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Erythrocyte Diaphorases DIA_1 and DIA_2 in Bloodstains

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Summary. The ability to determine the phenotypes of erythrocyte NADH diaphorase (DIA_1) was demonstrated in bloodstains, the utility of the system extending about two weeks. Electrophoretic variants representing three uncommon phenotypes (DIA_1 2-1, DIA_1 4-1 and DIA_1 7-1) were found in five of 785 individuals tested. The NADPH diaphorase DIA_2 isozymes could not be resolved sufficiently for practical use.

Key words: Diaphorases, DIA_1 variants - Bloodstains, DIA_1 variants

Zusammenfassung. Die Möglichkeit des Nachweises der Erythrozyten-NADH-Diaphorase (DIA_1) in Blutspuren bis zu ungefähr 2 Wochen Liegezeit wurde gezeigt. Elektrophoretische Varianten einschließlich der drei seltenen Phänotypen DIA_1 2-1, DIA_1 4-1 und DIA_1 7-1 wurden in fünf von 785 getesteten Individuen gefunden. Dagegen konnte die NADPH-Diaphorase DIA_2 für den praktischen Gebrauch nicht ausreichend aufgetrennt werden.

Schlüsselwörter: Diaphorase (DIA_1), in Blutspuren - Blutspuren, Diaphorasenachweis

The presence of a rare genetic variant in a bloodstain makes the linking of that stain to a suspect individual much easier. While the routine analysis of bloodstains for rare variants may not be practical, an occasional analysis of this type may be important when other more conventional systems do not yield useful information.

Three gene loci were originally postulated for the diaphorase system, two of which were present in the red cell, DIA_1 and DIA_2, the NADH and NADPH diaphorase [2].

Red cell NADH diaphorase (DIA_1) is believed to prevent the formation of high concentrations of methemoglobin and dysfunctions of this enzyme have been associated with methemoglobinemia [1]. The red cell NADH diaphorase

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system has rare (<1%) electrophoretic variants which have been described in a number of papers [1–5].

The purpose of this study was to determine if the erythrocyte diaphorases could be detected in dried stains for any useful period and if the electrophoretic variants could be distinguished in these stains. The approximate frequency of variants was also determined.

Materials and Methods

Hemolysates were prepared from washed red cells lysed with an equal volume of distilled water, and stains were prepared on cotton cloth with whole blood.

Hemolysates were added to slits in the gel by means of capillary tubes, while cloth threads 1 cm long were inserted in the gel for dried stains.

Electrophoresis was carried out in starch using a Tris-EDTA-Borate buffer at pH 8.6 [1]. The bridge buffer was a 1 : 7 dilution of a stock solution 0.9 *M* in Tris (Tris(hydroxymethyl)-aminomethane), 0.5 *M* in boric acid and 0.02 *M* in disodium EDTA. The gel buffer was a similar 1 : 10 dilution, and an 11% 2 mm thick starch gel was used. Electrophoresis was carried out on horizontal plates using a potential of 5 V/cm for 17 h with cooling plates 5–7°C.

The staining mixture was 50 ml of a 25 m*M* Tris/HCl pH 8.5 buffer containing 10 mg NADH or NADPH (for DIA_2) 2.5 mg. Thiazolyl blue (MTT) and 1 mg 2,6-dichloroindophenol sodium salt. The mixture was poured over the anodic area of the starch gel and incubated at 37°C in the dark for up to 2 h. Purple areas defined the enzyme activity.

Results and Discussion

Electrophoresis was carried out for erythrocyte NADH diaphorase (DIA_1) and NADPH diaphorase (DIA_2) on a series of blood samples submitted for analysis. The samples were predominantly from white people, and three previously reported phenotypes [1, 3] were found in addition to the usual DIA_1 1 phenotype in a series of 785 samples; two DIA_1 2-1, two DIA_1 4-1, and one DIA_1 7-1. Figure 1 shows the results of the DIA_1 and DIA_2 separations. Figure 2 shows the DIA_1 phenotypes found in this study. The DIA_2 isozymes could not be separated and all attempts produced a smeared area of unresolved enzyme activity.

Hemolysates left at room temperature produced a spurious band anodal to the DIA_1 1 isozyme mimicking the 2-1 phenotype, and on further standing these samples produced diffuse banding cathodic to the hemoglobin, rendering interpretation uncertain.

Variants which were stained on cloth showed the same phenotypic pattern as the samples run as hemolysates although the enzyme activity was noticeably less. Stains examined at intervals of up to 2 weeks showed a progressive weakening of the enzyme pattern until finally the staining pattern was unreadable, although no "wrong" phenotypes were observed because of selective degradation.

The electrophoretic buffer system has been used to separate many variant hemoglobins and will easily resolve hemoglobin types A/S, A/C, S/C, and several others which may be of interest if a non-white population sample is studied. Because of low stability and rare occurrence the erythrocyte NADH dia-

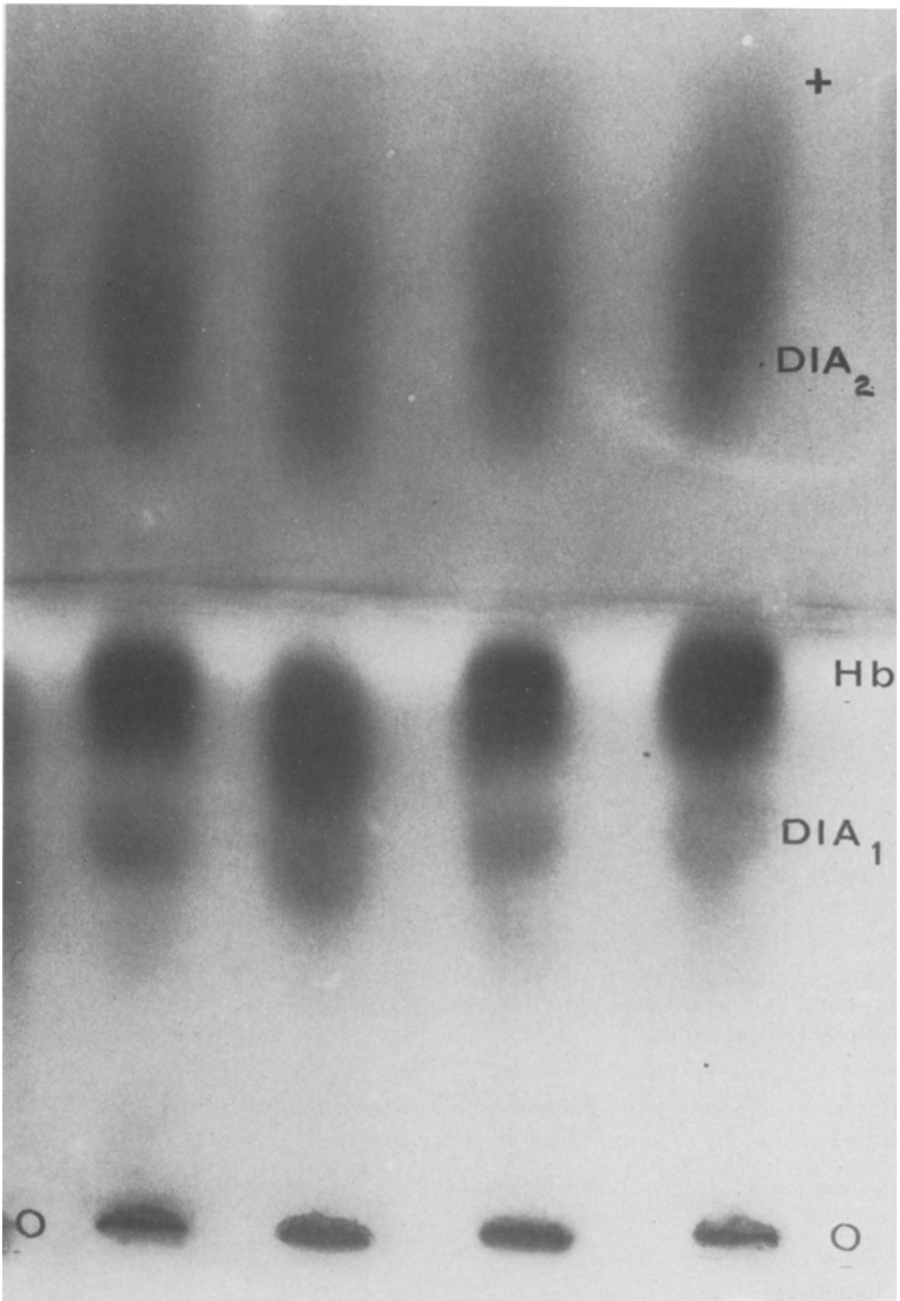

Fig. 1. The erythrocyte NADPH diaphorase (DIA_2) isozymes in relation to the NADH diaphorase (DIA_1) type 1 isozymes

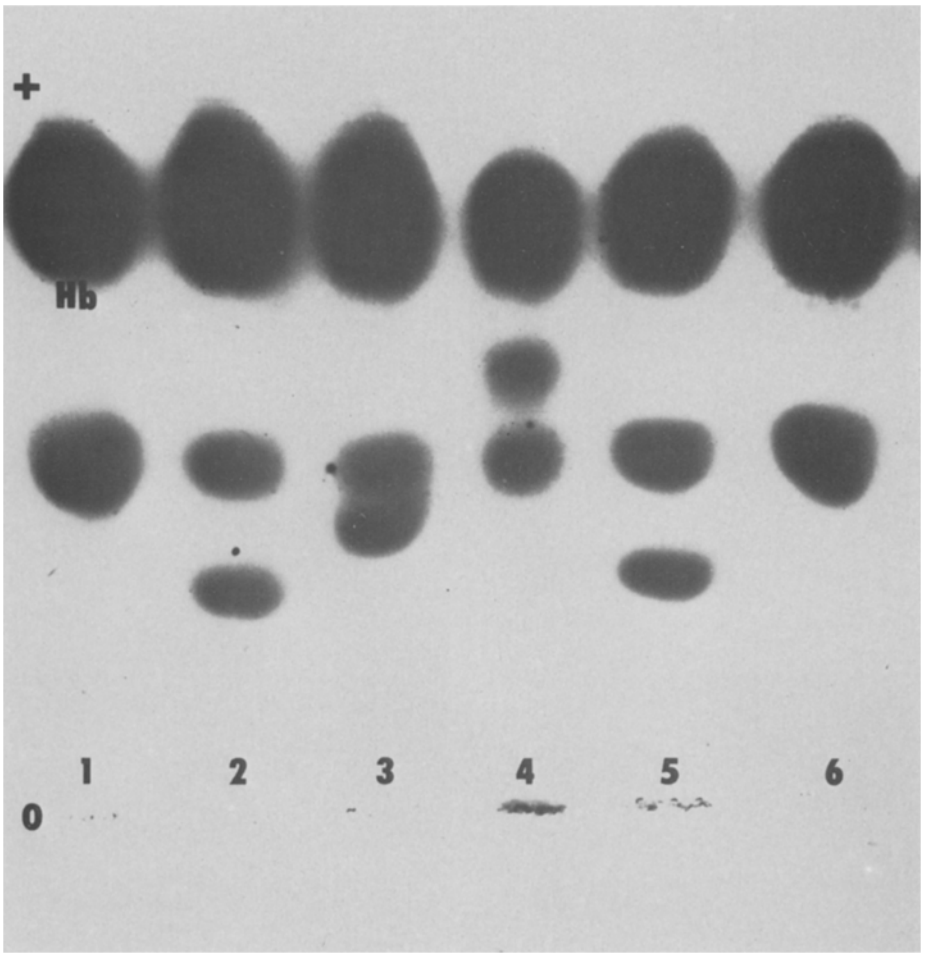

Fig. 2. The DIA_1 (erythrocyte NADH diaphorase) phenotypes. *Track 1, 6,* DIA_1 1; *tracks 2, 5,* DIA_1 4–1; *track 3,* DIA_1 7–1; *track 4,* DIA_1 2–1. The origin is at the *bottom* with hemoglobin (Hb), the dark band at *top*

phorase system appears to have little use in the routine examination of bloodstains but may be of some value in unusual cases.

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