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

TECHNICAL NOTE

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Alteration of Electrophoretic Mobility of Hemoglobin in Bloodstains

Phenotyping of hemoglobin variants from whole blood and dried bloodstains is an important procedure for the forensic scientist. Although electrophoretic techniques for hemoglobin phenotyping have been described [*1-5*], there has been to our knowledge no published report of alterations in the electrophoretic mobility of hemoglobin in dried bloodstains. In the present study, observations were made on changes after electrophoresis on cellulose acetate membranes in the mobility of hemoglobin from bloodstains 1 to 28 days old. A procedure has been worked out to prevent these changes which may cause errors in the phenotyping of hemoglobin variants.

Materials and Methods

Equipment and Reagents

Electrophoresis Equipment—The Beckman Microzone Electrophoresis System (Beckman Instruments, Inc., Fullerton, Calif.) was used with Sartorius 5.5- by 14.5-cm cellulose acetate membranes (Sartorius Filters, Inc., South San Francisco, Calif.). A one- to eight-sample applicator previously described [*5*] was used to apply samples. A standard single-sample applicator can also be used.

Electrophoresis Buffers—The cell buffer consisted of 21.8 g glycine (0.28*M*) and 4.5 g tris(hydroxymethyl)aminomethane to 1 litre with water, pH 8.4. The cell buffer was used full strength as the membrane buffer.

Reaction Mixtures—The ethanol soak consisted of 95% ethyl alcohol. For the staining solution, 70 mg *o*-dianisidine was dissolved in 50 ml 95% ethanol. To this were added 30 ml of acetate buffer at pH 4.7 (2.92 g sodium acetate and 0.52 ml glacial acetic acid in 1 litre of water) and 1 ml 30% hydrogen peroxide.

Method

Samples—Whole blood samples, preserved anaerobically with acid-citrate-dextrose (ACD), were obtained from a local blood bank.

The preparation of these materials was financially assisted through a federal grant from the Law Enforcement Assistance Administration (LEAA) and the California Office of Criminal Justice Planning (OCJP) and under Title I of the Crime Control Act of 1973. The opinions, findings, and conclusions in this publication are those of the authors and not necessarily those of OCJP or LEAA, and OCJP reserves a royalty-free, nonexclusive, and irrevocable license to reproduce, publish, and use these materials, and to authorize others to do so. Received for publication 22 Aug. 1978; revised manuscript received 11 Oct. 1978; accepted for publication 13 Nov. 1978.

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Preparation of Bloodstains and Extracts—Bloodstains from the whole blood samples were prepared on pieces of clean white cotton cloth and were kept at ambient temperature and pressure for 1 to 28 days. To prepare extracts, a part of each bloodstain was soaked with a minimal volume of water, and another portion of the same bloodstain was soaked with a solution of Cleland's reagent (7.8 mg 1,4-dithiothreitol in 1 ml water). All stains were allowed to stand for 30 min and then centrifuged at 10 000 *g* for 1 min. The eluates were used for electrophoresis.

Standards—Standards were prepared from hemolysates of the same blood samples as used for the bloodstains and were kept frozen at −20°C between uses. Prior to electrophoresis, some hemolysates were diluted with water, and some were diluted with the same concentration Cleland's reagent as mentioned before. During the study, only bloodstains and standards of hemoglobin A, AS, and AC were used, as these were the most frequently found variants in random samples analyzed in the present study.

Electrophoresis and Staining Procedures—The operating conditions were 500 V, for 12 or 13 min, at 1.5 to 2.0 mA, with application to the cathode side of the cell.

After the electrophoresis, the membrane was placed for 30 s in 95% ethanol to displace most of the water in order to prevent the precipitation of *o*-dianisidine during staining. The membrane was then placed in the staining solution for 1 or 2 min. Following staining, the membrane was again placed in 95% ethanol to remove the excess *o*-dianisidine. The membrane was finally washed thoroughly with water and dried between blotters.

Results and Discussion

Even for bloodstains only one day old, the electrophoretic pattern for hemoglobin following water extraction showed a cathodal shift of all bands relative to standards (Fig. 1). Hemolysates were prepared for electrophoresis in different ways in a series of experiments to determine whether or not the altered electrophoretic mobility could be due to an artifact. Hemoglobin A was used as a standard. Hemolysates were deposited on both cloth and glass for drying. Prior to drying, one portion of the hemolysate was treated with ACD according to our usual procedure, and the other portion remained free of ACD. Two sources of distilled water were used for extraction, one from a quartz still (our usual source) and the other from a Millipore filtering apparatus, which produces water of very high electrical resistance. None of these experimental variations changed the observed electrophoretic mobility shift of the hemoglobin.

The shift of the electrophoretic patterns was absent for extracts from stains extracted with Cleland's reagent (Fig. 1). Similar results were seen with bloodstains seven days old (Fig. 2). For the 28-day-old stains extracted with Cleland's reagent, the alteration of the electrophoretic patterns for hemoglobin was still reversed to allow accurate phenotyping (Fig. 3). The electrophoretic patterns of standards showed no detectable changes with time. Standards run with and without the addition of Cleland's reagent showed identical mobilities (Fig. 4). It is generally known that Cleland's reagent reduces the disulfide SS bonds to the sulfhydryl SH, thus restoring their original electrophoretic mobility. Cleland's reagent appears to have a similar effect on the dried hemoglobin molecules.

Summary

The study shows that deterioration and shifting of the mobility occur in electrophoretic patterns of hemoglobin from bloodstains 1 to 28 days old. The use of Cleland's reagent reverses such changes in stains up to 28 days old. It is recommended that all bloodstains and all standards kept frozen at −20°C be treated with Cleland's reagent prior to use for electrophoretic phenotyping of hemoglobin.

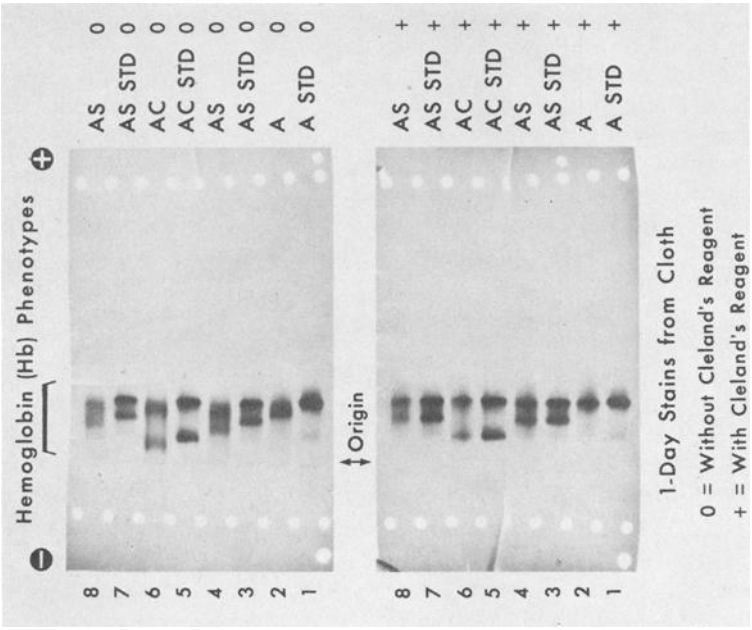

FIG. 1—*Electrophoretograms of 1-day-old hemoglobin standards and dried bloodstains. All samples in upper figure were treated with water; all those in lower were treated with Cleland's reagent.*

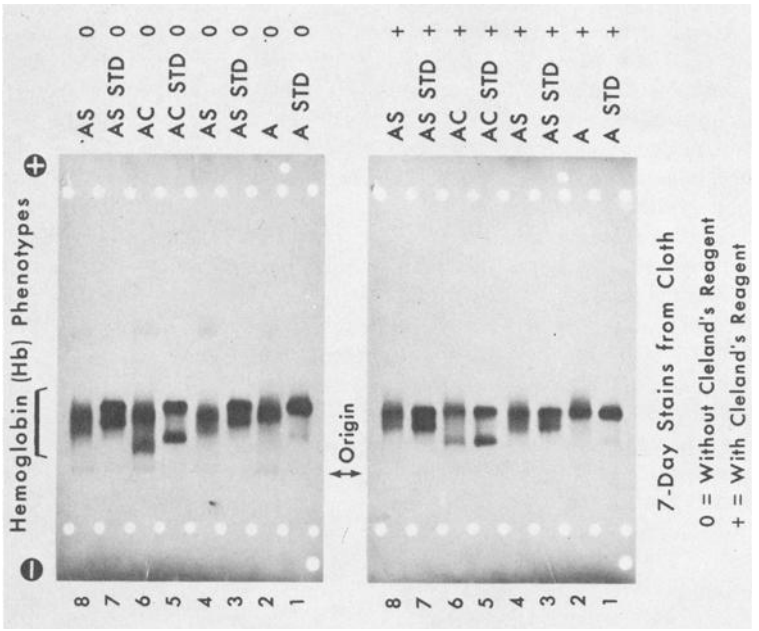

FIG. 2—*Electrophoretograms of 7-day-old hemoglobin standards and dried bloodstains. All samples in upper figure were treated with water; all those in lower were treated with Cleland's reagent.*

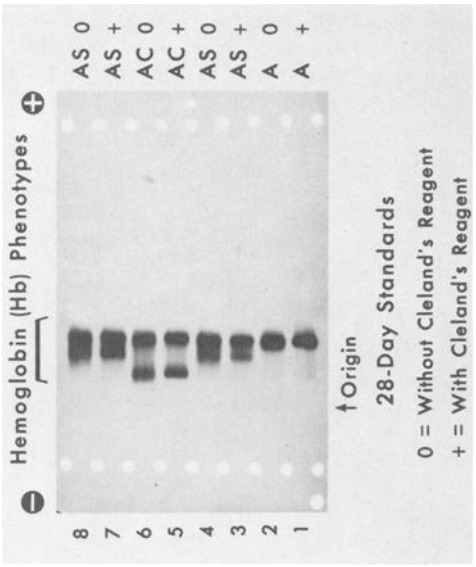

FIG. 4—*Electrophoretogram of 28-day-old hemoglobin standards, treated with water or Cleland's reagent as indicated.*

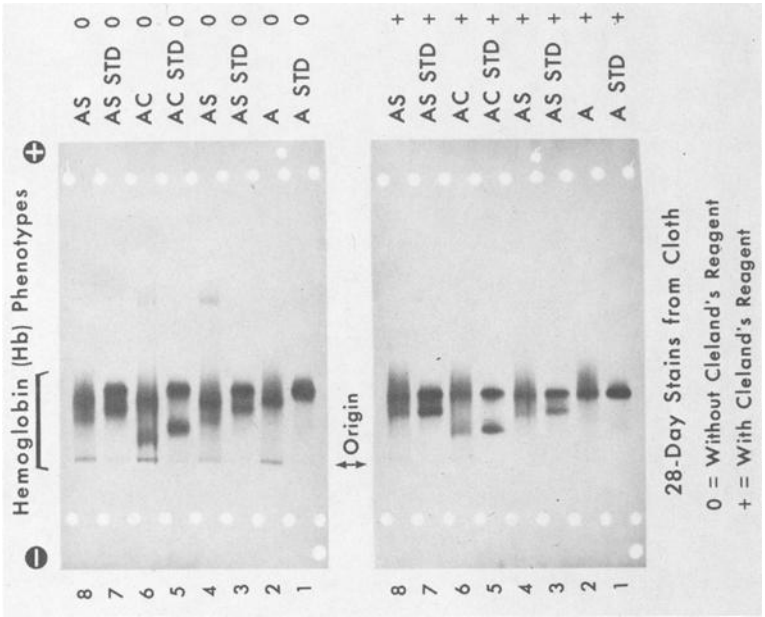

FIG. 3—*Electrophoretograms of 28-day-old hemoglobin standards and dried bloodstains. All samples in upper figure were treated with water; all those in lower were treated with Cleland's reagent.*

References

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