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]

Induction of blueing reaction in rabbit skin by anti-rabbit colostral IgA and anti-rabbit IgG

Material injected	Dilution of antisera							
	Rabbit No. 3				Rabbit No. 4			
	1:20	1:40	1:80	1:2560	1:20	1:40	1:80	1:160
Specific anti IgA	+++	++	±	—	+++	+++	++	—
Specific anti IgM	—	—	—	—	—	—	—	—
Specific anti IgG	+++	+++	+++	+	+++	+++	+++	±
Normal guinea pig serum	—	—	—	—	—	—	—	—
Normal goat serum	—	—	—	—	—	—	—	—

was absorbed with an ethylchloroformate copolymer of rabbit Fab, macroglobulin fraction (Sephadex G-200 first peak) and colostral IgA (prepared according to CEBRA et al.[10]). Goat anti-rabbit IgM[9] was made specific by absorption with an ethylchloroformate copolymer of rabbit Fab and rabbit colostrum. Guinea-pig anti-rabbit colostral IgA was prepared according to the immunization procedure of BINAGHI et al.[11], using rabbit colostral IgA as the immunogen. The guinea-pig serum was absorbed with an ethylchloroformate copolymer of rabbit Fab and rabbit macroglobulin.

All copolymerization procedures were carried out according to AVRAMEAS and TERNYNCK[12] in 0.2 *M* acetate buffer pH 4.5. Absorption was carried out with polymerized rather than with soluble antigens, in order to avoid any possible skin reaction due to non-precipitating antibodies or soluble antigen-antibody complexes.

The specificity of the antisera was verified by immunoelectrophoresis and by double gel diffusion analysis. Specific guinea-pig anti-rabbit colostral IgA reacted with rabbit colostral IgA and faintly also with rabbit serum. In the latter instance, the faint precipitation line was much intensified by including 2% dextran in the agar plates[13].

The precipitin content of the antisera, as determined by the quantitative precipitin test[14], was 1.8 mg/ml for the anti-rabbit IgG and 0.8 mg/ml for the anti-rabbit colostral IgA. The precipitin content of the anti-rabbit IgM could not be determined owing to a shortage of this antiserum.

Representative results of the blueing ('reverse-type') reaction in the skin of rabbits are given in the Table.

Results. As can be seen from the Table, both specific goat anti-rabbit IgG and specific guinea-pig anti-rabbit colostral IgA are capable of evoking the blueing reaction. No such reaction was obtained with specific anti-rabbit IgM. Neither did normal goat serum or normal guinea-pig serum provoke the reaction (except for a non-specific reaction which occurred with normal goat serum in dilution up to 1:5).

Comparable results were obtained also in rabbits which had been immunized with various immunogens. The same general pattern of results was observed in all rabbits tested; however, the final dilution of antiserum capable of evoking the blueing reaction varied in individual rabbits. This may be partly due to individual differences in rabbit skin structure (in this connection we have noted that moderately thick, highly vascularized skin is the most suitable for the demonstration of the skin reaction).

The present results suggest that both IgG and IgA are capable of fixing onto skin target cells, conceivably mast cells. The reaction with the appropriate antiserum probably triggers the release of pharmacologically active substances which contribute to the appearance of an immediate-type skin reaction.

The reaction of the specific anti-rabbit IgG was more pronounced than that of the specific anti-rabbit colostral IgA. This may be attributable to a quantitative preponderance, or alternatively to more effective skin fixation of the skin-fixing IgG ('γ_1G') as compared to skin-fixing IgA.

It is worth mentioning in this connection that the total serum concentration of rabbit IgG is about 10 mg/ml[15] whereas that of IgA is estimated as only 0.2 mg/ml[10]. In the last analysis, however, one cannot yet completely rule out the possibility that either or both the specific anti IgG serum and the specific anti IgA serum contain antibodies which are capable of reacting with a still unidentified immunoglobulin (a counterpart of human IgE ?) presumably present on skin target cells.

Résumé. Un sérum spécifique de chèvre anti-IgG de lapin ou un sérum de cobaye anti-IgA de collostrum de lapin inoculés dans le derme de lapins normaux provoquent chez ces derniers une réaction d'hypersensibilité immédiate. Par contre, le sérum spécifique de chèvre anti-IgM de lapin ne provoque pas de réaction. Les mêmes résultats ont été observés chez tous les lapins testés.

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