

Immunological Investigation of Possible Structural Similarities between Pollen Antigens and Antigens in Apple, Carrot and Celery Tuber

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Crossed line immunoelectrophoresis (CLIE) was used to show that a minor allergen present in birch pollen and another present in timothy pollen shared common epitopes with antigens in apple, carrot and celery tuber. Major pollen allergens were not involved. Structural similarities were also noticed in some mugwort pollen antigens and antigens in apple, carrot and celery, but none of these mugwort antigens acted as an allergen. In crossed immunoelectrophoresis (CIE) of samples of apple, carrot or celery extracts towards antibodies against birch pollen, grass pollen or mugwort pollen, four to 14 distinct precipitates were observed and shown to be specific. In crossed radioimmunoelectrophoresis (CRIE) none of the precipitates could be shown to act as an allergen. Using allergen detection on nitrocellulose paper, the apple, carrot and celery extracts were shown to possess IgE-binding capacity.

Key words: apple; birch pollen; carrot; celery; crossed immunoelectrophoretic techniques; food hypersensitivity; mugwort pollen; timothy pollen.

Accepted for publication 19 October 1984

The observation that patients allergic to birch pollen suffer from hypersensitivity to fresh fruits and vegetables more often than patients allergic to other pollens has led to several clinical and experimental studies in recent years. It has been found that 35% of those sensitive to birch pollen show a positive skin reaction, when tested with fresh fruit and vegetable material (10). The fruits and vegetables giving frequent positive reactions were parsnip, apple, potato, carrot and celery. A study, based on a questionnaire, reports 40% of birch pollen-sensitive patients being sensitive to certain foodstuffs (6); more recently the percentage has been assessed at 70 (7), while the figure for allergic patients not suffering from birch pollen allergy was about 20%. Foods related to birch pollen in this manner were various nuts, fruits and vegetables. Structural similarities in the aller-

gens in question have been proposed as one explanation for the observed co-occurrence of allergies (6).

The occurrence of partial immunological identity between birch pollen and hazel nut allergens has been demonstrated by immunodiffusion and passive hemagglutination inhibition (3) and by crossed line immunoelectrophoresis (2). In RAST-inhibition experiments birch pollen extract can inhibit the binding of corresponding specific IgE to apple, carrot or celery tuber allergens bound to a disc (4, 9, 13) indicating identical or partially identical structures. On the other hand, the efforts to show partial immunological identity between birch pollen antigens and some fruit and vegetable antigens by means of crossed immunoelectrophoretic techniques have not been successful (2, 16).

The purpose of this investigation was to study whether the observed relationship between birch pollen allergy and hypersensitivity to apple, carrot or celery is due to partial identity in the structures of antigens from these sources. A study reporting severe allergic reactions caused by celery and parsley in patients with mugwort pollen allergy (12), as well as a study describing IgE antibodies that crossreact with vegetable foods, pollen (especially grass pollen) and *Hymenoptera* venom (1) led us to investigate also the possible existence of similarities in the structures of some timothy pollen antigens or mugwort pollen antigens, and in those of apple, carrot or celery tuber antigens.

MATERIAL AND METHODS

Allergen extracts. The apple extract was prepared mainly as described by Björkstén et al. (4) and the carrot and celery tuber extracts were prepared essentially as described by Halmepuro et al. (9). The extracts obtained were dialysed twice for 24 h at +5°C against 20 mmol/l ammonium bicarbonate. The dialysed products were lyophilised and stored at +5°C. For the present purposes the extracts were used in solutions (10 mg/ml, w/v). The birch pollen (batch 1079), timothy pollen (batch 0180) and mugwort pollen (batch 979) extracts used as standard extracts were in 50% glycerol and originated from *Betula verrucosa* (Bv), *Phleum pratense* (Pp) and *Artemisia vulgaris* (Av) pollen (Soluprick® SQ, ALK, Copenhagen, Denmark). Birch pollen extract denoted as Bv 0782 and timothy pollen extract denoted as Pp 0581 used in some CLIE-experiments were prepared as described for timothy (14). The other extracts used in control experiments were from *Dermatophagoides pteronyssinus* (Dp), *Alternaria alternata* (Aa) and horse hair and dander, all obtained from ALK, Copenhagen, Denmark.

Antibodies. All the antibodies used were long-term immunization antibodies available from ALK, Copenhagen, Denmark. We used rabbit antibodies against *Betula verrucosa* pollen (aBv),

grass pollen (aNG) (a mixture of antibodies against *Avena elatior*, *Dactylis glomerata*, crude *Phleum pratense* and *Phleum pratense* antigens 19/25 in relations 1:1:4:2), *Artemisia vulgaris* pollen (aAv), *Dermatophagoides pteronyssinus* (aDp), *Alternaria alternata* (aAa) as well as horse hair and dander (aHorse). ¹²⁵I-labelled, immunoabsorbed, polyclonal anti-IgE was prepared as described by Løwenstein & Weeke (17). Normal rabbit immunoglobulin fraction (lot 051E) (nonsense-ab) was purchased from DAKO-immunoglobulins, Copenhagen, Denmark.

Patient sera. For crossed radioimmuno-electrophoresis and allergen detection on nitrocellulose paper different individual and pooled sera were used. The Bv+ pool was made up of sera from 20 birch pollen-allergic patients, the Pp+ pool consisted of sera from 20 timothy pollen-allergic patients, the P+ pool comprised sera from 20 patients simultaneously allergic to birch, timothy and mugwort pollens, whereas the A+ pool was from 13 patients allergic to dog and cat epithelium but not to pollens. These pooled sera were collected from patients visiting the Hospital for Allergic Diseases in Helsinki, Finland. The pools were made from equal volumes of the individual sera, and their concentration of IgE specific to the antigens ranged from 3 to 4 on the RAST-score scale. The fifth pool consisted of sera from 20 timothy pollen-allergic patients collected at Diagnoselaboratorium, Copenhagen, Denmark. The individual sera used had specific IgE against birch, timothy or mugwort pollen (RAST score from 3 to 4), or a very high IgE concentration (myeloma patient serum). The control serum pool, denoted as WE, was made up of sera from 1000 voluntary donors, collected in 1974 in Denmark and stored at -20°C until used.

Immuno-electrophoretic methods. Rocket immuno-electrophoresis, crossed immuno-electrophoresis (CIE), crossed line immuno-electrophoresis (CLIE) and crossed radioimmuno-electrophoresis (CRIE) were performed essentially as described by Løwenstein (15). The experiments were performed on 5 × 5 cm², 5 × 7 cm², or 5

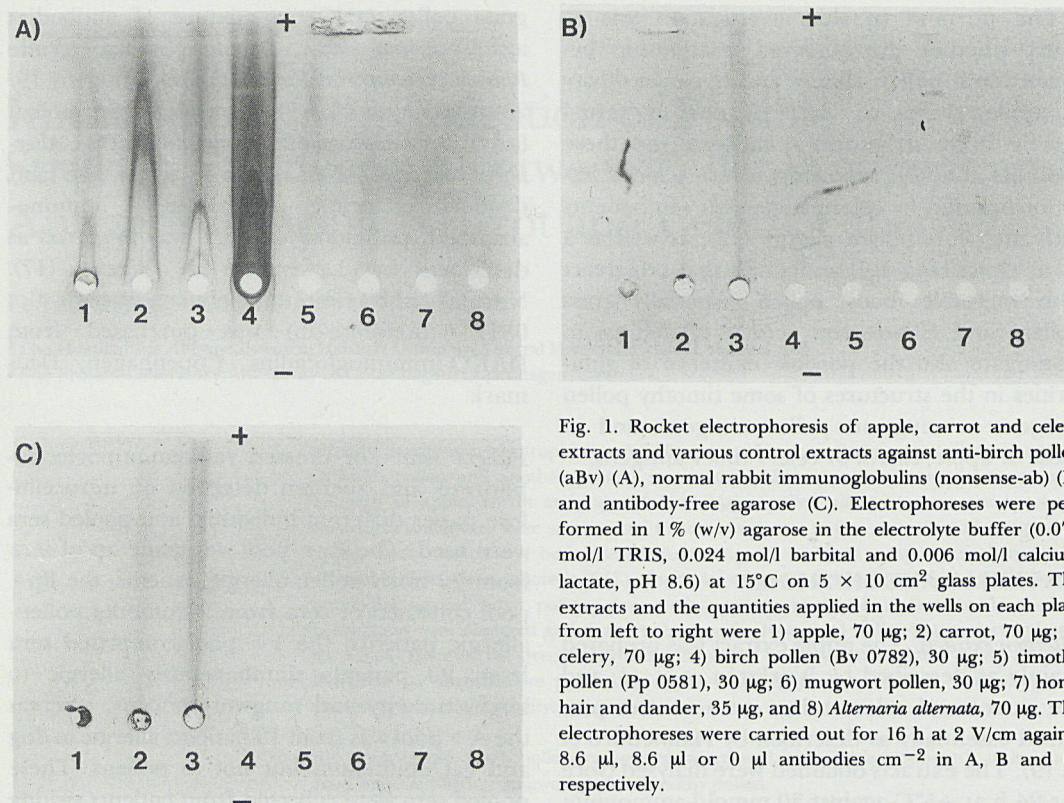


Fig. 1. Rocket electrophoresis of apple, carrot and celery extracts and various control extracts against anti-birch pollen (aBv) (A), normal rabbit immunoglobulins (nonsense-ab) (B) and antibody-free agarose (C). Electrophoreses were performed in 1% (w/v) agarose in the electrolyte buffer (0.073 mol/l TRIS, 0.024 mol/l barbital and 0.006 mol/l calcium lactate, pH 8.6) at 15°C on 5×10 cm² glass plates. The extracts and the quantities applied in the wells on each plate from left to right were 1) apple, 70 µg; 2) carrot, 70 µg; 3) celery, 70 µg; 4) birch pollen (Bv 0782), 30 µg; 5) timothy pollen (Pp 0581), 30 µg; 6) mugwort pollen, 30 µg; 7) horse hair and dander, 35 µg, and 8) *Alternaria alternata*, 70 µg. The electrophoreses were carried out for 16 h at 2 V/cm against 8.6 µl, 8.6 µl or 0 µl antibodies cm⁻² in A, B and C, respectively.

$\times 10$ cm² glass plates. The quantities used ranged from 40 µg to 150 µg antigen, from 200 µl to 400 µl antiserum and, in CLIE, from 300 µg to 2000 µg of interacting antigen in the intermediate gel. Further data is given in the legends to the figures.

Allergen detection on nitrocellulose paper. A method combining the property of nitrocellulose to bind proteins and the reaction of IgE with immobil-

Table 1

The number of CIE-precipitates visible in different antigen/antibody-systems, the quantities of antigens and antibodies are as described in the legend of Fig. 2

Extract	Antiserum		
	aBp	aNG	aAv
Apple	4	7	6
Carrot	9	9	9
Celery	9	14	9

ized antigens was used to prove the allergenicity of apple, carrot and celery extracts. The procedure was performed according to Lise Nyholm (personal communication): 50 µg of each extract to be studied as well as control extracts in 10 mg/ml solutions were applied on a spot as a series of drops on a sheet of nitrocellulose paper, and the sheet was incubated for 2 h at room temperature. The binding capacity of nitrocellulose was blocked by incubating the sheet in a solution of 1% Tween 20 in Sørensen buffer (0.067 mol/l K-Na-phosphate buffer, pH 7.4) for 1 h at room temperature. The washing of the sheet twice with Sørensen buffer was followed by the addition of 300 µl of control serum or patient serum, which contained specific IgE against birch pollen, timothy pollen or mugwort pollen, diluted with Sørensen buffer to a final volume of 8 ml, and the sheet was incubated overnight at room temperature. After a new wash with the buffer ¹²⁵I-labelled anti-IgE (0.25 µCi in 8 ml Sørensen buffer) was

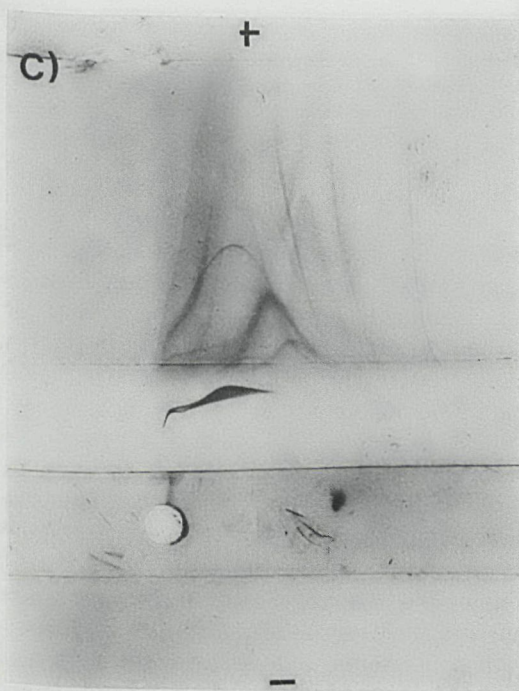
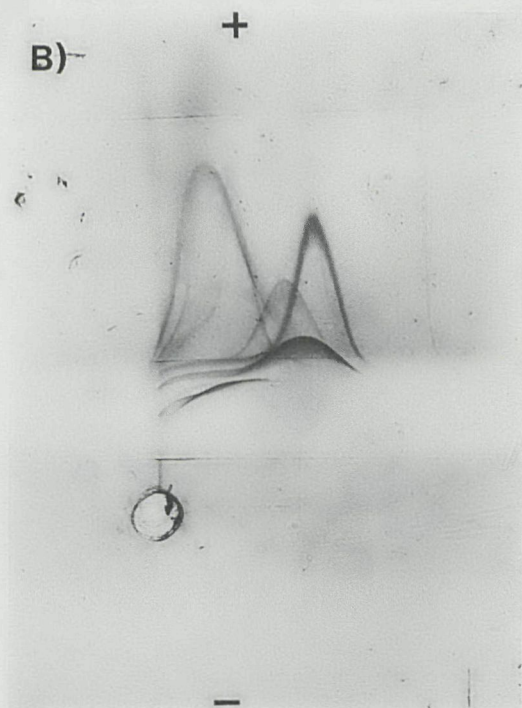
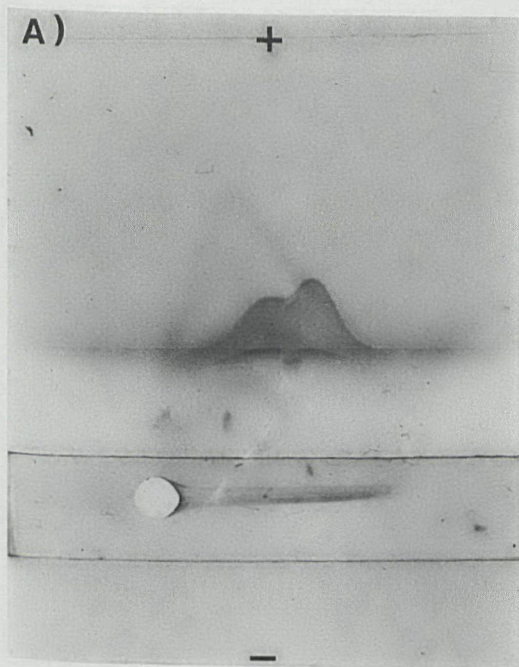


Fig. 2. Examples of crossed immunoelectrophoresis of 150 μg apple, carrot or celery extracts against different pollen antibodies performed on $5 \times 7 \text{ cm}^2$ glass plates in 1% (w/v) agarose in the electrolyte buffer (see legend for Fig. 1) at 15°C . First dimension electrophoreses were carried out for 30 min at 10 V/cm and second dimension electrophoreses for 16 h at 2 V/cm using the following quantities of extracts and antibodies: 150 μg apple extract against 17.1 μl aBv/ cm^2 in the anodic and 13.3 μl aBv/ cm^2 in the cathodic antibody-containing gel (A); 150 μg carrot extract against 8.6 μl aAv/ cm^2 in the anodic and 10.0 μl aAv/ cm^2 in the cathodic antibody-containing gel (B); 150 μg celery extract against 12.9 μl aNG/ cm^2 in the anodic and 10.0 μl aNG/ cm^2 in the cathodic antibody-containing gel (C).

added, and the sheet was again incubated overnight at room temperature. Autoradiography lasting 8 days or 14 days was performed.

RESULTS

Rocket immunoelectrophoresis. Several distinct rocket precipitates could be seen, when electrophoresis with samples of apple, carrot or celery extracts was carried out against aBv, aNG or

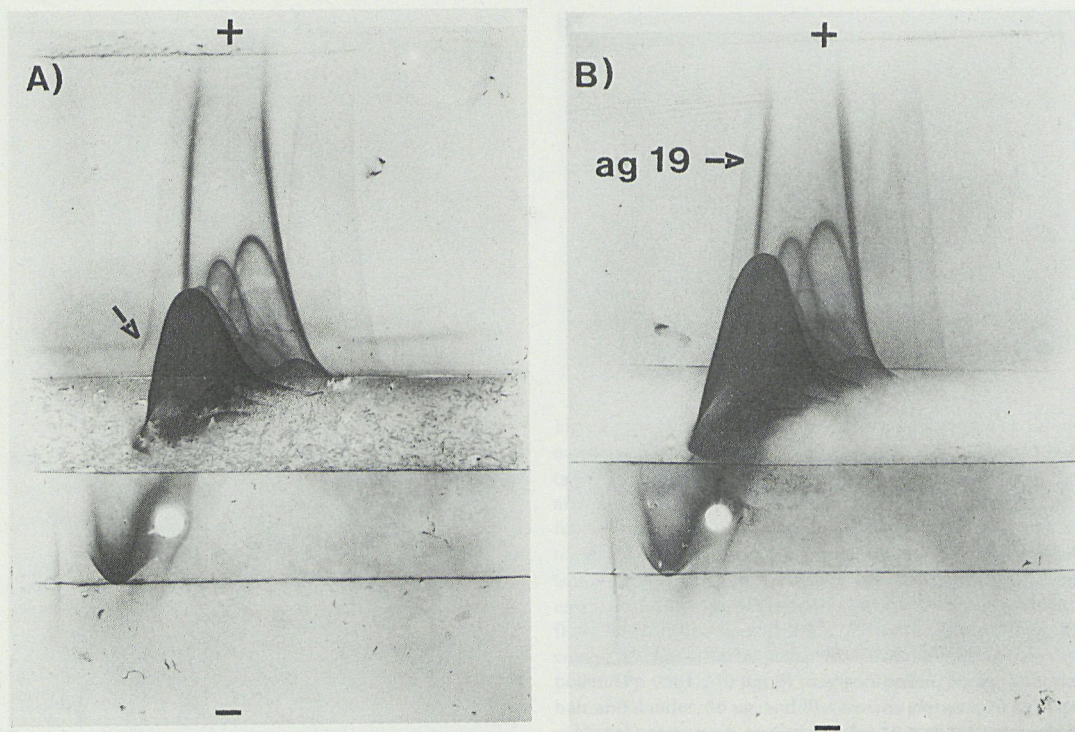


Fig. 3. Crossed line immunoelectrophoresis of 40 μ g standard birch pollen extract against aBv with apple extract in the intermediate gel performed on 5×7 cm² glass plates in 1% agarose in the electrolyte buffer (see legend for Fig. 1) at 15°C. First dimension electrophoreses were carried out for 25 min at 10 V/cm and second dimension electrophoreses for 16 h at 2 V/cm using 17.1 μ l aBv/cm² in the anodic and 13.3 μ l aBv/cm² in the cathodic antibody-containing gel. The plate to the left (A) contained 300 μ g of apple extract in the intermediate gel and the control plate to the right (B) contained no additional antigens in the intermediate gel.

aAv. Only anodic-migrating antigens were present. When the electrophoresis was performed in aHorse-, aAa- or nonsense-ab-containing gel, no distinct rocket precipitates could be recognised. Only vague tails of stain-absorbing substances running through the gel could be seen, and these artefacts were visible even when the electrophoresis was performed against antibody-free agarose (Fig. 1).

Crossed immunoelectrophoresis. The more detailed study, by means of CIE, of the precipitates seen in rocket immunoelectrophoresis revealed four to 14 antigens in different antigen/antibody-systems as shown in Table 1. Some examples of CIE-patterns are shown in Fig. 2.

In order to reveal which precipitates were

caused by interaction between proteins in apple, carrot, or celery and their corresponding antibodies produced by rabbits after normal intake of these foodstuffs, absorption experiments were performed with normal rabbit immunoglobulins in an intermediate gel. Only in the carrot/aBv-system did two antigens react with normal rabbit immunoglobulins. In the other systems the precipitation patterns were not affected.

Crossed line immunoelectrophoresis. One birch pollen antigen became clearly elevated and fused with a horizontal precipitate, when apple extract was introduced into the intermediate gel in second dimension electrophoresis of standard Bv-extract (Fig. 3). Simultaneously, the

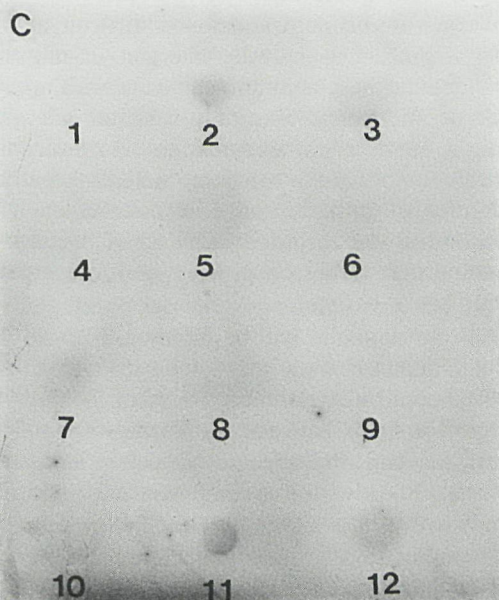
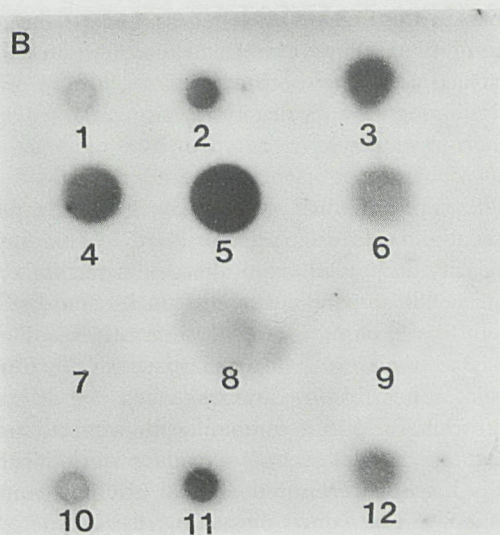
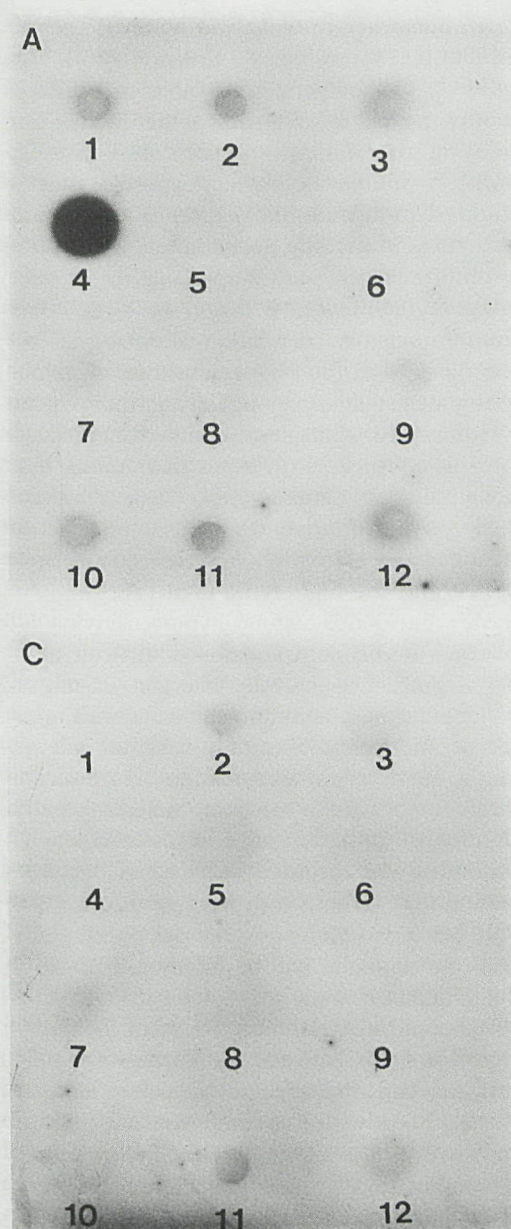


Fig. 4. Allergen detection on nitrocellulose paper (for procedure, see Material and Methods). The extracts on each sheet were 1) apple; 2) carrot; 3) celery; 4) birch pollen (Bv 0782); 5) timothy pollen (Pp 0581); 6) mugwort pollen; 7) *Dermatophagoides pteronyssinus*; 8) *Alternaria alternata*; 9) horse hair and dander; 10) apple; 11) carrot; 12) celery. The sera used in incubation were A) Bv + pool; B) P + pool; C) control serum WE.

In CLIE experiments of standard Pp-extract against aNG with apple, carrot or celery extract in the intermediate gel one to seven timothy pollen antigens became elevated and the restricted migration of some timothy pollen antigens could be observed with the apple extract in the intermediate gel. Again, only one allergen, Pp ag 1, classified earlier as minor allergen by CRIE (18), was affected by all the extracts.

In CLIE, several antigens were affected in the Av/aAv-system by introduction of apple, carrot or celery extract into the intermediate gel. None of these Av-antigens was able to bind IgE in CRIE.

Crossed radioimmuno-electrophoresis. None of the precipitates visible in apple, carrot or celery extract/anti-pollen-systems proved to be allergens in CRIE. After 24 days' exposure only some weak radiostained precipitates could be

migration of some birch pollen antigens was restricted by the apple extract. With carrot or celery extract in the intermediate gel seven birch pollen antigens were elevated. In each case only one of the birch pollen antigens involved was an allergen, minor allergen Bv ag 19 according to an earlier CRIE-classification of the Bv-extract (11).

seen in the extracts/aBv-systems and no radiostaining at all in extracts/aNG-systems, or in extracts/aAv-systems. Furthermore, where radiostaining was visible, it was also observed in the control plates.

Allergen detection on nitrocellulose paper. Apple, carrot and celery extracts possessed IgE-binding capacity as judged from allergen detection on nitrocellulose paper, though to a lesser degree than Bv, Pp or Av extracts used as controls (Fig. 4). Radiostaining no heavier than with the control serum could be observed when one of the nitrocellulose sheets containing the extracts studied was incubated with a patient serum with very high concentration of total IgE (myeloma patient serum).

DISCUSSION

The association between birch pollen allergy and hypersensitivity to certain fruits and vegetables has been verified in many studies (4, 5, 6, 7, 9, 13, 16). The RAST-inhibition experiments (4, 9, 13) have suggested the presence of common epitopes in the allergens concerned. In the present investigation the precipitates demonstrated in CIE of the samples of apple, carrot or celery extracts against aBv could be shown, by CLIE, to share a common molecular structure with some Bv-antigens, out of which one was a minor allergen (11).

Previously, Andersen & Løwenstein (2) have investigated the possible relationship between birch pollen and apple antigens using CLIE-technique, but failed to show partial immunological identity between the antigens studied. This result, contradictory to that of the present study, might be due to different kinds of apple extracts used. In their study Andersen & Løwenstein used apple peel extract or fresh apple peels, whereas our extract was made out of apple fruit tissue, and the reactions between apple proteins and phenolic compounds present in apple tissue were inhibited in the extraction procedure (4).

RAST-inhibition results obtained by Aalberse et al. (1) indicate immunological identity between allergens in grass pollen (*Dactylis glome-*

rata), buckwheat, potato and honeybee venom. Similarly, Løwenstein & Eriksson (16), using selected patient sera, were able to inhibit timothy pollen RAST with some foods. Considering the findings of these two investigations, it should not be unexpected that we could demonstrate the existence of common structures in timothy pollen antigens, including a minor allergen, and in apple, carrot or celery antigens. However, Eriksson et al. (7) have found a negative correlation between grass pollen allergy and food hypersensitivity when analysing selected birch pollen-allergic patients. Furthermore, the latest report from Eriksson and his co-workers (8) states that among birch pollen-allergic patients, who were simultaneously hypersensitive to grass pollen, the stronger the skin prick test reaction to grass pollen and the higher the total IgE, the lower was the incidence of food hypersensitivity. The reason for this contradiction is difficult to explain. The fact that the allergen in timothy pollen sharing a common structure with apple, carrot and celery was only a minor allergen might serve as an explanation. However, no direct investigation of immunological partial identity of antigens or of crossreacting IgE antibodies was included in either of the above mentioned studies, and the statistical comparison between results of skin prick test, RAST and questionnaire will be highly influenced by the patient selection.

We could also demonstrate partial immunological identity between some mugwort pollen antigens and apple, carrot or celery antigens, but as none of these mugwort antigens are known to act as allergens, this finding offered no explanation for the possible co-occurrence of mugwort pollen allergy and celery hypersensitivity (12).

In CIE of the samples of apple, carrot and celery extracts against aBv, aNG, or aAv, four to 14 antigens were distinguished. Apples, carrots and celeries are foodstuffs eaten by rabbits, and rabbit serum might contain antibodies against antigens in different fruits and vegetables. Therefore it was necessary to demonstrate the specificity of the observed reactions. The rocket precipitates, obtained when electro-

phoreses of apple, carrot or celery extracts were carried out in the three pollen antibodies containing gels, and the lack of distinct precipitates in gels containing non-pollen antibodies gave evidence in favour of specificity of reactions between antigens in apple, carrot or celery and the pollen antibodies. Further evidence was obtained, as no prominent changes in the CIE-patterns of extracts were observed after introducing normal rabbit immunoglobulins into the intermediate gel.

Antigens in apple, carrot and celery showed partial identity with several antigens present in birch, timothy or mugwort pollen in CLIE. In birch and timothy pollen only one of these antigens has been characterized as a minor allergen. Major allergens were not involved. This could explain the result that the apple-RAST (4, 9) and carrot- and celery-RAST (9) could be inhibited by birch pollen extract, whereas the efforts to inhibit Bv-RAST with apple, carrot or celery extract have been only partially successful (4, 9, 13). An additional explanation might be that the common minor allergen involved was underrepresented in the paper discs used.

In the CLIE experiments with apple extract in the intermediate gel, restrictions in the migration of some antigens in each antigen/antibody-system were observed. This can be explained by electroendosmosis caused by viscosity of the apple extract, which in turn is due to high pectin concentration in the apple fruit tissue.

In CRIE, none of the antigens in apple, carrot or celery extracts were identified as allergens in any antigen/antibody-system used. This might be because the serum pools or the individual sera used contained too little specific IgE against the respective minor allergen. Another explanation could be that CRIE-technique may fail to identify some antigens as allergens. Thus, some of the rabbit antibodies used for the preparation of the native CIE-plates could have reacted with exactly the same epitopes as the human IgE antibodies and thereby masked some of the allergens. The nitrocellulose paper technique where no rabbit antibodies were used could detect IgE-binding capacity in the apple, carrot and celery extracts, though less so than in the extracts of Bv, Pp or Av. Also, to per-

form a CRIE experiment in an appropriate manner, antibodies against apple, carrot and celery should have been used.

Lectins, a group of plant proteins with the capacity to bind to mono- and polysaccharides and glycoproteins, have been suggested to be the active agents causing fruit and vegetable hypersensitivity (2, 16). The present study gives evidence against the lectin hypothesis, as in CRIE and in allergen detection on nitrocellulose paper of the samples of apple, carrot and celery extracts the use of a patient serum with high total IgE concentration did not give heavier radiostaining than the control serum.

The present study suggests that the observed co-occurrence of birch pollen allergy and hypersensitivity to certain fruits and vegetables results from structural similarities in allergens. The subject should be studied further, because these results are not sufficient to explain the high percentage of the incidence of food hypersensitivity among patients with birch pollen allergy found by Eriksson et al. on the basis of a questionnaire (7, 8). Besides, the relationship between other pollen allergies and food hypersensitivity should be further investigated with a view to the possibility of a common structure in fruits and vegetables and other pollen allergens supported by the present study. The apple, carrot and celery tuber extracts used in this study were crude extracts and had rather low protein concentrations. Purification of the extracts and further experiments might give more information on the structures.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the Nordic Council and the Finnish Foundation of Allergy Research for scholarships, which made this study possible.

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