THE DISTRIBUTION OF RED CELL ENZYME GROUPS AMONG CHINESE AND MALAYS IN SINGAPORE

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SYNOPSIS

Samples from 378 Chinese and 259 Malay blood donors in Singapore have been studied for electrophoretic variants in 13 red cell enzyme systems and for abnormal haemoglobins. Variants were detected in 8 of the enzyme systems, and the frequencies were polymorphic for acid phosphatase, 6 phosphogluconate dehydrogenase phosphoglucomutase (locus 1) among both Chinese and Malays, and for adenylate kinase also among Malays. Rare variants were detected in the phosphohexose, NADH diaphorase and lactate dehydrogenase systems. A new 6 PGD phenotype and three new LDH phenotypes have been described. Electrophoretic variants of haemoglobin were more frequent among Malays than among Chinese.

During the last few years an impressive number of genetically controlled variations in red cell enzyme groups have been described and many of these variants achieve polymorphic frequency in human populations (see Giblett, 1969; Harris, 1970 for review). So far, however, with the exception of studies of the incidence of abnormal haemoglobins and of glucose-6-phosphate dehydrogenase (G6PD) deficiency (Vella, 1961; Saha, 1969; Lie-Injo, 1969) and also of the distribution of red cell acid phosphatase variants (Lai and Kwa, 1968) no studies of these enzyme systems have been undertaken on populations in Singapore. To fill this gap we have examined recently blood samples from 378 Chinese and 259 Malay donors attending the Singapore Blood Bank.

METHODS

Portions of blood from the donor pilot tubes were separated and washed cells were transported at dry ice temperature to Canberra, where they were stored in a liquid nitrogen refrigerator until used for testing.

Red cell enzyme groups were determined using the buffers, electrophoretic conditions and substrate procedures outlined previously by Blake *et al* (1971) and Malcolm *et al* (1972).

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RESULTS AND DISCUSSION

The distribution of phenotypes in those systems revealing variation for both Chinese and Malays is given in Table I. The corresponding gene frequencies are presented in Table II. X^2 values have been calculated from the gene frequencies assuming Hardy-Weinberg equilibrium and the appropriate values are shown in Table I. Only one of the values, that for the acid phosphatase system among Chinese, reaches the $P = 0{\cdot}05$ level of significance for departure from random mating. The reason for this departure is unclear and a further investigation is being undertaken.

(i) Red Cell Acid Phosphatase

In Europeans three acid phosphatase alleles, p^a, p^b and p^c are present, but for the majority of populations in the world only the first two occur. This is true for the Chinese and the frequencies of p^a and p^b in the present series (21·43 and 78·57 per cent respectively) correspond closely with those given by Lai and Kwa (1968). The Malays, as found also by Lai and Kwa, have a higher frequency of the p^a allele (32·63 per cent) with a lower frequency of the p^b allele (67·18 per cent). In addition we found a single CB Malay individual, and Lai and Kwa found a single CA individual, indicating that the p^c allele does occur among Malays, even though with low frequency.

(ii) 6-Phosphogluconate Dehydrogenase

No previous results are available for this system among Chinese or Malays in Singapore, though Shih *et al* (1968) have studied 228 Chinese in Taiwan. In the present series the 'Plaistow' variant,

TABLE I

RED CELL ENZYME SYSTEMS SHOWING PHENOTYPIC VARIATIONS IN SINGAPORE BLOOD DONORS

System		Chinese		Malays	
		No.	Per Cent	No.	Per Cent
Acid Phosphatase					
AcPh	A	24	6·35	32	12·36
	AB	114	30·16	105	40·54
	B	240	63·49	121	46·72
	CB	0	0·00	1	0·38
	Total	378	100·00	259	100·00
	χ^2		4·12*		1·99
6 - Phosphogluconate Dehydrogenase					
PGD	AA	327	86·51	228	88·02
	AC	50	13·23	29	11·20
	CC	0	0·00	1	0·39
	Others	1	0·26	1	0·39
	Total	378	100·00	259	100·00
	χ^2		1·98		0·07
Phosphoglucomutase (Locus 1)					
PGM_1	1 - 1	204	53·97	150	57·92
	2 - 1	143	37·83	93	35·91
	2 - 2	27	7·14	14	5·41
	3 - 1	0	0·00	2	0·76
	8 - 1	2	0·53	0	0·00
	8 - 2	2	0·53	0	0·00
	Total	378	100·00	259	100·00
	χ^2		1·24		0·63
Adenylate Kinase					
AK	1 - 1	378	100·00	252	97·30
	2 - 1	0	0·00	6	2·32
	2 - 2	0	0·00	1	0·38
	Total	378	100·00	259	100·00
	χ^2				0·11
Phosphohexose Isomerase					
PHI	1 - 1	377	99·74	257	99·23
	2 - 1	1	0·26	0	0·00
	3 - 1	0	0·00	2	0·77
	Total	378	100·00	259	100·00
Diaphorase					
DIA	1 - 1	376	99·73	256	100·00
	4 - 1	1	0·27	0	0·00
	Total	377	100·00	256	100·00

TABLE I *(Continued)*

System		Chinese		Malays	
		No.	Per Cent	No.	Per Cent
Peptidase A					
Pep. A	1 - 1	252	66·67	156	60·23
	1 - 1 weak	108	28·57	71	27·41
	"0"	18	4·76	32	12·36
	Total	378	100·00	259	100·00
Lactate Dehydrogenase					
LDH	Normal	375	99·21	257	99·22
	Chinese - 1	2	0·53	0	0·00
	Chinese - 2	1	0·26	0	0·00
	Malay - 1	0	0·00	1	0·39
	Malay - 2	0	0·00	1	0·39
	Total	378	100·00	259	100·00

*Significant at the P = 0·05 level.

or heterozygote AC, was frequent among both Chinese and Malays, and in the latter one case of the 'Canning' variant, or homozygous CC, was detected. The PGD^C gene frequency of 6·61 per cent in Chinese is similar to the value of 5·98 per cent in Malays, and both values are not significantly different from the frequency of 6·58 per cent for Chinese in Taiwan (Shih *et al*, 1968). In addition to the 'Plaistow' and 'Canning' variants one example of the 'Richmond' phenotype was found in the Malays and a completely novel phenotype was found in a single Chinese individual. This phenotype is an electrophoretic slow variant (illustrated in Fig. 1) and the band spacing corresponds to that of the fast "Elcho" variant (Blake and Kirk, 1969). Dr. C. W. Parr (personal communication) has confirmed that the variant described here has not been reported previously and has suggested that it be given the trivial name PGD—'Singapore'. The allele controlling the heterozygote phenotype in combination with PGD^A has been designated PGD^S.

(iii) Phosphoglucomutase (Locus 1)

No previous studies have been carried out for this system in Singaporeans, but Lie-Injo *et al* (1968) has reported PGM_1 frequencies among Chinese in San Francisco and Kuala Lumpur, Lie-Injo and Poey-Oey (1970) have studied PGM in Indonesians, and Welch *et al* (1972) have given PGM frequencies for Malayan Aborigines. In the present study the common phenotypes PGM 1-1, 2-1, 2-2 were present in Chinese and Malays and the PGM_1^1 frequency is 73·15 and 76·26 per cent respectively, being almost the same as Lie-Injo *et al*'s frequencies in San Francisco (76·4) and Kuala Lumpur (74·8). In the present series 2 persons among the Malays were identified as PGM_1 3-1, whilst two Chinese were PGM_1 8-1 and a further two PGM_1 8-2. These latter cases give an incidence of the PGM_1^8 allele in Chinese of 0·5 per cent. Although this is a low frequency the PGM_1^8 gene appears to be present in northern Mongoloid populations, having been reported from several localities in Japan (Omoto and Harada, 1970; Shinoda and Matsunaga, 1970a and 1970b, and Harada *et al*, 1971). The original 8-1 variant was found in England (Harris *et al*, 1968; Hopkinson and Harris, 1968) and another example has been reported in a Jewish family in the Middle East (Tomashewsky and Szeinberg, 1970).

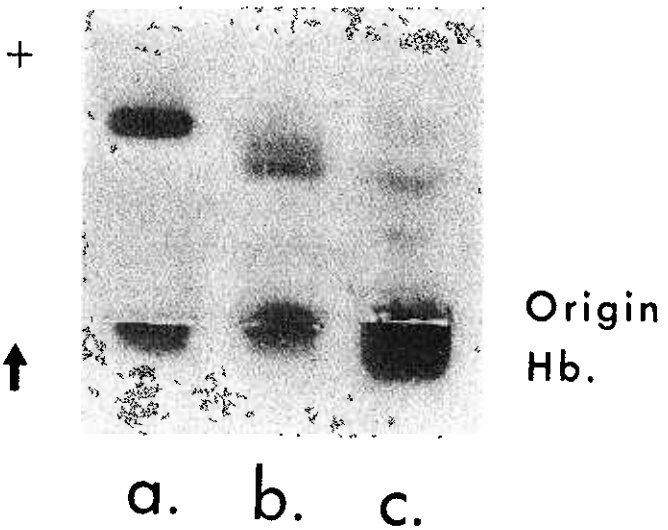

Fig. 1. Starch el gelectrophoresis of haemolysates, stained for 6—PGD activity.
(a) normal pattern; (b) 'Canning' variant;
(b) 'Singapore' variant.

TABLE II

GENE FREQUENCIES FOR RED CELL ENZYME SYSTEMS SHOWN IN TABLE I

System	Alleles	Chinese	Malays
Acid Phosphatase			
	P^a	0·2143	0·3263
	P^b	0·7857	0·6718
	P^c	0·0000	0·0019
6-Phosphogluconate Dehydrogenase			
	PGD^A	0·9325	0·9382
	PGD^C	0·0661	0·0598
	PGD^R	0·0000	0·0019
	PGD^S	0·0013	0·0000
Phosphoglucomutase (1)			
	$PGM\frac{1}{1}$	0·7315	0·7625
	$PGM\frac{2}{1}$	0·2632	0·2336
	$PGM\frac{3}{1}$	0·0000	0·0039
	$PGM\frac{8}{1}$	0·0053	0·0000
Adenylate Kinase			
	AK^1	1·0000	0·9846
	AK^2	0·0000	0·0154
Phosphohexose Isomerase			
	PHI^1	0·9987	0·9961
	PHI^2	0·0013	0·0000
	PHI^3	0·0000	0·0039
Diaphorase			
	DIA^1	0·9987	1·0000
	DIA^4	0·0013	0·0000
Lactate Dehydrogenase			
	LDH^N	0·9960	0·9961
	LDH^{Chi-1}	0·0026	0·0000
	LDH^{Chi-2}	0·0013	0·0000
	LDH^{Mal-1}	0·0000	0·0019
	LDH^{Mal-2}	0·0000	0·0019

(iv) **Adenylate Kinase**

Shih *et al* (1968), Shih and Hsia (1969), Welch *et al* (1971) and Chan (1971) have given frequencies for the adenylate kinase system in Taiwan and West Malaysia respectively. The Chinese are almost invariant for AK, only the 1-1 phenotype being observed in the present investigation and among 318 persons sampled by Chan in Kuala Lumpur and 210 reported by Shih *et al* (1968) and Shih and Hsia (1969) from Taiwan. Only one Chinese person with the AK 2-1 phenotype has been reported so far, in a person originating in Mainland China (Shih *et al*, 1968). The situation among the Malays is very different. Gordon (1966) reported AK 2-1 persons among Malays in South Africa, Chan (1971) found a frequency of the AK^2 gene of 1·87 per cent among Malays, and Welch *et al* (1971) also found an AK^2 frequency of 1·5 per cent among Malays and 1·3 per cent among Aborigines in West Malaysia and McDermid *et al* (1973) have reported an AK2-1 person among the Batak of Samosir Island in northern Sumatra.

Our present frequency for AK^2 of 1·54 per cent among Malays in Singapore is similar to those of Chan and of Welch *et al* for Malays in West Malaysia quoted above. Giblett (1969) gives a value of 2·2 per cent for the AK^2 gene in Thailand.

(v) **Phosphohexose Isomerase**

Variants of PHI are rare in all parts of the world, the highest frequencies reported so far approximating 1 per cent among populations in India (Omoto and Blake, 1972). In the present investigation only a single PHI 2-1 variant was found among the 378 Chinese donors, and 2 PHI 3-1 persons among the 259 Malay donors.

(vi) **NADH Diaphorase**

Deficiency of NADH diaphorase (methaemoglobin reductase) is associated with methaemoglobinaemia (Jaffe *et al*, 1966) and this deficiency is sometimes associated with a change in the electrophoretic mobility of the enzyme (Kaplan and Beutler, 1967; West *et al*, 1967) and electrophoretic variants of diaphorase not associated with clinical abnormality have been reported also and the frequency of such variants approximates 1 per cent in a number of populations (Hopkinson *et al*, 1970). Only a single person, a DIA 4-1 was found among the Chinese, and no variants were found among the Malays in the present study.

(vii) **Peptidase A**

The Pep A enzyme is unstable and tests on samples not completely fresh show varying strengths of reaction. Among the Singaporeans tested here, all were of the Pep A 1-1 phenotype, but approximately 28 per cent were weak in their

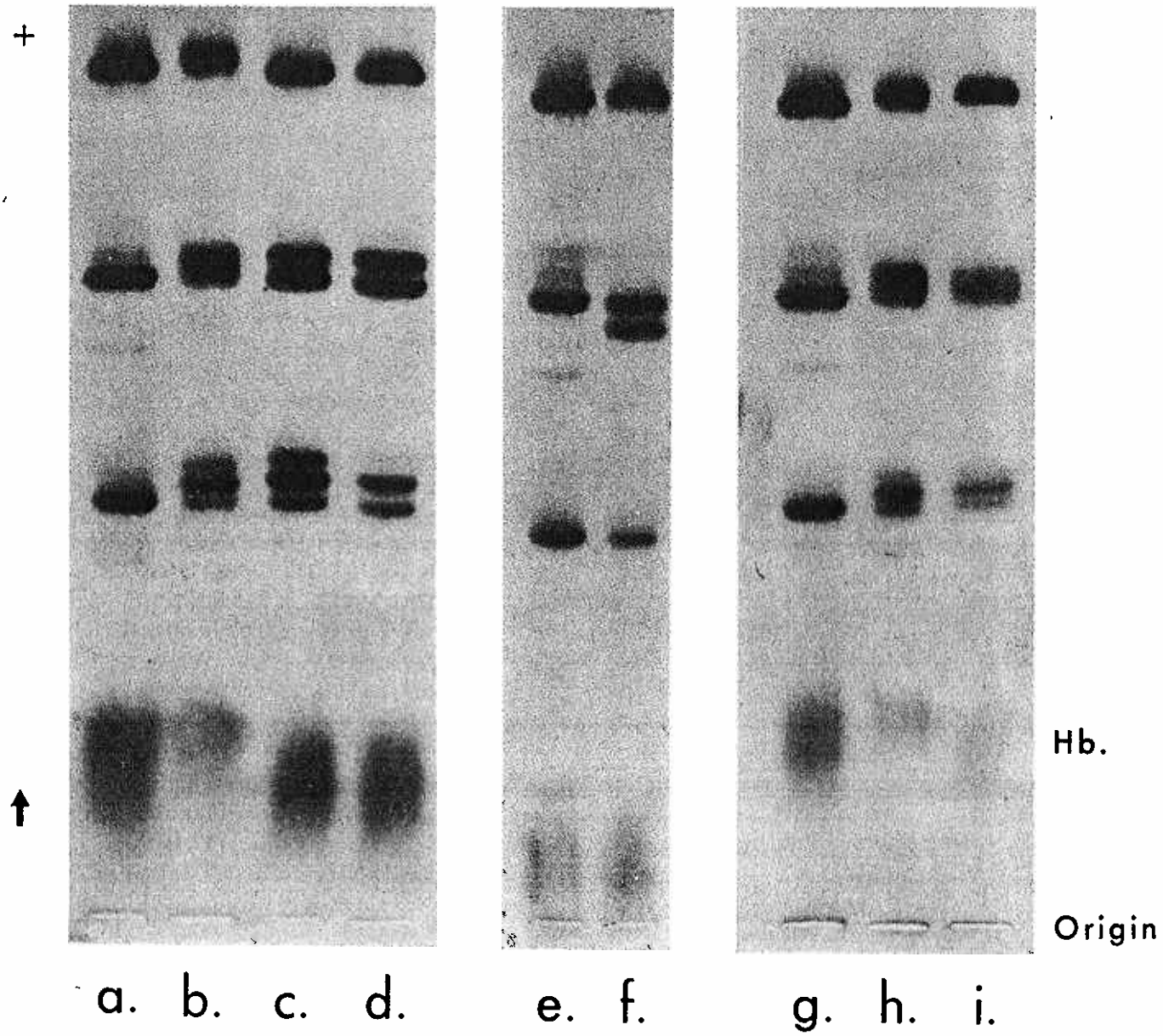

Fig. 2. Starch gel electrophoresis of haemolysates, stained for LDH activity.
(a), (e), (g), normal pattern; (b), (h), 'Calcutta—1';
(c), 'Chinese—1'; (f) 'Chinese—2'; (i), 'Malay—1'; (d) 'Malay—2'.

TABLE III

HAEMOGLOBIN VARIANTS IN SINGAPORE BLOOD DONORS

		Chinese		Malays	
		No.	Per Cent	No.	Per Cent
Phenotypes: Hb	A	376	99·47	245	94·59
	AE	2	0·53	12	4·63
	EE	0	0·00	1	0·39
	AD	0	0·00	1	0·39
	TOTAL	378	100·00	259	100·00
Gene frequencies					
	Hb^A	0·9974		0·9710	
	Hb^E	0·0026		0·0270	
	Hb^D	0·0000		0·0019	

reactivity and in 4·76 per cent of the Chinese and 12·36 per cent of the Malays no activity could be detected. No particular significance is attached to these results.

(viii) **Lactate Dehydrogenase**

Genetic variants of LDH in red cells and other tissues occur sporadically in many populations, but they occur with the highest frequency, from 1-4 per cent, among populations in India (Das *et al*, 1970). The present survey was of interest because it revealed four different types of LDH variants, two among the Chinese donors and two among the Malays. The variants have been given trivial names corresponding to the ethnic group in which they were observed, and they are illustrated in Fig. 2.

LDH "Chinese-1" is an electrophoretically fast A sub-unit variant showing the characteristic pattern expected for such variants (Vesell, 1965), and occurred in two individuals. LDH "Chinese-2" is atypical. Two bands are present in the position of isozyme-2, with no other change being detectable. No similar variant in LDH has been described previously. Among the Malays one individual, LDH "Malay-1" reveals a pattern which is indistinguishable from the common LDH "Calcutta-1" variant widespread in India. The other person with LDH "Malay-2" has a pattern similar to, but not identical with another Indian variant, LDH "Calcutta-2" described recently by Das *et al* (1972). The combined frequency of LDH phenotypic variants is almost the same in Chinese and Malays (0·79 and 0·78 per cent respectively).

(ix) **Enzyme Systems Showing No Variation**

All 378 Chinese and 259 Malay samples were tested for electrophoretic variants in the malate dehydrogenase, phosphoglucomutase (locus 2), peptidase B, phosphoglycerate kinase and 'oxidase' systems. No variants were detected. Chen and Giblett (1972) have recently reported the absence of phosphoglycerate kinase variants among 134 Chinese in Taiwan, in agreement with our own findings for Chinese in Singapore.

(x) **Abnormal Haemoglobins**

A number of investigators have reported the frequency of abnormal haemoglobins in Chinese and Malays, both in Singapore and West Malaysia (see Lie-Injo, 1969). The distribution of variants among the donors studied in the present investigation is shown in Table III. Among the Chinese two persons were heterozygous for the Hb^A and Hb^E genes, but the incidence among Malays of AE heterozygotes was 4·63 per cent. One Malay sample was homozygous EE and one was heterozygous AD. These differences between Chinese and Malays in the frequency of abnormal haemoglobins is in accord with the observations of other workers.

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