

RESPONSE OF NONALLERGIC PERSONS TO INJECTED CASTOR BEAN ALLERGEN, CB-1A

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STUDIES have been carried out in this Laboratory to determine the conditions for the inactivation of the castor bean allergen, CB-1A, by various physical and chemical treatments. Part of the evaluation of this inactivation was determination of destruction of the reagin neutralizing capacity of CB-1A by a method¹ which involved injection of recipients with various amounts of CB-1A.

This paper describes the response of 161 nonallergic persons to intracutaneous and subcutaneous injections of CB-1A at various intervals, with respect to development of blocking antibodies, reagins, skin sensitivity and/or clinical reactions.

It is the consensus of allergists that castor beans contain an allergen or allergens with unusually potent sensitizing capacity. The first recorded case of hypersensitivity to castor beans was described in 1914 by Alilaire who attributed the allergenic activity to ricin.² The first case of occupational castor bean sensitivity was that of a chemist who worked in the United States Department of Agriculture (described by Bernton³). Since then many cases of hypersensitivity have been reported. Figley and Elrod⁴ first described endemic asthma caused by castor beans within a one mile radius of an oilseed processing mill in Toledo. Ordman⁵ described an outbreak of asthma in South Africa affecting over 200 persons caused by dust from a castor oil processing plant. Castor bean allergy due to contamination of burlap bags⁶ and of green coffee^{7, 8} has also been reported. Other literature on this subject is reviewed by Ordman.⁵

The isolation and properties of the principal allergen or allergens of castor beans, CB-1A, have been described in previous papers from this Laboratory.⁹⁻¹⁴ CB-1A is a polysaccharidic protein belonging to the natural proteose classification which amounted to 1.8 per cent of ether-defatted domestic castor beans and 0.33 per cent of a commercial pomace by isolation. The CB-1A contents of several varieties of decorticated, defatted castor beans ranged from 6 to 9 per cent, as determined by a quantitative precipitin method.¹⁵ The allergenic and antigenic specificities of CB-1A are attributed to protein components. CB-1A is soluble in water and in basic lead acetate solution but is precipitated by 75 per cent ethanol. CB-1A is composed of amino acids with relatively high

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arginine and glutamic acid contents but no tryptophan. CB-1A contains no ricin and is nontoxic. CB-1A is extremely stable to heating, even in alkaline solution.¹

MATERIAL USED

Castor Bean Allergen, CB-1A.—CB-1A was isolated from defatted castor beans, as described before.^{9, 14} Two preparations were used in this study which had nitrogen contents of 13.6 and 16.6 per cent on an air-dried basis. The preparations were regarded as equivalent for purposes of this study.

Reaginic Serum.—Serum was obtained from a castor bean-sensitive person, E. McL, and stored at 5° C.

Recipients for Passive Transfer Tests.—Recipients for passive transfer tests were volunteer nonreactors to castor bean selected from among male inmates of the District of Columbia Workhouse, Occoquan, Virginia. For the most part, they had no family history of allergy. The recipients were free from antihistaminic medication.

EXPERIMENTAL

Although the reagin neutralization method has been described in detail,¹ it is repeated here with the slight modifications adopted during a subsequent usage because of its pertinence to the subject under consideration.

Reagin Neutralization Method.—Sensitized sites were located as follows: sites 1 and 2, on the anterior aspect of the forearm, 3½ and 11½ inches below the bend of the elbow; sites 3 and 4, on the upper aspect of the biceps, 2½ and 4½ inches above the bend of the elbow on each arm. Sites were used in pairs of 1 and 2, and 3 and 4 on the same arm so that 4 pairs were available, 1 pair being used for each complete test.

The reagin neutralization method is described on a day-by-day basis, 4 days being required for the test.

First Day, Sensitization.—Each recipient, in groups of approximately 25, was passively sensitized by intracutaneous injection of 0.05 ml. of E. McL. serum at site 1 when pair 1 and 2 was used, or at site 3 when pair 3 and 4 was used.

Second Day, Challenge of Site.—Site 1 or 3 was challenged by direct intracutaneous injection with 0.05 ml. of CB-1A of known concentration or with 0.05 ml. of a solution of CB-1A that had been subjected to various physical or chemical treatments. The size of the wheal produced in 30 minutes was measured.

Third Day, Resensitization.—Each recipient was passively sensitized by intracutaneous injection of 0.05 ml. of E. McL. serum at site 2 or 4 of the same arm used on the first day of the test. This site was a positive control in the test for neutralization on the fourth day. This site was not sensitized on the first day because of the possibility of reagin neutralization by migration of allergen from sites 1 or 3 injected on the second day.¹⁶

Fourth Day, Test for Reagin Neutralization.—Each recipient was injected

subcutaneously with 1.0 ml. of saline solution containing 1.0 mg. of CB-1A on the outer aspect of the upper arm, opposite that having the sensitized sites. This method of challenge eliminated need for a control test, inasmuch as the sites were challenged via the circulation, there was no trauma of sites by needle or irritation by solvent. The challenge dose was in excess of that required to produce reaction with all residual reagins under the conditions of the tests.

Reagin Neutralization With CB-1A.—The threshold amount of CB-1A required to neutralize reagins in sensitized sites 1 or 3 was determined by direct intracutaneous injection of twofold serial dilutions of CB-1A on the second day of testing. A range of concentrations, usually from 0.016 to 0.13 μg (μg = 0.000 001 Gm.) of CB-1A per 0.05 ml., was used concurrently in each series of tests with the treated CB-1A solutions to be certain that the threshold value did not change appreciably.

Reagin Neutralization With Treated CB-1A.—The reagin neutralizing capacities of the treated CB-1A solutions were determined by direct intracutaneous injection of sites 1 or 3 on the second day of the test with 0.05 ml. of solution containing 0.13 or 0.25 mg. of treated CB-1A.

RESULTS AND DISCUSSION

Subcutaneous injections of CB-1A into 161 nonallergic men have been correlated with regard to development of blocking antibodies, reagins, skin sensitivity and/or clinical symptoms. These injections were made with another primary objective, namely, determination of the inactivation of the reagin neutralizing property of CB-1A resulting from various physical and chemical treatments of CB-1A. Nevertheless, correlation and interpretation of the responses of the recipients are of interest because of the alleged potent sensitizing property of the castor bean allergen.

A few slightly positive or doubtful skin reactions were encountered in the initial scratch test screening of potential recipients. Although none of these reactions was regarded as indicative of clinical castor bean sensitivity, nevertheless, subjects showing slight or doubtful skin sensitivity were not used in the tests.

In general, each weekly test period involved 4 days. Two days were used for passively sensitizing sites, one day for challenge of sites by direct intracutaneous injection and one day for challenge of sites by subcutaneous injection of 1.0 mg. of CB-1A. The material injected in the intracutaneous tests ranged from approximately 0.016 to 0.13 μg of CB-1A to 0.25 mg. of treated CB-1A. Because of the great variation in dosage of the intracutaneous injections, it was impossible to correlate their effects which probably would be of lesser immunologic importance than the 1.0 mg. doses of CB-1A injected subcutaneously.

Generally, each recipient was used for only one 4-week (or less) series of tests. However, a few were used for two or three series of tests with intervals of 10 to 95 weeks between each series. There were 2 apparently refractory recipients who gave weak or doubtful passive transfer reactions.

There were 132 recipients, or 82 per cent of the total, who had one series of tests ranging from one to 5 consecutive 1.0 mg. injections of CB-1A weekly and who had no apparent immunologic response as measured by development of blocking antibodies or clinical evidence of hypersensitivity as shown in Table I. Seventy-four (46 per cent) of this group had four or five consecutive weekly doses of 1.0 mg. of CB-1A each. There were 8 recipients, or 5.0 per cent of the total, who had two series of tests with intervals of from 10 to 95 weeks between series, and who had no response, as shown in Table II. Thus, 87 per cent of the total had no apparent response from one to a maximum of eight 1.0 mg. injections of CB-1A.

TABLE I. SUMMARY OF TESTS ON RECIPIENTS RECEIVING ONE SERIES OF SUBCUTANEOUS INJECTIONS OF CB-1A AT WEEKLY INTERVALS WHO SHOWED NO APPARENT IMMUNOLOGIC RESPONSE*

NO. OF RECIPIENTS	NO. OF INJECTIONS OF CB-1A (1.0 MG. EACH)	PER CENT OF TOTAL
4	5	2.5
70	4	43.5
20	3	11.2
33	2	20.5
5	1	3.1

*A few doses of CB-1A were given 2 days apart instead of 7. This was done only once in a series on any recipient.

TABLE II. SUMMARY OF TESTS ON RECIPIENTS RECEIVING TWO SERIES OF SUBCUTANEOUS INJECTIONS OF CB-1A AT WEEKLY INTERVALS WHO SHOWED NO IMMUNOLOGIC RESPONSE

RECIPIENT	FIRST SERIES	INTERVAL BETWEEN SERIES (WEEKS)	SECOND SERIES
	NO. OF INJECTIONS OF CB-1A (1.0 MG. EACH)		NO. OF INJECTIONS OF CB-1A (1.0 MG. EACH)
H. B.	4	90	1
C. B.	4	11	1
E. B.	3	85	4
W. H.	2	10	4
N. McD	2	95	4
R. S.	3	20	4
E. S.	2	10	4
E. P.	4	90	4

Four recipients (2.5 per cent) had slight clinical reactions in the first series of tests, as shown in Table III. One of these had itching and a rash on the neck, a second had swelling at the site after the first injection of CB-1A, a

TABLE III. SUMMARY OF TESTS ON RECIPIENTS WHO HAD SLIGHT REACTIONS IN FIRST SERIES OF TESTS

RECIPIENTS	NO. OF INJECTIONS OF CB-1A (1.0 MG. EACH)	CLINICAL REACTIONS FOLLOWING LAST SUBCUTANEOUS INJECTION OF CB-1A
C. I.	1	Itching, rash on neck
W. P.	1	Swelling at site of injection
M. P.	3	Wheal at site of injection
B. W.	3	Itching and rash on injected arm, duration 4 hours

third had a wheal at the site of the injection, and the fourth had itching and a rash on the injected arm after the third injection. These persons were not used in further tests after the first clinical reaction in each case. Unfortunately, they were not retested for skin sensitivity.

Fifteen (9.3 per cent) recipients developed some symptoms attributed to injection of CB-1A during the second or third series of tests, as shown in Table IV. Nine of this group lost their ability to give positive passive transfer reaction and 2 more became doubtful reactors during the second series of tests. One recipient, H. Sha, appeared to lose his passive transfer capacity in the third series after a total of ten 1.0 mg. injections of CB-1A. It is noteworthy that 2 recipients, D. R. and M. McD., who lost passive transfer capacity in the second series of tests, regained their passive transfer capacity in the third series, about 77 weeks later, and gave four positive passive transfer reactions in 4 consecutive weeks. Two recipients, C. R. and J. S., complained of sore arms after the last injection of the second series but they gave positive passive transfer reactions in this final test.

The subject of reaginic and blocking antibodies and its early history is well reviewed by Sherman,¹⁷ who, speaking of injected antigen, states: "During this passage through the blood stream, the blocking antibody has ample opportunity to inactivate the antigen." We have attributed the loss of passive transfer capacity of the above-mentioned recipients to neutralization of CB-1A by blocking antibodies while the subcutaneously injected CB-1A traveled to the sensitized site on the opposite arm. Sherman further states: "In clinical hay fever, on the other hand, the pollen reaches the sensitized nasal membranes directly, and the question is: does blocking antibody have any chance to inactivate it?" Seemingly pertinent to this question is the observation that the recipients who had lost passive transfer capacity when challenged by the subcutaneous route retained this capacity when the sites were challenged by direct intracutaneous injection. Thus, it appears that neutralization of CB-1A by blocking antibody occurred in the blood stream when CB-1A was injected into the arm opposite that sensitized, but neutralization did not occur when CB-1A was injected directly into the sensitized site. It is noteworthy that of the recipients who lost their capacity for passive transfer tests and, therefore, were presumed to have developed blocking antibodies, none developed clinical symptoms. Conversely, all those who developed clinical symptoms, those in Table III, and C. R., J. B., and J. S. in Table IV, retained their passive transfer capacity, even on receiving the subcutaneous injection which produced the symptoms and, therefore, they were presumed not to have developed blocking antibodies.

The presence of blocking antibodies was determined by the tanned red cell method¹⁸ with 5 recipients listed in Table IV who had lost passive transfer capacity. Serum from these recipients was collected within 5 days to 3 weeks after they lost passive transfer capacities. Results of the tanned red cell titration (Table V) showed that one serum agglutinated tannic acid-treated human type O, Rh negative red blood cells coated with CB-1A, 1 mg. per milliliter at

TABLE IV. SUMMARY OF TESTS ON RECIPIENTS RECEIVING TWO OR THREE SERIES OF SUBCUTANEOUS INJECTIONS OF CB-1A AT WEEKLY INTERVALS WHO DEVELOPED CLINICAL SYMPTOMS OR LOST PASSIVE TRANSFER CAPACITY (PRESUMABLY DEVELOPED BLOCKING ANTIBODIES) DURING SECOND SERIES

RECIPIENT	FIRST SERIES		INTERVAL WEEKS	SECOND SERIES			CLINICAL SYMPTOMS	INTERVAL (WEEKS)	THIRD SERIES†	
	NO. OF DOSES OF CB-1A	NO. OF DOSES OF CB-1A		NO. OF POS. P-K TESTS	BLOCKING ANTIBODIES	NO. OF DOSES OF CB-1A			NO. OF POS. P-K TESTS	
A. H.	3	4	10	4	2	+	Neg.			
C. E.	4	3	87	3	3	Neg.	S*			
D. R.	3	4	10	4	1	+	Neg.	77	4	4
H. Sha.	5	2	11	2	2	Neg.	Neg.	82	4	3
H. She.	3	4	11	4	1	+	Neg.			
J. B.	2	2	89	2	2	Neg.	GR†			
J. Ba.	2	4	10	4	3	+	Neg.	80	4	4
J. D.	2	4	10	4	1	+	Neg.			
J. S.	4	1	10	1	1	Neg.	S*			
L. T.	3	4	10	4	3	±	Neg.			
M. McD.	2	4	10	4	2	+	Neg.			
R. C.	2	3	17	3	1	+	Neg.	78	4	4
R. W.	5	4	10	4	1	+	Neg.			
T. M.	5	4	14	4	2	+	Neg.			
W. V.	2	4	10	4	3	±	Neg.			

*Sore arm.

†Generalized reaction, hives, itching.

‡No clinical symptoms.

maximum dilution of 1/320, two at 1/125, one at 1/5, and one \pm at 1/5. Control tests were negative with a normal serum and with a serum from E. McL. who was clinically sensitive to castor beans and had high reagin titer.

TABLE V. TANNED RED CELL AGGLUTINATION WITH SERUM OF SOME RECIPIENTS (TABLE IV) WHO APPEARED TO HAVE DEVELOPED BLOCKING ANTIBODIES DURING SECOND SERIES OF INJECTIONS WITH CB-1A

RECIPIENT	TITER GIVING AGGLUTINATION TANNED RED CELL*
J. D.	1/125
M. McD.	1/125
D. R.	1/320
A. H.	1/5
J. Ba.	1/5 (\pm)
E. McL.†	Neg.
C. N.‡	Neg.

*Highest fivefold serial dilution of serum giving agglutination.

†Castor bean reaginic serum used in previous studies, undiluted.

‡Cottonseed reaginic serum, undiluted.

The most interesting case was that of J. B. (Table IV) who had no family history of allergy and was a good recipient. J. B. had generalized itching and hives over the whole body following the second subcutaneous injection of CB-1A in the second series of tests. The chronology of tests on J. B. is shown in Table VI. In the first series of tests, J. B. gave a negative scratch test with

TABLE VI. CHRONOLOGY OF TESTS ON J. B. WHO DEVELOPED REAGINS, SKIN SENSITIVITY, AND CLINICAL SYMPTOMS FROM INJECTIONS OF CB-1A

DATE	TYPE OF TEST	AMOUNT OR CONCENTRATION OF CB-1A	RESULTS I AND COMMENT‡
11/12/58	Cutaneous	1:100	Neg.
11/18/58	Sensitize*		
11/19/58	Intracutaneous	0.13 mg. CB-1A (T)†	2+ Passive transfer reaction
11/20/58	Sensitize*		
11/21/58	Subcutaneous	1.0 mg. CB-1A	3+, 3+ Passive transfer reactions
12/ 2/58	Sensitize*		
12/ 3/58	Intracutaneous	0.016 μ g CB-1A	3+ Passive transfer reaction
12/ 4/58	Sensitize*		
12/ 5/58	Subcutaneous	1.0 mg. CB-1A	2+, 3+ Passive transfer reactions
8/25/60	Cutaneous	1:200	Neg.
8/30/60	Sensitize*		
8/31/60	Intracutaneous	0.25 mg. CB-1A (T)†	4+ Passive transfer reaction
9/ 1/60	Sensitize*		
9/ 2/60	Subcutaneous	1.0 mg. CB-1A	4+ Passive transfer reaction
9/ 6/60	Sensitize*		
9/ 7/60	Intracutaneous	0.25 mg. CB-1A (T)†	3+ Passive transfer reaction
9/ 8/60	Sensitize*		
9/ 9/60	Subcutaneous	1.0 mg. CB-1A	4+ Passive transfer reaction and generalized itching and hives

*Sensitized with 0.05 ml. of E. McL. castor bean reaginic serum.

†(T) = Treated.

‡Size of reaction: 1+, wheal up to 6 mm.; 2+, wheal 7 to 12 mm.; 3+, wheal 13 to 20 mm.; 4+, wheal, over 20 mm.

1:100 CB-1A in 50 per cent glycerin on Nov. 12, 1958. In the first series of tests he had two intracutaneous injections of 0.13 mg. and 0.16 μ g. of treated and untreated CB-1A, respectively, and two 1.0 mg., subcutaneous, injections of CB-1A. Eighty-nine weeks later, on Aug. 25, 1960, he gave a negative scratch test with 1:200 CB-1A. The second series of tests consisted of two intracutaneous injections of 0.25 mg. of treated CB-1A each and two weekly 1.0 mg. subcutaneous injections of CB-1A. Clinical symptoms of hives and itching were not apparent one hour after the last subcutaneous injection when the positive passive transfer reaction was read, but developed in the following one or 2 hours. This reaction occurred on Sept. 9, 1960.

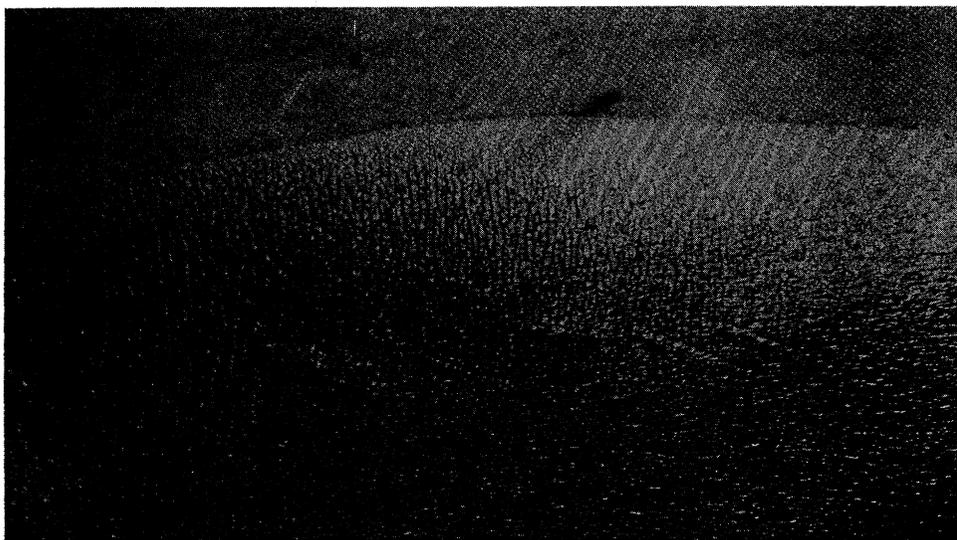


Fig. 1.—Reaction on J. B. to cutaneous test with 1:100 CB-1A. Negative control at *right*.

J. B. gave a positive scratch test with 1:100 CB-1A on September 25 and October 14, 16 and 35 days after the general reaction, respectively. The test on October 14 is shown in Fig. 1, after 30 minutes.

Blood serum was obtained from J. B. on September 15, 6 days after the general reaction. The presence of reagins was shown by sensitizing sites on 5 recipients with 0.05 ml. of J. B. serum on the left arm, site 2. Twenty-four hours later the sites were challenged by subcutaneous injection of 1.0 mg. of CB-1A into the right arm of each recipient. The diameters (in millimeters) of the passive transfer reactions were: 17 by 13, 8 by 10, 7 by 11, and 4 by 4, with one negative. The passive transfer reaction with J. B. serum on recipient I. B., 57 minutes after subcutaneous challenge, is shown in Fig. 2. The case of J. B. is the first recorded example of sensitization to injected castor bean allergen, CB-1A, with development of skin sensitivity, reagins, and clinical sensitivity, but apparently no blocking antibodies. According to Sherman,¹⁷ in general, injection of pollen antigens into nonallergic persons does not produce skin-sensitizing antibody. But skin-sensitizing antibodies are produced by the injection of heterologous antisera,¹⁹ insulin,²⁰ *Ascaris* antigen,²¹ and

diphtheria toxoid.²² J. B. became sensitized with essentially 4 1.0 mg. injections of CB-1A but with an 89 week interval between the first two and the last two injections. Whether or not he would have become sensitive to 4 consecutive weekly injections of CB-1A is not known. But, of the 161 recipients used, 95 had as much or more CB-1A than J. B. without becoming sensitized.

According to others, artificially induced sensitivity is transitory as compared with naturally acquired sensitivity. Cooke and Spain¹⁹ reported that serum taken 20 days after sensitization to horse serum contained reagins, but that taken 28 days after the first sample did not contain them. Sensitivity to insulin disappeared 4 months after its occurrence, according to Tuft.²⁰ Brunner²¹ observed that persons sensitized by injections of *Ascaris* had lost their sensitivity when tested 2 years after acquiring sensitivity. Likewise,

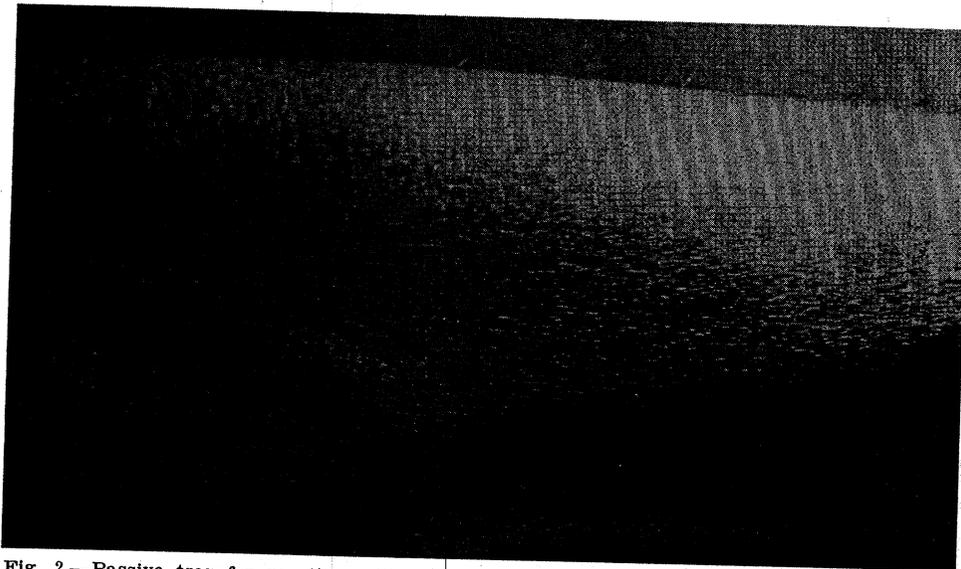


Fig. 2.—Passive transfer reaction with 0.05 ml. of J. B. serum challenged by subcutaneous injection of 1.0 mg. of CB-1A on opposite arm.

reagins in J. B. serum were transitory. Serum taken 7 weeks after J. B.'s clinical response to CB-1A gave negative passive transfer reactions when tested on 2 recipients on whom 4+ reactions were obtained at the same time in sites sensitized with castor bean reaginic serum from E. McL.

Of the 23 recipients receiving two or more series of injections of CB-1A, 15 (65 per cent) developed some evidence of immunologic response. None of the recipients who developed clinical reactions had respiratory symptoms such as asthma which is the type of castor bean sensitivity usually encountered as a result of natural sensitization and challenge. This difference may be due to the effect of different routes of entry of the allergen on the shock tissues; one by inhalation of dust and the other by subcutaneous injection.

SUMMARY

The immunologic responses of development of blocking antibodies, reagins, skin sensitivity and/or clinical symptoms of hypersensitivity of 161 nonallergic

men to 1.0 mg. subcutaneous injections of the castor bean allergen, CB-1A, have been studied. These data were obtained in previously described studies in which the men served as recipients for passive transfer tests. One hundred and thirty-two recipients (82 per cent), who had one series of tests consisting of from one to 5 consecutive weekly injections of 1.0 mg. of CB-1A, had no apparent response. Four (2.5 per cent) had slight clinical response in the first series, two of which followed the first and two the third injection of CB-1A. Eight recipients (5 per cent) had two series of injections of CB-1A with intervals of 10 to 95 weeks between series with no response. Fifteen recipients (9.3 per cent) had responses attributed to CB-1A in the second or third series of weekly injections of CB-1A. Twelve of these developed blocking antibodies as shown by loss of their ability to give passive transfer reactions but developed no clinically evident hypersensitivity. Two complained of soreness at the site of the injection. One of this group (J. B.) had hives and generalized itching after the fourth injection of CB-1A. Two of the injections of J. B. were given in one series and two in a second series, 89 weeks after the first. J. B. developed skin sensitivity to CB-1A and reagins in his blood serum. Of the 23 who had two or more series of tests, 15, or 65 per cent, developed blocking antibodies or some clinical response. None who developed blocking antibodies had clinical symptoms and those who developed clinical symptoms did not develop blocking antibodies. None of the clinical reactions were respiratory as is the case with naturally occurring castor bean allergy, a difference which may be due to different routes of entry of the allergen to shock tissues; one, naturally by inhalation of dust and the other by injection.

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