

Flow cytometry *with vision*

Introducing FlowSight

- > Capable
- > Intuitive
- > Affordable



amnis
www.amnis.com



This information is current as of May 23, 2012.

The Immunochemistry of Allergens : IX. The Relationship of Carbohydrate to the Antigenic Properties of the Allergenic Protein from Cottonseed

E. J. Coulson, Joseph R. Spies and Henry Stevens

J Immunol 1949; 62:171-182; ;
<http://www.jimmunol.org/content/62/2/171>

-
- | | |
|----------------------|--|
| Subscriptions | Information about subscribing to <i>The Journal of Immunology</i> is online at:
http://jimmunol.org/subscriptions |
| Permissions | Submit copyright permission requests at:
http://www.aai.org/ji/copyright.html |
| Email Alerts | Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/cgi/alerts/etoc |

The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 9650 Rockville Pike, Bethesda, MD 20814-3994. Copyright © 1949 by The American Association of Immunologists, Inc. All rights reserved. Print ISSN: 0022-1767 Online ISSN: 1550-6606.



THE IMMUNOCHEMISTRY OF ALLERGENS

IX. THE RELATIONSHIP OF CARBOHYDRATE TO THE ANTIGENIC PROPERTIES OF THE ALLERGENIC PROTEIN FROM COTTONSEED^{1, 2}

E. J. COULSON, JOSEPH R. SPIES, AND HENRY STEVENS

Received for publication December 31, 1948

Previous investigations of the composition of the allergenic fraction, CS-1A (1), from cottonseed have indicated the presence of at least 2 protein antigens (2) and 2 different polysaccharides (3). The principal allergenic protein of CS-1A is precipitable by picric acid (1). The second antigen is not precipitable by picric acid (2, 3) and probably is not commonly allergenic (3). With regard to the 2 polysaccharides present in CS-1A, there is convincing evidence that the less readily hydrolyzable one is combined with variable proportions of the allergenic protein (3). Thus, upon electrophoresis of CS-1A, fractions contained increased proportions of carbohydrate as they approached the anode. The readily hydrolyzable polysaccharide was separated from the polysaccharide-protein fractions by precipitation of the latter with picric acid. The anode fraction, CS-56R³, contained 13.2 per cent nitrogen and 35.2 per cent carbohydrate while the cathode fraction, CS-51R, contained 19.8 per cent nitrogen and 0.9 per cent carbohydrate. Fraction CS-51R consists principally of low-molecular-weight protein (4, 5).

No difference in antigenic specificity between fractions CS-56R and CS-51R could be detected by means of the Schultz-Dale test (2). Moreover, these fractions were equally effective in eliciting fatal shock in sensitized guinea pigs when comparison was based on nitrogen-content (6). Hence, protein components of these 2 fractions are believed to be immunologically identical. However, fraction CS-56R was 8 times more effective than CS-51R in inducing fatal anaphylactic sensitivity (6). The high sensitizing capacity of CS-56R was therefore tentatively attributed to association of the low-molecular-weight protein with the large polysaccharide molecule.

Dialysis through semi-permeable membranes effected a separation of the protein and carbohydrate components of CS-1A which was qualitatively similar to that obtained by electrophoresis (7, 8). Most of the polysaccharide components were retained within the dialyzing sac. A protein fraction possessing antigenic specificity identical to CS-51R was recovered from the dialyzate⁴ of an aqueous extract of cottonseed (7). This latter observation showed that the allergenic protein was native to cottonseed and was not formed by the procedure of isolation.

¹ Contribution from Allergen Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

² For Paper VIII of this series see Ref. (12).

³ The letter "R" in the symbol designation of a fraction denotes picric-acid precipitation of the preparation with subsequent removal of the picric acid.

⁴ The term "dialyzate" designates the substance which passes through the membrane.

In an attempt to elucidate further the relationship between carbohydrate and antigenic activity of the cottonseed allergen, this report presents a study of the antigenic potency (shocking and sensitizing capacities) and specificity of the electrophoretic fractions of CS-1A and of dialytic fractions from CS-1A, CS-56R, and CS-51R.

EXPERIMENTAL

Comparative dialysis of CS-51R and CS-56R

Dialytic fractions of CS-51R and CS-56R were prepared as follows:

A solution containing 180 mg of fraction CS-51R⁵ in 10 ml of saline was transferred to a cellophane sac and dialyzed against 10 ml of saline. A solution containing 903 mg of CS-56R and 50 ml of saline was likewise dialyzed against 50 ml of saline. The 2 solutions represented equal dilutions of each fraction on a weight-basis. All solutions were preserved by saturation with toluene. The dialyzate from each fraction was removed daily for the first 3 days and then dialysis of the residues was continued for 27 days against running water. The solids in the dialyzates and residues were recovered by alcohol-precipitation.

During the first 3 days of this experiment a total of 21 mg of solids, containing 19.8 to 19.3 per cent nitrogen, was recovered from the dialyzates of CS-51R; 88 mg of solids, containing 19.5 to 19.0 per cent nitrogen, was recovered from CS-56R. The nitrogen-content of the dialyzate-solids tended to decrease progressively during dialysis. During the 30-day dialytic period 88.1 per cent of the total solids of CS-51R, representing 88.6 per cent of the total protein and 70.7 per cent of the carbohydrate; and 50.8 per cent of the solids of CS-56R, representing 67.2 per cent of the protein and 20.7 per cent of the carbohydrate, diffused through the membranes. Total nitrogen of fractions CS-51R and CS-56R diminished from 19.8 and 13.2 per cent in the original materials to 18.9 and 8.8 per cent, respectively, in the dialyzed residues.

A 267-mg portion of the dialyzed residue from CS-56R from the foregoing experiment was dissolved in 30 ml of saline and redialyzed against 30 ml of saline for a period of 7 days. The dialyzate yielded 9.0 mg of solid containing 18.0 per cent nitrogen. The residue was dialyzed against running water for 24 days. The final residue weighed 184 mg and contained 6.5 per cent nitrogen.

Finally, a solution containing 361 mg of the original fraction, CS-56R, in 25 ml of saline was dialyzed against frequent changes of saline (600 ml volumes) for a period of 6 months. Dialysis was then continued for 5 weeks against distilled water. The dialyzates were discarded. The dialyzed residue (after 220 days) contained 80 mg of solids; the nitrogen content was 4.3 per cent.

The results of the dialytic experiments showed that the membrane used, though relatively more permeable to the protein was not impermeable to the polysaccharide. Thus 21 per cent of the carbohydrate from CS-56R diffused through the membrane during the 30-day dialytic period; 35 per cent diffused during the 61-day period, and 50 per cent diffused during the 220-day period.

⁵ All weights and calculations are expressed on an ash-water-free basis.

On the other hand, 67 per cent of the protein from CS-56R diffused during the 30-day period; 83 per cent during the 61-day period, and 93 per cent diffused during the 220-day period. It is apparent, therefore, that the early dialyzates from CS-56R contained very little more carbohydrate than those from CS-51R and that the protein of both fractions diffused at about the same rate. As dialysis continued the rate of diffusion of protein from CS-56R diminished more than that of CS-51R; and the later dialyzates from CS-56R contained the larger proportions of carbohydrate.

Immunological studies

It seemed probable that if fraction CS-56R contained antigens of markedly different molecular size or specificity the dialytic experiments might have accomplished a sufficient degree of fractionation so that differences in potency or specificity might be demonstrated between some of the fractions. Accordingly, experiments designed to compare the antigenic capacities and specificities of the different dialytic fractions were conducted. Dialytic fractions of CS-1A and electrophoretic fractionation products of CS-1A, described in earlier reports (7, 3) were included for comparative purposes.

Table 1 records the symbol designations and composition of the dialytic and electrophoretic fractions. The protein component of the fractions was assumed to contain 20.4 per cent nitrogen; this was the composition of the carbohydrate-free protein, CS-60C (5), isolated from CS-1A. Justification for considering the protein in these preparations as identical with CS-60C will become evident during presentation of the data. The carbohydrate-contents of the fractions were calculated by difference. The last column records the carbohydrate-content of some of the fractions determined by the method of M. Sørensen and Haugaard as modified by Heidelberger and Kendall (9). The carbohydrate found by analysis agreed well with the calculated content in most of the electrophoretic fractions, but the analytical method gave high results in the dialyzed-residue fractions.

Schultz-Dale experiments with the dialytic products of CS-56R and with CS-51R and CS-60C

Possible differences in antigenic specificity were investigated by the Schultz-Dale method, detailed in earlier papers of this series (2). In these experiments guinea pigs were sensitized with dialyzate fractions of CS-51R obtained in early stages of dialysis. The uterine muscles from these animals were then tested with dialyzed-residue fractions, with dialyzates, and with CS-51R. Other guinea pigs were sensitized with the residue fractions and the sensitized muscles were tested with the dialyzates, with dialyzed-residue fractions and with CS-51R or CS-60C. Results obtained with products from the first dialytic experiment, table 2, show that irrespective of the sensitizing agent, the dialyzed residue, the dialyzate, and CS-51R cross-reacted and desensitized the muscles to each other. Hence, no differences in antigenic specificity were observed in any of these fractions.

Table 3 records the results of Schultz-Dale experiments with the exhaustively dialyzed residue, CS-56R-ExD, from CS-56R and an early dialyzate, CS-56R-D2. By applying the law of multiple proportions (10, 11), the comparative

TABLE 1

*Nitrogen-protein- and carbohydrate-content of the dialytic fractions of CS-1A, CS-51R and CS-56R and of the electrophoretic fractionation products of CS-1A**

FRACTION DESIGNATION	DESCRIPTION	NITROGEN CONTENT	PROTEIN (N $\times$ 4.9)	CARBOHYDRATE BY DIFFERENCE	CARBOHYDRATE FOUND†
Dialytic fractions					
CS-1A-D	Dialyzate from CS-1A (15 days)	19.4	95.1	4.9	2.7
CS-1A-E	Dialyzed residue from CS-1A (6 weeks)	3.9	19.1	80.9	100
CS-51R-D1	Dialyzate from CS-51R, 1st day	19.8	97.0	3.0	—
CS-51R-D2	Dialyzate from CS-51R, 2nd day	19.3	94.6	5.4	—
CS-51R-D3	Dialyzate from CS-51R, 3rd day	19.5	95.6	4.4	—
CS-51R-E	Dialyzed residue from CS-51R, 30 days	18.9	92.6	7.4	—
CS-56R-D1	Dialyzate from CS-56R, 1st day	19.5	95.6	4.4	—
CS-56R-D2	Dialyzate from CS-56R, 2nd day	19.2	94.1	5.9	—
CS-56R-D3	Dialyzate from CS-56R, 3rd day	19.0	93.1	6.9	—
CS-56R-E1	Dialyzed residue from CS-56R, 30 days	8.8	43.1	56.9	—
CS-56R-E2	Dialyzed residue from CS-56R-E1, 31 days	6.5	31.9	68.1	84.4
CS-56R-ExD	Exhaustively dialyzed residue from CS-56R, 7 mo.	4.3	21.1	78.9	87.4
CS-1A and electrophoretic subfractions					
CS-1A	Original allergenic fraction	11.8	57.8	42.2	39.9
CS-51R	Cathodic fraction, cell—	19.8	97.0	3.0	0.9
CS-52R	Cathodic fraction, cell 1—	20.2	99.0	1.0	0.9
CS-53R	Cathodic fraction, cell 2—	19.7	96.5	3.5	3.0
CS-54R	Anodic fraction, cell 2+	19.1	93.6	6.4	6.3
CS-55R	Anodic fraction, cell 1+	15.3	75.0	25.0	23.8
CS-56	Anodic fraction, cell +	10.0	49.0	51.0	48.0
CS-56R	Anodic fraction, cell +	13.2	64.7	35.3	35.2
CS-60C	Carbohydrate-free allergenic protein	20.4	100.0	0.0	0.0

* All analyses and calculations are on ash-water-free basis.

† Carbohydrate found by method of Heidelberger and Kendall (9).

capacity of CS-56R-ExD and CS-56R-D2 to induce contractions in sensitized uterine muscles was determined. Previous experience has shown that when a sensitized muscle has responded to a dose of CS-1A, a second response can usually be induced by a twenty-fold multiple of the preceding dose. Accordingly, table 3 shows the results obtained with 2 animals sensitized with CS-56R-ExD and with one animal sensitized with CS-56R-D2. Tissues from the 2 animals

sensitized with CS-56R-ExD exhibited first submaximal contraction with 400 PNU of CS-56R-ExD and likewise with 400 PNU of CS-56R-D2. The muscles

TABLE 2
Antigenic relationship of the dialytic products of fraction CS-56R

ANIMAL NO.	SENSITIZING ANTIGEN	INCUBATION- ARY PERIOD	HORN NO.	PREPARATION ADDED	PROTEIN N. UNITS	UTERINE CON- TRACTIONS
4064 and 4069	CS-56R-D1 0.5 mg P.N. Alum-pptd.	days 28-30	1	CS-56R-E2 CS-56R-D1 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-D1 CS-56R-E2 Histamine	1000 1000	++++ 0 ++++
	s.c.*		1	CS-56R-D1 CS-56R-E2 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-D1 CS-56R-E2 Histamine	1000 1000	++++ 0 ++++
4070 and 4078	CS-56R-E2 0.5 mg P.N. Alum-pptd.	30-31	1	CS-56R-D1 CS-56R-E2 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-56R-D1 Histamine	1000 1000	++++ 0 ++++
	s.c.		1	CS-56R-D1 CS-56R-E2 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-56R-D1 Histamine	1000 1000	++++ 0 ++++
4107 and 4110	CS-56R-E2 0.5 mg P.N. Alum-pptd.	31-32	1	CS-51R CS-51R-E2 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-51R Histamine	1000 1000	++++ 0 ++++
	s.c.		1	CS-51R CS-56R-D1 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-D1 CS-51R Histamine	1000 1000	++++ 0 ++++
4068	CS-56R-D1 0.5 mg P.N. Alum-pptd.	29	1	CS-51R CS-56R-D1 Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-D1 CS-51R Histamine	1000 1000	++++ 0 ++++
	s.c.		1	CS-56R-D1 CS-51R Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-51R Histamine	1000 1000	++++ 0 ++++
4144	CS-51R 0.9 mg P.N. i.a.†	23	1	CS-56R-D1 CS-51R Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-51R Histamine	1000 1000	++++ 0 ++++
			1	CS-56R-D1 CS-51R Histamine	1000 1000	++++ 0 ++++
			2	CS-56R-E2 CS-51R Histamine	1000 1000	++++ 0 ++++

* Subcutaneous injection.

† Intraabdominal injection.

were thereafter insensitive to the homologous fraction and insensitive, also, to the alternate fraction. Similar results were obtained with the uterine muscles

TABLE 3
Antigenic relationship of the exhaustively dialyzed-residue fraction of CS-56R to the dialyzate and to CS-60C

ANIMAL NO.	SENSITIZING ANTIGEN	INCUBATION- ARY PERIOD	HORN NO.	PREPARATION ADDED	PROTEIN N. UNITS	UTERINE CONTRAC- TIONS
6470	CS-56R-ExD 0.02 mg P.N. i.a.	days 29	1	CS-56R-ExD	1	0
				CS-56R-ExD	20	0
				CS-56R-ExD	400	++
				CS-56R-ExD	4000	0
				CS-56R-D2	4000	0
				Histamine		++++
			2	CS-56R-D2	1	0
				CS-56R-D2	20	0
				CS-56R-D2	400	++
				CS-56R-D2	4000	0
				CS-56R-ExD	4000	0
				Histamine		++++
6577	CS-56R-ExD 0.16 mg P.N. i.a.	27	1	CS-56R-ExD	1	0
				CS-56R-ExD	20	0
				CS-56R-ExD	400	+++
				CS-56R-ExD	4000	±
				CS-56R-D2	4000	0
				Histamine		++++
			2	CS-56R-D2	1	0
				CS-56R-D2	20	±
				CS-56R-D2	400	+++
				CS-56R-D2	4000	±
				CS-56R-ExD	4000	0
				Histamine		++++
6253	CS-56R-D2 0.33 mg P.N. i.a.	25	1	CS-56R-ExD	1	0
				CS-56R-ExD	20	+
				CS-56R-ExD	400	+++
				CS-56R-D2	400	0
				Histamine		++++
			2	CS-56R-D2	1	0
				CS-56R-D2	20	+
				CS-56R-D2	400	+++
				CS-56R-ExD	400	0
				Histamine		++++
6496	CS-1A 0.5 mg P.N. i.a.	28	1a	CS-56R-D2	1	0
				CS-56R-D2	20	++++
				CS-56R-D2	400	+++
				CS-60C	400	0
				Histamine		++++
			2a	CS-60C	1	0
				CS-60C	20	++++
				CS-60C	400	++++
				CS-56R-D2	400	0
				Histamine		++++
			1b	CS-60C	1	0
				CS-60C	20	++++
				CS-60C	400	++++
				CS-56R-ExD	400	0
				Histamine		++++
			2b	CS-56R-ExD	1	0
				CS-56R-ExD	20	+++
				CS-56R-ExD	400	++++
				CS-60C	400	0
				Histamine		++++

from guinea pig no. 6253, previously sensitized with CS-56R-D2, except that the first contractions were induced in both horns at the 20 PNU dosage-level and again at the 400 PNU level.

The comparative potencies of CS-56R-ExD, CS-56R-D2, and CS-60C to elicit contractions in sensitized muscle are shown in the last experiment recorded in table 3. In this experiment the two horns from guinea pig no. 6496, previously

TABLE 4

Data for calculation of median sensitizing dose of the electrophoretic and dialytic fractions

FRACTION	SENSITIZING DOSAGES: μ g AIR-DRIED SOLIDS								
	50	100	200	400	800	1,600			
	Mortality ratio*								
CS-52R.....		1/10	1/10	5/10	8/10	9/10			
CS-53R.....	0/20	5/20	9/20	13/20	15/20				
CS-54R.....	0/8	1/10	3/10	6/10	8/10				
CS-55R.....	0/10	3/10	5/10	6/10					
CS-56R.....	4/21	6/21	14/21	15/21	11/11				
CS-1A-D.....	0/9	2/10	3/10	4/10	8/10				
CS-1A-E.....	3/10	2/10	7/10	8/10	10/10				
	SENSITIZING DOSAGES: μ g AIR-DRIED SOLID								
	30	60	120	240	480				
	Mortality ratio								
CS-56R-E2.....	1/10	3/10	6/10	9/10	10/10				
	SENSITIZING DOSAGES: μ g AIR-DRIED SOLID								
	8	16	32	64	128	256	512	1,024	2,048
	Mortality ratio								
CS-56R-ExD.....	0/3	0/3	1/3	0/3	1/3	2/3	2/3	1/1	1/1

* The mortality-ratio indicates the number of animals dying in anaphylactic shock divided by the number of animals tested. The guinea pigs weighed 300 ± 10 g when sensitized (i.a.) and were challenged, intravenously, 21 days later with 0.5 mg of fraction CS-52R.

sensitized with CS-1A, were each cut into two sections, providing four segments for the test. Contractions of the muscle were induced with 20 PNU and again with 400 PNU with each of the 3 fractions. Fractions CS-56R-ExD and CS-60C desensitized to each other and fractions CS-56R-D2 and CS-60C likewise desensitized to each other.

These experiments indicated that the dialyzed-residue fraction, the dialyzate, and CS-60C were all of equal potency when tested on the basis of equal nitrogen-content and the antigenic specificity of all 3 fractions was the same.

Precipitin-reactions of the dialytic fractions

The precipitin reaction was employed in a further effort to detect possible differences in antigenic characteristics of the dialytic fractions. The CS-1A

antiserum used was a high-titer rabbit serum, previously described (12). Precipitin tests were conducted by the antigen-dilution method. For comparison of precipitative capacity, dilutions of the fractions were prepared on the basis of equal nitrogen-concentration. Fractions CS-51R, CS-60C, and the dialyzed-residue fractions from CS-51R and CS-56R all titrated out to the same endpoint; $1.73\mu\text{g N}$ per ml. However, the dialyzate from CS-51R (CS-51R-D3) and the

TABLE 5
Calculated median sensitizing dose of the electrophoretic and dialytic fractions

FRACTION	SD50*	SD50 _N †
	μg	μg
CS-1A§.....	$140 \pm 28\ddagger$	$14 \pm 3\ddagger$
CS-51R§.....	515 ± 88	92 ± 16
CS-52R.....	429 ± 92	78 ± 17
CS-53R.....	278 ± 47	49 ± 8
CS-54R.....	339 ± 71	58 ± 12
CS-55R.....	245 ± 69	33 ± 9
CS-56R.....	155 ± 26	19 ± 3
CS-56§.....	136 ± 21	12 ± 2
CS-1A-D.....	379 ± 97	64 ± 16
CS-1A-E.....	133 ± 31	4.6 ± 1.1
CS-56R-E2.....	89 ± 16	5.1 ± 0.9
CS-56R-ExD.....	195 ± 92	7.7 ± 3.6

* On the basis of air-dried solid.

† On basis of nitrogen-content.

§ See reference (6).

‡ Average standard error.

TABLE 6
Data for calculation of LD50 for dialyzate, CS-1A-D, and dialyzed-residue fraction, CS-56R-ExD, in guinea pigs sensitized with CS-1A

DIALYTIC FRACTION	CHALLENGING DOSES: μg AIR-DRIED SOLID							
	12.5	25.0	37.5	50	100	200	400	800
	Mortality-ratios							
CS-1A-D.....	0/4	1/5	3/6	5/5	3/3	4/5	4/5	2/2
CS-56R-ExD.....		0/5		2/5	1/5	4/5	4/5	2/2

3 dialyzates from CS-56R gave the least visible precipitates in tubes containing $3.47\mu\text{g N}$ per ml. Therefore, the dialyzates had only about one-half the precipitative capacity of the original fractions.

Anaphylactogenic potency of the dialytic and electrophoretic fractions

Results of a previous study (6) of the anaphylactogenic potencies of CS-1A and two of its electrophoretic fractions, CS-51R and CS-56R, indicated that the sensitizing capacities of these fractions were markedly influenced by their carbohydrate-content. Accordingly, since dialysis effected an even greater concen-

tration of carbohydrate than resulted from electrophoretic fractionation, the sensitizing capacities of two of the dialyzed-residue fractions of CS-56R and the dialyzed-residue of CS-1A⁶ were determined. Also included for comparative

TABLE 7
Calculated median lethal dose for dialytic and electrophoretic fractions

FRACTION	LD50*	LD50 _N †
	μg	μg
CS-56R-ExD.....	125 ± 38‡	4.9 ± 1.5‡
CS-1A-D.....	33.9 ± 3.5	5.71 ± 0.69
CS-1A§.....	33.5 ± 3.5	3.42 ± 0.35
CS-51R§.....	31.5 ± 2.2	5.61 ± 0.39
CS-56§.....	62.2 ± 9.7	5.60 ± 0.87
CS-56R§.....	55.8 ± 6.3	6.75 ± 0.77

* On the basis of air-dried solid.

† On the basis of nitrogen-content.

‡ Average standard error.

§ See reference (6).

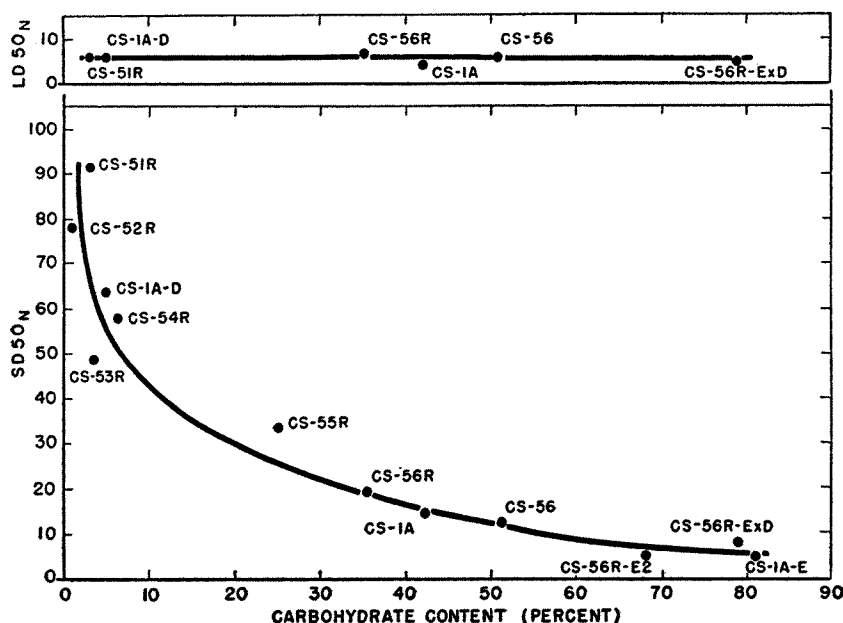


FIG. 1. RELATIONSHIP OF CARBOHYDRATE-CONTENT OF THE ALLERGENIC FRACTIONS TO THE SD50_N AND TO THE LD50_N

purposes were the dialyzate from CS-1A (7), and electrophoretic fractions (3) which had not been investigated previously.

⁶ The dialyzed-residue from CS-1A contained both types of polysaccharide and protein, whereas the residue fraction from CS-56R contained only the difficultly hydrolyzable polysaccharide and allergenic protein.

The sensitizing capacity was measured by determining the median sensitizing dose (SD50) of each of the fractions. Details of the procedure and the method of calculation of the results appeared in a previous paper (6). Data for calculation of the SD50 are assembled in table 4.

Table 5 records the calculated SD50 of the different fractions including those previously reported (6). The results are expressed on the basis of the air-dried solid (SD50) and also on the basis of nitrogen-content (SD50_N). For all fractions the general trend was increased sensitizing capacity (SD50_N decreased) as the proportion of carbohydrate in the fraction increased.

Table 6 records data obtained in determination of the median lethal dose (LD50) in sensitized guinea pigs for the dialyzate from CS-1A(CS-1A-D) and for the dialyzed-residue fraction CS-56R-ExD. For determination of LD50, guinea pigs weighting 300 ± 10 g were each sensitized with 5 mg of CS-1A. Twenty-one days later the animals were challenged with intravenous injections at dosage-levels⁷ indicated in table 6.

Table 7 records the calculated LD50 and LD50_N for CS-1A, for the dialytic fractions, and for the electrophoretic fractions previously recorded (6). The results show that there were no significant differences in the shocking capacities of any of these fractions when comparison was based on nitrogen-content.

Graphic representation of the relationship of carbohydrate-content of the different fractions to the SD50_N and to the LD50_N is shown in figure 1. The curves cover a range from 1 to 81 per cent carbohydrate. It is apparent that the influence of carbohydrate on the sensitizing capacity of the protein reached a maximum when the fraction contained about 70 per cent carbohydrate. In contrast, however, carbohydrate had no significant effect on the shocking capacities of these fractions.

DISCUSSION

On the basis of chemical and clinical evidence, Spies, Bernton and Stevens (3) suggested that the allergenic cottonseed-fractions consisted of protein of small molecular weight combined with varying proportions of polysaccharide. The investigations recorded in this paper were undertaken to obtain more complete evidence for or against this hypothesis.

The Schultz-Dale experiments clearly demonstrated the antigenic identity of the carbohydrate-free protein, CS-60C, and the polysaccharide-protein fraction contained in the exhaustively dialyzed residue from CS-56R. Moreover, the electrophoretic fractions and the dialytic fractions, containing widely different concentrations of carbohydrate, were all equally effective, on a nitrogen-basis, in eliciting contractions in sensitized muscles or in inducing shock in sensitized guinea pigs. The precipitative reactivities of these fractions were likewise more

⁷ In these experiments the challenging dosage-levels were administered on the basis of body-weight. Subsequent studies (13) have shown that the LD50 in sensitized guinea pigs is not proportional to body-weight. However, for comparative purposes the LD50, as determined in these experiments, are valid because the animals were of uniform age and size and weighed approximately 500 g when challenged.

closely correlated with their nitrogen-content than with their carbohydrate-concentration. The conclusion is justified, therefore, that the carbohydrate-component plays no role in determination of immunological specificity or shocking capacity of these fractions. On the other hand, however, carbohydrate had a marked influence on the capacity of these fractions to induce antibody-formation. The relationship of the carbohydrate-concentration of the fractions to their median sensitizing doses, recorded in figure 1, shows that increased concentration of carbohydrate was associated with increased sensitizing capacity. It is significant that this relationship was observed with the dialytic fractions as well as with the electrophoretic fractions in which separation of proteins on the basis of molecular size was not a factor.

Further evidence of the role of carbohydrate in stimulating antibody-formation by the cottonseed allergen is found in the previously recorded observations on the sensitizing and shocking capacities of the two electrophoretic fractions CS-51R and CS-56 (14). Fraction CS-56 was a carbohydrate-rich fraction and was nearly 8 times more effective than CS-51R as a sensitizing agent when compared on a nitrogen basis. However, after treatment with dilute acid—a procedure designed to disrupt carbohydrate linkages—the two fractions exhibited the same sensitizing capacity.

The preponderance of evidence obtained in this study supports the view that the allergenic protein and the difficultly hydrolyzable carbohydrate are chemically combined, but further studies should be made to determine the nature of the chemical linkage before a final conclusion can be reached.

Evidence recorded by others indicates that carbohydrate contributes to the antigenic capacity of certain other native proteins even aside from its role of determining specificity as in certain bacterial antigens. Native, carbohydrate-free crystalbumin from bovine serum has been reported to exhibit a much lower degree of antigenic activity than its non-crystalline counterpart (15). The carbohydrate played no role in the determination of serological specificity (16).

Gay (17) mentions the fact that some haptens may be restored to full antigenicity by simply mixing the hapten with protein *in vitro*. Gay believes it to be debatable whether this activation depends upon a real chemical union between the hapten and activator or whether the phenomenon is merely due to an absorptive effect resulting in larger molecular size. However, *in vitro* mixtures of the carbohydrate-free cottonseed allergen in solution with carbohydrate from other sources (potato starch and inulin) does not result in enhanced sensitizing capacity.⁸

SUMMARY

Evidence is presented to show that the carbohydrate, which is associated with protein in the allergenic fractions, separated from cottonseed, enhances the anaphylactogenic sensitizing capacity of the allergenic protein but does not determine antigenic specificity and does not influence the shocking capacity of the protein.

⁸ Unpublished data from this laboratory.

REFERENCES

1. SPIES, J. R., COULSON, E. J., BERNTON, H. S., AND STEVENS, H. 1940 The chemistry of allergens. II. Isolation and properties of an active protein component of cottonseed. *J. Amer. Chem. Soc.*, **62**: 1420-1423.
2. COULSON, E. J., SPIES, J. R., AND STEVENS, H. 1943 The immunochemistry of allergens. II. Antigenic studies by the Dale method of the electrophoretic fractionation products of the protein-carbohydrate fraction, CS-1A, from cottonseed. *J. Immunol.*, **46**: 347-365.
3. SPIES, J. R., BERNTON, H. S., AND STEVENS, H. 1941 The chemistry of allergens. IV. An electrophoretic fractionation of the protein-polysaccharide fraction, CS-1A, from cottonseed. *J. Amer. Chem. Soc.*, **63**: 2163-2169.
4. SPIES, J. R., BERNTON, H. S., AND STEVENS, H. 1940 The chemistry of allergens. III. The solubility behavior of an active protein picrate from cottonseed. *J. Amer. Chem. Soc.*, **62**: 2793-2799.
5. SPIES, J. R., AND UMBERGER, E. J. 1942 The chemistry of allergens. VI. Chemical composition and properties of an active carbohydrate-free protein from cottonseed. *J. Amer. Chem. Soc.*, **64**: 1889-1891.
6. COULSON, E. J., AND SPIES, J. R. 1943 The immunochemistry of allergens. III. Anaphylactogenic potency of the electrophoretic fractionation products of CS-1A from cottonseed. *J. Immunol.*, **46**: 367-376.
7. COULSON, E. J., SPIES, J. R., AND STEVENS, H. 1943 The immunochemistry of allergens. V. Comparison of the rates of dialysis of crystalline ovalbumin and of the cottonseed allergen, CS-1A. *J. Immunol.*, **47**: 443-452.
8. SPIES, J. R., BERNTON, H. S., AND STEVENS, H. 1939 The chemistry of allergens. I. Isolation and properties of an active fraction from cottonseed. *J. Allergy*, **10**: 113-129.
9. HEIDELBERGER, M. AND KENDALL, F. E. 1936 Carbohydrate-containing proteins of the hemolytic streptococcus. *J. Immunol.*, **30**: 267-273.
10. COCA, A. F. AND KOSAKAI, M. 1920 Studies in anaphylaxis. *J. Immunol.*, **5**: 297-319.
11. WALZER, M. AND GROVE, E. F. 1925 Studies in specific hypersensitiveness. XVI. On antigens. A comparative study of the antigenic properties of pollens, egg white, and glue, in guinea pigs as determined by the intravenous and Dale methods of testing in anaphylaxis. *J. Immunol.*, **10**: 483-554.
12. COULSON, E. J., SPIES, J. R., JANSEN, E. F., AND STEVENS, H. 1946 The immunochemistry of allergens. VIII. Precipitin formation and passive transfer reactions with allergenic proteins from cottonseed and castor beans. *J. Immunol.*, **52**: 259-266.
13. COULSON, E. J., AND STEVENS, H. 1949 Quantitative studies in anaphylaxis. I. Influence of age and body-weight of guinea pigs on the sensitizing and shocking dose. *J. Immunol.*, **61**: 1-10.
14. COULSON, E. J., AND SPIES, J. R. 1943 The immunochemistry of allergens. IV. Effect of dilute acid on anaphylactogenic activity, specificity, and reagin-neutralization capacity of cottonseed allergenic fractions. *J. Immunol.*, **46**: 377-389.
15. MARTIN, D. S., ERICKSON, J. O., AND NEURATH, H. 1943 Antigenic properties of native and regenerated bovine albumin. *Fed. Proc.*, **2**: 66.
16. MARTIN, D. S., ERICKSON, J. O., PUTNAM, F. W., AND NEURATH, H. 1943 Native and regenerated bovine albumin. II. Immunological properties. *J. Gen. Physiol.*, **26**: 533-539.
17. GAY, F. P. 1935 *Agents of Disease and Host Resistance*. Charles C. Thomas, Baltimore, p. 332.