

Fruit allergy: Demonstration of IgE antibodies to a 30 kd protein present in several fruits

Ahmed A. Wadee, PhD, Lee-Ann Botting, BSc, and

Arthur R. Rabson, MB, BCh, MRCPATH Johannesburg, Republic of South Africa

A patient who had experienced allergic responses to various fruits developed an acute anaphylactic reaction after the ingestion of a local strain of cling peaches. The patient's serum, but not control sera, contained IgE antibodies reactive to extracts from peaches, guavas, bananas, mandarins, and strawberries in an ELISA. The patient's serum, however, did not demonstrate elevated levels of IgE antibodies to extracts from apples, pears, and nectarines. Adsorption of the patient's serum with extracts from peaches, strawberries, and mandarins resulted in a decline of detectable IgE antibodies to these fruits. Adsorption with extracts from apples and pears had no such effect. These results demonstrate the specificity of the patient's IgE antibodies to selected fruits only. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting revealed a 30 kDa of protein that was recognized by the IgE antibodies in the patient's serum. This protein was not present in extracts from pears or apples. Our results highlight the importance of specific allergens in considering acute allergic reactions to individuals exhibiting sensitivity to various foods or fruits. (J ALLERGY CLIN IMMUNOL 1990;85:801-7.)

Fruits are a vast and heterogeneous group of foods that may cause symptoms of hypersensitivity sometimes resulting in severe reactions.¹ Food allergy is essentially diagnosed by clinical observations, including exclusion diets, food challenge, and sometimes by laboratory investigations.² The exact incidence of food allergy and the nature of the responsible food allergen are not always known.³

The allergens causing immediate hypersensitivity are generally proteins,⁴ and immediate skin reactions to the labile fruit and vegetable allergens have been demonstrated with freshly crushed material and a scratch-chamber technique.^{5, 6} Patients with allergic reactions to several different fruits and vegetables have been described. These include patients in whom the diagnosis was confirmed by the demonstration of IgE antibodies to apple,^{3, 4} carrot, potato, celery,^{7, 8} and pawpaw.² In other patients the confirmation of allergy to citrus fruit was made by elimination and challenge.⁹

Abbreviations used

PRU:	Phadebas RAST unit
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
OD:	Optical densities

We describe in the present study a patient who had developed an acute anaphylactic reaction after ingestion of a local strain of cling peaches and in whom serum IgE antibodies against a 30 kDa fraction derived from a number of different fruits could be demonstrated with classic Western blot analysis.

MATERIAL AND METHODS

Patient

The patient was a 33-year-old white woman who, on a number of occasions, had experienced allergic reactions after the ingestion of various fruits. In particular, she had developed rhinitis and pharyngeal itching after ingestion of peaches, strawberries, bananas, guavas, and mandarins. On two occasions she had developed severe anaphylactic reactions, including bronchospasm within 30 minutes of eating cling peaches. After the most recent episode, she required admission to hospital for resuscitation, and since this attack, she had avoided all fruits other than pears and apples. The patient had no history of allergic rhinitis, asthma, or other allergic problems and was otherwise in good health. The patient gave her informed consent for blood to be drawn for these studies but refused to have skin prick or

From the Department of Immunology, School of Pathology of the South African Institute for Medical Research, and University of the Witwatersrand, Johannesburg, South Africa.

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Reprint requests: A. A. Wadee, PhD, SAIMR, PO Box 1038, Johannesburg, Republic of South Africa 2000.

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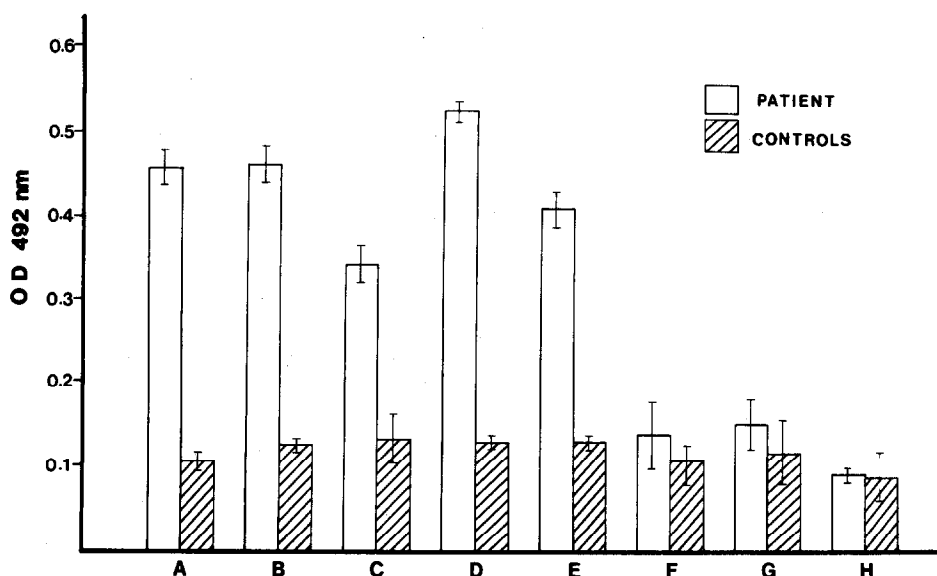


FIG. 1. Detection of IgE antibodies in the sera of the patient and 10 normal individuals against extracts from cling peach (*lane A*), guava (*lane B*), banana (*lane C*), strawberry (*lane D*), mandarin (*lane E*), nectarine (*lane F*), apple (*lane G*), and pear (*lane H*). (Mean OD $\pm$ SD of three experiments.)

patch/skin window tests performed. For this reason only in vitro tests were performed in the present studies.

Preparation of fruit extracts

Fruits (cling peaches, nectarines, strawberries, apples, pears, guavas, bananas, and mandarins) were purchased and their extracts were prepared from (1) the outer covering of each fruit (hereafter referred to as the skin) and (2) the soft juicy edible pulp minus the seeds (flesh). Each sample was diced and pulped with a Waring commercial juice extractor (Waring Products Division, New Hartford, Conn.).

Antigens were extracted by incubating the various fruit juices with preserving fluid containing 0.399 mol/L NaCl (Labchem, Johannesburg, Republic of South Africa), 0.13 mol/L NaHCO₃ (Hopkins & Williams, Johannesburg), and preserving fluid containing 1.86% phenol in a 1:5 (vol/vol) ratio. The resultant mixture was incubated at room temperature for 48 hours. The solution was then filtered through a piece of muslin cloth and filter paper. The pH of the filtered juice extract was then adjusted to 5.4 by the addition of 1 N NaOH (Saarchem, Krugersdorp, Republic of South Africa). All assays were performed with freshly prepared fruit extracts.

ELISA

The ELISA was performed in flat-bottomed, 96-well microtiter plates (Nunc, Gibco Ltd., Uxbridge, England). The wells were coated with 100 μ l of various fruit extracts at a concentration of 10 μ g/ml in sodium carbonate buffer, pH 9.6. Plates were incubated for 1 hour in a humidified chamber at room temperature, after which they were washed four

times with PBS-Tween. The unbound sites were blocked with 100 μ l of 0.5% bovine serum albumin (Gamma Biologicals, Inc., Houston, Texas) for an additional hour. Plates were washed once more and incubated with 100 μ l of varying dilutions of serum from the patient and normal volunteers for 2 hours.

After an additional wash, the wells were coated with 100 μ l of peroxidase-conjugated goat antihuman IgE (dilution, 1:3000) (Miles-Yeda, Ltd., Rehovot, Israel) for 1 hour. After a final wash, 100 μ l of peroxidase substrate was added to each well, and the reaction was terminated after 15 minutes with 50 μ l of 2.5 mol/L of sulfuric acid (Labchem, Boksburg, Republic of South Africa). ODs were read at 492 nm in a Dynatech multiscan ELISA reader (Flow Laboratories, Mclean, Va.).

In some experiments, the ELISA to detect specific IgE was performed on the patient's serum after it had been adsorbed with cling peach or other fruit extracts. In these experiments, fruit extracts in coating buffer were plated onto plastic Petri dishes (50 mm in diameter; Sterilin Laboratories, Feltham, Middlesex, England) for 1 hour at room temperature. Unbound sites were blocked for 1 hour with 0.5% bovine serum albumin, and the Petri dishes were washed with PBS-Tween. In subsequent experiments, a 1:20 dilution of patient's serum was added to plates coated with various fruit extracts, and the plates were incubated for 2 hour at room temperature. At the end of this period, the adsorbed serum was collected and used in ELISA to detect specific IgE as described above.

To ascertain whether the patient's serum contained cross-reacting antibodies to a variety of other allergens, the com-

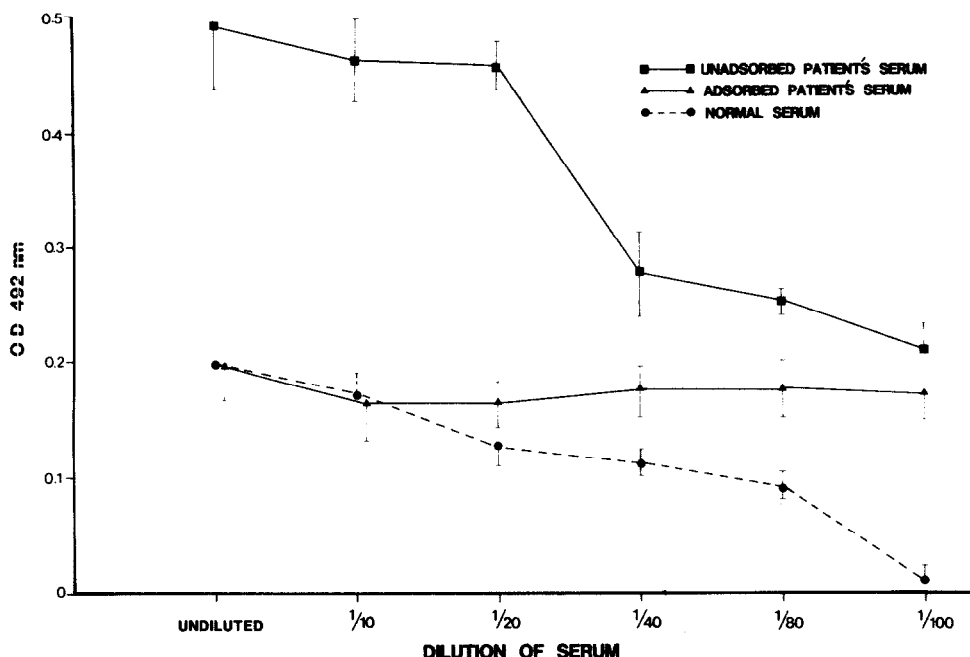


FIG. 2. The effects of adsorption on the levels of IgE to peach extracts. The patient's serum was adsorbed with peach extracts and then used in ELISA to detect remaining IgE to peaches. (Mean OD $\pm$ SD of three experiments.)

mercial Phadebas RAST (Pharmacia Diagnostics, Uppsala, Sweden) was used. The commercial allergoid disks used were egg white, wheat, rye, peanut, a combination of hazel nut, almond, and brazil nut, cheese, cat epithelium, dog dander, bee venom (*Apis mellifera*), banana, strawberry, and apple. The RAST radioimmunoassay was performed according to the instructions of the supplier. Reactions were read in comparison with four known standards supplied in kit form. Reactions from these standards were recorded as 1+ (0.35 PRU/ml), 2+ (0.70 PRU/ml), 3+ (3.50 PRU/ml), and 4+ (17.50 PRU/ml).

Separation of fruit extract proteins by SDS-PAGE and Western blotting

To detect specific antigenic components, SDS-PAGE analysis of fruit extracts was performed by loading 125 μ g of protein from each fruit extract onto gradient gels of 5% to 20% acrylamide with 4% stacking gels as described by Laemmli.¹⁰ One part of the gel was stained with Coomassie brilliant blue (Searle Diagnostics, Berkshire, England), and the other part was blotted onto a nitrocellulose sheet by applying 60 V across the membrane for 3 hours in a Bio-Rad transblotting cell with a 250/2.5 power supply (Bio-Rad Laboratories, Richmond, Calif.).

These latter strips were quenched with 3% gelatin for 1 hour in quenching buffer containing 2 mmol/L of Tris base (Koch-Light Laboratories, Ltd., Coinbrook, Berkshire, England) and 100 mmol/L of glycine (BDH Chemicals, Poole, England) at room temperature and were washed in Tris-buffered saline containing Tween 20. Thereafter, mem-

brane strips were incubated with a 1:100 dilution of patient's or control subject's sera for 2 hours at room temperature with gentle agitation. Membranes were then washed twice with Tris-buffered saline containing Tween 20 and incubated with a 1:1000 dilution of goat antihuman IgE horseradish-peroxidase conjugate (Sigma Chemical Co., St. Louis, Mo.). Color was developed with a horseradish-peroxidase substrate (Bio-Rad Laboratories).

Chemical modification of fruit antigens

Carbohydrate moieties were degraded according to the method of Owhashi et al.¹¹ Lyophilized proteins were incubated in 0.05 mol/L of sodium metaperiodate (Merck Frosst Laboratories, Darmstadt, West Germany) in the dark with gentle mixing for 72 hours at 4° C. The reaction was terminated by dialysis against phosphate-buffered saline (four changes of 2 L each at 4° C). Protease treatment was performed by incubating active fractions with 1 μ g/ml of proteinase K (Boehringer-Mannheim GmbH, Penzburg, West Germany) for 1 hour at room temperature, followed by extensive dialysis at 4° C. Dilipidation of active fractions was achieved by treating fractions with 2 vol of anesthetic ether. The mixture was frozen at -70° C and thawed at 37° C, and the interface containing any lipid was removed. This process was repeated three times until lipid was no longer observed at the interface. Lipid samples were pooled and the ether was removed by evaporation over nitrogen. The remaining lipid was reconstituted in 1 ml of RPMI 1640 containing 0.1 ml of ethanol by sonication in an MSE Soniprep (MSE Scientific Instruments, Sussex, England) 150

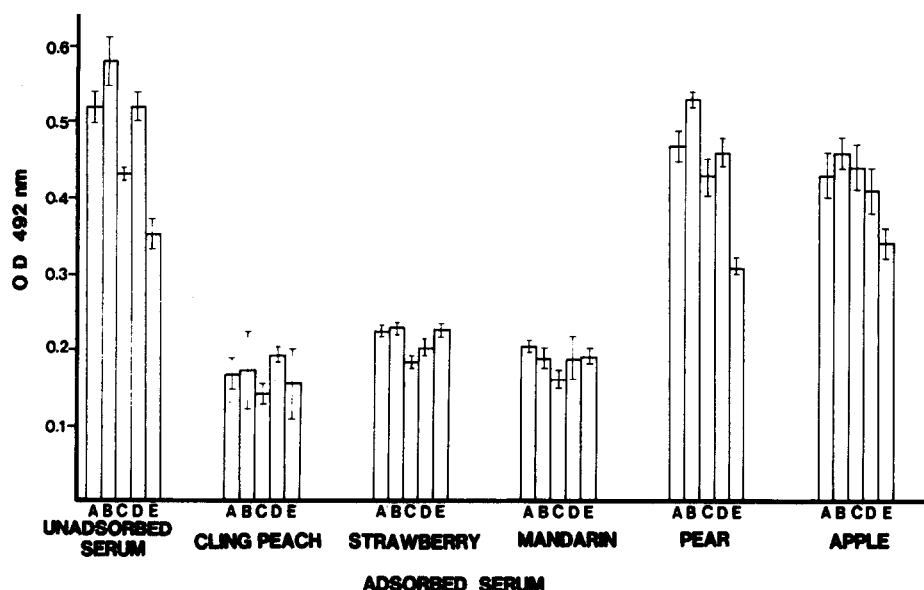


FIG. 3. IgE antibodies in the patient's serum after adsorption with cling peach (lane A), strawberry (lane B), mandarins (lane C), guavas (lane D), and bananas (lane E). The patient's serum was assessed against a variety of fruits as indicated. (Mean OD $\pm$ SD of three experiments.)

TABLE I. IgE antibodies in the patient's serum after chemical modification of fruit extracts (mean OD (at 492 nm) $\pm$ SD of three experiments)

	Cling peach	Guava	Strawberry
Untreated extract	0.505 $\pm$ 0.04	0.488 $\pm$ 0.03	0.496 $\pm$ 0.02
Extracts depleted of			
Protein	0.106 $\pm$ 0.23	0.172 $\pm$ 0.082	0.152 $\pm$ 0.07
Carbohydrate	0.478 $\pm$ 0.08	0.414 $\pm$ 0.055	0.538 $\pm$ 0.05
Lipid	0.498 $\pm$ 0.07	0.520 $\pm$ 0.04	0.472 $\pm$ 0.09

ultrasonic disintegrator. Reconstituted materials were then brought to the original volume in RPMI 1640. The remaining delipidated fractions were also reconstituted to the original starting volume in RPMI 1640.

RESULTS

Serum from the patient and from 10 different normal volunteers were tested for the presence of specific IgE antibodies on three different occasions with the ELISA. Significant amounts of IgE antibody against a variety of fruit extracts, including peach, guava, banana, strawberry, and mandarin, were demonstrated in the patient's serum (Fig. 1). Raised IgE antibodies could not be detected against nectarines, pears, or apples. Serum from normal volunteers did not contain significantly detectable levels of specific IgE antibodies to any of the fruit extracts tested. Further investigations revealed that the IgE antibodies were recognizing antigens from both the skin and the flesh of cling peaches (results not presented).

To determine the specificity of these antibodies, initial experiments were designed to detect IgE in various dilutions of the patient's unadsorbed serum and serum absorbed with peach extracts. The IgE antibody could be absorbed by incubating the patient's serum with extracts from cling peaches (Fig. 2). Even though raised levels of IgE to peaches could still be detected in a 1/20 dilution of the patient's unadsorbed serum, this dilution was sufficient for use in adsorption studies, as evidenced by a significant decline of antibody levels (Fig. 2).

In these experiments, OD values read at 492 nm decreased from a mean of 0.491 ± 0.05 for untreated serum to 0.175 ± 0.02 for adsorbed serum (Fig. 2). To economize on serum therefore and without prejudicing the sensitivity of the test, similar adsorption experiments were performed with other fruit extracts. When the patient's serum was adsorbed with cling peaches, strawberries, and mandarins, and then, in crossover studies, assessed for antibodies to various

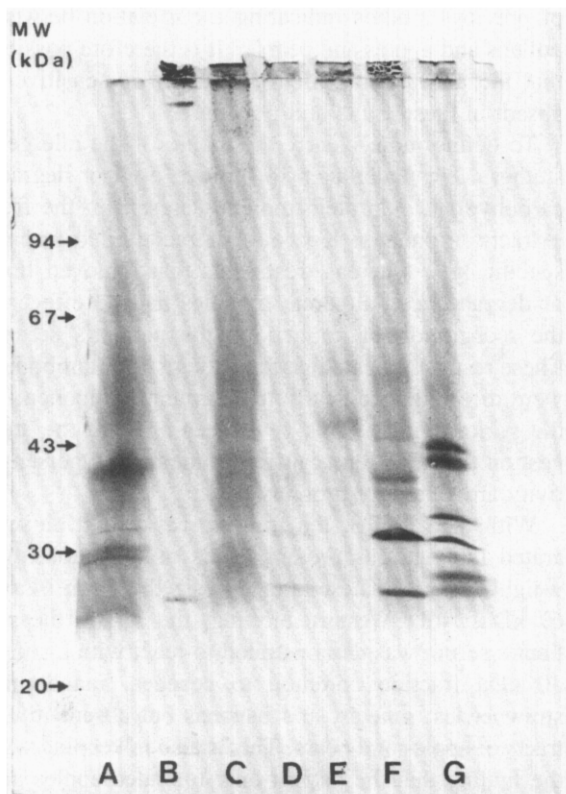


FIG. 4. SDS-Page analysis of various fruit extracts. Peach (*lane A*), apple (*lane B*), mandarin (*lane C*), guava (*lane D*), pear (*lane E*), banana (*lane F*), and strawberry (*lane G*).

fruit extracts, a significant reduction in detectable IgE was observed (Fig. 3). However, adsorption of the patient's serum with extracts from pears or apples had no effect on the ability of this serum to react to the other fruit extracts (cling peaches, strawberries, mandarins, guavas, and bananas) (Fig. 3). In addition experiments to ascertain cross-reactivity of the patient's IgE antibodies, RASTs were carried out with commercial disks and kits. These assays indicated that the patient's serum contained high levels of antibody to strawberry (4+) and banana (3+) but not to apples or to several other allergens (results not presented).

To characterize the antigenic component recognized by the patient's IgE antibodies, extracts from peaches, guavas, and strawberries were subjected to treatment that would delipidate or degrade carbohydrate or polypeptide moieties. Treatment with proteinase K resulted in a significant reduction of detectable IgE levels (Table I). Delipidation of the molecule or degradation of carbohydrate moieties, however, was without effect. The results would therefore suggest that the IgE antibodies are directed specifically against a protein component present in these extracts.

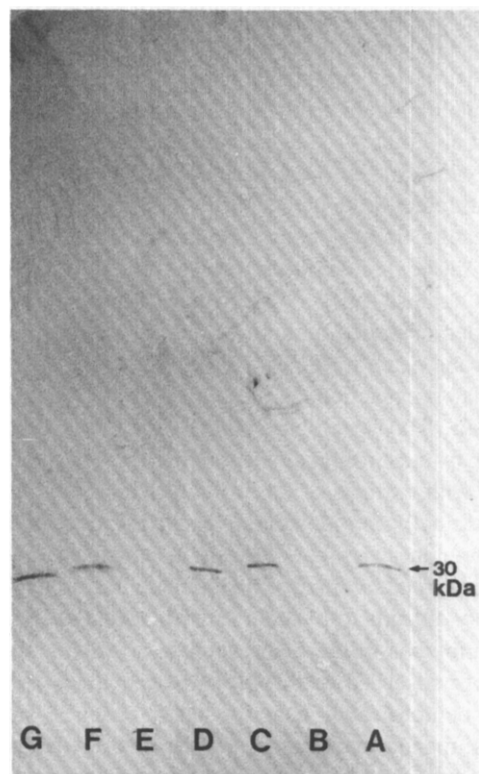


FIG. 5. Western blot analysis to detect IgE antibodies to a 30 kDa fraction from various fruit extracts. Strawberry (*lane G*), banana (*lane F*), pear (*lane E*), guava (*lane D*), mandarin (*lane C*), apple (*lane B*), and peach (*lane A*).

To characterize further these antigenic proteins, extracts were separated on SDS-PAGE, several distinct protein bands were observed (Fig. 4, *lane A*). Similarly, protein bands were observed in extracts from mandarins, guavas, bananas, and strawberries (Fig. 4, *lanes C, D, F, and G*), but extracts of apples (Fig. 4, *lane B*) and pears (*lane E*) demonstrated few protein bands. No additional protein bands were observed from these latter extracts, even when higher concentrations (250 μ g/ml) of these extracts were used for SDS-PAGE analysis (results not presented).

When these proteins were transferred onto nitrocellulose and incubated with the patient's serum, IgE antibodies were only detected against a 30 kDa fraction from cling peaches, mandarins, strawberries, guavas, and bananas (Fig. 5). No IgE antibodies could be detected against any proteins separated from pears or apples (Fig. 5).

DISCUSSION

As patients with immediate hypersensitivity reactions to fruits and vegetables usually demonstrate allergic reactions to various plant pollens,^{6, 7, 12} it was

unusual to observe a patient with no previous atopic history who presented with a variety of fruit-related allergies. It has previously been demonstrated that allergens extracted from fruits contain low levels of protein and that these extracts are not useful for skin prick tests.⁷ In the present study, therefore, specific IgE antibodies were detected by an ELISA technique. The results of this assay demonstrated that significantly raised IgE antibody levels were only demonstrated against fruits to which the patient had previously demonstrated a clinical reaction. Antibodies to apples and pears were not present in keeping with the fact that no reaction to those fruits was previously recorded. The specificity of the assay was demonstrated by adsorbing the antibodies in the patient's serum with extracts of the fruits to which the patient had originally demonstrated raised IgE levels. Furthermore, in crossover studies, in which adsorption was performed by incubating the patient's serum with a variety of fruits followed by measurement of specific IgE antibodies in these adsorbed sera, only sera that had been adsorbed with apple or pear extracts continued to demonstrate IgE antibodies to peaches, strawberries, mandarins, guavas, and bananas. Adsorption of sera with any of these latter fruits, however, significantly reduced the amount of IgE that would react with any one of the other fruits, suggesting that a common allergen was present in each of these fruit extracts. It has been suggested that allergens causing immediate hypersensitivity are generally proteins and that more than one allergenic protein is usually found.¹³ The results of the present study, however, indicated that the patient's serum reacted to a single allergen common to peaches, mandarins, strawberries, guavas, and bananas.

Cross-reactions between unrelated allergens have previously been reported.^{7, 14-16} These include an association between ragweed pollinosis and banana- or melon-induced pruritus, cross-reactivity between ragweed and cantaloupe,¹⁶ and cross-reactivity between rice or wheat and buckwheat.¹⁷ Studies have also indicated that most individuals hypersensitive to pollens may be additionally sensitive to a variety of raw fruits, vegetables, and nuts,^{7, 15, 18, 19} whereas ragweed-pollen hypersensitivity is generally accompanied by sensitivity to melon and banana.^{7, 16} The present study, however, describes an unusual finding in that our patient appears to be sensitive to a particular allergen found only in several fruits. This was not only true on ELISA (Fig. 2), but the patient's serum did not demonstrate IgE to other allergens on RAST as well. Of interest also is the finding that the patient does not appear to be sensitive to any of the pollen allergens tested or to apples and pears, especially in the light

of previous studies indicating a correlation between pollens and apples and pears.¹⁵ It is therefore possible that the antigen to which the patient is sensitive is absent in these fruits and pollens.

To define more clearly the nature of the allergen, studies were undertaken to remove lipid or degrade carbohydrate or protein moieties present in the fruit extracts. Proteinase K-treated extracts failed to bind specific IgE, whereas treatments that removed lipid or degraded carbohydrate moieties had no effect on the recognition of antigen by the patient's serum. These results indicate that the patient's IgE antibodies were directed specifically to a protein component in the various fruits. This is in keeping with the suggestion that allergens causing immediate hypersensitivity are generally proteins.^{4, 13}

With SDS-PAGE, the fruit extracts could be separated into various protein bands of low molecular weight (Fig. 4). These ranged in size between 14 and 67 kDa. After Western blotting, the IgE in the patient's serum was demonstrated to react with a single 30 kDa fraction common to peaches, mandarins, strawberries, guavas, and bananas but absent in extracts of apples and pears. This is also in keeping with the finding that the patient only tolerated apples and pears but reacted to all the former fruits. It has been suggested that low protein concentrations in extracts from apples and pears may result in antigens not being detected by these techniques.⁷ This is unlikely, since even when high (>250 $\mu\text{g/ml}$) concentrations of the fruit extracts were used in electrophoresis studies, the 30 kDa fraction was still not observed in lanes containing apple and pear extracts (results not presented). To eliminate the possibility of the use of denatured or altered antigens caused by binding with phenols,⁴ extracts of the various fruits, including apples and pears, were prepared according to the technique of Bjorksten et al.⁴ IgE to apples or pears could still not be detected in our ELISA (results not presented). These findings are supported by further ELISA studies (Fig. 2) demonstrating that adsorption with pears or apples does not significantly reduce the amount of IgE antibodies to the 30 kDa of protein found in peaches and other fruits. It was not surprising, therefore, to detect similar allergens in extracts of such a wide variety of presumably unrelated fruits. Of particular interest was the observation that the protein, although it is present in peaches, could not be demonstrated in nectarines: a fruit that is generally considered to be closely related. Our results, however, do correlate with the clinical history of the patient in whom allergic reactions to peaches, bananas, guavas, mandarins, and strawberries had been experienced, whereas pears and apples caused no reactions.

Our experiments suggest the use of ELISA and SDS-PAGE in identifying allergenic proteins in fruits to which individuals may have a sensitivity. Such detection of IgE to specific antigens common to several fruits may prove useful for patients with such sensitivities.

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