TECHNICAL NOTE

Randall C. Baselt,[1] *Ph.D.; Richard F. Shaw,*[2] *B.S.; and Robert McEvilly,*[2] *B.S.*

Effect of Sodium Fluoride on Cholinesterase Activity in Postmortem Blood

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ABSTRACT: Thirty-two postmortem blood specimens, with and without sodium fluoride as preservative, were analyzed for cholinesterase activity by the Michel method. The fluoridated specimens, which contained from 0.7 to 31 mg/mL (average 6.3) of sodium fluoride, were found to exhibit cholinesterase activities that were 5 to 59% (average 25%) lower than the duplicate unfluoridated specimens. We concluded that, while this decrease is quite significant, a fluoridated postmortem blood specimen may be used for the measurement of cholinesterase activity when a nonfluoridated specimen is unavailable.

KEYWORDS: pathology and biology, toxicology, cholinesterase, blood, sodium fluoride, pesticides

Sodium fluoride is commonly used as a preservative and anticoagulant in specimens of whole blood intended for toxicological analysis, particularly alcohol analysis. Concentrations of this chemical believed to be effective for these purposes range from 5 to 10 mg/mL (0.5 to 1.0% by weight). In a number of medicolegal jurisdictions in the United States, it has been the practice for many years to use fluoridated whole blood for all routine toxicological investigations, including cholinesterase determination.

Yet, this practice is not without controversy. Two compendia of analytical techniques frequently relied on by forensic toxicologists caution against the use of fluoride in specimens for cholinesterase determination [*1,2*], while a third states that cholinesterase is not inhibited by fluoride [*3*]. Fluoride inhibition of serum cholinesterase has been employed to differentiate between the different phenotypes of this enzyme [*4,5*], but red cell cholinesterase has been shown to be considerably more resistant to fluoride inhibition than the variety found in serum [*6*]. The purpose of this brief communication is to resolve the question of the effect of sodium fluoride on cholinesterase activity in postmortem specimens of whole blood.

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[1]Director, Chemical Toxicology Institute, Foster City, CA.
[2]Chief toxicologist and assistant toxicologist, respectively, Office of the Coroner, San Diego, CA.

Methods

Specimen Collection

Specimens of whole blood were obtained at autopsy from 32 individuals with no history of pesticide exposure, whose deaths fell under the coroners' jurisdiction in San Diego County, during July 1983. The time elapsed from death (or time found) to blood collection varied from 2.5 to 39 h. The specimens, from 10 to 100 mL in volume, were collected from an internal jugular vein incision. The blood was allowed to flow into a 120-mL (4-oz.) glass bottle containing roughly 1 g of sodium fluoride, with a lesser amount of blood placed into a 10-mL green-stoppered glass tube that contained sodium heparin, 143 USP units (Becton-Dickinson Vacutainer®). The specimens were placed into a refrigerator at 4°C within 1 to 2 h and maintained there until the cholinesterase determination could be performed, usually within one to three weeks.

Cholinesterase Determination

All specimen pairs (with and without fluoride) were analyzed at the same time, and all specimens were analyzed over a three-day period. Whole blood was analyzed by the Michel method as described by Curry [*3*], using acetylcholine as substrate. Reference values for this method were stated to fall within a range of 0.80 to 1.60 pH units/h for whole blood and 0.50 to 1.00 pH units/h for plasma [*3*].

Sodium Fluoride Determination

Specimens of whole blood (0.5 mL) were diluted to 50 mL with deionized water. A 30-mL aliquot of this solution was placed into a 50-mL beaker and analyzed directly with a fluoride-specific electrode (Orion Research, Cambridge, MA 02139) attached to a pH meter with a millivolt scale (Beckman Instruments, Fullerton, CA 92634). Results were compared to a standard curve, prepared from blood spiked to contain 0, 1, 5, 10, 20, and 50 mg/mL of sodium fluoride and analyzed in the same manner, that was drawn on semi-log graph paper.

Results and Discussion

As shown in Fig. 1, each of the 32 postmortem blood specimens showed a significant decrease in cholinesterase activity, roughly proportional to fluoride content. The enzyme activities of the heparinized blood specimens ranged from 0.41 to 1.59 pH units/h (average 0.98), while fluoride treatment caused a decline to 0.33 to 1.36 pH units/h (average 0.89), with individual decreases ranging from 5 to 59% (average 25%). The large fluctuation in blood sodium fluoride concentration, 0.7 to 31 mg/mL (average 6.3), was the result of variation in both the volume of blood placed into the specimen bottles and the amount of solid sodium fluoride added.

While these results suggest that the use of sodium fluoride as a preservative in whole blood is an impediment to a meaningful cholinesterase determination, this is not necessarily so. It is generally accepted that a 70% decrease in whole blood cholinesterase activity is indicative of a serious exposure to a cholinesterase inhibitor [*7*]. Of 47 routine cholinesterase assays performed over the years 1971 to 1982 in San Diego County on fluoridated postmortem blood, 5 cases fell below 0.27 pH units/h (a 70% decrease from the average of 0.89 pH units/h found in this study for "normal" fluoridated postmortem blood). Of these five fatal cases, two were determined to result from suicidal administration of organophosphate insecticides (diazinon and malathion), two were of undetermined cause, and one was the result of adrenal neuroblastoma with liver metastases, a condition known to cause reduced blood cholinesterase ac-

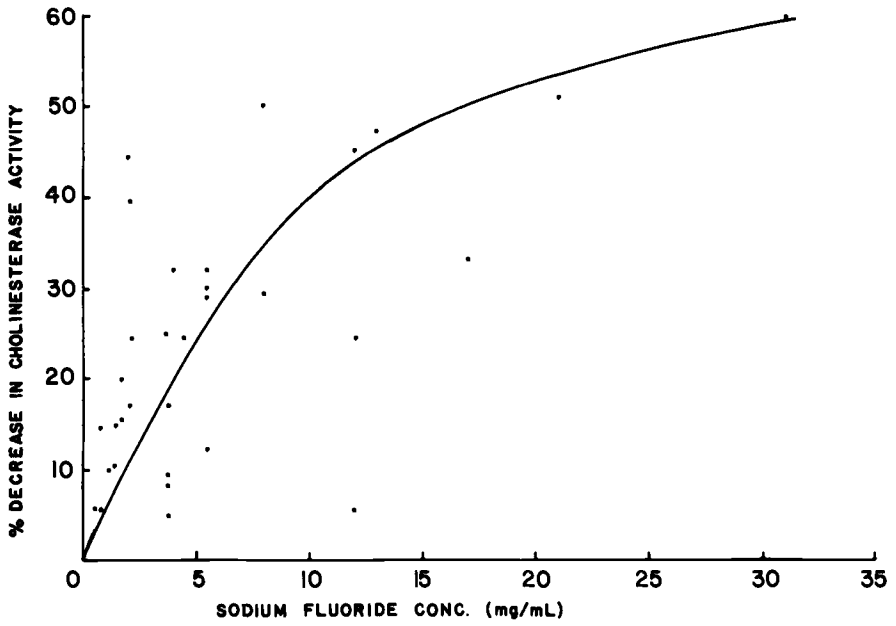

FIG. 1—*Correlation of percent of decrease in postmortem blood cholinesterase activity with sodium fluoride concentration.*

tivity [*8*]. Two further cases with apparently normal blood cholinesterase activities of 0.40 and 0.43 delta pH units/mL were determined by investigation to be the result of suicidal ingestion of pesticides. However, the toxic agents in these cases were carbamates, a form of cholinesterase inhibitor for which reactivation of the enzyme is known to occur *in vitro* within hours [*9*].

We have not considered the effect of aging of the blood specimen upon cholinesterase activity in the present study. In previous studies, investigators have found that postmortem blood cholinesterase, inhibited or not, is stable for up to three or four weeks when refrigerated at 4°C [*10,12*]. Edson et al [*10*] found that spuriously low values may be due to abnormally low erythrocyte content of certain postmortem blood specimens, and recommended that a hemoglobin determination be routinely performed in conjunction with cholinesterase assays.

Conclusion

We conclude that a fluoridated postmortem blood specimen may be used for the measurement of cholinesterase activity when a nonfluoridated specimen is unavailable. However, the presence of the preservative causes a significant decrease in the activity of this enzyme, averaging 25% loss in blood containing on average 6.3 mg/mL of sodium fluoride.

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Address requests for reprints or additional information to
Dr. R. C. Baselt, Director
Chemical Toxicology Institute
P.O. Box 8209
Foster City, CA 94404