7. VITAMINS AND HORMONES

In addition to carbohydrate, lipid, protein and inorganic substances, some organic nutrients called vitamins are needed for the living organism to maintain a healthy life. The word meaning of vitamin is the amine of life. The difference between the vitamin and the other organic nutrients is that they do not enter into the tissue structure and do not provide energy to the organism. Generally, they are exogen which means they are from external sources.

Vitamins are complex chemicals that work as regulators. They participate in the structure of coenzyme or an enzyme. Nicotinamide Adenine Dinucleotide (NAD), Nicotinamide (Niacin) Adenine-Dinucleotide Phosphate (NADP), Flavin Adenine Dinucleotide (FAD), Flavin Mono Nucleotide (FMN), Thiamine Pyrophosphate (TPP) and Coenzyme-A which contains pantothenic acid are the important vitamins that can be used as coenzymes. Additionally, there are also many coenzymes including pyridoxine, lipoic acid, biotin, folic acid and vitamin B12. Coenzyme-Q is an organic compound that exhibits similar activity to α-tocopherol activity.

If a vitamin is not taken to the organism enough, some disorders can be occured, called as hypovitaminosis. In addition to some vitamins themselves, the precursors are taken to the organism and are converted into vitamins. These precursors are called as provitamines.

Vitamins are examined in two groups as water-soluble and fat-soluble according to their solubility;

Water Soluble Vitamins:

- Thiamine (vitamin B1)
- Riboflavin (vitamin B2)
- Niacin (Nicotinamide)
- Pyridoxine (vitamin B6)
- Biotin
- Pantothenic acid
- Para-aminobenzoic acid
- Folic acid
- Vitamin B12
- Lipoic acid
- Vitamin C

Fat Soluble Vitamins:

- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K

Water-soluble vitamins vary by chemical structures, but they are all polar molecules thus, they are soluble in water. The water-soluble vitamins can be synthesized by plants except Vitamin B_{12}. Most of water-soluble vitamins are precursors to the coenzymes of enzymes in intermediate metabolism. The water soluble vitamins can be excreted in the urine when they are taken in large quantities because they cannot be stored.

Among the fat soluble vitamins, only Vitamin K has the coenzyme function. These vitamins which are absorbed together with fatty foods and stored in the liver and adipose tissue cannot be excreted in the urine. Toxic effects can be seen when the vitamin A and vitamin D are taken in high doses. Vitamin D is also considered as a hormone because it has a function in the regulation of calcium and phosphorus metabolism.

7.1. Vitamin C (Ascorbic Acid)

Ascorbic acid is the endiol lactone of glucuronic acid:

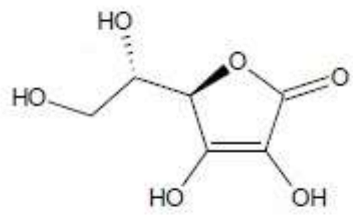
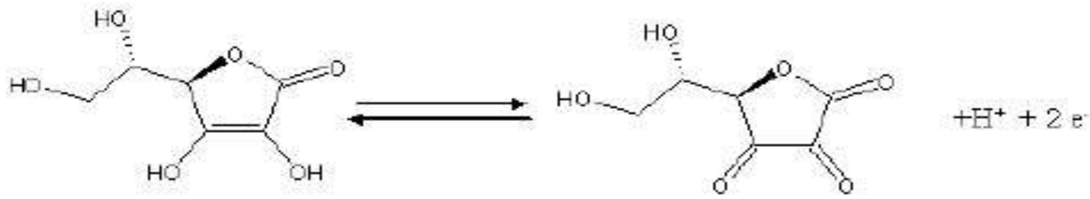


Figure 7.1. Ascorbic acid



Ascorbic acid Dehydroascorbic acid

Figure 7.2. Conversion of ascorbic acid to dehydroascorbic acid

Ascorbic acid is the least stable vitamin among the water-soluble vitamins. Microorganisms do not need and synthesize ascorbic acid. Animal tissues such as adrenal gland, liver and milk have the highest ascorbic acid concentration. The most important sources of ascorbic acid are green vegetables, fruits, tomatoes and citrus fruits.

Ascorbic acid is easily absorbed from intestines. Ascorbic acid passes through the cell membrane in the form of dehydroascorbic acid which is its fat soluble form.

The amount of ascorbic acid in blood plasma in humans is about 1% mg/dL. Excessive amounts of ascorbic acid may exceed the kidney threshold of 1.5% mg/dL. Ascorbic acid can be converted to oxalate in human and calcium salt of oxalate, excreted in urine, can form kidney stones. After 9 g of ascorbic acid is taken orally, the excreted oxalic acid amounts doubles in urine.

The recommended daily amount of ascorbic acid for human is 30-40 mg. During pregnancy, lactation, stress and fever the need of ascorbic acid increases. The ascorbic acid deficiency leads to scurvy. In scurvy disease, changes in bone formation and growth, loose tooth, and cracked skin can be seen due to collagen metabolism disorder.

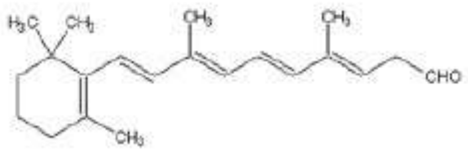
Most commonly used analytical methods for the ascorbic acid analysis are the titrations with iodine and 2,6-dichlorophenol-indophenol, fluorometric analysis, photochemical reactions with methylene blue and gas chromatography.

7.2. Vitamin A (Retinol)

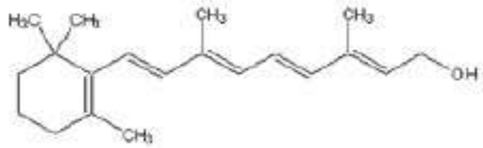
β-carotene, a tetraterpene, is synthesized by plant cells and acts as a provitamin A. β-carotene, an antioxidant, has conjugated alkyl structure and is effective in eliminating the free organic peroxide radicals. Like all other carotenoids, β-carotene is found in the esterified structure and in the different concentrations in vegetable and animal cells. β-carotene is broken down in the intestines and as a result of this two molecules of retinal are produced. Then, retinal is converted to retinol (Vitamin A) and retinoic acid. These products are transported to the liver by lymphatic chylomicrons.

Retinol, retinal and retinoic acid have spesific biological functions. Retinol usually acts as a hormone. Retinal is the precursor of visual pigment rhodopsia. Retinoic acid is absorbed through the portal system. It is excreted in the urine by metabolizing it to more polar compounds such as epoxides and it is not stored in liver and other tissues.

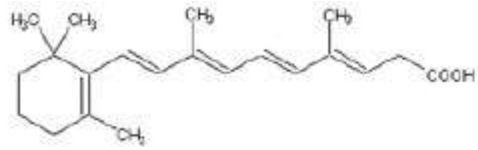
Beta carotene



Retinal



Retinol



Retinoic acid

Figure 7.2. β-carotene and its products

The recommended daily amount of ascorbic acid is up to 2-3 mg. The highest vitamin A concentration is found in the liver oil of marine fish. Milk, butter and egg yolk are also important sources of vitamin A. Carrots, herbs, green and dried clover are very rich sources in terms of β-carotenes. Vitamin A is found as Vitamin A_2 in freshwater fishes (a second double bond exists between 3^{rd} and 4^{th} carbon atoms of the ionan ring).

One of the early symptoms of the deficiency of vitamin A is night blindness (nyctalopia) which is characterized by adaptation disorder towards dark and problems in epithelial tissue development.

7.3. Vitamin E (Tocopherol)

Vitamin E is generally called as tocopherols. The most important of them are α-, β- and γ-tocopherols. Among them, α-tocopherol has the greatest effect as vitamin. Tocopherols have the isoprene units and a ring system in their structure. This ring called as Kroman kernel and it consists of benzene and pyran. The differences between different tocopherols are related to the number of methyl groups bound to the benzene ring.

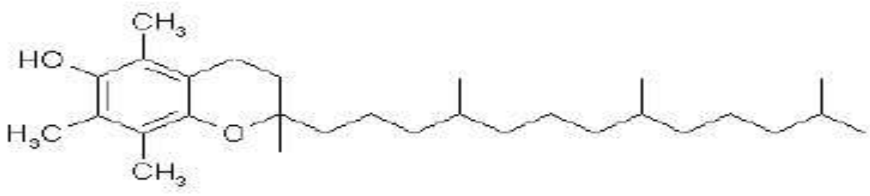


Figure 7.2. α-tocopherols (5,7,8-trimethyl tocol)

The daily requirement dose for vitamin E is 0.1-0.2 mg per kg of body weight for adults and 0.5 mg for infants. Eggs, banana, butter, beef, carrots, bran, beef liver, strawberries, cheese, dry beans, peanuts, soya and olive oil, oil of tuna and swordfish, milk, orange, fresh peas, apple, potatoes, chicken, rice and tomato are the food sources rich in Vitamin E.

α-Tocopherol is synthesized by mevalonic acid in plants, in particular the germinated grain seed is very rich in terms of tocopherol.

Tocopherols as fat-soluble vitamins are taken into body through the nutrients. αTocopherol is easily absorbed through the small intestines, and then it is transported into the liver and peripheral tissues into lipoproteins with chylomicrons. Mitochondrial phospholipids, endoplasmic reticulum and plasma membrane have specific affinity for α-tocopherol. Vitamin E is stored here as concentrated.

Vitamin E deficiency causes infertility in men. The symptoms of vitamin E deficiency in laboratory animals vary. The common symptom of the vitamin E deficiency in rats is infertility. Acute liver necrosis occurs in rats fed with a diet rich in unsaturated fatty acids, lacking protein and vitamin E. Vitamin E deficiency occurs in rats with progressive paralysis of the hind legs, a decrease in muscle creatinine concentration, creatinuria, and dystrophy associated with a slight decrease in creatinine excretion. Herbivores (grass-eating animals) such as rabbits are more susceptible to vitamin E deficiency, the dystrophy in these species occurs very quickly and animals can die in a few weeks.

7.4. Hormones

Hormones are organic substances that the name of the 'hormone' is derived from the meaning of the 'stimulation'. Hormones are produced in the body that controls and regulates the activity of certain cells or organs. Many hormones are secreted by special glands, such as thyroid hormone produced by the thyroid gland. Hormones are essential for every activity of life, including the processes of digestion, metabolism, growth, reproduction, and mood control. Many hormones, such as neurotransmitters, are active in more than one physical process. Hormones can be divided into two classes by means of structure as protein and steroid.

Hormones are very similar to enzymes because they act with very small amounts and act as biological catalysts, but they are different in some ways. These can be summarized as follows:

1) Hormones are synthesized in another organ than the organ that they act on.
2) Hormones are secreted into the bloodstream before use.
3) Hormones do not have to be in the protein structure. They can be small polypeptide, a single amino acid or a chemical substance in the steroid structure.

7.4.1. Adrenaline (Epinephrine)

Adrenaline is an important hormone secreted from the interior of the kidney glands. Another important hormone secreted from the kidney glands is noradrenaline (norepinephrine). Both hormones are in the class of catecholamines, and the adrenaline is the first hormone to be synthesized in laboratories. Similar substances in terms of both structure and effect were synthesized and used as medicines in the field of medical treatment such as metaraminol etc. The effect of these hormones (adrenaline and noradrenaline) can increase heart rate, pulse rate, arterial blood pressure, respiratory rate and depth, metabolism, the amount of blood to muscles, the contraction force. The ratio of these two hormones secreted by the adrenal glands in human and various mammals is different. It is understood from the examinations that the more noradrenaline is released during the nerving. Adrenaline treatment in medicine is used for many purposes; it is used in the operation in order to make heart work which has stopped due to some reasons, in the treatment of bronchial asthma especially during seizures (but only by a physician and in his control), and in the removal of excessive blood supply and swelling in the bronchial wall.

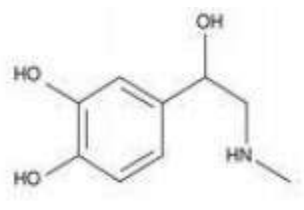


Figure 7.4 The structure adrenaline

7.5. Experimental Studies

Experiment 7.5.1. Titrimetric Determination of Ascorbic Acid in Lemon Juice

Principle of the experiment: It is based on the principle of calculating the amount of ascorbic acid by titrating with lime juice in 2,6-dichlorophenol-indophenol (dye) solution. While the 2,6-dichlorophenol-indophenol solution is reduced by ascorbic acid during titration, the ascorbic acid is oxidized to form dehydroascorbic acid. At the end of the titration, pink colour of the unreacted 2,6-dichlorhorphenol-indophenol is seen due to the fact that ascorbic acid is completely consumed in the medium.

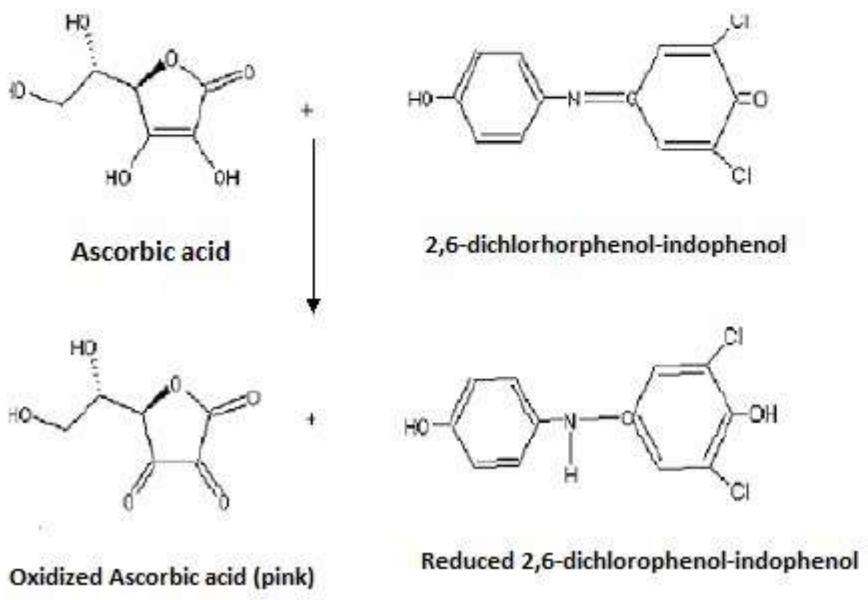


Figure 7.6. The reaction of ascorbic acid with 2,6 dichlorophenol-indophenol

Experimental Procedure: Lemon juice is diluted with water by 5 times of its volume.

1) Take 5 mL of the diluted lemon juice solution to a flask and add 1 mL of glacial acetic acid. This solution is titrated with the 2,6-dichlorophenol-indophenol solution until a permanent pink colour is obtained. The volume of the 2,6-dichlorophenolindophenol solution spent from the burette is recorded. This value is the value T (example) in the formula.

2) Add 5 mL of distilled water and 1 mL of glacial acetic acid to another flask. Titrate this solution in the same manner. The volume of 2,6-dichlorophenolindophenol which is spent on the burette is read and recorded. This value gives the blank (BL) value in the formula.

3) Add 5 mL of ascorbic acid and 1 mL of glacial acetic acid to another flask and titrate this solution in the same manner. The volume of solution spent in titration is read. This value is the St (standard) value in the formula.

The amount of vitamin C in the sample is calculated in the unit of mg / 100 mL.

$$\frac{T-BL}{St-BL} \times 2 \times \text{Dilution factor} = \text{C vit. (mg/100mL)}$$

Solutions:

2,6-Dichlorophenol-indophenol solution: The powdered 100 mg of 2,6-dichlorophenol-indophenol is dissolved in pure water by gentle heating and the total volume is completed to 500 mL and stored in a brown bottle. If 2,6-dichlorophenol-indophenol is in tablet form, a tablet is dissolved in 40 mL of hot water, the solution is cooled and the volume is completed to 50 mL.

Standard ascorbic acid solution: Ascorbic acid solution is prepared with water in the concentration of 0.02 mg / mL.

Experiment 7.5.2. Qualitative Determination of Vitamin A

Principle of the experiment: Vitamin A gives the colored complexes with chloroform and antimony III chloride.

Experimental Procedure: Add a few drops of antimony III chloride solution onto 0.3 mL of vitamin A solution and mix. Dark blue color is observed.

Solutions:

Vitamin A: 1 tablet of vitamin A / 5 mL of chloroform

Experiment 7.5.3. Extraction of Carotene

Principle of the experiment: Carotene is obtained from carrot by extraction method.

Experimental Procedure:

1) Crush 10 grams of carrots which are shredded and kept in the freezer. Extract the crushed carrot with 50 mL of 95% hot ethanol for 30 min. Use the water bath for this process.
2) Filter the yellow extract through the filter paper. To reduce the ethanol concentration in the extract from 95% to 85%, add 5.9 mL of distilled water in 50 mL of extract. Cool the extract to room temperature.
3) Transfer the extract into a separating funnel and rinse with 25 mL of petroleum ether. Wait for the separation of layers. The top yellow orange oil ether layer contains carotenes; the ethanol layer carries xanthophylls.
4) When the yellow-orange layer at the top of the separating funnel is separated as much as possible, this portion is collected in a beaker. The carotene in the carrot is then obtained.

Experiment 7.5.4. Qualitative Determination of Vitamin E

Principle of the experiment: Vitamin E is soluble in absolute alcohol and gives a red complex when heated with HNO_3.

Experimental Procedure: Take 3 mL of vitamin E solution, then add 1 mL of 65% HNO_3 by dropwise and heat the tube is heated until boiling. The red color is observed.

Solutions:

E Vitamini: 300 mg Vitamin E / 25 mL ethyl alcohol

Experiment 7.5.5. Determination of Adrenaline in Adrenal Glands

Principle of the experiment: The phosphotungstic acid is reduced by adrenaline in the basic medium and forms a blue color.

Experimental Procedure: The adrenal gland is divided into small pieces with scissors. Then, it is conveyed into a grinder, a few drops of 10% trichloroacetic acid is added and the crushed thoroughly. Then, 3 mL of 10% trichloroacetic acid is added and mixed well. After centrifugation (5 minute 1500 rpm), the supernatant is separated. In this way the adrenaline solution is obtained.

Add 1 mL Folin and Denisin reagent over 2 mL of this obtained adrenaline solution. Na_2CO_3 is then added until the mixture is basic. CO_2 output is observed. As a result, a dark blue color is formed by reduction of phosphotungstic acid by adrenaline to tungsten blue. The formation of this color indicates the presence of adrenaline.

Solutions:

Folin-Denis Indicator (phosphotungstic acid reagent): 5 g of sodium tungustate is dissolved in 40 mL of pure water and mixed with 4 mL of 85% H_3PO_4 and heated under reflux for 4 hours. Then, diluted to 50 ml with pure water.
10% TCA: 10 g of trichloroacetic acid is diluted to 100 mL.

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