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Increased Responsiveness of Electric Precipitation (Electrosynerese) by Means of a Chemical Method

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Summary. Electric precipitation is a method in serological diagnostics (determination of species-specificity). Since serum protein fractions (especially immunoglobulins) and extracts from blood stains migrate in agar-gel at pH 8.2—8.6 towards the cathode they cannot be used for the determination of the species specificity. By a chemical method using glutaraldehyde authors have produced an albumin-immunoglobulin complex, altering in this way the direction of electrophoretic mobility. Treatment of the blood samples with glutaraldehyde increases the sensitivity and reliability of the method.

Key words: Blood stains, Electric precipitation – Electric precipitation

Zusammenfassung. Die electric precipitation (Überwanderungselektrophorese) stellt ein Verfahren zur Diagnostik von Blutflecken (Bestimmung der Artspezifität) dar. Da die zum negativen Pol wandernden Fraktionen (insbesondere die Immunoglobuline) der Serum- und Blutflecken-Extrakte entsprechend der Ladungsverhältnisse der Eiweiße bei einem pH von 8,2–8,6 in Agargel für die Bestimmung nicht nutzbar sind, wurde von den Verfassern durch ein chemisches Verfahren, unter Verwendung von Glutaraldehyd, ein Komplex zwischen Albumin und Immunoglobulinen erzeugt, wodurch die Wanderungsrichtung der Immunoglobuline verändert wurde. Die Empfindlichkeit der Methode wird durch die Glutaraldehyd-Behandlung wesentlich erhöht. Mit deren Hilfe kann die Artidentifizierung der Eiweiße aus Blut und Körpersäften, selbst aus geringen Mengen, mit großer Sicherheit durchgeführt werden.

Schlüsselwörter: Blutspuren, Überwanderungselektrophorese – Überwanderungselektrophorese

Electric precipitation ("electrosynerese," Bussard) is known as a valuable instrument of criminological blood stain diagnostics, being a simple, fast and responsive method for determining the species specificity of blood proteins [1, 2]. It is

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essentially based on the rules of agargel-electrophoresis; at pH values of 8.2–8.6 the beta-2 and gamma fractions of the blood proteins are wandering towards the negative pole and all the rest towards the positive pole. Let us cut two holes in the gel and put the blood or the blood stain extract into the hole next to the negative pole and the immunoserum into the hole next to the positive one. Under the influence of electric current, the protein molecules of the blood or of the stain extract—except the beta-2 and gamma globulins—will approach the positive pole, while the immunoserum's gamma globulins of antibody specificity begin to move towards the negative pole. "Forced" to one another by the electric current, the protein molecules bring about a "provoked" precipitation reaction on the area between the two holes. However, there is an insufficiency of the method: the beta-2 and gamma globulins of the test material do not take part in the reaction! This is a major loss indeed: If the protein content of a normal blood serum amounts to 7–7.5 g%, then the concentration of the immunoglobulins wandering in the beta-2 and gamma fractions may be about 2–2.5 g%, and so we were unable so far to utilize about one third of the serum proteins.

This protein amount is especially missing when a small quantity of stain material must be investigated.

In order to eliminate this deficiency we must either change the electric charge of the proteins in question (make them negative) or link them chemically to a protein which is swiftly wandering towards the positive pole, building a complex with the immunoglobulins and "transporting" them as a carrier.

For quantitative determination of serum proteins Kingdon and Bowers [3] have published a method—mainly of clinical importance—which measures the concentration of immunoglobulins in agarose gel mixed with antibody by establishing an intermolecular linkage with glutaraldehyde between them and the albumin; since the intermolecular bonds do not damage the molecule, the immunological reaçtivity remains intact.

Material and Methods

This phenomenon was used according to the aims of electric precipitation and stain diagnostics. Based upon an earlier work [4], the authors modified the method so that the addition of the reagent hardly dilutes the test material—presumably of low protein content—while the immunoglobulins wandering in the beta-2 and gamma fractions and not utilized so far are building a complex with albumin and moving towards the positive pole present visible precipitation reactions.

The examinations were carried out on glass sheets of 10×7.6 cm size, with layers of 8 ml of Difco (Special Agar Noble (agar) conc. 1%) dissolved in pH 8.2 veronal buffer placed on them. The electric precipitation took 20 min at a tension adjusted according to 20 mA. After the electric current was cut off, the sheets were placed for 1 h into a moist chamber in order to reinforce the precipitation reaction; after moistening them for 1 h (physiological sodium chloride), they were dried and stained with Amido-black. For the experiments the authors used normal human serum and dilution series of a 1-month-old blood stain extract with antihuman immuno-sera developed in horse and rabbit. A 2.5% glutaraldehyde solution was made in veronal buffer and $^1/_{10}$ volumen part was added to the test material (10 parts protein solution, 1 part glutaraldehyde).

Results and Discussion

Under the influence of glutaraldehyde, a complex is brought about between immunoglobulins and albumin at room temperature within 30 min in agarose and agargel without impeding the immunological reaction.

Every fraction wanders towards the positive pole and takes part in the precipitation reaction.

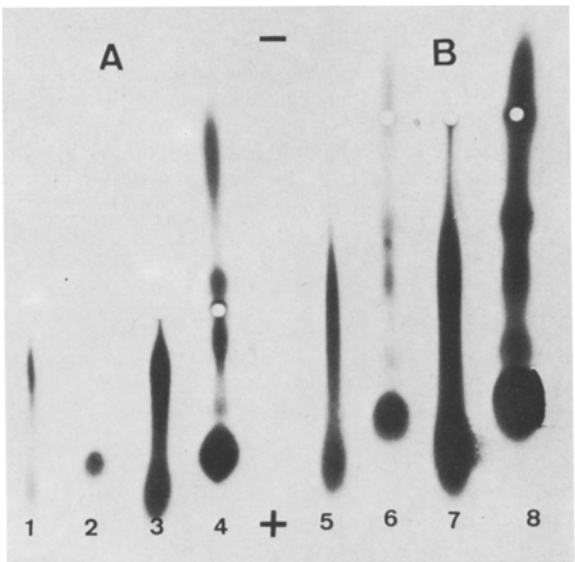

Fig. 1A and B. Electrophoresis of human serum in agar and agarose gel. Agar on part (**A**) of the sheet, agarose on (**B**). The samples 1, 3, 5, and 7 are treated with glutaraldehyde, the others are not. The samples 1, 2, 5, and 6 are sera diluted at a rate of $^1/_{10}$, the others are not diluted

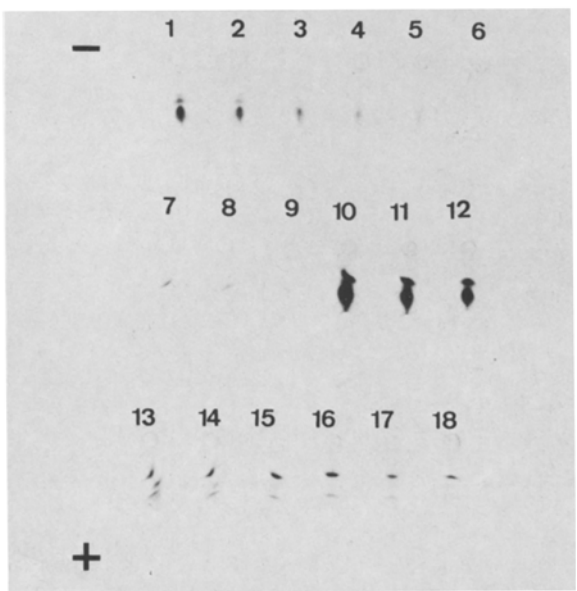

Fig. 2. Electric precipitation in agar-gel: Dilution series ($^1/_{50}$–$^1/_{12800}$) of human serum not treated with glutaraldehyde were poured into the members next to the (−) pole of the pair of holes 1–9; the same dilution series treated with glutaraldehyde were poured into the holes 10–18. The holes nearer to the (+) pole contained antihuman serum

A comparison of the treated and untreated dilution series of blood protein solutions by means of electric precipitation shows that the material treated with glutaraldehyde produces a stronger reaction and can be evaluated even at a dilution rate of $^{1}/_{12800}$ (Figs. 1 and 2).

Thus, the method offers more safety for the species identification of proteins in blood and body humours even in a small quantity of test material, than without the described treatment.

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