TECHNICAL NOTE

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The Effects of Heat Upon the Glyoxalase I Isoenzyme

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ABSTRACT: It was observed during the course of routine casework that different bloodstains from the same individual could produce anomalies in the glyoxalase I band patterns. Bloodstains were heated at different temperatures for periods of 4 and 6 h and then examined using electrophoretic techniques. It was demonstrated that upon heating, band alterations in the glyoxalase I Type 1 phenotype can occur, causing the analyst to render the results inconclusive.

KEYWORDS: forensic science, glyoxalase, electrophoresis, genetic typing, phenotype, polymorphism, isoenzyme

In the forensic science laboratory, glyoxalase I (GLO I) polymorphism has been used in the typing of dried bloodstains. Three distinct phenotypes of GLO I (GLO 1, GLO 2-1, and GLO 2) have been identified in the population and are directed by two common autosomal alleles, GLO^1 and GLO^2 [*1,2*].

The phenotypes of GLO I can be demonstrated from dried bloodstains by the use of electrophoretic methods. Alterations in their band pattern may be seen with thermal degradation, and such an alteration was experienced in an actual case sample (Fig. 1), prompting research relative to the change observed.

Materials and Methods

Blood samples used for this study were stains prepared on clean cotton sheeting from whole blood. Portions of each stain were subjected to temperatures of 0, 37, 56, and 65°C for periods of 4 and 6 h.

A total of 21 tests were initiated. The first group consisted of eight bloodstains incubated for 4 h at 0, 37, 56, and 65°C. All stains were analyzed using the Group 1 procedure [*3*]. The second group consisted of nine bloodstains incubated for 6 h at 0, 37, 56, and 65°C.

Tank buffer was composed of 0.10*M* Tris, 0.10*M* maleic acid, 0.01*M* ethylenediamine tetraacetic acid (EDTA) free acid, and 0.01*M* magnesium chloride ($MgCl_2 \cdot 6\ H_2O$, pH 7.4 [*4*]. Gel buffer (pH 7.4) was a 1:14 dilution of the tank buffer.

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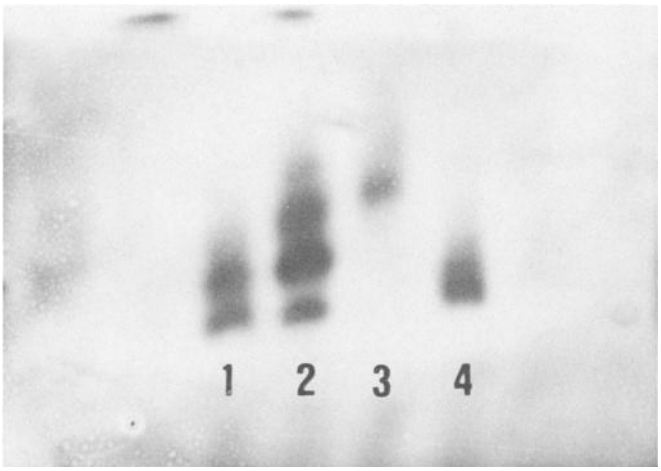

FIG. 1—*GLO I isoenzymes. Lanes 2 through 4 are control samples representing Types 2-1, 2, and 1, respectively. Lane 1 is a known GLO I Type 1 from a case sample showing a second band forming above or anodic to the "1" band position.*

Cotton threads were prepared for electrophoresis by dipping 1-cm-long portions of stained thread into single drops of 0.05*M* Cleland's reagent (dithiothreitol) and inserted into a 1% agarose, 2% hydrolyzed starch gels as described by Wraxall [*4*].

For electrophoresis, two layers of Whatman No. 1 filter paper were used to connect the anodal and cathodal buffers to the gel. Electrophoresis was carried out at a constant voltage of 400 V for 2 h at 5°C, with initial milliamperage ranging from 35 to 45, using an LKB 2103 power supply.

After electrophoresis, the GLO phenotype was developed by soaking a mixture of 12-mg reduced glutathione, 50 μL of 40% methyl glyoxal in 10 mL of 0.20*M* phosphate buffer, pH 6.2, onto a 10- by 14-cm Whatman No. 3 filter paper [*5*]. After any excess reagent was removed, the paper was laid on the anodic portion of the gel. The gel was incubated at 37°C for 30 min. After incubation, the overlay was removed and 0.5 g of agarose was dissolved in 25 mL of water, followed by the addition of 200 μL of iodine solution to the dissolved agarose, which was then poured onto the gel where the overlay had been placed earlier. The GLO I isoenzymes appeared as blue areas on a yellowish/clear background.

Results and Discussion

Eighty-eight results were obtained from the first group of tests run on the bloodstains; one hundred and fifty-three results were obtained from the second group.

Some of the results of the GLO I isoenzyme separations are shown in Fig. 2. GLO I types can be resolved and are fairly linear across the gel (Fig. 3). Problems, however, can occur.

It should be remembered that if no GLO I isoenzymes develop, one of the following possibilities exist: (a) the enzymes have degraded to the point of being nondetectable or (b) procedural errors may have occurred. Analytical parameters must be properly maintained to achieve regular, reproducible results.

Figures 4 and 5 demonstrate the appearance of a second band forming directly anodic to the No. 1 band position in the known GLO I Type 1. This band appears as a possible degradation product, and intensifies as the heating process continues. With the appear-

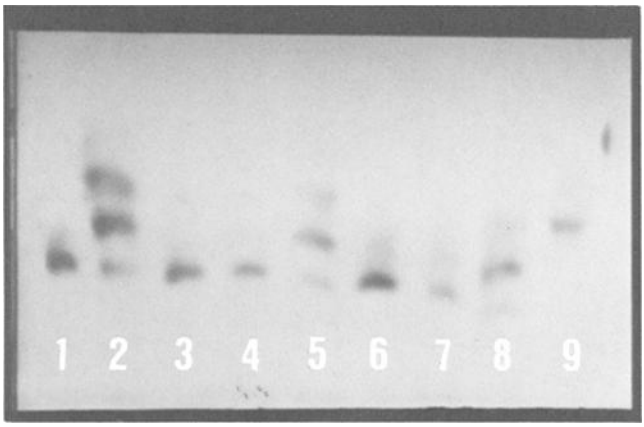

FIG. 2—*GLO I band patterns. Lanes 1, 2, 5, 8, and 9 are control samples representing Types 1, 2-1, 2-1, 2-1, and 2, respectively. Lanes 3, 4, 6, and 7 are known GLO I Type 1 samples incubated at 0, 37, 56, and 65°C for 4 h. Note: shadow of anodic band forming in Lanes 6 and 7.*

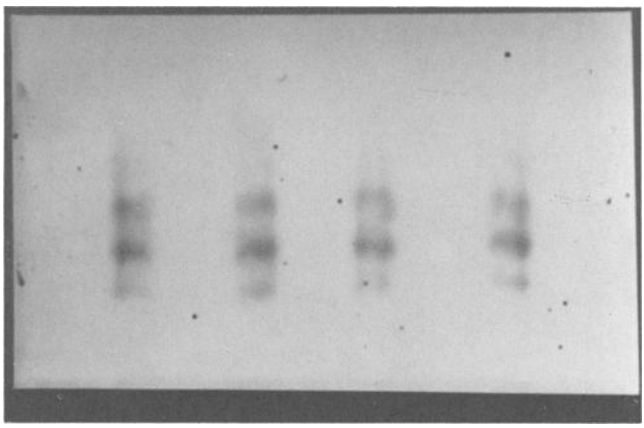

FIG. 3—*GLO I Type 2-1 band patterns. Band positions are fairly linear across the gel.*

ance of this second band, an inconclusive result will be recorded. Note that this second band is of equal or less intensity than the bottom band, but never more intense.

A second observation was made concerning the gradual disappearance of the No. 1 band on the GLO I Type 2-1. The middle band still remained more intense than the No. 2 band, so the missing band should not cause any erroneous interpretations. Complete loss of activity can be seen as the heating process continued (Fig. 6).

Experiments with temperatures inside automobiles were conducted at this laboratory. Temperatures of bloodstains exposed to direct sunlight were found to reach 73°C inside closed automobiles with an outside temperature of 22°C. Therefore, denaturation of bloodstains subjected to these conditions can be anticipated.

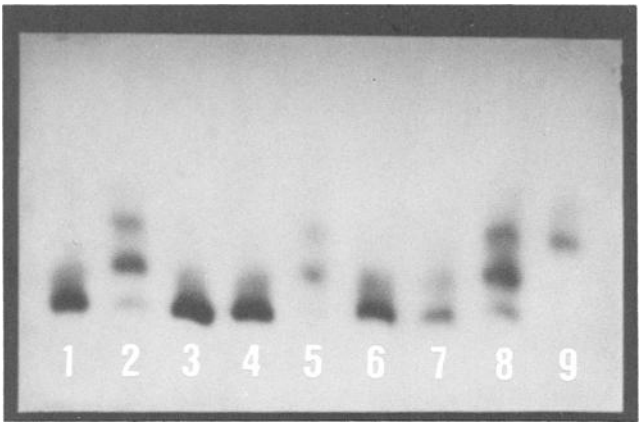

FIG. 4—*Known samples developed for GLO I. Note the appearance of second band forming in Lane 7. This sample is a known GLO I Type 1 heated at 65°C for 6 h. Lanes 1, 2, 5, 8, and 9 are control samples representing Types 1, 2-1, 2-1, 2-1, and 2, respectively.*

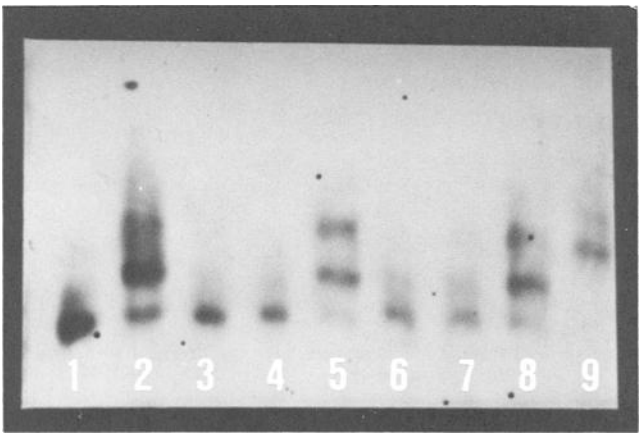

FIG. 5—*Known GLO I types. Lanes 1, 2, 5, 8, and 9 are control samples representing Types 1, 2-1, 2-1, 2-1, and 2, respectively. Lanes 3, 4, 6, and 7 are known GLO I Type 1 samples incubated at 0, 37, 56, and 65°C for 6 h.*

Since the majority of problems in Group 1 tend to be confined to the GLO I system, careful consideration should be given to the possibility of partial degradation of the sample, with the formation of a storage band to produce an anomalous result.

Conclusion

As shown in the figures, a GLO I Type 1 phenotype may develop an additional band when a bloodstain has undergone some heat denaturation. This will render the results inconclusive. Care should be taken to ensure that no misinterpretations are made when analyzing old or degraded samples.

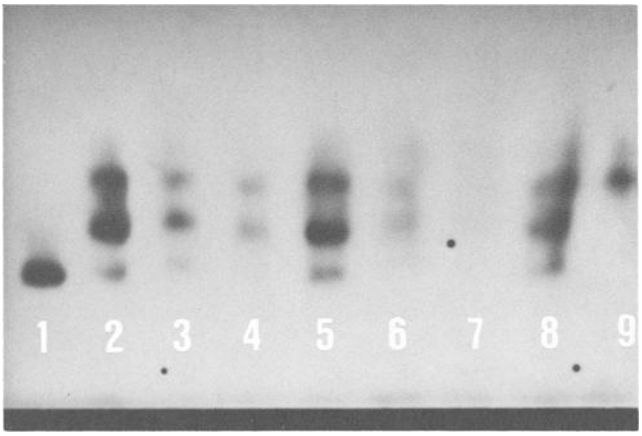

FIG. 6—*GLO I band patterns. Lanes 1, 2, 5, 8, and 9 are control samples representing Types 1, 2-1, 2-1, 2-1, and 2, respectively. Lanes 3, 4, 6, and 7 are known GLO I Type 2-1 samples incubated at 0, 37, 56, and 65°C for 6 h. Note gradual disappearance of the 1 (bottom) band while the middle band still remains more intense than the other bands. Complete loss of activity is demonstrated in Lane 7.*

References

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