

Basophil histamine release in patients with birch pollen hypersensitivity with and without allergic symptoms to fruits

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Histamine release (HR) studies were performed in 40 birch pollen-allergic patients (positive case history, positive SPT, positive birch pollen-specific serum IgE: RAST ≥ 3) with ($n = 20$, A) and without ($n = 20$, B) fruit hypersensitivity, and 10 nonatopic volunteers (C). Several fruit allergens were used and characterized by protein determination and immunoblot techniques. Dose-dependent HR (apple peel = apple pulp > peach = cherry) was demonstrated in both allergic groups, but to a higher extent in patients with fruit allergy ($P < 0.01$). Increased basophil sensitivity to birch pollen was found in the group with fruit allergy ($P < 0.001$). Strong correlations between the mediator response induced by several fruits indicate common allergens within the extracts. We conclude that fruit-related symptoms require not only high specific serum IgE, but a strong cellular sensitization to birch pollen allergens together with an increased cellular reactivity to fruit allergens.

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IgE-mediated allergic symptoms by pollen antigens are often combined with sensitizations to food allergens from various kinds of plants (5, 7, 18). These observations were found predominantly in patients allergic to pollen antigens from birch trees, which are one of the main inducers of seasonal respiratory symptoms of hypersensitivity in northern and central Europe. It has been shown that 70% of birch pollen-allergic patients developed symptoms of hypersensitivity after ingestion of raw vegetable, nut and fruit products (5, 7, 8, 11, 13). Local disorders, such as oral itching and paresthesia are the symptoms most often reported by birch pollen-allergic patients after ingestion or handling of raw fruits. Also such systemic reactions as rhinoconjunctivitis, urticaria and asthma have been documented. A study of 600 pollen-allergic patients claimed that 70% of them suffered from local symptoms after ingestion of one or more types of fruits (7). The demonstration of common antigens in birch pollen and hazelnut (2) favors the hypothesis of cross-allergenicity, which has also been proposed by clinical observations and results of RAST and RAST-inhibition studies (11, 12). As experiments based on crossed-immuno-electrophoresis (CIE) failed to detect any common allergenic proteins of birch pollen and apple, the authors speculated that lectins from plants may cross-link membrane-bound IgE antibodies through

their high affinity to specific polysaccharide patterns on the surface of strongly glycosylated IgE molecules (1). The existence of at least three distinct cross-reactive structures – responsible for cross-reactive phenomena of IgE antibodies among birch pollen antigens and fruit – has recently been shown (4). One of these proteins with a molecular mass of 17–20 kDa was found in apple, peach and cherry. The cross-linking of IgE antibodies by glycoproteins able to recognize specific glycosylated side chains of the IgE molecules – the lectin theory – was disputed by Halmeppuro (10), who demonstrated structural similarities between pollen antigens and antigens in apple, carrot, and other vegetables. In the present study we performed histamine release experiments with standardized birch pollen and fruit extracts using basophil-containing peripheral leukocytes from birch pollen-allergic patients with and without symptoms of fruit hypersensitivity.

Material and methods

Patients

The study comprised patients with a positive case history of such birch pollen-related symptoms, as seasonal rhinoconjunctivitis and/or asthma, who had given their informed consent. Further, a positive skin prick test (SPT) to a birch pollen extract

(ALK SQ Soluprick®, Denmark, allergen-induced wheal $\geq$ positive control induced by 10 mg/ml histamine), and birch pollen-specific serum IgE (Phadebas RAST-class ≥ 3 , Pharmacia, Sweden) were required as criteria of inclusion. The patients were divided into two groups according to their positive or negative case history of fruit allergy besides their birch pollen-related symptoms. None of the patients had received immunotherapy or taken medications which might interfere with the SPT reactions. All the patients were examined out of season. Ten individuals without allergic symptoms and a negative prick skin test to birch pollen allergens and without any birch pollen-specific serum IgE served as negative controls.

Material

The following materials were purchased: piperazine-*N,N'*-bis 2-ethanesulphonic acid (PIPES) (Serva, Germany); human serum albumin (Red Cross, Germany); 6% dextrane in physiological saline (Knoll, Germany); 50% glucose (Braun, Germany).

Buffers

PIPES-albumin-glucose buffer (PAG) contained 25 mmol PIPES, 110 mmol NaCl, 5 mmol KCl, 0.003% human serum albumin, and 0.1% glucose. PAG supplemented with 1 mmol of CaCl_2 and 1 mmol of MgCl_2 (PAG-CM) was used during the release experiments.

Cell isolation

Venous blood was collected from normal and atopic volunteers. Mixed leukocyte preparations were obtained, employing dextrane sedimentation as previously described (17). In brief, 20 ml of blood were mixed with a solution containing 5 ml of 6% dextrane, 2 ml 0.1 mmol ethylene diamine tetraacetic acid and 0.8 ml 50% glucose. After sedimentation of red cells and polynuclear leukocytes (90–120 min), the basophil-containing supernatant was carefully sampled and centrifuged for 8 min with $200 \times g$ at room temperature. The cell pellet was washed twice with PAG by centrifugation (8 min, $200 \times g$, 4°C). Finally, the cells were resuspended in PAG-CM to a volume required for the following experiments.

Challenge

The cells were challenged with different concentrations of the birch pollen and fruit extracts for 40 min at 37°C in a waterbath. The release process was stopped by centrifugation ($400 \times g$, 15 min, 4°C).

The supernatants were removed for histamine analysis. Total histamine content was determined by lysing aliquots of the cells with 2% of HClO_4 . Cells incubated in PAG-CM alone served as a measure of spontaneous histamine release ($<5\%$ of the total histamine content). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous release. All experiments were performed in duplicate.

Histamine assay

Histamine release was measured according to the automated fluorometric method (17). The extracts did not fluoresce, nor did they effect the fluorescence of known amounts of histamine.

Allergens

The food extracts (apple peel and apple pulp of "Granny Smith"; peach and cherry) were prepared according to the method described by Björkstén (3). Additionally, all extracts were concentrated by polyethylene glycol to half of the starting volume and dialysed against 10 mmol potassium phosphate buffer, pH 7.2. Protein concentrations, determined after dialysis by the Lowry method (15), yielded the following amounts: apple peel 0.9 mg/ml; apple pulp 0.56 mg/ml, cherry 1.5 mg/ml, and peach 1 mg/ml. The extracts were stored in appropriate aliquots at -20°C until used. Birch pollen-related histamine release was triggered by a standardized, lyophilized extract (ALK SQ aquagen birch pollen extract, 100 000 SQ/ml, ALK Diagnostics, Denmark). Dilutions of all allergen extracts were prepared fresh daily in PAG-CM buffer.

The fruit extracts were further characterized by immunoblot techniques. Approximately 280–650 μg of the extracts (15–50 $\mu\text{g}/\text{cm}$ gel) were separated on a 17.5% homogeneous sodium dodecylsulfate-polyacrylamide gel by electrophoresis (SDS-PAGE) (16) and electrophoretically transferred to nitrocellulose (NC). The NC was blocked with 0.1% Tween 20 in 0.1 mol Tris-HCl, pH 7.4, 0.1 mol NaCl, 2.5 mmol MgCl_2 , cut in strips and incubated overnight with 1:4 diluted sera of birch pollen-allergic fruit-positive patients. For detection of specific IgE an alkaline phosphatase linked second goat-antihuman-IgE antibody (Jackson, Inc. Dianova, Hamburg, Germany) was used. The intensity of IgE binding to each protein band was optically determined and expressed by a score of 1–4 for semi-quantitative evaluation. The scores of each protein band were summarized and divided by the number of the sera used, to estimate the average intensity of antibody binding.

Statistical analysis

The statistical significance of differences in allergen-induced histamine amounts between the groups was analyzed by the Mann-Whitney-U test for unpaired data. Differences between the fruit extracts within the same group were determined by the Wilcoxon signed rank test for paired data. Values were considered significant at the $P < 0.05$ level. Correlations between the mediator responses to birch and fruit extracts were calculated by the Spearman rank test.

Results

Peripheral, washed leukocytes obtained from patients with birch pollen-related symptoms either with or without accompanying fruit allergy were challenged with birch pollen and different fruit extracts. The freshly prepared fruit extracts were characterized by protein determination and immunoblot studies. The frequency of antibody binding to the fruit extract and its distribution pattern to different protein bands were calculated by a semiquantitative scale. Table 1 summarizes the relative intensity of IgE binding to fruit-related proteins with different molecular weights. These fruit extracts were used as well as the standardized birch pollen extract to challenge washed leukocytes of different patient groups. Group A consisted of 20 persons (8 females, 12 males; age 20–59, average 32 years) who suffered from seasonal, birch-related allergic disorders plus fruit-induced symptoms of hypersensitivity (frequency in Table 2). Twenty individuals with birch pollen allergy but without fruit hypersensitivity (6 females, 14 males, age 16–65, average 35 years) comprised Group B. Ten nonallergic individuals (4 females, 6 males, age 16–49, mean: 31 years) proven by case history, SPT and RAST served as negative controls (Group C). Histamine release induced by the birch pollen allergen extract is shown in Fig. 1. The range and average of maximum response – obtained by each single dose-response curve – are

Table 2. Frequency of fruit-related symptoms in 20 birch pollen-allergic patients with fruit hypersensitivity

Fruit-related symptoms	Frequency <i>n</i>
Oral-pharyngeal itching	14
Lip edema	11
Intraoral aphthous lesions	7
Bronchial asthma	4
Conjunctivitis	4
Pharyngitis	4
Glossitis	2
Rhinitis	1
Urticaria	1

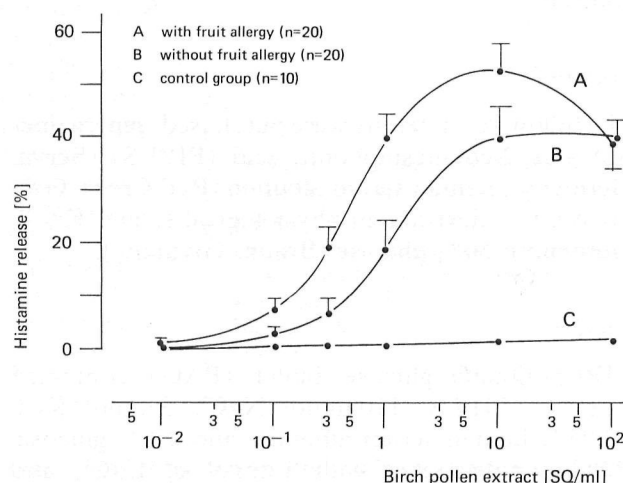


Fig. 1. Birch pollen-induced histamine release from patients with birch pollen allergy with (A, $n = 20$), and without (B, $n = 20$) fruit hypersensitivity. No release was obtained in Group C (10 non-atopics). Histamine values are expressed as a percentage (means $\pm$ SEM) of the total cellular histamine content.

Table 1. Binding of specific IgE to the single proteins of fruit extracts determined by immunoblotting techniques. Molecular mass (MM) range of the electrophoretically separated proteins and the relative intensity of binding determined from 12 sera of birch pollen-allergic patients with additional fruit hypersensitivity

Apple		Peach		Cherry	
MM range (kDa)	Relative intensity	MM range [kDa]	Relative intensity	MM range [kDa]	Relative intensity
22–28	1.59	15–25	0.54	20–25	0.33
30–38	1.04	38–45	1.20	35–41	1.29
43–56	1.86	72–98	2.25	66–76	1.25
60–90	1.27			80–90	1.25

depicted in Table 3. No significant difference between groups A and B was found, comparing the maximum of histamine release. Nevertheless, a tendency to higher histamine values was found in Group A. In contrast, Ag10 – representing birch pollen-related cell sensitivity – differed between Groups A and B (ranges and medians depicted in Table 3) with high significance ($P < 0.001$). Figs. 2 and 3 demonstrate the dose-dependent histamine release curves induced by apple peel and apple pulp extracts (ranges, averages, and medians of HR_{max} and Ag10 in Table 3). The maximum response to both stimuli differed highly significantly between Groups A and B ($P < 0.001$). The apple pulp extract caused a small release ($< 10\%$ of total histamine) at its highest concentration in Group C. Peach- and cherry-induced histamine release appeared in a dose-dependent fashion (Figs. 4 and 5); the maximum release was obtained with $100 \mu\text{g/ml}$ of both fruit extracts and differed significantly between Groups A and B

Table 3. Fruit- and birch pollen-induced histamine release (HR) from peripheral leukocytes of birch pollen allergics with (fruit+) or without (-) fruit hypersensitivity

Antigen for basophil HR	Birch allergics	n	Cell reactivity HR _{max} (%)		Cell sensitivity Ag10[μg/ml * SQ/ml]	
			Range	Mean	Range	Median
Apple peel	fruits +	20	18-95	47	0.01-3.7	0.13
	-	20	8-76	29	0.01-65	0.65
Apple pulp	fruits +	20	13-85	43	0.04-8.5	0.3
	-	20	2-93	24	0.01-3.5	1.2
Peach	fruits +	20	19-86	45	0.16-22	2.3
	-	20	8-72	39	0.16-44	13
Cherry	fruits +	20	19-93	47	0.6-42	4.8
	-	20	4-95	34	0.13-60	16
Birch pollen (ALK SQ aquagen)	fruits +	20	15-86	52	0.03-1*	0.18*
	-	20	12-79	48	0.08-20*	1.2*

n: number of patients. Cell reactivity reflects the individual maximum of release (range and average of HR_{max}) detected from the histamine dose-response curves. Cell sensitivity (Ag10) represents antigen concentration (range, median) required for 10% release of total histamine.

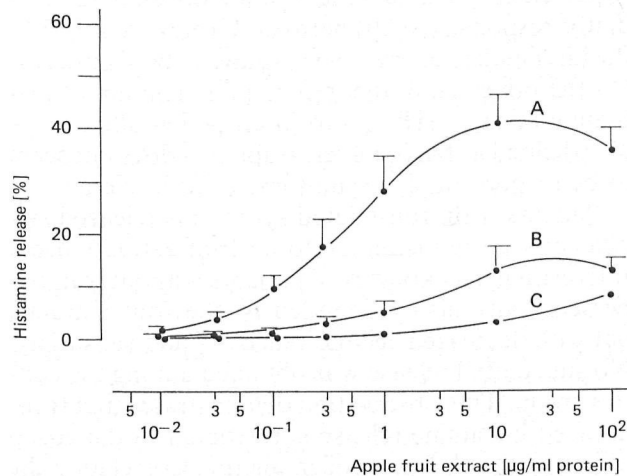


Fig. 2. Apple pulp-induced histamine release (means $\pm$ SEM) from patients with birch pollen allergy with (A, $n = 20$), and without (B, $n = 20$) fruit hypersensitivity, and non-atopic individuals (C, $n = 10$). Histamine values are expressed as means $\pm$ SEM.

($P < 0.05$). Furthermore, significant differences between the Ag10 of Groups A and B in peach-induced histamine release was detectable ($P < 0.01$). A small amount of histamine ($< 10\%$) was released by 100 $\mu\text{g/ml}$ of the peach and cherry extract in Group C (Figs. 4 and 5).

To gain further insight into the relationship between birch pollen and fruit-induced mediator release, we correlated the maximum histamine release for each extract within Groups A and B (Table 4a, Table 4b) and the allergen concentration required for 10% release within both groups. While the Ag10 to birch pollen was not well correlated to the Ag10 of the fruit extracts, significant correlations between the maximum of each response curve were found

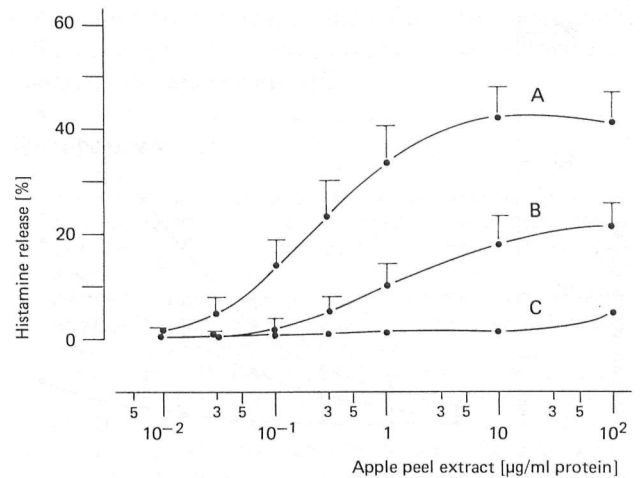


Fig. 3. Apple peel-induced histamine release from peripheral leukocytes (means $\pm$ SEM, groups in Fig. 1).

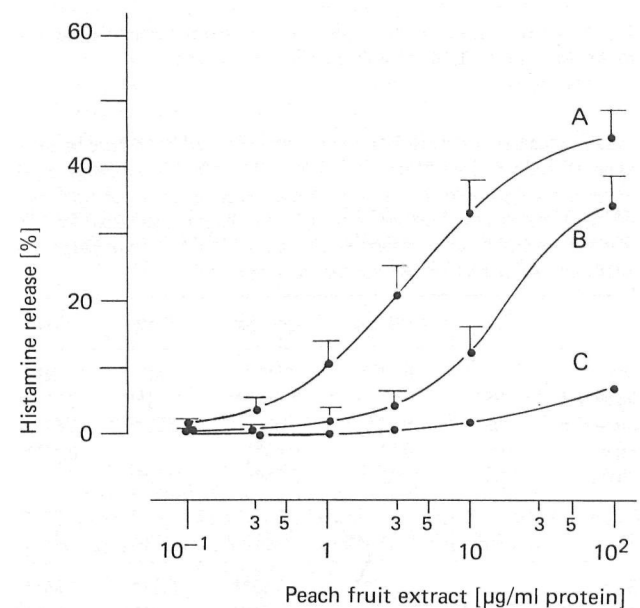


Fig. 4. Peach-induced histamine release from peripheral leukocytes. (means $\pm$ SEM, groups in Fig. 1).

between birch-pollen- and fruit-induced histamine release, especially in the group without fruit allergy (Table 4b). Highly significant correlations were determined not only between the histamine release response curves to the different fruits with respect to HR_{max} but also to Ag10. The correlations between Ag10 of the different fruits within Group B without fruit allergy were much more pronounced than the appropriate coefficients of correlation in Group A (Table 4a). However, a similar correlation between the individual maximum of fruit-induced histamine release was found in both groups, with and without fruit allergy.

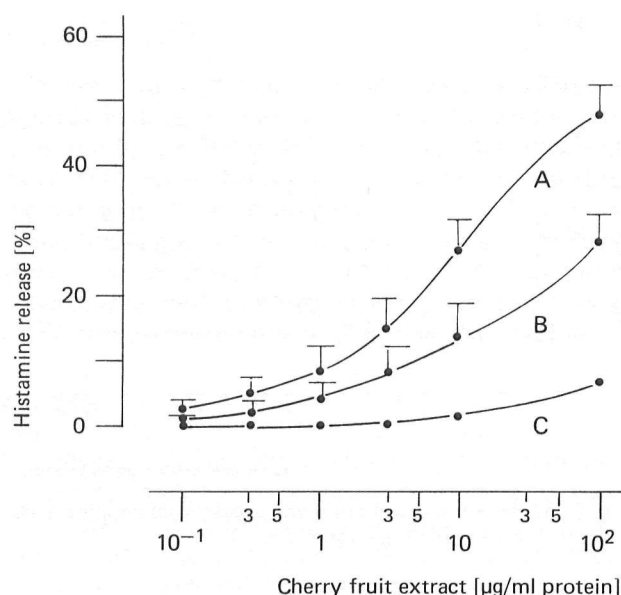


Fig. 5. Cherry-induced histamine release from peripheral leukocytes. (means $\pm$ SEM, groups in Fig. 1).

Table 4. Spearman rank correlations (ρ) of birch-pollen- and fruit-induced histamine release (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P < 0.001$). Correlations of cell reactivity – maximum of individual histamine release – are presented on upper right; lower left depicts correlations of cell sensitivity Ag10, the allergen concentration for 10% release of the total histamine content. Results obtained from birch-pollen-allergic individuals with (4a) and without (4b) fruit hypersensitivity

a	Birch	Apple peel	Apple pulp	Peach	Cherry
Birch		0.5*	0.5*	NS	NS
Apple peel	NS		0.96***	0.64**	0.78***
Apple pulp	NS	0.7**		0.65**	0.67**
Peach	0.5*	0.5*	0.69**		0.74**
Cherry	NS	NS	0.56*	0.79**	
b	Birch	Apple peel	Apple pulp	Peach	Cherry
Birch		0.71**	0.63**	0.57*	0.65**
Apple peel	0.79**		0.9***	0.8***	0.81***
Apple pulp	NS	0.8**		0.7**	0.86**
Peach	NS	0.9**	0.87**		0.54*
Cherry	NS	0.57*	0.83**	0.77**	

Discussion

Different hypotheses exist to explain the phenomenon of fruit hypersensitivity in people with birch pollen-related allergic symptoms. Several investigations underline a theory based on the existence of birch pollen-specific IgE antibodies, which might cross-react with antigenic proteins in some fruits (1, 4, 5, 12). It has also been speculated that lectins in fruits might mediate fruit hypersensitivity through their cross-linking of heavily glycosylated IgE molecules, with subsequent degranulation of mast cells or basophils (9, 14). We tried to evaluate both groups

of birch-allergic patients (with and without fruit hypersensitivity) by determining histamine release from peripheral leukocytes triggered by a characterized and standardized birch pollen extract and self-prepared fruit extracts. The group with fruit hypersensitivity was characterized by a more pronounced cellular sensitivity to birch pollen (about seven times higher, comparing Ag10). However, neither specific IgE classes (RAST class averages: 3.7 vs 3.6) nor total IgE levels (medians of total IgE: 361 vs 348) differed between the groups, as high birch pollen-specific IgE levels (RAST class > 3) had been chosen as criteria of inclusion for the present study. These results are in contrast to earlier findings, which favored the role of birch pollen-specific and total serum IgE levels in the development of fruit-induced hypersensitivity (10). Nevertheless, we would suggest that basophil sensitivity to specific birch pollen allergens might play a more important role than specific and total serum IgE in the phenomenon of fruit hypersensitivity. Differences in the dose-related mediator response (Ag10) between Groups A and B to the birch pollen allergen were found to be significant. On the other hand, the individual maximum of histamine release (HR_{max}) to birch pollen allergens – also defined as basophil releasability – does not seem to be responsible for symptoms of fruit allergy.

Patients with fruit-related symptoms released significantly more histamine to all fruit extracts used. Interestingly, leukocytes of patients without fruit hypersensitivity also responded to the fruit extracts, but with decreased cellular reactivity and sensitivity. No substantial release was obtained among the control group. This observation demonstrates that fruit-induced histamine release is restricted to the group of patients with birch pollen allergy. Correlating the mediator response to birch pollen and fruit extracts did not indicate a close relationship between both kinds of stimuli. Thus, fruit hypersensitivity might not be only a simple function of cellular reactivity to birch pollen allergens. However, in the group without fruit allergy, positive correlations were determined between the maximum response to birch and to each fruit extract. Highly significant correlations were found within both groups (not only with, but also without fruit allergy) in regard to fruit-related cellular sensitivity and reactivity. The high concordant relation, especially in apple peel- and apple pulp-induced histamine release, favors the existence of common antigens in all fruit extracts, possibly inducing similar mediator release. There is probably no evidence for lectin binding as a reason for fruit-induced mediator release, otherwise this should also appear in the nonallergic group.

The results of an *in vitro* method like histamine release are largely dependent on the quality of the allergens used. The proteins inducing fruit-related

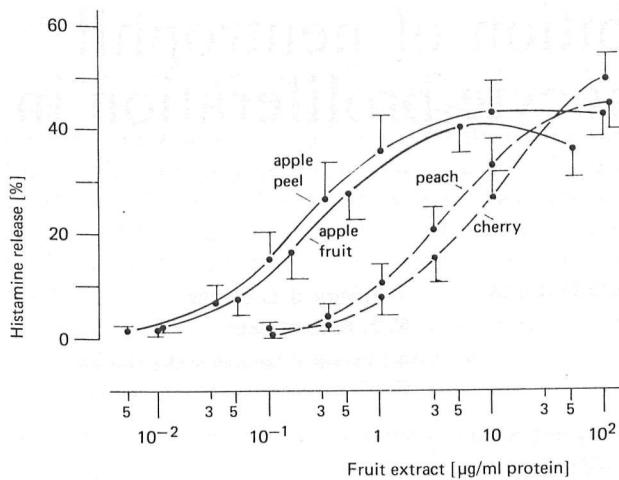


Fig. 6. Fruit-induced histamine release (means $\pm$ SEM) from basophil-containing, peripheral leukocytes of birch-allergic patients with fruit hypersensitivity ($n = 20$).

symptoms tend to be heat labile. Nevertheless, the fruit-induced histamine release – only found among the birch pollen-allergic group – indicates highly allergenic activities of the self-prepared fruit extract. Fig. 6 compares the allergenic activities of the fruit extracts to induce histamine release from peripheral leukocytes of patients with fruit allergy. The extracts of apple – creating similar dose-response curves – tend to be 10 times more potent than the extracts from peach and cherry, whose dose curves also paralleled each other. The molecular range of the single proteins did not include an IgE binding at 17 kDa, which represents Bet v I, the major allergen of birch pollen (with the exception of the cherry extract).

Recently, this predominant allergen has been demonstrated in both birch pollen and fruit extracts (6). It has been speculated that different types of apples might contain varying amounts of the major birch allergen. Therefore, the weak relationship between the cellular sensitivity to birch pollen and the fruit extracts in our study might be due to at least a small amount of Bet v I in our fruit extracts. Positive test results (i.e. SPT, specific serum IgE) are not generally equated with a relevant allergy. These findings are also confirmed by our study, since basophils of patients – not allergic to fruits – are capable of releasing significant amounts of histamine to fruit extracts. However, the response of mediator-containing cells is much less than in birch allergic-patients with accompanying fruit allergies. Since no differences in birch pollen-specific IgE levels were found between the groups, *in vitro* histamine release induced by birch

pollen and fruit extract might far more accurately reflect the patient's condition than measurements of allergen-specific serum IgE.

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