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TECHNICAL NOTE

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Further Alleles of Phosphoglucomutase in Human Semen Detected by Isoelectric Focusing

The polymorphism of the phosphoglucomutase (PGM) system was first demonstrated by starch gel electrophoretic studies conducted by Spencer et al [*1*] on the enzyme from the human red cell. Pedigree studies [*2*] have shown that two common autosomal alleles PGM_1^1 and PGM_1^2 situated on the same locus of chromosome 1 [*3*] are responsible for determining the three common phenotypes PGM_1 1, PGM_1 2, and PGM_1 2-1 that are observed by starch gel electrophoresis.

Subsequent investigations [*4–6*] have shown two further loci, PGM_2 and PGM_3, located on different chromosomes [*7,8*], each of which determines a separate set of isoenzymes and all being inherited in a Mendelian fashion.

Isoelectric focusing of PGM from red blood cells produces a far more complex band pattern [*9,10*] than that observed by starch gel electrophoresis. Bark et al [*11*] examined 150 blood samples and proposed that the more complex band patterns, which gave ten distinct phenotypes instead of the three normally seen by starch gel electrophoresis, were due to the presence of four isoenzymes which they called 1+, 1−, 2+, and 2−. They suggested that the 1+1− and 2+2− isoenzymes were equivalent to the "a" and "b" isoenzymes, respectively, formerly proposed by Spencer et al [*1*]. Similar studies conducted by Kühnl [*12*] confirmed these observations, and Sutton and Burgess have shown that these alleles are inherited in a Mendelian fashion.

Semen, like blood, also shows PGM activity [*14*], but unlike blood only the isoenzymes determined by the PGM_1 locus can be detected by starch gel electrophoresis [*15*]. In view of the limited number of polymorphic systems available for typing human semen it was considered that the new PGM_1 alleles, if they could also be demonstrated in human semen, would be invaluable to the practicing forensic serologist.

Materials and Methods

All isoelectric focusing work was conducted on the LKB 2117 Multiphor apparatus supplied by LKB Instruments, England. The preparation of the electrofocusing gels, run-

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ning conditions, and the final enzyme visualization have been previously described in our work on this enzyme from the human red cell [*13*].

It was found that direct application of liquid semen to the isoelectric focusing gels produced distortions in the isoenzyme patterns which may be due to the proteolytic or the viscous nature, or both, of the material. The difficulty was overcome by preparing stains from these semen samples on linen cloth and storing them at room temperature until required. The stains (5 by 3 mm) were then moistened in 0.1% mercaptoethanol solution in distilled water and applied at a point approximately 2 cm from the anode; this procedure prevents any residual material from the stain masking the area in which the isoenzymes will subsequently focus.

Results and Discussion

The pattern obtained following electrofocusing of semen specimens earlier typed by conventional starch gel electrophoresis as PGM_1 1, 2-1, and 2 are illustrated in Fig. 1, with a corresponding diagram in Fig. 2. The patterns are identical to those observed following electrofocusing of red cells [*11,13*] except that the "c" and "d" isoenzymes are not so intense; the second locus isoenzymes were not visible. The identity of each phenotype was based on the resolution of the "a" and "b" bands, all of which were clearly visible.

In this survey we examined 100 semen stains. The observed frequencies of nine of the ten common phenotypes are shown in Table 1. Although this survey was relatively small we were able to obtain close agreement between the observed and expected gene frequencies on the basis that four and not two alleles are determined by the first locus [*2*]. We were unable to find a 2+2− phenotype in the survey, but we predict from the expected frequency data that it will have a frequency of approximately 3% in the British population.

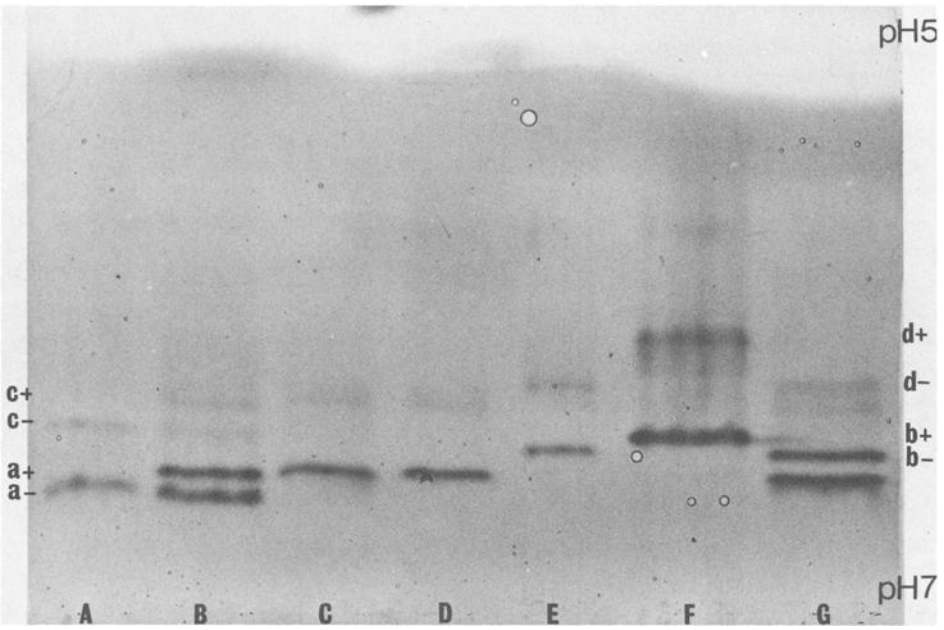

FIG. 1—*Isoelectric band patterns of six of the ten PGM_1 phenotypes from human seminal plasma;* A = *1*−, B = *1+1*−, C = *1*+, D = *1*+, E = *2*−, F = *2*+, *and* G = *2*−*1*+. (*An example of a 2+2− phenotype was not found in this survey.*)

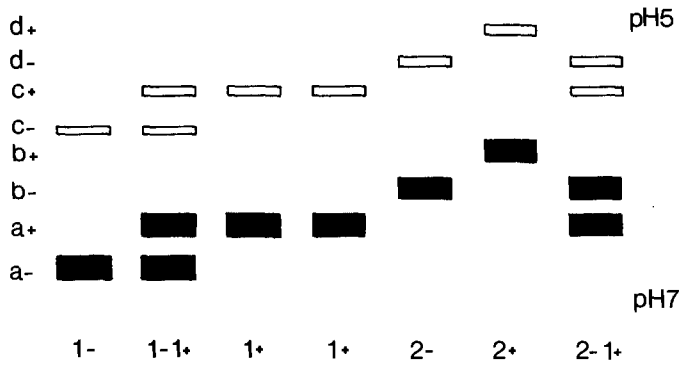

FIG. 2—*Diagram showing six of the ten PGM$_1$ phenotypes from human seminal plasma. The proposed relationship of the new PGM$_1$ isozymes to those described by Spencer et al* [1] *using starch gel electrophoresis is given.*

TABLE 1—*Frequency of PGM$_1$ phenotypes in semen.*

Old Phenotype	Observed Frequency (Blood), % [*1*]	New Phenotype	Observed Number[a]	Observed Frequency, %	Expected Frequency, %
1	55	1+	37	37	40
		1+1−	19	19	15.7
		1−	1	1	1.5
2−1	37	2+1+	18	18	16.4
		2+1−	2	2	3.2
		2−1+	15	15	14.5
		2−1−	2	2	2.8
2	7	2+	3	3	1.7
		2+2−	...	...	3
		2−	3	3	1.3

[a]Total = 100.

A PGM$_1$ 1 phenotype observed by starch gel electrophoresis may therefore be one of three possible phenotypes by isoelectric focusing designated as either PGM$_1$ 1+, PGM$_1$ 1−, or PGM$_1$ 1+1−. Similarly, a PGM$_1$ 2 phenotype could be either a PGM$_1$ 2+, PGM$_1$ 2−, or a PGM$_1$ 2+2−. The PGM$_1$ 2-1 heterozygote could therefore be one of the four possible phenotypes PGM$_1$ 2+1+, PGM$_1$ 2−1+, PGM$_1$ 2+1−, or PGM$_1$ 2−1−. At no time have we observed a heterozygote with all four isoenzymes or any triplet combination of these isoenzymes.

We have found that the frequency distribution of the isoenzymes of PGM separated by isoelectric focusing are in very close agreement with those reported in blood [*11–13*]. The discriminating power[2] [*16,17*] of the PGM system has now been increased from 0.56 for the three phenotypes observed by starch gel electrophoresis to 0.77 for the ten phenotypes now observed by isoelectric focusing.

At present the grouping of semen stains in forensic science laboratories is not so advanced as that of blood; usually only two systems are used: ABO and PGM (starch gel). The use of these new genetic variants of PGM will considerably enhance the investigation of semen stains in forensic science.

[2]The discriminating power is defined as the probability that two semen samples taken at random from an infinitely large population will be discriminated by a given test.

Summary

A technique for identifying further alleles of PGM_1 in human semen detected by isoelectric focusing has been described. A survey of 100 semen samples has shown that there was close agreement between the observed and expected gene frequencies of these new alleles on the basis that there were four common alleles determined by the PGM_1 locus and not two as originally proposed [*1,2*]. The use of these new genetic variants of PGM will considerably enhance the investigation of semen stains in forensic science.

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