different samples were extracted and analyzed. Results are recorded in Table II.

Other Elements. Manganese is probably completely extracted from plant tissue by salts of EDTA, but attempts to use the 403-mμ Mn line for flame analysis were unsuccessful owing to the proximity of the 404-mμ potassium line. Sodium analysis by this procedure is not recommended, since the calcium normally present in plant tissue causes a positive error in the flame analysis of sodium. Sodium is more conveniently extracted from plant tissue with ammonium oxalate (*4*) which simultaneously precipitates calcium.

Phosphorus is not completely extracted by this procedure. A nearly colorless extract is obtained by addition of activated carbon. EDTA in low concentrations does not interfere with the molybdovanadophosphoric acid method for phosphorus so that "soluble" phosphorus might be determined by this method. A test of some samples showed that approximately 65% of the total phosphorus was extracted by ammonium EDTA at pH 8.0.

No attempt was made to extract other elements, although presumably any metals complexed by EDTA should be extractable. Since EDTA is used in the determination of copper (*1*), that element might be determined by this procedure if the organic matter extracted by EDTA does not interfere with the copper determination.

The simplicity and rapidity of this procedure suggest that it might be useful as the basis of a quick test system of plant analysis.

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CASTOR BEAN COMPONENTS

The Chemistry of Allergens. Inactivation of the Castor Bean Allergens and Ricin by Heating with Aqueous Calcium Hydroxide

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Castor oil with its derivatives has scores of industrial uses, while the pomace is used only as a fertilizer and this usage has some hazard because of the potent allergens it contains (*11, 16*). Maximum safe utilization of castor bean pomace, as fertilizer and even more so as livestock feed, requires inactivation of not only the allergens and ricin, the two principal harmful components, but also a substance which causes necrosis on injection of castor bean extracts. The toxicity of ricin is destroyed when its water solution is heated to boiling (*6*) or even to the coagulation temperature of the protein (*5*). However, the principal castor bean allergen retains immune-precipitating and allergenic properties after heating at 100° C. even in alkaline solution (*7, 16*).

The isolation and chemical and immunological properties of the principal allergen or allergens of castor beans, CB-1A, have been described in previous papers from this laboratory (*2, 4, 15, 17–19*). CB-1A is a mixture of proteins and polysaccharidic proteins, classified as natural proteoses. Layton, Moss, and DeEds (*10*) have described separation of six components of CB-1A by ion exchange chromatography and further study of relationships of antigenic and allergenic specificities of these components is in progress. Defatted, domestic castor beans and a commercial pomace yielded 1.8 and 0.33% of CB-1A, respectively. Isolation methods give minimal yields, and the allergen contents of several varieties of castor bean meals ranged from 6 to 9% as determined by a quantitative precipitin method (*3*). The allergenic and antigenic specificities of the components of CB-1A are attributed to the protein components (*4, 17*). CB-1A is soluble in water and in basic lead acetate solution but is precipitated by 75% ethyl alcohol. CB-1A is composed of amino acids with relatively high arginine and glutamic acid contents and no tryptophan (*18*). CB-1A contains no ricin and is nontoxic.

In a previous report the effects of heating the castor bean allergen in solutions of pH 4 to 10 for various times on its immune-precipitating and reagin-neutralizing properties were determined (*16*). Gardner and associates (*7*) made practical exploratory experiments with several chemicals on the detoxification and deallergenization of castor beans. They determined destruction of ricin toxicity by agglutination, and allergen content of treated material by an antigen dilution method with rabbit antiserum. Alkaline heat treatments were effective in destroying the precipitating property of the allergen, but no tests of products of inactivation were made with a castor bean–sensitive person either directly or with serum by the passive-transfer method. Gardner worked mostly with castor bean meats (undefatted) of low moisture content; since he gave no pH values, comparison with the present work is not possible. Calcium hydroxide was used by Gardner in one experiment, but under conditions of the test it was not as effective as sodium hydroxide in reducing allergen content. Kodras, Whitehair, and MacVicar (*9*) studied the effects of heating castor bean pomace with water, sodium hydroxide, and hydrochloric acid slurries on the oral toxicity to rats and chicks.

This paper describes the conditions of temperature, time, and pH for the inactivation of the castor bean allergen, ricin, and the necrotizing agent (*4*) by heating with aqueous calcium hydroxide. After inactivation, excess calcium hydroxide is neutralized with phosphoric acid to yield a safe-to-handle mixture suitable for plant nutrition and possibly for livestock feed. Both castor bean meal and the isolated allergen were studied. Inactivation of ricin was determined by toxicity tests with guinea pigs. Destruction of immune-precipitating property of the allergen was determined with rabbit antiserum. Destruction of the allergenic properties of reagin neutralization and skin reactivity were determined with

Castor beans contain two principal harmful components, an unusually stable allergen and ricin, a less-stable, extremely toxic protein. The conditions of time, temperature, and pH have been determined for the inactivation of the allergen and ricin with calcium hydroxide to yield a safe-to-handle mixture of pomace and calcium acid phosphate. A relationship between destruction of the immune-precipitating and reagin-neutralizing properties of the allergen was observed. At pH 5.9 to 8.7, heating destroyed reagin-neutralizing property before precipitating property but at pH 10.8 to 11.9 the reverse was observed. Castor beans yield approximately 50% each of oil and pomace. The pomace is now utilized as fertilizer, which has some hazard because of its residual allergen content. The market value of the castor bean crop could be enhanced if inactivation of the allergens extended the usefulness of the pomace as a fertilizer or livestock feed.

serum from a castor bean–sensitive person by a passive-transfer method and by direct cutaneous testing on a castor bean–sensitive person, respectively.

Materials

Castor Bean Allergen (CB-1A)E. CB-1A was isolated from castor beans as described previously (*15*, *18*). (CB-1A)E was obtained from CB-1A by dialysis. Sixty-two grams of CB-1A was dissolved in 620 ml. of water and dialyzed in a Visking cellulose casing against five 1500-ml. changes of water at intervals of 24, 68, 168, 168, and 167 hours (total time of dialysis was 595 hours). The total nitrogen removed was 1, 2.8, 5.4, 4.3, and 3.8% in respective dialysis intervals. The fraction remaining inside the membrane was precipitated with four volumes of ethyl alcohol, isolated by centrifuging, and washed with 500 ml. of ethyl alcohol. The precipitate was dried over calcium chloride and equilibrated with air. The yield of (CB-1A)E was 44.4 grams. (CB-1A)E contained 16.6% nitrogen and 5.7% carbohydrate (*8*), air-dried basis. CB-1A and (CB-1A)E are regarded as equivalent for the purpose of this study (*4*, *15*).

Castor Bean Meal. The castor bean meal was sample SL-33 (Argentina 1938) prepared as described previously (*3*). SL-33 contained 7.7% CB-1A as determined by a quantitative precipitin method (*3*).

Rabbit Antiserum. Rabbits were immunized to a castor-bean allergen fraction, CB-13E, with Freund's adjuvant as described before (*16*). Fraction CB-13E contained the principal antigens of (CB-1A)E (*3*).

Reaginic Serum. Blood serum was obtained from a castor bean–sensitive person. All tests were conducted with the same lot of serum, stored at 3° to 5° C.

Recipients, Passive-Transfer Tests. Recipients for the passive-transfer tests were nonallergic, castor bean nonreactors, who were free from antihistamine medication.

Cutaneous Tests. Conventional cutaneous testing was done on the forearms of an adult male, at George Washington University Hospital, who was clinically sensitive (asthma) to castor beans and whose blood serum gave positive passive-transfer reactions when tested with (CB-1A)E.

Methods

Heating. Suspensions or solutions were heated in 15 × 120 mm. heavy-walled, borosilicate glass tubes. The tubes were closed by a gasketed screw cap or by flame sealing, depending on the temperature used. The tubes were placed in 15-ml. metal centrifuge cups which were already at the temperature of the test. These cups were placed upright in an aluminum block, 7 cm. high and 14 cm. in diameter, bored to hold eight tubes and a thermometer. The block was at the temperature of the test at the start and heating for 1-hour intervals was carried out in an oven at constant temperature ±1° C. Hea ing for shorter periods, as in the rate studies, was done in a stirred water or oil bath at constant temperature ±1° C.

Treatment of Castor Bean Meal with Calcium Hydroxide. To 78-mg. samples of castor bean meal were added calculated amounts of water and a freshly prepared aqueous suspension of calcium hydroxide (100 or 10 mg. per ml.) to give a total volume of 3.0 ml. and percentages of calcium hydroxide (based on meal) of 0, 1, 2, 4, 8, and 16. The concentrations of calcium hydroxide in these suspensions were 0, 0.0035, 0.007, 0.014, 0.028, and 0.056*M*, respectively. Meal and water were left standing 15 minutes before calcium hydroxide was added to dissolve soluble components. Then the contents of the tubes were mixed; and the tubes were sealed and heated. The tubes were cooled to room temperature and pH was determined. Then calculated amounts of water and 0.1*M* orthophosphoric

Table I. Effect of Heating Castor Bean Meal with Aqueous $Ca(OH)_2$ on Toxicity and Immune-Precipitating Capacity of (CB-1A)E

(Heating time, 1 hour at indicated temperature)

$Ca(OH)_2$		60° C.			80° C.			100° C.		
M	%[a]	pH[b]	Precipitin[c]	Toxicity[d]	pH[b]	Precipitin[c]	Toxicity[d]	pH[b]	Precipitin[c]	Toxicity[d]
0	0	6.3	4+	0.2 D	6.2	4+	0.8 N	6.4	4+	12.8 NS
0.0035	1	8.0	4+	0.2 D	7.7	4+	12.8 I	7.5	4+	12.8 NS
0.007	2	10.1	4+	0.8 D	9.5	3+	12.8 I	9.0	1+	12.8 NS
0.014	4	11.0	3+	1.6 D	10.4	1+	12.8 I	10.3	1+	12.8 I
0.028	8	12.2	1+	6.4 I	11.8	0	12.8 I	11.8	0	12.8 I
0.056	16	12.5	±	12.8 NS	12.5	0	12.8 I	12.3	0	12.8 I

[a] Based on weight of meal used per test (78 mg.).
[b] After heating and cooling, before addition of phosphoric acid.
[c] Precipitin reaction with 1 to 10^4 dilution of (CB-1A)E, based on analysis of meal.
[d] NS, no symptoms with the quantity of meal (mg.) indicated; I, induration (approximately 20 mm.) with the quantity of meal (mg.) indicated but no symptoms with smaller amounts and no deaths with 12.8 mg.; N, necrosis with the quantity of meal indicated but no symptoms with smaller amounts and no deaths with 12.8 mg.; D, death with indicated quantity of meal, lesser amounts usually produced necrosis.

acid were added (one mole of acid per mole of calcium hydroxide) so that the total volume was 6.0 ml. The pH of the neutralized slurries was 6 ± 0.5. After several minutes with occasional shaking, the solid was removed by centrifuging. The supernatant solution was filtered, if necessary. This solution, or dilution thereof, was used for toxicity and precipitin tests. The solutions used for the cutaneous tests were prepared as described above, except that they were heated in closed tubes of about 4-ml. capacity and were turned upside down and mixed frequently while immersed in the heated oil bath. This ensured intimate contact of calcium hydroxide with meal and left no trace of untreated meal on the sides of the tube. The pH of suspensions of calcium hydroxide and meal became progressively lower with longer time of heating and their pH values were lower than suspensions of calcium hydroxide and (CB-1A)E heated corresponding periods of time as shown in Table VI.

Table II. Effect of Time of Heating Castor Bean Meal at 100° C. with 0.028*M* $Ca(OH)_2$ on Its Toxicity and on Immune-Precipitating Capacity of Its (CB-1A)E

Time, Min.	pH[a]	Precipitin[a]	Toxicity[b]
0	12.5	4+	0.4 D
1	12.6	3+	1.6 I
2	12.6	1+	3.2 I
4	12.5	1+	6.4 I
8	12.3	1+	6.4 I
16	12.0	±	12.8 I
32	11.2	0	12.8 I
64	11.2	0	12.8 I

[a] See footnotes [a] and [b], Table I.
[b] See footnote [d], Table I.

Table III. Determination of Quantity of (CB-1A)E Required to Neutralize Reagins in Passively Sensitized Sites

(CB-1A)E per Test Site, Mμg.[a]	No. of Tests	Reagins in Sensitized Site: Neutralized	Reagins in Sensitized Site: Not neutralized
16	3	0	3
32	8	0	8
64	8	2	6
128	8	7	1
256	8	8	0

[a] Quantity of (CB-1A)E in 0.05 ml. injected into site previously sensitized with 0.05 ml. of serum.

Treatment of (CB-1A)E with Calcium Hydroxide. Samples (30 mg.) of (CB-1A)E were dissolved in the calculated volumes of water, and a freshly prepared aqueous suspension of calcium hydroxide (100 or 10 mg. per ml.) was added to give a total volume of 3.0 ml. and 0, 0.0035, 0.007, 0.014, 0.028, and 0.056*M* concentrations of calcium hydroxide. The solutions or suspensions were heated in sealed tubes and cooled, and the pH was determined. Excess calcium hydroxide was neutralized with 0.1*M* orthophosphoric acid, mole for mole, and the final volume was adjusted to 6.0 ml. with water. The pH of resulting solutions or suspensions was 6 ± 0.5. After occasional shaking for several minutes, the precipitate of calcium acid phosphate, if any, was separated by centrifuging. The clear solutions or their dilutions were used for precipitin and allergen tests.

Toxicity Tests. Toxicity was determined by subcutaneous injection in guinea pigs of 1 ml. of treated or untreated meal solution. Twofold serial dilutions were injected through a maximum dosage of extract from 12.8 mg. of castor bean meal. Guinea pigs were observed for 7 days. Time of death ranged from less than 24 hours to several days. The average lethal dose of untreated meal was 0.1 mg. per 300 ± 20-gram guinea pig, the range being from 0.05 to 0.2 mg.

Precipitin Tests. Clarified rabbit antiserum (0.15 ml.) was mixed with 0.15 ml. of test solution in a 5 × 45 mm. tube and incubated 30 minutes at 37° C. The tubes were placed at 5° C., and the precipitate was read visually after 24 hours. Dilutions were made with a buffered saline solution, pH 7.0. Control tests with normal rabbit serum were negative with all dilutions of untreated (CB-1A)E and with 1 to 10^4 dilutions of treated or untreated castor bean meal. The precipitin readings of twofold dilutions of untreated (CB-1A)E starting at 1 to 10^3 through 1 to 512,000 were: 1+, 2+, 3+, 4+, 3+, 3+, 2+, 1+, 1+, and 0, respectively. Treated meal and (CB-1A)E solutions were tested at 1 to 10^4 with respect to their original (CB-1A)E content to determine destruction of precipitating capacity. Therefore, absence of a precipitate with 1 to 10^4 dilution indicated destruction of 98%, or more, of the precipitating capacity.

Preparation of Solutions for Passive Transfer Tests. Four-milliliter samples of undiluted, treated (CB-1A)E solutions were sterilized by filtration through a borosilicate glass bacterial filter into 6-ml. sterile bottles.

Reagin Neutralization Method. The method described before (*16*) was used, with one modification: Sensitized site pairs were 1 and 3 and 2 and 4, instead of 1 and 2 and 3 and 4. Also, 0.05 ml. instead of 0.025 ml. of solution was used for the intracutaneous challenge on the second day of the test. Fundamental principles of this method are described under Results and Discussion.

Results and Discussion

The effects of heating castor bean meal with aqueous calcium hydroxide for 1 hour at 60°, 80°, and 100° C. on its toxicity to guinea pigs and its immune-precipitating capacity with castor bean allergen rabbit antiserum are shown in Table I. The pH values of the suspensions ranged from 6.2 without calcium hydroxide to 12.5 with the maximum concentration of calcium hydroxide (0.056*M* or 16% based on weight of meal). The toxicity of meal heated at 60° C. at pH 6.3, 11, and 12.2 was approximately 100, 6, and 0%, respec-

Table IV. Effect of Heating (CB-1A)E with Aqueous $Ca(OH)_2$ on Immune-Precipitating Capacity and Allergenic Property (Reagin Neutralization)

(Heating time, 1 hour at indicated temperature)

$Ca(OH)_2$, *M*	80° C. pH[a]	80° C. Precipitin[b]	80° C. Allergenic property[c]	100° C. pH[a]	100° C. Precipitin[b]	100° C. Allergenic property[c]	120° C. pH[a]	120° C. Precipitin[b]	120° C. Allergenic property[c]
0	6.2	4+	+		4+	+	6.0	2+	+
0.0035	9.8	1+	+	9.3	1+		8.7	1+	0
0.007	11.0	1+	+	10.8	0	+	9.8	0	0
0.014	11.9	0	+	11.4	0	+	10.6	0	0
0.028	12.4	0	+	12.0	0	0	12.1	0	0
0.056	12.5	0	+	12.4	0	0	12.7	0	0

[a] pH of solution or suspension after heating and cooling, before addition of phosphoric acid.
[b] Precipitin reaction with 1 to 10^4 dilution of (CB-1A)E.
[c] +, less than 99.95% destruction; 0, destruction of 99.95% or more reagin neutralization capacity of (CB-1A)E.

tively, of that of untreated meal. Toxicity was destroyed by heating at 80° C. at pH 6.2, but the necrotic factor was not destroyed, as 0.8 mg. of meal produced necrosis. Heating at 80° and 100° C. at pH 7.7 and 6.4, respectively, destroyed both toxicity and the necrotic factor. Induration sometimes produced at sites of injection was caused, in part at least, by the calcium phosphate. The precipitating property of meal extracts was destroyed at 60°, 80°, and 100° C. at pH 12.5, 11.8, and 11.8, respectively.

The rate of destruction of ricin toxicity and the immune-precipitating property of the allergen when meal was heated at 100° C. in 0.028*M* calcium hydroxide are shown in Table II. The temperature of the meal–calcium hydroxide suspension inside the borosilicate glass tube was 70°, 85°, 91°, 98°, and 100° C. after 30, 45, 60, 90, and 120 seconds, respectively, in the water bath at 100° C. Thus, heating at 91° C. at pH 12.6 for 1 minute destroyed the toxicity and necrotizing agent of the meal, as there were no symptoms with dosages of 0.1, 0.2, 0.4, and 0.8 mg. of meal and only slight induration with dosages through 12.8 mg. The immune-precipitating property was destroyed in 32 minutes at pH 11.2.

The Prausnitz-Küstner or passive-transfer test (*12, 13*) was used to evaluate destruction of the allergenic property of reagin neutralization, because not enough hypersensitive individuals were available for direct testing. Blood serum from a castor bean–sensitive person contains reagins or allergic antibodies capable of reacting specifically and quantitatively with the castor bean allergen. When the serum is injected intracutaneously into a normal, nonsensitive person, the reagins become fixed in the tissues and impart a local sensitivity to castor bean allergen. An area surrounding the site of the injection ranging from 20 to 40 mm. in diameter becomes sensitized with 0.05 ml. of this serum. When allergen is injected into a site sensitized 24 hours previously, it reacts to form a wheal. There is a quantitative relationship between the quantity of allergen injected and the wheal-producing capacity of the reagins present. Thus, if an excess of allergen is injected into the site to combine with all the reagins, no further reaction can be obtained by a second injection of allergen 24 hours after the first reaction has subsided. But if insufficient allergen is injected to neutralize all the reagins present, a further reaction can be obtained by a second injection of allergen 24 hours after the first reaction has subsided. Therefore, the amount of (CB-1A)E (in 0.05 ml. of solution) required to neutralize the reagins in a site sensitized with 0.05 ml. of serum was determined.

Results in Table III show that under the conditions of the test, 128 mμg. of (CB-1A)E was the threshold amount required to neutralize the reagins. Treated test solutions of (CB-1A)E injected into sensitized sites contained 250 μg. per 0.05 ml. Therefore, if 250 μg. of treated (CB-1A)E did not neutralize the reagins, as shown by further reaction on subsequent testing, 99.95% or more of the allergen had lost the property of specifically reacting with reagins. If no further reaction was obtained on second test after first testing with the 250 μg., it indicated that at least 128 mμg. of (CB-1A)E remained in the treated solution and that less than 99.95% of the allergen was destroyed. Determination of this degree of completeness of destruction of the allergen was necessary because of the extremely small amount of castor bean allergen capable of provoking an asthmatic attack in hypersensitive persons. The possibility that an allergic response may be provoked by allergen too far degraded to neutralize reagins has been recognized and discussed (*16*). To settle this question definitely would require further study.

Comparison of the effects of heating (CB-1A)E with concentrations of calcium hydroxide from 0 to 0.056*M* for one hour at 80°, 100°, and 120° C. on its immune-precipitating capacity and on the allergenic property of reagin neutralization is shown in Table IV. At 80° C., the precipitating property was destroyed at pH 11.9, but the reagin-neutralizing property was not destroyed even at pH 12.5. At 100° and 120° C., the precipitating capacity was destroyed at pH 10.8 and 9.8 and reagin neutralizing property at pH 12.0 and 8.7, respectively.

The rates of destruction of reagin-neutralizing and immune-precipitating capacities of (CB-1A)E at 100° and 120° C. in 0.028*M* calcium hydroxide are shown in Table V. At 100° and 120° C., the immune-precipitating capacity was destroyed in 4 and 2 minutes at pH 12.6, and 12.4, respectively, and the reagin-neutralizing capacity was destroyed in 32 and 8 minutes at pH 12.4, and 12.2, respectively.

The effects of heating both (CB-1A)E and castor bean meal with calcium hydroxide on the skin reactivity on a castor bean–sensitive person are shown in Table VI. Cutaneous test solutions of

Table V. Effect of Time of Heating (CB-1A)E, with 0.028*M* Aqueous $Ca(OH)_2$ on Immune-Precipitating Capacity and Allergenic Property (Reagin Neutralization)

Time, Min.	100° C.			120° C.		
	pH[a]	Precipitin[b]	Allergenic[c] property	pH[a]	Precipitin[b]	Allergenic[c] property
0	12.6	4+		12.6	4+	
1	12.5	1+	+			
2	12.5	1+	+	12.4	±	+
4	12.6	0	+	12.3	0	+
8	12.5	0	+	12.2	0	0
16	12.5	0	+	12.1	0	0
32	12.4	0	0	12.0	0	0
64	12.3	0	0	11.6	0	0

[a,b,c] See footnotes of Table IV for explanation of column heads.

Table VI. Cutaneous Tests with Treated (CB-1A)E and Castor Bean Meal Solutions

(pH of neutralized test solutions 6.0 ± 0.5)

Temp., ° C.	Time Heating, Min.	$Ca(OH)_2$ M	$Ca(OH)_2$ %[a]	(CB-1A)E pH[b]	(CB-1A)E Cutaneous[c] test	Meal pH[b]	Meal Cutaneous[c] test
100	64	0.028		12.3	0		
	32	0.028		12.4	0		
	16	0.028		12.5	3+		
	32	0.056	16			12.1	0
120	32	0.028	8	12.0	0	10.4	0
	16	0.028	8	12.1	0	10.7	0
	8	0.028	8	12.2	2+[d]	11.2	0
	32	0.056	16			11.9	0
	16	0.056	16			11.9	0
	8	0.056	16			11.8	0
130	60			5.9	3+		
140	60			5.9	±		
150	60			5.9	0		

[a] Based on weight of meal.
[b] After heating and cooling to room temperature before neutralization with phosphoric acid.
[c] Readings in 15 to 20 minutes.
[d] 2+ reaction obtained twice and 0 (negative) reaction once.

Table VII. Effect of Heating (CB-1A)E on Immune-Precipitating Capacity and Allergenic Property (Reagin Neutralization)

(Heated 1 hour at indicated temperature at pH 5.9)

Temp., °C.	Precipitin[a]									Allergenic[b] Property
	2	4	8	16	32	64	128	256	512	
25	2+	3+	4+	3+	3+	2+	1+	1+	0	
90	2+	3+	4+	4+	3+	2+	1+	1+	0	
100	2+	3+	4+	3+	2+	1+	1+	1+	0	
110	2+	4+	3+	2+	2+	1+	1+	1+	0	+
120	±	1+	1+	2+	1+	1+	1+	0	0	+
130	1+	±	1+	1+	1+	1+	1+	0	0	+
140	0	0	1+	1+	1+	1+	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0
170	0	0	0	0	0	0	0	0	0	0

[a] Precipitin reaction of twofold serial dilutions of (CB-1A)E. Concentration of (CB-1A)E expressed as reciprocal of dilution times 10^{-3}—e.g., 2 equals 1/2000 dilution.
[b] See footnote, Table IV.

treated (CB-1A)E and meal were diluted 1 to 200 and 1 to 1000, respectively, on the basis of their original (CB-1A)E contents. The highest dilution of (CB-1A)E giving negative cutaneous tests was 1 to 2,000,000. Therefore, a negative cutaneous test with treated solutions of (CB-1A)E and meal indicated 99.99 and 99.95% or more destruction of skin reactivity, respectively. The results show good agreement between destruction of reagin-neutralizing and skin reactivity properties of heated (CB-1A)E solutions. At 100° and 120° C., the reagin-neutralizing capacity of (CB-1A)E was destroyed in 32 and 8 minutes and the skin reactivity was destroyed in 32 and 16 minutes, respectively, at pH 12 or over. The skin reactivity of treated meal likewise was destroyed by heating at 120° C. for 8 minutes and at 100° C. for 32 minutes, at pH values of 11.2 and 12.1, respectively, after the heating periods.

The effects of heating (CB-1A)E at pH 5.9 on the destruction of reagin-neutralizing and immune-precipitating capacities are shown in Table VII and the effects on skin reactivity over the range 130° to 150° C. are shown in Table VI. (CB-1A)E is not coagulated at temperatures up to 170° C. at pH 5.9 through >12.0. The results serve as a basis of comparison of the increased inactivation effect of pH values higher than 5.9 at the various temperatures. Heating (CB-1A)E at 90° and 100° C. had no effect on the precipitating capacity. At 110° C., one half of the precipitating capacity was destroyed. At 120° C., destruction was marked and from 130° to 150° C., precipitating property disppeared gradually rather than abruptly (*16*). Reagin-neutralizing and skin-reacting capacities were destroyed on heating at 140° C., but heating at 150° C. was required for complete destruction of precipitating capacity.

The relationship between destruction of reagin-neutralizing and immune-precipitating properties of (CB-1A)E by heating appears to depend on the pH. Thus, at pH 5.9 to 8.7, the immune-precipitating property appears to be more stable than the reagin-neutralizing property. At pH 5.9, heating for 1 hour at 150° and 140° C. was required to destroy the immune-precipitating and reagin-neutralizing properties, respectively (Table VII). And at pH values up to 8.7 at 120° C. the immune-precipitating property was also more stable than the reagin-neutralization property (Table IV). But at pH values above 10.8 to 11.9 at 100° and 80° C., respectively, the reagin-neutralizing property was significantly more persistent than the immune-precipitating property (Table IV). This observation is not entirely unexpected, because immune precipitation is dependent on both the molecular size of the antigen and a specific grouping capable of reacting with the antibody. The specificity determinant groupings responsible for immune precipitation and reaction with reagins are not necessarily the same and they may have different stabilities under different conditions. Furthermore, in reagin neutralization the specific grouping of the molecule alone would be necessary for reaction with reagins and this specific grouping might well remain intact as a polypeptide after heating in alkaline solution had split the molecule into fragments too small to precipitate. The allergen molecule undoubtedly ruptures at the disulfide linkage of its cystine component. This idea is supported by the qualitative observation that the amount of hydrogen sulfide liberated from heated (CB-1A)E solutions (on their acidification) is greater the higher the pH of heating. On the basis of the limited evidence available, destruction of reagin-neutralizing and skin-reacting properties seems to be more closely correlated than does destruction of reagin-neutralizing and immune-precipitating properties of (CB-1A)E. These results show that determination of loss of precipitating capacity of an allergen cannot be used as final criterion in determination of the destruction of allergenic properties.

The results of this study confirm and extend knowledge about the remarkable stability of the castor bean allergen. Retention of antigenic and allergenic properties after such drastic treatment, particularly heating in alkaline solution, is unique so far as published results are concerned. The other allergens of the natural proteose group—almond nut-1A, Brazilnut-1A, cottonseed-1A, filbert nut-1A, flaxseed-1A, kapok-1A, and mustard-1A—also are stable because they retain their antigenic and allergenic properties after being heated 1 hour at 100° C., in water, a treatment used in their isolation (*18*). The cottonseed allergen retained allergenic and antigenic properties after refluxing for 4 hours in 0.1*N* sulfuric acid (*1, 14*).

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