

# Bronchoprovocation studies in coffee worker's asthma

Reynold M. Karr, M.D. Everett, Wash.

Occupational asthma in affected workers is generally defined as asthma resulting from an inhalant exposure to a specific etiologic agent used in industry. The offending agent may occur in 1 of 2 forms, either as a particulate or as a gas. Procedures for human bronchoprovocation (BP) studies have been developed for both forms, and I will use the example of coffee worker's asthma to illustrate such testing with a particulate occupational antigen. Following my article, Drs. Butcher and Gerblich et al. will use the example of toluene diisocyanate (TDI)-induced asthma to illustrate the application of bronchoprovocation techniques where an offending gas or vapor is involved. It should be emphasized at this point that the technique of bronchoprovocation, when performed with appropriate controls, is at present the single best way to prove a direct "cause and effect" relationship between a suspect antigen and occupational asthma.

The study of any form of occupational asthma requires a stepwise approach. The first step is to identify those industrial agents most likely to be important in the production of disease. Studies reported in the literature and an epidemiologic analysis of affected workers provide the basis for making this determination. Using coffee worker's asthma as an example, work dating back to the 1950s suggests a reagin-mediated reaction to inhaled green coffee and contaminating castor bean allergens.<sup>1-5</sup> In a recent evaluation of a local New Orleans coffee processing plant, all symptomatic subjects worked in areas of the plant concerned with the processing of the raw green beans. In contrast, there was a striking lack of allergic symptoms in workers who processed only roasted beans. Processing of beans begins with the manual transfer of beans from their sacks into a dump bin (Fig. 1). After jets of air in the coffee cleaner remove most of the stems, sack remnants, and other large debris, the beans are transferred to hold bins where the chaff is removed. The beans are then roasted, cooled, and then transferred to another hold bin from which they may be processed further by decaffeination, extrac-

tion and freeze-drying, grinding, or direct packaging. All of the symptomatic subjects worked in either the dumping or roasting areas. Workers dumping the green beans appeared to complain of the most severe symptoms. We therefore selected green coffee beans, castor beans, and a dust sample from the dumping area for antigen preparation.

The second step is to properly prepare the test materials. All antigens should be sterile and in a water-soluble form. Insoluble particles should not be used in human bronchoprovocation studies at the present time. Since most occupational antigens are not available commercially, it will therefore be instructive to briefly describe a method for antigen preparation. Green coffee bean (GCB) and castor bean (CaB) antigens were prepared from bean samples obtained from the plant and commercial sources (Table I). Beans were homogenized in a Waring blender for 3 min and homogenized for an additional 5 min with 1 l phosphate buffered saline (PBS). A factory dust (FD) sample, 100 gm, was taken from the dumping area and directly homogenized in 1 l PBS. All extracts were mixed at 24° C for 18 hr, filtered through gauze, and centrifuged at 44,000 × g. The extracts were concentrated to about 20 ml (Amicon UM 10), dialyzed against PBS at 4° C, and centrifuged at 105,000 × g. Dry weights were determined, cultures taken, and extracts stored at -20° C.

The third step is to determine whether reagenic antibody, specific for the prepared suspect antigen, is detectable in symptomatic workers. This may be done by skin testing or use of the radioallergosorbent test. Such studies were performed on the 8 symptomatic coffee workers from the processing plant previously mentioned<sup>5</sup> (Fig. 2). Six of these workers described symptoms of asthma, allergic rhinitis, conjunctivitis, and urticaria with occupational exposure, while the other 2 workers described only nonspecific respiratory symptoms aggravated by both occupational and nonoccupational factors. Coffee workers with allergic symptoms demonstrated significantly increased skin reactivity to GCB and FD antigens. They also demonstrated elevated serum RAST ratios for both GCB and CaB antigens. These procedures not only identified these workers as hypersensitive to these occu-

Reprint requests to: Dr. Reynold M. Karr, Clinical Immunology Laboratory, 3030 Hoyt Ave., Everett, WA 98201.

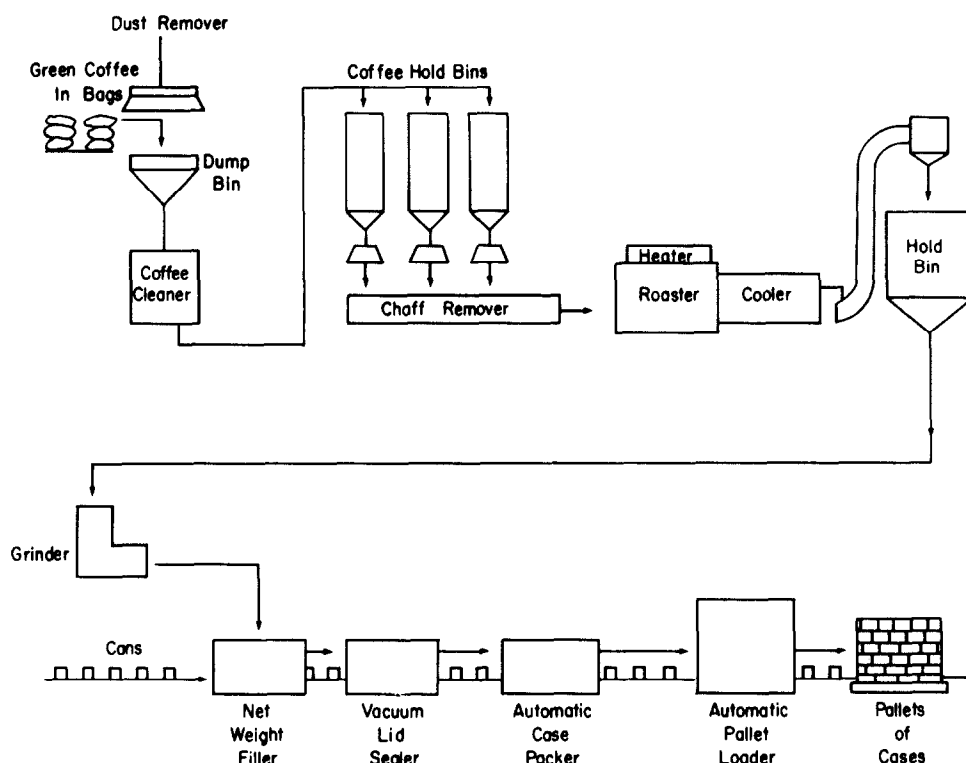


**FIG 1.** Vacuum-packed coffee processing. Bags of green coffee beans are emptied into a large dump bin, which feeds the beans to the coffee cleaner. Jets of air clean away stems, sack remnants, and other large debris. After storage in hold bins, the beans are passed into the chaff remover. They are then roasted, allowed to cool, and stored in another hold bin. Further processing may include grinding, extraction and freeze-drying, decaffeination, or direct packaging. All affected workers developed allergic symptoms with exposure to those areas of the plant concerned with dumping, cleaning, and roasting green coffee beans.

pational agents but additionally showed the bean components to be antigenically active in man.<sup>6</sup> It should be mentioned that this kind of immunologic analysis may not always be rewarding in the study and diagnosis of occupational asthma, as will be seen in the case of TDI exposure.

The fourth step is the BP procedure itself.<sup>7</sup> I will use a study of coffee workers to illustrate how the technique may be applied to any type of occupational asthma where a particulate antigen is suspected. BP tests with extracted GCB antigen were performed on 3 symptomatic, allergic coffee workers and on a non-sensitive, previously unexposed asthmatic subject who served as a negative control. Pulmonary function parameters, specifically 1-sec forced expiratory volume (FEV<sub>1</sub>) and peak flow, were required to be within 80% of predicted values before challenge. Twenty consecutive inhalations of PBS or GCB antigen were administered by a Bird IPPB\* nebulizer, with the subject inhaling from functional residual capacity to total lung capacity. Any suitable pressurized delivery sys-

**TABLE I.** Extraction of allergens from beans

1. Homogenization of beans in PBS
2. Extraction overnight at 24° C
3. Filtration of extract through gauze
4. Centrifugation of filtrate (44,000 × g)
5. Concentration of supernate on Amicon UM 10
6. Dialysis of concentrate against PBS
7. Centrifugation of dialyzed material (105,000 × g)
8. Culture and storage of supernate at -20° C

tem such as a dosimeter or Maximyst is adequate. Recent data would indicate that ultrasonic nebulization and delivery of a low-flow mist may maximize distribution to small airways and minimize deposition of antigen in the oropharynx. Accordingly, my co-workers and I recently began using a DeVilbiss ultrasonic 35B nebulizer with low-flow delivery and have obtained results that appear to be somewhat more reproducible. Further studies will be needed to determine whether this innovation represents a real advantage. Spirometry was done at 2 and 8 min following the 20 inhalations. A 20% fall in FEV<sub>1</sub> (from post-PBS

\*Intermittent positive pressure breathing.

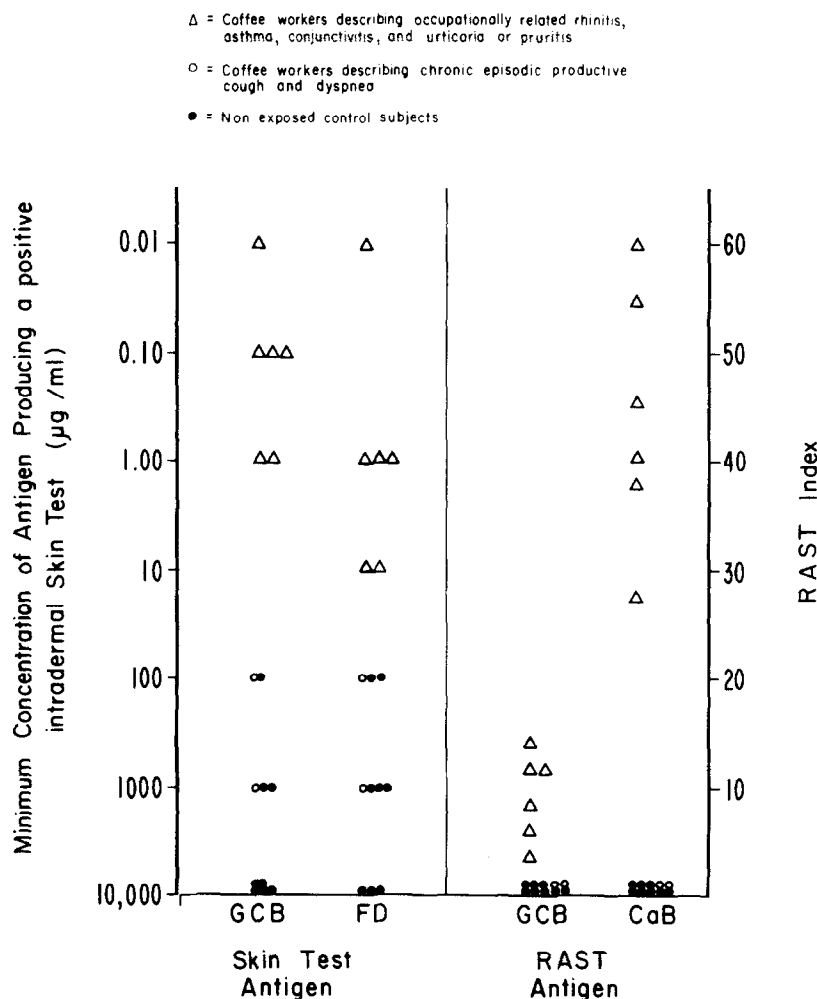


**FIG. 2.** Skin reactivity and RAST with antigens from the coffee industry. Coffee workers, describing occupationally related manifestations of conjunctivitis, asthma, rhinitis, and urticaria or itching, all demonstrated significantly greater skin reactivity to GCB and FD antigens than did 2 coffee workers denying these symptoms and 8 unexposed control subjects. 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.

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 two-fold and fivefold multiples to a maximum concentration of 10 mg/ml. Spirometry and peak flow measurements were obtained at regular intervals for 24 hr following completion of the procedure in order to detect late reactions. The results may commonly be expressed in either of 2 ways. First, the absolute value of  $FEV_1$  or the percent fall in  $FEV_1$ , may be plotted against time (Fig. 3). In the second method,  $FEV_1$  is plotted against the total amount of antigen administered, which is in cumulative breath units (CBU) (Fig.

4). One CBU may be arbitrarily defined as that amount of antigen administered by 1 inhalation of a 2 mg/ml nebulized solution. Fig. 3 illustrates a small drop in  $FEV_1$  following administration of the PBS control. It is important to subtract this value when determining the baseline from which to calculate the 20% fall in  $FEV_1$ . Fig. 4 indicates a positive challenge in subjects L. T. and J. R. This means of expressing the data also allows for the calculation of  $PD_{20}FEV_1$  (provocation dose inducing 20% fall in  $FEV_1$ ) values for each subject. These values, which inversely reflect relative airway sensitivity to antigen, were 163 for L. T. and 1,170 for J. R., indicating that L. T. demonstrated greater relative airway sensitivity. Challenge of a previously unexposed asthmatic sub-

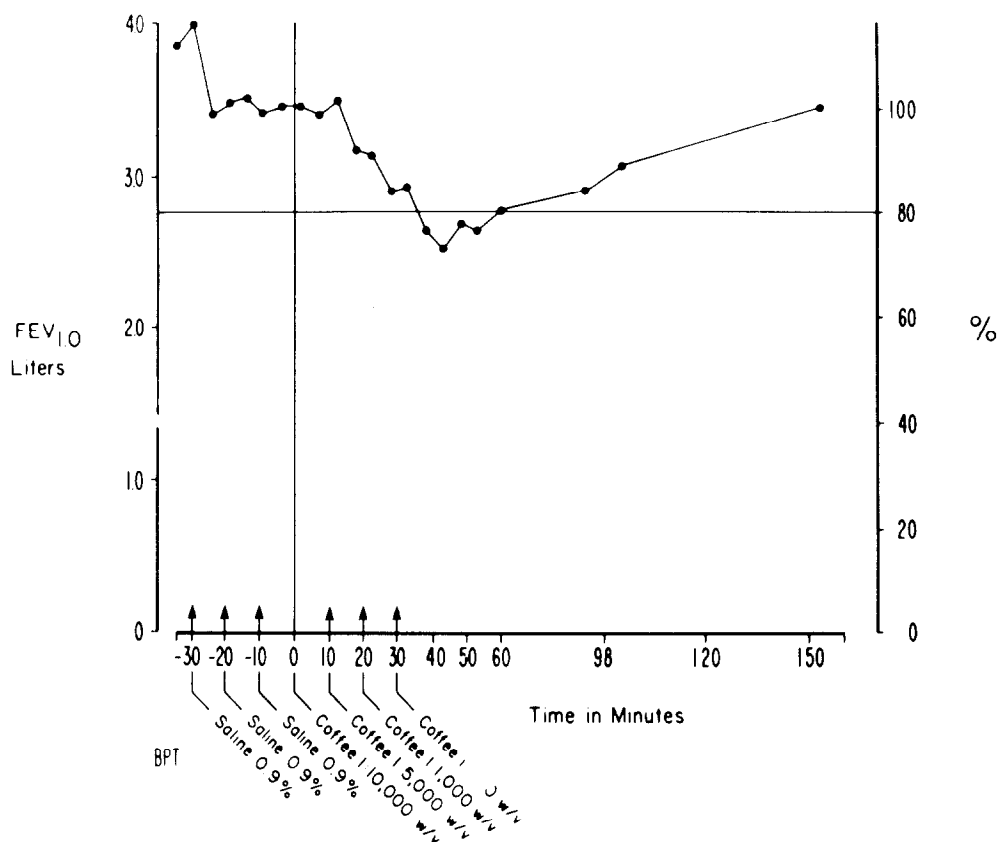


**FIG. 3.** BP with increasing concentrations of soluble green coffee bean extract.  $FEV_1$  was plotted against time. Arrows indicate administration of antigen or PBS. Each antigen concentration was delivered in 20 consecutive inhalations (functional residual capacity to total lung capacity) by a Bird IPPB nebulizer. The resultant decremental change in  $FEV_1$  reached a nadir of 27% following challenge with a 1:500 w/v concentration of antigen.  $FEV_1$  spontaneously returned to baseline values by 2½ hr after challenge. There were no late reactions.

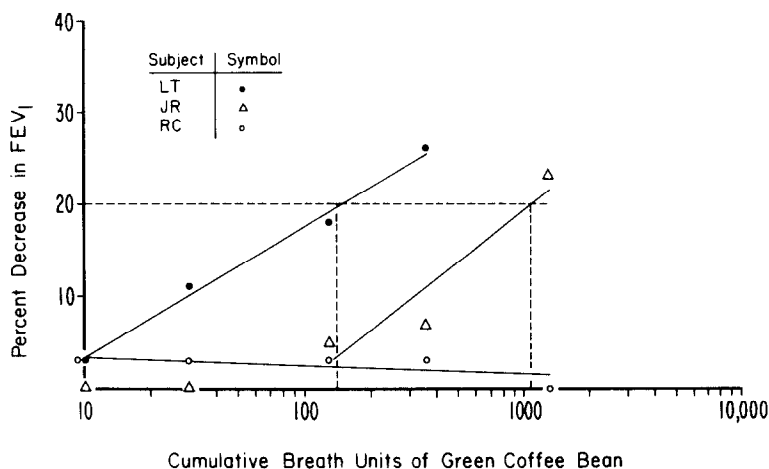


**FIG. 4.** Bronchoprovocation with green coffee bean antigen. Percent baseline  $FEV_1$  was plotted against CBU of GCB antigen. (One CBU has been arbitrarily defined as that amount of antigen delivered by 1 inhalation of a 2.0 mg/ml concentration from a Bird IPPB nebulizer.) Skin test- and RAST-positive coffee workers L. T. and J. R. demonstrated progressive decreases in  $FEV_1$  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 a history of green coffee bean exposure.

ject, R. C., resulted in no significant airway response and excluded a nonspecific irritant or pharmacologic effect of the antigen.

In conclusion, bronchoprovocation with a soluble extract of particulate antigen is an important tool in the investigation of occupational asthma as illustrated by the coffee worker study. It is the most direct way to prove the pathogenic potential of an occupational agent and to identify sensitive subjects.

#### REFERENCES

1. Figley KD, Rawling FA: Castor bean: An industrial hazard as a contaminant of green coffee dust and used burlap bags. *J ALLERGY* **21**:545, 1950.
2. Coulson EJ, Spies JR, Stevens H: Identification of castor bean allergen in green coffee. *J ALLERGY* **21**:554, 1950.
3. Bernton HS: On occupational sensitization—A hazard to the coffee industry. *JAMA* **223**:1146, 1973.
4. Layton LL, Panzani R, Cortese TA: Coffee-reaginic human sera tested in human volunteers and macaque monkeys. Absence of reactions to chlorogenic acid. *Int Arch Allergy* **33**:417, 1968.
5. Lehrer SB, Karr RM, Salvaggio JE: Analysis of allergens present in green coffee bean. *Clin Allergy* **8**:217, 1978.
6. Karr RM, Lehrer SB, Butcher BT, Salvaggio JE: Coffee worker's asthma: A clinical appraisal using the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* **62**:143, 1978.
7. Karr RM, Davies RJ, Butcher BT, Lehrer SB, Wilson MR, Dharmarajan V, Salvaggio JE: Occupational asthma. *J ALLERGY CLIN IMMUNOL* **61**:54, 1978.