

Immune Sera in the Study of Food Antigens

II. Crustaceans

Sheldon G. Cohen, M.D.,* and Charles B. Reif, Ph.D.,** Wilkes-Barre, Pa.

INTRODUCTION

IN A previous study¹ the potential value of immune rabbit sera in the study of component antigenic fractions of fish extracts and fish-derivative preparations was demonstrated. It was decided, therefore, to extend these experimental techniques to an investigation of some problems related to the crustaceans, including (1) cross-antigenicity between skin-test extracts of those shellfish (arthropods) responsible for allergic reactions in man, (2) antigenic relationships between taxonomically related "insect-type" (entomostracan) crustaceans and larger edible shellfish (malacostracans), and (3) uniformity of qualitative antigenic content of shellfish (crustacean) extracts prepared for skin-testing purposes in different laboratories.

MATERIALS AND METHODS

Extracts of the edible muscle of common commercially available crustaceans, including lobster (genus *Homarus*), crab (genus *Cancer*), shrimp (genus *Panilurus*) were prepared† by standard methods of extraction in glycerinated buffered saline. Similar extraction techniques were employed in our laboratory with abdominal muscles of fresh-water crayfish obtained on the open food market‡ and also collected by us from local lakes and streams.

Crustaceans of the "insect type" (entomostracan) employed were of two varieties. Water fleas (*Daphnia pulex*), taken with a fine-mesh net from a depth of 40 feet in a local oligotrophic lake between 10 A.M. and 2 P.M., were obtained in practically a pure collection. They were immediately frozen to prevent deterioration prior to extraction. Brine shrimp (*Artemia salina*),

From the Department of Biology, Wilkes College.

Presented in part at the fifteenth annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9 to 11, 1959.

This study was sponsored by the Committee on Food Allergy of the Research Council, American Academy of Allergy.

Received for publication Dec. 31, 1958.

*Associate Professor of Biological Research.

**Professor of Biology.

†These extracts were prepared and supplied through the courtesy of Margaret Strauss, Director of the Allergy Laboratory, University Hospital, New York University—Bellevue Medical Center.

‡Supplied through the courtesy of Dr. Vincent J. Derbes, Tulane University School of Medicine, New Orleans, Louisiana.

gathered from Great Salt Lake in a pure collection, were shipped to us in the frozen state from Utah.* Whole-body extracts of these tiny crustaceans (phyllopods) were prepared in buffered saline immediately upon thawing.

Immunizing dosages and schedules for the preparation of rabbit antisera and the agar-gel diffusion precipitin tests (Ouchterlony technique) for qualitative antigenic analyses were identical with those previously reported for the study of fish extracts.¹

RESULTS

The results of multiple agar-gel diffusion precipitin tests employing each crustacean extract with each homologous and heterologous antiserum are summarized in Table I.

TABLE I

EXTRACT	NUMBER OF PRECIPITIN BANDS ANTISERA TO:						
	LOBSTER	CRAB	SHRIMP	ROCK LOBSTER	CRAW- FISH	DAPHNIA	BRINE SHRIMP
Lobster	6-8	3	4	2	2	0	1
Crab	4	7-9	4	1	2	0	0
Shrimp	2-4	3-4	6	1	1	0	1
Rock lobster	2	1	3	5-7	2	0	1
Crayfish	3	3	2	3	4	2	2
Daphnia	0	0	0	0	1	6-7	1
Brine shrimp	1	0	1	1-2	1	1	3-4

The results of agar-gel diffusion precipitin tests employing randomly selected samples of skin-test crustacean extracts prepared in different laboratories, and presumably by the same methods, with homologous species antisera to the crustaceans are summarized in Table II.

TABLE II

ANTISERUM TO: + EXTRACT		NUMBER OF PRECIPITIN BANDS			
		A	B	C	D
Crab	Crab	7-9	8-9	0	0
Lobster	Lobster	6-7	1	5	
Shrimp	Shrimp	6	0-1	2-3	

COMMENT

The multiplicity of distinct antigenic components⁴⁻⁹ within the edible muscles of crustaceans has been demonstrated by their precipitin-producing properties in rabbits. It is to be pointed out that these figures may not include the total number of potential antigens for either lobster, crab, shrimp, rock lobster, or crayfish, for the respective immunizing extracts did not include either related blood hemocyanins²⁻³ or hepatopancreatic organs. Among the five common groups studied, only a partial degree of cross-antigenicity could be noted with these in vitro techniques. No more than two-thirds of

*Collected by and supplied through the courtesy of Mr. C. C. Saunders of Ogden, Utah.

the total antigenic fractions identifiable in any one species were demonstrated in reactions of identity, and in most instances the number of antigens common to any two species of crustaceans was found to be considerably less. Furthermore, in searching for reactions of identity between component antigens of more than two species, it was impossible to find bands of precipitate common to the five representative crustaceans studied. Thus, even though the taxonomic classification (Table III) of the class *Crustacea* places lobster, shrimp, crab, rock lobster, and crayfish in the same order, a relationship that might be characterized by a common antigenic denominator for their muscle proteins could not be established. Such a relationship could explain variations in uniformity of skin-test patterns and clinical manifestations of hypersensitivity. Determinants of such reactions would then include not only a quantitative factor of group antigen⁴ but also variations in content of qualitatively distinct serum reagins to species-specific antigens in crustacean-allergic patients. At this stage, however, the evidence for relating allergenic constituents of a food or food extract to its precipitin-producing antigenic components can be considered only presumptive.

TABLE III. CLASSIFICATION OF CRUSTACEANS INCLUDED IN THIS STUDY

Phylum Arthropoda	
Class Crustacea	
Subclass Entomostraca	
Order Branchiopoda	
Suborder Phyllopoda	<i>Artemia salina</i>
Suborder Cladocera	<i>Daphnia pulex</i>
Subclass Malacostraca	
Division Eumalacostraca	
Order Decapoda	
Genus Homarus	lobster
Genus Penaeus	commercial shrimp
Genus Panilurus	rock lobster
Genus Cambarus	fresh-water crayfish
Genus Cancer	crab

The importance of insect allergy is receiving increasing attention. The current status of this intriguing subject has been recently reviewed and summarized by Perlman.⁵ It is to be noted that organisms placed in the classes *Crustacea* and *Insecta* are collectively members of the same phylum (*Arthropoda*) and that entomostracans (daphnia and brine shrimp), because of their small size, have often been too loosely categorized as "insects." Therefore, a study of the antigenic relationship of daphnia and brine shrimp to larger edible shellfish appeared to be of more than academic interest in the consideration of crustacean allergy.

The usual diurnal habitat of daphnia in fresh-water lakes places them at greater depths than are likely to be encountered by the average swimmer. However, it is possible that they might come into contact with shellfish-allergic human beings in shallow ponds and drainage ditches or by the inhalation of daphnia "dust" resulting from the handling of compressed dried cakes of these organisms which are available commercially as a source of fish food. Instances of allergic reactions in such circumstances have been reported.⁶⁻⁷ Frozen brine

shrimp have also been made available to tropical fish hobbyists, and Perlman⁵ points out possible additional sources of contact to ocean swimmers and to residents in areas where the tiny eggs of the brine shrimp could conceivably be part of the atmospheric dust.

Among members of the order *Decapoda* (lobster, shrimp, rock lobster, crayfish) possessing multiple common antigens, only crab failed to exhibit cross-antigenicity with brine shrimp by the common bond of at least one antigenic fraction. *Daphnia pulex*, on the other hand, demonstrated antigenic constituents which were common only to brine shrimp and crayfish. Its closer antigenic relationship to brine shrimp might be suspected from their taxonomic relationship within the same order, *Branchiopoda* (Table III). It is also to be pointed out that, whereas extracts of daphnia and brine shrimp were prepared from whole organisms including their hemocyanins and hepatopancreatic organs, extracts of the larger crustaceans included only those antigens characteristic of edible abdominal muscle. The exact reason for selective cross-antigenicity of daphnia with only one member of the order *Decapoda* (crayfish) is not apparent. However, one might suspect this to exist on an ecologic basis, since they both are the only fresh-water crustaceans represented in this study. In further consideration of ecologic possibilities, it is of interest that the brine shrimp (*Artemia salina*) studied here evolved from fresh-water progenitors which were able to adapt to the increased salt content of the Great Salt Lake in Utah. That potential clinical allergy to daphnia and brine shrimp in shellfish-sensitive human beings may be related to a variable presence of reagins to specific crustacean antigenic fractions is further suggested by the studies of Perlman.^{5, 8} Crab- and lobster-sensitive patients often reacted to skin testing with daphnia and brine shrimp extracts, but in a number of circumstances positive reactions were produced for one crustacean species and not for another.

The wide variation in content of precipitin-producing antigens demonstrated among species-identical crustacean extracts prepared in different laboratories resembles a similar situation previously noted among fish extracts.¹ Although the instability of shellfish extracts has been mentioned,⁹ other possible factors under consideration and study by the Committee on Food Allergy of the Research Council of the American Academy of Allergy include the effect of preliminary freezing of the raw material, variations in technique of extraction and storage, and the addition of glycerin preservative. The importance of possible variations in qualitative antigenic content of shellfish extracts influencing results and interpretation of diagnostic skin tests is self-evident and a matter for our further attention and evaluation.

SUMMARY

1. Sera from specifically immunized rabbits were employed in agar-gel diffusion precipitin tests in the qualitative antigenic analyses of crustacean extracts.

2. Multiple antigenic components (4-9) were demonstrated in the edible abdominal muscles of lobster, crab, shrimp, lobster tail, and fresh-water crayfish and in the bodies of entomostracans ("insect type" crustaceans), daphnia and brine shrimp.

3. Only a partial degree of qualitative cross-antigenicity was noted among various taxonomically related edible shellfish, and an antigen common to all five could not be demonstrated.

4. Partial qualitative cross-antigenicity was noted among taxonomically related entomostracans ("insect-type" crustaceans), *Artemia salina* (brine shrimp) and *Daphnia pulex* (water fleas). Some antigenic components of brine shrimp were common to those of the edible malacostracans (shellfish), whereas daphnia contained antigen common only to ecologically related fresh-water crayfish.

5. A wide qualitative variation among individual component antigens of crustaceans was demonstrated in homologous species skin-testing extracts of different laboratory sources.

REFERENCES

1. Cohen, S. G.: Immune Sera in the Study of Food Antigens. I. Fish, J. ALLERGY 30: 250, 1959.
2. Leone, C. A.: Comparative Serology of Some Brachyuran Crustaceae and Studies in Hemocyanin Correspondence, Biol. Bull. 97: 273, 1949.
3. Boyden, A.: The Measurement and Significance of Serological Correspondence Among Proteins. In Cole, W. H. (editor): Serological Approaches to Studies of Protein Structure and Metabolism, New Brunswick, 1954, Rutgers University Press, pp. 74-97.
4. Tuft, L., Blumstein, G. I., and Usher, S.: Studies in Food Allergy. I. Antigenic Relationship of Shellfish, J. ALLERGY 11: 475, 1940.
5. Perlman, F.: Insects as Inhalant Allergens; Consideration of Aerobiology, Biochemistry, Preparation of Material, and Clinical Observations, J. ALLERGY 29: 302, 1958.
6. Way, K. D.: Water Flea Sensitivity; Case Report, J. ALLERGY 12: 495, 1940.
7. Charpin, I.: Semaine d. hôp. Paris, Oct. 26, 1957, cited in J. A. M. A. 163: 762, 1957.
8. Perlman, F.: Personal Communication.
9. Sherman, W. B., and Kessler, W. R.: Allergy in Pediatric Practice, St. Louis, 1957, The C. V. Mosby Company, p. 104.