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Investigations on the Decay of Acid Phosphatase Types in Stored Blood Stains and Blood Samples

BERND BRINKMANN, MARTHA GÜNNEMANN, and ERWIN KOOPS

Institut für gerichtliche Medizin und Kriminalistik der Universität Hamburg (BRD)

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Summary. A method is given for determining red cell acid phosphatase types in stored blood stains and blood samples. The time limits for determination in stored blood stains (20—25°C) vary from 6—8 weeks. In stored blood samples (4°C) the periods for determination were found to be approximately 15 months. The devised method has some advantages compared with conventional methods.

Zusammenfassung. Es wird eine Methode beschrieben für den Nachweis der Typen der sauren Erythrocytenphosphatase aus gelagerten Blutspuren und Blutproben. Die Grenzen des zeitlichen Nachweises aus gelagerten Blutspuren (20—25°C) variieren — je nach Spurenträger — zwischen 6—8 Wochen. Aus gelagerten Blutproben (4°C) ließen sich die Typen noch nach 15 Monaten nachweisen. Hinsichtlich der erforderlichen Spurenmenge und der zeitlichen Nachweisgrenzen ist die gewählte Methode vorteilhafter als die bisher angewendeten Techniken.

Key words: Red cell acid phosphatase polymorphism — Stored blood stains — Stored blood samples.

Rather discrepant data are available from the literature about the limits of determining erythrocyte acid phosphatase types (EAP) in stored samples and stains. The limits of time described for the decay in stored blood stains vary from 2 to 3 days (Nagata and Dotzauer, 1970) to 30 days (Heidel, 1968). Similar differences were observed in stored blood samples, where time limits vary from 10 days (Fiedler, 1967) to 12 months (Smerling, 1968).

These investigations were done in order to find effective methods in defining the time limits of these determinations more precisely.

Materials and Methods

Stains: Fresh drops of venous blood from persons with known phenotypes A, B, C, CB, CA, BA were pipetted on glass, plastic, cotton, and paper, grouped according to size, divided in different series: 10 μl, 20 μl, 50 μl, and 100 μl of blood, and allowed to dry at room temperature. One series was stored at —18 to —20°C, the other was stored in the room at 20—25°C (varying temperatures).

The samples were redissolved in different ways: stains that could be scraped off, were dissolved about three hours before they were inserted in a 0.06 m phosphate buffer, pH 6.2, containing mercapto aethanol 0.06 m. The same solution was used for the extraction of absorbed stains, which was performed as described (Brinkmann and Dirks, 1971). The lyophilized

extract was redissolved by adding 0.03 m phosphate buffer, containing 0.03 m mercapto aethanol, pH 6.2.

Fresh stains (1—3 days old) were dissolved in about 4/5 of the original blood volume, older samples in smaller volumes.

Blood samples: Clotting of blood samples was prevented by the addition of sodium fluoride (final concentration 0.6%; w/v) and these were stored at 4°C. Since the red cells were haemolysed completely at the first time of reinvestigation (4 weeks after withdrawal), the filter papers were soaked with the haemolytic sample. 2 to 4 hrs prior the electrophoresis, one tenth of the volume of the blood sample of a solution of 0.3 m phosphate buffer containing mercapto aethanol 0.3 m, final pH 6.2, was added.

The samples were reinvestigated every 3 months up to a final storage age of 12 months. 120 samples of the five commonest types were investigated alltogether.

Electrophoresis was carried out on horizontal polyacrylamide gels which were prepared according to Hennig *et al.* (1968). The gel buffer used was a 0.006 m sodium — potassium phosphate buffer containing 6×10^{-3} m EDTA, final pH 6.0. The bridge buffer was a citrate-phosphate buffer, 0.245 m in NaH_2PO_4 and 0.15 m in trisodium citrate (Karp and Sutton, 1967), pH 5.9. The samples were brought to different filter papers (seize 7×9 mm).

For fresh samples (1—3 day old stains; up to one month old blood samples), Schleicher & Schüll No. 2043b papers were used, preferably one to two-fold. Older stains and samples needed thicker papers (Schleicher & Schüll No. 598 and No. 5703) which were used up to two-fold.

The substrate solution used was twice the usual concentration. The incubation time was 4 hours.

Results and Discussion

Blood stains stored at room temperature showed different limits of determination of the EAP types. These depended on the phenotypes and the kind of stains: The maximum time limit of all six EAP types was found in stains which consisted of crusts. All types could still be determined after 60 days. After this time interval it became difficult increasingly to diagnose the types CA, BA and A reliably, because the anodic "a" isozyme became more and more faint, blurred, and finally disappeared. The slow gene product of P^c was still determined after 12 weeks and the types B, CB and C showed these time limits too.

In absorbed stains, the time limits were found slightly different: *All* phenotypes still could be determined after six weeks of storage, and the more stable types B, CB and C after 8—9 weeks.

Considerably high determination limits were found in stains that were stored in the deep freezer: All types could be determined after 7½ months, and types B and CB even after 10½ months of storage (type C was not present in this series).

Blood samples stored at 4°C showed a determination limit of 15 months for all phenotypes, except type C, which was not included in this series. After this period the same changes on storage were observed as in the blood stains.

The size of the necessary sample was increased continnously. In fresh stains a minimum of 10 μl of a haemolysate containing about 20 g% haemoglobin was needed. In the oldest stains and samples a considerable larger sample was necessary; that is 50—60 μl of a haemolysate containing about 30 g% haemoglobin. The types BA, CA and A sometimes had to be reinvestigated with larger samples inserted than the other types.

The devised procedures were developped systematically: Starch methods (Radam and Strauch, 1966; Karp and Sutton, 1967) turned out to be disadvantageous

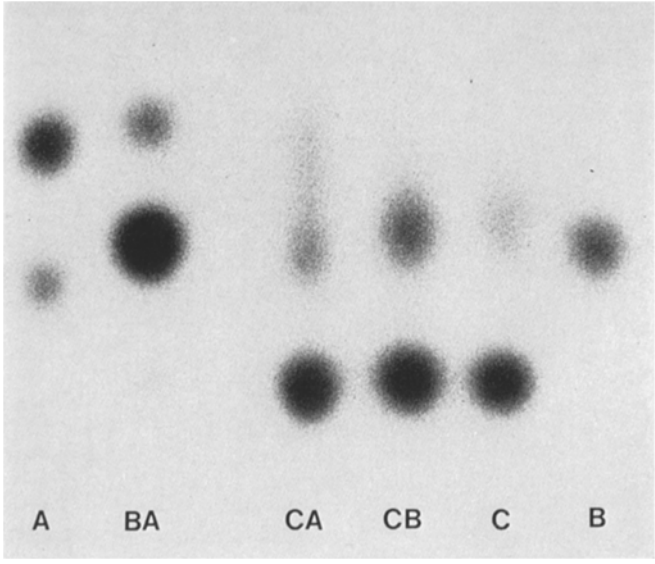

Fig. 1. EAP types in stains (on glass, plastic) stored at 20—25°C for 6 weeks

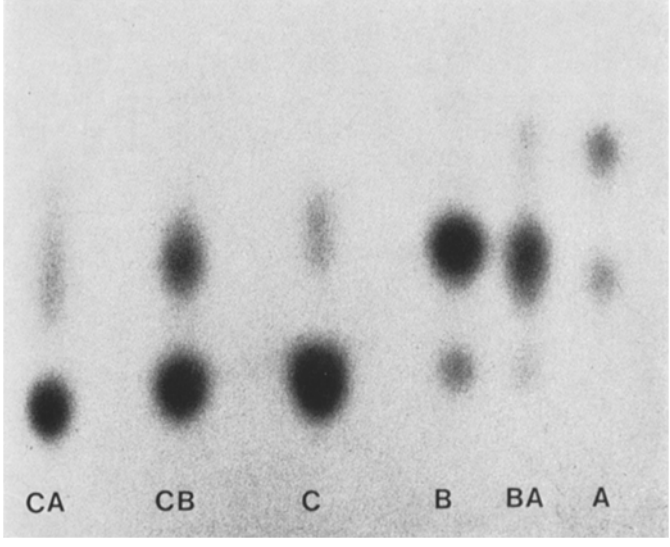

Fig. 2. EAP types in stains (on glass, plastic) stored at 20—25°C for 8 weeks

for this purpose, because the limit of determination was reached after 2—3 weeks. Furthermore, they needed much larger samples, i.e.a. 6 to 8-fold volume of the polyacrylamide method. Reimann and Willner (1968) recommended the use of a mangan solution for the activation of the enzyme in stored samples. This solution was used in different concentrations. No striking effect was observed. The method of Hennig et al. (1968) resulted in intensively stained patterns. However, as the

fast "a" isozyme became blurred first and since this isozyme shows low migration differences to the fast "b" isozyme, it was rather difficult to diagnose the heterozygous conditions of the P^a gene. Therefore, the described buffer which results in a better separation in this critical zone was preferred. When mercapto aethanol was omitted, the time limits were found to be approximately 3 weeks (Brinkmann, 1971). They could be prolonged considerably by adding mercapto aethanol and phosphate as described above. This observation is in agreement with results from the literature and with the enzyme's biochemical properties (Fisher and Harris, 1969; Giblett and Scott, 1965).

The observed differences in time regarding the kinds of stains (blood crusts or absorbed spots) are in accordance with similar observations on other SH-enzymes in stains (Brinkmann and Dirks, 1971).

The time differences of the different phenotypes correspond to the differences in stability and activity described by various authors (Spencer et al., 1964).

Since the amount of the sample has to be increased considerably in old stains and since this is the limiting factor regularly it is concluded that early enzyme typings only yield a higher resolution power of a stain.

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Dr. med. B. Brinkmann
Institut für gerichtliche Medizin
und Kriminalistik der Universität
D-2000 Hamburg 54, Butenfeld 34
Deutschland