CHAPTER I

Introduction

Cholinesterases (ChE) are a group of enzyme capable of hydrolyzing choline esters at a higher rate than other ester to yield free choline and the corresponding acid. The general reaction of this enzyme is:-

$$RCO_2R' + H_2O \longrightarrow RCO_2H + R'OH$$

Where the organic radical R is commonly a choline moiety, R' may be acetate, butyrate, propionate or benzoate (MacQueen, 1973).

Cholinesterases constitute a group of esterases with widely divergent properties. Some of them are specific in their nature, i.e., acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), propionylcholinesterase (PrChE) and intermediate types between specific cholinesterases also exist (Berry, 1960). Cholinesterases are specific since they serve to catalyze the hydrolysis of acetylcholine which is the neurohumoral transmitter of the parasympathetic nervous system.

Cholinesterase was first isolated from horse serum in 1932 and it was identified in human serum in 1935 (Searcy, 1969). It has been known since 1943 that, in man and other mammalian species, there are two principal groups of cholinesterase; i.e., "acetyl-

cholinesterase" and "cholinesterase". They are formerly known as "true cholinesterase" and "pseudocholinesterase" respectively. (See Table 1). Acetylcholinesterase is abundant in red blood cell and neurons at the neuromuscular junction and in certain other tissue whereas pseudocholinesterase is rich in serum, liver and other organs. It now seems appropiate to use the terms acetylcholinesterase for enzyme in red cells and pseudocholinesterase or serum cholinesterase for enzyme in serum.

The formula structures of cholinesterase have not been clearly known. Here both histochemical and pharmacological findings suggest that the neuronal acetylcholinesterase consists of two fractions: a functional portions, with its active sites directed externally to the surface of the cell membrane and directly concerned in the hydrolysis of acetylcholine, and an internal or reserve portion, representing enzyme more recently synthesized within the endoplasmic reticulum and serving as a source of replacement for the former in the course of the cell's cycle of protein turnover (Mclsaac and Koelle, 1959).

Table 1 Nomenclature of cholinesterases

acetylcholinesterase	serum cholinesterase
acetylcholine acetyl-hydrolase (3.1.1.7)	acylcholine acyl-hydrolase (3.1.1.8)
(specific cholinesterase, true cholinesterase, "e"-type cholinesterase) acetocholinesterase	(butyrylcholinesterase, propionylcholinesterase, nonspecific cholinesterase, pseudocholinesterase, "s"-type cholinesterase)

After Holmstedt, 1971 and Searcy, 1969.

The active sites of both acetylcholinesterase and pseudocholinesterase are ester binding group ("esteratic site"), a second active site that is negative charged "(anionic site") in acetylcholine and that is a van der Waals centre in pseudocholinesterase. This non-esteratic site explains why cholinesterases are much more sensitive to quaternary ammonium salts than are other esterases. This affinity for cationic substrates (and inhibitors) is the most characteristic feature of these enzymes. The second site which controls binding and orientation of the substrate, is frequently responsible for the action specificity of the enzyme. Consequently, the dominant reactive forces of this site in pseudocholinesterase

are van der Waals forces, whereas the predominant ones in the anionic site of acetylcholinesterase are coulombic (Augustinsson, 1971).

There is little doubt that serine and histidine are the basic groups in the esteratic site of all cholinesterases that have been studied. The strongest evidence for the presence of serine has been provided by degradation studies of $DF^{32}P$-inhibited enzymes. The evidence for the presence of histidine is indirect, as are the indications that the acidic group of the esteratic site is the tyrosine hydroxyl. The carrier for the negative charge in the anionic site of acetylcholinesterase is most likely glutamic acid. It was sustained that the carrier of pseudocholinesterase is an acid with no free carboxyl group (Augustinsson, 1971).

The substrate, acetylcholine, combines with an active unit of the enzyme to form a complex, by electrostatic attraction between the guaternary N^+ atom of the choline moiety and the anionic site of the enzyme and by interaction between the electrophilic C atom of the carbonyl group and the serine hydroxyl group of the esteratic site as shown in figure 1. Choline is then split off, leaving the acetylated enzyme. The latter reacts rapidly with water to produce acetic acid and the regenerated active enzyme.

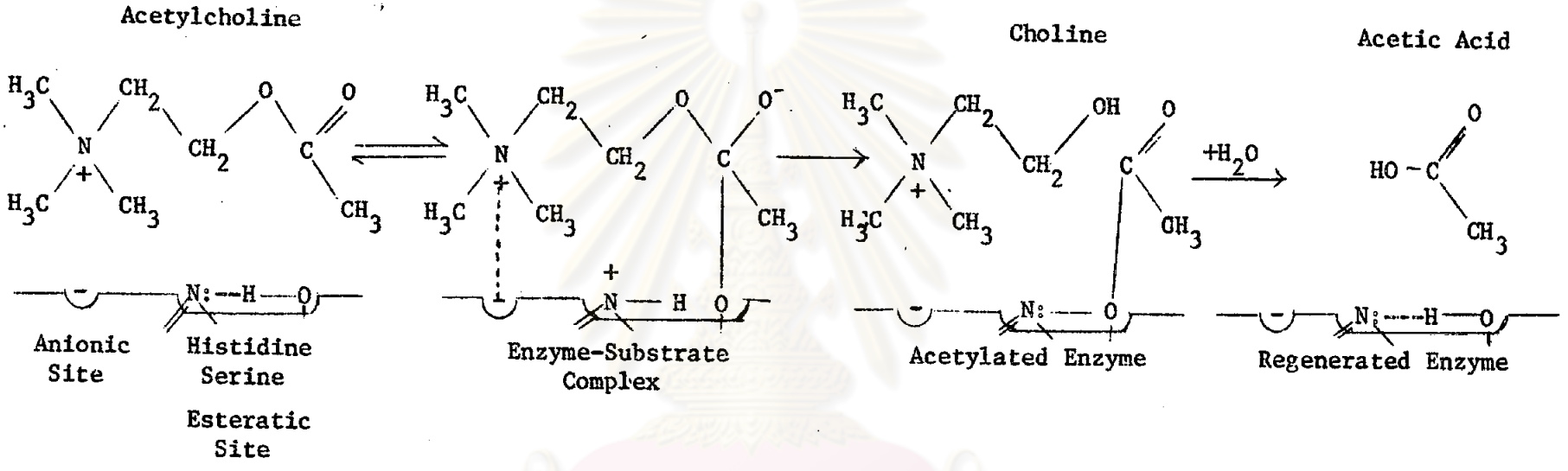


Fig 1 Steps involved in the hydrolysis of acetylcholine by acetylcholinesterase (AChE) (After Goodman and Gillman, 1975).

Distribution of Cholinesterases

The cholinesterases can also be characterized by their occurrence and distribution in different organs. Histochemical techniques have been used in order to reveal the cells or parts of cells in which the activity of the enzyme is localized. It was found that various types of cholinesterases are present in almost all tissues together with other esterases enzymes and different quantities. Acetylcholinesterase appears to be the integral parts of certain electrogenic membranes and other isoluble cell structures. The main sources are brain, nervous tissues, erythrocytes, and electric organs. In central nervous system, acetylcholinesterase activity is particularly abundant in most neurones of a large number of region especially in the gray matter of central nervous tissue. The concentration of the enzyme appeared to be consistently high in cholinergic neurones especially in postsynaptic but were lower in the adrenergic and sensory types.

Studies on tissue homogenates have revealed that striated muscle contains approximately 95% of acetylcholinesterase and 5% of pseudocholinesterase localized in various places. The cholinesterases in the end-plates consists predominantly of acetylcholinesterase, but cholinesterase is also present.

Acetylcholinesterase is also found in a number of other tissues, such as the smooth muscles of the bronchioles and the urinary bladder, the effector cells of the salivary glands, the diaphram and the adrenal gland (Holmstedt, 1971).

Pseudocholinesterase are riched in blood plasma of humans and higher vertebrates. In central nervous system, pseudocholinesterase is present in highest concentration in the white fibre tracts and is localized in the glial cells. It is also found around the supraoptic nucleus and around the third ventricle in the region of the paraventricular nucleus. The neurones of Auerbach's plexus and the associated interstitial cells were found to contain, in addition, uniformly high concentrations of pseudocholinesterase, which in other ganglia occurs only in the glial cells. Very little of pseudocholinesterases were found in the striated muscle. They are also present in various tissues e.g., liver cells, the carotid body, the muscularis mucosa of the intestinal canal, and the carotid cells of the zona glomerulosa of the adrenals.

Some Properties of Cholinesterase

The specificity and properties of these enzymes has not been studied thoroughly due to the fact that the cholinesterases have not been highly purified. The purification of cholinesterase are very difficult because of the insolubility of acetylcholinesterase from most sources, and serum cholinesterase is sensitive to denaturation by organic solvents and the high molecular weight of most of the enzymes. In view of these difficulties, it is not surprising that the literature and nomenclature of these enzymes are very confused.

As other enzymes, the 2 groups of cholinesterases can be differentiated by the use of either specific substrates or selective inhibitors and by the properties of isolated protein and by studying kinetic behavior. The general properties of cholinesterases are listed in Table 2.

Cholinesterases specificity

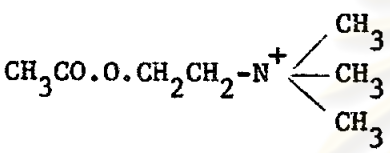
It is generally accepted that there were no cholinesterases which have absolute specificity for choline esters. In fact, all cholinesterases also split ordinary esters and the various enzymes having distinct specificity patterns. Thus for example, acetyl-cholinesterase splits acetic acid esters more rapidly than propionic, or butyric acid esters whereas human plasma butyrylcholinesterase catalyses the hydrolysis of butyric acid esters at a higher rate than the esters of the lower homologous acids. As mentioned above, there are other types that split propionic esters at the highest rate. This rule is valid for choline as well as for non-choline esters.

Table 2 Some general properties of cholinesterases.

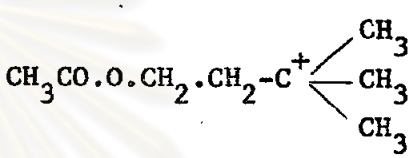
Property	Acetylcholinesterases	Cholinesterases
source	electric organ of the electric eel, brain, erythrocytes, cobra venom	serum, pancreas, heart, liver
optimum substrate	acetylcholine	butyrylcholine, propionylcholine, or benzoylcholine
utilization of acetyl-β-methyl-choline	substrate	non-substrate
species differences	not significant	significant
inhibition by:		
quaternary ammonium compound	+++	+
bis-N, N'-diiso-propyl phospho-rodiamidic anhydride	+	+++
phenothiazine derivatives	+++	+

After Augustinsson, 1971.

Many factors suggest that cholinesterases have a special affinity for methyl groups, hence branching of the alkyl chain (except at the carbon atom next to the ester group) increases the susceptability of esters. The optimum alkyl group in both cases appear to be 3, 3-dimethyl-butyl, which is in fact a carbon analogue of choline as the structure is shown below.



acetylcholine



3, 3-dimethyl-butyl

It is interesting that the charged nitrogen atom of choline is not essential for either enzymes, and the shape of tne molecule is clearly one of the most importance factors.

The cholinesterases, like carboxyesterases, can hydrolyse aromatic as well as aliphatic esters, often quite rapidly.

Molecular weight

Different values have been reported for the molecular weight of purified acetylcholinesterase and pseudocholinesterase (Augustinsson, 1971). Besides species difference, the molecular weight values are also depended on the methods used. The molecular size seems to be dependent on the pH and ionic strength of the medium used in isolating the enzymes. Acetylcholinesterase is polydispersed in media of low ionic strength, but exhibit only one sedimentation coefficient (14$) in solutions of higher ionic strength. The moiety

sedimenting at 4 S is probably the monomer of acetylcholinesterase, that sedimenting at 8-10 S the dimer, and that sedimenting at 12-14 S the trimer.

The molecular weight of purified human serum butyrylcholinesterase has been shown to exceed 200,000. Ultracentrifugation studies gave a value of approximately 300,000. Augustinsson (1971) suggested that there are several active sites per molecule and the multiplicity of serum cholinesterases. He demonstrated that difference in various mammalian species, is probably attributed to reversible polymerization.

Electrophoretic properties

Serum cholinesterases have been more thoroughly studied electrophoretically than the acetylcholinesterases. They usually have the isoelectric point of pH 3-5. However, this is dependent on the electrophoretic technique used and the source and purity of the preparation.

Serum cholinesterases from man has been seperated into 2-7 distinct bands by mean of different techniques of gel electrophoresis. Each of these bands may be referred to as an isoenzyme. There is much less evidence for the existence of isoenzymes among acetylcholinesterases than among pseudocholinesterases, but various electrophoretic studies indicate that they do exist.

Amino acid composition

The amino acid composition and sequences of both acetylcholinesterase and pseudocholinesterase are now fairly well established. However, the active site of cholinesterases was sustained to contain the sequence Glu-Ser-Ala, that is similar to other hydrolytic enzymes,

Function of Cholinesterases

Acetylcholine is served as the neuro-humoral agent in peripheral junctional transmission by being removed or inactivated within the time limits imposed by the response characteristic of visceral neuroeffector junctions, motor endplates, and various types of neurons. This limits range from over a second to less than a millisecond. Enzyme Cholinesterase, especially acetylcholinesterase that present in both inside and outside the cell membrane of tissues and in body fluids, are capable of rapidly hydrolyzing acetylcholine to choline and acetic acid. The choline produced is pharmacologically weak in comparision with its acetylated precursor (Goodman and Gillman, 1975). Thus, it is likely that acetylcholine and acetylcholinesterase participate in the maintenance of the excitable state in those fibers in which they are present in significant concentrations.

The physiological function of pseudocholinesterase or cholinesterase is unknown. It can hydrolyze acetylcholine and certain other aliphatic and aromatic esters. Pseudocholinesterase may serve to prevent acetylcholine from reaching undesirable levels in the

body. The postulation that pseudocholinesterase may protect acetylcholinesterase from inhibitors is only supported by the two enzymes occur in close association in the body.

It was sustained by some investigators that the cholinesterases in red blood cell especially acetylcholinesterase exerts a considerable influence on the cellular membrane stability of the erythrocyte (Greig and Holland, 1947, Greig and Faulkner, 1953; Holland and Greig, 1951). They suggested that cholinesterase may be mediated through its effect on the normal sodium and potassium gradients of the erythrocyte.

Cholinesterases activity and diseases

Decreased cholinesterase activity

Cholinesterase levels in serum or red cells can be reduced by an impairment of their synthesis. This may relate to an acquired disease or a genetic defect. Reductions in cholinesterase activity sometimes reflect the presence of a specific substance, such as certain organophosphorus or carbamate insecticides, that inhibit the action of the enzymes.

Cholinesterases have been primary known as an index of exposure to anticholinesterase insecticides. Following exposure to organophosphate and carbamate insecticide, the level of serum cholinesterase falls and then the amount of cholinesterase decrease (MacQueen et al, 1973). The measurements of serum cholinesterase and red cell acetylcholinesterase are the practical method than any

other method for measuring the absorption of anticholinesterase insecticides, according to its sensitivity, rapidity and simplicity. It is necessary to determine enzyme cholinesterase activity for all personnel who come in contact with those compounds. It is advised that worker who shows a weak enzyme activity should not continue to work in situation where exposure is likely to occur.

The level of serum cholinesterase is useful in the diagnosis and evaluation of patients with several diseases i.e. infectious hepatitis, cancer, anemia, etc. For infectious hepatitis serum cholinesterase is depressed because of the decreased capacity of the liver, the organ of origin and synthesize it (Wetstone and LaMotta, 1965; Molander et al, 1954; Kaufman, 1954). It may be helpful in indicating prognosis, and beginning of recovery from hepatitis or failure of therapy (MacQueen et al, 1973; Sider et al, 1968). Serial determinations of serum bilirubin and cholinesterase were suggested as the most sensitive indices of liver damage following transplantation (Evans and Lehman, 1971).

The level of cholinesterase activity has been evaluated in parenchymal disease. In the presence of a known carcinoma, normal cholinesterase levels suggest no liver metastasis (Mac Queen et al, 1973; Molander et al, 1954). Vaccarezza and his colleagues (1964) found that plasma, whole blood and red blood cell cholinesterase were significantly lower in patients with pulmonary cancer than in healthy persons.

It was observed that cholinesterase was also useful in the differential diagnosis of jaundice (Alcalde, 1950). Normal values were found in benign extrahepatic obstruction and low values in malignant obstruction. Dermatomyositis may also be differentiated from other collagen diseases by decreased activity of the enzymes (MacQueen, 1973).

Serum cholinesterase assay has been found to be helpful in diagnosis, prognosis, and management in all the categories of malnutrition from maramus to the severest form of kwashiorkor (Baclay, 1973; Begum and Prathapkumar, 1969). Begum and Prathapkumar (1969) demonstrated that patients with protein calory malnutrition had low levels of both serum albumin and cholinesterase but there was no correlation between these two parameters on recovery. They also found that there were significantly rise in the level of red cell acetylcholinesterase and serum cholinesterase on giving vitamin A to the children on low vitamin intake.

Milstoc (1970) recommened to use the ratio between cholinesterase activities of the red cell and plasma in evaluating the pathologic process of rheumatoid arthritis in which the ratios increased with the severity of the disease.

Acetylcholinesterase activity in red cells may be useful for supporting the diagnosis of the newborn ABO hemolysis since acetylcholinesterase activity are frequently reduced in babies with ABO incompatability with hemolytic disease and acetylcholinesterase was higher in babies with ABO incompatibility with no hemo-

lytic disease when compared to normal babies (Kaplan et al, 1964; Herz et al, 1972).

Low activity of acetylcholinesterase are noted in patients with thalassemia or autoimmune hemolytic anemia (Searcy, 1969). Most authorities agree that cholinesterase regulates the resistance of the red cell membrane to hemolytic agents. This may be mediated through its effect on the normal sodium and potassium gradients of the erythrocyte.

One of the most important use for cholinesterase assay clinically is presurgical screening for sensitivity to succinylcholine. Succinylcholine, a short-acting muscle relaxant, is composed of two acetylcholine molecules. Normally this drug is hydrolyzed in vivo by pseudocholinesterase in two to four minutes. The prolonged paralysis following exposurer to succinylcholine was associated with low total pseudocholinesterase activity due to genetic abnormality (Evans et al 1952, Lehmann and Ryon 1956).

Besides the diseases and genetic defect as mentioned above, cholinesterase activity in serum and red cells appears to be affected due to the alteration of physiological factors i.e. age, sex, pregnancy etc. There is a general tendency for serum cholinesterase levels to be lower during infancy than in adulthood. The activity of this enzyme normally increase during the first month, approaching the normal adult range (Searcy, 1969). The activity of serum cholinesterase increases with the age of the subject until the sixth decade of life (Sidell and Kaminskis, 1975).

Some observers have noted that women had lower serum cholinesterase activity than men (Sidell and Kaminskis, 1975; Wetstone and LaMotta, 1965; Kaufman, 1954, Garry, 1971). However, some observers found that there were no significantly difference in both sexes (Callaway et al, 1951; Vorhaus and Kark, 1953; Augustinsson, 1955).

The activity of cholinesterases in women taking oral contraceptive pills were found to be decreased (Sidell and Kaminskis, 1975; Redderson, 1973). It was suggested that the female sex steroids depress hepatic function.

It is now well established that cholinesterase activity declines during pregnancy (Friedman and Lapan, 1961; Shnider, 1966; Robertson, 1966; Howard et al, 1978). The fall in enzyme activity occurs early in pregnancy, reaches its lowest level on the third postpartum day, and may not return to normal levels until 6 weeks postpartum. Various explanations such as hemodilution, malnutrition and hepatic dysfunction during pregnancy have been postulated.

Increased cholinesterase levels

Elevated cholinesterase activity in the serum or red cells is occasionally encountered, but at present this finding has only limited diagnostic usefulness. The increase may reflect a stimulation in synthesis or some cellular destructive process. For example, an accelerated turnover of red cells will increase blood level of cholinesterase since the young erythrocytes, which become more plentiful, are very rich in the enzyme.

Elevated levels of cholinesterase in serum have been found in patients ill with nephrotic syndrome (Vorhaus and Kark, 1953; Vincent, 1958). Enzyme levels in serum may be increased as much as three folds above normal . In the nephrotic syndrome, liver cells are stimulated to produce albumin to replace the excess amount of this protein lost in the urine, and therefore synthesis the excess amount of plasma cholinesterase.

Patients with manic depressive psychosis, anxiety and depressive neurosis or schizophrenia frequently exhibit modest degrees of hyperpseudocholineterasemia. Minor increases are also associated with electroshock therapy. Spastic children exhibit high serum levels of pseudocholinesterase, presumably a reflection of increased activity at the neuromuscular junction (Searcy, 1969).

Hyperthyroidism complicated with heart failure or atrial fibrillation has increased red cell acetylcholinesterase activity. The turnover rate of the enzyme is accelerated, but there is not result of the direct action of thyroid hormones. This phenomenon is ascribed to the shortened life span of the erythrocyte and to the fact that young red cells are richer in acetylcholinesterase than those of the old cells (Searcy, 1969).

Hyperpseudocholinesterase has been frequently found in diabetics (Searcy, 1969). The elevated serum pseudocholinesterase levels are evident primarily in cases where obesity accompanies diabetes. There is no adequate explanation for this association

other than the possibility that pseudocholinesterase plays some role in fat metabolism.

Anticholinesterase agents

Drugs that inhibit or inactivate acetylcholinesterase and pseudocholinesterase are called anticholinesterase (anti-cholinesterase) agents. They cause acetylcholine to accumulate at cholinoceptive sites and thus are potentially capable of producing effects equivalent to continuous stimulation of cholinergic fibers throughout the central and peripheral nervous systems.

Reversible and irreversible cholinesterase inhibitors have been distinguished by the type of bonding between enzyme and inhibitor-the irreversible inhibitors would react chemically with the enzyme, while the reversible inhibitors would bind to the enzyme by much weaker bonds (hydrogen bonds, electrostatic bonds, etc.). Competetion for the enzyme between substrate and inhibitor can occur with either reversible or irreversible anticholinesterase and reversible inhibitors can protect the enzyme from irreversible inhibitors (Zeller and Fouts, 1962).

Many substances, natural and synthetic were found to be the reversible cholinesterase inhibitors. The more powerful inhibitors have a quaternary or basis nitrogen atom, they often contain some ester-like grouping such as urethane group and are highly methylate. All of these features might be expected to produce competetion with the substrate acetylcholine, which has a highly methylate nitrogen

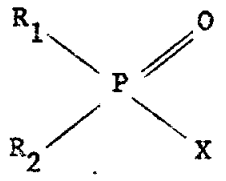
atom in the choline residue.

Most of irreversible cholinesterase inhibitors are organophosphorous or thiophosphorous derivatives. This group of substances is collectively known as "the nerve gases", since the higher sensitivity of the cholinesterase makes them highly toxic to central nervous system. These compounds have widely used as agricultural insecticides and frequently caused poisoning in humans and other animals. The general formular for this group of cholinesterase inhibitors is shown in Table 3.

The various cholinesterases are not always equally inhibited by these compounds. In particular the acetylcholinesterases differ considerably from the cholinesterases in their sensitivity towards the difference competitive inhibitors; so that acetylcholinesterase is inhibited selectively by low concentrations of several bis-quaternary ammonium bases and by other agents but pseudocholinesterase is more sensitive to inhibition by several organophosphorous agents such as DFP and mipafox, and certain quaternary compounds than in acetylcholinesterase (Goodman and Gillman, 1975).

Table 3 Chemical classification of representative organophosphorus compounds of particular pharmacological or toxicological interest

General formula (Schrader, 1952):



Group A, X = halogen, cyanide, or thiocyanate; group B, X = alkyl, alkoxy, or aryloxy; group C, thiol- or thionophosphorus compounds; group D, pyr. . nosphates and similar compounds; group E, quaternary ammonium compounds.

GROUP	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
A	DFP Diisopropyl phosphoro-fluoridate	Potent, irreversible inactivator
	Mipafox, Isopestox N, N-Diisopropylphospho-rodiamidic fluoride	Early insectide; selective inhibitor of nonspecific cholinesterase (butyryl-cholinesterase)
	Tabun Ethyl N-dimethylphospho-ramidocyanidate	Extremely toxic "nerve gas"
	Sarin (GB) Isopropyl methylphospho-nofluoridate	Extremely toxic "nerve gas"
	Soman Pinacolyl methylphospho-nofluoridate	Extremely toxic "nerve gas"

Table 3 (Continued)

GROUP	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
B	Paraoxon, Mintacol, E 600 Diethyl 4-nitrophenyl phosphate	Active metabolite of parathion
C	Parathion, Thiophes, E 605 (see list of trade names in text) Diethyl O-(4-nitrophenyl) phosphorothioate	Widely employed agricultural insecticide, resulting in numerous cases of accidental poisoning
	EPN O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate	Widely employed agricultural insecticide
	Malathion O, O-Dimethyl S-(1, 2-dicarbethoxyethyl) phosphorodithioate	Widely employed insecticide of greater safety than parathion or EPN because of rapid metabolism by higher organisms
D	TEPP Tetraethyl pyrophosphate	Early insecticide
	OMPA, Schradan Octamethyl pyrophosphoramide	Insecticide; inactive in vitro, but metabolized by animals and plants to potent anti-cholinesterase agent

Table 3 (Continued)

GROUP	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
E	Echothiophate, Phospholine, 217MI Diethoxyphosphinylthio-choline iodide	Extremely potent choline derivative; employed in treatment of glaucoma; relatively stable in aqueous solution

After Goodman and Gillman, 1975.

Many methods of cholinesterase determination have been suggested and compared but only a few of them are appropriate for work in the field. Such methods should be simple, rapid, specific and precise. The diversity of methodology has encouraged the introduction of a number of different units for expressing activity. Some confusion may be avoided by adoption of the International Unit, which expresses activity in μM acetylcholine hydrolyzed/min/ml. at 37°C.

Different methods for determining cholinesterase are listed below:-

1. Manometric method (Mandel and Rudney, 1943; Chiou, (1973); Kekwick, 1960; Callaway et al, 1951).

2. Tritrimetric method (Stedmen, Stedman and White, 1953; Nabb and Whitfield, 1967; Aldrich et al, 1969, Sawitsky et al, 1948).

3. Electrometric techniques (Michael, 1949)

4. Colorimetric methods (Ellman, 1961; Garry and Routh, 1965; Carraway, 1956; Biggs et al, 1959; Rappaport and Finto, 1959; Gerard et al, 1965; Stein and Lewis, 1966; MacQueen et al, 1971; Tetsuo et al, 1972; Augustinsson et al, 1978)

5. Radiometric methods (Johnson and Russell, 1975; McCaman et al, 1968).

The studies of cholinesterase activity in several diseases and anticholinesterase insecticide poisoning have been investigated by many investigators. Cholinesterase activity assay was evaluated as a useful tool for differential diagnosis of some diseases as mentioned above. However, studies on enzyme activity in patients with tropical disease such as malaria and certain diseases in Thailand have not been done previously. Besides, the measurements of serum cholinesterase and red cell acetylcholinesterase in people exposed to anticholinesterase insecticide are very important since Thailand is a agricultural country which use anticholinesterase insecticide very frequently. The purpose of the present study are to determine the cholinesterase activity in serum and red blood cell of normal Thais, people who exposed to anticholinesterase insecticide, i.e., DFP, parathion, malathion, carbamate etc., patients with certain diseases such as infectious hepatitis, malarial infection, thalassemia, and congenital heart disease, pregnant women and cord blood.