

ANTIGENIC SPECIFICITY
RELATIONSHIPS OF CASTOR
BEAN MEAL, POLLEN, AND
ALLERGENIC FRACTION, CB-1A,
OF RICINUS COMMUNIS

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Antigenic specificity relationships of castor bean meal, pollen, and allergenic fraction, CB-1A, of *Ricinus communis*

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Guinea pigs could not be sensitized to castor bean pollen antigens by sensitization with CB-1A. CB-1A had less than 0.025 per cent cross-reactive potency with guinea pigs sensitized to the pollen when tested by the Schultz-Dale method and by quantitative precipitin tests. The cross-reactive potency of castor bean pollen and castor bean meal antigens was less than 0.4 per cent by the Schultz-Dale test and was 1.6 per cent by a gel diffusion method of testing. The analogous relationships of CB-1A and CS-1A to sensitivity to castor beans and cottonseed, respectively, are discussed.

The object of this paper is to determine the specificity relationships of the antigens of the bean meal, the pollen, and allergenic fraction, CB-1A, of *Ricinus communis* by gel diffusion, absorption, the Schultz-Dale technique, and the cutaneous test.

CB-1A is a complex mixture of low molecular weight proteins and polysaccharidic proteins which contains the principal allergenic components of castor bean meal, immunologically distinct from other allergens and antigens in the meal. The nature and status of our present concept of CB-1A have been described recently.¹

Layton and associates² have reported that CB-1A and castor bean pollen cross-react as shown by the Schultz-Dale technique. By means of the passive cutaneous anaphylaxis test on monkeys with human reaginic serum, Layton and others^{3, 4} have reported that castor bean pollen and castor beans have common allergenic components and that castor bean pollen may be involved in sensitization of man to castor bean meal proteins.

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MATERIALS USED

CB-1A

CB-1A was isolated from defatted castor beans as previously described.^{5, 6}

Castor bean meal

Baker 296, California 1957 (designated SL-30)⁷ castor beans were used. The beans were decorticated by hand and defatted with ether.

Castor bean pollen

The castor bean pollen samples were provided by Dr. C. A. Thomas, Oilseed and Industrial Crop Research Branch, U. S. Department of Agriculture, Beltsville, Maryland. Mixed pollen was collected randomly in an experimental field containing the following varieties: Baker 296, Hale, Nebraska 145-4, 3/415-9 hybrid, Cimarron, and Dawn. A sample of Nebraska 145-4 was collected free from contamination with pollen from the other varieties. The pollen samples were defatted with ether.

EXPERIMENTAL

Rabbit antisera

Rabbits were immunized with the aid of Freund's adjuvant by a process similar to that described for preparation of CB-13E antiserum.⁸ Pooled samples of antisera were used as follows: CB-1A, five bleedings from 12 rabbits; castor bean meal, six bleedings from 3 rabbits; and mixed pollen, eight bleedings from 2 rabbits.

Preparation of solutions

Solutions of CB-1A and dilutions of castor bean pollen and meal extracts were made with buffered saline, pH 7.0, containing 0.01 per cent Merthiolate. Castor bean meal and pollen samples were extracted with physiological salt solution. Protein nitrogen (P.N.) was determined by precipitation with phosphotungstic acid.⁹

Dialysis of mixed castor bean pollen extract

Mixed pollen extract was dialyzed against 1,000 ml. of water for seven days. The inner solution was designated "dialyzed residue," MP (DR). The outer solution was concentrated by lyophilization and designated mixed pollen dialysate, MP (D).

Gel double-diffusion technique

The Ouchterlony technique^{10, 11} was used. A single filling of antiserum was added 24 hours before a single filling of antigen solution. The diffusion patterns were allowed to develop at $24 \pm 1^\circ$ C. for 4 to 6 days in a moist atmosphere.

Schultz-Dale technique

The Schultz-Dale procedure used has been described in a previous report from this laboratory.¹² The capacity of the Dale baths was 50 ml.

RESULTS

The term "cross-reactive potency" (CRP) is used to designate the response of a complex mixture of antigens from one source reacting specifically with a complex mixture of antibodies which were produced by a complex mixture of antigens from another source. We have no evidence as to whether the reactions observed were cross-reactions in the usual sense or whether the reactions were induced by identical antigens in the two sources. CRP is the ratio (expressed as per cent) of the quantity of protein nitrogen in the homologous extract which will produce a response approximately equivalent to a determined quantity of protein nitrogen in the heterologous extract. CRP as determined by precipitin and by Schultz-Dale reactions is designated (CRP)P and (CRP)S-D, respectively.

The minimum concentrations of antigens of CB-1A, castor bean mixed pollen extract dialyzed residue, castor bean pure pollen extract (Nebraska 145-4), and castor bean meal extract which gave a precipitate with each of CB-1A antiserum, mixed pollen antiserum, and castor bean meal antiserum, and with normal rabbit serum are shown in Table I. Also shown in Table I are the maximum concentrations of these antigens which gave no precipitate when similarly tested for cross-reactions. No nonspecific precipitate occurred with normal rabbit serum tested with mixed pollen extract dialyzed residue, 0.1 mg. P.N. per milliliter; mixed pollen extract dialysate, 0.2 mg. P.N. per milliliter; castor bean pollen extract (Nebraska, 145-4) 0.24 mg. P.N. per milliliter; and CB-1A, 1.6 mg. P.N. per milliliter. The minimum concentration of castor bean meal extract which gave a nonspecific precipitate with normal rabbit serum was 0.2 ml. P.N. per milliliter.¹³ Mixed pollen dialysate gave no precipitate with 0.2 mg. P.N. per milliliter when tested with mixed pollen antiserum.

Table I. *Precipitinogenic potencies* of CB-1A, unfractionated castor bean meal, mixed castor bean pollen, and a pure castor bean pollen, each tested against CB-1A, castor bean meal, and mixed pollen antisera†*

Antigen	Rabbit antisera Lowest concentration of antigens giving visible precipitate when tested against indicated antiserum. (Mg. P.N./ml.)			Rabbit serum normal
	A-MP	A-1A	A-U	
MP(DR)	0.0008	None (0.1)‡	0.05	None (0.1)‡
MP(D)	None (0.2)‡			None (0.2)‡
1A	None (1.6)‡	0.0004	0.0008	None (1.6)‡
U	0.05	0.0008	0.0008	0.20
PP	0.0016	None (0.24)‡	0.10	None (0.24)‡

*Relative precipitinogenic potencies determined as described in Table II of Spies and Coulson.¹

†The following abbreviations are used in the tables. CB-1A, 1A; mixed castor bean pollen extract, MP; components which remained inside the membrane on dialysis of mixed castor bean pollen, MP(DR); components which passed through the membrane on dialysis of mixed castor bean pollen, MP(D); pure castor bean pollen (Nebraska 145-4) extract; PP; unfractionated castor bean meal extract, U; CB-1A rabbit antiserum, A-1A; mixed castor bean pollen rabbit antiserum, A-MP; unfractionated castor bean meal rabbit antiserum, A-U; protein nitrogen, P.N.

‡Maximal concentration tested which did not give a precipitate.

(CRP)P values of antigens in castor bean meal, pollen, and CB-1A, were estimated from data in Table I. The (CRP)P values of mixed pollen and pure pollen (Nebraska, 145-4) were less than 0.8 to 0.7 per cent respectively, vs. CB-1A antiserum. The (CRP)P of CB-1A was less than 0.025 per cent vs. mixed pollen antiserum. The (CRP)P values of both pollen samples were 1.6 per cent vs. castor bean meal antiserum. The (CRP)P value of CB-1A was 50 per cent vs. castor bean antiserum.

The gel diffusion pattern of antigens of mixed pollen dialyzed residue, mixed pollen dialysate, pure pollen (Nebraska 145-4), castor bean meal, and CB-1A when diffused against a mixture of equal parts of mixed pollen antiserum and castor bean meal antiserum is shown in Fig. 1. The crossing of the lines of precipitate of the pollen antigens with those of castor bean meal and of CB-1A show the nonidentity of all of the principal antigens of the pollen with those of CB-1A and castor bean meal. Lines of precipitate of castor bean meal and CB-1A join showing identity as expected. The additional lines of precipitate near well *U* show the antigens present in the meal but not in CB-1A. The lines of precipitate of mixed pollen and pure pollen join showing no major antigens in the mixed pollen not present in the pure pollen. Mixed pollen dialysate gave no precipitate.

Specificity relationships of the antigens of CB-1A and castor bean meal by the absorption technique are shown in Fig. 2. The lines of precipitate between wells *A-U* and *1A* show the reaction of CB-1A with antibodies in castor bean

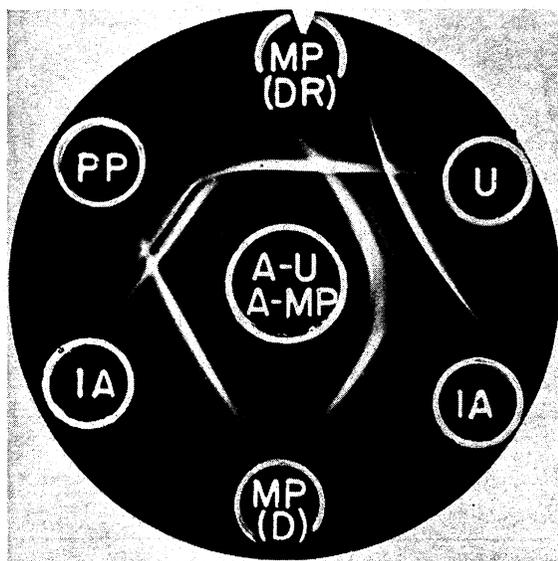


Fig. 1

Specificity relationships of mixed pollen dialyzed residue and dialysate, pure pollen, castor bean meal extract and CB-1A by gel diffusion. Well *A-U* *A-MP*, equal volumes (0.07 ml. each) of castor bean meal antiserum and mixed pollen antiserum; well *MP(DR)*, 0.05 mg. P.N. of mixed pollen dialyzed residue per milliliter; well *MP(D)*, 0.2 mg. P.N. of mixed pollen dialysate per milliliter; well *PP*, 0.1 mg. P.N. of pure pollen (Nebraska 145-4) per milliliter; well *U*, 0.05 mg. P.N. of unfractionated castor bean meal extract per milliliter; well *1A*, 0.05 mg. P.N. of CB-1A per milliliter.

meal antiserum specific for antigens of CB-1A (positive control). The space between wells *1A* and *ABS A-U* show that absorption of CB-1A antibodies in castor bean meal antiserum was complete (negative control). The lines of precipitate between wells *ABS A-U* and *U* are produced by antigens of castor bean meal other than those antigens present in CB-1A. The lines of precipitate between wells *U* and *A-U* are produced by all of the antigens in castor bean meal (positive control).

Six antigenic components were demonstrated in mixed pollen extract when mixed pollen dialyzed residue diffused against mixed pollen antiserum. Three major antigens were distinguished by separation of the ends of a heavy band of precipitate. There were two minor lines of precipitate outward from the major lines and one minor line of precipitate between the major lines and the antiserum well. Pure pollen (Nebraska 145-4) tested against mixed pollen antiserum showed the presence of five and possibly six antigens.

Contractions of uterine muscles (Schultz-Dale) from nonsensitized guinea pigs did not occur with test doses of the following preparations: mixed pollen extract, 310 μg P.N.; mixed pollen extract dialysate, 100 μg P.N.; castor bean meal extract, 1000 μg P.N.; ground pollen suspension, 2 mg. in 0.2 ml. of saline solution. Nonspecific contractions occurred with mixed pollen dialysate, 310 μg P.N.

An attempt was made to show cross-reactions of castor bean pollen and CB-1A with guinea pigs sensitized in various ways to CB-1A using the Schultz-

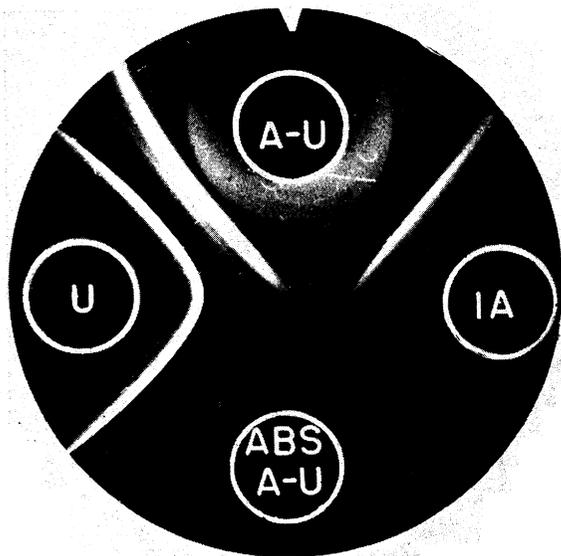


Fig. 2

Specificity relationships of CB-1A and unfractionated castor bean meal extract by absorption technique. Unabsorbed castor bean meal antiserum and CB-1A absorbed castor bean meal antiserum were placed in wells designated *A-U* and *ABS A-U*, respectively. After 24 hours, a solution containing 0.025 mg. of CB-1A P.N. per milliliter and a solution containing 0.050 mg. of unfractionated castor bean meal P.N. per milliliter were placed in wells *1A* and *U*, respectively. The photograph was taken after 5 days' diffusion at $24 \pm 1^\circ \text{C}$.

Dale test. Six guinea pigs were sensitized to CB-1A as follows: two to the alum precipitate; three with a dose of 100 mg. of CB-1A, as was done by Layton and associates;² and one was sensitized passively with 2 ml. of CB-1A rabbit antiserum. One segment of uterine muscle of each animal was challenged with a saline suspension containing 2 mg. of ground pollen as was done by Layton and associates.² With other segments of the same uteri, the minimal responsive dose of CB-1A was found to range from 0.06 to 0.6 μ g. None of these uterine muscles reacted to the pollen suspensions and test with the pollen suspension did not diminish the reaction to subsequent minimal responsive doses of CB-1A. No response was obtained with 100 to 310 μ g P.N. of the mixed pollen or the mixed pollen dialyzed residue. Minimal response doses of CB-1A ranged from 0.01 to 0.1 μ g of P.N. These data showed that the (CRP)S-D of CB-1A and pollen was less than 0.03 per cent when a suspension of pollen was used to challenge and less than 0.03 per cent when extracts of the pollen were used.

Results of Schultz-Dale tests for cross-reactions between pollen, castor bean

Table II. *Schultz-Dale, cross-reaction tests of castor bean pollen, castor bean meal, and CB-1A in guinea pigs actively sensitized with mixed pollen**

Test No.	Sensitizing substance	Incubation period (days)	Uterine strip No.	Test substance	Dose P.N. (μ)	Response†			
1	MP	25	1	1A	1,000	0			
				MP(DR)	1	++++			
			2	1A	4,000	+++			
				MP(DR)	1	++++			
			3	U	100	+++			
				MP(DR)	1	0			
2	MP	26	1	1A	1,000	++			
				MP(DR)	0.2	0			
				U	100	0			
			2	MP(DR)	0.2	+++			
				1A	1,000	0			
			3	1A	250	0			
				U	100	++++			
			4	MP(DR)	0.1	0			
				MP(DR)	1.0	++++			
			3	MP	26	1	MP(DR)	0.2	++++
							MP(DR)	0.1	++
						3	MP(DR)	0.05	±
1A	1,000	0							
4	MP(DR)	0.2				++++			
	1A	2,000				±			
5	1A	4,000				0			
	MP(DR)	0.2				++++			
4	MP	25	1	1A	1,000	0			
				MP(DR)	1	++++			
				U	1,000	++++			
			2	MP(DR)	1	++++			
				U	100	0			

*See Table I, second footnote.

†Comparative responses produced by the test doses as related to the subsequent, final response to histamine.

Table III. *Schultz-Dale, cross-reaction tests of pollen, castor bean meal, and CB-1A in guinea pigs passively sensitized to mixed pollen or castor bean meal antisera**

<i>Test No.</i>	<i>Sensitizing substance</i>	<i>Incubation period (days)</i>	<i>Uterine strip No.</i>	<i>Test substance</i>	<i>Dose P.N. (μg)</i>	<i>Response†</i>
1	MP	2	1	MP(DR)	2	+++
				U	1,000	++++
			2	MP(DR)	50	++++
				U	1,000	0
			3	U	500	+
				MP(DR)	3	±
			4	1A	2,000	0
				U	1,000	+++
2	MP	1	1	MP(DR)	0.5	0
				U	1,000	+++
			2	1A	1,000	0
				U	1,000	+++
			3	MP(DR)	2	++
			4	1A	2,000	0
				U	500	0
				MP(DR)	4	++++
3	U	2	1	MP(DR)	135	++++
				U	0.1	++++
			2	MP(S)‡	2 mg.	0
				U	0.1	++++

*See Table I, second footnote.

†Comparative responses produced by the test doses as related to the subsequent, final response to histamine.

‡Mixed pollen suspension.

meal, and CB-1A with guinea pigs sensitized with alum-precipitated mixed pollen are shown in Table II. Results of these tests show that the (CRP)S-D of CB-1A and pollen was less than 0.025 per cent and that the (CRP)S-D of castor bean meal and pollen was more than 10 times the (CRP)S-D of CB-1A and pollen.

Results of Schultz-Dale tests for cross-reactions between pollen, castor bean meal, and CB-1A with guinea pigs passively sensitized with mixed pollen, or with castor bean meal rabbit antisera, are shown in Table III. Test No. 1 shows that the minimal responsive doses of mixed pollen dialyzed residue and castor bean meal were in the approximate ratio of 2:500; hence, the (CRP)S-D of castor bean meal and pollen is approximately 0.4 per cent. Test No. 2 shows that the (CRP)S-D of castor bean meal and pollen is 0.2 per cent. No response was obtained with a dose of 2,000 μg P.N. of CB-1A which confirmed results of previous tests with guinea pigs actively sensitized with pollen. Test No. 3 shows that castor bean meal antiserum contained antibodies cross-reacting with pollen antigens.

DISCUSSION

The observation of Layton and associates² that guinea pigs sensitized to CB-1A cross-reacted with castor bean pollen by the Schultz-Dale technique was not

confirmed. None of six CB-1A sensitized guinea pigs (three with 100 mg. of CB-1A as was done by Layton, two with alum-precipitated CB-1A which is 400 times more effective in sensitization of guinea pigs than is aqueous CB-1A¹⁴, and one passively sensitized with CB-1A rabbit antiserum) gave any response in the Schultz-Dale test with 2 mg. of pollen suspension. Furthermore, negative Schultz-Dale responses were obtained in the uterine strips from these CB-1A sensitized guinea pigs with dosages of mixed pollen dialyzed residue from 100 to 310 μg P.N. which represented soluble extractives from 52 to 160 mg. of pollen. In contrast, Layton and associates,² found that eight of ten guinea pigs sensitized with 100 mg. of aqueous CB-1A gave maximal Schultz-Dale responses when challenged with 2 mg. of castor bean pollen suspension.

However, a trace of CB-1A antigen cross-reactive with mixed pollen antibodies was detectable by the Schultz-Dale technique using six guinea pigs sensitized with castor bean mixed pollen (Table II and III). Four of the animals exhibited no sensitivity to maximal test doses of 1,000, 2,000 and 4,000 μg of CB-1A nitrogen. In one test a submaximal response to 1,000 μg of CB-1A nitrogen was obtained. In another test a submaximal response was obtained with 4,000 but not 1,000 μg of CB-1A nitrogen. On a solid basis these are test doses of 6 to 24 mg. of solid CB-1A. Previously it has been shown that the dose of CB-1A required to induce anaphylactic sensitivity in guinea pigs is 6,000 times larger than that detectable by the Schultz-Dale technique.¹⁵ Accordingly, it was concluded that the trace of antigen in CB-1A cross-reacting with pollen antibodies would not be sufficient to induce sensitivity. Layton and associates² reported positive Schultz-Dale responses to 2 mg. of CB-1A in three of ten guinea pigs sensitized to pollen.

In contrast to the difficulty in demonstrating cross-reactions between CB-1A and castor bean pollen, the cross-reacting antigen(s) in the pollen and castor bean meal were readily demonstrated. Although easily demonstrable, nevertheless, the CRP of castor bean meal and pollen was relatively low. Uterine strips from all four pollen-sensitive guinea pigs exhibited sensitivity to castor bean meal in doses ranging from 100 to 1,000 μg P.N. which are equivalent to 2.9 to 29 mg. of castor bean meal. From data in Tables II and III it was estimated that the maximum (CRP)S-D values of castor bean meal and pollen dialyzed residue were from 0.1 to 0.4 per cent. The true values are undoubtedly less. Antibodies cross-reactive with pollen also were demonstrated in castor bean meal antiserum (Table III).

There was no evidence of cross-reactions between the antigens of castor bean pollen and those of CB-1A or castor bean meal by the gel diffusion method (Fig. 1) and none was expected under the conditions of the tests because of the trace proportions of the cross-reactive antigens in the test solutions. The faint circle of precipitate near the serum well in Fig. 1 may be caused by a cross-reacting antigen in pollen, CB-1A, and castor bean meal, but it appears more likely to be an artifact.

One major and one minor antigen in castor bean meal which are not present in CB-1A are shown in the gel diffusion pattern in Fig. 1. The major castor bean meal antigen distinct from CB-1A is undoubtedly globulin and the minor one

may be ricin. The presence of one major and two minor antigens in castor bean meal which are not present in CB-1A is shown by the lines of precipitate between wells *ABS A-U* in Fig. 2 where castor bean meal antiserum absorbed with CB-1A was used.

The dialysate of mixed pollen gave no Schultz-Dale response with uterine muscles from guinea pigs sensitized with the pollen. The pollen dialysate at a dosage of 310 μg P.N. was the only material which produced nonspecific uterine contractions at the dosage levels used. Layton and associates² reported that substances in castor bean flour, pollen, and blossoms gave histaminelike contractions in nonsensitized uterine muscles. Layton removed or destroyed the nonspecific substance from these materials by exhaustive extraction with hot methanol, a procedure likely to have denatured or altered some of the antigens present in these materials.

In the present study a comparative cutaneous test with CB-1A and mixed pollen was made on a Type II castor bean-sensitive person whose serum contained reagins for CB-1A. A 2 plus reaction with redness and pseudopods was obtained with CB-1A, 0.17 μg nitrogen per milliliter, whereas mixed pollen, 100 μg P.N. per milliliter, gave a nontypical 1 plus reaction with redness but no pseudopods. On this basis castor bean pollen contained less than 0.17 per cent of CB-1A.

Layton and others⁴ have described a study of 60 castor bean-sensitive or persons suspected of sensitivity. All exhibited some degree of skin sensitivity when tested with a crude aqueous extract of castor beans. Of the sera from the 60 persons, 34 gave negative tests for reagins and 26 gave strongly positive tests for reagins in the PCA test with monkeys. These results of Layton and others are analogous to those obtained in extensive studies on the relationship of cutaneous and clinical sensitivities of CS-1A from cottonseed.¹⁶⁻¹⁹ In these studies it was observed that cutaneous sensitivity to cottonseed meal was of two types: (a) Type I, cutaneous sensitivity to cottonseed meal but not to CS-1A, and (b) Type II, cutaneous sensitivity to cottonseed meal and to high dilutions (approximately $1:10^6$) of CS-1A. Individuals with Type I usually had no reagins and either no or doubtful clinical sensitivity. Those with Type II almost always had reagins to CS-1A associated with clinical sensitivity to cottonseed, and sometimes, also, reagins to other allergens of cottonseed meal. CB-1A has a relationship to castor bean meal similar to that of CS-1A to cottonseed meal. One type I castor bean-sensitive subject has been observed (Table VI of Spies and co-workers⁶) as well as many Type II subjects.

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