatty acid to sphingosine produces a ceramide. Addition of

phosphorylcholine produces the phospholipid sphingomyelin. Phospholipids are the predominant lipids of cell membranes. Nonmembrane phospholipids serve as components of lung surfactant and bile. Dipalmitoylphosphatidylcholine, also called dipalmitoyl lecithin, is the major lipid component of lung surfactant.Insufficient surfactant production causes respiratory distress syndrome Phosphatidylinositol (PI) serves as a reservoir for arachidonic acid in membranes. The phosphorylation of membrane-bound PI produces phosphatidylinositol 4,5-bisphosphate (PIP2). This compound is degraded by ***phospholipase C*** in response to the binding of various neurotransmitters, hormones, and growth factors to membrane G protein–coupled receptors. The products of this degradation, inositol 1,4,5-trisphosphate (IP3) and DAG, mediate the mobilization of intracellular calcium and the activation of ***protein kinase C***, which act synergistically to evoke cellular responses. Specific proteins can be covalently attached via a carbohydrate bridge to membrane-bound PI, forming a glycosyl phosphatidylinositol (GPI) anchor. A deficiency in GPI synthesis in hematopoietic cells results in the hemolytic disease paroxysmal nocturnal hemoglobinuria. The degradation of phosphoglycerides is performed by ***phospholipases*** found in all tissues and pancreatic juice. Sphingomyelin is degraded to a ceramide plus phosphorylcholine by the lysosomal enzyme ***sphingomyelinase***, a deficiency of which causes Niemann-Pick (A and B) disease. Glycosphingolipids are derivatives of ceramides to which carbohydrates have been attached. Adding one sugar molecule to the ceramide produces a cerebroside, adding an oligosaccharide produces a globoside, and adding an acidic N-acetylneuraminic acid molecule produces a ganglioside. Glycosphingolipids are found predominantly in cell membranes of the brain and peripheral nervous tissue, with high concentrations in the myelin sheath. They are antigenic. Glycolipids are degraded in the lysosomes by ***acid hydrolases***. A deficiency of any one of these enzymes causes a sphingolipidosis, in which a characteristic sphingolipid accumulates. Prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), the eicosanoids, are produced in very small amounts in almost all tissues, act locally, and have an extremely short half-life. They serve as mediators of the inflammatory response. Arachidonic acid is the immediate precursor of the predominant class of human PG (those with two double bonds). It is derived by the elongation and desaturation of the essential fatty acid linoleic acid and is stored in the membrane as a component of a phospholipid, generally PI. Arachidonic acid is released from the phospholipid by ***phospholipase A2*** (inhibited by cortisol).Synthesis of the PG and TX begins with the oxidative cyclization of free arachidonic acid to yield PGH2 by ***PGH2 synthase*** (or, ***prostaglandin endoperoxide synthase***), an endoplasmic reticular membrane protein that has two catalytic activities: ***fatty acid cyclooxygenase*** (***COX***) and ***peroxidase***. There are

two isozymes of ***PGH2 synthase***: ***COX-1*** (constitutive) and ***COX-2*** (inducible). Aspirin irreversibly inhibits both. Opposing effects of PGI2 and TXA2 limit clot formation. LT are linearmolecules produced from arachidonic acid by the ***5-lipoxygenase*** (***5-LOX***)pathway. They mediate allergic response.Their synthesis is inhibited by cortisol and not by aspirin.

## Complex lipid metabolism.

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and, in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. Therefore, it is critically important that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the hepatic cholesterol pool from a number of sources including dietary cholesterol as well as that synthesized de novo by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins that carry lipids to the peripheral tissues. In humans, the balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-, cerebro-, and peripheral vascular disease.summarizes the major sources of liver cholesterol and the routes by which cholesterol leaves the liver

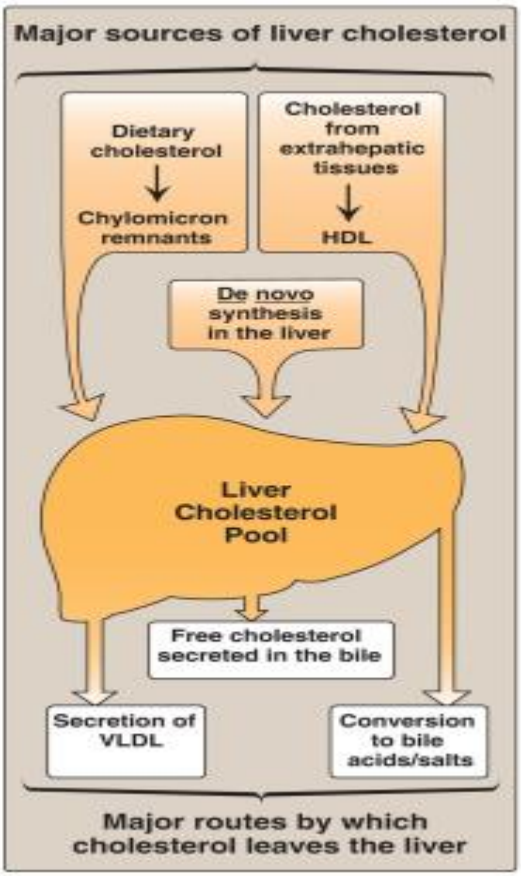



Figure: Sources of liver cholesterol (influx) and routes by which cholesterol leaves the liver (efflux). HDL and VLDL = high- and very-low-density lipoproteins.

Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings (A–D) called the steroid nucleus, and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring. Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbon 5 and carbon 6

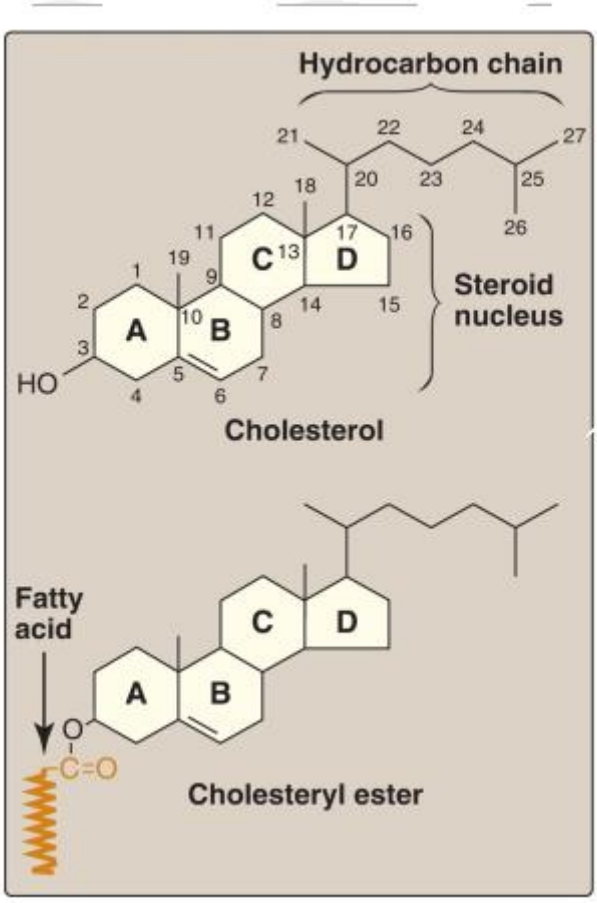



Figure:Structure of cholesterol and its ester.

**A. Sterols**

Steroids with 8 to 10 carbon atoms in the side chain at carbon 17 and a hydroxyl group at carbon 3 are classified as sterols. Cholesterol is the major sterol in animal tissues. It arises from de novo synthesis and absorption of dietary cholesterol. Intestinal uptake of cholesterol is mediated by the Niemann-Pick C1-like 1 protein, the target of the drug ezetimibe that reduces absorption of dietary cholesterol. [Note: Plant sterols (phytosterols), such as β-sitosterol, are poorly absorbed by humans (5% absorbed as compared to 40% for cholesterol). After entering the enterocytes, they are actively transported back into the intestinal lumen. Defects in the efflux transporter (ABCG5/8) result in the rare condition of sitosterolemia. Because some cholesterol is transported back as well, plant sterols reduce the absorption of dietary cholesterol. Daily ingestion of plant sterol esters supplied, for example, in spreads, is one of a number of dietary strategies to reduce plasma cholesterol levels ]

**B. Cholesteryl esters**

Most plasma cholesterol is in an esterified form (with a fatty acid [FA] attached at carbon 3, as shown in, which makes the structure even more hydrophobic than free (nonesterified) cholesterol. Cholesteryl esters are not found in membranes and are normally present only in low levels in most cells. Because of their hydrophobicity, cholesterol and its esters must be transported in association with protein as a component of a lipoprotein particle or be solubilized by phospholipids and bile salts in the bile

CHOLESTEROL SYNTHESIS

Cholesterol is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta, make the largest contributions to the cholesterol pool. As with FA, all the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA), and nicotinamide adenine dinucleotide phosphate (NADPH) provides the reducing equivalents. The pathway is endergonic, being driven by hydrolysis of the highenergy thioester bond of acetyl CoA and the terminal phosphate bond of ATP. Synthesis requires enzymes in the cytosol, the membrane of the smooth endoplasmic reticulum (SER), and the peroxisome. The pathway is responsive to changes in cholesterol concentration, and regulatory mechanisms exist to alance the rate of cholesterol synthesis against the rate of cholesterol excretion. An imbalance in this regulation can lead to an elevation in circulating levels of plasma cholesterol, with the potential for vascular disease. A. 3-Hydroxy-3-methylglutaryl coenzyme A synthesis The first two reactions in the cholesterol biosynthetic pathway are similar to those in the pathway that produces ketone bodies. They result in the production of 3-hydroxy-3-methylglutaryl CoA ([HMG CoA], First, two acetyl CoA molecules condense to form acetoacetyl CoA. Next, a third molecule of acetyl CoA is added by ***HMG CoA synthase***, producing HMG CoA, a six-carbon compound. [Note: Liver parenchymal cells contain two isoenzymes of the ***synthase***. The cytosolic enzyme participates in cholesterol synthesis, whereas the mitochondrial enzyme functions in the pathway for ketone body synthesis.]

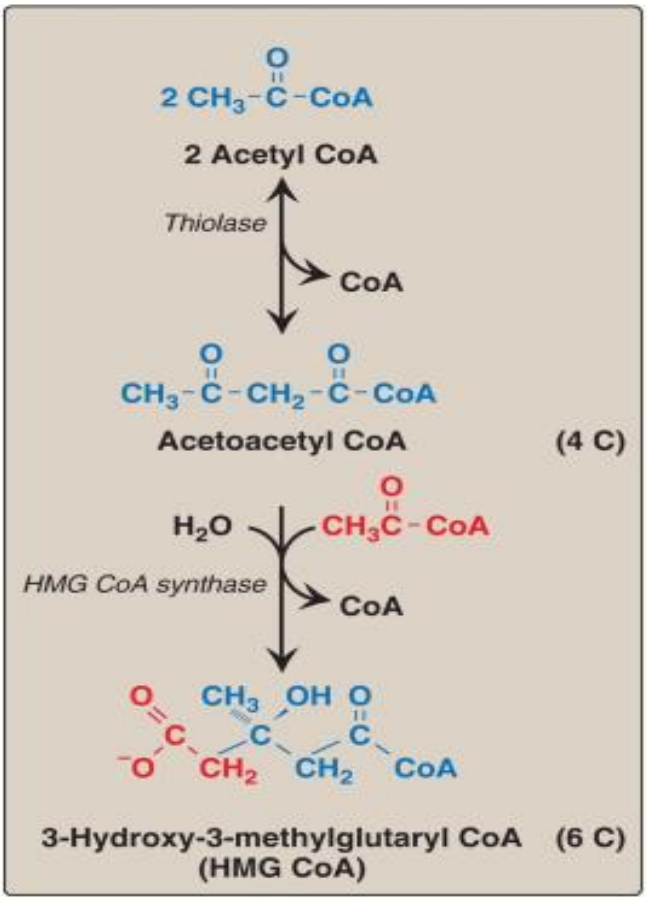



Figure: Synthesis of HMG CoA. CoA = coenzyme A

**B. Mevalonate synthesis**

HMG CoA is reduced to mevalonate by ***HMG CoA reductase.*** This is the rate-limiting and key regulated step in cholesterol synthesis. It occurs in the cytosol, uses two molecules of NADPH as the reducing agent, and releases CoA, making the reaction irreversible. [Note: ***HMG CoA reductase*** is an integral membrane protein of the SER, with its catalytic domain projecting into the cytosol. Regulation of ***reductase*** activity is discussed in D. below.]

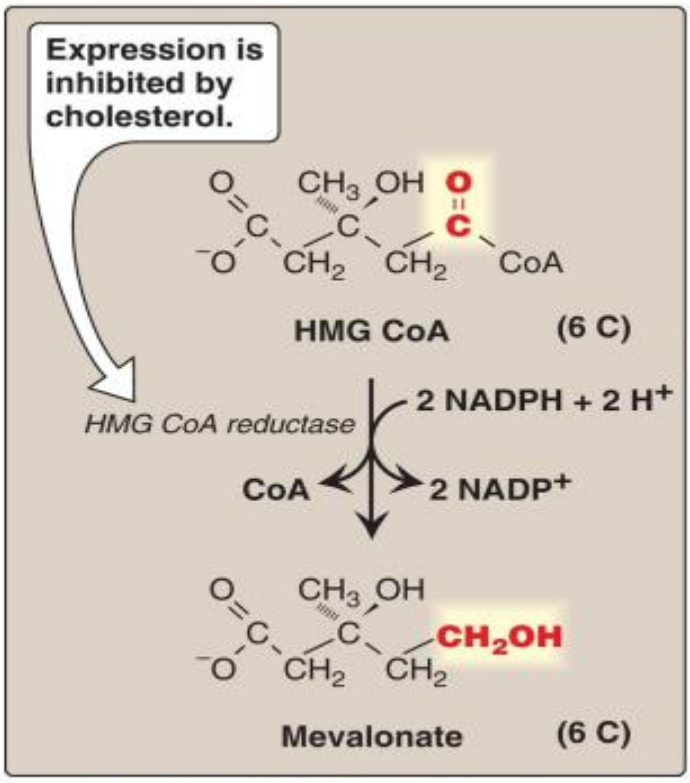



Figure 18.4 Synthesis of mevalonate. HMG CoA = hydroxymethylglutaryl coenzyme A; NADP(H) = nicotinamide adenine dinucleotide phosphate.

### C. Cholesterol synthesis from mevalonate

The reactions and enzymes involved in the synthesis of cholesterol from mevalonate are illustrated in. [Note: The numbers shown in brackets below correspond to numbered reactions shown in this figure.]

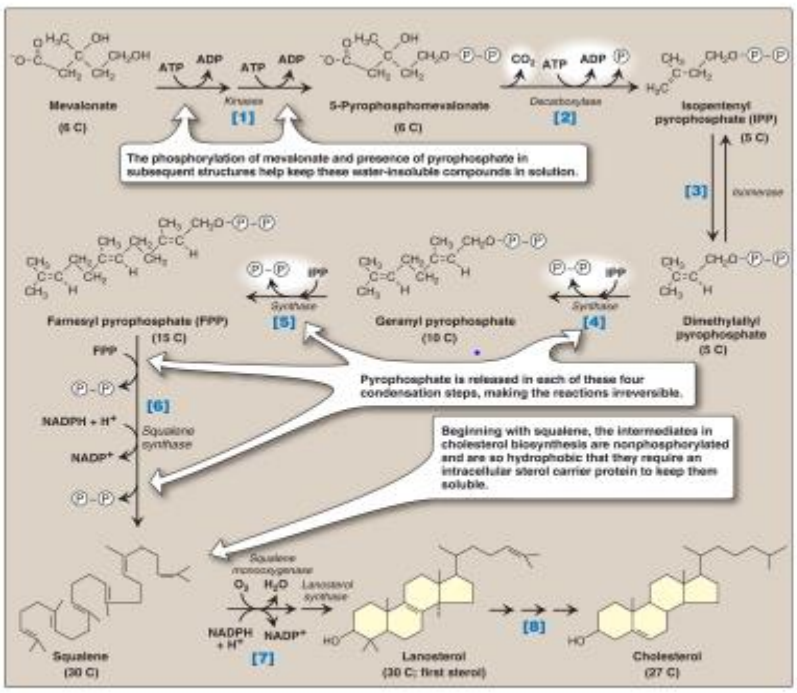



Figure 18.5 Synthesis of cholesterol from mevalonate. ADP = adenosine diphosphate; = phosphate; ~ = pyrophosphate; NADP(H) = nicotinamide adenine dinucleotide phosphate.

[1] Mevalonate is converted to 5-pyrophosphomevalonate in two steps, each of which transfers a phosphate group from ATP.

[2] A five-carbon isoprene unit, isopentenyl pyrophosphate (IPP), is formed by the decarboxylation of 5-pyrophosphomevalonate. The reaction requires ATP. [Note: IPP is the precursor of a family of molecules with diverse functions, the isoprenoids. Cholesterol is a sterol isoprenoid.Nonsterol isoprenoids include dolichol and ubiquinone or, coenzyme Q;

[3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).

[4] IPP and DPP condense to form 10-carbon geranyl pyrophosphate (GPP).

[5] A second molecule of IPP then condenses with GPP to form 15-carbon farnesyl pyrophosphate (FPP). [Note: Covalent attachment of farnesyl to proteins, a process known as prenylation, is one mechanism for anchoring proteins (for example, ras) to the inner face of plasma membranes.]

[6] Two molecules of FPP combine, releasing pyrophosphate, and are reduced, forming the 30-carbon compound squalene. [Note: Squalene is formed from six isoprenoid units. Because 3 ATP are hydrolyzed per mevalonate residue converted to IPP, a total of 18 ATP are required to make the polyisoprenoid squalene.]

[7] Squalene is converted to the sterol lanosterol by a sequence of two reactions catalyzed by SER-associated enzymes that use molecular oxygen (O2) and NADPH. The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.

[8] The conversion of lanosterol to cholesterol is a multistep process involving shortening of the side chain, oxidative removal of methyl groups,reduction of double bonds, and migration of a double bond. Smith-LemliOpitz syndrome (SLOS), an autosomal-recessive disorder of cholesterol biosynthesis, is caused by a partial deficiency in ***7-dehydrocholesterol-7-reductase***, the enzyme that reduces the double bond in 7-dehydrocholesterol (7-DHC), thereby converting it to cholesterol. SLOS is one of several multisystem, embryonic malformation syndromes associated with impaired cholesterol synthesis. [Note: 7-DHC is converted to vitamin D3 in the skin.

D. Cholesterol synthesis regulation

***HMG CoA reductase*** is the major control point for cholesterol biosynthesis and is subject to different kinds of metabolic control. 1. Sterol-dependent regulation of gene expression: Expression of the gene for ***HMG CoA reductase*** is controlled by the trans-acting factor, sterol regulatory element–binding protein-2 (SREBP-2), which binds DNA at the cis-acting sterol regulatory element (SRE) upstream of the ***reductase*** gene. Inactive SREBP-2 is an integral protein of the SER membrane and associates with a second SER membrane protein, SREBP cleavage–activating protein (SCAP). When sterol levels in the SER are low, the SREBP-2–SCAP complex moves from the ER to the Golgi. In the Golgi membrane, SREBP-2 is sequentially acted upon by two ***proteases***, which generate a soluble fragment that enters the nucleus, binds the SRE, and functions as a transcription factor. This results in increased synthesis of ***HMG CoA reductase*** and, therefore, increased cholesterol synthesis. However, if

sterols are abundant, they bind SCAP at its sterolsensing domain and induce the binding of SCAP to yet other ER membrane proteins, the insulin-induced gene proteins (INSIG). This results in the retention of the SCAP–SREBP complex in the SER,thereby preventing the activation of SREBP-2 and leading to downregulation of cholesterol synthesis. [Note: SREBP-1c upregulates expression of enzymes involved in FA synthesis in response to insulin.]

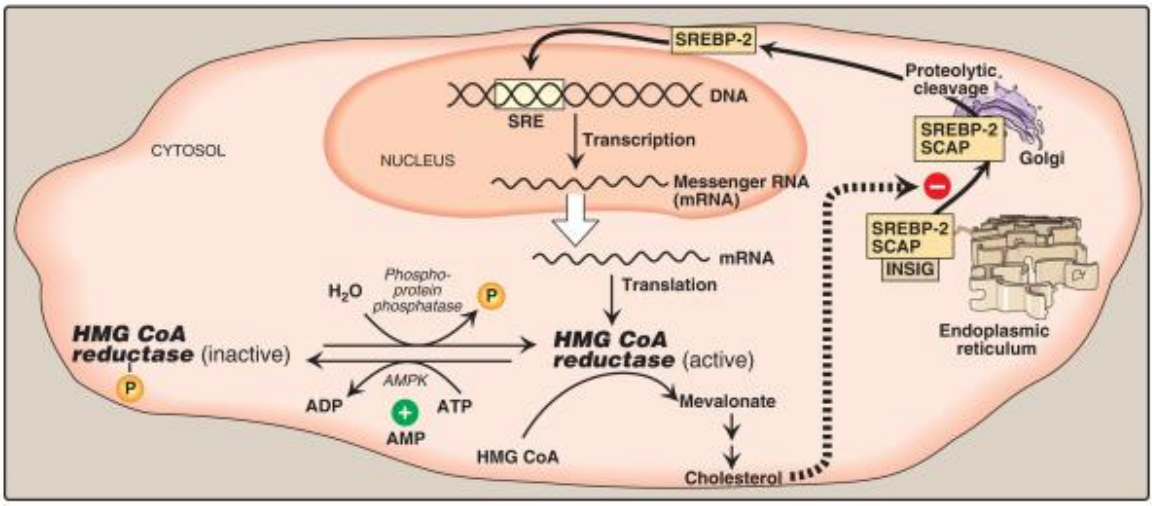



Figure 18.6 Regulation of ***hydroxymethylglutaryl coenzyme A*** (***HMG CoA reductase***. SRE = sterol regulatory element; SREBP = SRE-binding protein SCAP = SREBP cleavage–activating protein; ***AMPK*** = ***adenosine monophosphate–activated protein kinase***; ADP = adenosine diphosphate; phosphate; INSIG = insulin-induced gene protein.

2. Sterol-accelerated enzyme degradation:

The ***reductase*** itself is a sterolsensing integral protein of the SER membrane. When sterol levels in the SER are high, the enzyme binds to INSIG proteins. Binding leads to cytosolic transfer, ubiquitination, and proteasomal degradation of the ***reductase***

3. Sterol-independent phosphorylation/dephosphorylation: ***HMG CoA reductase*** activity is controlled covalently through the actions of ***adenosine monophosphate*** (***AMP***)–***activated protein kinase*** ([***AMPK***] and a ***phosphoprotein phosphatase***. The phosphorylated form of the enzyme is inactive, whereas the dephosphorylated form is active. [Note: Because ***AMPK*** is activated by AMP, cholesterol synthesis, like FA synthesis, is decreased when ATP availability is decreased.]

4. Hormonal regulation: The activity of ***HMG CoA reductase*** is controlled hormonally. An increase in insulin favors dephosphorylation (activation) of the ***reductase***, whereas an increase in glucagon and epinephrine has the opposite effect.

5. Drug inhibition: The statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin) are structural analogs of HMG CoA

and are (or are metabolized to) reversible, competitive inhibitors of ***HMG CoA reductase***. They are used to decrease plasma cholesterol levels in patients with hypercholesterolemia.

**CHOLESTEROL DEGRADATION**

Humans cannot metabolize the cholesterol ring structure to carbon dioxide andwater. Rather, the intact steroid nucleus is eliminated from the body byconversion to bile acids and bile salts, a small percentage of which is excreted inthe feces, and by secretion of cholesterol into the bile, which transports it to theintestine for elimination. [Note: Some of the cholesterol in the intestine ismodified by bacteria before excretion. The primary compounds made are theisomers coprostanol and cholestanol, which are reduced derivatives ofcholesterol. Together with cholesterol, these compounds make up the bulk ofneutral fecal sterols.] V. BILE **ACIDS AND BILE SALTS**

Bile consists of a watery mixture of organic and inorganic compounds. Phosphatidylcholine (PC), or lecithin, and conjugated bile salts are quantitatively the most important organic components of bile. Bile can either pass directly from the liver, where it is synthesized, into the duodenum through the common bile duct, or be stored in the gallbladder when not immediately needed for digestion.

A. Structure

The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group. The carboxyl group has a pKa (see p. 6) of ~6. In the duodenum (pH ~6), this group will be protonated in half of the molecules (the bile acids) and deprotonated in the rest (the bile salts). The terms bile acid and bile salt are frequently used interchangeably, however. Both forms have hydroxyl groups that are α in orientation (they lie below the plane of the rings) and methyl groups that are β (they lie above the plane of the rings). Therefore, the molecules have both a polar and a nonpolar surface and can act as emulsifying agents in the intestine, helping prepare dietary fat (triacylglycerol [TAG]) and other complex lipids for degradation by pancreatic digestive enzymes.

B. Synthesis

Bile acids are synthesized in the liver by a multistep, multiorganelle pathway in which hydroxyl groups are inserted at specific positions on the steroid structure; the double bond of the cholesterol B ring is reduced; and the hydrocarbon chain is shortened by three carbons, introducing a carboxyl group at the end of the chain. The most common resulting compounds, cholic acid (a triol) and chenodeoxycholic acid (a diol), as shown in, are called primary bile acids. [Note: The rate-limiting step in bile acid synthesis is the introduction of a hydroxyl group at carbon 7 of the steroid nucleus by ***7-α-hydroxylase***, a SER-associated ***cytochrome P450 monooxygenase*** found only in liver. Expression of the enzyme is downregulated by bile acids

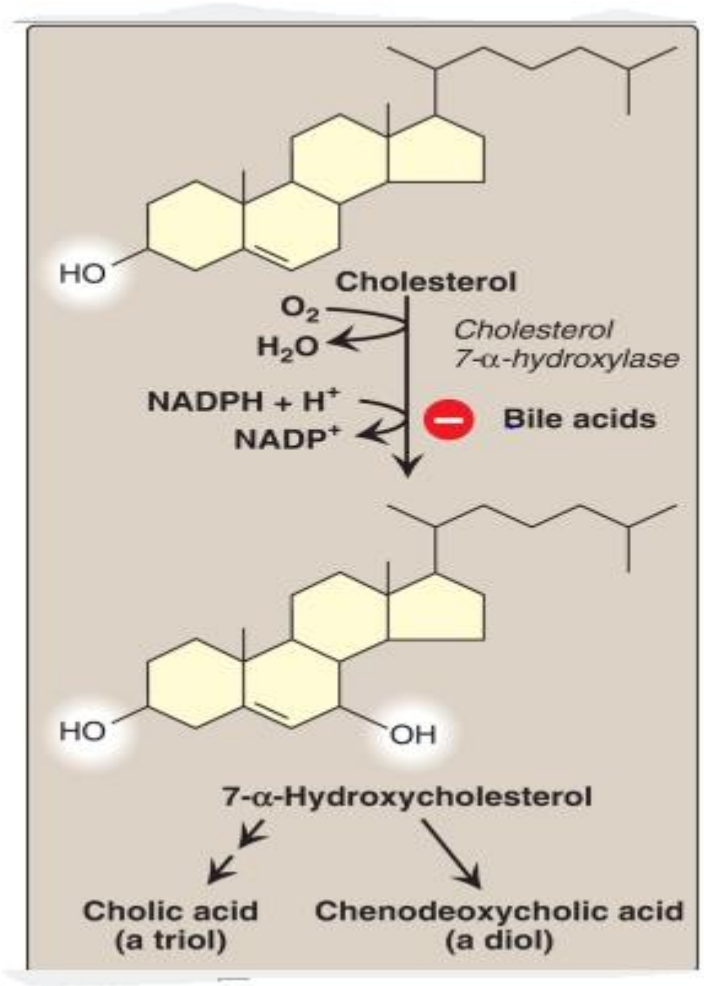



Figure: Synthesis of the bile acids cholic acid and chenodeoxycholic acid from cholesterol.

**C. Conjugation**

Before the bile acids leave the liver, they are conjugated to a molecule of either glycine or taurine (an end product of cysteine metabolism) by an amide bond between the carboxyl group of the bile acid and the amino group of the added compound. These new structures include glycocholic and glycochenodeoxycholic acids and taurocholic and taurochenodeoxycholic acids. The ratio of glycine to taurine forms in the bile is ~3/1. Addition of glycine or taurine results in the presence of a carboxyl group with a lower pKa (from glycine) or a sulfonate group (from taurine), both of which are fully ionized (negatively charged) at the alkaline pH of bile. The conjugated, ionized bile salts are more effective detergents than the unconjugated ones because of their enhanced amphipathic nature. Therefore, only the conjugated forms are found in the bile. Individuals with genetic deficiencies in the conversion of cholesterol to bile acids are treated with exogenously supplied chenodeoxycholic acid.

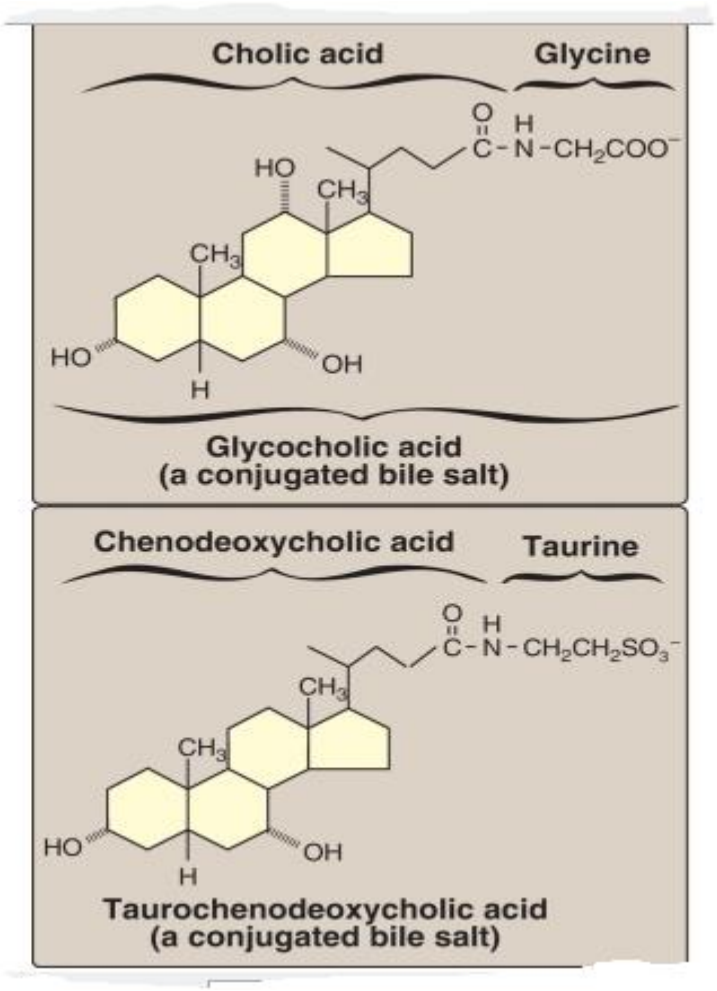



Figure: Conjugated bile salts. Note “cholic” in the names.

**Bile salts provide the only significant mechanism for cholesterol excretion,both as a metabolic product of cholesterol and as a solubilizer of cholesterol in bile**

D. Bacterial action on bile salts

Bacteria of the intestinal microbiota can deconjugate (remove glycine and taurine) bile salts. They can also dehydroxylate carbon 7,producing secondary bile salts such as deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid.

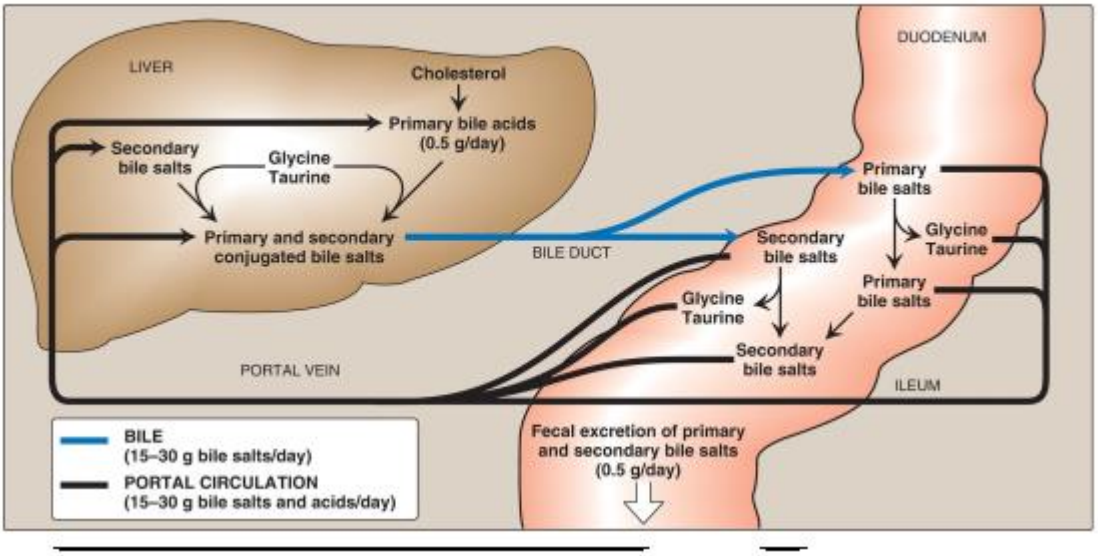



Figure: Enterohepatic circulation of bile salts. [Note: Ionized bile acids are called bile salts.]

E. Enterohepatic circulation Bile salts secreted into the intestine are efficiently reabsorbed (>95%) and reused. The liver actively secretes bile salts via the bile salt export pump. In the intestine, they are reabsorbed in the terminal ileum via the apical sodium (Na+)–bile salt cotransporter and returned to the blood via a separate transport system. [Note: Lithocholic acid is only poorly absorbed.] They are efficiently taken up from blood by the hepatocytes via an isoform of the cotransporter and reused. [Note: Albumin binds bile salts and transports them through the blood as was seen with FA.] The continuous process of secretion of bile salts into the bile, their passage through the duodenum where some are deconjugated then dehydroxylated to secondary bile salts, their uptake in the ileum, and their subsequent return to the liver as a mixture of primary and secondary forms is termed the enterohepatic circulation. Between 15 and 30 g of bile salts are secreted from the liver into the duodenum each day, yet only ~0.5 g (<3%) is lost daily in the feces. Approximately 0.5 g/day is synthesized from cholesterol in the liver to replace the amount lost. Bile acid sequestrants,such as cholestyramine, bind bile salts in the gut and prevent their reabsorption, thereby promoting their excretion. They are used in the treatment of hypercholesterolemia because the removal of bile salts relieves the inhibition on bile acid synthesis in the liver, thereby diverting additional cholesterol into that pathway. [Note: Dietary fiber also binds bile salts and increases their excretion.

F. Bile salt deficiency:

Cholelithiasis The movement of cholesterol from the liver into the bile must be accompanied by the simultaneous secretion of phospholipid and bile salts. If this dual process is disrupted and more cholesterol is present than can be solubilized by the bile salts and PC present, the cholesterol may precipitate in the gallbladder, leading to cholesterol gallstone disease or cholelithiasis. This disorder is typically caused by a decrease of bile acids in the bile. Cholelithiasis also may result from increased secretion of cholesterol into bile, as seen with

the use of fibrates (for example,gemfibrozil) to reduce cholesterol (and TAG) in the blood. Laparoscopic cholecystectomy (surgical removal of the gallbladder through a small incision) is currently the treatment of choice. However, for patients who are unable to undergo surgery, oral administration of chenodeoxycholic acid to supplement the body's supply of bile acids results in a gradual (months to years) dissolution of the gallstones. [Note: Cholesterol stones account for >85% of cases of cholelithiasis, with bilirubin and mixed stones accounting for the rest].

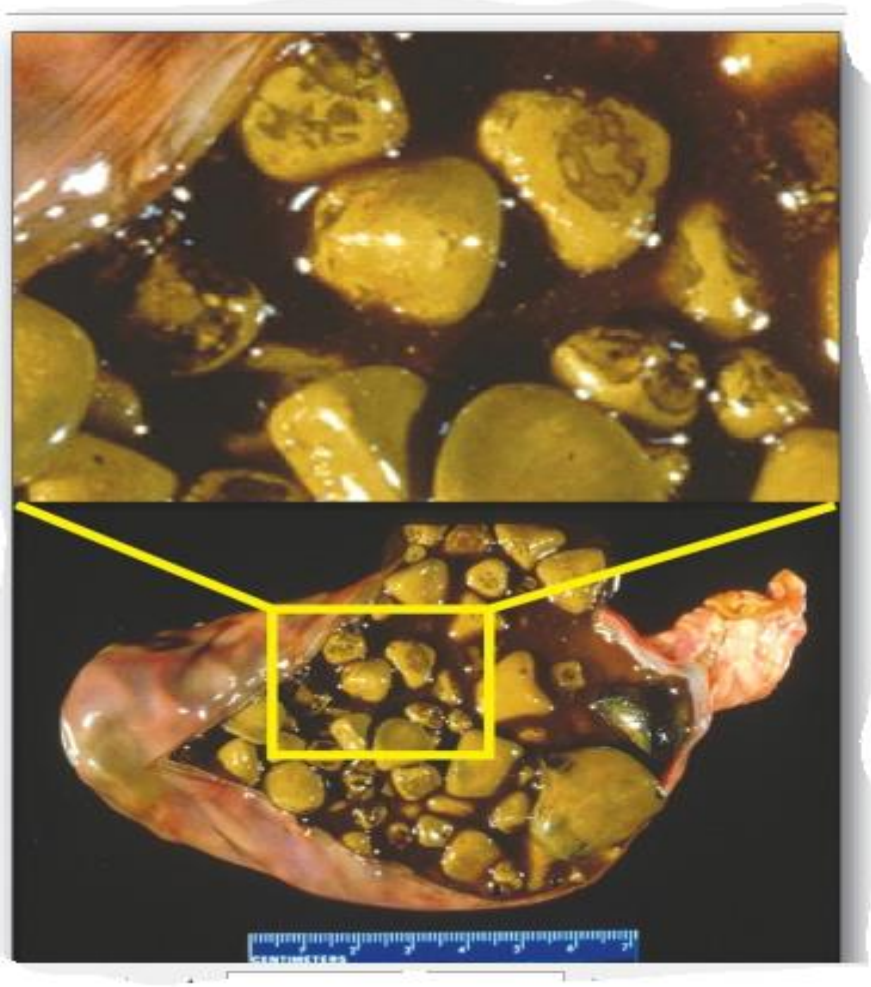


Figure: Gallbladder with gallstones.

A. CompositionLipoproteins are composed of a neutral lipid core (containing TAG andcholesteryl esters) surrounded by a shell of amphipathic apolipoproteins,phospholipid, and nonesterified (free) cholesterol. Theseamphipathic compounds are oriented such that their polar portions areexposed on the surface of the lipoprotein, thereby rendering the particlesoluble in aqueous solution. The TAG and cholesterol carried by thelipoproteins are obtained either from the diet (exogenous source) or from denovo synthesis (endogenous source). [Note: The cholesterol (C) content ofplasma lipoproteins is now routinely measured in fasting blood. Total C =LDL-C + HDL-C + VLDL-C, where VLDL-C is calculated by dividingTAG by 5 because the TAG/cholesterol ratio is 5/1 in VLDL. The goalvalue for total cholesterol is <200 mg/dl.]

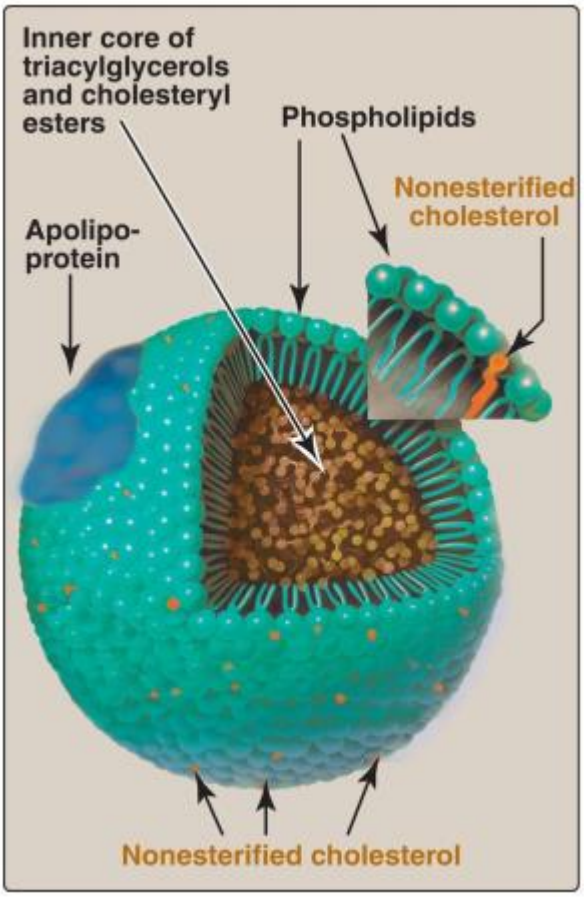



Figure: Structure of a typical lipoprotein particle.

1. Size and density: Chylomicrons are the lipoprotein particles lowest indensity and largest in size and that contain the highest percentage of lipid(as TAG) and the lowest percentage of protein. VLDL and LDL aresuccessively denser, having higher ratios of protein to lipid. HDLparticles are the smallest and densest. Plasma lipoproteins can beseparated on the basis of their electrophoretic mobility, as shown in, or on the basis of their density by ultracentrifugation.

2. Apolipoproteins: The apolipoproteins associated with lipoproteinparticles have a number of diverse functions, such as providingrecognition sites for cell-surface receptors and serving as activators orcoenzymes for enzymes involved in lipoprotein metabolism. Some of theapolipoproteins are required as essential structural components of theparticles and cannot be removed (in fact, the particles cannot be producedwithout them), whereas others are transferred freely betweenlipoproteins. Apolipoproteins are divided by structure and function intoseveral major classes, denoted by letters, with each class havingsubclasses (for example, apolipoprotein [apo] C-I, apo C-II, and apo CIII). [Note: The functions of all the apolipoproteins are not yet known.]B. Chylomicron metabolismChylomicrons are assembled in intestinal mucosal cells and carry dietary(exogenous) TAG, cholesterol, fat-soluble vitamins, and cholesteryl estersto the peripheral tissues. [Note: TAG account for close to 90%of the lipids in a chylomicron

1. Apolipoprotein synthesis: Apo B-48 is unique to chylomicrons. Itssynthesis begins on the rough ER (RER), and it is glycosylated as itmoves through the RER and Golgi. [Note: Apo B-48 is so namedbecause it constitutes the N-terminal 48% of the protein encoded by thegene for apo B. Apo B-100, which is synthesized by the liver and foundin VLDL and LDL, represents the entire protein encoded by this gene.Posttranscriptional editing of a cytosine to a uracil inintestinal apo B-100 messenger RNA (mRNA) creates a nonsense (stop)codon, allowing translation of only 48% of the mRNA.]

2. Chylomicron assembly: Many enzymes involved in TAG, cholesterol,and phospholipid synthesis are located in the SER. Assembly of theapolipoprotein and lipid into chylomicrons requires microsomaltriglyceride transfer protein ([MTP], which loads apo B-48with lipid. This occurs before transition from the ER to the Golgi, wherethe particles are packaged in secretory vesicles. These fuse with theplasma membrane releasing the lipoproteins, which then enter thelymphatic system and, ultimately, the blood. [Note: Chylomicrons leavethe lymphatic system via the thoracic duct that empties into the leftsubclavian vein.]

3. Nascent chylomicron modification: The particle released by the intestinalmucosal cell is called a nascent chylomicron because it is functionallyincomplete. When it reaches the plasma, the particle is rapidly modified,receivingapo E (which is recognized by hepatic receptors) and apoC.The latter includes apo C-II, which is necessary for the activation of***lipoprotein lipase*** (***LPL***), the enzyme that degrades the TAG contained inthe chylomicron. The source of these apolipoproteins is circulating HDL. [Note: Apo C-III on TAG-rich lipoproteins inhibits***LPL***.]

4. Triacylglycerol degradation by lipoprotein lipase: ***LPL*** is an extracellularenzyme that is anchored to the capillary walls of most tissues butpredominantly those of adipose tissue and cardiac and skeletal muscle.The adult liver does not express this enzyme. [Note: A ***hepatic lipase*** isfound on the surface of endothelial cells of the liver. It plays a role inTAG degradation in chylomicrons and VLDL and is important in HDLmetabolism] ***LPL***, activated by apo C-II on circulatingchylomicrons, hydrolyzes the TAG in these particles to FA and glycerol.The FA are stored (in adipose) or used for energy (in muscle). Theglycerol is taken up by the liver, converted to dihydroxyacetonephosphate (an intermediate of glycolysis), and used in lipid synthesis orgluconeogenesis. [Note: Patients with a deficiency of ***LPL*** or apo C-II(type I hyperlipoproteinemia or familial chylomicronemia) show adramatic accumulation (≥1,000 mg/dl) of chylomicron-TAG in theplasma (hypertriacylglycerolemia) even in the fasted state. They are atincreased risk for acute pancreatitis. Treatment is the reduction of dietaryfat.]

5. Lipoprotein lipase expression: ***LPL*** is synthesized by adipose tissue andby cardiac and skeletal muscle. Expression of the tissue-specificisozymes is regulated by nutritional state and hormonal level. Forexample, in the fed state (elevated insulin levels), ***LPL*** synthesis isincreased in adipose but decreased in muscle tissue. Fasting (decreasedinsulin) favors ***LPL*** synthesis in muscle.

[Note: The highestconcentration of ***LPL*** is in cardiac muscle, reflecting the use of FA toprovide much of the energy needed for cardiac function.]

6. Chylomicron remnant formation: As the chylomicron circulates, and>90% of the TAG in its core is degraded by ***LPL***, the particle decreasesin size and increases in density. In addition, the C apolipoproteins (butnot apo B or E) are returned to HDL. The remaining particle, called aremnant, is rapidly removed from the circulation by the liver, whose cellmembranes contain lipoprotein receptors that recognize apo E. Chylomicron remnants bind to these receptors and are taken intothe hepatocytes by endocytosis. The endocytosed vesicle then fuses witha lysosome, and the apolipoproteins, cholesteryl esters, and othercomponents of the remnant are hydrolytically degraded, releasing aminoacids, free cholesterol, and FA. The receptor is recycled. [Note: Themechanism of receptor-mediated endocytosis is illustrated for LDL in.

**C. Very-low-density lipoprotein metabolism**

VLDL are produced in the liver. They are composedpredominantly of endogenous TAG (~60%), and their function is to carrythis lipid from the liver (site of synthesis) to the peripheral tissues. There,the TAG is degraded by ***LPL***, as discussed for chylomicrons.[Note: Nonalcoholic fatty liver (hepatic steatosis) occurs in conditions inwhich there is an imbalance between hepatic TAG synthesis and thesecretion of VLDL. Such conditions include obesity and type 2 diabetesmellitus]

1. Release from the liver: VLDL are secreted directly into the blood by theliver as nascent particles containing apo B-100. They must obtain apo CII and apo E from circulating HDL. As withchylomicrons, apo C-II is required for activation of ***LPL***. [Note:Abetalipoproteinemia is a rare hypolipoproteinemia caused by a defect inMTP, leading to an inability to load apo B with lipid. Consequently, fewVLDL or chylomicrons are formed, and TAG accumulates in the liverand intestine. Absorption of fat-soluble vitamins is decreased. LDL arelow.]

2. Modification in the circulation: As VLDL pass through the circulation,TAG is degraded by ***LPL***, causing the VLDL to decrease in size andbecome denser. Surface components, including the C and Eapolipoproteins, are returned to HDL, but the particles retain apo B-100.Additionally, some TAG are transferred from VLDL to HDL in anexchange reaction that concomitantly transfers cholesteryl esters fromHDL to VLDL. This exchange is accomplished by ***cholesteryl estertransfer protein*** (***CETP***), as shown in .

3. Conversion to low-density lipoproteins: With these modifications, theVLDL is converted in the plasma to LDL. Intermediate-densitylipoproteins (IDL) of varying sizes are formed during this transition. IDLcan also be taken up by liver cells through receptor-mediated endocytosisthat uses apo E as the ligand. Apo E is normally present in threeisoforms, E-2 (the least common), E-3 (the most common), and E-4. ApoE-2 binds poorly to receptors, and patients who are homozygotic for apoE-2 are deficient in the clearance of IDL and chylomicron remnants.These individuals have familial type III hyperlipoproteinemia (familialdysbetalipoproteinemia or broad beta disease),

withhypercholesterolemia and premature atherosclerosis. [Note: The apo E-4isoform confers increased susceptibility to an earlier age of onset of thelate-onset form of Alzheimer disease. The effect is dose dependent, withhomozygotes being at greatest risk. Estimates of the risk vary.]

**D. Low-density lipoprotein metabolism**

LDL particles contain much less TAG than their VLDL predecessors andhave a high concentration of cholesterol and cholesteryl esters.About 70% of plasma cholesterol is in LDL.

1. Receptor-mediated endocytosis: The primary function of LDL particles isto provide cholesterol to the peripheral tissues (or return it to the liver).They do so by binding to plasma membrane LDL receptors thatrecognize apo B-100 (but not apo B-48). Because these LDL receptorscan also bind apo E, they are known as apo B-100/apoE receptors. Asummary of the uptake and degradation of LDL particles is presented in. [Note: The numbers in brackets below refer tocorresponding numbers on that figure.] A similar mechanism of receptormediated endocytosis is used for the uptake and degradation ofchylomicron remnants and IDL by the liver.

[1] LDL receptors are negatively charged glycoproteins that are clusteredin pits on cell membranes. The cytosolic side of the pit is coated withthe protein clathrin, which stabilizes the pit.

[2] After binding, the LDL–receptor complex is endocytosed. [Note:Defects in the synthesis of functional LDL receptors causes asignificant elevation in plasma LDL-C. Patients with suchdeficiencies have type IIa hyperlipidemia (familialhypercholesterolemia [FH]) and premature atherosclerosis.Autosomal dominant hypercholesterolemia can also be caused bydefects in apo B-100 that reduce its binding to the receptor and byincreased activity of a ***protease***, ***proprotein convertasesubtilisin/kexin type 9*** (***PCSK9***), which promotes internalization andlysosomal degradation of the receptor. ***PCSK9*** inhibitors are nowavailable for the treatment of hypercholesterolemia.]

[3] The vesicle containing LDL loses its clathrin coat and fuses with othersimilar vesicles, forming larger vesicles called endosomes.

[4] The pH of the endosome falls (due to the proton-pumping activity ofendosomal ***ATPase***), which allows separation of the LDL from itsreceptor. The receptors then migrate to one side of the endosome,whereas the LDL stay free within the lumen of the vesicle.

[5] The receptors can be recycled, whereas the lipoprotein remnants in thevesicle are transferred to lysosomes and degraded by lysosomal ***acidhydrolases***, releasing free cholesterol, amino acids, FA, andphospholipids. These compounds can be reutilized by the cell. [Note:Lysosomal storage diseases result from rare autosomal-recessivedeficiencies in the ability to hydrolyze lysosomal cholesteryl esters(Wolman disease) or to transport free cholesterol out of the lysosome(Niemann-Pick disease, type C).]2. Endocytosed cholesterol and cholesterol homeostasis: The chylomicronremnant–, IDL-, and LDL-derived cholesterol affects cellular cholesterolcontent in several ways. First, expression of the gene for***HMG CoA reductase*** is inhibited by high

cholesterol, and de novocholesterol synthesis decreases as a result. Additionally, degradation ofthe ***reductase*** is accelerated. Second, synthesis of new LDL receptorprotein is reduced by decreasing the expression of the LDL receptorgene, thus limiting further entry of LDL-C into cells. [Note: As was seenwith the ***reductase*** gene, transcriptional regulation of theLDL receptor gene involves an SRE and SREBP-2. This allowscoordinate regulation of the expression of these proteins.] Third, if thecholesterol is not required immediately for some structural or syntheticpurpose, it is esterified by ***acyl CoA:cholesterol acyltransferase*** (***ACAT***).***ACAT*** transfers a FA from a fatty acyl CoA to cholesterol, producing acholesteryl ester that can be stored in the cell The activity of***ACAT*** is enhanced in the presence of increased intracellular cholesterol.

3. Uptake by macrophage scavenger receptors: In addition to the highlyspecific and regulated receptor-mediated pathway for LDL uptakedescribed above, macrophages possess high levels of scavenger receptoractivity. These receptors, known as scavenger receptor class A (SR-A),can bind a broad range of ligands and mediate the endocytosis ofchemically modified LDL in which the lipid or apo B component hasbeen oxidized. Unlike the LDL receptor, the scavenger receptor is notdownregulated in response to increased intracellular cholesterol.Cholesteryl esters accumulate in macrophages and cause theirtransformation into "foam" cells, which participate in the formation ofatherosclerotic plaque. LDL-C is the primary cause ofatherosclerosis.

**E. High-density lipoprotein metabolism**

HDL comprise a heterogeneous family of lipoproteins with a complexmetabolism that is not yet completely understood. HDL particles areformed in the blood by the addition of lipid to apo A-1, an apolipoproteinmade and secreted by the liver and intestine. Apo A-1 accounts for ~70% ofthe apolipoproteins in HDL. HDL perform a number of important functions,including the following.

1. Apolipoprotein supply: HDL particles serve as a circulating reservoir ofapo C-II (the apolipoprotein that is transferred to VLDL andchylomicrons and is an activator of ***LPL***) and apo E (the apolipoproteinrequired for the receptor-mediated endocytosis of IDL and chylomicronremnants).

2. Nonesterified cholesterol uptake: Nascent HDL are disc-shaped particlescontaining primarily phospholipid (largely PC) and apo A, C, and E.They take up cholesterol from nonhepatic (peripheral) tissues and returnit to the liver as cholesteryl esters. [Note: HDL particles areexcellent acceptors of nonesterified cholesterol as a result of their highconcentration of phospholipids, which are important solubilizers ofcholesterol.]

3.Cholesterol esterification: The cholesterol taken up by HDL is immediately esterified by the plasma enzyme ***lecithin:cholesterol acyltransferase*** (***LCAT***, also known as ***PCAT***, in which P stands for phosphatidylcholine, the source of the FA). This enzyme is synthesized and secreted by the liver. ***LCAT*** binds to nascent HDL and is activated by apo A-I.

***LCAT*** transfers the FA from carbon 2 of PC to cholesterol.This produces a hydrophobic cholesteryl ester, which is sequestered in the core of the HDL, and lysophosphatidylcholine, which binds to albumin. [Note: Esterification maintains the cholesterol concentration gradient, allowing continued efflux of cholesterol to HDL.] As the discoidal nascent HDL accumulates cholesteryl esters, it first becomes a spherical, relatively cholesteryl ester–poor HDL3 and, eventually, a cholesteryl ester–rich HDL2 particle that carries these esters to the liver. ***Hepatic lipase***, which degrades TAG and phospholipids, participates in the conversion of HDL2 to HDL3. ***CETP*** transfers some of the cholesteryl esters from HDL to VLDL in exchange for TAG, relieving product inhibition of ***LCAT***. Because VLDL are catabolized to LDL, the cholesteryl esters transferred by ***CETP*** are ultimately taken up by the liver

4. Reverse cholesterol transport: The selective transfer of cholesterol from peripheral cells to HDL and from HDL to the liver for bile acid synthesis or disposal via the bile is a key component of cholesterol homeostasis.This process of reverse cholesterol transport (RCT) is, in part, the basis for the inverse relationship seen between plasma HDL concentration and atherosclerosis and for the designation of HDL as the "good" cholesterol carrier. [Note: Exercise and estrogen raise HDL levels.] RCT involves efflux of cholesterol from peripheral cells to HDL, esterification of the cholesterol by ***LCAT***, binding of the cholesteryl ester–rich HDL (HDL2) to liver (and, perhaps, steroidogenic cells), selective transfer of the cholesteryl esters into these cells, and release of lipid-depleted HDL (HDL3). The efflux of cholesterol from peripheral cells is mediated primarily by the transport protein ABCA1. [Note: Tangier disease is a very rare deficiency of.

ABCA1 and is characterized by the virtual absence of HDL particles due to degradation of lipid-poor apo A-1.] Cholesteryl ester uptake by the liver is mediated by the cell-surface receptor SR-B1 (scavenger receptor class B type 1) that binds HDL for SR-A receptors). The HDL particle itself is not taken up. Instead, there is selective uptake of the cholesteryl ester from the HDL particle. [Note: Low HDL-C is a risk factor for atherosclerosis.] ABCA1 is an ATP-binding cassette (ABC) protein. ABC proteins use energy from ATP hydrolysis to transport materials, including lipids, in and out of cells and across intracellular compartments. In addition to Tangier disease, defects in specific ABC proteins result in sitosterolemia, cystic fibrosis, X-linked adrenoleukodystrophy, respiratory distress syndrome due to decreased surfactant secretion, and liver disease due to decreased bile salt secretion.

**F. Lipoprotein (a) and heart disease** Lipoprotein (a), or Lp(a), is nearly identical in structure to an LDL particle. Its distinguishing feature is the presence of an additional apolipoprotein molecule, apo(a), which is covalently linked at a single site to apo B-100. Circulating levels of Lp(a) are determined primarily by genetics. However,factors such as diet may play some role, as trans FA have been reported to increase it. The physiologic function of Lp(a) is unknown. When present in large quantities in the plasma, Lp(a) is associated with an increased risk of coronary heart disease. [Note: Apo(a) is structurally

homologous to plasminogen, the precursor of a blood ***protease*** whose target is fibrin, the main protein component of blood clots. It is hypothesized that elevated Lp(a) slows the breakdown of blood clots that trigger heart attacks because it competes with plasminogen for binding to fibrin.] Niacin reduces Lp(a), as well as LDL-C and TAG, and raises HDLC.

**Topic: Protein metabolism and function.**

Unlike fats and carbohydrates, amino acids are not stored by the body. That is,no protein exists whose sole function is to maintain a supply of amino acids forfuture use. Therefore, amino acids must be obtained from the diet, synthesizedde novo, or produced from the degradation of body protein. Any amino acids inexcess of the biosynthetic needs of the cell are rapidly degraded. The first phaseof catabolism involves the removal of the α-amino groups (usually bytransamination and subsequent oxidative deamination), forming ammonia andthe corresponding α-keto acids, the carbon skeletons of amino acids. A portionof the free ammonia is excreted in the urine, but most is used in the synthesis of urea, which is quantitatively the most important route for disposingof nitrogen from the body. In the second phase of amino acid catabolism, described in, the carbon skeletons of the α-keto acids are convertedto common intermediates of energy-producing metabolic pathways. Thesecompounds can be metabolized to carbon dioxide ($CO_2$) and water ($H_2O$),glucose, fatty acids, or ketone bodies by the central pathways of metabolism described in

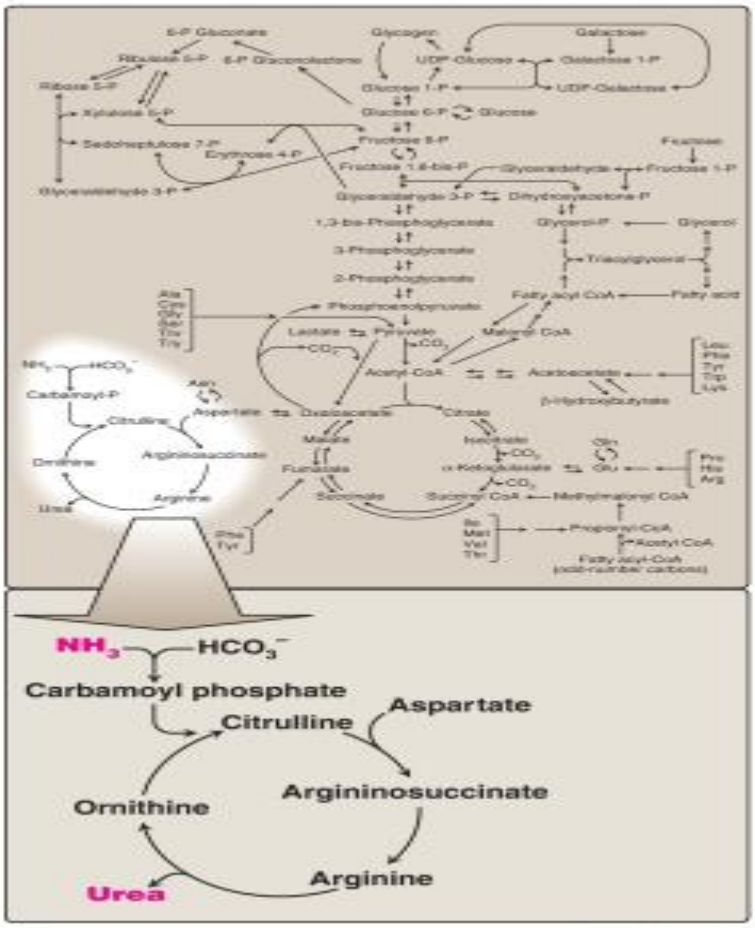



Figure: Urea cycle shown as part of the essential pathways of energymetabolism. NH3 = ammonia; CO2 = carbon dioxide.

## OVERALL NITROGEN METABOLISM

Amino acid catabolism is part of the larger process of the metabolism ofnitrogen-containing molecules. Nitrogen enters the body in a variety ofcompounds present in food, the most important being amino acids contained indietary protein. Nitrogen leaves the body as urea, ammonia, and other productsderived from amino acid metabolism. The role ofbody proteins in these transformations involves two important concepts: theamino acid pool and protein turnover.

A. Amino acid poolFree amino acids are present throughout the body, such as in cells, blood,and the extracellular fluids. For the purpose of this discussion, envision allof these amino acids as if they belonged to a single entity, called the aminoacid pool. This pool is supplied by three sources:

1) amino acids providedby the degradation of endogenous (body) proteins, most of which arereutilized;

2) amino acids derived from exogenous (dietary) protein; and

3)nonessential amino acids synthesized from simple intermediates of metabolism. Conversely, the amino acid pool is depleted bythree routes:

1) synthesis of body protein,

2) consumption of amino acids asprecursors of essential nitrogen-containing small molecules, and

3)conversion of amino acids to glucose, glycogen, fatty acids, and ketonebodies or oxidation to $CO_2$ + $H_2O$. Although the amino acidpool is small (comprising ~90–100 g of amino acids) in comparison withthe amount of protein in the body (~12 kg in a 70-kg man), it isconceptually at the center of whole-body nitrogen metabolism.

In healthy, well-fed individuals, the input to the amino acid pool isbalanced by the output. That is, the amount of amino acids contained in thepool is constant. The amino acid pool is said to be in a steady state, and theindividual is said to be in nitrogen balance.

B. Protein turnoverMost proteins in the body are constantly being synthesized and thendegraded (turned over), permitting the removal of abnormal or unneededproteins. For many proteins, regulation of synthesis determines theconcentration of protein in the cell, with protein degradation assuming aminor role. For other proteins, the rate of synthesis is constitutive (that is,essentially constant), and cellular levels of the protein are controlled byselective degradation.

1. Rate: In healthy adults, the total amount of protein in the body remainsconstant because the rate of protein synthesis is just sufficient to replacethe protein that is degraded. This process, called protein turnover, leadsto the hydrolysis and resynthesis of 300–400 g of body protein each dayThe rate of protein turnover varies widely for individual proteins. Shortlived proteins (for example, many regulatory proteins and misfoldedproteins) are rapidly degraded, having half-lives measured in minutes orhours. Long-lived proteins, with half-lives of days to weeks, constitutethe majority of proteins in the cell. Structural proteins, such as collagen,are metabolically stable and have half-lives measured in months or years.

2. Protein degradation: There are two major enzyme systems responsiblefor degrading proteins: the ATP-dependent ubiquitin (Ub)–proteasomesystem of the cytosol and the ATP-independent degradative enzymesystem of the lysosomes. Proteasomes selectively degrade damaged orshort-lived proteins. Lysosomes use ***acid hydrolases*** tononselectively degrade intracellular proteins (autophagy) andextracellular proteins (heterophagy), such as plasma proteins, that aretaken into the cell by endocytosis.a. Ubiquitin–proteasome system: Proteins selected for degradation by thecytosolic ubiquitin–proteasome system are first modified by thecovalent attachment of Ub, a small, globular, nonenzymic protein thatis highly conserved across eukaryotic species. Ubiquitination of thetarget substrate occurs through isopeptide linkage of the α-carboxylgroup of the C-terminal glycine of Ub to the ε-amino group of a lysinein the protein substrate by a three-step, enzyme-catalyzed, ATPdependent process. [Note: ***Enzyme 1*** (***E1***, an activating enzyme)activates Ub, which is then transferred to ***E2*** (a conjugating enzyme).***E3*** (a ***ligase***) identifies the protein to be degraded and interacts with***E2***-Ub. There are many more ***E3*** proteins than there are ***E1*** or ***E2***.]The consecutive addition of four or more Ub molecules to the targetprotein generates a polyubiquitin chain. Proteins tagged with Ubchains are recognized by a large, barrel-shaped,

macromolecular,proteolytic complex called a proteasome. The proteasomeunfolds, deubiquitinates, and cuts the target protein into fragments thatare then further degraded by cytosolic ***proteases*** to amino acids, whichenter the amino acid pool. The Ub is recycled. It is noteworthy that theselective degradation of proteins by the ubiquitin–proteosome complex(unlike simple hydrolysis by proteolytic enzymes) requires ATPhydrolysis.

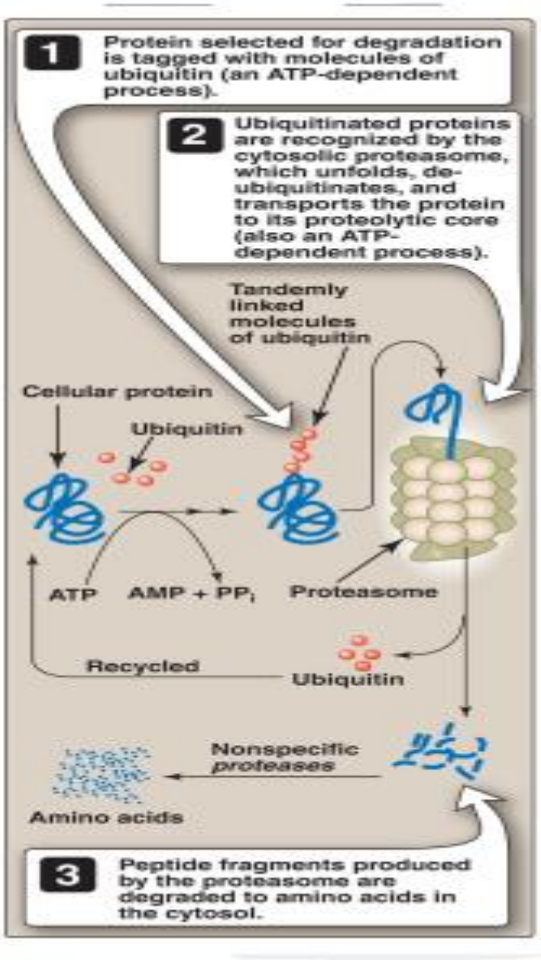



Figure: The ubiquitin–proteasome degradation pathway of proteins. AMP =adenosine monophosphate; PPi = pyrophosphate.

b. Degradation signals: Because proteins have different half-lives, it isclear that protein degradation cannot be random but, rather, isinfluenced by some structural aspect of the protein that serves as adegradation signal, which is recognized and bound by an ***E3***. The halflife of a protein is also influenced by the amino (N)-terminal residue,the so-called N-end rule, and ranges from minutes to hours.Destabilizing N-terminal amino acids include arginine andposttranslationally modified amino acids such as acetylated alanine. Incontrast, serine is a stabilizing amino acid. Additionally, proteins richin sequences containing proline, glutamate, serine, and threonine(called PEST sequences after the one-letter designations for theseamino acids) are rapidly ubiquitinated and degraded and, therefore,have short half-lives.

DIETARY PROTEIN DIGESTIONMost of the nitrogen in the diet is consumed in the form of protein, typicallyamounting to 70–100 g/day in the American diet. Proteins aregenerally too large to be absorbed by the intestine.

[Note: An example of anexception to this rule is that newborns can take up maternal antibodies in breastmilk.] Therefore, proteins must be hydrolyzed to yield di- and tripeptides as wellas individual amino acids, which can be absorbed. Proteolytic enzymesresponsible for degrading proteins are produced by three different organs: thestomach, the pancreas, and the small intestine.

A. Digestion by gastric secretionThe digestion of proteins begins in the stomach, which secretes gastricjuice, a unique solution containing hydrochloric acid (HCl) and theproenzyme pepsinogen.

1. Hydrochloric acid: Stomach HCl is too dilute (pH 2–3) to hydrolyzeproteins. The acid, secreted by the parietal cells of the stomach, functionsinstead to kill some bacteria and to denature proteins, thereby makingthem more susceptible to subsequent hydrolysis by ***proteases***.

2. Pepsin: This acid-stable ***endopeptidase*** is secreted by the chief cells ofthe stomach as an inactive zymogen (or proenzyme), pepsinogen. [Note:In general, zymogens contain extra amino acids in their sequences thatprevent them from being catalytically active. Removal of these aminoacids permits the proper folding required for an active enzyme.] In thepresence of HCl, pepsinogen undergoes a conformational change thatallows it to cleave itself (autocatalysis) to the active form, ***pepsin***, whichreleases polypeptides and a few free amino acids from dietary proteins.

B. Digestion by pancreatic enzymesOn entering the small intestine, the polypeptides produced in the stomachby the action of ***pepsin*** are further cleaved to oligopeptides and amino acidsby a group of pancreatic ***proteases*** that include both ***endopeptidases*** (thatcleave within) and ***exopeptidases*** (that cut at an end). [Note: Bicarbonate(HCO3-), secreted by the pancreas in response to the intestinal hormonesecretin, raises the intestinal pH.]

1. Specificity: Each of these enzymes has a different specificity for theamino acid R-groups adjacent to the susceptible peptide bond. For example, ***trypsin*** cleaves only when the carbonyl group of thepeptide bond is contributed by arginine or lysine. These enzymes, like***pepsin*** described above, are synthesized and secreted as inactivezymogens.

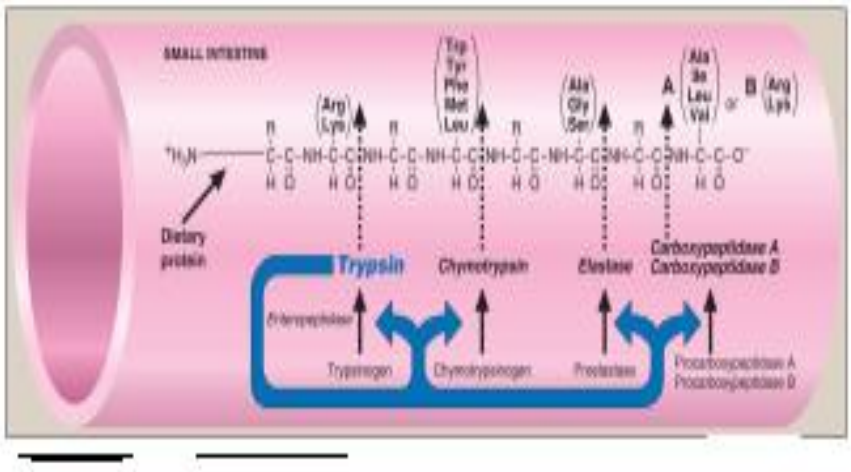


Figure: Cleavage of dietary protein in the small intestine by pancreatic***proteases***. The peptide bonds susceptible to hydrolysis are shown for each of thefive major pancreatic ***proteases***. [Note: The first three are serine ***endopeptidases***,whereas the last two are ***exopeptidases***. Each is produced from an inactivezymogen.]

2. Zymogen release: The release and activation of the pancreatic zymogensare mediated by the secretion of cholecystokinin, a polypeptide hormoneof the small intestine.

3. Zymogen activation: ***Enteropeptidase***(also called ***enterokinase***), a***serine protease*** synthesized by and present on the luminal (apical)surface of intestinal mucosal cells (enterocytes) of the brush border,converts the pancreatic zymogen trypsinogen to ***trypsin*** by removal of ahexapeptide from the N-terminus of trypsinogen. ***Trypsin*** subsequentlyconverts other trypsinogen molecules to ***trypsin*** by cleaving a limitednumber of specific peptide bonds in the zymogen. Thus, ***enteropeptidase***unleashes a cascade of proteolytic activity because ***trypsin*** is the commonactivator of all the pancreatic zymogens.

4. Digestion abnormalities: In individuals with a deficiency in pancreaticsecretion (for example, because of chronic pancreatitis, cystic fibrosis, orsurgical removal of the pancreas), the digestion and absorption of fat andprotein are incomplete. This results in the abnormal appearance of lipidsin the feces as well asundigested protein.Celiac disease (celiac sprue) is a disease of malabsorption resulting fromimmune-mediated damage to the small intestine in response to ingestion ofgluten (or gliadin produced from gluten), a protein found in wheat, barley,and rye.

C. Digestion of oligopeptides by small intestine enzymesThe luminal surface of the enterocytes contains ***aminopeptidase***, an***exopeptidase*** that repeatedly cleaves the N-terminal residue fromoligopeptides to produce even smaller peptides and free amino acids.

D. Amino acid and small peptide intestinal absorptionMost free amino acids are taken into enterocytes via sodium-dependentsecondary active transport by solute carrier (SLC) proteins of the apicalmembrane. At least seven different transport systems with overlappingamino acid specificities are known. Di- and tripeptides, however, are takenup by a proton-linked peptide transporter (PepT1). The peptides are thenhydrolyzed to free amino acids. Regardless of their source, free amino acidsare released from enterocytes into the portal system by sodium-independenttransporters of the basolateral membrane. Therefore, only free amino acidsare found in the portal vein after a meal containing protein. These aminoacids are either metabolized by the liver or released into the generalcirculation. [Note: Branched-chain amino acids (BCAA) are notmetabolized by the liver but, instead, are sent from the liver to muscle viathe blood.]

E. Absorption abnormalitiesThe small intestine and the proximal tubules of the kidneys have commontransport systems for amino acid uptake. Consequently, a defect in any oneof these systems results in an inability to absorb particular amino acids intothe intestine and into the kidney tubules. For example, one system isresponsible for the uptake of cystine and the dibasic amino acids

ornithine,arginine, and lysine (represented as COAL). In the inherited disordercystinuria, this carrier system is defective, and all four amino acids appear in the urine. Cystinuria occurs at a frequency of 1 in 7,000individuals, making it one of the most common inherited diseases and themost common genetic error of amino acid transport. The disease expressesitself clinically by the precipitation of cystine to form kidney stones(calculi), which can block the urinary tract. Oral hydration is an importantpart of treatment for this disorder.

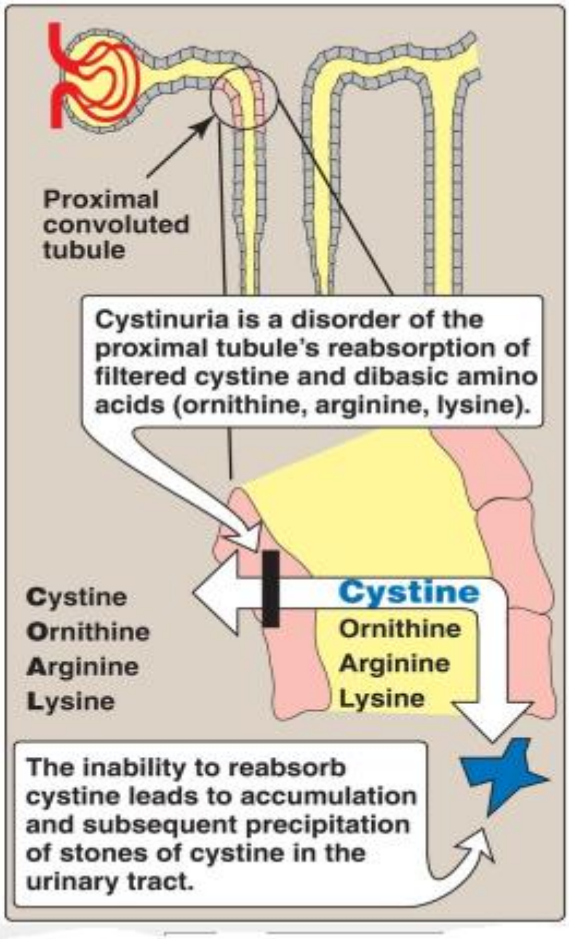



Figure: Genetic defect seen in cystinuria. [Note: Cystinuria is distinct fromcystinosis, a rare defect in the transport of cystine out of lysosomes that resultsin the formation of cystine crystals within the lysosome and widespread tissuedamage.]

NITROGEN REMOVAL FROM AMINOACIDS

The presence of the α-amino group keeps amino acids safely locked away fromoxidative breakdown. Removing the α-amino group is essential for producingenergy from any amino acid and is an obligatory step in the catabolism of allamino acids. Once removed, this nitrogen can be incorporated into othercompounds or excreted as urea, with the carbon skeletons being metabolized.This section describes transamination and oxidative deamination,

reactions thatultimately provide ammonia and aspartate, the two sources of urea nitrogen.

Transamination: Funneling amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of theirα-amino group to α-ketoglutarate, producing an α-keto acid(derived from the original amino acid) and glutamate. α-Ketoglutarate playsa pivotal role in amino acid metabolism by accepting the amino groupsfrom most amino acids, thereby becoming glutamate. Glutamate producedby transamination can be oxidativelydeaminatedor used asan amino group donor in the synthesis of nonessential amino acids. Thistransfer of amino groups from one carbon skeleton to another is catalyzedby a family of enzymes called ***aminotransferases*** (also called***transaminases***). These enzymes are found in the cytosol and mitochondriaof cells throughout the body. All amino acids, with the exception of lysineand threonine, participate in transamination at some point in theircatabolism. [Note: These two amino acids lose their α-amino groups by deamination.]

1.Substrate specificity: Each ***aminotransferase*** is specific for one or, at most,a few amino group donors. ***Aminotransferases*** are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important ***aminotransferase*** reactions are catalyzed by

a.***alanine aminotransferase*** (***ALT***) and ***aspartate aminotransferase*** (***AST***), as shown in The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most ***aminotransferases***) functions in the direction of glutamate synthesis. [Note: In effect, glutamate acts as a collector of nitrogen from most amino acids.]

b. Aspartate aminotransferase: ***AST*** is an exception to the rule that ***aminotransferases*** funnel amino groups to form glutamate. During amino acid catabolism, ***AST*** primarily transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle . Like other transaminations, the ***AST*** reaction is reversible.

2. Mechanism: All ***aminotransferases*** require the coenzyme pyridoxal phosphate (a derivative of vitamin B6;), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. ***Aminotransferases*** act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme.shows these two component reactions for the transamination catalyzed by ***AST***.

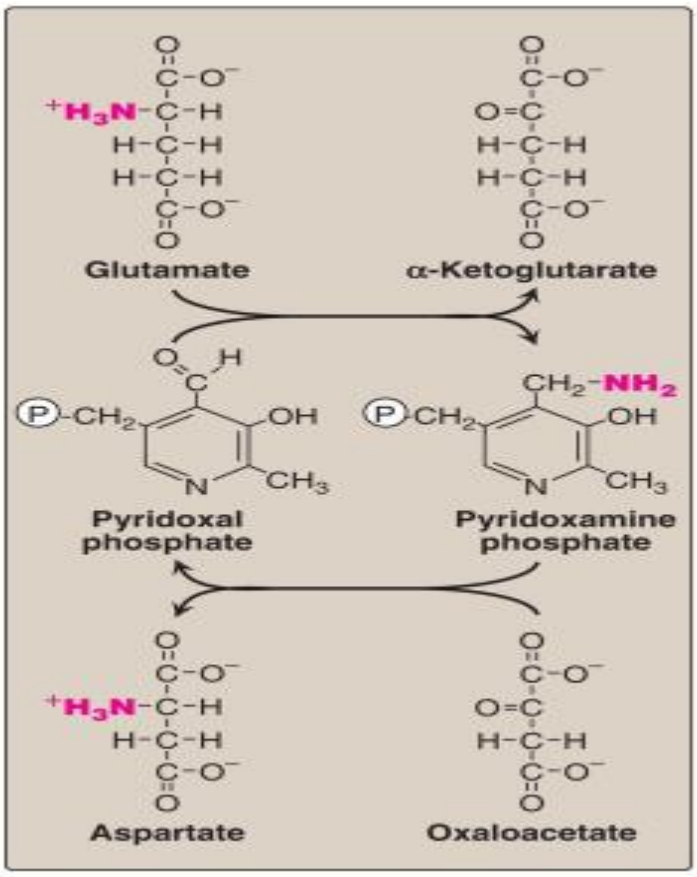



Figure: Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the ***aspartate aminotransferase*** reaction. = phosphate group.

3. Equilibrium: For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

4. Diagnostic value: ***Aminotransferases*** are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of ***aminotransferases*** indicate damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two ***aminotransferases***, ***AST*** and ***ALT***, are of particular diagnostic value when they are found in the plasma. a. Hepatic disease: Plasma ***AST*** and ***ALT*** are elevated in nearly all hepatic diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ***ALT*** is more specific than ***AST*** for liver disease, but the latter is more sensitive because the liver contains larger amounts of ***AST***. Serial measurements of ***AST*** and ***ALT*** (liver function tests) are often useful in determining the course of liver damage.shows the early release of ***ALT*** into the blood, following ingestion of a liver toxin. [Note: The elevation in bilirubin results from

hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin.]

b. Nonhepatic disease: ***Aminotransferases*** may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle.However, these disorders can usually be distinguished clinically from liver disease.

B. Oxidative deamination: Amino group removal In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as freeammonia. These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis. [Note: Ammonia exists primarily as ammonium (NH4+) in aqueous solution, but it is the unionized form (NH3) that crosses membranes.]

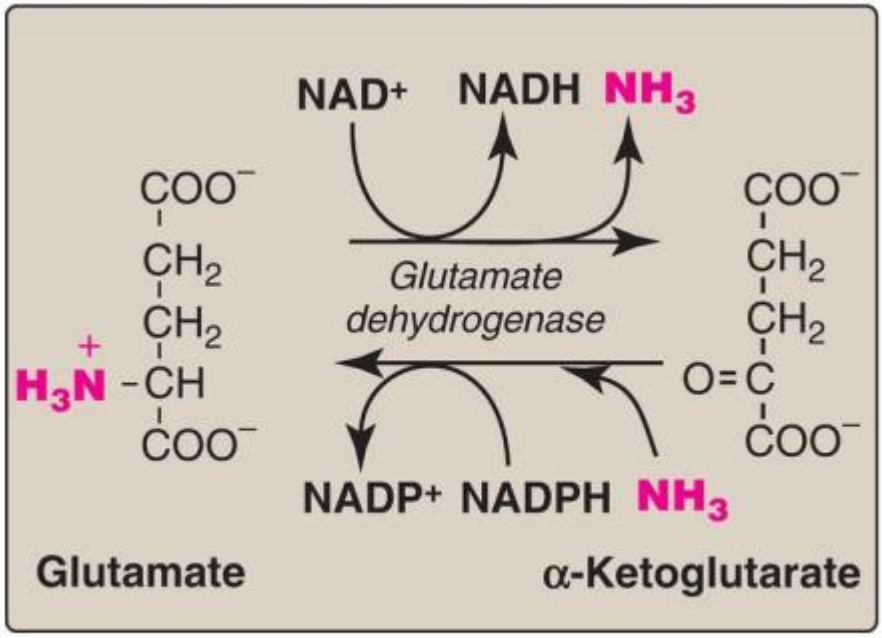



Figure 19.11 Oxidative deamination by ***glutamate dehydrogenase***. [Note: The enzyme is unusual in that it uses both $NAD^+$ (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate).] $NH_3$ = ammonia.

1. Glutamate dehydrogenase: As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by ***glutamate dehydrogenase*** ([***GDH***]. Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia. a. Coenzymes: ***GDH***, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD+) or its phosphorylated reduced form (NADPH) as a coenzyme. NAD+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, as shown in ), whereas NADPH is used in

reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in ).

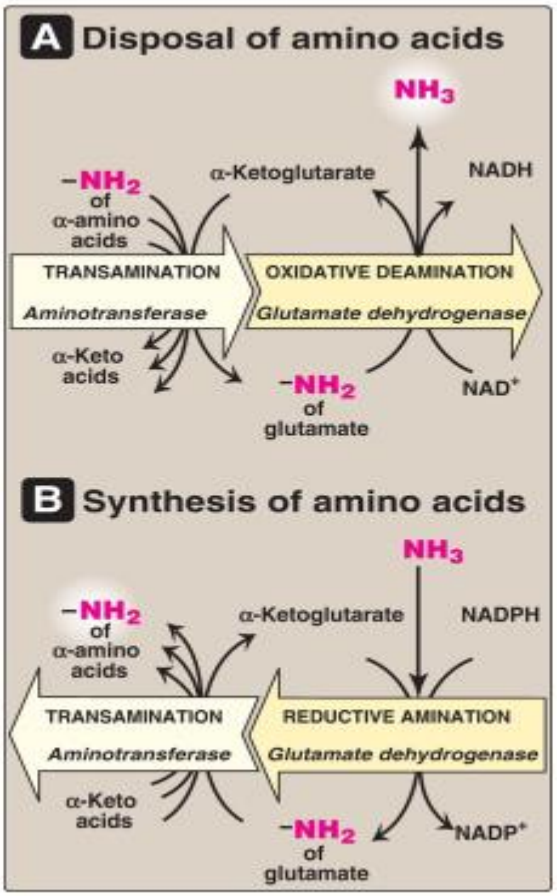



Figure: A, B. Combined actions of ***aminotransferase*** and ***glutamate dehydrogenase*** reactions. [Note: Reductive amination occurs only when ammonia (NH3) level is high.] NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate

b. Reaction direction: The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia. High ammonia levels are required to drive the reaction to glutamate synthesis.

c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of ***GDH***, whereas adenosine diphosphate is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by ***GDH*** is high, facilitating energy production from the carbon skeletons derived from amino acids.

2. d-Amino acid oxidase: D-Amino acids are supplied by the diet but are not used in the synthesis of mammalian proteins. They are, however, efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by flavin adenine dinucleotide–dependent ***D-amino acid oxidase*** (***DAO***). The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. [Note: ***DAO***

degrades D-serine, the isomeric form of serine that modulates ***N***-methylD-aspartate (NMDA)-type glutamate receptors. Increased ***DAO*** activity has been linked to increased susceptibility to schizophrenia. ***DAO*** also converts glycine to glyoxylate (see p. 263).] ***L-Amino acid oxidases*** are found in snake venom

C. Ammonia transport to the liver

Two mechanisms are available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses ***glutamine synthetase***to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by ***glutaminase***to glutamate and ammonia. The glutamate is oxidativelydeaminated to ammonia and α-ketoglutarate by ***GDH***. The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the BCAA isoleucine and valine. Alanine is transported in the blood to the liver, where it is transaminated by ***ALT*** to pyruvate. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle. The glutamate product of ***ALT*** can be deaminated by ***GDH***, generating ammonia. Thus, both alanine and glutamine carry ammonia to the liver.

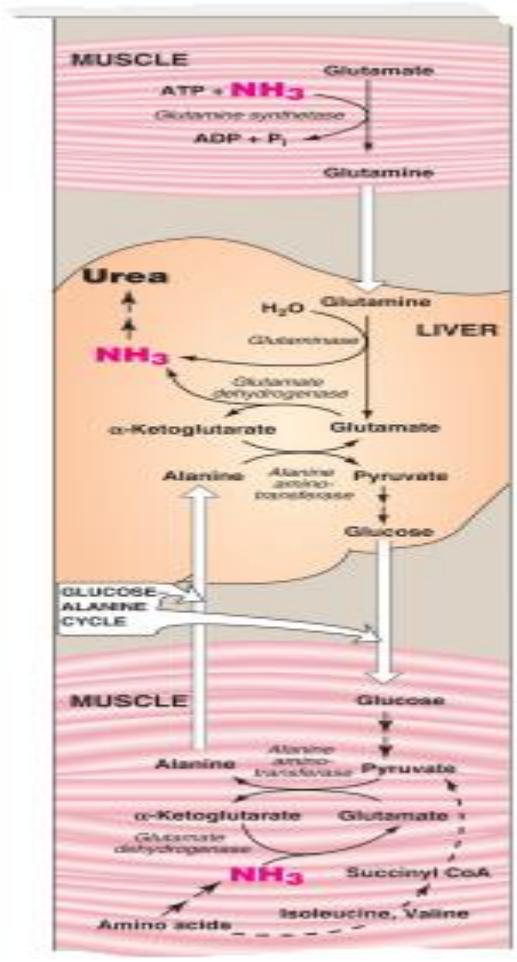



Figure: Transport of ammonia (NH3) from muscle to the liver. ADP = adenosine diphosphate; Pi = inorganic phosphate; CoA = coenzyme A.

Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. Free amino acids in the body are produced by hydrolysis of dietary protein by ***proteases*** activated from their zymogen form in the stomach and intestine, degradation of tissue proteins, and de novo synthesis. This amino acid pool is consumed in the synthesis of body protein, metabolized for energy, or its members used as precursors for other nitrogen-containing compounds. Free amino acids from digestion are taken up by intestinal enterocytes via sodium-dependent secondary active transport. Small peptides are taken up via proton-linked transport. Note that body protein is simultaneously degraded and resynthesized, a process known as protein turnover. The concentration of a cellular protein may be determined by regulation of its synthesis or degradation. The ATPdependent, cytosolic, selective ubiquitin–proteasome and ATPindependent, relatively nonselective lysosomal ***acid hydrolases*** are the two major enzyme systems that are responsible for degrading proteins. Nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are quickly degraded. The first phase of catabolism involves the transfer of the α-amino groups through transamination by pyridoxal phosphate–dependent ***aminotransferases*** (***transaminases***), followed by oxidative deamination of glutamate by ***glutamate dehydrogenase***, forming ammonia and the corresponding α-keto acids. A portion of the free ammonia is excreted in the urine. Some ammonia is used in converting glutamate to glutamine for safe transport, but most is used in the hepatic synthesis of urea, which is quantitatively the most important route for disposing of nitrogen from the body. Alanine also carries nitrogen to the liver for disposal as urea. The two major causes of hyperammonemia (with its neurologic effects) are acquired liver disease and congenital deficiencies of urea cycle enzymes such as X-linked ***ornithine transcarbamylase***.

**Topic: Protein metabolism and function.**

Aminotransferases (Transaminases) Both muscle and liver have aminotransferases, which, unlike deaminases, do not release the amino groups as free ammonium ion. Th s class of enzymes transfers the amino group from one carbon skeleton (an amino acid) to another (usually α-ketoglutarate, a citric acid cycle intermediate). Pyridoxal phosphate (PLP) derived from vitamin B 6 is required to mediate the transfer Aminotransferases are named according to the amino acid donating the amino group to α-ketoglutarate. Two important examples are alanine aminotransferase (ALT, formerly GPT) and aspartate aminotransferase (AST, formerly GOT). Although the aminotransferases are in liver and muscle, in pathologic conditions these enzymes may leak into the blood, where they are

useful clinical indicators of damage to liver or muscle. The reactions catalyzed by aminotransferases are reversible and play several roles in metabolism: • During protein catabolism in muscle, they move the amino groups from many of the different amino acids onto glutamate, thus pooling it for transport. A portion of the glutamate may be aminated by glutamine synthetase (as in other tissues) or may transfer the amino group to pyruvate, forming alanine using the aminotransferase ALT.• In liver, aminotransferases ALT and AST can move the amino group from alanine arriving from muscle into aspartate, a direct donor of nitrogen into the urea cycle

Amino acid degradation involves removal of the α-amino group, followed by thecatabolism of the resulting α-keto acids (carbon skeletons). These pathwaysconverge to form seven intermediate products: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, andacetoacetate. The products directly enter the pathways of intermediarymetabolism, resulting either in the synthesis of glucose, ketone bodies, or lipidsor in the production of energy through their oxidation to carbon dioxide ($CO_2$)by the tricarboxylic acid (TCA) cycle.provides an overview of thesepathways, with a more detailed summary presented in. Nonessential amino acids can be synthesized in sufficient amountsfrom the intermediates of metabolism or, as in the case of cysteine and tyrosine,from essential amino acids. In contrast, because the essential amino acids cannotbe synthesized (or synthesized in sufficient amounts) by humans, they must beobtained from the diet in order for normal protein synthesis to occur. Geneticdefects in the pathways of amino acid metabolism can cause serious disease.

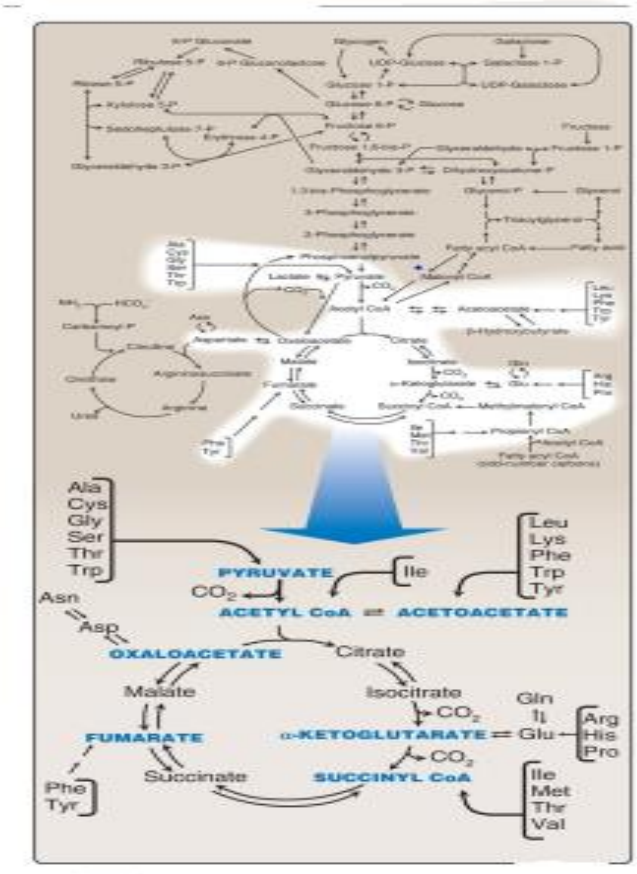


Figure: Amino acid metabolism shown as a part of the essential pathways of energy metabolism. CoA = coenzyme A; CO2 = carbon dioxide.

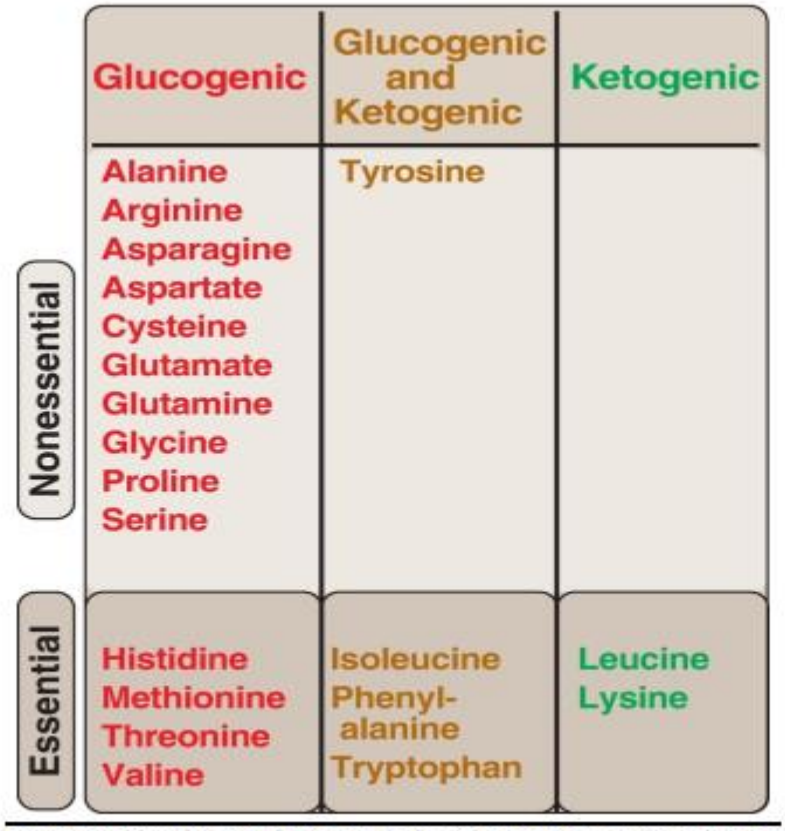



Figure 20.2 Classification of amino acids. [Note: Some amino acids can become conditionally essential. For example, supplementation with glutamine and arginine has been shown to improve outcomes in patients with trauma,

Amino acid degradation involves removal of the α-amino group, followed by the catabolism of the resulting α-keto acids (carbon skeletons). These pathways converge to form seven intermediate products: oxaloacetate, pyruvate, α-ketoglutarate, fumarate, succinyl coenzyme A (CoA), acetyl CoA, and acetoacetate. The products directly enter the pathways of intermediary metabolism, resulting either in the synthesis of glucose, ketone bodies, or lipids or in the production of energy through their oxidation to carbon dioxide (CO2) by the tricarboxylic acid (TCA) cycle. Nonessential amino acids can be synthesized in sufficient amounts from the intermediates of metabolism or, as in the case of cysteine and tyrosine, from essential amino acids. In contrast, because the essential amino acids cannot be synthesized (or synthesized in sufficient amounts) by humans, they must be obtained from the diet in order for normal protein synthesis to occur. Genetic defects in the pathways of amino acid metabolism can cause serious disease.

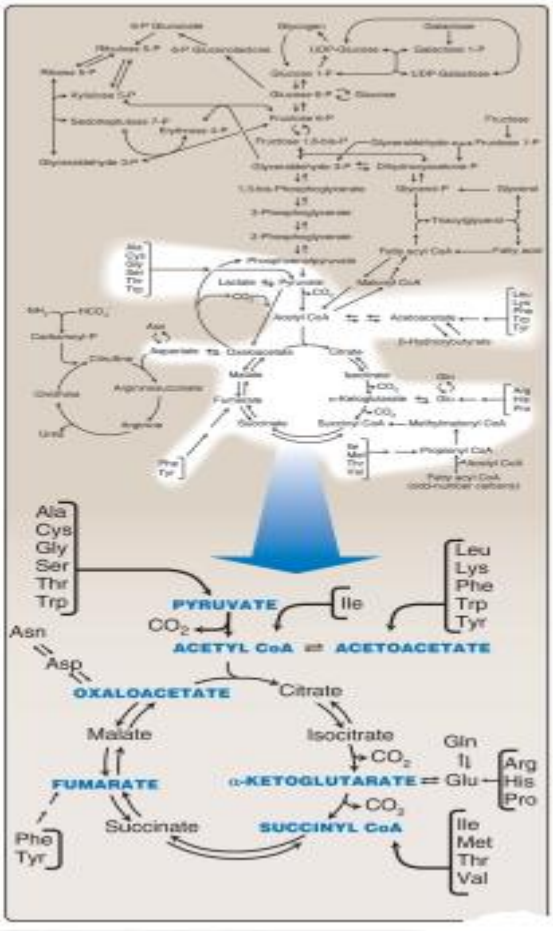



Figure: Amino acid metabolism shown as a part of the essential pathways ofenergy metabolism. CoA = coenzyme A; CO2 = carbon dioxide

Amino acids can be classified as glucogenic, ketogenic, or both, based on which of the seven intermediates are produced during their catabolism

A. Glucogenic amino acids Amino acids whose catabolism yields pyruvate or one of the intermediates of the TCA cycle are termed glucogenic. Because these intermediates are substrates for gluconeogenesis, they can give rise to the net synthesis of glucose in the liver and kidney. B. Ketogenic amino acids Amino acids whose catabolism yields either acetoacetate or one of its precursors (acetyl CoA or acetoacetyl CoA) are termed ketogenic. Acetoacetate is one of the ketone bodies, which also include 3-hydroxybutyrate and acetone. Leucine and lysine are the only exclusively ketogenic amino acids found in proteins. Their carbon skeletons are not substrates for gluconeogenesis and, therefore, cannot give rise to the net synthesis of glucose.

NITROGEN REMOVAL FROM AMINO ACIDS

The presence of the α-amino group keeps amino acids safely locked away from oxidative breakdown. Removing the α-amino group is essential for producing energy from any amino acid and is an obligatory step in the catabolism of all amino acids. Once removed, this nitrogen can be incorporated into other compounds or excreted as urea, with the carbon skeletons being metabolized. This section describes transamination and oxidative deamination, reactions that ultimately provide ammonia and aspartate, the two sources of urea nitrogen.

Transamination: Funneling amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of their α-amino group to α-ketoglutarate, producing an α-keto acid (derived from the original amino acid) and glutamate. α-Ketoglutarate plays a pivotal role in amino acid metabolism by accepting the amino groups from most amino acids, thereby becoming glutamate. Glutamate produced by transamination can be oxidativelydeaminated or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called ***aminotransferases*** (also called ***transaminases***). These enzymes are found in the cytosol and mitochondria of cells throughout the body. All amino acids, with the exception of lysine and threonine, participate in transamination at some point in their catabolism. [Note: These two amino acids lose their α-amino groups by deamination.]

1.Substrate specificity: Each ***aminotransferase*** is specific for one or, at most, a few amino group donors. ***Aminotransferases*** are named after the specific amino group donor, because the acceptor of the amino group is almost always α-ketoglutarate. Two important ***aminotransferase*** reactions are catalyzed by

a.***alanine aminotransferase*** (***ALT***) and ***aspartate aminotransferase*** (***AST***), as shown in The enzyme catalyzes the transfer of the amino group of alanine to α-ketoglutarate, resulting in the formation of pyruvate and glutamate. The reaction is readily reversible. However, during amino acid catabolism, this enzyme (like most ***aminotransferases***) functions in the direction of glutamate synthesis. [Note: In effect, glutamate acts as a collector of nitrogen from most amino acids.]

b. Aspartate aminotransferase: ***AST*** is an exception to the rule that ***aminotransferases*** funnel amino groups to form glutamate. During amino acid catabolism, ***AST*** primarily transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle . Like other transaminations, the ***AST*** reaction is reversible.

2. Mechanism: All ***aminotransferases*** require the coenzyme pyridoxal phosphate (a derivative of vitamin B6;), which is covalently linked to the ε-amino group of a specific lysine residue at the active site of the enzyme. ***Aminotransferases*** act by transferring the amino group of an amino acid to the pyridoxal part of the coenzyme to generate pyridoxamine phosphate. The pyridoxamine form of the coenzyme then reacts with an α-keto acid to form an amino acid, at the same time regenerating the original aldehyde form of the coenzyme.shows these two component reactions for the transamination catalyzed by ***AST***.

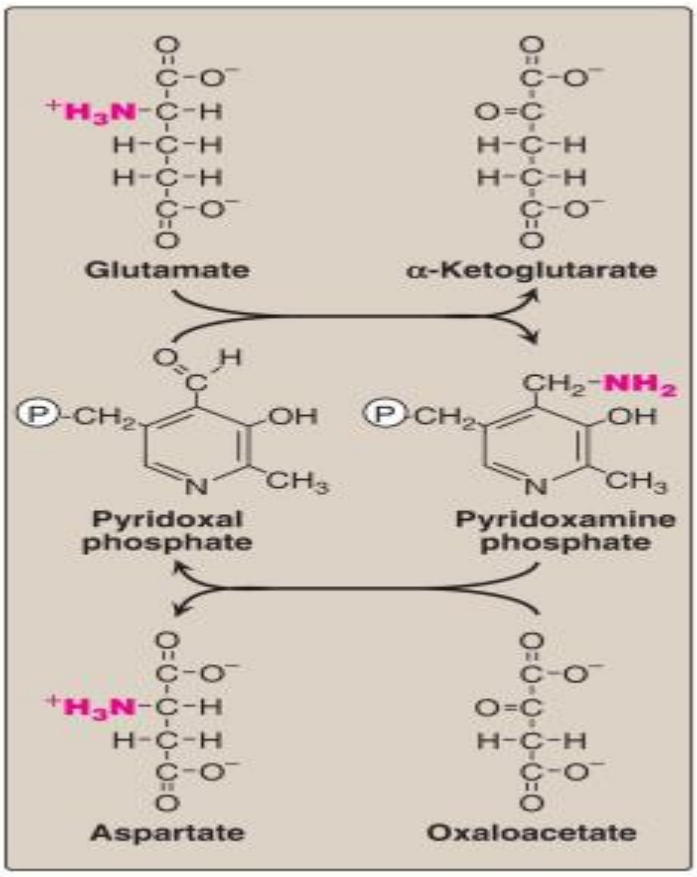



Figure: Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the ***aspartate aminotransferase*** reaction. = phosphate group.

3. Equilibrium: For most transamination reactions, the equilibrium constant is near 1. This allows the reaction to function in both amino acid degradation through removal of α-amino groups (for example, after consumption of a protein-rich meal) and biosynthesis of nonessential amino acids through addition of amino groups to the carbon skeletons of α-keto acids (for example, when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells).

4. Diagnostic value: ***Aminotransferases*** are normally intracellular enzymes, with the low levels found in the plasma representing the release of cellular contents during normal cell turnover. Elevated plasma levels of ***aminotransferases*** indicate damage to cells rich in these enzymes. For example, physical trauma or a disease process can cause cell lysis, resulting in release of intracellular enzymes into the blood. Two ***aminotransferases***, ***AST*** and ***ALT***, are of particular diagnostic value when they are found in the plasma. a. Hepatic disease: Plasma ***AST*** and ***ALT*** are elevated in nearly all hepatic diseases but are particularly high in conditions that cause extensive cell necrosis, such as severe viral hepatitis, toxic injury, and prolonged circulatory collapse. ***ALT*** is more specific than ***AST*** for liver disease, but the latter is more sensitive because the liver contains larger amounts of ***AST***. Serial measurements of ***AST*** and ***ALT*** (liver function tests) are often useful in determining the course of liver damage.shows the early release of ***ALT*** into the blood, following ingestion of a liver toxin. [Note: The elevation in bilirubin results from

hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin.]

b. Nonhepatic disease: ***Aminotransferases*** may be elevated in nonhepatic diseases such as those that cause damage to cardiac or skeletal muscle.However, these disorders can usually be distinguished clinically from liver disease.

B. Oxidative deamination: Amino group removal In contrast to transamination reactions that transfer amino groups, oxidative deamination reactions result in the liberation of the amino group as freeammonia. These reactions occur primarily in the liver and kidney. They provide α-keto acids that can enter the central pathways of energy metabolism and ammonia, which is a source of nitrogen in hepatic urea synthesis. [Note: Ammonia exists primarily as ammonium (NH4+) in aqueous solution, but it is the unionized form (NH3) that crosses membranes.]

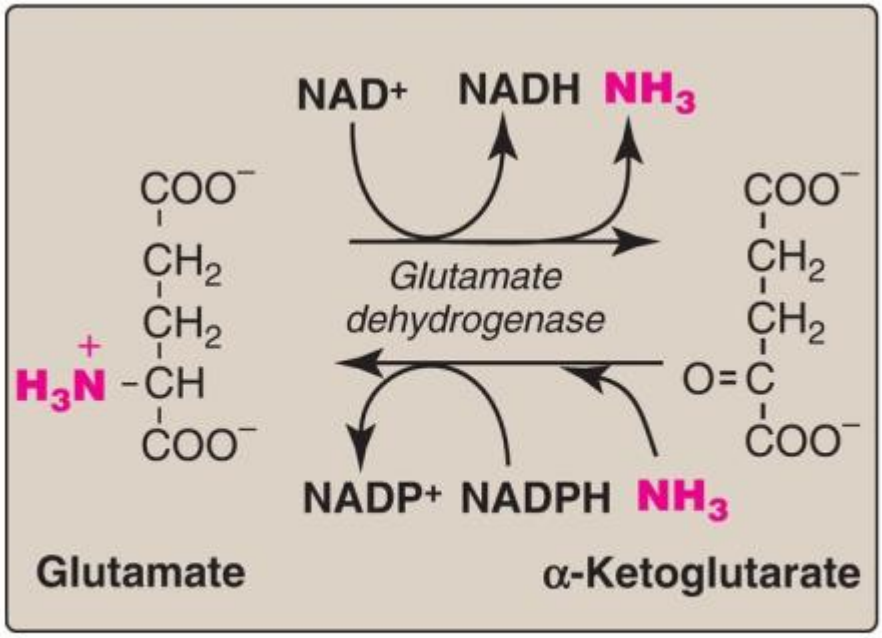



Figure 19.11 Oxidative deamination by ***glutamate dehydrogenase***. [Note: The enzyme is unusual in that it uses both $NAD^+$ (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate).] $NH_3$ = ammonia.

1. Glutamate dehydrogenase: As described above, the amino groups of most amino acids are ultimately funneled to glutamate by means of transamination with α-ketoglutarate. Glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination, a reaction catalyzed by ***glutamate dehydrogenase*** ([***GDH***]. Therefore, the sequential action of transamination (resulting in the transfer of amino groups from most amino acids to α-ketoglutarate to produce glutamate) and the oxidative deamination of that glutamate (regenerating α-ketoglutarate) provide a pathway whereby the amino groups of most amino acids can be released as ammonia. a. Coenzymes: ***GDH***, a mitochondrial enzyme, is unusual in that it can use either nicotinamide adenine dinucleotide (NAD+) or its phosphorylated reduced form (NADPH) as a coenzyme. NAD+ is used primarily in oxidative deamination (the simultaneous loss of ammonia coupled with the oxidation of the carbon skeleton, as shown in ), whereas NADPH is used in

reductive amination (the simultaneous gain of ammonia coupled with the reduction of the carbon skeleton, as shown in ).

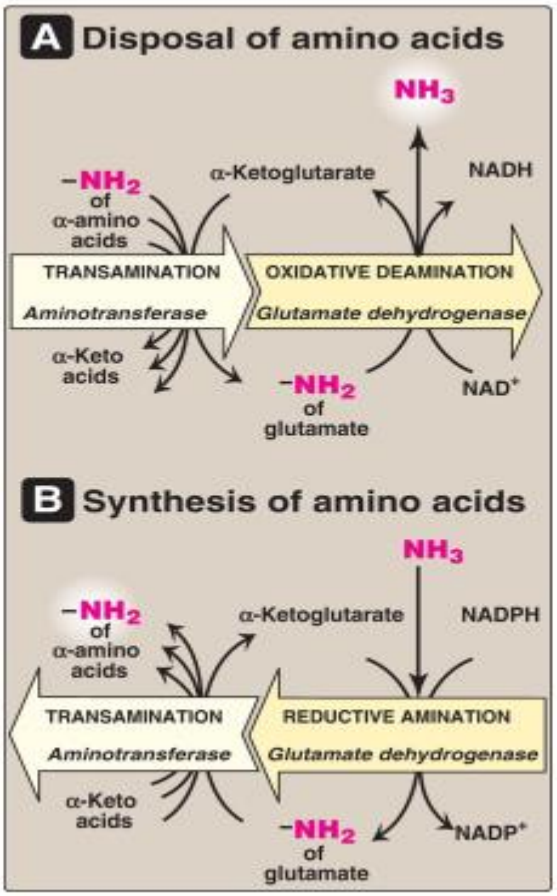



Figure: A, B. Combined actions of ***aminotransferase*** and ***glutamate dehydrogenase*** reactions. [Note: Reductive amination occurs only when ammonia (NH3) level is high.] NAD(H) = nicotinamide adenine dinucleotide; NADP(H) = nicotinamide adenine dinucleotide phosphate

b. Reaction direction: The direction of the reaction depends on the relative concentrations of glutamate, α-ketoglutarate, and ammonia and the ratio of oxidized to reduced coenzymes. For example, after ingestion of a meal containing protein, glutamate levels in the liver are elevated, and the reaction proceeds in the direction of amino acid degradation and the formation of ammonia. High ammonia levels are required to drive the reaction to glutamate synthesis.

c. Allosteric regulators: Guanosine triphosphate is an allosteric inhibitor of ***GDH***, whereas adenosine diphosphate is an activator. Therefore, when energy levels are low in the cell, amino acid degradation by ***GDH*** is high, facilitating energy production from the carbon skeletons derived from amino acids.

2. d-Amino acid oxidase: D-Amino acids are supplied by the diet but are not used in the synthesis of mammalian proteins. They are, however, efficiently metabolized to α-keto acids, ammonia, and hydrogen peroxide in the peroxisomes of liver and kidney cells by flavin adenine dinucleotide–dependent ***D-amino acid oxidase*** (***DAO***). The α-keto acids can enter the general pathways of amino acid metabolism and be reaminated to L-isomers or catabolized for energy. [Note: ***DAO***

degrades D-serine, the isomeric form of serine that modulates ***N***-methylD-aspartate (NMDA)-type glutamate receptors. Increased ***DAO*** activity has been linked to increased susceptibility to schizophrenia. ***DAO*** also converts glycine to glyoxylate (see p. 263).] ***L-Amino acid oxidases*** are found in snake venom

C. Ammonia transport to the liver

Two mechanisms are available in humans for the transport of ammonia from peripheral tissues to the liver for conversion to urea. Both are important in, but not exclusive to, skeletal muscle. The first uses ***glutamine synthetase***to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by ***glutaminase***to glutamate and ammonia. The glutamate is oxidativelydeaminated to ammonia and α-ketoglutarate by ***GDH***. The ammonia is converted to urea. The second transport mechanism involves the formation of alanine by the transamination of pyruvate produced from both aerobic glycolysis and metabolism of the succinyl coenzyme A (CoA) generated by the catabolism of the BCAA isoleucine and valine. Alanine is transported in the blood to the liver, where it is transaminated by ***ALT*** to pyruvate. The pyruvate is used to synthesize glucose, which can enter the blood and be used by muscle, a pathway called the glucose–alanine cycle. The glutamate product of ***ALT*** can be deaminated by ***GDH***, generating ammonia. Thus, both alanine and glutamine carry ammonia to the liver.

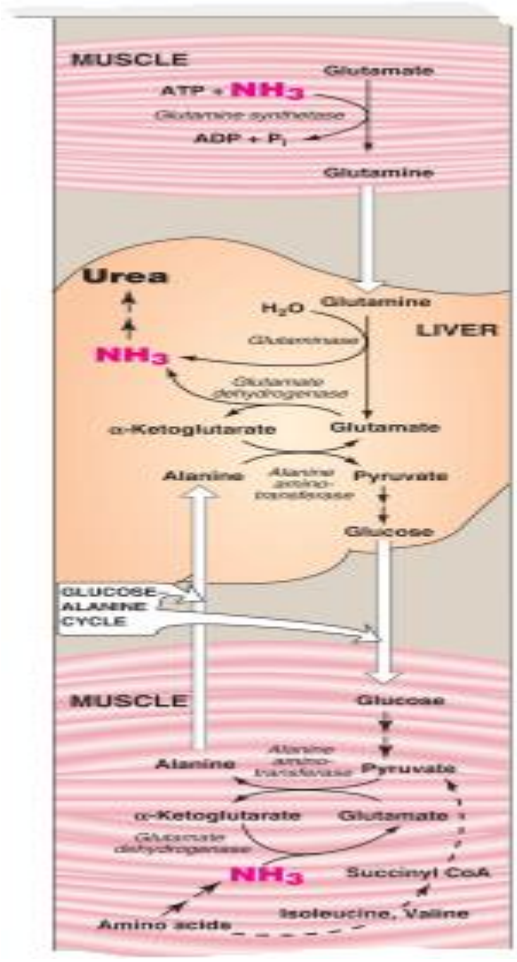



Figure: Transport of ammonia (NH3) from muscle to the liver. ADP = adenosine diphosphate; Pi = inorganic phosphate; CoA = coenzyme A.

Nitrogen enters the body in a variety of compounds present in food, the most important being amino acids contained in dietary protein. Nitrogen leaves the body as urea, ammonia, and other products derived from amino acid metabolism. Free amino acids in the body are produced by hydrolysis of dietary protein by ***proteases*** activated from their zymogen form in the stomach and intestine, degradation of tissue proteins, and de novo synthesis. This amino acid pool is consumed in the synthesis of body protein, metabolized for energy, or its members used as precursors for other nitrogen-containing compounds. Free amino acids from digestion are taken up by intestinal enterocytes via sodium-dependent secondary active transport. Small peptides are taken up via proton-linked transport. Note that body protein is simultaneously degraded and resynthesized, a process known as protein turnover. The concentration of a cellular protein may be determined by regulation of its synthesis or degradation. The ATPdependent, cytosolic, selective ubiquitin–proteasome and ATPindependent, relatively nonselective lysosomal ***acid hydrolases*** are the two major enzyme systems that are responsible for degrading proteins. Nitrogen cannot be stored, and amino acids in excess of the biosynthetic needs of the cell are quickly degraded. The first phase of catabolism involves the transfer of the α-amino groups through transamination by pyridoxal phosphate–dependent ***aminotransferases*** (***transaminases***), followed by oxidative deamination of glutamate by ***glutamate dehydrogenase***, forming ammonia and the corresponding α-keto acids. A portion of the free ammonia is excreted in the urine. Some ammonia is used in converting glutamate to glutamine for safe transport, but most is used in the hepatic synthesis of urea, which is quantitatively the most important route for disposing of nitrogen from the body. Alanine also carries nitrogen to the liver for disposal as urea. The two major causes of hyperammonemia (with its neurologic effects) are acquired liver disease and congenital deficiencies of urea cycle enzymes such as X-linked ***ornithine transcarbamylase***.

**TOPIC: Protein metabolism and function.**

Protein obtained from the diet or from body protein during prolonged fasting orstarvation may be used as an energy source. Body protein is catabolized primarilyin muscle and in liver. Amino acids released from proteins usually lose their aminogroup through transamination or deamination. Te carbon skeletons can be converted in the liver to glucose (glucogenic amino acids), acetyl CoA, and ketone bodies (ketogenic), or in a few cases both may be produced (glucogenic and ketogenic).

**REMOVAL AND EXCRETION OF AMINO GROUPS**Excess nitrogen is eliminated from the body in the urine. Te kidney adds smallquantities of ammonium ion to the urine in part to regulate acid-base balance, butnitrogen is also eliminated in this process. Most excess nitrogen is converted to ureain the liver and goes through the blood to the kidney, where it is eliminated in

urine.Amino groups released by deamination reactions form ammonium ion (NH4+),which must not escape into the peripheral blood. An elevated concentration ofammonium ion in the blood, hyperammonemia, has toxic eff ects in the brain(cerebral edema, convulsions, coma, and death). Most tissues add excess nitrogen to the blood as glutamine by attaching ammonia to the g-carboxyl group ofglutamate. Muscle sends nitrogen to the liver as alanine and smaller quantities ofother amino acids, in addition to glutamine. Figure I-17-1 summarizes the fl wof nitrogen from tissues to either the liver or kidney for excretion.

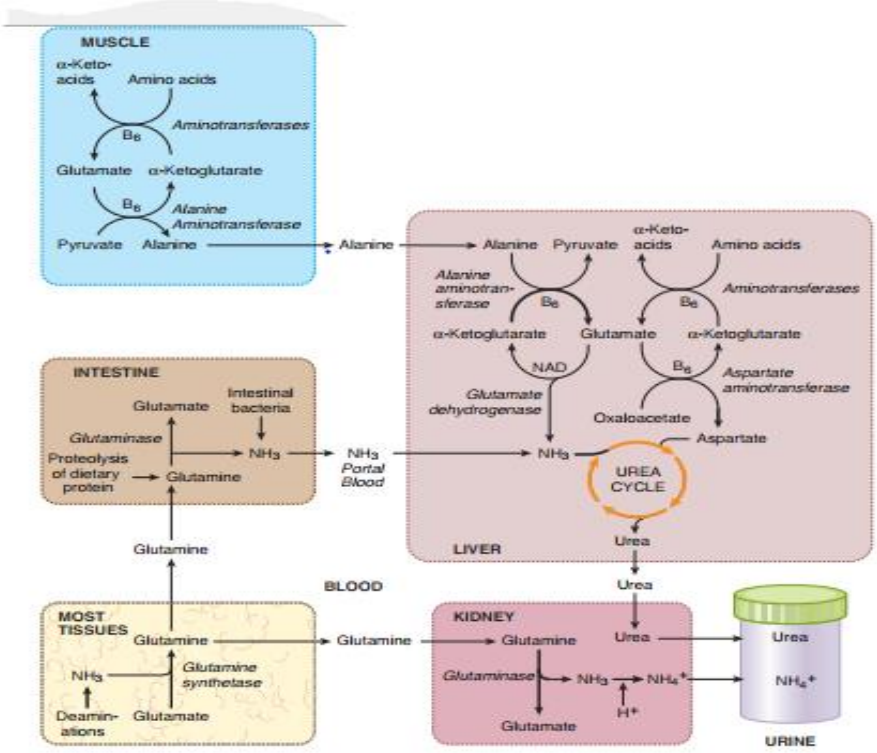



Figure I-17-1. Amino Group Removal for Elimination as Urea and Ammonia

**Glutamine Synthetase**Most tissues, including muscle, have glutamine synthetase, which captures excess nitrogen by aminating glutamate to form glutamine. Te reaction is irreversible. Glutamine, a relatively nontoxic substance, is the major carrier of excess nitrogen from tissues. GlutaminaseTe kidney contains glutaminase, allowing it to deaminate glutamine arriving in the blood and to eliminate the amino group as ammonium ion in urine. Te reaction is irreversible. Kidney glutaminase is induced by chronic acidosis, in which excretion of ammonium may become the major defense mechanism. Te liver has only small quantities of glutaminase; however, levels of the enzyme are high in the intestine where the ammonium ion from deamination can be sent directly to the liver via the portal blood and used for urea synthesis. Te intestinal bacteria and glutamine from dietary protein contribute to the intestinal ammonia entering the portal blood. Aminotransferases (Transaminases) Both muscle and liver have aminotransferases, which, unlike deaminases, do not release the amino groups as free ammonium ion. Th s class of enzymes transfers the amino group from one carbon skeleton (an amino acid) to another (usually α-ketoglutarate, a citric acid cycle intermediate). Pyridoxal phosphate (PLP) derived from vitamin B 6 is required to mediate the transfer Aminotransferases are named according to the amino acid donating the

amino group to α-ketoglutarate. Two important examples are alanine aminotransferase (ALT, formerly GPT) and aspartate aminotransferase (AST, formerly GOT). Although the aminotransferases are in liver and muscle, in pathologic conditions these enzymes may leak into the blood, where they are useful clinical indicators of damage to liver or muscle. The reactions catalyzed by aminotransferases are reversible and play several roles in metabolism: • During protein catabolism in muscle, they move the amino groups from many of the different amino acids onto glutamate, thus pooling it for transport. A portion of the glutamate may be aminated by glutamine synthetase (as in other tissues) or may transfer the amino group to pyruvate, forming alanine using the aminotransferase ALT.• In liver, aminotransferases ALT and AST can move the amino group from alanine arriving from muscle into aspartate, a direct donor of nitrogen into the urea cycle Glutamate Dehydrogenase Th s enzyme is found in many tissues, where it catalyzes the reversible oxidative deamination of the amino acid glutamate. It produces the citric acid cycle intermediate α-ketoglutarate, which serves as an entry point to the cycle for a group of glucogenic amino acids. Its role in urea synthesis and nitrogen removal is still controversial, but has been included in.

The pathways by which amino acids are catabolized are conveniently organizedaccording to which one (or more) of the seven intermediates listed above isproduced from a particular amino acid

A. Amino acids that form oxaloacetateAsparagine is hydrolyzed by ***asparaginase***, liberating ammonia andaspartate .Aspartate loses its amino group by transamination toform oxaloacetate. [Note: Some rapidly dividing leukemiccells are unable to synthesize sufficient asparagine to support their growth.This makes asparagine an essential amino acid for these cells, which,therefore, require asparagine from the blood. ***Asparaginase***, whichhydrolyzes asparagine to aspartate, can be administered systemically totreat leukemia. ***Asparaginase***lowers the level of asparagine in the plasma,thereby depriving cancer cells of a required nutrient.]

B. Amino acids that form α-ketoglutarate via glutamate

1. Glutamine: This amino acid is hydrolyzed to glutamate and ammonia bythe enzyme ***glutaminas.***Glutamate is converted to α-ketoglutarate by transamination or through oxidative deamination by***glutamate dehydrogenase***.

2. Proline: This amino acid is oxidized to glutamate. Glutamate istransaminated or oxidativelydeaminated to form α-ketoglutarate.

3. Arginine: This amino acid is hydrolyzed by ***arginase*** to produceornithine (and urea). [Note: The reaction occurs primarily in the liver aspart of the urea cycle.] Ornithine is subsequently convertedto α-ketoglutarate, with glutamate semialdehyde as an intermediate.

4. Histidine: This amino acid is oxidativelydeaminated by ***histidase***tourocanic acid, which subsequently forms N-formiminoglutamate([FIGlu], FIGlu donates its formimino group totetrahydrofolate (THF), leaving glutamate, which is degraded asdescribed above. [Note: Individuals deficient in folic acid excreteincreased amounts of FIGlu in the urine, particularly after ingestion of alarge dose of histidine. The FIGlu excretion test has been used indiagnosing a deficiency of folic

acid. for a discussion of folicacid, THF, and one-carbon metabolism.]Degradation of histidine. NH3 = ammonia.

C. Amino acids that form pyruvate

1. Alanine: This amino acid loses its amino group by transamination toform pyruvate [Note: Tryptophan catabolism producesalanine and, therefore, pyruvate .]Transamination of alanine to pyruvate. PLP = pyridoxal phosphate.

2. Serine: This amino acid can be converted to glycine as THF becomesN5,N10-methylenetetrahydrofolate (N5,N10-MTHF), as shown in. Serine can also be converted to

3. Glycine: This amino acid can be converted to serine by the reversibleaddition of a methylene group from N5,N10-MTHF oroxidized to CO2 and ammonia by the glycine cleavage system. [Note:Glycine can be deaminated to glyoxylate (by a ***d-amino acid oxidase***;), which can be oxidized to oxalate or transaminated to glycine.Deficiency of the ***transaminase*** in liver peroxisomes causesoverproduction of oxalate, the formation of oxalate stones, and kidneydamage (primary oxaluria type 1).]

4. Cysteine: This sulfur-containing amino acid undergoes desulfurization toyield pyruvate. [Note: The sulfate released can be used to synthesize 3′-phosphoadenosine-5′-phosphosulfate (PAPS), an activated sulfate donorto a variety of acceptors.] Cysteine can also be oxidized to its disulfidederivative, cystine.5. Threonine: This amino acid is converted to pyruvate in most organismsbut is a minor pathway (at best) in humans.

D. Amino acids that form fumarate

1. Phenylalanine and tyrosine: Hydroxylation of phenylalanine producestyrosine. This irreversible reaction, catalyzed bytetrahydrobiopterin-requiring ***phenylalanine hydroxylase*** (***PAH***),initiates the catabolism of phenylalanine. Thus, phenylalaninemetabolism and tyrosine metabolism merge, leading ultimately tofumarate and acetoacetate formation. Therefore, phenylalanine andtyrosine are both glucogenic and ketogenic.Degradation of phenylalanine.

2. Inherited deficiencies: Inherited deficiencies in the enzymes ofphenylalanine and tyrosine metabolism lead to the diseasesphenylketonuria (PKU), tyrosinemia, andalkaptonuria as well as the condition of albinism.

E. Amino acids that form succinyl CoA: MethionineMethionine is one of four amino acids that form succinyl CoA. This sulfurcontaining amino acid deserves special attention because it is converted to***S***-adenosylmethionine (SAM), the major methyl group donor in one-carbonmetabolism. Methionine is also the source of homocysteine(Hcy), a metabolite associated with atherosclerotic vascular disease andthrombosis.

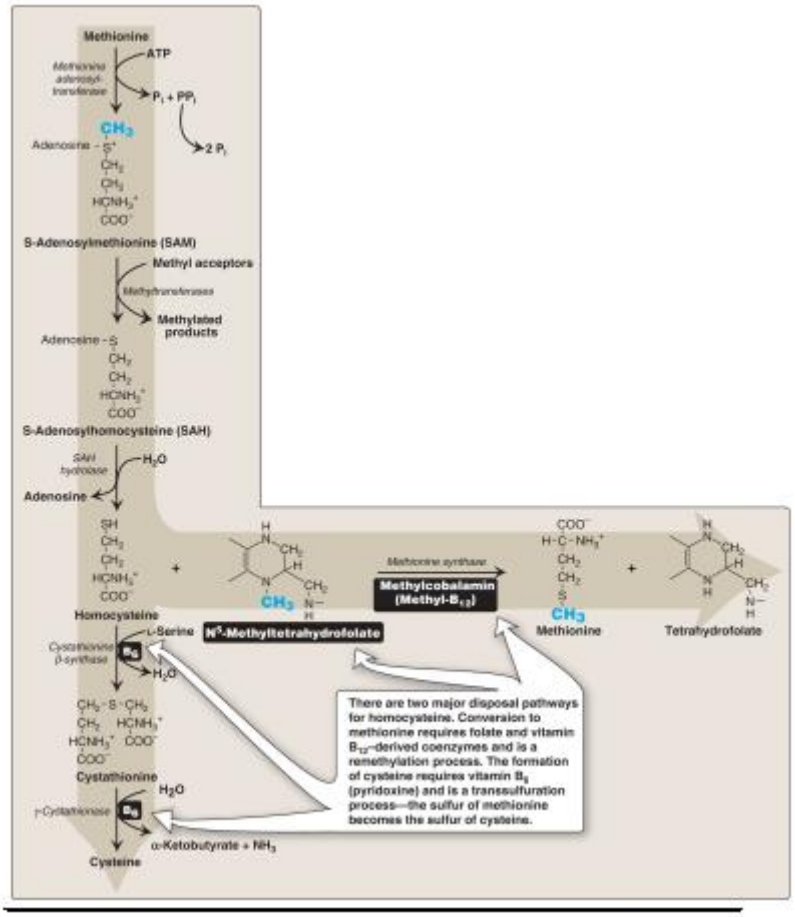



Figure: Degradation and resynthesis of methionine. [Note: The resynthesisof methionine from homocysteine is the only reaction in which tetrahydrofolateboth carries and donates a methyl (-CH3) group. In all other reactions, SAM isthe methyl group carrier and donor.] PPi = pyrophosphate; Pi = inorganicphosphate; NH3 = ammonia.

1. S-Adenosylmethionine synthesis: Methionine condenses with ATP,forming SAM, a high-energy compound that is unusual in that it containsno phosphate. The formation of SAM is driven by hydrolysis of all threephosphate bonds in ATP.

2. Activated methyl group: The methyl group attached to the sulfur in SAMis activated and can be transferred by ***methyltransferases***to a variety ofacceptors such as norepinephrine in the synthesis of epinephrine. Themethyl group is usually transferred to nitrogen or oxygen atoms (as withepinephrine synthesis and degradation, respectively;) andsometimes to carbon atoms (as with cytosine). The reaction product, ***S***adenosylhomocysteine (SAH), is a simple thioether, analogous tomethionine. The resulting loss of free energy makes methyl transferessentially irreversible.

3. S-Adenosylhomocysteine hydrolysis: After donation of the methyl group,SAH is hydrolyzed to Hcy and adenosine. Hcy has two fates. If there is adeficiency of methionine, Hcy may beremethylated to methionine. If methionine stores are adequate, Hcy may enter thetranssulfuration pathway, where it is converted to cysteine.a. Methionine resynthesis: Hcy accepts a methyl group from N5-methyltetrahydrofolate (N5-methyl-THF) in a reaction requiringmethylcobalamin, a coenzyme derived from vitamin B12.[Note: The

methyl group is transferred by ***methionine synthase*** fromthe B12 derivative to Hcy, regenerating methionine. Cobalamin isremethylated from N5-methyl-THF.]b. Cysteine synthesis: Hcy condenses with serine, forming cystathionine,which is hydrolyzed to α-ketobutyrate and cysteine.This vitamin B6–requiring sequence has the net effect of convertingserine to cysteine and Hcy to α-ketobutyrate, which is oxidativelydecarboxylated to form propionyl CoA. Propionyl CoA is converted tosuccinyl CoA. Because Hcy is synthesizedfrom the essential amino acid methionine, cysteine is not an essentialamino acid as long as sufficient methionine is available.4. Relationship of homocysteine to vascular disease: Elevations in plasmaHcy levels promote oxidative damage, inflammation, and endothelialdysfunction and are an independent risk factor for occlusive vasculardiseases such as cardiovascular disease (CVD) and stroke.Mild elevations (hyperhomocysteinemia) are seen in ~7% of thepopulation. Epidemiologic studies have shown that plasma Hcy levels areinversely related to plasma levels of folate, B12, and B6, the threevitamins involved in the conversion of Hcy to methionine and cysteine.Supplementation with these vitamins has been shown to reducecirculating levels of Hcy. However, in patients with established CVDvitamin therapy does not decrease cardiovascular events or death. Thisraises the question as to whether Hcy is a cause of the vascular damageor merely a marker of such damage. [Note: Large elevations in plasmaHcy as a result of rare deficiencies in ***cystathionine β-synthase*** of thetranssulfuration pathway are seen in patients with classic homocystinuria(resulting from severe hyperhomocysteinemia [>100 μmol/l]] Deficiencies in the remethylation reaction also result in a rise inHcy..Association between cardiovascular disease mortality and totalplasma homocysteine.Elevated homocysteine and decreased folic acid levels in pregnant womenare associated with increased incidence of neural tube defects (improperclosure, as in spina bifida) in the fetus. Periconceptual supplementationwith folate reduces the risk of such defects.

F. Other amino acids that form succinyl CoADegradation of valine, isoleucine, and threonine also results in theproduction of succinyl CoA, a TCA cycle intermediate and gluconeogeniccompound. [Note: It is metabolized to pyruvate.]1. Valine and isoleucine: These amino acids are branched-chain aminoacids (BCAA) that generate propionyl CoA, which is converted tomethylmalonyl CoA and then succinyl CoA by biotin- and vitamin B12–requiring reactions.2. Threonine: This amino acid is dehydrated to α-ketobutyrate, which isconverted to propionyl CoA and then to succinyl CoA. Propionyl CoA,then, is generated by the catabolism of the amino acids methionine,valine, isoleucine, and threonine. [Note: Propionyl CoA also is generatedby the oxidation of odd-numbered fatty acids.]

G. Amino acids that form acetyl CoA or acetoacetyl CoATryptophan, leucine, isoleucine, and lysine form acetyl CoA or acetoacetylCoA directly, without pyruvate serving as an intermediate. As noted earlier,phenylalanine and tyrosine also give rise to acetoacetate during theircatabolism. Therefore, there are a total of six partly orwholly ketogenic amino acids.1. Tryptophan: This amino acid is both glucogenic and ketogenic, becauseits catabolism yields alanine and acetoacetyl

CoA Quinolinate from tryptophan catabolism is used in the synthesis ofnicotinamide adenine dinucleotide (

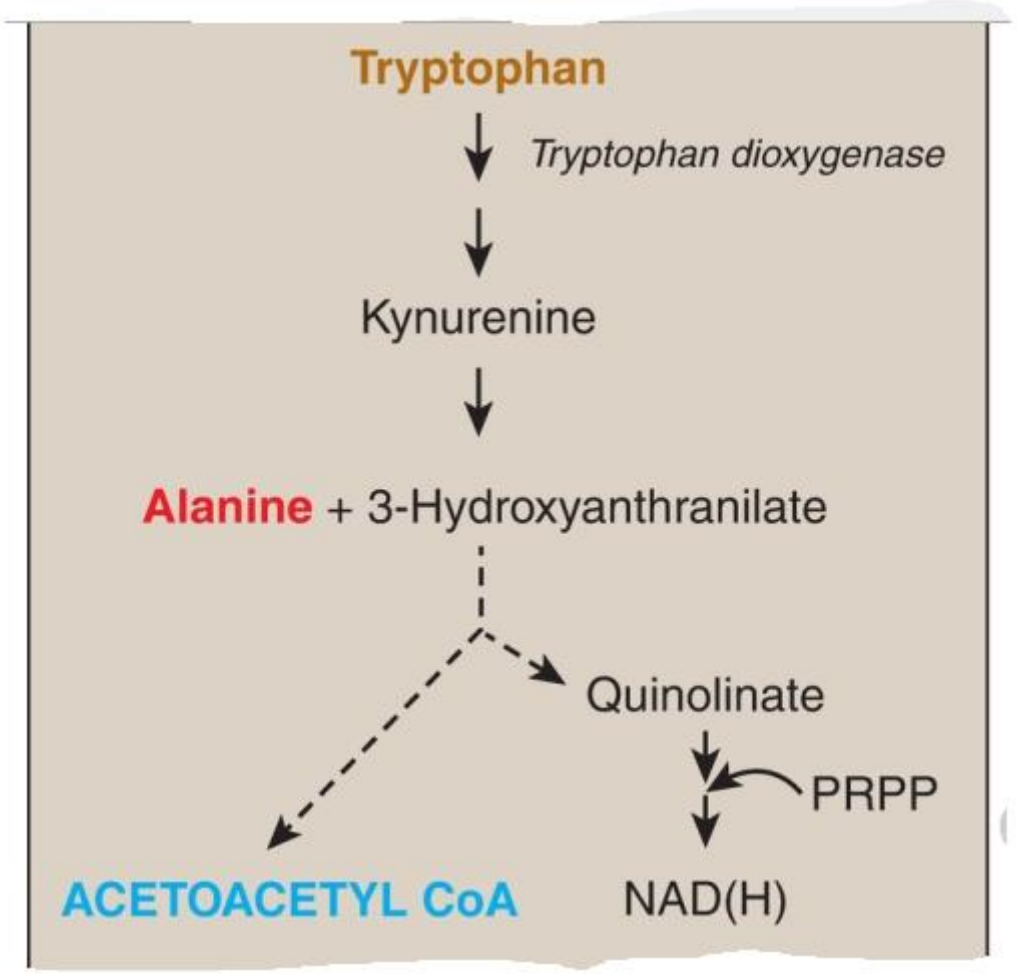



Figure 20.10 Metabolism of tryptophan by the kynurenine pathway(abbreviated). CoA = coenzyme A; PRPP = phosphoribosyl pyrophosphate;NAD(H) = nicotinamide adenine dinucleotide.2. Leucine: This amino acid is exclusively ketogenic, because its catabolismyields acetyl CoA and acetoacetate

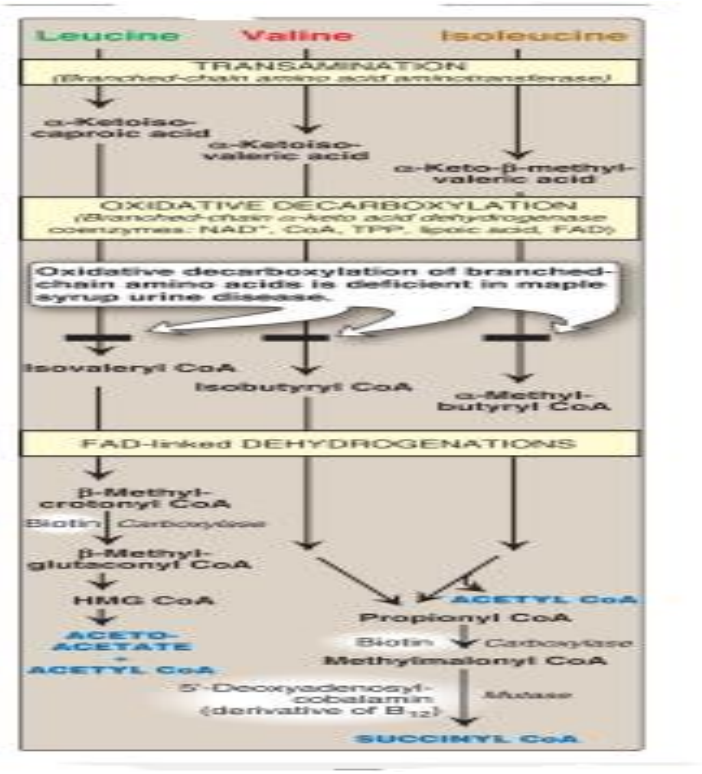



Figure: Degradation of leucine, valine, and isoleucine. [Note: ***β-Methylcrotonyl CoA carboxylase*** is one of four biotin-requiring ***carboxylases***discussed in this book. The other three are ***pyruvate carboxylase***, ***acetyl CoAcarboxylase***, and ***propionyl CoA carboxylase***.] TPP = thiamine pyrophosphate;FAD = flavin adenine dinucleotide; CoA = coenzyme A; NAD = nicotinamideadenine dinucleotide; HMG = hydroxymethylglutarate.

3. Isoleucine: This amino acid is both ketogenic and glucogenic, because itsmetabolism yields acetyl CoA and propionyl CoA.

4. Lysine: This amino acid is exclusively ketogenic and is unusual in thatneither of its amino groups undergoes transamination as the first step incatabolism. Lysine is ultimately converted to acetoacetyl CoA.

H. Branched-chain amino acid degradationThe BCAA isoleucine, leucine, and valine are essential amino acids. Incontrast to other amino acids, they are catabolized primarily by theperipheral tissues (particularly muscle), rather than by the liver. Becausethese three amino acids have a similar route of degradation, it is convenientto describe them as a group.

1. Transamination: Transfer of the amino groups of all three BCAA to α-ketoglutarate is catalyzed by a single, vitamin B6–requiring enzyme,***branched-chain amino acid aminotransferase***, that is expressedprimarily in skeletal muscle.

2. Oxidative decarboxylation: Removal of the carboxyl group of the α-ketoacids derived from leucine, valine, and isoleucine is catalyzed by a singlemultienzyme complex, ***branched-chain α-keto acid dehydrogenase(BCKD) complex.*** This complex uses thiamine pyrophosphate, lipoicacid, oxidized flavin adenine dinucleotide (FAD), NAD+, and CoA as itscoenzymes and produces NADH. [Note: This reaction is similar to theconversion of pyruvate to acetyl CoA by the ***pyruvate dehydrogenase***(***PDH***) ***complex*** and α-ketoglutarate to succinylCoA by the***α-ketoglutarate dehydrogenase complex***. The

***dihydrolipoyldehydrogenase*** (***Enzyme 3***, or ***E3***) component is identical in all threecomplexes.]

3. Dehydrogenations: Oxidation of the products formed in the ***BCKD***reaction produces α-β-unsaturated acyl CoA derivatives and FADH2.These reactions are analogous to the FAD-linked dehydrogenation in theβ-oxidation of fatty acids. [Note: Deficiency in the***dehydrogenase*** specific for isovaleryl CoA causes neurologic problemsand is associated with a "sweaty feet" odor in body fluids.]

4. End products: The catabolism of isoleucine ultimately yields acetyl CoAand succinyl CoA, rendering it both ketogenic and glucogenic. Valineyields succinyl CoA and is glucogenic. Leucine is ketogenic, beingmetabolized to acetoacetate and acetyl CoA. In addition, NADH andFADH2 are produced in the decarboxylation and dehydrogenationreactions, respectively. [Note: BCAA catabolism also results inglutamine and alanine being synthesized and sent out into the blood from muscle FOLIC ACID AND AMINO ACIDMETABOLISMSome synthetic pathways require the addition of single-carbon groups that existin a variety of oxidation states, including formyl, methenyl, methylene, andmethyl. These single-carbon groups can be transferred from carrier compoundssuch as THF and SAM to specific structures that are being synthesized ormodified. The "one-carbon pool" refers to the single-carbon units attached tothis group of carriers. [Note: CO2, coming from bicarbonate (HCO3–), is carriedby the vitamin biotin, which is a prosthetic group for mostcarboxylation reactions but is not considered a member of the one-carbon pool.Defects in the ability to add or remove biotin from ***carboxylases*** result inmultiple ***carboxylase*** deficiency. Treatment is supplementation with biotin.]

A. Folic acid and one-carbon metabolismThe active form of folic acid, THF, is produced from folate by***dihydrofolate reductase*** in a two-step reaction requiring two nicotinamideadenine dinucleotide phosphate (NADPH). The one-carbon unit carried byTHF is bound to N5 or N10 or to both N5 and N10.shows thestructures of the various members of the THF family and theirinterconversions and indicates the sources of the one-carbon units and thesynthetic reactions in which the specific members participate. [Note: Folatedeficiency presents as a megaloblastic anemia because of decreasedavailability of the purines and of the thymidine monophosphate needed for DNA synthesis.]

**TOPIC: Ammonia neutralization. Disorders of the synthesis and excretion of urine**.

UREA CYCLE

Urea is the major disposal form of amino groups derived from amino acids and accounts for ~90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia

(through oxidative deamination by ***GDH***) and aspartate nitrogen (through transamination of oxaloacetate by ***AST***).] The carbon and oxygen of urea are derived from CO2 (as HCO3-). Urea is produced by the liver and then is transported in the blood to the kidneys for excretion in the urine.

Reactions The first two reactions leading to the synthesis of urea occur in the mitochondrial matrix, whereas the remaining cycle enzymes are located in the cytosol. [Note: Gluconeogenesis and heme synthesis also involve both the mitochondrial matrix and the cytosol.]

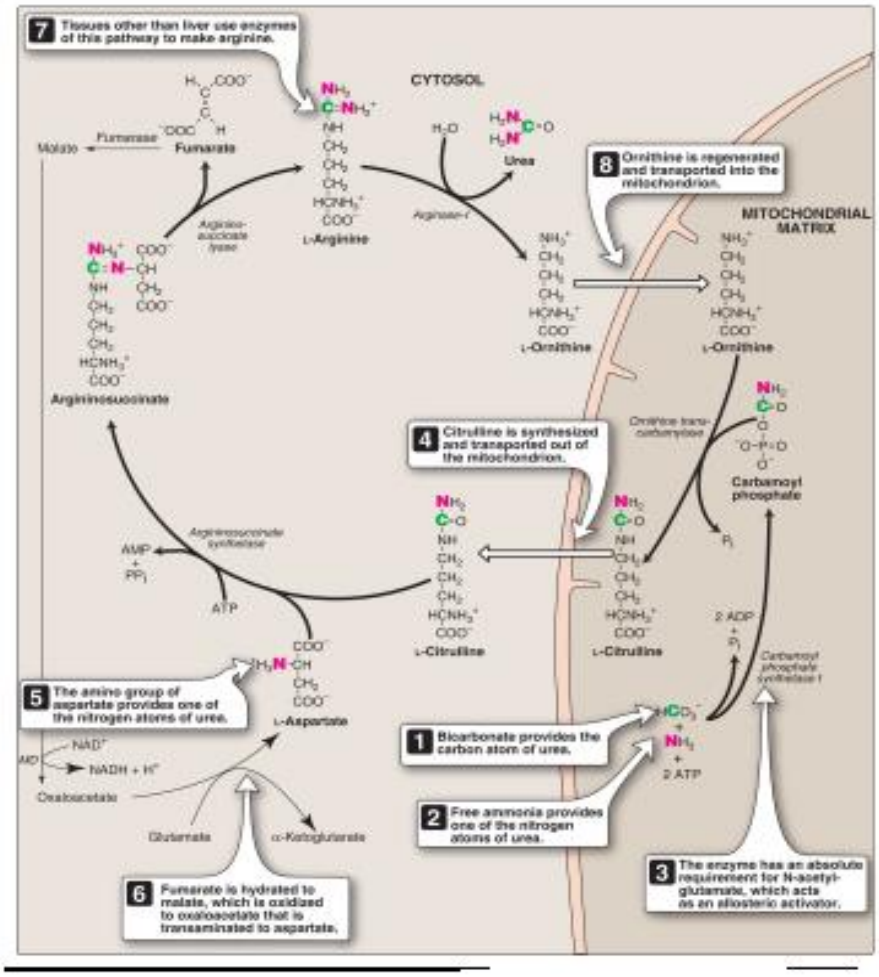



Figure: Reactions of the urea cycle. [Note: An antiporter transports citrulline and ornithine across the inner mitochondrial membrane.] ADP =denosine diphosphate; AMP = adenosine monophosphate; PPi = pyrophosphate;Pi = inorganic phosphate; NAD(H) = nicotinamide adenine dinucleotide; ***MD =alate dehydrogenase***.

1. Carbamoyl phosphate formation: Formation of carbamoyl phosphate by***carbamoyl phosphate synthetase I (CPS I)*** is driven by cleavage of twomolecules of ATP. Ammonia incorporated into carbamoyl phosphate isprovided primarily by the oxidative deamination of glutamate bymitochondrial

***GDH***. Ultimately, the nitrogen atomderived from this ammonia becomes one of the nitrogens of urea. ***CPS I***requires N-acetylglutamate (NAG) as a positive allosteric activator. [Note: ***Carbamoyl phosphate synthetase II*** participates inthe biosynthesis of pyrimidines. It does not require NAG,uses glutamine as the nitrogen source, and occurs in the cytosol.]

2. Citrulline formation: The carbamoyl portion of carbamoyl phosphate istransferred to ornithine by ***ornithine transcarbamylase***(***OTC***) as thephosphate is released as inorganic phosphate. The reaction product citrulline, is transported to the cytosol. [Note: Ornithine and citrullinemove across the inner mitochondrial membrane via an antiporter. These basic amino acids are not incorporated into cellular proteins because there are no codons for them.] Ornithine is regenerated with each turn of the urea cycle, much in the same way that oxaloacetate is regenerated by the reactions of the tricarboxylic acid (TCA) cycle.

3. Argininosuccinate formation: ***Argininosuccinatesynthetase***combines citrulline with aspartate to form argininosuccinate. The α-amino group of aspartate provides the second nitrogen that is ultimately incorporated into urea. The formation of argininosuccinate is driven by the cleavage of ATP to adenosine monophosphate and pyrophosphate. This is the third and final molecule of ATP consumed in the formation of urea

4. Argininosuccinate cleavage: Argininosuccinate is cleaved by ***argininosuccinatelyase***to yield arginine and fumarate. The arginine serves as the immediate precursor of urea. The fumarate is hydrated to malate, providing a link with several metabolic pathways. Malate can be oxidized by ***malate dehydrogenase*** to oxaloacetate, which can be transaminated to aspartate and enter the urea cycle. Alternatively, malate can be transported into mitochondria via the malate–aspartate shuttle, reenter the TCA cycle, and get oxidized to oxaloacetate, which can be used for gluconeogenesis. [Note: Malate oxidation generates NADH for oxidative phosphorylation, thereby reducing the energy cost of the urea cycle.]

5. Arginine cleavage to ornithine and urea: ***Arginase-I*** hydrolyzes arginine to ornithine and urea and is virtually exclusive to the liver. Therefore, only the liver can cleave arginine, thereby synthesizing urea, whereas other tissues, such as the kidney, can synthesize arginine from citrulline. [Note: ***Arginase-II*** in kidneys controls arginine availability for nitric oxide synthesis.]

6. Fate of urea: Urea diffuses from the liver and is transported in the blood to the kidneys, where it is filtered and excreted in the urine. A portion of the urea diffuses from the blood into the intestine and is cleaved to CO2 and

ammonia by bacterial ***urease***. The ammonia is partly lost in the feces and is partly reabsorbed into the blood. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of ***urease*** on this urea becomes a clinically important source of ammonia,contributing to the hyperammonemia often seen in these patients. Oral administration of antibiotics reduces the number of intestinal bacteria responsible for this ammonia production.

B. Overall stoichiometry

$$\text{Aspartate} + NH_3 + HCO_3^- + 3\,\text{ATP} + H_2O \rightarrow$$
$$\text{urea} + \text{fumarate} + 2\,\text{ADP} + \text{AMP} + 2\,P_i + PP_i$$

Because four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea, the synthesis of urea is irreversible, with a large, negative ΔG (see p. 70). One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. Glutamate is the immediate precursor of both ammonia (through oxidative deamination by ***GDH***) and aspartate nitrogen (through transamination of oxaloacetate by ***AST***). In effect, both nitrogen atoms of urea arise from glutamate, which, in turn, gathers nitrogen from other amino acids.

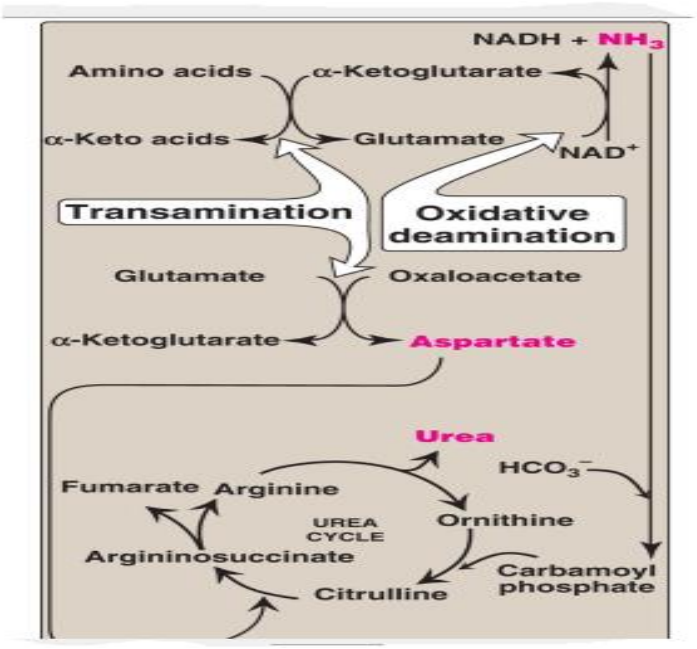



Figure: Flow of nitrogen from amino acids to urea. Amino groups for urea synthesis are collected in the form of ammonia (NH3) and aspartate. NAD(H) =nicotinamide adenine dinucleotide; HCO3– = bicarbonate.

C. Regulation

NAG is an essential activator for ***CPS I***, the rate-limiting step in the ureacycle. It increases the affinity of ***CPS I*** for ATP. NAG is synthesized fromacetyl CoA and glutamate by ***N-acetylglutamate synthase*** (***NAGS***), as shown in, in a reaction for which arginine is an activator. Thecycle is also regulated by substrate availability (short-term regulation) andenzyme induction (long term)

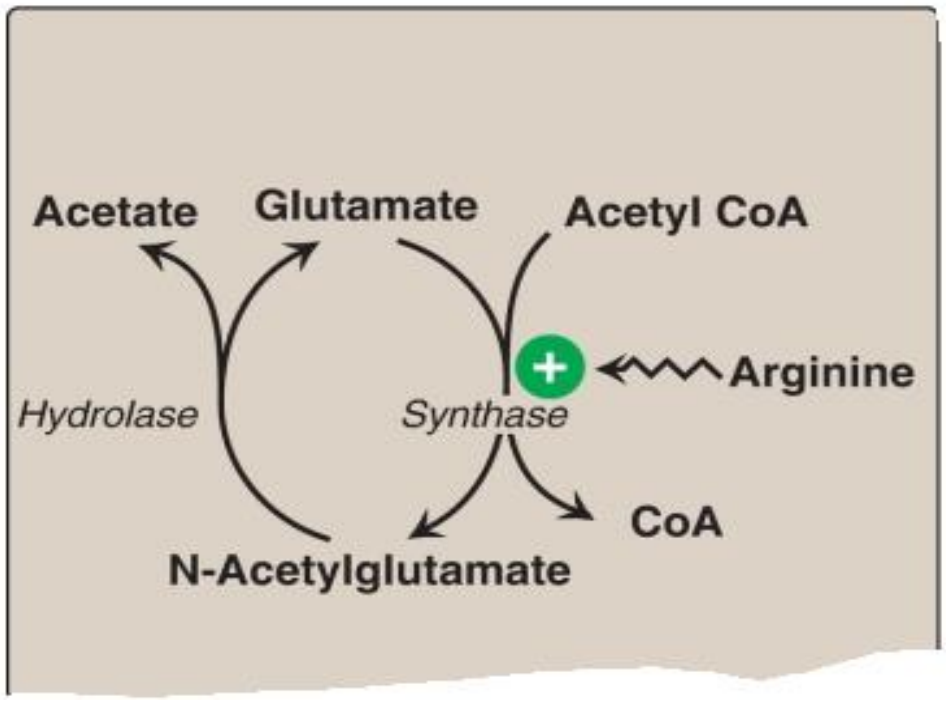



Figure: Formation and degradation of ***N***-acetylglutamate, an allosteric activator of ***carbamoyl phosphate synthetase I***. CoA = coenzyme A.

AMMONIA METABOLISMAmmonia is produced by all tissues during the metabolism of a variety ofcompounds, and it is disposed of primarily by formation of urea in the liver.However, the blood ammonia level must be kept very low, because even slightlyelevated concentrations (hyperammonemia) are toxic to the central nervoussystem (CNS). Therefore, a mechanism is required for the transport of nitrogenfrom the peripheral tissues to the liver for ultimate disposal as urea whilekeeping circulating levels of free ammonia low.

A.Sources

Amino acids are quantitatively the most important source of ammoniabecause most Western diets are high in protein and provide excess aminoacids, which travel to the liver and undergo transdeamination (that is,

thelinking of the ***aminotransferase*** and ***GDH*** reactions), producing ammonia.[Note: The liver catabolizes straight-chain amino acids, primarily.]However, substantial amounts of ammonia can be obtained from othersources.

1. Glutamine: An important source of plasma glutamine is from thecatabolism of BCAA in skeletal muscle. This glutamine is taken up bycells of the intestine, the liver, and the kidneys. The liver and kidneysgenerate ammonia from glutamine by the actions of ***glutaminase*** and ***GDH***. In the kidneys, most of this ammonia is excreted intothe urine as NH4+, which provides an important mechanism formaintaining the body's acid–base balance through the excretion ofprotons. In the liver, the ammonia is detoxified to urea and excreted.[Note: α-Ketoglutarate, the second product of ***GDH***, is a glucogenicprecursor in the liver and kidneys.] Ammonia is also generated byintestinal ***glutaminase***. Enterocytes obtain glutamine either from theblood or from digestion of dietary protein. [Note: Intestinal glutaminemetabolism also produces alanine, which is used by the liver forgluconeogenesis, and citrulline, which is used by the kidneys tosynthesize arginine.]

2. Intestinal bacteria: Ammonia is formed from urea by the action ofbacterial ***urease*** in the lumen of the intestine. This ammonia is absorbedfrom the intestine by way of the portal vein, and virtually all is removedby the liver via conversion to urea.

3. Amines: Amines obtained from the diet and monoamines that serve ashormones or neurotransmitters give rise to ammonia by the action of***monoamine oxidase***.

4. Purines and pyrimidines: In the catabolism of purines and pyrimidines,amino groups attached to the ring atoms are released as ammonia

B. Transport in the circulationAlthough ammonia is constantly produced in the tissues, it is present at verylow levels in blood. This is due both to the rapid removal of blood ammoniaby the liver and to the fact that several tissues, particularly muscle, releaseamino acid nitrogen in the form of glutamine and alanine, rather than asfree ammonia.

1. Urea: Formation of urea in the liver is quantitatively the most importantdisposal route for ammonia. Urea travels in the blood from the liver tothe kidneys, where it passes into the glomerular filtrate.

2. Glutamine: This amide of glutamate provides a nontoxic storage and transport form of ammonia. The ATP-requiring formation of glutamine from glutamate and ammonia by ***glutamine synthetase***occurs primarily in skeletal muscle and the liver but is also important in the CNS, where it is the major mechanism for the removal of ammonia in the brain. Glutamine is found in plasma at concentrations higher than other amino acids, a finding consistent with its transport function. [Note: The liver keeps blood ammonia levels low through ***glutaminase***, ***GDH***, and the urea cycle in periportal (close to inflow of blood) hepatocytes and through ***glutamine synthetase***as an ammonia scavenger in the perivenous hepatocytes.] Ammonia metabolism is summarized in.

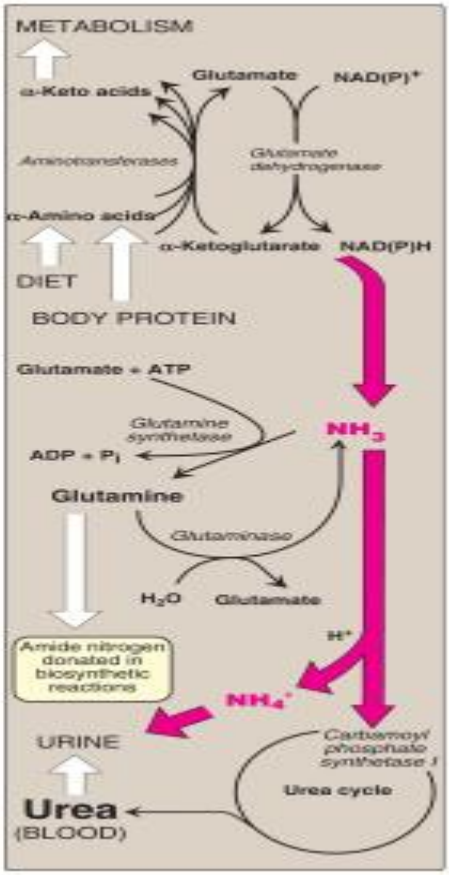



Figure: Ammonia (NH3) metabolism. Urea content in the urine is reported as urinary urea nitrogen, or UUN. Urea in blood is reported as BUN (blood urea nitrogen). [Note: The enzymes ***glutamate dehydrogenase***, ***glutamine synthetase***, and ***carbamoyl phosphate synthetase I*** fix NH3 into organic molecules.]

C. **Hyperammonemia** The capacity of the hepatic urea cycle exceeds the normal rates of ammonia generation, and the levels of blood ammonia are normally low (5–35 μmol/l). However, when liver function is compromised, due either to genetic defects of the urea cycle or liver disease, blood levels can be >1,000 μmol/l. Such hyperammonemia is a medical emergency, because ammonia has a

direct neurotoxic effect on the CNS. For example, elevated concentrations of ammonia in the blood cause the symptoms of ammonia intoxication, which include tremors, slurring of speech, somnolence (drowsiness), vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. There are two major types of hyperammonemia.

1. Acquired: Liver disease is a common cause of acquired hyperammonemia in adults and may be due, for example, to viral hepatitis or to hepatotoxins such as alcohol. Cirrhosis of the liver may result in formation of collateral circulation around the liver. As a result, portal blood is shunted directly into the systemic circulation and does not have access to the liver. Therefore, the conversion of ammonia to urea is severely impaired, leading to elevated levels of ammonia.

2. Congenital: Genetic deficiencies of each of the five enzymes of the urea cycle (and of ***NAGS***) have been described, with an overall incidence of 1:25,000 live births. X-linked ***OTC*** deficiency is the most common of these disorders, predominantly affecting males, although female carriers may become symptomatic. All of the other urea cycle disorders follow an autosomal-recessive inheritance pattern. In each case, the failure to synthesize urea leads to hyperammonemia during the first weeks following birth. [Note: The hyperammonemia seen with ***arginase*** deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine.] Historically, urea cycle defects had high morbidity (neurologic manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent protein catabolism. Administration of compounds that bind covalently to nonessential amino acids, producing nitrogencontaining molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses with glutamine to form phenylacetylglutamine, which is excreted.

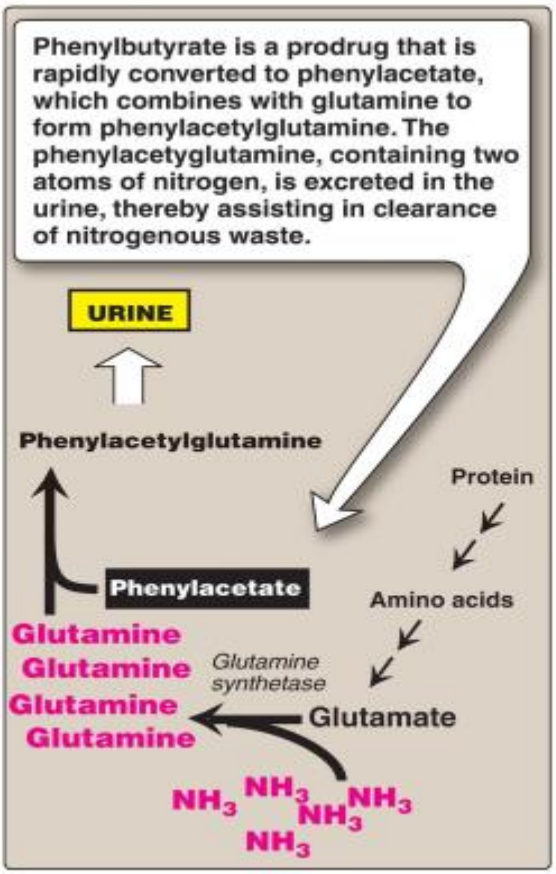



Figure: Treatment of patients with urea cycle defects by administration of phenylbutyrate to aid in excretion of ammonia (NH3).

## Nitrogen metabolism

It is the polymeric nitrogen containing compounds proteins and nucleic acids that define the major attributes of organism such as function and structure. Operation and mechanism of metabolic pathways is provided by proteins. Genetic information is stored in nucleic acid polymers. Each of the monomer of these macromolecules has an individual metabolic pathway. In addition, the monomeric nucleotides are essential for energy turnover as key intermediates in *all* metabolic pathways and also as second messenger molecules, often in form of cyclic nucleotides.

Amino acids contribute to carbohydrate synthesis via gluconeogenesis, to fat synthesis or energy production via acetyl-CoA, and special nitrogen compounds such as catecholamines (neurotransmitters), thyroid hormones, creatine(-phosphate), the protoporphyrin ring (heme), and contribute to nucleic acid and phospholipid synthesis as nitrogen group donor.

Microbial nitrogen fixation (KEGG pathway MAP00910)

All nitrogen metabolism is based on a recycling of ammonia NH3 in its neutral or charged form NH4+(ammonium ion). Ammonia, however, is not a major form of nitrogen on earth, instead it has to be replenished to support a growing need of life

forms. As simple as it may sound, a growing bacterial culture needs raw material in form of small organic and inorganic molecules. NH3 ultimately is derived from atmospheric N2. In a process called nitrogen fixation, some bacterial species, the symbiotic eubacteria *Rhizobium* (in plant root nodules) and the archaea *cyanobacteria* (formerly blue-green algae) contain an enzyme complex for the reduction of molecular nitrogen to ammonia. This is the *nitrogenase complex* and contains Fe-S and Mo-Fe cofactors for the transfer of electrons from ferredoxin to N2. The process of nitrogen reduction is extremely energy dependent. The triple bond energy in molecular nitrogen is 225kcal/mol and the industrial production of ammonia requires temperatures of 500 degrees Celsius and a pressure of 300 atmospheres. Rhizobium uses 8 reducing equivalents and 16 ATPs:

N2 + 8e- + 8H+ + 16ATP + 16H2O = 2NH3 + H2 + 16ADP + 16Pi + 8H+

This reaction is catalyzed by the hetero-oligomeric protein complex composed of a reductase and a nitrogenase part. The *reductase* is a homodimer containing a 4Fe-4S cluster and an ATP binding site at the subunit interface. The *nitrogenase* (EC 1.18.6.1; PDB entry 1N2C) is a hetero-tetramer with a subunit stoichiometry of □2□2. The □□ interface contains the so called P cluster (containing two 4Fe-4S clusters) which oxidizes the *reductase* and is oxidized by the Mo-Fe cofactor which contains two Mo-3Fe-3S clusters comprising the N2 binding site. For a complete structure of nitrogenase complex from *Azotobacter Vinelandii* click here.

The *reductase* (PDB structure from Clostridium Pasterianum; EC 1.19.6.1) contains an 4Fe-4S complex used to oxidize ferredoxin, which is supplied either by photosynthetic membranes (PSI) or from oxidative catabolism. The reductase donates 8 electrons in succession to the nitrogenase cofactor, a molybdenum-iron containing active center, where one molecule of N2 is reduced in the presence of protons to 2 NH3, and H2 as a byproduct. The reduction catalysis is powered by sixteen ATP molecules hydrolyzed by the reductase subunit. Molecular oxygen is a strong

inhibitor of the nitrogenase Mo-Fe cofactor and is removed by the plant oxygen binding protein *leghemoglobin* in the root nodules.

Dietary nitrogen

The majority of useful nitrogen for animal metabolism comes from proteins in the form of reusable ammonia (NH3). Nitrogen is fixated in form of ammonia by microorganisms (see chapter on amino acid synthesis) and all 'higher' forms of life (eukaryotes) depend on this primordial source of nitrogen extracted from the air. The 'usefulness' of proteins depends on four distinct properties:

1. total amount of protein ingested 2. digestibility of proteins 3. amino acid composition of proteins 4. total caloric intake

Digestibility and amino acid composition define the biological value of dietary proteins. Hair and skin keratin is non-digestible and useless as such. Pretreatment like heating can improve protein usefulness. Animal proteins are a better mix for our diet than plant proteins based on their amino acid composition. Nucleic acids, on the other hand, are not needed as dietary supplement. Excess nucleic acid in the diet is degraded and secreted and most nucleic acid synthesis in cells is provided by protein degradation (in form of amino acid precursors). Since all nitrogen containing compounds are dependent on protein supply, protein deficiency is one of the major nutritional problems in the world. This is specifically important for children and pregnant women, since the developing organism has a several fold higher need for proteins in the diet than the adult. Low protein intake results in lowered protein synthesis and thus in a lower supply of essential proteins involved in food digestion (proteases) and blood plasma transport (lipoproteins).

Essential amino acids

Some amino acids may be synthesized in human cells, some however cannot. The latter are referred to as essential amino acids meaning that they are required dietary components. The non-essential amino acids, however, can be interconverted into each other or synthesized *de novo* from carbohydrate, nucleic acid, or lipid intermediates, provided that an **adequate source of total nitrogen** is available. For essential amino acids there is no metabolic pathway for *de novo* synthesis except in bacteria and plants. Humans therefore need a daily balanced intake of those essential amino acids. Meat and milk provide such a balanced amino acid diet by virtue of the evolutionary relationship with between animals and humans. The list of the essential amino acids for human protein synthesis includes the branched amino acids *Isoleucine*, *Leucine*, and *Valine*, the sulfur containing *Methionine*, the hydrophilic amino acids *Lysine* and *Threonine*, and the aromatic amino acids *Phenylalanine* and *Tryptophan*. The amino acids *Arginine* and *Histidine* are synthesized in human cells, but only slowly and thus can be considered essential, if they become rate limiting factors for protein synthesis.

Nitrogen balance

The nitrogen balance is an indication of protein synthesis and degradation. *A positive nitrogen balance* indicates that the intake of nitrogen containing compound exceeds the nitrogen lost from the body. A positive nitrogen balance correlates with a net synthesis of proteins and nucleic acids. This obviously describes a state of growth of an organism - childhood, pregnancy, recovering from

illness. The opposite results in a net degradation of proteins. Less nitrogen is taken up than is lost, is a state of *negative nitrogen balance*. The omission of essential amino acids, and this needs to affect only one type, results in a negative nitrogen balance, since it will be rate limiting for protein synthesis. A healthy body is characterized by a nitrogen equilibrium (steady state equilibrium) where intake and loss of nitrogen are equal. Here is a quote from Murray's "Essentials of human metabolism":

" In a way this equilibrium description satisfies our common sense observation of non growing adults. The interpretation would be that the body uses only as much protein nitrogen from the diet as necessary to replace digestive enzymes, gastrointestinal cells (GI) lost in the feces, or any degenerated tissue components, such as skin cells or erythrocytes that wear out during normal use. ... Why then does the body have such high requirements specifically for protein as well as for essential amino acids? Why could these energy requirements not be met almost entirely by increased carbohydrate and lipid intakes? The answer to these questions requires a different concept of metabolism than is implied by the terms 'chemical equilibrium' or the replacement of components due to wear and tear."

The answer lies in protein turn over, continuous exchanges of material and energy with our surroundings. The body requires energy to transport metabolites, needs to make sure the proteins are in good shape and that the structures inside and outside of cells are not compromised by hazardous materials. The entire human body is in a true **steady-state**, a flow of components recycled through rounds of synthesis and degradation. The non-growing body is essentially constantly renewing itself creating stability and the illusion of non activity, while in fact its components are being continuously exchanged. Turn-over rates of proteins are measured in minutes, hours, or days depending on the protein and its cellular location. Not only can the organism replace damaged enzymes, it can also quickly adjust the levels and types of proteins according to metabolic needs. Indeed, extracellular transport mechanisms (lipoproteins) are coupled to intracellular protein synthesis and degradation pathways.

The key regulatory element of this turn-over process is the nitrogen balance reflected as the free amino acid pool. This pool is regenerated by dietary proteins and tissue protein degradation and is the source of protein synthesis as well as nitrogen secretion while maintaining nitrogen level homeostasis.

Turnover rates are best described as biological half-life time. An estimated 2 to 3 weeks has been given for a complete turnover of all body proteins (with a considerable variation). The turnover rate of individual proteins or specific families of proteins may be less than an hour. In actual numbers the rate of protein synthesis every day is estimated at about 500g or nearly five times the average dietary intake. There is obviously a highly efficient amino acid recycling machinery at work. This, in short, is the significance of amino acid metabolism.

Liver nitrogen metabolism

The liver is the main metabolic organ utilizing amino acids for tissue protein synthesis, heme formation, pyrimidine and purine synthesis (nucleotide precursors), ketone body and carbohydrate formation, *de novo* synthesis of non-

essential amino acids, and finally excrete surplus nitrogen via the urea cycle. The liver thus is the gatekeeper of the nitrogen balance in animals, its intake and excretion. Because of its central role of regulating and coordinating body metabolism, protein turnover in liver is particularly fast. This ensures a reliable supply of (intact) blood plasma proteins, and liver resident proteins obviously affect liver metabolism which affects all other tissues, too. Finally, some proteins may be rapidly degraded to provide a constant level of free amino acids for the formation of ketone bodies, carbohydrates, nucleic acids, and heme. Hormonal control (glucocorticoids) makes sure that a starving body first breaks down proteins from non-essential organs like skeletal muscle, while liver enzymes for gluconeogenesis and urea cycle (nitrogen decontamination of the body) are enhanced. The liver acts as an **aminostat**. Free amino acid levels in blood plasma as well as plasma proteins are maintained at constant levels despite fluctuations in intake and tissue demand.

Glutamate (C00025) and glutamine (C00064) are the two important amino acids in recycling ammonia in our body instead of excreting it as waste in form of urea (C00086). Glutamine is synthesized from glutamate by incorporation of an NH3 into the carboxyl group forming an amide. This step requires ATP and is catalyzed by *glutamine synthetase* (EC 6.3.1.2). The coupling of glutamine synthesis with ATP hydrolysis renders the reaction irreversible. The back reaction - the regeneration of glutamate from glutamine - is catalyzed by *glutaminase* (EC 3.5.1.2), which deaminates glutamine via a hydrolysis reaction. The concerted control of these two enzymes is responsible for the maintenance of the glutamine pool in blood. An example of controlling the NH3 levels is enhanced gluconeogenesis in specialized organs such as muscle and brain. Carbohydrates are synthesized from amino acid sources increasing the cellular ammonia levels. They are secreted by the peripheral tissues in form of glutamine (to avoid that nitrogen is excreted from the body) which is taken up by hepatocytes where the NH3 is re-used for amino acid and nucleotide synthesis.

Aminotransferases

Aminotransferases are a class of enzymes responsible to attach and remove amino groups from alpha-carbons of *amino acids* and *keto acids*. Aminotransferases (or transaminases) link amino acid metabolism with other pathways, most importantly the citric acid cycle. The reaction catalyzes the transfer from an alpha amino acid to an alpha keto acid. The transferase using alpha-ketoglutarate and alpha-glutamate as acceptor and donor group, respectively, takes a central role in the linkage between amino acid metabolism and citric acid cycle. This reaction is coupled with the enzyme *glutamate dehydrogenase* which catalyzes the amination-deamination equilibrium between alpha-ketoglutarate and glutamate. Thus, the interplay of the two enzymes *glutamate transaminase* (EC 2.6.1.1; transferase) and *glutamate dehydrogenase* (EC 1.4.1.2) is essential in the control of nitrogen balance in the body.

Aminotransferase reactions involve little change in free energy (they catalyze the reaction close at its chemical equilibrium) and the direction of the catalysis is essentially controlled by the concentration levels of the reactants. The

dehydrogenase activity is controlled by the redox potential of the cell in form of NADH. The amination (NH3) reaction is coupled to a reduction step using NADH/H+ (oxidized) and alpha-ketoglutarate (reduced) while producing glutamate, NAD+ and water.

Aminotransferase reactions depend on vitamin B6, namely its derivative pyridoxal-phosphate (C00018), which acts as a coenzyme in the reaction, temporarily binding the transferred amino group. The pyridoxal phosphate group converts to pyridoxamine phosphate during the catalysis. Pyridoxal phosphate, however, is quite a versatile coenzyme being used in enzymes catalyzing the following reactions by temporarily accepting the transferred reactant (hint: click on the link above to pyridoxal phosphate and explore the long list of enzymes (117) that use this functional group):

- transamination - decarboxylation - deamination - racemization - aldole cleavage - elimination and replacement reactions at  carbons and  carbons  Glutamate is the major partner for many amino acids during aminotransferase activity. Among those amino acids are aspartate (aspartate-glutamate aminotransferase), tyrosine (tyrosine-glutamate aminotransferase), and alanine (alanine-glutamate aminotransferase EC 2.6.1.2). The respective keto acids are oxaloacetate (aspartate), pyruvate (alanine), and hydroxyphenylpyruvate (tyrosine). The central step in glutamate/alpha-ketogluatarate amination and transamination can be illustrated by the amination of pyruvate to alanine. The net reaction

pyruvate + NH3 + NADH + H+  alanine + NAD+

is catalyzed in two steps. First by *glutamate dehydrogenase*:

alpha-ketoglutarate + NH3 + NADH + H+  glutamate + NAD+

followed by *alanine-glutamate aminotransferase*:

pyruvate + glutamate  alanine + alpha-ketoglutarate

All steps are reversible. The dehydrogenase reaction occurs in the mitochondrial matrix where it directly interacts with NAD+ and alpha-ketoglutarate. The dehydrogenase is under allosteric control of the energy charge of the cell. High levels of ATP and GTP inactivate the enzyme while high levels of ADP and GDP activate it.

Non-essential amino acids

All non-essential amino acids except for tyrosine and cysteine are derived and are dependent on transamination from glutamate. **Proline, ornithine, arginine** obtain their carbon units and amino nitrogen from glutamate. **Alanine, serine, glycine** obtain their C3 carbon units from glycolytic intermediates and the amino nitrogen from glutamate (Note: glycine is a C2 amino acid derived from serine by decarboxylation; see one carbon metabolism). **Aspartate** derives its carbon backbone from oxaloacetate (C4) and amino nitrogen from glutamate. In fact, glutamate-dehydrogenase in combination with any aminotransferase is capable of forming any non-essential amino acid, given the occurrence of the proper alpha-keto acid and a source for ammonia. This process is called **reductive amination** (see above formation of alanine from pyruvate). The main purpose of reductive amination is to recycle NH3 instead of excreting it in form of urea and to preserve other amino acids which could serve as amino group donor.

Reductive amination is the first of three processes in liver for ammonia incorporation. The second important process is the formation of glutamine, which serves as a reservoir for ammonia for all organs and is maintained as blood glutamine levels by liver cells. Glutamine serves as transport mechanism of NH3 between organs. Third, liver can form carbamoyl-phosphate, which is necessary for the formation of pyrimidine bases of nucleotides and the production of urea via the enzymes of the **urea cycle**.

Urea cycle (KEGG pathway MAP00220)

The urea cycle is a liver resident process removing nitrogen in form of ammonia to be excreted from the body. The cycle involves two amino acids which are not used for protein synthesis. These are *ornithine* and *citrulline*. Ornithine has a role analogous to that of oxaloacetate in the citric acid cycle. It provides the carbon backbone and works as a *catalytic carrier*, but is not itself used up in the cyclic reaction. Ornithine has a terminal amino group that serves as a hook or handle for the incoming carbamoyl phosphate (C00169), a small molecule formed from CO2 and NH3 and ATP as phosphate donor. Carbamoyl-phosphate is catalyzed by *carbamoyl-phosphate synthetase* (EC 6.3.4.16; forming carbamoyl- phosphate) in the mitochondrial matrix and requires the hydrolysis of 2 molecules of ATP. Carbamoyl is transferred to the ornithine amino group driven by the hydrolysis of its phosphate ester bond. Citrulline (C00327) is the product of this reaction and will be transported, together with aspartate, out of the mitochondria and into the cytoplasm. There, aspartate and citrulline are combined into the metastable intermediate argininosuccinic acid (C03406) using one molecule of ATP as energy source. Argininosuccinate is cleaved into arginine and fumarate. The latter is recycled back into the mitochondria for use by the citric acid cycle, while most of the arginine is converted by *arginase* (EC 3.5.3.1) to urea (C00086) and ornithine, thus completing the cycle. Like ornithine-carbamoyl transferase, arginase is a liver specific enzyme (in the cytoplasm) and only in those animals (mammals) which convert their nitrogen waste to urea. Note that in muscle most arginine synthesized is instead used for protein synthesis and creatine (C00300) formation. The phosphorylated creatine-P is used as an intermediate energy storage device under anaerobic conditions in skeletal muscle.

Fumarate is used to regenerate aspartate used up by urea formation. This is done by funneling fumarate back into the citric acid cycle and removing it in form of oxaloacetate (alpha keto acid) which can equilibrate with aspartate (alpha amino acid) catalyzed by an amino transferase reaction. Both the Krebs and urea cycle are thus strictly interrelated with the former providing essential intermediates plus carbon dioxide and energy in form of ATP (from oxidative phosphorylation, strictly speaking).

The urea cycle is part of two cellular compartments, the mitochondrial matrix which performs the biosynthetic part of the precursors citrulline and aspartate, and the cytoplasm, which after formation of arginino-succinate cleaves this intermediate into three different products, one of which is the net product (urea), the other two (ornithine and fumarate) are recycled into the matrix compartment and their respective cycle to start a new round of urea formation

Ammonium ion metabolism

Nitrogen can also be excreted as ammonium. This process is controlled by the kidney and is used to control the blood plasma pH. The blood plasma pH, however, is determined by other factors as well, such as organic acids (amino acids) and carbonic acid ($CO_2$ levels). Ammonium metabolism in kidney functions to depose $H^+$ in urine. In a first reaction, kidney enzymes deaminate glutamine in two steps to □□ketoglutarate. The first side chain deamination is catalyzed as simple hydrolysis and is *not reversible*.

This process is stimulated by inorganic phosphate. The free ammonia equilibrates with protons to ammonium:

$NH_3 + H^+ \rightleftharpoons NH_4^+$

and is trapped in the charged form inside kidney cells. The non-electrolytic ammonia is freely diffusible across cell membranes. Glutamine is the nontoxic form of $NH_3$ and shuttles it between liver and kidney in the blood plasma. The kidney functions as $H^+$ sink and protons are disposed in form of $NH_4^+$ while maintaining charge homeostasis using phosphate or acetoacetate.

**THE UREA CYCLE**

SYNTHESIS OF CARBAMOYL PHOSPHATE: This is how we "activate" a free ammonia before we subsequently make urea.

□ Enzyme = ***Carbamoyl Phosphate Synthetase***.

□ This reaction occurs in the *mitochondrion*.

□ 2 ATP are required. Basically these are used to "charge" or "activate" ammonia with a high-energy phosphate bond, before we subsequently start urea synthesis.

□ **N-Acetylglutamate is absolutely required as a cofactor**. This compound also serves a regulatory role in urea synthesis.

o The rate of carbamoyl phosphate synthesis is dependent on the levels of N-Acetylglutamate in the mitochondria (I'm not sure whether this is a linear relationship).

□ Usually, the free ammonia is derived directly from Glutamate Dehydrogenase, but it could come from anywhere.

THE UREA CYCLE:

□ REQUIRED STARTING MATERIALS:

o *Carbamoyl Phosphate:* It donates a free $NH_3$ to urea, per turn of the cycle.

o *Ornithine:* It is regenerated each turn of the cycle

o *Aspartic Acid:* It donates an $NH_3$ to urea from aminotransferases, per turn of the cycle.

□□Aspartic Acid has the *same carbon skeleton as Oxaloacetate*:

o 4 ATP are required per molecule of urea

□□□

□□2ATP required to synthesize Arginosuccinate, via 1 ATP and a pyrophosphatase

□ **Carbamoyl Phosphate + Ornithine ------> Citrulline**

o This is THE COMMITTED STEP
o One of the two NH3's of urea comes to us from Carbamoyl Phosphate, essentially the same as starting with a charged **free NH3**.
o This step occurs *in the mitochondria*. Citrulline is then transferred from mitochondria to the cytosol for the rest of the steps.
o Enzyme: ***Ornithine Transcarbamoylase***
 **Citrulline + Aspartic Acid ------> Arginosuccinate**
o **ATP ------> AMP + PPi**. Metabolic equivalent of 2ATP required, with pyrophosphatase driving the reaction forward, making the reaction irreversible.
o **Aspartic Acid** carries with it the *other NH3 of urea*. This NH3 has come to us via Aspartic Acid, courtesy of *Aspartate Aminotransferase*.
o This step, and all subsequent steps, occurs *in the cytosol.*
 **Arginosuccinate ------> Arginine + Fumarate**
o Catalyzed by ***Arginosuccinase***
o Fumarate then goes through the **TCA Cycle**, in order to *regenerate the carbon skeleton of Aspartic Acid*:
 *Fumarase:* **Fumarate ------> L-Malate**
 *L-Malate Dehydrogenase:* **L-Malate ------> Oxaloacetate**
 ***Aspartate Aminotransferase:*** **Oxaloacetate + NH3 ------> Aspartic Acid**
 **Arginine ------> Urea + Ornithine**
o Catalyzed by ***Arginase***. Know this enzyme, because this is the step that regenerates ornithine.
 END PRODUCTS:
o *Fumarate*, which is recycled, via the TCA cycle and transamination, to Aspartate
o Urea
o *Ornithine*, which is recycled to the first step.

REGULATION OF THE UREA CYCLE:
 Short-Term Regulation (metabolic regulation):
o The primary regulated step is Carbamoyl Phosphate Synthetase.
o **Increased levels of Arginine** promotes the formation of **N-Acetyl Glutamate**, which is required for carbamoyl phosphate synthesis.
 **Glutamate + Acetyl-CoA ------> N-Acetylglutamate**
o So when Arginine levels goes up, carbamoyl phosphate is made and urea synthesis occurs.
 Long-Term Regulation (diet)
o In the long-term, a *high-protein diet* will increase overall levels of urea synthesis.
o People who are protein derived would be ill-equipped to handle a sudden massive increase in the amount of protein in their diet, because enzyme levels would not be high enough. That's why you change a diet gradually as a rule of thumb, especially for the malnourished.

AMMONIA DETOXIFICATION: Ways of getting rid of excess ammonia in the bloodstream.

 From Benzoic Acid: HIPPURATE

o **Benzoic Acid + Coenzyme-A ------> Benzoyl-CoA**

 2 ATP are required (coupled with pyrophosphatase), as usual, to make a CoA derivative.

o **Benzoyl-CoA + Glycine ------> Hippurate**

o Hippurate is innocuous and is readily excreted in the urine.

o This form of detoxification will *only work if we can transaminate an NH3 group onto glyoxalase* (the keto-acid form of glycine). If we can't get the NH3 into glycine, then we can't get rid of it by this means!

 From Phenylacetate: PHENYLACETYLGLUTAMINE

o **Phenylacetate + Glutamine ------> Phenylacetylglutamine**

o **Coenzyme-A** is required as a cofactor, but *there is no free Coenzyme-A intermediate*. In other words, it is a concerted reaction.

o 2 ATP are required (coupled with pyrophosphatase), as usual.

o Phenylacetylglutamine is readily excreted in the urine.

**Hyperammonemia** is a metabolic disturbance characterised by an excess of ammonia in the blood. It is a dangerous condition that may lead to brain injury and death. It may be primary or secondary.

Ammonia is a substance that contains nitrogen. It is a product of the catabolism of protein. It is converted to the less toxic substance urea prior to excretion in urine by the kidneys. The metabolic pathways that synthesize urea involve reactions that start in the mitochondria and then move into the cytosol. The process is known as the urea cycle, which comprises several enzymes acting in sequence. It is greatly exacerbated by common zinc deficiency, which raises ammonia levels further.[1]

**Complication[**edit]

Hyperammonemia is one of the metabolic derangements that contribute to hepatic encephalopathy, which can cause swelling of astrocytes and stimulation of NMDA-receptors in the brain. Overstimulation of NMDA-receptors induces excitotoxicity.

**Diagnosis[edit]**

**Types[**edit]

*Primary vs. secondary[**edit]***

 Primary hyperammonemia is caused by several inborn errors of metabolism that are characterised by reduced activity of any of the enzymes in the urea cycle. The most common example is ornithine transcarbamylase deficiency, which is inherited in an X-linked fashion.

 Secondary hyperammonemia is caused by inborn errors of intermediary metabolism, which are characterised by reduced activity of enzymes that are not part of the urea cycle or dysfunction of cells that make major contributions to metabolism. Examples of the former are propionic acidemia and methylmalonic

acidemia, and examples of the latter are acute liver failure and hepatic cirrhosis with liver failure.

*Acquired vs. congenital[**edit]***

 Acquired hyperammonemia is usually caused by diseases that result in either acute liver failure, such as overwhelming hepatitis B or exposure to hepatoxins, or cirrhosis of the liver with chronic liver failure. Chronic hepatitis B, chronic hepatitis C, and excessive alcohol consumption are common causes of cirrhosis. The physiologic consequences of cirrhosis include shunting of blood from the liver to the inferior vena cava, resulting in decreased filtration of blood and removal of nitrogen-containing toxins by the liver, and then hyperammonemia. This type of hyperammonemia can be treated with antibiotics to kill the bacteria that initially produce the ammonia, though this doesn't work as well as removal of protein from the colon prior to its digestion to ammonia, achieved by lactulose administration for frequent (3-4 per day) bowel movements.

 Medication induced hyperammonemia can occur with valproic acid overdose, and is due to a deficiency in carnitine. Its treatment is carnitine replacement.

 Severe dehydration and small intestinal bacterial overgrowth can also lead to acquired hyperammonemia.

 Glycine toxicity causes hyperammonemia, which manifests as CNS symptoms and nausea. Transient blindness can also occur. [2]

 Congenital hyperammonemia is usually due to genetic defects in one of the enzymes of the urea cycle, such as ornithine transcarbamylase deficiency, which leads to lower production of urea from ammonia.

CLINICAL CASE-STUDY: HYPERAMMONEMIA

 The patient had a deficiency in *Ornithine Transcarbamoylase*.

o Blood-levels of NH3 were almost twice normal.

o The enzyme was not completely absent, however. If you gave the patient protein, it would take him 120 hours to completely metabolize the protein to urea, which is far longer than normal.

 CLINICAL PRESENTATION: Bizarre behavior: crying, agitation, babbling, loss of sense of reality.

 Lab results suggest a deficiency in ornithine transcarbamoylase:

o High levels of Alanine, Glutamine, and Orotic Acid (a metabolite of purine synthesis)

o NH3 of course was way high

o The Carbamoyl Phosphate builds up and must be shunted off to another pathway.

 TREATMENT:

o A high carbohydrate, low protein diet (but not no protein)

o Treat with benzoic acid or phenylacetate for ammonia detoxification.

***Topic:*** **Nucleotide exchange**

Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essentialfor all cells. Without them, neither ribonucleic acid (RNA) nor deoxyribonucleicacid (DNA) can be produced, and, therefore, proteins cannot be synthesized orcells proliferate. Nucleotides also serve as carriers of activated intermediates inthe synthesis of some carbohydrates, lipids, and conjugated proteins (forexample, uridine diphosphate [UDP]-glucose and cytidine diphosphate [CDP]-choline) and are structural components of several essential coenzymes, such ascoenzyme A, flavin adenine dinucleotide (FAD[H2]), nicotinamide adeninedinucleotide (NAD[H]), and nicotinamide adenine dinucleotide phosphate(NADP[H]). Nucleotides, such as cyclic adenosine monophosphate (cAMP) andcyclic guanosine monophosphate (cGMP), serve as second messengers in signaltransduction pathways. In addition, nucleotides play an important role as energysources in the cell. Finally, nucleotides are important regulatory compounds formany of the pathways of intermediary metabolism, inhibiting or activating keyenzymes. The purine and pyrimidine bases found in nucleotides can besynthesized de novo or can be obtained through salvage pathways that allow thereuse of the preformed bases resulting from normal cell turnover. [Note: Little ofthe purines and pyrimidines supplied by diet is utilized and is degraded instead.]

**STRUCTURE**Nucleotides are composed of a nitrogenous base; a pentose monosaccharide; andone, two, or three phosphate groups. The nitrogen-containing bases belong totwo families of compounds: the purines and the pyrimidines.

A. Purine and pyrimidine basesBoth DNA and RNA contain the same purine bases: adenine (A) andguanine (G). Both DNA and RNA contain the pyrimidine cytosine (C), butthey differ in their second pyrimidine base: DNA contains thymine (T),whereas RNA contains uracil (U). T and U differ in that only T has a methyl group. Unusual (modified) bases are occasionally foundin some species of DNA (for example, in some viral DNA) and RNA (forexample, in transfer RNA [tRNA]). Base modifications includemethylation, glycosylation, acetylation, and reduction. Some examples of unusual bases are shown in. [Note: The presence of an unusualbase in a nucleotide sequence may aid in its recognition by specificenzymes or protect it from being degraded by ***nucleases***.]

B. NucleosidesThe addition of a pentose sugar to a base through an N-glycosidic bond produces a nucleoside. If the sugar is ribose, a ribonucleoside isproduced, and if the sugar is 2-deoxyribose, a deoxyribonucleoside is produced. The ribonucleosides of A, G, C, and U are named adenosine, guanosine, cytidine, and uridine, respectively. The deoxyribonucleosides of A, G, C, and T have the added prefix deoxy- (for example, deoxyadenosinee). [Note: The compound deoxythymidine is often simply called thymidine, with the deoxy- prefix being understood, because it is incorporated into DNA only.] The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately.[Note: Carbons in the pentose are numbered 1′ to 5′. Thus, when the 5′-carbon of a nucleoside (or nucleotide) is referred to, a carbon atom in the pentose, rather than

an atom in the base, is being specified.] systems for purine- and pyrimidine-containing nucleosides.

**C. Nucleotides** The addition of one or more phosphate groups to a nucleoside produces a nucleotide. The first phosphate group is attached by an ester linkage to the 5′-OH of the pentose, forming a nucleoside 5′-phosphate or a 5′-nucleotide. The type of pentose is denoted by the prefix in the names 5′-ribonucleotide and 5′-deoxyribonucleotide. If one phosphate group is attached to the 5′-carbon of the pentose, the structure is a nucleoside monophosphate, like adenosine monophosphate (AMP, or adenylate). If a second or third phosphate is added to the nucleoside, a nucleoside diphosphate (for example, adenosine diphosphate [ADP] or triphosphate, for example, ATP) results. The second and third phosphates are each connected to the nucleotide by a "high-energy bond" (a bond with a large, negative change in free energy [–ΔG,] of hydrolysis.

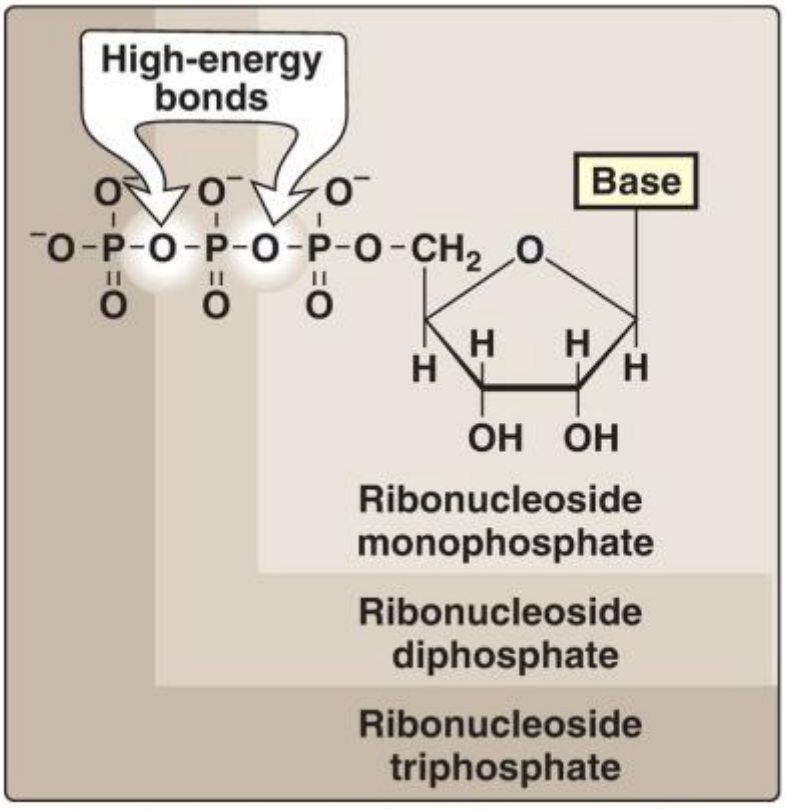



Figure 22.4 Ribonucleoside monophosphate, diphosphate, and triphosphate.

**PURINE NUCLEOTIDE SYNTHESIS** The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartate, glycine, and glutamine), carbon dioxide (CO2), and N10-formyltetrahydrofolate (N10-formyl-THF), as shown in. The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate. [Note: Synthesis of ribose 5-phosphate from glucose 6-phosphate by the pentose phosphate pathway is discussed on p.]

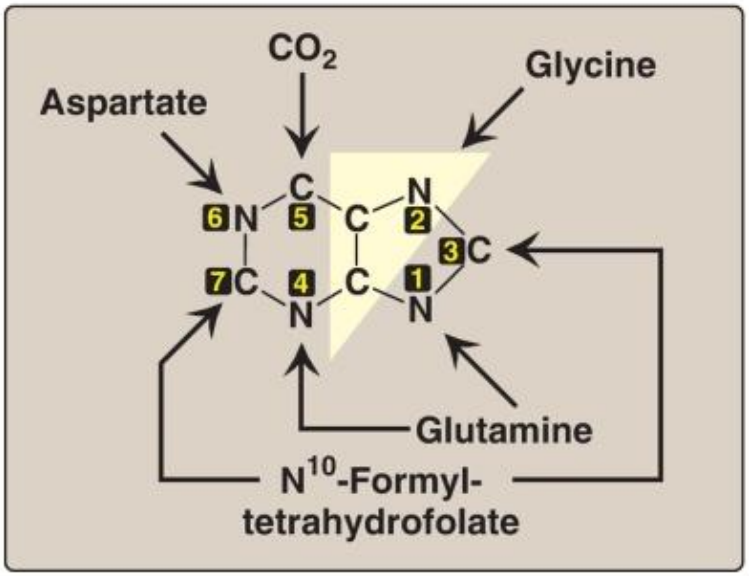



Figure 22.5 Sources of the individual atoms in the purine ring. The order in which the atoms are added is shown by the numbers in the black boxes (see Fig. 22.7). $CO_2$ = carbon dioxide.

A. 5-Phosphoribosyl-1-pyrophosphate synthesis 5-Phosphoribosyl-1-pyrophosphate (PRPP) is an activated pentose that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by ***PRPP synthetase***. This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end-product inhibition). [Note: Because the sugar moiety of PRPP is ribose, ribonucleotides are the end products of de novo purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced. Synthesis of PRPP, showing the activator and inhibitors of the reaction. [Note: This is not the committed step of purine synthesis.

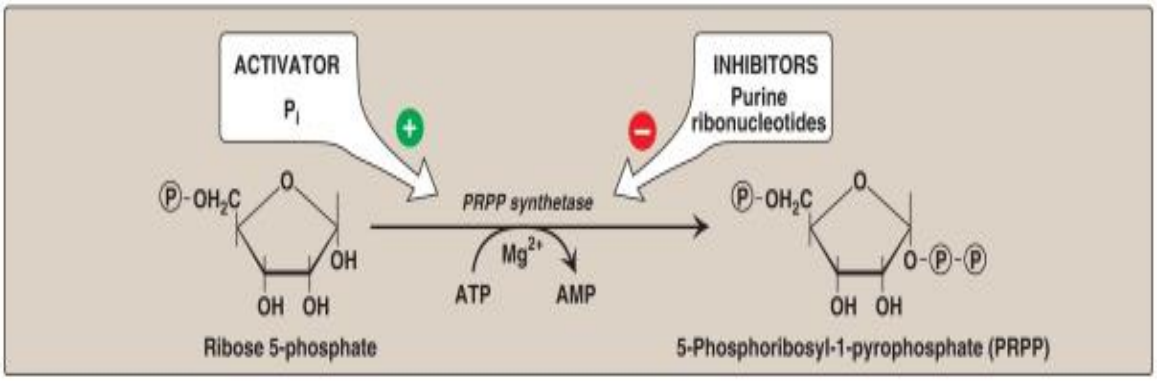



Figure: Synthesis of PRPP, showing the activator and inhibitors of thereaction. [Note: This is not the committed step of purine synthesis because PRPPis used in other pathways such as salvage.] = phosphate; Pi =inorganic phosphate; AMP = adenosine monophosphate; Mg = magnesium.

B. 5-Phosphoribosylamine synthesis. Synthesis of 5-phosphoribosylamine from PRPP and glutamine is shown in. The amide group of glutamine replaces the pyrophosphategroup attached to carbon 1 of PRPP. This is the committed step in purinenucleotide biosynthesis. The enzyme that catalyzes the reaction,***glutamine:phosphoribosylpyrophosphateamidotransferase***(***GPAT***), isinhibited by the purine 5′-nucleotides AMP and guanosine monophosphate(GMP, or guanylate), the end products of the pathway. The rate of thereaction is also controlled by the intracellular concentration of PRPP.[Note: The concentration of PRPP is normally far below the Michaelisconstant (Km) for the ***GPAT***. Therefore, any small change in the PRPPconcentration causes a proportional change in rate of the reaction.]

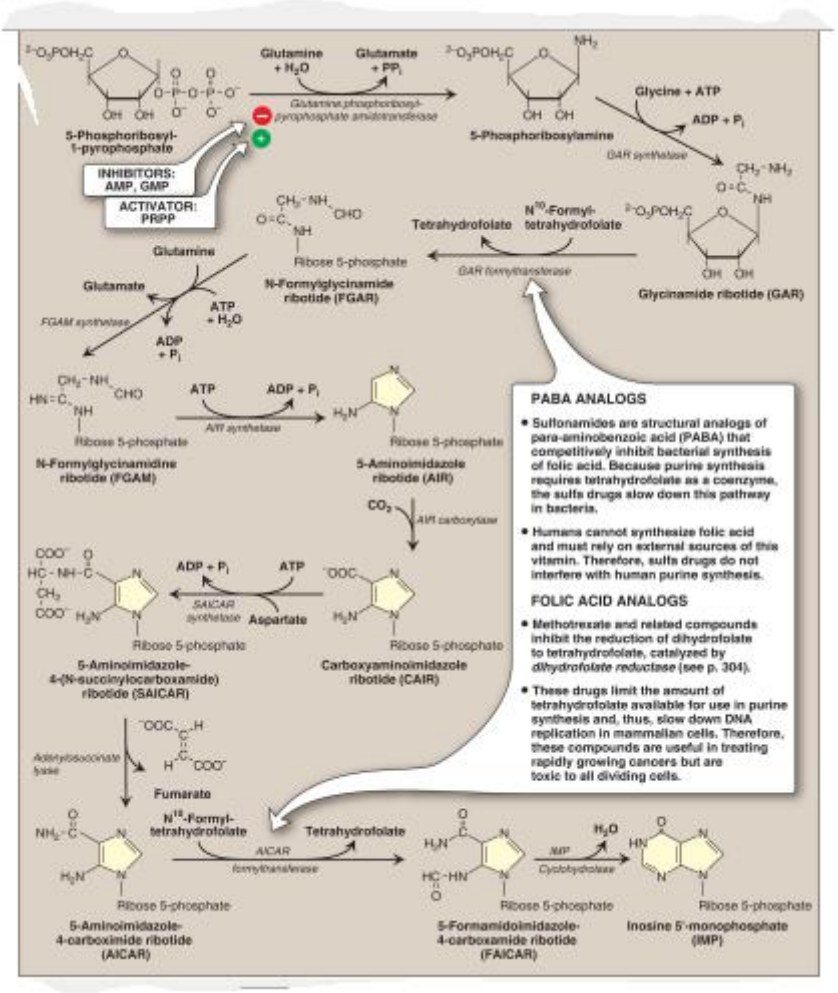



Figure: De novo synthesis of purine nucleotides, showing the inhibitoryeffect of some structural analogs. AMP and ADP = adenosine mono-anddiphosphates; GMP = guanosine monophosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; Pi = inorganic phosphate; PPi= pyrophosphate; CO2 = carbondioxide.

C. Inosine monophosphate synthesis The next nine steps in purine nucleotide biosynthesis leading to the synthesis of inosine monophosphate ([IMP] whose base is hypoxanthine) are illustrated in. IMP is the parent purine nucleotide for AMP and GMP. Four steps in this pathway require ATP as an energy source,

and two steps in the pathway require N10-formyl-THF as a one-carbon donor. [Note: Hypoxanthine is found in tRNA.

D. Synthetic inhibitors Some synthetic inhibitors of purine synthesis (for example, the sulfonamides) are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions. Other purine synthesis inhibitors, such as structural analogs of folic acid (for example, methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA. Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, gastrointestinal (GI) tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiency, and hair loss.

E. Adenosine and guanosine monophosphate synthesis The conversion of IMP to either AMP or GMP uses a two-step, energy- and nitrogen-requiring pathway. [Note: AMP synthesis requires guanosine triphosphate (GTP) as an energy source and aspartate as a nitrogen source, whereas GMP synthesis requires ATP and glutamine.] Also, the first reaction in each pathway is inhibited by the end product of that pathway. This provides a mechanism for diverting IMP to the synthesis of the purine present in lesser amounts. If both AMP and GMP are present in adequate amounts, the de novo pathway of purine nucleotide synthesis is inhibited at the ***GPAT*** step. Conversion of IMP to AMP (or, adenylate) and GMP (or, guanylate).

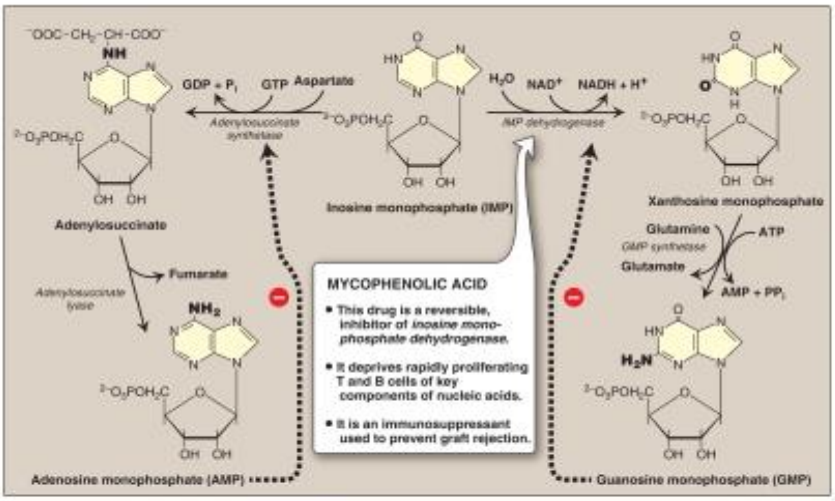



Figure 22.8 Conversion of IMP to AMP (or, adenylate) and GMP (or, guanylate) showing feedback inhibition. NAD(H) = nicotinamide adenine dinucleotide; GDP and GTP = guanosine di- and triphosphates; $P_i$ = inorganic phosphate; $PP_i$ = pyrophosphate.

**F. Nucleoside di- and triphosphate synthesis**Nucleoside diphosphates are synthesized from the corresponding nucleosidemonophosphates by base-specific ***nucleoside monophosphate kinases***. [Note: These ***kinases*** do not discriminate between ribose ordeoxyribose in the substrate.] ATP is generally the source of the transferredphosphate because it is present in higher concentrations than the

othernucleoside triphosphates. ***Adenylate kinase*** is particularly active in the liverand in muscle, where the turnover of energy from ATP is high. Its functionis to maintain equilibrium among the adenine nucleotides (AMP, ADP, andATP). Nucleoside diphosphates and triphosphates are interconverted by***nucleoside diphosphate kinase***, an enzyme that, unlike the ***monophosphatekinases***, has broad substrate specificity.

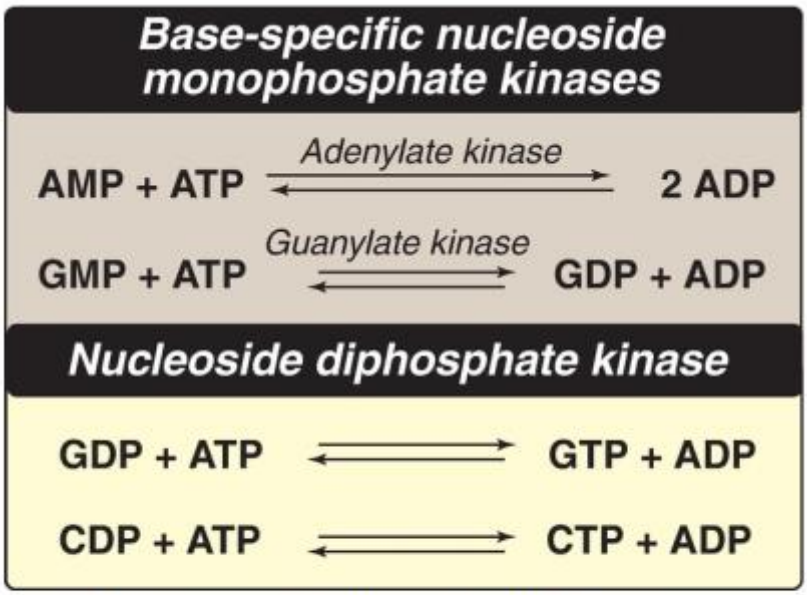



Figure 22.9 Conversion of nucleoside monophosphates to di- and triphosphates. AMP and ADP = adenosine mono- and diphosphates; GMP, GDP, and GTP = guanosine mono-, di-, and triphosphates; CDP and CTP = cytidine di- and triphosphates.

G. Purine salvage pathwayPurines that result from the normal turnover of cellular nucleic acids, or thesmall amount that is obtained from the diet and not degraded, can beconverted to nucleoside triphosphates and used by the body. This is referredto as the salvage pathway for purines. [Note: Salvage is particularlyimportant in the brain.]

1. purine base salvage to nucleotides: Two enzymes are involved: ***adeninephosphoribosyltransferase***(***APRT***) and X-linked ***hypoxanthine–guanine phosphoribosyltransferase***(***HGPRT***). Both use PRPP as thesource of the ribose 5-phosphate group. The release ofpyrophosphate and its subsequent hydrolysis by ***pyrophosphatase***makesthese reactions irreversible. [Note: Adenosine is the only purinenucleoside to be salvaged. It is phosphorylated to AMP by ***adenosinekinase***.][Note: Virtuallycomplete deficiency of ***HGPRT*** results in Lesch-Nyhan syndrome. Partialdeficiencies of ***HGPRT*** are known. As the amount of functional enzymeincreases, the severity of the symptoms decreases.] IMP, GMP, and AMP =inosine, guanosine, and adenosine monophosphates; PRPP = 5-phosphoribosyl-1- pyrophosphate; PPi = pyrophosphate.

2. Lesch-Nyhan syndrome: This is a rare, X-linked recessive disorder associated with a virtually complete deficiency of ***HGPRT***. The deficiency results in an inability to salvage hypoxanthine or guanine, from which excessive amounts of uric acid, the end product of purine degradation, are then produced. In addition, the lack of this salvage pathway causes increased PRPP levels and decreased IMP

and GMP levels. As a result, ***GPAT*** (the regulated step in purine synthesis) has excess substrate and decreased inhibitors available, and de novo purine synthesis is increased. The combination of decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid, making ***HGPRT*** deficiency an inherited cause of hyperuricemia. In patients with Lesch-Nyhan syndrome, the hyperuricemia frequently results in the formation of uric acid stones in the kidneys (urolithiasis) and the deposition of urate crystals in the joints (gouty arthritis) and soft tissues. In addition, the syndrome is characterized by motor dysfunction, cognitive deficits, and behavioral disturbances that include selfmutilation (for example, biting of lips).

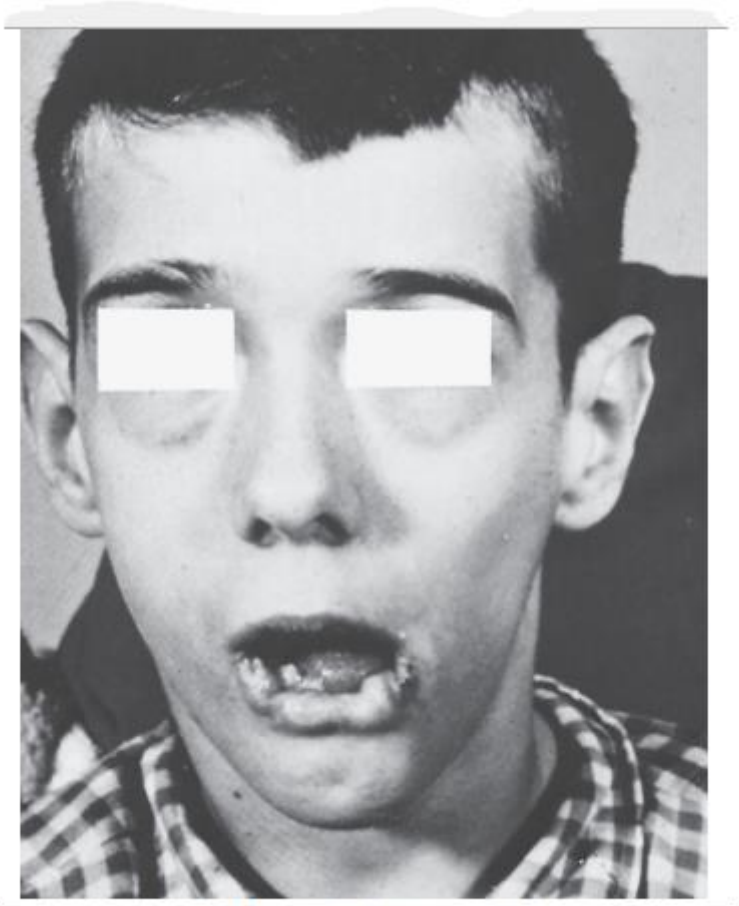


Figure 22.11 Lesions on the lips of a patient with Lesch-Nyhan syndrome.

DEOXYRIBONUCLEOTIDE SYNTHESIS The nucleotides described thus far all contain ribose (ribonucleotides). DNA synthesis, however, requires 2′-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ***ribonucleotide reductase*** during the S-phase of the cell cycle. [Note: The same enzyme acts on pyrimidine ribonucleotides.] A. Ribonucleotide reductase ***Ribonucleotide reductase*** (***ribonucleoside diphosphate reductase***) is a dimer composed of two nonidentical subunits, R1 (or, α) and the smaller R2 (or, β), and is specific for the reduction of purine nucleoside diphosphates (ADP and GDP) and pyrimidine nucleoside diphosphates (CDP and UDP) to their deoxy forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2′-hydroxyl group are two sulfhydryl (–SH)

groups on the enzyme itself (R1 subunit), which form a disulfide bond during the reaction. [Note: R2 contains the stable tyrosyl radical required for catalysis at R1.] Reduced enzyme regeneration: In order for ***ribonucleotide reductase*** to continue to produce deoxyribonucleotides at R1, the disulfide bond created during the production of the 2′-deoxy carbon must be reduced. The source of the reducing equivalents is thioredoxin, a protein coenzyme of ***ribonucleotide reductase***. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two –SH groups of thioredoxin donate their hydrogen atoms to ***ribonucleotide reductase***, forming a disulfide bond in the process.

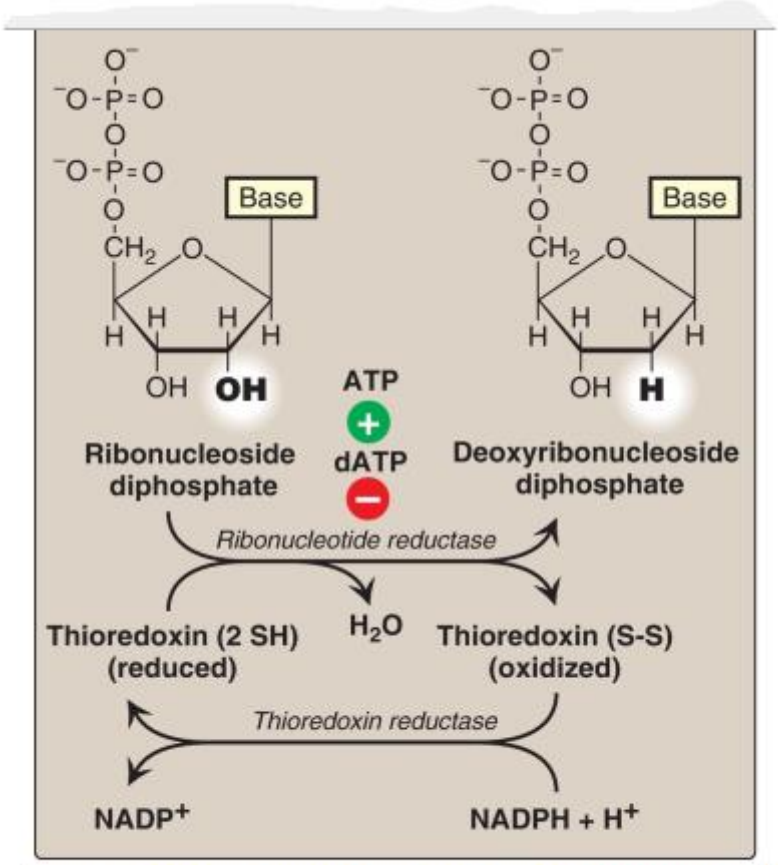



Figure 22.12 Conversion of ribonucleotides to deoxyribonucleotides. NADP(H) = nicotinamide adenine dinucleotide phosphate; dATP = deoxyadenosine triphosphate.

2. Reduced thioredoxin regeneration: Thioredoxin must be converted backto its reduced form in order to continue performing its function. Thereducing equivalents are provided by NADPH + H+, and the reaction iscatalyzed by ***thioredoxin reductase***, a selenoprotein. B. Deoxyribonucleotide synthesis regulation***Ribonucleotide reductase*** is responsible for maintaining a balanced supplyof the deoxyribonucleotides required for DNA synthesis. Consequently, theregulation of the enzyme is complex. In addition to the catalytic site, R1contains two distinct allosteric sites involved in regulating enzymic activity. Regulation of ***ribonucleotide reductase***. dATP, dTTP, and dGTP =deoxyadenosine, deoxythymidine, and deoxyguanosine triphosphates. [Note:The R1 subunit is also referred to as α and the R2 as β.]

1. Activity sites: The binding of dATP to allosteric sites (known as activitysites) on R1 inhibits the overall catalytic activity of the enzyme

and,therefore, prevents the reduction of any of the four nucleosidediphosphates. This effectively prevents DNA synthesis and explains thetoxicity of increased levels of dATP seen in conditions such as***adenosine deaminase (ADA)*** deficiency. In contrast, ATPbound to these sites activates the enzyme.

2. Substrate specificity sites: The binding of nucleoside triphosphates toadditional allosteric sites (known as substrate specificity sites) on R1regulates substrate specificity, causing an increase in the conversion ofdifferent species of ribonucleotides to deoxyribonucleotides as they arerequired for DNA synthesis. For example, deoxythymidine triphosphatebinding at the specificity site causes a conformational change that allowsreduction of GDP to dGDP at the catalytic site when ATP is at theactivity site.The drug hydroxyurea (hydroxycarbamide) inhibits ***ribonucleotidereductase***, thereby inhibiting the generation of substrates for DNAsynthesis. The drug is an antineoplastic agent and is used in the treatment of cancers such as melanoma. Hydroxyurea is also used in the treatment of sickle cell anemia. However, the increase in fetal hemoglobin seen with hydroxyurea is because of changes in gene expression and not to ***ribonucleotide reductase*** inhibition.

**PURINE NUCLEOTIDE DEGRADATION**Degradation of dietary nucleic acids occurs in the small intestine, where pancreatic ***nucleases*** hydrolyze them to nucleotides. The nucleotides are sequentially degraded by intestinal enzymes to nucleosides, phosphorylated sugars, and free bases. Uric acid is the end product of intestinal purine degradation. [Note: Purine nucleotides from de novo synthesis are degraded in the liver primarily. The free bases are sent out from the liver and salvaged by peripheral tissues.] A. Degradation in the small intestine ***Ribonucleases*** and ***deoxyribonucleases***, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides that are further hydrolyzed by pancreatic ***phosphodiesterases***, producing a mixture of 3′-yand 5′-mononucleotides. At the intestinal mucosal surface, ***nucleotidases***remove the phosphate groups hydrolytically, releasing nucleosides that are taken into enterocytes by sodium-dependent transporters and degraded by ***nucleosidases***(***nucleoside phosphorylases***) to free bases plus (deoxy) ribose 1-phosphate. Dietary purine bases are not used to any appreciable extent for the synthesis of tissue nucleic acids. Instead, they are degraded to uric acid in the enterocytes. Most of the uric acid enters the blood and is eventually excreted in the urine. A summary of this pathway is shown in. [Note: Mammals other than primates express ***urate oxidase*** (***uricase***), which cleaves the purine ring, generating allantoin. Modified recombinant ***urate oxidase*** is now used clinicallyto lower urate levels.]

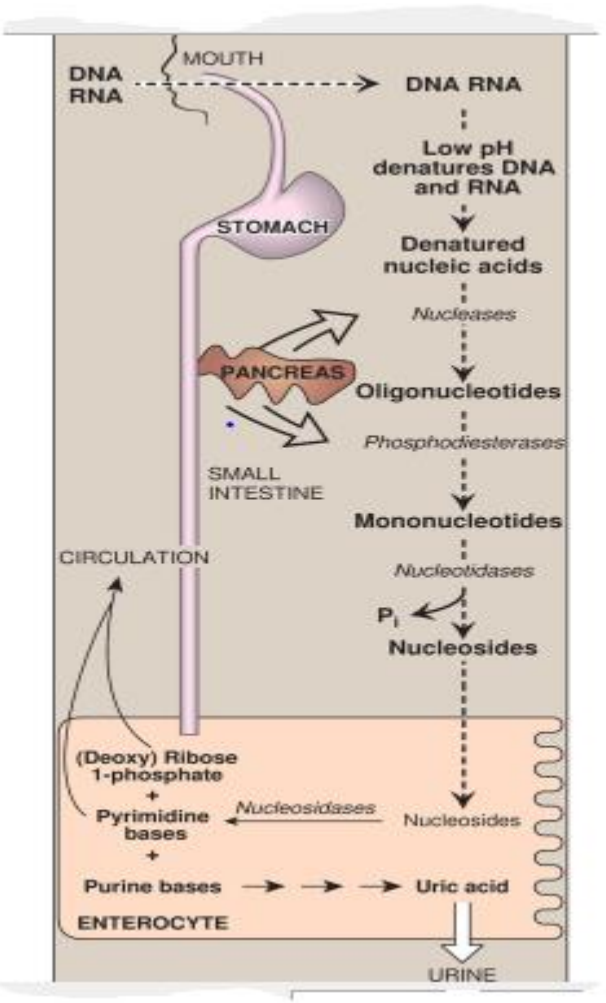



Figure: Digestion of dietary nucleic acids. Pi = inorganic phosphate.

Uric acid formation A summary of the steps in the production of uric acid and the genetic diseases associated with deficiencies of specific degradative enzymes are shown in. [Note: The bracketed numbers refer to specific eactions in the figure.]

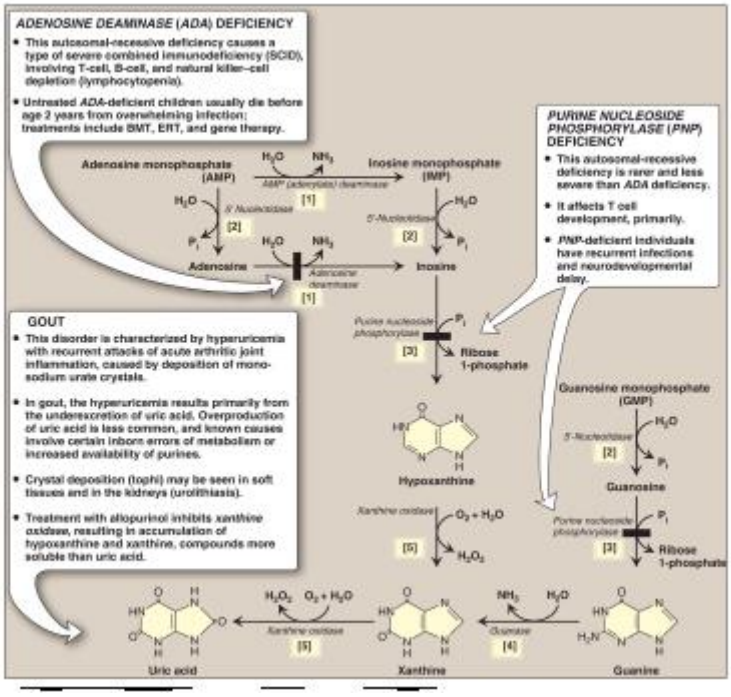


Figure: The degradation of purine nucleotides to uric acid, illustratingsome of the genetic diseases associated with this pathway. [Note: The numbersin brackets refer to the corresponding numbered citations in the text.] BMT =bone marrow transplantation; ERT = enzyme replacement therapy; Pi =inorganic phosphate; H2O2 = hydrogen peroxide; NH3 = ammonia.

[1] An amino group is removed from AMP to produce IMP by ***AMP*** (***adenylate***) ***deaminase*** or from adenosine to produce inosine (hypoxanthine-ribose) by ***adenosine deaminase***.
[2] IMP and GMP are converted into their respective nucleoside forms, inosine and guanosine, by the action of ***5′-nucleotidase***.
[3] ***Purine nucleoside phosphorylase*** converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine. [Note: A ***mutase*** interconverts ribose 1- and ribose 5-phosphate.]
[4] Guanine is deaminated to form xanthine.
[5] Hypoxanthine is oxidized by molybdenum-containing ***xanthine oxidase*** (***XO***) to xanthine, which is further oxidized by ***XO*** to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.

C. Diseases associated with purine degradation 1. Gout: Gout is a disorder initiated by high levels of uric acid (the end product of purine catabolism) in blood (hyperuricemia), as a result of either the overproduction or underexcretion of uric acid. The hyperuricemia can lead to the deposition of monosodium urate (MSU) crystals in the joints and an inflammatory response to the crystals, causing first acute and then progressing to chronic gouty arthritis. Nodular masses of MSU crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout. Formation of uric acid stones in the kidney (urolithiasis) may also be seen. [Note: Hyperuricemia is not sufficient to cause gout, but gout is always preceded by hyperuricemia. Hyperuricemia is typically asymptomatic but may be indicative of comorbid conditions such as hypertension.] The definitive diagnosis of gout requires aspiration and examination of synovial fluid from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped MSU crystals.

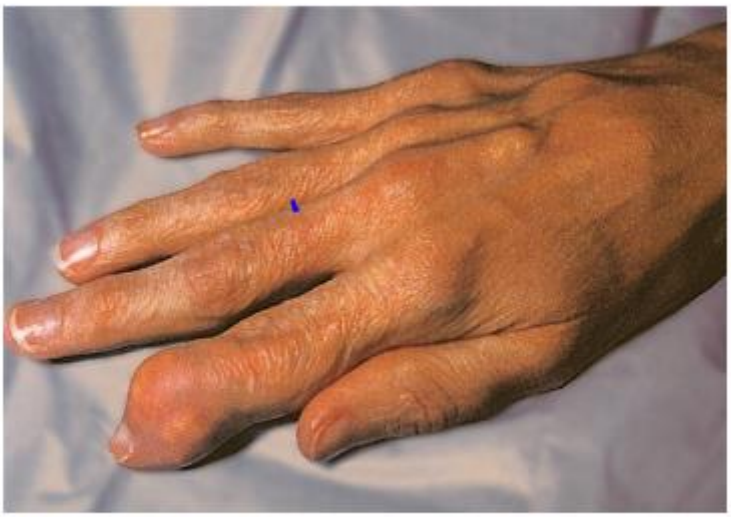


Figure 22.16 Tophaceous gout.

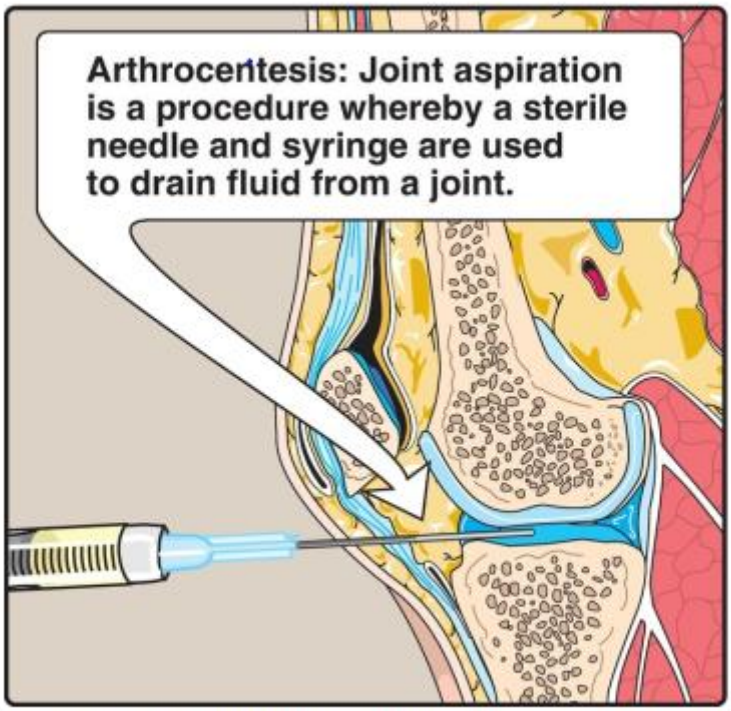



Figure 22.17 Analysis of joint fluid can help to define causes of joint swelling and arthritis, such as infection, gout, and rheumatoid disease.

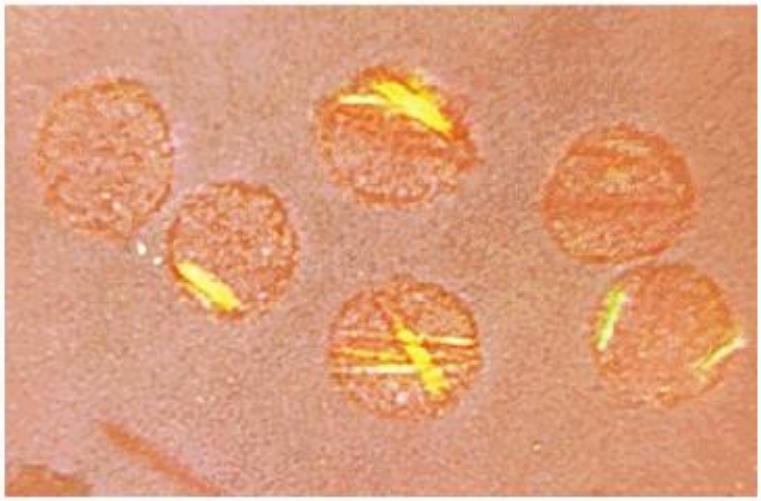


Figure 22.18 Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized light microscopy. Here, crystals are seen within polymorphonuclear leukocytes.

Uric acid underexcretion: In >90% of individuals with hyperuricemia,the cause is underexcretion of uric acid. Underexcretion can beprimary, because of as-yet-unidentified inherent excretory defects, orsecondary to known disease processes that affect how the kidneyhandles urate (for example, in lactic acidosis, lactate increases renalurate reabsorption, thereby decreasing its excretion) and toenvironmental factors such as the use of drugs (for example, thiazidediuretics) or exposure to lead (saturnine gout).b. Uric acid overproduction: A less common cause of hyperuricemia isfrom the overproduction of uric acid. Primary hyperuricemia is, for themost part, idiopathic (having no known cause). However, severalidentified mutations in the gene for X-linked ***PRPP synthetase***resultin the enzyme having an increased maximal velocity ([Vmax] for the production of PRPP, a lower Km for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides, itsallosteric inhibitors. In each case, increased availability ofPRPP increases purine production, resulting in elevated levels ofplasma uric acid. Lesch-Nyhan syndrome also causeshyperuricemia as a result of the decreased salvage of hypoxanthine andguanine and the subsequent increased availability of PRPP. Secondaryhyperuricemia is typically the consequence of increased availability ofpurines (for example, in patients with myeloproliferative disorders orwho are undergoing chemotherapy and so have a high rate of cellturnover). Hyperuricemia can also be the result of seemingly unrelatedmetabolic diseases, such as von Gierke disease or hereditary fructose intolerance.A diet rich in meat, seafood (particularly shellfish), and ethanol isassociated with increased risk of gout, whereas a diet rich in low-fat dairyproducts is associated with a decreased risk.c. Treatment: Acute attacks of gout are treated with anti-inflammatoryagents. Colchicine, steroidal drugs such as prednisone, andnonsteroidal drugs such as indomethacin are used. [Note: Colchicineprevents formation of microtubules, thereby decreasing the movementof neutrophils into the affected area. Like the other anti-inflammatorydrugs, it has no effect on uric acid levels.] Long-term

therapeuticstrategies for gout involve lowering the uric acid level below itssaturation point (6.5 mg/dl), thereby preventing the deposition of MSUcrystals. Uricosuric agents, such as probenecid or sulfinpyrazone, thatincrease renal excretion of uric acid, are used in patients who areunderexcretors of uric acid. Allopurinol, a structural analog ofhypoxanthine, inhibits uric acid synthesis and is used in patients whoare overproducers of uric acid. Allopurinol is oxidized to oxypurinol, along-lived inhibitor of ***XO***. This results in an accumulation of hypoxanthine and xanthine, compounds more solublethan uric acid and, therefore, less likely to initiate an inflammatoryresponse. In patients with normal levels of ***HGPRT***, the hypoxanthinecan be salvaged, reducing the levels of PRPP and, therefore, de novopurine synthesis. Febuxostat, a nonpurine inhibitor of ***XO***, is alsoavailable. [Note: Uric acid levels in the blood normally are close to thesaturation point. One reason for this may be the strong antioxidanteffects of uric acid.]

2. Adenosine deaminase deficiency***ADA*** is expressed in a variety of tissues, but, in humans, lymphocytes have thehighest activity of this cytoplasmic enzyme. A deficiency of ***ADA*** results in an accumulation of adenosine, which is converted to its ribonucleotide or deoxyribonucleotide forms by cellular ***kinases***. As dATP levels rise, ***ribonucleotide reductase*** is inhibited, thereby preventing the production of alldeoxyribose-containing nucleotides. Consequently, cells cannotmake DNA and divide. [Note: The dATP and adenosine that accumulate in ***ADA***deficiency lead to developmental arrest and apoptosis of lymphocytes.] In itsmost severe form, this autosomal-recessive disorder causes a type of severecombined immunodeficiency disease (SCID), involving a decrease in T cells, Bcells, and natural killer cells. ***ADA*** deficiency accounts for ~14% of cases ofSCID in the United States. Treatments include bone marrow transplantation,enzyme replacement therapy, and gene therapy. Without appropriatetreatment, children with this disorder usually die from infection by age 2 years.[Note: ***Purine nucleoside phosphorylase*** deficiency results in a less severe munodeficiency primarily involving T cells.]

**CHAPTER SUMMARY**Nucleotides are composed of a nitrogenous base (adenine = A, guanine =G, cytosine = C, uracil = U, and thymine = T); a pentose sugar; and one,two, or three phosphate groups (Fig. 22.24). A and G are purines, and C, U,and T are pyrimidines. If the sugar is ribose, the nucleotide is aribonucleoside phosphate (for example, adenosine monophosphate [AMP]),and it can have several functions in the cell, including being a componentof RNA. If the sugar is deoxyribose, the nucleotide is adeoxyribonucleoside phosphate (for example, deoxyAMP) and will befound almost exclusively as a component of DNA. The committed step inpurine synthesis uses 5-phosphoribosyl-1-pyrophosphate ([PRPP], anactivated pentose that provides the ribose 5-phosphate for de novo purineand pyrimidine synthesis and salvage) and nitrogen from glutamine toproduce phosphoribosylamine. The enzyme is***glutamine:phosphoribosylpyrophosphateamidotransferase***and isinhibited by AMP and guanosine monophosphate (the end products of thepathway) and activated by PRPP. Purine nucleotides can also be producedfrom preformed purine

bases by using salvage reactions catalyzed by***adenine phosphoribosyltransferase***(***APRT***) and ***hypoxanthine–guaninephosphoribosyltransferase***(***HGPRT***). A near-total deficiency of ***HGPRT***causes Lesch-Nyhan syndrome, a severe, inherited form of hyperuricemiaaccompanied by compulsive self-mutilation. All deoxyribonucleotides aresynthesized from ribonucleotides by the enzyme ***ribonucleotide reductase***.This enzyme is highly regulated (for example, it is strongly inhibited bydeoxyadenosine triphosphate [dATP], a compound that is overproduced in bone marrow cells in individuals with ***adenosine deaminase*** [***ADA***] deficiency). ***ADA*** deficiency causes severe combined immunodeficiency disease. The end product of purine degradation is uric acid, a compound of low solubility whose overproduction or undersecretion causes hyperuricemia that, if accompanied by the deposition of monosodium urate crystals in joints and soft tissues and an inflammatory response to those crystals, results in gout. The first step in pyrimidine synthesis, the production of carbamoyl phosphate by ***carbamoyl phosphate synthetase II***, is the regulated step in this pathway (it is inhibited by uridine triphosphate [UTP] and activated by PRPP). The UTP produced by this pathway can be converted to cytidine triphosphate. Deoxyuridine monophosphate can be converted to deoxythymidine monophosphate by ***thymidylate synthase***, an enzyme targeted by anticancer drugs such as 5-fluorouracil. The regeneration of tetrahydrofolate from dihydrofolate produced in the ***thymidylate synthase*** reaction requires ***dihydrofolate reductase***, an enzyme targeted by the drug methotrexate. Pyrimidine degradation results in soluble products.