

Proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children

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Summary

This study was undertaken to determine the proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children. Cutaneous reactions evoked by graded amounts of food extracts were compared with results of double-blind food challenge and *in vitro* release of histamine from leucocytes. A 3 mm or greater weal reaction in skin tests by puncture technique using food extracts of 1:20 w/v concentration was found to indicate the degree of hypersensitivity likely to be associated with clinically significant hypersensitivity reactions to food. Proper use of this simple technique will facilitate accurate diagnosis of food hypersensitivity in children by identifying the group among whom all positive reactions to food challenges will be found. Nevertheless, double-blind food challenge is essential to establish a diagnosis of symptomatic hypersensitivity to food.

Introduction

Textbook discussions of allergy to food are replete with statements that reactions produced by skin tests with food extracts are often a result of irritants and are non-specific. Furthermore, there is said to be poor correlation between reactions to skin tests and clinical manifestations of hypersensitivity to foods. Thus skin testing with food extracts is generally considered to be of little value. No studies adequate to serve as the basis of this opinion were found in the literature; entirely lacking is quantitative comparison of reactions to food extracts in skin tests with other objective identification of specific hypersensitivity. The present investigation in children is a step toward remedying this defect; cutaneous reactions evoked by various quantities of food extracts are compared with the results of double-blind food challenges and *in vitro* release of histamine by the corresponding food substances. Both allergic and non-allergic persons were studied.

With the food extracts studied fully, a 3 mm or larger weal in skin tests by puncture technique with 1:20 w/v concentration of extract served to identify a relatively small group of children among whom were found all those exhibiting symptoms in double-

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blind food challenges. Thus proper performance and interpretation of skin tests with the particular food extracts studied proved helpful by narrowing the need for double-blind food challenges. However, double-blind food challenge is essential for accurate diagnosis of clinically significant or symptomatic hypersensitivity to food. Findings with the food extracts studied less completely point to the same conclusions and so the proposed procedure for evaluation and proper use of food extracts should have general applicability.

Materials and methods

Non-hypersensitive individuals

Twenty-seven children aged 2–15 years and twenty-seven adults aged 15–60 years, all of whom denied manifestations of hypersensitivity to common allergens, served as a control population receiving cutaneous tests.

Asthmatic subjects

105 asthmatic children aged 3–15 years evaluated at the National Jewish Hospital participated in the investigation. For each subject a stable medication regimen was achieved before the various tests were administered; the dosages of bronchodilators and steroids were of the magnitudes found to have no significant inhibitory effects on skin tests with allergens (Galant *et al.*, 1973; Slott & Zweiman, 1974). In addition, antihistamines were withheld for at least 12 hr before skin testing. No patients with histories of anaphylactic reactions were included in the investigation because accurate diagnosis by history alone usually presents no difficulties.

Extracts of foods

For practical reasons extracts of twenty foods were chosen for investigation, some for their reputation as dependable skin testing agents and others for their common incrimination as instigators of non-specific cutaneous reactivity. Aqueous extracts of the twenty foods, listed in Table 1, were obtained from Greer Laboratories, Lenoir, North Carolina. Fish mix consisted of a mixture of cod, flounder, halibut, mackerel and tuna. The shellfish mix was composed of crab, lobster and shrimp. Using phenol-buffered saline as a diluent, fresh dilutions of extracts were prepared not less often than every 6 weeks. Throughout the course of the investigation three different lots of the commercial extracts were employed to perform skin testing.

Non-hypersensitive individuals were tested with extracts of peanut, peas, corn, chocolate, fish mix, wheat, milk, eggs, Timothy grass, *Alternaria* mold and short ragweed.

Techniques of cutaneous testing

In order to introduce a range of quantities of food extract into the skin, four techniques of application were employed. Puncture tests were administered with a dissecting needle available from surgical suppliers. One drop of a 1:20 w/v solution of extract was placed on the skin of the subject's back or arm and the dissecting needle was passed through the drop and into the skin until a small indentation was visible. Discomfort was rare and erythema, induration and bleeding were never produced by this technique.

Scratch testing was accomplished with a 27-gauge needle. A 1 cm intra-epidermal

scratch was produced and one drop of a 1:20 w/v solution of extract was applied over the scratch.

Two concentrations of extract were employed for intradermal testing: 1:1000 w/v and 1:100 w/v solutions. A 27-gauge disposable needle and tuberculin syringe were employed to administer approximately 0.01 ml of extract intradermally, raising a small bleb. Intradermal tests were placed only on the arm so that in the event of a systemic reaction occurring a tourniquet could be placed proximal to the site of injection.

The non-hypersensitive control subjects initially received puncture tests with 1:20 w/v concentration and intradermal tests with solutions of 1:100 w/v concentration. Any control subject with a positive intradermal test at this concentration was retested with the weaker 1:1000 w/v concentration of that extract to ascertain the smallest amount required to elicit a response.

The control employed to measure skin reactivity to histamine was 1 mg/ml histamine for puncture and scratch testing and 0.1 mg/ml for intradermal tests. Testing with food extracts was only performed if the patient responded to histamine. Phenol-buffered saline, identical to that used as the diluent, was administered concurrently by each technique to determine non-specific skin reactivity.

Reactions were measured 15–20 min after application, and any bleb remaining from the diluent control was subtracted from the weal response to obtain the net weal reaction. Only net weal reactions are reported in this investigation. A net weal diameter of 3 mm or greater was considered to be significant. Patient's skin test sites were examined at 4, 24 and 48 hr for delayed responses.

Erythema surrounding the weals was not measured because the boundary is often too vague. Erythema without a weal is not so clearly indicative of an immunological reaction.

Release of histamine from leucocytes in vitro

The ability of five of the food extracts (peanut, wheat, milk, ovalbumin and chocolate) to release histamine from leucocytes *in vitro* was measured using techniques previously described (May *et al.*, 1970). These five foods were chosen because of their frequent incrimination and the availability of extracts not containing phenol (phenol interferes in the leucocyte histamine release procedure).

Food challenges (FC)

The procedure and materials for food challenges are described in detail elsewhere (May, 1976). Briefly, dried or crushed foods were placed in opaque colorless capsules and administered in a double-blind manner to patients suspected of hypersensitivity to foods. If 8 g given blindly caused no symptoms, the food was introduced into the diet openly in ordinary portions of undehydrated foods. No-one who did not react to 8 g of dried food showed any symptoms upon open consumption of the parent food.

Results

Non-hypersensitive subjects

Of the twenty-seven control children whose history did not reveal manifestations of hypersensitivity, nineteen exhibited no cutaneous responses by puncture test with 1:20

w/v concentration or by intradermal technique with 1:100 w/v concentration of extracts. Eight children exhibited a small but significant (3 mm) response to the intradermal test with one of the food extracts at 1:100 w/v concentration, but intradermal testing with 1:1000 w/v concentration was negative. Two of these children also exhibited a positive (3 mm) response to intradermal testing with one of the extracts of inhalant allergen at 1:1000 w/v concentration.

Twenty of the twenty-seven non-hypersensitive adults manifested no cutaneous responses to the extracts applied. Seven of the twenty-seven subjects had one or more significant responses to intradermal tests at 1:100 w/v concentration, but when tested with 1:1000 w/v no responses were elicited. One subject, completely unresponsive to testing with food extracts, exhibited a positive (3 mm) response to an inhalant allergen at 1:1000 w/v concentration, and two of the seven subjects, responding only to 1:100 w/v solutions of food extracts, exhibited a positive (3 mm) response to an inhalant allergen.

None of the non-hypersensitive children or adults manifested cutaneous responses to puncture tests (1:20 w/v) with the food or the inhalant extracts.

Asthmatic subjects

Table 1 presents the incidence of all positive (significant net reaction: 3 mm or larger weal) and all negative skin test responses with twenty food extracts in 105 asthmatic

Table 1. Skin tests* with twenty food extracts in 105 asthmatic children

Extract	No. positive (and percentage)	No. negative (and percentage)	Extract	No. positive (and percentage)	No. negative (and percentage)
Peanut	74 (70)	31 (30)	Wheat	72 (68)	33 (32)
Walnut	61 (58)	44 (42)	Orange	55 (52)	50 (48)
Almond	65 (62)	40 (38)	Tomato	69 (66)	36 (34)
Cashew	64 (61)	41 (39)	Potato	46 (44)	59 (56)
Filbert	64 (61)	41 (39)	Beef	37 (35)	68 (65)
Peas	79 (75)	26 (25)	Pork	35 (33)	70 (67)
Soybean	78 (74)	27 (26)	Fish mix	53 (50)	52 (50)
Corn	65 (62)	40 (38)	Shell fish mix	54 (51)	51 (49)
Chocolate	63 (60)	42 (40)	Whole milk	50 (48)	55 (52)
Pepper	53 (50)	52 (50)	Whole egg	46 (44)	59 (56)

Positive = significant net weal, 3mm or larger.

* All puncture tests with 1:20 w/v plus all intradermal tests with 1:1000 and 1:100 w/v.

children, including all puncture and intradermal tests. From this table it is evident that none of the extracts caused positive cutaneous responses in all patients, which means the extracts were not generally non-specific irritants. Furthermore, among the 105 asthmatic patients, there were thirteen who had no cutaneous reactions to any of these extracts of foods. Seven of these thirteen patients exhibited significantly positive cutaneous responses only to extracts of inhalant antigens.

Table 2 shows the distribution of the 105 patients according to the least amount of each extract required to first elicit a significant net weal reaction. Using peanut as an example: sixteen subjects exhibited a significant positive response to the quantity of

Table 2. Distribution of subjects according to amount of extract required to first elicit a significant *weal reaction

Extract	Puncture		Scratch		Intradermal		Intradermal	
	(1:20 w/v)		(1:20 w/v)		(1:1000 w/v)		(1:100 w/v)	
	No.	(%)†	No.	(%)	No.	(%)	No.	(%)
Peanut	16	22	20	27	29	39	9	12
Walnut	6	10	13	21	30	49	12	20
Almond	5	8	20	31	24	37	16	24
Cashew	7	11	14	22	25	39	18	28
Filbert	8	12	13	28	25	41	12	19
Peas	9	11	18	23	31	39	21	27
Soybean	5	6	23	29	38	49	12	16
Corn	1	2	15	23	31	48	18	27
Chocolate	1	1	10	16	30	48	22	35
Pepper	0	0	2	4	21	39	30	57
Wheat	3	4	15	21	40	56	14	19
Orange	4	7	10	18	25	46	16	29
Tomato	4	6	11	16	30	43	24	35
Potato	0	0	12	26	15	33	19	41
Beef	1	3	7	19	13	35	16	43
Pork	1	3	10	29	12	34	12	34
Fish mix	6	11	3	6	18	34	26	49
Shell fish mix	7	13	5	9	17	32	25	46
Whole milk	2	4	10	20	22	44	16	32
Whole eggs	10	22	6	13	17	37	13	28

* Per cent of subjects first reacting to extract at the specified concentration.

† Significant = net wheal, 3mm or larger.

extract introduced by the puncture test; twenty additional patients reacted significantly to scratch test; twenty-nine patients first reacted significantly to intradermal test at 1:1000 w/v concentration; and nine patients reacted significantly only when receiving extract intradermally at 1:100 w/v concentration. For all of the extracts it is evident that in general only a small number of patients were sufficiently hypersensitive for a significant net weal reaction to be evoked by the small amount of extract provided by the puncture technique.

No delayed cutaneous responses were noted after 4, 24 and 48 hr, and no systemic reactions occurred in any of the patients.

Release of histamine from leucocytes in vitro

Shown in Table 3 is the *in vitro* release of histamine from leucocytes compared with the cutaneous response for five of the food extracts. A number of patients exhibited significant weal responses, but their leucocytes failed to release histamine. No patient's leucocytes released histamine in the absence of a positive skin test. It should be noted that the extracts did not produce non-specific release of histamine from leucocytes. These data corroborate the immunological specificity of the cutaneous reactions.

Food challenges

Peanut and egg were selected for extensive testing. In the seventy-four patients

Table 3. Comparison of *in vitro* release of histamine with skin test results* for five of the extracts of foods

Extract	No. subjects	Skin test positive, histamine release positive	Skin test positive, histamine release negative	Skin test negative, histamine release negative
Peanut	59	28 (47)†	12 (20)	19 (32)
Milk	58	16 (28)	9 (15)	33 (57)
Egg	55	4 (7)	18 (33)	33 (60)
Chocolate	55	10 (18)	24 (44)	21 (38)
Wheat	54	35 (65)	4 (7)	15 (28)

* Skin test positive = significant net wheal 3mm or larger with any of the techniques and concentrations of food extracts employed (see text).

† All results given as number of subjects, and the percentage in parentheses.

exhibiting significant weal responses to peanut extract at any concentration (Table 1), a total of thirty-one double-blind food challenges were performed employing crushed peanut. Results of the double-blind food challenges compared with significantly positive skin tests are displayed in Table 4. Fifteen of the thirty-one patients subjected to peanut challenge manifested a positive puncture test (one additional patient who was not subjected to a food challenge exhibited a positive puncture test). Nine of the sixteen patients (56%) responding significantly to the puncture test exhibited a clinically symptomatic reaction to the food challenge. Table 4 also shows that the findings were similar in the case of egg.

The nature and distribution of the symptoms elicited by the food challenge in various systems are also shown in Table 4. Gastrointestinal symptoms included abdominal pain, vomiting, and diarrhoea. Skin manifestations included urticaria and exacerbation of eczema. Respiratory symptoms were wheezing and rhinorrhoea. Individual patients manifested one or more symptoms, all of which occurred within 2 hr of the challenge.

Eggs, milk, fish, shell fish and nuts are generally accepted as the more common causes of hypersensitivity in childhood. Extracts of the other eleven foods in Table 2 evoked only twenty-nine significant weal reactions in the 1155 puncture tests

Table 4. Double-blind food challenges in patients with significantly positive puncture tests

Extract	Positive puncture tests	FC	FC* positive		G.I.	System affected	
			No.	(%)†		Skin	Respiratory
Peanut	16	15‡	9	(56)	7	5	4
Egg	10	8‡	5	(50)	4	1	0
Total	26	23	14	(53)	11	6	4

*FC = Double-blind food challenge.

†Positive FC as percentage of positive puncture test.

‡ Not every patient with a positive puncture test had an FC.

performed on the 105 children using 1:20 w/v concentration. None of these significantly positive tests were associated with symptoms after food challenges or inclusion of the foods in the diet. Thus in this population of 3- to 15-year-old children asymptomatic hypersensitivity to those eleven foods was infrequent, and clinically significant or symptomatic hypersensitivity did not occur.

Discussion

The goal of the present study was to examine the usefulness of a selection of commercially prepared extracts of food as aids in identification of hypersensitivity to food in children. The procedures employed serve to illustrate the means whereby the proper use of food extracts can be ascertained. Keeping in mind that the study is of children over 3 years of age and not of infants, and of one source of food extracts, the findings bear on questions commonly raised.

Do extracts of food frequently contain irritants which evoke non-specific 'false positive' reactions in the skin?

No evidence was found that any of the diverse assortment of extracts with the concentrations employed gave misleading reactions. In fact, numerous negative reactions were seen with each of the food extracts, even in persons shown to be hypersensitive to extracts of other foods or inhalants (Table 1). On the contrary, with the amounts of extract injected by the techniques used in this study, only weals indicative of hypersensitivity were elicited. In a number of examples the immunological significance of the weal reactions was confirmed by *in vitro* release of histamine from leucocytes (Table 3). None of the extracts caused non-specific release of histamine from leucocytes.

What is the proper amount of food extract to inject into the skin to identify persons likely to have symptoms after eating the food?

The smallest amount of antigen from food was introduced into the skin by the puncture technique with 1:20 w/v concentration of extract, and the largest with intradermal technique using 1:100 w/v. The greater the amount of extract required to evoke a significant weal reaction, the less the degree of hypersensitivity may be assumed to be (Table 2).

None of the fifty-four persons who denied symptoms of hypersensitivity exhibited weal reactions with 1:20 w/v by the puncture technique or 1:1000 w/v intradermally, but weal reactions to a few food extracts were elicited by intradermal injection of 1:100 w/v concentration. Because the extracts were shown not to be irritants, in these asymptomatic individuals the few weals from the largest amount of extract may be presumed to indicate presence of specific antibody, but in insufficient quantity to be the basis of symptoms.

By contrast, significant reactions (net weal 3 mm or larger) elicited by the small amount of extract introduced by the puncture technique appear to indicate so high a degree of hypersensitivity that occurrence of symptoms when the food is eaten would not be surprising. For example, in the case of peanuts, double-blind challenge was carried out with thirty-one children. Sixteen of these thirty-one exhibited significant weal reactions with the puncture technique, and nine of the sixteen (56%) had symptoms when peanuts were administered by double-blind technique (Table 4). Furthermore, the remaining fifteen of the thirty-one children had negative puncture tests with peanut and none reacted to peanuts in the double-blind challenges, although significant

which introduces larger amounts of antigen. Indeed, in our experience, lack of weals were evoked in intradermal skin tests with 1:1000 or 1:100 w/v peanut extract, significant weal response by the puncture technique with 1:20 w/v extract is invariably accompanied by absence of symptoms of immediate-type hypersensitivity in double-blind food challenges.

Thus, in the example given, the amount of food antigen introduced by the puncture technique using 1:20 w/v extract proved to be the proper amount to identify those individuals who may exhibit symptoms when the food is eaten. Of course weal reactions in the skin within minutes after injection of antigen are related only to clinical manifestations of immediate-type hypersensitivity.

What is the role of food challenges in clinical diagnosis?

Testing the effect of eating the food is unnecessary and contraindicated when the history indicates severe anaphylactic reactions. Otherwise symptoms ascribed to food are notoriously prone to psychological influences. Objective confirmation that food causes an adverse reaction requires administration of the suspected food by double-blind procedure. Appearance of symptoms under these circumstances means the food caused an adverse reaction.

Before the adverse reaction may be attributed to hypersensitivity immunological study is required. The food extracts examined in this study, when applied by the puncture technique, proved to be useful for detection of the relatively few persons with a high degree of hypersensitivity that may result in symptoms when the food is eaten.

Net weals of 3 mm or greater in puncture tests with 1:20 w/v extracts serve to detect the relatively few persons with whom a double-blind food challenge should be conducted for objective identification of clinically significant, symptomatic hypersensitivity to food.

Skin testing with intradermal injection of 1:1000 w/v extracts identified the larger number of individuals who are less hypersensitive and will not develop symptoms in a double-blind food challenge; in other words, they are examples of asymptomatic hypersensitivity.

Conclusion

There is no justification for the term 'false positive' with respect to skin tests with extracts of foods properly performed and evaluated in the manner presented. Clear comprehension of the distinction between symptomatic and asymptomatic hypersensitivity will avoid confusion about the clinical significance of weal reactions to food extracts, which we found are truly of immunological import in the examples presented.

While the results and interpretations strictly apply only to the particular food extracts studied, and to immediate-type hypersensitivity, the simple steps taken for verification of the usefulness and proper use of food extracts could be generally applied.

The conclusions reached are comparable to those of recent studies of hypersensitivity to codfish and of inhalant allergens (Aas & Johansson, 1971; Aas & Lundkvist, 1973; Bryant, Burns & Lazarus, 1975; Bruce *et al.*, 1975). The simple skin test by puncture technique, when properly performed and interpreted, is a convenient office procedure for the practitioner, and confirmatory double-blind food challenges are

easy to arrange. The findings in this study corroborate early studies (Freedman & Sellare, 1959; Goldman *et al.*, 1963; Hill, 1948), and the recommendations are probably in agreement with the long-standing practice of some clinicians with respect to proper use and interpretation of skin tests with extracts of food.

Proper performance and interpretation is of major importance to usefulness of skin tests with verified food extracts as aids in diagnosis of hypersensitivity to food in children.

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