J Forensic Sci, Apr. 1979, Vol. 24, No. 2

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An Improved Means of Enzyme Typing of Hair Roots Using Isoelectric Focusing

A recent publication [*1*] described the characterization of human hair using a technique dependent on starch gel electrophoresis of phosphoglucomutase (PGM) from hair root sheath cells. Similar, independent work has been reported elsewhere [*2,3*]. Phosphoglucomutase has three common phenotypes, PGM_1 1, PGM_1 2-1, and PGM_1 2, observable by starch gel electrophoresis and hence typing of hair roots in this manner is a valuable aid to discriminating human hairs.

Concurrent with this work on hair have been developments in the typing of blood based on the PGM system. Using the technique of isoelectric focusing it has been shown that the "a" and "b" isoenzymes observed by the starch gel electrophoresis of PGM may each be resolved into either of one or two extra bands [*4-6*]. This gives rise to ten PGM phenotypes, instead of three as originally described by Spencer [*7*] using the starch gel method. The technique of isoelectric focusing has also been shown to be applicable to semen with this system [*8*].

In this paper we report the application of isoelectric focusing to the study of human hair roots, based on the PGM system, and demonstrate an improved means of human hair discrimination.

Materials and Methods

Collection of Hair Roots

Plucked Hairs—Hairs were plucked (pulled rapidly) in a random manner from various regions of the scalps of eight individuals who had previously been blood-typed by isoelectric focusing and whose PGM_1 phenotypes were therefore established. Pubic and chest hairs were also taken from certain donors; these hairs were used immediately after plucking. In addition, some hairs were removed from the scalp of some donors by firm but slow pulling. This procedure causes anagen hairs to be obtained that are free of sheath cells [*9*].

Aging of Plucked Hairs—Plucked head hairs were attached to a glass plate with adhesive tape in such a way that the roots were completely exposed to the air. The hairs were stored at room temperature and individual hairs were typed at intervals up to six weeks.

Isoelectric Focusing

Single hair roots were applied directly to an isoelectric focusing plate of pH 5 to 7 [*4*]. The applications were made approximately 10 mm from the anode.

Received for publication 18 Sept. 1978; accepted for publication 18 Oct. 1978.

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The electrofocusing was performed on the LKB 2117 Multiphor apparatus (LKB Instruments, England) with settings of 1600 V, 20 W, and 15 mA. These settings produced an initial voltage and current of 400 V and 15 mA, respectively, which altered to 1600 V and approximately 10 to 12 mA during the 2-h running period.

Enzyme Visualization—Enzyme visualization was carried out by using an agar overlay method with the reaction mixture as previously described [*6*].

Results

Freshly Plucked Hairs

The results obtained from the roots of head, pubic, or chest hairs were similar to those obtained for blood after isoelectric focusing and PGM visualization. In each instance the PGM phenotype from hair matched that obtained from blood. Figure 1 illustrates results obtained from a number of hairs of different phenotypes. The classification of the phenotypes PGM_1 2−1+, 2+1−, and so forth and the labeling of the bands a−a+, b−b+, and so on follows that recommended for blood [*4*].

It is important that the running period does not exceed 2 h because beyond this period we observed denaturation of the bands with the appearance of extra "shadow" bands at a more cathodic position.

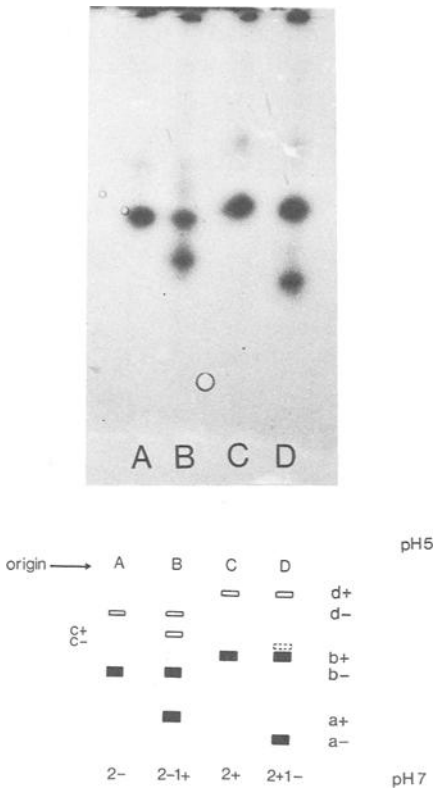

FIG. 1—*The isoelectric focusing pattern obtained from sheath cells from human head hair showing PGM phenotypes as follows:* (A) *2*−, (B) *2*−*1*+, (C) *2*+, *and* (D) *2*+*1*−. *Single hairs were applied to the surface of the gel at the origin mark.*

Aging Studies

Good results have been obtained from hair roots dried and maintained at room temperature (mean, 18°C) for six weeks (Fig. 2).

Anagen Hairs Without Sheath Cells

Figure 3 illustrates clearly that PGM activity is associated only with hair roots bearing sheath cells; no activity was detected from hair roots alone.

Discussion

The discovery of PGM activity in hair root sheath cells has provided a valuable means of discriminating between different hair samples. Although the three common PGM_1 phenotypes can readily be observed by starch gel electrophoresis the demonstration of the ten new PGM_1 phenotypes by isoelectric focusing has provided an even more valuable means of discriminating among such samples. This increase in discrimination is more precisely assessed in terms of the "discriminating power" (DP) advocated by Fisher [*10*] and later by Jones [*11*]:

$$DP = 1 - \sum_{i=1}^{n} p_i^2$$

where n is the number of PGM_1 phenotypes and p is the frequency of these phenotypes in the population.

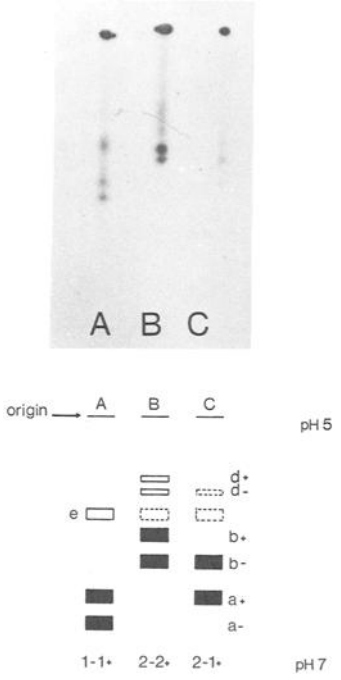

FIG. 2—*Isoelectric focusing of hair root sheath cells, after storage at room temperature for six weeks, showing PGM phenotypes as follows:* (A) *1−1+*, (B) *2−2+*, *and* (C) *2−1+*.

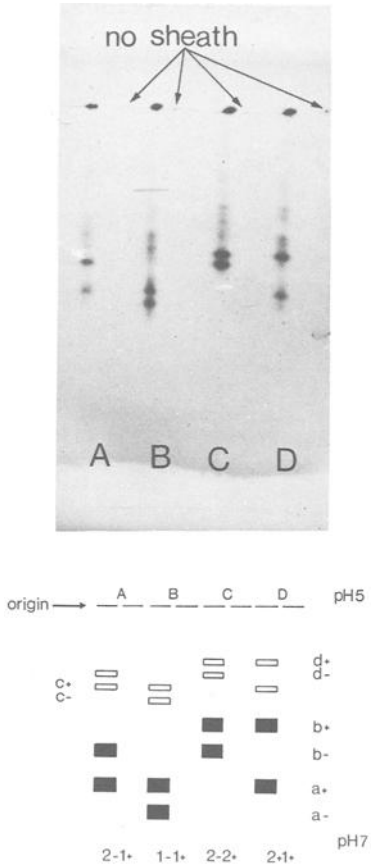

FIG. 3—*Isoelectric focusing of paired hairs from donors* A *through* D, *with and without sheath cells. The blood phenotypes of the donors were as follows:* (A) *2−1+*, (B) *1−1+*, (C) *2−2+*, *and* (D) *2+1+*. *Only those hairs with sheath cells show the corresponding PGM phenotypes after isoelectric focusing of the hair roots.*

Discriminating power is defined as the ability to discriminate between two individuals taken at random from a large population when a given blood-grouping system is used. With this system DP_{PGM} for isoelectric focusing is 0.77, while DP_{PGM} for starch gel is 0.56. The DP of the new isoelectric focusing system is very close to that achieved by such complex systems as Rhesus (DP = 0.79) calculated by using the five antigens c, C, D, E, and e.

As reported in our earlier work using starch gel electrophoresis [*1*], the PGM activity of plucked hair roots appears to be associated only with the sheath cells. However, hairs without sheath cells (those removed by slow but firm pulling) did not show PGM activity. We confirm that plucked hairs with sheath cells attached give good results even after having been dried at room temperature for many weeks.

The application of enzyme typing of hair roots in case work should therefore be of most value in those cases of assault, including murder and rape, in which hair is snatched or pulled from the scalp during the fracas between the parties involved. The enzyme typing of hair roots using starch gel electrophoresis is already being used in case work in the

United Kingdom.[2] We may expect the use of isoelectric focusing to extend the usefulness of this new technique for characterizing hair.

Summary

An improved method of grouping hair, based on the alleles of PGM observed by isoelectric focusing, has been described. The increased discriminating power of this system (0.77) compared to that obtained by the starch gel technique (0.55) provides a new and more sensitive means of typing hair.

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[2]Mrs. L. Fereday and her colleagues in our sister laboratory, the Aldermaston Forensic Science Laboratory, are now using this technique when necessary in case work. They hope to publish their experiences with the method in the *Journal of the Forensic Science Society* as soon as possible.