

An investigation of the possible immunological relationship between allergen extracts from birch pollen, hazelnut, potato and apple

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In a retrospective study on a selected group of patients, the coincidence of birch pollen allergy and a clinically relevant positive prick test reaction to apples and potatoes was confirmed.

Immunochemical comparison using the crossed line immunoelectrophoresis technique (CLIE) confirmed partial identity between birch pollen and hazelnut. By the same method no partial immunological identity between birch pollen and extracts and fresh peel from apples and potatoes was found. However, both apples and potatoes gave rise to non-immunological affinity precipitates. On this basis it is discussed, whether the active agents from apples and potatoes were extremely labile allergens, plant lectins, or low molecular weight substances with a direct or indirect histamine release effect.

Key words: Apples – birch pollen – contact dermatitis (immediate) – immunoelectrophoresis partial immunological identity – potatoes.

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Belin (1972) demonstrated the occurrence of partial immunological identity between birch pollen and hazelnut, and mentioned the possibility of cross reacting allergens between birch pollen and the peel from fresh potatoes and apples. Such type I-like sensitivity may be relevant to the development of hand eczema in many atopic individuals (Cronin 1973, Maibach 1976). Hannuksela & Lahti (1977) recently demonstrated the coincidence of hypersensitivity to birch pollen and immediate positive responses to apples, potatoes and other fruits and vegetables. The nature of the cross reacting allergens could not be determined.

The aim of the present study was to investigate the results of the prick test with birch pollen, hazelnut, apple and potato extract and their clinical relevance; further by immunochemical methods to study any partial immunological identity between birch pollen extracts and the peel of apples and potatoes.

The coincidence of positive reactions may also be explained by frequent contamination of birch pollen, apples and potatoes with micro fungi. We therefore considered whether there was any partial immunological identity between birch pollen extract and these micro fungi.

Materials and Methods

The material is retrospective and comprises 169 patients collected from 1974 to 1977 with various dermatological and allergological disorders in whom the 41 routine prick tests, including birch and hazelnut, were supplemented with prick tests with fresh peel and freeze-dried extract of apples and potatoes. The potato peel consists of two distinct layers, of which the green inner layer was used for prick tests. These substances were tested in food handlers, housewives, and in many patients who reacted to birch pollen.

Control prick tests with birch pollen, hazelnut, apple and potato extract and fresh apple and potato peel were performed on healthy persons without an atopic history or manifestations.

Prick tests were carried out on the abdominal skin, using histamine dihydrochloride solution (1 mg/ml) as a positive control. As negative controls 0.05 M sodium hydroxide and 0.15 M sodium chloride/glycerol 50 % (v/v) were used. The reactions were read after 15–20 min. Reactions were counted as positive when the wheal diameter exceeded 4 mm for both histamine and the extracts.

For the prick tests freeze-dried extracts of birch pollen, hazelnut, apples and potatoes were obtained from Allergologisk Laboratorium A/S (Copenhagen, Denmark). Up to May 1976 the extracts were used dissolved on the skin in a drop of sodium hydroxide 0.05 M; later we used Soluprick® (the extracts dissolved in 0.15 M sodium chloride/glycerol 50 % v/v). The peel of fresh potatoes and apples was used in its natural state, placed on the skin and pierced with the lancet.

Immunoelectrophoretic methods

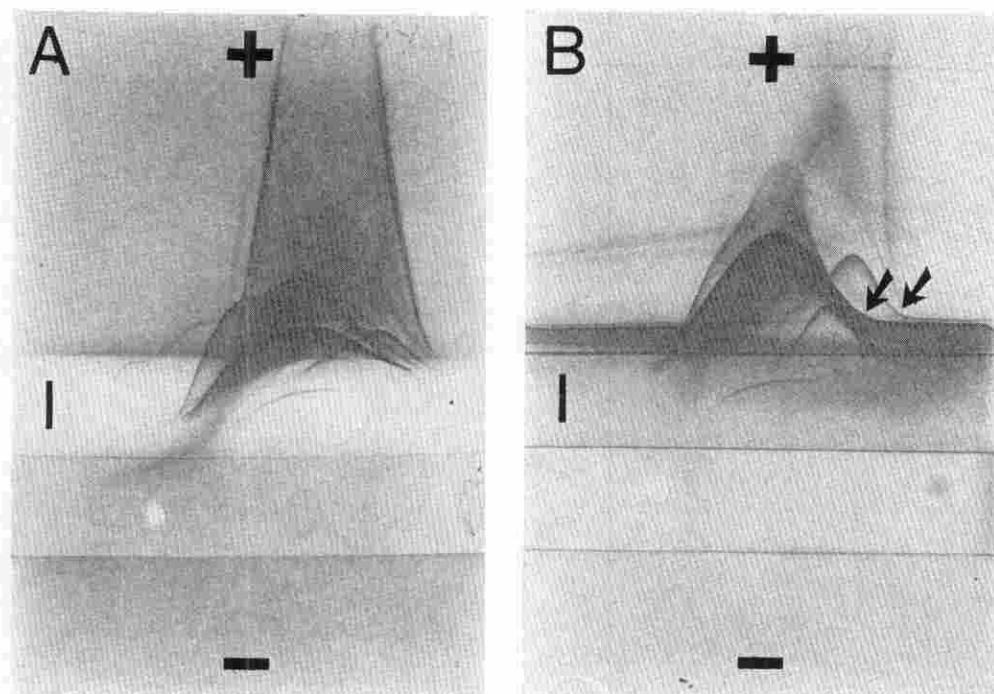
Equipment, reagents and methods were as described by Axelsen et al. (1973). The

electrophoreses were performed in 1 % (w/v) agarose gel (Litex, Glostrup, Denmark) containing a buffer of 0.073 M Tris, 0.024 M barbital, 0.006 M calcium lactate, and 0.003 M sodium azide (pH 8.6, 25° C). Crossed line immunoelectrophoresis (CLIE) was performed using both an anodic and cathodic second dimension gel. The first and second dimension electrophoreses were performed in a 0.15-cm thick gel at 10 V/cm for 30 min and in a 0.10-cm thick gel at 2 V/cm for 15 h, respectively. Glass plates 7×5 cm were used. The thickness of the intermediate gels was 0.12 cm. In the intermediate gel were included extracts from hazelnut, potatoes and apples as well as potato and apple peel in its natural state. When using the peelings directly on the intermediate gel, they were in the first two experiments placed on the gel for 30 min and 60 min before the electrophoresis was started. In a third experiment the peel was placed overnight during the run of the second dimension electrophoresis.

Allergen extracts for immunoelectrophoresis

Birch pollen allergen extracts (Bp 0275) were prepared and immunoelectrophoretically characterized mainly as described for phleum pratense (Pp 0375) (Løwenstein, in press). Freeze-dried extract from hazelnut, apple and potato peelings were obtained from Allergologisk Laboratorium. The extracts were gently agitated at 5° C overnight in a 25 % (w/v) aqueous solution containing 0.125 M ammonium bicarbonate and 0.015 M sodium azide (pH 7.6). After filtration at 5° C the solution was centrifuged at 0° C for 60 min at 50,000 g. The supernatant was filtered through a 0.65 μ pore filter and frozen in small amounts.

To obtain a soluble apple extract it was necessary to add 3 volume glycerol. Peelings from 1 kg of new potatoes and 1 kg of apples purchased in a supermarket were blend-



ed with a minimum of buffer (as used above) containing 1 % (v/v) Aprotinin (Novo, Mainz, BRD). The centrifugation and filtration was performed as described for the other extracts. The supernatants were divided into two parts, one was frozen and the other was dialyzed twice against a 50-fold volume of 0.005 M ammonium

bicarbonate, 0.015 M sodium azide, and then once against water for 24 h at 5° C. The dialyzed products were then freeze dried and stored at 5° C. Furthermore apple and potato peels were used in their natural state as a 'simplified' allergen extract.

Micro fungi allergen extracts from some micro fungi normally living on apples and

Table 1. Prick test results in 169 selected patients

Total	169	Positive prick test to birch pollen and/or hazelnut	Negative prick test to birch pollen and/or hazelnut
Atopic patients	116	86	30
Non-atopic patients	53	15	38
Positive prick test to birch pollen extract	83		
Positive prick test to hazelnut extract	73	101	68
Positive prick test to potato extract	24	23	1
Positive prick test to apple extract	8	8	0
Positive prick test to fresh potato peel	35	33	2
Positive prick test to fresh apple peel	28	27	1

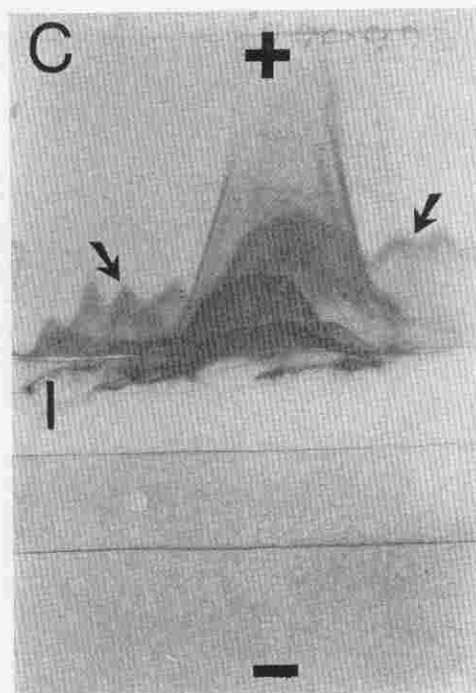


Fig. 1. Immunochemical analysis of birch, hazelnut and potato using birch/rabbit anti-birch as a reference system. Gel: 1 % (w/v) agarose containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate and 0.003 M sodium azide (pH 8.6, 25° C). First dimension electrophoresis: 10 V cm⁻¹ in a 15×50×1.5 mm³ gel for 25 min at 15° C; anode to the right. Second dimension electrophoresis: 2 V cm⁻¹ for 15 h (15° C) in the anodic (+) 40×50×1.0 mm³ and the cathodic (-) 20×50×1.0 mm³ antibody containing gels. Intermediate gel (I): 15×50×1.2 mm³ gel. Antigens: 3 µl Bp 0275 were applied in the wells in A, B and C. Antibodies: 300 and 100 µl anti-Bp 0275 in the + and - antibody containing gels in A, B and C. In B 100 µl extract from hazelnut was incorporated and in C fresh peel from potato was placed (60 min) on the intermediate gel. The arrows in B mark the fused precipitation lines of birch and hazelnut antigens and in C the non-immunological affinity precipitate.

potatoes were obtained from Allergologisk Laboratorium (phoma exigua, trichotecium roseum, mucor species, cladosporium herbarum, alternaria species and penicillium species). All were used in the concentration 10 mg/ml.

Antibodies

Antibodies against the birch pollen allergen extract (Bp 0275) were raised by immunizing rabbits. The antibodies were denoted a-Bp 0275, blend 1-13. The immunization and subsequent purification were performed according to the methods of Harboe & Ingild (1973).

Results

The prick test results are shown in Table 1. Among the 169 patients, 116 had a personal or family history (parents, children, brother or sister) of atopic dermatitis, allergic rhinitis and/or asthma.

A total of 101 patients had a positive reaction to birch pollen and/or hazelnut extract. Twenty-seven per cent of these had a wheal response when tested with fresh apple peel; 33 % reacted in a similar way to fresh potato peel. Only three of the positive reactions to fresh peel from apple and potato occurred in patients with negative reactions to birch pollen and/or hazelnut extract.

Fifteen patients had positive reactions to both birch pollen, hazelnut, apple and potato. Among these 15 patients, the reactions to fresh peelings from apples and potatoes were judged relevant in eight cases (53 %) because the patients noted itching on the hands, aggravation of hand eczema, itching/swelling of the lips or tongue, rhinitis and/or conjunctivitis when handling or eating fresh apples and potatoes.

In eight cases the patients gave a positive history, but the prick tests were nega-

tive. A positive prick test to potato and apple peel occurred in 33 cases with a negative history and was judged not relevant. In five patients with a previous relevant positive prick test to apple and potato peels, we could confirm the sensitivity in four cases with the actual apples and potatoes, whose peel was placed directly on the intermediate gel performing the CLIE. Prick tests with fresh peel gave more positive reactions than testing with extract as shown in Table 1.

The control series comprises 30 persons of whom three had a positive reaction to birch pollen and one to hazelnut. The 27 other persons showed no reactions.

Immunoelectrophoretic results

The comparisons between antigens of birch pollen and hazelnut by means of CLIE showed fusion between individual precipitation lines and the corresponding crossed immunoelectrophoretic precipitation lines, thus demonstrating the antigenic relationship between birch pollen and hazelnut (Fig. 1 B).

CLIE when performed using the same birch pollen antigen/anti-birch pollen rabbit antibody system with various amounts of extracts of potato peel and apple peel in the intermediate gel, did not demonstrate any partial immunological identity. However, horizontal precipitates were found when extracts or fresh peel from apple and potato were added to the intermediate gel (Fig. 1 C). Furthermore, the CLIE using mould extracts in the intermediate gel did not show any partial identity.

Discussion

The patient material is retrospective and selected. The clinical information is based on the case records and was often incomplete. The figures of false positive and false negative prick tests are minimum figures,

because it is uncertain if all were questioned. Eight patients with a positive history had negative prick tests; they may have been false negatives since a prick test is a weak challenge. Nevertheless our prick test results have shown that the overwhelming majority of positive reactions to fresh potato and apple occurs in atopic patients, consistent with the results of Hannuksela & Lahti (1977).

The finding of partial identity between birch pollen and hazelnut shows that CLIE is a valuable method for detecting immunological cross reactivity and confirms the finding of Belin (1972). We were, however, unable to demonstrate partial immunological identity between birch pollen and the peel from apples and potatoes, as suspected from the botanical relationship between these species. Furthermore no micro fungi species common for the investigated extracts were demonstrable in the birch pollen. The history of the patients and the results of the prick tests convincingly points to a positive correlation between birch pollen allergy and reactions to apples and potatoes. If cross reacting allergens were responsible, they were perhaps lost in the preparation of the extracts of apples and potatoes for the CLIE. Extraction procedures used were close to physiological conditions (pH 7.6–8.6 and ionic strength 0.1–0.25) and should mimic the conditions when patients are handling fruits and vegetables. To avoid the loss of allergenicity we used natural fresh peelings, but even these gave negative reactions in the CLIE. The active substances are obviously labile, because they are destroyed by cooking or digestive processes (Pearson 1966, Hannuksela & Lahti 1977).

The finding of horizontal precipitates might be explained as affinity precipitates caused by the interaction between lectins from the apple and potato peel and glycoproteins still available for reaction in the

antibody-containing gel (Bøg-Hansen et al. 1977). Lectins are plant proteins which react against certain polysaccharides in glycoproteins, for example, human immunoglobulins. Lectins have been found in potatoes (Allen & Neuberger 1973) and Siraganian & Siraganian (1975) have shown that lectins can cause a higher histamine release from basophil leucocytes from allergic patients compared with normal persons. Thus, lectins could cause a non-specific histamine-like reaction by prick testing with potato and apple peel, but not demonstrable by the CLIE.

As a third possibility low molecular weight substances could cause the hypersensitivity-like reaction, either directly as histamine or indirectly through intracellular mechanisms in the mast cells, thus giving rise to a vasoactive histamine release.

Most of the patients with reactions to raw potatoes and apples are atopics, as found by Hannuksela & Lahti (1977); but patients without any evidence of atopy may develop immediate-type-like reactivity to protein-like materials (Hjorth & Roed Petersen 1976). Cross reactivity between various vegetables and fruits has been stressed by Rowe & Rowe (1972) and recently by Hannuksela & Lahti (1977). We have concentrated on apples and potatoes but have not in general studied reactions against other fruits and vegetables.

In occupational medicine it is important to direct persons with an atopic predisposition to jobs in which they need not handle food and other products giving rise to hypersensitivity reactions. Further studies are needed to show the nature of the active substances and what causes their instability. These questions are important for the inactivation of the substances in daily life, and for the production of food extracts for test procedures.

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References

- Allen, A. K. & Neuberger, A. (1973) The purification and properties of the lectin from potato tubers, a hydroxyproline-containing glycoprotein. *Biochemical Journal* 135, 307-314.
- Axelsen, N. M., Krøll, J. & Weeke, B. (1973) A manual of quantitative immunoelectrophoresis. Methods and applications. *Scandinavian Journal of Immunology* 2, suppl. 1.
- Belin, L. (1972) Immunological analyses of birch pollen antigens, with special reference to the allergenic components. *International Archives of Allergy* 42, 300-322.
- Bøg-Hansen, T. C., Bjerrum, O. J. & Brogren, C. H. (1977) Identification and quantification of glycoproteins by affinity electrophoresis. *Analytical Biochemistry* 81, 78-87.
- Cronin, E. (1973) Immediate type hypersensitivity to potato. *Contact Dermatitis Newsletter* 13, 358.
- Hannuksela, M. & Lahti, A. (1977) Immediate reactions to fruits and vegetables. *Contact Dermatitis* 3, 79-84.
- Harboe, M. H. G. & Ingild, A. (1973) Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scandinavian Journal of Immunology* 2, suppl. 1, pp. 161-164.
- Hjorth, N. & Roed Petersen, J. (1976) Occupational protein contact dermatitis in food handlers. *Contact Dermatitis* 2, 28-42.
- Løwenstein, H. Isolation and partial characterization of 3 allergens of timothy pollen. *Allergy*, in press.
- Maibach, H. (1976) Immediate hypersensitivity in hand dermatitis. *Archives of Dermatology* 112, 1289-1291.
- Pearson, R. S. B. (1966) Potato sensitivity, an occupational allergy in housewives. *Acta allergologica* 21, 507-514.
- Rowe, A. H. & Rowe, A. jr. (1972) *Food Allergy*. Its manifestations and control and elimination diets, 1st ed., pp. 23-40 and 534-593. Springfield: Charles C. Thomas.

- Siraganian, R. P. & Siraganian, P. A. (1975) Mechanism of action of concavalin A on human basophils. *Journal of Immunology* 114, 886-893.
- Tuft, L. & Mueller, H. L. (1970) *Allergy in Children*, pp. 128-154. Philadelphia, London, Toronto: W. B. Saunders Company.
- Weeke, B. (1973) Crossed immunoelectrophoresis. *Scandinavian Journal of Immunology* 2, suppl. 1, pp. 47-56.
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