

Coffee worker's asthma: A clinical appraisal using the radioallergosorbent test

R. M. Karr, M.D., S. B. Lehrer, Ph.D., B. T. Butcher, Ph.D.,
and J. E. Salvaggio, M.D. New Orleans, La.

Eight coffee workers with job-related respiratory symptoms were studied with water-soluble green coffee bean (GCB), castor bean (CaB), and factory dust (FD) antigens. Six workers described occupationally related asthma, rhinitis, conjunctivitis, and urticaria or pruritis and demonstrated positive wheal and flare skin tests with GCB and FD antigens. Serum radioallergosorbent test (RAST) indices ranged from 3 to 15 for GCB and 28 to 60 for CaB specificities. The other 2 coffee workers, who denied allergic symptoms, and 8 atopic and sex-matched control subjects demonstrated negative skin tests and RAST indices <2 with these same antigens. Provocative inhalation challenge (PIC) with GCB in 2 skin test- and RAST-positive subjects resulted in significant immediate asthmatic reactions, while PIC in a skin- and RAST-negative asthmatic subject failed to produce an airway response. GCB and CaB antigen characteristics and industrial sources were studied by RAST inhibition analysis. Lack of crossed RAST inhibition with GCB and CaB preparations showed these antigens to be distinct. Several industrial dust and sack samples produced significant RAST inhibition for GCB or CaB determinants. Chlorogenic acid produced no RAST inhibition for either determinant. The results indicated that coffee workers with occupational allergic disease demonstrate serum IgE antibodies specific for etiologic GCB and CaB antigens and that these antigens are distinct, unrelated to chlorogenic acid, present in certain industrial dust and sack samples, and capable of producing asthma in sensitized subjects.

In 1950, Rawling and Figley¹ described 8 workers in the coffee industry who developed occupational asthma, rhinitis, or both. Positive wheal and flare skin tests to castor bean antigen in 7 of these subjects suggested a major etiologic role for this known potent allergen. Most of these workers also demonstrated a positive wheal and flare skin reaction to an extract of washed green coffee beans. In the same year, Coulson, Spies, and Stevens,² using the Schultz-Dale technique, analyzed samples of green coffee dust and other coffee products from various sources and found significant castor allergen contamination. These stud-

ies seemed to implicate a castor bean component as the likely offending allergen in the occupational allergic disease of coffee workers. Freedman, Krupey, and Sehon,³ in 1961, suggested that a small molecular weight compound, chlorogenic acid, was a major allergen present in both coffee and castor beans. Their findings were challenged by Layton and co-workers,^{4, 5} who showed large molecular weight coffee allergen contamination of the former group's chlorogenic acid preparation. Spirited debate ensued,⁶⁻⁸ which culminated generally in the rejection of any etiologic role for chlorogenic acid in the production of allergic disease.

Although disputes existed over offending allergens, disease pathogenesis proved less controversial. Characteristic immediate-type wheal and flare skin reactions with certain occupational materials⁹ such as green coffee bean, castor bean, and burlap sack and the rapid appearance of symptoms following natural green coffee dust exposure suggested a reagin mediated hypersensitivity reaction. Other evidence came from the work of Layton, Panzani, and Cortese¹⁰ who showed that allergic coffee worker sera could passive-

From the Department of Medicine, Clinical Immunology Section, Tulane University School of Medicine.

Supported in part by the Allergic Disease Center Grant No. AI-13401 from the National Institute of Allergy and Infectious Disease and by the Specialized Center of Research Grant No. HL-15092 from the National Heart, Lung, and Blood Institutes.

Received for publication Feb. 6, 1978.

Accepted for publication May 3, 1978.

Reprint requests to: Reynold M. Karr, M.D., Tulane University School of Medicine, Clinical Immunology Section, 1700 Perdido St., New Orleans, LA 70112.

ly transfer cutaneous green coffee bean hypersensitivity to Macaque monkeys and nonatopic humans.

Nevertheless, the precise nature of these reactions has not been clearly determined, and the relative importance of green coffee and castor allergens has not been established. Indeed, a direct "cause and effect" relationship between these allergens and the asthma of coffee workers has been assumed but never proved. The current study was undertaken to address these issues with the use of the radioallergosorbent test (RAST), RAST inhibition analysis, and provocative inhalation challenge (PIC).

METHODS

Study subjects

Eight coffee workers were seen at the Tulane Medical Center Allergy Clinic because of respiratory symptoms which they associated with their work environment. They were all employed by the same local processing plant and were chosen for study on a basis of their occupation, the occupational nature of their symptoms, and their availability through the clinic. All were men and ages ranged from 27 to 57 yr. Two subjects were coffee roasters, 2 were decaffeinated beans, 2 dumped beans, one was an electrician, and 1 was a foreman. All were exposed to green coffee bean dust. Only 1 worker gave no history of cigarette smoking. Pulmonary function tests demonstrated irreversible obstructive airways disease in 1 worker and mild reversible obstructive airways disease in 2 other workers. The chest roentgenogram of the first worker showed flattening of the diaphragm, but chest roentgenograms of the other workers were normal. Atopic status was determined with a battery of prick tests with 10 common inhalant allergens. Two or more positive tests indicated the presence of atopy in 7 of the 8 coffee workers tested. Prior to investigation, these subjects were divided into two categories based on the historic presence or absence of 4 allergic occupational manifestations: asthma, rhinitis, conjunctivitis, and urticaria or pruritis. Occupational asthma was defined as chest tightness, cough, wheezing, and shortness of breath with work exposure. Occupational rhinitis, conjunctivitis, and urticaria or pruritis were determined in a similar historic fashion. Eight atopic and sex-matched control subjects with no history of exposure to green coffee bean or castor bean products were evaluated in parallel.

Preparation of antigens

Green coffee bean (GCB) and castor bean (CaB) samples were obtained from a separate commercial source and antigens were prepared by initially homogenizing beans in a Waring blender for 3 min and continuing homogenization for an additional 5 min in 1 L phosphate-buffered saline (PBS). Factory dust (FD) antigen was prepared from a dust sample taken from the green bean processing area of the coffee factory. Dust, 100 gm, was directly homogenized in 1 L PBS. Three sack antigens (grass sack from Mexico, burlap sack from Africa, and Santos sack from Brazil) were

prepared by extracting 500 gm of sack material with PBS.

All extracts were stirred at 24° C overnight, filtered through gauze, and centrifuged at 64,000 × G. Pellets were discarded and the supernatants concentrated to approximately 20 ml (Amicon UM10), dialyzed against PBS at 4° C, and centrifuged at 105,000 × G. Dry weights were determined after evaporation of H₂O, and extracts were stored at -20° C.

Detection of antibodies

Skin tests. Intradermal skin test endpoint titration was performed with 0.02-ml volumes of GCB and FD antigens beginning at a 0.01 µg/ml concentration. Production of edema greater than 5 mm in diameter over saline control at 15 min was considered a positive reaction.

Radioallergosorbent test (RAST). Filter paper discs were activated with CNBr according to the method of Ceska and Lundkvist.¹¹ GCB or CaB antigen 20 ml (20 mg/ml in borate buffer, pH 8.0), was added to 500 mg of activated discs in a 50-ml screw-top test tube and incubated for 6 hr at 24° C on a rotator. Fluid was decanted and discs washed 3 times in 1 M ethanolamine, pH 8.0, and left in ethanolamine at 4° C overnight. The next morning, discs were washed 3 times and then frozen at -20° C in assay buffer (500 ml 0.2 M tris buffer, pH 7.5; 500 ml 1.8% NaCl; 10 ml 5% NaN₃; 5 ml Tween 20; 8 gm BSA). Antigen coupled discs were thawed as needed. RAST was performed on duplicate serum samples according to standard procedure (Pharmacia Diagnostics, Piscataway, N. J.). After subtracting background counts, indices of activity were calculated by dividing test sample counts by counts obtained with a nonatopic human serum pool. Indices ≥ 2 were arbitrarily considered positive results.

Paper radioimmunosorbent test (RAST). Total serum IgE antibody was determined by the commercially available PRIST (Pharmacia Diagnostics, Piscataway, N. J.).

Provocative inhalation challenge (PIC)

Pulmonary function parameters were required to be within 80% predicted values before PIC. Twenty consecutive inhalations of PBS or GCB antigen from a Bird IPPB nebulizer (functional residual capacity to total lung capacity) was followed by spirometry at 2 and 8 min. Baseline spirometric values were obtained following PIC with PBS. A 20% fall in 1-sec forced expiratory volume (FEV₁) from baseline following antigen challenge was considered a positive reaction. If no reaction occurred by 8 min, the procedure was repeated with a higher antigen concentration. Beginning concentration of GCB antigen was 0.1 mg/ml and was increased by alternating 2x and 5x multiples to a maximum concentration of 10 mg/ml. Spirometry was obtained at regular intervals for 12 hr following completion of the procedure in order to detect late reactions.

Antigen analysis

The RAST inhibition technique as described by Gleich and associates¹² was used to analyze samples for the presence of either GCB or CaB antigens. The initial competitive

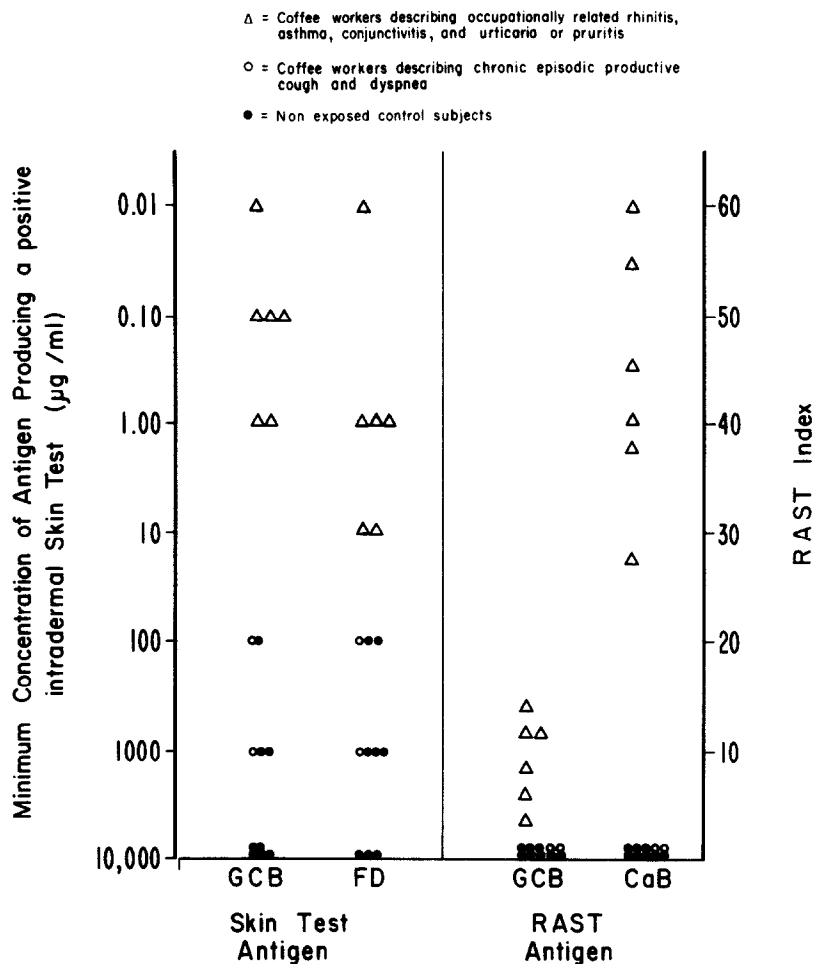


FIG. 1. Skin reactivity and RAST with antigens from the coffee industry. Coffee workers describing occupationally related manifestations of conjunctivitis, asthma, rhinitis, and urticaria or itching all demonstrated significantly greater skin reactivity to GCB and FD antigens than did 2 coffee workers denying these symptoms and 8 unexposed control subjects. A concentration of 1 µg/ml GCB and 10 µg/ml FD appeared to effectively differentiate the 2 groups, with GCB antigen producing the better discrimination. IgE antibodies specific for GCB or CaB antigens as measured by RAST were present in the sera of the 6 allergic coffee workers, but not in the sera of the 2 nonallergic coffee workers or the 8 unexposed control subjects.

inhibition step was performed by incubating an assay serum, containing high-titer IgE antibodies specific for GCB or CaB antigens, with either PBS standard or test sample and with a GCB or CaB antigen coupled disc. The second step was the same as that described for RAST. Inhibition by a given concentration of sample was expressed as a percentage of the PBS standard.

RESULTS

Clinical correlations

Skin tests and RAST. Six coffee workers reported symptoms characteristic of asthma, rhinitis, conjunctivitis, and urticaria or pruritis with occupational exposure. These subjects demonstrated significant skin reactivity to GCB and FD antigens, and serum RAST indices ranging from 3 to 15 for GCB and 28 to

60 for CaB specificities (Fig. 1). Total serum IgE antibody ranged from 170 to 240 IU/ml which was within control group values. The other 2 coffee workers denied allergic symptoms but described chronic intermittent productive cough and shortness of breath. Although these symptoms were aggravated by factory dust, nonoccupational exposure to house dust, smog, cooking odors, or cold weather equally provoked exacerbations. One of these 2 subjects was the only coffee worker demonstrating negative skin reactivity to the screening battery of atopic allergens, irreversible obstructive airways disease by pulmonary function testing, and flattening of the diaphragm on chest roentgenogram. Skin reactivity to GCB and FD antigens in these 2 subjects, as well as titers of serum IgE antibody specific for GCB and CaB antigens,

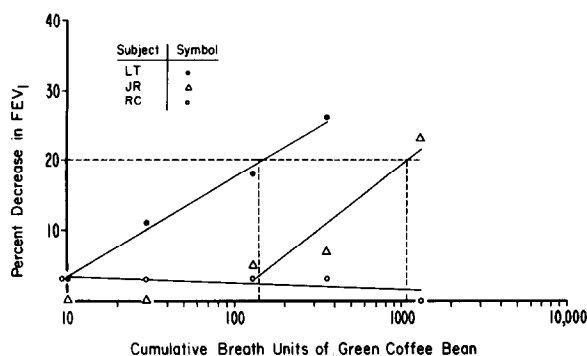


FIG. 2. Provocative inhalation challenge with GCB antigen. Percent fall in FEV₁ was plotted against cumulative breath units of GCB antigen. (One cumulative breath unit has been arbitrarily defined as that amount of antigen delivered by one inhalation of a 0.2 mg/ml concentration from a Bird IPPB nebulizer.) Skin test and RAST-positive coffee workers L. T. and J. R. demonstrated progressive decrease in FEV₁ from baseline (postsaline challenge) with increasing quantities of GCB antigen. In contrast, no significant reaction occurred in Subject R. C., a skin test- and RAST-negative asthmatic subject without history of green coffee bean exposure.

were comparable to values obtained for the 8 control subjects.

Provocative inhalation challenge. PIC with GCB antigen was performed in 2 GCB skin test- and RAST-positive subjects and in one GCB skin test- and RAST-negative asthmatic subject. Data were tabulated by the method of Chai and co-workers¹³ and linear regression lines calculated. The 2 sensitive coffee workers, L. T. and J. R., demonstrated a greater than 20% fall in FEV₁ following PIC (Fig. 2). PD₂₀-FEV₁ values for L. T. and J. R. were 163 and 1,170 cumulative breath units (CBU), respectively. The nonsensitive asthmatic subject, R. C., did not react even at the maximum antigen dose of 1,330 CBU. There were no late reactions up to 12 hr following PIC.

Antigen analysis

GCB antigen inhibited IgE antibodies specific for GCB but not those specific for CaB ($p < 0.0005$). Likewise, CaB antigen inhibited IgE antibodies specific for CaB but not those specific for GCB ($p < 0.005$). Analyses of covariance¹⁴ demonstrated no crossed inhibition between GCB and CaB antigens (Fig. 3 A and B). Chlorogenic acid (Sigma Chemical Corp., St. Louis, Mo.) failed to inhibit IgE antibodies specific for either GCB or CaB specificities (Table I). Inhibition of IgE antibodies specific for GCB was produced by GCB and FD antigens, but not by grass sack, burlap sack, or Santos sack antigens. Inhibition of IgE antibodies specific for CaB was produced by

TABLE I. RAST inhibition analysis for GCB and CaB antigens in samples from the coffee industry

Test sample	Concentration (mg/ml)	Percent inhibition of GCB RAST	Percent inhibition of CaB RAST
GCB	25	89	0
	2.5	83	0
CaB	25	0	88
	2.5	0	78
Chlorogenic acid	5	0	0
	1	0	0
FD	25	25	20
	2.5	0	14
Grass sack	10	0	0
	2	0	0
Burlap sack	10	0	0
	2	0	0
Santos sack	10	0	26
	2	0	22

CaB, FD, and Santos sack, but not by grass sack or burlap sack antigens.

DISCUSSION

Results of the study indicate that serum IgE antibodies specific for either GCB or CaB antigens were unique to the 6 coffee workers describing occupational allergic symptoms. Skin reactivity to GCB and FD antigens in concentrations of 1 and 10 $\mu\text{g/ml}$, respectively, and serum RAST indices greater than 2 with GCB and CaB antigens best discriminated this group from the nonallergic coffee workers and unexposed control subjects. Skin test results confirmed the findings of others^{1, 4, 5, 9} but additionally demonstrated a correlation with elevated titers of IgE antibodies specific for GCB and CaB antigens as determined by the RAST. Preliminary evidence that these tests may identify symptomatic subjects is provided by positive challenges with GCB antigen in 2 skin-reactive and RAST-positive coffee workers. However, PD₂₀-FEV₁ values, which reflect relative airway sensitivity to GCB antigen, did not parallel serum RAST indices with GCB. This finding is not unexpected and may mean that airway lability rather than serum reagent titer is the more important factor determining airway sensitivity to antigen. Also, serum reagent levels may not directly reflect amount of reagent bound to bronchial mast cells. Nevertheless, the PIC response demonstrated the asthmogenic potential of GCB and supported its importance as a significant occupational antigen. Although PIC with CaB antigen was not performed (due to its known potent allergenic characteristics), it is likely that positive challenges

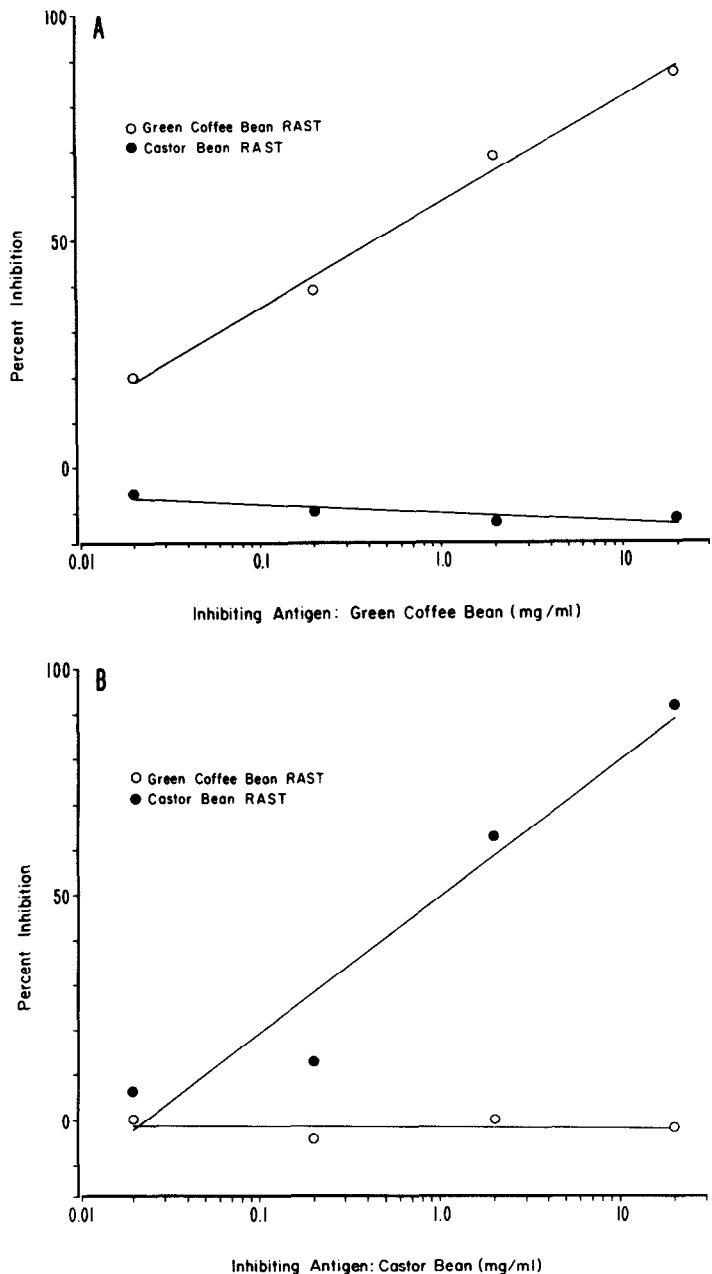


FIG. 3. Analysis of GCB and CaB antigens for common antigenic determinants. *A*, RAST inhibition with GCB antigen. Serial increases of GCB antigen inhibited RAST-detecting IgE antibodies specific for GCB; however, GCB antigen did not inhibit RAST-detecting IgE antibodies specific for CaB ($p < 0.0005$). *B*, RAST inhibition with CaB antigen. Serial increases of CaB antigen inhibited RAST-detecting IgE antibodies specific for CaB; however, CaB antigen did not inhibit RAST-detecting IgE antibodies specific for GCB ($p < 0.005$).

would have also occurred since serum titers of IgE antibodies specific for CaB antigen were even higher than those for GCB antigen in the sensitive subjects tested. Furthermore, allergic asthma resulting from exposure to castor bean dust has been previously recognized and well described.^{9, 15, 16}

RAST technology has been used to investigate

other types of occupational asthma. IgE antibodies specific for *Bacillus subtilis* have been detected in the sera of workers from the detergent industry,¹⁷ and a similar radioimmunoassay with polystyrene tubes as the solid phase has enabled demonstration of IgE antibodies specific for trimellitic anhydride conjugates in sera of workers from the plastics industry.¹⁸ This

report expands the list of occupational exposures in which RAST has been successfully used to study allergic disease. It has also provided another basic tool for epidemiologic surveys of respiratory disease in coffee workers.

Antigen analysis by the RAST inhibition technique with a single high-titer patient serum provided good evidence that GCB and CaB preparations were antigenically distinct. Although other investigators had reached the same conclusion with bioassays using sensitized guinea pig ileum² and mouse PCA,¹⁹ our data extended these findings to man and provided a quantitative immunochemical technique for analyzing industrial samples. It is possible that common antigenic determinants exist in quantities less than those detectable by the microgram limits of RAST inhibition sensitivity, but such log-fold differences in antigenic potency do not exist in any comparable system making this possibly unlikely. We have, therefore, concluded that GCB and CaB antigens are distinct.

RAST inhibition analyses with chlorogenic acid in significant concentrations failed to demonstrate common antigens with the GCB or CaB preparations and supported the findings of Lowell⁶ and Layton and co-workers^{4, 8}, who have dismissed any role for chlorogenic acid as an allergen in this system. Analysis of the FD antigen preparation, used in skin testing, revealed the presence of small but significant quantities of both GCB and CaB antigens and suggested another source of industrial exposure. None of the 3 sack preparations contained GCB antigen and CaB antigen was demonstrated only in the Santos sack from Brazil. The discovery of CaB antigen in FD and in coffee sack is consistent with the results of investigations with other techniques.^{1, 2, 9} Brazil produces castor beans, and it has been recognized that sacks used in their transport are often reused for the transport of coffee beans.⁹ Since the allergic coffee workers described significantly greater symptoms with exposure to green coffee produce from Brazil, it is intriguing to speculate that CaB contamination of these Brazilian sacks is responsible. Thus, by use of RAST inhibition to analyze industrial samples for etiologic antigens, patterns of occupational disease may be better understood and etiologic agents more effectively monitored.

We wish to express our thanks to Mr. Vincent Anicetti for his technical assistance and Miss Velda Force for her aid in the preparation of the manuscript.

REFERENCES

1. Figley, K. D., and Rawling, F. A.: Castor bean: An industrial hazard as a contaminant of green coffee dust and used burlap bags, *J. ALLERGY* **21**:545, 1950.
2. Coulson, E. J., Spies, J. R., and Stevens, H.: Identification of castor bean allergen in green coffee, *J. ALLERGY* **21**:554, 1950.
3. Freedman, S. O., Krupey, J., and Schon, A. H.: Chlorogenic acid: An allergen in green coffee bean, *Nature* **192**:241, 1961.
4. Layton, L. L., Greene, F. C., Panzani, R., and Corse, J. W.: Allergy to green coffee. Failure of patients allergic to green coffee to react to chlorogenic acid, roasted coffee, or orange, *J. ALLERGY* **36**:84, 1965.
5. Layton, L. L., Panzani, R., Greene, F. C., and Corse, J. W.: Atopic hypersensitivity to a protein of the green coffee bean and absence of allergic reactions to chlorogenic acid, low-molecular-weight components of green coffee, or to roasted coffee, *Int. Arch. Allergy* **28**:116, 1965.
6. Lowell, F. C.: Chlorogenic acid again. *J. ALLERGY* **38**:276, 1966. (Letter.)
7. Freedman, S. O., Krupey, J., and Schon, A. H.: Further comments on the allergenicity of chlorogenic acid, *J. ALLERGY* **38**:277, 1966. (Letter.)
8. Layton, L. L.: Reply, *J. ALLERGY* **38**:279, 1966. (Letter.)
9. Bernton, H. S.: On occupational sensitization—A hazard to the coffee industry, *J. A. M. A.* **223**:1146, 1973.
10. Layton, L. L., Panzani, R., and Cortese, T. A.: Coffee—reagin human sera tested in human volunteers and macaque monkeys. Absence of reactions to chlorogenic acid, *Int. Arch. Allergy* **33**:417, 1968.
11. Ceska, M., and Lundkvist, V.: A new and simple radioimmunoassay method for the determination of IgE, *Immunochemistry* **9**:1021, 1972.
12. Gleich, G. J., Larson, J. B., Jones, R. T., and Baer, H.: Measurement of the potency of allergy extracts by their inhibitory capacities in the radioallergosorbent test, *J. ALLERGY CLIN. IMMUNOL.* **53**:158, 1974.
13. Chai, H., Farr, R. S., Froelich, L. A., Mathison, D. A., McLean, J. A., Rosenthal, R. R., Sheffer, A. L., Spector, S. L., and Townley, R. G.: Standardization of bronchial inhalation challenge procedures, *J. ALLERGY CLIN. IMMUNOL.* **56**:323, 1975.
14. Dixon, W. J., and Massey, F. J., Jr.: Introduction to statistical analysis, New York, 1957, McGraw-Hill Book Company, Inc., p. 209.
15. Bernton, H. S.: On occupational sensitization to the castor bean, *Am. J. Med. Sci.* **165**:196, 1923.
16. Layton, L. L., Yamanaka, E., Lee, S., and Green, T. W.: Multiple allergies to the pollen and seed antigens of *Ricinus communis* (castor bean), *J. ALLERGY* **33**:232, 1962.
17. Pepys, J., Wells, I. D., D'Souza, M. F., and Greenberg, M.: Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers, *Clin. Allergy* **3**:143, 1973.
18. Zeiss, C. R., Patterson, R., Pruzansky, J. J., Miller, M. M., Rosenberg, M., Suszko, I., and Levitz, D.: Immunologic and clinical aspects of trimellitic anhydride respiratory disease, *J. ALLERGY CLIN. IMMUNOL.* **60**:96, 1977.
19. Lehrer, S. B., Karr, R. M., and Salvaggio, J. E.: Analysis of allergens present in green coffee bean, *Clin. Allergy* (In press.)