
Standardized Extracts, Foods

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Historical Review and Epidemiology

To date, food allergen extracts have not been standardized. Many problems would have to be overcome to obtain reliable, standardized food extracts, including the identification of adequate source materials, the preparation of suitable reference standards, the development of reliable assay procedures, the development of acceptable storage procedures, and the resolution of potential problems resulting from the existence of multiple and cross-reacting allergens. These problems are not unique to food allergen extracts, but such problems have not been carefully addressed with food systems and considerable research will be necessary to resolve certain of these issues.

The prevalence of food allergies is not precisely known. Estimates range from 0.3 to 7.5% of the population.¹ The most common allergenic foods include peanuts, crustacea, fish, cow's milk, eggs, tree nuts, and soybeans. Allergies, however, can occur to virtually all foods, especially those with appreciable quantities of protein. The prevalence of individual types of food allergy will depend in part on the eating habits of the affected population. Cow's milk is the most common allergenic food for infants because it is the most commonly consumed food in this age-group. Fish allergies are more prevalent in Scandinavian countries than in the United States, probably because fish consumption is more frequent in Scandinavia.² Soybean allergy may be more common in Japan than in the United States for similar reasons.³ Standardized food allergen extracts first should be developed for the most common allergenic foods. Yunginger and Jones⁴ proposed a method for the preparation of standardized peanut extracts, which will be reviewed in greater detail in later sections. They defined the source of the peanuts and the chemical and physical procedures for the preparation of the extracts. They lyophilized the extract to insure adequate stability and considered protein content, electrophoretic patterns, and immunologic methods [radioallergosorbent test (RAST), skin tests, crossed-radioimmuno-electrophoresis (CRIE)] as procedures for the characterization of the final extract.

The persistence and nature of food sensitivities may complicate the development and utilization of standardized food extracts. Infants with food

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allergies tend to outgrow their sensitivities.⁵⁻⁷ Allergies to certain foods, such as cow's milk and eggs, are more frequently outgrown than other types of food allergy.^{5,7} The transient nature of certain food allergies and the age of the afflicted patient population could complicate the development of standardized food extracts. For example, finding an adequate (pediatric population) and stable group of patients with cow's milk allergy for the evaluation of standardized extracts might be difficult. Finally, although standardized food extracts would be useful in the diagnosis of IgE-mediated food sensitivities, many adverse food reactions appear to have no immunologic basis or, at least, are not IgE-mediated. Thus, the diverse nature of food sensitivity can further complicate the selection of patient groups that could be used to evaluate the efficacy of various standardized extracts in the diagnosis and treatment of various food-related disorders.

Collection of Material

The identification of source materials for food extracts is the first problem to overcome. With many allergenic foods, multiple species, varieties, and strains exist. Yunginger and Jones⁴ proposed that a mixture of Virginia, Runner, and Spanish peanuts be used in the preparation of a standardized peanut extract. While these are the 3 most important commercial varieties of peanuts, numerous strains exist within each variety. Protein electrophoretic patterns are known to change as a function of variety, strain, growing area, and even climate.^{8,9} These alterations in protein profile could have important implications in allergen extract preparation. Similar problems with multiple varieties and strains would occur with most food plants.

The existence of cross-reacting allergens in related species can reduce the number of different food extracts required but further complicates the selection of adequate source materials. It is common practice to use combined extracts in the diagnosis of fish and shellfish allergies. However, these combined extracts do not contain all of the possible species in each category (Tables 1 through 3). Cross-reactions would not necessarily be predicted to occur between distantly related fish or between crustacea and molluscs. The use of combined extracts is desirable, but these extracts should contain only those species likely to cross-react. For example, a crustacean extract should probably contain a mixture of the crustacean species listed in Table 2. Alternatively, it might be desirable to have separate mixed extracts for lobsters, crabs, and shrimp using mixtures of all of the species in each group (Table 3). With fish, mixtures of closely related species, such as *salmonids* and *gadoids*, would be most useful (Table 1), although mixtures containing representatives of each common subgroup would provide a more general diagnostic tool.

Another factor to be considered in the selection of source materials is the use of cooked versus uncooked foods. With some foods, such as peanut,¹⁰ the allergens are heat-stable and the choice of raw versus cooked material is not particularly important. However, even with peanuts, some of the multiple allergens are more heat-stable than others.¹⁰ The quantity of allergens and proteins is higher in raw peanut extracts than in roasted peanut extracts.¹⁰⁻¹³ Yunginger and Jones⁴ suggested the use of raw peanut extracts for this reason.

Table 1. Taxonomic Relationships Among the Edible Fishes

Taxonomic classification		
Class	Subclass or order	Common names of fishes in the orders
Condrichthyes	Elasmobranchii	Sharks
Osteichthyes	Acipenseriformes	Sturgeons, paddlefishes
	Elopiformes	Tarpons, ten-pounders, bonefishes
	Anguilliformes	Common eels, morays
	Clupeiformes	Herrings, sardines, alewives, shad, menhadens, anchovies
	Salmoniformes	Trouts, salmons, whitefishes, graylings, ciscoes, lake herrings, charrs, smelts, capelins, eulachons, pikes, saugers muskelunges, pickerels
	Gonorynchiformes	Milkfishes or awa
	Cypriniformes	Minnows, carps, suckers, catfishes
	Beloniformes	Sauries, needlefishes, flying fishes
	Mugiliformes	Mulletts, barracudas, threadfishes, silversides
	Lambridiformes	Opah or mariposas
	Tetraodontiformes	Pufferfishes, boxfishes or trunkfishes
	Pleuronectiformes	Flounders, halibuts, soles, dabs, turbots
	Perciformes	
	Suborder Percoidei	Basses, crappies, bluegills, sea basses, sunfishes, perches, bluefishes, jacks, pompanos, dolphin fishes, snappers, groupers, grunts, scups, porgies, sheepsheads, pomfrets, snooks, robalos, bigeyes, catalufas, croakers, redfishes, drums, butterfishes, spade fishes, wrasses, tautogs, goatfishes, cichlids, mojarras, rudderfishes, surffishes, roaches, spots, weakfishes
	Perciformes	
	Suborder Scombroidei	Mackerels, tunas, cutlassfishes, albacores, bonitos, kingfishes
	Perciformes	
	Suborder Xiphoidei	Swordfishes, marlins, sailfishes, spearfishes
	Perciformes	
	Suborder Ammodytoidei	Sand lances
	Perciformes	
	Suborder Stromateoidei	Butterfishes
	Scorpaeniformes	Rockfishes, scorpionfishes, greenlings
	Gadiformes	Codfishes, ling cods, pollocks, haddock, tomcads, hakes or codlings
	Percopsoidi	Trout-perches or sand rollers

However, humans never consume raw peanuts so an argument could be made that inclusion of the heat-labile allergens might be of little value diagnostically. The situation becomes more complex with other foods. Some foods are allergenic only in the raw state.^{14,15} If these foods are commonly consumed raw, then the use of raw extracts seems obvious. Some cases have been described of individuals allergic to cooked but not raw food.¹⁶ This finding

Table 2. Taxonomic Relationships Among the Edible Molluscs and Crustacea

Taxonomic classification			Common names of edible species
Phylum	Class	Subclass	
Mollusca	Gastropoda	Prosobranchia	Marine snails, abalone, periwinkles, whelks
		Pulmonata	Terrestrial and freshwater snails
	Bivalvia	Lamellibranchii	Mussels, cockles, oysters, scallops, clams
Arthropoda	Cephalopoda	Coleoidea	Squid, octopus
	Crustacea	Malacostraca	Shrimp, crabs, lobsters, crayfish

Table 3. List of the Edible Crustacea

Group	Common name	Scientific name
Lobster	Atlantic lobster	<i>Homarus vulgaris</i>
	European lobster	<i>Homarus gammarus</i>
	Northern lobster	<i>Homarus americanus</i>
	Spiny lobster	<i>Panulirus argus</i>
Crabs	Blue crab	<i>Callinectes sapidus</i>
	Deep sea blue crab	<i>Portunus pelagicus</i>
	Dungeness crab	<i>Cancer magister</i>
	Jonah crab	<i>Cancer borealis</i>
	King crab	<i>Paralithodes camtschatica</i>
	Snow crab	<i>Chionoectes bairdi</i>
	Tanner crab	<i>Chionoectes tanneri</i>
	Spider crab	<i>Talvia maticum</i>
	Stone crab	<i>Menippe mercenaria</i>
Prawns/shrimp	Common prawn	<i>Palaemon serratus</i>
	Deepwater prawn	<i>Pandalus borealis</i>
	Pink Maine shrimp	<i>Pandalus borealis</i>
	Coon Stripe shrimp	<i>Pandalus hypsinotus</i>
	Brown shrimp	<i>Panaeus aztecus</i>
	Pink shrimp	<i>Panaeus duorarum</i>
	White shrimp	<i>Panaeus indicus</i>
	Gamba shrimp	<i>Panaeus longirostris</i>
	Karuma prawn	<i>Panaeus japonicus</i>
	Giant tiger prawn	<i>Panaeus monodon</i>
	Common tiger prawn	<i>Panaeus esculentus</i>
	Indian prawn	<i>Panaeus indicus</i>
	Eastern king prawn	<i>Panaeus plebejus</i>
	Banana prawn	<i>Panaeus merguensis</i>
	Brine shrimp	<i>Artemia salina</i>
	Rock shrimp	<i>Silyornia brevirostris</i>
	Sea bob shrimp	<i>Xiphopenaeus kroyeri</i>
	Royal red shrimp	<i>Hymenopenaeus robustus</i>
	Freshwater prawn	<i>Macrobrachium carcinus</i>

is not always consistent for all patients with that particular food allergy. Therefore, in some cases, it may be necessary to consider extract mixtures of raw and cooked product.

Characterization of Various Food Allergens

Following purification, most food allergens have been found to be glycoproteins with molecular weights between 10,000 to 40,000 daltons. Although exceptions exist, the majority of food allergens tend to be resistant to protease digestion, heat treatment, and denaturation. All food allergens that have been described have been purified from water extracts of foods and, therefore, little is known regarding the antigenicity or the potential biologic relevance of water-insoluble allergens. Finally, as in the case of ragweed pollen, numerous allergens can be isolated from a single food such as milk. This feature has complicated the identification and characterization of individual allergens since not all individuals who respond clinically to challenge with certain foods will demonstrate the same RAST or skin test pattern when they are tested with individual components of the food. Since more in-depth reviews have been published,¹⁷ the following section will briefly highlight some of the information that is known regarding the characterization of the most common and best characterized food allergens (Table 4).

Milk

A variety of clinical symptoms have been attributed to milk protein intolerance including anaphylaxis, urticaria, vomiting and diarrhea, colic, enterocolitis, atopic dermatitis, pulmonary hemosiderosis, and more.¹⁸ Thus, in addition to allergen-induced IgE-antibody formation, it is likely that antigen activation of other immunologic pathways also plays a role in milk hypersensitivity. It is therefore important to keep in mind that, by the nature of the assays, RAST or skin testing are only helpful in identifying and characterizing milk constituents that are responsible for immediate hypersensitivity reactions.

Cow's milk is a complex mixture of inorganic compounds, lipids, proteins, and carbohydrates. Caseins occur in many genetic variants and are the principal proteins found in cow's milk. β -lactoglobulin and α -lactalbumin are the 2 principal whey proteins. β -lactoglobulin contains 2 identical 18,000-molecular weight polypeptide chains, α -lactalbumin is a single 14,200 molecular weight peptide, and bovine serum albumin is composed of a single polypeptide chain with a molecular weight of 67,000. Lactoperoxidase is the most common enzyme and IgA, IgM, and IgG immunoglobulin constitute about 2% of the protein content. Since human IgG and IgM antibodies to milk proteins can be found in virtually all individuals who consume milk, their relationship to the pathogenesis of milk hypersensitivity seems remote. In contrast, there appears to be a reasonable correlation between the results of immediate skin testing with milk antigen and clinical histories of immediate hypersensitivity. Using a variety of milk antigens, Goldman and co-workers¹⁹

Table 4. Characteristics of Major Food Allergens

Source	Name	Molecular weight	Physicochemical properties	Special characteristics
Milk	Casein	$\beta + \alpha$: 23,600 κ : 20,000	Heat-stable	In presence of lactase, pasteurization increases allergenicity
	β -lactoglobulin	Dimer: 18,000 each	Partially heat-stable	
Codfish Shrimp	α -lactalbumin	14,200	Heat-labile	Calmodulin Isolated from raw shrimp extracts Isolated from cooked shrimp extracts
	Bovine serum albumin	67,000	Heat-labile	
	Bovine γ -globulin	150,000	Heat-labile	
	Allergen M	12,328	Heat-stable	
	Antigen I	Dimer: 21,000 each	Heat-labile	
	Antigen II	38,000 (May exist as polymer)	Heat-stable	Nucleic acid-like molecule
	Nuclear material	>100,000	Ribonuclease-sensitive	
Chicken egg	Ovalbumin		Protease-resistant	
	Ovomucoid		Heat-stable	
Soybeans	Conalbumin		Heat-stable	Allergenicity enhanced with heating
	2S fraction		Heat-stable	
	7S fraction		Heat-labile	
	11S fraction		Heat-labile	
	Kunitz trypsin inhibitor	21,000	Acid- and proteolysis-resistant	
Peanuts	Peanut I	Polymer composed of 3-5 subunits	Acidic glycoprotein	May represent a subfraction of arachin or conarachin

skin tested 3 groups of children: normal controls, a group of children allergic to substances other than milk, and a group allergic to milk by history. In milk-allergic children, 50 of 85 had positive skin tests to 1 or more proteins: 34 were positive to casein, 22 to α -lactalbumin, 20 to β -lactoglobulin, and 21 to serum albumin. Six of 102 normal children reacted to no more than 1 milk protein and 21 of 31 allergic (but not to milk) reacted to 1 or more proteins. Positive oral challenges occurred to all fractions tested in approximately equal frequency, although other studies have suggested that casein and β -lactoglobulin are the most frequent offenders.^{20,21} Bovine γ -globulin also can produce positive skin tests in milk-sensitive children.²² Preparations of pure milk proteins are difficult to obtain; indeed, cross-contamination by other milk proteins can occur in amounts up to 2 to 3% of the total protein.¹⁹ Although heating denatures some proteins (bovine serum albumin, bovine γ -globulin, and α -lactalbumin), pasteurization of milk may actually render some molecules, such as β -lactoglobulin, more antigenic.²³ The creation of new allergens by proteolytic digestion of milk protein has been demonstrated in animal models²⁴ and also in humans.²⁵ However, the relevance of these to human disease states is unknown. These results indicate that milk allergen extracts should contain at least casein, β -lactoglobulin, α -lactalbumin, bovine serum albumin, and bovine γ -globulin to maximize the sensitivity of the skin testing diagnostic procedure for the evaluation of immediate hypersensitivity due to milk protein.

Fish

Fish allergy, especially to cod, is common and, as previously indicated, reported to be more prevalent in Scandinavian countries where large quantities of fish are consumed.² Codfish contains 1 major allergen (allergen M) that can be isolated from the myogen fraction within the white meat. Allergen M is heat-stable and resistant to proteolytic digestion and several denaturation procedures. It has a molecular weight of 12,328 daltons, an isoelectric point (pI) of 4.75, and composed of 113 amino acids and one glucose residue.²⁶ Allergen M belongs to a group of calcium-binding proteins and therefore is a calmodulin.²⁷ The 3-dimensional structure of M protein is known and it is apparently arranged in 3 domains, 2 of which can bind calcium.²⁸

Seafood

Allergens in other seafoods also have been preliminarily characterized. Two allergens, termed antigens I and II, have been described in extracts of shrimp (species not specified).²⁹ Antigen I was obtained from extracts of raw shrimp whereas antigen II was isolated from cooked shrimp. Antigen I is a dimer composed of 2 polypeptide chains with molecular weights of 21,000 daltons. Antigen II has a molecular weight of over 200,000 using Sephadex G200 chromatography and this material gives a single band on polyacrylamide gel electrophoresis (PAGE) or sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with a molecular weight of 38,000. Although skin testing with these antigens was not performed, 11 serum samples reacted

with antigen II, and 7 of 11 with antigen I by direct RAST. Antigen II also inhibited the RAST using raw or cooked shrimp extracts with 4 serum samples from anaphylactic reactors to shrimp. Additional protein peaks also were described but not further characterized in this study. Of interest is the report that a nuclear constituent, probably RNA, from shrimp extracts (*Paenaeus indicus*) also may be a major allergen in some individuals.³⁰ Using pooled serum from crustacea-allergic individuals, CRIE has revealed at least 5 crawfish and 3 lobster allergens.³¹ Immunologic and clinical cross-reactivity among crustacean-allergic individuals and even oyster-allergic individuals also has been reported.^{32,33}

Egg

Chicken egg is a food commonly implicated in allergic disease. The egg can be divided into the white and the yolk; the allergenicity of the yolk protein is less than the egg white protein³⁴ and contains lipoproteins and a water-soluble substance, livetin. The white contains ovalbumin (50% of total protein), ovomucoid, ovomacroglobulin, conalbumin (ovotransferrin), ovomucin, lysozyme, and trace proteins including catalase, flavoprotein, ovoinhibitor, avidin, and ribonuclease. Using skin testing and RAST, ovalbumin and ovomucoid appear to be the major allergens,³⁵ although conalbumin reactivity also can be demonstrated in some patients.³⁶ Both ovomucoid and ovalbumin can be found in raw or cooked eggs.

Soybeans

Allergic reactions to legumes including soybean, peanut, castor bean, and green peas are common. Soybean protein is used in many commercial foods and consists of a globulin fraction (85%) and a whey fraction. The globulin fraction has been separated into 4 components with sedimentation constants of 2, 7, 11, and 15S. The allergenicity of globulin fractions 2, 7, and 11S have been examined by RAST and RAST-inhibition experiments using sera from soybean-sensitive (asthma and atopic dermatitis) patients. Reactivity with specific IgE antibody was found in all fractions with considerable cross-reactivity among the 3 fractions; the 2S-globulin had the highest allergen potency to inhibit the RAST against 11S-, 7S-, and 2S-globulin. When the fractions were heat treated at 80, 100, and 120°C for 30 minutes, the RAST activity of the 2S-globulin heated at 80°C for 30 minutes was slightly enhanced, whereas that of the others was reduced to 40 to 75% of that of the native globulin.³

Approximately 10% of soybean protein consists of the whey fraction, which also contains a trypsin inhibitor and a hemagglutinin. A proteinase inhibitor obtained from soybeans, termed Kunitz soybean trypsin inhibitor, has been incriminated in 1 patient's severe allergic reactions to soybean products.³⁷ The inhibitor was found to be homogeneous by cellulose acetate electrophoresis and gave a single band with an apparent molecular weight of 20,000 to 21,000 by disc PAGE. The purified protein elicited positive immediate skin tests in the patient, gave a positive RAST test using the patient's serum, and could inhibit the RAST when whole soybean extract was used as the solid-

phase antigen. Two other soybean-allergic patients were not found to have IgE antibody to this protein.¹¹ Thus, at least 4 components of soybean have been found to be allergenic indicating the need to include at least these fractions in prepared extracts. Interestingly, soybean-allergic individuals have been found to be unresponsive to oral challenge with soybean oil, indicating that the major allergenic activities reside in the water-extractable fractions.³⁸

Peanuts

Peanuts are one of the most allergenic foods. Radioallergosorbent test analysis has demonstrated IgE antibodies to both raw and roasted allergen preparations.¹¹ While RAST inhibition studies³⁹ have indicated that peanut butter syrup, peanut butter powder, peanut butter chips, peanut flours, and pressed defloured peanuts are allergenic in sensitive patients, peanut oil is not.⁴⁰ Peanut proteins have been traditionally classified as albumins (water soluble) or globulins (saline soluble). Globulins can be further separated into arachin and conarachin fractions. Arachin, contained within the protein bodies of the peanut seed, can be separated by SDS-PAGE into 7 subunits,⁴¹ the most prominent between molecular weights of 18,000 to 30,000. Conarachin, contained within the cytoplasm of the peanut seed, can be separated by ultracentrifugation into 7.8S and 12.6S components with corresponding molecular weights of 142,000 and 295,000, respectively. The 12.6S component has been designated as Conarachin II or α -conarachin and can be dissociated into 7 subunits with molecular weights ranging from 18,000 to 62,000.

A number of other components have been isolated. One heat-labile peanut lectin or agglutinin has been purified that is a tetramer composed of four 27,000-molecular weight subunits. A 66,000-dalton concanavalin A-reactive protein comprises about 1% of soluble peanut proteins. Multiple protease inhibitors also are present in peanuts including a heat-stable trypsin and chymotrypsin inhibitor and an α -amylase inhibitor. Phospholipase D, phytic acid, and aflatoxins also have been demonstrated in peanut extracts.⁴¹

In addition to the information available regarding the chemistry of peanut constituents, progress also has been made in the area of the isolation and characterization of peanut allergens. Using serum from peanut-sensitive individuals, Heiner and Neucere⁴² performed RAST to document the allergenicity of multiple peanut preparations including extracts of cotyledons, hypocotyls, axial germ tissue, and skins, as well as the allergenicity of arachin and conarachin fractions of peanut extracts. Sachs et al.,⁴³ using pooled serum from peanut-allergic individuals, identified allergenic components in fractionated peanut extracts by RAST inhibition. Following ammonium bicarbonate extraction, raw defatted peanut meal was chromatographed on anion exchange resins and 8 absorbance peaks, several of which were allergenic, were identified. One peak was further characterized and found to be a protein (called Peanut I), which by SDS-PAGE was composed of 2 major bands (20,000 and 30,000) along with at least 3 minor bands of lower and higher molecular weight. In peanut-allergic individuals skin testing with Peanut I elicited positive responses, and isolated basophils from these patients released histamine in response to in vitro challenge with Peanut I. Sachs and co-workers⁴³ con-

cluded that Peanut I was an acidic glycoprotein with nonidentical subunits and they speculated that Peanut I may represent a subfraction of either arachin or conarachin.

The allergenicity of other purified and well-characterized peanut constituents also has been assessed. Alpha-arachin, conarachin I, concanavalin A-reactive protein, peanut agglutinin, and phospholipase D have been assessed by RAST using serum from the peanut-allergic patients.¹⁰ Whereas peanut agglutinin and phospholipase D gave poor RAST responses, α -arachin, conarachin I, and concanavalin A-reactive protein all gave significant RAST results, albeit lower than those obtained with the crude extracts. The extent of RAST inhibition obtained with these materials was inversely related to their abundance in the total peanut protein. Crossed-immunoelectrophoresis (CIE) with extracts from raw and roasted peanut indicated the presence of 22 and 10 anodically migrating antigens, respectively. Crossed-radioimmunoassay analysis using pooled serum demonstrated 16 IgE-binding antigens for raw peanut and 7 for roasted peanut. Crossed-radioimmunoassay plates treated with individual serum samples showed that all of the patients had specific IgE for the major antigen peak, which was tentatively identified as α -arachin, the main storage peanut protein that is particularly heat-resistant. Finally, Barnett et al⁴⁴ have shown by RAST inhibition that the concanavalin A-reactive glycoprotein is a major allergen in approximately 50% of peanut-sensitive individuals. Using CIE, this glycoprotein comigrates with arachin, and further studies are needed to determine if it is a subunit of arachin.

With this as a background, Yunginger and Jones⁴ have proposed the following scheme to prepare a standard or reference extract for peanuts: Peanuts selected for extraction should be Virginia, Spanish, and Runner varieties from the most recent harvest and should be obtained from a United States Department of Agriculture (USDA) research station to assure maximum quality (lowest aflatoxin levels). Each variety should be processed separately. Selected kernels should be heated in water at 40 to 45°C for 5 minutes to facilitate manual removal of the skins. Although nearly all peanuts consumed by humans are roasted during processing, several studies^{10,30} indicate that the numbers or quantities of allergens or proteins are higher in raw extracts than in roasted extracts. Split cotyledons (hearts should be removed) from each variety should be ground into flour separately, defatted, cultured for bacteria and fungi, assayed for aflatoxin levels, and stored sealed under vacuum at -20°C. Equal parts of the 3 peanut flours should be mixed and extracted 1:20 weight per volume (wt/vol) in 1.0 M NaCl-20 mM phosphate buffer (pH 7.0) for 4 hours at 4°C with continual pH monitoring. The extract should be isolated by centrifugation and extensively dialyzed to remove phytic acid. Mannitol (3% final) should be added and the dialyzed extracts should be frozen at -20°C for shipping. Thawed extracts should be warmed and clarified by coarse membrane filtration, separated into sterile ampules, freeze-dried, and stored at -20°C. Measurements of dry weight, residual moisture protein content, and physicochemical characterization by PAGE (acidic and alkaline buffer), SDS-PAGE, 2D-PAGE, and thin layer isoelectric focusing should be made. Immunochemical characterization should include CIE and CRIE; allergenic activity should be documented by direct RAST and RAST-inhibition

assays as well as by skin testing and leukocyte histamine release in peanut-sensitive individuals.⁴⁴

Clinical Data, Safety, and Efficacy

Food extracts now are commonly used as skin test reagents. The pioneering work of Bock et al⁴⁵ provided useful guidelines that addressed the issues of concentration, appropriate technique, and diagnostic usefulness for food skin testing. From their work, Bock et al⁴⁵ concluded that water soluble extracts of foods (1:20 wt/vol) preserved in 50% glycerine with phenol or phenol-buffered saline gave the optimal results in terms of safety, specificity, and sensitivity. Sixty-eight children, aged 5 months to 15 years, were studied because of histories of adverse symptoms related to food ingestion.⁴⁶ These symptoms included wheezing, rash, eczema, urticaria, vomiting, and diarrhea. In children over 3 years, 20 of 39 gave positive histories, positive skin tests, and negative challenges with the suspected food. The foods all were tolerated when reintroduced openly into the diet. In contrast, all children 3 years of age or older who responded with an adverse reaction during double-blind food challenge demonstrated a significant wheal response to puncture skin tests with the concomitant food extract. The results were slightly different in children under 3 years of age. In each of 9 children exhibiting an adverse reaction to food within 2 hours of challenge (immediate hypersensitivity), skin tests with the relevant food were positive. Only 1 of 4 infants with the onset of symptoms more than 4 hours after challenge exhibited a significant wheal reaction to the relevant food. Only 4 of 12 gave positive histories, positive skin tests, and negative challenge with the suspected food. No adverse reactions to skin testing were reported. While these studies were not performed using standardized extracts, they indicated that crude food extracts could be helpful in the diagnostic evaluation of children with food hypersensitivity.

Sampson and Albergo⁴⁷ extended these earlier studies by evaluating skin testing and RAST with commercial food extracts to determine their usefulness in the management of pediatric patients with atopic dermatitis and a suspected food trigger for their eczema. Eight food antigens including egg, peanut, milk, wheat, fish, soy, chicken, and pork were evaluated. Defining a positive skin test as a wheal 3 mm in diameter or larger (using commercial extracts) than the negative control, and a positive RAST as a Phadebas RAST score of 3 or 4, the sensitivity, specificity, and predictive accuracies of these tests were evaluated. Both were found to be comparable except for wheat antigen where skin testing was clearly superior to the RAST. Accepting a RAST score of 2 or more as a positive result slightly improved sensitivity in some cases but dramatically decreased specificity. Combining results of skin tests and RASTs did not improve the diagnostic accuracy over results of the tests used individually. In 40 children, although the skin prick test was found to be generally a valuable test for excluding immediate hypersensitivity, sole reliance on a positive skin test would have resulted in missing 15% of clinically significant challenge reactions (23% with RAST alone).⁴⁷ Furthermore, the positive predictive accuracies (positive skin test = clinical hypersensitivity) of both skin

tests and RAST were found to be poor and quite variable: 25 to 75% for skin tests and 0 to 57% for RAST. These results indicate that food skin tests and RAST using commercially available food extracts should only be used as a guide for identifying possible food allergens that could be used in more definitive diagnostic procedures (double-blind challenge).

Summary

While progress has been made in the areas of food allergen characterization, both the complexity of the biochemical constituents of food and the body's normal physiologic (digestion) and immunologic responses to food ingestion provide challenging obstacles to efforts aimed at developing standardized food extracts. As indicated above, while currently available food extracts can be useful in the evaluation of food hypersensitivity, the results obtained using these reagents are far from optimal. Indeed, in some cases, skin testing after rubbing a small amount of the food on the skin may be more inciteful than using commercially prepared extracts.¹⁴ While this approach may provide physicians with help in terms of diagnosis, purified food components will be necessary to comprehensively study the complex issues of food allergen digestion and absorption; immune response and tolerance to food allergens; and hopefully, treatment and prevention of food hypersensitivity.

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