A Second Heterozygote for 'Silent' and 'Fluoride Resistant' Genes for Serum Cholinesterase

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Genetically controlled variation is well established for cholinesterase in human serum, now known as acetylcholine acetylhydrolyase (A.C.A.H., Enzyme Commission No. 3.1.1.8). Stimulus for investigation of variants of the enzyme arose from the observation that one genetic variant designated as atypical was responsible for prolonged apnoea following a standard dose of the muscle relaxant, succinylcholine (Kalow and Staron, 1957). Details of classification of phenotypes and genotypes for variants of cholinesterase and their relation to succinylcholine-sensitivity are summarized elsewhere (Motulsky, 1964; Lehmann and Liddell, 1964; Simpson and Kalow, 1966).

The atypical variant of the enzyme is recognized by a low percentage inhibition of activity by dibucaine (known as dibucaine number or DN) when benzoylcholine is used as substrate compared to high inhibition for the usual type. The two forms of enzyme are controlled by two allelic genes known as \( E_1^a \) (for the atypical) and \( E_1^u \) (for the usual variant). Heterozygotes \( (E_1^a E_1^u) \) are recognized by an intermediate DN.

Subsequently, Liddell, Lehmann, and Silk (1962) and Hodgkin, Giblett, Levine, Bauer, and Motulsky (1965) described serum with no enzyme activity towards benzoylcholine, the gene for which the former authors named 'silent' \( (E_1^s) \). Goedde, Gehring, and Hofmann (1965a, b), however, have evidence for minimal activity with benzoylcholine from sera from two subjects who were thought to be homozygous for the 'silent' gene. There is evidence for allelism of the \( E_1^s \) gene with \( E_1^a \) and \( E_1^u \) genes (Simpson and Kalow, 1964).

Differential inhibition by sodium fluoride combined with dibucaine inhibition has distinguished a third variant of the enzyme known as 'fluoride resistant' resulting from a gene known as \( E_1^f \), with evidence that it is allelic to \( E_1^a \) and \( E_1^u \) (Harris and Whittaker, 1961, 1962). As Whittaker (1967) states, evidence for allelism of the \( E_1^f \) gene has been meagre because of its rarity and complicated enzyme kinetics rendering its recognition open to errors.

In addition to enzyme variation controlled by the four alleles at the \( E_1 \) locus, Harris, Robson, Glen-Bott, and Thornton (1963b) have described a variant controlled by a dominant gene at a second locus known as \( E_2 \) and recognized by differences in migration during starch gel electrophoresis (Harris, Hopkinson, and Robson, 1962). The phenotypes at the \( E_2 \) locus are known as \( C5^- \) for the commonly occurring type and \( C5^+ \) for the dominant variant, and are not related to succinylcholine-sensitivity. Other rare electrophoretic variants have been reported (Neitlich, 1966; Ashton and Simpson, 1966).

The description of the following family ascertained through an \( E_1^f E_1^s \) propositus, who had had prolonged apnoea after a standard dose of succinylcholine, is reported here as a second observation of this rare heterozygote (the first is described by Whittaker, 1967) and as additional evidence that the \( E_1^f \) gene is at the \( E_1 \) locus.

Methods and Material

Cholinesterase activity was measured spectrophotometrically using benzoylcholine as substrate under standard conditions at 26°C. (Kalow and Lindsay, 1955) and expressed for convenience as micromoles of acetylcholine hydrolysed by one ml. serum at 37°C during one hour. Percentage inhibition of activity by dibucaine (DN) and sodium fluoride (FN) were determined under conditions described by Kalow and Genest (1957) and Harris and Whittaker (1961). Classification of phenotypes at \( E_1 \) locus were made according to Table I in a paper by Simpson (1966). All determinations were made at least in duplicate.

Classification of phenotype at the \( E_2 \) locus was made after one-dimensional vertical starch gel electrophoresis at pH 5.3, using a discontinuous buffer system as described by Harris, Hopkinson, Robson, and Whittaker (1963a).

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The proposita was a 40-year-old woman born in Greece who had 20 minutes' apnoea after 30 mg. succinylcholine. Sera from other available members of her family who are now living in Canada were studied.

Results

The propositus was classified as F phenotype with a mean DN of 69 and a mean FN of 34 from four determinations. A pedigree of the family (Fig.) shows the genotypes and phenotypes of the family members. Table I gives means for two to four determinations of cholinesterase activity, DN, and FN, as well as classification of phenotypes at both loci and genotypes at the E1 locus for each of the family members who were tested. Three offspring (III. 5, 7, and 8) of the propositus (II. 7) by her first husband (II. 6) were UF phenotype, and her daughter (III. 6) was classified as U phenotype. Assuming allelism, the mother or propositus was classified as an E1+/E1+ heterozygote to explain the UF and U phenotypes in her children, and the daughter who was U phenotype was classified as an E1−/E1+ heterozygote. Fortuitously, the propositus remarried a man (II. 8) who was heterozygous for the atypical and usual genes (E1−/E1+) and their son (III. 9) was atypical phenotype which was classified as E1−/E1− genotype. A brother (II. 2) of the propositus was usual phenotype and a sister (II. 10) was IF phenotype (heterozygous for E1− and E1+) genes; thus all four alleles E1−, E1+, E1−, and E1+ occurred in the sibship of the propositus. Sera from the two parents (I. 1 and I. 2) were unfortunately not available for testing.

In addition, the C5+ variant was observed in the second husband (II. 8) of the propositus and his son (III. 9). All other members of the family were C5−.  

Discussion

The distinction of UF (E1+E1+) heterozygotes from U (E1−E1−) homozygotes and E1−E1− heterozygotes is not always satisfactory. For 39 UF phenotypes in this laboratory the mean DN is 75 and the mean FN is 44 (Table II). These means are derived from inhibition data for UF phenotypes for which there is also genetic evidence for the UF phenotype such as an IF or F parent or an individual who has a parent and child who are unequivocally heterozygous for the E1− gene. The upper ranges

![Fig. Segregation of four alleles at the E1 locus for serum cholinesterase in a single family. Transmission of the C5 variant at the E2 locus for serum cholinesterase occurs from II. 8 to III. 9.](image-url)
of DN (79) and FN (51) for UF phenotypes, however, overlap with the lower ranges for U phenotypes which are taken from Canadian families in which there is no genetic evidence that an \( E_1t \) gene is in the family (N. E. Simpson, 1967, unpublished data). The lower ranges of 71 for DN and 38 for FN overlap with those for I phenotype in Canadian families in which there is distinct evidence for the \( E_1a \) gene in the family. Classification of F phenotype is distinct from other phenotypes.

Classification of III. 6 as usual phenotype and III. 5, 7, and 8 as UF phenotypes, however, is unequivocal from inhibition data, and the phenotypes of the offspring may be explained if \( E_1E_1 \) is the genotype of the propositus. In addition, DN sharply distinguishes the atypical phenotype in the son (III. 9) by her second husband who was intermediate phenotype, confirming that the propositus has an \( E_1t \) gene. Atypicals who are \( E_1E_1 \) heterozygotes, such as the son (III. 9), are well known, resulting from \( E_1E_1 \times E_1E_1 \) matings or as parents of children with usual esterase (Kalow, 1959; Harris, Whittaker, Lehmann, and Silk, 1960; Liddell et al., 1962; Simpson and Kalow, 1964; Szeinberg, Pipano, Osterfeld, and Evtatar, 1966; Dietz, Lubran, and Rubinstein, 1965; Thompson and Whittaker, 1966).

Allelism of the \( E_1t \) gene to the \( E_1a \) and \( E_1u \) genes was suggested by Harris and Whittaker (1962) based on data from two IF \( \times \) U (\( E_1E_1 \times E_1E_1 \)) matings; one type of mating which is critical for testing the hypothesis. Table III shows that the ratio of \( 17 \) UF (\( E_1E_1 \)) : \( 17 \) I (\( E_1E_1 \)) offspring has now been observed by pooling published data and data from this laboratory from \( 11 \) IF \( \times \) U matings (Harris and Whittaker, 1962; Liddell, Lehmann, and Davies, 1963; Lehmann and Liddell, 1964; Whittaker, 1967; N. E. Simpson, 1967, unpublished data). The offspring of the sister (II. 10) of the propositus who is phenotype IF are included in Table III. Additional evidence for allelism comes from the observation of five F homozygotes (Liddell et al., 1963; Whittaker, 1964; Simpson and Kalow, 1965; Griffiths, Davies, and Lehmann, 1966).

Since \( E_1t \) was established as an allele in this system (Liddell et al., 1962; Simpson and Kalow, 1964), the \( E_1E_1 \) genotype was predicted and direct observations of the phenotype, one by Whittaker (1967) and the present one, are additional confirmation of the allelic hypothesis.

The genotypes of generation II in the present family led us to conclude that one parent was \( E_1E_1 \) and the other \( E_1E_1 \) genotype, assuming that the allelic hypothesis is correct. Unfortunately, their sera were not available to confirm the prediction.

The propositus had moderate apnoea (20 minutes) after 30 mg. succinylcholine, which is the type of reaction which Lehmann and Liddell (1964) predicted for the genotype. Whittaker's (1967) example of \( E_1E_1 \) genotype was not selected by the drug reaction and hence her succinylcholine-sensitivity is not known.

**Summary**

A family is described in which six genes for serum cholinesterase occur.

The propositus is the second person reported who is heterozygous for the silent \( E_1t \) and fluoride-resistant \( E_1t \) genes, and the first reported to have prolonged apnoea following a standard dose of succinylcholine.

Difficulties in classification of the fluoride types are discussed.

The genotype of the propositus along with other pertinent data are discussed as evidence for the fluoride-resistant gene being allelic to the usual, atypical, and silent genes for serum cholinesterase.

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