College of Veterinary Medicine
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Veterinary practical Pharmacology Part 2

Determination of plasma cholinesterase activity by an electrometric method

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• Acetylcholinesteras

• an enzyme present in nervous tissue, muscle, and red blood cells that catalyzes the hydrolysis of **ACETYLCHOLINE** to choline and acetic acid.

• This enzyme is present throughout the body, but is particularly important at the **myoneural JUNCTION**, where the nerve fibers terminate. Acetylcholine is released when a nerve impulse reaches a myoneural junction. It diffuses across the synaptic cleft and binds to cholinergic receptors on the muscle fibers, causing them to contract. **CHOLINESTERASE** splits acetylcholine into its components, thus stopping stimulation of the muscle fibers. The end products of the metabolism of acetylcholine are taken up by nerve fibers and

resynthesized into acetylcholine.

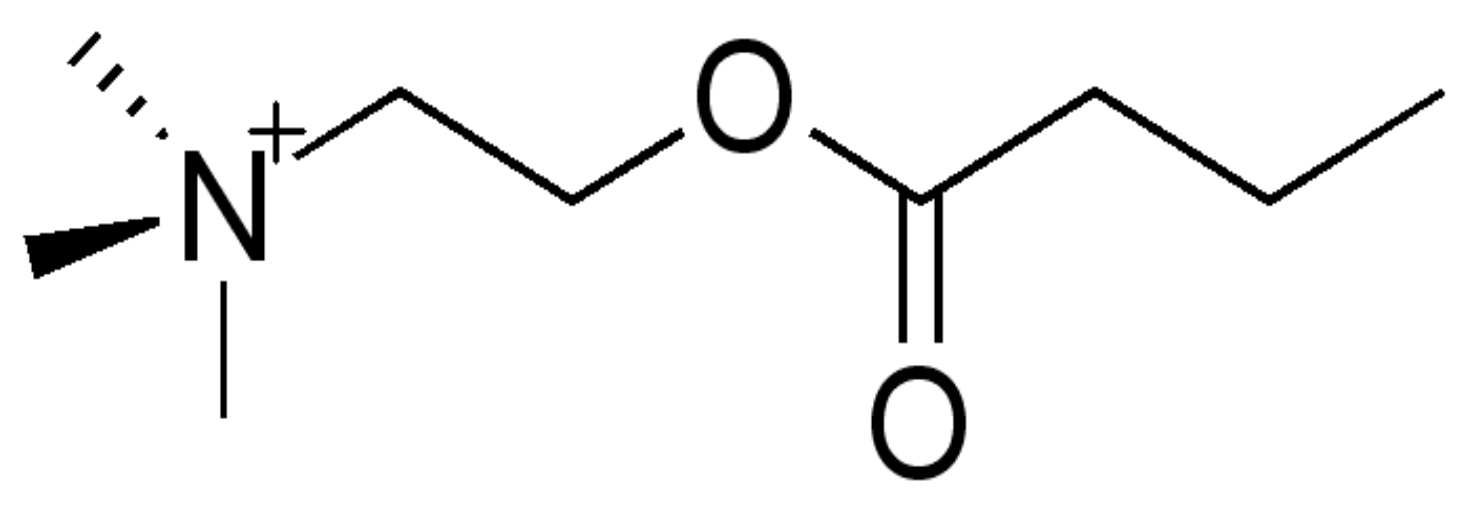
Types of cholinesterase:

1.Acetylcholinesterase (EC 3.1.1.7), also known as AChE, choline esterase I, RBC cholinesterase, or erythrocyte cholinesterase, **true cholinesterase**, choline esterase I, or (most formally) acetylcholine acetylhydrolase, is found primarily in the blood on red blood cell membranes, in neuromuscular junctions, and in other neural synapses. Acetylcholinesterase exists in multiple molecular forms. In the mammalian brain the majority of AChE occurs as a tetrameric.

1. **2. Butyrylcholinesterase** (EC 3.1.1.8) (BCHE), also known as cholinesterase, choline esterase II, BChE, BuChE, **pseudocholinesterase**, plasma cholinesterase, serum cholinesterase, butylcholinesterase, or (most formally) acylcholine acylhydrolase, is produced in the liver and found primarily in blood plasma..

Acetylcholinesterase(true cholinesterase)

Butyrylcholinesterase(pseudocholinesterase)



The enzyme is inhibited **by organophosphates and carbamate insecticides**. Approximately **30%** decrease in enzyme activity indicates exposure of the animal or man to cholinesterase inhibitors.

Techniques for determination of cholinesterase activity in the plasma , R.B.C and brain :

1- **Electrometric Technique** (e.g. Michel ´s method) : it measures PH change in the reaction medium following hydrolysis of acetylcholine into acetic acid and choline .
2- **Hestrin method** : it utilizes the reaction of acetylcholine with hydroxylamine and ferric chloride producing areddish- purple complex which is measured at 515 nm by a spectrophotometer .

3- **Ellman ´s procedure**: Acetylcholine iodide is hydrolyzed by acetylcholinesterase producing thiol group . quantification of the sulfhydryl groups can be achieved by coupling the reaction to 5,5- dithiobis –(2-nitibenzoic acid) (DTNB) and measuring the colored product spectrophotometrically at 412nm.

Procedure:

A modified electrometric method is outlined below for measuring plasma or serum cholinesterase activity in laboratory animals (e.g. rats, mice or chickens).

0.2 ml plasma

+

3ml distilled water

+

3ml phosphate buffer

1.237g sodium barbital , 0.163g potassium dihydrogen phosphate , 35.07 g Na Cl/ 1 L distilled water , PH 8.1)

↓

Determination PH of the reaction mixture by a PH meter (PH1)

↓

Add 0.12 ml acetylcholine iodide (7.5 %)

↓

Incubation at 37 C° for 30 minutes

↓

Determination PH of the reaction mixture (PH2)

↓

Cholinesterase activity (Δ PH / 30 min)
= (PH1- PH2) - Δ PH of blank (without plasma)

Sample	PH1	PH2	Δ PH
0.2 ml plasma			
Blank (no plasma)			

Δ PH /30 min = Δ PH sample - Δ PH blank

PH-meter