

IDENTIFICATION OF CASTOR BEAN ALLERGEN IN GREEN COFFEE*

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THIS report supplements the clinical evidence which led Figley and Rawling¹ to recognize apparent sensitiveness to green coffee dust as actually castor bean allergy. Our collateral interests relevant to identification of castor bean allergen² prompted examination of additional specimens of green coffee dust and other coffee products collected from different sources. The results amplify Figley and Rawling's observations¹ and imply that contamination of green coffee with some castor bean derivative is not exceptional.

DESCRIPTION OF SAMPLES

Green Coffee Dusts

Sample T.—Furnished by Dr. Karl D. Figley of Toledo, Ohio, as representative of the material which he used in his clinical studies and described in his report.¹

Sample C.—Furnished by Dr. Leon Unger of Chicago, Ill., from his stock of various by-products of green and roasted coffee. This sample was identical in appearance with Sample T.

Sample W.—Collected at a coffee roasting plant in Washington, D. C. In appearance it was like Samples T and C, except that it contained a minor proportion of whole and broken beans.

Green Coffee Beans

Sample F.—Selected at random from a collection of samples of cleaned and blended green coffee that had been submitted to the Food and Drug Administration for grading in connection with government purchases. This sample was composed entirely of cleaned, sound, whole beans.

Sample P.—Parchment coffee selected from a specimen collected at an agricultural experiment station in Guatemala, by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. Parchment coffee is separated from the coffee cherry by fermentation of the residual cherry pulp, washing in water, and drying. At this stage of processing for export, the coffee bean is enclosed in two membranes: the adherent silverskin (spermoderm), and a surrounding, friable shell suitably called the parchment (endocarp). The specimen of parchment coffee, from which beans with unbroken parchment were selected for Sample P, provided green coffee that had not been exposed directly or indirectly to any contaminant. Sample P was dissected and separated into three parts: the parchment membrane, the silverskin, and the coffee bean.

Sample G.—Cleaned, green coffee beans obtained especially for this investigation by the Office of Foreign Agricultural Relations from Instituto Agro-

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pecuario Nacional, Guatemala, and certified by Rolland C. Lorenz, Director, to be "absolutely free of any contact with castor beans."

Sample GP.—Parchment coffee from the same source as Sample G.

Powdered Soluble Coffee

Sample SC-1.—Brand A.

Sample SC-2.—Brand B.

Powdered soluble coffees are made by rapid drying of a concentrated and filtered water extract of roasted coffee. Accordingly, Samples SC-1 and SC-2 represented a substantial concentration of the water extract of roasted coffee.

PREPARATION OF TEST SOLUTIONS

One part by weight of each of the green coffee dusts was extracted with 9 parts of physiologic saline solution, saturated with toluene. The 1:10 extracts were filtered through filter paper and used for the Dale tests without further treatment. These extracts contained from 0.6 to 0.7 mg. total nitrogen and from 0.4 to 0.5 mg. protein nitrogen (phosphotungstic acid) per ml.

Green coffee beans were ground to pass a 20-mesh sieve before extraction. Saline 1:10 extracts of ground beans contained from 1.3 to 1.9 mg. total nitrogen and from 0.8 to 1.2 mg. protein nitrogen per ml. One preparation of coffee bean extract (Sample F) contained a nonspecific muscle irritant. This was completely removed by dialysis of the extract for 15 hours in running water. Before use in the Schultz-Dale tests, the dialyzed extract was equilibrated with Tyrode solution. About 50 per cent of the nitrogenous components of the extract was removed by dialysis. Experiments with CB-1A had shown that less than 10 per cent of the allergen would be lost under comparable conditions.²

A 1:10 extract of the parchment membrane from Sample P contained 0.06 mg. total nitrogen and 0.03 mg. protein nitrogen per ml. A 1:20 extract of silverskin from Sample P contained 0.4 mg. total nitrogen and 0.3 mg. protein nitrogen per ml.

Ten grams of each of the powdered soluble coffees was dissolved in hot water, cooled, and made up to 50 ml. with cold water. The 1:5 dilutions of Samples SC-1 and SC-2 contained 6.9 and 3.2 mg. total nitrogen and 4.6 and 2.1 mg. protein nitrogen per ml., respectively. Both preparations were dialyzed and equilibrated with Tyrode solution before use. About 80 per cent of the nitrogenous components of each preparation was removed by dialysis.

IDENTIFICATION AND DETERMINATION OF CB-1A IN COFFEE PRODUCTS

Identification and determination of the castor bean allergen, CB-1A, in the extracts of coffee products were conducted by Moody's³ modification of the Schultz-Dale technique as illustrated in Figley and Rawling's report.¹ Each extract was tested first with uterine muscles from nonsensitized guinea pigs to prove the absence of nonspecific, muscle irritants. The smallest dose of each extract required to induce anaphylactic contraction of uterine muscle segments from an animal sensitized with CB-1A was then determined. This dose was

compared with the smallest dose of CB-1A required to produce an equivalent response in uterine tissue from the same animal. From this comparison the approximate concentration of CB-1A in the extract was calculated. Finally, the capacities of the extract and of CB-1A to desensitize to each other were tested for each sample. Without exception, extracts that induced anaphylactic contractions in CB-1A-sensitized muscle desensitized the tissue to subsequent tests with maximal doses of CB-1A. Likewise, reaction with and desensitization to CB-1A desensitized the tissue to subsequent maximal doses of the reactive extracts. On the other hand, nonreactive extracts did not desensitize to minimal doses of CB-1A.

The sensitivity of the quantitative Dale technique is dependent on 3 factors: (1) the sensitivity of the muscle tissue to CB-1A; (2) the concentration of the extract; and (3) the volume of the extract that may be used satisfactorily in the test. For example, if a muscle tissue is sensitive to a minimal dose of 0.011 μg CB-1A and is not responsive to a dose of 5 ml. of a 1:10 extract of coffee beans, then the extract contains less than 0.002 μg CB-1A per ml., and the original sample contains less than 0.02 μg CB-1A per Gm. The limit of sensitivity for this test would be 0.02 p.p.m. of CB-1A.

Previous work in this laboratory⁴ has shown that the sensitizing dose of CB-1A is more than 25 times larger than the shock dose. The present work shows that the Schultz-Dale method is more than 200 times as sensitive for detecting CB-1A as by demonstration of anaphylactic shock and more than 6,000 times as sensitive as by demonstration of sensitization.

The maximal dosage of extract used in these tests was 5 ml. (in 50 ml. Dale baths) except in 1 instance; Sample G was tested at both 5 ml. and 10 ml. dosage levels. Specifically reactive dosages for the green coffee dust extracts ranged from 0.02 to 0.5 ml. Dosages up to 10 ml. induced no response in tissues from control animals.

RESULTS

Table I records the castor bean allergen content of various coffee products calculated as p.p.m. of CB-1A in the original sample. The results are averages of duplicate or triplicate determinations. The last column records the limit of sensitivity of the test for detecting CB-1A in the sample.

Each of 3 green coffee dust samples examined contained castor bean allergen. Concentration of the allergen, calculated as CB-1A, ranged from 1 to 5 p.p.m.

The 1 sample of cleaned, green coffee beans obtained from commercial sources (Sample F) contained castor bean allergen equivalent to 0.08 p.p.m. of CB-1A. It seemed pertinent to determine whether the castor bean allergen in this sample was a surface contaminant or whether it permeated the whole bean. Accordingly, 20 Gm. of the whole bean was washed 4 times with 25 ml. portions of saline. The combined washings were filtered and concentrated in vacuo to about 30 ml. This concentrated extract was dialyzed and equilibrated with Tyrode solution. The beans were then thoroughly washed in running water for 30 minutes, dried, ground, and extracted in the usual manner.

The dialyzed washings from the original whole beans contained allergen equivalent to 0.6 μ g CB-1A which represented 0.03 p.p.m. in the whole beans. On the other hand, no castor bean allergen could be detected in the extract from the washed and ground beans. Evidently, the castor bean allergen was a surface contaminant.

Castor bean allergen was not detectable in extracts from any of the green coffee products obtained from the experiment station in Guatemala. Hence, green coffee does not contain antigen similar to CB-1A.

No castor bean allergen was detected in the powdered soluble coffee.

Attempts to demonstrate castor bean allergen in the green coffee dusts by sensitization were unsuccessful. Uterine muscles from animals sensitized with alum-precipitated coffee dust extracts reacted with the dust extracts but none of them reacted with 0.5 mg. doses of CB-1A. These results coincide with the observation that detection of CB-1A by sensitization is less sensitive than detection by demonstration of anaphylaxis in sensitized animals or tissues.

TABLE I. RESULTS OF ASSAY OF CASTOR BEAN ALLERGEN (CB-1A) IN COFFEE PRODUCTS

SAMPLE NO. AND DESCRIPTION	CB-1A FOUND P.P.M.	LIMIT OF SENSITIVITY P.P.M.
<i>Green Coffee Dusts</i>		
Sample T	1.4	
Sample C	1.0	
Sample W	5.0	
<i>Commercial Green Coffee—Sample F</i>		
Untreated beans, ground and extracted	0.08	
Saline extract of whole beans	0.03	0.004
Washed beans, ground and extracted	None	0.03
<i>Parchment Coffee—Sample P</i>		
Parchment membrane (endocarp)	None	0.03
Silverskin (spermoderm)	None	0.08
Bean (endosperm and embryo)	None	0.03
<i>Green Coffee From Guatemala Experiment Station</i>		
Sample G (cleaned green coffee)	None	0.01
Sample GP (coffee in parchment)	None	0.02
<i>Powdered Soluble Coffee</i>		
Sample SC-1 (Brand A)	None	0.01
Sample SC-2 (Brand B)	None	0.01

DISCUSSION

Unequivocal evidence of castor bean allergen as a contaminant of green coffee has been established. The 3 samples of green coffee dust and 1 sample of cleaned, green coffee beans, in each of which the castor bean allergen was identified, were from green coffee delivered to, or offered for delivery to, wholesale purchasers in Toledo, Chicago, and Washington. Conditions of collection of these samples permit the inference that the 4 samples were not from the same lot or shipment, but evidence to support that inference is lacking. The place of origin and relevant history is known only for the 3 samples of green coffee supplied by the Office of Foreign Agricultural Relations. None of them contained detectable castor bean allergen.

Detection of castor bean allergen in green coffee dust also implies the presence of the active toxalbumin, ricin, of castor bean. In the sensitization experiments animals injected with 2 ml. of the coffee dust extracts showed no evidence of ricin toxicity during the incubational period. However, the dosage used for sensitization would not have contained more than one-tenth of a lethal dose of ricin and, accordingly, would not be likely to induce recognizable symptoms of ricin toxicity. This speculative calculation was based on the assumption that ricin might accompany the castor bean allergen in the proportion found in castor bean. Actually, neither the origin nor identity of the castor bean contaminant is known. Nevertheless, it would be reasonable to assume that ricin would be present whether the contaminant were castor bean or castor pomace.

Extensive examinations of roasted coffee did not appear to be justified by available clinical evidence. Moreover, the experiments recorded here showed that the castor bean contaminant detected in cleaned green coffee was on the surface of the unroasted beans. Observation of the conventional commercial roasting process showed that the surfaces of individual beans are thoroughly exposed to radiation from bare, red-hot, heating elements at a temperature which causes ignition of loosened particles of coffee dust. Presumably, the roasting process would completely inactivate superficial castor bean contaminant. Examination of 2 brands of powdered, soluble coffee failed to reveal any trace of castor bean allergen. However, there was no evidence that these products were derived from contaminated coffee.

SUMMARY

Castor bean allergen was identified in 3 samples of green coffee dust from different coffee roasting plants. Castor bean allergen was also found in 1 sample of cleaned, green coffee beans from a commercial source.

No castor bean allergen was detected in samples of parchment coffee or cleaned, green coffee beans which were produced at a Guatemalan experiment station and were known to have had no contact with castor bean.

Available evidence indicates that hazards resulting from contamination of green coffee with a castor bean derivative are significant only to those who are exposed to green coffee.

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