**Lesson 4**

**Analysis of medicinal preparations containing pyrimidine-thiazole derivatives (group B1 vitamins): thiamine chloride,**

**cocarboxylase and benfotiamine medicinal forms.**

The basis of the chemical structure of thiamine is pyrimidine and thiazole heterocycles:

N

N

N

S

pyrimidine thiazole

In the thiamine molecule, pyrimidine and thiazole nuclei are connected to each other through a methyl group. Therefore, thiamine refers to pyrimidinediazole or pyrimidylmethylthiazole vitamins.

The disease, called beriberi, occurs with vitamin B1 hypovitaminosis. People suffering from this disease feel unusual heaviness in their legs, it seems as if they are being replaced by prostheses. At the initial stage, the patient tries to walk on his toes, as he feels a sharp pain in the calf muscles when his heels touch the ground. Then the legs and hands become paralyzed, the patient is so upset that he looks like a skinned skeleton. When the disease is severe, it often ends with a fatal outcome.

Observations and experiments conducted by many scientists at the end of the 19th and the beginning of the 20th century have shown that there is a factor that cures this disease in rice bran and some plants belonging to the leguminous family. Even the active preparation concentrate was obtained from rice bran. Vitamin B1 in crystalline form was first isolated from yeast in 1931 by the German chemist Adolf Windaus. Since the molecule of this substance contains sulfur and an amino group, it was called thiamine.

Thiamine or vitamin B1 forms the coenzyme part of thiamine enzymes involved in the metabolism of sugars in the form of phosphate acid ester (cocarboxylase, thiamine diphosphate). Enzymes of thiamine are involved in reactions of decarboxylation of pyruvic acid (CH3COCOOH) and other α-ketoacids. Lack of thiamine in the body causes the accumulation of pyruvic acid in the cells. Polyneuritis occurs due to the toxic effect of pyruvic acid on nerve cells and cardiac muscle. Beriberi disease is a severe form of peripheral polyneuritis and is associated with damage to motor and sensory neurons.

Thiamine is contained in products of plant origin and microorganisms. In plants (wheat, rye, peas, rice, etc.) thiamine is mainly in free form, and in microorganisms (brewer's yeast) in phosphated form. In cereals, thiamine is mainly found in bran. Therefore, the removal of bran leads to a decrease in vitamin content in food products.

Since the thiamine molecule has an oxyethyl (R-CH2-CH2OH) group, it forms simple and complex esters. At the present time, many derivatives with thiamine activity are known. One group of them is based on thiaminthiol. Thiamine bromide and thiamine chloride are used in medical practice. Thiamine-thiol can easily be converted into thiamine-disulfide (RS-S-R) based on the sulfhydryl group (R-SH). Thiamine-disulfide is equal to thiamine in biological activity and less toxic.

So, along with thiamine, many of its derivatives possess the activity of vitamin B1. Therefore, these substances form vitamins of the B1 group and have the property of turning into thiamine in the body.

There is a very close connection between the structure of the thiamine molecule and its biological activity. Thus, with certain changes in pyrimidine and thiazole residues in the thiamine molecule, the vitamin property is lost and even antivitaminoactive substances are formed. For example, when replacing the NH2 group in the 4'-position of the pyrimidine core with the OH group, the substance oxythiamine not only does not have vitamin activity, but is even a strong antivitamin of vitamin B1. When replacing the thiazole part in the thiamine molecule with dihydro- and tetrahydrothiazol residues, it loses its vitamin properties. When replacing the thiazole part with a derivative of pyridine with the preservation of functional groups, the pyritamine substance does not have vitamin properties, it is even an antivitamin of vitamin B1.

For the synthesis of thiamine preparations, pyrimidine and thiazole fragments are first synthesized, and then they are condensed.

Acetamide and α-acetoxymethylene-β-

The cis-form of ethoxypropionitrile is condensed:



acetamidine α-acetoxymethylene- 2-methyl-4-amino-5- 2-methyl-4-amino-5-bromo-

β-ethoxypropionitrile ethoxymethylpyrimidine methylpyrimidinehydro-bromide

b) Synthesis of thiazole ring based on thioformamide and bromoacetopropylacetate they do:



thioformamide bromacetopropyl- 4-methyl-5β- 4-methyl β-

 acetate acetoxyethylthiazole acetoxyethylthiazole

Pyrimidine and thiazole parts obtained in previous stages at 100-120℃

combined into one molecule by melting at room temperature or by heating in an organic solvent:

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2-methyl-4-amino-5-bromo(chloro)- 4-methyl-5β-oxyethyl-thiazole

-methylpyrimidine-hydrobromide

(hydrochloride)

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**Thiamine-bromide - Thiamine Bromide**

**Thiamine chloride - Thiamine Chloride**

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4-Methyl-5-β-oxyethyl-N-(2-methyl-4-amino-5-methylpyrimidyl)-thiazolium chloride (bromide)-hydrochloride (hydrobromide)

Purchase

It should be noted that thiamin is also contained in groats, walnuts, peanuts, etc. d. are available in natural raw materials, and these sources can be used to obtain thiamine. However, obtaining thiamine from these sources is a complicated process, and its yield is very low. For example, only 0.25 g of thiamine can be obtained from 1 t of yeast. Therefore, at present, thiamine preparations are obtained by the synthesis method.

Both preparations (thiamine bromide and thiamine chloride) are white crystalline powder. Thiamine bromide has a pale yellow color. They have a characteristic smell (the smell of yeast). They are easily soluble in water, moderately soluble in alcohol, practically insoluble in ether, acetone, benzene and chloroform. Thiamine is a diacid base (the basicity is determined by an amino group in the pyrimidine core and a nitrogen atom in the thiazole core). Therefore, they form two types of salts - chloride and hydrochloride (bromide and hydrobromide).

Definition of personality

1) The thiochrome reaction characteristic of these preparations is carried out. 0.05 g of the drug is dissolved in 25 ml of water. 1 ml of potassium hexacyanoferrate (III), 1 ml of NaOH solutions, 5 ml of butyl or isoamyl alcohol are added to 5 ml of solution, shaken and left to rest. Blue fluorescence in UV rays is observed in the upper layer; When the solution is acidified, the fluorescence disappears, and when it is alkalized, it reappears. It is assumed that the thiochrome reaction proceeds in several stages. At the first stage, 1 mol of HCl or HBr is neutralized with alkali. At the 2nd stage, under the action of alkali, 2 moles of thiamine hydroxide are formed (due to the nitrogen of the thiazole nucleus). At the 3rd stage, the thiamine-thiol form is obtained by the action of 3 moles of alkali:

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2) 1 ml of hydrochloric acid, 1 ml of chloramine solution, 1 ml of chloroform are added to 5 ml of the solution prepared in step 1 and shaken; no yellow color should be obtained in the chloroform layer. This reaction refers to the bromide ion and distinguishes thiamine chloride from thiamine bromide:

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2HBr + Cl2→ Br2 + 2HCl

3) In 5 ml of the solution prepared in the 1st determination, they perform a chloride reaction.

4) A solution of the preparation in water gives a white precipitate with a saturated solution of HgCl2, a red-brown precipitate with a 0.1 M iodine solution, and a yellow precipitate with a saturated solution of picric acid (melting point 206-2080C).

5) A 1% solution of the drug in water gives a yellow color with a 15% alkaline solution.

6) When thiamine solution is treated with 6-aminothymol in alkali, a dark yellow color is formed.

In an alkaline medium, thiamine is converted to thiamine thiol, and 6-amynthimoyl to thymoquinone. Thiaminthiol (R-SH) then reacts with thymoquinonimine to give a yellow indophenol-type derivative.

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6-Amintymol thymoquinone

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R

of thymoquinone with thiamine-thiol

the aurin dye it produces

7) Thiamine with its ammonium rheinate salt gives a 1:1 pink precipitate soluble in acetone at pH 4-5:

(C12H17N4OS)Cl-· HCl + NH4[Cr(NH3)2(SCN)4] → NH4Cl + HCl +

+ [C12H17N4Os]· [Cr(NH3)2(SCN) 4]

Using this determination, the amount of thiamine can be determined by photocolorimetry.

8) Like other ternary amines, thiamine is colored red when heated in a water bath with acetic anhydride and citric acid crystals. As a result of the pyrolysis of thiamine, condensation occurs due to the single amine group in its molecule:

2R$-$ NH2 $\rightarrow $ R $-$ NH $–$ R + NH3$\uparrow $

Therefore, when thiamine is heated with dimethyloxalate in the presence of thiobarbituric acid, a red substance is obtained.

9) UV-spectrophotometry: a 0.0015% solution of thiamine-bromide in 0.1 M hydrochloric acid gives maximum absorption at a wavelength of 246 nm, and a 0.0025% solution of thiamine-chloride in water gives a maximum absorption at a wavelength of 237 and 262 nm.

10) IR-spectroscopy: the spectrum of thiamine-bromide and thiamine-chloride taken in the fields of 4000-700 cm-1 should be the same as the spectrum of the standard sample.

Determination of cleanliness

Thiothiamine (an intermediate in thiamine metabolism) is assayed. 0.2 g of the drug is dissolved in 5 ml of water, 1 ml of clear HCl solution is added; yellow color should not be obtained and turbidity should not form in the solution.

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Thiothiamine

Quantification

It is done in several ways.

1. Aqueous titration method

About 0.1 g (d.c.) of thiamine chloride (or bromide) is dissolved in 20 ml of anhydrous acetic acid with low heating. After cooling the solution, add 5 ml of mercury 2-acetate solution and titrate with 0.1 M perchloric acid to emerald-green color (indicator-violet crystal).

In parallel, a control experiment is performed (T=0.01688 g/ml thiamine-chloride and T=0.02176 g/ml thiamine-bromide)

Tiamin-xlorid + Hg(CH3COO)2 + 2HClO4→

(və ya (C12H17N4OS)+Cl- $∙$ HCl)

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2) Neutralization (alkalimetry) method

Dissolve about 0.05-0.1 g (d.c.) of thiamine chloride (or bromide) in 10-15 ml of water and dissolve it with 0.1 M NaOH solution to a blue color (indicator bromthymol blue) or to a red color (indicator -phenolphthalein)) are titrated (T=0.03373 g/ml for thiamine-chloride and T=0.04352 g/ml for thiamine-bromide).

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3) Argentometry (Tile) method

(C12H17N4OS)+Cl- · HCl + 2AgNO3→

→2AgCl↓ + (C12H17N4OS)+NO3- · HNO3

Dissolve about 0.05 g of thiamine chloride (or thiamine bromide) in 10 ml of water and add 2-3 drops of bromphenol blue and acetic acid drop by drop until a greenish-yellow color is obtained. The obtained solution is titrated with 0.02 M AgNO3 solution until purple color. The titer is the same as in the 2nd determination.

4) Fluorometric method (based on thiochrome reaction).

5) Determination of the amount of thiamine-bromide is also carried out by the gravimetric method. The determination is based on the precipitation of thiamine with silicon tungstic acid (SiO2·12WO3). The obtained sediment is filtered through a glass filter and dried at 100-1050C. The composition of the precipitate: SiO2 · 12WO3 · 2C12H17BrN4OS. N.k. obtained by multiplying the amount of sediment by 0.25. calculate the percentage of the drug:

 çöküntünün qr-la miqdarı $∙$ 0,25 $∙$ 100

X% =

 0,05%

The amount of the preparation should not be less than 98%.

Vitamin B1 preparations are used in the prevention and treatment of B1 hypo- and avitaminosis. In addition, B1 vitamins are used to treat many diseases (neuritis, radiculitis, neuralgia, peripheral paralysis, gastric and duodenal ulcers, intestinal atony, liver diseases, myocardial dystrophy, spasm of peripheral vessels, itching of various etiologies, pyoderma, eczema, psoriasis, furunculosis, etc. ) is widely used in treatment.

It is not recommended to inject vitamin B1 at the same time as pyridoxine (vitamin B6) and cyanocobalamin (vitamin B12). Because cyanocobalamin increases the allergy-causing effect of thiamine, and pyridoxine makes it difficult to convert thiamine into a biologically active form - thiamine-diphosphate.

Thiamine chloride 0.002; 0.005 and 0.01 g tablets; Injection solutions of 2.5 and 5% are released in an ampoule in the amount of 1 ml.

Thiamine-bromidine 0.00258; 0.00645 and 0.0129 g tablets; 3% and 6% injection solutions are released in the amount of 1 ml. The release of thiamine-bromide in slightly higher doses is due to its molecular mass (M.k=435.2) being higher than the molecular mass of thiamine-chloride (M.k. 337.27). Therefore, 0.00129 g (1.29 mg) of thiamine bromide corresponds to 0.001 g (1 mg) of thiamine chloride.

Vitamin B1 (0.014 g/l) and vitamin B2 (0.003 g/l) are present together with protein in purified dry brewer's yeast (Faex medicinalis).

Thiodin solution (Solutio "Tiodinum") contains 12.5 mg of thiamine bromide and 10 mg of sodium iodide in 1 ml (used in radiculitis, CNS diseases).

Vitamin B1 is included in many multivitamin preparations (Pentovit, Heptavit, Decamevit, Aerovit, Hexavit, Undevit, etc.).

In one syringe, vitamin B1 should not be mixed with penicillin or streptomycin (antibiotics break down), including nicotinic acid (vitamin B1 breaks down).

Thiamine preparations are stored in tightly closed containers and protected from light.

**Cocarboxylase-hydrochloride-**

**Cocarboxylase Hydrochloride**

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3-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]-5-(2-hydroxyethyl)-4-

methylthiazolio-0-disphosphonate hydrochloride

or Thiamine-diphosphate

Thiamine is a coenzyme preparation. Participates in carbohydrate metabolism.

It is a white crystalline powder with a weak characteristic odor. Easily soluble in water, practically insoluble in alcohol.

Determination of identity

1) Thiochrome reaction is carried out.

2) A reaction related to phosphates is carried out.

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H3PO4 + 12(NH4)2MoO4 + 21HNO3→

→ 12H2O + 21NH4NO3 + (NH4)3PO4 ·12MoO3↓

(yellow crystal)

3) Chlorides are determined.

4) UV-spectrophotometry: a 0.002% solution of the drug in water gives a maximum absorption at a wavelength of 246 nm.

5) IR-spectroscopy: the most objective method to distinguish between cocarboxylase, thiamine, phosphothiamine and benfotiamine. In the IR-spectrum of these substances, including cocarboxylase, seven main zones are observed in the areas of 3500-2500 cm-1. The IR spectra of the substance and the standard sample must be identical.

Determination of cleanliness

The mixture of phosphothiamine (not more than 3%) in the preparation is checked.

Quantification

1) It is carried out by the method of neutralization (alkalimetry).

0.25 g of the drug is dissolved in 100 ml of water in a 250 ml flask, 8

add a drop of thymolphthalein solution and titrate with 0.1 M NaOH solution until a faint blue color.

In parallel, a control experiment is performed (T=0.01536 g/ml).

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It is carried out by the UV-spectrophotometry method (based on the 4th identity determination).

0.05-0.1 g is injected into a muscle or a vein in case of cardiovascular system function and coronary artery disorders.

The preparation is stored at a temperature not exceeding +5℃, protected from light. The maximum storage period is 1 year.

**Benfothiamine – Benfothiamine**

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N-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]-N(2-hydroxy-2-mercapto-

-1-methyl-1-butenyl) formamide-S-benzoate-0-phosphate

It is a white crystalline powder with a weak characteristic odor. Practically insoluble in water and ethanol. It is soluble in 1% NaOH solution.

Determination of identity

1) UV-spectrophotometry: a solution of benfotiamine in a phosphate buffer with a pH of 4.9-5.1 gives a maximum absorption at a wavelength of 244 nm and a minimum at a wavelength of 225 nm.

2) Benfotiamine does not give the thiochrome reaction. However, after heating 0.025 g of the drug together with a solution of 0.05 g of cysteine hydrochloride in 10 ml of water in a boiling water bath for 20 minutes, they conduct the thiochrome reaction in 5 ml of the filtered solution:

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3) It gives the 2nd reaction in cocarboxylase-hydrochloride.

Quantification

It is carried out by the spectrophotometry method. The optical density of its solution in phosphate buffer with a pH of 4.9-5.1 is determined at a wavelength of 244 nm. Phosphate buffer solution is taken as a control solution.

It is used as an analogue of thiamine. It is released in 0.005 and 0.025 g tablets.

The drug is stored at room temperature, protected from light.