

ON ALLERGY TO COTTONSEED OIL

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The question of specific sensitiveness to edible cottonseed oil in contradistinction to cottonseed is worthy of recognition and more critical study than it has received. Discussions of cottonseed sensitiveness in textbooks on allergy generally imply that cutaneous sensitiveness to allergenic extracts of cottonseed or cottonseed meal signifies clinical sensitiveness to foods containing salad oil or vegetable shortening made from cottonseed oil. A pronounced difference of opinion concerning this concept is displayed in the testimony of six allergists who appeared as expert witnesses at a public hearing¹ in relation to label declaration of vegetable oils in salad dressings.

Opportunity to study allergy to cottonseed oil is rare.¹ Hence, worthy of record are results of a concurrent comparison of two cottonseed-sensitive allergic subjects, one of whom disclaimed and the other proclaimed a demonstrable sensitiveness to edible cottonseed oil.

REPORT OF CASES

CASE 1.—A male asthmatic patient (S.), age 34, exhibited multiple sensitiveness in cutaneous tests with several allergens, including cotton and cottonseed. His clinical history showed convincingly negative reactions to cottonseed oil. This conclusion was confirmed by oral administration, under observation, of 1 fluidounce (29.6 cc.) of cottonseed salad oil. Ingestion of this dose three hours after breakfast induced no immediate or delayed symptoms. At a later visit a specimen of blood was taken from this patient, the source of serum S.

CASE 2.—An allergic subject (O.) was not examined in person, but his own diagnosis of his sensitiveness to cottonseed was accepted and is reported without prejudice in acknowledgment of his respected standing as an allergist of extensive clinical and research experience.

The family history of this subject is positive for allergy. Hay fever, identified with pollen sensitivity, developed in 1930. Two years later he was afflicted with hives, which could be induced by trial contact with nonabsorbent cotton or by sleeping on a cotton-filled mattress. At this time, abdominal discomfort, which he attributed to ingestion of foods containing hydrogenated vegetable oil shortenings, led him to suspect a pronounced sensitiveness to edible cottonseed oil. This diagnosis, he concluded, was confirmed by his positive reactions to cutaneous tests with cottonseed extract. Clinical sensitiveness to several other foods became evident in 1943, proved by dietary trial and elimination from the diet. However, only his clinical evidence in support of the diagnosis of his acute allergic sensitiveness to cottonseed oil is pertinent to the present study.

This subject endeavored to exclude from his diet any food that might contain cottonseed oil or hydrogenated cottonseed oil even as an ingredient of the least proportion. Allergic symptoms resulting from unintentional ingestion of such foods were hives, prolonged abdominal distress, including acute gripping pain, and diarrhea. These symptoms could be relieved or diminished with antihistaminic drugs.

In contrast to the invariable allergic reaction resulting from eating foods containing cottonseed oil, this subject stated that

ingestion of corn oil or olive oil caused him no distress. These oils were regularly used in his home. He was confident, therefore, that he could distinguish cottonseed oil from corn oil or olive oil by the consistent occurrence of perceptible allergic symptoms whenever he ingested even a comparatively small quantity of cottonseed oil. But he was reluctant to undergo the discomfort required to demonstrate this capacity. Fortunately, however, this subject was keenly interested in our studies of allergens of cottonseed products and complied with our request for his collaboration. On Jan. 3, 1948, we received from him a quantity of his blood serum, which is identified as serum O. Subsequently, he served as subject for a succession of discrimination trials to test his capacity to distinguish cottonseed oil by his symptoms of allergy.

PASSIVE TRANSFER TESTS (SERUM S AND SERUM O)

Serum S and Serum O were investigated in comparative tests for reagins for cottonseed oil and for cottonseed. Reactivity of passively sensitized sites was determined by intracutaneous tests with cotton seed oil and with allergenic extracts of defatted cottonseed and, also, by oral administration of both oil and defatted cottonseed.

Nine recipients were given an intracutaneous injection in the left upper arm with 0.1 cc. of serum O. After an interval of three or more days, four sites were tested with 0.025 cc. of sterile cottonseed oil; three sites were tested with 0.01 mg. of CS-1A (an active protein component of cottonseed)²; and two sites were tested with unfractionated cottonseed extract (1:50,000). The skin of the anterior surface of the lower arm served for control injections. The controls and all passively transferred sites gave negative reactions in these tests.

Five recipients were sensitized with 0.1 cc. each of serum S and serum O in each upper arm. The sites were separated by about 2 inches (5 cm.). Four days later one serum S site and one serum O site on each of the 5 recipients were tested with an intracutaneous injection of cottonseed oil. All results were negative. One serum S site and one serum O site on each of 3 recipients were tested with cottonseed extract. The three serum S sites gave vigorous positive reactions. All three serum O sites were inactive. One serum S site and one serum O site on each of the 2 remaining recipients were tested with CS-1A. The two serum S sites gave vigorous positive reactions; both serum O sites gave negative reactions.

For the tests by oral administration, 5 subjects were sensitized in the left upper arm with 0.1 cc. of serum S and in the right upper arm with 0.1 cc. of serum O. Three subjects, members of our laboratory staff, were similarly sensitized with 0.05 cc. of the respective serums. All subjects were advised to avoid food products containing cottonseed oil for the duration of the experiment. One to three days later, with fasting stomach, 3 of the subjects drank 60 cc. and 3 subjects drank 100 cc. of cottonseed oil. They were kept under observation for one hour or more. There was no itching, swelling, or redness at the passively sensitized sites.

Two of the subjects refused to take the oil. Accordingly, their sensitized sites were tested intracutaneously with cottonseed oil, with negative results.

Six days after the ingestion of the 100 cc. dose of cottonseed oil, each of the 3 laboratory personnel

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1. Allergy to Cottonseed and Other Oil Seeds and Their Edible Derivatives, excerpts from testimony before the Administrator, Federal Security Agency, Docket FDC-51, Public Hearings Held at Washington, D. C., Nov. 18, 1947 and Jan. 6-8, 1948 (Memphis, Tenn., National Cottonseed Products Association, Inc., 1948).

2. Spies, J. R.; Coulson, E. J.; Bernton, H. S., and Stevens, H.: The Chemistry of Allergens: II. Isolation and Properties of an Active Protein Component of Cottonseed, *J. Am. Chem. Soc.* **62**: 1420 (June) 1940.

ingested 10 Gm. of defatted cottonseed in water. Whealing became perceptible on the left arm, at the serum S site, eight, fifteen and twenty-seven minutes, respectively, after ingestion. The serum O sites remained inactive.

TABLE 1.—*Discrimination Trials, Series I*

Vial	Submitted	Response	Net Dose and Identity of Sample
E	April 1	Positive	No cottonseed oil; 4.1 Gm. corn oil
H	April 5	Negative	No cottonseed oil; 4.0 Gm. corn oil
F	April 12	Negative	0.6 Gm. cottonseed oil; 3.5 Gm. corn oil
A	April 19	Positive	No cottonseed oil; 3.9 Gm. corn oil
D	April 26	Negative	No cottonseed oil; 3.9 Gm. corn oil
B	May 3	Negative	0.6 Gm. cottonseed oil; 3.3 Gm. corn oil
C			No cottonseed oil; corn oil
G			No cottonseed oil; corn oil

Results of the passive transfer tests were consistent. The presence of reagin for cottonseed was demonstrated in serum S; the absence of this reagin was proved for serum O. Each site sensitized with serum S reacted positively whether the water-soluble allergen of the cottonseed embryo was administered by direct injection into the sensitized site or by ingestion, while none of the sites sensitized with serum O showed any perceptible response. The tests employed failed to show the presence of a reagin for cottonseed oil in either serum S or serum O. After these tests were completed subject O (case 2) reported that he reacted negatively to cutaneous tests with corn oil, cottonseed oil and CS-1A, but an intracutaneous test with his own cottonseed extract elicited a positive reaction.

TESTS OF DISCRIMINATIVE CAPACITY

Three series of ingestion trials were conducted to test the capacity of subject O to discriminate between samples of vegetable oil that were alike in color, viscosity, flavor and odor, but which differed with respect to the presence or absence of cottonseed oil. Series I and II were designed to yield statistically significant evidence of discrimination, or lack of it. Series III

TABLE 2.—*Discrimination Trials, Series II*

Vial	Submitted	Response	Net Dose and Identity of Sample
K	May 18	Negative	2.1 Gm. corn oil; 2.1 Gm. olive oil
N	May 24	Negative	1.9 Gm. corn oil; 1.9 Gm. olive oil
O	June 1	Negative	2.2 Gm. cottonseed oil; 2.2 Gm. olive oil
J	June 7	Positive	2.0 Gm. corn oil; 2.0 Gm. olive oil
M	June 14	Negative	1.9 Gm. cottonseed oil; 1.9 Gm. olive oil
I	June 21	Negative	2.0 Gm. corn oil; 2.0 Gm. olive oil
P	June 28	Negative	1.9 Gm. corn oil; 1.9 Gm. olive oil
L	July 6	Negative	1.2 Gm.* cottonseed oil; 1.2 Gm.* olive oil

* About one third of sample spilled; net dose was estimated.

was designed to find the subject's threshold of allergic response to cottonseed oil.

Corn oil, cottonseed oil and olive oil used in these trials were purchased at retail and represent familiar brands of these salad oils. The manufacturer's representation of identity of each oil was confirmed. Each oil met the standards prescribed for it by the United States Pharmacopeia XIII.

Individual samples of each series were matched in appearance by addition of butter color. Samples comprising each series were judged by three chemists to be indistinguishable in taste and appearance. Series I and II were submitted in 4 cc. vials, each containing a single trial dose of about 4 Gm. of oil. For series III, 7.5 cc. vials were used to provide a range of larger doses of cottonseed oil.

In each series both the distribution of the samples containing cottonseed oil and the order of presentation of samples were randomized. The subject was told in advance of the trials the number of samples to be submitted in each series, the approximate dose and the number of times cottonseed oil might be encountered. Neither the subject nor the dispenser of the vials was informed of the composition of the individual samples until the last report of each series had been recorded.

The subject complied with our instructions to ingest each sample without other food when free from symptoms of allergy. He returned each emptied vial with

TABLE 3.—*Discrimination Trials, Series III**

Subject's Comment	Subject's Decision	Vial	Cottonseed Oil	
			Gm.	Percentage
Not the slightest discomfort (no. 4, 5, 6) or no recollection of discomfort (no. 8). Definitely negative	Definitely negative, no. 4,5,6,8	4	None	
		5	3.6	50
		6	None	
Slight, transient abdominal discomfort; ignored unless looking for symptoms. Not quite definitely negative	Qualified negative, no. 1,2,9	8	1.9	25
		1	7.3	100
		2	3.6	50
Abdominal pain mild but noticeable, lasting to one-half hour. Reaction from no. 0 was more than from no. 3. Considered doubtful positive reactions	Qualified positive, no. 0,3	9	5.6	75
		0	1.9	25
Definitely positive reactions. Prompt appearance (10 min.) of moderate to severe abdominal pain, persisting for 1 hour or more. Unquestionably positive reactions	Definitely positive, no. 7,10	3	5.6	75
		7	1.9	25
		10	3.6	50

* Order of ingestion of samples: No. 10, 6, 7, 4, 2, 1, 9, 3, 5, 8, 0.

a report of his decision, positive or negative, to indicate presence or absence of cottonseed oil, according to his perception of allergic response. Each succeeding sample, after the first one of series I and II, was submitted on return of the emptied vial of the preceding sample. The net weight of each sample ingested and the net dose of cottonseed oil were calculated from the difference in weights of each filled and emptied vial.

The last two samples of series I were not submitted because the results of the preceding six trials provided convincing evidence of inability of the subject to distinguish cottonseed oil in dosage of 0.6 Gm. When this evidence was presented to the subject he concluded that he was less sensitive to cottonseed oil than he had presumed. Accordingly, he agreed to try another series in which a larger dose of cottonseed oil would be included in two or three of eight samples. For benefit of flavor the samples of series II were composed either of equal parts of olive oil and corn oil or olive oil and cottonseed oil.

Results of series II provided conclusive evidence that the subject did not distinguish cottonseed oil when ingested in doses corresponding to quantities of this fat commonly encountered in a dietary portion of staple

food—salad or bread, for example. This evidence prompted the subject to propose a trial to establish his threshold of allergic sensitiveness to cottonseed oil. Series III was designed for that purpose.

Eleven samples of series III were submitted to the subject with the information that they were composed of samples of corn oil, and mixtures of corn and cottonseed oil that provided net doses of cottonseed oil in a range within and above the quantities ingested in the preceding series. Because series III was designed to establish the threshold of allergic response to cottonseed oil, the subject chose to qualify his response to each trial. His responses are classified and summarized in table 3.

Results of series III provided no evidence of discrimination consistently relatable to net dosage of cottonseed oil. Therefore, no evidence is deducible from these data of a threshold of sensitiveness to an allegedly allergenic component of the samples composed of corn and/or cottonseed oil. This series provided two samples of 7.3 Gm. of corn oil and the following net doses of cottonseed oil: three of 1.9 Gm., three of 3.6 Gm., two of 5.6 Gm. and one of 7.3 Gm.

COMMENT

Two cottonseed-sensitive allergic subjects have been studied. They both exhibited cutaneous sensitiveness to aqueous extracts of defatted cottonseed and to cotton. With respect to evidence of clinical sensitiveness to edible cottonseed oil these subjects, S and O, represent opposite extremes. Subject S reported no symptoms suggestive of clinical sensitiveness to cottonseed oil. On the other hand, subject O supported his own positive diagnosis by a clinical history clearly indicative of a high degree of allergic sensitiveness to cottonseed oil. A unique opportunity was afforded by the coincident availability of these subjects to investigate relationships between cutaneous sensitiveness to allergenic extracts of the cottonseed embryo, and clinical sensitiveness to the edible oil derived from this part of the seed.

Comparison of serums of subject S and subject O by passive transfer technic demonstrated the reagin for cottonseed only in serum S. No evidence of sensitiveness to cottonseed oil was manifested in either subject by direct cutaneous or passive transfer tests with the oil. Oral administration of cottonseed oil to subject S confirmed his opinion that ingestion of this oil caused no perceptible reaction. Collaboration of subject O enabled us to conduct discrimination trials designed to test the validity of the clinical evidence of his sensitiveness to ingested cottonseed oil. These trials yielded conclusive evidence that he could not distinguish cottonseed oil from corn oil or olive oil by perception of allergic symptoms when he ingested samples containing cottonseed oil. Therefore, his diagnosis of clinical sensitiveness to cottonseed oil was not substantiated.

Results of our comparison of 2 cottonseed-sensitive subjects are relevant to certain of our previously reported investigations dealing with immunologic properties of cottonseed products. In 1940, Bernton, Spies and Stevens³ described a series of experiments which led to the following conclusions: (1) that edible cottonseed oil does not contain the water-soluble cottonseed allergen which is readily extractable from the defatted

cottonseed kernel (embryo) or from commercial cottonseed flour or meal and, (2) that cutaneous sensitiveness to such extracts does not imply clinical sensitiveness to cottonseed oil. Later, Coulson and Stevens⁴ reported the preparation of water-soluble antigenic fractions from widely different grades of cotton linters. Neither the higher grade of linters, which was essentially free from visible hull material, nor the lower grade, which was heavily laden with hull particles, contained the cottonseed antigen. These observations clearly distinguished the water-soluble antigen of the seed hairs from that of the cottonseed embryo. This distinction between antigens of different tissues of the entire ginned cottonseed justifies the inference that a corresponding distinction may be anticipated with respect to clinical sensitiveness to cotton linters and to cottonseed flour or meal. At least, substantial confirmatory evidence should be expected to support a claim of immunologic interrelationship in clinical sensitiveness to essentially different commodities derived from cottonseed.

In 1942 Figley⁵ reported his study of 2 cases of suspected sensitiveness to cottonseed and cottonseed oil. A tentative diagnosis of sensitiveness to the water-soluble seed allergens was not substantiated for either case by direct or by passive transfer tests. For both cases, however, the diagnosis of clinical sensitiveness to cottonseed oil appeared to be substantiated by results of a few ingestion trials, which, for one of the patients, were conducted in collaboration with one of us (H. S.). Subsequent study of both patients by Figley generated doubt about this diagnosis. Justifiable skepticism has since been expressed by Figley¹ concerning the adequacy of the published data⁵ for proof of clinical sensitiveness to cottonseed oil in either of these cases. Consequently, these 2 cases must be classed with those reported in the literature, and in legend, for which the diagnosis of clinical sensitiveness to ingested cottonseed oil lacks substantial confirmatory evidence. While allergy to ingested cottonseed oil may exist, no case on record has been found for which this diagnosis has been confirmed by conclusive, scientific evidence.

SUMMARY AND CONCLUSIONS

Two cottonseed-sensitive allergic cases have been compared with reference, principally, to diagnostic criteria of clinical sensitiveness to edible cottonseed oil. In 1 case, the diagnosis of specific sensitiveness to cottonseed, with no implied sensitiveness to ingested cottonseed oil, was corroborated by evidence derived from passive transfer studies and demonstration of tolerance to a liberal dose of cottonseed oil. In the other case, the diagnosis of clinical sensitiveness to ingested cottonseed oil was not confirmed. No evidence of discrimination was displayed by the subject when he endeavored to distinguish cottonseed oil solely by his perception of allergic reaction to ingested doses in the range of 0.6 to 7.3 Gm. Comparative study of these cases adds supporting evidence to the conclusion that specific sensitiveness to water-soluble, allergenic extracts of defatted cottonseed (embryo) does not imply clinical sensitiveness to edible cottonseed oil.

3. Bernton, H. S.; Spies, J. R., and Stevens, H.: Significance of Cottonseed Sensitiveness, *J. Allergy* **11**: 138 (Jan.) 1940.

4. Coulson, E. J., and Stevens, H.: Antigenic Relationship of Cotton Linters, Dust, and Dust Precursors, *J. Allergy* **11**: 537 (Sept.) 1940.

5. Figley, K. D.: Cottonseed Oil Sensitivity, *Letters Internat. Correspond. Club of Allergy* **5**: 30, 1942.