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Biological Chemistry Department

BIOCHEMISTRY OF VITAMINS

Textbook for students of international faculty
Speciality: 7.120 10001 «General Medicine»

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This textbook is recommended to use for students of international faculty (the second year of study) for independent work at home and in class. It is created as additional manual for study of Biochemistry for students of international faculty.

Александрова К.В.

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INTRODUCTION

Sometimes it is difficult for students to find out the main important notions for study of biochemistry in basic literature that is recommended. The educational process for students of medical department requires the use not only the basic literature but also that one which is discussed as additional literature sources. This is because each day we have new scientific researches in biochemistry, later which can improve our understanding of theoretical questions this subject. This manual is proposed by authors as additional one for study of water-soluble and fat-soluble vitamins: their structure, properties, functions and metabolism in human organism.

Authors

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GENERAL INFORMATION ABOUT VITAMINS

Vitamins are a group of organic nutrients of various nature required in small quantities for multiple biochemical reactions for the growth, survival and reproduction of the organism, and which, generally, cannot be synthesized by the body and must therefore be supplied by the diet. The most prominent function of the vitamins is to serve as coenzymes (or prosthetic group) for enzymatic reactions.

The discovery of the vitamins began with experiments performed by Hopkins at the beginning of the twentieth century; he fed rats on a defined diet providing the then known nutrients: fats, proteins, carbohydrates, and mineral salts. The animals failed to grow, but the addition of a small amount of milk to the diet both permitted the animals to maintain normal growth and restored growth to the animals that had previously been fed the defined diet. He suggested that milk contained one or more “accessory growth factors” – essential nutrients present in small amounts, because the addition of only a small amount of milk to the diet was sufficient to maintain normal growth and development. The first of the accessory food factors to be isolated and identified was found to be chemically an amine; therefore, in 1912, Funk coined the term *vitamine*, from the Latin *vita* for “life” and amine, for the prominent chemical reactive group. Although subsequent accessory growth factors were not found to be amines, the name has been retained—with the loss of the final “-e” to avoid chemical confusion. The decision as to whether the word should correctly be pronounced “vitamin” or “veitamin” depends in large part on which system of Latin pronunciation one learned – the Oxford English Dictionary permits both. During the first half of the twentieth century, vitamin deficiency diseases were common in developed and developing countries. At the beginning of the twenty-first century, they are generally rare, although vitamin A deficiency is a major public health problem throughout the developing world, and there is evidence of widespread subclinical deficiencies of vitamins B₂ and B₆. In addition, refugee and displaced populations are at risk of multiple B vitamin deficiencies, because the cereal foods used in emergency rations are not usually fortified with micronutrients.

Vitamins are grouped together according to the following **general biological characteristics**:

1. Vitamins are not synthesized by the body and must come from food. An exception are vitamin B₃ (PP), which active form NADH (NADPH) can be synthesized from tryptophan and vitamin D₃ (cholecalciferol), synthesized from 7-dehydrocholesterol in the skin. Amount of those ones and vitamins partially synthesized by intestinal microflora (B₁, B₂, B₃, B₅, B₆, K, and others) is normally not sufficient to cover the body's need them.

2. Vitamins are not plastic material. Exception is vitamin F.

3. Vitamins are not an energy source. Exception is vitamin F.

4. Vitamins are essential for all vital processes and biologically active already in small quantities.

5. They influence biochemical processes in all tissues and organs, i.e. they are not specific to organs.

6. They can be used for medicinal purposes as a non-specific tools in high doses for: diabetes mellitus - B₁, B₂, B₆; colds and infectious diseases - vitamin C; bronchial asthma - vitamin PP; gastrointestinal ulcers - vitamin-like substance U and nicotinic acid; in hypercholesterolemia - nicotinic acid.

Since only a few vitamins can be stored (A, D, E, B₁₂), a lack of vitamins quickly leads to **deficiency diseases (hypovitaminosis or avitaminosis)**. These often affect the skin, blood cells, and nervous system. The causes of vitamin deficiencies can be treated by improving nutrition and by administration vitamins in tablet form. An overdose of vitamins leads to **hypervitaminosis state** only, with toxic symptoms, in the case of vitamins A and D. Normally, excess vitamins are rapidly excreted with the urine.

Lack of vitamins leads to the development of pathological processes in the form of specific hypo- and avitaminosis. Widespread hidden forms of vitamin deficiency have not severe external manifestations and symptoms, but have a negative impact on performance, the overall tone of the body and its resistance to various adverse factors.

Avitaminosis is a disease that develops in the absence of a particular vitamin. Currently avitaminosis are not commonly found, but hypovitaminoses are observed with vitamin deficiency in the body. Numerous examples you can see in the table 1.

Table 1. **Vitamin functions and manifestations of hypo- and avitaminoses**

Vitamin		Functions	Hypovitaminosis symptoms
B ₁	Thiamin	Functional part of coenzyme TPP in pyruvate and α -ketoglutarate dehydrogenases, transketolase; poorly defined function in nerve conduction	Peripheral nerve damage (polyneuritis beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome) Concentration of pyruvate is increased in the patient's blood, the most of which is excreted with urine
B ₂	Riboflavin	Functional part of coenzymes FAD, FMN in oxidation-reduction reactions	Epithelial, mucosa, cutaneous, corneal lesions: lesions of corner of mouth, lips, and tongue; seborrheic dermatitis
B ₃ (PP)	Niacin, nicotinic acid, nicotinamide	Functional part of coenzymes NAD ⁺ , NADP ⁺ in oxidation-reduction reactions	Pellagra: photosensitive dermatitis, glossitis (tongue inflammation), alopecia (hair loss), edema (swelling), diarrhea, depressive psychosis, aggression, ataxia (lack of coordination), dementia, weakness
B ₅	Pantothenic acid	Functional part of coenzyme CoA (universal acyl carrier in Krebs cycle, fatty and other carboxylic acid metabolism) and phosphopantetheine (acyl carrier protein in fatty acid synthesis)	Numbness in the toes, burning sensation in the feet, the defeat of mucous membranes of internal organs, early graying, hair loss, various disorders of the skin: the development of small cracks in the corners of the mouth, the appearance of white patches on various parts of the body. There may also be depressed mood, fatigue.
B ₆	Pyridoxine, pyridoxal, pyridoxamine	Functional part of coenzyme PLP in transamination and decarboxylation of amino acids and glycogen phosphorylase	Dermatitis of the eyes, nose, and mouth. There is mental confusion, glossitis and peripheral neuropathy, convulsions (due to lack of inhibitory neurotransmitter GABA)
B ₇ (H)	Biotin	Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis	Seborrheic dermatitis, anemia, depression, hair loss, high blood sugar levels, inflammation or pallor of the skin and mucous membranes, insomnia, loss of appetite, muscle aches, nausea, sore tongue, dry skin, high blood cholesterol
B ₉	Folic acid	Functional part of coenzyme THFA in transfer of one-carbon fragments	Megaloblastic anemia: red tongue, anemia, lethargy, fatigue, insomnia, anxiety, digestive disorders, growth retardation, breathing difficulties, memory problems. Deficiency during pregnancy is associated with neural tube defects

B ₁₂	Cobalamin	Functional part of coenzymes adenosylcobalamin (Methylmalonyl Co A mutase) and methylcobalamin (Methionine synthase) in transfer of one-carbon fragments and metabolism of folic acid	Vitamin B ₁₂ -deficiency anemia (in other words pernicious anemia or Addison–Biermer anemia) is one of many types of megaloblastic anemias with degeneration of the spinal cord, anemia, fatigue, depression, low-grade fevers, diarrhea, weight loss, neuropathic pain, glossitis (swollen, red and smooth appearance of the tongue), angular cheilitis (sores at the corner of the mouth) Possible manifestations are also hypochromic anemia, splitting hair and loss of hair, increased nail bottling and taste alteration
C	Ascorbic acid	It serves as a donor of protons in hydroxylation reaction for: - collagen synthesis (prolyl- and lysyl residues are hydroxylated by prolyl 3(4)-hydroxylase and lysyl 5-hydroxylase respectively; - catecholamines and steroid hormone synthesis; It has properties of antioxidant; enhances absorption of iron	Scurvy: general weakness, subcutaneous hemmorrhages (frequent hemorrhages from internals and mucous membranes), gingival hemmorrhages, loss of teeth, formation of spots on the skin, spongy gums, yellow skin, fever, neuropathy Multiple hemorrhages in the places of clothes friction are possible if a person often experiences acute respiratory infections
A	Retinol	Functional part of visual pigments (rhodopsins and iodopsins) in the retina; regulation of gene expression and cell differentiation; β-carotene (provitamin A) is an antioxidant	Vision impairment hemeralopia (night blindness), xerophthalmia; keratinization of skin
D	Calciferol	Stimulation of Ca ²⁺ absorption through intestinal wall, maintenance of calcium balance and mobilization of bone mineral	Rickets = poor mineralization of bone; osteomalacia = bone demineralization Osteoectasia of the lower extremities and delayed mineralization of cranial bones are onserved in infants
E	Tocopherols	Antioxidant, especially in cell membranes	Extremely rare is a serious neurologic dysfunction
K	Phyllo-quinone, mena-quinones	Coenzyme in formation of γ-carboxyglutamate residues in structure of: - factors II (prothrombin), VII, IX, X, XIV, protein S (blood coagulation system); - bone matrix proteins	Impaired blood clotting, hemorrhagic disease, osteoporosis and coronary heart disease Intestinal dysbacteriosis occurs hemorrhagic syndrome

External causes for hypovitaminosis

1. Lack of the vitamin in the diet or presence of food factors hindering the absorption of vitamin. For example, use of large amounts of raw eggs (they contain protein avidin binds vitamin H (biotin)) as a result may develop a state of hypovitaminosis H.

2. Do not take into account the need for a particular vitamin. For example, in protein-free diet is increasing demand for vitamin PP (with normal diet it may be partially synthesized from tryptophan). If a person consumes much protein, it can increase the need for vitamin B₆ and reduce the need for vitamin PP.

3. Social reasons: urbanization, power and extremely high purity of canned food; antivitamin presence in food. People are not enough exposed to sunlight in large cities - so it can be hypovitaminosis D. In such cases, the medicine uses ultraviolet radiation in the form of different physical treatments, which activate the synthesis of vitamin D₃ from 7-dehydrocholesterol in the skin cells.

Internal causes of hypovitaminosis

1. Physiological increased need for vitamins, for example, during pregnancy, with heavy physical labor.

2. Long-term severe infectious diseases, as well as during the recovery period.

3. Disturbance of vitamin absorption in some diseases of the digestive tract, for example impaired absorption of fat-soluble vitamins is observed at cholelithiasis; vitamin B₁₂ is done with atrophy of the gastric mucosa and a deficiency of Castle intrinsic factor. Another case if a person who hadn't been consuming fats but had been getting enough carbohydrates and proteins for long time revealed dermatitis, poor wound healing, vision impairment. Lack of vitamins A, D, E, K, F (linoleic, linolenic, arachidonic acids) is probable cause of the metabolic disorder.

4. Intestinal dysbacteriosis. It has the meaning as some vitamins are synthesized by the intestinal microflora (these vitamins are B₃, B₆, B₇ (H), B₉, B₁₂, and K).

5. Cirrhosis. The liver is the major depot of many vitamins, particularly fat-soluble (especially high hepatic reserves of fat soluble vitamins A, D), but also certain water-soluble, such as B₉, B₁₂, etc. In case of vitamin consumption increase and reducing their dietary intake, which is usually the case, for example, in alcoholism, megaloblastic anemia is developed in a short time as a characteristic sign of hypovitaminosis B₉. Patients with cirrhosis may experience blurred vision in the twilight due to malabsorption of vitamin A in the intestine and its reduced deposit in the liver.

6. Genetic defects of some enzymatic systems. For example, vitamin D-resistant rickets occurs in children lack the enzymes involved in the formation of the active form of vitamin D - calcitriol (1, 25-dihydroxycholecalciferol).

CLASSIFICATION AND NOMENCLATURE OF THE VITAMINS

In addition to systematic chemical nomenclature, the vitamins have an apparently illogical system of accepted trivial names arising from the history of their discovery. For several vitamins, a number of chemically related compounds show the same biological activity, because they are either converted to the same final active metabolite or have sufficient structural similarity to have the same activity. Different chemical compounds that show the same biological activity are collectively known as vitamers. Where one or more compounds have biological activity, in addition to individual names there is also an approved generic descriptor to be used for all related compounds that show the same biological activity.

When it was realized that milk contained more than one accessory food factor, they were named A (which was lipid-soluble and found in the cream) and B (which was water-soluble and found in the whey). This division into fat- and water-soluble vitamins is still used, although there is little chemical or nutritional reason for this, apart from some similarities in dietary sources of fat-soluble or water-soluble vitamins. Water-soluble derivatives of vitamins A and K and fat-

soluble derivatives of several of the B vitamins and vitamin C have been developed for therapeutic use and as food additives.

As the discovery of the vitamins progressed, it was realized that “Factor B” consisted of a number of chemically and physiologically distinct compounds. Before they were identified chemically, they were given a logical series of alphanumeric names: B₁, B₂, and so forth.

Table 2. Classification of vitamins. Group B.

Alphanumeric name of vitamin	Chemical and other names of vitamin
Vitamin B ₁	thiamine
Vitamin B ₂	riboflavin
Vitamin B ₃	niacin, niacinamide, niacin, niacinamide, RR
Vitamin B ₄	choline
Vitamin B ₅	pantothenic acid
Vitamin B ₆	pyridoxine, pyridoxal
Vitamin B ₇	biotin, vitamin H
Vitamin B ₈	inositol, myo-inositol, vitamin U
Vitamin B ₉	folic acid, foliatsin, vitamin B _C , M
Vitamin B ₁₀	para-aminobenzoic acid, PABA, vitamin H ₁
Vitamin B ₁₁	L-carnitine, vitamin T, vitamin D
Vitamin B ₁₂	cyanocobalamin, cobalamin
Vitamin B ₁₃	orotic acid
Vitamin B ₁₄	pyrrolo-quinoline quinone, metoksantin, coenzyme of PQQ (Pyrroloquinoline Quinone)
Vitamin B ₁₅	pangamic acid, sometimes referred to as vitamin B ₁₆
Vitamin B ₁₆	sometimes pangamic acid - B ₁₅ , and sometimes cyanocobalamin - B ₁₂
Vitamin B ₁₇ (misnomer)	L-citral, letral, letril, amygdalin (The structure - a 2glucose + mandelonitrile)

And such vitamins or vitamin similar compounds as:

Vitamin C - ascorbic acid;

Vitamin P - bioflavonoids: quercetin, rutin, myricetin, apigenin, hesperin, hesperidin, luteolin, catechin, eriodictyol, cyaniding and others;

Vitamin N - lipoic acid;

Vitamin U (ulcus - ulcer) - a derivative of methionine-methionine-methyl sulfonium (pharmacology known as "metiosulfoniya chloride")

As can be seen from Table 2, a number of compounds were assigned vitamin status, and were later shown either not to be vitamins, or to be compounds that had already been identified and given other names.

For a compound to be considered a vitamin, it must be shown to be a dietary essential. Its elimination from the diet must result in a more-or-less clearly defined deficiency disease, and restoration must cure or prevent that deficiency disease. Demonstrating that a compound has pharmacological actions, and possibly cures a disease, does not classify that compound as a vitamin, even if it is a naturally occurring compound that is found in foods. Equally, demonstrating that a compound has a physiological function as a coenzyme or hormone does not classify that compound as a vitamin. It is necessary to demonstrate that endogenous synthesis of the compound is inadequate to meet physiological requirements in the absence of a dietary source of the compound. There is some evidence that premature infants and patients maintained on long-term total parenteral nutrition may be unable to meet their requirements for carnitine, choline, and taurine unless they are provided in the diet, and these are sometimes regarded as "marginal compounds," for which there is no evidence to estimate requirements.

The rigorous criteria outlined here would exclude niacin and vitamin D from the list of vitamins, because under normal conditions endogenous synthesis does indeed meet requirements. Nevertheless, they are considered to be vitamins, even if only on the grounds that each was discovered as the result of investigations into once common deficiency diseases, pellagra and rickets.

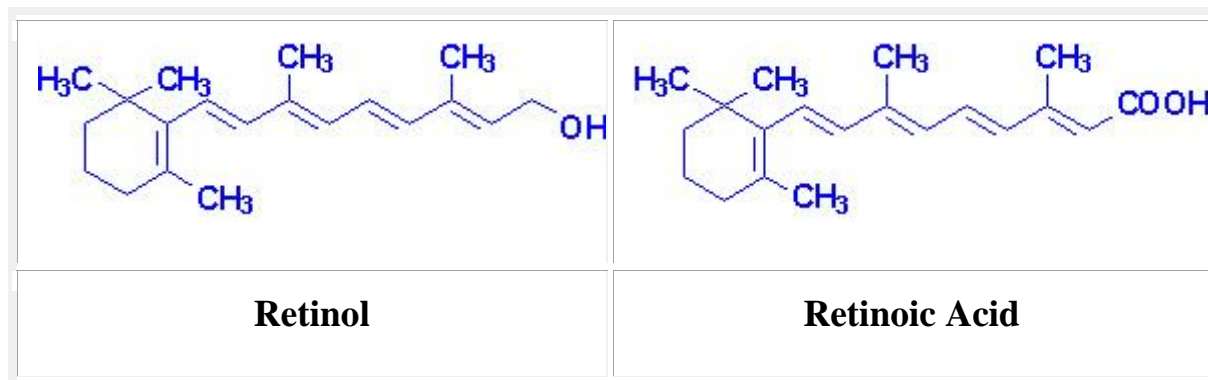
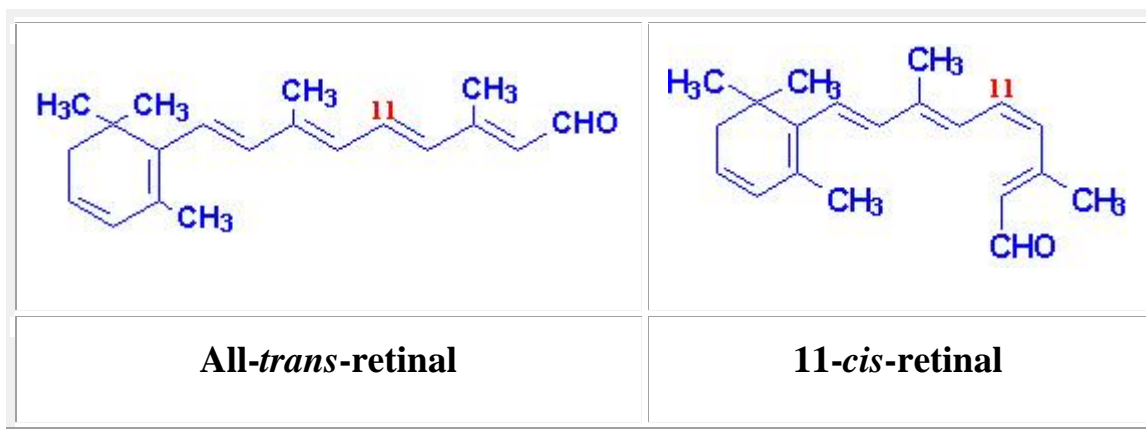
In addition to the marginal compounds like carnitine choline, there are a number of compounds present in foods of plant origin that are considered to be beneficial, in that they have actions that may prevent the development of atherosclerosis and some cancers, although there is no evidence that they are dietary essentials, and they are not generally considered as nutrients.

GROUP I. FAT-SOLUBLE VITAMINS

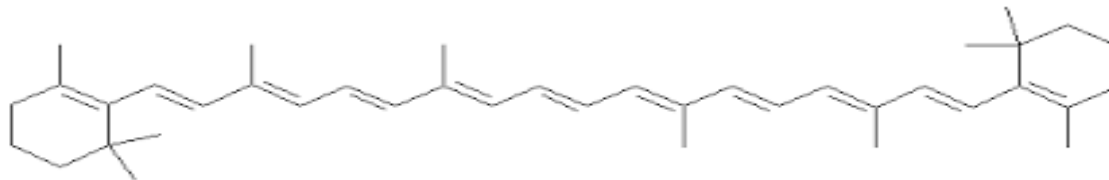
Group I is Fat-soluble vitamins: A (retinol), D (calciferol), E (tocopherol), K (naphthoquinone), F (polyunsaturated fatty acid: linoleic, linolenic, arachidonic).

VITAMIN A

Vitamin A includes two vitamers: retinol and dehydroretinol but group of vitamin A consists and their biologically active molecules retinal (retinaldehyde) and retinoic acid.



Each of these compounds are derived from the plant produced molecule carotene (a member of a family of molecules known as carotenoids). Beta-carotene structure:



Beta-carotene, which consists of two molecules of retinal linked at their aldehyde ends, is also referred to as the provitamin form of vitamin A.

Ingested β -carotene is cleaved in the lumen of the intestine by beta-carotene dioxygenase to yield retinal. Retinal is right here reduced to retinol by retinaldehyde reductase, an NADPH requiring enzyme within the intestines. Retinol is esterified to palmitic or stearic acids 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 esters. Transport of retinol from the liver to extrahepatic tissues occurs by binding of retinol to retinol 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. One protein else is capable to transport of vitamin A: it is transthyretin. Transthyretin (TTR) is a serum and cerebrospinal fluid carrier of the thyroid hormone thyroxine (T_4) and retinol-binding protein bound to retinol. This is how transthyretin gained its name, **trans**ports **thy**roxine and **retinol**. The liver secretes transthyretin into the blood, and the choroid plexus secretes TTR into the cerebrospinal fluid.

TTR was originally called *prealbumin* (or thyroxine-binding prealbumin) because it ran faster than albumin on electrophoresis gels.

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 α -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 cGMP to GMP. cGMP 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.

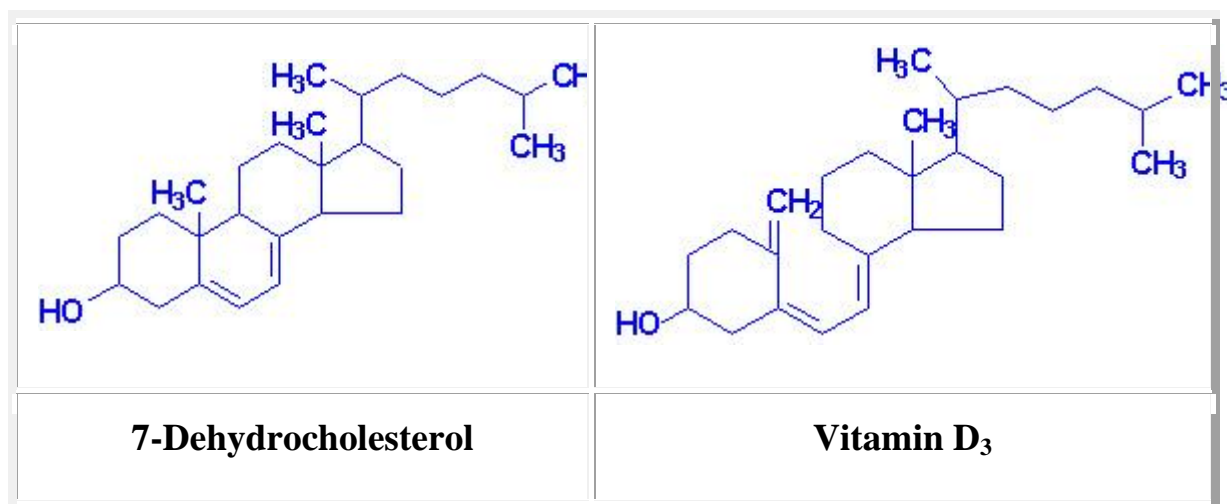
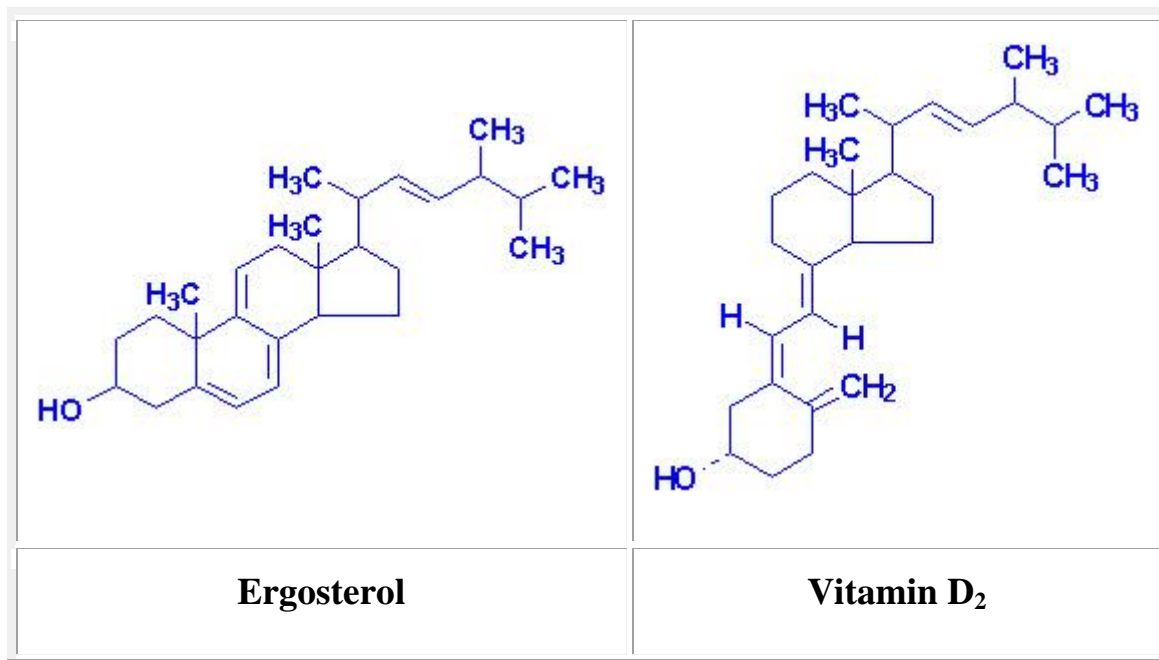
Clinical Significances of Vitamin A Deficiency

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 a depletion in beta-carotene. β -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 beta-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.

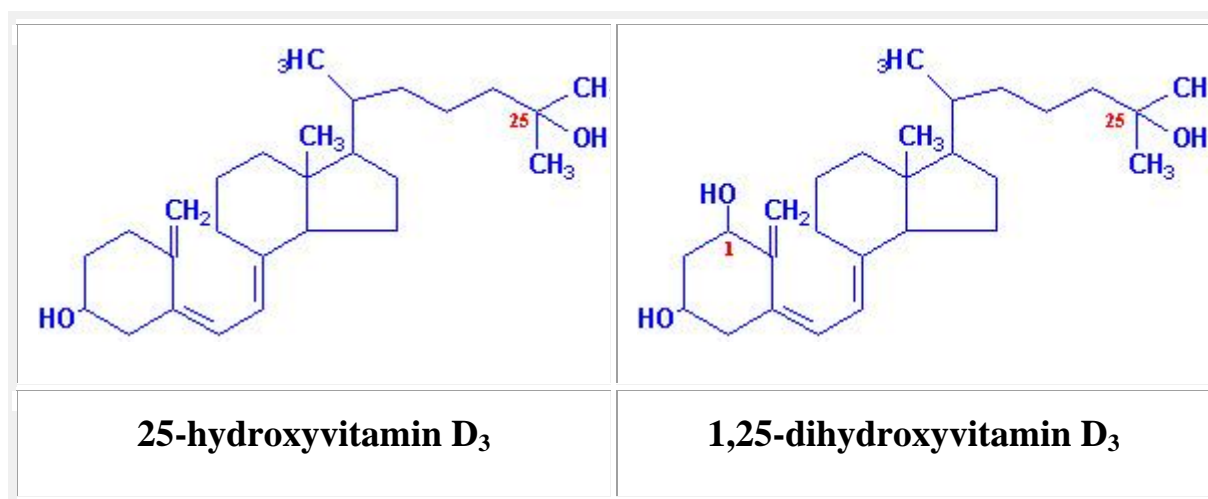
VITAMIN D

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 D₃ (1,25-(OH)₂D₃, also termed calcitriol). Calcitriol functions primarily to regulate calcium and phosphorous homeostasis.



Active calcitriol is derived from ergosterol (produced in plants) and from 7-dehydrocholesterol (produced in the skin). Ergocalciferol (vitamin D₂) is formed by UV (ultraviolet) irradiation of ergosterol. In the skin 7-dehydrocholesterol is converted to cholecalciferol (vitamin D₃) following UV irradiation.

Vitamin D₂ and D₃ are processed to D₂-calcitriol and D₃-calcitriol, respectively, by the same enzymatic pathways in the body. Cholecalciferol (or ergocalciferol) 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 D₃-25-hydroxylase generating 25-hydroxy-D₃ [25-(OH)D₃] which is the major circulating form of vitamin D. Conversion of 25-(OH)D₃ to its biologically active form, calcitriol, occurs through the activity of a specific D₃-1-hydroxylase present in the proximal convoluted tubules of the kidneys, and in bone and placenta. 25-(OH)D₃ can also be hydroxylated at the 24 position by a specific D₃-24-hydroxylase in the kidneys, intestine, placenta and cartilage.



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)₂D₃. 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

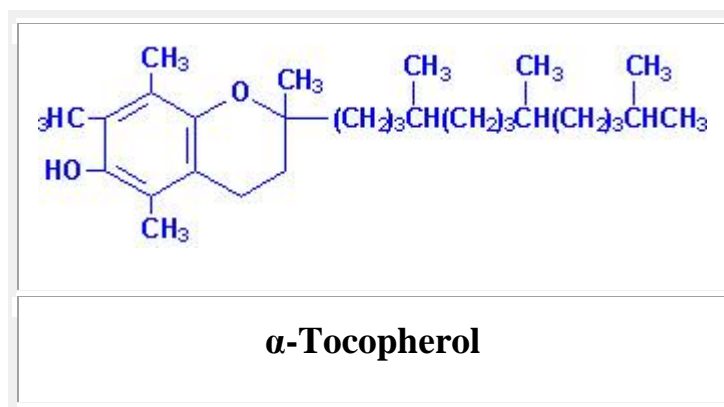
calcitriol and PTH are bone where they stimulate bone resorption and the kidneys where they inhibit calcium excretion by stimulating reabsorption by the distal tubules. The role of calcitonin in calcium homeostasis is to decrease elevated serum calcium levels by inhibiting bone resorption.

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.

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.



α -Tocopherol is the main source found in supplements and in the European diet, where the main dietary sources are olive and sunflower oils, while γ -tocopherol is the most common form in the American diet due to a higher intake of soybean and corn oil.

Tocotrienols, which are related compounds, also have vitamin E activity. All of these various derivatives with vitamin activity may correctly be referred to as

"vitamin E". Tocopherols and tocotrienols are fat-soluble antioxidants but also seem to have many other functions in the body.

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.

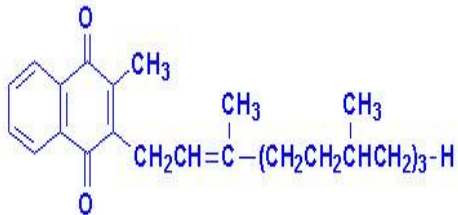
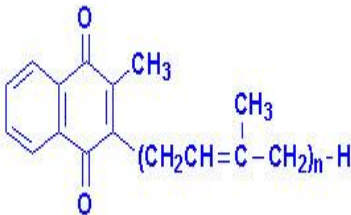

The major function of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen. In particular vitamin E is important for preventing peroxidation of polyunsaturated membrane fatty acids. The vitamins E and C are interrelated in their antioxidant capabilities. Active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

VITAMIN K

The K vitamins exist naturally as K₁ (phylloquinone) in green vegetables and K₂ (menaquinone) produced by intestinal bacteria and K₃ is synthetic menadione (vicasol). When administered, vitamin K₃ is alkylated to one of the vitamin K₂ forms of menaquinone.

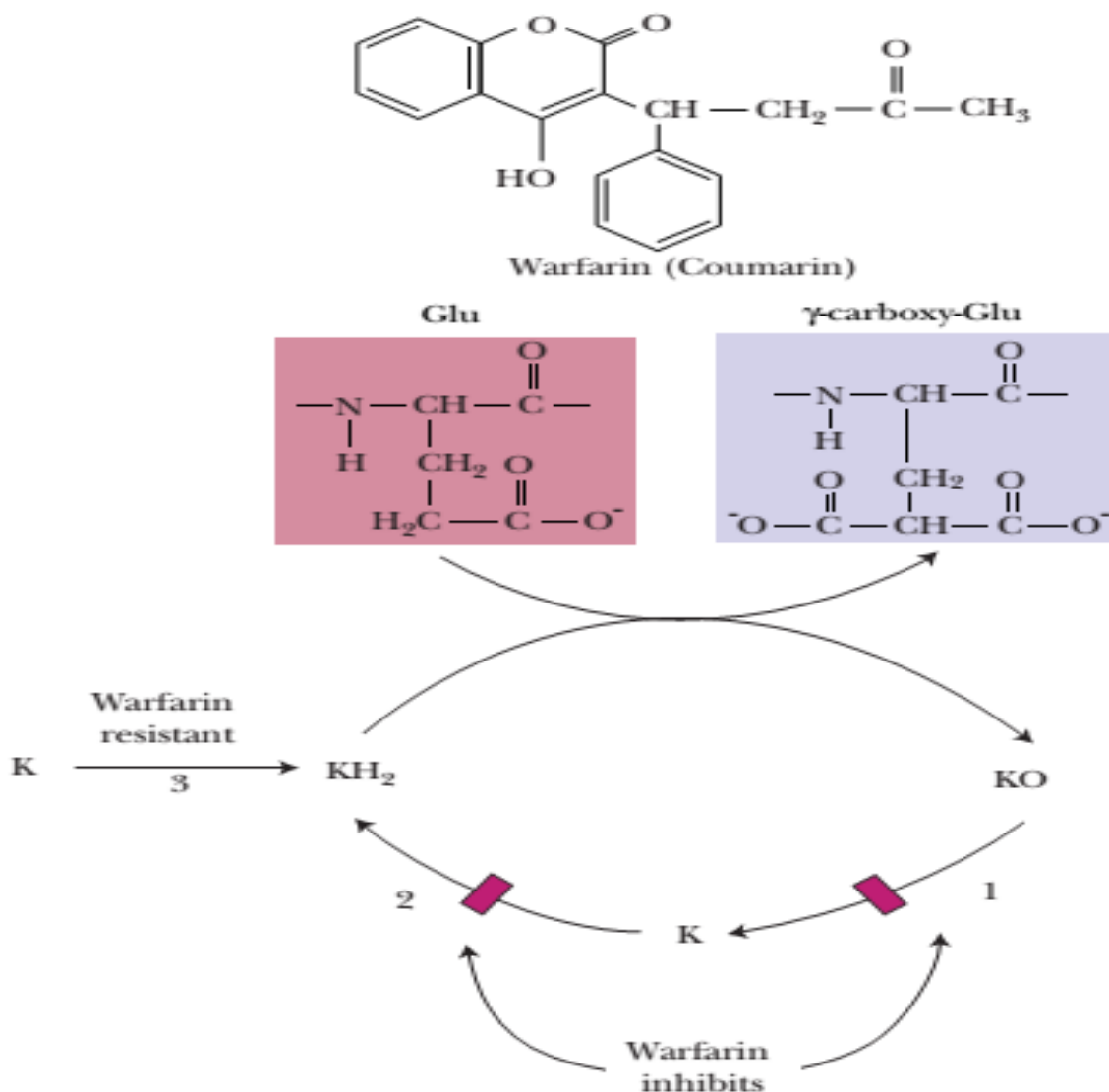
		
<p>Vitamin K₁</p>	<p>Vitamin K₂ "n" can be 6, 7 or 9 isoprenoid groups</p>	<p>Vitamin K₃ (Vicasol)</p>

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 residues. This modification is a carboxylation and the enzyme responsible requires vitamin K as a cofactor. The resultant modified protein residues are λ -carboxyglutamate. This process is most clearly understood for factor II, also called prothrombin. Prothrombin is modified preprothrombin. The λ -carboxyglutamate 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 dicoumarol based anticoagulants such as warfarin.

Mechanism action of warfarin is following:



The isoprene-derived molecule whose structure is shown here is known alternately as Coumarin and warfarin. By the former name, it is a widely prescribed anticoagulant. By the latter name, it is a component of rodent poisons. How can the same chemical species be used for such disparate purposes? The key to both uses lies in its ability to act as an antagonist of vitamin K in the body. Vitamin K stimulates the carboxylation of glutamate residues on certain proteins, including some proteins in the bloodclotting cascade. Carboxylation of these coagulation factors is catalyzed by a carboxylase that requires the reduced form of vitamin K

(vitamin KH₂), molecular oxygen, and carbon dioxide. KH₂ is oxidized to vitamin K epoxide, which is recycled to KH₂ by the enzymes vitamin K epoxide reductase (1) and vitamin K reductase (2, 3). Coumarin/warfarin exerts its anticoagulant effect by inhibiting vitamin K epoxide reductase and possibly also vitamin K reductase. This inhibition depletes vitamin KH₂ and reduces the activity of the carboxylase. Coumarin/warfarin, given at a typical dosage of 4 to 5 mg/day, prevents the deleterious formation in the bloodstream of small blood clots and thus reduces the risk of heart attacks and strokes for individuals whose arteries contain sclerotic plaques. Taken in much larger doses, as for example in rodent poisons, Coumarin/warfarin can cause massive hemorrhages and death.

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 K₃ is water soluble and absorbed irrespective of the presence of intestinal lipids and bile. Since the vitamin K₂ form is synthesized by intestinal bacteria, 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.

GROUP II. WATER-SOLUBLE VITAMINS

Group II is water-soluble vitamins:

- Group B: B₁ (thiamine), B₂ (riboflavin), B₃ or PP (nicotinamide, niacin), B₅ (pantothenic acid), B₆ (pyridoxine), B₇ or H (biotin), B₉ or Bc (folic acid), B₁₂ (cyanocobalamin) ;
- Vitamin C (ascorbic acid);
- Vitamin P (rutin and other bioflavonoids).

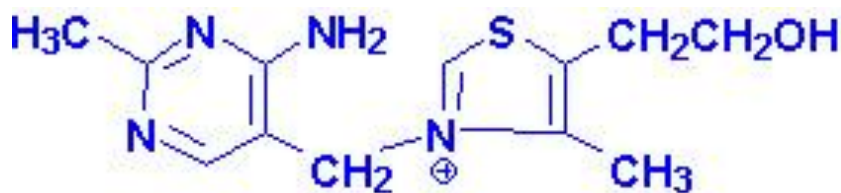
Water-soluble vitamins are usually functioning as precursors of coenzymes and prosthetic groups of enzymes. For example, coenzyme form of:

- Vitamin B₁ is TPP (thiamine pyrophosphate) (trade name - cocarboxylase);
- Vitamin B₂ is FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide);
- Vitamin B₃ is NAD⁺ (nicotinamide adenine dinucleotide) or NADP⁺ (nicotinamide adenine dinucleotide phosphate);
- Vitamin B₅ is Coenzyme A (coenzyme of acylation);
- Vitamin B₆ is PLP (pyridoxal phosphate);
- Vitamin B₉ is THFA (tetrahydrofolic acid);
- Vitamin B₁₂ is adenosylcobalamin and methylcobalamin.

Holoenzymes containing coenzymes (as its non-protein part) which are often vitamin derivatives perform multiple functions. For example, the first enzyme in gluconeogenesis pyruvate carboxylase uses biotin for carboxylation of pyruvate; but the transformation of the pyruvate to acetyl-CoA by pyruvate dehydrogenase complex requires five coenzymes: TPP, lipoic acid, CoA, FAD, NAD⁺. Since TPP is involved in this conversion first, pyruvate accumulation in cells of the nervous system (primarily) and then increase in pyruvate content in the blood and urine of patients in the case of vitamin B₁ deficiencies becomes obvious.

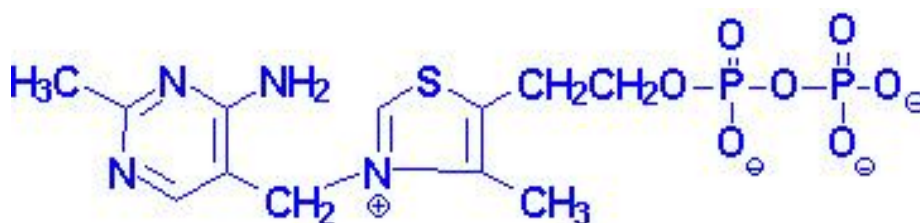
VITAMIN B₁

Vitamin B₁ is also known as thiamine.



Thiamine structure

Thiamine is derived from a substituted pyrimidine and a thiazole which are coupled by a methylene bridge. Thiamine is rapidly converted to its active form, **Thiamine Pyrophosphate (TPP)**, in the brain and liver by a specific enzymes, thiamine diphosphotransferase.



Thiamine pyrophosphate

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 thiamine 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 thiamine 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.

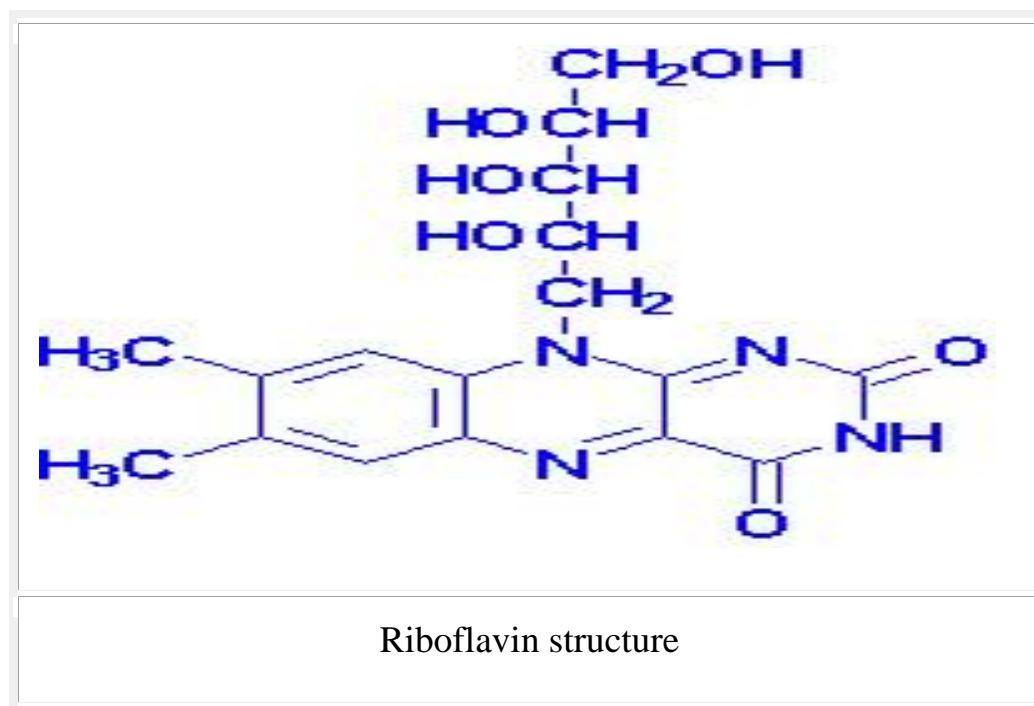
Clinical Significances of Thiamine Deficiency

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

The severe thiamine deficiency disease known as Beriberi, is the result of a diet that is carbohydrate rich and thiamine deficient. An additional thiamine deficiency related disease is known as Wernicke-Korsakoff syndrome. This disease is most commonly found in chronic alcoholics due to their poor dietetic lifestyles.

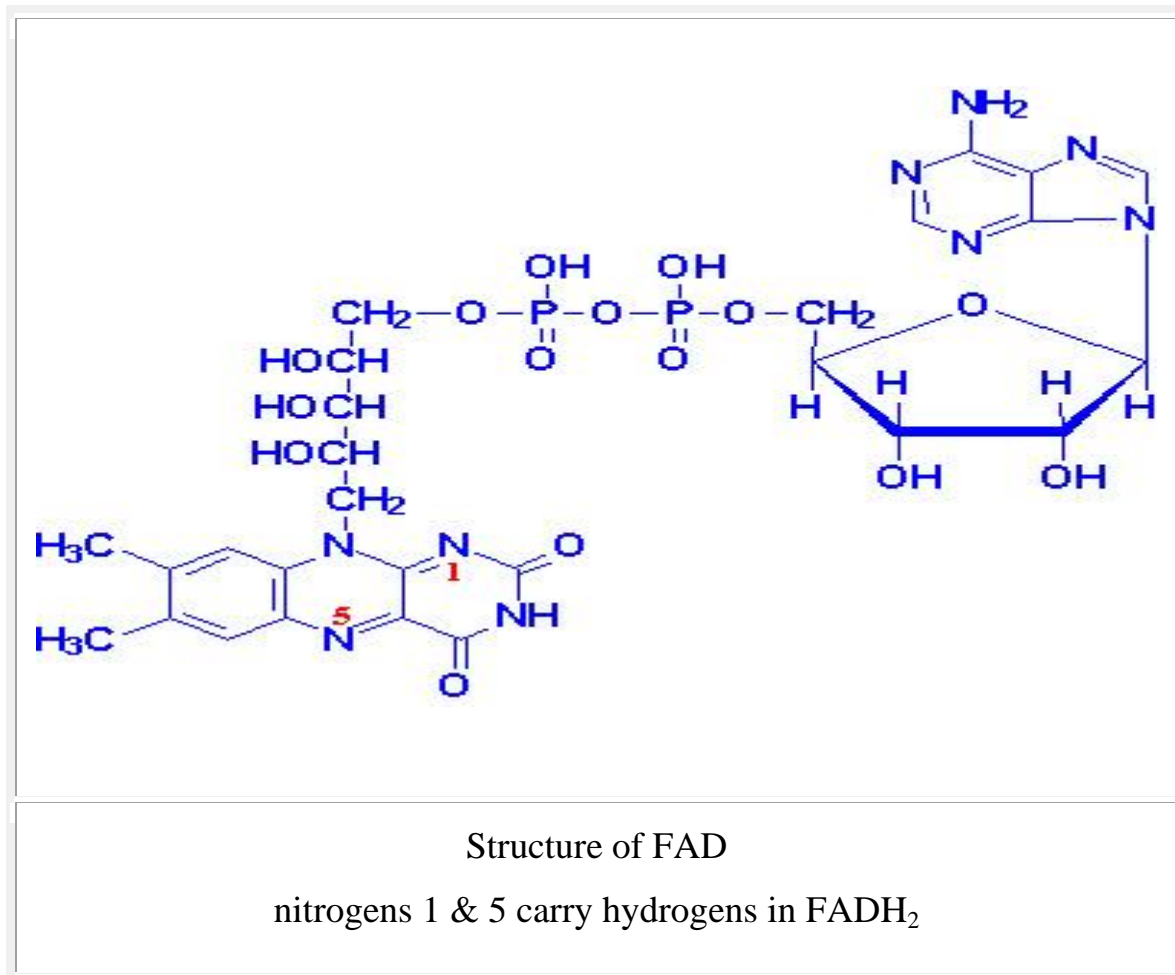
VITAMIN B₂

Vitamin B₂ is also known as riboflavin.



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, FMNH₂ and FADH₂, respectively.



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

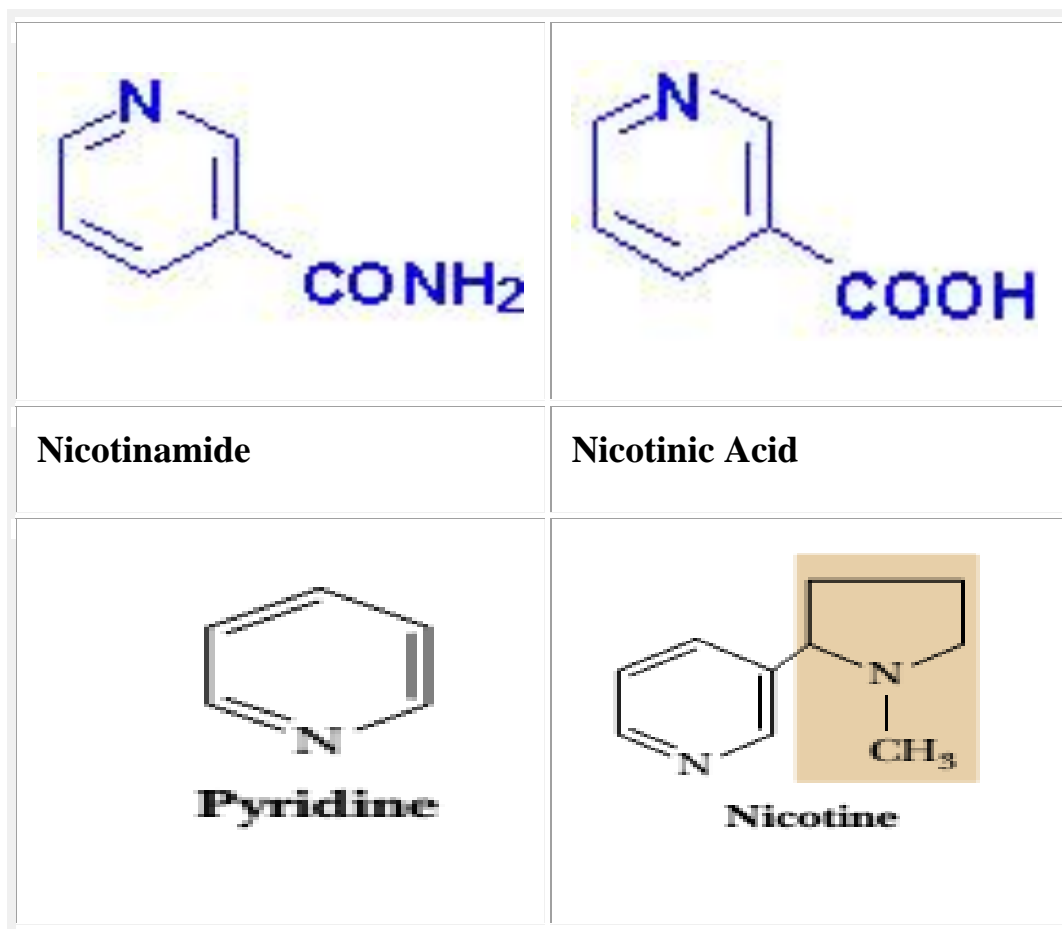
Clinical Significances of Riboflavin Deficiency

Riboflavin deficiencies are rare 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.

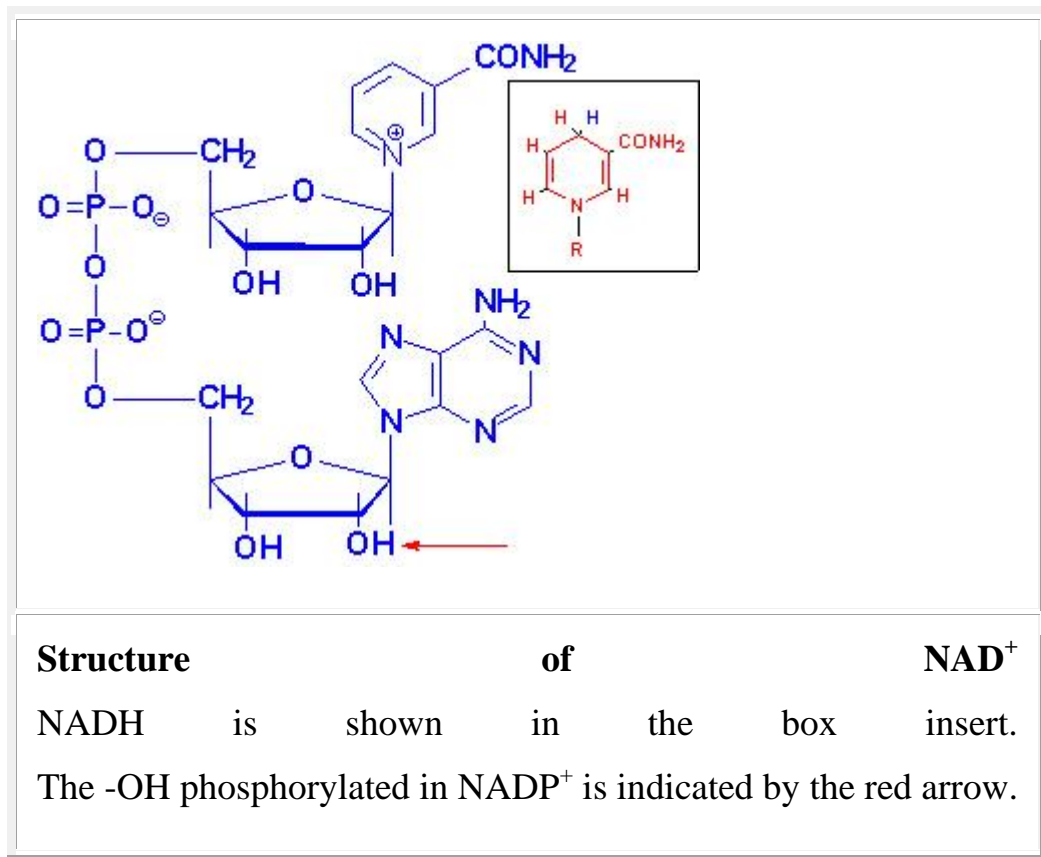
VITAMIN B₃.

Vitamin B₃ is also known as niacin (nicotinic acid and nicotinamide).



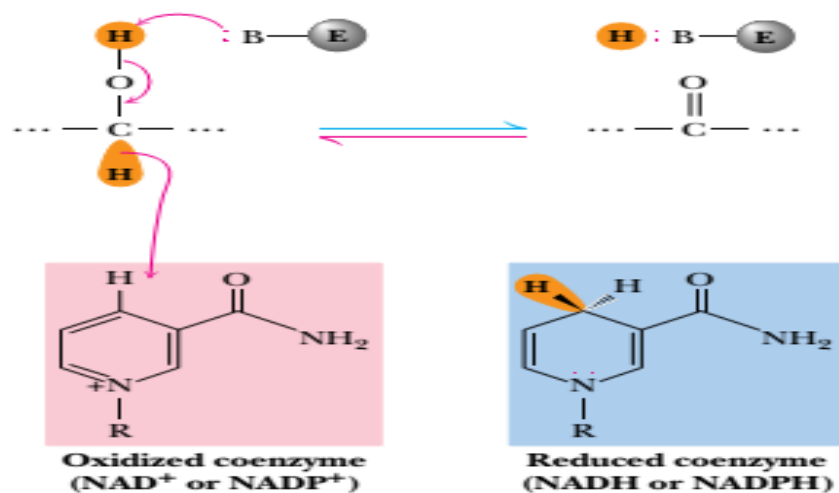
Both nicotinic acid and nicotinamide can serve as the dietary source of vitamin B₃. Niacin is required for the synthesis of the active forms of vitamin B₃, nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺). Both NAD⁺ and NADP⁺ function as cofactors for numerous dehydrogenase, e.g., lactate and malate dehydrogenases.

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 B₁, B₂ and B₆ which would be limiting in themselves on a marginal diet.



Examination of the structures of NADH and NADPH reveals that the 4-position of the nicotinamide ring is pro-chiral, meaning that while this carbon is not chiral, it would be if either of its hydrogens were replaced by something else.

As shown in following figure the hydrogen “projecting” out of the page toward you is the “pro-R” hydrogen because, if a deuterium is substituted at this position, the molecule would have the R-configuration. Substitution of the other hydrogen would yield an S-configuration.



An interesting aspect of the enzymes that require nicotinamide coenzymes is that they are stereospecific and with draw hydrogen from either the pro-R or the pro-S position selectively. This stereospecificity arises from the fact that enzymes (and the active sites of enzymes) are inherently asymmetric structures. These same enzymes are stereospecific with respect to the substrates as well.

The NAD- and NADP-dependent dehydrogenases catalyze at least six different types of reactions: simple hydride transfer, deamination of an amino acid to form an α -keto acid, oxidation of α -hydroxy acids followed by decarboxylation of the α -keto acid intermediate, oxidation of aldehydes, reduction of isolated double bonds, and the oxidation of carbon–nitrogen bonds (as with dihydrofolate reductase).

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**.

Pellagra is a disease characterized by dermatitis, diarrhea, and dementia, has been known for centuries. It was once prevalent in the southern part of the United States and is still a common problem in some parts of Spain, Italy, and Romania. Pellagra was once thought to be an infectious disease, but Joseph Goldberger showed early in this century that it could be cured by dietary actions. Soon thereafter, it was found that brewer's yeast would prevent pellagra in humans. Studies of a similar disease in dogs, called black tongue, eventually led to the identification of nicotinic acid as the relevant dietary factor. Elvehjem and his colleagues at the University of Wisconsin in 1937 isolated nicotinamide from liver, and showed that it and nicotinic acid could prevent and cure black tongue in dogs. That same year, nicotinamide and nicotinic acid were both shown to be able to cure

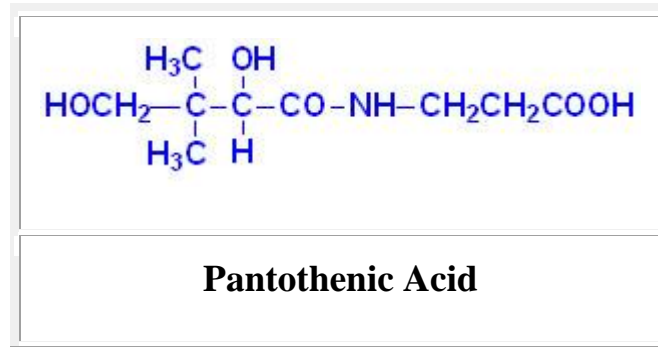
pellagra in humans. Interestingly, plants and many animals can synthesize nicotinic acid from tryptophan and other precursors, and nicotinic acid is thus not a true vitamin for these species. However, if dietary intake of tryptophan is low, nicotinic acid is required for optimal health. Nicotinic acid, which is beneficial to humans and animals, is structurally related to nicotine, a highly toxic tobacco alkaloid. In order to avoid confusion of nicotinic acid and nicotinamide with nicotine itself, niacin was adopted as a common name for nicotinic acid. Cowgill, at Yale University, suggested the name from the letters of three words—nicotinic, acid, and vitamin.

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.

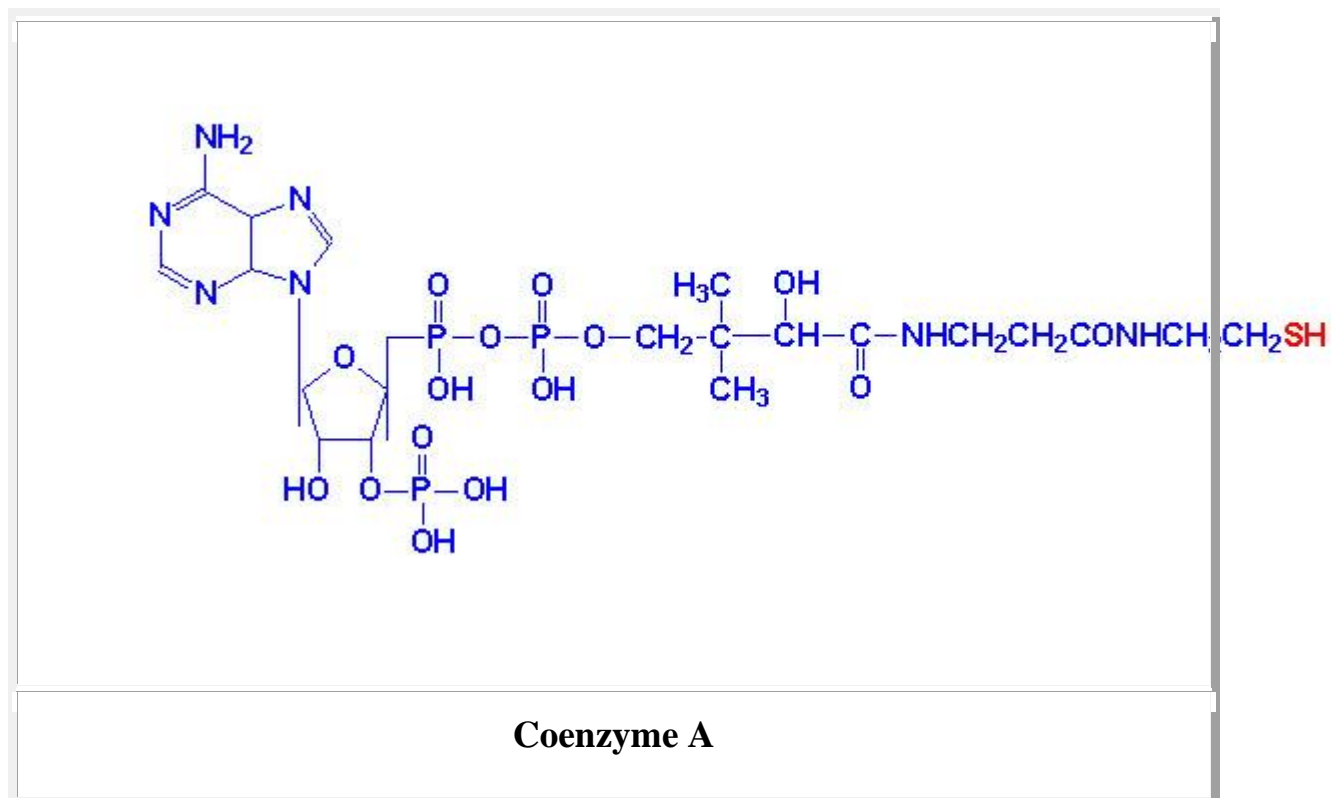
VITAMIN B₅.

Vitamin B₅ is also known as pantothenic acid.



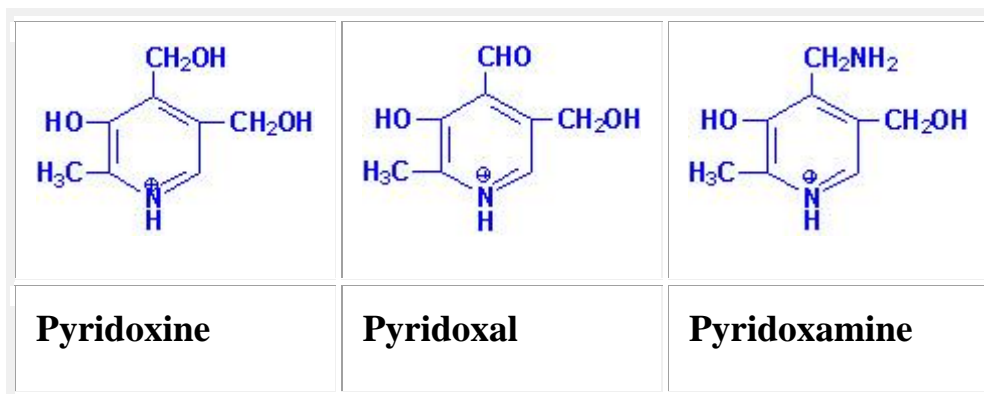
Pantothenic acid is formed from β-alanine and pantoic acid. Pantothenate is required for synthesis of coenzyme A, CoA and is a component of the acyl carrier protein (ACP) domain of fatty acid synthase. Pantothenate is, therefore, required for the metabolism of carbohydrate via the TCA cycle and all fats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

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.

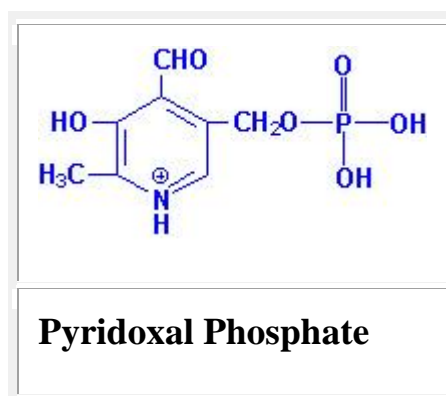


VITAMIN B₆

Vitamin B₆ are collectively known as pyridoxal, pyridoxamine and pyridoxine.

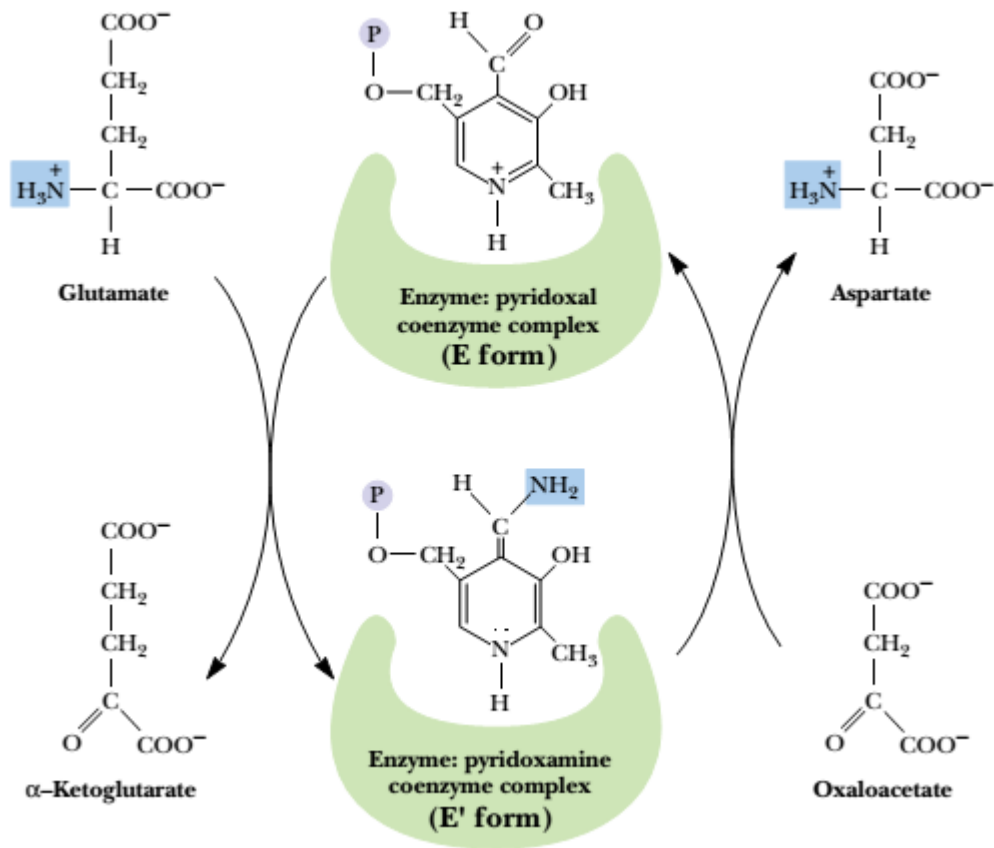


All three compounds are efficiently converted to the biologically active form of vitamin B₆, pyridoxal phosphate. This conversion is catalyzed by the ATP requiring enzyme, pyridoxal kinase.



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.

A specific example would be glutamate : aspartate aminotransferase. It is a pyridoxal phosphate – dependent enzyme. Glutamate : aspartate aminotransferase is an enzyme conforming to a double-displacement bisubstrate mechanism. The pyridoxal serves as the -NH₂ acceptor from glutamate to form pyridoxamine. Pyridoxamine is then the amino donor to oxaloacetate to form aspartate and regenerate the pyridoxal coenzyme form.

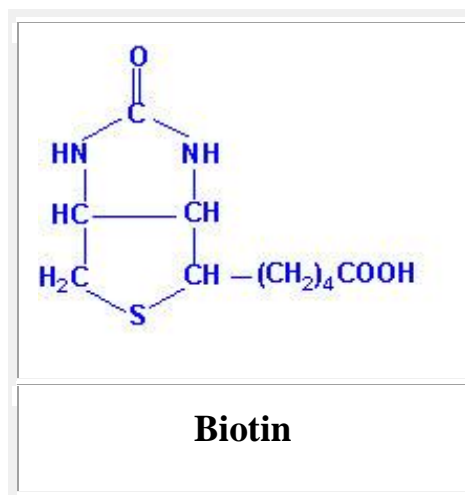


The requirement for vitamin B₆ in the diet is proportional to the level of protein consumption ranging from 1.4 - 2.0 mg/day for a normal adult. During pregnancy and lactation the requirement for vitamin B₆ increases approximately 0.6 mg/day.

Deficiencies of vitamin B₆ 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.

VITAMIN B₇

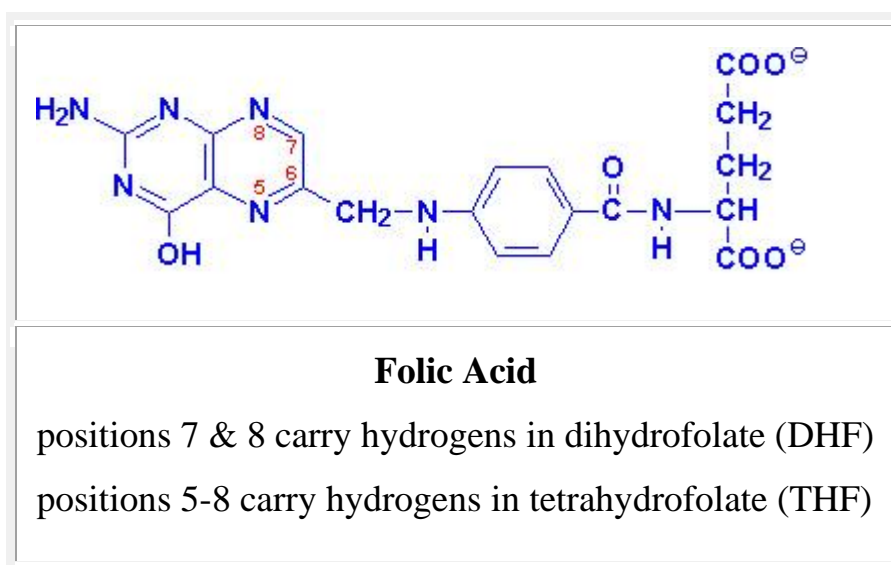
Vitamin B₇ is known as biotin.



Biotin is the cofactor required of 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.

VITAMIN B₉

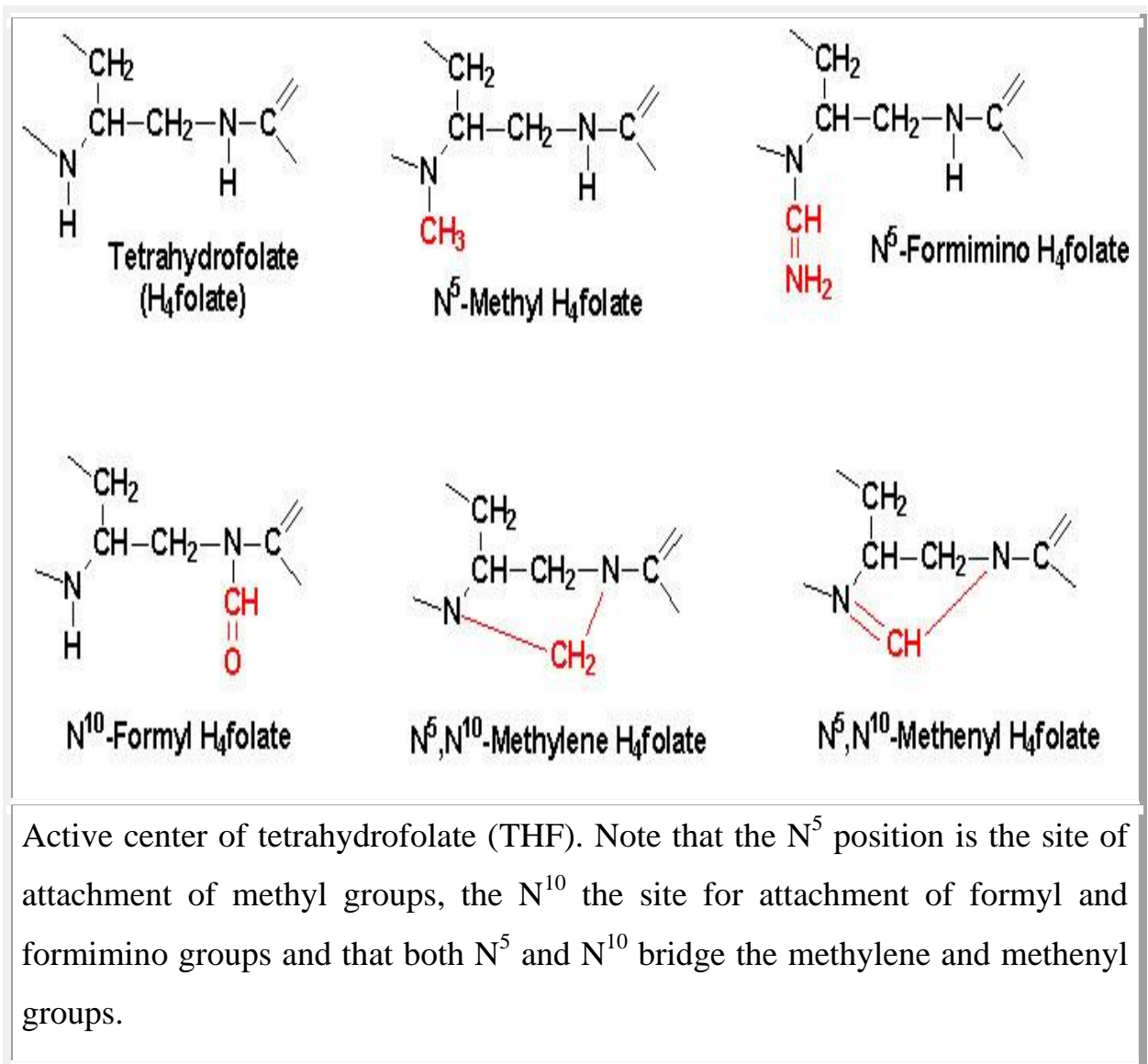
Vitamin B₉ is known as folic acid.



Folic acid is a conjugated molecule consisting of a pteridine ring structure linked to para-aminobenzoic acid (PABA) that forms pteronic acid. Folic acid itself is then generated through the conjugation of glutamic acid residues to pteronic 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 pteronic acid, thus, requiring folate intake in the diet.

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 laminal 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 H₄folate) through the action of dihydrofolate reductase (DHFR), an NADPH-requiring enzyme.

The function of THF derivatives is to carry and transfer various forms of one carbon units during biosynthetic reactions. The one carbon units are either methyl, methylene, methenyl, formyl or formimino 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 N⁵,N¹⁰-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin. The role of vitamin B₁₂ and N⁵-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 B₁₂ deficiency. The most pronounced effect of folate deficiency on

cellular processes is upon DNA synthesis. This is due to an 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 B₁₂ 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.

VITAMIN B₁₂

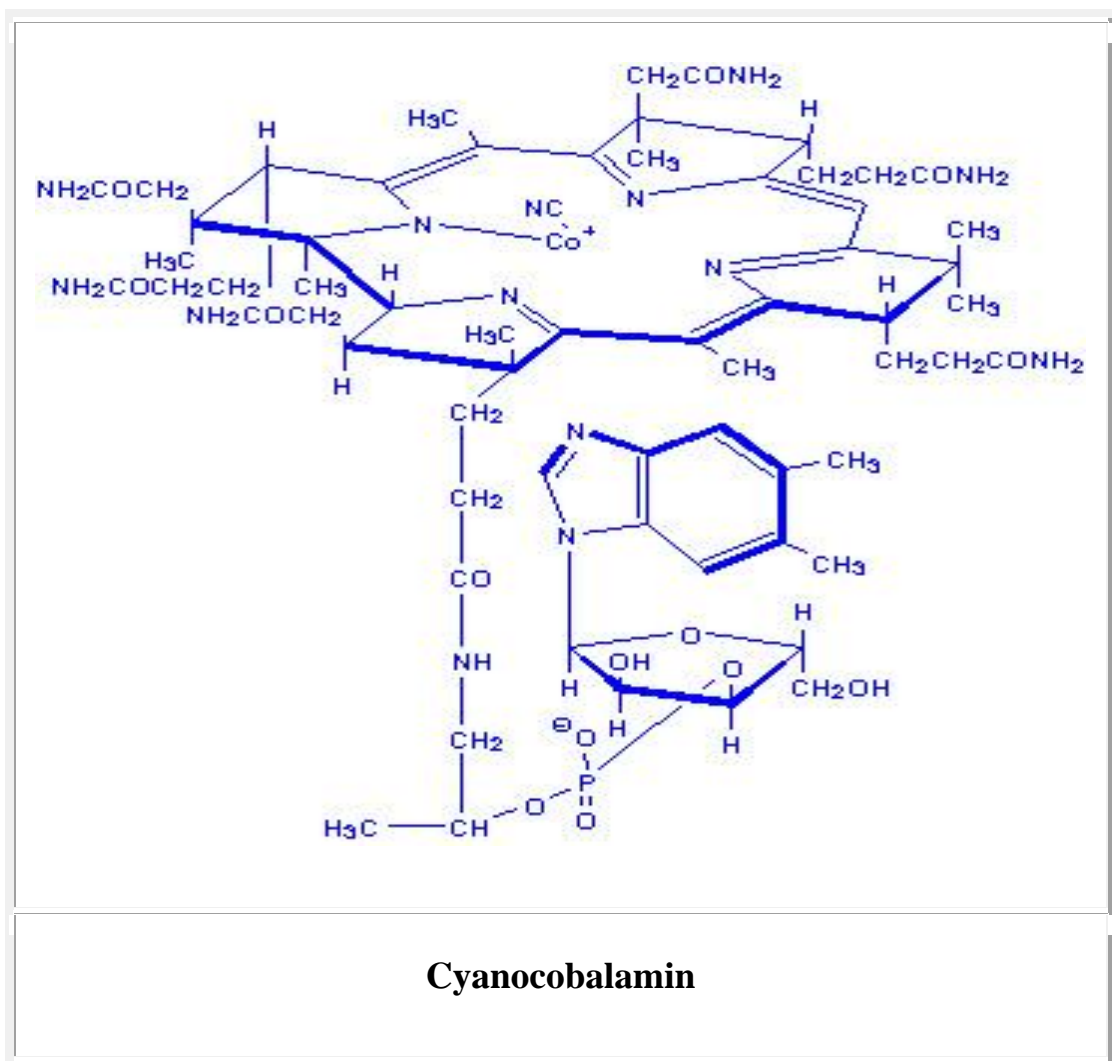
Vitamin B₁₂ is known as cobalamin.

Cobalamin is more commonly known as vitamin B₁₂. Vitamin B₁₂ is composed of a complex tetrapyrrol ring structure (corrin ring) and a cobalt ion in the center. Vitamin B₁₂ 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 B₁₂ 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 B₁₂ 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 B₁₂ catalyzes the conversion of homocysteine to methionine and is catalyzed by methionine synthase. This reaction results in the transfer of the methyl group from N⁵-methyltetrahydrofolate to hydroxycobalamin generating tetrahydrofolate (THF) and methylcobalamin during the process of the conversion.



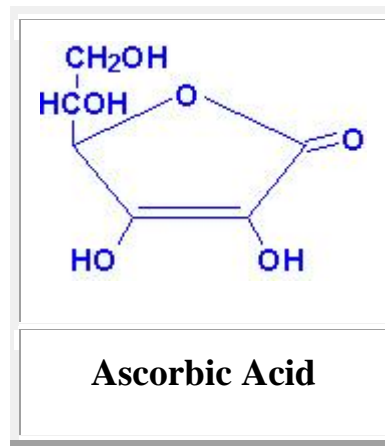
Clinical Significances of B₁₂ Deficiency

The liver can store up to six years worth of vitamin B₁₂, hence deficiencies in this vitamin are rare. Pernicious anemia is a megaloblastic anemia resulting from vitamin B₁₂ 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 B₁₂ on folate metabolism. When vitamin B₁₂ is deficient essentially all of the folate becomes trapped as the N⁵-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 B₁₂ deficiency and result from a progressive demyelination of nerve cells. The demyelination is thought to result from the increase in methylmalonyl-CoA that result from vitamin B₁₂ 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

VITAMIN C

Vitamin C is more commonly known as ascorbic acid. 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.

Scurvy results from a dietary vitamin C deficiency and involves the inability to form collagen fibrils properly. This is the result of reduced activity of prolyl hydroxylase, which is vitamin C-dependent. Scurvy leads to lesions in the skin and blood vessels, and, in its advanced stages, it can lead to grotesque disfiguration and eventual death. Although rare in the modern world, it was a disease well known to sea-faring explorers in earlier times who did not appreciate the importance of fresh fruits and vegetables in the diet.

Hydroxylation of proline residues is catalyzed by prolyl hydroxylase. The reaction requires α -ketoglutarate and ascorbic acid (fig 1).

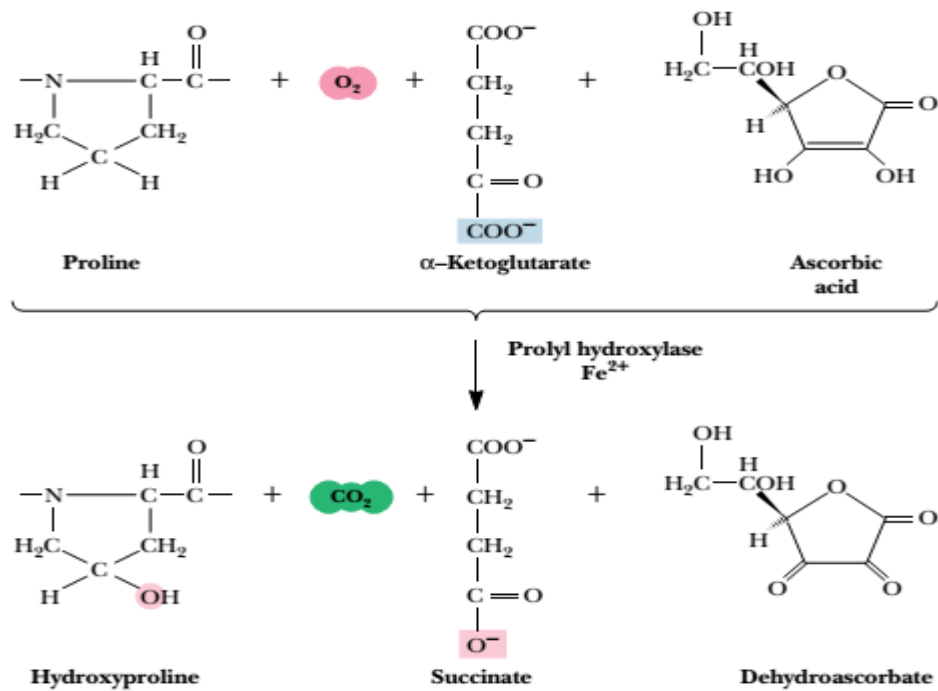


Figure 1. Hydroxylation of proline residues is catalyzed by prolyl hydroxylase. (The reaction requires α -ketoglutarate and ascorbic acid (vitamin C).

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 adrenocorticotrophic 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.

GROUP III. VITAMIN-LIKE SUBSTANCES

Group III is vitamin-like substances. They are separated:

- **fat-soluble:** Coenzyme Q (ubiquinone),
- **water-soluble** vitamins: B₄ (choline), B₈ (inositol), B_T or B₁₁ (carnitine), B₁₃ (orotic acid), B₁₅ (pangamic acid), U (S-methylmethionine), N (lipoic acid).

Most water-soluble vitamins must be supplied regularly with food, as they are quickly removed or destroyed in the body. Fat-soluble vitamins can be deposited in the body. Furthermore, they are poorly excreted, therefore, hypervitaminosis as diseases associated with high doses of fat-soluble vitamin intoxication of organism are observed. Such diseases are described for vitamins A and D.

Choline

Choline appears to be an essential nutrient for a number of animals and microorganisms that cannot synthesize adequate quantities to satisfy their requirements. Choline is a constituent of an important class of lipids called phospholipids, which form structural elements of cell membranes; it is a component of the acetylcholine molecule, which is important in nerve function. Choline also serves as a source of methyl groups ($-CH_3$ groups) that are required in various metabolic processes. The effects of a dietary deficiency of choline itself can be alleviated by other dietary compounds that can be changed into choline. Choline also functions in the transport of fats from the liver; for this reason, it may be called a lipotropic factor. A deficiency of choline in the rat results in an accumulation of fat in the liver. Choline-deficiency symptoms vary among species; it is not known if choline is an essential nutrient for humans since a dietary deficiency has not been demonstrated.

***Myo*-inositol**

The biological significance of *myo*-inositol has not yet been established with certainty. It is present in large amounts—principally as a constituent of phospholipids—in humans. Inositol is a carbohydrate that closely resembles glucose in structure; inositol can be converted to phytic acid, which is found in grains and forms an insoluble (and thus unabsorbable) calcium salt in the intestines of mammals. Inositol has not been established as an essential nutrient for humans; however, it is a required factor for the growth of some yeasts and fungi.

Para-aminobenzoic acid

Para-aminobenzoic acid (PABA) is required for the growth of several types of microorganisms; however, a dietary requirement by vertebrates has not been shown. The antimicrobial sulfa drugs (sulfanilamide and related compounds) inhibit the growth of bacteria by competing with PABA for a position in a coenzyme that is necessary for bacterial reproduction. Although a structural unit of folic acid, PABA is not considered a vitamin.

Carnitine

Carnitine is essential for the growth of mealworms. The role of carnitine in all organisms is associated with the transfer of fatty acids from the bloodstream to active sites of fatty acid oxidation within muscle cells. Carnitine, therefore, regulates the rate of oxidation of these acids; this function may afford means by which a cell can rapidly shift its metabolic patterns (e.g., from fat synthesis to fat breakdown). Synthesis of carnitine occurs in insects and in higher animals; therefore, it is not considered a true vitamin.

Lipoic acid

Lipoic acid has a coenzyme function similar to that of thiamin. Although it is apparently an essential nutrient for some microorganisms, no deficiency in mammals has been observed; therefore, lipoic acid is not considered a true vitamin.

Bioflavonoids

The bioflavonoids once were thought to prevent scurvy and were designated as vitamin Pc, but additional evidence refuted this claim.

HEALTH EFFECTS OF VITAMINS AND ANTIVITAMINS

Currently, vitamins and antivitamins widely used to prevent and treat a variety of disorders of metabolism. For example:

- vitamin K or menadione, or vicasol (both are synthetic water-soluble analogue of vitamin K) are prescribed to stimulate the synthesis (specifically post-translational γ -carboxylation of glutamic acid residues) such enzymes of coagulation system as factors II (prothrombin), VII, IX and X in the liver. They are usually used after long-term antibiotic treatment (if there is increased bleeding with small injuries, increase in blood clotting time) and in the preoperative period;

- vitamin K antagonist (antivitamin K) dicumarol reduces the efficiency of the blood coagulation promoting blood thinning thereby it use for the treatment of blood clotting diseases, in particular, thrombosis, thrombophlebitis;

- Vitamin A and its derivatives like retinol acetate are used for treating of vitamin A deficiency. For example, they can be administered a patient in order to restore his vision if the patient suffers from vision impairment hemeralopia (night blindness, twilight vision impairment), age-related glaucoma, cataracts etc. Vitamin A drug is also used for skin conditions including acne, eczema, psoriasis, cold sores, wounds, burns, sunburn. It is also used for gastrointestinal ulcers, gum disease, urinary tract infections, diseases of the nervous system;

- drug isoniazid which is antivitamin nicotinic acid and pyridoxine is used In the treatment of patients with pulmonary tuberculosis;

- the structural analogue of vitamin B₂ acrichine is formerly widely used as an antimalarial drug but superseded by chloroquine in recent years. It has also been used as an anthelmintic (in enterobiasis) and in the treatment of giardiasis and malignant effusions. The mechanism action of the drug is based on preventing of

microorganism FAD(FMN)-dependent dehydrogenases;

- Ascorutinum is recommended to use as a more effective drug in comparison with ascorbic acid for patients with reduced immunity and frequent colds. Vitamin C (Ascorbic acid) is involved in the hydroxylation of prolyl- and lysyl residues by prolyl 3(4)-hydroxylase and lysyl 5-hydroxylase during collagen synthesis. Effect of the vitamin C is enhanced by vitamin P, which stabilizes the ground substance of fibrous connective tissue in way of hyaluronidase inhibition. Ascorutinum can be recommended in case of bleeding gums, petechial hemorrhages;

- Sulfonamide drugs are folic acid antivitamin. They are structurally resemble paraaminobenzoic acid and due to this similarity it is displaced from its complex with the enzyme synthesizing folic acid. This leads to the inhibition of bacterial growth. This mechanism of action of sulfonamides allows their use as antibacterial agents;

- Pregnant women with a history of several miscarriages is assigned the therapy including α -tocopherol (vitamin E) vitamin supplements using, It contributes to the childbearing. Furthermore, tocopherol acetate, vitamin preparation is usually given in the course of radiation therapy, since this substance has a distinct radioprotective membrane stabilizing action due to its antioxidant activity;

- Derivatives of pyridoxine (vitamin B₆) are used as neurotrophic agents for the correction of mental retardation in childre; in cases of mental disorders in adults; as neuroprotective agents in rehabilitation of patients with stroke and other pathological conditions. The positive effects of pyridoxine is explained by its use as a precursor of PLP that is prosthetic group of the enzyme glutamate decarboxylase in neurons. The enzyme carries out inhibitory neurotransmitter GABA formation.

- Cabbage and potato juices rich in vitamin U are recommended to drink for patient with duodenal ulcer after the therapy course. Whether taken as a supplement or from foods, vitamin U has been shown to be able to treat a variety

of gastrointestinal conditions, including ulcerative colitis, acid reflux, and peptic ulcers. It may also be able to treat skin lesions, improve the symptoms of diabetes, and strengthen the immune system. Some studies show that it can also help prevent liver damage by protecting the organ from the effects of high doses of acetaminophen. Additionally, it may be able to reduce allergies and sensitivities to cigarette smoke and improve cholesterol levels.

The aforementioned examples are only a small part of the use of vitamins and their derivatives in medicine.

Therefore, knowledge of the biochemical basis of vitaminology is of great importance for future doctors.

METHODS USED IN VITAMIN RESEARCH

Determination of vitamin requirements

If a specific factor in food is suspected of being essential for the growth of an organism (either by growth failure or some other clinical symptoms that are alleviated by adding a specific food to the diet) a systematic series of procedures is used to characterize the factor.

The active factor is isolated from specific foods and purified; then its chemical structure is determined, and it is synthesized in the laboratory. Structural determination and synthesis, which may be achieved only after long and intensive research, must be completed before the function and the quantitative requirements of the factor can be established accurately. Established organic and analytical chemical procedures are used to determine the structure of the factor and to synthesize it.

Biological studies may be performed to determine functions, effects of deprivation, and quantitative requirements of the factor in various organisms. The development in an organism of a deficiency either by dietary deprivation of the vitamin or by administration of a specific antagonist or compound that prevents the normal function of the vitamin (antivitamin) often is the method used. The obvious

effects (e.g., night blindness, anemia, dermatitis) of the deficiency are noted. Less obvious effects may be discovered after microscopic examination of tissue and bone structures. Changes in concentrations of metabolites or in enzymatic activity in tissues, blood, or excretory products are examined by numerous biochemical techniques. The response of an animal to a specific vitamin of which it has been deprived usually confirms the deficiency symptoms for that vitamin. Effects of deprivation of a vitamin sometimes indicate its general physiological function, as well as its function at the cellular level. Biochemical function often is studied by observing the response of tissue enzymes (removed from a deficient host animal) after a purified vitamin preparation is added. The functions of most of the known vitamins have been reasonably well defined; however, the mechanism of action has not yet been established for some.

The procedure for determining the amount of a vitamin required by an organism is less difficult for microorganisms than for higher forms; in microorganisms, the aim is to establish the smallest amount of a vitamin that produces maximal rate of multiplication of the organisms when it is added to the culture medium. Among vertebrates, particularly humans, a number of procedures are used together to provide estimates of the vitamin requirement. These procedures include determinations of: the amount of a vitamin required to cure a deficiency that has been developed under controlled, standard conditions; the smallest amount required to prevent the appearance of clinical or biochemical symptoms of the deficiency; the amount required to saturate body tissues (i.e., to cause “spillover” of the vitamin in the urine; valid only with the water-soluble vitamins); the amount necessary to produce maximum blood levels of the vitamin plus some tissue storage (applicable only to the fat-soluble vitamins, particularly vitamin A); the amount required to produce maximum activity of an enzyme system if the vitamin has a coenzyme function; the actual rate of utilization, and hence the requirement, in healthy individuals (as indicated by measuring the excreted breakdown products of radioisotope-labeled vitamins).

The above procedures are practical only with small groups of animals or human subjects and thus are not entirely representative of larger populations of a particular species. A less precise, but more representative, method used among human populations involves comparing levels of dietary intake of a vitamin in a population that shows no deficiency symptoms with levels of intake of the vitamin in a population that reveals clinical or biochemical symptoms. The data for dietary intakes and incidence of deficiency symptoms are obtained by surveys of representative segments of a population.

Determination of vitamin sources

A quantitative analysis of the vitamin content of foodstuffs is important in order to identify dietary sources of specific vitamins (and other nutrients as well). Three methods commonly used to determine vitamin content are described below.

Physicochemical methods

The amount of vitamin in a foodstuff can be established by studying the physical or chemical characteristics of the vitamin—e.g., a chemically reactive group on the vitamin molecule, fluorescence, absorption of light at a wavelength characteristic of the vitamin, or radioisotope dilution techniques. These methods are accurate and can detect very small amounts of the vitamin. Biologically inactive derivatives of several vitamins have been found, however, and may interfere with such determinations; in addition, these procedures also may not distinguish between bound (i.e., unavailable) and available forms of a vitamin in a food.

Microbiological assay

Microbiological assay is applicable only to the B vitamins. The rate of growth of a species of microorganism that requires a vitamin is measured in growth media that contain various known quantities of a foodstuff preparation containing unknown amounts of the vitamin. The response (measured as rate of growth) to the unknown amounts of vitamin is compared with that obtained from a known quantity of the pure vitamin. Depending on the way in which the food

sample was prepared, the procedure may indicate the availability of the vitamin in the food sample to the microorganism.

Animal assay

All of the vitamins, with the exception of vitamin B₁₂, can be estimated by the animal-assay technique. One advantage of this method is that animals respond only to the biologically active forms of the vitamins. On the other hand, many other interfering and complicating factors may arise; therefore, experiments must be rigidly standardized and controlled. Simultaneous estimates usually are made using a pure standard vitamin preparation as a reference and the unknown food whose vitamin content is being sought; each test is repeated using two or more different amounts of both standard and unknown in the assays listed below.

In a growth assay, the rat, chick, dog (used specifically for niacin), and guinea pig (used specifically for vitamin C) usually are used. One criterion used in a vitamin assay is increase in body weight in response to different amounts of a specific vitamin in the diet. There are two types of growth assay. In a prophylactic growth assay, the increase in weight of young animals given different amounts of the vitamin is measured. In a curative growth assay, weight increase is measured in animals first deprived of a vitamin and then given various quantities of it. The curative growth assay tends to provide more consistent results than the prophylactic technique.

In a reaction time assay, an animal is first deprived of a vitamin until a specific deficiency symptom appears; then the animal is given a known amount of a food extract containing the vitamin, and the deficiency symptom disappears within a day or two. The time required for the reappearance of the specific symptoms when the animal again is deprived of the vitamin provides a measure of the amount of vitamin given originally. The graded response assay, which may be prophylactic or curative, depends on a characteristic response that varies in degree with the vitamin dosage. An example of this technique is an assay for vitamin D in which the measured ash content of a leg bone of a rat or chick is used to reflect the amount of bone calcification that occurred as a result of administration of a

specific amount of vitamin D. In an all-or-none assay, the degree of response cannot be measured; an arbitrary level is selected to separate positive responses from negative ones. The percent of positively reacting animals provides a measure of response; i.e., vitamin E can be measured by obtaining the percent of fertility in successfully mated female rats.

Clinical and laboratory testing of vitamin deficiency

1. Quantitative determination of ascorbic acid.

THE PRINCIPLE OF THE METHOD:

The quantitative determination of vitamin C is based on its capacity to restore 2, 6-dichlorophenolindophenole (DCIP). While vitamin C is in titrated solution, the poured DCIP will become colourless due to the formation of the restored form. As soon as all the quantity of vitamin C being in the solution is oxidized the titrated solution becomes pink due to the formation of the DCIP restorative form.

1.1. The determination of ascorbic acid concentration in vegetables (a potato, cabbage).

THE COURSE OF THE WORK:

Crush 5 g of potato (cabbage or other product) with a scalpel and pound it in a mortar, add 3 drops of 10 % hydrochloric acid solution and gradually 15 ml of distilled water. The mass received pour into a flask for titration. Titrate by 0.001 N DCIP solution up to the appearance of pink coloring, which will not disappear within 30 sec.

The calculation will be carried out according to the formula:

$$X = \frac{0.088 \cdot A \cdot 100}{5} \text{ mg \%}, \text{ where}$$

X - the content of vitamin C, mg %;

0.088 - the equivalent of ascorbic acid, which is titrated by 0,001N DCIP solution;

A - the quantity of DCIP (ml), used for titration;

100 - recalculation at 100 g of the product;

5 - quantity (g) of the product taken for the analysis.

Compare the received results with the content of vitamin C in foodstuff: in potatoes (6-20 mg %), cabbage (20-50 mg %), apples (20-40 mg%), lemons (40-55 mg%), needles (150-250 mg %), onions (30 mg %), parsley (150 mg %), cauliflower (70 mg %).

1.2. Determination of ascorbic acid content in the urine.

THE COURSE OF THE WORK:

Pour 10 ml of the urine into a flask for titration and add 10 ml of distilled water, then add 20 drops of 10 % hydrochloric acid. Titrate from the tube 0.001N of DCIP solution up to permanent pink coloring. Calculate daily excretion of vitamin C according to the formula:

$$X = \frac{0.088 \cdot A \cdot B}{C}, \text{ where}$$

X - daily excretion of vitamin C, in mg;

0.088 -the equivalent of an ascorbic acid, which is titrated with 1 ml 0.001 N DCIP solution

A – the volume of the indicator spent for titration;

B - daily average volume of the urine: men - 1500ml, women -1200 ml;

C - urine volume taken for titration.

The significance of vitamin C determination in the blood plasma and urine:

In norm the content of vitamin C in the urine is 20-30 mg / daily.

It is very important to define this index during the stage: the latent form of vitamin C Hypovitaminosis. The patient drinks the ascorbic acid - glucose solution, containing a daily norm of vitamin C (correlated with the patient's age). In 2-3 hours later the vitamin C concentration is determined in the patient's urine. If the result correlates with normal value, you can say about the latent form of vitamin C Hypovitaminosis.

2. Qualitative reactions for vitamins C and B₁.

2.1. Qualitative reaction for vitamin C.

THE PRINCIPLE OF THE METHOD:

The qualitative revealing of vitamin C is based on its capacity to restore $K_3[Fe(CN)_6]$ and the methylenic blue.

THE COURSE OF THE WORK:

a) Add 1 drop of 10 % NaOH solution into 5 drops of 1% vitamin C solution, some grains of $K_3[Fe(CN)_6]$ and mix. Then add 3 drops of 10 % hydrochloric acid solution and 1 drop of 1 % $FeCl_3$ solution. The residue $Fe_4[Fe(CN)_6]_3$ drops out. For the control test: repeat the course of the work, using distilled water instead of vitamin C.

b) Add 1 ml of methylenic blue into 1 ml of 1 % ascorbic acid solution. Put the test tube in a boiling water bath. Some time later you can see the decolorizing of the mixture.

2.2. Qualitative reaction for vitamin B₁.

THE PRINCIPLE OF THE METHOD:

Thiamine is oxidized by action of $K_3[Fe(CN)_6]$ solution to thiochrome. There is a fluorescence of thiochrome solution under UV-rays.

THE COURSE OF THE WORK:

Pour into a test tube: 1 drop of 5% thiamine solution, 5-10 drops of 10% NaOH solution, 1-2 drops of $K_3[Fe(CN)_6]$ solution and shake. Then use a fluoroscope to see the blue fluorescence of obtained thiochrome solution.

3. The determination of pyruvate content in the urine

THE PRINCIPLE OF THE METHOD:

Pyruvic acid (PA), reacting with 2,4-dinitrophenylhydrazine in alkaline environment, forms hydrazone derivatives coloured yellow. The intensity of colouring is proportional to PA concentration.

THE COURSE OF THE WORK:

Use dry test tubes, pipettes and cuvettes. Take 3 test tubes, add 1 ml of distilled water into each of two test tubes, and add to the 3-rd one 1 ml of the urine. Then pour 1 ml of 2,5% KOH alcoholic solution into every test tube, mix the content of all test tubes for 1 minute, pour 0,5 ml of 0,1% 2,4-dinitrophenylhydrazine solution into each of them. Mix and let them stay for 15 minutes on the table. After that the optical density of a test sample is measured against control test in cuvettes (5 mm) using a blue colour filter. The content of PA (mcg/ml) is determined using the graph (A).

Calculation by the formula:

$$[PA] \text{ mg/day} = A \cdot 1,5 \text{ (or } 1,2), \text{ where}$$

A - index of PA according to the graph;

1,5 (or 1,2) - the factor that correlates with diuresis for men or for women.

Normal PA content in the urine is 10-25 mg / day (113,7-283,9 $\mu\text{mol/day}$).

Clinical significance:

A large quantity of PA is accumulated in blood plasma and is excreted with urine during B1 hypovitaminosis in human organism. The content of this acid increases in the urine during diabetes mellitus, cardiac insufficiency, pituitary-

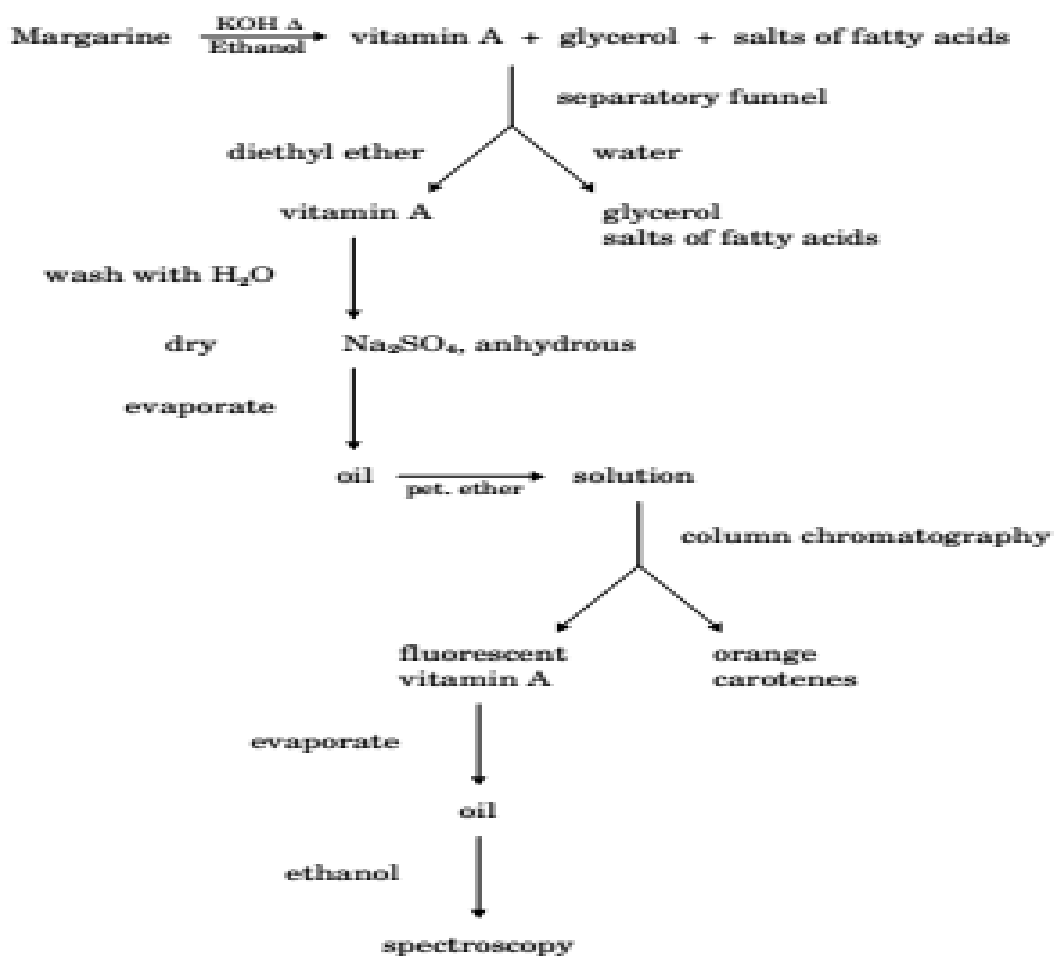
adrenal system superstimulation. The quantity of pyruvic acid increases during the drugs treatment: camphor, strychnine, adrenalin.

The content of pyruvic acid reduces during anesthesia.

4. Analysis of vitamin A in margarine

THE COURSE OF THE WORK:

The analysis of vitamin A requires a multistep process. In order that you should be able to follow the step-by-step procedure, a flow chart is provided here:



1. Margarine is largely fat. In order to separate vitamin A from the fat in margarine, first the sample must be saponified. This converts the fat to water-soluble products, glycerol and potassium salts of fatty acids. Vitamin A can be extracted by diethyl ether from the products of the saponification process. To start, weigh a cover glass to the nearest 0.1 g.

Report this weight on the Report Sheet (1). Add approximately 10 g of margarine to the watch glass. Record the weight of watch glass plus sample to the

nearest 0.1 g on your Report Sheet (2). Transfer the sample from the watch glass into a 250-mL Erlenmeyer flask with the aid of a glass rod, and wash it in with 75 mL of 95% ethanol. Add 25 mL of 50% KOH solution. Cover the Erlenmeyer flask loosely with a cork and put it on an electric hot plate. Bring it gradually to a boil. Maintain the boiling for 5 min. with an occasional swirling of the flask using tongs. The stirring should aid the complete dispersal of the sample. Remove the Erlenmeyer from the hot plate and let it cool to room temperature (approximately 20 min.).

CAUTION!

50% KOH solution can cause burns on your skin. Handle the solution with care, do not spill it. If a drop gets on your skin, wash it immediately with copious amounts of water.

Use gloves when working with this solution.

CAUTION!

Diethyl ether is very volatile and flammable. Make certain that there are no open flames, not even a hot electrical plate in the vicinity of the operation.

2. While the sample is cooling, prepare a chromatographic column. Take a 25-mL buret.

Add a small piece of glass wool. With the aid of a glass rod, push it down near the stopcock. Add 15–16 mL of petroleum ether to the buret. Open the stopcock slowly, and allow the solvent to fill the tip of the buret. Close the stopcock. You should have 12–13 mL of petroleum ether above the glass wool. Weigh about 20 g of alkaline aluminum oxide (alumina) in a 100-mL beaker. Place a small funnel on top of your buret. Pour the alumina slowly, in small increments, into the buret. Allow it to settle to form a 20-cm column. Drain the solvent but do not allow the column to run dry.

Always have at least 0.5 mL clear solvent on top of the column. If the alumina adheres to the walls of the buret, wash it down with more solvent.

3. Transfer the solution (from your reaction in step no. 1) from the Erlenmeyer flask to a 500-mL separatory funnel. Rinse the flask with 30 mL of

distilled water and add the rinsing to the separatory funnel. Repeat the rinsing two more times. Add 100 ml of diethyl ether to the separatory funnel. Close the separatory funnel with the glass stopper. Shake the separatory funnel vigorously. (See Exp. 37 Fig. 37.1 for technique.)

Allow it to separate into two layers. Drain the bottom aqueous layer into an Erlenmeyer flask. Add the top (diethyl ether) layer to a second clean 250-mL Erlenmeyer flask. Pour back the aqueous layer into the separatory funnel. Add another 100-mL portion of diethyl ether. Shake and allow it to separate into two layers. Drain again the bottom (aqueous) layer and discard. Combine the first diethyl ether extract with the residual diethyl ether extract in the separatory funnel. Add 100 mL of distilled water to the combined diethyl ether extracts in the separatory funnel. Agitate it gently and allow the water to drain. Discard the washing.

4. Transfer the diethyl ether extracts into a clean 300-mL beaker. Add 3–5 g of anhydrous

Na_2SO_4 and stir it gently for 5 min. to remove traces of water. Decant the diethyl ether extract into a clean 300-mL beaker. Add a boiling chip or a boiling stick. Evaporate the diethyl ether solvent to about 25 mL volume by placing the beaker in the hood on a steam bath. Transfer the sample to a 50-mL beaker and continue to evaporate on the steam bath until an oily residue forms. Remove the beaker from the steam bath. Cool it in an ice bath for 1 min. Add 5 mL of petroleum ether and transfer the liquid (without the boiling chip) to a 10-mL volumetric flask. Add sufficient petroleum ether to bring it to volume.

5. Add 5 mL of extracts in petroleum ether to your chromatographic column. By opening the stopcock drain the sample into your column, but take care not to let the column run dry. (Always have about 0.5 mL liquid on top of the column.) Continue to add solvent to the top of your column. Collect the eluents in a beaker. First you will see the orange-colored carotenes moving down the column. With the aid of a UV lamp, you can also observe a fluorescent band following the carotenes. This fluorescent band contains your vitamin A. Allow all the orange color band to

move to the bottom of your column and into the collecting beaker. When the fluorescent band reaches the bottom of the column, close the stopcock. By adding petroleum ether on the top of the column continuously, elute the fluorescent band from the column into a 25-mL graduated cylinder. Continue the elution until all the fluorescent band has been drained into the graduated cylinder. Close the stopcock, and record the volume of the eluate in the graduated cylinder on your Report Sheet (4). Add the vitamin A in the petroleum ether eluate to a dry and clean 50-mL beaker. Evaporate the solvent in the hood on a steam bath. The evaporation is complete when an oily residue appears in the beaker.

Add 5 mL of absolute ethanol to the beaker. Transfer the sample into a 10-mL volumetric flask and bring it to volume by adding absolute ethanol.

6. Place your sample in a 1-cm length quartz spectroscopic cell. The control (blank) spectroscopic cell should contain absolute ethanol. Read the absorbance of your sample against the blank, according to the instructions of your spectrophotometer, at 325 nm.

Record the absorption at 325 nm on your Report Sheet (5).

7. Calculate the amount of margarine that yielded the vitamin A in the petroleum ether eluate. Remember that you added only half (5 mL) of the extract to the column. Report this value on your Report Sheet (6). Calculate the grams of margarine that would have yielded the vitamin A in 1 mL absolute ethanol by dividing (6)/10 mL. Record it on your Report Sheet (7). Calculate the vitamin A in a pound of margarine by using the following formula:

$\mu\text{g vitamin A/lb of margarine} = \text{Absorption} \times 5.5 \times [454/(7)].$

Record your value on the Report Sheet (8).

Chemicals and Equipment

1. Separatory funnel (500 mL)
2. Buret (25 mL)
3. UV lamp
4. Spectrophotometer (near UV)
5. Margarine

6. Petroleum ether (30–60 °C)
7. 95% ethanol
8. Absolute ethanol
9. Diethyl ether
10. Glass wool
11. Alkaline aluminum oxide (alumina)

TEST-TASKS FOR SELFCONTROL

1. The avitaminosis of ascorbic acid is named as:
 - A. Cushing`s syndrome
 - B. Addison`s disease
 - C. Kwashiorkor
 - D. Hemolytic anemia
 - E. Scurvy
2. Find out the vitamin whose deficiency is associated with disturbed transamination of amino acids:
 - A. Pyridoxine
 - B. Rutin
 - C. Thiamine
 - D. Folic acid
 - E. Ascorbic acid
3. The glycolysis duration is in need for one vitamin, only. Name it:
 - A. Pyridoxal phosphate
 - B. Riboflavin
 - C. Thiamine
 - D. Nicotinic acid
 - E. Ascorbic acid
4. Examination of a patient with frequent hemorrhages from internals and mucous membranes revealed proline and lysine being a part of collagen fibers. What vitamin absence caused disturbance of their hydroxylation?
 - A. Vitamin A
 - B. Thiamine
 - C. Vitamin K
 - D. Vitamin E
 - E. Vitamin C

5. A woman who has been keeping to a clean-rice diet for a long time was diagnosed with polyneuritis (beri-beri). What vitamin deficit results in development of this disease?
- A. Folic acid
 - B. Thiamine
 - C. Ascorbic acid
 - D. Riboflavin
 - E. Pyridoxine
6. Most participants of Magellan expedition to America died from avitaminosis. This disease declared itself by general weakness, subcutaneous hemorrhages, falling of teeth, gingival hemorrhages. What is the name of this avitaminosis?
- A. Biermer's anemia
 - B. Polyneuritis (beri-beri)
 - C. Pellagra
 - D. Rachitis
 - E. Scurvy
7. Pyruvate concentration in the patient's urine has increased 10 times from normal amount. What vitamin deficiency can be the reason of this change:
- A. Vitamin B₆
 - B. Vitamin A
 - C. Vitamin E
 - D. Vitamin C
 - E. Vitamin B₁
8. Hydroxylation of endogenous substrates and xenobiotics requires a donor of protons. Which of the following vitamins can play this role?
- A. Vitamin C
 - B. Vitamin E
 - C. Vitamin P
 - D. Vitamin A
 - E. Vitamin B₆

9. A 10-year-old girl often experiences acute respiratory infections with multiple hemorrhages in the places of clothes friction. Hypovitaminosis of what vitamin is in this girl organism?

A. A

B. B₂

C. B₁

D. B₆

E. C

10. A 9-month-old infant is fed with artificial formulas with unbalanced vitamin B₆ concentration. The infant presents with pellagra dermatitis, convulsions, anaemia. Convulsions development might be caused by the disturbed formation of:

A. Dopamine

B. Histamine

C. Serotonin

D. DOPA

E. GABA

11. Vitamin C is in need for certain type of reaction. Choose it.

A. Carboxylation

B. Dehydrogenation

C. Hydroxylation

D. One-carbon transfer

E. Transamination

12. Which of the following vitamins requires the intrinsic factor for absorption?

A. Folic acid

B. Vitamin B₁₂

C. Vitamin C

D. Vitamin E

E. Vitamin K

13. Choose the vitamin which is important in non-oxidative decarboxylation, transamination and transsulfuration reactions from the following list:

- A. Riboflavin
- B. Thiamine
- C. Pyridoxine
- D. Pantothenic acid
- E. Folic acid

14. Point out vitamin which is the most indispensable during mitosis:

- A. Folic acid
- B. Pantotenic acid
- C. Ascorbinic acid
- D. Aspartic acid
- E. Thiamine

15. Which vitamin is related to cofactor in glycine metabolism?

- A. Tocopherol
- B. Folic acid
- C. Thiamine
- D. Cobalamin
- E. Pantothenic acid

16. What vitamin takes part in flavoprotein formation?

- A. Vitamin B₆
- B. Vitamin B₂
- C. Vitamin B₁
- D. Vitamin A
- E. Vitamin PP

17. Point out the vitamin that is essential for transamination:

- A. B₁
- B. B₂
- C. B₆
- D. B₁₂
- E. B₉

18. Choose the name of phase for vitamin K use in the formation of clotting factors:
- A. Post-transcription processing
 - B. Post-translation modification
 - C. Transcription
 - D. Reparation
 - E. Replication
19. Choose the vitamin required for carboxylation reactions in human tissues:
- A. Vitamin B₂
 - B. Vitamin B₆
 - C. Vitamin H
 - D. Vitamin B₁₂
 - E. Vitamin K
20. Which enzyme activity is measured in diagnostic of Beri-beri?
- A. Transketolase
 - B. Glucose-6-phosphste dehydrogenase
 - C. Alanine transaminase
 - D. Deaminase
 - E. Glutamate decarboxylase
21. People eating only maize as staple diet develop vitamin PP deficiency due to low content in maize of:
- A. Niacin
 - B. Leucine
 - C. Tryptophan
 - D. Isoleucine
 - E. Positions A & C are right
22. Choose the correct statement about vitamin K:
- A. It increases a coagulation time in infants with hemorrhagic diseases
 - B. It is helpful in preventing thrombosis
 - C. It is synthesized by intestinal bacteria

- D. It is present in increased amount in cows and breast milk
- E. It is derived to coenzyme of α -decarboxylases
23. Point out a similarity between vitamin C and vitamin K:
- A. Both help in conversion of proline to hydroxyproline
- B. Both help in post-translational modification of polypeptide chains
- C. Both are fat soluble vitamins
- D. Both are involved in coagulation cascade
- E. Both are water soluble vitamins
24. Point out the function for vitamin C:
- A. Coenzyme in the synthesis of clotting factors
- B. Coenzyme for enzymes of post translational modification of procollagen
- C. Anticoagulant
- D. The component of electron transport chain in mitochondria
- E. Coenzyme for beta-oxidation of HFA
25. Choose the vitamin, whose antivitamin is named as Dicoumarol:
- A. Vitamin A
- B. Vitamin B₆
- C. Vitamin C
- D. Vitamin D
- E. Vitamin K
26. Choose the vitamin, whose deficiency leads to osteomalacia at adults:
- A. Vitamin C
- B. Vitamin E
- C. Vitamin D
- D. Vitamin K
- E. Vitamin PP
27. Choose the vitamin, which is a powerful natural antioxidant:
- A. Retinal
- B. Tocopherol
- C. Ergocalciferol

D. Riboflavin

E. Pyridoxine

28. Name the blood plasma index whose low value will prove the deficiency of vitamin K in patient:

A. Urea

B. Albumins

C. Immunoglobulin G

D. Prothrombin

E. C-reactive protein

29. Name the active form of vitamin whose level in the blood is depended on the secretion rate of parathyroid hormone:

A. Ascorbic acid

B. Calcitriol

C. Thiamine

D. Tocopherol

E. Naphtoquinone

30. Find out the fat-soluble vitamin whose function is hormone-similar one:

A. Vitamin C

B. Vitamin E

C. Vitamin D

D. Vitamin K

E. Vitamin PP

31. Vitamin A group contains substance whose function is associated mainly with stimulation of proliferation and differentiation processes in tissues. Name it:

A. Retinal

B. Pantothenic acid

C. Retinoic acid

D. Nicotinic acid

E. Nicotine amide

32. A patient suffers from vision impairment – hemeralopia (night blindness). What vitamin preparation should be administered the patient in order to restore his vision?

- A. Pyridoxine
- B. Retinol acetate
- C. Vicasol
- D. Thiamine chloride
- E. Tocopherol acetate

33. A 6 y.o child was administered vicasol to prevent postoperative bleeding. Vicasol is a synthetic analogue of vitamin K. Name post-translation changes of blood coagulation factors that will be activated by vicasol:

- A. Carboxylation of glutamic acid residues
- B. Polymerization
- C. Partial proteolysis
- D. Glycosylation
- E. Phosphorylation of serine radicals

34. A patient who was previously ill with mastectomy as a result of breast cancer was prescribed radiation therapy. What vitamin preparation has marked radioprotective action caused by antioxidant activity?

- A. Tocopherol acetate
- B. Riboflavin
- C. Folic acid
- D. Ergocalciferol
- E. Thiamine chloride

35. There is an inhibited coagulation in the patients with bile ducts obstruction, bleeding due to the low level of absorption of vitamin. What vitamin is in deficiency?

- A. K
- B. E
- C. D

D. A

E. Carotene

36. A 2-year-old child has got intestinal dysbacteriosis, which results in hemorrhagic syndrome. What is the most likely cause of hemorrhage of the child?

A. Activation of tissue thromboplastin

B. PP hypovitaminosis

C. Fibrinogen deficiency

D. Vitamin K insufficiency

E. Hypocalcemia

37. Deficiency of which of the following vitamins can lead to anemia?

A. Folic acid

B. Vitamin B₁₂

C. Vitamin C

D. Vitamin E

E. All of the above

38. Choose the most active form of vitamin D₃:

A. 25-Hydroxycholecalciferol

B. 25-Hydroxyergocalciferol

C. 24, 25-Dihydroxycholecalciferol

D. 1, 25-Dihydroxycholecalciferol

E. Calcidiol

39. What reaction is in need of vitamin K?

A. Gamma-carboxylation

B. Oxidation

C. Methylation

D. Hydroxylation

E. Alpha-decarboxylation

40. All of the following are antioxidants except one. Choose it.

A. Tocopherol

- B. Beta-Carotene
- C. L-Ascorbic acid
- D. Cholecalciferol
- E. Retinol

41. During examination of an 11-month-old infant a pediatrician revealed osteoectasia of the lower extremities and delayed mineralization of cranial bones. Such pathology is usually provoked by the deficit of the following vitamin:

- A. Thiamine
- B. Riboflavin
- C. Bioflavonoids
- D. Pantothenic acid
- E. Cholecalciferol

42. A patient presents with twilight vision impairment. Which of the following vitamins should be administered?

- A. Cyanocobalamin
- B. Ascorbic acid
- C. Nicotinic acid
- D. Retinol acetate
- E. Pyridoxine hydrochloride

43. In clinical practice tuberculosis is treated with izoniazid preparation – that is an antivitamin able to penetrate into the tuberculosis bacillus. Tuberculostatic effect is induced by the interference with replication processes and oxidation-reduction reactions due to the buildup of pseudo-coenzyme:

- A. FMN
- B. NAD
- C. CoQ
- D. FAD
- E. TPP

44. Some infectious diseases caused by bacteria are treated with sulfanilamides, which block the synthesis of bacteria growth factor. What is the mechanism of their action?
- A. They inhibit the absorption of folic acid
 - B. They are allosteric enzyme inhibitors
 - C. They are allosteric enzymes
 - D. They are anti-vitamins of para-amino benzoic acid
 - E. They are involved in red-ox processes
45. A patient complains of photoreception disorder and frequent acute viral diseases. He has been prescribed a vitamin that affects photoreception processes by producing rhodopsin, the photosensitive pigment. What vitamin is it?
- A. Cyanocobalamin
 - B. Tocopherol acetate
 - C. Pyridoxine hydrochloride
 - D. Thiamine
 - E. Retinol acetate
46. A 6-year-old child suffers from delayed growth, disrupted ossification processes, decalcification of teeth. What can be the cause?
- A. Vitamin D deficiency
 - B. Hyperthyroidism
 - C. Vitamin C deficiency
 - D. Decreased glucagon production
 - E. Insulin deficiency
47. A patient, who has been suffering for a long time from intestine disbacteriosis, has increased hemorrhaging caused by disruption of posttranslational modification of blood coagulation factors II, VII, IX and X in the liver. What vitamin deficiency is the cause of this condition?
- A. K
 - B. B₁₂
 - C. B₉

D.C

E.P

48. During regular check-up a child is detected with interrupted mineralization of bones. What vitamin deficiency can be the cause?

A. Calciferol

B. Riboflavin

C. Tocopherol

D. Folic acid

E. Cobalamin

49. Choose uncharacteristic symptom of vitamin A deficiency in humans among the listed ones below:

A. Growth retardation

B. Malformation of the long bones

C. Loss of body mass

D. Affected mucous epithelium and eyes

E. Night blindness

50. Point out the vitamin, whose deficiency leads to pellagra:

A. Vitamin P

B. Vitamin A

C. Vitamin C

D. Vitamin B₃

E. Vitamin B₂

ANSWERS TO TEST-TASKS FOR SELF-CONTROL:

1	2	3	4	5	6	7	8	9	10
E	A	D	E	B	E	D	A	E	E
11	12	13	14	15	16	17	18	19	20
C	B	C	A	B	B	C	B	C	A
21	22	23	24	25	26	27	28	29	30
E	C	B	B	E	C	B	D	B	D
31	32	33	34	35	36	37	38	39	40
C	B	A	A	A	D	E	C	A	D
41	42	43	44	45	46	47	48	49	50
E	D	B	D	E	A	A	A	B	D

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