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Determination of Phenotypes of Phosphoglucomutase (PGM_1) in Bloodstains by Cellulose Acetate Electrophoresis

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Summary. A good separation of PGM_1 isoenzymes from bloodstains has been obtained with an adapted Sonneborn method of electrophoresis on cellulose acetate. This method requires very small quantities of the examined material, a short time of electrophoresis, and is cheaper and simpler than hitherto described. A correct determination of PGM_1 phenotypes from bloodstains was easy up to 24 weeks. Occasionally it was possible to determine phenotypes in bloodstains as old as 47 weeks, and in one case even in bloodstains 126 weeks old. This method may be useful in the forensic serology for determination of PGM_1 phenotypes from bloodstains.

Zusammenfassung. Es wird eine gute Trennung der Isoenzymmuster von PGM_1 aus Blutspuren durch Adaptierung der Sonneborn-Methode mit der Celluloseacetat-Elektrophorese erreicht. Nur sehr kleine Mengen von untersuchtem Material und kurze Zeit der Elektrophorese sind bei dieser Methode erforderlich, die Technik ist billiger und einfacher als die bisher angewendete. Eindeutige Bestimmung der Phänotypen PGM_1 aus den Blutspuren bis zu 24 Wochen stößt auf keine Schwierigkeiten. Gelegentlich war die Phänotypenbestimmung auch in älteren Blutspuren möglich: bis 47 Wochen, und in 1 Fall sogar bis 126 Wochen. Die Methode kann in der forensischen Serologie bei der Blutspurenuntersuchung brauchbar sein.

Key words. Bloodstains, Phosphoglucomutase-PGM_1 – Cellulose Acetate Electrophoresis, PGM, Determination in Bloodstains

The polymorphism of phosphoglucomutase (PGM_1) was discovered by Spencer, Hopkinson and Harris [9]. The authors worked out the method of electrophoresis in starch gel using a TEMM buffer (TRIS-EDTA, maleic-acid, $MgCl_2$), pH 7.4. In the following years a number of the new methods of separation were introduced. In 1968 Monn [5] worked out the method of electrophoresis in agar gel using a phosphate buffer pH 7.5. In 1970 Hummel [4] presented the method of electrophoresis in the discontinuous system using phosphate and citrate buffers at pH 6.0. A year later

Wrede, Koops and Brinkmann [12] described the method of electrophoresis in horizontal polyacrylamide gel in the discontinuous system using a phosphate buffer, pH 6.2, and a TRIS-maleate buffer, pH 7.2. In 1972 Hoppe, Henning and Brinkmann [3] worked out the method of electrophoresis in horizontal polyacrylamide gel using a TEMM buffer, pH 7.2. The same year Sonneborn [8] used the electrophoresis on cellulose acetate and a TEMM buffer at pH 7.4. for the separation of PGM_1. The above-mentioned methods were used for the determination of PGM_1 phenotypes from fresh red cell lysates. Because of an advantageous distribution of the gene frequencies, attempts at determining PGM_1 phenotypes in bloodstains have been undertaken as well. In 1967 Culliford adopted the original method of Spencer, Hopkinson and Harris for the determination of PGM_1 phenotypes in bloodstains [2]. In 1968 Wraxall and Culliford [11] worked out the method of electrophoresis in thin-layer starch gel. This method, after some modifications, has been accepted by a number of authors. Oepen [6] determined PGM_1 phenotypes in bloodstains 8–10 weeks old, and Rothwell [7] in bloodstains 8–16 weeks old. Turowska [10] determined PGM_1 phenotypes in bloodstains 8 weeks old. In 1969 Brinkamnn employing his own method (electrophoresis in starch gel using a TEMM buffer at pH 7.4) determined PGM_1 phenotypes obtaining positive results in bloodstains 3–12 weeks old [1]. All the presented methods of determining PGM_1 phenotypes in bloodstains requires either a long time of electrophoresis and staining or considerable amounts of reagents, or the obtained electrophorograms are insufficiently readable. For this reason we have attempted to adopt the Sonneborn method of electrophoresis on cellulose acetate [8] for determination of PGM_1 phenotypes.

Materials and Methods

In the preliminary investigation the quality of separation obtained on "Cellogel" cellulose acetate foils from Chemetron of Milan and on foils from Sartorius, Biotest and Oxoid were compared with each other. The best separations were obtained on "Cellogel" foils and on foils produced by Biotest and these were used for further investigations.

Preparation of experimental stains. 50 samples of blood with known PGM_1 phenotypes were poured on strips of white cloth and dried and preserved at room temperature. Depositing samples of bloodstains on the foil. Strips of the stained cloth of the size of about 1 square millimeter were put directly on the foil with a small drop of the electrophoresis buffer. The strips were removed after the first 15 minutes of separation.

The electrophoresis was carried out according to the Sonneborn method using a TEMM buffer at pH 7.4, with slight modifications. The isoenzymes were stained by the sandwich method according to Sonneborn [8].

Results

In the material investigated by us the correct determination of the PGM_1 phenotypes was possible in all the cases of absorbed bloodstains up to 24 weeks old. Occasionally it was also possible to determine PGM_1 phenotypes in older bloodstains – up to 47 weeks old, and in one case in a bloodstains 126 weeks old. In some cases in bloodstains more than one year old it was only possible to prove the activity of the PGM_1, without the possibility of a sure determination of phenotypes. The obtained separations are shown on Figures 1–2. The results obtained by us entitle us to recommend this method for the practical determining PGM_1 phenotypes in bloodstains the more

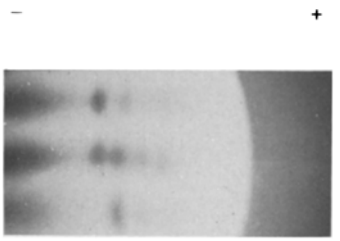

Fig. 1. Phenotypes of PGM_1 isoenzymes from the absorbed experimental bloodstains 11 days old ("Cellogel" cellulose acetate)

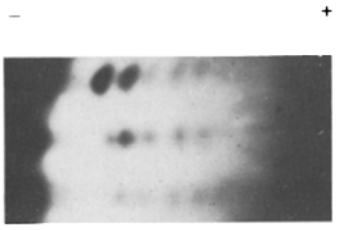

Fig. 2. Phenotypes of PGM_1 isoenzymes from the absorbed experimental bloodstains 24 weeks old ("Cellogel" cellulose acetate)

so that it requires only very small quantities of the examined material and is cheaper and simpler as well as less timeconsuming (total time-2 hours) than the hithero used methods. At present the interdependence between the detectability of PGM_1 isoenzymes and the conditions under which the bloodstains are preserved (the temperature of the environment, humidity, light, etc) and the ground on which they are found (wood, metal, cloth) is being investigated in detail and on a larger material.

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