TITLE: A RAPID, SEMI-AUTOMATED METHOD FOR DETERMINING DIBUCAINE NUMBERS.

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INTRODUCTION: The Dibucaine Number (DN), a comparison of the ratio of plasma cholinesterase (PchE) activities with and without dibucaine using benzoylcholine as substrate, differentiates genetically normal PchE from an abnormal variant[1]. However, this assay is time consuming and the results, used to predict sensitivity to succinylcholine (Sch), are not rapidly available to the clinician. The goal of this study was to adapt an existing, widely available, automated assay for PchE activity performed by the DuPont Automatic Clinical Analyzer (ACA) to provide DN's quickly, and with little effort.

METHODS: Blood samples were obtained by venipuncture (consent approved by committee). After clot retraction, samples were centrifuged, and serum was removed for assay or stored at -20°. Aliquots of serum were assayed without and with dibucaine by each of three methods: a) Serum samples diluted 100x were assayed for PchE at 25° without and with dibucaine (1×10^{-5}M) using the method of Kalow and Genest[1]. b) Serum samples diluted 1400x were assayed manually for PchE at 37° with butyrlthiocholine (BTC, 5.4×10^{-4}M) as substrate using the method of Ellman[2] as modified by Klingman[3]. Reactions were done in duplicate, were linear and were terminated after 10 minutes with either neostigmine (2.4×10^{-4}M) or echothiophate (7.2×10^{-5}M). PchE activity was determined from the change in optical density at 412 nm and expressed as µmol of substrate hydrolyzed/min/ml of serum. Some assays were carried out in the presence of dibucaine (1×10^{-6}M to 6×10^{-4}M). c) Serum samples were also assayed without and with several concentrations of dibucaine (7.2×10^{-6}M to 7.6×10^{-5}M) using the ACA, a system that reports PchE results in less than 10 minutes and uses BTC as substrate. To achieve the desired dibucaine concentrations, 500 µl aliquots of serum were added to tubes containing crystalline dibucaine that previously had been added in aqueous solution and then lyophilized to dryness. The amount of dibucaine present in representative tubes following lyophilization was confirmed by gas chromatography. Dibucaine numbers are reported as:

$$DN = \left(1 - \frac{\text{inhibited activity}}{\text{uninhibited activity}}\right) \times 100$$

RESULTS: Using the method of Kalow and Genest[1] DN's determined with normal sera ranged from 71 to 81. With the same method, sera from two patients with documented abnormal responses to Sch were identified (DN 15 and 19).

PchE activity assayed manually with BTC was inhibited by dibucaine in a dose dependent manner. Inhibition was 18.5 ± 2.2% (SEM) at 1×10^{-6}M dibucaine and increased to 97.3 ± 0.5% with 6×10^{-4}M. The percent inhibition attained a plateau of 80.9 ± 2.2% with 3×10^{-5}M dibucaine and this concentration was selected for the determination of DN's with this manual method.

Similarly, a dose-related inhibition of PchE activity with dibucaine was detectable using the automated assay system. Inhibition in normal sera ranged from 24.8 ± 1.6% at a dibucaine concentration of 7.2×10^{-6}M to 77.3 ± 1.4% at 7.6×10^{-5}M. The latter concentration was then selected for determination DN's by the automated method.

In comparison to the inhibition response of normal sera to dibucaine, both of the abnormal sera tested were markedly less sensitive to dibucaine. That the lack of sensitivity could be detected by both the manual and automated assays using BTC is shown in tabular form with the more classical BeCh assay for comparison.

DIBUCAINE NUMBER (SEM)

	Benzoylcholine	Butyrylthiocholine	
SERUM	MANUAL	MANUAL	AUTOMATED
Normal (n=6)	74.8±2.9	80.9±2.2	77.5±1.4
Abnormal #1	15.3	22.9	22.8
Abnormal #2	19.2	20.0	16.4

DISCUSSION: The results of this study suggest that butyrylthiocholine is as effective a substrate for PchE when used in manual determination of dibucaine numbers as is the more classical substrate, benzoylcholine. The results further suggest than an existing, rapid, automated assay for plasma cholinesterase that utilizes BTC can easily be modified to provide DN's in short order. With the latter method, these data can be obtained within one half to one hour, do not require specially trained laboratory personnel and may in the future, be available in any hospital having access to a DuPont Clinical Analyzer.

REFERENCES:

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3) Klingman, GI, Klingman, JD, and Poliszczuk, Acetyl and pseudocholinesterase activities in sympathetic ganglia of rats. J. Neurochem. 15: 1121-1130, 1968.