

Hypersensitivity to Apple and Carrot Can Be Reliably Detected with Fresh Material

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Hypersensitivity to birch pollen was linked with allergy to apple and carrot, apple seeds and carrot seeds and also with their fractions. Skin reactions among other atopics were rare, and non-atopic patients showed no reactions to fresh fruits and vegetables.

Apple and carrot as well as apple seeds and carrot seeds were fractionated using column chromatography. Two major fractions were obtained, one with sugar and proteins, and one with proteins, the latter giving skin reactions more often than the former. The correlation between clinical symptoms and skin test results with the fractionated material was somewhat lower than that obtained with the non-fractionated fresh material. The allergens of fruits and vegetables might be proteins (enzymes).

Key words: contact urticaria; fruit allergy; scratch-chamber test; urticaria, vegetable allergy.

Using the scratch-chamber test technique, reactions to common fruits, edible roots and vegetables have been recorded in about 30% of atopic patients allergic to birch pollen, but in only 2-6% of other atopic patients. An agreement between clinical symptoms and test results was found with carrot and apple in over 85% of the cases (2).

In order to characterize the nature of the allergens and to find a standardizable antigen, we fractionated fresh apple and carrot, apple seed and carrot seed using column chromatography. The clinical responsiveness of the tests performed with fresh material was somewhat better than that of the tests performed with fractionated allergens, which were obviously proteins.

PATIENTS AND METHODS

Atopic patients with a positive scratch test result to birch pollen (Dome Laboratories) and with one or more positive scratch-chamber reaction to fresh apple, carrot,

potato, tomato, swede, peach or plum were chosen for further testing with three varieties of apple (*Granny Smith* from Argentina, *Sturmer* from New Zealand and *Punahaneli* from Finland), carrot (*Nantes* cultivated in Finland), apple seed (*Granny Smith*), carrot seed (*Nantes*), and chromatographic fractions of apple flesh and seed (*Granny Smith*) and carrot flesh and seed (*Nantes*). Twenty-five of the patients were men and 41 women, and their mean age was 17.2 years (range 6-41 years).

The comparison series consisted of 22 atopic patients without birch pollen allergy, nine of whom were men and 13 women (mean age 36.0 years, range 14-69 years). Apple, carrot, potato and tomato were also tested using the scratch-chamber technique in 105 non-atopic persons, 39 of whom were men and 66 women (mean age 34.2 years, range 7-71 years).

Columns, 2 x 20 cm, were prepared using Sephadex® QAE-50 (Pharmacia Fine Chemicals, Sweden) in phosphate buffer (PB)

containing KH_2PO_4 3.64 g and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 7.11 g and water up to 1,000 ml, pH 7.0. The crude antigen, 15 cm³ of crushed apple or carrot (peel excluded) or 2 g of seeds homogenized in PB with Ultra Turrax®, was carefully placed on the column, and the elution performed with buffered saline. Increasing the content of sodium chloride in PB from 0.0 to 1.0 M stepwise (0, 0.04, 0.09, 0.17, 0.34, 0.86, 1.0 M), 7 × 8 × 4 ml aliquots were gathered. Protein was measured qualitatively with ninhydrin (Nin spray®, Spray Master AB, Sollentuna, Sweden) and sugar with Clinistix® (Ames Company) in each aliquot.

Two major fractions were obtained from apple flesh and carrot flesh as well as from the seeds. The first fraction contained both sugar and protein (Fraction 1) and the other protein only (Fraction 2). The fractions were sterilized with a Millipore® type GS 0.22 µm filter and the sterility was checked for both aerobic and anaerobic bacteria. Phenol 0.4% was used as the antimicrobial agent throughout the procedure.

Fresh apple and carrot were tested with the scratch-chamber technique described earlier (2); Scratches approximately 5 mm long were made on the skin of the upper back or of the arms. A small amount of test material (about 20 µg) was put into an aluminium epicutaneous test chamber (Finn Chamber, Epitest Ltd OY, Fredrikinkatu 61, SF-00100 Helsinki 10). The chambers were fixed with porous tape (Scanpor®, Norgesplaster A/S, Oslo, Norway). The chambers and test materials were removed after 15 min, when the test results were read. Histamine 1:100 (scratch test) was used as a positive control, and physiological saline as a negative control. Reactions of at least half of the area produced by histamine were regarded as positive, but minor reactions as negative.

The seeds, homogenized with Ultra Turrax®, were tested by the ordinary scratch test method using 0.1 N NaOH as the vehicle, and the apple and carrot flesh and seed fractions were given intracutaneously. Histamine 10 mg/ml in glycerosaline for

scratch test and 1 mg/ml for intracutaneous test was used as a positive control, and 0.1 N NaOH and phosphate buffered saline as negative control. Reactions of at least half of the area produced by histamine were regarded as positive, but minor reactions as negative.

Inquiry concerning the patients' symptoms when eating or handling apples and carrots was made in each case. Only clear-cut symptoms were considered "positive". The patients who had not noticed symptoms from apple or carrot, and those who did not know whether or not they had symptoms, were regarded as "negative" cases.

The investigation was performed during a 2-week period in October 1977, just after the allergen fractions were made. The allergenicity of the fractions did not diminish during these 2 weeks.

RESULTS

Homogenized apple seeds elicited reactions slightly more often than fresh apple but the opposite result was found with carrot and carrot seeds (Fig. 1). The agreement between the skin test results with raw apple and apple seeds was 77% and the corresponding figure was 70% for raw carrot/carrot seed. The ability of raw carrot and apple to cause skin reactions was stronger than that of the corresponding carrot and apple flesh fractions with the exception of apple flesh fraction 2. Seed fractions 1 and 2 gave positive skin reactions more frequently than the homogenized seeds themselves (Fig. 1).

The correlation between the skin test results and clinical symptoms was calculated on the basis of the patient histories only and no challenge tests were made. The inquiry concerning symptoms after handling or eating raw apples revealed symptoms in 49 of 66 patients with birch pollen allergy, namely lip, mouth or throat symptoms in 37, abdominal symptoms in seven, rhinoconjunctivitis in nine, dermatitis in five, and general symptoms in six cases. The corresponding figures for carrot were: total 23,

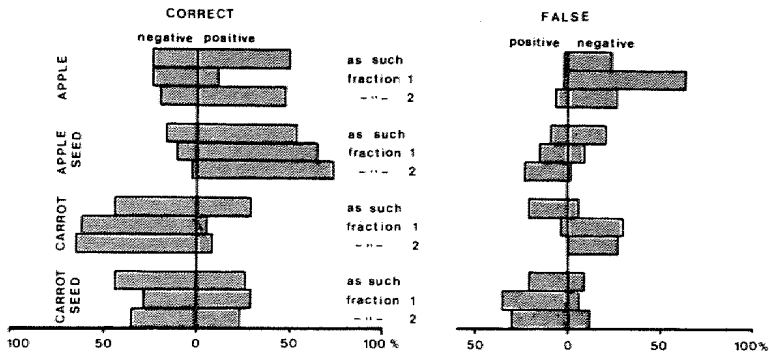


Fig. 1. Correlation between clinical symptoms and the results of skin tests with apple and carrot in 66 patients with birch pollen allergy. "Correct positive" means that skin test was positive and the patient had noticed some symptoms when eating or handling fresh apple (carrot). "Correct negative" means that skin test was negative and the patient had no harm from fresh apple (carrot). Correspondingly, "false-negative" means: skin test negative but clinical symptoms present, and "false-positive": skin test positive but no clinical symptoms.

lip/mouth/throat, 20, abdominal, one, rhinoconjunctivitis, three, dermatitis, three and general symptoms, four.

The agreement between skin tests and clinical symptoms was best in 3/4 of the cases (Fig. 1). Seeds and seed fractions frequently elicited false-positive results while the apple and carrot fractions gave many false-negative skin test results.

Of the three varieties of apple, *Sturmer* elicited most reactions, but those produced by *Granny Smith* were on average a little stronger (Table 1).

Of the 22 birch pollen-negative patients, one reacted positively to apple as such, two to the apple seed fractions but none to apple seed as such nor to carrot, carrot seed and their fractions. All the 105 non-atopic subjects showed a negative skin test result to apple, carrot, potato and tomato as such.

DISCUSSION

The present results confirmed our earlier observations on the close link between birch pollen hypersensitivity and allergy to various

Table 1
Immediate reactions to three varieties of apple in 66 atopic patients with birch pollen allergy

Apple variety	Skin reaction (scratch-chamber test)			Total n	%
	+	++	+++		
n	n	n	n	n	%
<i>Granny Smith</i> (Argentina)	4	18	10	32	48.5
<i>Sturmer</i> (New Zealand)	4	28	7	34	51.5
<i>Punakaneli</i> (Finland)	2	13	1	16	24.2

fruits and vegetables. The number of positive skin reactions was even higher when corresponding seeds and seed fractions were used. These reactions were also linked with birch pollen allergy. Until now, studies on cross reactivity between various fractions of birch pollen and fresh fruits and vegetables have not been published.

Although the fractions of carrot seeds and apple seeds gave the most frequent positive skin test results, the number of false-positive reactions was too high to make these allergens suitable for screening fruit and vegetable allergy. As to apple itself, the Finnish variety, *Punakaneli*, elicited fewer reactions than the foreign ones, a fact that was clearly revealed in the patient histories. There were distinctly more false-positive reactions to raw carrot than to raw apple, reflecting mainly the number of "not known" answers to the question of whether or not the patient had noticed symptoms after peeling or eating raw carrots. To avoid allergic reactions to fresh fruits and vegetables the patients with birch pollen allergy should be told to eat them cooked or as juices. These patients should be especially careful with fresh celery which can cause anaphylactic shock (2).

It has been suggested that allergens in various fruits and vegetables could be proteins or protein-like substances (1), proteinaceous molecules (3), and enzymes (6). The extreme lability of these allergens has been the most confusing factor, and this would seem to speak in favour of enzymes and other easily destroyable compounds. We found one fraction containing polypeptides and another containing polypeptides and sugar not only in apple and carrot themselves but also in their seeds. Skin reactions were elicited by the polypeptide fraction (Fraction 2) more often than by the other fraction, supporting the theory that enzymes are the most important allergens.

Chromatographic separation of the allergens of carrot and apple made the antigens retain their allergenicity for at least 2 weeks. Obviously the allergenic base was

somehow purified in the fractionation although the skin test results obtained with these fractions were somewhat less reliable than those with fresh non-fractionated material.

A scratch test with soluble or dry food allergens has been found superior to other test methods, e.g. Black's cytotoxic test, the skin window technique, determination of the leukopenic index, tanned red cell hemagglutination, and the gel diffusion test (4). On the other hand, the allergenicity of fruits and vegetables is easily destroyed through juice-making, deep-freezing and cooking (2, 5), and the present results showed the slight superiority of the fresh material over the chromatographic fractions. Thus, the best way to study fruit and vegetable allergy still seems to be the scratch-chamber test with fresh material.

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