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Enzyme Typing of Human Hair Roots

At present there exists no reliable means of characterizing human hair. Although several attempts have been made to provide improved techniques for the examination of hair, relatively little progress has been made [*1*]. In the present study, the possibility of detecting isoenzymes in hair roots after electrophoresis was investigated as a means of characterizing human hair. The enzymes chosen were those for which genetically determined isoenzyme systems are well documented (phosphoglucomutase, acid phosphatase, adenosine deaminase, adenylate kinase, and esterase D) and which are easily demonstrable after starch-gel electrophoresis.

Methods

Collection of Hair Roots

Plucked Hairs—Hairs were plucked randomly from various regions of the scalps of 34 donors who were staff at the Home Office Central Research Establishment, Aldermaston or the Metropolitan Police Laboratory, London.

Brushed Hairs—Hairs which had been removed from the head by brushing, generally within the previous 24 h, were collected from the hairbrushes of three donors.

As soon as conveniently possible after collection, the hairs were cut 1 cm from the root end and the latter was frozen for periods of up to two weeks before use. The hair roots were frozen and thawed for a second time in an attempt to enhance the solubility of the proteins before electrophoresis.

Aging Studies—Plucked hair roots were stored in a dry condition in a paper envelope at room temperature (15 to 20°C), in an oven at 37°C, or in a moist chamber at 37°C. After storage the hair roots were frozen and thawed twice before electrophoresis.

Electrophoresis

After freezing and thawing were completed, hair roots were applied directly to 1-mm thick starch gels and subjected to electrophoresis. For determination of phosphoglucomutase (PGM) types, the method of Culliford and Wraxall [*2*] was used and the enzyme visualized with the overlay method described by Spencer et al [*3*]. Electrophoresis of acid phosphatase was carried out according to the method of Wraxall and Emes [*4*] and of adenosine deaminase by the method of Spencer et al [*5*]. Esterase D isoenzymes were separated with a tris(hydroxymethyl)aminomethane (Tris)-maleate buffer system and stained with 4-methylumbelliferyl acetate [*6*]. Adenylate kinase isoenzymes were separated by electrophoresis with a succinic acid buffer system described by Culliford [*7*].

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The results of electrophoresis were recorded photographically and, for the aging studies, photographs of the starch gels were scanned with a Chromoscan densitometer. The PGM activity was estimated semiquantitatively since it was not possible to make allowance for the different sizes of hair roots.

Results

Plucked Hairs

Of the five enzymes chosen for study PGM seemed to be present in greatest quantities in hair roots. Starch-gel electrophoresis of single plucked hair roots with either complete or fragmentary outer root sheaths produced isoenzyme patterns in accordance with the PGM type obtained with red cell lysates from the same individuals. The PGM isoenzyme patterns obtained from single hair roots are shown in Fig. 1. In all, 71 hair roots from 34 individuals were grouped successfully. Of these donors, 19 were PGM Type 1, four PGM Type 2, and 11 PGM Type 2-1. Most of the PGM activity appeared to be in the outer root sheath, and the intensity of the bands of activity obtained on electrophoresis depended on the amount of follicular tissue adhering to each hair root. On five occasions no PGM activity was present despite the fact that fragments of sheath could be seen on these hair roots.

Adenylate kinase, adenosine deaminase, and esterase D activities were also detected in plucked hair roots and behaved in the same way on starch-gel electrophoresis as the red

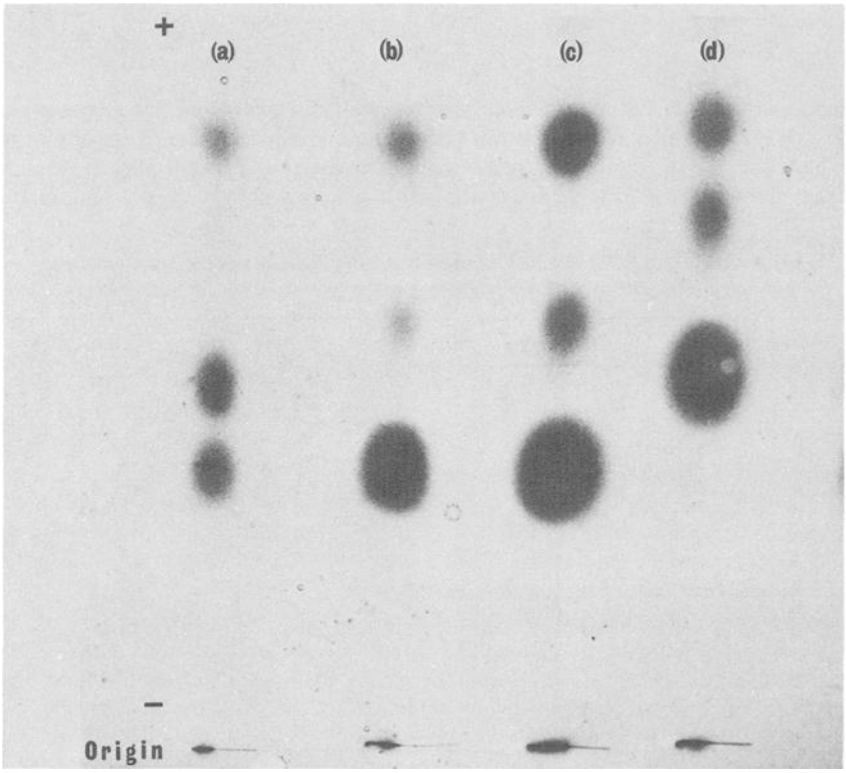

FIG. 1—*Starch-gel electrophoresis of PGM from single plucked hair roots;* (a) *PGM 2–1,* (b) *and* (c) *PGM 1, and* (d) *PGM 2.*

cell enzymes from the same individuals. These enymes seemed to be present in much smaller quantities than PGM, and activity could not be detected in hair roots bearing only fragments of outer sheath. Acid phosphatase activity was found to be present in hair roots but produced isoenzyme patterns different from those obtained on starch-gel electrophoresis of erythrocyte acid phosphatase.

Brushed Hairs

Attempts to type PGM in the roots of brushed hairs with varying amounts of outer root sheath adhering to them were mostly unsuccessful. Out of a total of 68 hairs from three donors in which PGM typing was attempted, only 2 had sufficient enzyme activity to enable identification of the PGM type and a further 2 had slight activity that was insufficient to enable typing. In approximately half of these hair roots a small amount of PGM activity could be seen at the point of application of the hair root to the starch gel.

Aging Studies

It was possible to identify PGM types in plucked hair roots that had been stored in a dry condition at room temperature for up to six weeks (Table 1) when hairs with large amounts of sheath material were used. With increasing periods of storage, a greater part of the PGM activity remained at the origin and failed to migrate on electrophoresis (Fig. 2). A more rapid decrease in PGM activity in hair roots stored at 37°C was observed, but storage in a moist chamber at 37°C almost completely eliminated PGM activity after a period of two days.

Discussion

Although enzyme activity in hair roots was detected for each of the five enzymes considered, only PGM seemed to be present in sufficient quantities to enable typing of plucked hairs. The reason for the presence of relatively large amounts of PGM activity in the outer root sheath is not clear. It may be associated with the large amounts of glycogen found in

TABLE 1—*Activity of PGM in hair roots stored in a dry condition at room temperature. Plucked hair roots bearing relatively large amounts of outer root sheath were selected.*

Donor	PGM Type	Period of Storage, days	PGM Activity
A	1	0	+ + +
A	1	4	+ + +
A	1	8	+ + +
A	1	10	+ +
A	1	15	+ +
A	1	29	+ +
A	1	39	+
A	1	40	+
B	1	0	+ + +
B	1	3	+ + +
B	1	10	+ +
B	1	45	+ +
C	2	0	+ + +
C	2	3	+ + +
C	2	10	+ +

[a] + + + = PGM activity approaching maximum seen from a single hair root.
+ + = Sufficient activity remaining to be easily typed.
+ = Only just possible to type.

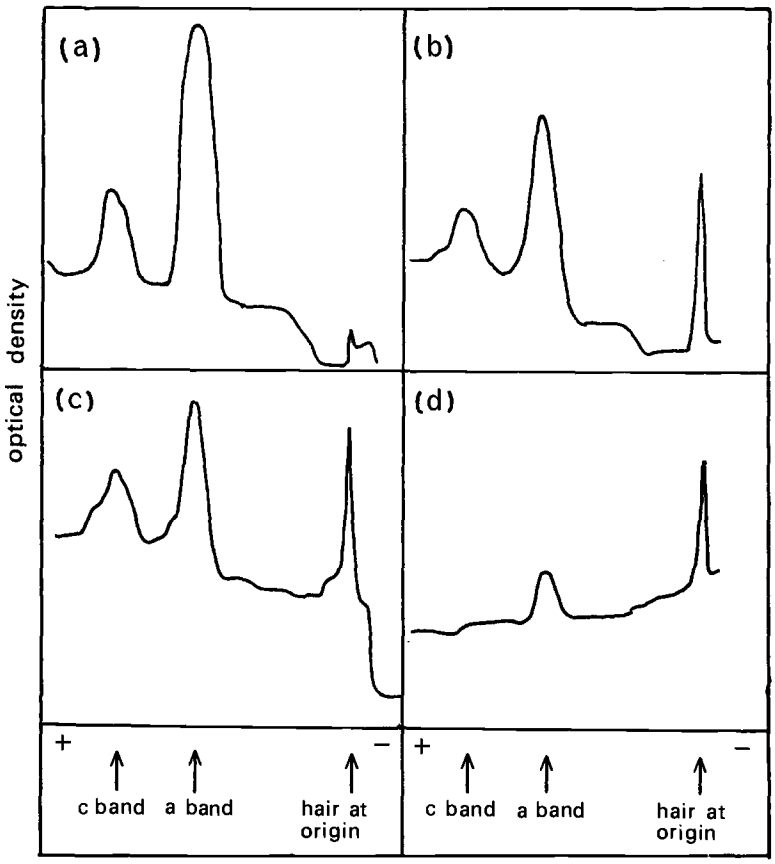

FIG. 2—*Densitometer tracings of starch gel electrophoresis of PGM from single hair roots stored at room temperature, in a dry condition, for* (a) *0 days,* (b) *15 days,* (c) *29 days, and* (d) *40 days. Plucked hair from a PGM-1 donor was used.*

the outer root sheath, since PGM catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate, an important step in glycogen metabolism.

Very low levels of PGM activity were detected in brushed hairs when compared with plucked hair roots of similar age and with comparable amounts of sheath material. This great reduction in activity may be attributed to a difference in growth phase between plucked and brushed hairs, although all hairs with outer root sheaths may be considered to be in the anagen phase [*8*]. It is possible that only hairs reaching the end of their anagen phase are dislodged by brushing and PGM activity in the root sheaths may be reduced naturally in these hairs. However, Adachi and Uno [*9*] found little difference in enzyme levels in sheath cells of anagen and telogen hairs, although PGM was not included in their study.

Alternatively, the difference in PGM activities may be due to the environment of the root after removal from the scalp. It was shown that increased temperature and humidity rapidly reduced the amount of PGM activity. It is conceivable that the roots of hairs collected by brushing may have been detached from the scalp at an earlier date, and the hair remained trapped within the mass of hair until removal some time later. The hair root would then be maintained in conditions of temperature and humidity higher than the storage conditions used for plucked hair roots, accounting for the rapid deterioration of PGM in hair roots collected by brushing.

Of the other enzymes detected in hair roots, adenylate kinase, adenosine deaminase, and esterase D were present in insufficient quantities to make typing of hair roots a practical proposition with present techniques.

At present PGM typing of hair is limited to plucked hair roots bearing at least a fragment of outer root sheath. Analysis of PGM types may therefore find application in hair samples obtained from certain crimes of violence when hair has been removed forcibly from the scalp. Since single hair roots may be grouped, the technique could be used to establish the identity of individual hairs in a mixed sample.

Summary

Sufficient phosphoglucomutase activity was found to be present in plucked hair roots bearing either fragmentary or complete outer root sheaths to enable typing of individual roots by starch-gel electrophoresis. Hair roots collected by brushing were found to contain very little PGM activity. Other isoenzyme systems were detected in hair roots but in insufficient quantities to make typing feasible.

References

[1] Porter, J. and Fouweather, C., "An Appraisal of Human Head Hair as Forensic Evidence," *Journal of the Society of Cosmetic Chemists*, Vol. 26, 1975, pp. 299-313.

[2] Culliford, B. J. and Wraxall, B. G. D., "A Thin-Layer Starch Gel Method for Enzyme Typing of Bloodstains," *Journal of the Forensic Science Society*, Vol. 8, 1968, pp. 81-82.

[3] Spencer, N., Hopkinson, D. A., and Harris, H., "Phosphoglucomutase Polymorphism in Man," *Nature* (London), Vol. 204, 1964, pp. 742-745.

[4] Wraxall, B. G. D. and Emes, E. G., "Erythrocyte Acid Phosphatase in Bloodstains," *Journal of the Forensic Science Society*, Vol. 16, 1976, pp. 127-132.

[5] Spencer, N., Hopkinson, D. A., and Harris, H., "Adenosine Deaminase Polymorphism in Man," *Annals of Human Genetics* (London), Vol. 32, 1968, pp. 9-14.

[6] Hopkinson, D. A., Mestriner, M. A., Cortner, J., and Harris, H., "Esterase D: A New Human Polymorphism," *Annals of Human Genetics* (London), Vol. 37, 1973, pp. 119-137.

[7] Culliford, B. J., *The Examination and Typing of Bloodstains in the Crime Laboratory*, U.S. Government Printing Office, Washington, D.C., 1971, p. 139.

[8] Orentriech, N., in *Hair Growth*, W. Montagna and R. L. Dobson, Eds., Pergamon Press, London, 1967, p. 99.

[9] Adachi, K. and Uno, M., in *Hair Growth*, W. Montagna and R. L. Dobson, Eds., Pergamon Press, London, 1967, p. 528.

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