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Improved Method for the Identification of the Fluoride-Resistant Plasmacholinesterase Genotypes[1]

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This investigation was prompted by the findings that (1) dibucaine-resistant homozygotes and heterozygotes for plasmacholinesterase also exhibit resistance to fluoride inhibition, (2) the differentiation of dibucaine-resistant from the fluoride-resistant genotypes is ambiguous with the method of Harris and Whittaker, (3) the plasmacholinesterase inhibition by Na fluoride (FN) is markedly influenced by the temperature. Therefore, we modified their method by increasing (1) the temperature of the reaction from 25C to 37C and (2) the concentration of Na fluoride from 5.0×10^{-5} M to 2.5×10^{-4} M. With this method, genetically normal individuals have a mean FN ± SD = 77.0 ± 3.22 while atypical dibucaine-resistant homozygotes have a mean FN ± SD = 43.0 ± 10.0 and atypical dibucaine-resistant heterozygotes 67.0 ± 5.37. Since a linear correlation was observed between DN and FN by our new method, a fluoride number 2 SD lower than the predicted FN from the DN can distinctly identify the fluoride-resistant plasmacholinesterase genotype E_1^f.

KEY WORDS: FN determination; plasmacholinesterase; E_1^f genotype; enzyme assay; modified FN.

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INTRODUCTION

Harris and Whittaker (1961) claimed that a new phenotype of human serum cholinesterase was recognized by sodium fluoride inhibition. Since this original report, the method of Harris and Whittaker has been accepted as a standard method for the determination of fluoride-resistant plasmacholinesterase (Whittaker, 1967, 1968; Thompson and Whittaker, 1966; Whittaker and Vickers, 1970). These authors utilized 5.0×10^{-5} M concentration of Na fluoride at 25C. Under these conditions of essay, a normal person has FN < 64 while a fluoride-resistant homozygote $E_1^f E_1^f$) has FN < 34.

After the adaptation of the method for FN determination according to Harris and Whittaker, we early recognized that the narrow percentage ranges of inhibition in the various genotypes with the use of 5.0×10^{-5} M Na fluoride concentration did not allow a clear differentiation of the genotypes. Therefore, a great overlap existed between the various genotypes based on the concomitant determination of DN by the method of Kalow and FN by the method of Harris and Whittaker. Subsequently we recognized that individuals with dibucaine resistance also exhibited fluoride resistance and *vice versa* at both 25 and 37 C utilized by Foldes *et al.* (1963) for the determination of DN. Although the increase in temperature from 25 to 37 C caused only predictable and minor changes in DN, it markedly influenced the FN. In order to improve the selectivity and accuracy of FN determination, our study was undertaken.

MATERIALS AND METHODS

Source of Enzymes

Freshly obtained venous heparinized blood samples were separated by centrifugation at 2600 rpm into red cells and plasma. The supernatant plasma was immediately separated from the buffy coat and stored at −20 C or immediately utilized. The hydrolysis rate of benzoylcholine chloride commercially obtained from Calbiochem (Pasadena, California) was determined by Kalow's ultraviolet spectrophotometric method at 25, 31, and 37 C as indicated in the figures. The influence of dibucaine hydrochloride, sodium fluoride, and RO2-0683 [2-hydroxy-5-phenylbenzyl trimethylammonium (bromide) dimethylcarbamate] on the hydrolysis of benzoylcholine chloride was determined, and *p*I–inhibition curves were plotted as shown in Results. Each point represents the mean of three determinations checking within 5%. Based on the *p*I–inhibition curves, a concentration of Na fluoride was selected which gives 75% inhibition of benzoylcholine hydrolysis rate. In addition,

RO2-0683 was utilized to confirm the dibucaine resistant genotypes as reported by Foldes *et al.* (1963). Three plasma samples of suspected or claimed fluoride-resistant plasma cholinesterase genotypes were also studied and the *p*I–inhibition curves of the three inhibitors determined at 37 C.

RESULTS

The *p*I inhibition curves of Na fluoride at 37 C and 25 C in the plasma of normal $E_1^uE_1^u$ and atypical dibucaine-resistant homozygotes $E_1^aE_1^a$ are shown in Fig. 1. It is evident that increasing the reaction temperature from 25 to 37 C results in a markedly reduced FN. It can also be observed that there is a marked difference in the I_{50} between the $E_1^uE_1^u$ and $E_1^aE_1^a$. It is evident from the curve that the use of 5.0 × 10^{-5} M concentration at either 25 or 37 C may result in a very large experimental error because this concentration falls on the mid portion of the *p*I–inhibition curve where the slope is the greatest.

Figures 2 and 3 summarize the results obtained with dibucaine and RO2-0683. As can be observed in Fig. 2, the influence of temperature on the *p*I–inhibition curve of dibucaine is small. Consequently, changing the temperature from 25 to 37 C results in only a few units of change in DN. It should also be noted that 1 × 10^{-5} M dibucaine concentration falls on the flattening portion of the *p*I–inhibition curve; therefore, errors in dibucaine concentration will lead to a little experimental error in DN determination. Similarly the *p*I–inhibition curve of RO2-0683 was little affected by temperature change, as Fig. 3 illustrates.

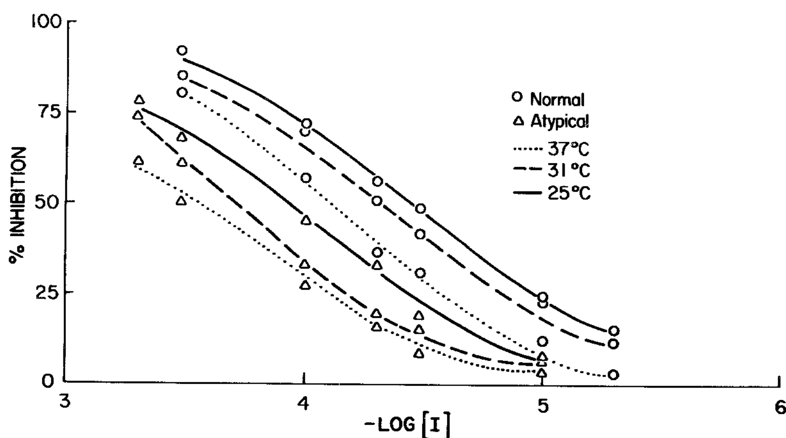

Fig. 1. Effect of temperature on the *in vitro* inhibition of normal and atypical homozygous plasmacholinesterase by sodium fluoride.

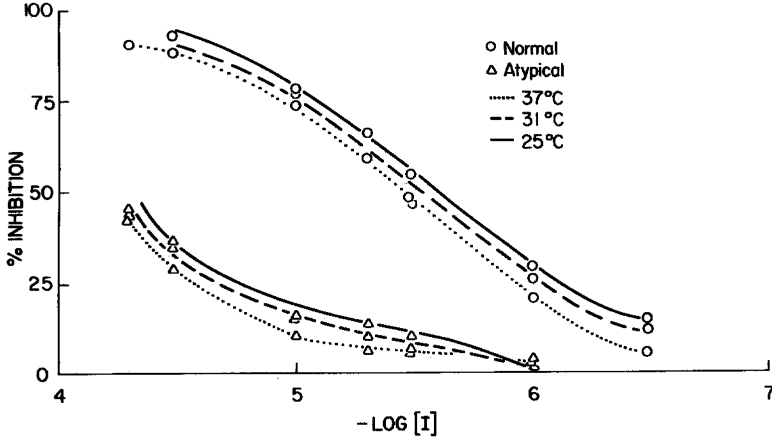

Fig. 2. Effect of temperature on the *in vitro* inhibition of normal and atypical homozygous plasmacholinesterase by dibucaine hydrochloride.

A distinct differentiation of the various genotypes can be achieved by the utilization of the *p*I–inhibition curves of dibucaine, sodium fluoride, and RO2-0683 in combination. In this way, three claimed $E_1^uE_1^f$ variants were unquestionably identified as $E_1^aE_1^a$ or $E_1^uE_1^a$. Figure 4 shows such an example of $E_1^aE_1^a$ who was erroneously classified as a fluoride-resistant heterozygote $E_1^aE_1^f$ (compare Fig. 2 to Fig. 4). Figure 5 shows the effect of sodium fluoride on the cholinesterase of the same $E_1^aE_1^a$ (compare Fig. 5 to Fig. 1).

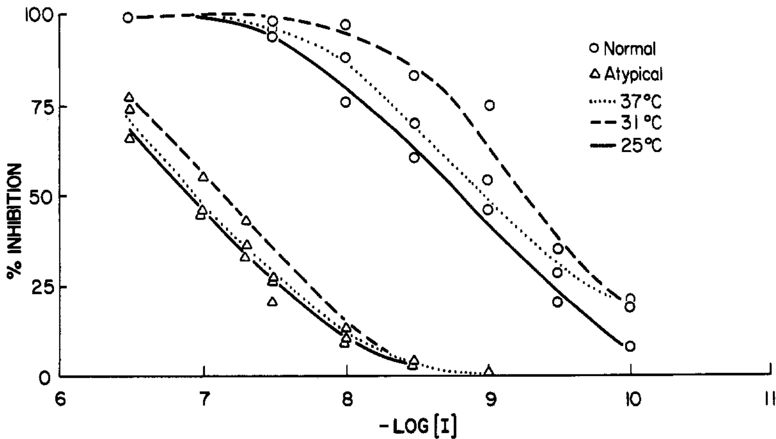

Fig. 3. Effect of temperature on the *in vitro* inhibition of normal and atypical homozygous plasmacholinesterase by RO2-0683.

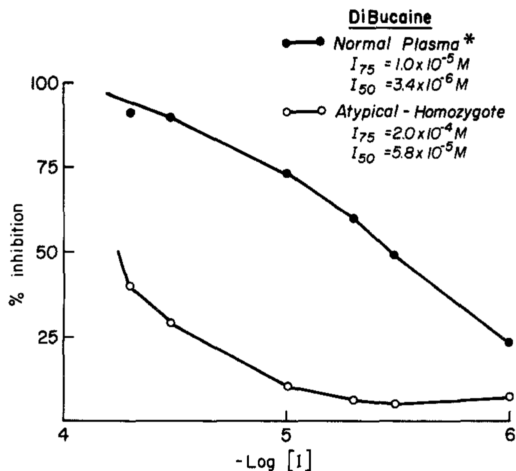

Fig. 4. Identification of the genotype of plasma cholinesterase by the *p*I–inhibition curve for dibucaine in a questionable subject.

DISCUSSION

Since the discovery of fluoride-resistant genotype by Harris and Whittaker (1961), it has become increasingly evident that the differentiation of fluoride resistant from the dibucaine-resistant genotypes by this method was inaccu-

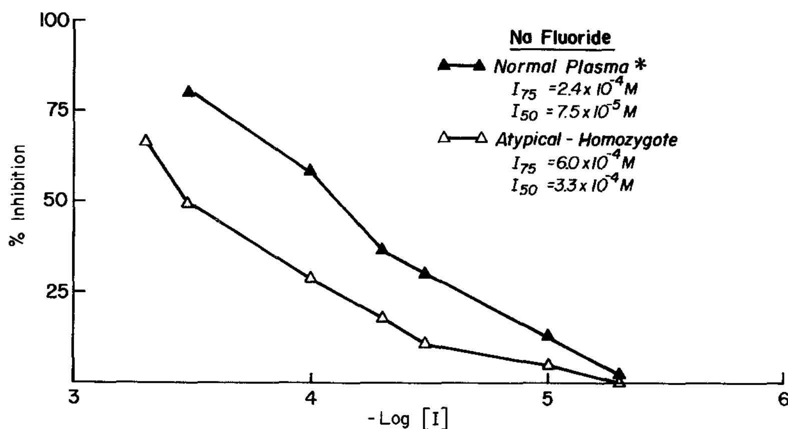

Fig. 5. Identification of the genotype of plasmacholinesterase by the *p*I–inhibition curve for sodium fluoride in a questionable subject.

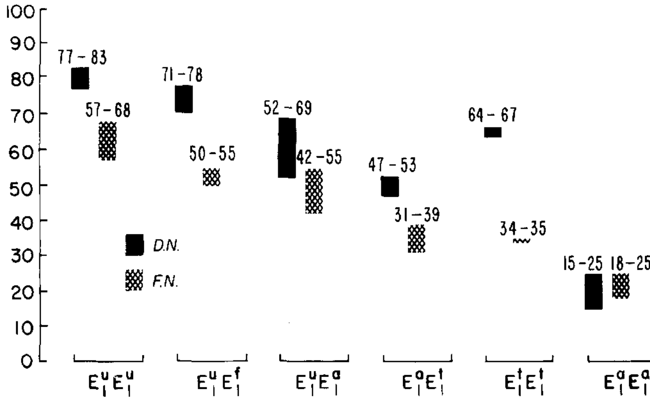

Fig. 6. DN and FN reported in 390 apneic patients with various atypical genotypes of plasmacholinesterase. Data plotted from Whittaker and Vickers (1970).

rate as is illustrated in Fig. 6. This figure contains all data points reported by Whittaker and Vickers (1970) from 390 apneic patients collected from the world literature. When these results were replotted, it became evident that based only on the DN and FN by the Harris and Whittaker method, genotypes $E_1^uE_1^a$ and $E_1^aE_1^f$ can be erroneously identified because of the overlap DN and FN values in these two groups. Furthermore, $E_1^fE_1^f$ cannot be distinguished from $E_1^aE_1^f$, since both FN and DN are intermediary, and the expected FNs fall between the values for normal homozygotes ($E_1^uE_1^u$) and dibucaine-resistant homozygotes ($E_1^aE_1^a$), as seen in Fig. 6.

That dibucaine-resistant homozygotes and heterozygotes also have an abnormally low FN was recently well established by Kothary *et al.* (1979). By the aid of a correlation equation between DN and FN, FN = 0.59 DN + 32, one can predict the FN in a dibucaine-resistant E_1^a variant. No such fluoride-resistant variant (E_1^f) was observed in 144 probands and their relatives referred to our laboratory, since none deviated from this equation. In order to clearly identify the genotypes containing an E_1^f component, one is forced to resort to the plotting of the *p*I–inhibition curve of dibucaine, Na fluoride, and RO2-0683 in the plasma samples of each individual claimed to be a fluoride-resistant phenotype. In three questionable E_1^f variants, the *p*I–inhibition curves using dubucaine, Na fluoride, and RO2-0683 clearly established that they were atypical dibucaine-resistant genotypes; one example is shown in Fig. 4.

Although it was claimed that phenotypes with an E_1^f variant have prolonged apnea following succinylcholine by Harris and Whittaker (1961) and Whittaker and Vickers (1970), one should confirm the existence of such an E_1^f variant by the *p*I–inhibition curve, and/or simply by the determination of FN by our improved method, before one accepts such a cause–effect

relationship. Erroneous identification of a dibucaine-resistant variant as fluoride-resistant may lead to an erroneous assumption that the latter variant is responsible for the prolonged apnea following succinylcholine. In order to facilitate the correct identification of E_1^f variant, we recommend the utilization of our modified method for the determination of FN.

CONCLUSION

Based on the enzyme kinetic studies reported in this article, we recommend the use of 2.5×10^{-4} M Na fluoride concentration which causes 75% inhibition of the plasmacholinesterase in $E_1^uE_1^u$ at 37C or body temperature instead of room temperature in order to clearly identify the so-called fluoride-resistant genotypes. The improved selectivity of our modified method for FN allows an accurate identification of the fluoride-resistant genotypes of plasmacholinesterase.

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