

The diagnosis of hypersensitivity to ingested foods

Reliability of skin prick testing and the radioallergosorbent test with different materials

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Summary

The diagnostic reliability in food allergy of skin prick tests (SPT) and the radioallergosorbent test (RAST) was investigated in paediatric patients with respiratory and skin allergies. SPT and RAST were found to be reliable for the diagnosis of allergy to codfish, peas, nuts, peanuts and egg white. Positive SPT and RAST to cereals were common, but were most often without clinical significance or were correlated with respiratory allergy to the inhalation of flour dust. SPT and RAST were only partly reliable with regard to allergy to cow's milk, and were mostly reliable when used together and showing corresponding results. Experimental allergosorbents for RAST with soy beans and white beans were not reliable. The study shows the need to improve the diagnostic materials and to establish the diagnostic reliability of the material and tests used for each food item in question.

Introduction

The diagnosis of food allergy with prick tests and RAST

Much controversy exists as regards the importance and incidence of food allergy in atopic dermatitis and bronchial asthma. The main cause of this controversy is that the diagnosis of food allergy may be quite difficult. Indeed, many reports on food allergy appear to be based on unconvincing diagnostic evidence (May, 1974, 1976). Strict diagnostic criteria have to be satisfied for an allergy diagnosis to be convincing, and this applies as much to the diagnosis of allergy to foods as to inhalants (Table 1).

For the diagnosis of allergy to inhalants, skin prick tests (SPT) and radioallergosorbent tests (RAST) have been shown to be immunologically specific and relevant for the pathophysiology of hypersensitivity mediated by immunoglobulin E (IgE) antibodies, but with varying reliability, depending on the allergenic material used (Aas, 1975b; Aas & Johansson, 1971). This study was undertaken to investigate to what extent SPT and RAST could be used to predict clinical allergy to different foods. The reliability of SPT and RAST was evaluated for allergenic test materials

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Table 1. Diagnostic criteria for specific allergy

Criteria
(1) The disease or major symptoms and signs of it are provoked by moderate or low amounts of a given substance
(2) The reaction is convincing and reproducible
(3) The substance in question reacts with tissue or serum from the patient in immunologically specific systems which are relevant for the main pathophysiology of the symptoms in question
(4) The substance does not react in a corresponding way or degree in similar immunological systems with tissues or serum from healthy and allergic individuals who tolerate moderate amounts of the substance.

considered to represent different stages of knowledge and technical development in this field.

Materials and methods

Patients and controls

The investigation comprised eighty-four children, 1–16 years old, with chronic or subchronic atopic dermatitis, bronchial asthma or a combination of both, and with known or suspected food allergies. Food allergy was suspected when the allergic symptoms could not be fully explained by inhalant allergies, infections or other known trigger factors (Aas, 1975a), and when the parents indicated particular suspicion of certain food items. On the other hand, if the patient during the last year had had a period of well-being of 4 weeks or more without medication, and during that period had eaten moderate or large amounts of the particular food two or more times, this was accepted as evidence that the food in question was tolerated.

Since some of the food items were tolerated by individual patients, these patients served also as control individuals for that particular food. Furthermore, twenty-seven hay fever subjects who tolerated all foods and were symptom-free outside the pollen season were used as controls. Fifteen of them had serum IgE concentrations below 100 u/ml and twelve had serum IgE concentrations higher than 200 u/ml.

The patients were admitted to the clinical department of the Allergy Institute for several weeks, to facilitate repeated elimination and challenge diets under close and qualified observation. The parents answered a detailed questionnaire with respect to observed and suspected reactions to a number of inhalants and common food items, and were thoroughly interviewed by experienced physicians. The information obtained was given a reliability or probability score ranging from –3 to +3 (–3 indicating convincing tolerance, +3 convincing intolerance, and 0 that no conclusions could be drawn from the case history) (Aas, 1975a).

Test material

Reagents for skin testing were as follows: crude codfish allergen extract 1:1000 w/v

(from Allergologisk Laboratorium, Copenhagen) and/or purified codfish allergen (DS 22N from Nyegaard & Co., Oslo, Norway), 10 µg/ml corresponding to 1 HEP quality (1 mg/ml histamine-HCl equivalent in prick testing), according to the Scandinavian reference system (Aas, 1975b; Aas & Belin, 1972); cereals were applied in skin tests as freshly made saline suspensions of the flour in question corresponding to 1–5 mg/ml w/v concentrations; skimmed cow's milk was used undiluted and in a ten-fold dilution; the same soy bean preparation (Soyasemp from Semper, Sweden) was used undiluted in skin testing and food challenges; frozen and thawed green peas were squeezed to deliver a tiny drop of juice on the skin for puncture tests (Ancoma & Schumacher, 1950) and allergen extracts of nuts and white beans (1 : 100 w/v) were purchased from Allergologisk Laboratorium, Copenhagen, and Bencard, Beecham, respectively.

Venous blood was drawn from each patient shortly after admission to the hospital. After clotting, serum was separated and stored at -20°C until used.

Reagents for RAST were those available in Phadebas RAST kits (Pharmacia, Uppsala, Sweden) except for a few of the allergosorbents. Phadebas allergosorbents from codfish, four cereals (barley, oats, wheat, rye), cow's milk, hazelnut, coconut, almond, peanut, egg white and green peas were provided by Pharmacia. Pharmacia also provided experimental allergosorbents with corn, rice, walnut, soy bean and white bean, with the reservation that the experimental allergosorbents were not considered qualified for release as Phadebas reagents.

Skin prick testing

Prick (puncture) tests were performed as described before (Frosted *et al.*, 1977) and were recorded according to the Scandinavian allergy reference system (SARS), using histamine-HCl 1 mg/ml as the positive +++ reference (Aas & Belin, 1972). Reactions of ++ or more were recorded as positive.

The radioallergosorbent test

RAST was carried out according to the instructions given by the manufacturers. The results of the RAST analyses were plotted against a reference system and expressed in RAST classes as recommended by the manufacturer. RAST class 2 or more was considered positive.

Elimination diets and food challenges

Since elimination diets followed by dietary provocation tests are very time-consuming and demanding, they were restricted to one, or a few, food items in patients suspected of being allergic to the particular food item or with positive results to prick tests or RAST to a food item scoring +2 or less in the case history, or giving a total score of less than +7.

Dietary provocation tests were thought unnecessary and potentially dangerous when a convincing history with a score +3 was confirmed by positive skin tests and/or RAST results giving a total score of +7 or more.

Depending on the type of patient, the case history, the food item in question and the degree of hypersensitivity of the patient, dietary provocations were carried out with increasing quantities of allergen from a low dose level that was considered safe in each case. If no reaction occurred, increasing quantities were given up to an amount considered normal for a daily intake, when feasible. The food item in question

was usually given concealed as a component of another easily digested food. In two patients provocation testing was carried out with the food item given in a 'blind' manner, concealed in gelatin capsules. This procedure was abandoned, however, since it was found quite impracticable in most of the children and the use of purified material was found to be too expensive for the purpose.

The patient was kept under close observation. Any changes in symptoms or signs which could be attributed to the dietary provocation were recorded and scored accordingly by a trained nurse. When positive reactions were suspected, a trained physician was called to examine the patient. For patients suffering from bronchial asthma and old enough to co-operate, lung function testing was carried out two or three times daily by means of peak expiratory flow and forced expiratory volume in 1 sec (FEV₁), using a Wright peak flow meter and an Ohio electronic spirometer. Only reductions of 20% or more in these readings were accepted as positive evidence for bronchial obstruction (Aas, 1975a). Most dietary provocations were carried out twice, unless the results of the first one were unequivocally positive and fully correlating with the observations reported by the parents as well as with the results of SPT and RAST. For white beans only the findings reported by the parents were recorded.

Results

Sixteen patients had to be excluded from the records because the skin condition (atopic eczema) was so labile that no impression whatsoever could be made as regards correlation between the diet and the disease, or because skin tests could not be performed, or due to insufficiently reliable observations.

The major symptoms observed and recorded were exacerbations of asthma (A), atopic eczema (E), nasal symptoms (N) and urticaria or angioedema (U). In a few patients these symptoms were accompanied by malaise, vomiting and/or diarrhoea (G), and in a few children irritability and uneasiness were also observed. The latter type of subjective symptoms were not recorded as evidence of allergy, however, since they may be precipitated by so many uncontrollable factors.

The exacerbations of atopic eczema were usually initiated by an urticarial reaction and/or angioedema. In all patients with eczema in whom peas, fish or egg white elicited nausea, exacerbations of the eczema occurred within a few hours after the acute urticarial reaction had subsided. However, in a number of other children, eczema also became worse following food challenges without any urticarial symptoms being observed. Multiple allergies, including inhalant allergies, were found in most of the patients.

Prominent differences were found in the diagnostic reliability for the different kinds of food and diagnostic material, both in SPT and RAST. The control sera from the twenty-seven hay fever subjects were negative in RAST with all allergosorbents except those from cereals, soy bean and white beans.

Codfish

Twenty patients with clinical allergy to codfish demonstrated combinations of respiratory reactions with asthma and skin reactions with urticaria, angiodema and eczema. This study confirmed previous reports (Aas & Lundkvist, 1973) that codfish allergy can be diagnosed in a completely reliable way with the use of SPT and—with a few exceptions—with RAST (Fig. 1).

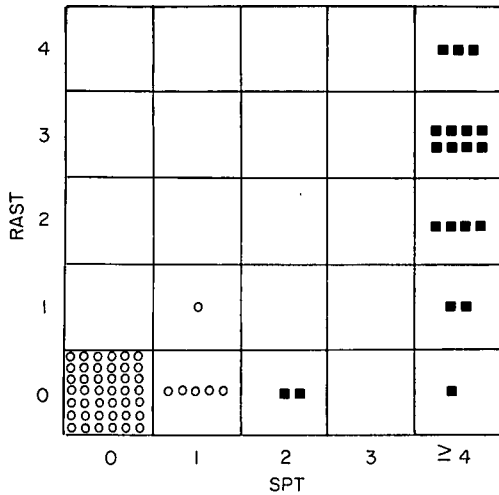


Fig. 1. Correlation between results of SPT and RAST with codfish. (○) No clinical allergy; (■) clinical allergy.

Peas, nuts, peanuts and egg white

Peas. Peas elicited reactions both in the skin alone (10 EU and 4 U) and in the skin and respiratory passages at the same time (9 AEU and 3 AU). Respiratory symptoms were reported also on inhalation of steam or smell from cooking peas.

Nuts. Angioedema or urticaria were elicited in all patients allergic to nuts. Five of them also suffered from bronchial obstruction.

Peanuts. A convincing clinical allergy to peanuts was reported in sixteen patients. Eleven of them showed a positive RAST. SPT was carried out with peanuts in only five of them, and was positive in four.

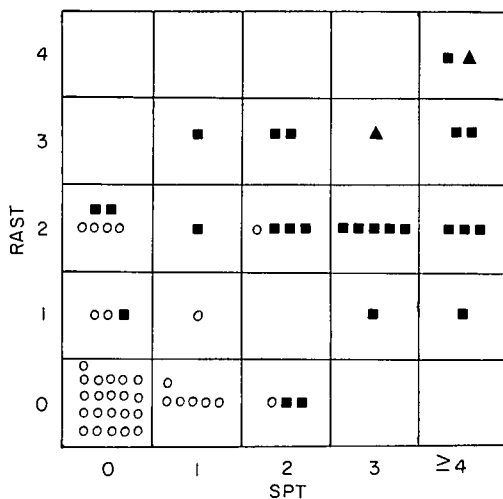


Fig. 2. Correlation between results of SPT and RAST with green peas. (○) No clinical allergy; (■) clinical allergy; (▲) possible clinical allergy, but not convincing.

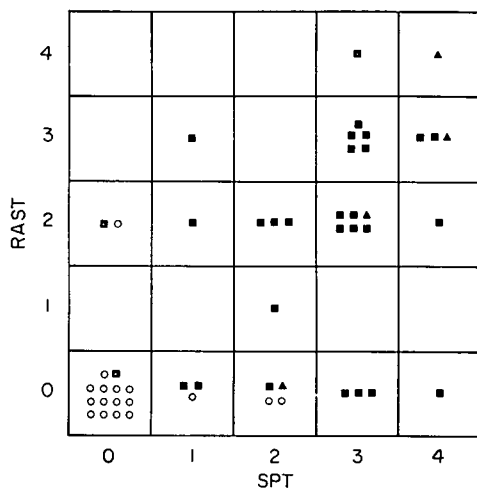


Fig. 3. Correlation between results of SPT and RAST for the five nuts tested. For legend, see Fig. 2.

Table 2. Food items and test material classified with respect to diagnostic reliability in arbitrarily chosen diagnostic quality groups

Class	Classification
(I)	Highly reliable in SPT and/or RAST
(II)	Moderately reliable in SPT and/or RAST
(III)	Not reliable in SPT and/or RAST

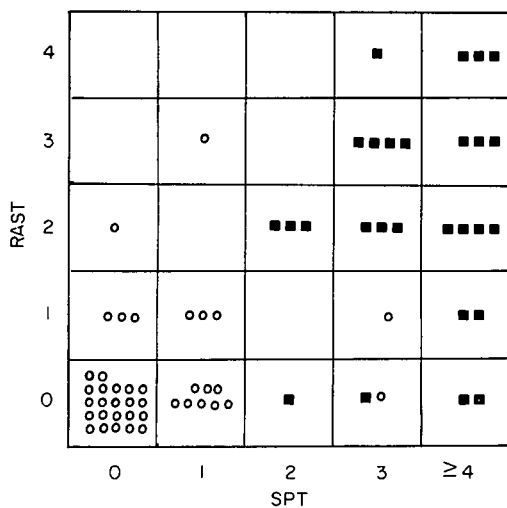


Fig. 4. Correlation between results of SPT and RAST with egg white. For legend see Fig. 1.

Egg white. Twenty-seven patients showed clinical allergy to egg white which manifested itself with urticaria, angioedema and/or exacerbations of atopic eczema. Eleven of them also reacted with bronchial obstruction. Positive SPT with negative reactions in RAST were found in two patients with previous clinical allergy to egg white, but who were now tolerating this food item. Two other patients without clinical allergy to egg had positive RAST with negative SPT.

Allergy to peas (Fig. 2) and to several nuts (Fig. 3) could also be diagnosed by means of SPT and/or RAST in the majority of instances, and in this study clinical allergy to egg white was found to be present in most individuals demonstrating positive reactions to SPT and/or RAST (Fig. 4). These allergens were classified accordingly as being of high quality as far as diagnostic reliability of SPT and RAST is concerned (Table 2). A high degree of specificity was found for nuts, particularly in the RAST system, but not from the results of skin testing or the history. Since clinical reactions to nuts were particularly fierce, provocation tests were deemed dangerous and unnecessary in most SPT-and/or RAST-positive instances—particularly because these patients regularly presented convincing (+3) case histories, reporting immediate reactions on encounter with minute amounts of nuts.

Cereals

Four patients reacted to ingested cereals (1 AEU, 1 EU and 2 E?) and five to inhaled flour dust, one of whom also reacted to ingested cereals (1 N, 1 AN and 3A). As

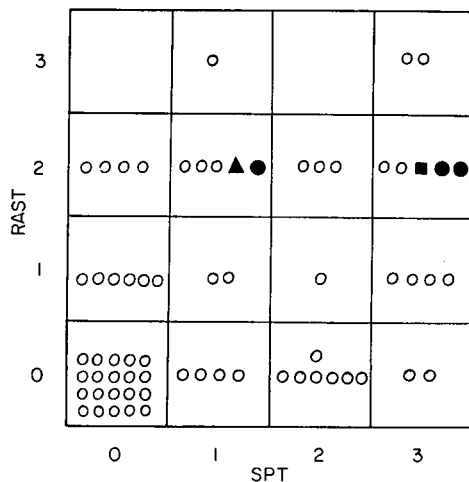


Fig. 5. Correlation between results of SPT and RAST with wheat. (○) No clinical allergy; (■) clinical allergy to ingested wheat; (●) clinical allergy only to inhaled wheat; (▲) possible clinical allergy but not convincing.

illustrated by the results for wheat (Fig. 5), prick test reactions with cereals correlated quite well with the results of RAST. This suggests that SPT reactions and the results of RAST are equally specific. However, in several instances no clinical allergy could be found in SPT- and RAST-positive patients following food challenges with wheat

and other cereals. When a correlation with the clinical allergy was found, it was more often due to allergy to the inhalation of the cereal dust while the food was tolerated when ingested. In spite of positive skin test or RAST results to the cereals in question, many children could eat cereals and inhale cereal dust without any discomfort whatsoever. In eighteen of the patients, RAST with six different cereals gave results differing by more than one class. Many positive RAST reactions were found with the allergosorbent to rice, which is considered particularly hypoallergenic. In our study, rice elicited clinical allergy reactions in only one case and in that case even the clinical reaction was considered unconvincing. RAST with corn was regularly among the lowest in patients with positive cereal RAST. RAST to cereals was positive also in five of the hay fever control patients.

According to the results found, cereals were classified in quality group II with respect to reliability of SPT and RAST for the diagnosis of food allergy (Table 2).

Cow's milk

Ten patients demonstrated convincing allergy to cow's milk (2 with systemic reactions (S), 2 A, 1 AU, 1 AEU, 2 AE, 1 EU and 1 AEG). Three patients had reactions not

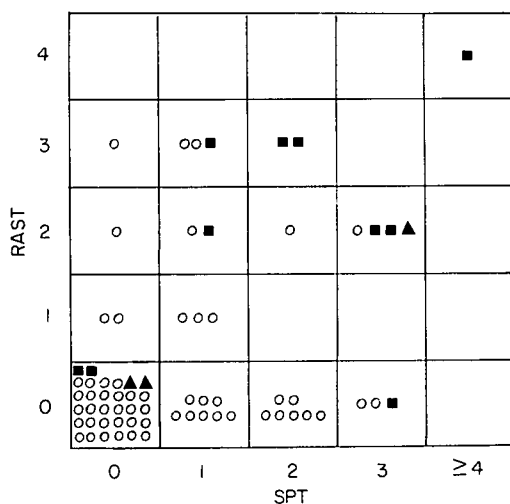


Fig. 6. Correlation between results of SPT and RAST with cow's milk. For legend, see Fig. 2.

considered convincing (2 E? and 1 A?) (Fig. 6). In seven of the children obviously tolerating cow's milk, a positive RAST result was found and two of them also had positive SPT reactions. Positive SPT was found in nine other RAST-negative children tolerating cow's milk. These results qualified cow's milk for classification in quality group II (Table 2).

White beans and soy beans

Beans elicited convincing clinical reactions only in three individuals reacting with UE in combination. In five additional children, beans were said to elicit eczema but this was not quite convincing.

Unconvincing reactions to soy beans in the form of exacerbations of eczema

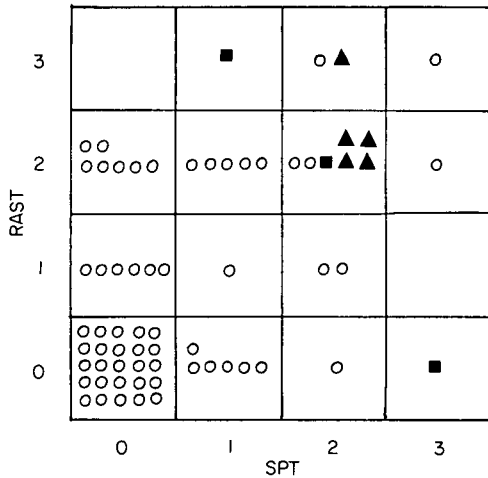


Fig. 7. Correlation between results of SPT and RAST with white beans. For legend, see Fig. 2.

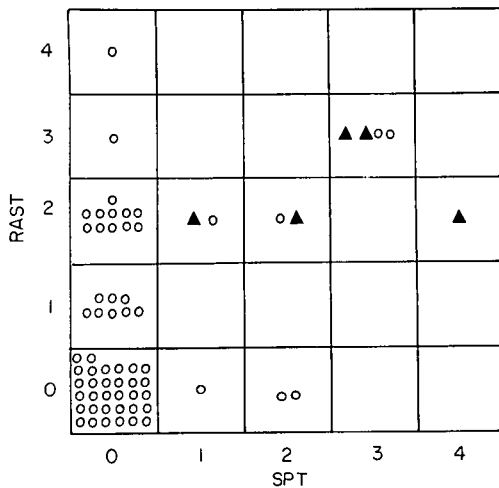


Fig. 8. Correlation between results of SPT and RAST with soy bean. For legend, see Fig. 2.

were reported in five children. A high number of patients had positive RAST to white beans (Fig. 7) and to soy beans (Fig. 8), most of them being without clinical significance. Slightly better results were obtained by means of SPT, but the results were not unequivocal, since the clinical reactions were not considered convincing. All reported and observed exacerbations, following the introduction of white beans or soy beans in the food, occurred in patients with atopic eczema.

There was a close correlation between the white bean and soy bean RAST results.

Discussion

In general, positive reactions were demonstrated with SPT and RAST in most patients with clinical allergy to the food item in question. In 119 instances of clinical hypersensitivity, both tests were below 2 in only ten cases (8.4%). Conversely,

negative reactions were found both with SPT and RAST in the majority of patients tolerating the food item in question. In 327 such instances, both tests were below 2 in 239 (73.1%). However, the reliability of SPT and RAST, respectively, was found to vary much for the different food items investigated. The reliability was found to be determined in a negative direction, mostly by the number of positive test results found in patients tolerating the substance, showing that our fourth diagnostic criterion (Table 1) is not satisfied.

The highest diagnostic reliability was obtained with SPT with the purified codfish allergen. With only very few exceptions, positive RAST was found in most of the same patients. Since IgE antibodies may episodically be absent from serum, even when present on the tissue mast cells in the skin and the affected tissues (Aas, 1975a; Aas & Lundkvist, 1973), the diagnosis of codfish allergy represents the highest attainable degree of precision and immunological specificity for RAST. This allergen then satisfies all our diagnostic criteria (Table 1).

Almost the same can be said about the diagnosis of allergy to green peas and to nuts of different kinds. The diagnostic reliability as regards egg white allergy was also good in our patient material, consisting of children more than 1 year old. The demonstration of both positive SPT and positive RAST to egg white predicted clinical allergy in all twenty-one instances. In contrast to the results discussed above, the diagnosis of allergy to other food items may be much more difficult, as illustrated by the findings with the cereals and cow's milk. Many positive results in both RAST and SPT without demonstrable clinical significance were found with both.

A few positive RAST to cereals were found in the control individuals with hay fever as well. Positive reactions to cereals have been found in such individuals by others, and a certain degree of cross-reactivity between cereals and grass pollen allergens has been confirmed by crossed immunoelectrophoresis studies of wheat extracts shown to contain forty-four antigenic components, some of which cross-react with antigens present in grass pollen extracts (Blands *et al.*, 1976; Hoffman, 1975).

Positive skin test and RAST reactions to cereals may be of no direct clinical significance, or they may demonstrate the presence of IgE antibodies of importance for respiratory allergy only (Kallos & Kallos-Deffner, 1971). With allergens of this type, it is then not sufficient to demonstrate that the individual patient is allergic to the substance by means of SPT and/or RAST. For a convincing diagnosis of food allergy to cereals dietary manipulations appear mandatory.

At the present time, it seems impossible to satisfy our diagnostic criteria for cow's milk allergy either, except possibly in the rare case of anaphylactic reactions showing positive SPT to cow's milk in so low dilutions that similar reactions are never seen in control individuals, and at the same time, with positive RAST in so low serum dilutions that similar reactions are not observed in the controls. There was only one such patient in this group.

The demand stated that the substance in question should give specific and positive responses in relevant immunological tests with tissues or serum from the patient, and not from those of control individuals, including allergic control individuals, is a critical one. If it is not satisfied for the diagnosis of cow's milk allergy, for instance, a number of non-immunological intolerance reactions to cow's milk may erroneously be considered as allergies.

Until adequate biologically related standardization is supplied for allergen extracts, the immunological specificity of skin tests are highly dependant on the quality and

concentration of the allergenic material used, and the mode of application of it (Aas, 1975a,b).

The need to establish the degree of reaction which convincingly indicates immunological specificity for each allergen seems also to apply to RAST, since the specificity and reliability of RAST depends on the allergen extracts used to prepare the allergosorbent (disc), as well as of a number of other variables. This may not be a problem for some allergens, but obviously for a number of food allergens such as cereals, cow's milk and, as shown here, for the experimental allergosorbents from white beans and soy beans. Here is a pitfall which must be seriously considered by all who try to produce allergosorbents by their own. Further development of reliable methods and refinement of both skin testing and RAST is needed for a number of food allergens. Progress probably depends on purification and isolation of the major allergenic fractions to be used in the diagnostic tests.

A large number of clinically insignificant positive reactions to soy beans and white beans were found in RAST and also in SPT. The RAST reactions may be due to non-specific binding of IgE or may possibly be due to the existence of IgE antibodies without clinical significance in allergy. Both possibilities need further elucidation.

A number of other basic problems in food allergy need clarification. First of all, it is quite unclear which role may be played by antigens arising or unmasked during digestion. Furthermore, the role of non-IgE versus IgE antibodies in food allergy is not well enough understood. We cannot expect to master the problems of non-IgE-mediated allergy before we do better in the field of IgE-mediated hypersensitivity, where we now can apply more and better tools.

With some food allergens, SPT and RAST are quite dependable adjuncts to the diagnosis of food allergy, whereas neither of the tests are reliable for other foods (Chua *et al.*, 1976; Hoffman & Haddad, 1974; Schur, Hyde & Wypych, 1974). This study shows the need to improve the diagnostic material and to establish the diagnostic reliability of the material and tests used for each food item in question.

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