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Complete Pseudocholinesterase Deficiency: Genetic and Immunologic Characterization *

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The plasma enzyme pseudocholinesterase (acylcholine acyl hydrolase) is composed of a series of related glycoproteins that comprise no more than 0.01% of the total plasma proteins (1). Its biological function in normal metabolism is not fully understood (2). Pseudocholinesterase is essential for the rapid breakdown of the muscle relaxant suxamethonium (succinylcholine), which is frequently used in surgery and electroshock therapy. Thus, if pseudocholinesterase activity is defective or markedly decreased, prolonged muscle relaxation and apnea may follow the administration of the drug (3, 4).

Pseudocholinesterase activity is markedly inhibited by a number of compounds. There are, however, two variant forms of the enzyme, which are characterized by decreased susceptibility to inhibition by the local anesthetic dibucaine (5, 6), on the one hand, and by fluoride (7) on the other. The formation of these enzymes is presumably controlled by allelic mutants of the normal gene, E_1^u, which determines the usual esterase (8, 9). The dibucaine-resistant enzyme is produced by the gene called E_1^a, for which 3 to 4% of the population are heterozygous (10). The other gene E_1^f, associated with fluoride-resistant esterase production, has a much lower frequency. Individuals who are either homozygous for these genes (i.e., E_1^a/E_1^a and E_1^f/E_1^f) or who possess both genes (E_1^a/E_1^f) are susceptible to prolonged apnea after suxamethonium administration.

A fourth gene, probably situated at the pseudocholinesterase locus, is associated with complete absence of enzyme activity in the homozygote and has been referred to as the "silent" gene (E_1^s). Only two homozygotes have been described (11, 12). These otherwise normal subjects had prolonged apnea when given suxamethonium. This report describes a kindred containing two additional individuals who are homozygous for the "silent" gene. Study of these patients and their relatives provided further data of the genetics of the silent gene. New data are presented to suggest that in such rare homozygotes no significant amount of antigenically related cross-reacting material is produced.

Methods

Case reports

Case 1. Patient G. Cl. (II-1; Figure 1) a 53-year-old white male of Irish origin was hospitalized in 1958 for elective repair of a ventral hernia. His parents were not related. Past history revealed hypertension of 3 years' duration, auricular fibrillation, and digitalis therapy for 2 years. Physical examination revealed an obese white male with a blood pressure of 180/110, moderate cardiomegaly, and a grade one apical systolic murmur. Aside from a ventral hernia, no other abnormality was noted.

Laboratory studies showed a hemoglobin of 16.4 g per 100 ml, hematocrit 50%, and leukocytes 6,600 with normal differential count. Urinalysis was normal. Nonprotein nitrogen was 25 mg per 100 ml, and cholesterol was 320 mg per 100 ml. The electrocardiogram showed auricular fibrillation and suggested left ventricular hypertrophy.

Anesthesia preparatory to the hernia repair was induced with intravenous sodium pentothal. Cyclopropane was followed by nitrous oxide, ether, and oxygen. A total of 235 mg of succinylcholine was given intravenously. Muscle relaxation and apnea were prolonged to approximately 3 hours, during which artificial ventilation

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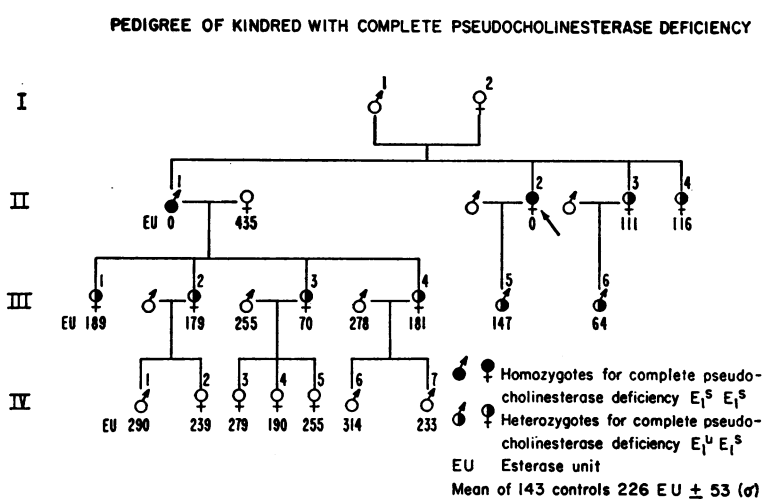

FIG. 1. PEDIGREE OF KINDRED STUDIED. Dibucaine and fluoride numbers were normal in all individuals.

was required. Intermittent hypotension occurred for 24 hours postoperatively and was treated with intravenous metaraminol and with 500 ml of whole blood. The remaining hospital course was uneventful, and several liver function tests before discharge were normal. The patient subsequently expired as the result of a cerebral vascular accident.

Case 2. Patient G. Co. (II-2: Figure 1), a 52-year-old white female and sister of patient II-1, entered the hospital on May 16, 1961, for treatment of an acute traumatic injury to her right hand. Physical examination showed no abnormalities other than injury. Anesthesia was induced with intravenous sodium pentothal, then maintained with cyclopropane. A total of 300 mg of succinylcholine was given intravenously. Muscle relaxation and apnea persisted for approximately 4 hours. During the period of apnea, pulmonary congestion appeared and was treated with 8 mg of lanatoside D intravenously. Her condition gradually improved, all signs of pulmonary congestion disappearing within 24 hours. She was maintained on digoxin for several weeks and has continued in excellent health since.

Serum specimens from these two patients and their relatives were used in the experiments described in this paper.

Pseudocholinesterase activity

Enzymatic activity of serum cholinesterase was measured by the method of Kalow and Lindsay (13) using benzoylcholine as the substrate.

Dibucaine number

Inhibition of enzyme activity was determined by the method of Kalow and Genest (5) where the "dibucaine number" represents the per cent inhibition of enzyme activity.

Fluoride number

Inhibition of enzyme activity was determined by the method of Harris and Whittaker (7) where the "fluoride number" represents the per cent inhibition.

Starch gel electrophoresis

Vertical starch gel electrophoresis was performed for 6 to 18 hours at 4 v per cm at 4° C by the method of Smithies (14) using the discontinuous buffer system of Poulik (15). One-half of the gel was stained for protein with amido black, and the other half stained for esterase activity by the method of Harris, Hopkinson, and Robson (16) using as substrate α-naphthyl or β-naphthyl acetate and, as the diazo salt, fast red TR or diazo blue B.

Preparation of antipseudocholinesterase

Five mg of a human pseudocholinesterase preparation estimated to contain 90% impurities[1] was mixed with Freund's adjuvant and injected in 0.5-ml amounts into the foot pads of two rabbits. One week later a subcutaneous injection of 1 mg of the enzyme preparation in Freund's adjuvant was administered, followed in 2 weeks by a similar injection intraperitoneally and, 1 day later, intravenously.

Immunodiffusion

The technique of double diffusion in agar (17) was employed using human serum as the antigen in the peripheral wells and rabbit antipseudocholinesterase in the central well. The observed precipitin bands were shown to be due to antibodies against human globulins having no esterase activity. Because of its very small content of cholinesterase, it was possible to use human

[1] Obtained from Sigma Chemical Company, St. Louis, Mo.

serum to absorb out the unwanted antiglobulin antibodies from the rabbit serum, which thus retained most of its antipseudocholinesterase activity. The reaction of this antibody with its corresponding antigen was not visible as a precipitin band until the agar plate was stained with α-naphthyl acetate and fast red TR (see above).

Immunoelectrophoresis

With a method similar to that described by Grabar (18) and staining techniques described by Uriel (19), immunoelectrophoresis of normal serum was carried out on 3¼- × 4-inch photographic glass plates, employing 1% Ionagar dissolved in 0.1 M barbital buffer, pH 8.6. After electrophoresis, the linear troughs were first filled with 100 μl of normal serum in dilutions of 1:4 to 1:64. When this diluted serum had diffused into the agar, the troughs were charged with 100 μl of anti-human pseudocholinesterase rabbit serum diluted 1:8, and the plates were incubated at 4° C for 72 hours. Washing in two changes of phosphate buffer pH 7.1 during a 48-hour period was followed by staining for esterase as described above. When the antipseudo-cholinesterase antibodies had been neutralized by the previously added human serum, no enzyme activity was visible. On the other hand, if the diluted human serum contained little or no antigenic material to neutralize the antibody, a brilliantly stained esterase band became apparent.

Results

Pseudocholinesterase levels

The mean serum esterase activity of 142 apparently normal random blood bank donors was 226 U with a SD of ± 53, as shown in Figure 2. Esterase levels determined on the family members are indicated in Figure 1. All members of the kindred had normal dibucaine and fluoride numbers. The index cases II-1 and II-2 had no detectable pseudocholinesterase activity and were therefore considered homozygotes for the "silent" gene. Their five children, III-1 through III-5, obligate heterozygotes for the "silent" gene, had a mean esterase activity of 153 ± 49.2, a value significantly different from the control mean at the 1% level. However, only III-3 had a level

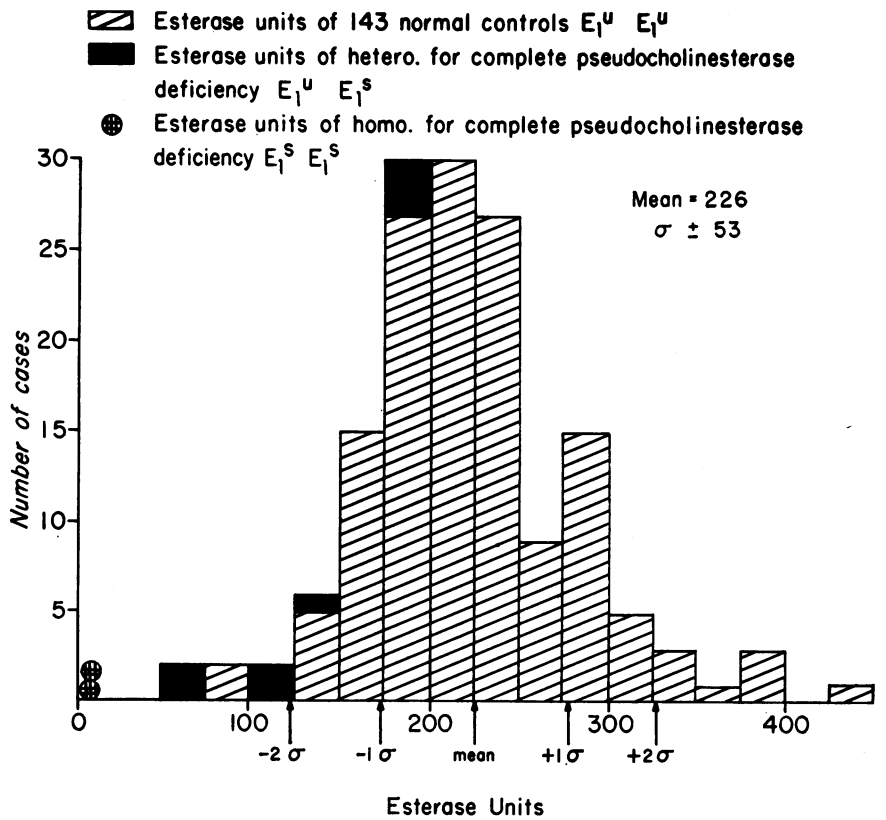

FIG. 2. ESTERASE LEVELS OF 143 BLOOD BANK DONORS WITH NORMAL DIBUCAINE AND FLUORIDE NUMBERS. Heterozygotes for silent gene ($E_1^uE_1^s$) include the five obligatory heterozygotes (III-1 through III-5) plus II-3, II-4, and III-6, who had esterase levels below 2 SD of the mean.

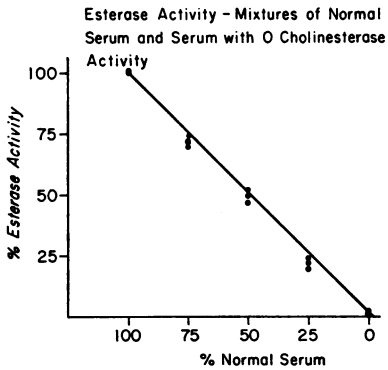

FIG. 3. ESTERASE ACTIVITY AS PER CENT OF TOTAL ACTIVITY OBSERVED WHEN DILUTED WITH SERUM OF INDEX CASES. Note lack of inhibition of normal esterase activity.

deviating by more than 2 SD from the normal mean. Other family members, II-2, II-4, and III-6, had esterase activities below 2 SD of the mean of normals and undoubtedly were also heterozygotes for the "silent" gene.

Effect of mixing serum specimens

In order to determine whether the sera lacking any enzyme activity might contain an inhibitor substance, mixtures of enzyme-deficient serum with normal serum were prepared in the proportions indicated in Figure 3. Absence of an inhibitor is demonstrated by the linear decrease in esterase activity that followed a curve expected from dilution alone. Fluoride and dibucaine numbers were similarly unaffected.

Starch gel electrophoretic pattern

As previously described by Harris and his co-workers (16), DFP (diisopropyl fluorophosphate) inhibitable cholinesterase activity of normal serum appeared in one major and three minor zones on starch gel (Figure 4). Essentially identical patterns were obtained with the commercial pseudocholinesterase preparation as well as with the sera of individuals homozygous and heterozygous for the dibucaine-resistant type of enzyme variant. None of these bands was demonstrable in the sera of the two subjects homozygous for the "silent" gene. Three additional zones of esterase activity, previously described by Uriel (19) and Harris and his coworkers (16) in the zones corresponding to α- and β-lipoprotein and albumin were detected in all serum specimens from normal individuals, homozygotes for the atypical allele, E_1^a, and homozygotes for the silent gene. The bands associated with lipoprotein were more clearly visualized when β-naphthyl acetate was utilized as the enzyme substrate.

Immunologic studies

Immunodiffusion. Serum specimens from homozygotes for the silent gene (S) were alternated with normal serum (U) in the peripheral wells of agar immunodiffusion plates, and the central well was filled with rabbit antihuman pseudocholinesterase (AB). Figure 5 shows that the esterase-stained precipitation bands formed with the normal serum (U) as antigen were not deflected from entry into the wells containing enzyme-deficient serum (S). Since the esterase

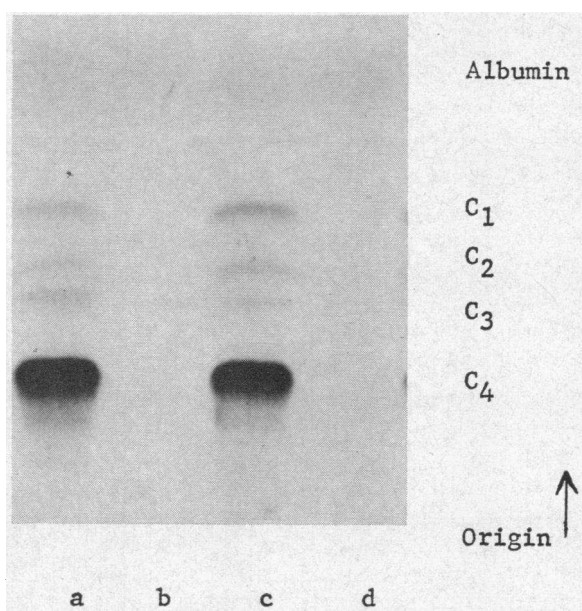

FIG. 4. STARCH GEL ELECTROPHORESIS OF SERUM. Slot c is serum from a normal individual ($E_1^uE_1^u$). C_4 represents the principal isozyme of pseudocholinesterase. This band and three minor pseudocholinesterase bands (C_1–C_3) are normally observed. Slot a: note identical electrophoretic mobility of C_1–C_4 in serum from homozygotes ($E_1^aE_1^a$) for the atypical, presumably structurally mutant, enzyme. Slots b and d are from patients II-2 and II-1, respectively; these homozygotes for the silent allele ($E_1^sE_1^s$) lack all pseudocholinesterase bands. Note that albumin has esterase activity under these experimental conditions. As expected, homozygotes for the silent allele preserve the esterase activity of albumin.

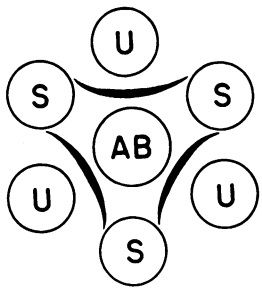

FIG. 5. IMMUNODIFFUSION. (Drawn after photograph.) Normal serum (U) is alternated in the round wells with serum of Patient II-2 (S) who has complete pseudocholinesterase deficiency. Antipseudocholinesterase rabbit serum (AB) is placed in the center well. The arc-like bands represent the enzyme-stained precipitin bands indicating a reaction of normal serum (U) with the anticholinesterase rabbit serum (AB). Lack of deflection of the bands from the wells with patient's serum (S) is consistent with absence of cross-reacting material (CRM) in this serum. If any CRM were present, the precipitin bands would not tend to terminate in the wells containing the sera without pseudocholinesterase activity.

activity localized the protein, this pattern is consistent with the absence of cross-reacting protein without enzymatic activity from the abnormal serum.

No precipitating lines were observed between the wells containing normal and acholinesterasemic serum consistent with the absence of serum antibody against pseudocholinesterase in homozygotes for the silent gene.

"Blocking" experiments. The possible presence of a gene product with neither enzymatic nor antigenically cross-reactive activity, which might block the reaction between cholinesterase and anticholinesterase, was tested in the following manner (20). Antihuman cholinesterase serum was placed in the central well. Human serum with normal pseudocholinesterase activity, to which had been added an equal quantity of serially diluted serum from the patients with no pseudocholinesterase activity, was placed in the peripheral wells. The serum from the patients failed to affect the equal intensity of the resulting precipitin lines. This finding is consistent with the absence of an immunologically related substance blocking the antigen-antibody reaction.

Immunoelectrophoresis

Immuoelectrophoretic experiments were designed to demonstrate the presence in human serum of antibody-neutralizing activity. Normal human serum in dilutions up to 1:8 contained sufficient cholinesterase antigen to neutralize the rabbit antihuman cholinesterase. Similar results were obtained when dilutions of serum from homozygous individuals ($E_1^aE_1^a$) with the most common type of mutant enzyme were used to precharge the troughs. Thus it appears that although the enzyme in such individuals is structurally altered, it reacts with the antinormal cholinesterase and is present in normal quantities. Other kinds of studies on the nature of the mutant (21, 22) have provided similar evidence. In an identical experiment, serum (from II-2) with-

TABLE I

Pseudocholinesterase E_1 mutants

Genotype	Phenotype	Esterase level Relative %	Dibucaine number	Fluoride number	Frequency*
		Normal response to suxamethonium			
$E_1^uE_1^u$	U	100	71–83	57–88	96%–97%
$E_1^uE_1^a$	I	78	52–69	42–55	3%– 4%
$E_1^uE_1^s$	U	65	71–83	57–68	~1/150
$E_1^uE_1^f$	UF	80	71–78	50–55	?
		Prolonged response to suxamethonium			
$E_1^aE_1^a$	A	25	15–25	20–25	~1/3,000
$E_1^sE_1^s$	S	0			1/40,000-1/160,000
$E_1^fE_1^f$	F	50	64–67	34–35	Very rare
$E_1^aE_1^s$	A	20	15–25	20–25	~1/8,000
$E_1^aE_1^f$	IF	60	47–53	41–39	?
$E_1^fE_1^s$	F		Not described yet		

* These frequencies are based on actual observation for the $E_1^aE_1^u$ genotype and have been calculated for the others as discussed by Simpson and Kalow (8) and Motulsky (9).

out pseudocholinesterase activity was substituted for normal serum. Even when this specimen was used undiluted, there was no evidence of antibody neutralization, again indicating the absence of any demonstrable cross-reacting material.

Discussion

The complete absence of pseudocholinesterase activity from the sera of these two patients and of the other two reported cases (11, 12) was not associated with any detectable impairment of health. Liver tissue obtained by biopsy of one patient contained no detectable enzyme (12). Since that organ is the site of pseudocholinesterase production, the total lack of enzyme in individuals homozygous for the silent allele is confirmed. The enzyme is therefore completely dispensable, and its normal physiological role, if any, can be compensated by other systems.

Two variants of pseudocholinesterase (the dibucaine and the fluoride-resistant enzyme) have been previously well characterized (4) and their heredity studied. Family data fit the hypothesis that both variants are determined by mutant genes (E_1^a and E_1^f) allelic to the gene determining the normal enzyme (E_1^u) (4, 6, 23, 24). Table I presents all of the known phenotypes and genotypes of the pseudocholinesterase system along with their associated laboratory findings and population frequencies. Individuals sensitive to suxamethonium are either homozygous or mixed heterozygous for the mutant genes E_1^a and E_1^f or the "silent" gene. All presently available pedigree data and considerations based on population genetics suggest that the "silent" gene is an allele of the normal esterase gene and its two allelic mutants (8, 9, 11, 23). The kindred presented here provides further evidence against the alternative hypothesis (8) that the gene determining complete absence of pseudocholinesterase is nonallelic but specifically suppresses the E_1^a gene rather than the E_1^u gene. In that case the genotype of silent gene homozygotes would be E_1^a/E_1^a, ss and its frequency rare. Silent gene heterozygotes would be of genotype E_1^a/E_1^u, Ss. One quarter of the children of matings from such heterozygotes with normals (E_1^a/E_1^uSs × E_1^u/E_1^uSS) would be expected to be of genotype E_1^a/E_1^u,SS and have the characteristics of the atypical heterozygote with intermediate dibucaine resistance. Among 14 offspring of such matings [one reported by Harris, Whittaker, Lehmann, and Silk (23), one by Liddell, Lehmann, and Silk (11), five by Simpson and Kalow (8), and seven (i.e., IV-1–IV-7) in the present paper], no individual of this type was found. The probability of not finding such individuals under the nonallelic hypothesis is $(3/4)^{14} = 2\%$. Although more offspring from the critical matings are required to disprove this more remote hypothesis, allelism of the silent gene with the E_1^u and E_1^a gene is the most likely possibility, both statistically and biologically. The silent gene is therefore designated in Table I as E_1^s.

Liddell and associates (4, 11) demonstrated inhibition of normal pseudocholinesterase activity by serum from their patient with complete pseudocholinesterase deficiency. These findings are unlike those shown in Figure 3 where no inhibition of normal pseudocholinesterase could be shown. This discrepancy raises the problem of a different type of mutation or possibly of antibody production against pseudocholinesterase by transfusion in Liddell and associates' patient (4, 11). Such antibodies might inhibit normal pseudocholinesterase activity. No antipseudocholinesterase antibodies could be demonstrated in our two sibs with complete pseudocholinesterase deficiency.

A number of genetic systems with enzyme deficiency have been studied with immunological methods. In *Neurospora* as well as in *Escherichia coli,* there are many mutants with no tryptophane synthetase enzymatic activity. These have been clarified on the basis of serological cross-reactivity with the wild type enzyme as cross-reacting material (CRM) positive or CRM negative (25, 26).

In studies on human subjects, no CRM was detected in muscle phosphorylase deficiency (27) and in the Japanese type of acatalasemia (28, 29). The results of a very recent investigation suggested that in the Swiss type of acatalasemia, trace amounts of catalase with the same physicochemical properties as normal catalase could be demonstrated (30). In the case of glucose-6-phosphate dehydrogenase deficiency, Marks and Tsutsui (31) have shown that the enzyme isolated from the red cells of subjects with the two major variants have immunological identity with the normal enzyme. The immunological studies described in this paper indicate that individuals

with an inherited complete absence of serum pseudocholinesterase do not produce an antigenically similar protein detectable by the methods used.

A variety of genetic mechanisms could lead to the present findings. Considering that the silent gene mutation is allelic, an operator negative mutation (32–34) at the pseudocholinesterase locus is an attractive hypothesis. However, at the present time, proof for this type of mutation does not exist yet in mammalian systems.

Structural gene mutation affecting directly or indirectly both antigenic and enzymatic "active" sites would also lead to complete absence of enzyme activity. The problem is similar to that presented by other human mutations causing absence of gene product. A variety of genetic mechanisms including structural mutations can lead to the absence of a protein or enzyme. At our present state of knowledge, a definite conclusion regarding the specific nature of the mutation is not yet possible in such cases.

The mean pseudocholinesterase activity of five obligatory heterozygotes was 67% of normal in our study and 71% of normal in six obligatory heterozygotes of Simpson and Kalow (8). Similar observations were made by Harris and his coworkers (35). It appears that the control of pseudocholinesterase activity, as that of other enzymes (36), is exerted on the genic level and not by feedback repression (37), so that individuals with only one mutant gene for pseudocholinesterase have less enzyme activity than normals. However, these individuals, unlike most heterozygotes in other systems, produce two-thirds, rather than one-half, of the enzyme activity of the normal homozygote.

Summary

Two sibs with complete absence of pseudocholinesterase activity were discovered in an American-Irish family. In both instances, prolonged apnea following succinylcholine administration was observed, but no other pathologic effect of the enzyme deficiency was apparent.

The family data were compatible with the hypothesis that "a pseudocholinesterasemia" represents the homozygous state for a gene that determines complete absence of pseudocholinesterase activity. Genetic considerations from this and previously reported families indicate that this gene is allelic with the two more frequently observed structural gene mutations affecting pseudocholinesterase.

Starch gel electrophoresis of serum from the index cases showed absence of the four isozyme bands associated with normal pseudocholinesterase activity. The electrophoretic pattern of these isozymes in subjects with the common structural ("dibucaine resistant") mutation of this enzyme was indistinguishable from normal.

Heterozygotes for the rare "silent" allele had significantly less but more than half of normal cholinesterase activity.

Serum with complete absence of pseudocholinesterase activity failed to inhibit normal pseudocholinesterase activity.

Potential gene products in the sera with no enzyme activity were tested by immunologic methods. No cross-reacting or blocking material could be demonstrated by immunodiffusion and immunoelectrophoretic techniques. Although these findings would be expected with an operator type of mutation, certain structural mutations could lead to similar results.

Acknowledgments

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