Biochemical Pharmacology, 1966, Vol. 15, pp. 145-154. Pergamon Press Ltd., Printed in Great Britain.

THE SUBSTRATE SPECIFICITY OF ATYPICAL CHOLINESTERASE IN RELATION TO PHENOTYPES

RICHARD L. IRWIN and MANFRED M. HEIN

Section on Applied Pharmacology,
National Institute of Neurological Diseases and Blindness, Bethesda, Md., U.S.A.

(*Received* 21 *July* 1965; *accepted* 5 *October* 1965)

Abstract—The activities of plasma esterase and phenotypes were studied in a family of four in which the mother had previously responded to the neuromuscular blocking agent succinylcholine with a prolonged paralysis. The plasma of the mother contained atypical esterase which hydrolyzed succinylcholine and other esters at reduced rates. The increased response to succinylcholine thus relates to an atypical cholinesterase and a lowered total esterase activity.

Three phenotypes were indicated by data obtained from dibucaine and fluoride inhibition studies, but each of the four family members had different substrate activity patterns for plasma esterase, as determined by K_m values. The study reported here presents an example of two siblings with the same phenotype but with markedly different plasma esterase activity toward a variety of choline and noncholine esters. The father and the one son who have the least difference in enzyme activity patterns have distinctly different phenotypes, as determined by fluoride and dibucaine inhibition.

The study emphasizes the need for additional characterizations of atypical esterase within families so that inheritance in relation to plasma esterase may be further understood.

SOON after the neuromuscular blocking agent succinylcholine began to be used during anesthetic procedures, it was observed that certain individuals failed to recover promptly from its paralytic affects.[1-3] The prolongation of paralysis often could be attributed to a low cholinesterase activity in the plasma which failed to reduce the concentration of succinylcholine to a nonparalytic level.[4-5] Consequential investigations established that in certain individuals with low cholinesterase activity there exists a genetically determined atypical cholinesterase.[6] The identification of the atypical enzyme was based on the selective inhibition of plasma esterase by dibucaine with benzoylcholine as the substrate. Further familial and inhibition studies using fluoride in addition to dibucaine, have led to the description of seven phenotypes and ten possible genotypes in the general population.[7-16] The presence of a non-allelic genetically determined C_5 factor, identified as an additional electrophoretic band, has been correlated with an increased plasma esterase activity.[17-18]

Individuals homozygous for the atypical esterase, phenotype A, have low plasma cholinesterase activity against benzoylcholine and certain other choline esters.[19] Those heterozygous for atypical and normal esterase, phenotype I, are intermediate and usually have a reduced amount of cholinesterase; the normal homozygotes, phenotype U, have on the average the most plasma cholinesterase. Much overlap occurs between the heterozygotes and the other two groups. The amount of cholinesterase, by itself, is thus not a valid indication of an individual's phenotype. Little is known about

the kind of esterase activity of each phenotype except as characterized by dibucaine and fluoride inhibition. A further characterization of activity of atypical esterase within families seemed indicated since the plasma esterase isozymes can be identified by electrophoretic and chromatographic techniques. The present study deals with the atypical esterase activity in a family whose members were clearly identified as to phenotypes by dibucaine and fluoride inhibition studies.

METHOD

Plasmas for cholinesterase determinations were obtained from a woman who previously had responded to succinylcholine by a prolonged paralysis, her husband and two sons aged 5 and 8. The plasmas from five randomly selected blood donors in apparent good health were pooled and used as a control.

The hydrolysis rates of succinylcholine chloride (SuCh) were estimated at room temperature in beakers containing 1 or 2 ml of undiluted plasma and 0·004 or 0·04 M SuCh in a total volume of 5 or 10 ml. The diluent was a 0·04 M $MgCl_2$–0·0175 M NaCl solution. The amount of NaOH needed to return the pH to 7·0 was used as the index of the hydrolysis rate over a period of at least 18 hr. Experimental values were corrected for nonenzymatic hydrolysis of SuCh without enzyme and with enzyme strongly inhibited by 10^{-5} M neostigmine.

Dibucaine numbers were determined spectrophotometrically in 10-mm quartz cells at 240 mμ according to the method of Kalow and Genest[6] and fluoride numbers by the method of Harris and Whittaker.[10] The hydrolysis rate of 5×10^{-5} M benzoylcholine chloride (BzCh) was measured with and without 10^{-5} M dibucaine HCl (Ciba) or 5×10^{-5} M sodium fluoride, and the percent inhibition was expressed as dibucaine or fluoride number. A phosphate buffer (0·066 M, pH 7·4) was used throughout for dilution of the enzyme (1:100) and as solvent for the substrate and inhibitors. Dibucaine numbers were also obtained by the titration method, with 0·01 M butyrylcholine β-toluene sulfonate and 10^{-5} M dibucaine in the $MgCl_2$–NaCl salt solution.

The hydrolysis rates of each substrate, except BzCh and SuCh, were determined with a recording pH stat at 7·4 with 0·005 M NaOH as titrant. Samples with a final volume of 5 ml were magnetically stirred in water-jacketed reaction vessels maintained at 37°. Plasma was used at a final dilution of 1:250 with the $MgCl_2$–NaCl solution. The same solution was also used as diluent for the substrates. After at least 5 min of nonenzymatic hydrolysis of the substrate, the enzyme was added, and a further period of hydrolysis was recorded for at least 5 min. The initial enzymatic hydrolysis rates were corrected for nonenzymatic hydrolysis and calculated in terms of micromoles substrate hydrolyzed per milliliter plasma per hour. The following esters were used: acetylcholine iodide (ACh), proprionylcholine iodide (PrCh), butyrylcholine β-toluene sulfonate (Buch), lauroylcholine chloride (LaurCh), myristoylcholine chloride, and β-carbonapthoxycholine iodide. The noncholine esters used included triacetin, tripropionin, tributyrin, trihexanoin, trilaurin, β-naphthol acetate, α-naphthol butyrate, indoxyl acetate, *p*-nitrophenyl acetate, and *o*-nitrophenyl butyrate.

Estimation of V_{max}, K_m and S_{V0} was made from either substrate–activity curves or by graphical analysis of the data by plotting V against V/S.[20] These terms are defined as follows: V = velocity of hydrolysis at specified substrate concentration; S = substrate concentration; V_{max} = the maximal rate of substrate hydrolysis; K_m = the

concentration of substrate at which the enzyme(s) are hydrolyzing the substrate at $V_{max}/2$. S_{V0} = the substrate concentration below which there is no measurable enzymatic hydrolysis. Indoxyl acetate and α-naphthol acetate are examples of substrates not soluble enough in the aqueous $MgCl_2$–NaCl solution to be studied over the entire substrate-activity range. K_m and V_{max} values thus could not be determined for each substrate used.

RESULTS

Hydrolysis of succinylcholine

Plasma obtained from the woman who had responded to succinylcholine by a prolonged paralysis failed to hydrolyze 0·004 M succinylcholine during an 18-hr period (Fig. 1). At 0·04 M her plasma hydrolyzed SuCh at only 5·8% of the rate of pooled plasma. At the end of 2 hr SuCh was not hydrolyzed at either 0·04 or 0·004 M. After 1 hr the pooled plasma gave measurable rates of hydrolysis at both concentrations. The prolonged paralysis experienced by B.T. after the administration of SuCh is thus correlated with the failure of her plasma to hydrolyze succinylcholine. Individuals homozygous for atypical esterase have plasma which hydrolyzes SuCh at a slower rate than normal.[11]

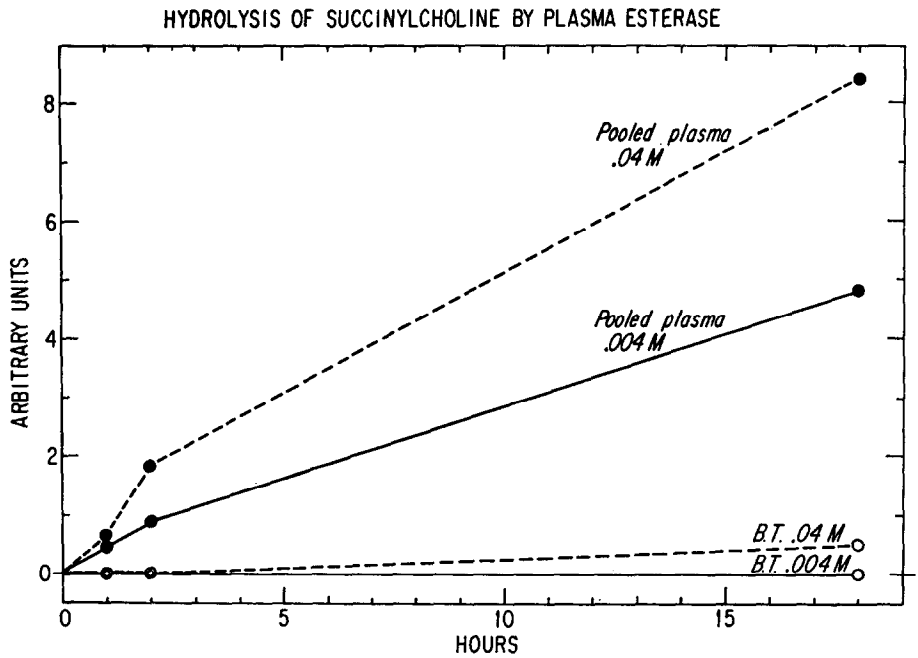

FIG. 1. Hydrolysis of succinylcholine chloride by plasma esterase. M indicates initial substrate concentration in moles per liter. B.T. indicates plasma from mother.

Phenotypes as determined by dibucaine and fluoride inhibition

In order to determine the phenotypes, the inhibition of plasma esterase activity by dibucaine was studied in each member of the family. The activity of the pooled sample was determined under the same conditions and compared to the experimental findings.

The data in Fig. 2 show that the plasma from the mother (B.T.) is markedly resistant to inhibition by either fluoride or dibucaine. According to the genetic classifications established by Kalow and Genest,[6] the mother can be considered as a phenotype A. The plasma from the father (N.T.) was strongly inhibited by either dibucaine of fluoride. The degree of inhibition was not different from that of the pool or from that of the general population reported by Kalow and Gunn.[21] The father can thus be considered the usual phenotype U.

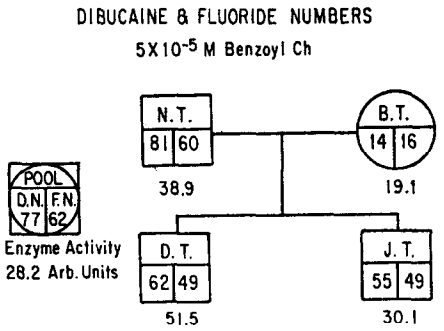

FIG. 2. Dibucaine and fluoride inhibition. B.T. is the mother, who responded to succinylcholine with a prolonged paralysis; J.T., younger son; D.T., older son; N.T., father. The same pool of plasma was used as control throughout all the experiments. At lower left, D.N. indicates per cent of plasma esterase activity inhibited by dibucaine (Dibucaine number). F.N. indicates fluoride inhibition (Fluoride number). Numbers below indicate activity of plasma esterase against benzoylcholine chloride (5×10^{-5} M) in arbitrary units.

The fluoride values of the two siblings were precisely the same and intermediate between those for the father and the mother. The dibucaine numbers (Kalow and Genest)[6] were also intermediate, but the younger son (J.T.) had a slightly lower value than the older son (D.T.). The two sons, therefore, can appropriately be classified as phenotype I according to dibucaine and fluoride inhibition data. Although plasma from D.T. had intermediate inhibition values for both fluoride and dibucaine, it showed greater cholinesterase activity with benzoylcholine than did the plasma from the father (N.T.) or the pool. Another unexpected finding was that, although the fluoride and dibucaine numbers classified the two siblings as the same phenotype, the cholinesterase activities determined with benzoylcholine were much less for the younger son (J.T.) than for the older (D.T.). The pool and the younger son, although different phenotypes, had nearly the same esterase activity. There is thus no complete correlation between cholinesterase activity and dibucaine and fluoride inhibition in this family.

According to genetic theory, the occurrence of the silent gene, E_1^s,[12, 16, 22, 23] in the two sons is precluded by the intermediate dibucaine number (genotype $E_1^uE_1^a$). The father has a dibucaine number indicating typical esterase and plasma esterase levels equal to the controls and, thus, must be considered homozygous for the usual esterase gene, E_1^u, since occurrence of E_1^u E_1^s reduces the esterase level to about 65%.[16] The mother could be genotype $E_1^aE_1^a$ or $E_1^aE_1^s$. However, her phenotype is A, and if E_1^s is present it was not transmitted to the sons.

The dibucaine numbers stated in Fig. 2 were determined by the method of Kalow

and Genest.[6] Additional tests of dibucaine inhibition were carried out by titration at 37° with 0·01 M BuCh as the substrate and 5×10^{-5} M dibucaine as the inhibitor. Although the values obtained were of the same relative order, they were 5%–10% lower than those obtained by the method of Kalow and Genest. Since a 10 per cent difference might lead to an erroneous phenotype classification, it is desirable to duplicate precisely the methods used by Kalow and Genest for the determination of dibucaine numbers when they are to be used for genetic typing or for direct comparison with other data.

Specificity of the enzymes found in different phenotypes

The cholinesterase activity of the plasma of each member of the family has been determined by use of a variety of substrates. Figure 3 shows that when acetylcholine was used as the substrate, the esterase activity differed for each of the family members and that each failed to match exactly the enzyme activity of the pooled plasma. The

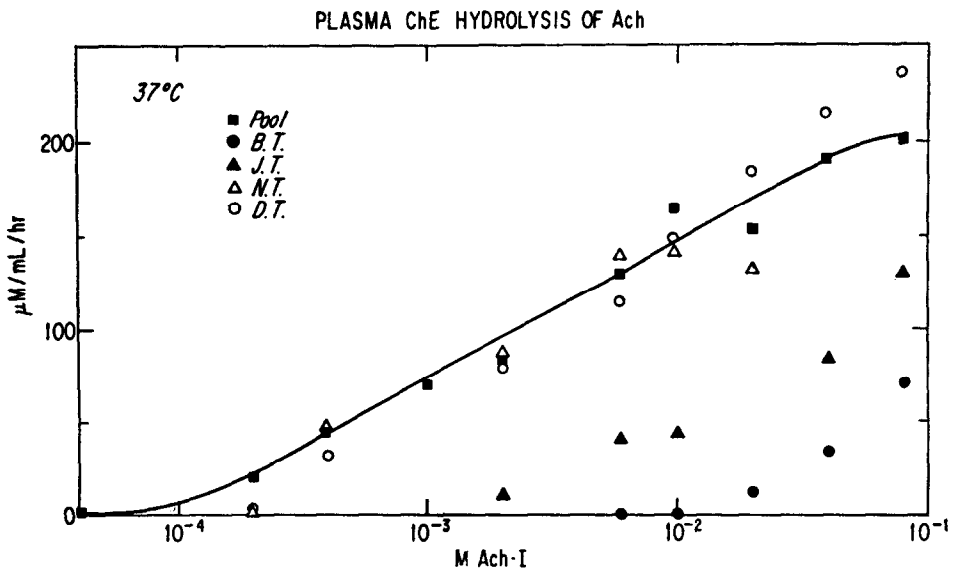

FIG. 3. Hydrolysis of acetylcholine iodide by plasma esterase from a family with atypical esterase. Letter designations as in Fig. 2. Each data point represents averages of two to six determinations.

least activity was found in plasma from the mother (B.T.). Somewhat more activity occurred with plasma from the younger son (J.T.). The older son (D.T.) and the father (D.T.) had plasma esterase activities like that of the pool for intermediate concentrations of substrate, but the V_{max} values were markedly different (Table 1). The shape of the curves with the mother's plasma and that from the younger son (J.T.) indicates that these plasmas may have enzyme activity which becomes operative only at the higher substrate concentrations. This response seems to be absent in plasma from the father (N.T.).

When butyrylcholine was used, the substrate velocity curve obtained with plasma from the mother (B.T.), while shifted to the right, was more nearly like the pooled control plasma than with ACh. No indication for additional enzyme activity occurred at higher substrate concentrations, since both the control and B.T. plasma activity

TABLE 1. PLASMA ESTERASE

V_{max} mM/l/min

	Pool	B.T.	J.T.	D.T.	N.T.
ACh	2·7	>1·1*	>2·2	4·2*	2·3
PrCh	6·3	2·5*			
BuCh	4·8	2·2*			
LaurCh	5·5	0·9*		2·5*	
AcSCh	4·7	2·5*	2·0*	3·6*	3·4*
BuSCh	10·3	5·6*			
α-NapthBu	2·5	2·7			
β-NapthAc	1·5	1·2			
o-nitro-φBu	6·3	4·3*			
p-nitro-φAc	2·1	0·5*			

* Difference from control > 1 mM/l/min.

curves were of similar shape. At 0·01 M the pooled plasma hydrolyzed BuCh at 272 μmoles/hr/ml, the D.T. plasma at 344, N.T. at 244, J.T. at 164, and B.T. at 90. This is the same relative order of activity as was found with ACh. Similar relative orders within the family were found with proprionylcholine, benzoylcholine, acetylthiocholine, and butyrylthiocholine. Thus within the family the mother always had, with a variety of substrates and concentrations, the least cholinesterase activity; the younger son (J.T.) had more activity than the mother but still an abnormally low hydrolysis rate. The father had control or near control rates, and the other son (D.T.) had activity nearly equal to or greater than the pooled plasma.

The results stated above present an example of why the amount of cholinesterase activity is misleading if it is to be used to assign phenotypes or genotypes since, as Fig. 3 shows, a different assignment could be made, dependent on the substrate concentration at which the amount of activity was measured. The substrate used also needs to be considered, since at 0·01 M ACh the mother might be considered "silent" for plasma cholinesterase. However, at 5×10^{-5} M benzoylcholine she was not homozygous "silent" but had more than one half the activity of the control plasma. Similarly, V_{max} values are worthless in genotype assignment, since a different V_{max} can be observed with a reduced amount of enzyme or with a partially inhibited enzyme. Simpson and Kalow[22] have discussed the difficulty of using cholinesterase activity for typing because of individual variations in the population. However, if K_m and S_{V0} values are used it can be readily determined whether the enzymes are qualitatively different in various individuals (Tables 2 and 3). By use of homologous series of substrates, it would be possible to determine the substrate specificity of the enzymes present. Whether such studies can, in general, be of benefit toward a further understanding of the inheritance of cholinesterase remains to be further tested. The K_m and S_{V0} data in Table 2 and 3 indicate that B.T., J.T., and D.T. do not have the same kind of esterase activity in their plasma as does N.T. or the pool. It can be seen that the enzymatic abnormality in the plasma from B.T. is more related to activity against acetylcholine than to higher homologues, and that the difference between B.T. and the pool is not present with lauroylcholine. The V_{max} data indicate, however, that there is less cholinesterase which will hydrolyze LaurCh in plasma from B.T. Similar findings for phenotype A have been described by Davies *et al.*[19]

When aromatic noncholine esters were used as substrates, the difference between

the plasma from B.T. and the pool was less than that observed with short-chain choline esters. The K_m and V_{max} values for α-naphthol butyrate and β-naphthol acetate were similar for plasma from B.T. and the pool. The S_{V_0} for β-naphthol acetate was somewhat higher for B.T. than for the pool. McComb *et al.*[24] tested serum from an atypical homozygote, phenotype A, with *o*-nitrophenyl butyrate and found the enzyme activity to be the same as with normal serum. When this substrate was

TABLE 2. PLASMA ESTERASE

K_m mM/l

	Pool	B.T.	J.T.	D.T.	N.T.
ACh	2·7	40·0*	22·0*	8·0*	1·0
PrCh	2·1	8·3*			
BuCh	0·39	0·90*			
LaurCh	0·15	0·25		0·23	
AcSCh	1·3	21·0	3·7	0·97	1·3
BuSCh	1·2	3·8			
α-NapthBu	0·50	0·87			
β-NapthAc	0·20	0·60			

* Difference from control > 0·5 magnitude.

TABLE 3. PLASMA ESTERASE

S_{V0} mM/l

	Pool	B.T.	J.T.	D.T.	N.T.
ACh	0·08	10·0*	1·0*	0·15	0·15
PrCh	0·01	1·5*			
BuCh	0·008	0·1*			
LaurCh	0·002	0·002		0·002	
AcSCh	0·006	3·0*	0·4*	0·045*	0·01
BuSCh	0·004	1·0*			
α-NapthBu	0·02	0·03			
β-NapthAc	0·05	0·2*			
o-nitro-φBu	0·02	0·1*			
p-nitro-φAc	0·2	2·0*			

* Difference from control > 0·5 magnitude S_{V_0} = Substrate concentration at V_0.

studied with plasma from B.T. the V_{max} and S_{V_0} values were different from those of the pooled plasma (Tables 1 and 3). This difference, and that seen with *p*-nitrophenyl acetate, shows that the mother has a somewhat unusual aromatic esterase pattern.

The plasma collected from B.T. on two occasions, about five weeks apart, gave similar experimental results. Cholinesterase determinations in two other laboratories also showed markedly reduced plasma CHE activity. Thus the unusual enzyme pattern was not temporary.

Determinations of cholinesterase activity of the washed, lysed erythrocytes from B.T., with 6×10^{-3} Ach as substrate, gave results which fell into the low normal range i.e. B.T. = 722; 13 controls = 844 with one standard deviation = 98 μmole /ml packed RBC/hr.

The marked difference in esterase activity between the two siblings, each of whom can clearly be considered phenotype I, posed the question of the existence of the C_5 component in the sibling with the greater plasma esterase activity.[17–18] Although an increase in total serum cholinesterase activity usually accompanies the presence of the C_5 component, there is no indication that the greater esterase activity found in one sibling (D.T.) is due to the C_5 factor. Unidirectional electrophoresis in starch gel [17–18] performed at pH 8·75 gave no indication that any member of the family had plasma which contained the C_5 component. However, the C_5 band is difficult to observe at an alkaline pH, and we thus have no conclusive data indicating whether the high cholinesterase activity seen in the one son with an intermediate I phenotype is due to the C_5 component. The electrophoresis patterns determined by using plasma obtained from the mother, phenotype A, of the "T" family appear elsewhere.[25]

Triglycerides

Plasma esterases from human beings are known to have activity in the hydrolysis of certain triglycerides.[26] Figure 4 shows that plasma from B.T. failed to hydrolyze triacetin at any of three different concentrations. The plasmas of the father and two sons had a markedly reduced activity of 0·05 M triacetin. The B.T. plasma hydrolyzed

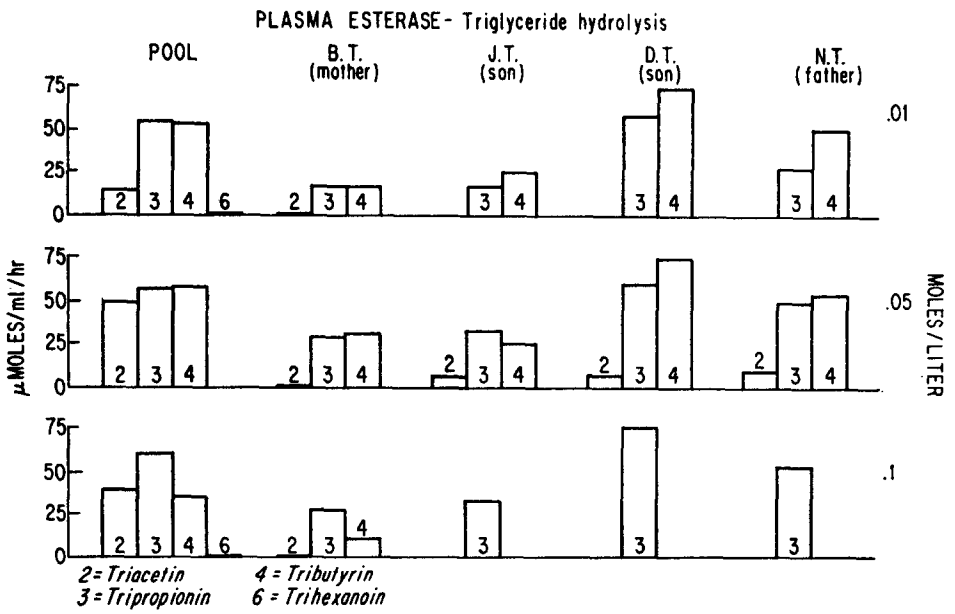

FIG. 4. Hydrolysis of triglycerides by plasma esterases. Moles/liter on right side of figure indicates substrate concentration.

tripropionin and tributyrin but at a low rate. The hydrolysis of tripropionin and tributyrin was also low for J.T., but the plasma from D.T. and N.T. were not different from that of the pool. Except for the reduced ability of each family member to split triacetin, the findings with the triglycerides were similar to those found when choline esters were used as substrates—i.e., activity in the plasma of B.T. and J.T. was less than that of D.T. and N.T. which are nearly like the pooled data.

DISCUSSION

The present study describes a disparate distribution within a family between cholinesterase activity of the plasma and the phenotype as determined by the fluoride and dibucaine inhibition of the esterase. One of the parents was classified as phenotype A, possibly homozygous for atypical esterase, the other as homozygous for the usual or typical plasma enzyme. According to genetic theory the two siblings should be heterozygous and have genotype $E_1^uE_1^a$ or $E_1^uE_1^s$. Tested for the heritable types of cholinesterase by dibucaine and fluoride determinations, the two sons showed the same phenotype and were unequivocally heterozygous for typical and atypical esterase. When the plasma esterase activities of the two were qualitatively tested by determining the K_m values, with acetycholine or acetylthiocholine as substrate, they were found to be markedly different. There is thus established in this family a qualitative difference in plasma esterase activity that is divergent from the established genetic pattern as determined by inhibition with dibucaine or fluoride.

Although there is much overlap in cholinesterase activity levels among phenotypes,[9] it was unexpected to find that one son with phenotype I had greater cholinesterase activity in his plasma for each substrate than had his father—a phenotype U—and that the other son with phenotype I had greatly reduced levels except with benzoylcholine. We have been able to locate in the literature eight instances in six families in which both dibucaine members and choline esterase levels are reported and which have one parent identified as phenotype U and the other as phenotype A: Families 1 (2 sets), 2, 3, 5, and 6 of Harris *et al.*;[9] two sets of parents from family "X" of Kalow and Staron.[27] One set of parents in family 1 is similar to the family described here. Each of the other families is different since in none of them is there a sibling who had equal or greater cholinesterase activity than the phenotype U parent. Except for the two families (No. 3 and one set of family 1[9]), the heterozygote siblings have nearly the same CHE activity as the average activity of their parents. Thus in eight reported instances, there are two exceptions to the amount of cholinesterase activity agreeing with the genetic patterns as established by inhibition. The "T" family reported here furnishes an additional exception in that both quantitatively and qualitatively the sons have different enzyme patterns but the same phenotype.

In a large family[23] without a homozygous atypical individual, in which 48 persons were tested, the cholinesterase activity of 6 individuals of phenotype I had a range of 52–63 units while 29 normals had a range of 68–123. Thus no individual in this family with a phenotype I exceeded any normal homozygote in amount of cholinesterase activity.

The findings of this study reinforce other studies[13, 27] which have shown that cholinesterase levels do not precisely follow the genetic pattern. The reduction in the level of plasma esterase activity by environmental factors, such as liver malfunction, is well known. It is unknown whether other genetically determined qualitative differences exist in plasma esterase which are unrelated to those identified by dibucaine and fluoride inhibition. The problem might be studied by concomitant familial experiments using dibucaine and fluoride genetic classification and determination of K_m values of plasma esterase. The problem is unrelated to the amount of CHE present, since the absolute amount of enzyme will not affect the K_m values. The present study shows that in at least one case the qualitative characteristics of plasma esterase are divergent from the known inheritance patterns for plasma esterase.

Acknowledgements—The cooperation of Dr. Walter Camp and Drs. Jerome Resnick and W. King Engel of the Medical Neurology Branch of the National Institute of Neurological Diseases and Blindness is gratefully acknowledged.

REFERENCES

1. F. T. EVANS, P. W. S. GRAY, H. LEHMANN and E. SILK, *Lancet* **1**, 1229 (1952).
2. F. T. EVANS, P. W. S. GRAY, H. LEHMANN and E. SILK, *Br. med. J.*, p. 136 (1953).
3. J. G. BOURNE, H. O. J. COLLIER and G. F. SOMERS, *Lancet* **1**, 1225 (1952).
4. A. FORBAT, H. LEHMANN and E. SILK, *Lancet* **136**, 1067 (1953).
5. H. LEHMANN and E. RYAN, *Lancet* **2**, 124 (1956).
6. W. KALOW and K. GENEST, *Can. J. Biochem.* **35**, 339 (1957).
7. E. N. ALLOTT and J. D. THOMPSON, *Lancet* **2**, 517 (1956).
8. W. KALOW, *Ciba Foundation Symposium on Biochemical Human Genetics*, p. 39. Little Brown, Boston (1959).
9. H. HARRIS, M. WHITTAKER, H. LEHMANN and E. SILK, *Acta genet. (Basel)* **10**, 1 (1960).
10. H. HARRIS and M. WHITTAKER, *Nature, Lond.* **191**, 496 (1961).
11. W. KALOW, *Pharmacogenetics*. Saunders, Philadelphia (1962).
12. J. LIDDELL, H. LEHMANN and E. SILK, *Nature, Lond.* **193**, 561 (1962).
13. H. LEHMANN and J. LIDDELL, *Progr. med. Genet.* **3**, 75 (1964).
14. N. E. SIMPSON and W. KALOW, *Am. J. hum. Genet.* **15**, 280 (1963).
15. N. E. SIMPSON and W. KALOW, *Am. J. hum. Genet.* **17**, 156 (1965).
16. W. E. HODGKIN, E. R. GIBLETT, H. LEVINE, W. BAUER and A. G. MOTULSKY, *J. clin. Invest.* **44**, 486 (1965).
17. H. HARRIS, E. B. ROBSON, A. M. GLEN-BOTT and J. A. THORNTON, *Nature, Lond.* **200**, 1185 (1963).
18. H. HARRIS, D. A. HOPKINSON, E. B. ROBSON and M. WHITTAKER, *Ann. hum. Genet.* **26**, 359 (1963).
19. R. O. DAVIES, A. V. MARTON and W. KALOW, *Canad. J. Biochem.* **38**, 545 (1960).
20. M. DIXON and E. C. WEBB, *Enzymes*, p. 58. Academic Press, New York (1964).
21. W. KALOW and D. R. GUNN, *Ann. hum. Genet.* **23**, 239 (1959).
22. N. E. SIMPSON and W. KALOW, *Am. J. hum. Genet.* **16**, 180 (1964).
23. H. W. GOEDDE, W. FUSS, D. GEHRING and H. BAITSCH, *Biochem. Pharmac.* **13**, 603 (1964).
24. R. B. MCCOMB, R. V. LAMOTTA and H. J. WETSTONE, *Fedn Proc. Fedn Am. Socs. Biol.* **23**, 280 (1964).
25. I. A. BRODY, J. S. RESNICK and W. K. ENGEL, *Archs. Neurol.* **13**, 126 (1965).
26. K. B. AUGUSTINSSON, *The Enzymes*, vol. 4, p. 526. Academic Press, New York (1960).
27. W. KALOW and N. STARON, *Canad. J. Biochem.* **35**, 1305 (1957).