CHAPTER IV

DISCUSSION

There are several methods for measurement of serum, whole blood and red cell cholinesterase activities. These methods based on the manometric, electrometric (potentiometric), colorimetric and radiometric processes. The most simple, rapid, accurate and sensitive methods for whole blood cholinesterase and serum cholinesterase were described by Ellman et al (1961) and Garry and Routh (1965) respectively. Both methods used acetylthiocholine as a substrate which was hydrolyzed by both plasma and red cell cholinesterase to acetic acid and thiocholine. Since the incubation time for measurement of serum and whole blood cholinesterase are 3 and 6 minutes respectively, and the amount of samples for assay is very small, therefore, they are suitable for using in the laboratory and in the field work.

Results in the present study showed that the reproducibility and the recovery of these 2 methods in our laboratory were quite satisfactory. We, therefore, used these methods throughout the study.

Results in the present study also showed that both serum and red cell cholinesterase levels in the blood sample were stable for more than 1 month. This finding was in accordance with results reported by other authors (Sider et al, 1968; Wetstone and LaMotta,

1965, Lanks and Sklar, 1976). All samples were therefore kept in the -4°C and were used for estimation of cholinesterase levels before one month.

Cholinesterase activity in Thai blood donors

Results in the present study showed that there was no significant difference between the mean values of serum and red cell cholinesterase levels in male and female blood donors, therefore, these data were grouped together as the normal value. These findings confirmed results reported by other authors (Vorhaus and Kark, 1953; Augustinsson, 1955). However, there were some reports showing that the mean value of serum and red cell cholinesterase in female subjects was significantly lower than that of male subjects (MacQueen, 1973; Sidell and Kaminski, 1975). They explained that the difference was probably due to the effect of the estrogen hormone in the female subjects.

The normal values of serum and red cell cholinesterase levels expressed as the international unit (I.U.) reported by various authors are shown in Table 26 in which data of the present study was also included. The mean value of serum cholinesterase in the present study was found to be slightly but not significantly lower than those of the other authers.

Results of the red cell cholinesterase activity in the normal subjects were slightly higher than those of MacQueen et al (1973) and Sidell and Kaminskis (1975) but were in accordance with the normal values of Knaak et al (1978).

Table 27 Red cell cholinesterase activity in normal human subjects determined by different methods.

No.	No. of Case studied	Method	Serum ChE (I.U.)		Red cell ChE (I.U.)		References
			Mean	Range or S.D.	Mean	Range or S.D.	
1	20	Manometric	4.03	2.37-5.43	-	-	Kekwick, (1960).
2	228	Manometric	3.95	-	-	-	Callaway et al, (1951).
3	15	Titrimetric	4.21	-	12.19	0.86	Sawitsky et al, (1948).
4	51	Colorimetric	3.40	2.58-4.80	-	-	Wetstone et al, (1957).
5	82	Colorimetric	3.51	2.13-5.60	-	-	Wetstone and Lamotta, (1965).
6	70	Colorimetric	4.89	2.80-6.61	-	-	Sider et al, (1968).
7	1842	Colorimetric	3.67	2.10-5.25	-	-	Garry, (1971).
8	47	Colorimetric	3.68	1.93-5.53	-	-	Garry and Routh, (1965).
9	50	Colorimetric	3.76	-	12.63[a] 10.87[b]		MacQueen, (1973).
10	-	Colorimetric	-	-	-	11.23-14.65[a] 10.08-15.11[b]	Sidell and Kaminski, (1975).

Table 27 (Continued)

No.	No. of Case studied	Method	Serum ChE (I.U.)		Red cell ChE (I.U.)		References
			Mean	Range or S.D.	Mean	Range or S.D.	
11	-	Colorimetric	-	-	27.5[a]	4.5	Knaak, et al, (1978).
					28.1[b]	3.8	
12	100	Colorimetric	3.07	1.62-4.49	21.28	12.73-33.5	present study.

a = male subjects.

b = female subjects.

Cholinesterase level in people exposed to anticholinesterase insecticides.

Results of serum cholinesterase levels in people directly or non-directly exposed to organophosphate insecticides were significantly lower than those of the normal values. However, there was no significant difference between the mean values of red cell cholinesterase in the exposed group and the normal group. These results were in accordance with results of other authors (Knaak et al, 1978; Simpson, 1974; Bicks, 1967). Since the serum cholinesterase level is more sensitive to anticholinesterase insecticide than red cell acetylcholinesterase. It is therefore an excellent tool for the measurement of exposure of insecticides in these workers (Goodman and Gillman, 1975).

Results in the present study also showed that the mean value or serum cholinesterase of the directly exposed group was significantly lower than that of the non-directly exposed group. Since the degree of exposure of man is presumably associated with the concentration of the insecticide and duration of exposure, the directly exposed workers had less serum cholinesterase activity than the non-directly exposed workers. Hayes (1971) found that the hazard of the dermal exposure is generally greater than the respiratory exposure. This is true even though only a small percentage of the material impinging on the skin may be absorbed.

A chronic low dose exposure to anticholinesterase insecticide may cause low cholinesterase activity in man and may produce symptoms

such as headache, blurred vision and mild muscarine signs. Results in the present study showed that the direct exposure workers showed no such symptoms. It was suggested by the Health Commission of New South Wales in 1979 that symptoms of poisoning may be expected to occur when the enzyme activity has been reduced to about 30% of normal value. However, this level may vary from person to person. Wamba (1971) noted that signs and symptoms of poisoning by organophosphates occured when more than 50% of the plasma or red cell cholinesterase is inhibited. Zavon (1965) reported that a prolonged low level exposure to organophosphate insecticide did not usually cause clinical illness until red cell cholinesterase activity decreased to 20% or 25% of the individual's pre-exposure level. Finding of low serum cholinesterase and normal red cell cholinesterase activities in both directly and non-directly exposure workers in the present study indicated that their exposure to anticholinesterase insecticides are not yet hazadous to their health.

Cholinesterase level in patients with acute anticholinesterase insecticide poisoning

Results in the present study showed that acute organophosphate and carbamate insecticides poisoning for suicide produced very low serum and red cell cholinssterase levels with severe symptoms and toxicity. The first symptom of acute organophosphates poisoning came within minutes. They are usually dizziness, fatique, salivation, blurred vision and pin-point pupils, muscle fasciculation and dyspnoea as shown in table 13.

Case 1 who ingested a large amount of organophosphate having undetectable serum cholinesterase level died after admission for 24 hours. Death in this case was probably due to the respiratory failure from paralysis of respiratory muscles and/or cardiac congestion.

Case 6 also had undetectable serum cholinesterase level but he survived after treatment. Other patients had low serum cholinesterase activity, i.e., less than 0.6 I.U. except case 8 and 11 who received a relatively small amount of carbamate and had serum cholinesterase 1.78 I.U. and 1.09 I.U. respectively. Since Carbamates are the reversible inhibitors, the recovery of enzyme activity is therefore comparatively rapid.

The recovery of blood cholinesterase level was very slowly in patients with acute poisoning. Since inhibition by organophosphate compounds is "irreversible", the recovery of cholinesterase is probably due to the replacement and not the reactivation. However, the low serum cholinesterase activities increased very slowly, i.e., from 0.04 I.U. to 0.16 I.U. after treatments for 4 days in case No. 2. This finding indicated that serum and red cell cholinesterase levels served as a good index for diagnosis the toxicity of organophosphate and carbamate insecticides but they were relatively not sensitive for follow up the treatment.

Cholinesterase activity in patients with infectious hepatitis

Results in the present study showed that patients with acute infectious hepatitis had markedly low serum cholinesterase. This finding was in accordance with results reported by many investigators (Kunkel, 1947; LaMotta and Wetstone, 1957; Kaufman, 1954, Sider et al, 1968; Molander et al, 1954). Low serum cholinesterase in these patients was possibly due to the impairment of the hepatic function involving cellular damage and impaired serum cholinesterase and albumin synthesis. Much evidence has supported the concept that serum cholinesterase is produced in the liver, probably in parallel with the albumin (Vorhaus, 1953). There is usually a striking parallel between reductions and the degree of albuminemia and circulating levels of cholinesterase. The enzyme is quickly restored to normal after optimal liver function is regained.

Serial determinations of serum cholinesterase in patients with acute infectious hepatitis in the present study indicated that the low serum cholinesterase level in these patients elevated considerably during the admission in the hospital in parallel to the improvement of their clinical symptoms. These findings confirmed the work of Vorhause et al (1951) who suggested that the determination of the serum cholinesterase was valuable for liver function test, differential diagnosis of jaundice and follow up the improvement of the liver.

Cholinesterase activity in patients with malarial infection.

Results in the present study showed that serum cholinesterase levels in patients with P. falciparum malaria were significantly lower than those of normal subjects and these values returned to the normal levels within 4-6 weeks after treatment. As acute malarial infection is usually associated with liver damage, the low serum cholinesterase activity in these patients is probably due to the impairment of the liver function. This finding was in accordance with results reported earlier. Alterations of many liver function tests indicating early inhibition of certain important liver functions have been observed during the acute phase of P. falciparum infection in man and chimpanzees (Sadun et al, 1966).

Since the physiological function of serum cholinesterase is not yet established, it is therefore difficult to evaluate the significance of the low serum cholinesterase activity in patients with malaria infection. The low serum cholinesterase activity in malarial patients in the present study indicated the possibility of staging the malarial attack by monitoring the serum cholinesterase activity.

Cholinesterase activity in patients with thalassemia.

In thalassemia trait, the erythrocytes life span in known to be shortened to a variable extent. The cause of hemolysis in thalassemia trait is obviously related to the instability of the circulating red cells. The mean values of serum cholinesterase in patients

with β/E thalassemia and Hb-H thalassemia in the present study were lower than that of the normal subjects. There was no previous report about serum cholinesterase in thalassemia and there is as yet no reasonable explanation for the decreased serum cholinesterase activity.

The present study also found that the mean value of red cell cholinesterase in β/E thalassemia was higher than that of normal value. The increased red cell cholinesterase is possibly due to the accelerated turnover of red cells in these patients with the increased production of cells which are very rich in these enzymes.

Cholinesterase activity in patients with congenital heart disease

There was no significant difference between the serum and red cell cholinesterase levels of patients with congenital heart disease and that of the normal subjects. Thus, there was no changes in both red cell and serum cholinesterase activities in patients with congenital heart disease.

Cholinesterase activity in pregnant women

Results in the present study showed that serum cholinestrese levels were low during pregnancy. This finding confirmed the results reported by previous investigators (Blitt et al, 1977; Friedman and Lapan, 1961; Robertson, 1966; Shnider, 1966; Howard et al, 1978). The possible reasons for the fall in cholinesterase

level might be due to the hemodilution, impairment of hepatic function and the effect of estrogen which is increased during pregnancy. Estrogen, female sex steroids, depressed hepatic synthesis or release of this enzyme. In women taking oral contraceptive also had low serum cholinesterase activity (Redderson, 1973). Some investigators have reported the relationship between the low serum cholinesterase activity and the prolonged apnea following succinylcholine given during caesarian section (Evan et al, 1953; Scholler et al, 1977; Pritchard, 1955). Thus serum cholinesterase activity should be monitored in every pregnant woman who was going to be given succinylcholine before the caesarian section.

Results in the present study showed that red cell cholinesterase level in pregnant women was not significantly different from those of non-pregnant women. This finding was in accordance with results reported by Pritchard and Weisman (1956).

Cholinesterase activity in cord blood.

Results in the present study showed that the activity of red cell cholinesterase in cord blood was significantly lower than that of adult value. This finding was in accordance with results reported earlier by other authors (Burman, 1961; Kaplan and Tildon, 1963). Burman (1961) noted that the adult level of serum cholinesterase was reached in infant between 3 and 5 months of age. Cholinesterase is probably similar to the other enzymes i.e., DPNH-dependent methaemoglobin reductrse, carbolic anhydrase catalase and glycoxylase which were low in the erythrocytes of newborn and had developed

fully after birth. The Low red cell cholinesterase activity found in the newborn was therefore the characteristic of this period of life.

The present study found that serum cholinesterase of cord bloods were lower than those of the adult bloods but this difference was not statistically significant. However, some investigators reported that there was significant difference between cord sera cholinesterase activity and that of adult serum (Jones and McCance, 1949; McCance, et al 1940). As the low serum albumin has been previously reported in newborns and there was a direct relationship between the low serum albumin concentration and the decreased serum cholinesterase in some subject (Dancis et al, 1960). The low serum cholinesterase activity and albumin concentration in the cord blood was therefore an indicative of the low liver function in this period of life.