A NEW METHOD FOR DETERMINATION OF THE CHOLINESTERASE ACTIVITIES

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Introduction. The cholinesterase (ChE) activity is an individual, stable characteristic and changes little throughout life. Row In the case of human diseases, changes in ChE in his blood. ChE activity decreases with stagnation in the liver (due to hemodynamic disturbances), obstructive jaundice, gallstone, disease, cholecystitis, cholangitis, liver cirrhosis, inflammatory processes in the liver , myocardial infarction, rheumatism, inflammatory Lesions of the skin and muscles (dermatomyositis), muscular dystrophy, chronic kidney disease, poisoning with some insecticides and pesticides, applied in agriculture and others. ChE activity increases with bronchial asthma, severe kidney disease (nephritis), uterine myoma, hypertensive disease nor, inflammatory diseases of the small intestine (exudative enteritis), exudative enteropathy, stomach ulcer, obesity, hyperlipidemia Teinemii, type II diabetes mellitus (in obese patients), alcoholism. It should be noted that a single analysis of ChE has limited diagnostic significance. More important is the laboratory monitoring ChE - data on changes in its activity - the results obtained during the systematic measurement of activity enzyme over a period of time. It causes requires a simple and inexpensive method of analysis ChE. Of the many methods developed so far for determining the activity of ChE, colorimetric methods are most popular, for which ease of analysis, high speed and sensitivity are characteristic. However they have some significant drawbacks. A new colorimetric enzymatic method for ChE determination has been proposed.

Aim. To develop a simple and express colorimetric method for determination of cholinesterase activities.

Materials and methods. All chemicals and reagents used were of analytical grade: Pharmacopoeial acetylcholine chloride (Ach) medication – 0.2 g per amp/5 ml; dry protein drug of ChE from horse serum–4 mg/ L (IV class) 28 AO/mg (SMU "Biomed", Russia), 3,3',5,5' -tetramethylbenzidine dihydrochloride monohydrate (TMB), 98,5% (Sigma). All the absorbence spectral measurements were made using photoelectric concentration colorimeter KPK-3-01 (Russia); filter №3, l = 3 cm). The rate of reactions described value of optical density of the solution for 10 minutes (by fixed time). Hydrogen Peroxide 30-40%" (LLC "Inter - Synthes", Boryslav, Ukraine).

Preparation solution of ChE: Standart stock solution was prepared in double distilled water by taking 0.0800g of dry *ChE* drug in a 20 ml volumetric flask and it

was diluted up to the mark. After that it was at thermostat 10 min at +38±0.5 ºC. The solutions of the test model samples are prepared similarly. The new photometric technique has been developed for determination ChE, based on the rate of enzymatic hydrolysis of acetylcholine ester by the enzyme sample (Fig 1.)

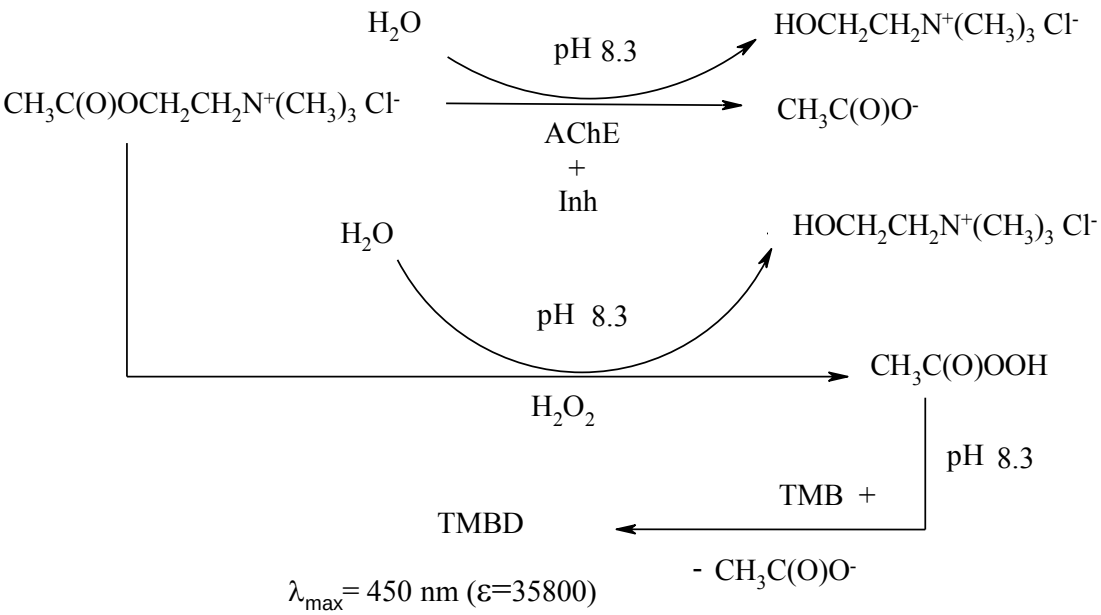

Fig 1. Scheme for determination ChE activity based on the use of conjugated perhydrolysis reactions and peroxy oxidation.

The activity of the enzyme is estimated from the concentration of acetylcholine, which is unreacted during the incubation with the enzyme, which is determined by the preliminarily constructed grading dependence of the absorption of the oxidation product of the indicator reaction (TMBD) on the concentration of the acetylcholine, taken after incubation in the absence of enzyme. For five averaged values of optical density using equation calibration curve found specific activity of the test sample enzyme (*U*), in international units (AO) - kmol/min to 1 mg of substance.

Results. The calibration graph the linear dependence of the activity of the enzyme (*U*, AO/mg) upon A was observed $U=(31.6\pm0.35)-(33.0\pm0.6)\cdot A$, (r=0,999). The results confirmed that the method is linear at concentrations ranging from 3.5 AO/mg to 28 AO/mg. Metrological characteristics of the proposed method is RSD= 1.8%. Accuracy is -0.5%.

Conclusion. A simple and inexpensive method of analysis ChE has been proposed.