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Demonstration of the Silent Allele PGM_1^0 in Three Families and Description of a New Variant

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Summary. The enzymic activity and the densitometric parameters of the red cell phosphoglucomutase are investigated on a randomly selected sample of blood donors and on three families showing opposite homozygous PGM_1 types of mother and child. The range of variability of the data is calculated and compared to those of the propositi. From the results it is concluded that opposite types in the mother/child-pairs are due to the existence of the silent allele PGM_1^0.

In order to exclude the silent allele in forensic cases, showing opposite types of man and child, the application of several methods is suggested.

Another family shows rather weak cathodic isozymes corresponding to PGM_1^s, but only about 20—30% as intensive as these. The existence of a new allele $PGM_1^{s\,w}$ is discussed.

Zusammenfassung. Die Enzymaktivität und die densitometrischen Parameter der erythrocytären Phosphoglucomutase wurden an einer Stichprobe von Blutspendern sowie an drei Familien mit entgegengesetzten PGM_1-Typen zwischen Mutter und Kind untersucht. Die berechnete Streubreite der Ergebnisse wird mit denen der Probanden verglichen. Es wird der Schluß gezogen, daß die entgegengesetzten elektrophoretischen Phänotypen durch die Existenz des stummen Allels PGM_1^0 zu erklären sind. Um in entsprechenden forensischen Fällen mit entgegengesetzten Phänotypen zwischen Mann und Kind sicher dieses Allel auszuschließen, wird die parallele Anwendung mehrerer Methoden empfohlen.

Eine weitere Familie weist zusätzliche schwache kathodische Isoenzyme auf, welche zu jenen des Allels PGM_1^s korrespondieren, aber nur 20—30% der Normalintensität aufweisen. Die Existenz eines neuen Allels $PGM_1^{s\,w}$ wird diskutiert.

Key words: PGM — Polymorphismus — Phosphoglucomutase, seltene Varianten.

The silent allele PGM_1^0 (phosphoglucomutase), first described in 1968 by Fiedler and Pettenhofer (1968, 1969), may lead to serious errors in paternity determination, if a defendant is excluded due to seemingly opposite homozygosity to the child. This investigation describes relevant observations in three German families and methods developed that permit to demonstrate the heterozygous state of this allele. In another family the occurrence of a PGM_1^8-like allele producing decreased activity of the corresponding isozymes is described.

Materials and Methods

Electrophoresis was performed on horizontal polyacrylamide gels according to Hoppe *et al.* (1972). The haemoglobin concentration of the haemolysates was adjusted to 20 g%, and 10 µl were applied to filter papers inserted, and incubated for 2 hrs.

Densitometry. The stained gels were measured with a Zeiss Chromatogram Spectrophotometer combined with an integrating recorder at 580 nm.

The absorbance of the stained bands was calculated following subtraction of the basic absorption of the unstained gel parts which were recorded simultaneously. The overlapping peaks were separated by two different methods, the choice of which depended on the extent of overlap: If the degree of superposition was less than 80%, a vertical line was drawn from the point of intersection thus dividing the corresponding integrating curve at this point. If the overlap was larger, the total integrated area was divided according to the relation of the altitudes of the peaks.

Enzyme Assays. Quantitative assays of the enzyme activity were performed as described elsewhere (Brinkmann *et al.*, 1972).

Blood Samples. A sample of 109 randomly selected healthy blood donors of all common blood types was submitted to quantitative studies. Enzyme assays were performed on all blood samples. Densitometric studies were performed with 67 blood samples (25 PGM_1 1; 12 PGM_1 2; 30 PGM_1 2—1). Another 12 PGM_1 2—1 samples were prepared artificially by mixing equal volumes of PGM_1 1 and PGM_1 2 haemolysates after they had been adjusted to 20 g% Hb. Four series of PAA (polyacrylamide) gels were used, which differed slightly according to the lot number of substances, time of polymerization and washing procedure. All assays of the enzyme activity were performed twice and independently. They were repeated, if the deviation was larger than 10% of the mean. All samples were investigated within 24 hrs after venipuncture, including the control samples, which were drawn simultaneously.

Family studies were performed on three families with apparent opposite homozygosity between mother and child (Families Ho., Wo., Be.). As these were legal cases, further family members were not available. Since the defendants were not excluded when testing various genetic markers, they are designated as "presumptive father" in this paper. Along with the controls another family (Fam. Li.) was investigated twice within 4 weeks, which showed additional cathodic isozymes corresponding to PGM_1^8, however, considerably weaker than normal.

Results

Random Samples. The results of enzyme assays are given in Fig. 1. The distribution of the individual values was apparently unimodal. The mean was 1.08 units per gram haemoglobin, the standard deviation 0.207, the range of two standard deviations 0.413. When separating the individual values according to phenotypes there was a small but not significant difference of the means (i.e. 0.03 units).

The *densitometric* intensities were compared by calculating the ratio PGM_1 : PGM_2. This ranged widely from 1.0 to 2.3.

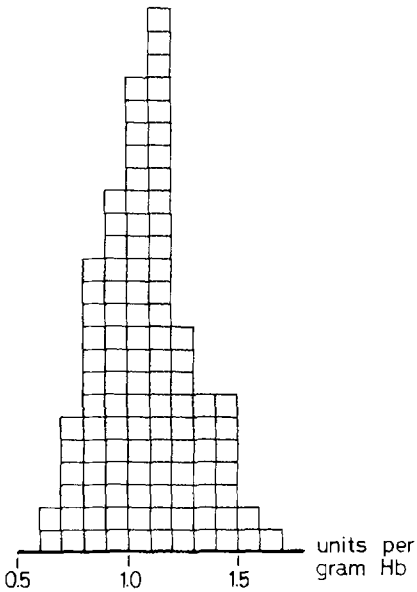


Fig. 1

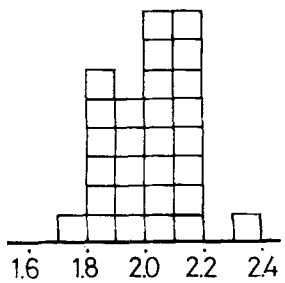


Fig. 2

Fig. 1. Red cell PGM activity in 109 blood donors. $\bar{x} = 1.08$. Standard deviation = 0.207. Each square represents one individual

Fig. 2. Densitometric ratio of isozymes $PGM_1 : PGM_2$ in 25 PGM_1 2—1 types. The lot numbers of PAA gels and the electrophoretic conditions were identical. Each square represents one individual

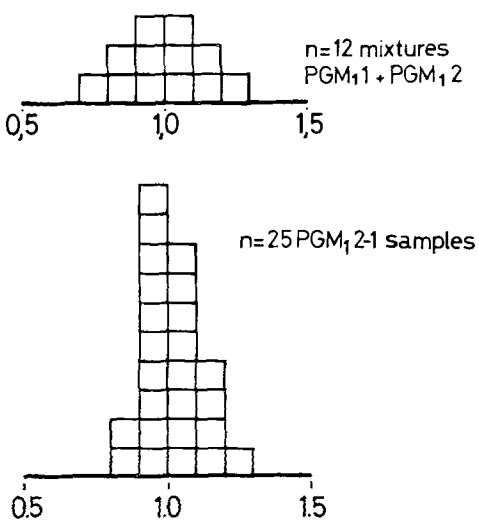


Fig. 3. Densitometric ratio of isozymes a + c : b + d in "natural" and in artificial PGM_1 2—1 patterns. $\bar{x} = 1.0$. Standard deviation = 0.09

When the ratios were grouped according to the phenotypes and lot number of gel series used, the extent of range was considerably reduced, as for instance in one series of 25 PGM_1 2—1 patterns (Fig. 2). The mean ratio $PGM_1 : PGM_2$ was slightly

higher in heterozygous samples than in homozygous ones. In the original as well as in the artificial PGM_1 2—1 zymograms the densitometric ratio of isozymes a + c : b + d was calculated and found to be rather constant in all series (Fig. 3). The mean ratio was 1.0, the standard deviation 0.09, the range of two standard deviations 0.18.

Family Studies. All blood group data of the family members are shown in the appendix. The reticulocytes, counted in some of the test persons, were normal. The haptoglobin concentrations were within the normal range.

In *family Ho.* mother and child showed opposite types (Fig. 4); no additional isozymes were detectable after long runs and prolonged incubation. The mean decrease in enzyme activity was 37.3% of that of concurrent controls (Table 1); the child showed a higher level of activity (Table 1) and densitometric expressivity (Table 2) of it's PGM_1 isozymes. The densitometric ratio PGM_1:PGM_2 indicated a clear deficiency of PGM_1 controlled enzymes in both test persons (Table 2, Fig. 5) as compared to simultaneous controls. The father showed a normal type 2—1.

The opposite types of mother and child *Wo.* were similar to those of family *Ho.* The enzyme activity in the child (PGM_1 2) was more reduced than in the mother (PGM_1 1); the mean decrease being 41.9%, in repeated determinations (Table 1). The densitometric ratios showed a clear deficiency of the PGM_1 isozymes in both test persons; the mother's PGM_1 isozymes were expressed slightly more than the child's (Table 2). The father had a normal PGM_1 1.

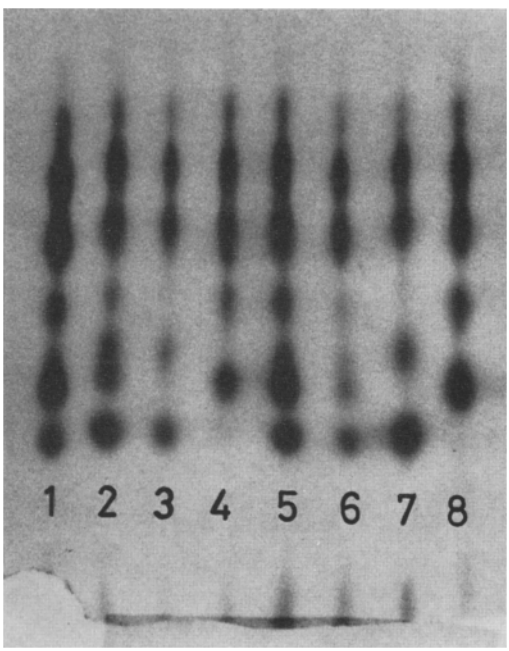


Fig. 4. Electrophoretic phenotypes in family Ho., together with controls. From the left: *1.* Mixture mother (type 1) + control (type 2); *2.* Mixture child (type 2) + control (type 1); *3.* Mother Ho.; *4.* Child Ho.; *5.* Mixture control (PGM_1 1) + control (PGM_1 2); *6.* Mixture mother (type 1) + child (type 2); *7.* control (type 1); *8.* control (type 2)

Table 1. PGM *in-vitro* activity in the propositi and in controls

Propositi and controls	Phenotype PGM_1	Activity (units per g Hb)		Mean decrease
		single values	mean	
Fam. Ho.:				
mother	1	0.49	0.555	37.3%
child	2	0.62	(propositi)	
father	2—1	n.t.		
controls ($n = 4$)	1 and 2	0.96; 0.75; 0.78; 1.05	0.885 (controls)	
Fam. Wo.:				
mother	2	0.8	0.6	41.9%
child	1	0.4	(propositi)	
father	1	n.t.		
controls ($n = 3$)	1 and 2	1.1; 1.08; 0.92	1.03 (controls)	
Fam. Be.:				
mother	1	0.48	0.53	48.0%
child	2	0.58	(propositi)	
father	2—1	n.t.		
controls ($n = 4$)	1 and 2—1	1.1; 1.2; 0.9; 0.85	1.01 (controls)	
Fam. Li.:				
1. mother[b]	1	0.92	0.655	30.0%
child	8[w]—1	0.69	(propositi)	
father	8[w]—2	0.68		
controls ($n = 2$)	1 and 2	0.85; 0.83	0.934 (controls)	
2. mother	1	0.96	0.625	
child[a]	8[w]—1	0.65	(propositi)	
father[a]	8[w]—2	0.60		
controls ($n = 3$)	1 and 2—1	0.98; 1.1; 0.9	0.96 (controls)	

[a] Values of the repeated test performed after 4 weeks.
[b] Mother Li. was treated as a control.
n.t. = not tested.

Opposite types in mother and child *Be.* were diagnosed even after prolonged electrophoresis and incubation period. The most pronounced decrease in enzymic activity was found in both test persons (average 48%), the densitometric values showing a strong reduction of the PGM_1 isozyme intensities (Tables 1 and 2).

Two propositi of *family Li.* showed rather weak additional isozymes after prolonged incubation, which were located at the sites of PGM_1^s (Fig. 6) (Hopkinson and Harris, 1966). The same results were obtained in another laboratory using a different technique (Dr. Hopkinson, London). The enzyme activity was reduced in the "father" and in the child of about 30% in repeated tests (Table 1). The densitometric ratio in the child's $a + c : a_1 + a_2$ (weak cathodic isozymes) was 1 : 0.26; the same ratio was observed in the father's pattern (Table 2, Fig. 7). Another PGM_1 8—1 pattern which was formerly investigated by the same PAA technique showed roughly equal amounts of $a + c : a_1 + a_2$.

Table 2. Densitometric data in the propositi and in controls

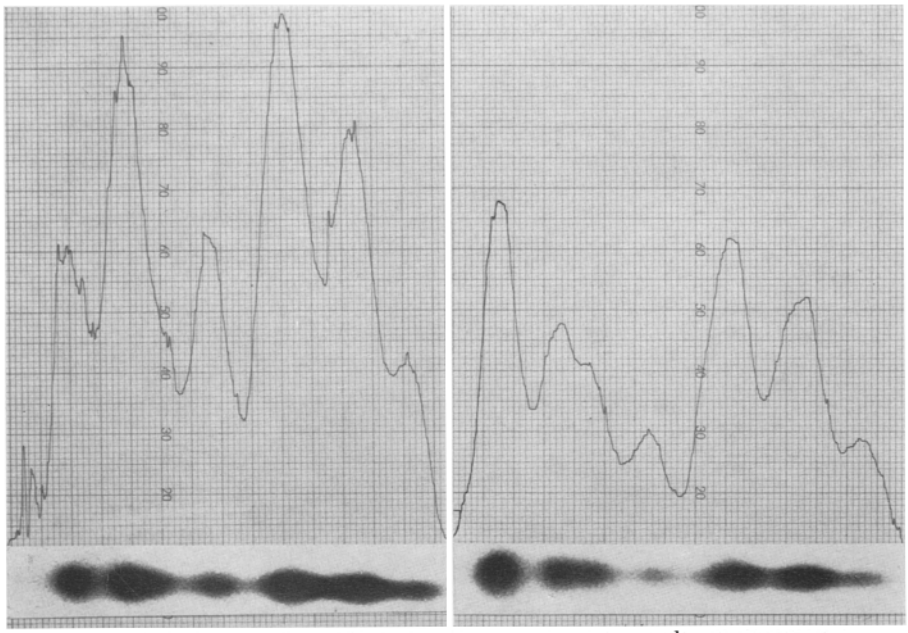
Propositi and controls	Phenotype PGM_1	Ratio PGM_1/PGM_2	Ratio a + c/b + d
Fam. Ho.:			
mother	1	0.64	
child	2	0.88	
control	1	1.1	
control	2	1.05	
mixture: mother + control PGM_1 2	"2—1"	0.91	0.50
mixture: child + control PGM_1 1	"2—1"	0.94	1.45
mixture: PGM_1 1 + control PGM_1 2	2—1	1.3	1.0
Fam. Wo.:			
mother	2	0.88	
child	1	0.62	
control	1	1.42	
control	2	1.38	
mixture: mother + control PGM_1 1	"2—1"	0.96	1.7
mixture: child + control PGM_1 2	"2—1"	0.8	0.55
Fam. Be.:			
mother	1	0.52	
child	2	0.63	
control	1	0.95	
control	2—1	1.1	1.05
mixture: mother + control PGM_1 2	"2—1"	0.8	0.44
mixture: child + control PGM_1 1	"2—1"	0.85	1.25
Fam. Li.:			
mother	1	n.t.	
child	8^w—1	n.t.	0.26[a]
father	8^w—2	n.t.	0.26[a]

[a] The ratio a + c : $a_1 + a_2$ (weak cathodic isozymes), resp. b + d : $a_1 + a_2$ was calculated.
n.t. = not tested.

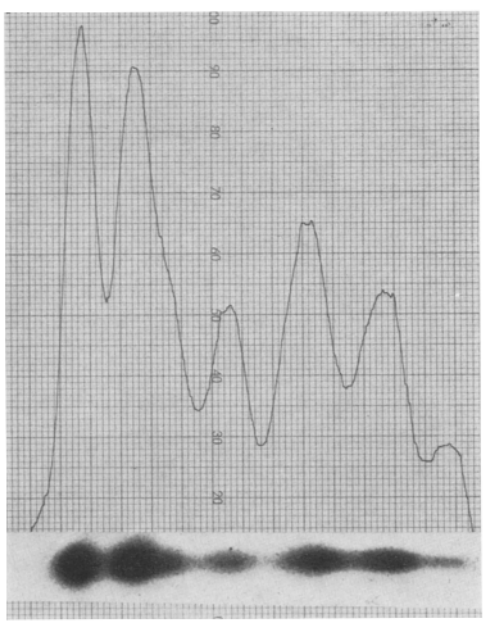
Discussion

The quantitative studies on the random sample were performed to obtain information on the extent of range of variation within this polymorphism. Modiano *et al.* (1970), using a different method of quantitative *in vitro* assay, observed a standard deviation of about 20%. Almost identical values have been found in this investigation. The range of two standard deviations of ± 0.4 units found in our series indicates that a degree of reduction of approx. 40% may be of random or systematical character. However, the finding of clearly reduced enzyme activity in two (or more) closely related persons, together with phenotypic irregularities indicates, that the reduction might be under genetic control.

The densitometric studies indicate rather large variability of the PGM_1:PGM_2 ratio, which is apparently due to variations of the electrophoretic parameters. Since the biochemical properties of the PGM_1 enzymes are different from PGM_2 (McAlpine *et al.*, 1970b), changes of electrophoretic and staining conditions may affect the kinetic in gel properties leading to unstable densitometric relations. This assumption is based on the observation of rather small variations of the same ratio



a b



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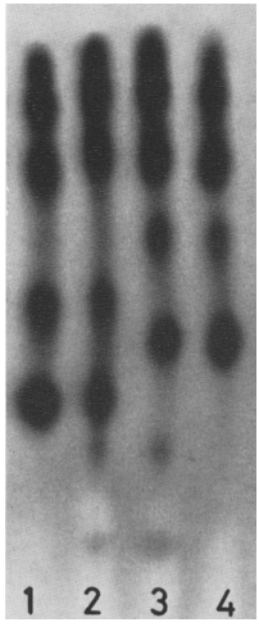

Fig. 6

Fig. 5a—c. Some densitograms and corresponding photographs in family Ho. For densitometric results see Table 2. a Mixture mother (type 1) + control (type 2). b Mixture child (type 2) + control (type 1). c Mixture control (type 1) + control (type 2)

Fig. 6. Electrophoretic phenotypes in family Li., together with controls. From the left: *1.* PGM_1 1; *2.* PGM_1 8^w—1; *3.* PGM_1 8^w—2; *4.* PGM_1 2

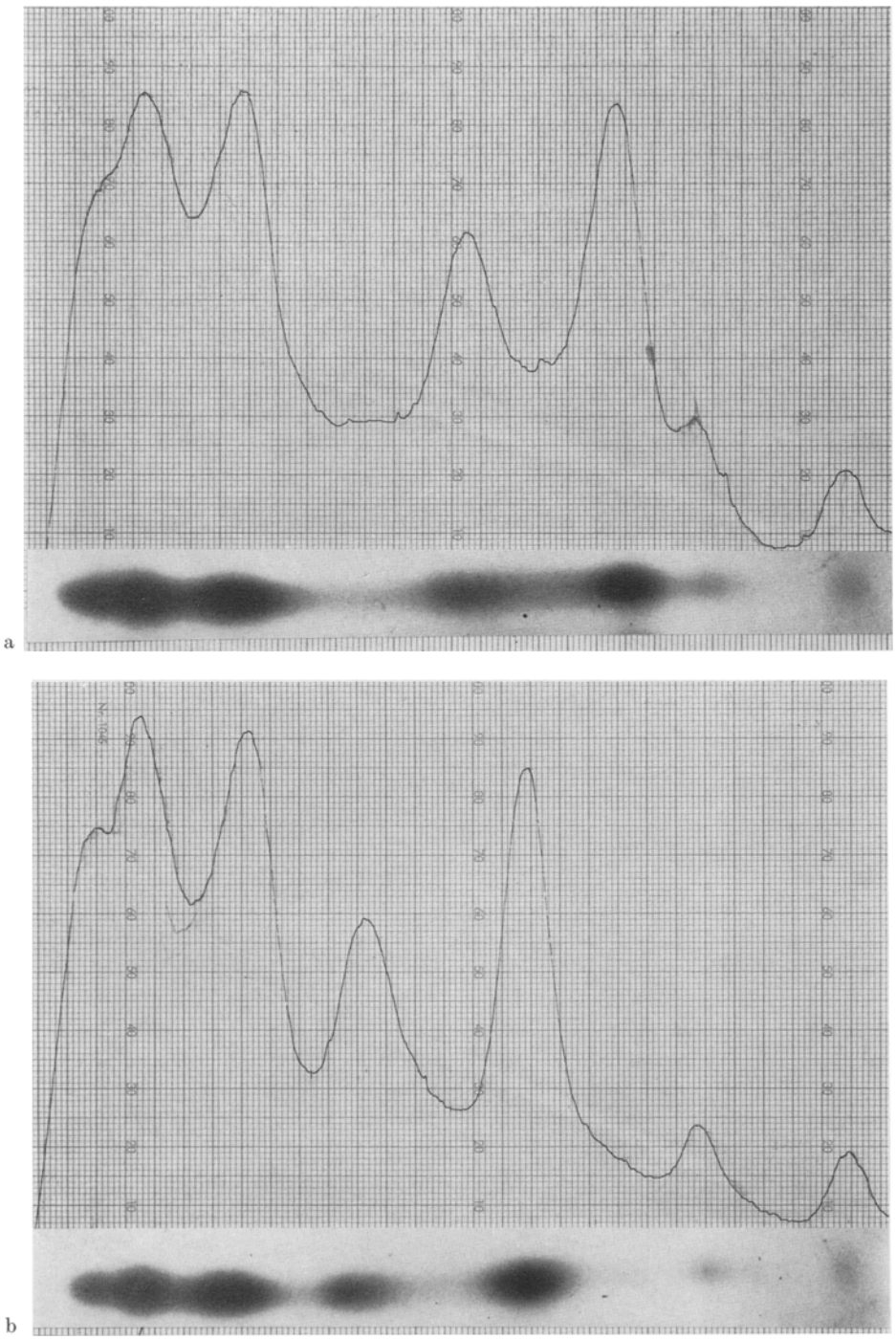

Fig. 7a and b. Densitograms and corresponding photographs in family Li. a Father Li. B Child Li.

Table 3. Appendix. Genetic markers in the families investigated

	System															
	AB0	MNSs	Rh-Ho	Kell	Duffy	Kidd	P	Hp	Gc	Gm	Inv	aPh	PGM_1	AK	ADA	6-PGD	GPT
Family																	
Ho.:																	
mother	0	NSs	ccddee	K+	(a+)	n.t.	P+	1	1	1—,2—	1+	BA	1	1	1	A	2—2
child	0	MNSs	CcD.ee	K—	(a+)	n.t.	P+	1	2—1	1—,2—	1—	A	2	1	1	A	2—2
father	0	MNss	$CC^wD.ee$	K—	(a+)	n.t.	P—	2—1	2—1	1—,2—	1—	BA	2—1	1	2—1	A	2—1
Wo.:																	
mother	0	MSs	ccD.ee	K—	(a+)	n.t.	P+	2—1	1	(1—,2—)	(1—)	BA	2	1	1	A	n.t.
child	A_1	MNSs	CcD.ee	K—	(a+)	n.t.	P—	1	2—1	(1—,2—)	(1—)	BA	1	1	1	A	n.t.
father	A_1	NSs	CCD.ee	K—	(a+)	n.t.	P+	2—1	2	(1—,2—)	(1—)	B	1	1	1	A	n.t.
Be.:																	
mother	A_2	NS	CCD.ee	K—	a+,b+	a+,b+	P+	2	1	1—,2—	1—	BA	1	1	1	A	2—1
child	0	NS	CCD.ee	K—	a+,b—	a+,b+	P+	2—1	1	1—,2—	1—	CB	2	1	1	A	2—1
father	A_1	NS	CcD.ee	K—	a+,b+	a+,b+	P—	2—1	1	1—,2—	1—	CB	2—1	2—1	2—1	A	2—1
Li.:																	
mother	A_1	MSs	ccD.Ee	K—	a+,b+	n.t.	P+	2—1	1	1+,2+	1—	B	1	1	1	A	2—2
child	A_2	MNss	ccD.Ee	K—	a+,b+	n.t.	P+	2—1	1	1+,2—	1—	B	8^w—1	1	1	A	2—1
father	0	Nss	ccD.EE	K—	a+,b+	n.t.	P+	2—1	1	1+,2—	1—	B	8^w—2	1	1	A	2—1

within one series (Fig. 2). The usefullness of measuring the PGM_1:PGM_2 ratio in presumed quantitative variants is restricted, therefore, to a comparison with controls of the same series. To observe a considerable decrease of this ratio (f.i. half the normal) in one or more persons may lead one to assume a systematical influence.

A more reliable method is obtained by measuring the ratio of isozymes controlled by one locus, as has been demonstrated by the rather low range of variability in the ratio a + c : b + d (Fig. 3). This agrees well with the observations of Terenato *et al.* (1970). There was no remarkable influence of the isozyme d′ in our series, since the ratio of 1.0 was established without considering d′. The extent of range was not considerably affected in artificial mixtures of opposite types, which would indicate that our results were reliable when clear deviations were observed in one family.

Family Studies. All findings in the families Ho., Wo. and Be. are consistent with the existence of a silent allele PGM_1^0. Some data, however, need further study: The degree of decrease in enzyme activity was variable, ranging from 37 to 48%. In family Wo. a difference of 0.4 units between mother and child was observed. It can be concluded from the densitometric findings that these differences are due to differences of the isozyme activities controlled by PGM_1.

In order to compare the normal range of quantitative data, the difference of 0.4 units in family Wo. has to be doubled, because two genes code for the total enzyme activity.

The difference of 0.8 units between two family members would rather appear to be of systematic than of random character. The same conclusion applies to the variable rate of decrease within the families observed. From the data of Fiedler and Pettenkofer (1969) an extent of decrease of 34% was calculated. All data of considerable quantitative variation within propositi concerning the silent allele lead one to suspect the existence of microheterogeneity. Further data and additional methods (inhibition studies, immunological studies) are required until definitive conclusions can be drawn on this subject.

Another interesting point arises from the fact that the mean decrease of enzyme activity was higher than expected. McAlpine *et al.* (1970b) pointed out that the relative activities of PGM_1 and PGM_2 are approx. equal in red cells. If this can be also attributed to the total PGM activity *in vitro*, the heterozygous state of the PGM_1^0 allele should be expected to show a mean decrease of 25%. From the observation of apparently stronger decreases in our families, including that described by Fiedler and Pettenkofer (1969), we would conclude that the proportion of the activity of PGM_1 *in vitro* is greater than predicted by the studies of McAlpine *et al.* (1970b). This difference is possibly due to the fact that kinetic reactions of separated isozymes cannot be compared quantitatively with the whole enzymic activity.

The finding of considerably weak isozymes corresponding to PGM_1^8 in family Li. leads us to assume the existence of a quantitative variant in this region under genetic control. The corresponding allele has therefore been designated PGM_1^{8w} ("w" for weak). Since these isozymes were detected only after prolonged incubation, the existence of this allele has to be taken into consideration in paternity determination.

The findings discussed above are also important in medico-legal cases. The data presented suggest that the heterozygous state of the silent allele can be demonstrated with a high degree of assurance, if different methods are used, including appropriate controls. If data in two or more family members are consistent, it may be concluded that a silent allele is present. The whole procedure should be performed in paternity exclusion due to apparent opposite homozygosity of man and child.

We are obliged to Dr. D. A. Hopkinson, University College London, for the investigation of blood samples of family Li.

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