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Increased Plasma Cholinesterase Activity and Succinylcholine Resistance: A Genetic Variant *

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Quantitative variations of plasma cholinesterase activity (also called serum cholinesterase, pseudocholinesterase, nonspecific cholinesterase, or cholinesterase II) are associated with several diseases. Slightly elevated levels have been observed in thyrotoxicosis (1), schizophrenia (2), hypertension (3), acute emotional disorders, and after concussion (4). Markedly elevated levels, two to three times normal, occur with the nephrotic syndrome (5). Plasma cholinesterase activity is decreased in various disorders of the liver (6), in carcinoma (7), and after anticholinesterase drugs (8).

Low plasma cholinesterase activity may signify the presence of a qualitatively different enzyme. Some surgical patients develop prolonged apnea after succinylcholine, a muscle relaxant normally hydrolyzed rapidly by cholinesterase; their plasma hydrolyzes cholinesterase substrates more slowly than usual (9). Several cholinesterase inhibitors such as dibucaine, physostigmine, and hexamethonium are weak inhibitors of the atypical cholinesterase from these patients (10). Under standardized conditions, dibucaine inhibits the usual enzyme more than 70%, but the atypical enzyme is inhibited only 20% (11). The per cent inhibition by dibucaine has become known as the dibucaine number. People with dibucaine numbers of about 20 are very sensitive to succinylcholine.

Kalow and Staron determined the dibucaine numbers of 1,556 healthy and mentally ill subjects (12) and demonstrated that two allelic, codominant, autosomal genes control the presence of the usual and atypical cholinesterase. Ninety-seven per cent of the population have dibucaine numbers higher than 70 and are homozygous for the usual cholinesterase; 0.03% have dibucaine numbers around 20 and are homozygous for the atypical enzyme; 3% are intermediate and have mixtures of the two enzymes.

Kalow's studies of the genetics of plasma cholinesterase inheritance have been expanded, and two further alleles have been reported. One is a "silent" gene that results in complete absence of cholinesterase activity in the homozygote (13); another cholinesterase genotype is characterized by a normal or slightly low dibucaine number but a resistance to sodium fluoride at standard conditions (14).

Harris, Hopkinson, Robson, and Whittaker detected a further phenotype that can only be demonstrated electrophoretically (15). Family studies suggested that the extra band they reported is due to a gene that is nonallelic to the previously described genes (16).

Recently, a normal man was found to have a plasma cholinesterase activity three times higher than usual. The inheritance, characterization, and significance of this cholinesterase form the subject of this report.

Methods

Subjects. The propositus is a 26-year-old male with normal physical and psychiatric examinations. He has had the usual childhood diseases, but has no history of severe illnesses and no operations. He is allergic to penicillin. Laboratory studies, including complete blood count, urinalysis, blood urea nitrogen, sulfobromophthalein retention, alkaline phosphatase, serum glutamic oxalacetic and pyruvic transaminases, cholesterol, protein-bound iodine, serum protein electrophoresis, electrocardiogram, electroencephalogram, and chest X-ray, were normal.

His relatives were examined, and blood was obtained for evaluation. They had no history of mental illness or physical abnormalities.

Plasma cholinesterase activity was determined on 1,029 military personnel between the ages of 17 and 35 (average, 22). The subjects selected as controls for the tests described in this paper had normal plasma cholinesterase activities and normal dibucaine and fluoride numbers.

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Experimental procedures. Diisopropyl fluorophosphate (DFP),[1] an irreversible noncompetitive inhibitor mainly of plasma cholinesterase, was administered intramuscularly in graded doses to the propositus and seven normal volunteers. DFP was diluted in saline and used immediately, since its half-life in solution is 16 hours. The total dose was 8 to 12 μg per kg divided into three or four injections. Each injection was separated by 45 to 60 minutes.

A continuous recording of the plasma cholinesterase activity of the propositus and one control subject was obtained to insure that the time interval between injections would be sufficient for the cholinesterase activity to stabilize. Intermittent blood samples were also drawn from the propositus and seven control subjects for cholinesterase activity assay before each injection and 1 hour after the last injection. After the last assay, an excess of DFP (0.15 mg, intramuscularly, per man) was given to inactivate the plasma cholinesterase activity completely. Blood was then drawn daily to measure the rate of cholinesterase regeneration.

Succinylcholine[2] (0.1 to 0.2 mg per kg) was given intravenously to the propositus and five normal volunteers to determine the pharmacological influence of elevated cholinesterase activity. The injection lasted 5 seconds. Grip strength was measured every 20 seconds with a Collins hand dynamometer until the strength returned to control levels. Oxygen saturation was measured continuously with a Waters ear oximeter. This experiment was conducted three times on each subject on successive days.

Laboratory methods. Individual blood samples were drawn from the antecubital vein and mixed with heparin [0.2 ml heparin (1,000 U.S.P. U per ml) per 8 ml blood]. Plasma was obtained immediately by centrifugation. The determinations were usually performed the same day. When this was not possible, the plasma was refrigerated at 4° C for less than 1 week or frozen. No change in activity occurred with these storage procedures.

The plasma cholinesterase activity of the volunteers was measured at least once by an automated procedure using De la Huerga, Yesenick, and Popper's modification of Hestrin's colorimetric technique (17). Undiluted plasma and acetylcholine (0.05 M) were used as reactants. Results are expressed in micromoles of acetylcholine hydrolyzed per hour by 1 ml of plasma at 37° C.

Another method for cholinesterase assay was the ultraviolet spectrophotometric determination described by Kalow and Lindsay (18). Plasma, diluted 1 to 50, was used, in contrast to the previous methods, which required undiluted plasma. Benzoylcholine (2×10^{-4} M) was used as the substrate. The dibucaine numbers and fluo-

[1] Merck Sharp and Dohme, Philadelphia, Pa.

[2] Abbott Laboratories, North Chicago, Ill.

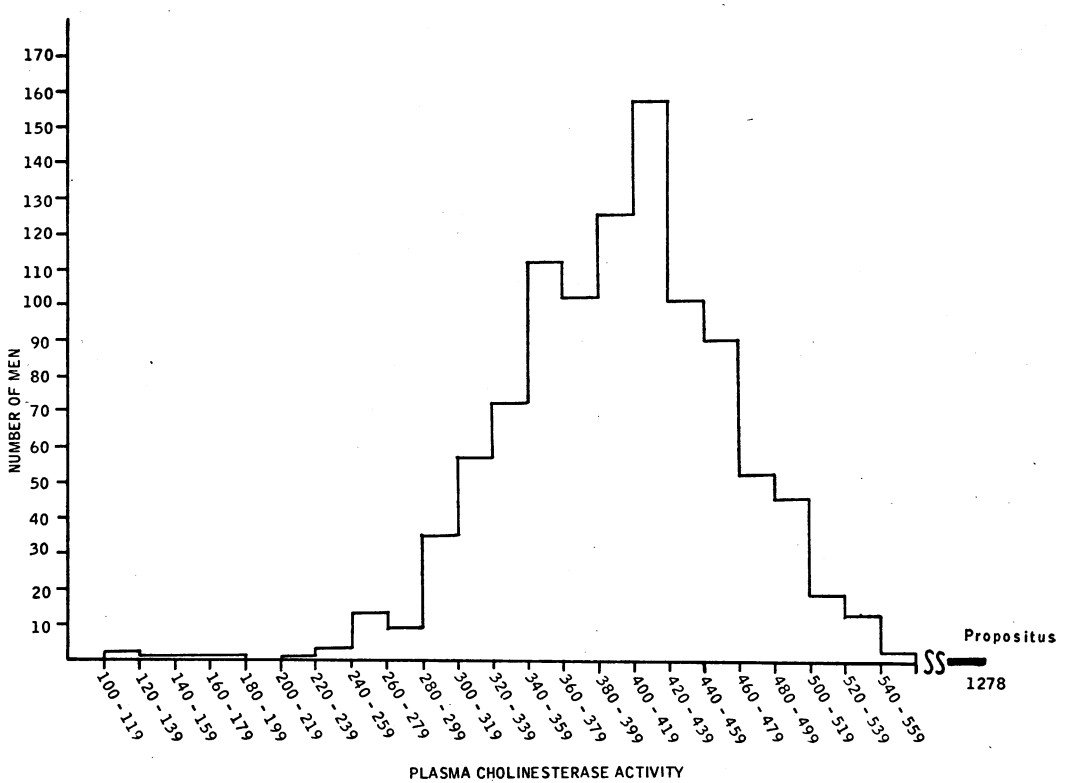

FIG. 1. DISTRIBUTION HISTOGRAM OF THE PLASMA CHOLINESTERASE ACTIVITY OF 1,029 MALE VOLUNTEERS. The mean is 391 and the SE 0.75. Results are expressed in micromoles of acetylcholine hydrolyzed per hour by 1 ml of plasma at 37° C.

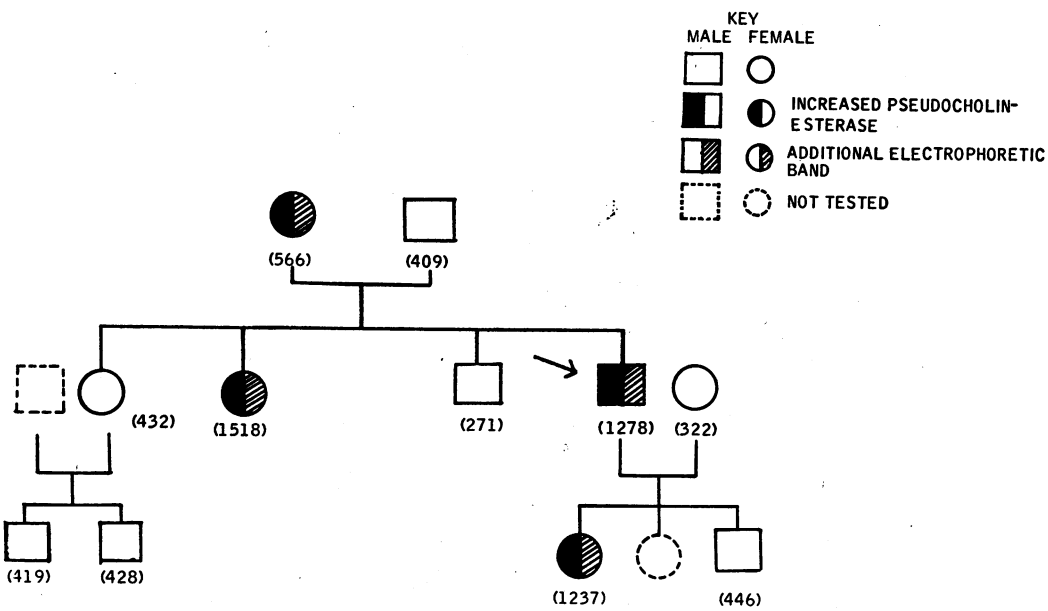

FIG. 2. FAMILY DISTRIBUTION OF INCREASED PLASMA CHOLINESTERASE ACTIVITY AND ADDITIONAL CHOLINESTERASE ELECTROPHORETIC BAND. The figures in parentheses indicate the cholinesterase activity; the arrow indicates the propositus. Note that each family member who has an increased cholinesterase activity also demonstrates an additional cholinesterase band with disc electrophoresis.

ride numbers were determined for the propositus, his family, and the control subjects (11, 14).

For the continuous monitoring of cholinesterase activity during the studies with DFP, 1 ml of blood per minute was drawn through an iv catheter leading from the antecubital vein to a multichannel analytic system that preferentially measured plasma cholinesterase since butyrylcholine was used as substrate (19).

Unidimensional disc electrophoresis was performed according to the method of Ornstein (20). Acrylamide gel (Canalco) was prepared with Tris-glycine buffer (pH 8.6). The material was stained for cholinesterase with α-naphthylacetate as substrate and fast blue B diazo coupler (21).

Results

A distribution histogram of the plasma cholinesterase activity in 1,029 male volunteers is shown in Figure 1. The mean was 391 and the SE 0.75. The mean was higher than Kalow's series because of the younger age and male sex of this group (22); the distribution was similar.

The propositus's activity, 1,278 U, was more than three times higher than the mean for the volunteers. Since no disease could be found to explain this elevation and since it remained constant for 6 months, his family was studied to determine whether the elevated cholinesterase activity might be familial. The family's cholinesterase activities are shown in Figure 2. The propositus's sister and daughter had very high enzyme activities, 1,518 and 1,237 U, respectively. His mother's cholinesterase activity, 566 U, was not so strikingly elevated, but was, nevertheless, higher than the other volunteers. Dibucaine numbers and fluoride numbers were normal in all members of the family.

The *in vivo* sensitivity of the propositus's cholinesterase activity was tested with DFP. This anticholinesterase was chosen because it reacts irreversibly and stoichiometrically with plasma cholinesterase (23). Therefore, a standardized dose should inhibit a constant amount of plasma cholinesterase activity. This relationship should be independent of the original concentration of the enzyme. The results are shown in Figure 3. The propositus's plasma cholinesterase activity was three to four times more sensitive to DFP than the controls'. DFP, 1 μg per kg intramuscularly, inhibited the propositus's plasma cholinesterase activity 93 U. The same dose inhibited the controls' cholinesterase activity by a mean of 25 U. After a total dose of 12 μg per kg, the propositus's cholinesterase activity was 124 U, and the mean of the controls was 66 U. The equations in Figure 3 show that 13 to 15 μg per kg would

have completely inhibited the cholinesterase activity of the propositus and the controls. The per cent of original cholinesterase activity inhibited by DFP was similar. Each microgram per kilogram inhibited the cholinesterase activity approximately 7% in the propositus and in the control subjects.

This experiment was repeated 15 days later, after the propositus had regenerated 70% of his original cholinesterase activity. Again, his enzyme activity was as sensitive to DFP as previously (Figure 3).

There is strong evidence that the regeneration of cholinesterase activity after inactivation by DFP represents resynthesis of new enzymes by the liver (8). The comparative rates of cholinesterase activity regeneration of three controls and the propositus are shown in Figure 4. The per cent of original activity regenerated daily was similar for the controls and propositus. After 4 days, the propositus had regenerated 27% of his cholinesterase activity; the controls, 40%. After 15 days, they had regenerated 70% and 80%, respectively. There was no statistical difference between the two slopes.

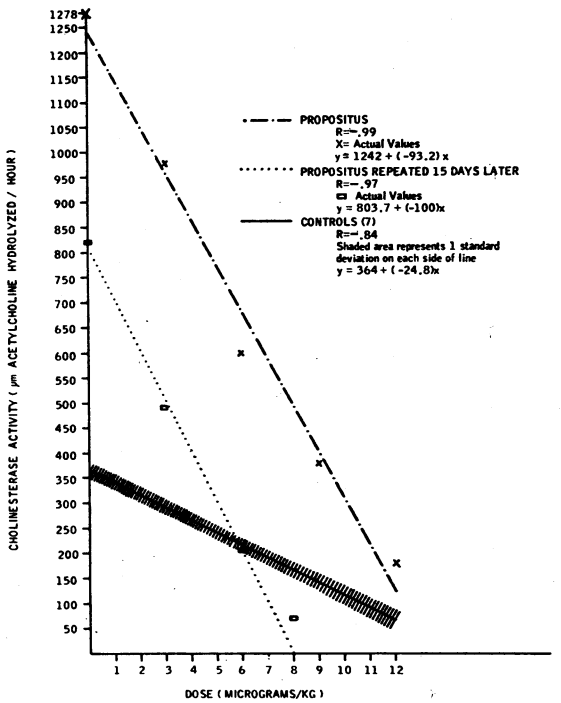

FIG. 3. THE INHIBITION OF PLASMA CHOLINESTERASE ACTIVITY AFTER ADMINISTRATION OF DIISOPROPYL FLUOROPHOSPHATE. R is the correlation coefficient; y is the equation for the line.

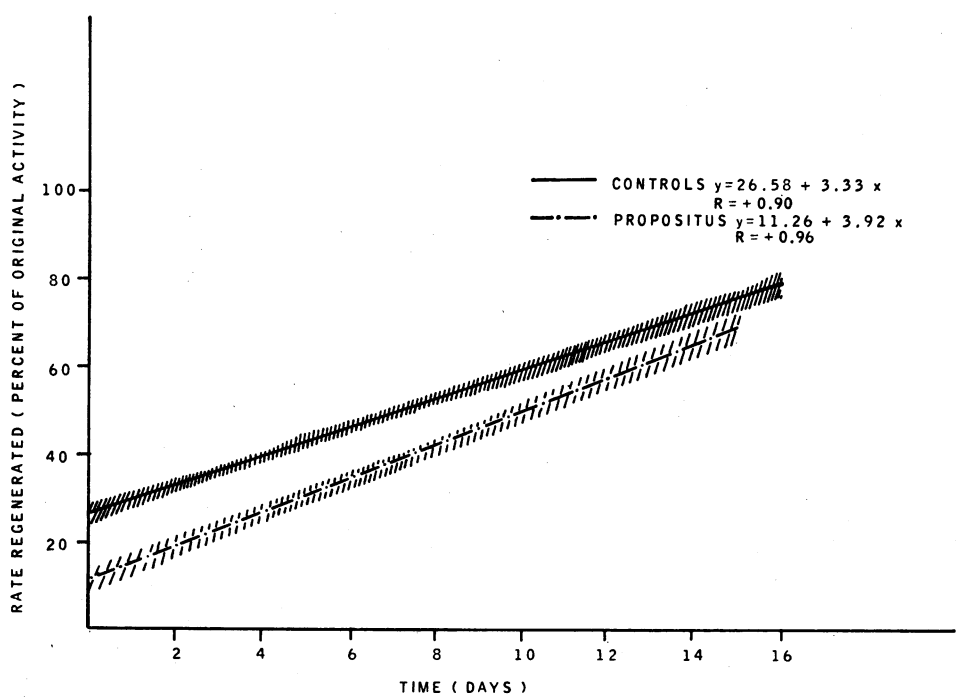

FIG. 4. RATE OF PLASMA CHOLINESTERASE REGENERATION (PER CENT OF ORIGINAL ACTIVITY). The shaded area represents 1 SE on each side of the line. There is no statistical difference between the two slopes.

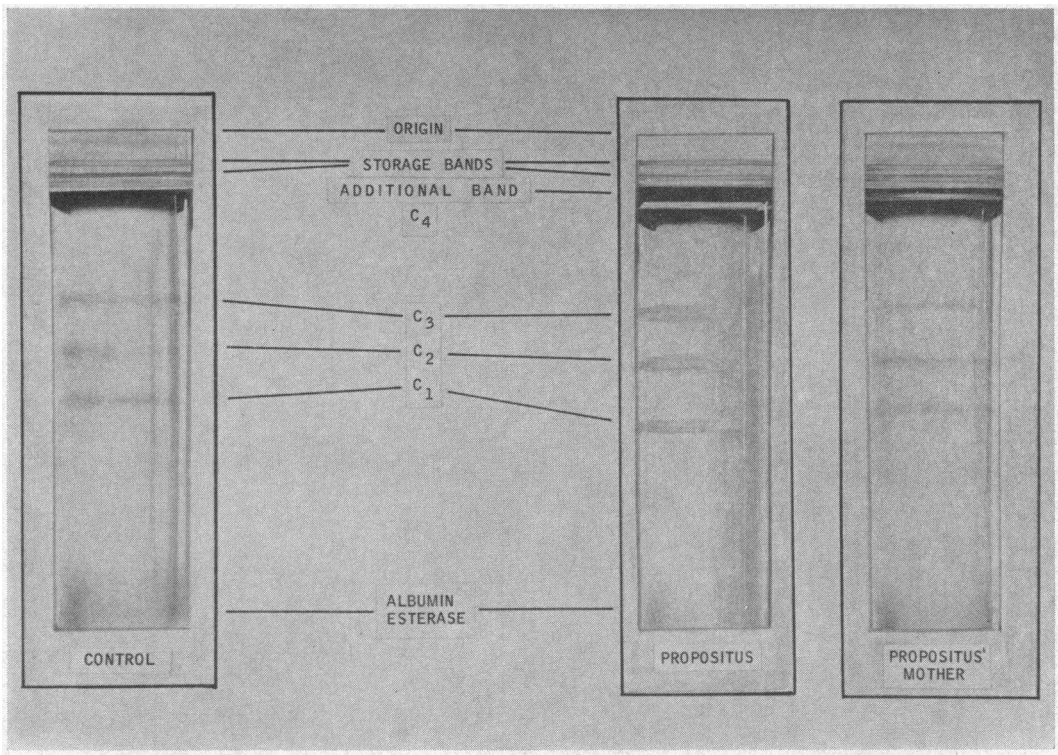

FIG. 5. SERUM DISC ELECTROPHORESES STAINED FOR CHOLINESTERASE. The propositus's additional cholinesterase band is thicker and more intense than his mother's.

The results of the disc electrophoreses stained for cholinesterase are shown in Figure 5. The two pale bands closest to the origin represent storage bands. Harris and associates believe that they may be derived from the more intense C_4 band after a few days' storage (15). They were not present when fresh specimens were used. Each sample demonstrated three light bands between the rapidly migrating albumin esterase and the slower C_4 band. These three bands, called C_1, C_2, and C_3, rapidly faded after the staining procedure. It has been suggested that the C_4 band contributes the greatest proportion of the cholinesterase activity in individuals with the usual cholinesterase genotype.

The propositus and each member of his family with increased cholinesterase activity had a densely staining additional band. This band was adjacent to the C_4 band, but was slightly closer to the origin. It was present in fresh and stored samples from the propositus. This band was not observed in the electrophoretic patterns of the members of the family with normal cholinesterase activity, nor was it present in the plasma obtained from numerous volunteers.

The staining characteristics of the additional band suggested that it contributed more to the total cholinesterase activity than the other bands. In three of the four affected family members, the extra band stained more intensely and diffusely than the C_4 band and was much darker than C_1, C_2, or C_3. The mother's additional band was slightly less intense than the C_4 band. This might be related to her lower cholinesterase activity relative to the other three.

The propositus's cholinesterase activity suggested that he might have an altered response to succinylcholine. His change in grip strength after iv administration of succinylcholine was measured and compared to that of five controls. Results are shown in Figure 6. One-tenth mg per kg affected the controls in 20 seconds. Grip strength, as measured by a hand dynamometer, decreased rapidly and then gradually recovered in the next 2 to 3 minutes. Oxygen saturation did not change, and there were no residual effects.

The propositus's strength was not affected by the same dose. He was then given twice the dose that the controls received with only a slight and

transient effect. Compared to the control subjects, the propositus was resistant to succinylcholine.

Discussion

There are two types of cholinesterase. These can be differentiated by substrate specificity, anatomic distribution, and biological function; their characteristics have been reviewed recently (24). Plasma cholinesterase hydrolyzes propionylcholine and butyrylcholine more rapidly than acetylcholine. Acetylcholinesterase (specific or true cholinesterase, cholinesterase I) hydrolyzes acetylcholine most rapidly and negligibly hydrolyzes butyrylcholine. Plasma cholinesterase is produced by the liver, and its main source is serum or plasma. Although the biological function of plasma cholinesterase is unknown, it is important in limiting the responses to hydrolyzable drugs such as succinylcholine. Acetylcholinesterase on the other hand, is found in the nervous system and red blood cells and is important in nerve impulse transmission. There are no biological or genetic similarities between the two types. The propositus's acetylcholinesterase activity was normal, but his plasma cholinesterase activity was very high.

Increased plasma cholinesterase activity is very rare. Kalow and his colleagues noted one individual with 2.5 times the average enzyme activity in a study of 1,556 subjects (11, 12). The subject had no evidence of disease and had a normal dibucaine number. No mention of family studies was made. If Kalow's group and the Army volunteers are combined, the prevalence of elevated cholinesterase activity more than 2.5 times the mean in normal individuals is approximately 2 per 2,600. The presence of this unusual characteristic in three members of the propositus's family supports the contention that the level is familial and not acquired. The family distribution of the increased cholinesterase and the additional electrophoretic band suggest that inheritance is autosomal dominant and that each member with increased activity is heterozygous for the phenotype, but X-linked dominant inheritance cannot be ruled out.

The elevated cholinesterase activities found in this family may be explained by increased enzyme production, slower catabolism of the enzyme, or a molecular alteration that resulted in a more active enzyme. The response of the propositus's cholinesterase to DFP suggests that the last hypothe-

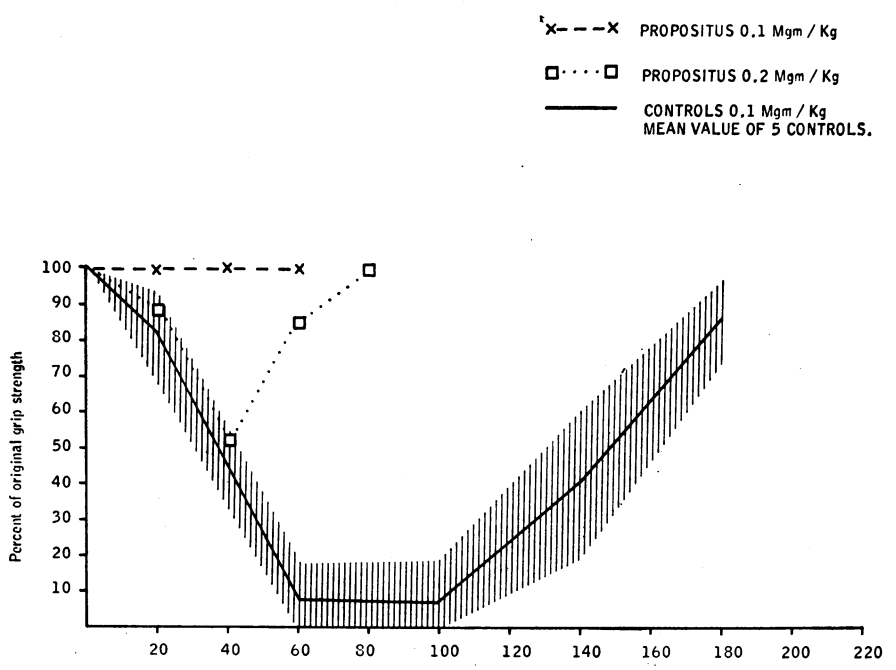

FIG. 6. MUSCLE STRENGTH AFTER IV SUCCINYLCHOLINE. The shaded area represents 1 SE on each side of the line. The propositus is relatively resistant to succinylcholine.

sis is correct. DFP is a potent anticholinesterase. It has been reported to inhibit the usual cholinesterase and the atypical enzyme described by Kalow and Davies in a 1:1 ratio (10). Therefore, the propositus's cholinesterase would be unlikely to be more sensitive to DFP than the usual cholinesterase. The more probable explanation for the results depicted in Figure 3 is that the propositus has a normal number of cholinesterase molecules, but some enzyme molecules are three to four times more active than usual. This would account for the similar dose of DFP required to inhibit completely the cholinesterase activity of the propositus and the controls. Also, this theory would explain the results in which the per cent of cholinesterase activity inhibited and the per cent regenerated after DFP were similar in the controls and the propositus despite very different enzyme activities.

The electrophoretic patterns of plasma cholinesterase further substantiate the hypothesis that the propositus has an additional enzyme that is different from the usual cholinesterase. The extra band that the propositus and affected family members possess migrated more slowly than the C_4 band. Using different electrophoretic techniques, Harris and associates described a band that also migrated more slowly than C_4, which he named C_5. This band was reported to be present in 10% of a random sample of the British population (15). However, the cholinesterase activity found in individuals with this phenotype, although slightly higher than usual, was much lower than for the subjects of the present report. Also, these individuals were as sensitive to succinylcholine as those with the usual cholinesterase genotype.

There are numerous examples to illustrate the relationship between genetic factors and the action of drugs (25). Drugs may exacerbate several inherited diseases. For example, an attack of acute intermittent porphyria may follow the ingestion of barbiturates. Drugs may detect genetic polymorphisms. Primaquine sensitivity is due to inherited glucose-6-phosphate dehydrogenase deficiency. The ability to taste phenylthiourea is genetically controlled, and this may be related to various thyroid disorders.

The plasma cholinesterase polymorphism was discovered after several surgical patients were found to be unusually sensitive to the muscle relaxant, succinylcholine. Enzyme kinetics and family studies suggested that their phenotype differed from the usual one for cholinesterase. The propositus of the present report probably is a heterozygote, with one allele leading to the production of a more active enzyme. The latter enzyme seems to provide increased resistance to succinylcholine.

Summary

Plasma cholinesterase activity has been determined in 1,029 men. Values ranged from 92 to 1,278 U with a mean of 391 U. One subject was found to have a consistently elevated plasma cholinesterase activity. Examination of his family revealed that this cholinesterase was probably inherited as an autosomal dominant. *In vitro* and *in vivo* studies suggest that the propositus is heterozygous for an enzyme molecule that is more than three times as active as the usual cholinesterase.

Acknowledgments

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ERRATUM

In the paper entitled, "Gastric Secretion in Relation to Mucosal Blood Flow Studied by a Clearance Technic," by Eugene D. Jacobson, Ray H. Linford, and Morton I. Grossman, published in the January issue, line 10 in the second column of page 11 is in error. Beginning with line 6, the text should read as follows: "If the extraction ratio for aminopyrine varied greatly with different experimental conditions, then the clearance of aminopyrine would not be a valid method for measuring gastric mucosal blood flow. In acute experiments we found that the gastric extraction of aminopyrine was 58%."