

A Clinical and Immunological Study of Allergy to Hen's Egg White

II. Antigens in Hen's Egg White Studied by Crossed Immunoelectrophoresis (CIE)

TOR LANGE LAND

Dept. of Dermatology, University Hospital, Rikshospitalet, Oslo, Norway

A CIE-reference precipitation pattern for hen's egg white was established, using rabbit anti-egg antiserum. It demonstrated 24 different immunoprecipitates. The precipitates corresponding to ovalbumin, ovomucoid and lysozyme were identified in the reference pattern.

Key words: antigens – crossed immunoelectrophoresis – hen's egg white.

Accepted for publication 29 December 1981

Hen's egg white represents a common food allergen among atopic individuals. Its biochemical composition is well known. The protein content constitutes approximately 11% of its total weight (18). Some major components (ovalbumin, ovomucoid, lysozyme, conalbumin and ovomucin) represent about 80% of the total protein amount, and the remaining 20% is composed of about 35 different minor proteins, many of which have not yet been characterized (18).

Studies of antigens in hen's egg white have previously been reported (2, 4, 9, 15, 17). Rodríguez-Burgos et al. (17) identified 22 different antigens by immunoelectrophoretic techniques.

Crossed immunoelectrophoresis (CIE) permits good separation of the different antigens, and a CIE-reference precipitation pattern for hen's egg white can be used as a basis for studies of *allergens* in hen's egg white.

MATERIAL

Antigens

A reference antigen solution was prepared by 4 h gentle stirring of crude egg white (less than

24 h old) of White Italians (*Gallus domesticus*) in an equal volume of 0.15 mol/l NaCl. After centrifugation (2500 r.p.m. for 30 min) the supernatant was stored at -20°C until used. The total protein concentration (TP) in the reference antigen solution was 70 mg/ml, measured according to Lowry, using bovine serum albumin as standard (13). When diluted, 0.15 mol/l NaCl was used. By centrifugation approximately 2% (v/v) sediment was obtained, which was stored at -20°C for further studies.

Ovalbumin was purified as described by Rhodes (16).^{*} Ovomucoid and lysozyme were obtained from Sigma Chem. Co., Missouri, U.S. (cat Nos. T2011 and L6875, respectively). These preparations were dissolved in 0.15 mol/l NaCl when used.

Human sera

Serum was collected from six patients with a history of allergy to hen's egg and a positive radioallergosorbent test (RAST) to egg white (Phadebas, Pharmacia, Sweden). As control,

^{*} This preparation was kindly supplied by B. Egelanddal, Norwegian Food Research Institute, Ås, Norway.

single sera from atopic patients tolerating egg white and with a negative RAST to egg white were used. RAST-kits were obtained from Pharmacia, Sweden.

Antisera were obtained by immunizing 10 rabbits as described by Harboe & Ingild (7). Seven rabbits were immunized with crude fresh egg white stirred gently for 4 h in an equal volume of physiological saline. Each injection of 1 ml consisted of 25 mg protein and 0.5 ml Freund's incomplete adjuvant. Two additional rabbits were injected with 10 mg lysozyme added to the crude egg white at each occasion. The last rabbit was immunized with lysozyme alone, each injection of 100 μ l containing 0.2 mg lysozyme and 50 μ l Freund's incomplete adjuvant. All rabbits were injected every fortnight for 3 months, whereafter monthly injections were given 10 days before bleeding.

The specific antibody concentrations of the rabbit antiserum were estimated by CIE as described below. Twenty-five sera, each of approximately 10 ml, considered most representative for the CIE-reference pattern, were pooled to make a reference antiserum. The serum was stored at -20°C in 1-ml aliquots. Sera from all rabbits were represented in the serum pool.

To obtain a suitable concentration of antibodies against lysozyme, the immunoglobulins from the rabbit which was immunized with lysozyme only was concentrated 4–5-fold by precipitation with ammonium sulphate (7), and added to the reference serum.

METHODS

SDS (sodium dodecyl sulphate)-polyacrylamide gel electrophoresis was performed as described by Weber & Osborne (19), preincubating the protein samples in an SDS urea phosphate-buffer containing 2-mercaptoethanol. After running the electrophoresis (18 h at 1.5 mA per tube, 20°C) in a phosphate buffer (pH 7.0), the gels were stained with Coomassie Brilliant Blue®, and with Schiff's reagent (5). Twenty-five μ l of the different protein samples was applied directly on each gel, the TP being as follows:

reference antigen solution: 1 mg/ml, ovalbumin, ovomucoid and lysozyme preparations: 0.5 mg/ml.

Analytical isoelectrofocusing was carried out using an Ampholin polyacrylamide gel (PAG-plate) (LKB, Bromma, Sweden), pH-range 3.5–9.5, and employed according to the producer (11). Fifteen μ l of the different protein samples was applied, the TP of the different preparations being as follows: reference antigen solution: 2 mg/ml, ovalbumin, ovomucoid and lysozyme preparations: 1 mg/ml.

Analytical ultracentrifugation. The ovalbumin, ovomucoid and lysozyme preparations were analyzed using a Beckman, Mod E ultracentrifuge with Schlieren optics for registration. TP of the different protein samples was as follows: ovalbumin: 7.8 mg/ml, ovomucoid: 8.5 mg/ml and lysozyme: 8.3 mg/ml, the proteins being dissolved in 0.1 mol/l phosphate buffer, pH 7.0. Partial specific volume was taken to be 0.72 ml/g for all three preparations. The molecular weight was determined by sedimentation equilibrium at 23,150 r.p.m. at 20°C .

Crossed immunoelectrophoresis (CIE) was performed on glass slides ($5 \times 7 \text{ cm}^2$) according to Clarke & Freedman (3), with equipment as described by Weeke (20), in 1% agarose gel (w/v) (HSB, batch 461, Litex, Glostrup, Denmark) containing 0.073 mol/l Tris, 0.024 mol/l barbital, 0.006 mol/l calciumlactate and 0.003 mol/l sodium acid (pH 8.6 at 25°C). The first and second dimensional electrophoreses were run at 20°C , at 10 V cm^{-1} for 35 min and 2 V cm^{-1} for 19 h, respectively.

CIE was also performed with the reference antigen solution and with ovalbumin, ovomucoid and lysozyme as antigens separately. Ten μ l of the different protein solutions were applied, the three latter with different TP from 0.1 mg/ml to 10 mg/ml. The CIE experiments with lysozyme as antigen were also performed with 2% (v/v) of a nonionic detergent (Nonidet P-40, Sigma, Missouri, U.S.) added to all gels, and in other experiments using rabbit serum without specific antibodies against lysozyme.

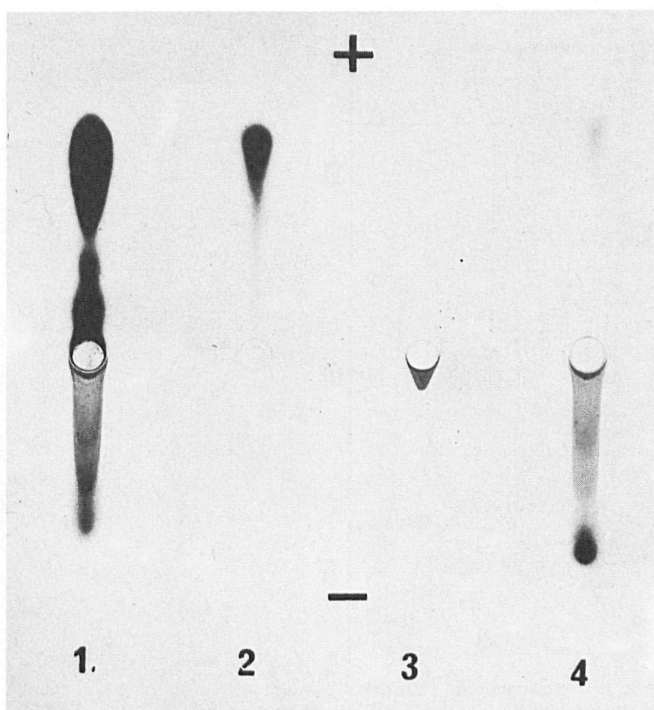


Fig. 1. Simple agarose gel electrophoresis. Agarose 1% (w/v) in diemal buffer, pH 8.6. 10 v cm^{-1} , 35 min. Stained with Coomassie Brilliant Blue. 1. Reference antigen solution. 2. Ovalbumin. 3. Ovomuroid. 4. Lysozyme.

First dimensional electrophoresis was performed as described for first dimensional electrophoresis in CIE. After running the electrophoresis, the proteins were precipitated with saturated picric acid, dried and stained with Coomassie Brilliant Blue. Ten μl of the different protein samples was applied in the wells. The reference antigen solution was diluted with 0.15 mol/l NaCl to 20 mg/ml TP, whereas the TP of the ovalbumin, ovomucoid and lysozyme solutions were 1 mg/ml.

Crossed-line immunoelectrophoresis (CLIE) was performed to identify ovalbumin and ovomucoid in the CIE-reference precipitation pattern for egg white. The conditions and the equipment were the same as used in CIE. In CLIE the protein to be identified was added to the intermediate gel next to the anodic primary gel. The experiments were repeated with different amounts of the ovalbumin and the ovomucoid preparations

in the intermediate gel, varying from 100 μg to 0.01 μg TP/ml agarosis.

Radio-allergo-sorbent test (RAST) was used to determine specific IgE-antibodies against rabbit serum proteins in sera from egg-allergic patients. Rabbit serum proteins (serum diluted 1:10 in 0.15 mol/l NaCl) were coupled to CNBr-activated paper discs according to Yman et al. (21). RAST assays were performed essentially according to the producer (Pharmacia, Sweden), incubating the discs with patient serum and 50 μl RAST-buffer and, after washing, with ^{125}I -anti IgE and 50 μl RAST buffer, both incubations lasting 18 h at 20°C. After repeating the washing procedure, the amount of radioactivity was estimated by means of a gamma-counter (Packard, 5220). The results were expressed as uptake of ^{125}I -anti-IgE in per cent of total amount applied. All analyses were performed in duplicate.

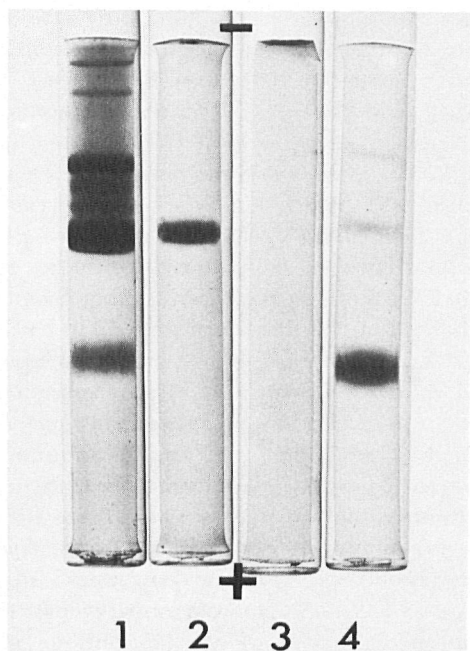


Fig. 2. SDS polyacrylamide gel electrophoresis (7% gels). Phosphate buffer, pH 7.0. Run for 18 h with 1.5 mA per tube. Stained with Coomassie Brilliant Blue. 1. Reference antigen solution. 2. Ovalbumin. 3. Ovomucoid. 4. Lysozyme.

RAST with the egg white sediment. The presence of allergenic proteins in the sediment obtained when preparing the reference antigen solution, was investigated by means of a modified RAST technique. The sediment was washed 10 times for 2 h in 0.15 mol/l NaCl. Each washing was followed by centrifugation of the sediment at 2500 r.p.m. for 30 min. After the last centrifugation the supernatant was discarded and the sediment divided into two parts of approximately 1 ml each. One part was then repeatedly incubated (four times overnight) with the reference antiserum. The incubations were performed at 20°C using 3 ml antiserum the first and last time and 10 ml the others.

The absorbed antisera from the first and last incubations were studied in CIE, using these sera as antisera, otherwise with conditions as described above.

The other part of the sediment was handled in the same manner, apart from using physiological saline instead of antiserum.

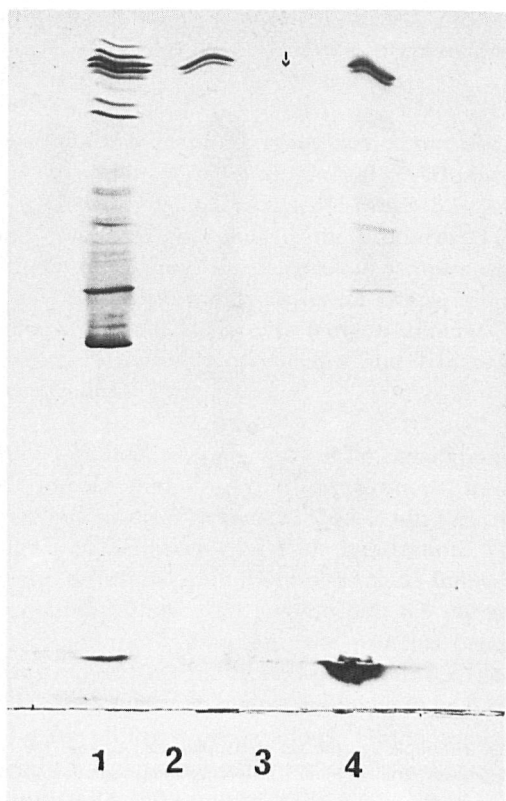


Fig. 3. Analytical isoelectric focusing. Gel slab with pH range 3.5–9.5 (lowest pH at top). Stained with Coomassie Brilliant Blue. 1. Reference antigen solution. 2. Ovalbumin. 3. Ovomucoid (arrow: faint staining corresponding to ovalbumin). 4. Lysozyme.

After the last incubation, both sediment samples were washed in 0.15 mol/l NaCl four times for 30 min, each washing followed by centrifugation at 2500 r.p.m. for 15 min. Thereafter the sediment was divided into portions of approximately 50 µl each, and incubated for 18 h at 20°C with different sera from egg-allergic individuals and controls, and with physiological saline (Table 2). Fifty µl of each serum and 50 µl RAST buffer was applied to two sediment parts, one which had been preincubated with antiserum, and one which had not.

The next day the sediments were washed four times for 30 min, each washing followed by centrifugation at 2500 r.p.m. for 15 min. All

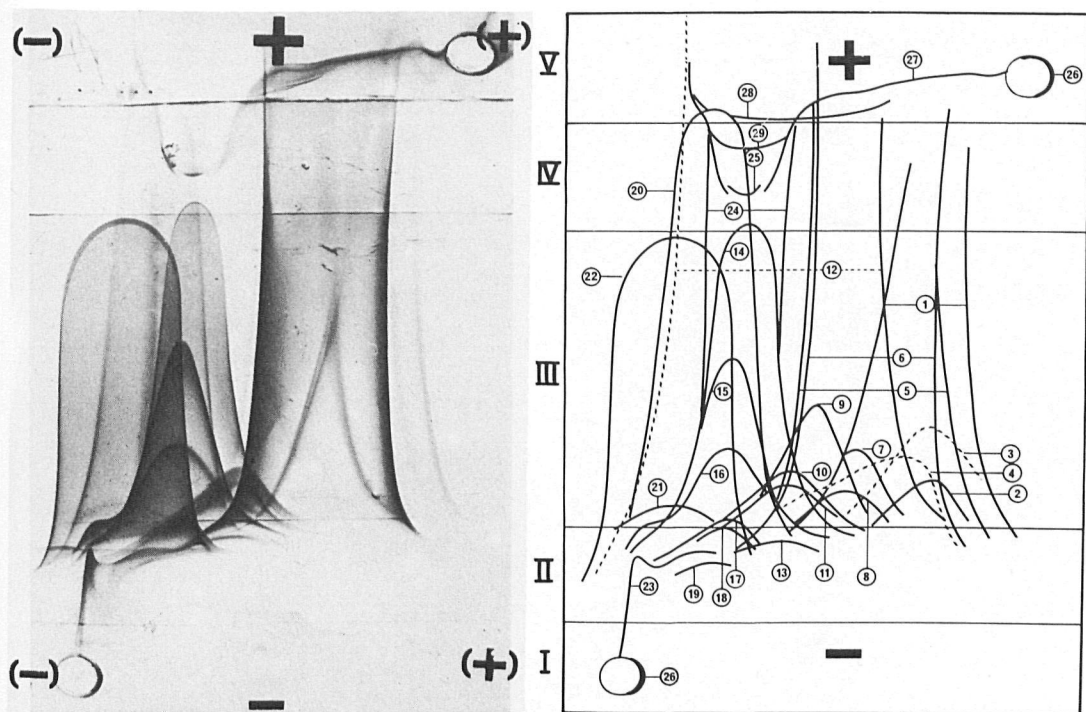


Fig. 4. CIE-reference pattern for hen's egg white. Left: Stained with Coomassie Brilliant Blue. Right: Drawing of Fig. 4A (dotted lines; precipitates only visible in CRIE with selected patient sera). Gels I and V: Primary gels ($0.16 \times 1 \times 5 \text{ cm}^3$). Gels II and IV: Intermediate gels ($0.15 \times 1 \times 5 \text{ cm}^3$). Gel III: Antibody-containing gel ($0.13 \times 3 \times 5 \text{ cm}^3$, containing $13 \mu\text{l}$ reference antiserum per cm^2 gel). Antigens: a) well of gel I: $10 \mu\text{l}$ of the reference antigen solution, diluted to 20 mg/ml TP. b) well of gel V: as in well of gel I with addition of $70 \mu\text{g}$ lysozyme. Poles in first dimensional electrophoresis designated (+) and (-), respectively, in second dimensional electrophoresis, anode at top.

specimens were then incubated for 18 h with $50 \mu\text{l}$ Phadebas RAST-isotope (Pharmacia, Sweden) and $50 \mu\text{l}$ RAST buffer. The next day the washing procedure was repeated, and the amount of radioactivity estimated by means of a gamma-counter (Packard, 5220). The counts of the different samples were divided by the dry weight of the sediments, and expressed in c.p.m./mg sediment.

RESULTS

Reference antigen solution

Isoelectrofocusing demonstrated 35 different bands (Fig. 3). Agarose gel electrophoresis and

SDS-polyacrylamide electrophoresis showed five and nine bands respectively (Figs. 1, 2).

CIE-reference precipitation pattern of hen's egg white

CIE of egg white showed 24 different immunoprecipitates (Fig. 4 left). Two of the precipitates (Nos. 3 and 4) were only detectable as radio-staining in crossed radio-immunoelectrophoresis (CRIE), using sera from egg allergic patients with IgE-antibodies against proteins in these precipitates. The precipitates corresponding to different antigens were numbered arbitrarily from 1 to 24 (Fig. 4 right). In addition there were five precipitates, designated Nos. 25, 26, 27, 28 and 29, appearing without the presence of antiserum.

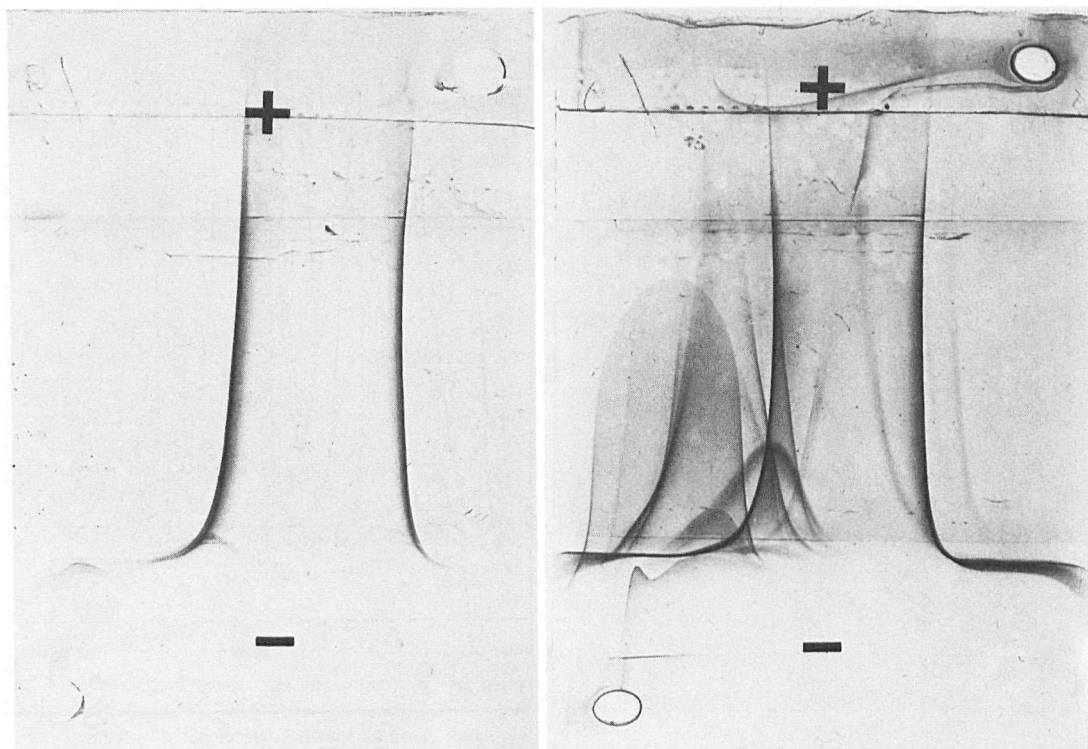


Fig. 5. a) Ovalbumin preparation examined in CIE. 10 μ g protein was applied in each well, otherwise conditions as described for the reference pattern for hen's egg white.

b) CLIE with 0.01 μ g ovalbumin added to the cathodic intermediate gel, otherwise conditions as described for the reference pattern for hen's egg white.

Additional precipitates did not appear, varying the amounts of antigen or antiserum. The presence of precipitates due to rapidly moving proteins were excluded by a short run (10 min) of the first dimensional electrophoresis on a larger glass slide ($10 \times 10 \text{ cm}^2$).

In the standard conditions most of the antigens migrated towards the anode, with those proteins corresponding to precipitate Nos. 24, 25, 27, 28 and 29 migrating towards the cathode. Precipitate Nos. 4, 6, 10, 15, 16, 17 and 23 were assymetrical.

Ovalbumin. Analysis of this preparation showed one band in agarose gel electrophoresis (Fig. 1), two bands in SDS polyacrylamide gel electrophoresis (Fig. 2), and four closely related bands, one major and three minor, in isoelectrofocusing (Fig. 3). The ovalbumin pre-

paration was found to have a molecular weight of 36,000 and $S_{20,w}$ of 2.9 S.

In CIE this preparation demonstrated four different precipitates. CLIE demonstrated an immunological identity-reaction with precipitate No. 6 in the CIE reference pattern (Fig. 5 a,b).

Ovomucoid. No bands were found in SDS-polyacrylamide gel electrophoresis staining with Coomassie Brilliant Blue or with Schiff's reagent (Fig. 2). Isoelectrofocusing showed the presence of ovalbumin (Fig. 3), and in agarose gel electrophoresis traces of lysozyme could be detected (Fig. 1). By ultracentrifugation the molecular weight was found to be 27,000 and the $S_{20,w}$ of 2.1 S.

In CIE this preparation demonstrated only one precipitate, and in CLIE an immunological

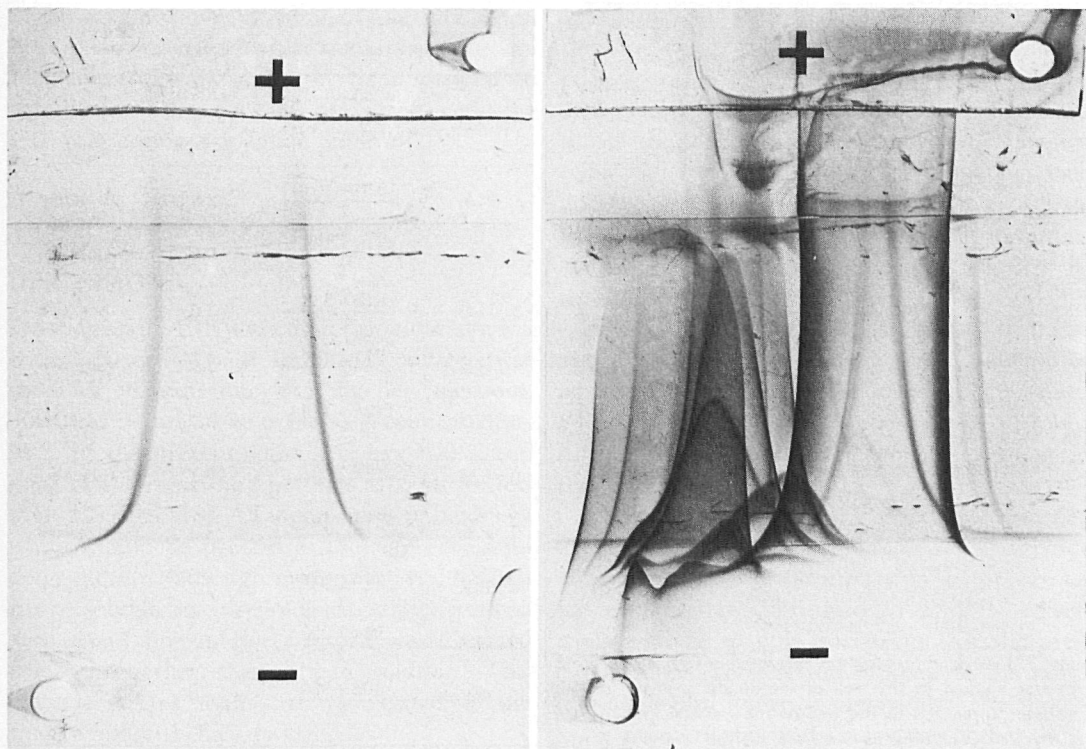


Fig. 6. a) Ovomucoid preparation examined in CIE. 10 μ g protein was applied in each well, otherwise conditions as described for the reference pattern for hen's egg white.

b) CLIE with 0.01 μ g ovomucoid added to the cathodic intermediate gel, otherwise conditions as described for the reference pattern for hen's egg white.

identity-reaction with precipitate No. 12 in the CIE-reference precipitation pattern was demonstrated.

Lysozyme. In this preparation anodically moving proteins were demonstrated by agarose gel electrophoresis (Fig. 1), judged as contamination. This was also found in isoelectrofocusing, demonstrating 15 bands (Fig. 3). SDS-polyacrylamide gel electrophoresis showed three bands, the fastest moving being the strongest (Fig. 2). The lysozyme preparation was found to have a molecular weight of 21,000.

This preparation demonstrated five precipitates in CIE, only one (No. 24) being immunologically specific. Three of the precipitates (Nos. 27, 28 and 29) appeared as well without the presence of antiserum, whereas precipitate No. 25 also appeared when using serum from a

rabbit which was not immunized with lysozyme. The CIE-precipitation pattern of this preparation was not changed by the addition of detergent to all gels.

RAST to rabbit serum protein

RAST results to rabbit serum proteins are given in Table 1. The result for patient No. 2 is considered positive. The other sera were negative with no more binding of ^{125} I-anti-IgE than in controls.

RAST with the egg white sediment

The results are given in Table 2. The counts for those specimens incubated with sera from egg-allergic patients were significant higher (13,463–38,469) than for those incubated with control

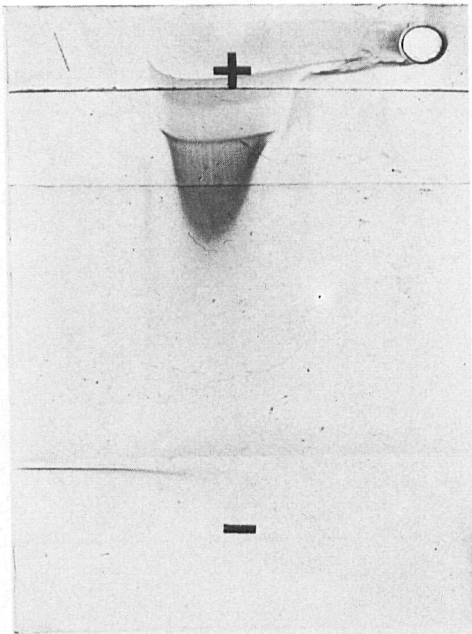


Fig. 7. Lysozyme-preparation examined in CIE. 70 µg protein was applied in the well of the anodic primary gel. No antigen applied in the well of the cathodic primary gel. Otherwise conditions as described for the reference pattern for hen's egg white.

sera (1,811–4,670), and those incubated with saline (2,645–4,670), all values expressed in c.p.m./mg sediment (dry weight).

Preincubation of the sediment with the reference antiserum extensively inhibited the IgE-binding to the sediment in sera from four of the six egg-allergic patients (Nos. 2, 4, 5 and 6). The inhibition was less pronounced with serum from patient No. 3, and no inhibition at all could be demonstrated with serum from patient No. 1. These two patients had negative RAST to rabbit serum proteins. The patient with positive RAST to rabbit serum proteins had a marked, but incomplete inhibition of the IgE-binding to the sediment, after this had been incubated with rabbit antiserum.

CIE, using antiserum from the first incubation with the sediment, demonstrated significant changes in all precipitates as compared with the CIE-reference pattern, most of the precipitates becoming higher and some of them disappearing, indicating a reduction in the antibody-titre

Table 1
Results of RAS Γ to rabbit serum proteins

Patient	c.p.m. ($\frac{X_1 + X_2}{2}$)	Per cent uptake of isotope	
1. E.A.	$\frac{1016 + 1030}{2}$	2.8	negative
2. S.A.	$\frac{3270 + 3936}{2}$	9.6	positive
3. H.B.	$\frac{818 + 338}{2}$	1.6	negative
4. H.C.	$\frac{911 + 1804}{2}$	3.6	negative
5. G.G.	$\frac{1428 + 1288}{2}$	3.7	negative
6. E.H.	$\frac{729 + 787}{2}$	2.1	negative
7. P.E.	$\frac{1512 + 1649}{2}$	4.3	negative
8. B.K.	$\frac{1108 + 896}{2}$	2.7	negative
9. Ø.A.	$\frac{511 + 623}{2}$	1.5	negative
10. Saline	$\frac{308 + 363}{2}$	0.9	—

(X₁ and X₂ = c.p.m. for each of the duplicates).
C.p.m. for 50 µl of the isotope (¹²⁵I-anti-IgE) = 37,304.

Table 2
Results of modified RAST-technique

Patients	Egg-allergy	c.p.m./mg sediment <i>not</i> pre-incubated with reference antiserum	c.p.m./mg sediment preincubated with reference antiserum
1. E.A.	Yes	23599	25002
2. S.A.	Yes	17050	7102
3. H.B.	Yes	20395	15186
4. H.C.	Yes	38469	12064
5. G.G.	Yes	13463	4003
6. E.H.	Yes	15961	5861
7. P.E.	No	1811	3363
8. B.K.	No	4280	4020
9. Ø.A.	No	4670	3679
9. Saline	—	2645	3534
10. Saline	—	6706	3946

to all antigens precipitating in the CIE-reference pattern. CIE with antiserum from the last incubation with the sediment, demonstrated no significant differences as compared with the CIE-reference precipitation pattern.

DISCUSSION

In studies with CIE and CRIE the antiserum is a critical factor. A high amount of antigen was used for immunization (35 mg per injection). This was because five proteins account for 80–90% of the protein content in egg white, and most of the remaining proteins account for less than 1% each (18). All the proteins in hen's egg white should be present in the reference antigen solution. Although most of the egg white is easily soluble in physiological saline, a small amount of non-soluble material was obtained when preparing the antigen solution. This material seems mainly to be related to the structure called chalazea (18).

CIE results, using rabbit antiserum incubated with the sediment, indicated that all proteins giving precipitates in the CIE-reference pattern are also present in the non-soluble part of the egg white. Correspondingly, the modified RAST demonstrated the presence of allergenic components in the sediment.

The attempt to block the allergenic determinants, by incubating the sediment with an excess of antiserum before performing the modified RAST, did not fully succeed. The presence of IgE-antibodies against rabbit serum proteins could not explain this, as the two sera which were only slightly or not inhibited at all, were negative in RAST to rabbit serum proteins. Another explanation for the incomplete inhibition could be that the rabbits may have responded to antigenic determinants in some proteins other than those which caused IgE-antibody production in the patients. Finally, the possibility that the rabbits have failed to respond to some of the proteins which are important allergens for the patients, can hardly be excluded on the basis of these experiments. However, results in CRIE using these patient

sera and the present CIE-reference pattern, demonstrated strong and specific radiostaining corresponding to several precipitates in the CIE-reference pattern (10). These results indicate that the rabbits have responded to proteins which are allergenic for these patients, and the strong degree of radiostaining suggests that those proteins which are most important as allergens for these patients are represented in the CIE-reference pattern.

The CIE-reference pattern shows that the antiserum contains precipitating antibodies against at least 24 antigens in hen's egg white. The asymmetry of certain precipitates is probably due to electrophoresis heterogeneity of some of the proteins.

The non-immunological precipitates, Nos. 25, 27, 28 and 29, may be related to lysozyme. Non-immunological precipitation of lysozyme has been reported previously (9, 14, 17). Precipitate No.25 may be due to non-specific precipitation of rabbit serum proteins in the non-immunological lysozyme-precipitate. The molecular weight of this preparation was higher than expected, most probably due to contaminations of various proteins with higher molecular weight than lysozyme, such as ovalbumin, demonstrated in other analyses of this preparation.

A separate staining is seen close to the wells (No. 26, Fig. 4B). When the reference antigen solution was applied immediately before running the first dimension electrophoresis, this staining was anodically localized. According to Vadehra (18), ovomucin is soluble in physiological saline, but precipitates at pH 8.2. Therefore this staining may probably represent ovomucin.

Under the conditions used in CIE, lysozyme moves towards the cathode with the same speed as ovalbumin moves towards the anode. In order to arrest the fastest moving proteins, using one primary gel and with the well centrally positioned, first dimensional electrophoresis must be run briefly. This results in poor separation of the different antigens. Therefore the modification of CIE with two different primary gels was chosen, one for anodically and one for cathodically moving proteins. This

modification permitted a better separation of the proteins during the first run, without loss of the fastest moving ones.

Previous studies of the allergens in hen's egg white suggest that ovomucoid (2) and ovalbumin (8) are essential allergens. It was therefore of particular interest to demonstrate the inclusion of these proteins in the CIE-reference pattern. Ovomucoid, however, acts as a weak antigen in rabbits (4), and may not give rise to production of antibodies that can be demonstrated in CIE.

CLIE is established as a useful method for the identification of a precipitated protein in CIE (12). The conclusions of these experiments are dependent on the quality of the purified egg white protein preparation used for the identity-reaction in CLIE. Therefore characterization of these preparations was carried out.

The ovalbumin preparation was demonstrated to be highly purified, following the major component of the reference antigen solution in the present analyses. The molecular weight and $S_{20,w}$ were found to be lower than expected (18), most probably due to some degree of denaturation of the ovalbumin preparation. Together with the results in CLIE, precipitate No. 6 is judged to contain ovalbumin.

The ovomucoid preparation seemed to be of high purity, traces of ovalbumin being the only detectable contamination. Ovomucoid could be demonstrated by ultracentrifugation, but not by staining in agarose gel electrophoresis, SDS-polyacrylamide gel electrophoresis or isoelectrofocusing.

This may be due to this protein's lack of precipitation in the gels, using ordinary staining procedures, which may depend on its being a glycoprotein with a high carbohydrate content (approx. 20%) and a relatively low molecular weight (approx. 28,000) (18). The lack of detection of ovomucoid by ordinary staining methods has been reported previously (1, 6).

However, CIE, with this preparation as antigen, demonstrated a protein with electrophoretic mobility similar to that of ovomucoid (2). Considering this, and the presence of an immunological identity-reaction in CLIE, pre-

cipitate No. 12 most probably represents ovomucoid.

There is a discrepancy between the number of precipitates in the CIE-reference pattern and the number of bands detected by isoelectrofocusing. This may be due to the high resolution obtained by isoelectrofocusing, revealing the microheterogeneity of a protein as several bands, whereas the molecular variants may appear as only one precipitate in CIE if they are immunologically identical. Ovalbumin illustrates this, giving four precipitates in isoelectrofocusing, but only one precipitate, with a slight asymmetry, in CIE.

Some proteins may have precipitated in CIE; however, the precipitates being so faint they would not be detectable in staining with Coomassie Brilliant Blue. Such precipitates may be demonstrated as radiostaining in CRIE, using sera from egg allergic patients with specific IgE-antibodies against these proteins. In absence of such sera these precipitates will remain undetected.

The CIE-reference system established appears suitable for further studies of antigens and allergens in hen's egg white. Such studies will be reported.

ACKNOWLEDGEMENTS

This work was supported by grants from the Anders Jahre's Foundation in Norway, and the Research Foundation of the Norwegian Asthma and Allergy Society.

Georg Rajka and Ole Fyrand are acknowledged for encouraging inspiration and advice. My thanks are also due to Kjell Aas and Lars Aukrust for valuable advice and fruitful discussions.

REFERENCES

1. Bleumink, E. & Young, E.: Studies on the atopic allergens in hen's egg. I. Identification of the skin reactive fraction in egg-white. *Int. Arch. Allergy* 35, 1-19, 1969.
2. Bleumink, E. & Young, E.: Studies on the atopic allergens in hen's egg-white. II. Further characterization of the skin reactive fraction in egg-white; immunoelectrophoretic studies. *Int. Arch. Allergy* 40, 72-88, 1971.
3. Clarke, H. M. G. & Freedman, T. A.: A quantitative immunoelectrophoresis method (Laurell electrophoresis). *Prot. Biol. Fluids* 14, 503-509, 1966.
4. Deutsch, H. F.: Immunochemical analyses of egg white. *Fed. Proc.* 12, 729-733, 1952.
5. Fairbanks, G., Stech, T. L. & Wallach, D. F. H.: Electro-

- phoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry* 10, 2606-2616, 1971.
6. Feeney, R. E., Abplanalp, H., Clary, J. J., Edwards, D. L. & Clark, J. R.: A genetically varying minor protein constituent of chicken egg white. *J. Biol. Chem.* 238, 1732-1736, 1963.
 7. Harboe, M. & Ingild, A.: Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 160-164, 1973.
 8. Hoffman, D. R.: Food allergy in children. Rast studies with milk and egg. In Evans, R. (ed.): *Advances in diagnosis of allergy: RAST*, pp. 165-169. Stratton Intercontinental Medical Book Corporation, New York, 1975.
 9. Kaminski, M.: Studies of egg white and its constituents by immunological techniques in gelified media. *J. Immunol.* 75, 367-376, 1955.
 10. Langeland, T.: A clinical and immunological study of allergy to hen's egg white. III. Allergens in hen's egg white studied by crossed radioimmuno-electrophoresis. (In preparation).
 11. LKB, Bromma Sweden. Publication No. 1804 - EO4.
 12. Løwenstein, H.: Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog. Allergy* 25, 1-62, 1978.
 13. Miller, G. L.: Protein determination for large number of samples. *Anal. Chem.* 31, 964, 1959.
 14. Miller, H. & Campbell, D. H.: Skin test reactions to various fractions of egg white and their possible clinical significance. *J. Allergy* 21, 522-524, 1950.
 15. Miller, H. & Feeney, R.: Immunochemical relationships of proteins of avian egg white. *Arch. Biochem. Biophys.* 108, 117-124, 1964.
 16. Rhodes, M. B., Azari, P. R. & Feeney, R. E.: Analysis, fractionation and purification of egg white proteins with cellulose cation exchanger. *J. Biol. Chem.* 30, 399-408, 1958.
 17. Rodriguez-Burgos, A. & Oteiza, J.: Antigenic composition of hen's (*Gallus domesticus*) egg white. *Comp. Biochem. Physiol.* 30, 649-656, 1969.
 18. Vadehra, D. V. & Nath, K. R.: Eggs as a source of protein. *C. R. C. Crit. Rev. Food Tech. Nov.* 193-309, 1973.
 19. Weber, K. & Osborne, M.: The reliability of molecular weight determination by dodecylsulphate polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244, 4406-4412, 1969.
 20. Weeke, B.: Crossed immunoelectrophoresis. *Scand. J. Immunol.* 2, Suppl. 1, 47-56, 1973.
 21. Yman, L., Ponterius, G. & Brandt, R.: RAST-based allergen assay methods. *Int. WHO-IABS symposium on standardization and control of allergens administered to man.* Geneva 1974. *Dev. Biol. Standard* 29, 151-165, 1975.

Address:

Tor Langeland
Dept. of Dermatology
University Hospital
Rikshospitalet
Oslo
Norway

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.