**199. A Consanguineous Kindred of Serum
Pseudocholinesterase Anomaly
found in Japan**

By Susumu SHIBATA, Iwao IUCHI, Yasuharu HASHIMOTO,
and Takaaki SHODA

Department of Medicine, the Yamaguchi University School of Medicine, Ube,
and the Kawasaki Institute for Tumor Research, Okayama

(Comm. by Taku KOMAI, M.J.A., Nov. 13, 1967)

About fifteen years ago, when suxamethonium was first introduced into anesthesiology as a short-acting muscle relaxant, several subjects were found who had a markedly low level of serum PsChE activity as a congenital defect. These individuals were highly sensitive to the drug, and fell into apnea that persisted for two or three hours instead of the usual two minutes. Such cases were called suxamethonium hypersensitivity, and put under intensive study from the pharmacogenetic standpoint in England, Canada, and the United States.^{1), 2)}

The activity of PsChE hydrolyzing benzoylcholine (5×10^{-5} M) is inhibited by dibucaine (10^{-5} M) and fluoride (5×10^{-5} M). The rate at which the enzyme is inactivated by one of these inhibitors is expressed in terms of its original activity and is referred to as D value for dibucaine and F value for fluoride. In hereditary anomalies of PsChE an abnormal enzyme of low activity resistant to dibucaine inhibition with unusually small D³⁾ or an abnormal enzyme resistant to fluoride with small F⁴⁾ is detected. Rarely cases of low enzymatic activity without apparent abnormality of these values⁵⁾ are encountered.

According to the current genetic interpretation the normal subjects are homozygous for the normal PsChE gene (E_1^u/E_1^u), the subjects of hereditary anomalies of PsChE are homozygous or heterozygous either for the gene controlling dibucaine-resistant enzyme (E_1^a), or for the gene controlling fluoride-resistant enzyme (E_1^f). The characteristic features of the PsChE anomaly may be classified as follows.^{1), 2)}

(1) Type of dibucaine-resistant enzyme (D). The homozygous individual (E_1^a/E_1^a) is hypersensitive to suxamethonium.

(2) Type of fluoride-resistant enzyme (F). The homozygote

This investigation was supported in part by a PHS research grant (GM 09469-05) from the Division of General Medical Sciences, U.S. Public Health Service.

(E_1^f/E_1^f) is sensitive to suxamethonium to some extent.

(3) Type of pseudocholinesterase (PsChE) suppressing gene⁵⁾ enzyme (S). The serum PsChE activity of the homozygote E_1^s/E_1^s is zero⁶⁾ or around 3 percent level of that in normal subjects;⁷⁾ his D and F numbers are unknown. The heterozygote E_1^u/E_1^s has a serum enzyme activity subnormal or low-normal with normal D and F values. However, the double heterozygotes for the PsChE suppressing gene and the abnormal enzyme, namely E_1^s/E_1^a and E_1^s/E_1^f , are hypersensitive or sensitive to suxamethonium, because both their serum PsChE activity and D and F are about equal to those of the corresponding homozygotes of the abnormal enzymes, namely E_1^a/E_1^a and E_1^f/E_1^f . Most previous genetic investigators on PsChE anomalies^{1), 2), 8)} favor the view that E_1^a , E_1^f , and E_1^s are the mutants of the autosomal gene E_1^u , and are mutually allelic.

For these ten years we have been looking for the cases of hypopseudocholinesterasemia which would cause suxamethonium hypersensitivity. Our effort had been fruitless until recently when, we happened to find an extremely low level of serum PsChE activity in a male patient, aged 62, who was admitted to the Kawasaki Hospital, Okayama, due to hypertension. His serum PsChE activity remained persistently almost zero, around 2 percent level of the normal range, throughout the seven examinations carried out for the period of observations extending over a year and 3 months. His hepatic tests except PsChE were all within the normal range, and he had not any neoplastic disease. He had a son and a daughter. To our surprise, both of them had equally marked hypopseudocholinesterasemia. Thus, pseudocholinesterase anomaly was suspected, and an extensive kindred study was conducted, employing Kalow-Lindsay's ultraviolet spectrophotometry (with benzoylcholine, 5×10^{-5} M, as substrate),⁹⁾ Kalow-Genest's method (dibucaine, 10^{-5} M, as inhibitor)¹⁰⁾ and Harris-Whittaker's procedure (fluoride, 5×10^{-5} M, as inhibitor)⁴⁾ for the estimation of serum PsChE activity, and D and F values.

The result of the study is presented in Fig. 1. Altogether fifteen subjects with subnormal serum PsChE level are included in the densely consanguineous pedigree, which may be divided into three branches, a, b, and c, characterized as follows:—

Branch a: —All members have normal D and F values. So, the hypocholinesterasemics may be heterozygous for the PsChE suppressing gene.

Branch b: —Two (IV_7 and IV_8) of the three mild hypocholinesterasemics of this branch have the normal (or low-normal) D and

F values. So, they are probably heterozygous for the PsChE suppressing gene. The remaining hypocholinesterasemic (IV₆) has a moderately small F value and a normal D value, and seems to be a double heterozygote for the PsChE suppressing gene and fluoride resistant enzyme gene.

Branch c: —All the individuals have hypopseudocholinesterasemia. They may be classified into three categories.

i) Mild hypocholinesterasemics with normal D and F values (IV₃, IV₅, V₆, and VI₅), and apparently heterozygous for the PsChE suppressing gene.

ii) Severe hypocholinesterasemics with normal D and F values (V₄ and VI₄). Their serum PsChE activity is extremely decreased, being much lower than that in the heterozygotes (IV₂, IV₇, IV₈, V₂, V₃, and VI₅). Accordingly, they are thought to be homozygous for the PsChE suppressing gene, although their serum PsChE level is not zero.

iii) Severe hypocholinesterasemics with a distinctly small F and a relatively small D (the propositus IV₄ and his daughter V₅). They are probably homozygous for the PsChE suppressing gene, because their serum PsChE level is almost zero. Moreover, they are heterozygous for E_1^f . The distinctly small F value and comparatively small D value in them lead us to conceive of the possibility of heterozygosity for the fluoride resistant enzyme gene under the influence of the homozygosity of the PsChE suppressing gene. If they were doubly heterozygous for the PsChE suppressing gene and E_1^a , their D value should be considerably smaller than those actually found, which is one-third of the normal value. If they were doubly heterozygous for the PsChE suppressing gene and E_1^f , their serum PsChE level should not be so low. If they were doubly heterozygous for E_1^a and E_1^f , the propositus (IV₄) and his wife (IV₅) who is heterozygous for the PsChE suppressing gene, would not have the son (V₄), who is apparently heterozygous for the PsChE suppressing gene. (The paternity and maternity relationships were checked by the examination of the ABO blood group trait.)

In Fig. 2 is summarized the probable genetic relationships of the subjects of PsChE anomalies in this pedigree which has been inferred from the above reasonings. However, there is an important problem to be solved before this interpretation is accepted. This concerns with the presence of the individuals (the propositus IV₄ and his daughter V₅) who are assumed to be homozygous for the PsChE suppressing gene and heterozygous for E_1^f . Such subjects seem to be incompatible with the current genetic conception of

PsChE anomaly, which postulates the allelism of the PsChE suppressing gene and E_1^u , E_1^a , and E_1^f genes.^{1),2)}

About ten instances of apparent homozygotes of the PsChE suppressing gene have been recorded thus far.⁶⁾ In most of them the serum PsChE activity was found to be zero, so that the determination of D and F values was not feasible. However, according to Goedde,⁷⁾ the enzyme activity is not entirely zero, but was around 3 percent level of the normal serum PsChE activity when measured by the micromanometric method with benzoylcholine as substrate. As a matter of fact, in the individuals who were suspected to be homozygous for the PsChE suppressing gene in our kindred (IV₄, V₄, V₅, and VI₄), the serum PsChE activity was considerably decreased, but the tendency had not gone to the extreme, and the measurement of D and F was feasible with a certain degree of precision, if the incubation was extended for a period ten times as long as the originally specified time. If the complete absence of serum enzyme activity were prerequisite to the homozygosity of PsChE suppressing gene which is assumed to be allelic to E_1^u , the individuals supposedly homozygous for the PsChE suppressing gene in our pedigree would not be of that category. There may be different kinds of PsChE suppressing gene, and it is conceivable that the one found in our kindred might be a new PsChE suppressing gene which is not allelic to E_1^u , in contrast to the classical PsChE suppressing gene which has been thought to be at the same locus with E_1^u , E_1^a , and E_1^f .

A few years ago Simpson and Kalow (1964)⁸⁾ conceived a hypothesis assuming that the PsChE suppressing gene might be a suppressor of the synthesis of PsChE, to be denoted as *s*, which is a mutant of the normal gene, *S*. This idea is comparable with the modern conception on the genetics of hemoglobin,¹¹⁾ in which a controller gene regulating the biosynthesis and a structural gene for determining the molecular structure (amino acid sequences) are assumed. With respect to PsChE, *S* and *s* may be a normal and an abnormal controller genes, while E_1^u , E_1^a , and E_1^f are normal and abnormal structural genes for the enzyme that occupy a locus different from that of *S* and *s*. This hypothesis has been abandoned, because the authors could not get any concrete material to substantiate it from their family study. However, this hypothesis conforms with the contemporary knowledge on the molecular structure of PsChE²⁾ better than the conception which assumes the allelism of all the E_1^u , E_1^a , E_1^f , and E_1^s (the PsChE suppressing gene). The molecule of PsChE has two active sites, the anionic and the esteratic. Dibucaine and fluoride are thought to block the activity of the enzyme through

Fig. 1. The pedigree. Numerals of each individual represent serum PsChE activity (top), D number (center) and F number (bottom). For instance, the subject IV₁ has serum PsChE activity of 45.0 units (Kalow-Lindsay), D number of 86.5% and F number of 54.8%. The underlined numerals show abnormal values. Arrow indicates the probitus.

Fig. 2. Genetic interpretation of the pedigree.
 Arrow indicates the probitus.

competitive acquisition of these sites with the substrate. There are evidences supporting the presumption that in the abnormal enzymes the active sites may be altered in a manner that substrate and inhibitors are not easily received.²⁾ If so, the dibucaine-resistant and fluoride-resistant enzymes possessing abnormal molecular structures may be the product of the aberrant structural genes E_1^a and E_1^f . In contrast, the idea that the PsChE suppressing gene E_1^s is a kind of structural gene does not seem to be convincing, unless a completely altered cistron resulting in the production of an enzyme lacking the proper activity is assumed for it. Such a completely altered cistron may not be impossible, and the cases of the PsChE suppressing gene homozygotes hitherto recorded are to be accounted for on this view point. However, it seems evident that the homozygotes for the PsChE suppressing gene in our family are different from these previous cases. The new PsChE suppressing gene can hardly be another allele of E_1^s ; instead, it seems to be a controller gene. Accordingly, it is concluded that the normal subject in this family may be expressed as $S/S, E_1^u/E_1^u$; while the mild hypopseudocholinesterasemics are $S/s, E_1^u/E_1^u$ or $S/s, E_1^u/E_1^f$, and the extreme hypopseudocholinesterasemics, including the propositus, his son, daughter, and grand-son are $s/s, E_1^u/E_1^f$ or $s/s, E_1^u/E_1^u$ (Fig. 2).

References

- 1) Lehmann, H., and Liddell, J.: Pseudocholinesterase deficiency and some other pharmacogenetic disorders. Stanbury, J.B., Wyngaarden, J.B., and Fredrickson, D. S.: *The Metabolic Basis of Inherited Disease* (2nd ed.). Blakiston-McGraw-Hill (New York, Toronto, Sydney, London 1966), pp. 1356-1369.
- 2) —: Genetical variants of human serum pseudocholinesterase. Steinberg, A. G., and Bearn, A. G.: *Progress in Medical Genetics*, vol. 3. Grune and Stratton (New York, London, 1964), pp. 75-105.
- 3) Kalow, W., and Genest, K.: *Canad. J. Biochem. Physiol.*, **35**, 339 (1957).
- 4) Harris, H., and Whittaker, M.: *Nature*, **191**, 496 (1961).
- 5) Liddell, J., Lehmann, H., and Silk, E.: *Nature*, **193**, 561 (1962).
- 6) Kattamis, C., Davies, D., and Lehmann, H.: *Acta Genet (Basel)*, **17**, 299 (1967).
- 7) Goedde, H. W.: Third International Congress of Human Genetics. Chicago, Sept. 5-10, 1966 (Abstract), p. 41.
- 8) Simpson, N. E., and Kalow, W.: *Amer. J. Human Gene.*, **16**, 180 (1964).
- 9) Kalow, W., and Lindsay, H. A.: *Canad. J. Biochem. Physiol.*, **33**, 568 (1955).
- 10) Kalow, W., and Genest, K.: *Canad. J. Biochem. Physiol.*, **35**, 339 (1957).
- 11) Ingram, V. M.: *The Hemoglobins in Genetics and Evolution*. Colombia University Press (New York, London, 1963).