STUDIES IN FOOD SENSITIVITY

II. The Effect of Protein Digestion on Antigenicity of Foods as Determined by Skin Tests and Clinical Food Trials

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IN THE diagnosis and treatment of allergic diseases, skin testing has been an important step in the attempts to determine the causative agents of any particular allergic disorder. In general, skin tests with pollens and inhalants, when properly performed and interpreted, have yielded sufficiently reliable information to be of diagnostic value. It is in skin testing with foods that we have encountered a stumbling block in our diagnostic approach to the etiology of allergic diseases. Here one finds so many inconsistencies in attempting to correlate the results of skin tests with the clinical symptoms that many allergists have come to the conclusion that skin testing with food extracts is of little or no value.

In 1923, Wacker, Wetmore, and Adkinson[1] studied the reactions in skin tests of artificial digests of food proteins as compared to standard extracts. They found that, with the exception of two cases, all cases that showed negative skin tests to the whole protein showed negative skin tests to the digests. From these observations they reached the conclusion that digestion products of proteins did not sensitize human beings and did not play a part in human allergy, and that, where sensitization took place, it was probably due to the assimilation into the blood stream of small quantities of unaltered protein.

However, as time went on, it became evident that positive skin tests could be obtained with substances other than unaltered proteins. In 1939 Winkenwerder, Buell and Howard[2] tested a group of ragweed cases with dilute solutions of nucleic acid, and in 50 cases obtained immediate wheal and erythema reactions. In ten of these patients, passive transfer reactions to nucleic acid were positive and in two of the ragweed patients, tested with nucleic acid, severe constitutional reactions developed.

Further studies along this line were made by Cooke and his co-workers[3] when they observed a fairly severe reaction occurring promptly in a patient after the administration of a second injection of tetanus toxoid. They found the reaction to be due to supposedly nonantigenic proteose, which was used in most tetanus culture media and which was present in tetanus toxoid. Subsequent studies with proteoses, from known sources, showed definite specific antigenicity in the guinea pig for primary proteoses of milk, whey, casein, beef, and chicken.[4]

These reports were followed by other studies regarding the significance of such findings as related to food sensitivities in human beings. It was assumed that, since the products of early protein digestion (primary proteoses) were antigenic, it might be possible to explain, as due to products of food digestion, certain clinical reactions which followed the ingestion of foods but in which

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skin tests to the original unaltered protein of such foods were negative. Using solutions of primary and secondary proteoses prepared from known sources, Cooke[5] tested a series of 39 allergic patients. In five patients he reported positive reactions to skin tests and passive transfer tests with these substances, whereas the standard extracts of these foods gave negative skin tests. These patients were all clinically sensitive to the foods in question. Cooke, therefore, arrived at the conclusion that clinical sensitization to protein digestion derivatives, at least at the proteose stage, does exist; and that, in some cases of food allergy, delayed clinical reactions can be explained on this basis. In such cases, skin tests with extracts of suitable preparations of artificially digested proteins give positive reactions.

Cooke's thesis was further substantiated by Blamoutier,[6] who studied a case of urticaria in which symptoms occurred approximately five to six hours after the ingestion of lamb, and lasted from twenty-four to thirty-six hours. Cutaneous (scratch) tests with standard extracts of lamb meat were repeatedly negative. However, when lamb meat was incubated with the patient's gastric and duodenal juice in succession, the resultant substance gave a positive reaction on skin test and on passive transfer, whereas tests with gastric and duodenal juice alone were negative.

However, there is also some evidence that failure to demonstrate positive skin tests in certain cases of clear cut clinical food allergy may not be due to changes in the antigens as a result of digestion but merely failure to employ, for testing, the antigens in an active form. Tuft and Blumstein[7] have demonstrated that the antigens of berries and certain other fruits were easily destroyed in the routine preparation of extracts for skin testing. In patients with negative skin tests to standard extracts, but with known clinical sensitivities to these foods, scratch tests with the fresh juice evoked positive skin tests.

More recently Ancona and Schumacher[8] have demonstrated a similar phenomenon in patients allergic to shellfish. In 14 patients definitely clinically allergic to shrimp, three different commercial extracts gave little or no reaction on skin test, but fresh or quick-frozen material gave positive reactions on scratch tests. They, therefore, concluded that fresh or quick-frozen materials are superior to standard extracts for skin testing. In a later report, Ancona and his associates[9] demonstrated positive scratch tests to crab meat in a case of purpura although tests with the corresponding commercial extracts by scratch and intradermal tests were negative. According to the authors, this may possibly be due to the presence of a themolabile factor in fresh food which either is removed by Seitz filtration and, therefore, absent in commercial extracts, or deteriorates in time at room temperature. It will be of interest to see this problem developed further with a greater variety of foods.

From the foregoing it is evident that there is a difference of opinion among investigators regarding the explanation for negative results of skin tests with standard extracts of foods which produce definite clinical reactions after ingestion. Since the majority of such clinical reactions associated with negative skin tests are of a delayed type, this investigation is concerned with the effect of digestion of foods on the antigenicity of such foods, as indicated by the skin

test, by the correlation of such skin test reactions with the results of intentional feeding trials, and the rapidity of such clinical reactions as occurred. We have attempted to determine whether or not digestion altered the antigenicity of a food so that the resulting complex was capable of producing a positive reaction on skin testing, which was not elicited by the unaltered food. If this occurred, the clinical reaction following the ingestion of the food should be of the delayed type, the delay theoretically being due to the lapse of time required for the production by digestion of the antigen to which the individual was sensitive and which was the actual cause of the clinical reaction.

MATERIALS USED

The following ten foods were utilized for the study: cocoa, orange, beef, wheat, milk, egg, chicken, corn, potato, and string bean. Extracts of these foods were made in the usual manner and standardized according to the protein nitrogen unit system. Digests of each of these foods, similarly standardized, were prepared* as follows:

Three hundred grams of food were placed in one liter of water and sodium hydroxide was added to pH 7.5. The extraction was continued for two hours. At this time the mixture was acidified to pH 2.5 with hydrochloric acid, 0.1 Gm. of pepsin was added and the mixture was incubated at 50° C. for 24 hours. A 300 ml. aliquot was then removed, the pH adjusted to 5, and the material boiled and filtered. The pH was then adjusted to 7 and Merthiolate was added in a 1-10,000 concentration. This was labeled the pepsin digest.

The remainder of the initial extraction was then adjusted to pH 8.5 and 10 ml. of chloroform and 1 Gm. of trypsin were added to it. Digestion proceeded for 24 hours at 40° C. The mixture was then boiled, centrifuged, filtered, and the pH adjusted to 8. Merthiolate was added in a 1-10,000 concentration, and this was designated the trypsin digest.

PROCEDURE

Thirty patients with various types of allergic disorders were picked at random for testing with the ten foods under study. Each patient was tested with the standard extract, pepsin digest, and trypsin digest in equal concentrations. Tests were made by the intracutaneous method and were read after an interval of twenty minutes. The skin test reactions were recorded as slight, moderate, or marked.

After the completion of all tests, each patient was given an intentional feeding test with each of the foods under study. Where the clinical trial was positive, the time interval between the ingestion of the food and the onset of clinical symptoms was noted. Differences in skin test reactions between the digests and the standard extracts were noted and correlated with the clinical reaction time. Of special importance in this study were positive skin reactions to one or both digests of a food of which the standard extract gave a negative skin test.

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FINDINGS

Of 125 negative tests with standard extracts, 56 were negative on tests with both the pepsin and trypsin digests, 26 were positive on test with the pepsin digest, 29 positive on test with the trypsin digest, and 14 were positive with both pepsin and trypsin digests. The majority of the positive reactions were slight reactions. Thus this group included many instances of negative skin reactions to standard extracts associated with positive reactions to the digests of the same foods.

Of the 195 positive reactions to skin tests with the standard extracts, 75 were positive to the standard extract alone, 34 were positive also with the pepsin digest, 41 to the trypsin digest, and 45 to both the pepsin and trypsin digests. Thus, 116 were negative to the pepsin digest, and 109 were negative to the trypsin digest. In this group, positive reactions to the artificial food digest extracts may be due to the incomplete digestion of the foods during the process of artificial digestion. These results are summarized in Table I.

TABLE I. RESULTS OF SKIN TESTS WITH STANDARD EXTRACTS OF 10 COMMON FOODS AND WITH THE PEPSIN AND TRYPSIN DIGESTS OF THESE FOODS IN 30 ALLERGY PATIENTS

Total Skin Tests Recorded	320	
Positive skin tests with standard extracts		195
Negative skin tests with standard extracts		125
Positive Skin Tests with Standard Extracts	195	
Positive also with pepsin digest extract		34
Positive also with trypsin digest extract		41
Positive also with trypsin and pepsin digest extracts		45
Positive with standard extract only		75
Negative Skin Tests with Standard Extracts	125	
With positive skin tests with pepsin digest extract		26
With positive skin tests with trypsin digest extract		29
With positive skin tests with pepsin and trypsin digest extracts		14
With negative skin tests with pepsin and trypsin digest extracts		56

Of 268 intentional feeding tests with the ten foods under study, 16 yielded positive clinical trials. Of the 16 positive clinical reactions, 11, or 4 per cent, occurred with foods giving positive skin reactions to the standard extracts, and 5, or 2 per cent, occurred with foods giving negative skin tests to the standard extracts. Of the 11 positive clinical trials with foods yielding positive skin reactions to standard extracts, 7 gave positive reactions on skin test to the digest extracts also, while in 4 cases, the skin test which was positive with the standard extract was negative with the digests. Of more importance, of the 5 positive clinical trials in which skin tests with the standard extracts were negative, the skin tests with the digest extracts were negative in 2 cases, but were positive in 3 cases. The results are summarized in Table II.

TABLE II. RESULTS OF 268 INTENTIONAL FEEDING TESTS IN 30 ALLERGY PATIENTS WITH POSITIVE OR NEGATIVE SKIN TEST REACTIONS TO STANDARD, PEPSIN DIGEST AND TRYPSIN DIGEST EXTRACTS OF 10 COMMON FOODS

Total Intentional Feeding Tests	268		
Negative feeding tests		252	
Positive feeding tests		16 (6%)	
Résumé of Positive Intentional Feeding Tests With Foods in Relation to Skin Tests:			
Number of positive feeding tests	16		
With positive skin tests to standard extracts		11 (4%)	
Together with positive tests with digest extracts			7 (2.6%)
Together with negative tests with digest extracts			4 (1.6%)
With negative skin tests to standard extracts		5 (1.9%)	
Together with positive tests with digest extracts			3 (1.1%)
Together with negative tests with digest extracts			2 (0.8%)

Of the 16 positive reactions to clinical trials, 8 were of the immediate type, with onset of symptoms in 5 to 30 minutes. In this study, clinical reactions, with the onset of symptoms within one hour, were considered immediate reactions, while those occurring after one hour, were considered delayed reactions. Of interest in this group is the fact that in 2 instances the skin tests were negative with the standard extracts, but positive to the digest extracts, whereas in 2 other instances the skin tests to all extracts, both natural and digests, were negative. The remaining 8 clinical trials were of the delayed reacting type. Of interest in this group is the fact that, in 5 cases, tests were positive to both the standard and digest extracts, and in two other instances there was a delayed clinical reaction in which positive skin tests were elicited with the standard extract only. The one remaining case in this group is the only one which conforms to all the criteria of the theory of specific allergy to products of digestion of foods, having a delayed clinical reaction, negative skin test with the standard extract and positive skin tests to the digests. These results are summarized in Tables III and IV.

TABLE III. RELATIONSHIP BETWEEN THE POSITIVE CLINICAL REACTIONS AND THE SKIN TESTS WITH STANDARD AND DIGEST EXTRACTS OF 10 FOODS

	IMMEDIATE REACTION	DELAYED REACTION
With positive skin tests to standard extracts only	2	2
With positive skin tests both to standard extracts and to digest extracts	2	5
With negative skin tests to standard extracts but with positive reactions to digest extract	2	1
With negative skin tests to both standard extracts and digest extracts	2	0
Number of Positive Clinical Reactions	16	

TABLE IV. POSITIVE REACTIONS TO FEEDING TESTS

PATIENT	DIAGNOSIS	FOOD TESTED	SKIN TEST RESULTS		TYPES OF CLINICAL REACTION	REACTION TIME	DURATION OF REACTION	SYMPTOMS OF REACTIONS
			STANDARD EXTRACT	DIGEST EXTRACT				
1. R.S.	Asthma	Cocoa	#	0	Immediate	10 min.	20 min.	Cough
2. N.G.	Asthma	Cocoa	0	#	Immediate	30 min.	30 min.	Itching of body
3. G.F.	Asthma Rhinitis	Cocoa	#	0	Immediate	5-10 min.	3-4 hrs.	Wheezing, nasal itching, and clogging
4. N.G.	Asthma	Orange	0	0	Immediate	30 min.	1 hour	Wheeze and cough
5. R.D.	Rhinitis	Milk	#	#	Immediate	30 min.	24 hours	Itching rash on neck rhinorrhea, cough
6. R.S.	Asthma	Wheat	0	#	Immediate	30 min.	5 min.	Cough
7. L.S.	Eczema	Orange	0	0	Immediate	10 min.	3-4 hrs.	Rash on face
8. E.H.	Asthma	Stringbean	# #	#	Immediate	30 min.	15 min.	Rhinorrhea after fresh and canned stringbeans
9. J.S.	Asthma	Cocoa	#	0	Delayed	3 hours	2-3 hrs.	Heaviness on chest
10. L.K.	Hay Fever Eczema	Orange	0	#	Delayed	6 hours	1 hour	Intense itching of eyes
11. N.G.	Asthma	Milk	#	#	Delayed	3 hours	12 hours	Itching scalp with scab formation
12. F.R.	Allergic arthritis	Orange	#	#	Delayed	12 hours	All Day	Painful, swollen joints of hand
13. L.G.	Asthma	Corn	#	#	Delayed	2 hours	24 hours	Sneezing, rhinorrhea
14. M.A.	Neuro-dermatitis	Corn	# #	#	Delayed	12 hours	3-4 hrs.	Body itch
15. S.B.	Asthma	Stringbean	# #	#	Delayed	2 hours	12 hours	Heaviness on chest
16. J.S.	Asthma	Stringbean	#	0	Delayed	5 hours	8 hours	Heaviness on chest, asthmatic attack relieved with ephedrine

0 = Negative Reaction; # = Slight Reaction; # # = Moderate Reaction.

DISCUSSION

It is conceded at the outset that the number of foods used in this study was limited and that the number of patients tested was small. Since, however, this study was concerned with the problem that digestion could alter the chemical composition of a food in such a way that the end result may prove to be the provocative agent in cases of food allergy, we sought confirmation of this from the effect of such food digestion on the skin test. The opinion has been expressed by others that certain delayed clinical reactions to foods are due to the effects of digestion upon foods and that the patient is not sensitive to the unaltered protein of the food eaten, but to the product of its digestion. We attempted to demonstrate sensitivity to digests of foods by means of the skin test and to confirm such sensitivity with the intentional feeding test, necessarily of the delayed type.

Changes in food skin test reactions from negative to positive, as a result of food digestion, suggest that a new antigen has been formed which is responsible for the clinical response. There were numerous instances in this study of changes in skin reactions with the digest extracts, but these were not confirmed clinically by intentional feeding tests. Of a total of 268 clinical trials, 16, or 6 per cent, were positive, compared to 3.1 per cent in our previous study.[10] Of these 16 positive trials, 11, or 4 per cent, occurred with foods giving positive skin reactions, compared with 4.5 per cent in our previous study.[10] Out of the 16 positive trials, 5 occurred to foods that yielded negative skin tests with standard extracts, an incidence of 2 per cent as compared to 1.9 per cent in our previous study.[10] In 3 of these 5 cases, the positive clinical food trials occurred with foods that reacted negatively in the skin with the standard extracts but gave positive reactions with the digest extracts. However, in only one of these 3 positive clinical trials did the clinical reaction appear as a delayed reaction. In the other 2 trials the reactions were of the immediate type. This emphasizes the infrequency of that type of food allergy resulting from sensitivity to products of food digestion.

However, some of the findings in this study are confusing and difficult of explanation. In two instances there occurred positive clinical reactions of the immediate type to foods giving negative skin tests to both standard and digest extracts. The mechanism of this type of reaction is unknown, and the explanation commonly offered is that the shock organ is sensitive and reactive on contact with the allergen, while the skin as an organ has not as yet become sensitive to provide the positive skin test. Tuft and Blumstein,[7] as well as Ancona and associates,[8, 9] regard these as a type of false negative reaction due either to the loss of the antigenic fraction during the routine preparation of extracts, or to the presence of a thermolabile fraction in the antigen which is destroyed on exposure to room temperature. On the other hand, there were 5 instances of positive clinical reactions of the delayed type to foods which gave positive skin reactions with both the standard and digest extracts. It is possible that the delayed reactions were due to the cumulative effect of the gradual absorption of

the unaltered food as well as the products of the digestion, since all extracts reacted positively in the skin and the individuals were presumably sensitive to a slight degree to the unaltered foods and their digestion products.

Two delayed reactions with foods yielding positive skin tests with the standard extracts only, may have been, in reality, immediate type of reactions, occurring three hours after ingestion of the foods. There is some confusion as to what constitutes a delayed reaction. Cooke[11] states that the immediate reaction is one that occurs within one hour after contact with an antigen, but in describing a delayed reaction[11] he states that the delayed reactions are those occurring several hours to several days after contact with the allergen. Again he states[11] that the skin sensitive group is characterized by a swift reaction time varying from a fraction of a second to several hours between contact with the exciting substance and the development of symptoms, while the food sensitive cases negative by skin test do not develop symptoms until an interval usually of 24 hours or longer has elapsed following the ingestion of the offending food. In this study, clinical reactions from intentional feedings occurring within one hour have been interpreted as immediate reactions, while those occurring beyond one hour, as delayed reactions.

That clinical symptoms can and do occur from foods yielding negative skin reactions is admitted by most observers. In many such instances the reaction is of the delayed type, and if due to the result of food digestion, the delay should be equal to the period of time necessary for the food digestion and the production of a new antigen. The incidence of such delayed clinical reactions, due to food digestion, is not stated by other observers but from the results of this study they are very few in number. From the results of this study, it is evident that the food digestion theory is not the explanation for the many cases of asthma, urticaria, rhinitis, or migraine with negative skin tests. Cases that can be proved to be due to specific sensitization to the products of food digestion appear to be limited in number.

The results of this study with 268 clinical food trials, when compared with our previous study[10] with 2,467 clinical food trials are so similar as to confirm our previous conclusions that foods play a minor role to pollens, inhalants and infection in the majority of allergic individuals.

SUMMARY

Thirty patients with various allergic manifestations were tested with standard extracts and with the pepsin and trypsin digest extracts of ten common foods.

Confirmatory clinical trials with the ten foods were made for each skin test, both positive and negative, by means of intentional feedings.

The results of the skin tests with the standard extracts, compared with their pepsin and trypsin digests, are suggestive of changes in the antigenicity of foods due to digestion.

Out of 16 positive feeding tests, however, clinical confirmation of a change in the antigenicity of foods due to digestion was obtained in only one instance

wherein the standard extract failed to react in the skin, the digest extracts reacted positively, and the positive clinical reaction with the intentional feeding was of a delayed type.

The results of this limited study suggest that changes in the antigenicity of foods as a result of digestion, as indicated by skin tests and intentional feeding tests, are an infrequent occurrence. In the search for the etiologic agent in nonreactive cases, the use of digest extracts for testing purposes will offer no advantage over a thorough and carefully taken history, confirmed by test feedings.

This study confirms our previous conclusions that foods play a minor role to pollens, inhalants, and infection in cases of allergy.

REFERENCES

1. Wacker, I. C., Wetmore, A., and Adkinson, J.: Sensitization Tests With Digestive Products of Protein, Arch. Int. Med. **32**: 323, 1923.
2. Winkenwerder, W. L., Buell, M. V., and Howard, J. E.: The Sensitizing Properties of the Nucleic Acids and Their Derivitives, Science **90**: 2337, 1939.
3. Cooke, R. A., Hampton, S. F., Sherman, W. B., and Stull, A.: Allergy Induced by Immunization With Tetanus Toxoid, J. A. M. A. **114**: 1854, 1940.
4. Stull, A., and Hampton, S. F.: Antigenic Studies of Proteoses, J. Immunol. **41**: 143, 1941.
5. Cooke, R. A.: Protein Derivitives as Factors in Allergy, Ann. Int. Med. **16**: 71, 1942.
6. Blamoutier, P.: Allergie Digestive: Sensibilisation a un Produit de Désintégration de la Proteine Alimentaire, Presse Med. **53**: 162, 1945.
7. Tuft, L., and Blumstein, G. I.: Studies in Food Allergy, J. ALLERGY **13**: 574, 1942; J. ALLERGY **15**: 346, 1944.
8. Ancona, G. R., and Schumacher, I. C.: The Use of Raw Foods as Testing Material in Allergic Disorders, Cal. Med. **73**: 473, 1950.
9. Ancona, G. R., Ellenhorn, M. J., and Falconer, E. H.: Purpura Due to Food Sensitivity —Use of Skin Testing in Etiological Diagnosis, J. ALLERGY **22**: 487, 1951.
10. Leibowitz, H., Chester, A., and Markow, H.: Importance of Foods in Patients as Determined by Skin Testing and Intentional Feeding, J. A. M. A. **144**: 990, 1950.
11. Cooke, R. A.: Allergy in Theory and Practice, Philadelphia, 1947, W. B. Saunders Co., p. 25, 27, 117.