A Fast and Simple Procedure for Routine Determinations of Plasma Cholinesterase Activities

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A simple, rapid and accurate method for routine plasma cholinesterase (ChE) determinations is described in the present paper. It is a modification of the acetylthiocholine procedure described by Ellman et al. (1) and may be applied for several purposes :

1. Detection of certain diseases which are connected with changes in ChE activity.

2. ChE activity surveys in human subjects being exposed to ChE inhibitors in factories where certain plant protection products are made.

3. Detection of possible organophosphate or carbamate intoxications in domesticated or wild animals.

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4. *In vivo* studies on the mode of action of ChE inhibitors in laboratory animals.

After adapting the colorimetric method of Ellman *et al.* (1) for routine tests, blood samples of 97 human beings of both sexes were collected in order to study the modified method in detail and to determine the variation of ChE activity. The data obtained were compared with those tabulated in an extensive literature review by Augustinsson (2).

Materials and Methods

Centrifuge fresh human blood at 3000 r.p.m. for 15 min. Pipette 0.02 ml of plasma into a graduated "Klett-Summerson" test tube which later serves as cell for measuring optical density in a "Klett-Summerson" photo-electric colorimeter equipped with blue filter No. 42. At zero time (stopwatch) add substrate reagent solution from a polyethylene spraying bottle until the 5-ml. mark of the test tube is reached. (Prepare this solution as follows : Dissolve 10 mg. of 5,5-dithio-bis-2-nitrobenzoic acid (DTNB, Aldrich Chem. Co., Milwaukee, USA) in 20 ml. of 1/15 M Sorensen phosphate buffer pH 7 ; add 20 mg. of acetylthiocholine iodide (Fluka, Switzerland) and 80 ml. of 1/15 M Sorensen phosphate buffer pH 8.) Incubate the enzyme-substrate-reagent-mixture for exactly 5 min. at 30°C. in a constant-temperature water bath before determining the optical density of the yellow color. Zero the instrument with substrate-reagent solution alone

(5 Min., 30°C.) to correct for non-enzymatic hydrolysis. The "Klett"-readings are proportional to the optical density and represent a direct measure of ChE activity.

Results

Basic requirements for quantitative enzyme activity measurements are proportionality between time of incubation and amount of substrate hydrolyzed on one hand and between enzyme concentration and activity on the other hand. Both fundamentals were checked and found to be fullfilled under the experimental conditions described above. Accuracy and reproducibility of the procedure were tested with 20 subsamples of one plasma sample. Ten subsamples were analysed on one day and the other ten the next day. The two groups had the following mean and standard deviation : Group 1, 221.2 and 4.44 ; Group 2, 221.0 and 4.35. The two standard deviations are not significantly different, hence a pooled t-Test was applied to compare the two means. The t-value of 0.11 with 18 d.f. indicated that no difference existed between the two groups (days). The grand mean amounted to 221.1 and the overall standard deviation to 4.4.

The data obtained from a statistical treatment of the original activity values (Klett-readings) are given in Table 1.

TABLE 1

Statistical evaluation of cholinesterase activity values.

Number of individuals (both sexes)	97
Number of ChE determinations	97
Mean activity value, $\bar{x}$	281
Range R	325
Standard deviation, s	60.4
Standard deviation of the mean, $s_{\bar{x}}$	6.1
Coefficient of variation	21.5 %
Confidence limits ; p = 0.05	281 ± 12.14
p = 0.01	281 ± 15.96

Augustinsson (2) presented the coefficients of variation obtained with 58 series of ChE determinations from 40 authors, employing titration, biological, gasometric, electrometric and colorimetric procedures. The coefficients of variation varied between 10 % and 46 %, but most of them were within the range of 15 and 25 %. The overall average of this 58 series was calculated by the present authors and was found to be 21.9 %. This figure is in close agreement with that (21.5 %) given in Table 1.

By applying the manometric technique Herzfeld and Stumpf (3) discovered that ChE activity values from a series of different persons do not follow a normal distribution. Consequently the original data were sub-

jected to a Test of Normality first to see if non-normality was involved and second, if such were the case, to perform a log-transformation. To test the hypothesis that the original data are indeed from a normal population, a normal density with mean 281 and standard deviation 60.4 was compared to the actual data. The criterion used for testing "Goodness of Fit" was the Chi^2 - test. Chi^2 was calculated to be 7.14 with 7 d.f. where there were ten categories. The probability of getting a Chi^2 of 7.14 or greater with 7 d.f. is approximately 0.6 (60 %), implying that the normal curve with mean 281 and standard deviation 60.4 is a very good fit. We may therefore safely assume the original data to be from a normal population.

One of the ChE determinations gave a value of 100 which seemed to be an outlier. It was not removed in all the above-described statistical treatments. However, it was felt that a measurement which departs completely from all others should be studied further as to whether it actually came from the population with mean 281 and standard deviation 60.4. Since the value of 100 is 3 standard deviations below the mean, we note from the table of the normal curve that the probability of having a value 3 or more standard deviations from the mean is only 0.00135 (0.135 %), or highly unlikely. We could with great assurance ignore it. The new mean would then be 283 and the standard deviation 57.

Discussion

The routine procedure of ChE determinations described above combines simplicity and high accuracy. The simplicity may be compared with that of semi-quantative methods using indicators on paper or in solution, and the accuracy of the ASCh/DTNB method can compete with that of the time-consuming manometric technique. The advantage over pH-indicator methods is constancy in pH during the incubation time, which, in addition, is short so that only initial hydrolysis rates are determined. The present procedure is also superior to Hestrin's (4) colorimetric acetylcholine determination because it may be applied for steady-state measurements. Furthermore the Hestrin method requires a substantial decrease in substrate concentration during the reaction period. Finally the method of Ellman et al. (1) is extremely well suited for precise and reproducible automated ChE determinations in large series (Voss (5), Voss and Geissbühler (6)).

ChE measurements of fresh plasmata of 97 individuals clearly demonstrated that the degree of variation, expressed by a coefficient of variation of 21.5 %, is well within the range found by other authors with different techniques. In contrast to reports from other authors the original, raw data of this study were normally distributed not requiring any transformation before applying statistical tests. One observation was found to be an outlier, but was not removed from the data ; it contributed little to the mean and to the standard deviation.

Summary

The present paper deals with a colorimetric procedure for routine determinations of plasma cholinesterase activity. The modified method of Ellman et al. (1) is described in detail and a statistical treatment of results obtained with plasma samples of almost 100 persons is presented to justify its routine application. The data were subjected to various statistical procedures and were found to compare favorably with those described by other authors. Finally the advantages of the method are discussed in terms of simplicity and accuracy.

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