

Appraisal of skin tests with food extracts for diagnosis of food hypersensitivity

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Summary

Seventy-six children aged 5 months to 15 years who exhibited a net weal of 3.0 mm or greater to a puncture skin test with one or more of fourteen foods were subjected to double-blind food challenge. Confirmed reactions to double-blind food challenge were found to occur only with peanut, milk, egg and soybean. Puncture skin tests with 1:20 w/v concentration of food extracts identified all subjects who exhibited an adverse reaction during the double-blind food challenge. Performance of intradermal skin tests did not identify any additional subjects who reacted clinically to double-blind food challenge.

Introduction

The detection of hypersensitivity to foods by using extracts of foods for skin testing has been criticized in many forums despite the lack of objective data either supporting or refuting the usefulness of food extracts for such testing. In a previous report from this institution it was found that extracts of some foods when properly used can identify those children in whom a clinically apparent adverse reaction may occur when they are challenged blindly with the food in question (Bock *et al.*, 1977). This work extends and expands the earlier study by: (a) using each of the foods under investigation in double-blind challenges; (b) administering a double-blind food challenge to each patient in whom there was a net weal response of 3.0 mm or greater to puncture skin tests with any of fourteen foods under study; (c) investigating adverse reactions to foods in children less than 3 years of age; and (d) measuring *in vitro* release of histamine from leucocytes of patients with net weals of 3.0 mm or greater to puncture skin tests for each of the fourteen extracted foods.

In this study, seventy-six children aged 5 months to 15 years who exhibited a net weal of 3.0 mm or greater to a puncture skin test with one or more of fourteen foods were subjected to a double-blind food challenge whether or not they reported a history

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of an adverse reaction to that food. Confirmed reactions to double-blind food challenge were found to be limited to peanut, milk, egg and soybean. Puncture skin tests with 1:20 w/v concentrations of food extracts which elicited a net weal 3.0 mm or greater identified all subjects who exhibited an adverse reaction within 2 hr of the administration of blind food challenge. Double-blind food challenge was found to be indispensable in eliminating subject and observer bias.

Materials and methods

Subjects

Seventy-six children aged 5 months to 15 years were the subjects of this investigation. These children were patients at the National Jewish Hospital and Research Center and were referred for evaluation of asthma, some having a history of adverse reactions to foods, or specifically for the evaluation of adverse reactions to foods. No children with a history of anaphylaxis after ingestion of a food were challenged with that food. Medication regimens were stabilized prior to performing skin testing or administering food challenges as previously described (Bock *et al.*, 1977). Antihistamines were withheld for at least 12 hr and usually longer prior to skin testing and double-blind food challenges.

Non-hypersensitive control subjects were investigated in the previous study (Bock *et al.*, 1977).

Techniques of cutaneous testing

Extracts of foods were applied to the skin by two techniques. All puncture tests were performed using 1:20 w/v concentration of extracts. Intradermal testing was accomplished with 1:1000 w/v concentration of extracts. The technique of application has been previously described (Bock *et al.*, 1977).

Histamine 1.0 mg/ml and 0.1 mg/ml was applied concurrently with puncture and intradermal tests respectively. 50% glycerine in sterile water with phenol or phenol-buffered saline, the diluents for the extracts, was administered to determine non-specific skin reactivity.

A net weal diameter of 3.0 mm or greater was considered a definite skin response. Net weal diameter was determined by subtracting any papule produced by the diluent control from the measured weal response produced by the skin test with extract. Only net weal diameters are reported. Reactions were measured 15–20 min after application of the extracts.

Extracts of foods

Extracts of the fourteen foods listed in Table 1 were investigated. Some of the extracts from the earlier study were eliminated because of their low incidence of eliciting definite skin responses and the absence of confirmable clinical symptoms associated with their ingestion. One food (strawberry) was added to this investigation. Extracts of those foods listed in Table 1 were stock preparations purchased from Greer Laboratories, Lenoir, North Carolina.

In vitro release of histamine from leucocytes

Release of histamine was measured during *in vitro* challenge of peripheral leucocytes with each food extract which had elicited a net weal of 3.0 mm or greater during

puncture skin testing. Preparation of extracts for use in the leucocyte histamine release procedure is described elsewhere (May, 1976a; Bock *et al.*, 1978). Extracts for skin testing and preparations for use in leucocyte histamine release are not identical because the presence of phenol in the former interferes with the *in vitro* procedure; the antigenic behavior of the materials has in our experience been qualitatively similar enough for practical purposes.

Blind food challenge

The technique of blind food challenge and the materials employed are reported elsewhere and are briefly summarized here (May, 1976a; Bock *et al.*, 1978). Dried or crushed foods were placed in opaque dye-free capsules and administered in a double-blind manner to subjects exhibiting a net weal of 3.0 mm or greater to puncture skin tests with extracts of the fourteen foods. Observations were recorded hourly for 24 hr. If 8.0 g of the food administered blindly did not evoke clinically apparent symptoms then usual portions of the food were added visibly to the diet. For subjects too young to consume capsules, foods to be tested were administered masked in another food known to be tolerated.

Results

Seventy-six children were included in this investigation. Table 1 shows the number of 3.0 mm or greater net weal responses to puncture tests elicited by a 1:20 w/v concentration of each of the fourteen food extracts. For each puncture test response 3.0 mm or greater, a double-blind food challenge was administered regardless of the history, unless the history was of an anaphylactic reaction (none of which were reported by

Table 1. Comparative usefulness of puncture and intradermal skin tests for identification of hypersensitivity to foods in seventy-six patients aged 5 months to 15 years

Food	Number of positive puncture tests*	Positive food challenge		Number of positive intradermal skin tests†
		Number	%	
Peanut	26	12	46	28
Egg	19	10	53	23
Cow milk	17	7	41	35
Soybean	10	2	20	37
Wheat	5	0	0	39
Peas	5	0	0	33
Fish mix	4	0	0	21
Tomato	3	0	0	27
Chocolate	2	0	0	27
Shellfish mix	2	0	0	21
Orange	1	0	0	25
Corn	1	0	0	16
Strawberry	1	0	0	20
Potato	0	0	0	19

* A positive skin test is a net weal skin response of 3.0 mm or greater, and the concentrations of extracts were 1:20 w/v.

† The concentrations of extracts were 1:1000 w/v.

these subjects). Correlation between history and skin testing is considered in a separate communication (Bock *et al.*, 1978).

Puncture skin test

The data in Table 1 shows that the puncture test was selective in identifying patients in whom a double-blind food challenge should be undertaken. Clinically observable adverse reactions were evoked in a blind food challenge with only four of the fourteen foods. The number of food challenges performed to the other foods was quite small because very few subjects exhibited a definite puncture skin test response. For peanut, egg, cow milk and soybean, 41%, 46%, 53% and 20% respectively of subjects with a puncture skin test response 3.0 mm or greater exhibited a clinically apparent response to a double-blind food challenge. As in the previous study, there was no significant correlation between the size of the weal reaction and the probability of a clinically apparent response to food challenge. Symptoms elicited by food challenges are discussed in a separate communication (Bock *et al.*, 1978).

Intradermal skin test

Results of skin testing by the intradermal technique are presented in column 3 of Table 1. It is apparent that reaginic sensitization of many individuals was detected by this procedure. Performing a food challenge in each of these subjects would have entailed considerably more effort in order to obtain the same information yielded by performance of the puncture skin test. For example, twenty-six children exhibited a 3.0 mm or greater net weal skin response to puncture test with 1:20 w/v concentration of peanut extract. Twenty-eight additional children had a 3.0 mm or greater net weal response to intradermal skin test with 1:1000 w/v concentration of peanut extract. Twelve of the fifty-four children exhibiting a definite reaction to either skin test with peanut extract had a clinical response during double-blind food challenge with peanut. The clinical responses to food challenge occurred only among those twenty-six subjects having a definite skin response in the puncture test. Therefore, identification of sensitization in twenty-eight additional subjects by the intradermal skin tests did not serve any clinically helpful purpose because the puncture skin tests identified all those subjects who exhibited a clinically apparent response to blind food challenge. All twenty-eight subjects in whom intradermal skin testing elicited a net weal 3.0 mm or greater were shown to tolerate the food in question when it was consumed in customary fashion.

Release of histamine from leucocytes

In vitro release of histamine from leucocytes was used as a method of confirming the presence of the reaginic sensitization demonstrated by skin testing. All patients exhibiting a 3.0 mm or greater net weal response to a puncture test with a food extract demonstrated release of histamine when their peripheral leucocytes were challenged with an extract of the food which produced the skin reaction. In some patients the histamine release was significant when the puncture skin test was negative and only the intradermal test elicited a 3.0 mm or greater weal. However, no patients exhibiting negative skin testing at both concentrations (1:20 and 1:1000 w/v) had significant histamine release. The phenomenon of high spontaneous release of histamine from the peripheral leucocytes was again strongly associated with confirmed

reactions to food challenges, as in the previous studies (May, 1976b), and is the subject of continuing investigations.

RAST assays were performed in some of the younger children and these results will be the subject of a separate communication.

Discussion

Combining the data from this and the previous study (Bock *et al.*, 1977) it is clear that properly performed skin testing with food extracts is a safe and useful procedure. Extracts of foods may be depended upon if their usefulness has been verified prior to general application to patients. Verification is achieved by demonstrating that the extracts do not produce responses in persons not sensitized and that they do contain material which will elicit a cutaneous response in sensitized subjects. Also the amount, technique and size of reaction associated with clinical symptoms needs to be ascertained.

As illustrated by study of these seventy-six children, verified extracts of these fourteen foods applied by the puncture technique identified all subjects requiring a food challenge. All reactions to food challenges occurred among those children displaying a net weal response 3.0 mm or greater to the puncture skin test. Furthermore, use of intradermal testing did not identify other subjects in whom a food challenge elicited a clinically apparent response. The intradermal test did identify other sensitized subjects and if used to identify patients to be challenged would have increased unnecessarily the number of double-blind food challenges required.

In view of these results the term 'false positive' skin test response seems confusing and unnecessary. If extracts are properly verified and used as described above, then subjects exhibiting a definite response to skin tests are sensitized with specific reagin. If a subject reacts clinically to double-blind food challenge then symptomatic sensitivity is present; if no clinical reaction results during challenge, then asymptomatic sensitivity obtains. These concepts are discussed more completely in recent reviews (May & Bock, 1978; May, 1978).

In conclusion, this study has shown that the fourteen extracts of food used, when properly verified as described and applied by the puncture technique with proper interpretation, will identify a group of children among whom will be found all those responding clinically during double-blind food challenge.

These results should not be extrapolated to other foods or extracts from other sources unless this type of investigation and evaluation is undertaken.

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