Atypical Cholinesterase Gene E_1^a: Rarity in Negroes and Most Orientals

Abstract. *Development of a rapid screening test for atypical cholinesterase in serum enabled large-scale surveys of populations. The frequency of the heterozygous trait among Greeks, Yugoslavs, and East Indians was similar to that among United States Caucasians (2.8 to 3.3 percent). In trait frequency, U.S. Negroes were intermediate (1.05 percent) between Congolese Africans (0.29 percent) and U.S. Whites (3.3 percent). The gene was absent from or very rare in populations originating in East Asia (Taiwanese, Japanese, Filipinos, and Eskimos). Prolonged apnea during anesthesia from administration of succinylcholine, caused by homozygosity for this gene, is expected to be extremely rare among populations of Negroes and East Asians.*

About 3 to 6 percent of Caucasian populations of Europe and North America are heterozygotes for a gene specifying an atypical cholinesterase in serum (acylcholine acyl hydrolase, 3.1.1.8; I.U.B. Commission on Enzymes) (*1–3*). The abnormal enzyme can be detected because various enzyme inhibitors do not diminish its activity to the same degree as they do that of the normal enzyme (*4*). Homozygotes for the abnormal enzyme may develop prolonged apnea when the frequently used muscle relaxant suxamethonium is administered (*5*). The gene specifying the abnormal enzyme is known as E_1^a; its normal allele, as E_1^u (*6*).

Development of a rapid screening method for the atypical enzyme, based on Harris and Robson's (*7*) agar-diffusion technique, enabled the examination of many serums. In the test, alpha naphthyl acetate is hydrolyzed by cholinesterase; the hydrolytic products form a purple color with 5-chloro-*o*-toluidine. The inhibitor RO2-0683 (dimethyl carbonate of 2-hydroxy-5-phenylbenzyltrimethylammonium bromide) strongly inhibits the normal but only slightly affects the abnormal enzyme.

On the basis of the resultant color differences, serums from normals ($E_1^uE_1^u$) can be visually distinguished in test tubes from those of heterozygotes ($E_1^uE_1^a$) and of abnormal homozygotes ($E_1^aE_1^a$). Details of the test and its validation in the detection of the atypical enzyme are being published (*8*). The test did not identify carriers of the fluoride-resistant allele but proved reliable for detecting heterozygotes and homozygotes for the atypical enzyme. Serums (3882) from various populations were tested by this method (Table 1).

Populations of European origin (Seattle blood donors, Greeks, and Yugoslavs) showed frequencies (2.8 to 3.3 percent) similar to those reported by other investigators using other methods (*1–3*). The frequency of the trait among 98 students from various areas of India was similar to the European frequency—3.1 percent. In contrast, the frequency among 666 American Negroes was distinctly lower (1.05 percent), and it was very low among 460 Congolese Negroes (0.22 percent). The trait was absent from 125 Ituri pygmies. No heterozygotes were encountered during an unpublished study of 300 Babinga pygmies, using the screening employed by us (*9*).

The difference in frequency between Seattle Caucasians and Seattle Negroes was statistically significant (chi-square, 1 d.f., $P < .025 > .01$) and became highly significant when Seattle and Congolese Negroes were pooled (chi-square, 1 d.f., $P < .001 > .0005$). If one assumes a very low frequency among African Negroes, our finding among Seattle Negroes could be explained by Caucasian admixture, which represents about 30 percent of the gene pool of the urban American Negro not living in the Deep South (*10*).

The gene has a still-lower frequency in various East Asian populations: no heterozygotes were found among our 140 Japanese subjects or among 371 other Japanese subjects of another investigation (*3*). Since the abnormality was detected four times among 426 Seattle Oriental blood donors who were largely Japanese, the gene presumably is not completely absent from this population. Taiwanese and Filipinos also yielded low frequencies, and no heterozygotes were detected among 145 Eskimos (Table 1). In another study of subjects from Alaska (*11*), no heterozygotes for the atypical cholinesterase were detected among 122 Eskimos and 141 Athabascan Indians, while one heterozygote was found among 58 Aleuts. Other investigators have reported absence of the abnormality among 700 Thais (*3*) and among 291 South American Indians (*12*). Lisker *et al.* (*13*), however, found a frequency of 1.9 percent among Mexican Indians. A study of 2138 Brazilians (which were estimated to have a gene pool of 48 percent Caucasian, 34 percent Negro, and 18 percent Indian genes) showed a frequency of 2.8 percent of heterozygotes (*2*).

Our results suggest a lower frequency of the atypical pseudocholinesterase gene among Negroes, and further document and extend data on the great rarity of this gene among populations originating in East Asia. The reasons for these racial differences remain obscure.

Our findings have practical significance. Since sensitivity to suxamethonium is often caused by the homozygous state for the abnormal enzyme, prolonged apnea due to this enzyme variant may be expected to be very rare among American Negroes (estimated frequency of the homozygous state, based on our data: $q^2 \sim 1/36{,}000$) and much rarer still in African and Oriental populations.

Table 1. Frequency of heterozygosity for the atypical cholinesterase gene in various populations; references cited describe in more detail populations previously studied for other traits. Abbreviations: BD, blood donors; SC, schoolchildren; AD, adults; HP, hospital patients. All data refer to this study.

Population	Subjects (No.)	Heterozygotes	
		No.	Percentage
Seattle Caucasians (BD)	246	8	3.3
Greeks (*14*)	561	16	2.85
Yugoslavs (*15*)	248	7	2.8
East Indians (*16*)	98	3	3.1
Seattle Negroes (236 SC, 430 BD)	666	7	1.05
Congolese Negroes (*17*)	460	1	0.22
Ituri pygmies (*17*)	125	0	0
Taiwanese (*18*)	340	1	0.29
Seattle Japanese (66 AD, 74 SC)	140	0	0
Seattle Orientals (BD, mostly Japanese)	426	4	0.94
Alaskan Eskimos (HP)	145	0	0
Filipinos (*19*)	427	2	0.47

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Selective Solubilization of a Protein Component of the Red Cell Membrane

Abstract. *Approximately 20 percent of the membrane-bound protein of erythrocyte ghosts can be solubilized and obtained free of other membrane components by dialysis against adenosine triphosphate and 2-mercaptoethanol. This protein forms one major band on polyacrylamide gels and a single boundary in free-boundary electrophoresis, and it undergoes polymerization in the presence of divalent cations to form coiled filaments visible by electron microscopy. Antibodies to this membrane protein react specifically with red blood cells or their membrane ghosts but do not react with serum, erythrocyte cytoplasm, or other blood cells. The functional role of this protein is unknown, but it appears to be involved in maintaining the structure of the red cell membrane. We suggest that this protein be called Spectrin since it is obtained from membrane ghosts.*

Although approximately one-half of the dry mass of most animal cell membranes consists of protein, very little is known about its chemical composition and physical properties. One of the major problems is the difficulty in separating proteins from the lipid and carbohydrate elements of membranes during solubilization without subjecting them to the denaturing effects of organic solvents or detergents.

It has been shown that trypsinized erythrocyte ghost membranes contain a protein or proteins capable of polymerizing into coiled filaments which are visible by electron microscopy (*1*). These filaments can be extracted in a soluble form from the digested membranes by dialysis against adenosine triphosphate (ATP) and reformed in vitro by incubating the extracted material with divalent cations. These findings suggested that part of the membrane protein was bound loosely to other membrane components which might be solubilized by extraction with ATP without treatment of the membranes with trypsin.

Membranes were prepared from guinea pig red blood cells by an osmotic lysis method (*2*). The washed membranes were extracted by dialysis against 0.3 m*M* ATP (*p*H 7.5) and 50 m*M* 2-mercaptoethanol at 4°C for 24 to 48 hours (*3*).

As a result of this treatment, the membranes break up into small fragments. Studies of thin sections by electron microscopy indicate that these fragments of membrane are made up of the usual three-layered structure, but that they differ from the original ghost membranes in two ways: (i) filamentous material normally present along the inner surface of intact membranes (*2*) is not attached to the fragments; and (ii) the edges of the fragments are "free" and do not form closed vesicles.

These fragments form a pellet after centrifugation at 78,000*g* for 2 hours, and the resulting supernatant contains approximately 20 percent of the original membrane-bound protein (*4*). This solubilized protein has a typical protein ultraviolet spectrum with a maximum absorbance at 282 mμ and contains no detectable lipid or carbohydrate moieties (*5*). The material forms one major band and one minor one on polyacrylamide gel electrophoresis in 8*M* urea (Fig. 1). A single boundary forms when the protein is run in free-boundary electrophoresis over a *p*H range of 6.5 to 9.8.

The extracted protein also forms coiled filaments similar in size and configuration to those in ghost membranes treated with trypsin (*1*). The filaments are produced by incubating the extracted protein with 0.5 m*M* ATP and 0.1 to 1.0 m*M* $MgCl_2$ or $CaCl_2$ at 37°C. They can be visualized directly by electron microscopy after negative staining with 2-percent phosphotungstic acid (PTA) (Fig. 2). The filaments measure approximately 40 to 60 Å in diameter and are of variable length.

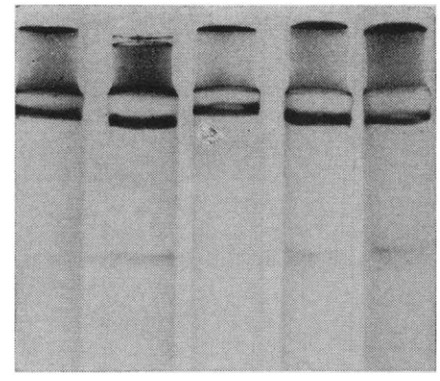

Fig. 1. Electrophoretic patterns of protein from red cell ghosts in 5-percent polyacrylamide gels containing 8*M* urea. The tubes represent protein extracted from five different red cell preparations. Gels were stained for protein with 0.5 percent Amido-Schwartz.

Antibodies to this protein were prepared by immunizing rabbits with the extracted material mixed with Freund's complete adjuvant. The antiserums obtained from these animals form a single precipitin zone with the extracted membrane protein after double diffusion in agar. No precipitin reaction occurs with either guinea pig serum or the red cell hemolyzate. Decomplemented antiserums agglutinate both intact red blood cells and their ghosts. Lysis of such antibody-treated cells occurs after the addition of serum containing active complement. Antiserums which agglu-

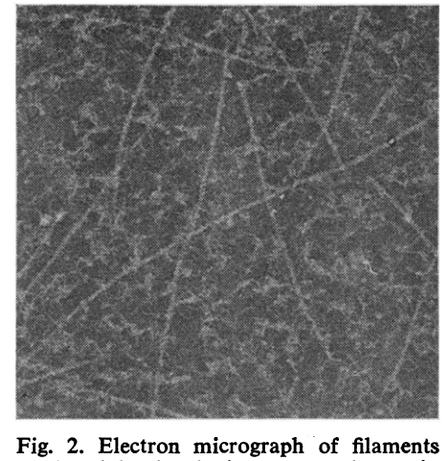

Fig. 2. Electron micrograph of filaments produced by incubating extracted protein with ATP (0.5 m*M*) and $MgCl_2$ (1.0 m*M*) at 37°C for 15 minutes. Preparation negatively stained with 2 percent PTA neutralized to *p*H 7.0 with KOH. The protein not organized in filaments appears as amorphous material in the background. We assume that this amorphous appearance is due to incomplete polymerization of the main component, because the extracted protein has this appearance when negatively stained without previous incubation with divalent cations (× 180,000).