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Polyacrylamide Electrophoresis Used for the Detection of C5+ Cholinesterase in Canadian Caucasians, Indians, and Eskimos

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Harris et al. [1] described a polymorphic variant of cholinesterase (E.C.3.1.1.8) which was recognized by the presence of an additional zone of activity of the enzyme in about 10% of the British population during both one- and two-dimensional starch gel electrophoresis. The mean enzyme activity was greater in sera with the variant. Individuals whose sera have the variant (phenotype C5+) are homozygous or heterozygous for a gene at the locus known as E_2, the second locus discovered which controls serum cholinesterase types in man.

The extra zone of activity was not demonstrable in sera from all persons who were known from family information to have the C5+ phenotype [1]. There were discrepancies and uncertainties in about 5% of 1,000 sera when two similar starch gel methods were compared by Ashton and Simpson [2].

This paper describes a polyacrylamide electrophoretic method for the detection of the variant and demonstrates its greater sensitivity than the starch gel methods.

MATERIALS AND METHODS

Experimental Procedures

Electrophoresis in polyacrylamide gels was performed essentially as described by Davis [3]. Plexiglas gel tubes were 90 mm in length, with an inner diameter of 5 mm. The 2.5% spacer gel (*p*H 6.7) was about 10 mm deep, and the 7% separating gel (*p*H 8.9) was about 70 mm deep. No sample gel was used. Routinely, 5λ of serum or plasma was laid on the spacer gel and covered with about a 6-mm layer of Sephadex G-200. The electrode buffer (*p*H 8.3) was as described by Davis [3], and the tracking dye was bromophenol blue. The current during electrophoresis was maintained at 2 ma per gel tube until the tracking dye front had run to the end of the separating gel, at which time the current was stopped. Running time was about 2 hr. Immediately following electrophoresis, the gels were removed from the gel tubes, washed in distilled water, and stained for esterases with α-naphthyl acetate and fast red TR salt. After staining for about 15 min at room temperature, the gels were rinsed in distilled water and stored in a 7% solution of acetic acid.

Vertical electrophoresis on starch gels of about 15% concentration at *p*H 5.3 was carried out using the discontinuous buffer system described by Harris et al. [1] and the enzyme was again detected using α-naphthyl acetate as substrate and fast red TR salt as the coupling dye.

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Selection of Samples

Sera from nine individuals who were known to be C5+ from the starch gel method were tested using the polyacrylamide method. Sera taken from a group of 258 Cree Indians from Moose Factory, Ontario, and 298 Eskimos from Igloolik, Northwest Territories, were tested by both the acrylamide and starch gel methods. Most of the Indians were unrelated, but there were a few sibs and parent-child groups in the series. The Eskimos were in family groups and their complex relationships will be described elsewhere. In addition, sera from 47 Caucasian Canadians from family groups were typed by both methods. Most of the Caucasian families had originally been ascertained through the atypical gene at the E_1 locus [4].

In order to test whether the acrylamide method was detecting all those who had the gene for the C5+ phenotype, nine families in which both parents were C5− when formerly tested by the starch method but had at least one C5+ child were tested by the acrylamide method. In addition, one mother who was C5− by the starch method and who had C5+ children by two different fathers was tested by the acrylamide method.

RESULTS

On acrylamide gels, the nine sera known to have the C5+ phenotype from previous starch gel electrophoresis had the same appearance as sample 4 in figure 1. They had been stored at −20° C for varying periods of time, and had the four "storage" bands, designated as "S" in figure 1. Fresh C5+ samples had the appearance of sample 2. Bands 1, 2, and 3 were faint in all samples.

Comparison of the Two Methods in the Random Samples

Table 1 shows that with the acrylamide method, 25% more examples of the C5+ phenotype were detected than by the starch method. Only one specimen with the

TABLE 1

COMPARISON OF TWO ELECTROPHORETIC METHODS FOR DETERMINATION OF C5+ CHOLINESTERASE TYPES

Phenotype by Starch Gel Method	Phenotype by Polyacrylamide Gel Method	
	C5−	C5+
C5−	538	16
C5+	1	48

C5+ phenotype was missed by the acrylamide method but detected by the starch method.

Of the 258 Indians tested by both methods, 15 (5.8%) were C5+ by the starch method and 19 (7.4%) by the acrylamide method. Of the 298 Eskimos, 31 (10.4%) were C5+ by the starch method and 39 (13.1%) by the acrylamide method. Since the Indian sample was essentially random, gene frequencies could be directly estimated from the above phenotype frequencies. The Eskimos were interrelated, and gene frequencies could not be accurately determined from the above phenotype frequencies.

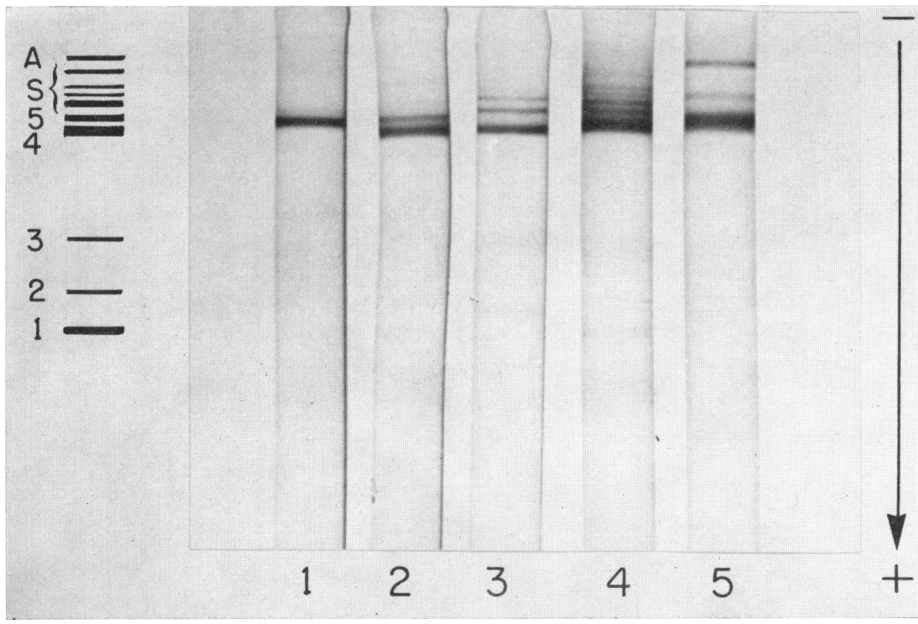

FIG. 1.—Acrylamide gels stained for serum cholinesterase. The diagram on the left illustrates all of the bands seen on the gels, and presence of band 5 indicates the C5+ phenotype. Gel 1 is C5— and gel 2 is C5+ before storage; gel 3 is C5— and gel 4 is C5+ after storage; gel 5 shows the A band which was observed in a few Indian and Eskimo samples (see text).

One variant was observed in a 27-year-old Indian woman. It was characterized by a very much slower band of activity, band A of sample 5 in figure 1. The results were reproduced a number of times on one sample and on sera taken on three different occasions from the woman over a period of 2 years. The variant also occurred in one of her daughters. The variant did not occur in sera from four other children, her husband, sibs, or parents. A similar but slower band was seen in several of the Eskimo samples, but Mendelian ratios were not evident. When the above samples were run on starch gels at both *p*H 5.3 and 8.6, the extra bands were not detectable.

Family Data for Testing the Reliability of the Polyacrylamide Method

By using the family data, it was possible to decide whether all of the C5+ phenotypes were being detected by the polyacrylamide method. Among the 19 parents who were C5— by the starch method and who had C5+ children, at least 10 would be expected to be C5+. Five of them were C5+ by the acrylamide method. Therefore, although the method is more capable than starch gel of detecting the C5+ phenotype (table 1), it cannot detect the phenotype in half of the specimens presumed to be C5+, but incorrectly designated as C5— when tested by the starch gel method.

DISCUSSION

The frequencies of the C5+ and C5— phenotypes in different populations are more variable than those of the cholinesterase variants at the E_1 locus [5]. The

frequencies of C5+ and C5— phenotypes for the Cree Indians and Eskimos in this study are within the range of frequencies for Caucasian, Jewish, Icelandic, Tristan da Cunla Islander, Brazilian, American Negro, Xavante Indian, and Saskatchewan Cree Indian populations previously reviewed [5].

Previous reports of the C5+ variant frequency may be slightly underestimated, since they were based on electrophoresis in starch gel. In our hands, at least, the acrylamide gel technique is 25% more sensitive than starch gel. However, if the hypothesis of dominant inheritance is correct, neither method can detect all of the individuals who carry the variant gene. In the family data, there were 18 families of a total of 28 which fit the dominant hypothesis by having at least one C5+ parent of one or more C5+ children as tested by starch gel electrophoresis. With the acrylamide method, the fit was enhanced by five more families, but there still remained another five with apparent discrepancies in inheritance of the variant.

The occurrence of the slowest-moving band A which appears in sample 5 of figure 1 did not segregate in Mendelian ratios but was observed in three serial samples from an Indian woman in a period of 2 years. The slow-moving band may be either an artifact or the method is not sensitive enough to detect all the samples with the variant.

SUMMARY

A polyacrylamide disc electrophoretic method for detecting the C5+ serum cholinesterase variant is described. This method appears to be 25% more sensitive than starch gel methods, although it fails to detect the C5+ type in 50% of sera typed as C5— by the starch method but expected from family information to have the variant. Frequencies of the C5+ variant by the acrylamide method were 7.4% for 258 Cree Indians from Moose Factory, Ontario, and 13.1% for 298 Eskimos from Igloolik, Northwest Territories. A slow-moving zone of cholinesterase was observed in sera from some individuals but was not apparently determined genetically.

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