TECHNICAL NOTE

T. E. Yeshion,[1] *M.S.*

Thermal Degradation of Erythrocyte Acid Phosphatase Isozymes in a Case Sample

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ABSTRACT: Analysis of bloodstains subjected to heat or improper collection techniques may yield expressions of erythrocyte acid phosphatase (EAP) phenotypes other than those originally coded. This report illustrates a case in which the deterioration of an EAP Type CB sample caused it to appear as an EAP Type C. Suggestions are offered to minimize such problems and to make the analyst aware of possible misinterpretations.

KEY WORDS: pathology and biology, phosphatases, genetic typing

Six distinct phenotypes of erythrocyte acid phosphatase (EAP) can be differentiated in white populations. Hopkinson et al [*1*] reported, in order of decreasing frequency, Types BA, B, A, CB, CA, and C. These types are controlled by three codominant, autosomal alleles: p^a, p^b, and p^c. Other rarely expressed phenotypes have been identified [*2-5*]. Because EAP typing provides a high potential for discrimination between individuals, it is commonly employed in forensic science analyses of bloodstains.

Blood samples subjected to heat or improper collection techniques may show alterations in their electrophoretic patterns. Analysis of such samples may yield an expression of phenotypes other than the "true" phenotype. This report illustrates a case involving the deterioration of an EAP Type CB sample.

Experimental Procedure

Electrophoretic techniques used in this study have been previously described by Wraxall and Emes [*6*].

Cleland's reagent (dithiothreitol), a reducing agent, was applied to all samples and incubated at room temperature for approximately 30 min prior to electrophoresis.

The nomenclature used for EAP band patterns has been adopted from Sensabaugh and Wraxall [*7*] and is based on theories offered by Fisher and Harris [*8*].

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[1]Serologist, North Carolina State Bureau of Investigation Crime Laboratory, Raleigh; presently, Florida Department of Law Enforcement Crime Laboratory, Tampa.

Case Summary

In a recent homicide case, several items of evidence from the victim, three suspects, and the crime scene were submitted for analysis. A "liquid" blood sample, collected from the ground upon which the victim was found, was submitted as a standard for comparison. This sample was hemolytic when it was received because of improper collection and transportation with exposure to temperatures exceeding 32°C (90°F) for several hours. Examination of this blood sample gave reactions for ABO Type O and phosphoglucomutase (PGM) Type 2-1. The EAP typing demonstrated a strong bc_1 (cathodic) band and a weak bc_2 (anodic) band. The location and relative intensity of these bands suggest the uncommon EAP Type C phenotype (Fig. 1).

Examination of the bloodstains from the victim's shirt gave reactions for ABO Type O, PGM Type 2-1, and EAP Type CB (Fig. 1). The victim's liquid blood sample obtained from autopsy also gave reactions for ABO Type O, PGM Type 2-1, and EAP Type CB (Fig. 1).

Discussion

Several studies on the thermostability of the EAP enzyme have noted various rates of denaturation [*6-11*]. All have concluded that the cathodic isozymes maintain greater stability than the anodic isozymes in the general order $bc_1 > a_1 > bc_2 > a_2$ or c > b > a. When the faster migrating components become weak, the slower components gain slightly in intensity [*6, 9, 10*]. Thus, as the anodal bands weaken, B types begin to appear as CB types and CB types begin to appear as C types.

Analysis of the case blood sample resulted in a strong bc_1 (cathodic) band and nearly complete degradation of the bc_2 (anodic) band in accordance with the thermostability studies noted above. This blood sample was recovered from the ground below the head of the victim by pipet into a stoppered test tube. This item, along with others, was transported to the investigating agency and retained there for several days at room temperature until subsequent submission to the laboratory. The deterioration of this sample could possibly have been avoided if (1) the sample had been delivered immediately to the

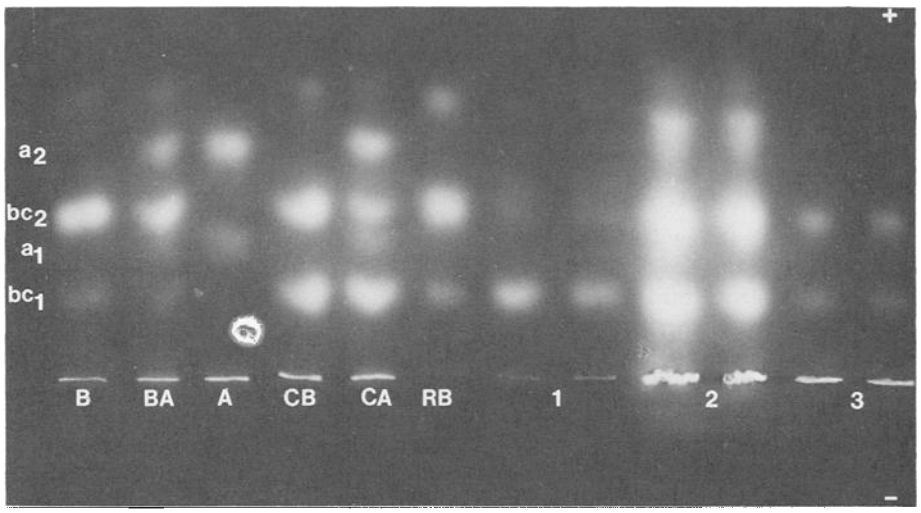

FIG. 1—*The common EAP phenotypes* (left to right): **B, BA, A, CB, CA,** *and the rare variant* RB; (1) *the case ground-blood sample,* (2) *stains from the victim's shirt, and* (3) *the autopsy sample.* (*Case samples are shown in duplicate.*)

laboratory as collected, or (2) the sample had been refrigerated until such time that it could be delivered, or (3) the sample had been absorbed onto a sterile cloth or gauze material and treated properly as a bloodstain.

It is important to note that the rarely occurring EAP Type C has a weak but well defined bc_2 (anodic) band. In those cases where there is a possibility of decomposition of the sample, identification of reactions for EAP Type C are not reliable and must be approached very cautiously. At least two distinct bands of activity should always be present before a particular phenotype is classified [*6*]. The importance of the presence of two or more bands is not only to distinguish a characteristic pattern, but also to compare relative intensities of the isozyme.

It is noted that no inconsistencies were encountered when the same case sample was analyzed for ABO or PGM types.

Summary

This paper has shown that deteriorated EAP Type CB blood samples may suffer decomposition of the bc_2 (anodic) band, leaving behind an intense bc_1 (cathodic) band, and thus appearing as an EAP Type C.

Generally, when a blood sample is exposed to excessive conditions it may undergo an alteration of the EAP isozyme patterns and appear to be some type other than that originally coded.

It is not uncommon to have bloodstained articles of evidence or autopsy samples from bodies that have been exposed to excessive conditions of heat or humidity, or both, to be submitted to the laboratory for analysis [*12*]. Problems such as those described here may at times be avoided by providing detailed training to field personnel on the collection and preservation of biological materials. Furthermore, in all cases it is good policy to obtain a "serological history" of the case samples from the officer and to approach these samples cautiously, especially those of questionable quality.

These points when applied by the forensic serologist will help to eliminate possible misinterpretations.

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Address requests for reprints or additional information to
T. E. Yeshion
Florida Department of Law Enforcement Crime Laboratory
P.O. Box 151776
Tampa, Fla. 33684