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ELECTROPHORETIC VARIANTS OF BLOOD PROTEINS IN JAPANESE

II. PHOSPHOGLUCOMUTASE-1 (PGM1)

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Summary Starch gel electrophoresis of erythrocyte phosphoglucomutase-1 (PGM1) of 17,126 Japanese from Hiroshima and Nagasaki was performed. Thirteen types of rare variants, 6 migrating anodally and 7 migrating cathodally to the *a*-band, were encountered in a total of 103 individuals. Family studies confirmed the genetic characteristics of most of them. The previous observation of the polymorphic occurrence of the *PGM1*7* allele in the Hiroshima and the Nagasaki populations was confirmed. A significant difference in the frequencies of rare variants between Hiroshima and Nagasaki was observed. A stronger influence of the migrating stream from the south through the Ryukyu Archipelago to Nagasaki is postulated on the basis of the frequencies of *PGM1*3NG1* and *PGM1*3OKINAWA*.

INTRODUCTION

The first paper in this series precisely describes the purpose of the study and circumstances under which the study was performed (Satoh *et al.*, 1984). The present paper describes electrophoretic variants of erythrocyte phosphoglucomutase-1 (PGM1) encountered in Japanese residents of Hiroshima and Nagasaki. Here we consider only those variants detectable by starch gel electrophoresis using Tris-EDTA-maleic acid-$MgCl_2$ of Spencer *et al.* (1964). Though variants with normal mobility but very weakly staining bands and hence suspected to be deficiency variants

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were encountered in our screening program, they are not included in this report. The populations and the method of family studies are described in the first paper of this series. Briefly, data are obtained from two populations, *i.e.*: the 'Adult' composed of A-bomb survivors and controls, and the 'Child' comprising children born to the proximally and distally exposed survivors. Since certain kinships are included in the two groups and within the offspring group, the 'Representative' population was selected from unrelated individuals in the first two populations. The frequencies of alleles are calculated from the third population. As before (Ferrell *et al.*, 1977), the convention in naming variants uses known similar types suffixed with city and order of discovery, abbreviating Hiroshima to HR and Nagasaki to NG.

MATERIALS AND METHODS

Preparation of the hemolysates for electrophoresis was carried out as described in the first paper in this series (Satoh *et al.*, 1984). For vertical starch gel electrophoresis for routine typing, the Tris-EDTA-maleic acid-$MgCl_2$ (TEMM) buffer system, pH 7.4, of Spencer *et al.* (1964) was used modifying the dilution ratio of gel buffer/bridge buffer to 1/15. Comparison of variants was performed as described previously (Satoh *et al.*, 1976, 1977) using both the TEMM buffer and a 0.005 M histidine–0.41 M citrate, pH 7.0, discontinuous buffer system of Fildes and Harris (1966). Although PGM1 isozymes were stained according to the method of Spencer *et al.* (1964) employing agar over-lay, 6,928 samples from the Child subjected to testing since September 1978 were stained by applying staining solution to the gel surface with a brush.

PGM activity of rare electrophoretic variants was determined using 20 μl of 1 : 20 diluted hemolysates, along with more than 10 control hemolysates, all PGM1 1 and PGM2 1 phenotypes. Hemolysates for PGM activity measurements were prepared as described previously (Satoh *et al.*, 1983). Methods for measuring PGM activity, in principle based on the methods recommended by Beutler (1975) and ICSH (Beutler *et al.*, 1977), are described in a separate paper concerning a low activity variant of PGM1 (Satoh *et al.*, in preparation).

Some rare electrophoretic variants of PGM1 have bands with very weak intensity, which is a characteristic of such variants. PGM activity in hemolysates is the sum of the activities of isozymes of PGM1 and PGM2, approximately half of which is considered to be derived from PGM1, the remainder from PGM2 (Terrenato *et al.*, 1970; McAlpine *et al.*, 1970). In the Japanese, PGM2 is not polymorphic and the frequency of rare variants is very low (Ishimoto, 1975; Satoh *et al.*, 1977; Satoh *et al.*, 1984). Therefore, it is assumed likely that there is little individual difference in PGM2 activity so that differences in PGM1 allozyme activity, arising from differences in phenotypes, would be reflected in total PGM activity. We have already examined PGM activity of samples for which the PGM2 phenotype was 1 and the

PGM1 phenotype was 1, 1-2, 2 or 1-7 (Satoh *et al.*, in preparation). There was little difference in activity among the four common PGM1 phenotypes. No difference in activity was observed between the Hiroshima and the Nagasaki samples though the condition and the length of time before processing samples were different. For normal activity of PGM, 1.84 IU/g Hb (International Unit per gram of Hemoglobin) was adopted, the mean value obtained from pooling 386 activity values obtained from 191 Hiroshima subjects (mean, 1.82 IU/g Hb; standard deviation, 0.23 IU/g Hb) and 195 Nagasaki subjects (mean, 1.85 IU/g Hb; SD, 0.19 IU/g Hb) all PGM1 1 and PGM2 1.

When possible, activity was determined for propositi having rare variants. Electrophoresis and determination of PGM activity were also made for their families. The results are shown in Tables 3, 4 and 5. The activity of each variant is also referred to when describing its characteristics.

RESULTS

In a previous paper (Satoh *et al.*, 1977) describing the results obtained for phosphoglucomutase-1 (PGM1) and phosphoglucomutase-2 (PGM2) in the Adult, data were presented for a total of 4,029 cases, 1,895 examined using TEMM buffer, pH 7.4 and 2,134 using histidine-citrate discontinuous buffer system, pH 7.0, but the data in this paper were obtained only from examinations using the TEMM buffer since it was found that some variants could not be detected by electrophoresis using the latter buffer system. Therefore, the results reported here are from 2,534 cases in the Adult, 1,895 reported previously, 620 tested subsequently, and 19 which, tested previously with the latter buffer system, were later reexamined with TEMM buffer. Grouped by city, 1,301 were from Hiroshima and 1,233 from Nagasaki.

Table 1 shows PGM1 phenotypes and the number of those phenotypes detected in the Adult and the Child along with the Representative composed of unrelated individuals selected from the first two populations, the total numbers of the Child and the Representative being 14,592 (7,596 from Hiroshima and 6,996 from Nagasaki) and 11,823 (6,670 from Hiroshima and 5,153 from Nagasaki), respectively. Characteristics of the variants have already been reported (Satoh *et al.*, 1976, 1977) except for those marked with an asterisk, which are shown here for the first time. Since approximately 30% of the Child comprises siblings, the same variant was often detected more than once. The figures in parentheses are the number of variants excluding those detected in siblings. Because $1\text{-}4_{HR2}$ and $1\text{-}5_{HR1}$ were detected in one sibling each of children who showed $2\text{-}4_{HR2}$ and $2\text{-}5_{HR1}$, respectively, numerals in parentheses for them are 0. When the phenotype could not be read clearly, 'no type' is indicated. Representatives with 'no type' were excluded in selecting the Representative. Those cases aside, the representatives were selected by the method described in " SAMPLES AND FAMILY STUDIES' of the first paper of this series (Satoh *et al.*, 1984). Sometimes, therefore, when there was a member with a normal type

Table 1. Various phenotypes of PGM1 among Japanese of two populations (Adult & Child) and the Representative population composed of selected members from the two populations.[a]

Phenotype	Population				
	Adult	Child	Representative		
			Combined	Hiroshima	Nagasaki
1	1,465	8,475	6,843	3,750	3,093
1-2	847	4,919	4,006	2,329	1,677
2	138	693	595	362	233
1-7	48	317	246	158	88
2-7	14	76	61	42	19
7	2	8	8	4	4
1-3$_{NG1}$	5	34 (28)	26	8	18
2-3$_{NG1}$	1	9 (8)	9	4	5
*1-4$_{HR1}$	1	0	1	1	0
*1-4$_{HR2}$	0	1 (0)	0[b]	0	0
*2-4$_{HR2}$	0	1 (1)	0[b]	0	0
*4$_{HR2}$	0	2 (1)	1	1	0
*1-4$_{NG1}$	0	3 (2)	1	0	1
*1-5$_{HR1}$	0	1 (0)	0[c]	0	0
*2-5$_{HR1}$	0	1 (1)	1	1	0
1-9$_{NG1}$	1	0	1	0	1
1-6$_{NG1}$	1	1 (1)	1	0	1
1-6$_{NG2}$	2	8 (5)	4	0	4
2-6$_{NG2}$	1	1 (1)	2	2	0
7-6$_{NG2}$	0	1 (1)	1	1	0
*2-6$_{NG3}$	0	1 (1)	1	0	1
1-6$_{HR1}$	1	0	1	1	0
2-6$_{HR1}$	0	1 (1)	1	1	0
1-6$_{HR2}$	2	0	2	0	2
2-6$_{HR2}$	1	4 (4)	2	2	0
1-6$_{HR3}$	0	3 (2)	2	2	0
*2-6$_{HR3}$	0	1 (1)	1	1	0
1-8$_{NG1}$	0	12 (8)	5	0	5
2-8$_{NG1}$	0	2 (2)	1	0	1
No type	4	17	0	0	0
Total	2,534	14,592	11,823	6,670	5,153

* Newly encountered variants in this study.

[a] See text for the description of the two populations and the Representative population.

[b] Father with PGM1 1-2 was selected as a representative of the family.

[c] The brother with PGM1 2-5$_{HR1}$ was selected.

and a member with a variant in the family, the former was selected and the latter excluded from the Representative.

Table 2 shows the frequency of alleles calculated from the number of each phenotype in the Representative of Table 1.

A. *Polymorphism*

Two alleles, *PGM1*2* and *PGM1*7*, already found in polymorphic proportions in the Adult (Satoh *et al.*, 1977), are also polymorphic, 0.223 and 0.014, respectively, in the Representative which is approximately 6 times larger than the previously reported population. The *PGM1*2* allele frequencies in both Hiroshima (0.233) and Nagasaki (0.210) were in the range of 0.191–0.249, frequencies also observed in various other Japanese populations (Ishimoto, 1975).

B. *Rare variants*

Of 2,534 individuals of the Adult, 2,530 were clearly typed, and seven kinds of variants, *i.e.*, 3_{NG1}, 4_{HR1}, 9_{NG1}, 6_{NG1}, 6_{NG2}, 6_{HR1}, and 6_{HR2} were encountered in 16 subjects. All of these were detected as phenotypes heterozygous with 1 or 2. Inasmuch as 13 of the 16 subjects were included among the 1,895 individuals who have already been reported, the characteristics of six of the variants, *i.e.*, 3_{NG1}, 9_{NG1}, 6_{NG1}, 6_{NG2}, 6_{HR1} and 6_{HR2}, have already been described along with results of family study for 5 of them (Satoh *et al.*, 1976, 1977). The result of family study for the remain-

Table 2. *PGM1* allele frequencies among 11,823 unrelated Japanese examined by starch gel electrophoresis using TEMM buffer, pH 7.4.

Allele	Population		
	Combined	Hiroshima	Nagasaki
*PGM1*1*	0.76047	0.74955	0.77460
*PGM1*2*	0.22308	0.23283	0.21046
*PGM1*7*	0.01370	0.01567	0.01116
*PGM1*3NG1*	0.00148	0.00090	0.00223
*PGM1*4HR1*	0.00004	0.00007	0
*PGM1*4HR2*	0.00008	0.00015	0
*PGM1*4NG1*	0.00004	0	0.00010
*PGM1*5HR1*	0.00004	0.00007	0
*PGM1*9NG1*	0.00004	0	0.00010
*PGM1*6NG1*	0.00004	0	0.00010
*PGM1*6NG2*	0.00030	0.00022	0.00039
*PGM1*6NG3*	0.00004	0	0.00010
*PGM1*6HR1*	0.00008	0.00015	0
*PGM1*6HR2*	0.00017	0.00015	0.00019
*PGM1*6HR3*	0.00013	0.00022	0
*PGM1*8NG1*	0.00025	0	0.00058

ing one, 6_{NG1}, is described in this paper (see below). The remaining three subjects were among 639 individuals examined subsequently in whom the variant phenotypes were 1-3_{NG1}, 2-6_{NG2}, and 1-4_{HR1} which is a new variant. Family study was not possible for them.

On the other hand, of the already reported 3_{NG2} (Satoh *et al.*, 1977) and 8_{NG1} (Satoh *et al.*, 1976, 1977), the latter was detected in three (one in Hiroshima, and two in Nagasaki) of 2,134 subjects who had been typed using a histidine-citrate discontinuous buffer system described in the previous paper, but this variant was not detected among the 2,530 individuals of the present report who were typed using TEMM buffer. The isozyme PGM1 3_{NG2} which was described in the previous paper as a PGM1 variant whose mobility was similar to that of PGM1 3_{NG1} but had weak activity, was subsequently shown to have phosphopentomutase activity, and therefore was considered to be a product of an allele at the *PGM2* locus. The variant therefore was renamed PGM2 9_{NG1} and the allele involved in the synthesis was named *PGM2*9NG1*. This variant is described in the first paper of the series concerning PGM2.

Of 15,141 samples from the Child, 14,592 were electrophoresed using TEMM buffer, and the phenotypes of 14,575 were recorded excluding 17 whose phenotypes could not be clearly typed. Four 'fast variants' of PGM1 whose major band migrates anodal to *a*-band, *i.e.*, 3_{NG1}, 4_{HR2}, 4_{NG1} and 5_{HR1} were detected among 52 children. Seven kinds of so-called 'slow variants' whose major band migrated cathodal to *a*-band, *i.e.*, 6_{NG1}, 6_{NG2}, 6_{NG3}, 6_{HR1}, 6_{HR2}, 6_{HR3}, and 8_{NG1}, were detected among 35 children. Two of the four propositi with 4_{HR2} seemed to be homozygotes, but the remaining 85 children were heterozygous for the rare variant alleles and the common alleles of *PGM1*1*, *PGM1*2* or *PGM1*7*. The variant detected and the number of children in whom these variants were found are shown in column 3 (Child) of Table 1. Of the 14,575 children whose phenotypes could be clearly typed, 87 had the variants, but if only unrelated children are selected, the total number on whom examinations were carried out is 10,484 and the number in whom variants were detected is 68.

Variants in the two populations were repeatedly compared by electrophoresis on the same starch gel. Five of the seven types of variants detected in the Adult, excepting 9_{NG1} and 4_{HR1}, were detected also in the Child. On the other hand, of the 11 types of variants detected in the Child, six types, 4_{HR2}, 4_{NG1}, 5_{HR1}, 6_{NG3}, 6_{HR3}, and 8_{NG1}, were not detected in the Adult. With the exception of 8_{NG1}, five of these variants and 4_{HR1} found in the Adult are previously unreported new variants, and therefore the characteristics of these six new variants are described below in detail. A total of 13 types of the variants, the six rare variants being reported for the first time here and the previously reported seven types, were electrophoretically tested simultaneously to compare mobility and the photographs and diagrams of 'fast variants' are shown in Figs. 1 and 2 and 'slow variants' in Figs. 4 and 5.

Family studies for rare variants encountered in the Child were conducted to

determine whether they had been transferred from the previous generation. Procedures for collecting family study data and their later treatment have been described in the first paper of this series. Results are compiled in Tables 3 and 4.

B-1. *Newly encountered variants*

PGM1 4_{HR1}. Since Hopkinson and Harris (1966) have named variant allozymes whose major band migrated between bands *a* and *b*, PGM1 4, we named the 3 types of variants detected in our populations whose major bands move between

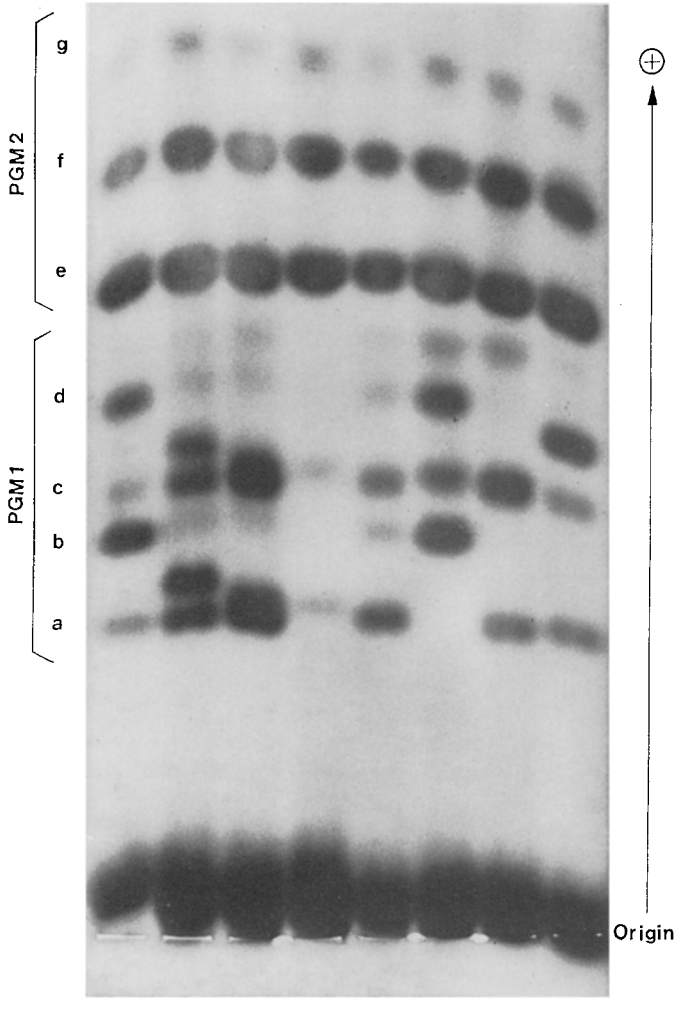

Fig. 1. Five types of PGM1 variants migrating faster than PGM1 1 on Electrostarch gel using TEMM buffer, pH 7.4. Hemolysates were treated with 2 mM 2-mercaptoethanol for 30 min at 37°C.

these two bands PGM1 4_{HR1}, PGM1 4_{NG1}, and PGM1 4_{HR2}, and have also designated the alleles which control them as *PGM1*4HR1*, *PGM1*4NG1*, and *PGM1*4HR2*. The migration shift to the anodal side is in the order of $4_{HR1} > 4_{NG1} > 4_{HR2}$. The order of the band intensity observed in the routine electrophoresis was $4_{NG1} \geqq 4_{HR1} > 4_{HR2}$, and even the major band of 4_{NG1} which has the strongest intensity of the three was weaker than that of *a*, *b*, *c*, and *d*-bands observed in a heterozygous phenotype. Nevertheless, when the hemolysates freshly prepared from the red cells preserved in the liquid nitrogen were treated with 2-mercaptoethanol (2 mM), the intensities of the major bands of 4_{HR1} and 4_{NG1} were almost the same as that of the *a*-band in the heterozygous phenotype and the mobilities of their bands decreased. These phenomena were observed on both Connaught-starch gel and Electrostarch gel.

A variant phenotype of PGM1 1-4_{HR1} was detected in a woman from Hiroshima in the Adult (ID No. 224948). The PGM activity was 1.76 IU/g Hb, which was 96% of the normal type (PGM1 1, PGM2 1). The two variant bands were located adjacent to the cathodal side of the *b*- and *d*-bands, respectively, under usual conditions, but they moved to a position midway between the *a*- and *b*-bands after 2-mercaptoethanol treatment as shown in Figs. 1 and 2. On the basis of these observations, we conclude that PGM1 4_{HR1} is a labile variant though its activity was maintained in the liquid nitrogen. Family study of the propositus has not yet been possible.

PGM1 4_{HR2}. In two Hiroshima brothers (variant Nos. 1 and 2), members of

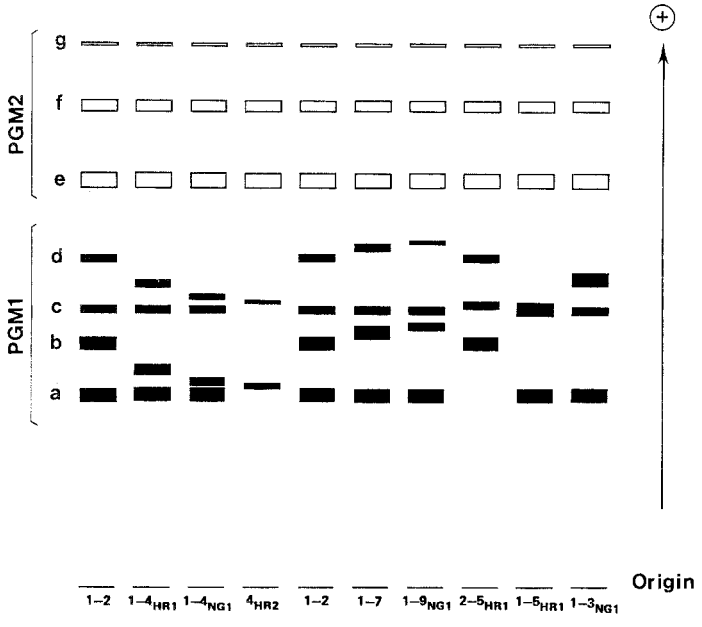

Fig. 2. Diagram of 6 types of PGM1 variants migrating faster than PGM1 1 on starch gel found in Hiroshima and Nagasaki. Conditions same as for Fig. 1.

the Child, only two very faint bands were observed in the PGM1 area of the zymogram, as shown in the well 4 of Fig. 1. The location of the major band was slightly anodal to and just adjacent to the position of *a*-band, while the minor band was slightly anodal to and just adjacent to the position of *c*-band. Positions and intensities of the bands did not alter after treatment with 2-mercaptoethanol. Despite the fact that the intensity of the three isozyme bands of PGM2, *e*, *f*, and *g*, were normal, the PGM activities of the brothers were merely 58% and 56% of the mean activity of the normal type (PGM1 1, PGM2 1). The brothers' parents had only *a*- and *c*-bands with very weak intensity as PGM1 isozymes suggesting they have heterozygous phenotypes. The intensity of their three isozyme bands of PGM2 was normal, whereas the total PGM activity was 81% of normal for mother and 67% for father. In naming the allele, which controls the PGM1 allozymes of the brothers, *PGM1*4HR2*, two possible phenotypes may be considered, 1) homozygous PGM1 4_{HR2} or 2) heterozygous PGM1 0-4_{HR2}. The two brothers probably have the same phenotype, because their PGM activities were almost identical. 1) If the homozygous phenotype PGM1 4_{HR2} is assumed, both parents would be phenotype 1-4_{HR2}. Judging from the mobility and the very weak intensity of the variant bands in the brothers, it may be surmised that for the heterozygous phenotype PGM1 1-4_{HR2}, variant bands probably would overlap the *a*- and *c*-bands, and it seems most likely that only *a*- and *c*-bands which have an intensity of approximately 1/2 of PGM1 1 would be detected. 2) If they are PGM1 0-4_{HR2}, which is a heterozygous phenotype, the parents would have 1-0 and 1-4_{HR2}, and thus in either case the intensity of the *a*- and *c*-bands would be 1/2 of that of PGM1 1. In the first case, both parents must possess the same rare variant allele, *PGM1*4HR2* and for the second case, they would have to possess two different and rare variant alleles, *PGM1*4HR2* and *PGM1*QO*. As shown in the family pedigree in Fig. 3, there were two instances of consanguineous marriage in this family, one involving the parents. As the grandmother on the brothers' paternal side was PGM1 1-2, if either I-1 or I-2 had *PGM1*4HR2*, it is possible that the phenotypes of both parents of the brothers could be PGM1 1-4_{HR2}. The frequency of *PGM1*4HR2* in the Child which excludes siblings and is composed of unrelated individuals is 2/10,000 at most, as shown in this report. On the basis of this frequency and the family pedigree, the probability for both parents to have PGM1 1-4_{HR2} is calculated to be

$$\left(\frac{1}{2}\right)^7 \times \frac{1}{5{,}000} = 0.16 \times 10^{-5}.$$

Although the frequency of *PGM1*QO* is not clear, when the mean value of 1/1,000 for frequency of deficiency variants of 11 types of red blood cell enzyme (Satoh *et al.*, 1983) is used, the frequency in the second case in which the phenotypes of parents are 1-0 and 1-4_{HR2} will be

$$(0.75)^2 \times \frac{1}{5{,}000} \times \frac{1}{1{,}000} = 0.11 \times 10^{-6},$$

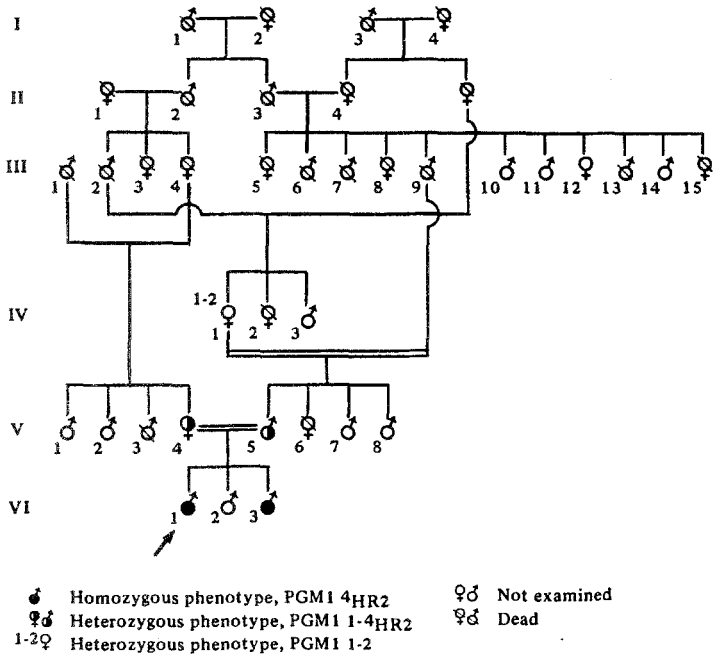

Fig. 3. Pedigree of family with a homozygous PGM1 4_{HR2}.

which demonstrates that the probability for the first case is approximately 10-fold higher than the second. Given the consanguinity it seems more reasonable to consider the phenotype as homozygous PGM1 4_{HR2} than to suppose two very rare phenotypes occur together in a family of the same generation.

A male child of Hiroshima (variant No. 3) in the Child who was unrelated to the brothers described above showed two very faint bands with the same mobility as that of PGM1 4_{HR2} together with *b*- and *d*-bands. This phenotype was named PGM1 2-4_{HR2}. Though the intensity of *e*-, *f*- and *g*-bands of this sample was normal, PGM activity was 76% of normal. A male child (variant No. 4) who is in the same population and is also a younger brother of the propositus had been detected as PGM1 1-0 through screening before the propositus was examined. The reason for suggesting this phenotype was that the intensities of the *a*- and *c*-bands of his PGM1 were as weak as those in the heterozygous phenotype, and the PGM activity was only 77% of normal. Since the elder brother (variant No. 3) was later found to have PGM1 2-4_{HR2} with reduced PGM activity, the younger brother's phenotype was assumed to be 1-4_{HR2}. The allozymes and intensity of PGM1 of the mother were identical to those of the younger brother and the PGM activity was 78% of normal. Thus her phenotype was assumed to be 1-4_{HR2}. The father was PGM1 1-2, and another brother was PGM1 1. Their PGM activities were 95% and 93%, respectively, of normal. For this family, again, it is possible to assume the presence of *PGM1* QO* in the younger brother and mother, and the assumption can be made

that the allele *PGM1*4HR2* of the elder brother was brought about by mutation. However, rather than assuming that two rare phenomena had occurred together it seems more plausible that the rare allele, *PGM1*4HR2*, had been transferred from the mother to her two children, but inasmuch as the mobility of 4_{HR2} differed only slightly from that of the *a*- and *c*-bands and the activity is very weak, the bands of 4_{HR2} of the mother and younger brother failed to separate and were not recognizable.

PGM1 4_{NG1}. When usually prepared hemolysates were used, the intensities of both the major and minor bands of the variant allozyme detected in a male child in Nagasaki (variant No. 5) and a female child (variant No. 6) and her elder brother (variant No. 7) in Hiroshima were weak, and the major band located on the anodal

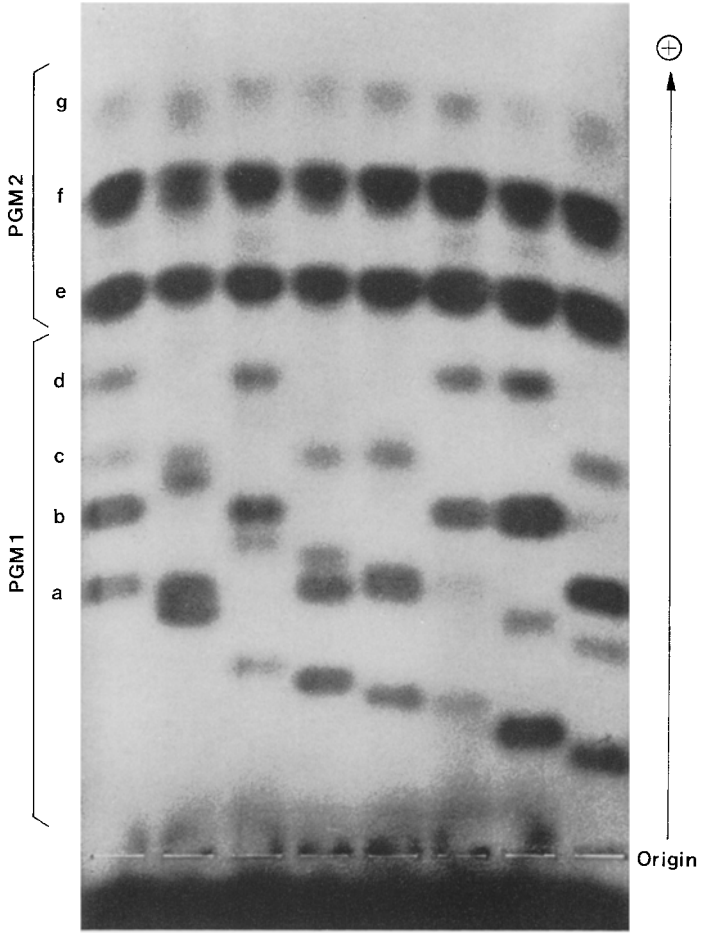

Fig. 4. Seven types of PGM1 variants with a major band migrating slower than the *a*-band on starch gel using TEMM buffer, pH 7.4.

Table 3. Family studies of variants of PGM1 in the Child population.

Propositus					Mother	Father	Other family member	Comments
Variant No.	City	Variant type	ID No.	Sex				
1	H	$^{4}_{HR2}$	909099	M	♀,♂	♂	♀Grandmother	*1
2	H	$^{4}_{HR2}$	910671	M				
3	H	$^{4}_{HR2}$	904896	M	♀,♂	♂	♂Brother	
4	H	$^{4}_{HR2}$	394424	M				
5	N	$^{4}_{NG1}$	721730	M	♀	♂		
6	H	$^{4}_{NG1}$	900780	F	♀,♂	♂	♂Brother	
7	H	$^{4}_{NG1}$	403411	M				
8	H	$^{5}_{HR1}$	307451	M	♀	♂		
9	H	$^{5}_{HR1}$	469650	M				
10	N	$^{6}_{NG1}$	722577	M	♀,♂	NT	[♂Brother ♀Grandmother	*2
11	N	$^{6}_{NG2}$	724250	M	♀	♂	♀Sister	
12	N	$^{6}_{NG2}$	083661	M				
13	N	$^{6}_{NG2}$	158787	F	♀,♂	NT		
14	N	$^{6}_{NG2}$	162010	M	♀,♂	Dead		
15	N	$^{6}_{NG2}$	726093	F				
16	N	$^{6}_{NG2}$	706390	M	♀,♂	♂		
17	N	$^{6}_{NG2}$	712385	F	♀	♂	♂Brother	
18	H	$^{6}_{NG2}$	911755	F	Dead	♂		
19	H	$^{6}_{NG2}$	909567	M	♀,♂	♂		
20	H	$^{6}_{NG2}$	905489	M				
21	N	$^{6}_{NG3}$	145273	M	♀	♂		
22	H	$^{6}_{HR1}$	418002	M	♀,♂	♂		*3
23	N	$^{6}_{HR2}$	705818	M	NT	♂	♂Grandfather	*4
24	H	$^{6}_{HR2}$	953506	M	♀,♂	♂		
25	H	$^{6}_{HR2}$	416310	F	♀	♂		
26	H	$^{6}_{HR2}$	923091	M	♀	♂		
27	H	$^{6}_{HR3}$	908905	F	♀	♂		
28	H	$^{6}_{HR3}$	288521	M				
29	H	$^{6}_{HR3}$	922635	F	NT	NT		
30	H	$^{6}_{HR3}$	915210	F	♀	♂		
31	N	$^{8}_{NG1}$	716985	M	♀	♂	♂Brother	
32	N	$^{8}_{NG1}$	759622	F				
33	N	$^{8}_{NG1}$	146416	F				
34	N	$^{8}_{NG1}$	715395	M				
35	N	$^{8}_{NG1}$	706113	M	♀	♂		
36	N	$^{8}_{NG1}$	706043	M	♀,♂	♂		
37	N	$^{8}_{NG1}$	708526	F	♀	♂		
38	N	$^{8}_{NG1}$	728002	M	♀	♂		
39	N	$^{8}_{NG1}$	725202	M	♀	Dead	♀Sister	
40	N	$^{8}_{NG1}$	711802	F	♀,♂	♂		
41	N	$^{8}_{NG1}$	723004	F	♀	♂	[♂Brothers ♂	
42	H	$^{8}_{NG1}$	417941	F	♀,♂	Dead	♀Sister	
43	H	$^{8}_{NG1}$	223858	M				
44	H	$^{8}_{NG1}$	469139	M	♀	♂	♀Sister	

Symbols used in Table 3:

♀,♂ Heterozygote for variant alleles at *PGM1* locus

♀,♂ Homozygote for normal *PGM1*1*

NT Not tested

][Sibship or other family member

*1 Male siblings homozygous for *PGM1*4HR2* (see Fig. 3). Paternal grandmother PGM1 1-2

*2 Mother in the Adult population, her variant previously reported. Maternal grandmother affected

*3 Mother in the Adult population, previously reported as PGM1 1-$^{6}_{HR1}$ using histidine-citrate buffer

*4 Father and paternal grandfather in the Adult population, both affected, previously reported

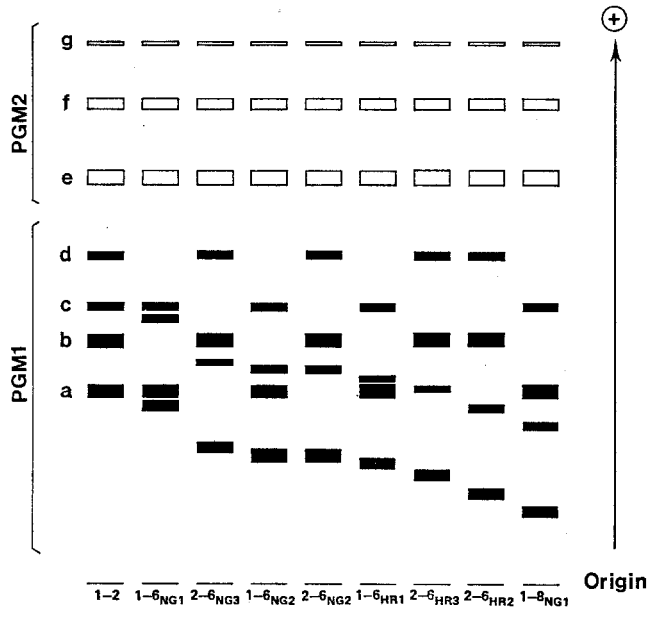

Fig. 5. Diagram of seven Hiroshima and Nagasaki PGM1 variants having a major band migrating slower than the *a*-band. Conditions same as for Fig. 4.

side to the center between the *a*- and *b*-bands and its anodal side was almost in contact with the cathodal side of *b*-band. The major band of this variant was separable from the *a*-band, whereas that of PGM1 4_{HR2} described above, was not. However, there were instances where complete separation was not possible due to slight differences in the electrophoretic conditions or to different lots of starch. In other words, there were instances where only a broad band was observed indicating the presence of a variant band, but not separable from one another into two bands. The mobility was similar to that of PGM1 4, reported by Hopkinson and Harris (1966), with a similarly weak intensity. The allozyme was named PGM1 4_{NG1}. But when hemolysates, freshly prepared from red cells stored in liquid nitrogen, treated with 2-mercaptoethanol were used, the major and the minor bands moved anodally to but could hardly be separated from the *a*-band or *c*-band, though their intensity was only slightly weaker than that of the *a*- or *c*-band (Figs. 1 and 2). The phenotypes of the Nagasaki child, his father, two siblings of Hiroshima and their mother were all 1-4_{NG1}. PGM activity of these five people was within 89–101% of normal.

PGM1 5_{HR1}. In a Hiroshima male child (variant No. 8), a variant band was detected slightly anodal to the *c*-band together with *b*- and *d*-bands. The intensity was about the same or weaker than that of the *c*-band in a heterozygous phenotype. This phenotype was named PGM1 2-5_{HR1}. Only two bands, the *a*-band and a broad *c*-band, were detected as the allozyme of PGM1 of the elder brother (variant No. 9) of the propositus. The intensity of his *a*-band was the same as that of the *a*-band

of heterozygous phenotype and the *c*-band was broad on the anodal side, the intensity of the two bands being almost equal. All of these observations suggested that he is a heterozygote for a variant allele and *PGM1*1* and his phenotype was assumed to be 1-5_{HR1}. The two bands observed in the father also demonstrated the same characteristics as those in the elder brother, and thus, he was likewise assumed to be 1-5_{HR1}. The mother was 1-2. The PGM activity of the two children was 88% of the normal value. The father's value was 94%, while the mother was 107%. In the case of phenotype 1-5_{HR1}, it is quite possible to overlook 5_{HR1} and consider it as 1 in the screening because the variant band does not separate from the *c*-band.

PGM1 6_{NG3}. Two variant bands detected in a male child from Nagasaki (variant No. 21) migrated slightly more to the anodal side than the two bands of PGM1 6_{NG2}. The minor band located closer to the *b*-band than midway between the *a*- and *b*-bands. Thus this variant was obviously different from 6_{NG2} whose minor band was closer to the *a*-band, and it was named 6_{NG3}. It was already reported that 6_{NG2} demonstrated the same mobility on starch gel electrophoresis as standard PGM1 6 provided us by Dr. Lie-Injo (Lie-Injo *et al.*, 1968) regardless of whether TEMM buffer system or histidine-citrate discontinuous buffer system was employed (Satoh *et al.*, 1976). The phenotype of the propositus and his father was PGM1 2-6_{NG3}, and of the mother, PGM1 2. The intensities of the two variant bands in both the propositus and his father were much weaker than those of the simultaneously detected *b*- and *d*-bands, and even the intensities of the major bands were weaker than those of any of the four bands, *a*, *b*, *c*, and *d*, of phenotype 1-2. Therefore, since the intensities of the two variant bands of 6_{NG2} are the same as those of the *a*- and *c*-bands, respectively, 6_{NG3} differs from 6_{NG2} not only in mobility but also in activity. When comparison of PGM activity is made with the normal type, the activity of the propositus and his father was 84%, and 71%, respectively, both of which were lower than that of the mother in whom it was 95%, whose phenotype was 2.

PGM1 6_{HR3}. A variant whose major band moved faster than 6_{HR2}, but slower than 6_{HR1} on electrophoresis in the TEMM buffer system, was detected in four children in Hiroshima (three unrelated children and a sibling of one), and was named 6_{HR3}. Two slow variants, 6_{HR1} and 6_{HR2} have already been reported. In phenotype 2-6_{HR3} (variant No. 30), a minor band with weak intensity was detected at the location of the *a*-band, but in three cases of another phenotype, 1-6_{HR3}, it was completely overlapped by *a*-band. Various alterations of electrophoretic conditions failed to separate it from *a*-band or to broaden the *a*-band which can suggest the existence of a variant band. In the three cases of 1-6_{HR3}, the intensity of the major band of 6_{HR3} was almost the same or weaker than that of the *c*-band, and the intensity of the minor band detected in 2-6_{HR3} was extremely weak. Of the four children, 1-6_{HR3} was detected in the father of two siblings (variant Nos. 27 and 28) and the father of a female child (variant No. 30), but for a remaining female child (variant No. 29), study of parents was not possible. Though the intensity of the bands of

6_{HR3} was weak, PGM activity in two cases of 1-6_{HR3} and a single case of 2-6_{HR3}, in whom the PGM activity could be determined, was 93%, 98% and 93% of normal, respectively.

B-2. *Variants already encountered in the Adult*

PGM1 3_{NG1}. It was reported earlier that five cases were detected in the Adult all in Nagasaki, but subsequently one case (ID No. 863215) was found in Hiroshima. In the Child, this variant was detected in 43 individuals; when seven cases among siblings are excluded, this variant was found in 36 unrelated individuals. Of the 36, 12 were born in Hiroshima and 24 in Nagasaki. The results of family studies are summarized in Table 4. Among the rare variant, 3_{NG1} is the most frequently encountered (Table 1), and the frequency in the Representative was 0.0015 (Table 2). However, the frequency in Nagasaki was 2.5 times higher than in Hiroshima (0.0022 vs. 0.0009).

When variants PGM1 $3_{OKINAWA}$ detected in Ryukyu islanders of Okinawa, the frequency of the allele which controls the isozyme PGM1 $3_{OKINAWA}$ being 0.0039 (Omoto *et al.*, 1973), and PGM1 3_{NG1} were compared on the same gel using TEMM buffer, the locations of the major bands on gels on both Connaught-starch and Electrostarch were the same, as were those of the minor bands for these two variants on Connaught-starch gel. No minor band could be detected on Electrostarch gel for these two variants.

Though the intensity of the major band of 3_{NG1} was much stronger than that of *a*- or *b*-bands, the intensity of the minor band was weaker than that of *c*- or *d*-bands. PGM activity of individuals having 1-3_{NG1} phenotype was normal: the mean activities for propositi, affected parents, non-affected parents were 1.98 IU/g Hb, 1.96 IU/g Hb, 1.90 IU/g Hb, respectively (Table 5). Thus, it appears that PGM1 3_{NG1} allozyme is more stable than PGM1 1 allozyme, that the degree of

Table 4. Family studies for PGM1 1-3_{NG1} and PGM1 2-3_{NG1} found in the Child population.

Propositus			Mother	Father
Combined	Hiroshima	Nagasaki		
12	3	9	♀	♂
13	5	8	♀	♂
2	2	0	♀	Dead
1	0	1	♀	NT
1	0	1	Dead	♂
1	0	1	Dead	♂
2	0	2	NT	Dead
3	2	1	NT	NT
1	0	1	Dead	Dead

♀,♂ Heterozygote for *PGM1*3NG1*
♀,♂ Homozygote for normal *PGM1*1*
NT Not tested

Table 5. PGM activities of children with phenotype PGM1 1-3_{NG1} and those of family members.

		n	PGM activity	
			Mean (IU/g Hb)	SD (IU/g Hb)
Propositus	Combined	15	1.98	0.21
	Hiroshima	8	2.01	0.23
	Nagasaki	7	1.95	0.20
Family	Affected (Hiroshima)	9	1.96	0.16
	Not affected (Hiroshima)	6	1.90	0.25

change from major to minor band is smaller than that from *a* to *c* or *b* to *d*, and that consequently, the major band of 3_{NG1} shows the strongest intensity of the three major bands.

PGM1 6_{NG1}. This is a variant detected as 1-6_{NG1} in a woman (ID No. 656337) of the Adult, living in Nagasaki. Since the variant was detected at the same time through family study in her son (ID No. 722577), a subject of the Child, he was regarded as the propositus and the results of the family study are shown in Table 3 (variant No. 10). 1-6_{NG1} was also detected in his maternal grandmother. The major band of 6_{NG1} located adjacent to the cathodal side of the *a*-band is the fastest variant among the so-called 'slow variants' (Satoh *et al.*, 1976). The PGM activity of the propositus was 1.96 IU/g Hb (107% of normal).

PGM1 6_{NG2}. This variant was the most frequently found slow variant. Its major and minor bands have exactly the same mobility as those of the PGM1 6 provided by Dr. Lie-Injo (Satoh *et al.*, 1976). In the Child, PGM1 6_{NG2} was found in 10 individuals, but if those detected in siblings are excluded, one case each of 2-6_{NG2} and 7-6_{NG2} were detected in Hiroshima and five cases of 1-6_{NG2} in Nagasaki, totalling seven 6_{NG2} cases. The results of family studies of these seven cases are shown in Table 3. The mean PGM activity of the 10 propositi with 6_{NG2} in the Child was 1.93 IU/g Hb (SD=0.26 IU/g Hb), and that of the five parents with variants was 1.80 IU/g Hb (SD=0.30 IU/g Hb), 105% and 98% of normal, respectively. In view of the fact that the value of the four parents with normal type was 1.92 IU/g Hb (SD=0.26 IU/g Hb), PGM1 6_{NG2} activity is assumed to be normal. Band intensities of 6_{NG2} suggested the same conclusion. The frequency of the allele *PGM1*6NG2* in Nagasaki was higher than in Hiroshima in the Representative (Table 2): 0.00039 vs. 0.00022.

PGM1 6_{HR1}. The minor band of this variant was observed to migrate slightly anodal to the position of the *a*-band in the phenotype PGM1 2-6_{HR1}, but in 1-6_{HR1}, it could not be separated from the *a*-band. The intensity of the major band was slightly weaker than that of the *a*-band. A single case each of 1-6_{HR1} and 2-6_{HR1} were detected in Hiroshima individuals in the Adult and the Child, respectively. The previous paper has already described the former (1-6_{HR1}, ID No. 226127). The

second individual, 2-6_{HR1} (variant No. 22), is in the Child, and at the same time the son of the Hiroshima woman (ID No. 249427) in the Adult who was described as 1-6_{HR1} in the previous report at the time of study using histidine-citrate discontinuous buffer system. Since only the results of studies using TEMM buffer are reported in this paper, it was decided to treat the son as the propositus and the mother a member of the family. Since the father is PGM1 1-2, the phenotype PGM1 2-6_{HR1} of the propositus is obviously inherited from his parents.

PGM1 6_{HR2}. The anodal side of the minor band of this variant was in contact with the *a*-band on the cathodal side. The intensities of both the major and minor bands of 6_{HR2} were clearly lower than those of the *a*, *b*, *c* and *d* bands. However, no difference in PGM activity was observed between these variant phenotypes and PGM1 1. A report was previously made of the three cases detected in the Adult and among them was father and son combination (ID Nos. 016475 and 093662). The four cases detected in the Child were all observed to have the 2-6_{HR2} phenotype. Among these, one individual in Nagasaki was a grandchild of the family of the above-mentioned father and son combination. The result of the family study centering around him as the propositus (variant No. 23), is shown in Table 3.

PGM1 8_{NG1}. Two cases of this variant were detected during study of the Adult using histidine-citrate discontinuous buffer, but in subsequent electrophoresis using TEMM buffer, none were found in the Adult though it was detected in the Child. In all, 14 cases of 8_{NG1} were encountered, but with siblings excluded, it was found in 10 unrelated individuals. In all eight families in which both parents were studied, it was detected in one of the parents in each family. Under our electrophoretic conditions, the minor band of this variant was always detected on the cathodal side of *a*-band and clearly separated from it. The mobility of this variant under various different conditions and the fact that it was confirmed to be electrophoretically identical with PGM1 8 by Dr. D.A. Hopkinson, have already been reported (Satoh *et al.*, 1976). The intensity of the major band of 8_{NG1} was slightly weaker than that of the *a*-band for almost all cases, but in the 10 children in whom PGM activity was measured, the mean value was 1.86 IU/g Hb (SD = 0.22 IU/g Hb), which is normal. Among these, the intensity of the variant band was extremely weak in variant No. 39 in two blood samples which were obtained in different occasions, thus it may be more labile than the others. However, since this is the case whose father was dead and his mother and sister did not have this variant, whether the lability is of a genetic nature or not could not be established.

C. *Effect of buffers on relative mobility of variants*

In a previous report on PGM1 of the Adult, it was stated that although the mobility of 6_{HR2} is greater (migrates a greater distance on the anodal side) than 8_{NG1} in TEMM buffer, in the histidine-citrate discontinuous buffer system, 8_{NG1} migrated further to the anodal side. This reverse phenomenon was observed also for 8_{NG1} and the newly detected variant 6_{HR3} (Fig. 6-B and Fig. 7), and for another new

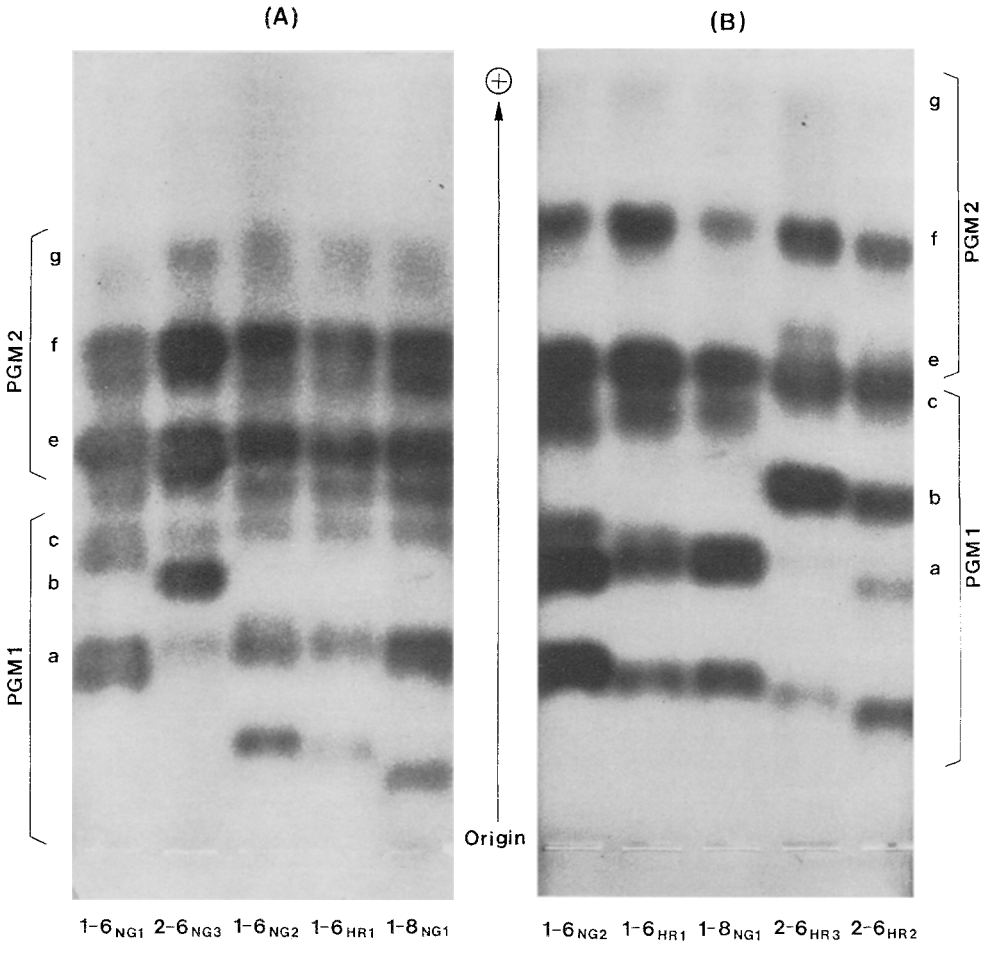

Fig. 6. The same seven PGM1 variants shown in Figs. 4 and 5 on starch gel using a histidine-citrate discontinuous buffer system, pH 7.0.

variant 6_{NG3} and 6_{NG1} (Fig. 6-A and Fig. 7). Further, as briefly reported (Takahashi and Satoh, 1982), the relative mobility of variants on polyacrylamide slab gel isoelectric focusing (IEF) was also different from the two kinds of relative mobilities obtained by the two buffer systems on starch gel electrophoresis. When the slow variants are listed according to mobility from the anodal side, they are:

In TEMM buffer,

$\underline{6_{NG1} \gg 6_{NG3}} > 6_{NG2} > 6_{HR1} > \underline{6_{HR3} > 6_{HR2}} > 8_{NG1}$,

In histidine-citrate buffer,

$\underline{6_{NG3} > 6_{NG1}} > 6_{NG2} > 6_{HR1} \geqq 8_{NG1} > \underline{6_{HR3} > 6_{HR2}}$,

By IEF, $6_{NG1} > 6_{HR1} > 6_{NG2} \geqq 6_{HR3} > 6_{NG3} \geqq 6_{HR2} > 8_{NG1}$.

Groups of underlined variants showed reversed relative mobility. Based on the

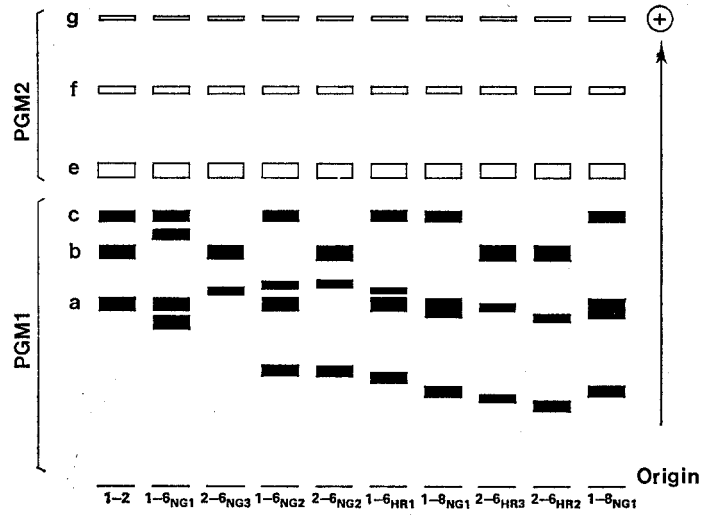

Fig. 7. Diagram of the same seven PGM1 variants shown in Figs. 4 and 5 on starch gel using histidine-citrate discontinuous buffer system, pH 7.0.

differences in mobility obtained by these three electrophoretic methods, variants can be distinguished which are otherwise difficult to separate because of only slight differences in mobility. The reason for the change in relative mobility caused by different electrophoretic conditions is now under study and it will be the subject of a separate paper.

DISCUSSION

Polymorphism of human PGM1 based on the presence of two alleles *PGM1*1* and *PGM1*2* has been observed in all populations in the world heretofore examined by starch gel electrophoresis and the allele *PGM1*7* has been encountered in polymorphic proportions in the Pacific area such as Western Caroline Islands (Blake *et al.*, 1973), west Malaysia (Welch *et al.*, 1972), Chinese (Lie-Injo *et al.*, 1968; Lie-Injo and Poey-Oey, 1970), Okinawa (Omoto *et al.*, 1973) and Japan (Satoh *et al.*, 1977; Nishigaki *et al.*, 1978). *PGM1*3* has been also found in polymorphic frequency in New Guinea (Blake and Omoto, 1975). In addition to these common alleles, at least ten kinds of rare electrophoretic variants have been reported (Hopkinson and Harris, 1966; Blake and Omoto, 1975; Satoh *et al.*, 1977). Therefore, human PGM1 is known as an enzyme of high genetic diversity even in the days when starch gel electrophoresis using TEMM buffer of Spencer *et al.* (1964) was the principal technique.

Later, isoelectric focusing (Bark *et al.*, 1976; Kühnl *et al.*, 1977; Sutton and Burgess, 1978; Dobosz and Kozioł, 1980; Dykes and Polesky, 1981) and acid starch gel electrophoresis (Bissbort *et al.*, 1978) classified the conventional PGM_1^1 into two

subtypes, PGM_1^{1+} and PGM_1^{1-} (or PGM_1^{a1} and PGM_1^{a3}), and PGM_1^{1S} and PGM_1^{1F}, respectively, and the PGM_1^{2} into two subtypes, PGM_1^{2+} and PGM_1^{2-} (or PGM_1^{a2} and PGM_1^{a4}), and PGM_1^{2S} and PGM_1^{2F}, respectively. Furthermore, Scozzari *et al.* (1981) reported an electrophoretically cryptic polymorphism of human PGM1 based on the sensitivity to heat denaturation. According to them, each of four 'isoelectric point alleles' was subtyped into a heat sensitive allele and a heat resistant allele, making eight common alleles in all. Thus, PGM1 is a very diversified protein and is potentially a system that may provide clues to the molecular evolution and origin of populations.

Recently, we reported that the conventional allele *PGM1*7* can be subtyped into *PGM1*7+* and *PGM1*7−* (Takahashi *et al.*, 1981) and *PGM1*3* into *PGM1*3+* and *PGM1*3−*, by isoelectric focusing (Takahashi *et al.*, 1982). Family studies confirmed these isoelectric point subtypes as real allels. Based on the isoelectric points of all of the 8 'isoelectric point alleles,' four of which are common to all the populations of three human races along with the four new alleles found in Japanese, and considering the distribution of the conventional alleles of *PGM1*7* and *PGM1*3* in the Pacific area, we proposed an evolutionary phylogeny of 'isoelectric point alleles' of PGM1; an enlargement of the hypothesis originally proposed by Carter *et al.* (1979).

The existence of the *PGM1*7* allele in polymorphic proportions in the Japanese was first described by us (Satoh *et al.*, 1977). Nishigaki *et al.* (1978) also observed this polymorphism of the *PGM1*7* allele in another Japanese population. When one of their samples whose starch gel electrophoretic phenotype was PGM1 7 was examined by IEF, Nishigaki detected two different bands one of which focused anodally to the secondary band of PGM2 1 and the other focused cathodally to the primary band of PGM2 1. He considered them to be the bands of PGM1 7^+ and PGM1 7^-, respectively (Nishigaki *et al.*, 1982). Later we were asked to compare his sample with our samples known to be heterozygous for PGM1 7^+ or PGM1 7^-, and found that the two bands from his sample migrated to the same positions as those of our samples, the primary bands of PGM1 7^+ and PGM1 7^- moving slightly anodally and cathodally to the primary band of PGM2 1, respectively, thus confirming the phenotype of his sample to be PGM1 7^+-7^- (Takahashi *et al.* in preparation). The migration position of PGM1 7 in a Japanese individual living in Tokyo area examined with IEF by Maneyama *et al.* (1978), was the same as that of our PGM1 7^-. Thus, the conventional PGM1 7 discovered in Japanese populations seems to be either PGM1 7^+ or PGM1 7^-. Nevertheless, those rare PGM1 7 variants described Kühnl and Spielmann (1978), Santachiara-Benereccetti *et al.* (1982), and Tipler *et al.* (1982) focused at various positions, all of which were different from those of the Japanese PGM1 7^+ and PGM1 7^-, though they were reported to migrate to the same position as that of PGM1 7 of Hopkinson and Harris (1966) on starch gel electrophoresis. One possibility is that they are different rare variants whose mobility is slightly different from that of PGM1 7, but without direct com-

parison it is difficult to distinguish them. A second possibility is that different variants showed the same mobility as that of PGM1 7 on starch gel electrophoresis and only IEF can detect the difference in mobility. A third possibility is sample deterioration. For the first possibility, we have found that though PGM1 9_{NG1} showed a slightly different mobility from that of PGM1 7 on starch gel electrophoresis using TEMM buffer, gel buffer being 1 : 15 diluted bridge buffer, they were indistinguishable on the gel using a 1 : 10 diluted bridge buffer.

Differences in variation of PGM1 between Hiroshima and Nagasaki, both in kinds and in frequency of alleles were noted previously (Satoh *et al.*, 1977; Neel *et al.*, 1978) and were again observed in the Representative described in this paper which is approximately 6 times larger than the previous population: *PGM1*8NG1* was observed in 6 unrelated Nagasaki individuals but none in Hiroshima, *PGM1*3NG1* was detected in 23 unrelated individuals from Nagasaki but only in 12 individuals from Hiroshima, and the total number of rare variants encountered in Nagasaki was 39 as opposed to 25 in Hiroshima. The differences between two cities in the frequencies of the *PGM1*3NG1* allele and of the total rare alleles are statistically significant ($p<0.01$ for both). Plainly, the populations of Hiroshima and Nagasaki are genetically different. One possible explanation, among others, for this phenomenon may lie in the migration into the Japanese Islands in prehistoric ages for which two different major routes are postulated: one from the south through the Ryukyu Archipelago, the other through the Korean Peninsula (Takahashi *et al.*, 1982). Since on starch gel electrophoresis the mobility of PGM1 $3_{OKIANWA}$ found in Ryukyuans from Okinawa Island is identical with that of PGM1 3_{NG1} and the allele frequency of the *PGM1*3OKINAWA* was reported to be 0.0039 while that for *PGM1*3NG1* was 0.0022 in Nagasaki and 0.0009 in Hiroshima, the difference seems to express the stronger influence of the southern migration stream into Nagasaki (Kyushu) compared to Hiroshima, on Japan's mainland (Honshu) northeast of Nagasaki.

Among slow variants, 6_{NG2} and 8_{NG1} showed the same mobility as that of 6 of Lie-Injo *et al.* (1968) and that of 8 of Hopkinson and Harris (1966), respectively, on the same starch gel of comparison run. In addition to these two variants at least 4 kinds of slow variants (6_{NG1}, 6_{HR1}, 6_{HR2}, 8_{Nara}) have been reported in Japanese (Satoh *et al.*, 1976) and two additional variants (6_{NG3} and 6_{HR3}) are described in this paper. Though Blake and Omoto (1975) discussed the heterogeneity of 6 and 8 detected in the various populations, in most of the reports slow variants were named 6 or 8 without precise characterizations and no data were shown to determine whether they are electrophoretically identical with one of Japanese variants or one of other slow variants discussed by Blake and Omoto (1975). Given the sporadic distribution and probable heterogeneity among the slow variants, at present they do not seem to be adequate markers for discerning population movements.

The work described here is the largest account of information yet reported for the PGM1 in the Japanese. The diversity of PGM1 among the Japanese is clearly

demonstrated in the results from screening of over 17,000 individuals, in contrast to PGM2 which is monomorphic and the occurrence of variants is very rare, despite probable common origin through gene duplication (Hopkinson and Harris, 1969). In a hypothetical evolutionary phylogeny for the PGM1 alleles (Takahashi *et al.*, 1982), we suggested that the *PGM1*7* allele played an important role in establishing PGM1 diversity in Japan. This hypothesis will be tested in the near future when probes for the gene encoding PGM1 become available. The variants described here should be excellent components for this investigation.

Recently, human population genetic studies have increasingly made use of the IEF since it is able to separate subtypes and variants of proteins not separable by SGE, while on the other hand, there are certain isozymes which cannot be separated by IEF but are effectively distinguished by SGE (Takahashi and Satoh, 1982; Fujita *et al.*, in preparation). An example is the combination of PGM1 2+ and PGM1 7−. Thus, though IEF is now a necessary tool, SGE too, is essential in human population genetics.

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