

Complement consumption in eggwhite and fish sensitivity

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Summary

A case of eggwhite allergy and a case of fish allergy, where complement-fixation by thermostable IgG-class antibody was positive at extremely high antigen dilutions are described. The antibody in eggwhite allergy was of the precipitating variety and the C-fixation phenomenon was not related to the presence or absence of IgE antibody.

Introduction

Hypersensitivity to hen's egg as food is known to occur more frequently in infants and children than in adults (Ratner & Untracht, 1952). The condition is often manifested in obviously predisposed infants in the form of urticarial eruptions, local or generalized eczema, wheezing, diarrhoea, and even shock reactions. Often there is evidence of increased susceptibility to bacterial and viral infections.

The diagnosis of eggwhite sensitivity usually depends on the clinical history, sometimes aided by skin tests, oral provocation, or an elimination diet. More recently, some investigators have employed RAST for the detection of specific anti-egg IgE in the blood serum (Schur, Hyde & Wypych, 1974). However, seemingly promising progress in the *in vitro* diagnosis of eggwhite and other cases of food sensitivity was made as early as 1930, when György, Moro & Witebsky (1930, 1932) described an exquisitely sensitive complement (C) fixation technique for the detection and quantification of antibodies against eggwhite or cow's milk in eczematous children. They clearly indicated the existence of two types of antibody, one 'classical', which was induced by active or inadvertent immunization and which fixed C at high antigen dosage; and the other, occurring 'spontaneously' (and solely) in the serum of children with eggwhite or cow's milk sensitivity. The presence of the latter antibody invariably coincided with a positive Prausnitz-Küstner test for eggwhite allergy (Woringer,

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1933). The peculiar feature of this 'reagin' antibody was that it fixed C in a very sharp and narrow zone only at extremely high antigen dilutions. Based on these findings, a positive C-fixation test for classical antibody was designated a 'left-hand zone phenomenon' (i.e. at high antigen concentration) and a positive test for 'PK-reagin'—allegedly non-precipitating—was termed a 'right-hand zone phenomenon' (at very low antigen concentration). Woringer (1933) even went so far as to consider the classical, precipitating antibody as 'unspecific' for the allergy condition.

In the years 1930–1935, this easy diagnostic test received ready recognition, not only for eggwhite, but also for cow's milk and fish allergy, and the above phenomena were confirmed and extended by other German (Jaffé, 1931; Moro, 1932; Strobl & Wasitzky, 1932; Müller & Brandt, 1932; Urbach, 1935), Scandinavian (Boström & Hellerström, 1935) and French authors (Woringer, 1932). In a search of the literature we have been unable so far to retrieve further reference to the use of C-fixation tests in food allergy after this amazingly short period of time. We recently had occasion to re-investigate and confirm the phenomenon.

Materials and methods

The eggwhite-sensitive patient in this investigation was a 10-month-old (male) child, referred to the paediatric ward because of urticaria and a possible asthmatic bronchitis. The child had a wheezy bronchitis, otitis media, persistent rhinitis and a diaper rash. The mother herself asserted that the urticarial eruption must be due to the consumption of hen's egg, possibly also of chocolate. Laboratory investigation gave normal values for haemoglobin, leucocytes and thrombocytes. Blood eosinophils were $330 \times 10^6/l$. The levels of immunoglobulins G, A and M were normal for his age; haemolytic complement (CH50) values were normal. At 12 months, the child had developed slight patches of infantile eczema on the trunk, and he had a dry skin. Blood eosinophils now were $100 \times 10^6/l$. On both occasions there were no eosinophils in the nose. A diagnosis of eggwhite sensitivity was made on the basis of this history and the results of the investigation recorded further on in this paper. The patient was repeatedly re-examined until the age of 22 months. Skin tests were not performed. During the period of observation, the child was not inoculated with egg-grown virus vaccines.

Blood of this and other subjects was obtained by venous puncture; after clotting in glass the sera were stored at -70°C . Total serum IgE was determined by RIST, using commercial tests sets (Phadebas®). Allergen-specific IgE was determined by RAST, using allergen-paper discs prepared in this laboratory, and commercially obtained radioactive anti IgE (De2-specific, Pharmacia Diagnostics AB, Uppsala, Sweden).

Eggwhite was prepared from fresh hens'-eggs by physical separation from the yolk. Eggwhite dilutions were made in veronal-saline buffer (VSB⁺⁺). Chocolate extracts were obtained by aqueous extraction of ether-defatted commercial cocoa powder, followed by dialysis and lyophilization. Fish extract was prepared by shaking minced white cod muscle with VSB⁺⁺, followed by filtration. A purified cod-fish allergen was also prepared to the stage of DS22 according to the method of Aas & Jebsen (1967). Dog and cat dander extracts were prepared as described (Berrens, Hénocq & Koers, 1974). House dust allergen was purified to the stage of fraction E (Berrens & Young, 1961), and human dander allergen similarly to the E-stage as before (Berrens, Morris & Young, 1966).

Insolubilized allergens for RAST were prepared by coupling the above prepa-

rations to Whatman no. 1 paper discs with cyanogen bromide according to the procedure of Ceska, Eriksson & Varga (1972). An exception was made for whole eggwhite, which was coupled, in 1:2 dilution in VSB⁺⁺, to *p*-aminobenzamide-ethyl-cellulose by a diazotation procedure according to Cuatrecasas & Anfinsen (1971).

The reactivity of haemolytic complement in human serum to house dust allergen, batch 72E, was determined and calculated as described (Weemaes, Stoop & Berrens, 1977). Plasma prekallikrein was determined with the chromogenic substrate α -N-benzoyl-L-phenylalanine-L-arginine-*p*-nitro-anilide (Pentapharm AG, Basle, Switzerland) by a slight modification of the method of Kluft (1978).

Results

During initial attempts to achieve meaningful C-consumption in cases of eggwhite sensitivity, we employed ovomucoid as an antigen in an extended dose range. This protein has been discussed by Bleumink & Young (1969) as the major skin-active allergen of chicken eggwhite. These experiments failed; indeed, in documented cases of eggwhite allergy we often observed an increase, up to a hundred-fold, in haemolytic C-efficiency. Therefore, the conditions of assay were adopted as originally proposed by György *et al.* (1930, 1932). Working with whole eggwhite, it was indeed found that a 'right-hand zone' phenomenon of C-fixation could be observed with the blood serum of our patient at very high eggwhite dilutions. The results of a typical experiment are shown photographically in Fig. 1, which also reproduces the original picture of a 'right-hand' and 'left-hand' zone phenomenon published by Moro (1932) and copied by Urbach (1935).

In our experiments, 80- μ l samples of patient's serum in 0.5 ml Ca⁺⁺-Mg⁺⁺-containing veronal-saline buffer (VSB⁺⁺) were incubated for 90 min at 37°C with 0.25 ml eggwhite dilution in VSB⁺⁺. The starting whole eggwhite dilution was 1:30 and further dilutions were sequentially made in steps of 1:3. After incubation, a 0.5 ml suspension of sheep red blood cells (E), optimally sensitized with rabbit amboceptor (A) in VSB⁺⁺ was added (5×10^8 cells EA), and incubation was prolonged for 20 min at 37°C. The suspension was then centrifuged, and haemoglobin in the supernatant was read in a spectrophotometer at 541 nm.

As shown in Table 1, the eggwhite dilution producing C-consumption in the patient's serum remained in a narrow range for about 1.5 years of observation, until at the age of 18 months the child's serum no longer displayed the phenomenon. During this period of observation, the child had only very occasional episodes of wheezy bronchitis, retained slight patches of infantile dermatitis and, twice, had an episode of otitis media. At the end of the series of consultations, blood eosinophilia was $450 \times 10^6/l$. From the first consultation, the child had been on a strict eggwhite-free diet which was well-guaranteed by his parents, who ran a baker's shop and were quite knowledgeable on food ingredients.

The high eggwhite dilutions required for detectable C-consumption (Table 1, Fig. 2) are not exceptional. Similar dilution ranges have been reported by György *et al.* (1930, 1932), Strobl & Wasitzky (1932), Müller & Brandt (1932), while Urbach (1935) even mentions dilutions of 1:10¹⁰ for eggwhite and 1:10⁷ in a case of cow's milk allergy. No follow-up studies have been reported so far. Figure 2 demonstrates a number of complete C-titration curves in our patient. The bell-shaped curves clearly show a prozone phenomenon. The antibody responsible for the phenomenon was thermostable (30 min at 56°C) and, in complex with antigen, consumed extraneous guinea pig

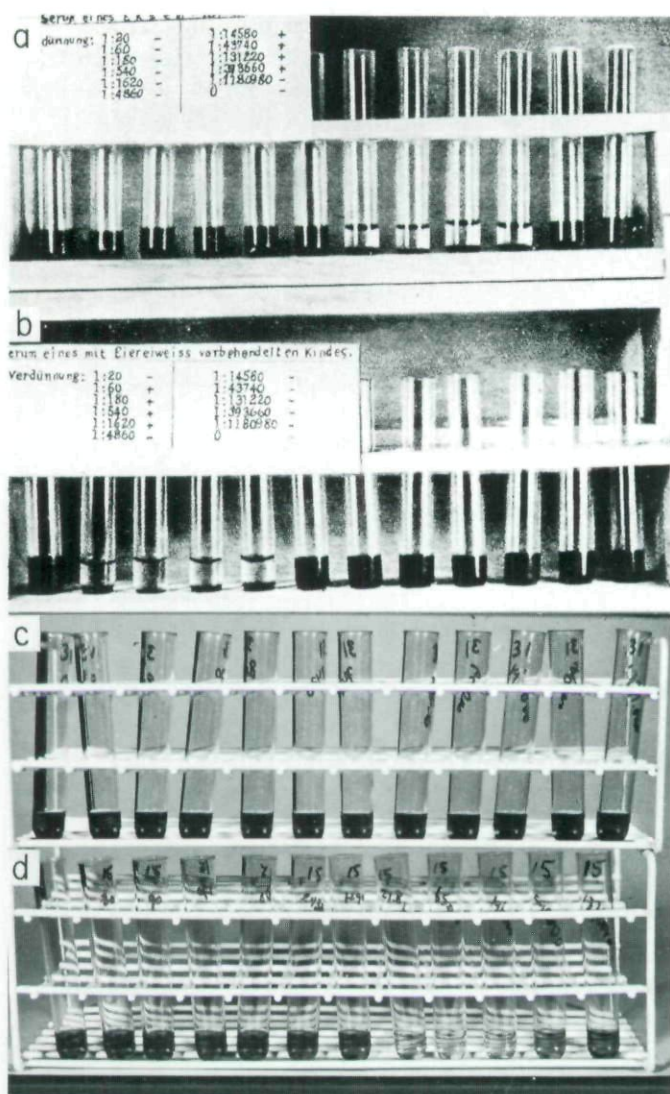


Fig. 1. Examples of 'right-hand' and 'left-hand' zone phenomena of C-fixation with eggwhite dilutions in egg-allergic children.

(a) C-fixation in the eggwhite dilution range of 1:14 580 to 1:393 660 in a child with exudative diathesis. (b) Left-hand zone phenomenon at dilutions of 1:60 to 1:1620 in a child deliberately immunized with eggwhite. Photographs (a) and (b) reproduced from Moro (1932) and Urbach (1935). (c) Negative C-fixation in a highly egg-allergic 6-year-old girl (RIST = 2600 iu/ml, RAST eggwhite 12%; RAST's also positive to house dust, human dandruff, cat, dog and horse danders). (d) Right-hand zone phenomenon of C-fixation with the serum of the child discussed in this paper at eggwhite dilutions between 1:21 870 and 1:590 490 (curve *a* in Fig. 2). Conditions of assay and serum concentrations identical for (a), (b), (c) and (d).

complement just as efficiently as intrinsic human C. This is in sharp contrast to allergens of the house dust group, which consume human, but not guinea pig C (Berrens, Van Rijswijk-Verbeek & Guikers, 1976).

At the indicated dilution range, the egg antigen protein concentration was in the picogram-nanogram order of magnitude. We found that dialysed eggwhite, lyophi-

Table 1. Values of total IgE, eggwhite-specific IgE (RAST), pre-kallikrein, and titre of eggwhite dilution for maximal complement consumption in sequential serum samples of an egg-allergic child (born 7 Jan. 1977)

Sample date	IgE iu/ml (RIST)	Eggwhite, IgE (RAST, percentage binding)	Pre- kallikrein (mU/ml)	Eggwhite dilution for C-consumption
26 Oct. 1977	125	25.8	398	1:65 610
30 Nov. 1977	86	22.1	428	N.D.
10 Feb. 1978	142	12.2	N.D.	1:196 830
4 April. 1978	178	13.6	460	1:196 830
2 Aug. 1978	152	12.6	414	1:590 490
3 Nov. 1978	118	5.2	491	1:65 610
24 Aug. 1979	280	4.6	N.D.	negative

lized eggwhite, and protein extracted from boiled eggs was just as effective in the C-consumption assay as the native material. In accordance with the early immunochemical studies of Bosch, György & Witebsky (1931) we also found that isolated ovalbumin was quite as effective as whole eggwhite, whereas ovomucoid was completely inactive in all the tests.

Total IgE values in the child's serum were not dramatically elevated (Table 1), and the prekallikrein levels were normal. Eggwhite-specific IgE by RAST was elevated quite considerably; other RAST's (with human dander, house dust, grass pollen, cod fish, cow's milk) were entirely negative. The egg IgE values, determined with the same reagents throughout the investigation, gradually decreased to low levels at the time the C-consumption phenomenon had become negative (Table 1). Dannaeus *et al.* (1977)

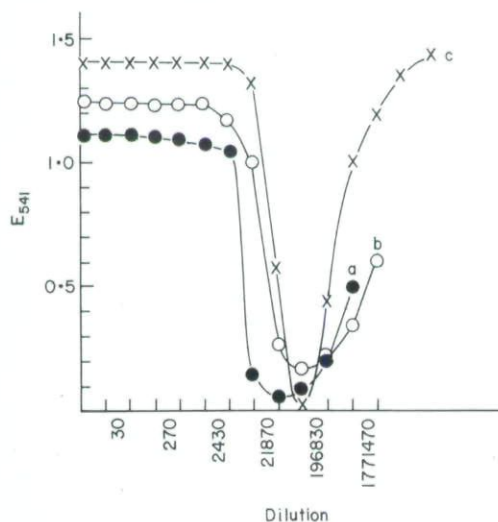


Fig. 2. Titration curves of C-consumption by eggwhite dilutions in sera of our patient taken at different intervals, viz: on 26 Oct. 1977 (a), 10 Feb. 1978 (b) and 4 Apr. 1978 (c); compare Table 1. Complement expressed in haemoglobin readings at 541 nm as discussed in the text.

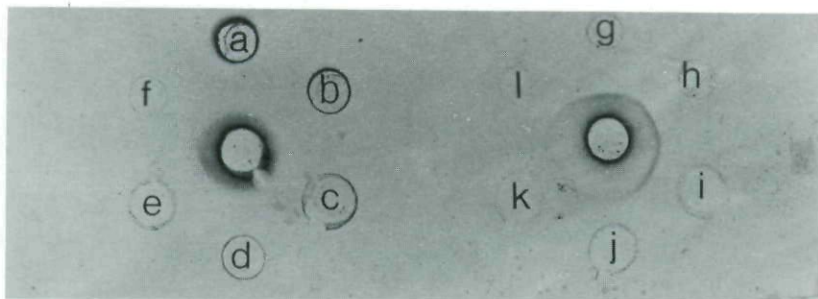


Fig. 3. Double diffusion in agar gel of undiluted serum of our egg-allergic patient (central well) against threefold serial dilutions of native eggwhite in veronal-saline buffer (peripheral wells). Dilutions range from 1:10 (a) to 1:2430 (f), and from 1:7000 (g) to 1:1.7 $\times 10^6$ (l). Stain: Coomassie brilliant blue.

have already shown that IgG antibody titres to food antigens parallel the IgE antibody-titre to the same food. C-consumption tests with soluble chocolate extract in a variety of techniques failed. Serum complement, moreover, was totally unresponsive to incubation with purified house dust allergen, a rather exceptional situation (Berrens *et al.*, 1976; Weemaes *et al.* 1977).

In contrast to the data in the early literature, we observed that the antibody responsible for the 'right-hand zone' phenomenon of C-consumption precipitates the antigen, again at extremely high dilutions and in prozone fashion. Figure 3 shows an assay by the Ouchterlony technique of eggwhite dilutions diffused against the patient's undiluted serum. In Fig. 4 the immunoelectrophoretic pattern of the serum developed with eggwhite dilutions show that the reaction partner in the serum is a slow γ -globulin.

Confirmation of the immunoglobulin nature of the reaction partner was sought by enzyme-linked immunosorbent assay (ELISA). In these tests, the patient's serum was

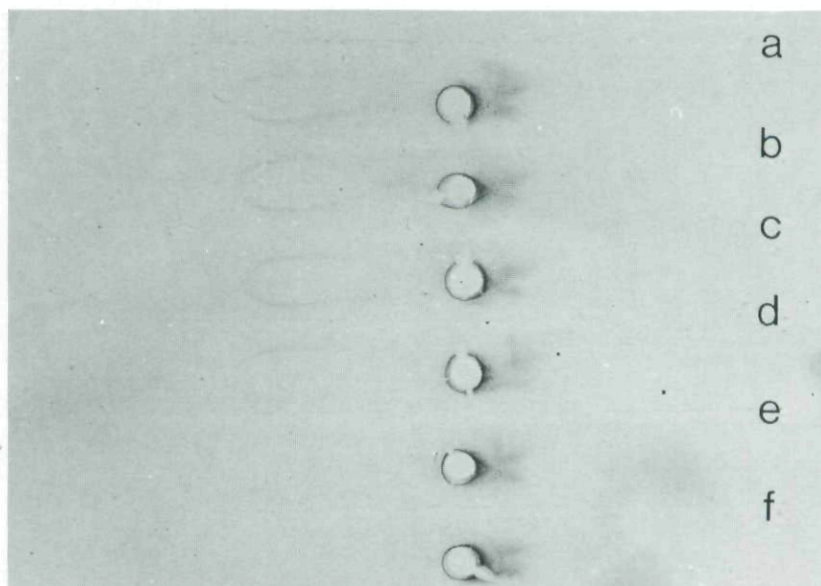


Fig. 4. Immunoelectrophoresis in agar gel of our patient's undiluted serum (5 μ l) of 10 Feb. 1978 (Fig. 2, Table 1) diffused against 90 μ l dilutions of native whole eggwhite in the grooves, ranging from 1:195 000 (a) to 1:4.7 $\times 10^7$ (f).

Table 2. Values of total IgE and codfish-specific IgE (by RAST to antigen DS22) in two fish-allergic male patients, R.R. (born 1966) and A.W. (born 1970). RAST-values corrected for aspecific anti-IgE binding

Patient	Total IgE (RIST, iu/ml)	RAST, percentage binding	
		Phadebas	Own discs
R.R.	575	26.0	34.8
A.W.	> 4000	37.3	53.1

incubated with paper discs containing covalently linked eggwhite proteins. After washing, the discs were incubated with rabbit anti-IgE, -IgM, -IgA or -IgG antisera, washed again and then treated with goat anti-rabbit IgG coupled to the enzyme alkaline phosphatase. The discs were finally incubated with the substrate *p*-nitrophenylphosphate and the supernatant colour was evaluated. In this fashion appreciable levels of bound IgG were detected, but no IgM, IgA, or IgE.

The above described phenomena were quite singular for the blood serum of our patient, but they are not typical for the diagnosis of egg allergy *per se*. Thus, we examined the sera of more than twenty different subjects with clinically proven eggwhite allergy (by history-taking, symptom score, skin tests, and provocation), several of whom were eczematous children. In the majority of these samples, positive RAST's to eggwhite were obtained, usually together with detectable IgE antibody against inhalant allergens. However, in none of these cases did we succeed in demonstrating either a 'right-hand zone' phenomenon of C-fixation, or precipitating antibodies against eggwhite dilutions. Negative reactions in this respect were also obtained with the blood serum of our patient's mother, father, and sister who was 2 years older.

Not only in cases of eggwhite allergy, but also in cases of fish allergy, the phenomenon of C-fixation at high dilution is of exceptional occurrence. More than thirty serum samples of patients with known fish allergy were investigated; none of these

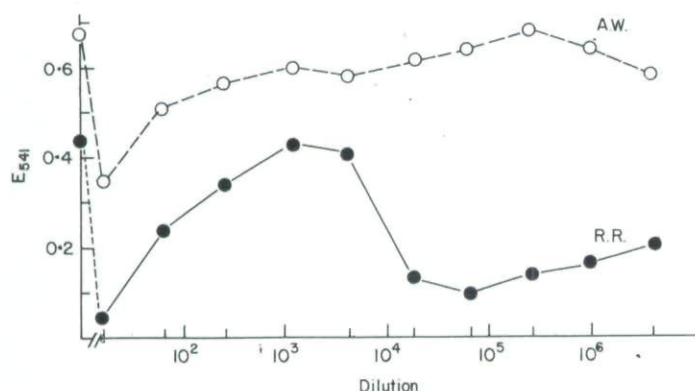


Fig. 5. Titration curves of fixation of guinea pig C (0.5 µl 1:100) by 50 µl samples of the heated (30 min 56°C) sera of two fish-allergic subjects (Table 2) upon incubation with 0.25 ml dilutions of fresh white cod muscle as indicated. Complement expressed in haemoglobin readings at 541 n.m. For further details see text.

displayed a C-fixation phenomenon with whole white cod muscle extract, and none had a positive RAST to cod allergen DS22. Through the courtesy of Professor Aas, Norway, we finally obtained two serum samples from cod-allergic patients who had a positive RAST to DS22. The analytical details are summarized in Table 2.

In one of these sera (patient R.R., Table 2) a 'right-hand zone' phenomenon of C-fixation at high dilution of white cod muscle extract was indeed observed. The titration curve is shown in Fig. 5. In this dilution range, no precipitating antibody against the fish proteins could be detected.

Discussion

In confirmation of earlier literature on C-fixation in cases of food allergy, we have observed C-consumption at very high dilutions of eggwhite (Györgi *et al.*, 1930, 1932; Moro, 1932; Strobl & Wasitzky, 1932) or fish protein (Jaffé, 1931; Boström & Hellerström, 1935). So far, we have been unable to reproduce C-fixation in cases of cow's milk allergy (György, Moro & Witebsky, 1931; Urbach, 1935). Even if reproducible, however, the phenomenon proved to be of rare occurrence among known cases of eggwhite-, fish- or milk-allergy. Though numerous examples of positive C-fixation are on record in the early literature, we have in 3 years succeeded in finding only two cases, *i.e.* those reported in the present paper.

Our studies on the eggwhite antigens also confirm the observations of Bosch, György & Witebsky (1931) on thermostable ovalbumin as the major antigen involved in the C-fixation phenomenon. More particularly, ovomucoid as described in egg allergy by Bleumink & Young (1969) did not react in the test, nor did ovomucin or the ovoglobulins.

Our experiments show the antibody involved to be an IgG immunoglobulin of the precipitating variety. It is extremely unlikely that the patient had received the antibody from the mother, because the mother's serum did not react and because the antibody persisted for almost 2 years without overt exposure to egg proteins. Furthermore, the one positive case of fish allergy was a 15-year-old subject on a strict fish-free diet. We have no further evidence on the subclass nature of the antibody, but it certainly is reminiscent of the IgS antibody arising early in immunization against ovalbumin in guinea pigs, as described by Van Es, Kwant-Den Harink & Pondman (1973). In view of the finding of several cases of positive IgE RAST against eggwhite in sera not displaying the C-fixation phenomenon, the antibody involved decidedly is not IgE. It may be related to the complement-dependent IgG-class 'short-term anaphylactic' antibody described by Parish (1970), which had already been implicated in a case of allergy to shellfish by Halpern (1977). In this connection, it is worthy of note that the inhibition of the Prausnitz-Küstner reaction by C3b inactivator reported by Torisu *et al.* (1968) was observed with the serum of a highly egg-sensitive patient upon challenge with egg albumin.

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References

- AAS, K. & JEBSEN, J.W. (1967) Studies of hypersensitivity to fish. Partial purification and crystallization of a major allergenic component of cod. *International Archives of Allergy*, **32**, 1.
- BERRENS, L., HENOCQ, E. & KOERS, W.J. (1974) The specificity of animal dander allergens: a semi-blind clinical and experimental study. *Acta Allergologica*, **29**, 10.

- BERRENS, L., MORRIS, J.H. & YOUNG, E. (1966) Studies on the human dandruff allergen. III. Further purification and comparison with the house dust allergen. *Dermatologica*, **132**, 433.
- BERRENS, L., VAN RIJSWIJK-VERBEEK, J. & GUIKERS, C.L.H. (1976) Characteristics of complement consumption by atopic allergens. *Immunochemistry*, **13**, 367.
- BERRENS, L. & YOUNG, E. (1961) Purification and properties of house dust allergen. *International Archives of Allergy*, **19**, 341.
- BLEUMINK, E. & YOUNG, E. (1969) Studies on the atopic allergen in hen's egg. I. Identification of the skin reactive fraction in eggwhite. *International Archives of Allergy*, **35**, 1.
- BOSCH, E., GYÖRGY, P. & WITEBSKY, E. (1931) Die immunochemische Analyse der Eiklarempfindlichkeit bei Eczema infantum. *Klinische Wochenschrift*, **10**, 2264.
- BOSTRÖM, G. & HELLERSTRÖM, S. (1935) Allergieprüfung bei Fall von Fischallergie. *Zentralblatt für Haut- und Geschlechtskrankheiten*, **50**, 104.
- CESKA, M., ERIKSSON, R. & VARGA, J.M. (1972) Radioimmunosorbent assay of allergens. *Journal of Allergy*, **49**, 1.
- CUATRECASAS, P. & ANFENSEN, C.B. (1971) Affinity chromatography. *Methods in Enzymology*, **31**, 345.
- DANNAEUS, A., JOHANSSON, S.G.O., FOUCARD, T. & ÖHMAN, S. (1977) Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatrica Scandinavia*, **66**, 31.
- GYÖRGY, P., MORO, E. & WITEBSKY, E. (1930) Eiklar-empfindlichkeit bei Eczema infantum. *Klinische Wochenschrift*, **9**, 1012.
- GYÖRGY, P., MORO, E. & WITEBSKY, E. (1931) Milchantikörper im Serum von Säuglingen. *Klinische Wochenschrift*, **10**, 821.
- GYÖRGY, P., MORO, E. & WITEBSKY, E. (1932) Weitere Erfahrungen über Trophallergie beim Eczema infantum. *Klinische Wochenschrift*, **11**, 1172.
- HALPERN, G.M. (1977) Sensitisation to shellfish. *Nouvelle Presse médicale*, **6**, 3111.
- JAFFÉ, K. (1931) Nachweis spezifischer Antikörper in vitro bei hochgradiger Fisch- und Hefeallergie. *Klinische Wochenschrift*, **10**, 304.
- KLUFT, C. (1978) Determination of prekallikrein in human plasma. Optimal conditions for activating prekallikrein. *Journal of Laboratory and Clinical Medicine*, **91**, 83.
- MORO, E. (1932) *Eczema infantum und Dermatitis seborrhoides*. *Klinik und Pathogenese*, p. 87. Springer, Berlin.
- MÜLLER, R. & BRANDT, R. Discussion remark to URBACH, E. (1932) Eczema infantum allergicum. Nachweis von Antikörpern auf biologischem (durch passive Übertragung) und serologischem Wege (Komplementbindung). *Zentralblatt für Haut- und Geschlechtskrankheiten*, **41**, 294.
- OEHLING, A. & SUBIRÄ, M.L. (1968) Brauchbarkeit des Komplementbindungstests in der Diagnose der Nahrungsmittel-Allergosen. *Allergie und Asthma*, **14**, 90.
- PARISH, W. (1970) Short-term anaphylactic IgG antibodies in human sera. *Lancet*, **ii**, 591.
- RATNER, B. & UNTRACHT, S. (1952) Egg allergy in children. *American Journal of Diseases of Childhood*, **83**, 309.
- SCHUR, S., HYDE, J.S. & WYPYCH, J.I. (1974) Egg-white sensitivity in atopic eczema. *Journal of Allergy and Clinical Immunology*, **54**, 174.
- STROBL, A. & WASITZKY, A. (1932) Untersuchungen über die alimentäre Allergie des Ekzemkinds. Mit besonderer Berücksichtigung der Eiklarempfindlichkeit. *Zeitschrift für Kinderheilkunde*, **54**, 53.
- TORISU, M., SONOZAKI, H., ITO, K. & SHIRAIISHI, S. (1968) The effect of C'3 Inactivator and Cu-chlorophyllin on the Prausnitz-Küstner reaction. *Journal of Immunology*, **101**, 1095.
- URBACH, E. (1935) *Klinik und Therapie der allergischen Krankheiten*, p. 150. Wilhelm Maudrich, Wien.
- VAN ES, L., KWANT-DEN HARINK, H. & PONDMAN, K.W. (1973) An unknown guinea-pig immunoglobulin with strong antibody activity to egg albumin. *Immunology*, **24**, 1087.
- WEEMAES, C.M.R., STOOP, J.W. & BERRENS, L. (1977) Inactivation of haemolytic complement by house dust allergen in the serum of children with atopic disease. *Clinical Allergy*, **7**, 75.
- WORINGER, P. (1932) Deux sortes d'anticorps ovalbuminiques dans le sang des nourrissons. *Comptes rendus de la Société de Biologie*, **109**, 206.
- WORINGER, P. (1933) Méthode de dosage de l'anticorps ovalbuminique dans le sang des sujets allergiques au blanc d'oeuf. *Annales de l'Institut Pasteur*, **50**, 270.

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