Erythrocytes - Abnormal Cells And Their Significance

Normal erythrocytes are disc shaped, biconcave cells which do not have nucleus. Appearance and morphology of normal erythrocytes may vary in various disorders or disease conditions.
In erythrocytes, following types of variations are observed:

1. Variation in size

- A normal erythrocytes has an average diameter of 7.2 micrometer (ranges between 6.8 - 7.5 micrometer. This normal size is known as normocytic.
- Increased variation in cell size is known as anisocytosis.
- Erythrocytes larger than the normal is known as macrocytic. It is occurs due to defect in either nuclear maturation resulting due to vitamin B12 deficiency or stimulated erythropoiesis which increase the synthesis of haemoglobin in developing cells.
- Erythrocytes smaller than the normal is known as microcytic. In microcytosis synthesis of haemoglobin decreases due to iron deficiency, impaired globulin synthesis and mitochondrial abnormality.

2. Variation in shape

A term poikilocytosis is used to denote a mature erythrocytes that have the shape other than the normal erythrocytes. Poikilocytosis are found in many shapes.

a. **Acanthocytes:** they have multiple thorny, spike like projections which are irregularly distributed around the cellular membrane and may vary in size. These are formed in abetalipoproteinaema and spur cell anaemia. Other reasons are heparin administration, in hepatic haemangioma and in neonatal hepatitis.
b. **Blister cells:** They have one or more vacuoles which look like bister on skin. These cells are appear in the case of severe burns, pulmonary emboli in sickle cell anaemia and microangiopathic haemolytic anaemia.
c. **Burr cells/ Echinocytes:** they have one or more spiny projections of cellular membrane. These cells appear in a variety of anaemia, bleeding gastric ulcers, gastric carcinoma, peptic ulcer, renal insufficiency and uraemia.
d. **Poikilocytes:** they are result from the production of abnormal cells by bone marrow or from damage to normal cells.
e. **Crenated erythrocytes:** they have short, scalloped or spike like projections and are distributed around the cell membrane. They appear in case of physical loss of intracorpuscular water.
f. **Elliptocytes**: they have rod, cigar or sausage-like shape and appear narrower and more elongated than megalocytes.
g. **Leptocytes**: these are thin or flattened RBCs, having central rounded area. Lepocytes occur mostly in sideropenic anaemia occurs due to disturbance in synthesis of haemoglobin.

h. **Oval macrocytes:** they are formed when there is a disturbance in the synthesis of DNA due to vitamin B12 or folic acid deficiency.
i. **Schistocytes:** these are fragments of red blood cells and are formed either by fragmentation of abnormal cells. They are seen in microangiopathic and mechanical haemolytic anaemia.
j. **Spherocytes**: these are spherical or nearly spherical in shape rather than biconcave disc shape.
k. **Sickle cells (drepanocytes):** these are found in sickle cell anaemia.
l. **Stomatocytes:** these cells have a central pallor or stoma on the stained blood film. These cells are rarely seen in the blood film of a healthy person.
m. **Target cells:** these cells are thinner than the normal cells and have an increased staining area which appear in the middle of the area of central pallor.
n. **Teardrop or pear shaped cells (dacrocytes):** they are formed in the case of bone marrow fibrosis or severe dyserythropoiesis and also in some haemolytic anaemia.

3. Alteration in color

RBCs have haemoglobin, which provides a characteristics red colour. Following alteration are observed in colour of RBCs during any disease or disorder:

a. **Hypochromia:** hypochromia means that the red blood cells have less color than normal when examined under microscope. This is usually occurs when there is not enough of the pigment that carries oxygen (haemoglobin) in the red blood cells.
b. **Hyperchromia:** hyperchromia means that the red blood cells have more color than normal when examined under microscope. This is usually occurs when there is more pigment that carries oxygen (haemoglobin) in the red blood cells.
c. **Anisochromasia**: it is a marked variability in the colour density of erythrocytes, which indicates the unequal haemoglobin content among the red blood cells. A major cause of anisochromasia is sideroblastic anaemia.
d. **Dimorphism**: it shows the presence of two types of RBCs. This term is mostly used when one population of RBCs is hypochromic, microcytic cells; and other population is normochromic cells, later which become normocytic or macrocytic.

4. Inclusions in erythrocytes

These are elements, present in RBCs. Each type of inclusions has specific appearance, composition and related physiology. Presence of these inclusions in RBCs indicates a disease or disorder. Following are the examples of common types of inclusion bodies:

a. **Howell-jolly bodies:** these are round, solid-staining, dark-blue to purple inclusions, having size of 1-2 micrometer. They are not seen in normal erythrocytes, and their presence is associated

with haemolytic anaemia, pernicious anaemia, particularly postsplenectomy, physiological atrophy of the spleen.

b. **Pappenheimer bodies (Siderotioc Granules):** these bodies are observed as purple dots in wright-stained smears and can be rarely seen in peripheral blood smear.
c. **Heinz body:** these inclusion bodies are 0.2-2.0 micrometer in size and can be observed on staining with crystal violet or brilliant cresyl blue. They indicated precipitated and denatured haemoglobin.
d. **Cabot rings:** these are loop-shaped, ring shaped, figure 8 shaped structures. They are present in cytoplasm of RBCs and like beads on a string. They attain red-purple colour under the wright stain.

5. Alteration in erythrocytes distribution

Two types of alterations agglutination and rouleaux formation are observed on a stained peripheral smear.

Agglutination is the clumping of erythrocytes, and in rouleaux formation, erythrocytes are arranged in groups that resemble stacks of coins.

Enzymes

Introduction

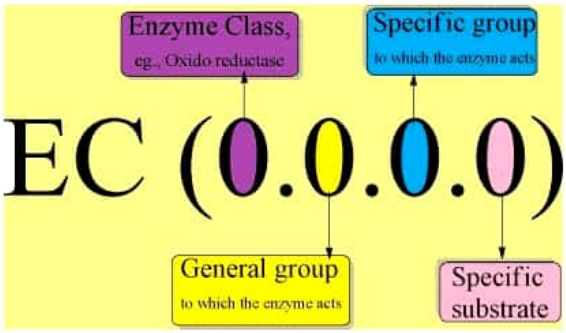
- Enzymes are the proteins that increase the rate of reaction by lowering the energy of activation.
- They catalyse nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable

Properties

- Enzymes are required only in small amounts.
- All enzymes are proteins, except the group of catalytic RNAs.
- The molecular weight of enzymes ranges from 12,000 to 1 million or more like other proteins.
- Like proteins, enzymes are colloidal in nature and precipitated by salt solution.
- They are inactivated by heat and alteration of pH.
- Enzyme activity can be regulated by some processes such as phosphorylation, glycosylation, etc.
- Enzymes are very specific in their action and thus separate enzymes are exist for different reactions.

Naming and enzyme classification (nomenclature)

In general many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity. In 1961, according to the report of the first Enzyme Commission (EC) of International Union of Pure and Applied Chemistry (IUPAC), Enzymes are classified in to six types on the basis of reaction they catalyze. They were assigned code numbers, prefixed by E.C., which contain four elements separated by points and have the following meaning as shown in **Scheme 1.1.**



- The name of an enzyme in many cases end in suffix -ase.
- For example, sucrase catalyses the hydrolysis of sucrose.
- The name describes the function of enzyme. For example, oxidase catalyse the oxidation reaction.
- Sometimes common names are used, particularly for the digestion enzymes such as pepsin and trypsin.
- Some names describe both the substrate and function. For example, alcohol dehydrogenase oxides ethanol.

Classification

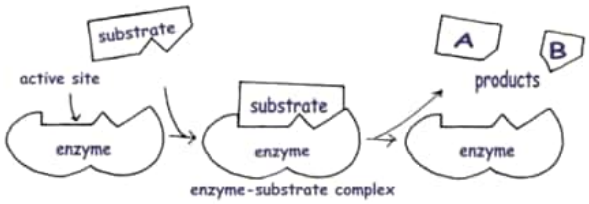
The enzyme can be classified in to six main classes as shown in the table.

s.no	Classification	Biochemical properties
1	**Oxidoreductases**	Act on many chemical groupings to add or remove hydrogen atoms. e.g. lactate dehydrogenase.
2	**Transferases**	Transfer functional groups between donor and acceptor molecules. e.g. aminotransferase.
3	**Hydrolases**	Add water across a bond, hydrolyzing it. e.g. acetyl choline esterase.
4	**Lyases**	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds e.g. aldolase .
5	**Isomerases**	transfer of groups within molecules to yield isomeric forms. e.g. triose phosphate isomerase.
6	**Ligases**	Catalyse reactions in which two chemical groups are joined with the use of energy from ATP. e.g. Acetyl CoA carboxylase.

Mechanism of enzyme action

Enzymes are macromolecules that help to accelerate (catalyze) chemical reactions in biological systems. Some biological reactions in the absence of enzymes may be as much as a million times slower. Any chemical reaction converts one or more molecules, called the **substrate,** into different molecule(s), called the **product**. Most of the reactions in biochemical processes require chemical events that are unfavorable or unlikely in the cellular environment, such as the transient formation of unstable charged intermediates or the collision of two or more molecules in the precise orientation required for reaction. In some of the Reactions like, digestion of food, send nerve signals, or contract a muscle simply do not occur at a useful rate without catalysis. Enzyme overcomes these problems by providing a specific environment within which a given reaction can occur more rapidly. Enzymes are usually proteins – each has a very specific shape or conformation. Within this large molecule is a region called an **active site**, which has properties allowing it to bind tightly to the substrate molecule(s).

As proposed by Charles-Adolphe Wurtz, an active site is a three dimensional cleft or crevice formed by groups that come from different parts of the amino acid sequence - residues far apart in the amino acid sequence may interact more strongly than adjacent residues in the sequence. The active site encloses a substrate and catalyzes its chemical transformation. The enzyme substrate complex was first discovered in 1880, is central to the action of enzymes. The enzyme–substrate interactions can be explained by the following theories.

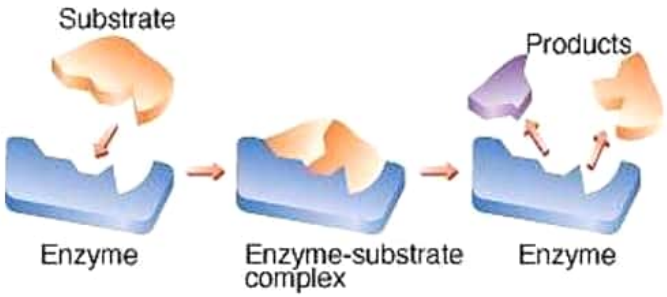


Lock and key model

- In lock and key model of enzyme action-

- The active site has a rigid shape.
- Only substrate with the matching shape can fit.
- The substrate is a key that fits the lock of active site.

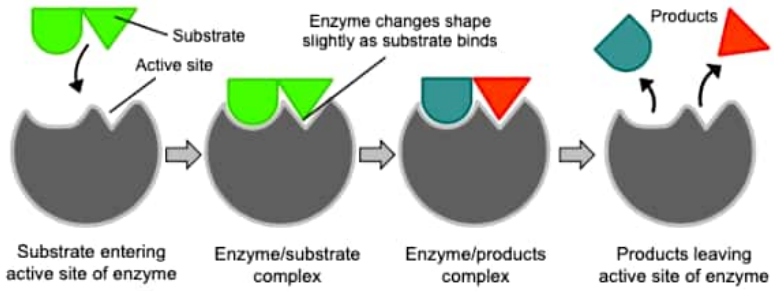
This is an older model, however and does not work for all enzymes.



Lock and key model for enzyme – substarte

Induced fit

Lock and key model does not explain the stability of the transition state for it would require more energy to reach the transition state complex. To explain this concept Koshland in 1958, first proposed the induced-fit model, this suggests that the enzyme active site is flexible, not rigid. Enzyme itself usually undergoes a change in conformation when the substrate binds, induced by multiple weak interactions and hydrophobic characteristics on the enzyme surface mold into a precise formation.



Transition state theory

According to this theory when an enzyme catalysis, the enzyme binds more strongly to its "*transition state complex rather than its ground state reactants*." This indicates, the transition state is more stable. A simple enzymatic reaction can be written as, $E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$
Where E, S, and P represent the enzyme, substrate, and product respectively; ES and EP are transient complexes of the enzyme with the substrate and with the product respectively.

Enzyme catalysed reactions

When a substrate (S) fits properly in an active site, an enzyme-substrate (ES) complex is formed.

$$E + S \leftrightarrow ES$$

Within the active site of the ES complex, the reaction occurs to convert substrate to product (P).

$$ES \leftrightarrow E + P$$

The products are then released, allowing another substrate molecule to bind the enzyme. This cycle can be repeated millions or even more times per minute.
The overall reaction for the conversion of substrate to product can be written as:

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$

Roles of enzymes

- Enzymes play an important role in metabolism, diagnosis and therapeutics.
- All biochemical reactions are enzyme catalysed in the living organism.
- Level of enzyme in blood are of diagnostic importance e.g. it is good indicator in disease such as myocardial infraction.
- Enzymes can be used therapeutically such as digestive enzymes.

Therapeutic uses of enzymes:

- enzymes like pepsin, papain, and amylase for improving digestion.
- The enzyme streptokinase, nattokinase and urokinase are used for dissolving blood clot.
- The enzyme asparaginase is used for the treatment of cancer.

Use of enzymes in the manufacture of bulk drugs:

- The enzyme papain is used in the production of protein hydrolysate.
- Amylase is needed for the production of dextrin.
- The enzymes glucose oxidase is needed for the production of fructose syrup.
- The enzyme penicillin amylase is used for the production of 6-amino penicillanic acid from penicillin G. 6-amino penicillanic acid is needed for the synthesis of several beta lactum antibiotics.

KINETICS OF ENZYME ACTION

Lowering of activation energy

To convert the reactant into the product, potential energy barrier needs to be overcome. To break this barrier, amount of energy needed is equivalent to activation energy. Enzyme helps in lowering down this activation energy during complex formation with substrate. Further this enzyme is released when the product is formed.

ES —- P+ E

E= enzyme
S= substrate
P= Product

Enzyme function by lowering the energy of activation like all other catalysis. The energy digram in figure illustrate this effect.

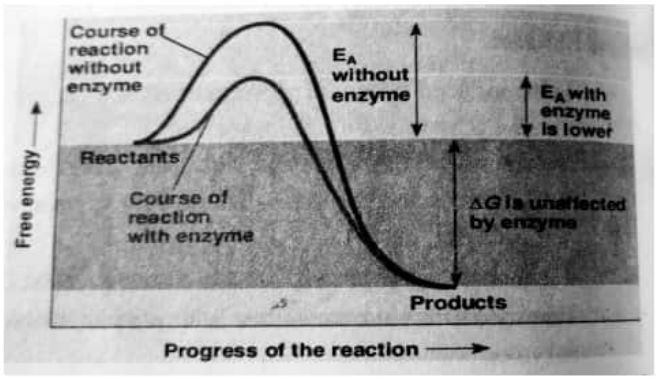


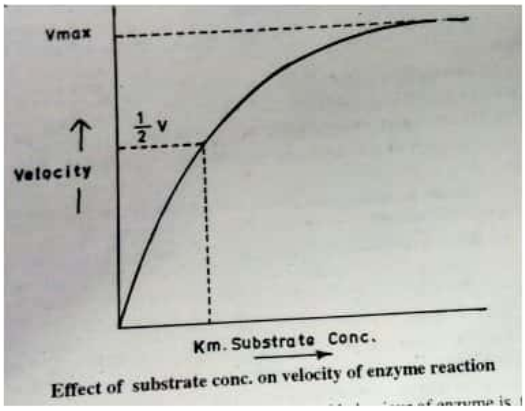
Figure shows the enzymes enhance reaction rates by lowering the energy of activation.

Factors affecting the enzyme activity

The velocity of the enzymes reaction is greatly influenced by certain parameters which affect the enzyme activity. These include:

1. Substrate concentration
2. pH
3. Temperature
4. co-enzyme & activator
5. Inhibitors
6. Time
7. Light and radiation

1. **Substrate concentration-** In an enzyme reaction if all other parameters are kept constant, the velocity of the reaction increases with increase in the substrate concentration to a certain limit after which it become constant and has no influence of the substrate concentration. If the velocity of the enzyme reaction is plotted against substrate concentration, a typical hyperbolic curve is obtained.



Effect of substrate conc. on velocity of enzyme reaction

The explanation of the above mentioned behaviour of enzyme is, that at low concentrations of substrate all enzyme molecules are not involved in the formation of enzyme substrate complex and as the substrate concentration is increased more and more enzyme molecules take part in the reaction with a proportional increased in the velocity. The maximum velocity Vmax is reached when all the enzyme molecules are saturated with substrate and the product is formed at a constant rate thus there is no increase in the velocity.The curv given below is called Michaelis CURV and the mathematical representation is given by Michaelis menten equation .

$$V = Vmax\ [S] / Km + [S]$$

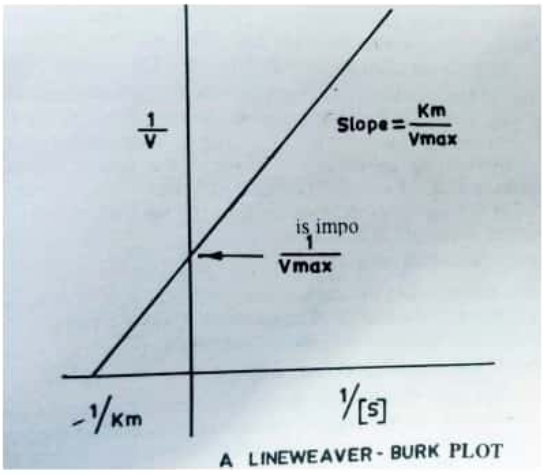
Where

V = velocity of the enzyme reaction at any time

Vmax= maximum velocity attained

S =substrate concentration

Km. = Michaelis constant, which is equivatent to the substrate concentration required to produce half maximum velocity. 1/km 1s called affinity factor between the substrate and the enzyme.



A LINEWEAVER - BURK PLOT

Some enzymes fail to exhibit the classic Michaelis Menten saturation kinetics, and instead show the Sigmoidal saturation kiņeties in accordance with the Hill equation This type of mechanism operates in those enzyme reactions where the enzyme has more than one binding site for the substrate molecules. The equation is (Hill Equation)

$$\text{Log } V / V\ max - V = n\ 1og\ (S) - log\ K$$

Where, **K = Complex constant.**

According to this equation where (S) is low Compared to 'K' the reaction velocity increases as the nth power of (S).

Most enzymes catalyze reactions in which more than one substrate is involved and produce more than one product. Some of these enzymes require the simultaneous presence of all substrates for reaction to occur, whereas for others there is an ordered system of introduction of one substrate at a

time. After the first substrate is altered, the second one is added so on. The latter kind of reactions are known to proceed through. Ping-Pong Mechanism.

It is so called because the enzyme alternates between E and E' form,

where E is the original enzyme and E' the one after releasing the first product.

2. Effect of pH- The enzyme catalysed reaction is greatly affected by the pH of the medium because enzymes are protein in nature and pH governed its bonding with substrate. Optimum pH is the pH possessed by each enzyme at which velocity of enzymes is maximum. Below optimum pH the activity of enzyme become low and above the optimum pH enzyme become inactive. Effect of pH is shown in figure.

3. Effect of temperature - An increase in the temperature leads to increase in the enzyme activity upto a maximum. An increase in activity of enzyme on increase temperature by 10'c is k/as the temperature coefficient or Q10. The temperature at which enzyme is optimally active is k/as optimum temperature. If temperature increase beyond optimum temperature denaturation occurs which decrease the enzyme activity. Effect of temperature is shown in figure.

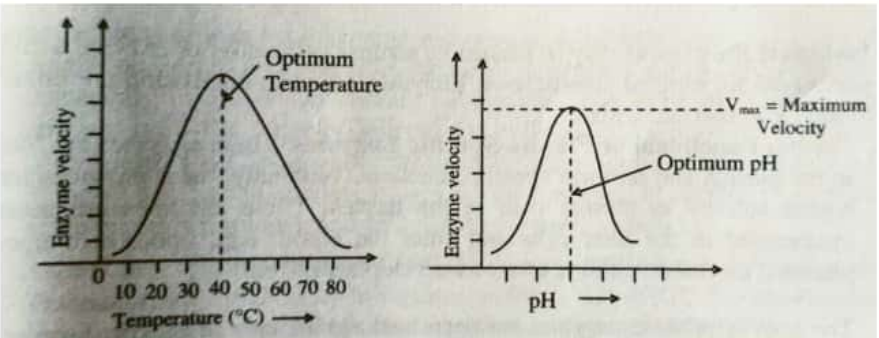


Figure 5.12: Effect of Temperature on Enzyme Velocity

Figure 5.13: Effect of pH on Enzyme Velocity

4. Effect of co-enzymes and activators- enzymes act most efficiently in the presence of certain co-enzymes and activators. co-enzymes are organic substance derived from the B-complex vitamins. Activators are the inorganic ions such as Cl, Mg and Ca which act as cofactor help to increase the enzyme activity.

5. Inhibitors- inhibitors are the substances that decreases the catalytic activity of the enzymes by preventing the formation of a normal enzyme substrate complex. E.g. ritonavir, disulfiram etc.

6. Time- time required for an enzymatic reaction is less provided for ideal and optimal conditions.

7. Light and radiation- on exposure to X-rays, gamma rays, UV and beta rays certain enzymes form peroxides which oxidise the enzymes and make them inactive.

Enzyme Inhibition

Inhibitors are the substances that decreases the catalytic activity of the enzymes by preventing the formation of a normal enzyme substrate complex. E.g. ritonavir, disulfiram etc.

Enzyme inhibition is classified as:

1. Reversible competitive inhibition.
2. Non- competitive inhibition.

1. Reversible competitive inhibition.

A competitive inhibitor has a structure like the substrate which compete with the substrate for the active site and thus diverts much of the enzyme to form the enzyme-inhibitor complex instead of enzyme substrate complex. The enzyme-inhibitor complex does not yield any product and remain stable and preventing further enzyme activity. This type of inhibition can be reversed by adding excess of substrate which will successfully dislodge the inhibitor molecules from enzymes.

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$
$$E + In \leftrightarrow E\text{-}In \leftrightarrow \text{no product}$$

2. Non- competitive inhibition.

A non- competitive inhibitior has a structure different than the substrate. It is not compete for the active site of enzyme, but affect the enzyme substrate complex. They distorts the shape of the enzyme, which alters the shape of active site. This type of inhibition cannot be released by increasing substrate concentration.

$$E + S \leftrightarrow ES + In \leftrightarrow ESIn \leftrightarrow \text{no product}$$

Few terms

1. **Zymogens-** some enzyme are produced by the living cell in an inactive form called zymogens or proenzymes. They are activated and converted into enzyme form. The activation is performed by some specific ions and other enzymes which are of proteolytic nature. E.g. pepsinogen into pepsin.

2. **Isoenzymes-** some enzymes having similar catalytic function but obtained from different sources exhibit different physical and chemical characteristics they are called isoenzymes or isozymes.

3. **Turn over number-** turn over number of an enzyme is the number of substrate molecules transferred per minute by single enzyme molecule when the enzyme concentration is the rate limiting factor. The carbonic anhydrase has the highest turn over number i.e. 36,000,000.

4. **Active site-** the substrate binding place at which catalysis occurs is known as the active site. This site is a cleft or big pocket shaped structure surrounded by amino acids.

5. **Substrate-** to catalyse a reaction an enzyme will bind to one or more reactant molecule known as substrate. In some reaction a single substrate is broken down into multiple products.

Vitamins and related compounds

Vitamins are organic molecules that are required in the diet for normal health and growth of an organism. This need results from the inability of cells to produce these compounds.

The name 'vitamin' was originally given to these accessory food factors because known to be vital for life and were all believed to be amine. When it became clear that some of them were not amines and did not even contain nitrogen, Drummond suggested the modification that led to the term vitamin.

Their minute quantity indicates a catalytic role in the cell.

The distinguishing feature of the vitamins is that they generally cannot be synthesized by mammalian cells and, therefore, must be supplied in the diet. The vitamins are of two distinct types (Table-1):

Table-1: Types of Vitamins

Water Soluble Vitamins	Fat Soluble Vitamins
• Thiamin (B_1) • Riboflavin (B_2) • Niacin (B_3) • Pantothenic Acid (B_5) • Pyridoxal, Pyridoxamine, Pyridoxine (B_6) • Biotin • Cobalamin (B_{12}) • Folic Acid • Ascorbic Acid	• Vitamin A • Vitamin D • Vitamin E • Vitamin K

WATER-SOLUBLE VITAMINS

Water-soluble vitamins consist of the **B vitamins** and **vitamin C**. With exception of vitamin B_6 and B_{12}, they are readily excreted in urine without appreciable storage, so frequent consumption becomes necessary. They are generally nontoxic when present in excess of needs, although symptoms may be reported in people taking mega doses of niacin, vitamin C, or pyridoxine (vitamin B_6). All the B vitamins function as coenzymes or cofactors, assisting in the activity of important enzymes and allowing energy-producing reactions to proceed normally. As a result, any lack of water-soluble vitamins mostly affects growing or rapidly metabolizing tissues such as skin, blood, the digestive tract, and the nervous system. Water-soluble vitamins are easily lost with overcooking.

A summary of water soluble vitamins is given in the table-2.

Table 2: List of Water Soluble Vitamins.

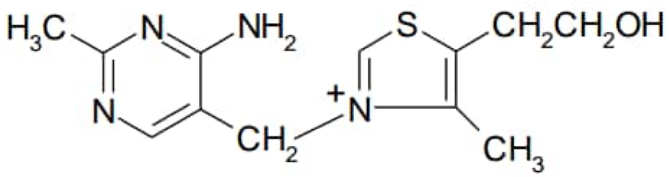
Vitamin	Deficiency	Recommended daily intake	Source
Thiamine (Vitamin B_1)	Beri Beri: anorexia, weight loss, weakness, peripheral neuropathy. Wernicke-Korsakoff syndrome: staggered gait, cross eyes, dementia, disorientation, memory loss.	Infants: 0.2 – 0.3 mg, Children: 0.5 – 0.6 mg, Adolescents: 0.9 – 1.2 mg, Men: 1.2 mg, Women: 1.1 mg, Pregnant/Lactating Women: 1.4 mg	Pork/pork products, beef, liver, yeast/baked products, enriched and whole grain cereals, nuts, and seeds

Riboflavin (Vitamin B_2)	Ariboflavinosis: inflammation of tongue (glossitis), cracks at corners of mouth (cheilosis), dermatitis, growth retardation, conjunctivitis, nerve damage	Infants: 0.3 – 0.4 mg, Children: 0.5 – 0.6 mg, Adolescents: 0.9 – 1.3 mg, Men: 1.3 mg, Women: 1.1 mg, Pregnant Women: 1.4 mg, Lactating Women: 1.6 mg	Milk, eggs, mushrooms, whole grains, enriched grains, green leafy vegetables, yeast, liver, and oily fish
Niacin (Vitamin B_3)	Pellagra: diarrhea, dematitis, dementia, and death	Infants: 2 – 4 mg, Children: 6 – 8 mg, Adolescents: 12 – 16 mg, Men: 16 mg, Women: 14 mg, Pregnant Women: 18 mg, Lactating Women: 17 mg	Meat, poultry, fish, yeast, enriched and whole grain breads and cereals, peanuts, mushrooms, milk, and eggs
Pantothenic acid (Vitamin B_5)	Rare	Infants: 1.7 – 1.8 mg, Children: 2 – 3 mg, Adolescents: 4 – 5 mg, Men & Women: 5 mg, Pregnant Women: 6 mg, Lactating Women: 7 mg	Widely distributed in foods
Biotin (Vitamin B_8)	Dermatitis, convulsions, hair loss (alopecia), neurological disorders, impaired growth	Infants: 5 – 6 µg, Children: 8 – 12 µg, Adolescents: 20 – 25 µg, Men & Women: 30 µg, Pregnant Women: 30 µg, Lactating Women: 35 µg	Whole grains, eggs, nuts and seeds, widely distributed in small amounts
Vitamin B_6	Dermatitis, anemia, convulsion, depression, confusion, decline in immune function	Infants: 0.1 – 0.3 mg, Children: 0.5 – 0.6 mg, Adolescents: 1.0 -1.3 mg, Men & Women: 1.3 mg, Pregnant Women: 1.9 mg,	Meat, fish, poultry, spinach, potatoes, bananas, avocados, sunflower seeds

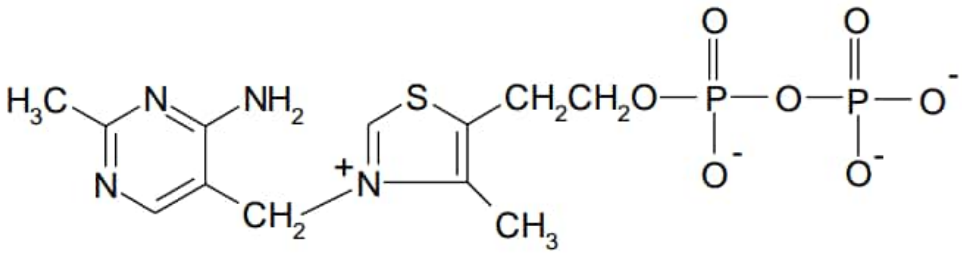
		Lactating Women: 1.2 mg	
Folate (Vitamin B_{12})	Megaoblastic (macrocytic) anemia, abdominal pain, diarrhea, birth defects	Infants: 65 – 80 µg, Children: 150 – 200µg, Adolescents: 300 – 400 µg, Men & Women: 400 µg, Pregnant Women: 600 µg, Lactating Women: 500 µg	Ready-to-eat breakfast cereals, enriched grain products, green vegetables, liver, legumes, oranges.

Thiamin

Thiamin is also known as vitamin B_1. Thiamin is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge. Thiamin is rapidly converted to its active form, thiamin pyrophosphate (TPP), in the brain and liver by specific enzymes, thiamin diphosphotransferase.



Thiamin



Thiamin pyrophosphate (TPP)

TPP is necessary as a cofactor for the pyruvate and α-ketoglutarate dehydrogenase catalyzed reactions as well as the transketolase catalyzed reactions of the pentose phosphate pathway. A deficiency in thiamin intake leads to a severely reduced capacity of cells to generate energy as a result of its role in these reactions.

The dietary requirement for thiamin is proportional to the caloric intake of the diet and ranges from 1.0 - 1.5 mg/day for normal adults. If the carbohydrate content of the diet is excessive then an in thiamin intake will be required.

Clinical Significances of Thiamin Deficiency

The earliest symptoms of thiamin deficiency include constipation, appetite suppression, nausea as well as mental depression, peripheral neuropathy and fatigue. Chronic thiamin deficiency leads to more severe neurological symptoms including ataxia, mental confusion and loss of eye coordination. Other clinical symptoms of prolonged thiamin deficiency are related to cardiovascular and musculature defects.

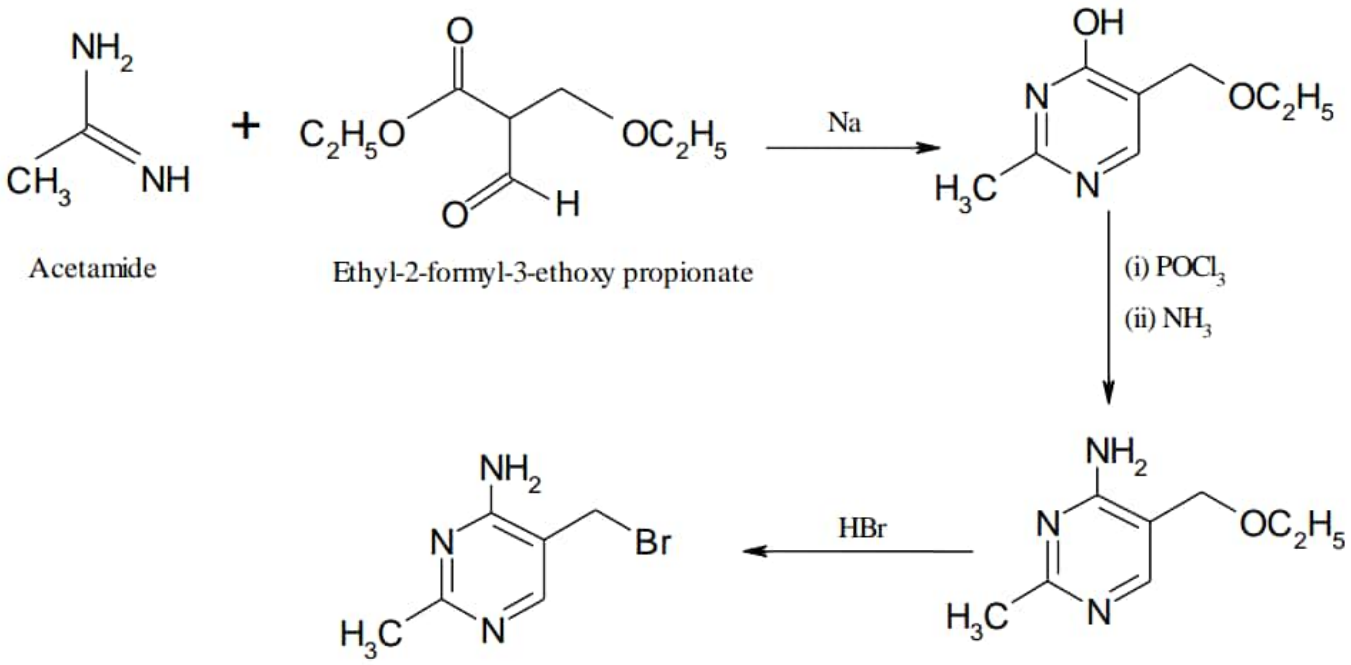
The severe thiamin deficiency disease known as **Beriberi** is the result of a diet that is carbohydrate rich and thiamin deficient. An additional thiamin deficiency related disease is

known as Wernicke-Korsakoff syndrome. This disease is most commonly found in chronic alcoholics due to their poor dietetic lifestyles.

Synthesis of Thiamin

The synthesis of thiamin is achieved in three steps.

(i) Synthesis of pyrimidine moiety (1) of thiamin

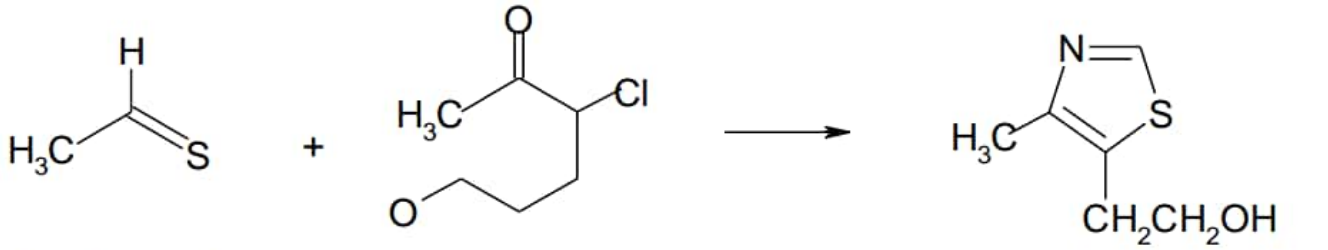


Acetamide

Ethyl-2-formyl-3-ethoxy propionate

4-Amino-5-bromomethyl-2-methylpyridine
(1)

(i) Synthesis of thiazole moiety (2) of thiamin

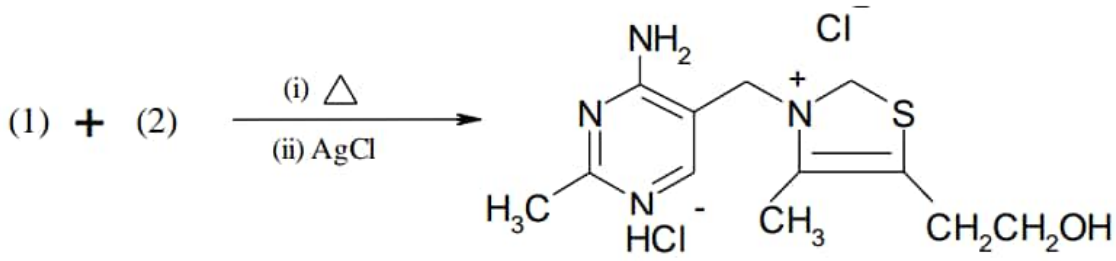


Thioformamide

3-chloro-1-hydroxy-4-pentanone

5-(ß-hydroxyethyl)-4-methylthiazole
(2)

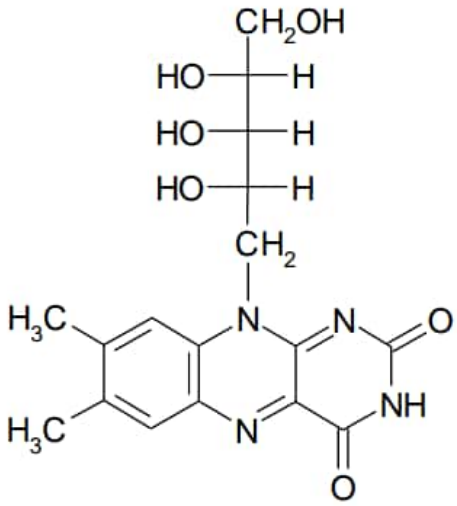
(ii) Condensation of (1) and (2) to form thiamin



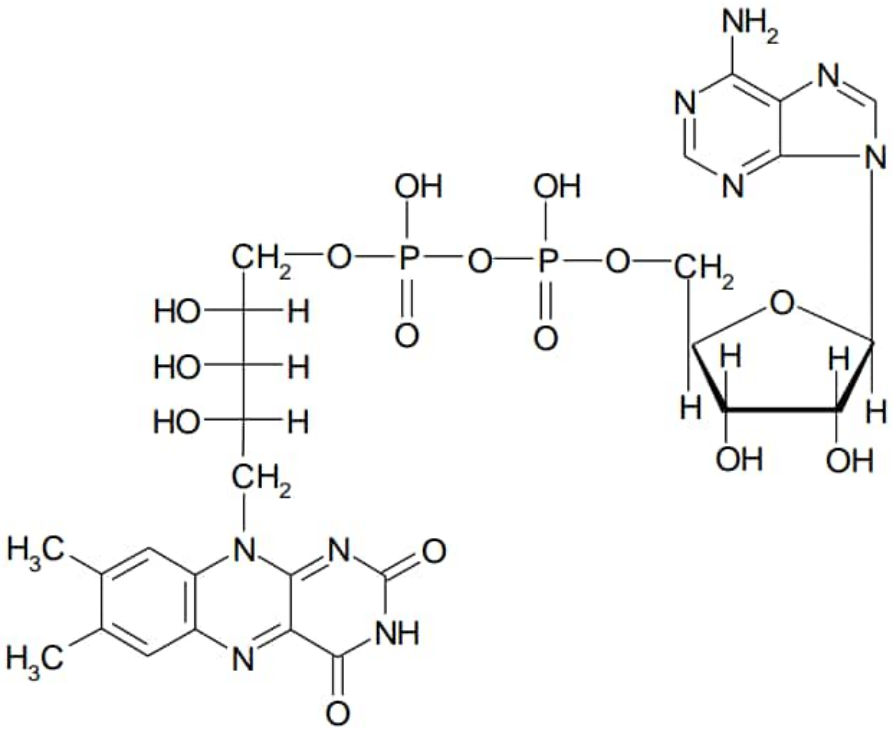
Thiamine Hydrochloride

Riboflavin

Riboflavin is also known as vitamin B_2. Riboflavin is the precursor for the coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The enzymes that require FMN or FAD as cofactors are termed flavoproteins. Several flavoproteins also contain metal ions and are termed metalloflavoproteins. Both classes of enzymes are involved in a wide range of redox reactions, e.g. succinate dehydrogenase and xanthine oxidase. During the course of the enzymatic reactions involving the flavoproteins the reduced forms of FMN and FAD are formed, $FMNH_2$ and $FADH_2$, respectively.



Riboflavin



Flavin adenine dinucleotide (FAD)

The normal daily requirement for riboflavin is 1.2 - 1.7 mg/day for normal adults.

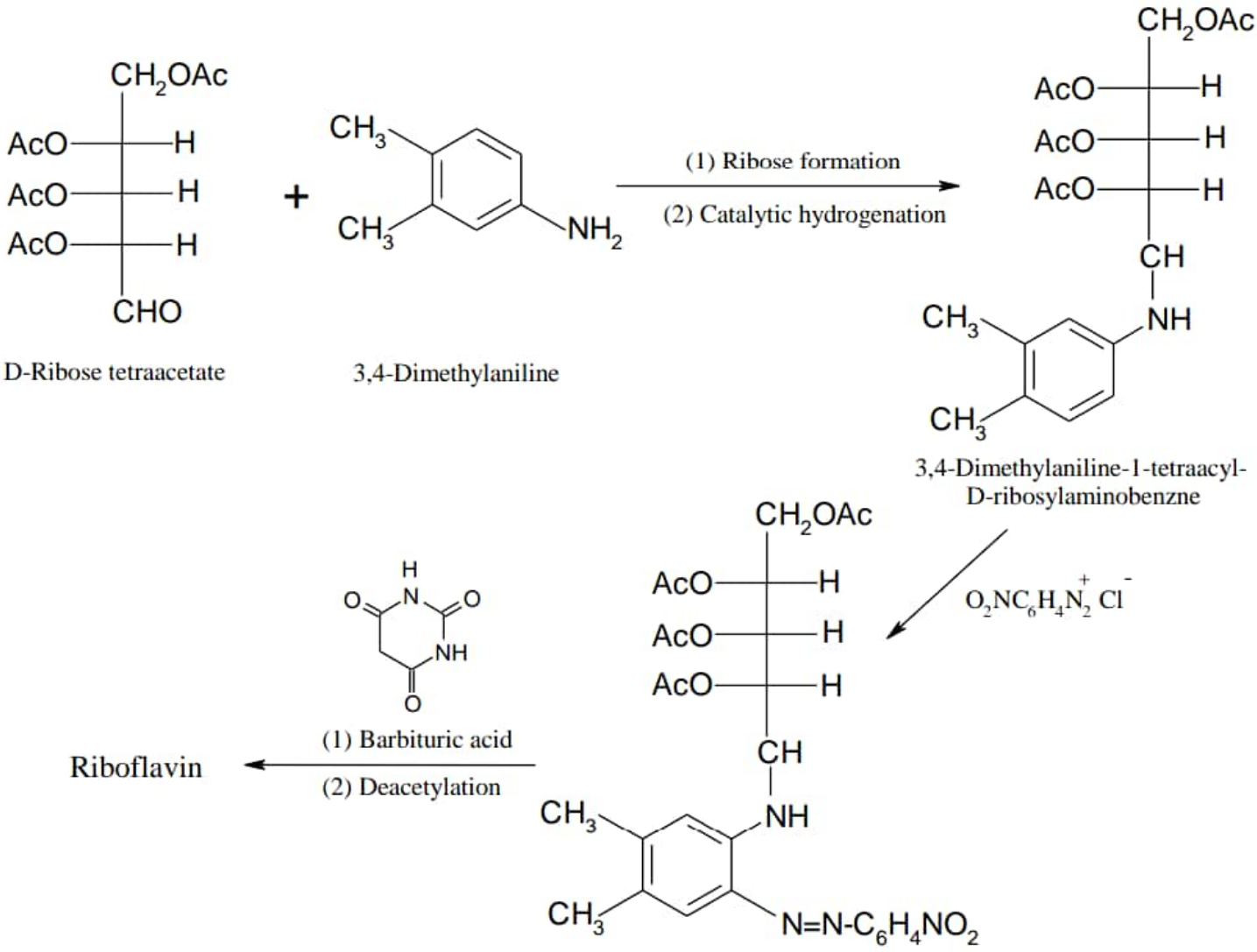
Clinical Significances of Flavin Deficiency

Riboflavin deficiencies are rare in the United States due to the presence of adequate amounts of the vitamin in eggs, milk, meat and cereals. Riboflavin deficiency is often seen in chronic alcoholics due to their poor dietetic habits.

Symptoms associated with riboflavin deficiency include, glossitis, seborrhea, angular stomatitis, cheilosis and photophobia. Riboflavin decomposes when exposed to visible light. This characteristic can lead to riboflavin deficiencies in newborns treated for hyperbilirubinemia by phototherapy.

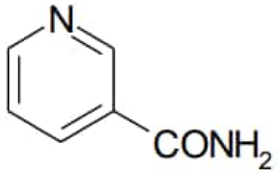
Synthesis of Riboflavin

Riboflavin can be synthesized by the following scheme.

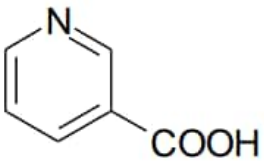


Niacin

Niacin (nicotinic acid and nicotinamide) is also known as vitamin B_3. Both nicotinic acid and nicotinamide can serve as the dietary source of vitamin B_3. Niacin is required for the synthesis of the active forms of vitamin B_3, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). Both NAD^+ and $NADP^+$ function as cofactors for numerous dehydrogenase, e.g., lactate and malate dehydrogenases.

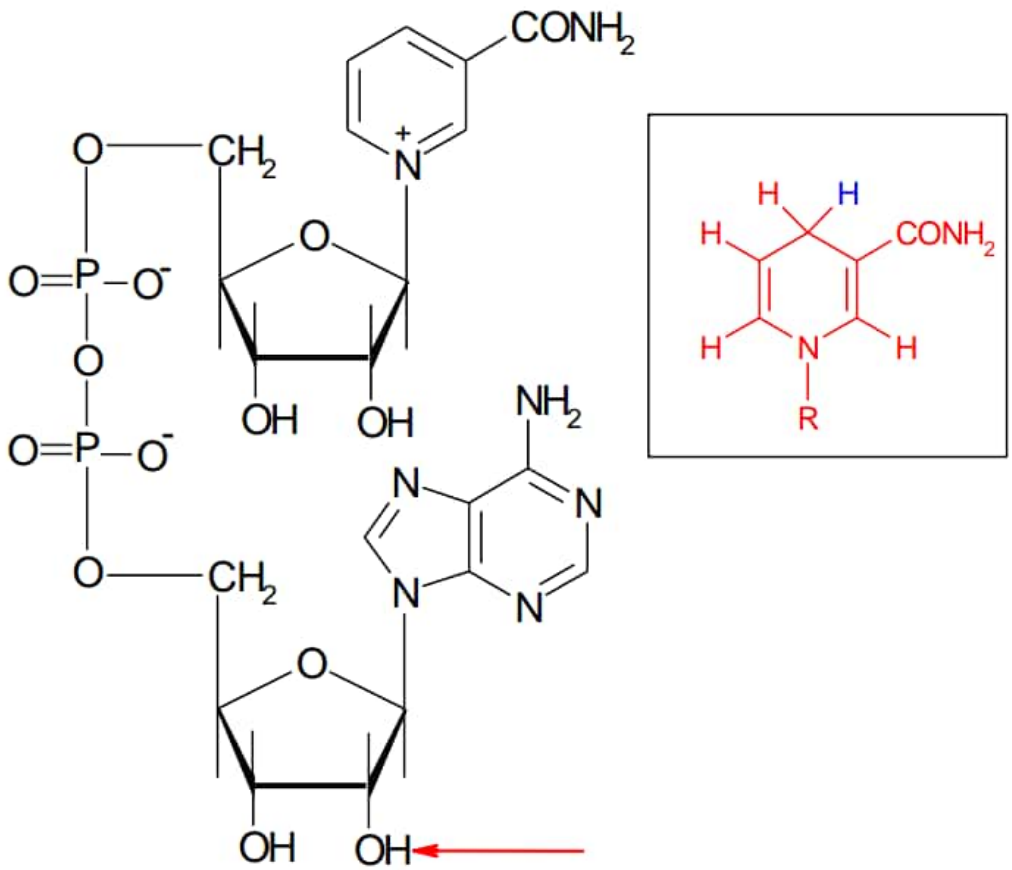


Nicotinamide



Nicotinic Acid

Niacin is not a true vitamin in the strictest definition since it can be derived from the amino acid tryptophan. However, the ability to utilize tryptophan for niacin synthesis is inefficient (60 mg of tryptophan are required to synthesize 1 mg of niacin). Also, synthesis of niacin from tryptophan requires vitamins B_1, B_2 and B_6 which would be limiting in them on a marginal diet.



Structure of NAD^+

NADH is shown in the box insert.

The -OH phosphorylated in $NADP^+$ is indicated by the red arrow.

The recommended daily requirement for niacin is 13 - 19 niacin equivalents (NE) per day for a normal adult. One NE is equivalent to 1 mg of free niacin).

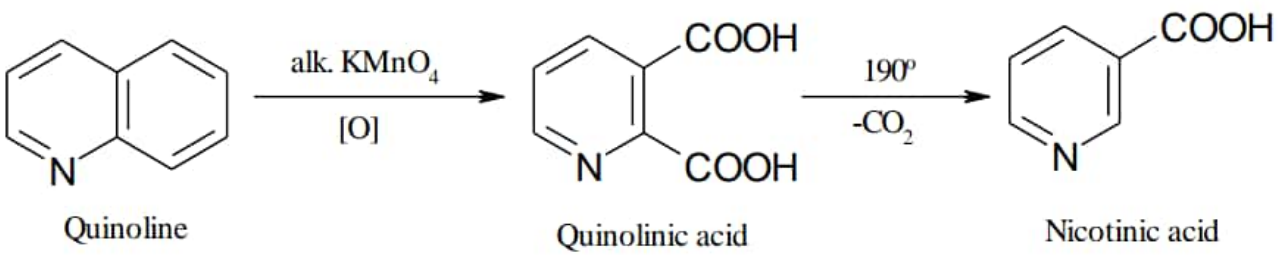
Clinical Significances of Niacin and Nicotinic Acid

A diet deficient in niacin (as well as tryptophan) leads to glossitis of the tongue, dermatitis, weight loss, diarrhea, depression and dementia. The severe symptoms, depression, dermatitis and diarrhea, are associated with the condition known as **pellagra**. Several physiological conditions (e.g. Hartnup disease and malignant carcinoid syndrome) as well as certain drug therapies (e.g. isoniazid) can lead to niacin deficiency. In Hartnup disease tryptophan absorption is impaired and in malignant carcinoid syndrome tryptophan metabolism is altered resulting in excess serotonin synthesis. Isoniazid (the hydrazide derivative of isonicotinic acid) is the primary drug for chemotherapy of tuberculosis.

Nicotinic acid (but not nicotinamide) when administered in pharmacological doses of 2 - 4 g/day lowers plasma cholesterol levels and has been shown to be a useful therapeutic for hypercholesterolemia. The major action of nicotinic acid in this capacity is a reduction in fatty acid mobilization from adipose tissue. Although nicotinic acid therapy lowers blood cholesterol it also causes a depletion of glycogen stores and fat reserves in skeletal and cardiac muscle. Additionally, there is an elevation in blood glucose and uric acid production. For these reasons nicotinic acid therapy is not recommended for diabetics or persons who suffer from gout.

Synthesis of Nicotinic acid

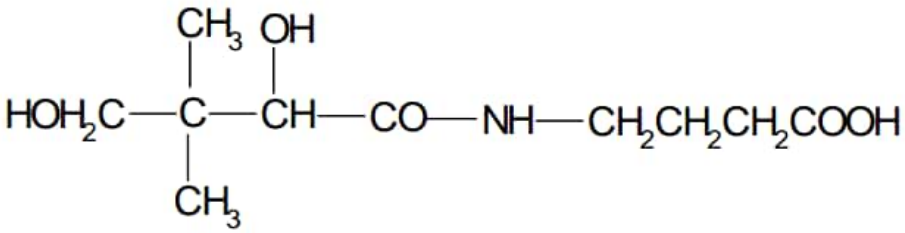
Nicotinic acid is synthesized as follows-



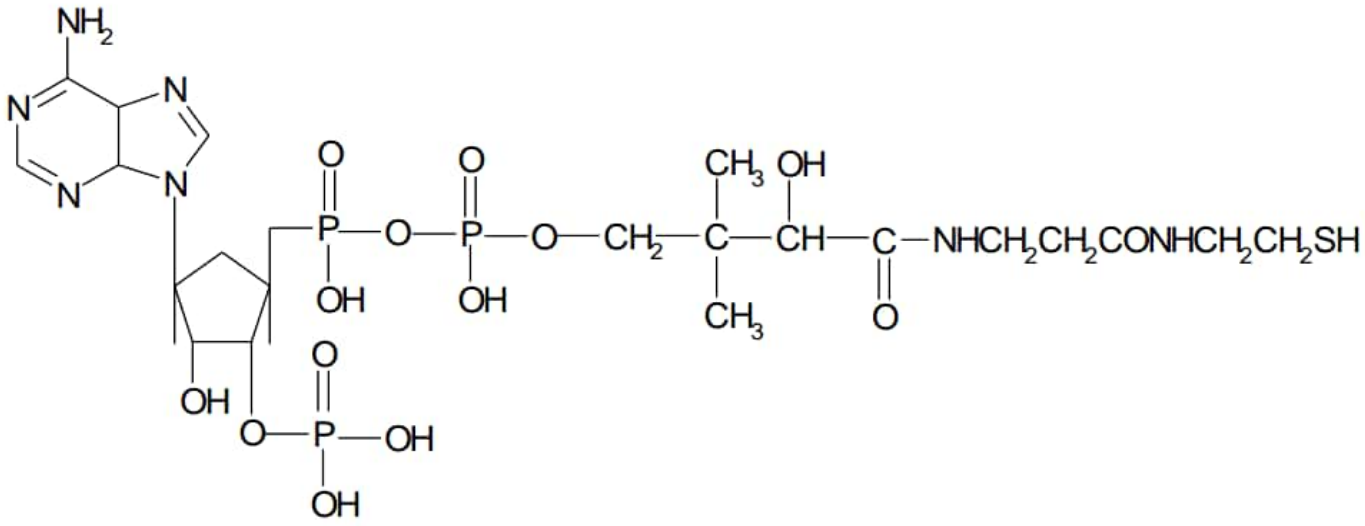
Quinoline | Quinolinic acid | Nicotinic acid

Pantothenic Acid

Pantothenic acid is also known as vitamin B_5. Pantothenic acid is formed from β-alanine and pantoic acid. Pantothenate is required for synthesis of coenzyme A, CoA and is a component of the acyl carrier protein (ACP) domain of fatty acid synthase. Pantothenate is, therefore, required for the metabolism of carbohydrate via the TCA cycle and all fats and proteins. At least 70 enzymes have been identified as requiring CoA or ACP derivatives for their function.



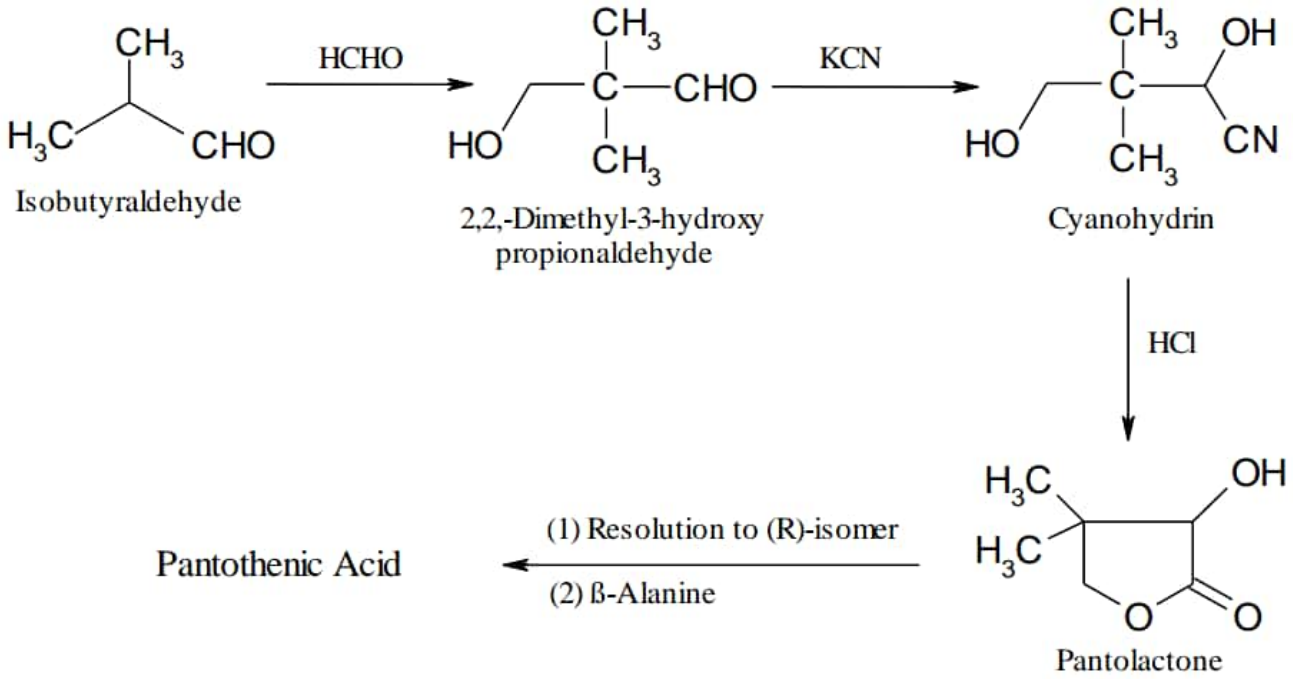
Pantothenic Acid



Coenzyme A

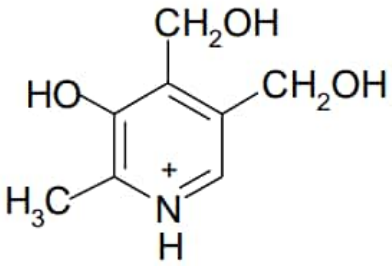
Deficiency of pantothenic acid is extremely rare due to its widespread distribution in whole grain cereals, legumes and meat. Symptoms of pantothenate deficiency are difficult to assess since they are subtle and resemble those of other B vitamin deficiencies.

Synthesis of Pantothenic acid

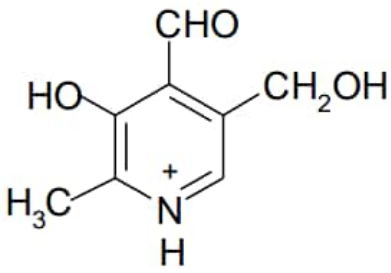


Vitamin B_6

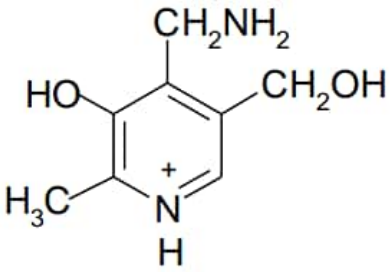
Pyridoxal, pyridoxamine and pyridoxine are collectively known as vitamin B_6. All three compounds are efficiently converted to the biologically active form of vitamin B_6, pyridoxal phosphate. This conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase.



Pyridoxine

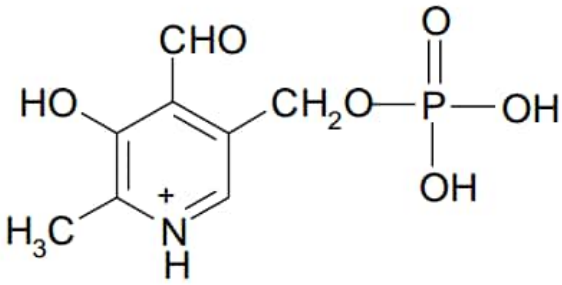


Pyridoxal



Pyridoxamine

Pyridoxal phosphate functions as a cofactor in enzymes involved in transamination reactions required for the synthesis and catabolism of the amino acids as well as in glycogenolysis as a cofactor for glycogen phosphorylase.

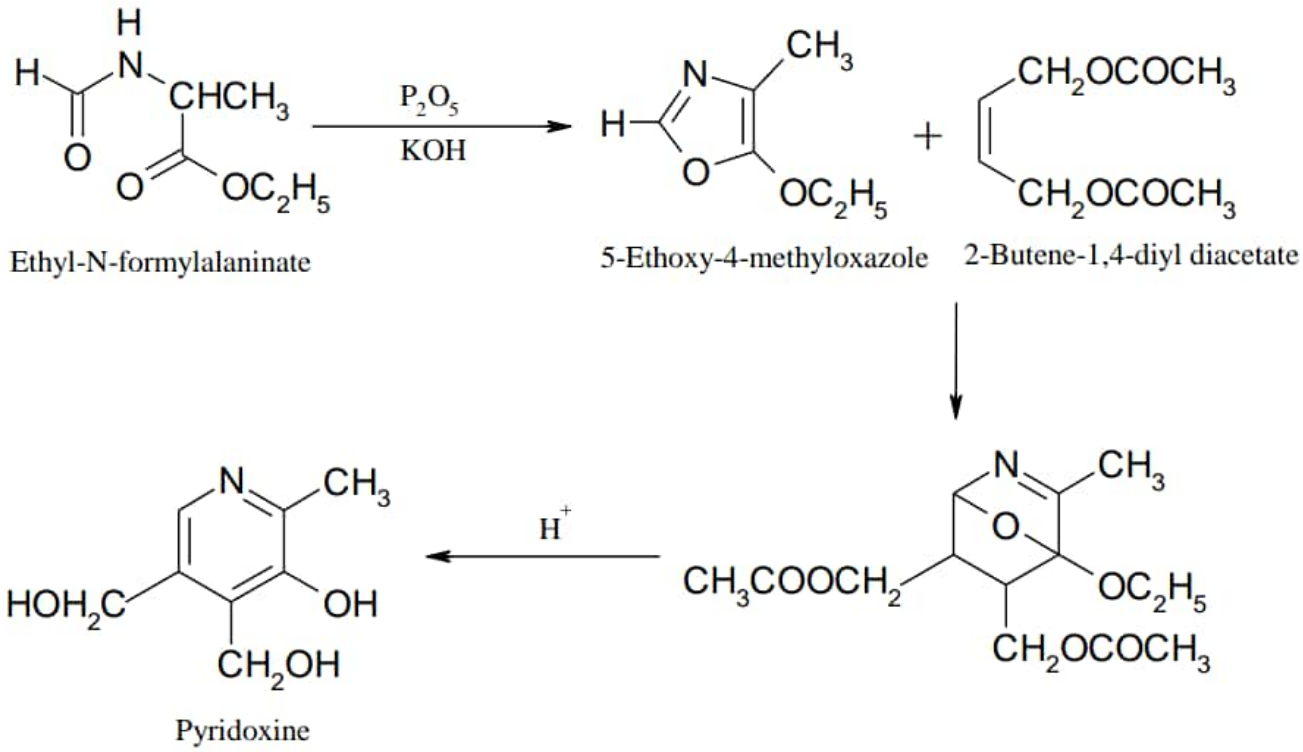


Pyridoxal phosphate

The requirement for vitamin B_6 in the diet is proportional to the level of protein consumption ranging from 1.4 - 2.0 mg/day for a normal adult. During pregnancy and lactation the requirement for vitamin B_6 increases approximately 0.6 mg/day.

Deficiencies of vitamin B_6 are rare and usually are related to an overall deficiency of all the B-complex vitamins. Isoniazid (see niacin deficiencies above) and penicillamine (used to treat rheumatoid arthritis and cystinurias) are two drugs that complex with pyridoxal and pyridoxal phosphate resulting in a deficiency in this vitamin.

Synthesis of Pyridoxine



Ethyl-N-formylalaninate

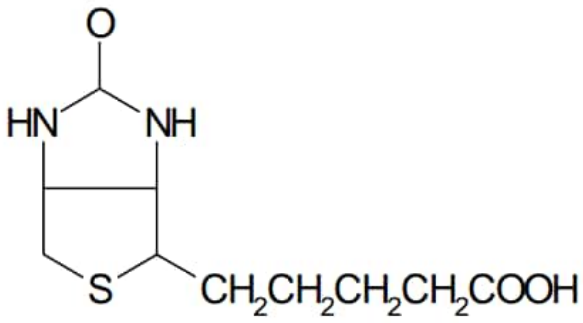
5-Ethoxy-4-methyloxazole

2-Butene-1,4-diyl diacetate

Pyridoxine

Biotin

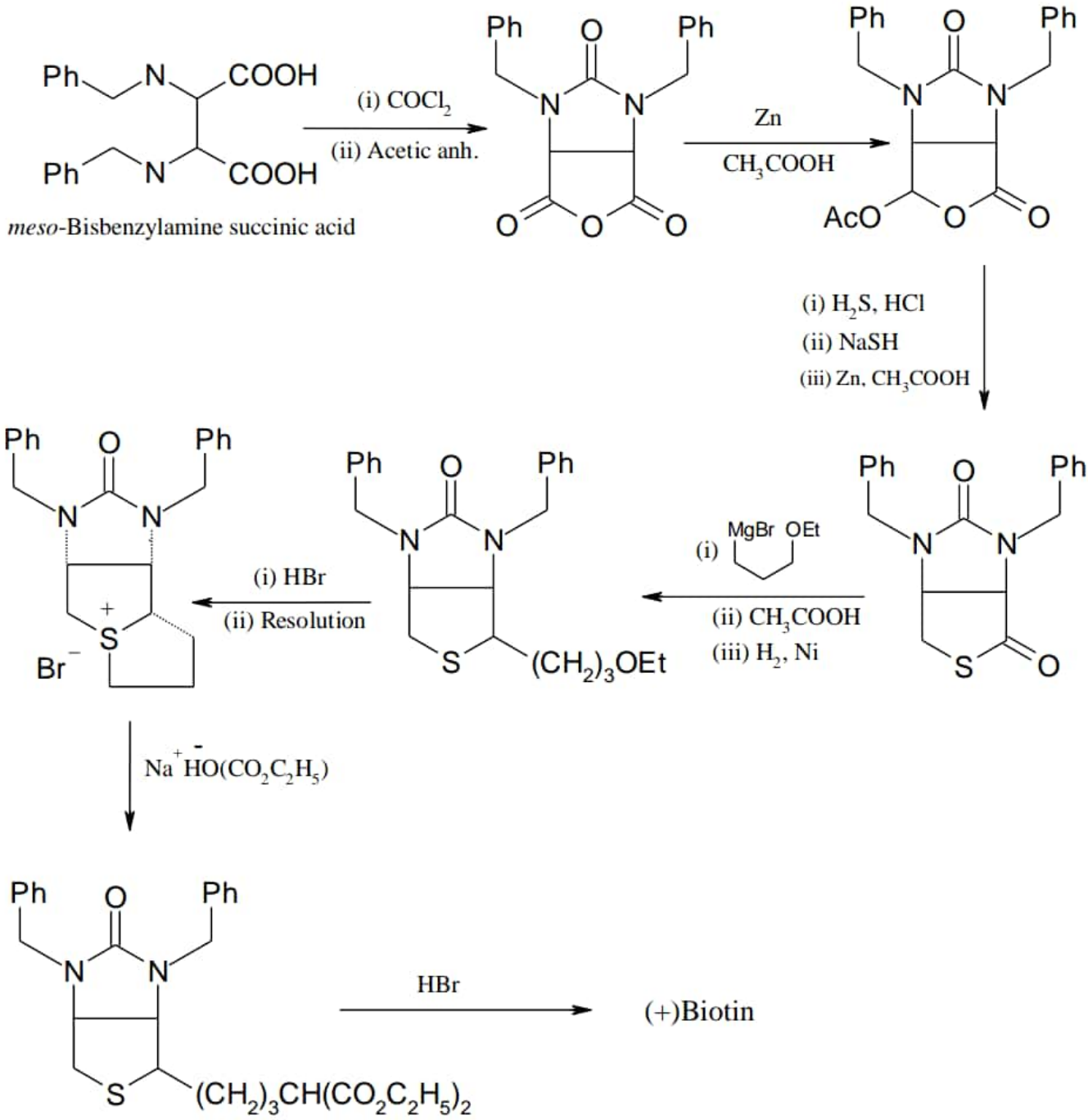
Biotin is the cofactor required for enzymes that are involved in carboxylation reactions, e.g. acetyl-CoA carboxylase and pyruvate carboxylase. Biotin is found in numerous foods and also is synthesized by intestinal bacteria and as such deficiencies of the vitamin are rare. Deficiencies are generally seen only after long antibiotic therapies, which deplete the intestinal fauna or following excessive consumption of raw eggs. The latter is due to the affinity of the egg white protein, avidin, for biotin preventing intestinal absorption of the biotin.



Biotin

Synthesis of Biotin

Biotin is synthesized by the following steps-



meso-Bisbenzylamine succinic acid

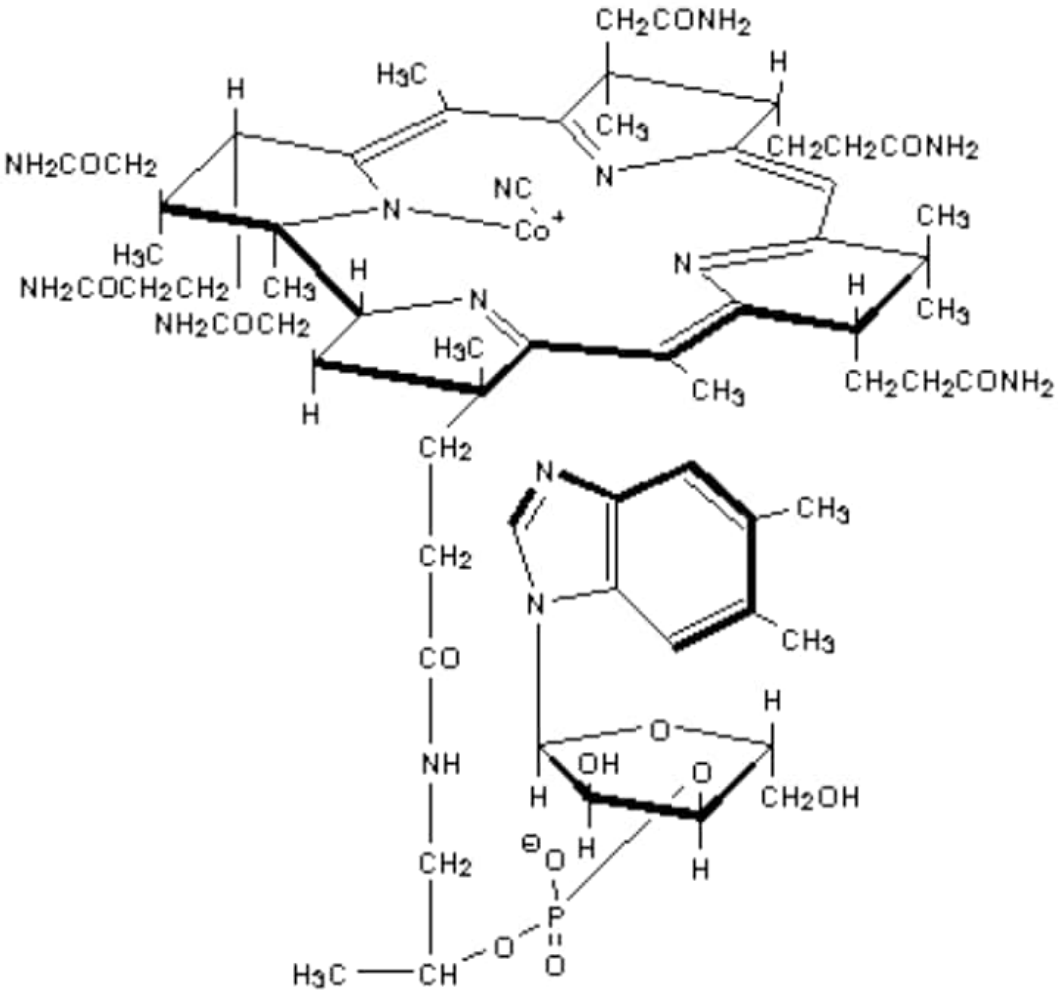
Cobalamin

Cobalamin is more commonly known as vitamin B_{12}. Vitamin B_{12} is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center. Vitamin B_{12} is synthesized exclusively by microorganisms and is found in the liver of animals bound to protein as methycobalamin or 5'-deoxyadenosylcobalamin. The vitamin must be hydrolyzed from protein in

order to be active. Hydrolysis occurs in the stomach by gastric acids or the intestines by trypsin digestion following consumption of animal meat. The vitamin is then bound by intrinsic factor, a protein secreted by parietal cells of the stomach, and carried to the ileum where it is absorbed. Following absorption the vitamin is transported to the liver in the blood bound to transcobalamin II.

There are only two clinically significant reactions in the body that require vitamin B_{12} as a cofactor. During the catabolism of fatty acids with an odd number of carbon atoms and the amino acids valine, isoleucine and threonine the resultant propionyl-CoA is converted to succinyl-CoA for oxidation in the TCA cycle. One of the enzymes in this pathway, methylmalonyl-CoA mutase, requires vitamin B_{12} as a cofactor in the conversion of methylmalonyl-CoA to succinyl-CoA. The 5'-deoxyadenosine derivative of cobalamin is required for this reaction.

The second reaction requiring vitamin B_{12} catalyzes the conversion of homocysteine to methionine and is catalyzed by methionine synthase. This reaction results in the transfer of the methyl group from N^5-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate (THF) and methylcobalamin during the process of the conversion.



Cyanocobalamin

Clinical Significances of B_{12} Deficiency

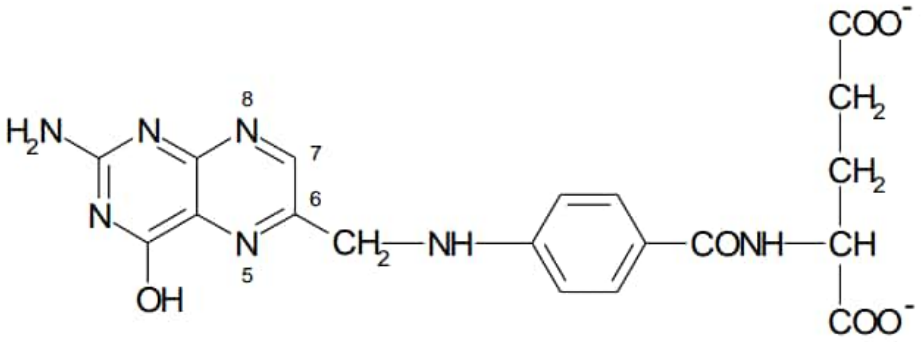
The liver can store up to six years worth of vitamin B_{12}, hence deficiencies in this vitamin are rare. Pernicious anemia is a megaloblastic anemia resulting from vitamin B_{12} deficiency that develops as a result a lack of intrinsic factor in the stomach leading to malabsorption of the vitamin. The anemia results from impaired DNA synthesis due to a block in purine and thymidine biosynthesis. The block in nucleotide biosynthesis is a consequence of the effect of vitamin B_{12} on folate metabolism. When vitamin B_{12} is deficient essentially all of the folate becomes trapped as the N^5-methylTHF derivative as a result of the loss of functional methionine synthase. This trapping prevents the synthesis of other THF derivatives required for the purine and thymidine nucleotide biosynthesis pathways.

Neurological complications also are associated with vitamin B_{12} deficiency and result from a progressive demyelination of nerve cells. The demyelination is thought to result from the increase in methylmalonyl-CoA that results from vitamin B_{12} deficiency.

Methylmalonyl-CoA is a competitive inhibitor of malonyl-CoA in fatty acid biosynthesis as well as being able to substitute for malonyl-CoA in any fatty acid biosynthesis that may occur. Since the myelin sheath is in continual flux the methylmalonyl-CoA-induced inhibition of fatty acid synthesis results in the eventual destruction of the sheath. The incorporation methylmalonyl-CoA into fatty acid biosynthesis results in branched-chain fatty acids being produced that may severely alter the architecture of the normal membrane structure of nerve cells.

Folic Acid

Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA) that forms pteroic acid. Folic acid itself is then generated through the conjugation of glutamic acid residues to pteroic acid. Folic acid is obtained primarily from yeasts and leafy vegetables as well as animal liver. Animal cannot synthesize PABA nor attach glutamate residues to pteroic acid, thus, requiring folate intake in the diet.

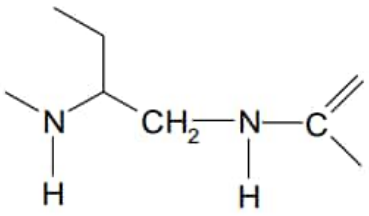


Folic Acid

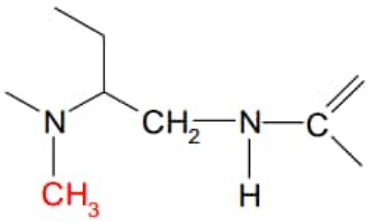
positions 7 & 8 carry hydrogens in dihydrofolate (DHF)

positions 5-8 carry hydrogens in tetrahydrofolate (THF)

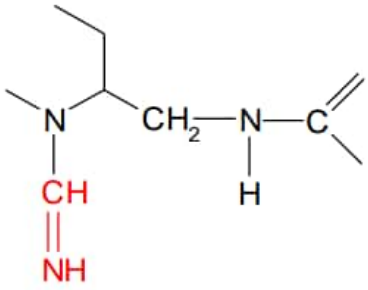
When stored in the liver or ingested folic acid exists in a polyglutamate form. Intestinal mucosal cells remove some of the glutamate residues through the action of the lysosomal enzyme, conjugase. The removal of glutamate residues makes folate less negatively charged (from the polyglutamic acids) and therefore more capable of passing through the basal lamenal membrane of the epithelial cells of the intestine and into the bloodstream. Folic acid is reduced within cells (principally the liver where it is stored) to tetrahydrofolate (THF also H_4folate) through the action of dihydrofolate reductase (DHFR), an NADPH-requiring enzyme.



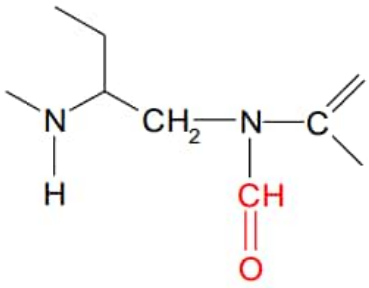
Tetrahydrofolate
(H_4 folate)



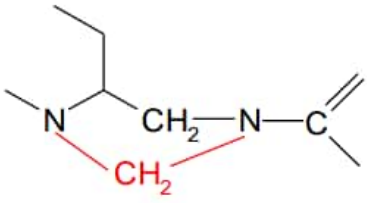
N^5-Methyl H_4 folate



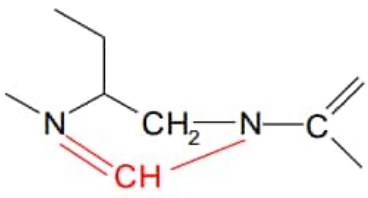
N^5-Formimino H_4 folate



N^{10}-Formyl H_4 folate



N^5,N^{10}-Methylene H_4 folate



N^5,N^{10}-Methenyl H_4 folate

The function of THF derivatives is to carry and transfer various forms of one-carbon units during biosynthetic reactions. The one carbon units are methyl, methylene, methenyl, formyl or formimino groups.

Active center of tetrahydrofolate (THF). Note that the N^5 position is the site of attachment of methyl groups, the N^{10} the site for attachment of formyl and formimino groups and that both N^5 and N^{10} bridge the methylene and methenyl groups. These one-carbon transfer reactions are required in the biosynthesis of serine, methionine, glycine, choline and the purine nucleotides and dTMP.

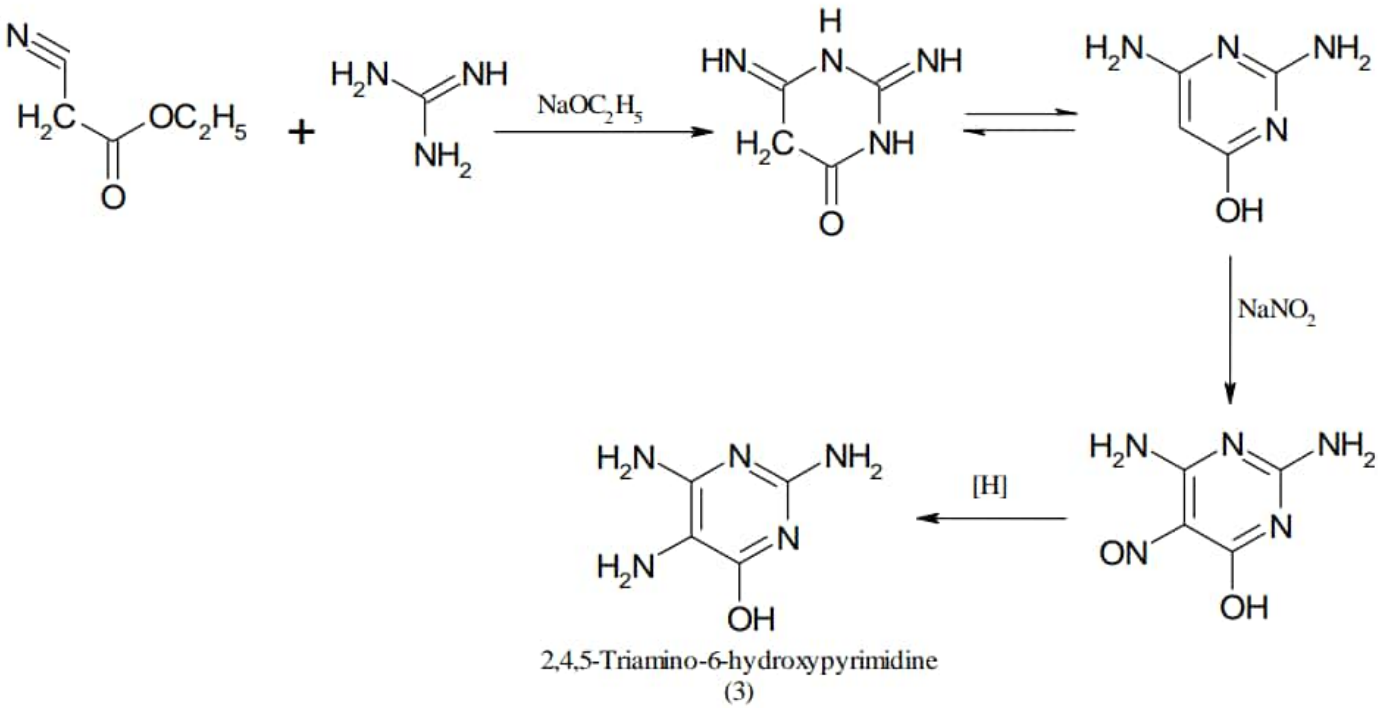
The ability to acquire choline and amino acids from the diet and to salvage the purine nucleotides makes the role of N^5,N^{10}-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin. The role of vitamin B_{12} and N^5-methyl-THF in the conversion of homocysteine to methionine also can have a significant impact on the ability of cells to regenerate needed THF.

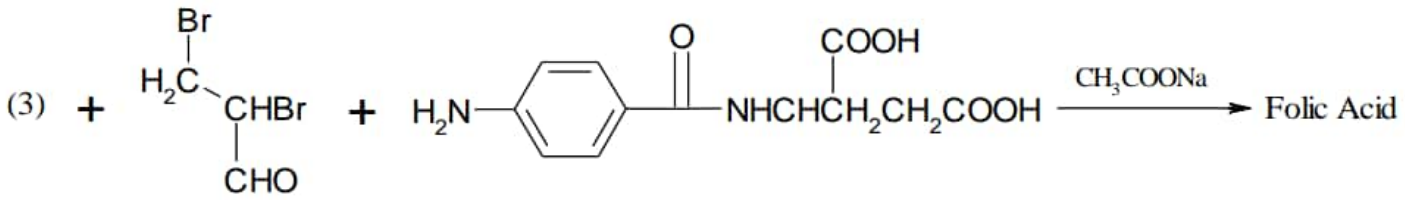
Clinical Significance of Folate Deficiency

Folate deficiency results in complications nearly identical to those described for vitamin B_{12} deficiency. The most pronounced effect of folate deficiency on cellular processes is upon DNA synthesis. This is due to impairment in dTMP synthesis which leads to cell cycle arrest in S-phase of rapidly proliferating cells, in particular hematopoietic cells. The result is megaloblastic anemia as for vitamin B_{12} deficiency. The inability to synthesize DNA during erythrocyte maturation leads to abnormally large erythrocytes termed macrocytic anemia.

Folate deficiencies are rare due to the adequate presence of folate in food. Poor dietary habits as those of chronic alcoholics can lead to folate deficiency. The predominant causes of folate deficiency in non-alcoholics are impaired absorption or metabolism or an increased demand for the vitamin. The predominant condition requiring an increase in the daily intake of folate is pregnancy. This is due to an increased number of rapidly proliferating cells present in the blood. The need for folate will nearly double by the third trimester of pregnancy. Certain drugs such as anticonvulsants and oral contraceptives can impair the absorption of folate. Anticonvulsants also increase the rate of folate metabolism.

Synthesis of Folic Acid



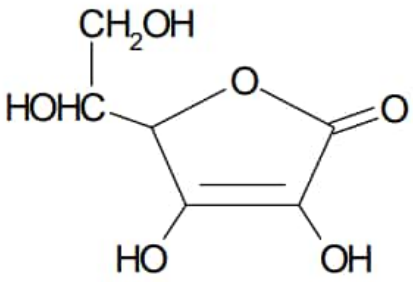


Ascorbic Acid

Ascorbic acid is more commonly known as vitamin C. Ascorbic acid is derived from glucose via the uronic acid pathway. The enzyme L-gulonolactone oxidase responsible for the conversion of gulonolactone to ascorbic acid is absent in primates making ascorbic acid required in the diet.

The active form of vitamin C is ascorbate acid itself. The main function of ascorbate is as a reducing agent in a number of different reactions.

Vitamin C has the potential to reduce cytochromes a and c of the respiratory chain as well as molecular oxygen. The most important reaction requiring ascorbate as a cofactor is the hydroxylation of proline residues in collagen. Vitamin C is, therefore, required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissue is the first event in wound tissue remodeling. Vitamin C also is necessary for bone remodeling due to the presence of collagen in the organic matrix of bones.



Ascorbic Acid

Several other metabolic reactions require vitamin C as a cofactor. These include the catabolism of tyrosine and the synthesis of epinephrine from tyrosine and the synthesis of the bile acids. It is also believed that vitamin C is involved in the process of steroidogenesis since the adrenal cortex

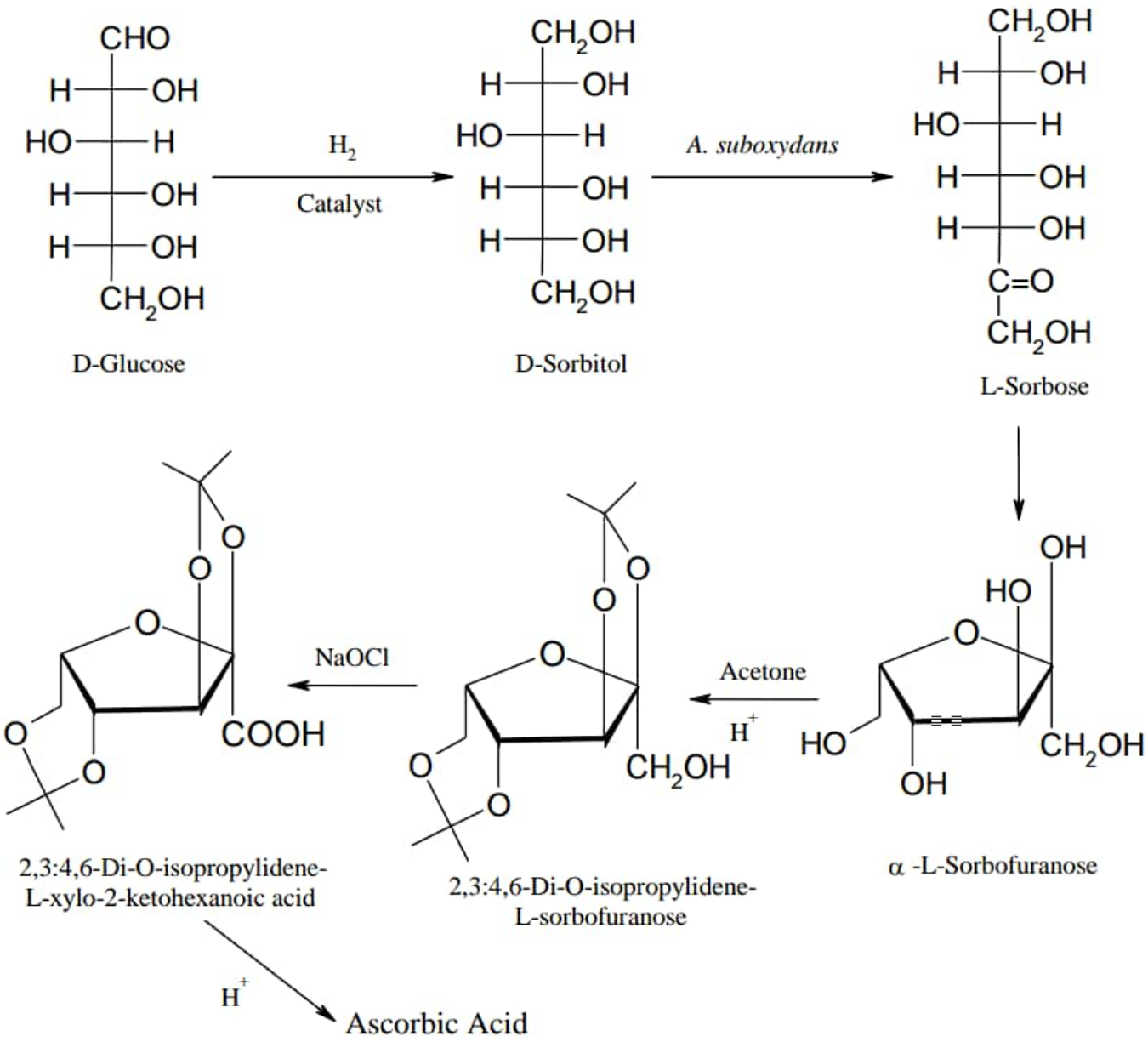
contains high levels of vitamin C which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland.

Deficiency in vitamin C leads to the disease **scurvy** due to the role of the vitamin in the post-translational modification of collagens. Scurvy is characterized by easily bruised skin, muscle fatigue, soft swollen gums, decreased wound healing and hemorrhaging, osteoporosis, and anemia.

Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement. The primary physiological state leading to an increased requirement for vitamin C is severe stress (or trauma). This is due to a rapid depletion in the adrenal stores of the vitamin. The reason for the decrease in adrenal vitamin C levels is unclear but may be due either to redistribution of the vitamin to areas that need it or an overall increased utilization.

Synthesis of Ascorbic Acid

Ascorbic acid is synthesized according to the following scheme-



FAT-SOLUBLE VITAMINS

Vitamins A, D, E, and K are soluble in fats, therefore, they are called ***fat-soluble*** vitamins. They are absorbed from the small intestines, along with dietary fat, which is why fat malabsorption resulting from various diseases (e.g., cystic fibrosis, ulcerative colitis, Crohn's disease) is associated with poor absorption of these vitamins. Fat-soluble vitamins are primarily stored in the liver and **adipose tissues**. With the exception of vitamin K, fat-soluble vitamins are generally excreted more slowly than water-soluble vitamins and vitamins A and D can accumulate and cause toxic effects in the body.

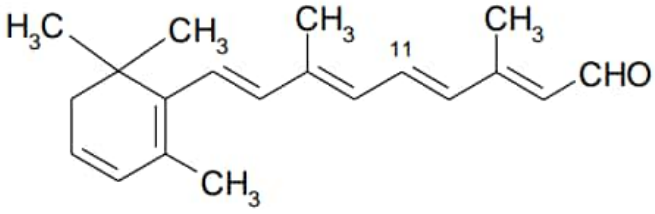
Table 3 List of Fat Soluble Vitamins

Vitamin	Deficiency	Recommended daily intakes	Source
Vitamin A Preformed retinoids and provitamin A carotinoids	Poor growth, night blindness, blindness, dry skin, Xerophthalmia	Infants: 400-500 mg, Children: 300-400 mg, Adolescents: 600-900 mg, Adult men & women: 700-900 mg, Pregnant women: 750-770 mg, Lactating women: 1200-1300 mg	Preformed vitamin A: liver, fortified milk, fish liver oils Provitamin A: red, orange, dark green, and yellow vegetables, orange fruits
Vitamin D Cholecalciferol Ergocalciferol	Rickets in children, osteomalacia in	0-50 years: 5 mg, 51-70 years: 10 mg, >70 years: 15 mg	Vitamin D fortified milk, fish oils

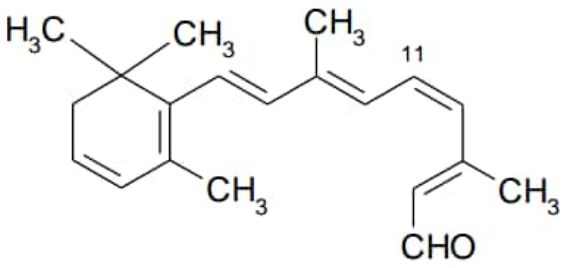
	older adults		
Vitamin E Tocopherols Tocotrienols	Hemolysis of red blood cells, degeneration of sensory neurons	Infants: 4-5 mg, Children: 6-7 mg, Adolescents: 11-15 mg, Adult men & women: 15 mg, Pregnant women: 15 mg, Lactating women: 19 mg	Plant oils, seeds, nuts, products made from oils
Vitamin K Phylloquinone Menaquinone	Hemorrhage, fractures	Infants: 2-2.5 mg, Children: 30-55 mg, Adolescents: 60-75 mg, Adult men: 90 mg, Adult women: 120 mg, Pregnant/lactating women: 75-90 mg	Green vegetables, liver, synthesis by intestinal micro-organisms

Vitamin A

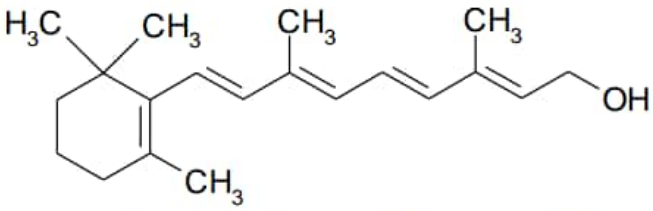
Vitamin A consists of three biologically active molecules, retinol, retinal (retinaldehyde) and retinoic acid. Each of these compounds is derived from the plant precursor molecule, β-carotene (a member of a family of molecules known as carotenoids). β-carotene, which consists of two molecules of retinal linked at their aldehyde ends, is also referred to as the provitamin form of vitamin A.



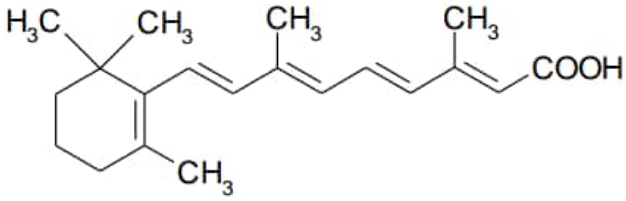
11-*trans*-retinal



11-*cis*-retinal



Retinol



Retinoic Acid

Ingested β-carotene is cleaved in the lumen of the intestine by β-carotene dioxygenase to yield retinal. Retinal is reduced to retinol by retinaldehyde reductase, an NADPH requiring enzyme within the intestines. Retinol is esterified to palmitic acid and delivered to the blood via chylomicrons. The uptake of chylomicron remnants by the liver results in delivery of retinol to this organ for storage as a lipid ester within lipocytes. Transport of retinol from the liver to extrahepatic tissues occurs by binding of hydrolyzed retinol to aporetinol binding protein (RBP). The retinol-RBP complex is then transported to the cell surface within the Golgi and secreted. Within extrahepatic tissues retinol is bound to cellular retinol binding protein (CRBP). Plasma transport of retinoic acid is accomplished by binding to albumin.

Gene Control Exerted by Retinol and Retinoic Acid

Within cells both retinol and retinoic acid bind to specific receptor proteins. Following binding, the receptor-vitamin complex interacts with specific sequences in several genes involved in growth and differentiation and affects expression of these genes. In this capacity retinol and retinoic acid are considered hormones of the steroid/thyroid hormone superfamily of proteins. Vitamin D also acts in a similar capacity. Several genes whose patterns of expression are altered by retinoic acid are involved in the earliest processes of embryogenesis including the differentiation of the three germ layers, organogenesis and limb development.

Vision and the Role of Vitamin A

Photoreception in the eye is the function of two specialized cell types located in the retina; the rod and cone cells. Both rod and cone cells contain a photoreceptor pigment in their membranes. The photosensitive compound of most mammalian eyes is a protein called opsin to which is covalently coupled an aldehyde of vitamin A. The opsin of rod cells is called scotopsin. The photoreceptor of rod cells is specifically called rhodopsin or visual purple. This compound is a complex between scotopsin and the 11-*cis*-retinal (also called 11-*cis*-retinene) form of vitamin A. Rhodopsin is a serpentine receptor imbedded in the membrane of the rod cell. Coupling of 11-*cis*-retinal occurs at three of the transmembrane domains of rhodopsin. Intracellularly, rhodopsin is coupled to a specific G-protein called transducin.

When the rhodopsin is exposed to light it is bleached releasing the 11-*cis*-retinal from opsin. Absorption of photons by 11-*cis*-retinal triggers a series of conformational changes on the way to conversion all-*trans*-retinal. One important conformational intermediate is metarhodopsin II. The release of opsin results in a conformational change in the photoreceptor. This conformational

change activates transducin, leading to an increased GTP-binding by the a-subunit of transducin. Binding of GTP releases the α-subunit from the inhibitory β- and γ-subunits. The GTP-activated α-subunit in turn activates an associated phosphodiesterase; an enzyme that hydrolyzes cyclic-GMP (cGMP) to GMP. Cyclic GMP is required to maintain the Na^+ channels of the rod cell in the open conformation. The drop in cGMP concentration results in complete closure of the Na^+ channels. Metarhodopsin II appears to be responsible for initiating the closure of the channels. The closing of the channels leads to hyperpolarization of the rod cell with concomitant propagation of nerve impulses to the brain.

Additional Role of Retinol

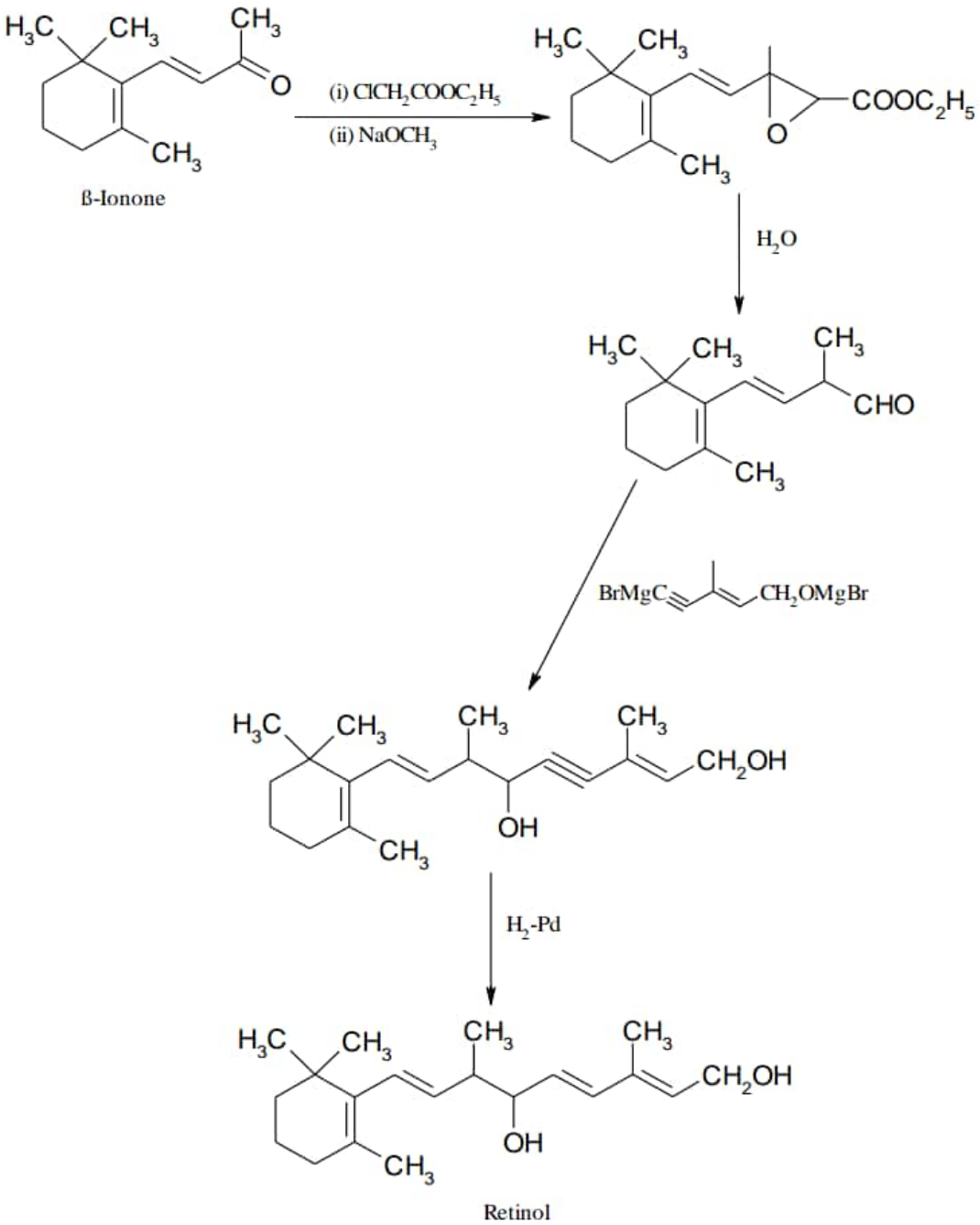
Retinol also functions in the synthesis of certain glycoproteins and mucopolysaccharides necessary for mucous production and normal growth regulation. This is accomplished by phosphorylation of retinol to retinyl phosphate which then functions similarly to dolichol phosphate.

Clinical Significances of Vitamin A Deficiency

Vitamin A is stored in the liver and deficiency of the vitamin occurs only after prolonged lack of dietary intake. The earliest symptoms of vitamin A deficiency are night blindness. Additional early symptoms include follicular hyperkeratinosis, increased susceptibility to infection and cancer and anemia equivalent to iron deficient anemia. Prolonged lack of vitamin A leads to deterioration of the eye tissue through progressive keratinization of the cornea, a condition known as xerophthalmia. The increased risk of cancer in vitamin deficiency is thought to be the result of depletion in β-carotene. Beta-carotene is a very effective antioxidant and is suspected to reduce the risk of cancers known to be initiated by the production of free radicals. Of particular interest is the potential benefit of increased β-carotene intake to reduce the risk of lung cancer in

smokers. However, caution needs to be taken when increasing the intake of any of the lipid soluble vitamins. Excess accumulation of vitamin A in the liver can lead to toxicity, which manifests as bone pain, hepatosplenomegaly, nausea and diarrhea.

Synthesis of Retinol: The following steps are involved in the synthesis of retinol-

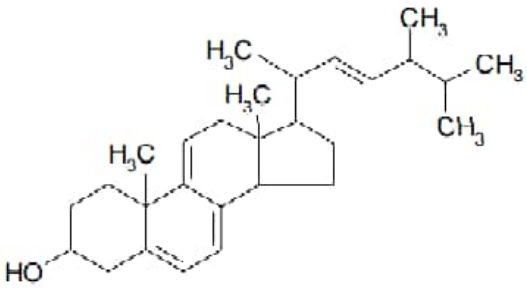


ß-Ionone

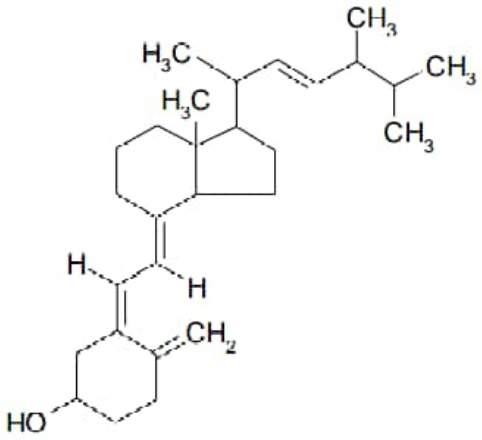
Retinol

Vitamin D

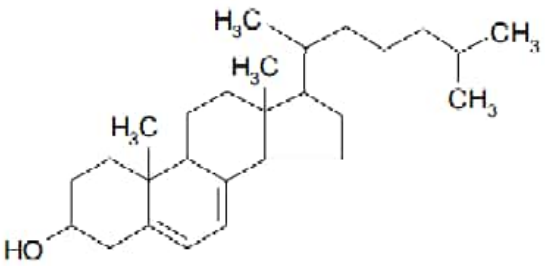
Vitamin D is a steroid hormone that functions to regulate specific gene expression following interaction with its intracellular receptor. The biologically active form of the hormone is 1,25-dihydroxy vitamin D_3 [1,25-$(OH)_2D_3$], also termed calcitriol. Calcitriol functions primarily to regulate calcium and phosphorous homeostasis.



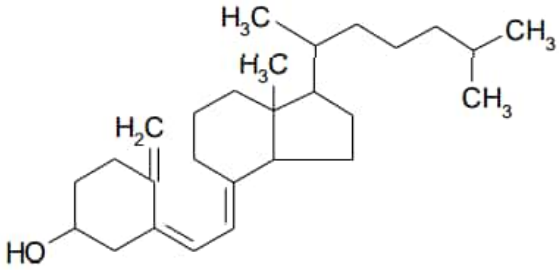
Ergosterol



Vitamin D_2



7-Dehydrocholesterol

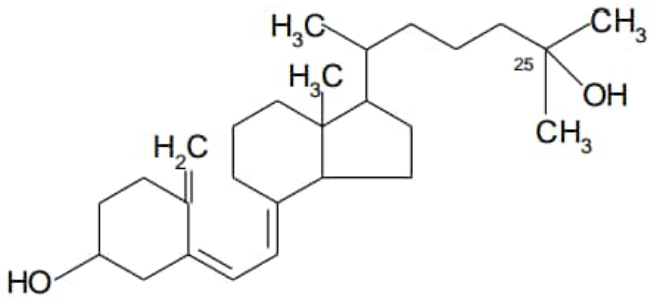


Vitamin D_3

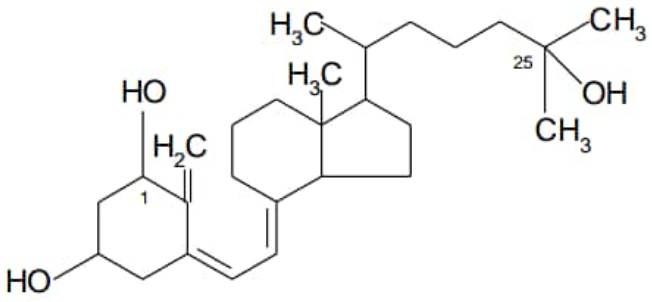
Active calcitriol is derived from ergosterol (produced in plants) and from 7-dehydrocholesterol (produced in the skin). Ergocalciferol (vitamin D_2) is formed by UV irradiation of ergosterol. In the skin 7-dehydrocholesterol is converted to cholecalciferol (vitamin D_3) following UV irradiation.

Vitamin D_2 and D_3 are processed to D_2-calcitriol and D_3-calcitriol, respectively, by the same enzymatic pathways in the body. Cholecalciferol (or egrocalciferol) are absorbed from the intestine and transported to the liver bound to a specific vitamin D-binding protein.

In the liver cholecalciferol is hydroxylated at the 25 position by a specific D_3-25-hydroxylase generating 25-hydroxy-D_3 [25-(OH)D_3] which is the major circulating form of vitamin D. Conversion of 25-(OH)D_3 to its biologically active form, calcitriol, occurs through the activity of a specific D_3-1-hydroxylase present in the proximal convoluted tubules of the kidneys, and in bone and placenta. 25-(OH)D_3 can also be hydroxylated at the 24 position by a specific D_3-24-hydroxylase in the kidneys, intestine, placenta and cartilage.



25-hydroxyvitamin D_3



1,25-dihydroxyvitamin D_3

Calcitriol functions in concert with parathyroid hormone (PTH) and calcitonin to regulate serum calcium and phosphorous levels. PTH is released in response to low serum calcium and induces the production of calcitriol. In contrast, reduced levels of PTH stimulate synthesis of the inactive 24,25-$(OH)_2D_3$. In the intestinal epithelium, calcitriol functions as a steroid hormone in inducing the expression of calbindinD_{28K}, a protein involved in intestinal calcium absorption.

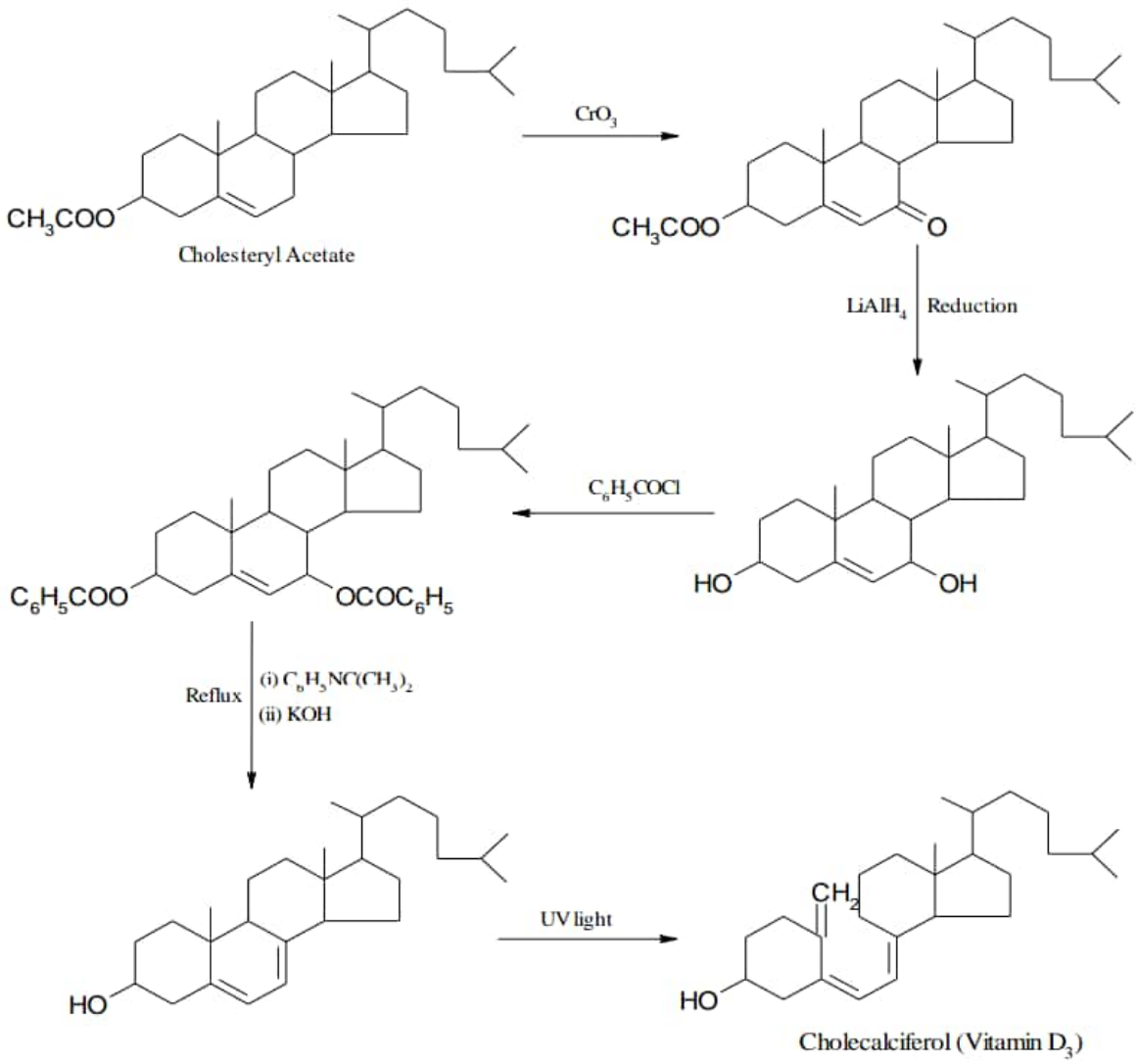
The increased absorption of calcium ions requires concomitant absorption of a negatively charged counter ion to maintain electrical neutrality. The predominant counter ion is Pi. When plasma calcium levels fall the major sites of action of calcitriol and PTH are bone where they stimulate bone resorption and the kidneys where they inhibit calcium excretion by stimulating reabsorption by the distal tubules. The role of calcitonin in calcium homeostasis is to decrease elevated serum calcium levels by inhibiting bone resorption.

Clinical Significance of Vitamin D Deficiency

As a result of the addition of vitamin D to milk, deficiencies in this vitamin are rare in this country. The main symptom of vitamin D deficiency in children is **rickets** and in adults is **osteomalacia**. Rickets is characterized improper mineralization during the development of the bones resulting in soft

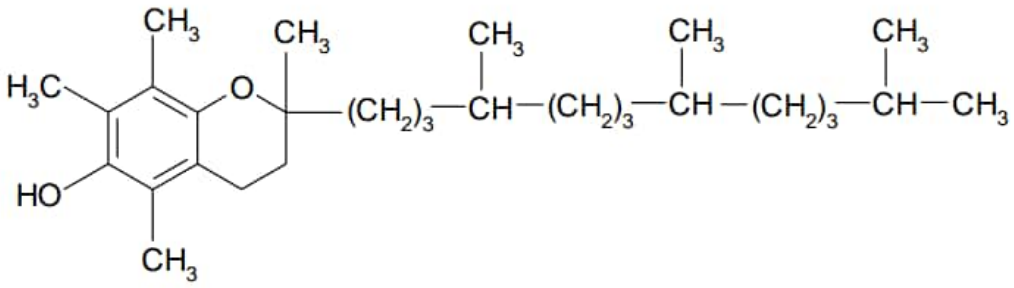
bones. Osteomalacia is characterized by demineralization of previously formed bone leading to increased softness and susceptibility to fracture.

Synthesis of Cholecalciferol (Vitamin D_3)



Vitamin E

Vitamin E is a mixture of several related compounds known as tocopherols. The α-tocopherol molecule is the most potent of the tocopherols. Vitamin E is absorbed from the intestines packaged in chylomicrons. It is delivered to the tissues via chylomicron transport and then to the liver through chylomicron remnant uptake. The liver can export vitamin E in VLDLs. Due to its lipophilic nature; vitamin E accumulates in cellular membranes, fat deposits and other circulating lipoproteins. The major site of vitamin E storage is in adipose tissue.



α-Tocopherol

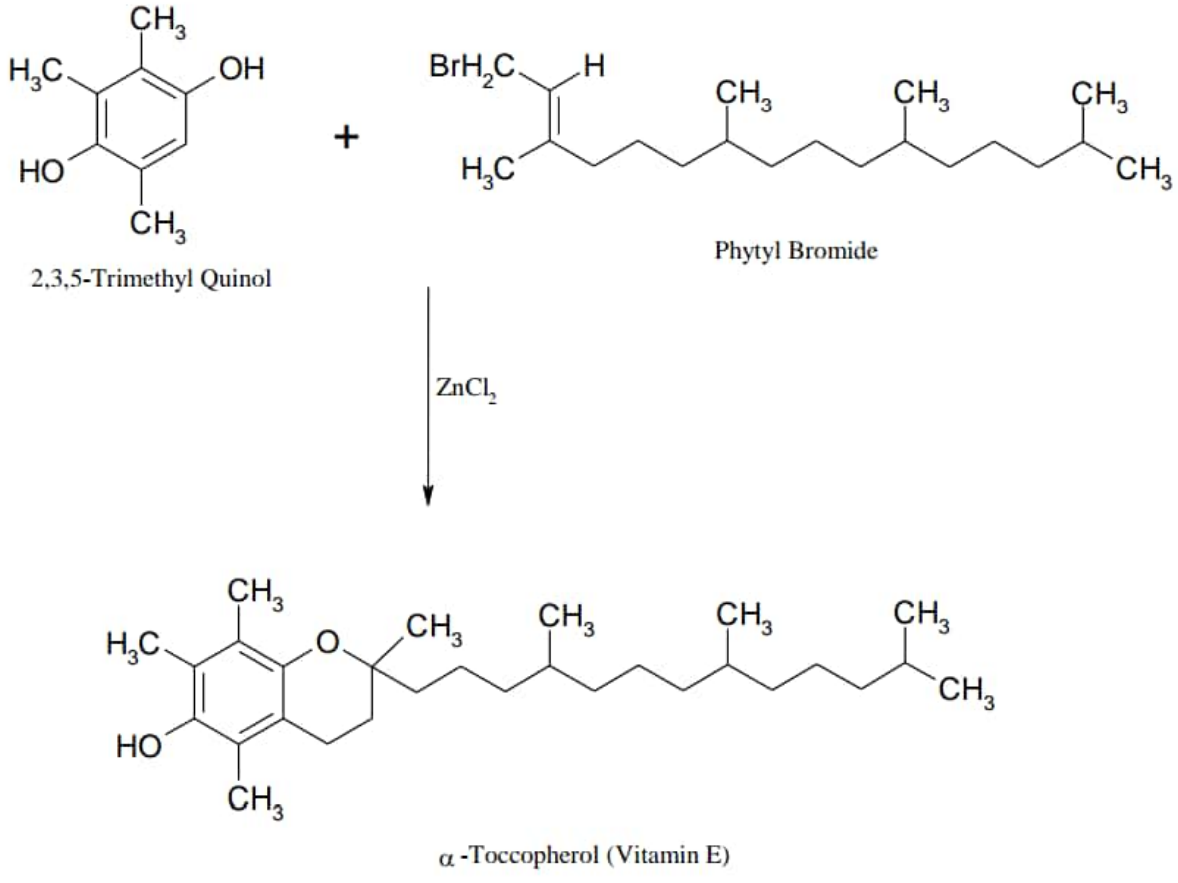
The major function of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen. In particular vitamin E is important for preventing peroxidation of polyunsaturated membrane fatty acids. The vitamins E and C are interrelated in their antioxidant capabilities. Active α-tocopherol can be regenerated by interaction with vitamin C following scavenge of a peroxy free radical. Alternatively, α-tocopherol can scavenge two peroxy free radicals and then be conjugated to glucuronate for excretion in the bile.

Clinical significances of Vitamin E Deficiency

No major disease states have been found to be associated with vitamin E deficiency due to adequate levels in the average American diet. The major symptom of vitamin E deficiency in

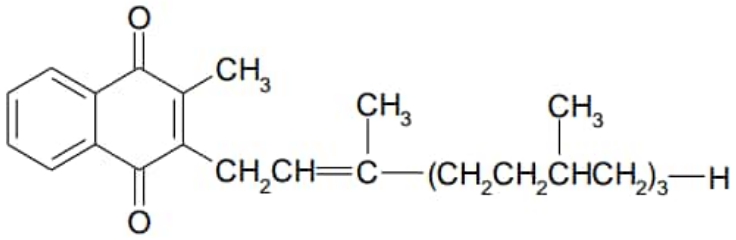
humans is an increase in red blood cell fragility. Since vitamin E is absorbed from the intestines in chylomicrons, any fat malabsorption diseases can lead to deficiencies in vitamin E intake. Neurological disorders have been associated with vitamin E deficiencies associated with fat malabsorptive disorders. Increased intake of vitamin E is recommended in premature infants fed formulas that are low in the vitamin as well as in persons consuming a diet high in polyunsaturated fatty acids. Polyunsaturated fatty acids tend to form free radicals upon exposure to oxygen and this may lead to an increased risk of certain cancers.

Synthesis of α-Toccopherol (Vitamin E)

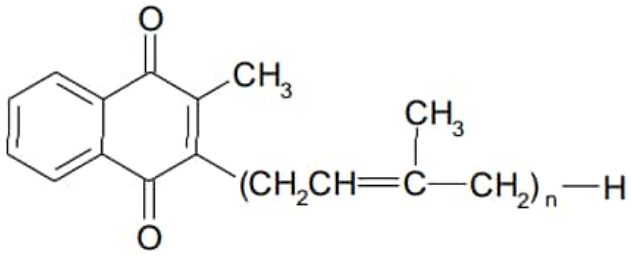


Vitamin K

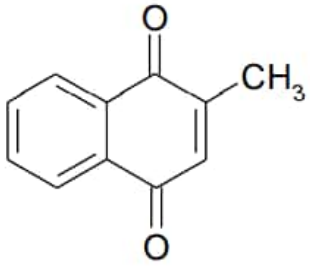
The K vitamins exist naturally as K_1 (phylloquinone) in green vegetables and K_2 (menaquinone) produced by intestinal bacteria and K_3 is synthetic menadione. When administered, vitamin K_3 is alkylated to one of the vitamin K_2 forms of menaquinone.



Vitamin K_1



Vitamin K_2
"n" can be 6, 7 or 9 isoprenoid groups



Vitamin K_3

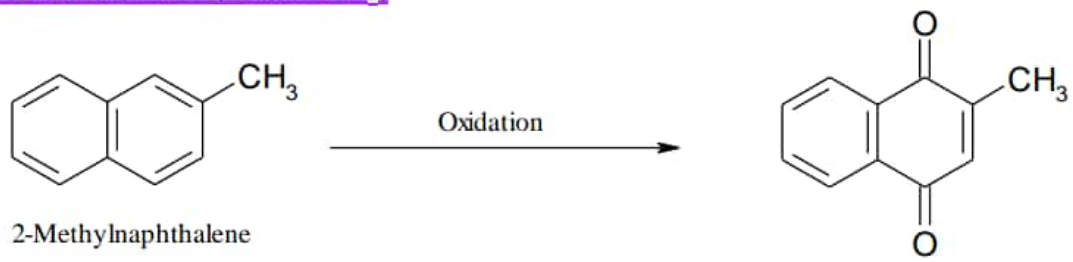
The major function of the K vitamins is in the maintenance of normal levels of the blood clotting proteins, factors II, VII, IX, X and protein C and protein S, which are synthesized in the liver as inactive precursor proteins. Conversion from inactive to active clotting factor requires a posttranslational modification of specific glutamate (E) residues. This modification is a carboxylation and the enzyme responsible requires vitamin K as a cofactor. The resultant modified E residues are γ-carboxyglutamate (**gla**). This process is most clearly understood for factor II, also called preprothrombin. Prothrombin is modified preprothrombin. The *gla* residues are effective calcium ion chelators. Upon chelation of calcium, prothrombin interacts with phospholipids in membranes and is proteolysed to thrombin through the action of activated factor X (Xa).

During the carboxylation reaction reduced hydroquinone form of vitamin K is converted to a 2,3-epoxide form. The regeneration of the hydroquinone form requires an uncharacterized reductase. This latter reaction is the site of action of the dicumarol based anticoagulants such as warfarin.

Clinical significance of Vitamin K Deficiency

Naturally occurring vitamin K is absorbed from the intestines only in the presence of bile salts and other lipids through interaction with chylomicrons. Therefore, fat malabsorptive diseases can result in vitamin K deficiency. The synthetic vitamin K_3 is water soluble and absorbed irrespective of the presence of intestinal lipids and bile. Since intestinal bacteria synthesize the vitamin K_2 form, deficiency of the vitamin in adults is rare. However, long-term antibiotic treatment can lead to deficiency in adults. The intestine of newborn infants is sterile, therefore, vitamin K deficiency in infants is possible if lacking from the early diet. The primary symptom of a deficiency in infants is a hemorrhagic syndrome.

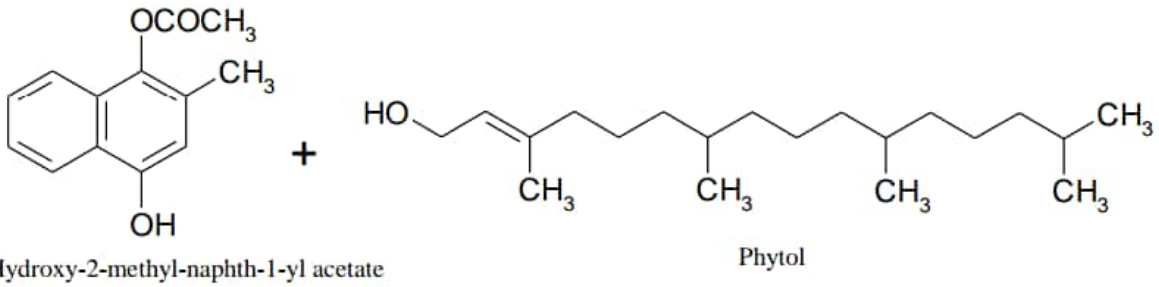
Synthesis of Menadione (Vitamin K_3)



2-Methylnaphthalene

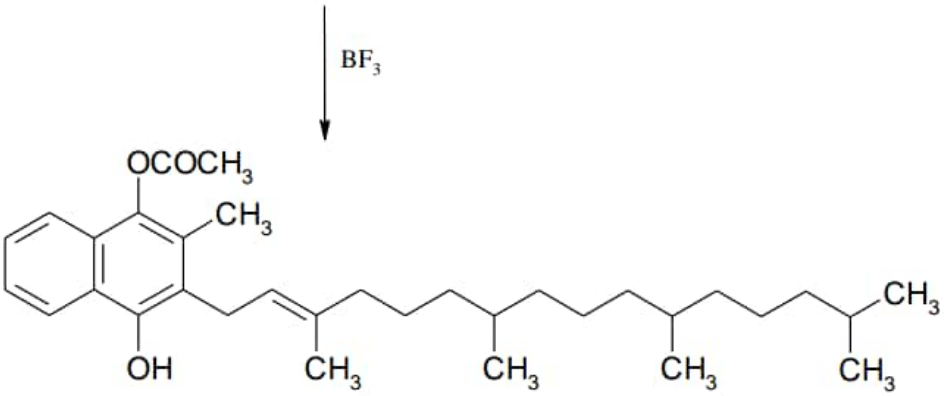
Menadione

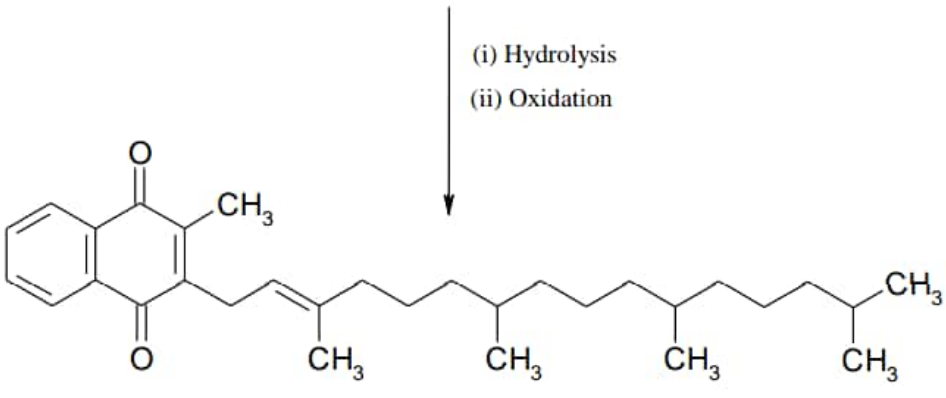
Synthesis of Phytomenadione (Vitamin K_1)



4-Hydroxy-2-methyl-naphth-1-yl acetate

Phytol

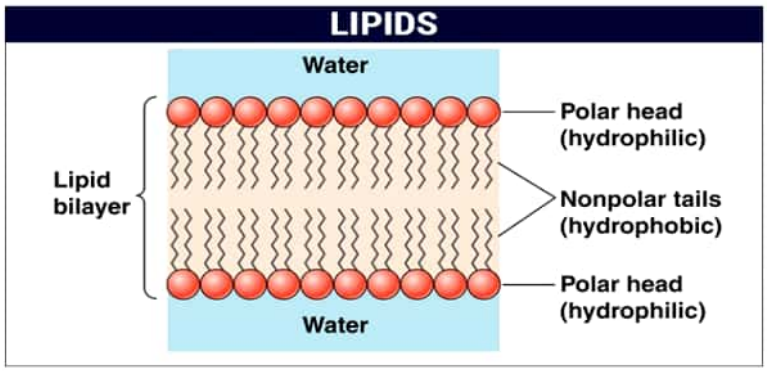




Phytomenadione (Vitamin K_1)

Unit- Lipids

These organic compounds are nonpolar molecules, which are soluble only in nonpolar solvents and insoluble in water because water is polar molecules. In the human body, these molecules can be synthesized in the liver and are and generally found in the oil, butter, whole milk, cheese, fried foods, and also in some red meats.



Properties of Lipids

Lipids are a family of organic compounds, composed of fats and oils. These molecules yield high energy and are responsible for different functions within the human body. Listed below are some important characteristics of Lipids.

Lipids are oily or greasy nonpolar molecules, stored in the adipose tissue of the body.

Lipids are a heterogeneous group of compounds, mainly composed of hydrocarbon chains.

Lipids are energy-rich organic molecules, which provide energy for different life processes.

Lipids are a class of compounds distinguished by their insolubility in water and solubility in nonpolar solvents.

Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment.

Classification of Lipids

Lipids can be classified into two major classes:

Nonsaponifiable lipids, and

Saponifiable lipids.

Nonsaponifiable Lipids

A nonsaponifiable lipid cannot be broken up into smaller molecules by hydrolysis, which includes triglycerides, waxes, phospholipids, and sphingolipids.

Saponifiable Lipids

A saponifiable lipid contains one or more ester groups allowing it to undergo hydrolysis in the presence of an acid, base, or enzymes. Nonsaponifiable lipids include steroids, prostaglandins, and terpenes.

Each of these categories can be further broken down into non-polar and polar lipids.

Nonpolar lipids, such as triglycerides, are used for energy storage and fuel.

Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids.

Fatty acids are important components of all of these lipids.

Types of Lipids

Within these two major classes of lipids, there are several specific types of lipids important to live, including fatty acids, triglycerides, glycerophospholipids, sphingolipids, and steroids. These are broadly classified as simple lipids and complex lipids.

Simple Lipids

Esters of fatty acids with various alcohols.

Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state.

Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols

Complex Lipids

Esters of fatty acids containing groups in addition to alcohol and a fatty acid.

Phospholipids: Lipids containing, in addition to fatty acids and alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.

Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine, and carbohydrate.

Other complex lipids: Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category

Precursor and Derived Lipids

These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids. These compounds are produced by the hydrolysis of simple and complex lipids.

Some of the different types of lipids are described below in detail.

Fatty Acids

Fatty acids are carboxylic acids (or organic acid), often with long aliphatic tails (long chains), either saturated or unsaturated.

Saturated fatty acids

When a fatty acid is saturated it is an indication that there are no carbon-carbon double bonds. The saturated fatty acids have higher melting points than unsaturated acids of the corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape.

Unsaturated fatty acids

If a fatty acid has more than one double bond then this is an indication that it is an unsaturated fatty acid.

"Most naturally occurring fatty acids contain an even number of carbon atoms and are unbranched."

Unsaturated fatty acids, on the other hand, have a cis-double bond(s) that create a kink in their structure which doesn't allow them to group their molecules in straight rod-like shape.

Role of Fats

Fats play several major roles in our body. Some of the important roles of fats are mentioned below:

Fats incorrect amounts are necessary for the proper functioning of our body.

Many fat-soluble vitamins need to be associated with fats in order to be effectively absorbed by the body.

They also provide insulation to the body.

They are an efficient way to store energy for longer periods.

Waxes

Waxes are “esters” (an organic compound made by replacing the hydrogen with acid by an alkyl or another organic group) formed from long-chain carboxylic acids and long-alcohols.

Waxes are seen all over in nature. The leaves and fruits of many plants have waxy coatings, which may protect them from dehydration and small predators.

The feathers of birds and the fur of some animals have similar coatings which serve as a water repellent.

Carnauba wax is valued for its toughness and water resistance(great for car wax).

Membranes are chiefly made of phospholipids which are Phosphoacylglycerols.

Triacylglycerols and phosphoacylglycerols are similar however the terminal OH group of the phosphoacylglycerol is esterified with phosphoric acid instead of fatty acid which leads to the formation of phosphatidic acid.

The name phospholipid comes from the fact that phosphoacylglycerols are lipids that contain a phosphate group.

Steroids Lipids

The chemical messengers in our bodies are known as hormones which are organic compounds synthesized in glands and delivered by the bloodstream to certain tissues in order to stimulate or inhibit the desired process.

Steroids are a type of hormone which is usually recognized by their tetracyclic skeleton, consisting of three fused six-membered and one five-membered ring, as shown in the diagram above. The four rings are designated as A, B, C & D as noted in blue, and the numbers in red represent the carbons.

Cholesterol

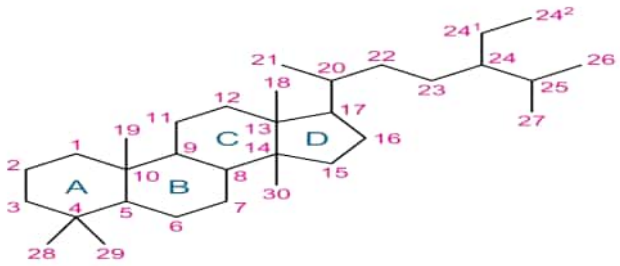
Cholesterol is waxy like substance, found only in animal source foods. Triglycerides, LDL, HDL, VLDL are different types of cholesterol found in the blood cells.

Cholesterol is an important lipid found in the cell membrane. It is a sterol, which means that cholesterol is a combination of steroid and alcohol. In the human body, cholesterol is synthesized in the liver.

These compounds are biosynthesized by all living cells and are essential for the structural component of the cell membrane.

In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps boost the rigidity of the cell membrane. Without cholesterol, the cell membrane would be too fluid.

It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D.



Qualitative Tests:

I. Physical Test:

1. Grease spot test:

Take a small amount of oil on a piece of paper, a greasy spot penetrating the paper will be formed. This happens because lipid does not wet paper unlike water.

2. Test for free fatty acids:

Take a few drops of phenolphthalein solution in a test tube and add to it one or two drops of very dilute alkali solution, just sufficient to give the solution a pink colour. Now add a few drops of the oil and shake. The colour will disappear as the alkali is neutralized by the free fatty acids present in the oil.

3. Emulsification

Oil or liquid fat becomes finely divided and is dispersed in water when shaken with water to form emulsification. Emulsification is permanent and complete in the presence of emulsifying agent. The important emulsifying agents are bile salts, proteins, soaps, mono- and diglycerides. Emulsification is important in the processes of fat digestion in the intestine. Emulsifying agents lower surface tension of the liquid.

Take 2 clean and dry test tubes, in one test tube added 2 ml water and in other 2ml dilute bile salt solution. Now to each tube added 2 drops of mustard oil and shaken vigorously for about one minute. Allow the tubes to stands for two minutes and note that the water, oil is broken in

small pieces and floats on the surface; where as in the bile salt solution, the oil can be seen in minute droplets suspended in the liquid (permanent emulsification).

4. Saponification test:

Esters can be hydrolysed by alkali to yield the parent alcohol and salt. When the fatty acid possesses a long chain the salt formed is a soap which we commonly use. This process is called saponification. Oils and fats usually contain long chain fatty acids and are, therefore, the starting materials for the preparation of soap.

Take 1 ml of the oil in a test tube and add an equal amount of alcoholic KOH solution, mix them thoroughly and keep the mixture during the course of warming and shake up gently with a little distilled water. Appearance of some oil drops will indicate the incomplete saponi- fication. After complete saponification no oil drops will appear.

5. Tests for unsaturation of fatty acids:

Unsaturated fatty acids like oleic acid can react with halogens like bromine and iodine due to presence of double bonds.The amount of Br_2 or I_2 taken up will indicate the amount of unsaturation present in a particu- lar acid. Approximate idea about the unsaturation in a different oils and fats can be obtained by the following test. Set up four clean and dry test tubes each containing 5 ml of CCl_4.

To the first, add one drop of shark liver oil, to the second, one drop of coconut oil, to the third, a drop of vegetable ghee and add nothing to the fourth tube. Now test for the unsaturation of the added oil by adding bromine water drop by drop to each tube followed by shaking.

Record the number of drops required to obtain a permanent yellowish red colour in each tube and infer the relative unsaturation in the three samples used. It may be mentioned here, vegetable ghee is prepared by hydrogenating vegetable oil. Hydrogenation means saturation of unsaturated fatty acid by hydrogen.

6. Tests for Glycerol:

I. Acrolein test:

Take pure glycerol in a dry test tube; add to it a few crystals of potassium hydrogen sulphate. Warm gently to mix and then heat strongly. A very pungent odour of acrolein is produced. Acrolein is formed due to removal of water from glycerol by potassium hydrogen sulphate.

II. Dichromate Test:

Take in a dry test tube 3 or 4 ml of glycerol solution, to it add a few drops of 5% potassium dichromate solution and 5 ml of conc. HNO_3, mix well and note that the brown colour is changed to blue. This test is given by the substances containing primary and secondary alcohol groups. The chromic ions oxidize the glycerol and in this process they are reduced to chromous

ions which give the blue colour. This test is also given by reducing sugars, so before confirming glycerol be sure that the reducing sugars are not present.

7.Tests for Cholesterol:

1. Salkowski's Test (H_2SO_4 Test):

Dissolve cholesterol in 2 ml of chloroform in dry test tube. Add equal amount of con. H_2SO_4. Shake gently. The upper layer turns red and the sulphuric acid layer shows a yellow colour with a green fluorescence.

2. Formaldehyde-H_2SO_4 Test:

Add 2 ml of formaldehyde-sulphuric acid solution (1 part of 40% formaldehyde to 50 parts of the acid) to 2 ml of chloroform solution in a dry test tube. The cherry colour is developed in the chloroform. Pour off the chloroform in another test tube and add 2-3 drops of acid anhydride. The blue colour develops.

Unit- Proteins

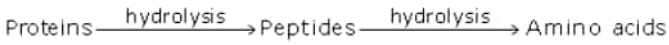
Proteins are the most abundant biological macromolecules, occurring in all cells.

It is also the most versatile organic molecule of the living systems and occur in great variety; thousands of different kinds, ranging in size from relatively small peptides to large polymers.

Proteins are the polymers of amino acids covalently linked by the peptide bonds.

The building blocks of proteins are the twenty naturally occurring amino acids.

Thus, proteins are the polymers of amino acids.



Properties of Proteins

Solubility in Water

- The relationship of proteins with water is complex.
- The secondary structure of proteins depends largely on the interaction of peptide bonds with water through hydrogen bonds.
- Hydrogen bonds are also formed between protein (alpha and beta structures) and water. The protein-rich static ball is more soluble than the helical structures.
- At the tertiary structure, water causes the orientation of the chains and hydrophilic radicals to the outside of the molecule, while the hydrophobic chains and radicals tend to react with each other within the molecule (hydrophobic effect).

Denaturation and Renaturation

- Proteins can be denatured by agents such as heat and urea that cause unfolding of polypeptide chains without causing hydrolysis of peptide bonds.
- The denaturing agents destroy secondary and tertiary structures, without affecting the primary structure.
- If a denatured protein returns to its native state after the denaturing agent is removed, the process is called renaturation.
- Some of the denaturing agents include

- Physical agents: Heat, radiation, pH
- Chemical agents: Urea solution which forms new hydrogen bonds in the protein, organic solvents, detergents.

Structure of protein

The linear sequence of amino acid residues in a polypeptide chain determines the three-dimensional configuration of a protein, and the structure of a protein determines its function.

All proteins contain the elements carbon, hydrogen, oxygen, nitrogen and sulfur some of these may also contain phosphorus, iodine, and traces of metals like ion, copper, zinc and manganese.

A protein may contain 20 different kinds of amino acids. Each amino acid has an amine group at one end and an acid group at the other and a distinctive side chain.

The backbone is the same for all amino acids while the side chain differs from one amino acid to the next.

The structure of proteins can be divided into four levels of organization:

1. Primary Structure

- The primary structure of a protein consists of the amino acid sequence along the polypeptide chain.
- Amino acids are joined by peptide bonds.
- Because there are no dissociable protons in peptide bonds, the charges on a polypeptide chain are due only to the N-terminal amino group, the C-terminal carboxyl group, and the side chains on amino acid residues.
- The primary structure determines the further levels of organization of protein molecules.

2. Secondary Structure

- The secondary structure includes various types of local conformations in which the atoms of the side chains are not involved.
- Secondary structures are formed by a regular repeating pattern of hydrogen bond formation between backbone atoms.
- The secondary structure involves α-helices, β-sheets, and other types of folding patterns that occur due to a regular repeating pattern of hydrogen bond formation.
- The secondary structure of protein could be :

Alpha-helix

Beta-helix

- The α-helix is a right-handed coiled strand.
- The side-chain substituents of the amino acid groups in an α-helix extend to the outside.
- Hydrogen bonds form between the oxygen of the C=O of each peptide bond in the strand and the hydrogen of the N-H group of the peptide bond four amino acids below it in the helix.
- The side-chain substituents of the amino acids fit in beside the N-H groups.
- The hydrogen bonding in a ß-sheet is between strands (inter-strand) rather than within strands (intra-strand).
- The sheet conformation consists of pairs of strands lying side-by-side.
- The carbonyl oxygens in one strand hydrogen bond with the amino hydrogens of the adjacent strand.
- The two strands can be either parallel or anti-parallel depending on whether the strand directions (N-terminus to C-terminus) are the same or opposite.
- The anti-parallel ß-sheet is more stable due to the more well-aligned hydrogen bonds.

3. Tertiary Structure

- Tertiary structure of a protein refers to its overall three-dimensional conformation.
- The types of interactions between amino acid residues that produce the three-dimensional shape of a protein include hydrophobic interactions, electrostatic interactions, and hydrogen bonds, all of which are non-covalent.
- Covalent disulfide bonds also occur.
- It is produced by interactions between amino acid residues that may be located at a considerable distance from each other in the primary sequence of the polypeptide chain.
- Hydrophobic amino acid residues tend to collect in the interior of globular proteins, where they exclude water, whereas hydrophilic residues are usually found on the surface, where they interact with water.

4. Quaternary Structure

- Quaternary structure refers to the interaction of one or more subunits to form a functional protein, using the same forces that stabilize the tertiary structure.
- It is the spatial arrangement of subunits in a protein that consists of more than one polypeptide chain.

Classification of Proteins

Based on the chemical nature, structure, shape and solubility, proteins are classified as:

- **Simple proteins**: They are composed of only amino acid residue. On hydrolysis these proteins yield only constituent amino acids. It is further divided into:
- **Fibrous protein**: Keratin, Elastin, Collagen
- **Globular protein**: Albumin, Globulin, Glutelin, Histones
- **Conjugated proteins**: They are combined with non-protein moiety. Eg. Nucleoprotein, Phosphoprotein, Lipoprotein, Metalloprotein etc.
- **Derived proteins**: They are derivatives or degraded products of simple and conjugated proteins. They may be :

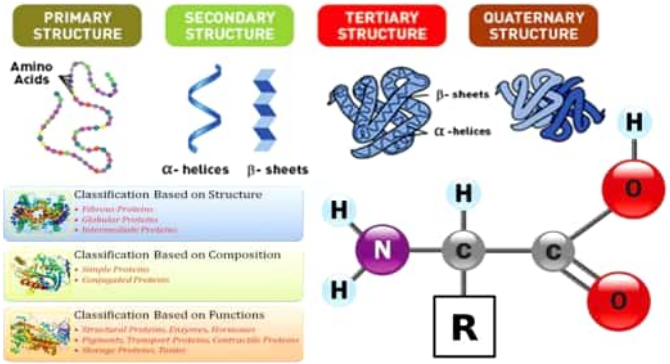
Primary derived protein: Proteans, Metaproteins, Coagulated proteins

Secondary derived proteins: Proteosesn or albunoses, peptones, peptides.

Functions of Proteins

- Proteins are vital for the growth and repair, and their functions are endless. They also have enormous diversity of biological function and are the most important final products of the information pathways.
- Proteins, which are composed of amino acids, serve in many roles in the body (e.g., as enzymes, structural components, hormones, and antibodies).
- They act as structural components such as keratin of hair and nail, collagen of bone etc.
- Proteins are the molecular instruments through which genetic information is expressed.
- They execute their activities in the trans- port of oxygen and carbon dioxide by hemoglobin and special enzymes in the red cells.

- They function in the homostatic control of the volume of the circulating blood and that of the interstitial fluids through the plasma proteins.
- They are involved in blood clotting through thrombin, fibrinogen and other protein factors.
- They act as the defence against infections by means of protein antibodies.
- They perform hereditary transmission by nucleoproteins of the cell nucleus.
- Ovalbumine, glutelin etc. are storage proteins.
- Actin, myosin act as contractile protein important for muscle contraction.



Qualitative tests for protiens.

The following are the simple tests carried to find the presence of proteins in the food given samples.

Biuret test

The compounds with peptide linkage undergo this test. Proteins are polypeptides of amino acids linked together by peptide bonds. An alkaline solution of protein is treated with a drop of aqueous copper sulfate and a bluish violet color is obtained.

Note: Formation of violet coloration confirms the presence of Proteins.

Xanthoproteic test

Proteins on treatment with nitric acid give a yellow or orange color. Concentrated nitric acid is used for nitration. On the treatment of nitric acid, proteins give yellow precipitate which turns to orange color on treatment with alkali.

Note: The appearance of a yellow color solution confirms the presence of proteins.

Millions test

Phenolic group of tyrosine of proteins reacts with mercuric sulfate in the presence of sodium nitrite and sulfuric acid to give red color. Millon's test is given by proteins containing phenolic amino acids. Gelatin does not give this test. First, a white precipitate is formed when proteins are treated with millions reagent and then turns to brick red color on boiling, this confirms the presence of proteins.

Note: The appearance of brick red color solution confirms the presence of proteins.

Ninhydrin test

Proteins react with pyridine solution of ninhydrin and change to a colored solution from a deep blue to violet-pink or sometimes even to a red color. Ninhydrin solution is prepared by dissolving 0.1gm of ninhydrin in about 100ml of distilled water. But this solution of ninhydrin is unstable and can be kept for two days.

Note: The appearance of violet color solution confirms the presence of proteins.

ESSENTIAL MINERALS

Your body uses minerals for a variety of important functions—from making red blood cells, to building bones, to supporting metabolism. They're considered essential because your body needs the minerals to stay healthy, but it cannot make enough of them on its own. Plants gets minerals from soil. Most of minerals in a human diet come from eating plants and animals and from drinking water. Fresh food aren't our only source of dietary minerals, however some processed foods like breakfast cereal, may be fortified with minerals.

- Without intake of minerals, nutritional deficiencies occur.
- All minerals are inorganic substance.
- All minerals are non caloric
- Minerals are needed in tiny amounts for cellular metabolism and structure.

TYPES OF ESSENTIAL MINERALS

There are two types of essential minerals: major and trace minerals. Both types are equally important for health, but major minerals are present in larger quantities in your body than trace minerals.

Major elements	Trace elements
calcium magnesium potassium sodium phosphorous sulphur chlorine	iron, iodine, copper, zinc, manganese, cobalt, selenium chromium fluorine.

MAJOR ELEMENTS

The major minerals are used and stored in large quantities in the body. These includes calcium, magnesium, potassium, sodium, phosphorous, sulphur and chlorine.

Major minerals

Mineral	Good Dietary Sources
Calcium	Milk, yogurt, cheese, paneer, broccoli, chickpeas and fish with soft bones.
Chloride	salt (sodium chloride), tomatoes, lettuce and pickles
Magnesium	Legumes, seeds, nuts, whole grains, fortified cereal, milk, yogurt and green, leafy vegetables like spinach
Phosphorus	Milk, dried fruit, whole grains and protein-rich foods, such as meat, poultry, eggs, fish, nuts and legumes
Potassium	Some fruits, such as blackberries, grapes, oranges, grapefruit and bananas; leafy greens like spinach; and root vegetables, such as carrots and potatoes
Sodium	Table salt (sodium chloride), cheese, salted nuts, and many processed foods, such as bread, cereal, pickles and sauces
Sulfur	Meat, fish, poultry, eggs, legumes, garlic, onions and asparagus

TRACE ELEMENTS-

Some minerals which act as cofactors for the enzymes and are needed in trace quantities are the trace elements. These elements are present in the living tissues in small amounts. They are subdivided into three groups.

- **Essential trace elements-** e.g. iron, iodine, copper, zinc, manganese, cobalt, selenium, chromium ad fluorine.
- **Possibly essential trace elements-** e.g. nickel, tin, vanadium and silicon.
- **Non - essential trace elements-** aluminium, boron, germanium, cadmium, arsenic, lead, and mercury.

.TRACE ELEMENTS

Mineral	Good Dietary Sources
Chromium	Whole grains, oatmeal, mushrooms, broccoli, potatoes, garlic, basil and grape juice
Copper	Shellfish, organ meats like liver, whole grains, nuts, beans, potatoes, dried fruit and dark, leafy green vegetables
Fluoride	Fluoridated water, tea and most seafood
Iodine	Iodized salt, seafood, milk, yogurt, cheese, eggs, bread, cereal and some fruits and vegetables, but the amount of iodine they contain depends on the soil and fertilizer used to grow them
Iron	Lean meat, seafood and poultry; iron- fortified bread and cereal; legumes, such as lentils, white beans, kidney beans and peas; nuts and some dried fruits like raisins
Manganese	Whole grains, nuts, seeds, legumes and pineapple
Molybdenum	Nuts, legumes, grains and milk
Selenium	Seafood, meat, poultry, eggs, bread, cereal and dairy products
Zinc	Oysters, crab, lobster, red meat, poultry, nuts, whole grains, beans and fortified cereal

- **Potassium-** it is major intracellular cation, and is generally found in tissues and body fluids.

Daily requirement- Adult- 2.4gm, child-1.3gm

Functions-

- it maintains intracellular osmotic pressure, acid base balance.
- Involves in cardiac & skeletal muscle activities, mainly potassium required is in depolarisation and contraction of heart.
- Involved in proper transmission of nerve impulse.
- It regulates enzymatic action.

Disease-

Hypokalaemia: It decreases serum potassium level it occurs in:

i. Serum K+ less than 5meq/L
ii. Cushing syndrome
ii. Renal tubular acidosis
iv. Metabolic alkalosis, Diarrhoea and vomiting
V. Muscle weakness, Tachycardia, Cardiac arrest.

Hyperkalaemia: It increases serum potassium level. It occurs in:

i. Renal failure Addison's disease
ii. Severe dehydration

ii. Depression of CNS
iv. Bradycardia

- **Sodium-** Sodium is the chief cation of the extracellular fluid. About one third of the sodium content of the body is present in the inorganic portion of skeleton.

Daily requirements: Infants: 100-700mg Children 300 - 2500mg, adults 100-330mg.

Functions:
i. Sodium regulates Osmotic pressure and fluid balance
ii. It regulates Acid-base balance in association with chloride and bicarbonate.
iii. It is involved in absorption of Glucose, Galactose & Amino acids.
iv. It helps in cell permeability.

Diseases:

Hyponatraemia: occurs due to:

I. Decrease Sodium levels.
II. Diarrhoea, vomiting-chronic renal failure.
III. Addisons disease: mild headache, moderate & severe low blood pressure & circulatory failure.
IV. Over hydration, administration of salt free fluids to patients.

Hypernatraemia: occurs due to:

I. Increase sodium level
II. Cushing's syndrome
III. Prolonged administration of steroid hormones (Cortisone, ACTH/ sex hormones)
IV. Severe dehydration(only water) as in case of Diabetes.

- **Chloride-** It is the chief anion in the body. Around 125 mmole/litre chloride is found in the Cerebrospinal Fluid (CSF) which more than in any other body fluid; approximately 80 gram of chloride ion are present in an average adult.

Daily Requirement: An average adult requires around 5-10 gram of chloride daily.

Functions: Chloride ions regulate osmotic pressure of extracellular fluids along with sodium cations. They help in the synthesis of hydrochloric acid in the stomach and also maintains blood pH.

Diseases:

Hyperchloraemia (or increased chloride concentration) occurs due to:

- Loss of excess fluid (dehydration),
- Adrenocortical hyperactivity of adrenal steroids which cause increased reabsorption from renal tubules,
- Diarrhoea which results in loss of bicarbonate and retention of chloride ions, and
- Respiratory alkalosis, metabolic acidosis, and renal tubular acidosis

Hypochloraemia (or decreased chloride concentration) occurs due to:

- Severe vomiting or loss of HCI, and so compensatory rise in bicarbonate occurs,
- In Addison's disease, mineralocorticoid depletion occurs which in turns impairs reabsorption of chloride from renal tubules,
- Prolonged gastric suction, and
- Respiratory acidosis and metabolic alkalosis.

- **Magnesium-** it is fourth most abundant cation in the body after sodium, potassium, and calcium. it is found in both intracellular and extracellular fluids. Today total body magnesium is about 20g, 70% of which is completed with calcium in bone and 30% in liver, muscle and body fluids.

Daily Requirement: adult man- 350mg/day, adult women- 300mg/day, children-150-250mg/day

Functions:

- Invoved in enzyme action. Magnesium is the cofactor of many enzymes requiring ATP. Alkaline phosphatase, hexokinase, fructokinase, adenylate cyclase, cAMP- dependent kinase need magnesium. Magnesium forms ATP-Mg complexes and binds to the enzymes.
- Required in neuronuscular activity.
- An important constituent of bone and teeth.

Diseases:

Hypomagnesaemia is a condition of low level of serum magnesium which contributes to a poor prognosis for the patient, especially when magnesium ion concentration is below 1.7mg/dl. Causes of hypomagnesaemia are diabetes, prolonged alcoholism, liver cirrhosis, hyperparathyroidism, aldosteronism, protein calorie malnutrition, and drug therapy. It may lead to many health problems, e.g., neuromuscular hyperirritability, tremors, increased vascular resistance, coronary vasospasm, and hypertension.

A Condition of increased level of serum magnesium is known as hypermagnesaemia which generally occurs in conditions of renal failure.

- **Calcium**: It is a chief constituent of bones and teeth; and in blood, half of the calcium is found in ionised form and rest in unionised form. A part of the unionised calcium bounds to citrate and some bounds to protein. Calcium is consumed in the diet in the form of calcium phosphate or carbonate. Dietary calcium is absorbed in the duodenum and proximal jejunum with the involvement of a calcium binding protein-calmodulin.

Daily Requirement:

Around 0.5gm/day adult man; and about 1.5gm and 1.0gm/day for pregnant women and children, respectively.

Function:

- It is required for the calcification of bones and teeth
- It plays a significant. role in the coagulation blood as it helps in the conversion of the prothrombin to thrombin and in the synthesis of thromboplastin.
- It acts as an activator for many enzymes (e-g., lipase, Succinic Dehydrogenase, ATPaSe, and some proteolytic enzymes).
- Calcium ions help in the muscle contraction.
- It is required for nerves excitation.
- It acts as an intracellular secondary messenger of different hormones.
- It facilitates the release of certain hormones such as insulin, PTH, and calcitonin.
- It increases the permeability of plasma membrane, and thus the effect is counterbalanced by the opposite action of sodium and potassium capillary permeability.

Diseases:

Hypercalcaemia: Symptoms of hypercalcaemia are nausea, lethargic (tiredness) conditions, loss of appetite, and tendency to fracture bones. Hypercalcaemia may occur in the following conditions:

- Hyperparathyroidism,
- Multiple myeloma,
- Metastatic carcinoma of bone,
- Milk-alkali syndrome,
- Treatment with drugs such as diuretics, and
- Hypervitaminosis D.

Hypocalcaemia: It may occur in the following conditions:

- Tetany calcium levels fall below 7mg/dl,
- Hypoparathyroidism thyroidism,
- Fanconi's syndrome (disorder of tubular reabsorption),
- Acute pancreatitis,
- Vitamin D deficiency,
- Chronic renal failure.

Phosphorus: It is one of the major components of bones and helps in bone mineralisation. It is also used for the production out 1kg 80% of energy. In a healthy adult human, phosphate concentration is about 1kg, 80% of which is present in bone and teeth, while 10% is in muscles. Phosphorous absorption mostly occurs in the jejunum. Its absorption is stimulated by PTH (parathormone) and vitamin D3.

Daily Requirement: Around 500mg/day for a healthy adult man, about 1gm and 400-600mg/day for pregnant women and children, respectively.

Functions:

- After calcium, phosphorous major constituent of bones and teeth.
- it is required for the production of high-energy phosphates like Adenosine Triphosphate (ATP), Cytidine Triphosphate (CTP), Guanosine Triphosphate (GIP), etc.
- The backbone of DNA and RNA is formed of phosphate diester linkages.
- Phosphorylation helps in the activation of Some enzymes.
- Phosphate is also required for the formation of various biochemical compounds like phospholipids, phosphoproteins, lipoproteins, and nucleotides.

i) Hyperphosphatemia is caused by:

a) Increased absorption of phosphate,
b) Increased cell lysis due to chemotherapy for cancer or due to bone secondaries,
c) Decreased excretion of phosphorus,
d) Renal impairment,
e) Massive blood transfusions, and
f) Drugs (e.g., chlorothiazide, nifedipine, and furosemide).

ii) Hypophosphatemia is caused by:

a) Decreased absorption of phosphate
b) Intracellular shift,
c) Increased urinary excretion of phosphate,
d) Hereditary hypophosphatemia,
e) Hypercalcaemia,
f) Chronic alcoholism, and
g) Drugs (e-g, antacids, diuretics, and salicylate intoxication)

- **Cobalt**: It is significant for the biosynthesis of vitamin B2 family of enzymes. The total body content of cobalt is around 1.1mg. It is mainly absorbed from the small intestine. Around 0.26mg of cobalt is eliminated per day through urine.

Functions:

- It is a constituent of vitamin B12 (around 4% of cobalt present). It is also required for the production of haemoglobin.
- It causes polycythaemia by either increasing or preventing the destruction of erythropoietin hormone (secreted by the kidneys). It may cause the development of macrocytic anaemia.
- It cause an increase in the number of RBCs.

- **Copper**: It is an important component of many redox enzymes (including cytochrome-c-Oxidase). In the body, copper exists in two forms, i.e., cuprous and cupric; but mainly cupric form is found in the body and these forms changes between each other from Cuprous to Cupric.

Functions:

Copper helps in oxidation-reduction and in scavenging of free radicals, as it has the capacity to accept and donates the electrons.

Copper toxicity has many consequences like nausea, vomiting, headache, dizziness, hypertension, hepatic cirrhoSIS, tremors, mental deterioration, Kayser-Fleischer rings, haemolytic anaemia, renal dysfunction (Fanconi-like syndrome), and even death of an individual.

- **Fluorine**: It helps in the formation of tooth enamel (highly mineralised part of the tooth that protects it from degradation), containing fluoroapatite. Around 10-20mg of fluorine is found in the blood in its ionised form. About 1-2 Ppm of fluorine is required per day. As fluorine is absorbed through water, it is expressed as ppm. It is absorbed in small intestine, and half of the ingested fluoride is eliminated through urine and remaining is deposited in bones. With ageing, this fluoride is collected in the bone.

Functions

- Fluorine is essential for tooth development.
- It prevents dental caries.
- Fluorine enhances calcium and phosphorus retention, thus promotes bone development.

- **Iodine**: It is a non-metallic trace element and helps in the synthesis of T3,(triiodothyronine) and T4 (thyroxine) thyroid hormones.

The iodine content in foods depends on the iodine content or the soil in which it was grown; for example, seafood is rich in iodine because they can concentrate the iodine from seawater. Some sea weed (e.g., Undaria pinnatifida or wakame) are also very rich in iodine. Addition of iodised salt and food additives (calcium iodate and potassium iodate) to processed foods increases the iodine content.

The free iodine and inorganic iodate are firstly converted into iodide which is simply absorbed in gastrointestinal tract. It can also be absorbed from mucous membrane, lungs, and skin.

Functions:

- Iodine is essential for the synthesis of thyroid hormones, i.e. triiodothyronine (T3) and tetraiodothyronine (T4).
- It involved in maintaining metabolic rate.

Disease :

Iodine deficiency or hypothyroidism causes stillbirths, abortions, congenital heart anomalies, endemic cretinism, mental retardation, and neurological defects. Treatment of iodine deficiency before pregnancy prevents disorders in children.

Hyperthyroidism leads to the following complications:

i) An enlarged and hyperactive thyrord is caused by exophthalmos.

ii) Thyroid-stimulating immunoglobulin synthesis is increased due to Grave's disease. It further activates the TSH (Thyroid Stimulating Hormone) and LATS (Long- Acting Thyroid Stimulating) factors.

ii) Hashimoto's disease may occur due to destruction of thyroid tissues, over production of anti-thyroid antibodies, due to overproduction of TSH, and hyperthyroidism.

- **Iron**: It is significant for many proteins and enzymes (particularly haemoglobin). Around 3-5gm iron is found in a healthy adult human body out of which around 75% is present in blood in the form of haemoglobin, 5% is in myoglobin, and 15% in ferritin.

Daily requirement

Around 20mg of iron is required per day for a healthy adult man, and about 40mg and 20-30mg of iron per day required by pregnant women and children respectively.

Disease:

Anaemia is commonly caused due to deficiency of iron. Due to anaemia, child loses his/her learning ability and in adults working capacity decreases

Haemosiderosis (or iron toxicity) is the condition in which body has excess amounts of iron. Haemosiderin pigments are found in spleen and liver. They are golden brown granules. Haemosiderosis is caused due to frequent blood transfusion in an individual.

- **Manganese**: it acts as a cofactor of antioxidant enzymes (superoxide dismutase). Around 30mg manganese is found in a healthy adult human body. It is absorbed through guts and transported in combination with B-globulin (called transmanganin). It is stored in the liver. Some amount of it is eliminated through urine, and the remaining through bile or faeces.

Daily requirement

Around 2-5mg per day for a healthy adult; and about 0.5-2mg per day is required by children.

Functions:

Manganese is required for the following metabolic processes:

- Deficiency of manganese causes sterility in animals and disturbance in citric acid cycle.
- It promotes synthesis and deposition of proteoglycans in many tissues, including bones due to glycosytransferase activity.
- It is a constituent of some porphyrins of erythrocytes.
- It is also required for bone growth and cholesterol syntheses.
- It acts as a cofactor for enzymes like arginase, isocitrate dehydrogenase, and leucine aminopeptidase.

Disease

In children, manganese toxicity causes encephalitis, and in adults psychosis and extrapyramidal syndrome may occur.

- **Molybdenum**: It is necessary for xanthine oxidase and other related oxidases. However, deficiency of molybdenum has not been yet observe in humans. It is readily absorbed in GIT, and excreted in urine and bile.

Daily requirement

Around 0.15-0.5mg/day for healthy adult.

Functions

- It takes part in uric acid metabolism.
- It is involved in the action of various enzymes
- In trace amounts, it helps in the utilisation of copper, Whereas in larger amounts it diminishes the same.

Disease

Molybdenum toxicity causes microcytic anaemia and low levels of tissue copper.

- **Selenium**: It is necessary for peroxidase (antioxidant proteins). Around 5-15mg selenium is found in the total body. It protects the cell from destruction.

Daily requirement

Around 0.2mg per day is required for a healthy adult and around 0.02-0.1mg per day for children.

Functions

- It 1s found in selenoproteins, e-g glutathione peroxidase, thioredoxin reductase, deiodinase, muscle selenoproteins, etc.
- It is an integral part of glutathione peroxidase, the following are the functions of it:

a) It guards vital cell components, for example, it protects cell membrane from hydrogen peroxide and other peroxides.

b) It increases the action of superoxIde dimutase which protects the cells against superoxide and other free radicals

- Selenium releases vitamin E in the following three methods:

a) Nornmal pancreatic function, and digestion and absorption of lipidsincluding vitamin E,

b) Component of glutathione peroxidase, and

c)Helps in retention of vitamin E in the blood.

- It reduces the risk of certain cancers.
- It helps in purine metabolism (thioredoxin reductase).
- It helps in sperm motility and muscle metabolism in the form of selenoproteins.

Disease

Toxicity of selenium occurs in human living in selenium-rich soil. In human selenium toxicity leads to cardiomyopathy.

- **Sulphur**: It acts as an enzyme cofactor and also is an essential component of cysteine and methionine (essential amino acids). Proteins contain about 1% sulphur by weight. Sulphates are not used in the form of inorganic sulphate salts of Na, K and Mg yet it is present in food.

Functions

- Compounds possessing phenolic groups may be detoxicated in liver by conjugation with sulphate from amino acids. Hydrocarbons are detoxicated by conjugation with esters of acetylated cysteine.
- Enzymes such as papain, urease, cathepsin depends on free sulphahydryl groups for their catalytic sites.

- **Zinc**: It is pervasive in nature and is essential fo many other enzymes (carboxypeptidase, liver alcohol dehydrogenase, and carbonic anhydrase) to complete their metabolic processes. Around 2.3gm zinc is found in the body, of which 80-110mg/dl is present in the plasma. High concentrations of zinc are found in choroid of eyes, prostate, kidneys, liver, and muscles. Around 5mg of zinc is required per day for a pregnant woman.

Absorption of zinc directly depends on the metallothionein (protein) level in intestinal mucosal cells; this protein also acts as a carrier for zinc. Its absorption is inhibited by copper, phosphate, phytate, and calcium. It is eliminated through faeces and some amount in urine and sweat.

Functions

- There are around 300 zinc-containing enzymes, e-g lactate dehydrogenase, carbonic anhydrase, alkaline phosphatase and carboxypeptidase.
- It is also found in cytosolic superoxide dismutase which contains copper also.
- It helps in the production of DNA and proteins.

WATER

WATER METABOLISM

- Essential for life
  It is possible to live without food than without water.
- Water makes up about 45-75% of your body weight

Water presents in the body may be classified:
1. Intracellular water-about 50% of body weight
2. Extracellular water- about 20% of body weight.

Extracellular water is further classified as:
- Plasma 7.5% of body weight.
- Interstitial fluid 20% of body weight.
- Connective tissues 15% of body weight.
- Transcellular fluids 5% of body weight.

Role of Water:
- Carry nutrients and wastes.
- It is the part of metabolic reactions.
- Solvent for many nutrients.
- Maintain structure of large molecules.
- Maintains body temperature.
- Maintains blood volume.
- Act as lubricant.

IMPORTANCE OF WATER
- Aids with transport
- Mechanical functions
- Helps to break substances down
- Helps to maintain body temperature/pH

1. **Water balance:** The body's content of water is maintained constant on the average. by the maintenance of balance between intake and output. This regularity mechanism prevents:
- Accumulation of water leading to edema.
- Loss of water leading to dehydration.

Body is said to maintain water balance when water gain is equal to water loss.

- **Adequate intake:**
- For men: 125 oz/ day
- For women: 91 oz/ day
- Ideally 80% of water should coming from drinking fluids.
- 20% of water intake should come from food

- **There is no "one-size-fits-all" water intake recommend.**
- Needs vary depending on activity & environmental conditions.

- **For water balance:**
- Consume 1ml water/Calorie expended.
- This is not an optimal level of water intake.

- **DRI for water for those >19 years:**
- Men: 3.7 L/day (approx. 15 cups)
- Women: 2.7 L/day (approx. 11 cups)

- **Functions:**
- Body temperature regulation
  - Water absorbs excess heat
  - Body secretes fluid via perspiration
  - Skin is cooled as perspiration evaporates
  - Humidity (bad) & fans (good) evaporation
- Removal of body waste via urine
  - Urea excretion (Nitrogen from protein breakdown)
  - Sodium excretion
  - Avoid concentrated urine (brownish)
- Amniotic fluid, joint lubricants, saliva, bile

2. Thirst Mechanism:

I. Not reliable
II. Concerns for infants, older adults, athletes
III. Athletes
- Weigh before and after training session
- Consume 3 cups for every pound lost

IV. Illness (vomiting, diarrhea, fever)
- Get additional water

- **Ignoring the Thirst Signal:**

I. Shortage of water increases fluid conservation
II. Antidiuretic hormone (vassopressin)
- Released by the pituitary gland
- Forces kidneys to conserve water (reduce urine flow)

III. Aldosterone
- Responds to drop in blood pressure
- Signals the kidney to retain sodium (water)

- **Hydration**

I. Loss of 1%-2% of body weight in fluid
- Thirst signal

II. Loss of 29% or more of body weight causes muscle weakness (stay hydrated -training)
- Lose significant strength and endurance

III. Loss of 10%-12%
- Heat intolerance

IV. Loss of 20%
- Coma and death

3. **Fluid Balance**

I. Water shifts freely in and out of cells
II. Controlled by electrolyte concentration
- Have electrical charges.. .Na, K, Cl, P, Mg. Ca

III. Osmosis (where an ion goes, H_2O flows)
IV. Intracellular water volume
- Depends on intracellular potassium and phosphate concentrations

V. Extracellular water volume
- Depends on extracellular sodium and potassium concentrations.

● **Dehydration**

Dehydration is a state in which loss of water exceeds intake resulting into reduced water body content. In the body negative water balance occurs.

Dehydration oceurrence due to:
- Primary depletion
- Secondary depletion
- Dehydration due to injectio of hypertonic solution.

1. Primary depletion (Water depletion): It occurs due to insufficient of water intake. It may occurs in:
- Illness like difficulty in swallowing.
- Non availability of water.

This type of dehydration raises the concentration and osmotic pressure of extracellular fluid as a result of which there is consequent out flow of the intracellular water to the ECF, thus ECF volume gets largely restored but there becomes deficiency of water inside the cells as a result of which they suffer from osocmoncenteration, symptoms are dry tongue, poor salivation, shrunken skin, nausea, reduce sweating and intense thirst.

2. Secondary dehydration (Salt depletion): It occurs when fluids with high Na or Cl are lost from the body. In the total electrolytes which affects the basic radicals chiefly Na (extracellular) and K"intracellular) and the acid radicals HCO_3 and Cl are accompanied by a corresponding increase or decrease in the volume of body water which is eventually the causes of intracellular edema. As a result of which there is slowing of circulation and impairment of urinal functions. All this causes an individual to become weak bodily.

3. Dehydration due to injection of hypertonic solution:

When highly concentration of solution and salt is injected into the body of an individual, the osmotic pressure of blood will be increase which results in the flow of fluid from the tissues into the blood unless an equilibrium is reached.

Consequently. the blood volume increases. This increased blood volume soon returns to normal by the loss of excess. through excretion which final losses the net loss of body water producing dehydration.

● **Facts about dehydration:**
- Around three-quarters of the human body is water.
- The causes of dehydration include diarrhea, vomiting.
- and sweating.
- Individuals more at risk of dehydration include athletes, people at higher altitudes, and older adults.

- Early symptoms of dehydration include dry mouth, lethargy, and dizziness.

- **Correction of dehydration:**

Ordinarily sodium chloride solution may be given parenterally to compensate the loss.
In several disorders like diarrhea gastroenteritis pancreatic or billary fistulas etc. a mixture of two thirds isotonic saline solution and one third sodium lactate solution (M/6) should be administered intravenously.

- **Water Intoxication:**

- It is the opposite of dehydration.
- It is caused by excessive retention of water in the body.
- Excessive intake of IV fluids.
- Excessive secretion of ADH or aldosterone

The symptoms of water intoxication are severe headache, confusion, coma, and death.

Carbohydrates and Biochemistry

• Carbohydrates are compounds of tremendous biological importance:
– they provide energy through oxidation
– they supply carbon for the synthesis of cell components
– they serve as a form of stored chemical energy
– they form part of the structures of some cells and tissues

• Carbohydrates, along with lipids, proteins, nucleic acids, and other compounds are known as biomolecules because they are closely associated with living organisms. Biochemistry is the study of the chemistry of biomolecules and living organisms.

Classification of Carbohydrates

Carbohydrates

• Carbohydrates are poly hydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis.
The term "carbohydrate" comes from the fact that when you heat sugars, you get carbon and water.

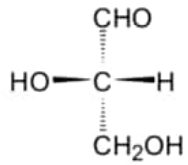
Classes of Carbohydrates

• Monosaccharides contain a single polyhydroxy aldehyde or ketone unit (saccharo is Greek for "sugar") (e.g., glucose, fructose).
• Disaccharides consist of two monosaccharide units linked together by a covalent bond (e.g., sucrose).
• Oligosaccharides contain from 3to10 monosaccharide units (e.g., raffinose).
Classes of Carbohydrates
• Polysaccharides contain very long chains of hundreds or thousands of monosaccharide units, which may be either in straight or branched chains (e.g., cellulose, glycogen, starch).

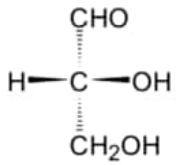
The chemistry of Carbohydrates

Stereoisomers

• Glyceraldehyde, the simplest carbohydrate, exists in two isomeric forms that are mirror images of each other:
•These forms are stereoisomers of each other.
• Glyceraldehyde is a chiral molecule—it cannot be superimposed on its mirror image. The two mirror- image forms of glyceraldehyde are enantiomers of each other.



L-glyceraldehyde



D-glyceraldehyde

Chiral Carbons

• Chiral molecules have the same relationship to each other that your left and right hands have when reflected in a mirror.
• A chiral object can be superimposed on the mirror images — for example, drinking glasses, spheres, and cubes.

- Any carbon atom which is connected to four different groups will be chiral, and will have two non superimposable mirror images; it is a chiral carbon or a centre of chirality.
- – if any of the two groups on the carbon are the same, the carbon atom cannot be chiral.
- Many organic compounds, including carbohydrates, contain more than one chiral carbon.

Optical Activity

• A levorotatory (–) substance rotates polarized light to the left. [E.g., l-glucose; (-)-glucose]

• A dextrorotatory (+) substance rotates polarized light to the right. [E.g., d-glucose; (+)-glucose]

• Molecules which rotate the plane polarized light are optically active.

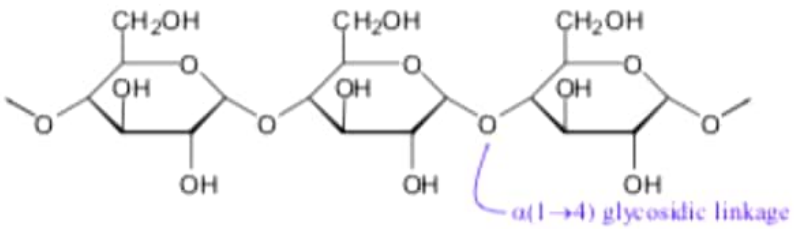
- Most biologically important molecules are chiral, and hence are optically active. Often, living systems contain only one of all of the possible stereochemical forms of a compound. In some cases, one form of a molecule is beneficial, and the enantiomer is a poison (e.g., thalidomide).

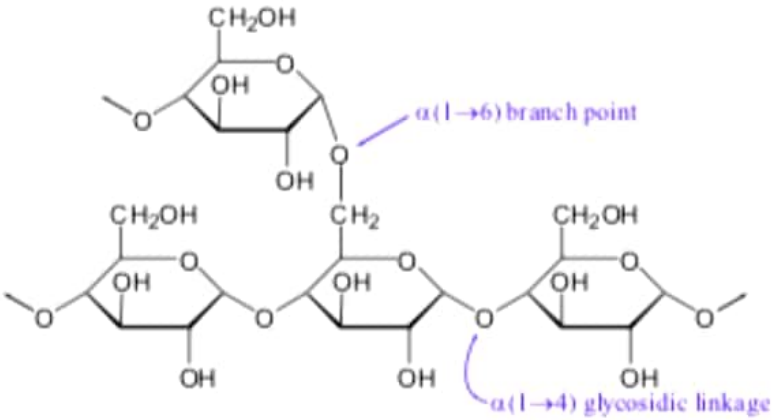
Starch

• Starch is a polymer consisting of D-glucose units.

• Starches(and other glucose polymers) are usually insoluble in water because of the high molecular weight, but they can form thick colloidal suspensions with water.

- There are two forms of starch: amylose and amylopectin.
- Amylose consists of long, unbranched chains of glucose (from 1000 to 2000 molecules) connected by α(1→4) glycosidic linkages.
- 10%-20% of the starch in plants is in this form
- Amylopectin consists of long chains of glucose (up to 105 molecules) connected by α(1→4) glycosidic linkages, with α(1→6) branches every 24 to 30 glucose units along the chain.
- 80%-90% of the starch in plants is in this form.





Glycogen

- Glycogen ,also known as animal starch, is structurally similar to amylopectin, containing both α(1→4) glycosidic linkages and α(1→6) branch points.
- Glycogen is even more highly branched, however, with branches occuring every 8 to 12 glucose units.
- Glycogen is abundant in the liver and muscles; on hydrolysis it forms glucose, which maintains normal blood sugar level and provides energy.

Cellulose is a polymer consisting of long, unbranched chains of D-glucose connected by β(1→4) glycosidic linkages; it may contain from 300 to 3000 glucose units in one molecule.

Functions of carbohydrates :

Carbohydrates have six major functions within the body:

- Providing energy and regulation of blood glucose
- Sparing the use of proteins for energy
- Breakdown of fatty acids and preventing ketosis
- Biological recognition processes
- Flavour and Sweeteners
- Dietary fiber
- Lactose is found in milk.
- Pectin forms the primary cell walls of terrestrial plants.
- Inulin is used for clinical purpose in clearance test.
- Pentosans are present in plant gums and mucilages.

Identification tests for carbohydrates

Molisch's Test:

This is a common test for all carbohydrates larger than tetroses. The test is on the basis that pentoses and hexoses are dehydrated by conc. Sulphuric acid to form furfural or hydroxymethylfurfural, respectively. These products condense with α-naphthol to form purple condensation product.

Fehling's Test:

This forms the reduction test of carbohydrates. Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or red cuprous oxide and is precipitated. Hence, formation of the yellow or brownish-red colored precipitate helps in the detection of reducing sugars in the test solution.

Benedict's Test:

As in Fehling's test, free aldehyde or keto group in the reducing sugars reduce cupric hydroxide in alkaline medium to red colored cuprous oxide. Depending on the concentration of sugars, yellow to green color is developed . All monosaccharides are reducing sugars as they all have a free reactive carbonyl group. Some disaccharides, like maltose, have exposed carbonyl groups and are also reducing sugars, but less reactive than monosaccharides

Barfoed's Test:

Barfoed's test is used to detect the presence of monosaccharide (reducing) sugars in solution. Barfoed's reagent, a mixture of ethanoic (acetic) acid and copper(II) acetate, is combined with the test solution and boiled. A red copper(II) oxide precipitate is formed will indicates the presence of reducing sugar. The reaction will be negative in the presence of disaccharide sugars because they are weaker reducing agents. This test is specific for monosaccharides . Due to the weakly acidic nature of Barfoed's reagent, it is reduced only by monosaccharides.

Seliwanoff's Test:

It is a color reaction specific for ketoses. When conc. HCl is added. ketoses undergo dehydration to yield furfural derivatives more rapidly than aldoses. These derivatives form complexes with resorcinol to yield deep red color. The test reagent causes the dehydration of ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural reacts with resorcinol present in the test reagent to produce a red product within two minutes (reaction not shown). Aldohexoses reacts so more slowly to form the same product.

Iodine Test:

This test is used for the detection of starch in the solution. The blue-black colour is due to the formation of starch-iodine complex. Starch contain polymer of α-amylose and amylopectin which forms a complex with iodine to give the blue black colour.

Osazone Test:

The ketoses and aldoses react with phenylhydrazine to produce a phenylhydrazone which further reacts with another two molecules of phenylhydrazine to yield osazone. Needle-shaped yellow osazone crystals are produced by glucose, fructose and mannose, whereas lactosazone produces mushroom shaped crystals. Crystals of different shapes will be shown by different osazones. Flower-shaped crystals are produced by maltose.

Diseases related to carbohydrate metabolisum

Galactose and fructose disorders

Galactosemia usually is caused by a defective component of the second major step in the metabolism of the sugar galactose. When galactose is ingested, as in milk, galactose-1-phosphate accumulates. Therefore, the clinical manifestations of galactosemia begin when milk feeding is started. If the feeding is not stopped, infants with the disorder will develop lethargy, jaundice, progressive liver dysfunction, kidney disease, and weight loss. They are also susceptible to severe bacterial infections, especially by *Escherichia coli*. Cataracts develop if the diet remains galactose-rich. Intellectual disability occurs in most infants with galactosemia if the disorder is left untreated or if treatment is delayed. Therapy is by exclusion of galactose from the diet and results in the reversal of most symptoms. Most children have normal intelligence, although they may have learning difficulties and a degree of intellectual disability despite early therapy.

Hereditary fructose intolerance (HFI) is caused by a deficiency of the liver enzyme fructose-1-phosphate aldolase. Symptoms of HFI appear after the ingestion of fructose and thus present later in life than do those of galactosemia. Fructose is present in fruits, table sugar (sucrose), and infant formulas containing sucrose. Symptoms may include failure to gain weight satisfactorily, vomiting, hypoglycemia, liver dysfunction, and kidney defects. Older children with HFI tend to avoid sweet foods and may have teeth notable for the absence of caries. Children with the disorder do very well if they avoid dietary fructose and sucrose.

Fructose 1,6-diphosphatase deficiency is associated with an impaired ability to form glucose from other substrates (a process called gluconeogenesis). Symptoms include severe hypoglycemia, intolerance to fasting, and enlargement of the liver. Rapid treatment of hypoglycemic episodes with intravenous fluids containing glucose and the avoidance of fasting are the mainstays of therapy. Some patients require continuous overnight drip feeds or a bedtime dose of cornstarch in order to control their tendency to develop hypoglycemia.

Glycogen storage disorders

The brain, red blood cells, and inner portion of the adrenal gland (adrenal medulla) depend on a constant supply of glucose for their metabolic functions. This supply begins in the small intestine, where transport proteins mediate the uptake of glucose into cells lining the gut. Glucose subsequently passes into the bloodstream and then the liver, where it is stored as glycogen. In times of starvation or fasting or when the body requires a sudden energy supply, glycogen is broken down into glucose, which is then released into the blood. Muscle tissue also has its own glycogen stores, which may be degraded during exercise. If enzymes responsible for glycogen degradation are blocked so that glycogen remains in the liver or muscle, a number of conditions known as glycogen storage disorders (GSD) can arise. Depending upon which enzyme is affected, these conditions may affect the liver, muscles, or both. In GSD type I (von Gierke disease), the last step in glucose release from the liver is defective, leading to hypoglycemia. Therapy consists of supplying continuous glucose to the digestive tract (e.g., by continuous drip feedings) during infancy and early childhood. As the child grows, an improvement in symptoms tends to occur. Adequate glucose is supplied by frequent feedings of carbohydrates and slow-release glucose (uncooked cornstarch) before bedtime. Liver transplantation may also be curative, but this drastic measure is reserved for the small percentage of patients who do not respond to the usual treatment or who develop liver cancer. For the muscular forms of the disease, avoidance of strenuous exercise is the usual therapy. Defects in earlier steps in glycogen breakdown in the liver cause GSD types III, IV, VI, and IX, which usually lead to milder versions of type I disease. Pompe disease (GSD type II) is discussed in the section Lysosomal storage disorders.