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Studies on the genetic and non-genetic (physiological) variation of human erythrocyte glutamic oxaloacetic transaminase

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SUMMARY

The thermostability profile of seven different electrophoretic variants of human erythrocyte GOT found in 13 different, unrelated families from a racially heterogeneous population was examined. The five different slow-variant and the two different fast-variant classes could be grouped into four different thermostability classes which were termed unstable, less stable, normal and more stable than normal. The thermostability differences among and within the electrophoretic variant classes permitted differentiation of the 13 individuals possessing an electrophoretic variant phenotype into a total of ten different variants.

INTRODUCTION

Human erythrocyte glutamic oxaloacetic transaminase (GOT; E.C. 2.6.1.1; aspartate aminotransferase) is a dimeric enzyme which catalyses the reversible reaction of L-aspartate and α-ketoglutarate to glutamate and oxaloacetate and requires pyridoxal phosphate as an obligatory cofactor (Jenkins, Yphantis & Sizer, 1959). Although both mitochondrial and cytoplasmic forms of the enzyme exist in animal, including human, tissues, only the cytoplasmic or soluble form occurs in the enucleated erythrocyte (Nisselbaum & Bodansky, 1964; Wada & Morino, 1964). Genetic variants of the soluble enzyme are relatively rare and have been described primarily from certain mongoloid populations, which, in addition to the common allele, contain two different electrophoretically identifiable alleles (Chen & Giblett, 1971; Ishimoto & Kuwata, 1974; Teng *et al.* 1978; Scott & Wright, 1981). These alleles occur at low frequencies, usually in heterozygous combination with the normal allele.

In this paper we report some observations resulting from studies on normal and ten different electrophoretic and thermostability variants of human erythrocyte GOT discovered in 13 families in various racial/ethnic groups.

MATERIALS AND METHODS

The study population consisted of all infants born at the University of Michigan Women's Hospital between January 1977 and January 1981 whose parents agreed to donate blood samples. The sample from the newborn infants was obtained from the umbilical cord at delivery, and from the parents by venipuncture. All samples were collected with ACD as the anticoagulant. For certain experiments, blood collected from various laboratory personnel served as controls. The erythrocytes were washed three times with saline and stored as packed cells in liquid nitrogen. Haemolysates for electrophoresis were prepared by diluting 1 volume of packed cells with 1 volume of distilled H_2O and extracting with 0·5 volume of toluene (Chen

et al. 1971). The stroma was removed by centrifugation at 20000 g for 20 min. Haemolysates for the activity and thermostability studies were prepared by diluting 1 volume of packed cells with 9 volumes of buffer (50 mM-HEPES (*N*-2-hydroxethyl piperazine-*N*-2-ethansulphonic acid), 2 mM dithiothreitol, 1 mM-EDTA, and 0·05% Triton-X 100, pH 7·2) and centrifuging at 40000 g for 30 min.

Enzyme activity and haemoglobin were assayed as described by Fielek & Mohrenweiser (1979) and Beutler (1975) utilizing a Beckman DU spectrophotometer equipped with a temperature controlled Gilford automatic cuvette positioner and chart recorder. The units of activity are μmole of product formed/g haemoglobin/min at 30 °C. Activity was measured in the presence of pyridoxal-5-phosphate following a 10 min preincubation period in reaction mix without α-ketoglutarate. The reaction was started by addition of α-ketoglutarate.

The thermostability studies were performed as outlined by Satoh & Mohrenweiser (1979) and Mohrenweiser & Neel (1981). The samples were incubated at 63 °C and 67 °C for varying times and/or for 20 min at varying temperatures between 63 °C and 78 °C.

Vertical starch gel electrophoresis, utilizing either 12·5% or 16% (w:v) Electrostarch (Otto Hiller, Madison, Wisconsin), was performed at 4 °C for 18 h using the equipment described by Brewer (1970). Five different buffer systems were employed. Electrophoretic system I was a continuous Tris-citrate buffer system at pH 6·2. The electrode buffer contained 0·223 M Tris and 0·086 M citric acid (monohydrate). The gel buffer contained 35 ml of electrode buffer diluted to 1 l with distilled H_2O. Electrophoretic system II was a continuous Tris-citrate buffer system at pH 7·0. The electrode buffer contained 0·155 M Tris and 0·043 M citric acid. The gel buffer was a 1:15 dilution of electrode buffer. Electrophoretic system III was a discontinuous buffer system at pH 7·5. The electrode buffer contained 0·44 M boric acid, 0·04 M Tris and 0·04 M lithium hydroxide. The gel buffer contained 0·0124 M Tris, 0·0036 M boric acid, 0·0033 M citric acid and 0·00033 M-LiOH. Electrophoretic system IV was the continuous Tris-citrate II buffer system at pH 8·0 of Selander *et al.* (1971) and electrophoretic system V was the discontinuous Tris-citrate/borate buffer system of Poulik (1957).

All samples were first screened for GOT variants with electrophoretic system I. In order to distinguish possible microheterogeneity within the various electrophoretic classes detected during the initial screening, all variants were subsequently electrophoresed on the four additional buffer systems indicated above.

Following electrophoresis, the gels were sliced and stained for GOT activity. The staining solution was modified from Banks *et al.* (1968) and contained 400 mg L-aspartic acid, 100 mg α-ketoglutaric acid, 5 mg pyridoxal-5-phosphate and 500 mg tetrazotized o-dianisidine dissolved in 400 ml of 0·2 M Tris-HCl, pH 8·0.

The electrophoretically identifiable variant alleles were assigned relative mobilities according to the system devised by Ayala *et al.* (1972). This system defines the mobility of the normal or reference allele as 1·00. The distance in mm that the mobility of a variant differs from that of the reference allele is then added to or deducted from 1·00 to give the final variant allele mobility (Table 1).

Table 1. *Mobilities of the erythrocyte* GOT *variant electromorphs relative to the mobility of the common electromorph*

Allele	Mobility	Buffer system	Number of individuals
3A	0·82	III	1
3B	0·83	III	1
3C	0·84	III	1
3D	0·86	III	1
3E	0·96	III	1
Normal	1·00	—	—
2A	1·11	II	5
2B	1·15	II	3

RESULTS

A total of 2219 cord blood samples and approximately 70 blood samples from Department of Human Genetics Laboratory personnel were screened for GOT variants. The ethnic distribution within the study population was 85% White, 10% Black, 1% Oriental and 4% unidentified or other. Seven different electrophoretic variants distributed among 13 different unrelated families in various racial/ethnic groups were ultimately resolved on starch gels (Fig. 1). The primary allozymes of the various phenotypes are depicted schematically in Fig. 2. The affected individuals were heterozygous for the common plus a variant allele.

The normal phenotype of erythrocyte GOT consists of a single, anodally migrating electromorph accompanied occasionally by a slightly faster, minor band. Depending on the buffer system employed, additional, faster migrating bands may be generated. The individuals with the variant phenotypes all possessed a 3-banded pattern of activity characteristic of dimeric enzymes. The mobilities of the variant electromorphs are indicated in Table 1.

Following the nomenclature of Chen & Giblett (1971) the variants with mobilities faster (more anodal) than the normal phenotype are designated as type 2 variants, whereas variants with mobilities slower (less anodal) than normal are designated as type 3 variants. Electrophoretic variants within the fast and slow classes are designated by letter and thermostability variants within an electrophoretic class are designated by an additional number.

We believe that alleles 3A and 2A correspond to the slow and fast alleles of Chen & Giblett (1971) since they occurred in individuals of Oriental ancestry. The other variants appear not to have been previously described.

During the initial electrophoretic screening which was conducted at pH 6·2 only four different (i.e. two different fast and two different slow) variant phenotypes were recognized. When it became apparent that thermostability differences existed within the various electrophoretic classes, all variants were re-electrophoresed on four additional buffer systems between pH 7·0 and pH 8·7 in an effort to obtain further resolution. The two slow phenotypes (5 individuals) originally recognized at pH 6·2 were thus differentiated into five different electrophoretic variants at pH 7·5. No electrophoretic differences within each of the two classes of fast variants were discerned in the pH range investigated with the five buffer systems employed in this study. Electrophoretic system II at pH 7·0, however, gave optimum separation of these two electrophoretic phenotypes.

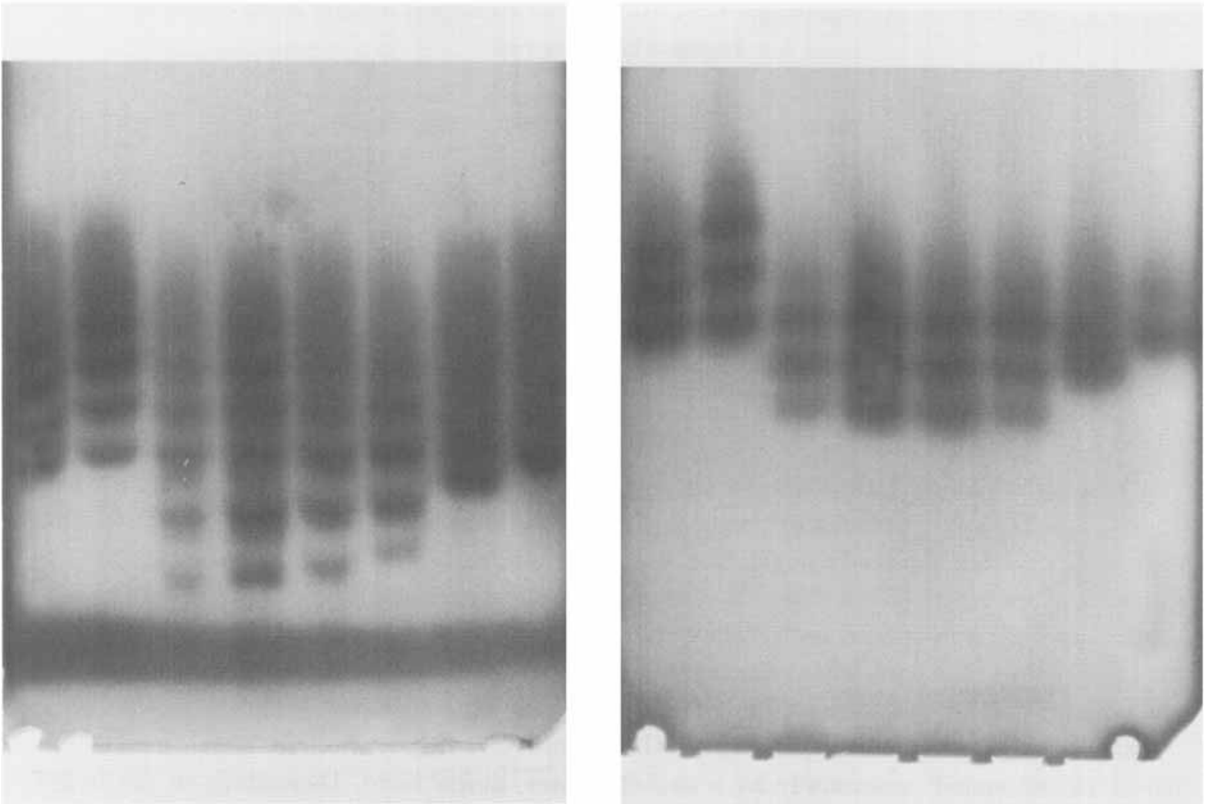

Fig. 1. Starch gel electrophoretic pattern of normal and seven different electrophoretic variant erythrocyte GOT phenotypes at pH 7·5 (Fig. 1 A) and at pH 7·0 (Fig. 1 B). The same samples are present on both gels. Well 1: 1/2A; well 2: 1/2B; well 3: 1/3A; well 4: 1/3B; well 5: 1/3C; well 6: 1/3D; well 7: 1/3E; well 8: 1/1.

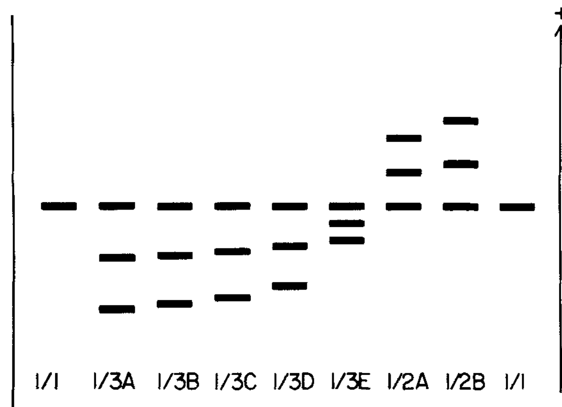

Fig. 2. Composite schematic representation of the common and the seven different electrophoretic variant phenotypes of erythrocyte GOT. Only the primary allozymes are illustrated.

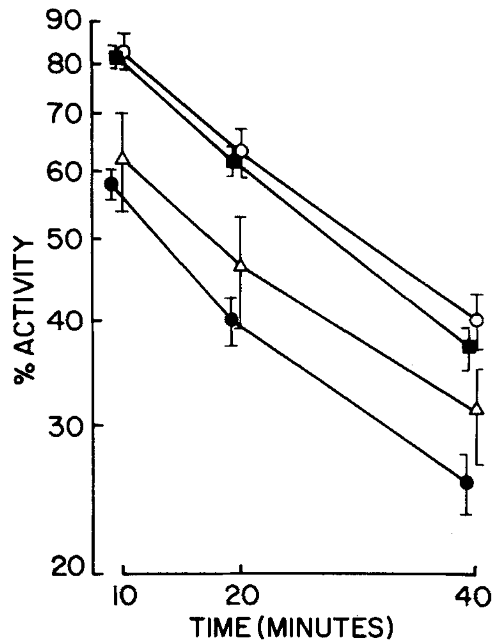

Fig. 3. Thermostability profile at 67 °C of the common erythrocyte GOT phenotype from different sample sources. ($\bar{X} \pm$ S.E.) ○—○ pregnant females, ■—■ cord bloods, △—△ non-pregnant females, ●—● males.

Thermostability

The erythrocyte GOT (common phenotype) from both cords and pregnant females was relatively thermostable compared to the enzyme from males and non-pregnant females (Fig. 3). The time for 50% inactivation (T-0·5) at 67 °C was 31 min for the enzyme from pregnant females and 28·5 min for the cord blood enzyme. This contrasts sharply to the values for the enzyme from non-pregnant females (17·5 min) and males (13·5 min).

The T-0·5 values for the cord blood electrophoretic variants ranged from a low of 14·5 min to a high of 40 min. Thus, several of the variants had thermostabilities which were significantly outside the normal range of 28·5 ± 1·3 min. These differences indicated that thermostability differences existed among the various electrophoretic classes. Additional studies also suggested that variants with differing thermostability profiles existed within several of the electrophoretic classes and indeed, when the various electrophoretic variants were re-electrophoresed at different pH's, the four individuals which possessed the same slow (type 3) electrophoretic phenotype at pH 6·2 were shown to differ electrophoretically at pH 7·5.

The five electrophoretically distinct type 3 variants fell into two thermostability classes (Table 2). These enzymes were either as stable as or were more stable than the normal enzymes. Figure 4 illustrates the thermostability profile of the three cord blood samples in this group. Two of the electrophoretic variants in this group were detected in the Laboratory personnel samples, thus cord bloods were not available for study. Since significant activity and thermostability differences exist in GOT from non-cord blood samples (Fig. 3; Table 4), it becomes important to account for both the sex and the reproductive state of the individuals, consequently the activity and stability of these latter two samples were compared with the appropriate normal adult samples.

Table 2. *Thermostability classification of* GOT *variants*

Allele	Sample ID	Stability class	T 0·5 (min)	T 0·5 (% of normal)	Comment
		Cord blood series			
2A–1	1904	+ + +	28	98	Normal
2A–2	973	+ +	18	63	Less stable
	863	+ +	20	70	Less stable
	1032	+ +	22	77	Less stable
2A–3	2312	+	15	53	Unstable
2B–1	972	+ + +	31	109	Normal
	1950	+ + +	30·5	107	Normal
2B–2	535	+	14·5	51	Unstable
1 (normal)	($N = 5$)	+ + +	$28·5 \pm 1·3$	100	Normal
3B	763	+ + +	25	88	Normal
3D	767	+ + + +	40	140	More stable
3E	2148	+ + + +	34	119	More stable
		Human Genetics miscellaneous samples			
3A	HG 11	+ + +	—	89	Normal
3B	HG 41	+ + + +	—	119	More stable

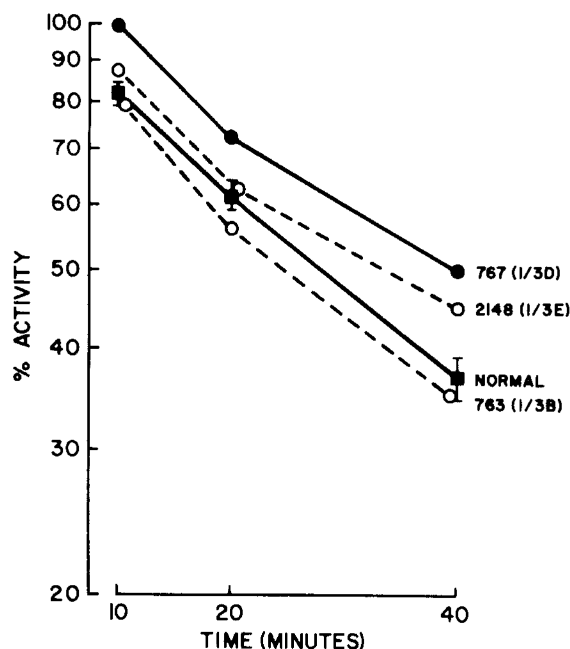

Fig. 4. Thermostability profiles at 67 °C of type 3 variants from cord blood samples compared with the common phenotype.

The eight variants which were distributed between the two electrophoretically distinct type 2 variants fell into three thermostability classes (Table 2). Thermostability differences were apparent not only between but also within each of the two fast electrophoretic classes. Taken as a group, the 2A variants are less stable than the 2B variants. Three thermostability classes are recognized within the 2A group (Fig. 5). Sample no. 973, which might well be placed into the 2A3 class based on the cord blood thermostability profile was placed into the 2A2 class based on the thermostability profile of enzyme from the affected parent. Two thermostability classes are recognized in the 2B group (Fig. 6).

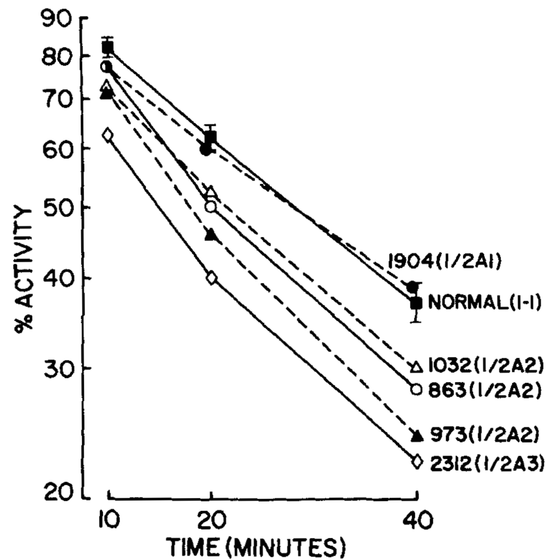

Fig. 5. Thermostability profiles at 67 °C of the 1/2A phenotypes from cord blood samples compared with the common phenotype.

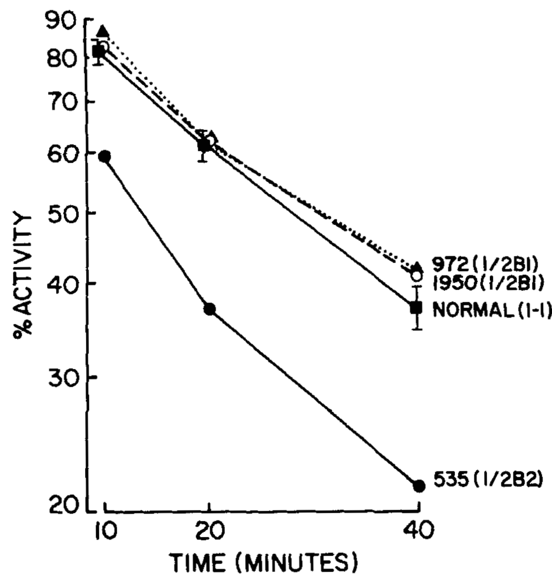

Fig. 6. Thermostability profiles at 67 °C of the 1/2B phenotypes from cord blood samples compared with the common phenotype.

Based on their thermostability profiles, the 13 GOT electrophoretic variants were placed into four different stability classes (Table 2). Two of the variants in two different electrophoretic classes were unstable, three variants in one electrophoretic class were less stable than normal, five variants in four different electrophoretic classes possessed normal stability and three different electrophoretic variants were more stable than normal. Family studies confirmed the inheritance of the electrophoretic as well as the thermostability variants (Table 3) except for variant 3A where additional family members were not available.

Table 3. *Inheritance of* GOT *variants*

Family	Ethnic group	Variant allele	Family Phenotypes		
			Mother	Father	Child
HG-11	Oriental	3A	—	1/3A	—
763	Caucasian	3B	1/1	1/3B	1/3B
HG-41	Caucasian	3C	1/3C	1/1	1/3C
767	Negro	3D	1/3D	1/1	1/3D
2148	Negro	3E	1/3E	1/1	1/3E
1904	Caucasian	2A1	1/2A1	1/1	1/2A1
973	Oriental	2A2	1/1	2A2/2A2	1/2A2
863	Caucasian	2A2	1/2A2	1/1	1/2A2
1032	Caucasian	2A2	1/2A2	1/1	1/2A2
2312	Caucasian	2A3	1/2A3	1/1	1/2A3
972	Caucasian	2B1	1/1	1/2B1	1/2B1
1950	Caucasian	2B1	1/2B1	1/1	1/2B1
535	Negro	2B2	1/1	1/2B2	1/2B2

Table 4. GOT *activity in samples from individuals with normal electrophoretic phenotypes*

Sample type	N	Activity ($\bar{X} \pm$ S.E.)*
Cords	8	$5.76 + 0.29$
Females		
Pregnant	9	4.49 ± 0.51
Non-pregnant	5	3.83 ± 0.37
Males	13	2.86 ± 0.23

* μm/min/g Hb.

Activity differences

Cord blood erythrocyte GOT activity was 5.76 ± 0.29 units which is 1·3 times the activity in erythrocytes from pregnant females, 1·50 times the activity in non-pregnant females and 2·0 times the activity in males (Table 4). The level of enzymatic activity measured in the individuals with the variant phenotypes fell within the range of activity observed in the phenotypically normal individuals in the appropriate control groups and were similar to levels of activity previously reported (Beutler, 1975; Mohrenweiser, 1981; Mohrenweiser, Fielek & Wurzinger, 1981), thus the *in vitro* lability observed during characterization of several of the variants is apparently not associated with an increased rate of enzyme degradation in the erythrocyte.

DISCUSSION

The differences in levels of erythrocyte GOT activity between cord and adult blood are usually explained in terms of differences in cell age. Young cells from either newborns or adults have been shown to have considerably higher GOT levels than old cells (Mohrenweiser *et al.* 1981; Sass, Vorsanger & Spear, 1964; Gahr, Meves & Schroter, 1979). The differences in erythrocyte GOT activity levels between pregnant families, non-pregnant females and males, however, is less easily explained. The differences between males and pregnant females undoubtedly reflect, at least partially, the almost universal vitamin supplementation of pregnant women in the population screened in this study. Dietary enrichment studies have shown that increased vitamin B_6 intake results not only in an increased activation of erythrocyte Apo-GOT, resulting

in an increased percentage of enzymatically active protein, but also in an increased synthesis of the Apo–enzyme in young erythroid cells (Folkers, Watanabe & Ellis, 1977; Solomon & Hillman, 1979). This, however, may not necessarily explain the activity differences between males and non-pregnant females. One may hypothesize that a causal relationship lies in the hormonal differences between males and females. There is, however, no direct data to support this hypothesis although induction of this enzyme by hormones is known in experimental animals (Hurvitz & Freedland, 1968; Gorin, Taylor & Shafres, 1969). Inferences regarding a hormonal influence on erythrocyte GOT levels may perhaps be drawn from studies which show that oral contraceptives (oestrogen & progesterone) taken in conjunction with oral supplementation of vitamin B_6 increases erythrocyte GOT levels to a greater degree than oral supplementation with vitamin B_6 alone, i.e. without concurrent oral contraceptive usage (Rose *et al.* 1972, 1973; Aly, Donald & Simpson, 1971; Bosse & Donald, 1979).

Differences in structure not demonstrable with electrophoretic techniques can often be detected as differences in thermostability as has been amply demonstrated for a variety of enzymes in various organisms (Singh, Hubby & Lewontin, 1974; Bonhome & Selander, 1978; Satoh & Mohrenweiser, 1979; Mohrenweiser & Neel, 1981). Thermostability differences, however, may also be a reflection of the physiological state of the individual, as is evidenced in the differences in the thermostability profile of erythrocyte GOT from males, non-pregnant females and pregnant females. This great difference in thermostability, as well as in levels of activity, may well reflect both the nutritional and the hormonal status of the individuals.

The cause of the greater thermostability of erythrocyte GOT from cord blood and pregnant females relative to males, and perhaps also non-pregnant females, in the presence of saturating levels of cofactor, is not known but may perhaps be due to a greater activation of enzyme during the heat treatment in the former group. Studies on cytoplasmic (as well as mitochondrial) GOT from other organisms indicate that, in addition to Apo- and Holo-GOT, there exists a considerable quantity of enzyme protein which binds pyridoxal phosphate in a catalytically inactive form (Martinez-Carrion *et al.* 1967; Banks *et al.* 1968; Magee & Phillips, 1971). It is possible that the heating step results in an activation of this latter form of the enzyme which could result in an apparently thermostable enzyme.

No differences in the thermostability profile of GOT from cord blood and maternal erythrocytes were observed. Since pyridoxal-5-phosphate concentrations in maternal and cord blood are highly correlated (Lumeng, *et al* 1976; Ejderhamn & Hamfelt, 1980) and since total (Apo- and Holo-) GOT levels are correlated with serum pyridoxal phosphate concentration (Hamfelt, 1967), the question arises as to whether the thermostability differences observed among the electrophoretic variants are solely a function of maternal nutritional status. Two pieces of information suggest that this is not correct. Firstly, several of the thermostability variants are paternal in origin. Secondly, the thermostability variants observed in the group of type 3 variants were shown to be electrophoretic variants when additional buffer systems were employed. Thus, although we realize that the thermostability profile of human erythrocyte GOT reflects both genetic and non-genetic influences, the data are sufficient to warrant sudividing the 2 electrophoretic classes of type 2 variants into 5 different genetic variants. This level of heterogeneity of 10 variant phenotypes occurring in 13 individuals in a sample of 2289 individuals is significantly higher than previously suggested and again indicates the need to utilize multiple techniques in studies of genetic variation.

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