

IMMUNE SERA IN THE STUDY OF FOOD ANTIGENS

III. Vegetable Seed Oils

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AN EXACT etiological role for the edible vegetable oils as allergens in hypersensitivity states of man has not been clearly delineated. This was again emphasized to the Committee on Food Allergy of the Research Council of the American Academy of Allergy in recent attempts to evaluate the conflicting clinical evidence offered in the literature¹ and by divergent opinions submitted to us by qualified allergists. We have therefore undertaken to study this still unresolved question through animal experimentation and clinical investigation. This report is concerned with an initial phase of the project in which the specific precipitinogenic activity of immune rabbit sera has been employed in the detection of antigenic vegetable seed fractions. The value of this type of experimental approach has been previously demonstrated in related studies concerned with the detection of antigenic fish components in pharmaceutical fish liver oil preparations.²

MATERIALS AND METHODS

Immunizing dosages and schedules for the preparation of rabbit antisera, techniques for agar-gel plate double-diffusion precipitin tests (Ouchterlony) for qualitative antigenic analyses, and the preparation of aqueous phase extracts of water-in-oil dispersions were identical to those previously reported in the study of fish extracts and fish liver oils.² Briefly these consisted of the following: (1) preparation of standard saline extracts of cottonseed, corn, olive, peanut, and soybean; (2) immunization of series of rabbits, each with 12 mg. protein nitrogen of a vegetable extract; (3) determination of the number of individual precipitating antibody-antigen systems for each vegetable extract and its corresponding antiserum by agar-gel double-diffusion techniques; (4) preparation of water-in-oil dispersions of each edible vegetable oil (cottonseed, corn, peanut, soybean); and (5) separation of the aqueous phase (extract) of the water-in-oil and its subsequent employment in agar-gel double-diffusion tests with corresponding antiserum to the whole vegetable extract.

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Two lots each of seven different commercial brands of cottonseed oil, five of corn oil, three of peanut oil, and two of soybean oil were purchased on the open market. These were then studied for the possible presence of antigenic constituents that might show reactions of identity with the precipitin-producing fractions of the vegetable seed of its derivation.

RESULTS

The results of multiple agar-gel diffusion precipitin tests employing each antiserum to a vegetable seed extract with (a) its corresponding extract and (b) the aqueous phase of the corresponding water-in-vegetable oil preparation are summarized in Table I.

TABLE I

EXTRACT OF:	ANTISERUM TO:	NUMBER OF PRECIPITIN BANDS
Cottonseed	Cottonseed	8*
Cottonseed oil		
Brand A	Cottonseed	3*
Brand B	Cottonseed	2*
Brands C-G	Cottonseed	0
Corn	Corn	2*
Corn oil		
Brand A	Corn	1*
Brands B-E	Corn	0
Peanut	Peanut	4
Peanut oil		
Brands A-C	Peanut	0
Soybean	Soybean	12
Soybean oil		
Brands A-B	Soybean	0

*Reactions of identity were demonstrated between precipitin bands developed in the same agar plate containing each extract diffusing against a common antiserum.

COMMENT

The multiplicity of distinct antigenic components within cottonseed, corn, peanut, and soybean is demonstrated by their serum precipitin-producing properties in rabbits. The presence of some of these same water-soluble antigens within some lots of derivative oils of cottonseed and corn, but not peanut or soybean, is also demonstrated by immunologic techniques of identification. An attempt to evaluate olive oil by similar study was not possible since we were unable to prepare an olive extract of sufficient antigenicity to induce the formation of precipitating serum antibody in the rabbit by the technique employed.

It is of special interest to take note of a study³ reporting quite similar differences in these oils by analytic chemical techniques. Peanut oil was found relatively free of non-oil constituents in contrast to other edible seed oils, especially cottonseed and corn, showing such contents of 2 per cent or more. While crude soybean oil was also found to contain large amounts of nonglycerid material, this was effectively reduced by premarketing treatment.

Proteins and protein fragments are known to be soluble in oil when in anhydrous form. It is therefore understandable that the extent of removal of

native seed proteinaceous substances is only proportional to the efficacy of "degumming" processes such as solvent extraction, steam, or weak alkali treatment of the vegetable oil.⁴ The variable presence of some seed antigenic contaminants in derivative oils thus becomes a distinct possibility. In further consideration of untoward reactions to cottonseed, Friedman⁵ has been studying the clinical importance of its gossypol content, a water-insoluble fraction with irritating properties to the gastrointestinal tract.⁶

It is to be pointed out that the identity of antigens of cottonseed and corn responsible for precipitin formation in the rabbit and allergens of similar origin for man have not been established. However, the possibility of relationship merits consideration in speculating on clinical applications of these findings. Interest is directed to the inconstant presence and the variability in number of individual water-soluble vegetable seed antigens within different lots of the final derivative oil product. Thus, along with a quantitative factor, perhaps another important determinant of allergic reactivity for any edible vegetable seed oil may be the variable presence or absence of that particular antigenic seed fraction for which the individual possesses reagins and/or clinical sensitivity. Qualitative and quantitative antigenic analyses of each lot of cottonseed oil employed may be prerequisite to evaluating clinical observations that only the infrequent cottonseed-sensitive patient is intolerant to cottonseed oil and that even the same individual may not react in a constant symptomatic pattern to each trial ingestion.

SUMMARY

1. Sera from specifically immunized rabbits were employed in agar-gel double-diffusion precipitin test techniques for the qualitative antigenic analyses of cottonseed, corn, peanut, and soybean extracts and their edible derivative oils.

2. Eight antigenic components were demonstrated in cottonseed extracts, two in corn, four in peanut, and twelve in soybean.

3. Two to three of these water-soluble seed antigens were demonstrated in two out of seven brands of commercial cottonseed oil and one antigenic fraction in one of five brands of corn oil by immunologic techniques of detection.

4. Water-soluble antigens of peanut and soybean could not be detected in the lots of their derivative oil preparations tested.

REFERENCES

1. Allergy to Cottonseed and Other Oilseeds and Their Edible Derivatives, Memphis, 1948, National Cottonseed Products Association, Inc.
2. Cohen, S. G.: Immune Sera in the Study of Food Antigens. I. Fish, *J. ALLERGY* 30: 250, 1959.
3. Jamieson, G. S., and Baughman, W. F.: *J. Oil & Fat Indust.* 3: 347, 1926 (cited by Bailey⁴).
4. Bailey, A. E.: *Industrial Oil and Fat Processes*, New York, 1945, Interscience Publishers, Inc., pp. 155-172, 501.
5. Friedman, H. T.: Personal communication to the Committee on Food Allergy, Research Council, American Academy of Allergy.
6. Merck Index, ed. 7, Rahway, 1960, Merck & Co., Inc., pp. 496-497.