Ann. Hum. Genet., Lond. (1977), **41**, 169
Printed in Great Britain

The frequency in Japanese of genetic variants of 22 proteins

III. Phosphoglucomutase-1, phosphoglucomutase-2, 6-phosphogluconate dehydrogenase, adenylate kinase, and adenosine deaminase

By C. SATOH,* R. E. FERRELL,†‡ R. J. TANIS,† N. UEDA,* S. KISHIMOTO,* J. V. NEEL,† H. B. HAMILTON* and K. BABA*

The present paper will describe the variants encountered in Japanese adults from Hiroshima and Nagasaki with respect to the following five erythrocyte enzymes: phosphoglucomutase-1 (PGM_1, EC 2.7.5.1), phosphoglucomutase-2 (PGM_2, EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.43), adenylate kinase (AK, EC 2.7.4.3) and adenosine deaminase (ADA, EC 3.5.4.2). The first paper in this series described the nature of the population and the circumstances under which the collections were made (Ferrell *et al.* 1977). As before, the convention in naming variants which do not clearly correspond to known types will be by city and order of discovery, abbreviating Hiroshima as HIR and Nagasaki as NGS.

MATERIALS AND METHODS

The preparation of the haemolysates for electrophoresis has been described in the second paper of the series (Ueda *et al.* 1977). Vertical starch-gel electrophoresis at 4 °C employing Electrostarch (13·3 %, lot nos. 146, 371 and 303) was used for the routine typing of all systems. The histidine–sodium citrate discontinuous buffer system, pH 7·0, of Fildes & Harris (1966), originally developed for the study of AK, was used for the AK, ADA and 6-PGD determinations. For the routine typing of these systems electrophoresis was at 9 V/cm for 4 h; comparison of the rare variants of 6-PGD was at 5 V/cm for 20 h. The citrate buffer system, pH 5·0, of Spencer, Hopkinson & Harris (1968) was used to confirm the ADA 2 phenotype. During the first half of this study (2134 samples), PGM_1 and PGM_2 were also typed using the histidine–sodium citrate buffer system mentioned above, with electrophoresis at 5 V/cm for 20 h. This permitted acid phosphatase determinations from a slice of the same gel. However, a tris-maleic acid buffer, pH 7·4 (Spencer, Hopkinson & Harris, 1964) was found to give superior resolution of the fast variants of PGM_1 and was employed for the last 1895 determinations. The resolution was further improved by using a 1:15 dilution of bridge buffer as the gel buffer, at 7 V/cm for 23 h. The results of the two PGM methods will be presented separately.

Staining of the isozymes of PGM was performed according to the method of Spencer *et al.* (1964). ADA, 6-PGD and AK were typed from three slices of a single gel using the staining methods

* Radiation Effects Research Foundation, Hiroshima, Japan. The Foundation, the successor to the Atomic Bomb Casualty Commission (ABCC), was established in April 1975 as a private non-profit Japanese Foundation, under the auspices of the Ministry of Health and Welfare and Finance Ministry, supported equally by funds from the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contracts with the Energy Research and Development Administration, the National Cancer Institute and the National Heart and Lung Institute.

† Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109. Financial support for the study derived from Contract E(11-1)-1552 with the Energy Research and Development Administration, Washington, D.C.

‡ Present address: Center for Demographic and Population Genetics, Graduate School of Biomedical Sciences at Houston, The University of Texas, Houston, Texas 77025.

Table 1. *The different phenotypes observed in the* PGM_1 1 *system*

(Data are presented by city and buffer system employed. See text for details regarding the differences between the histidine–sodium citrate and tris-maleic acid buffer system.)

	Hiroshima		Nagasaki		Combined	
Phenotype	His	Tris	His	Tris	His	Tris
1	1078	497	222	592	1300	1089
2–1	594	293	114	338	708	631
2	103	52	12	52	115	104
1–7	0	23	0	16	0	39
2–7	0	4	0	9	0	13
7	0	0	0	2	0	2
$1\text{–}3_{NGS\ 1}$	0	0	0	4	0	4
$2\text{–}3_{NGS\ 1}$	0	0	0	1	0	1
$1\text{–}3_{NGS\ 2}$	0	0	0	1	0	1
$1\text{–}6_{HIR\ 1}$	1	1	0	0	1	1
$1\text{–}6_{HIR\ 2}$	1	0	0	2	1	2
$2\text{–}6_{HIR\ 2}$	0	0	0	1	0	1
$1\text{–}6_{NGS\ 2}$	0	0	1	2	1	2
$1\text{–}6_{NGS\ 1}$	0	0	0	1	0	1
$1\text{–}8_{NGS\ 1}$	1	0	2	0	3	0
$1\text{–}9_{NGS\ 1}$	0	0	0	1	0	1
No type	3	2	2	1	5	3
Total number	1781	872	353	1023	2134	1895
	2653		1376		4029	

Table 2. *PGM_1 gene frequencies in* 1892 *Japanese blood specimens (tris-maleic acid buffer system)*

	Hiroshima	Nagasaki
PGM_1^1	0·7534	0·7578
PGM_1^2	0·2305	0·2216
$PGM_1^{3\ NGS\ 1}$	0·0000	0·0024
$PGM_1^{3\ NGS\ 2}$	0·0000	0·0005
PGM_1^7	0·0155	0·0142
$PGM_1^{6\ HIR\ 1}$	0·0006	0·0000
$PGM_1^{6\ HIR\ 2}$	0·0000	0·0015
$PGM_1^{6\ NGS\ 2}$	0·0000	0·0010
$PGM_1^{6\ NGS\ 1}$	0·0000	0·0005
$PGM_1^{9\ NGS\ 1}$	0·0000	0·0005

of Spencer *et al.* (1968), Fildes & Parr (1963) and Fildes & Harris (1966), respectively. For the typing of ADA, a cellulose acetate overlay technique was used instead of an agar overlay technique.

Some comparison runs of PGM_1 variants were also carried out with horizontal electrophoresis using Connaught starch gels (13·3 %, lot No. 314–2); the gel buffer was made by diluting the bridge buffer in a 1:15 ratio. In comparisons of variant zymograms of PGM_1, a gel buffer made by a 1:10 dilution of the bridge buffer was also used for both kinds of starch gel.

In any study of this nature, there are always a few isozyme patterns which for a variety of reasons – low enzyme activity, indistinct patterns which may be due to degradation, etc. – cannot be typed with confidence. We omit these from the figures for 'number typed', recognizing that this group has a disproportionately high frequency of containing poorly resolved variants.

RESULTS

Phosphoglucomutase-1

With respect to PGM_1, the results obtained with the two different buffer systems are presented separately (Table 1). There were eight specimens not typed in the total of 4029. We consider the histidine–sodium citrate system so unsatisfactory that although we describe the variants detected by its use, the calculation of variant frequencies is based solely on the results of the tris-maleic acid system.

The PGM_1 polymorphisms. Two alleles occur in polymorphic proportions, PGM_1^2 and PGM_1^7. There are over 20 reports which describe the PGM_1 polymorphism of Japanese; total samples examined amounted to 11680 from 26 different populations (Ishimoto, 1975). The gene frequencies for PGM_1^2 are in the range of 0·191–0·249 except in a few isolated populations. The values obtained in our studies fall on the high side of that range (Table 2).

The frequencies of the PGM_1^7 gene were 0·016 and 0·014 for the Hiroshima and Nagasaki residents, respectively. The two persons with PGM_1 7 phenotype were found in Nagasaki residents. No presumptive homozygotes for the PGM_1^7 allele were found in the routine samples comprising the population study in Hiroshima. However, in the course of a family study of a middle aged, healthy woman who showed the PGM_1 1–7 phenotype, the PGM_1 7 phenotype was found in her first son, the PGM_1 type of her husband and that of the second son being 1–7. In addition to this family, limited genetic studies were undertaken on 31 propositi who showed the PGM_1 1–7 and PGM_1 2–7 phenotypes. The results are shown in Table 3. In 23 cases other members of the family were found to have the variant. In most instances when the presence of the variant was not confirmed, the number of first degree relatives examined was quite small (average 1·5).

In the studies of 26 populations throughout Japan referred to earlier (Ishimoto, 1975), the PGM_1^7 gene frequency ranged from 0·0010 to 0·0026. The present frequency is approximately ten times greater. The difference could stem from the nature of the populations sampled or from technical reasons. In this connexion we note that Ishimoto and co-workers (Ishimoto, Kuwata & Kubota, 1973; Ishimoto & Kuwata, 1973), on the basis of 526 determinations, reported the PGM_1^7 allele to have a frequency of 0·0019 in Hiroshima and, on the basis of 608 determinations, a frequency of 0·0016 in Nagasaki. Although real differences in the populations being sampled cannot be excluded, the discrepancy between the results of Ishimoto and colleagues and ourselves forces us to consider technical factors carefully. With respect to the possibility that the difference rests in technique, most investigators, especially Japanese, employ Connaught starch and a gel buffer derived from a 1:10 dilution of bridge buffer, according to the original paper of Spencer *et al.* (1964). We have used Electrostarch with the gel buffer a 1:15 dilution of the bridge buffer.

When zymograms obtained with gels prepared from Electrostarch and the Connaught starch were compared, both made with a 1:10 dilution of the bridge buffer, the separation of the *a*, *b*, *c* and *d* bands associated with the PGM_1^1 and PGM_1^2 alleles and those produced by the PGM_1^3 and PGM_1^7 alleles was better on Electrostarch. The area between components *d* of PGM_1 and *e* of PGM_2 is wider on Electrostarch, so that the variant bands determined by the PGM_1^7 allele are more easily distinguished. Furthermore, a 1:15 gel buffer dilution results in clearer separation between the variant bands and the normal *b*, *c* and *d* bands on Electrostarch as shown on the left of Fig. 1. Blake & Omoto (1975) also recommended a 1:15 dilution of the bridge buffer for the gel buffer on the Connaught starch to produce better separation on the PGM_1 1–7 isozymes.

Table 3. *Tabular summary of the results of family studies on variants of PGM_1 and 6PGD*

Although the type 7 variant of PGM_1 has the frequency of a polymorphism, this was not apparent in the first portion of the investigation, and the family studies of this variant are included in this table. Solid symbols indicate the presence of a heterozygous variant (in one case, §, of a homozygous).

Variant	City	Propositus	Sex	Family studies: Mo	Fa	Sibs	Children
			System: PGM_1				
$3_{NGS\ 1}$	N	002396	M	—	—	—	*♀ ♂
$3_{NGS\ 1}$	N	011866	M	♀	—	—	*● ● ●
$3_{NGS\ 1}$	N	081961	F	—	*●	—	♂
$3_{NGS\ 1}$	N	164708	M	—	—	♂	*♂ ♀
$3_{NGS\ 1}$	N	028180	F	—	—	—	*● ●
$6_{NGS\ 2}$	N	003385	F	—	—	—	*●
$6_{NGS\ 2}$	N	014106	M	—	—	—	*♀ ●
$6_{HIR\ 1}$	H	226127	M	—	—	*♂	—
$6_{HIR\ 1}$	H	249427	F	—	—	—	*●
$6_{HIR\ 2}$	N	016475	M†	—	—	—	*●
$6_{HIR\ 2}$	N	018458	F	—	—	—	*♀ ♀ ● ●
$6_{HIR\ 2}$	N	093662	M‡	—	*●	—	—
$6_{HIR\ 2}$	H	235994	M	—	—	—	*●
$8_{NGS\ 1}$	N	037186	M	—	—	—	*● ♂ ● ● ●
$8_{NGS\ 1}$	N	120576	M	—	—	—	*● ♂ ♂
$9_{NGS\ 1}$	N	010166	M	—	—	—	*● ♂ ●
7	H	205214	F	—	—	—	*♂ ●
7	H	217724	M	—	—	—	*♂ ●
7	H	312826	M	*♀	—	♂	—
7	H	325054	F	—	—	—	*●
7	H	333203	F	—	—	—	*●
7	H	335221	F	—	—	—	§* ● ●
7	H	347423	F	—	—	—	*♂
7	H	845012	M	—	—	—	*♀
7	H	219485	F	—	—	—	*●
7	H	203481	M	—	—	—	*♀ ♀
7	H	276732	F	—	—	—	*♂ ● ♀
7	H	217501	F	—	—	—	*♂
7	H	227610	F	—	—	—	*♂
7	H	216645	F	—	—	—	*●
7	H	341359	F	—	—	—	*♂
7	N	041512	F	—	—	—	*♂ ♀
7	N	093824	F	—	—	●	*♂ ♀ ♂
7	N	097732	F	—	—	—	*♀ ●
7	N	016329	M	—	●	♀	*♂ ♂
7	N	020987	M	—	—	—	*♀ ● ♂
7	N	022815	M	—	—	—	*●
7	N	074446	F	—	—	—	*♀ ♂ ●
7	N	012819	F	—	—	♂ ♂ ●	*♀
7	N	021060	F	—	—	● ● ♀	*♂ ♀ ♀
7	N	034245	M	—	—	—	*♂ ●
7	N	009293	F	—	—	●	*♂ ♂ ♀ ♂
7	N	005758	F	●	♂	—	*♀ ●
7	N	021246	F	—	—	*● ●	♀ ♂
7	N	075532	F	●	—	♀	*● ●

* Indicates first primary relative tested in the family study.
† Father of 093662.
‡ Son of 016475.
§ Homozygous variant.

Table 3 (*cont.*)

Variant	City	Propositus	Sex	Family studies: Mo	Fa	Sibs	Children
7	N	007568	M	—	—	—	*♀
7	N	090727	F	—	—	*♀♀	—
7	N	089810	M	—	—	—	*♀♂
			System: 6PGD				
NGS 1	N	001572	M	—	—	—	*♀♀
HIR 1	H	286195	F	—	—	—	*♀♂
HIR 2	H	329045	F	—	—	—	*♀♂

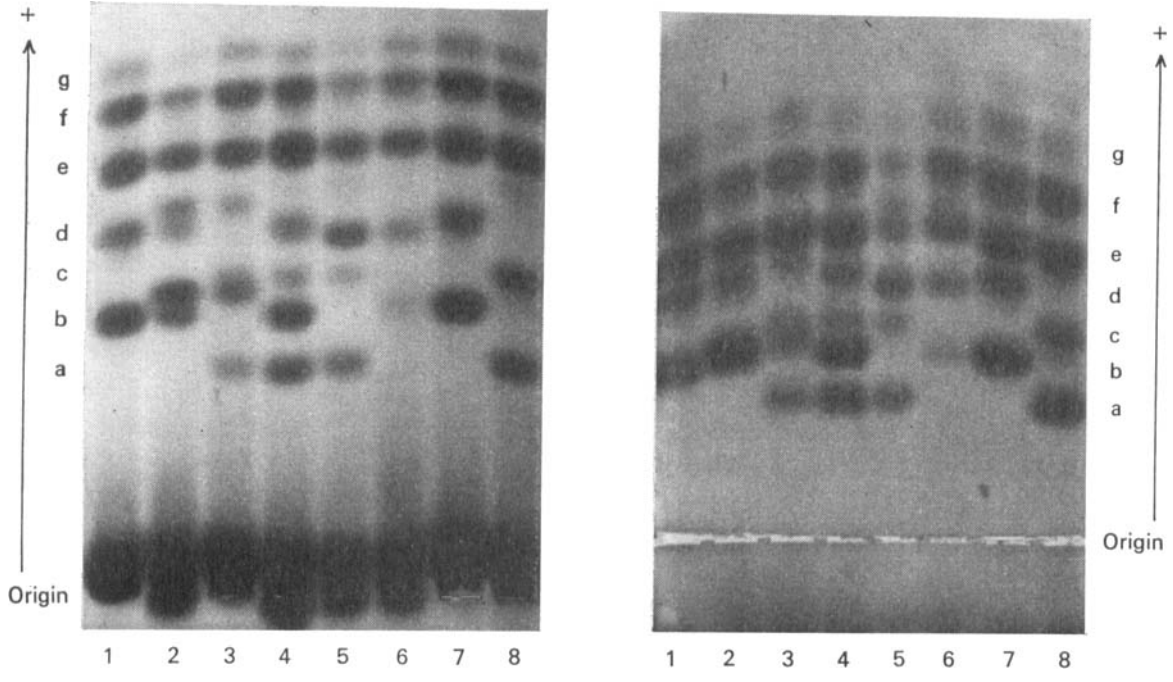

Fig. 1. Starch-gel electrophoresis of phosphoglucomutase. (A) Zymogram of PGM on the Electrostarch gel (left) and (B) Zymogram of PGM on the Connaught starch gel (right). Well 1, PGM_1 2; well 2, PGM_1 2–7; well 3, PGM_1 1–7; well 4, PGM_1 1–2; well 5, PGM_1 1–3; well 6, PGM_1 2–3; well 7, PGM_1 2; well 8, PGM_1 1.

However, note on the right of Fig. 1 the results obtained with Connaught starch using a 1:15 buffer dilution; clearly the PGM_1 2–7 phenotype is difficult to distinguish from the PGM_1 2 phenotype on Connaught starch, while these two phenotypes are easily distinguishable from one another on the Electrostarch gel.

In the case of the PGM_1 1 and 1–3 phenotypes two very faintly staining bands in addition to the usual bands were found on Electrostarch gels made with a 1:15 dilution. One was located in the position of the *d* band, while the other was located slightly anodal to *d* and cathodal to *e*. Examination of the PGM_1 1–2 or PGM_1 2 phenotypes revealed only the latter band, the former presumably being superimposed on the *d* band. The minor band associated with $PGM_1^?$ is located intermediate between these two bands. These minor bands provide a useful clue in avoiding an erroneous classification of PGM_1 1–7 or 2–7 as 1–2 or 2.

During the comparison of the PGM zymograms on the two kinds of the starch, haemoglobin was found to move cathodal relative to the origin on the Connaught starch, while it moved slightly anodal to or stayed at the origin on the Electrostarch. When the pH of the supernatant of a mixture of the starch and the buffer was examined after 30 min of stirring, the pH was 7·25 and 7·20 when Electrostarch (lot no. 371) was employed, at buffer dilutions of 1:10 and 1:15, respectively; however, it was 7·05 and 6·90 at the same dilutions when Connaught starch (lot no. 311–1) was used. This suggested that the difference in the zymograms obtained with the two types of starch might have arisen from the different pH of the starch gels. After adjustment of the pH of the mixture to 7·25, the PGM zymogram on the Connaught starch was similar to that on Electrostarch. PGM_1 2–7 showed four distinct isozyme bands even though haemoglobin migrated cathodally. The acidity of the Connaught starch was found to differ from lot to lot (pH range 6·8–7·05 with the 1:15 gel buffer dilution). PGM_1 was the most sensitive protein to the properties of the starch gel used to screen the 22 systems examined in our study. We believe that these slight technical differences, i.e. the kind and lot of starch, the pH and the concentration of the gel buffer, are a principal reason why we found the PGM_1^7 allele more frequently than it has been reported elsewhere in Japan (except in Okinawa Island, where the gene frequency for the PGM_1^7 allele was found to be 0·015 among a sample of 647 Ryukyuans; Omoto *et al.* 1973).

Frequencies of the PGM_1^7 allele comparable to those of this paper have been encountered in several other populations in the Western Pacific Region (review in Blake & Omoto, 1975): a frequency of 0·059 among 382 Micronesians in the Western Caroline Islands (Blake *et al.* 1973), and a lesser frequency of 0·011 among 88 Chinese-Indonesians in Indonesia (Lie-Injo & Poey-Oey, 1970) and of 0·010 among 920 aborigines from the west of Malaysia (Welch, Lie-Injo & Molton, 1972). Thus the allele, if it is identical throughout its distribution, appears to be widespread in the Pacific Islands and of considerable antiquity.

The PGM_1 rare variants. Six samples from Nagasaki showed a variant band on Electrostarch gel the electrophoretic mobility of which was similar to that of the major isozyme of the PGM_1 3 phenotype found by Hopkinson & Harris (1966); that is, slightly cathodal to the *d* band. For five of these six samples the intensity of the major variant band was stronger than bands *a* and *c*. When the specimens were examined on Connaught starch gel, a minor variant band could be seen slightly cathodal to the middle of the area between the bands *e* and *f*. Although gels made from Electrostarch have been found to be better than those made from Connaught starch for the detection of variant isozymes which appear around band *d*, the minor isozyme band mentioned above could not be seen when these five samples were processed on an Electrostarch gel. We suspect this minor band is superimposed on isozyme *e* in the Electrostarch preparations. The minor isozyme band was also not seen in runs on gels made from certain lots of Connaught starch. On the other hand, the sixth sample, even when it was very fresh, showed weak activity of the major variant band together with normal activity of the *a* and *c* isozymes, but the minor band described above could not be recognized at all, even on a Connaught starch gel. After 1 week of storage at −70 °C the major variant band of the sixth sample could not be seen, although it still showed strong activity in the other five samples. Although it has the same electrophoretic mobility, this labile variant seems different from the five other stable variants. Studies on the families of two propositi who showed the stable phenotype in combination with the PGM_1 1 phenotype and one propositus who exhibited the stable phenotype in combination with the PGM_1 2 phenotype confirmed that this phenotype has a genetic basis (cf. Fig. 2 and Table 3).

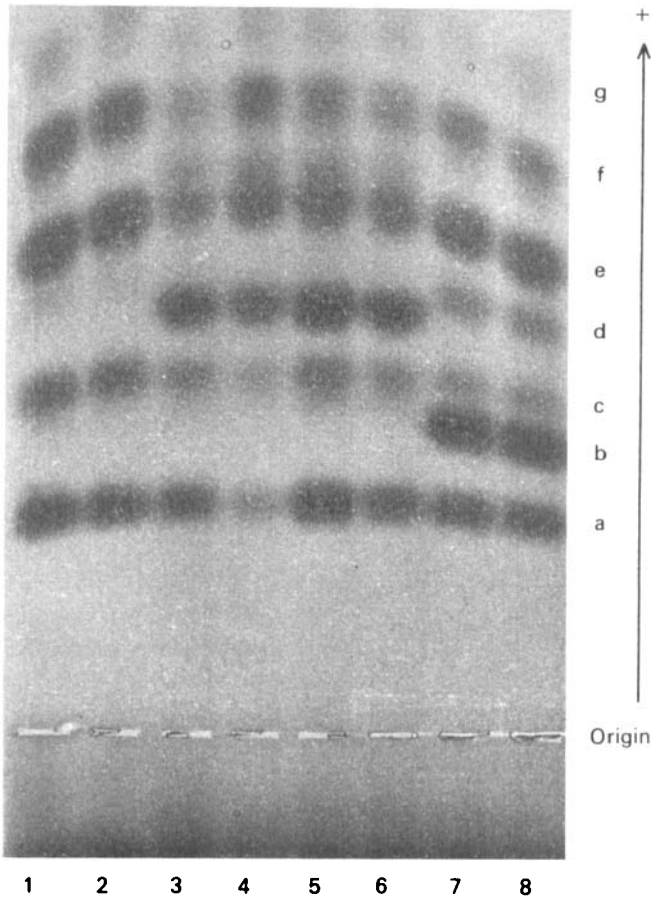

Fig. 2. Starch-gel electrophoretic patterns of normal and variant phosphoglucomutase on Connaught starch gel. Well 1 and 2, PGM_1 1; well 3, PGM_1 $1\text{–}3_{NGS\,1}$ (propositus); well 4, 5 and 6, PGM_1 $1\text{–}3_{NGS\,1}$ (the first daughter, the second daughter and the first son of the propositus); well 7 and 8, PGM_1 1–2.

Table 4. *Phenotypes and gene frequencies for the 6-PGD polymorphism in Hiroshima and Nagasaki*

Phenotype	Hiroshima	Nagasaki	Combined
A	2213	1117	3330
A–C	419	242	661
C	14	6	20
Variants	2	1	3
No type*	5	10	15
Total	2653	1376	4029
PGD^A†	0·916	0·907	0·913
PGD^C	0·084	0·093	0·087
χ^2	1·534	3·483	
P	$0{\cdot}20 < P < 0{\cdot}30$	$0{\cdot}05 < P < 0{\cdot}10$	

* These samples gave a 6-PGD pattern which could not be typed accurately. The sample which showed abnormal pattern which is described in page 179 is scored as 'no type'.

† Calculation of gene frequencies excludes no types and variants.

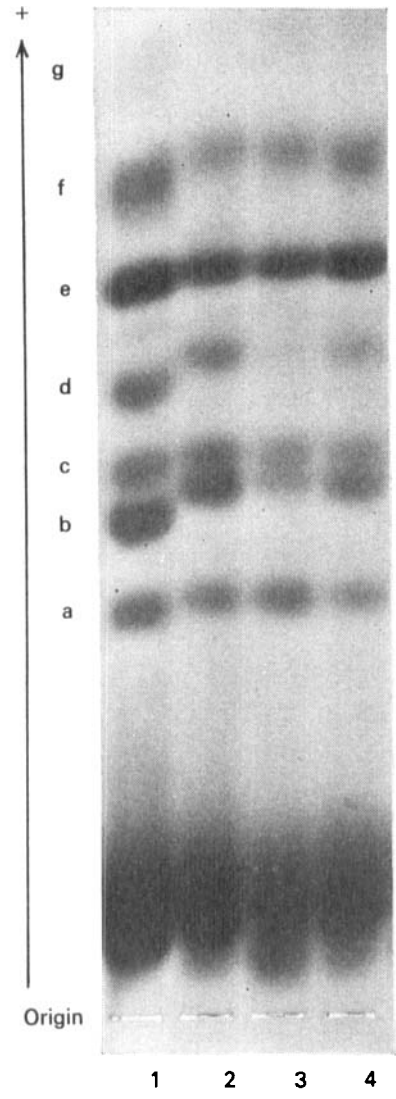

Fig. 3. Starch gel comparing the phenotypes of PGM_1 $1\text{–}9_{NGS\,1}$ and PGM_1 1–7; well 1, PGM_1 1–2; well 2 and 4, PGM_1 1–7; well 3, PGM_1 1–9.

We propose to designate this phenotype as $PGM_1\,3_{NGS\,1}$ and the responsible allele as $PGM_1^{3\,NGS\,1}$. Family studies have not been performed on the labile variant, which we shall designate as $PGM_1\,3_{NGS\,2}$. Since in any case it is an activity (rather than additional electrophoretic) variant, we shall not count it as an additional variant in the statistical considerations of our summary paper (Neel *et al.* 1977).

Hopkinson & Harris (1966) first encountered the rare PGM_1 1–3 phenotype in a single individual in a sample of 2115 English persons; another rare phenotype, PGM_1 2–5, was found in a single individual in this same sample. The major isozyme of $PGM_1\,3$ has a mobility slightly slower than *d* and the minor isozyme had a mobility slightly faster than *e*. On the other hand, one of the isozymes of $PGM_1\,5$ had a mobility intermediate between *c* and *d* and the other a mobility intermediate between *d* and *e*. There is in our opinion some uncertainty in the identification of the PGM_1 1–3 and PGM_1 1–5 phenotypes reported for the Japanese populations.

Ishimoto & Yada (1969), Ishimoto (1970), Omoto & Harada (1970), Shinoda & Matsunaga (1970*a*) and Harada *et al.* (1971) have all reported low frequencies of the PGM_1^5 allele in the central and eastern part of Japan (0·0007–0·0010). Shinoda & Matsunaga (1970*b*) reported the PGM_1^3 allele to occur in low frequency (0·0005 and 0·0003) in Tokyo and Shizuoka. Finally Omoto *et al.* (1973) found a PGM_1^3 gene frequency of 0·0039 in Okinawa. When the diagrams or the photographs presented by these authors were compared, the position of the major isozyme band in the pattern identified as PGM_1 5 was similar to that of PGM_1 3 while the position of the minor band was not clearly shown.

Recently, Blake & Omoto (1975) have discussed the possibility of heterogeneity in the PGM_1^3 alleles which have been detected in different populations. They proposed that the PGM_1^3 allele found in Okinawa should be named $PGM_1^{3\,\text{Okinawa}}$ because the major and minor isozyme bands associated with it were located slightly cathodal to those associated with the PGM_1^3 allele found in New Guinea. They also proposed that the PGM_1^5 allele type found in Japan should be named $PGM_1^{5\,\text{Japan}}$ because the minor isozyme band associated with it was located in the *e* position, thus distinguishing it from the PGM_1^5 allele reported in an English person by Hopkinson & Harris (1966). The gene frequency of $PGM_1^{3\,\text{NGS}\,1}$ in Nagasaki was found to be 0·0024. This value is less than that found for $PGM_1^{3\,\text{Okinawa}}$, but higher than that obtained in the central to eastern parts of Japan for the PGM_1^3 or PGM_1^5 alleles. A precise comparison of the several kinds of PGM_1 3 and PGM_1 5 phenotypes occurring in Japan will be presented in a separate paper (Omoto *et al.* in preparation).

A phenotype similar to that of PGM_1 1–7 was encountered in a single individual in Nagasaki. After repeated electrophoresis, it was concluded that the major isozyme in this pattern moved slower than the *c* band but faster than the major isozyme of the PGM_1 7 phenotype whereas the intensity of the minor isozyme was so weak that it could hardly be recognized (see Fig. 3). The phenotype is designated $PGM_1\,9_{\text{NGS}\,1}$. One of the daughters of the propositus showed the same phenotype, PGM 1–$9_{\text{NGS}\,1}$, as that of the propositus and another daughter showed a PGM_1 2–$9_{\text{NGS}\,1}$ phenotype (see Table 3 of the family study). The allele which can be inferred from these studies is designated $PGM_1^{9\,\text{NGS}\,1}$.

Five kinds of slow PGM_1 variants, in which the major variant band migrates cathodal to band *a*, were found in a total of 4029 samples from Hiroshima and Nagasaki (see Table 1). As mentioned earlier, 1895 samples (872 from Hiroshima, 1023 from Nagasaki) were examined in the tris-maleic acid buffer of Spencer *et al.* (1964). The remaining 2134 samples (1781 from Hiroshima, 353 from Nagasaki) were examined in the discontinuous histidine–sodium citrate buffer of Fildes & Harris (1966) as described in the section on Materials and Methods. Although we found this latter system unsatisfactory for the detection of fast variants, it did yield a number of slow variants, all of which could also be seen with the tris-maleic acid system. The slow variants found in both buffer systems were tentatively designated as $PGM_1\,8_{\text{NGS}\,1}$, $PGM_1\,6_{\text{HIR}\,2}$, $PGM_1\,6_{\text{HIR}\,1}$ $PGM_1\,6_{\text{NGS}\,2}$, $PGM_1\,6_{\text{NGS}\,1}$, listed in the sequence of the delay in the migration of the main isozyme band in tris-maleic acid buffer at pH 7·4. The corresponding allele symbols would be $PGM_1^{8\,\text{NGS}\,1}$, $PGM_1^{6\,\text{HIR}\,2}$, $PGM_1^{6\,\text{HIR}\,1}$, $PGM_1^{6\,\text{NGS}\,2}$, $PGM_1^{6\,\text{NGS}\,1}$. The patterns are shown diagrammatically in Fig. 4 and photographs of typical determinations of the various phenotypes are shown in Fig. 5.

The minor isozyme band of the $PGM_1\,8_{\text{NGS}\,1}$ pattern clearly migrated cathodally to the *a* band in tris-maleic acid buffer, while that of the $PGM_1\,6_{\text{NGS}\,1}$ and $PGM_1\,6_{\text{NGS}\,2}$ patterns clearly moved

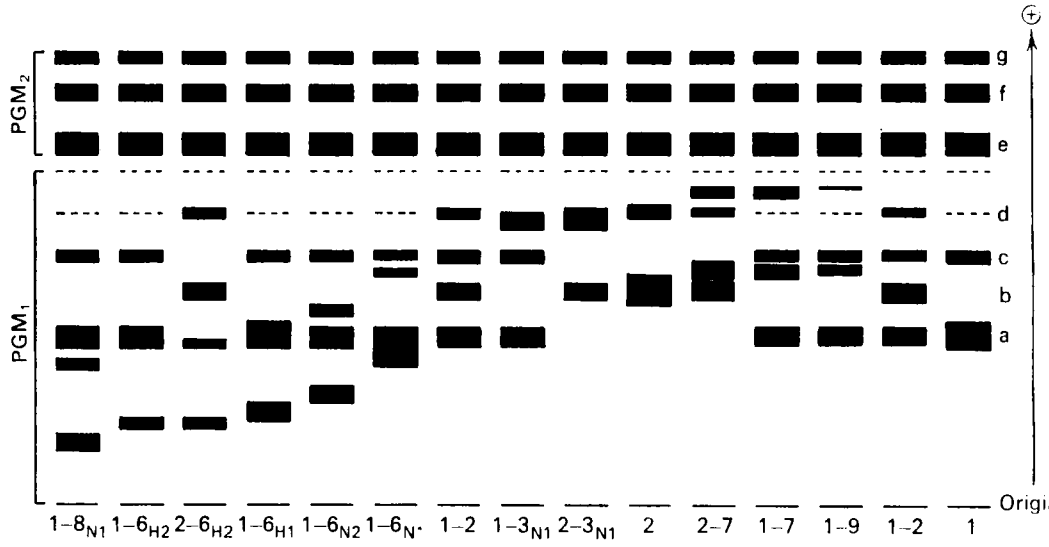

Fig. 4. Diagram of a composite starch gel showing the various phenotypes of PGM_1 found in the present study.

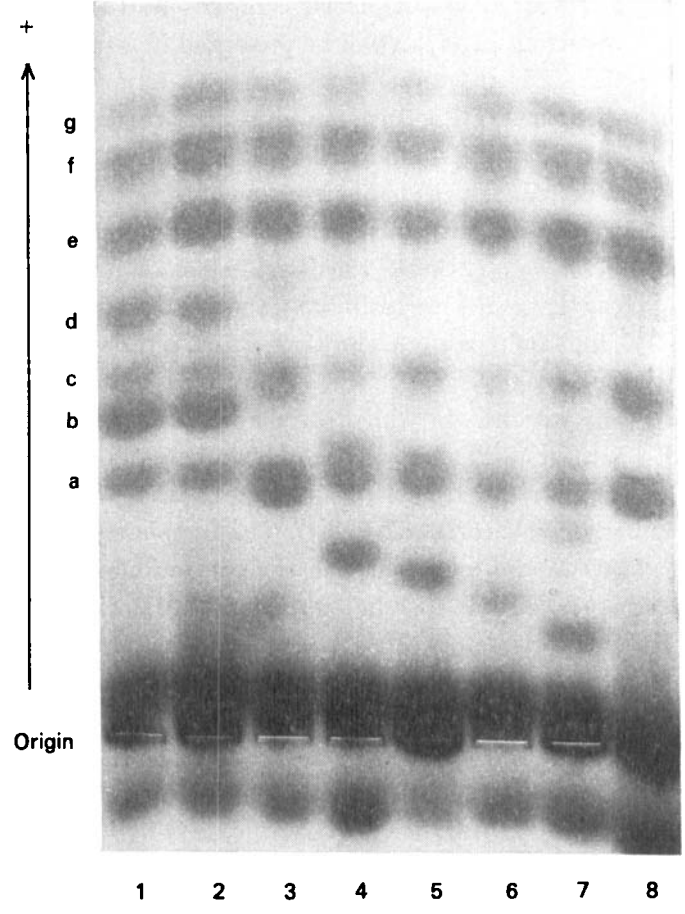

Fig. 5. Photograph of a starch gel showing the PGM_1 phenotypes which have a major isozyme band slower than the *a* band. Well 1 and 2, PGM_1 1–2; well 3, PGM_1 $1–6_{NGS\,1}$; well 4, PGM_1 $1–6_{NGS\,2}$; well 5, PGM_1 $1–6_{HIR\,1}$; well 6, PGM_1 $1–6_{HIR\,2}$; well 7, PGM_1 $1–8_{NGS\,1}$; well 8, PGM_1 1.

faster than *a* band. The minor isozyme of the $6_{HIR\,1}$ phenotype migrated slightly anodally to the *a* band and that of the $6_{HIR\,2}$ phenotype overlapped the *a* band.

When the electrophoretic resolution of a PGM_1 2–$6_{HIR\,2}$ phenotype was particularly sharp, the minor isozyme associated with the $PGM_1^{6\,HIR\,2}$ allele could be seen to be very slightly slower than band *a*. The minor isozyme bands associated with $PGM_1^{6\,NGS\,2}$ and $PGM_1^{6\,HIR\,1}$ alleles could not be recognized in a 1:10 dilution of bridge buffer. As reported previously (Satoh *et al.* 1974; Satoh *et al.* 1975), the mobility of the isozymes associated with the $PGM_1^{8\,NGS\,1}$ and $PGM_1^{6\,HIR\,2}$ alleles changed in the histidine–sodium citrate buffer. In that system the order of the migration of the isozymes from the cathodal side is $6_{HIR\,2}$, $8_{NGS\,1}$, $6_{HIR\,1}$, $6_{NGS\,2}$ and $6_{NGS\,1}$. This phenomenon is useful for the characterization of $6_{HIR\,2}$ and $8_{NGS\,1}$. The validity of the distinction between the phenotypes attributed to the $PGM_1^{8\,NGS\,1}$, $PGM_1^{6\,HIR\,2}$, $PGM_1^{6\,HIR\,1}$ and $PGM_1^{6\,NGS\,2}$ alleles has been confirmed by family studies. A family study for the $PGM_1^{6\,NGS\,1}$ allele has not been carried out. A detailed comparison of the differences and the similarities of these slow variants with the other slow variants reported thus far in Japanese was presented in a separate paper (Satoh *et al.* 1976).

Phosphoglucomutase-2

No variants of PGM_2 were detected in the total of 4029 samples, examined with either histidine–sodium citrate discontinuous buffer or tris-maleic acid buffer, the actual numbers being as shown in Table 1 for PGM_1. None of the other studies in Japan have detected variants of PGM_2 (Ishimoto, 1975).

6-*Phosphogluconate dehydrogenase*

The prevalence among 4014 samples (2648 from Hiroshima, 1366 from Nagasaki) of the phenotypes of the A–C polymorphism of 6-PGD obtained in the present study, and the gene frequencies, are summarized in Table 4. There were, in addition, 15 untypable specimens. The frequencies for the PGD^c allele were 0·084 and 0·093 in Hiroshima and Nagasaki residents, respectively. Ishimoto, Kuwata & Kubota (1973) reported a PGD^c frequency of 0·11 among 526 samples in Hiroshima and Ishimoto & Kuwata (1973) observed a value of 0·10 among 608 samples in Nagasaki. In a recent review (Ishimoto, 1975), the gene frequency of the PGD^c allele in 7221 Japanese is estimated to be 0·086, when all the data except for the isolates and the Ainu are combined. Thus, the allele frequency values obtained in the present study fall very close to the mean value for the Japanese.

We have encountered three different fast variants of 6-PGD, each in a single individual, one in Nagasaki and two in Hiroshima, all three in combination with the normal A phenotype. As shown diagrammatically in Fig. 6 each of the variant patterns consisted of three bands; the slowest band of the triplet was seen at the position of the normal *a* band, and the middle band was placed in the centre between *a* band and the most anodal band. As we have not yet compared these variants with any other known variants, they are tentatively named as 6-PGD NGS 1, 6-PGD HIR 2 and 6-PGD HIR 1 in the order of the migration toward anode. With respect to the intensity of the three bands, in the 6-PGD A-NGS 1 pattern, in which the variant band is the most rapidly migrating observed in the present study, the intensity of the *a* and the middle bands was equal, while the most anodal band stained only faintly. In the 6-PGD A-HIR 2 and 6-PGD A-HIR 1 patterns, the three bands stained with equal intensity after gel electrophoresis

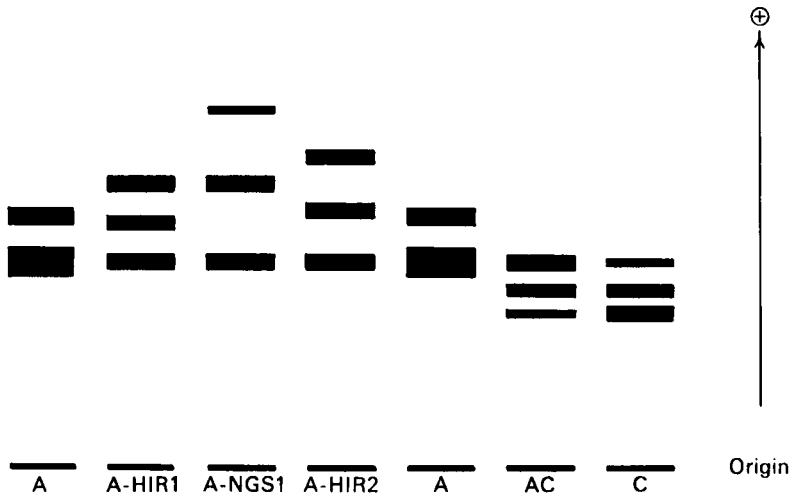

Fig. 6. Diagram of a starch gel showing phenotypes of 6-PGD variants. Electrophoresis was carried out for 20 h in the histidine–sodium citrate discontinuous buffer system.

for 20 h, but the *a* band stained most intensely when electrophoresis was limited to 4 h. Under either electrophoretic condition, all three of these variants differed from the intensity pattern of the C phenotype of the common polymorphism (the 'Canning' variant), in which the most cathodal, *c*, band is the most intense, the middle band is intermediate in intensity and the *a* band is faint. No change was observed in the pattern when these three variants were treated with 2-mercaptoethanol.

The distance between the *a* band and the most anodal band of 6-PGD A-HIR 1 is slightly greater than that between the *a* band and the most cathodal band of the common variant (phenotype 6-PGD A–C). The mobility of the most anodal band of 6-PGD A-HIR 1 is the same as that of the middle band of 6-PGD A-NGS 1. In the photographs of Blake *et al.* (1974) and Blake & Kirk (1969), the same relationship between the 'Richmond' variant and the 'Elcho' variant can be seen. Nevertheless, we are reluctant to classify the variants we have designated as 6-PGD A-HIR 1 and 6-PGD A-NGS 1 as electrophoretically identical to the 'Richmond' and 'Elcho' variants respectively until confirmed by a direct comparison. Limited family studies for these variants showed the same variant pattern in a child of each propositus (see Table 3).

Neither rare electrophoretic variants nor patterns with a deficiency in the activity of 6-PGD have been reported in the Japanese populations listed in the review of 6-PGD in Japanese (Ishimoto, 1975).

Adenosine deaminase

The prevalence among 4021 samples (2651 from Hiroshima, 1370 from Nagasaki) of the phenotypes of the well-known polymorphism of ADA, and the gene frequencies for ADA^1 and ADA^2, are summarized in Table 5. There were eight untypable samples. The frequencies for the ADA^2 allele were 0·025 and 0·032 for Hiroshima and Nagasaki residents, respectively. No rare variants of ADA were encountered.

Ishimoto & Kuwata (1970) observed an ADA^2 frequency of 0·028 among 448 samples in Hiroshima, and these same investigators (1973) obtained a value of 0·021 among 608 samples

Table 5. *Phenotypes and gene frequencies for the ADA polymorphism in Hiroshima and Nagasaki*

Phenotype	Hiroshima	Nagasaki	Combined
1	2523	1286	3809
2–1	126	79	205
2	2	5	7
No type*	2	6	8
Total	2653	1376	4029
ADA^1†	0·975	0·968	0·973
ADA^2	0·025	0·032	0·027
χ^2	0·155	9·634	
P	$0{\cdot}50 < P < 0{\cdot}70$	$0{\cdot}001 < P < 0{\cdot}01$	

* These samples gave an ADA pattern which could not be typed accurately.
† Calculation of gene frequencies excludes no types.

in Nagasaki. Ishimoto concluded in his review of ADA (Ishimoto, 1975) that there are no marked regional differences in the frequencies for the ADA^1 and ADA^2 alleles in the Japanese. The estimated frequency for the ADA^2 allele from the combined total of 5754 non-Ainu Japanese is 0·024.

Adenylate kinase

A total of 2250 samples (1098 from Hiroshima and 1152 from Nagasaki) were examined for AK. All the samples showed the AK 1 phenotype. All Japanese individuals so far examined are of phenotype 1 (Ishimoto, 1975).

DISCUSSION

The principal finding of this paper is the high frequency of rare variants of the PGM_1 system, especially in the face of a lack of variation in the PGM_2 system, despite their possible common origin through gene duplication (Hopkinson & Harris, 1969). Furthermore, a greater number of different PGM_1 variants, and a higher frequency for these variants, were encountered in the population of Nagasaki compared to the population of Hiroshima. Thus, in the tris-maleic acid buffer system, an allele designated $PGM_1^{3\,NGS\,1}$, which produces isozymes electrophoretically similar if not identical with those produced by the PGM_1^3 allele of other authors, was detected in five individuals in 1023 samples from Nagasaki though no such allele was detected in 870 samples from Hiroshima. The gene frequency for the $PGM_1^{3\,NGS\,1}$ allele is 0·0024, which is at least five times higher than that for the PGM_1^3 allele observed in the central part (Tokyo and Shizuoka) of Japan (0·0005 and 0·0003). Furthermore, four kinds of rare alleles which are associated with slow variants, designated $PGM_1^{6\,NGS\,1}$, $PGM_1^{6\,NGS\,2}$, $PGM_1^{6\,HIR\,2}$ and $PGM_1^{8\,NGS\,1}$ totalling nine in number, were found in 1373 samples from Nagasaki. Two of these four kinds of rare alleles, $PGM_1^{6\,NGS\,1}$ and $PGM_1^{6\,NGS\,2}$, were detected only in the Nagasaki population. In the population of Hiroshima, three kinds of alleles which produce slow variants, namely $PGM_1^{8\,NGS\,1}$, $PGM_1^{6\,HIR\,2}$ and $PGM_1^{6\,HIR\,1}$, totalling four in number, were detected in 2648 samples, $PGM_1^{6\,HIR\,1}$ being found only in Hiroshima. As mentioned previously, the calculation of the variant frequencies should be based on the results of the tris-maleic acid system. The combined frequency for the alleles associated with slow variants in Hiroshima is 0·0006 and that in Nagasaki is

0·0030. The value obtained in Nagasaki is about ten times higher than that (0·0003) which is calculated by us based on the review by Ishimoto (1975) of a combined sample of 10851 Japanese.

The occurrence of the PGM_1^7 allele in polymorphic frequencies (0·016 for Hiroshima and 0·014 for Nagasaki populations) is reported. Although the frequencies for the allele $PGM_1^{3\,NGS1}$ and the alleles which produce slow variants were different in Hiroshima and Nagasaki populations, the frequencies for the PGM_1^7 are almost the same in the two populations. Though the PGM_1^7 allele has been previously observed in the Japanese population, such a high frequency has not previously been encountered.

It should be mentioned that the Japanese studies have employed the tris-maleic acid buffer system, which we found best for the detection of fast variants. We believe the difference in our results with respect to fast variants is due to the starch and the gel buffer (a 1 : 15 dilution of the bridge buffer) employed. It will be interesting to extend the study of the PGM_1 system to populations in the eastern part of Japan, in order to determine whether this high frequency is unique to these two populations.

SUMMARY

Five enzyme systems, PGM_1, PGM_2, ADA, 6-PGD and AK, were examined by electrophoresis in over 4000 samples from Hiroshima and Nagasaki for the frequencies of common and rare variants. In the PGM_1 system, the PGM_1^2 allele and PGM_1^7 allele were found in polymorphic proportions. In addition, five kinds of slow variants and three types of fast variants of PGM^1 were detected. The $PGM_1^{3\,NGS1}$ allele was found in five individuals from Nagasaki, but was not observed in samples from Hiroshima. There were no variants of PGM_2. Three kinds of fast variants of 6-PGD were detected. No variation in AK was observed. There were no rare variants of ADA. The *6-PGD*^{c} allele had a frequency of 0·084 in Hiroshima and 0·093 in Nagasaki, and the ADA^2 allele frequencies of 0·025 in Hiroshima and 0·032 in Nagasaki.

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