# TECHNICAL NOTE

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# Cholinesterase Activity in Postmortem Blood As a Screening Test for Organophosphate/Chemical Weapon Exposure

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**ABSTRACT:** This study was undertaken to determine whether postmortem blood cholinesterase activity could be used as a screening test for exposure to nerve agents. Whole blood cholinesterase activity at 25°C was analyzed for a one week period in order to simulate the battle field collection problems of: hemolyzed blood samples, delayed recovery of the specimen, and unrefrigerated transfer to the testing facility. A total of 53 nonpreserved postmortem whole blood specimens were analyzed in triplicate for cholinesterase activity by the Δ pH method of Michel. There was a negligible loss of cholinesterase activity by the seventh day of the study. The enzyme activities of the specimens had a mean value (range) of 0.48 (0.20 to 0.74) initially and 0.45 (0.07 to 0.70) pH units after one week. Whole blood from five healthy adults remained essentially unchanged during this period, with an initial value 0.59 (0.52 to 0.67) and a final value of 0.52 (0.46 to 0.62) pH units. To compare postmortem and simulated nerve agent values, aliquots from 18 of the original 53 postmortem specimens were frozen during day one of the study, thawed on day seven and a cholinesterase inhibitor added. These specimens were then analyzed with the other specimens. All values from inhibited specimens were essentially zero (0.0 to 0.01) pH units compared to a range of 0.07 to 0.61 pH units for matched, uninhibited, day seven postmortem specimens. Fifteen actual nonpreserved specimens from the battlefield were analyzed as verification of screen performance. Their results fell within the uninhibited postmortem range above. All of the 53 day one postmortem, day seven postmortem, and 13 of 15 battlefield specimen cholinesterase activities were significantly greater than the levels found in the 18 specimens exposed to the cholinesterase inhibitor. We conclude that the cholinesterase activity in postmortem specimens collected within a one week period of death can be used as a screen for possible nerve agent exposure.

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Acetylcholine is a neurotransmitter appearing in postganglionic parasympathetic neurons and at the neuromuscular junction. It is rapidly inactivated by enzymatic hydrolysis to acetic acid and choline by cholinesterases. In man, there are two types of cholinesterases: 1) acetylcholinesterase, which appears in red blood cells, lung, spleen, nerve endings, and gray matter of the brain; and 2) pseudocholinesterase, which appears in the plasma and throughout the body. Acetylcholinesterase hydrolyzes acetylcholine and acetyl beta methylcholine while pseudocholinesterase hydrolyzes acetylcholine, butyrylcholine, and benzoylcholine. Acetylcholinesterase is responsible for hydrolysis of acetylcholine in the synapse while the role of pseudocholinesterase is unclear [*1*].

The role of cholinesterase in forensic toxicology is quite significant. For example, the use of organophosphate and carbamate pesticides, which are cholinesterase inhibitors, may result in accidental or intentional poisonings. The preferred method of detection of these agents is by measuring cholinesterase activity in postmortem blood. In addition, nerve agents such as Tabun, Sarin, and Soman, were prepared by Germany prior to the second World War and remain a threat in times of war. Some drugs such as cocaine are hydrolyzed by plasma cholinesterase in vivo and in vitro [*2*]. This instability in vitro complicates the interpretation of blood cocaine concentrations, therefore, cholinesterase activity may be relevant to poison identification and interpretation.

Because enzymes retain different activities outside the body, information about the in vitro stability of cholinesterase in blood becomes important. Studies have been performed on the stability of the enzymes in blood or plasma. Edson et al. [*3*] examined 28 specimens for whole blood cholinesterase. The interval between death and sampling ranged from 0 to 4 days, with the bodies being stored in the refrigerator during that time. The whole blood cholinesterase activity of the specimens averaged 1.17 with a range of 0.45 to 1.59. This corresponded to values obtained in live patients. Lanks and Sklar [*4*] found that pseudocholinesterase was stable for one month at 4°C in plasma obtained from the blood bank blood. Only 5 to 10% of the initial activity was lost after one month when unpreserved, heparinized-preserved, or EDTA-preserved plasma was stored at 4°C. Fluoride-preserved plasma demonstrated a 63% inhibition of pseudocholinesterase activity almost immediately. This decrease in pseudocholinesterase activity in the presence of fluoride was also documented by Baselt et al. [*5*]. This was expected because fluoride is a potent inhibitor of esterases. Pseudocholinesterase will retain its activity when plasma specimens are stored at −20°C for greater than one year [*6*].

The following is a series of studies performed on EDTA preserved and nonpreserved whole blood and red cells from both living and deceased individuals, respectively, to further document the potential use of cholinesterase measurements in postmortem specimens.

## Materials and Methods

### *Antemortem Blood Studies*

Antemortem blood was obtained from five normal healthy adult males. Whole blood, red blood cell (RBC), and plasma cholinesterase activity was determined on these samples from one to seven days at 4°C and 25°C. The initial stability study involved collection of two, eight mL EDTA tubes from each of the five volunteers. The blood from one tube was centrifuged to separate the RBCs and plasma. The RBCs and plasma were then aliquoted to provide cholinesterase determinations for a one week period at 4°C and 25°C. The blood from the second tube was divided in half. One half was used for whole

blood cholinesterase activity and the second was used for the preparation of the RBC, plasma and whole blood pool of the five individuals. Equal volumes of each volunteer's blood were combined, mixed, split, centrifuged and aliquoted into fractions sufficient for the one week stability study at both temperatures. All specimens were assayed in triplicate.

### *Postmortem Blood Studies*

Postmortem heart blood collected by dry syringe or scooped out of the pericardial sac was obtained from routine autopsy cases. Nonpreserved blood was collected within 24 h of death. Samples were immediately refrigerated and the analysis was performed within a period of two days after collection. Postmortem blood cholinesterase activity was assayed in triplicate for one week at 25°C to determine stability.

### *Postmortem Inhibited Blood Study*

To compare postmortem and simulated nerve agent values, aliquots from 18 of the original 53 postmortem specimens were frozen during day one of the study, thawed on day seven and 100 μL of a 1 mM mixture of two cholinesterase inhibitors, diisopropylfluorophosphate and phenylmethylsulfonyl fluoride, were added to three mL of each sample. The resulting concentration of the cholinesterase inhibitors was 5.94 $X(10)^{-6}$ g/mL and 5.62 $X(10)^{-6}$ g/mL, respectively. Cholinesterase activity of the treated blood samples were compared to the matched day seven untreated samples.

### *Postmortem Battlefield Blood Studies*

Battlefield postmortem blood was obtained from Desert Storm fatalities for routine medical examiner evaluations to determine cause and manner of death. Nonpreserved blood was collected within four to fourteen days of death. Single cholinesterase determinations were performed on a portion of the blood not used for other routine analyses.

### *Analytical Method*

The method for cholinesterase determination was a modification of that originated by Michel [7]. The method measures the change in pH per unit time, monitoring the formation of acetic acid from added acetylcholine, as an indication of cholinesterase activity. It allows for manual red blood cell cholinesterase determinations in 17 minutes and manual plasma determinations in one hour. The manual plasma method was used for plasma and whole blood cholinesterase determinations. The only modifications to the method was the use of a heated shaker water bath (Techne Tempettet Junior TE-85) for mixing and subtraction of blank readings, consisting of all reagents but the specimen, from the final pH values.

## Results

The initial antemortem experiment was undertaken to determine the stability of red blood cell, plasma and whole blood cholinesterase in five healthy male adults. A pool consisting of the five was also prepared for comparison with individual cholinesterase levels. The enzyme activity of cholinesterase at day one and at day seven was determined at 4°C and 25°C. Interassay precision was determined over a two week period using eel acetylcholinesterase Type VI in Tris buffer assayed in triplicate. The coefficient of variation (CV) was 4.0%, ($n$ = 17). Intraassay performance was determined using the same

acetylcholinesterase control. The CV was 5.6%, ($n$ = 13). The results are given in Table 1. Greater than 80% of the enzyme activity originally measured remained after one week.

The values obtained for plasma and RBC cholinesterase from the five volunteers were similar to previously reported values. Normal RBC activities ranged from 0.58 to 0.86 for men while normal plasma enzyme activities ranged from 0.61 to 1.28 [7]. The whole blood enzyme activities presented here are lower than the combined plasma levels previously reported [4]. Attempts were made to reconcile this, including checks of substrate concentration, assay pH and assay temperature. No explanation was ascertained, but measurements in this study were internally consistent.

There are several factors associated with battlefield conditions that must be addressed when looking at the testing of postmortem blood samples; the type of carrier and time required to ship the specimen to the assay laboratory, the temperature of the specimen before collection and during shipment to the testing facility, and the time that elapsed before collection. These factors suggest that blood samples taken from such a scenario will arrive hemolyzed. Because blood specimens received from the battlefield are expected to be hemolyzed the remaining experiments used whole blood as the specimen.

A total of 53 fresh postmortem whole blood specimens were analyzed in triplicate at room temperature to establish normal blood cholinesterase levels. The samples were then stored at room temperature and analyzed at day three, five, and seven. The average postmortem whole blood cholinesterase activity was 0.48 (0.20 to 0.74) pH units on day one and the enzyme activity was 0.45 (0.07 to 0.70) pH units after one week. There is an average decrease of less than 10% over the one week period, which is similar to the results obtained in the antemortem experiments.

In order to determine if the assay could distinguish between the low end of the postmortem range and true organophosphate poisoning it was necessary to simulate exposure to a nerve agent. To compare postmortem and simulated nerve agent values, aliquots from 18 of the original 53 postmortem specimens were frozen during day one of the study, thawed on day seven and a 1 mM solution of two cholinesterase inhibitors was added. These specimens were then analyzed with matched untreated day seven specimens.

TABLE 1—*Expected values of RBC, plasma, and whole blood cholinesterase activity. Each sample value is the mean of three measurements.*

| Group | Specimen | Temp °C | Cholinesterase (x + SD) | |
|---|---|---|---|---|
| | | | DAY 1 | DAY 7 |
| Antemortem | RBC | 4 | 0.77 ± 0.02 | 0.64 ± 0.01 |
| (Pooled) | | 25 | 0.77 ± 0.02 | 0.62 ± 0.01 |
| | Plasma | 4 | 1.03 ± 0.05 | 0.98 ± 0.05 |
| | | 25 | 1.03 ± 0.05 | 0.88 ± 0.01 |
| | Whole blood | 4 | 0.60 ± 0.005 | 0.59 ± 0.01 |
| | | 25 | 0.60 ± 0.005 | 0.58 ± 0.005 |
| Antemortem | RBC | 4 | 0.80 ± 0.07 | 0.68 ± 0.08 |
| ($n$ = 5) | | 25 | 0.80 ± 0.07 | 0.70 ± 0.12 |
| | Plasma | 4 | 0.98 ± 0.14 | 1.01 ± 0.16 |
| | | 25 | 0.98 ± 0.14 | 1.09 ± 0.22 |
| | Whole blood | 4 | 0.59 ± 0.05 | 0.64 ± 0.05 |
| | | 25 | 0.59 ± 0.05 | 0.52 ± 0.07 |
| Postmortem | Whole blood | 25 | 0.48 ± 0.14 | 0.45 ± 0.14 |
| ($n$ = 53) | | | range (0.2–0.74) | (0.07–0.7) |
| Untreated | Whole blood | 25 | 0.51 ± 0.11 | 0.42 ± 0.15 |
| ($n$ = 18) | | | range (0.32–0.68) | (0.07–0.61) |
| Inhibitor | Whole blood | 25 | — | 0.003 ± 0.005 |
| Treated | | | | range (0.0–0.01) |
| ($n$ = 18) | | | | |

TABLE 2—*Whole blood cholinesterase values from 15 battlefield fatalities.*

| Specimen | Activity | Time between death and assay (DAYS) |
|---|---|---|
| 1 | 0.60 | 4 |
| 2 | 0.56 | 4 |
| 3 | 0.44 | 7 |
| 4 | 0.39 | UNKNOWN |
| 5 | 0.16 | 5 |
| 6 | 0.38 | 14 |
| 7 | 0.46 | 8 |
| 8 | 0.40 | 14 |
| 9 | 0.49 | 12 |
| 10 | 0.30 | 11 |
| 11 | 0.60 | 12 |
| 12 | 0.42 | 4 |
| 13 | 0.25 | 13 |
| 14 | 0.06 | 12 |
| 15 | 0.34 | 13 |
| x = 0.39 range (0.06–0.6) | | |

All of the day seven treated values were essentially zero (0.0 to 0.01) pH units compared to a range of 0.07 to 0.61 pH units for matched, untreated, day seven postmortem specimens. Table 1 lists day one, day seven untreated and treated values for the 18 specimens. Day seven cholinesterase levels of the 18 untreated specimens are significantly greater than the levels resulting from organophosphate treatment.

Fifteen actual specimens from the battlefield were then analyzed as verification of screen performance. These specimens were collected and assayed to assist the medical examiner in determining cause and manner of death and there was no indication of nerve agent exposure. The average plasma enzyme activity was 0.39 (0.06 to 0.60) pH units. The time that elapsed before receipt of the samples ranged from four to 14 days. Table 2 lists the final whole blood cholinesterase levels and time of assay for the 15 specimens. For 13 of the 15 battlefield specimens, cholinesterase activities were significantly greater than the levels found in the 18 specimens treated with the organophosphate inhibitors.

## Discussion

This study was initiated to determine whether postmortem whole blood cholinesterase activity could be used as a screening test for exposure to nerve agents. The specific aims were to modify an existing method for cholinesterase activity determination using our laboratory's current instrumentation and to provide high volume testing of postmortem blood specimens received from the battlefield. Screen positive results would be confirmed by testing specimens for specific metabolites of nerve agents using gas chromatography/mass spectrometry. Although the whole blood postmortem enzyme activities were lower than previously published levels [*4*], the normal postmortem enzyme activity determined in this work was reproducible and was of sufficient magnitude to differentiate from organophosphate poisoning. There was no decrease in cholinesterase activity when antemortem whole blood, RBCs, or plasma was stored for one week at either 4°C or 25°C. There was also no difference between the whole blood enzyme activity of postmortem day one and day seven specimens at room temperature. Additionally 13 of the 15 battlefield specimens from regions of considerably higher temperature had retained enzyme activity that was within the normal postmortem range. The four to 14 day interval of these 15 specimens suggests that the stability of the enzyme may provide screening capability up to fourteen days. The World Health Organization has proposed that a

reduction of plasma or RBC cholinesterase activity to 50 or 70% of the initial value warrants removal of the worker from exposure to organophosphate pesticide [*8*]. The cholinesterase activities found in the 18 postmortem blood specimens exposed to the cholinesterase inhibitors demonstrated nearly a 100% reduction when compared with cholinesterase activities for the uninhibited medical examiner specimens and most of the battlefield specimens. Two of the 15 battlefield specimen enzyme activities fell outside of the normal postmortem range and would therefore require confirmation. We conclude that the nonpreserved whole blood cholinesterase activity in postmortem specimens collected within a one week period of death can be used as a screening test for nerve agent exposure.

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