Studies on the Sensitivity of Electrosyneresis

The possibility of detecting minute amounts of antigens is a current problem in the field of immunochemistry nowadays. Immuno-electrophoresis is the most frequently used method for this purpose. 'Counter-current' immuno-electrophoresis[1] and 'cross over immuno-electrophoresis', originally denominated as electrosyneresis[2-5], are also often used as rapid electrophoretic and immunological methods. These methods exploit the cathodic electro-endosmotic flow of the antibodies to throw them in the path of antigens having a higher mobility than the precipitins. The difficulty of employing antigens having a mobility as low as that of the IgG antibodies may be overcome by chemically modifying the charge of the antigens[6].

Electrosyneresis as a simple and sensitive method is used for detecting virus antigens[7,8] and also for the analysis of different blood spots[9]. It could be successfully employed also for the detection of ribonucleoprotein antigen components[10]. However, the results obtained were sometimes uncertain. In some cases, only immuno-electrophoresis, in others only electrosyneresis gave positive results. Therefore, the sensitivity of electrosyneresis has been investigated, comparing it with that of immuno-electrophoresis to determine the range of antigen-antibody ratios in which one or the other of them could be more successfully used.

Materials and methods. Purified human serum albumin (HSA) (Lot. 670312/K, Human, Budapest) was used as antigen. As impurity, an α-globulin was detected in this preparation by immuno-electrophoresis. However, only the HSA-antibody reaction was evaluated.

Two types of immune sera: hyperimmune horse serum precipitating the human serum proteins (aHS) (No. 249, Human, Budapest) and an individual rabbit anti HSA immune serum (aHSA) were tested. The antibody content of the immune sera was controlled by quantitative precipitation. The equivalent antigen concentrations for both of the immune sera were nearly the same (500 and 625 μg/ml, resp.).

Twofold dilutions of 1% HSA were prepared up to the dilution of 2^{-10}. These were examined at different levels of the immune sera (from undiluted sera up to a dilution of 1:128). Every proportion was investigated at least twice. 4 ml of 1% melted agar solution were poured on slides. Sodium veronal buffer was used (i.e. 0.05, pH 8.2). Electrophoresis was carried out at 4 V/cm for 2 h.

For electrosyneresis 2 oblong reservoirs were cut at a distance of 16 mm from each other (2 × 16 mm). The longitudinal axes were parallel to each other and perpendicular to the direction of electrophoresis (Figure 2a). The well nearer to the anodic end of the plate was filled with 0.025 ml immune serum dilution, the other with the same volume of an antigen dilution. Both substances were mixed with an equal volume of 2% molten agar at 45 °C prior to filling up the wells. During electrophoresis the time of appearance of the precipitates and sometimes also their dissolving were observed. The establishment of positivity and the measurement of the distance of the lines from the immune serum reservoir was made on slides stained with amidoblack.

In the electrophoretic preparations, the same amount of HSA was pipetted into the antigen wells. As much as 0.25 ml of immune serum was measured into the 3 × 57 mm troughs when the electrophoresis was terminated. The slides were washed after incubation for 18 h. The spot-like traces of stains were also taken for positive reactions.

Results. In the HSA-aHS system it could be stated (Figure 1) that, when employing undiluted immune serum, less HSA could be detected by electrosyneresis. Using higher dilutions of the immune serum, the zone of positivity in electrosyneresis narrows; and as long as the current was on, the precipitate disappeared or did not even appear. At this antibody level, the immuno-electrophoresis still gave positive reactions in a wide range of antigen dilutions. At 1:128 immune serum dilution, no precipitate could be observed by electrosyneresis, while spots of precipitate could be detected by immuno-electrophoresis.

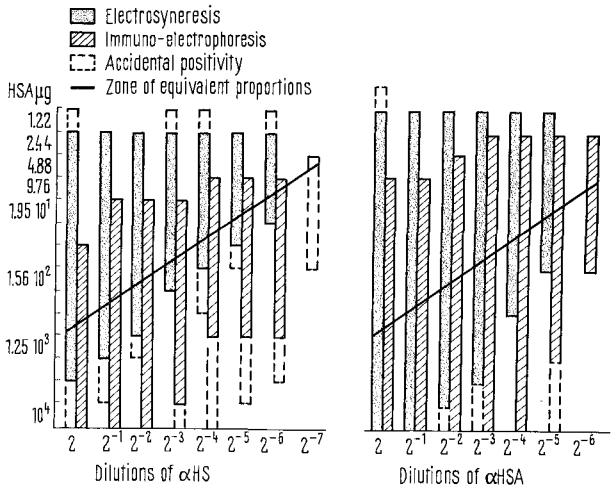

Fig. 1. Comparison of the sensitivity of electrosyneresis and immuno-electrophoresis.

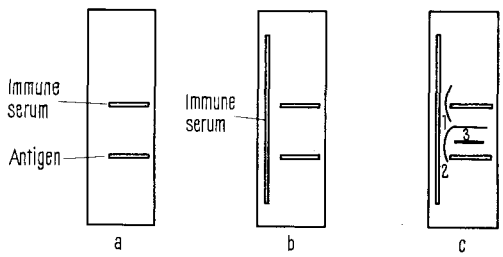

Fig. 2. Scheme of the method used for identifying the precipitates in electrosyneresis. (1) Electrosyneresis. (2) Application of reference immune serum. (3) The possible results, (a) positive reaction only by immuno-electrophoresis, (b) identity reaction of the 2 methods, (c) positive reaction only by electrosyneresis.

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Immune serum	Dilutions of 1% HSA solution													
	0[a]	−1	−2	−3	−4	−5	−6	−7	−8	−9	−10	−11	−12	−13	−14
aHs: undiluted	–	1.0[b]	1.8	2.0	*4.0*	4.2	5.0	5.8	6.0	6.5	6.5	6.5	7.0	–	–
aHSA: undiluted	7.5	8.0	8.1	8.1	*9.0*	9.0	9.0	9.0	9.0	9.0	9.0	9.2	9.5	10	10

Electrosyneresis. Localization of the precipitation lines at different antibody levels. Experiments 670,711 and 670,726. [a] The logarithms based to 2 of the dilutions of the HSA solution. [b] Distance in millimetres of the precipitation lines from the immune serum reservoir. The underlined results were obtained at nearly equivalent ratios.

Performing the experiments under the same conditions with rabbit aHSA immune serum, the results were almost identical (Figure 1b). The higher sensitivity of electrosyneresis at low dilutions of the immune serum (1.22 µg compared with 19.5 µg) was observed. At 1:64 aHSA dilution only the immuno-electrophoresis gave positive reaction.

Using electrosyneresis it is difficult to identify the precipitation lines. We tried to do this by observing the position of the lines. This was impossible, because their distance from the wells changed with the change of the antigen-antibody ratio (Table). At an antibody excess, the line was situated near to the antigen reservoir. In the zone of antigen excess, it appeared next to the immune serum well. At equivalent antigen-antibody ratios, the site of the line was strongly influenced by the origin of the immune serum. It may be that this method would be useful to identify the immunoglobulin type of the antibody studied. At ratios at which the precipitate seemed to dissolve after its appearance, a wide zone of precipitate was often obtained.

Another method proved to be successful in identifying the precipitates by electrosyneresis. After performing electrosyneresis, such troughs as are used in immuno-electrophoresis were cut along the longitudinal side of the slides (Figure 2c). These were filled with the same or some other immune serum. In the positivity zone of both methods, an identity reaction could be obtained between the 2 lines (Figure 2c). The impurities of the HSA solution used could be detected only as a line in the electrosyneresis part of the slide, and at high antigen excess, the HSA only by immuno-electrophoresis.

These experiments show that, at high antibody concentration, electrosyneresis may have an advantage over immuno-electrophoresis for the detection of minute amounts of antigens. Diluted immune serum gives sometimes positive results only in immuno-electrophoresis. It is of some interest that the sensitivity of electrosyneresis seems to be independent of the antibody level. For detecting as many components of a given antigen solution as possible, investigations made with both these methods are recommended.

Zusammenfassung. Beim methodischen Vergleich der Elektrosynerese mit der Elektrophorese wurde festgestellt, dass bei hoher Antikörperkonzentration die Methode der Elektrosynerese von Vorteil ist.

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Induction in Rabbits of Reverse-Type, Immediate Skin Reaction by Specific Anti-Rabbit IgG and Anti-Rabbit IgA

Homocytotropic antibody capable of evoking PCA- or P-K-type reactions in homologous skin, was described in rabbits immunized with hapten-conjugated proteins[1,2] or infected with various nematodes[3].

The homologous skin-sensitizing activity was attributed to various rabbit immunoglobulins. Such activity was found to be associated with a minor 7S IgA antibody component in hyperimmune sera[2] or with an immunoglobulin present in 'early' immune sera which differs from IgG in its faster electrophoretic mobility, greater sedimentation coefficient, its thermolability and its sensitivity to mercaptoethanol[1].

Studies carried out in this laboratory[4] have shown that hydrocortisone-hemisuccinate, when coupled to bovine serum albumin and injected intradermally into rabbits together with the complete Freund's adjuvant, elicits the formation of hapten-specific homocytotropic antibodies of 2 kinds: an 'early' antibody which seems to comprise a subclass ('γ_1G') of IgG, and a later homocytotropic antibody which is probably an IgA immunoglobulin. A further finding was that non-specific normal rabbit 'γ_1G' is capable of inhibiting the P-K type reaction obtained with 'early' homocytotropic antibody.

In guinea-pigs it has been established that immunoglobulin, of the class to which skin-sensitizing antibody belongs, blocks passive sensitization with an antibody and passively sensitizes the skin for reverse PCA reaction[5].

Recently Ishizaka et al.[6] have demonstrated a specific reaction in human or monkey skin injected intradermally with serial dilutions of anti-human IgE, but not with anti-human IgG, IgM or IgD. These results are in agreement with the known reaginic activity of IgE antibody and with the inhibiting capacity of the same immunoglobulin on the P-K reaction[7,8].

In the present study 6 normal, randomly bred white rabbits (2.5 kg each) were first injected i.v. with 2 ml of a 2% Evans blue solution and then intradermally with 0.1 ml samples of the appropriate dilutions of specific antisera against rabbit IgG, IgM and colostral IgA respectively. In cases of positive reaction, a blue spot appeared at the site of injection. The blueing was recorded after 30 min. Its intensity was arbitrarily graded as +++, ++, +, ± and −.

The specific anti-rabbit immunoglobulin sera were prepared in the following way. Goat anti-rabbit IgG[9]